

**HAEMATOLOGICAL AND SERUM BIOCHEMICAL VALUES OF SOME
SPECIES OF APPARENTLY HEALTHY FREE-LIVING WILD BIRDS IN ZARIA,
NIGERIA**

BY

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NIGERIA**

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NIGERIA**

BY

**James Enam SAMSON, DVM (ABU) 2010
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**DEPARTMENT OF VETERINARY PATHOLOGY,
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA**

APRIL, 2015

DECLARATION

I declare that the work in the thesis entitled, “**Haematological and Serum Biochemical Values of Some Species of Apparently Healthy Free-living Wild Birds in Zaria, Nigeria**” has been performed by me in the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria under the supervisions of Professors S. B. Oladele, P. A. Abdu and Dr. N. M. Useh. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree, diploma or certificate at any university.

James Enam SAMSON

Name of student

Signature

Date

CERTIFICATION

This Thesis entitled, “**HAEMATOLOGICAL AND SERUM BIOCHEMICAL VALUES OF SOME SPECIES OF APPARENTLY HEALTHY FREE-LIVING WILD BIRDS IN ZARIA, NIGERIA**” by James Enam, SAMSON meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This Thesis is dedicated to my late mother, Mrs. C. S. Yaks who planted in me the “seed” to always aim for greatness.

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ABSTRACT

This research was carried out to establish baseline haematological and serum biochemical parameters of some species of apparently healthy free-living wild birds in Zaria, Nigeria. A total of 240 free-living wild birds, belonging to 12 species, comprising 20 each of Laughing Dove (*Streptopelia senegalensis*), Bruce's Green Pigeon (*Treron waalia*), Speckled Pigeon (*Columba guinea*), Mourning Collar-Dove (*Streptopelia decipiens*), Senegal Parrot (*Poicephalus senegalus*), Rose-Ringed Parakeet (*Psittacula krameri*), Little Weaver Bird (*Ploceus luteolus*), Cattle Egret (*Bubulcus ibis*), White-Rumped Swift (*Apuscaffer*), Red-Billed Quelea (*Quelea quelea*), African Silver-Billed (*Euodicecantans*) and Northern Red Bishop (*Euplectes frascisca*) were used for the experiment. Haematocrit and Natt-Herrick's method were used for the packed cell volume, and total white and red cell counts, respectively, and thin blood smear technique was used for the differential leucocyte and estimated thrombocyte counts. The Audiocomb serum auto-analyser was used for biochemical analyses. The highest values for PCV ($46.25 \pm 1.43\%$), Hb concentration (17.75 ± 5.31 g/dl) and RBC count ($5.24 \pm 0.32 \times 10^{12}/l$) were obtained from the apparently healthy White-Rumped Swift (WRS), Cattle Egret (CE) and African Silver Billed (ASB), respectively. The lowest PCV ($31.98 \pm 1.67\%$) and Hb concentration (11.81 ± 0.56 g/dl) were obtained from the apparently healthy Bruce's Green Pigeon (BGP), while the apparently healthy CE had the lowest RBC count ($2.86 \pm 0.14 \times 10^{12}/l$). The apparently healthy Senegal Parrot (SNG) had the highest TWBC ($3.36 \pm 0.44 \times 10^9/l$), and also had similar value with the apparently healthy BGP for highest value for monocyte count ($0.13 \pm 0.03 \times 10^9/l$). The apparently healthy WRS had the highest eosinophil ($0.15 \pm 0.04 \times 10^9/l$) and basophil ($0.08 \pm 0.02 \times 10^9/l$) counts. The apparently healthy Rose-Ringed Parakeet (RRP) and CE had the highest lymphocyte ($2.47 \pm 0.23 \times 10^9/l$) and heterophil ($0.62 \pm 0.11 \times 10^9/l$) counts,

respectively. The apparently healthy ASB had the lowest TWBC ($0.63 \pm 0.08 \times 10^9/l$), lymphocyte ($0.54 \pm 0.08 \times 10^9/l$), heterophil ($0.04 \pm 0.02 \times 10^9/l$), basophil ($0.01 \pm 0.00 \times 10^9/l$) and monocyte ($0.01 \pm 0.00 \times 10^9/l$) counts, and also had similar lowest mean values with the apparently healthy Northern Red Bishop (NRB) and Red Billed Quelea (RBQ) for eosinophil ($0.01 \pm 0.00 \times 10^9/l$) count. The H/L ratio was highest in apparently healthy ASB (1.95 ± 1.90) and lowest in Morning Collar Dove (MCD) (0.08 ± 0.01). Apparently healthy SNG had the highest estimated thrombocyte count ($0.92 \pm 0.17 \times 10^9/l$), while apparently healthy ASB had the lowest mean estimated thrombocyte count ($0.06 \pm 0.01 \times 10^9/l$). The highest glucose (154.70 ± 5.72 mg/dl), creatinine (0.49 ± 0.02 mg/dl) and urea (5.53 ± 0.28 mg/dl) concentrations were obtained from the apparently healthy MCD, BGP and Speckled Pigeon (SP), respectively. Except for serum calcium concentration (2.64 ± 0.05 mg/dl) which was highest in the apparently healthy CE, the glucose (139.20 ± 4.64 mg/dl), urea (4.24 ± 0.15 mg/dl), total protein (6.65 ± 0.10 g/dl) and albumin (3.77 ± 0.10 g/dl) concentrations were lowest in this species of bird. The apparently healthy Laughing Dove (LD) and SNG had the lowest creatinine concentration (0.43 ± 0.01 mg/dl), but the latter recorded highest albumin concentration (3.99 ± 0.10 g/dl). The total protein (6.93 ± 0.10 g/dl) and globulin concentration (2.97 ± 0.04 g/dl) were highest in the apparently healthy LD, although it recorded lowest calcium (2.45 ± 0.02 mg/dl) and phosphorus (0.83 ± 0.06 mg/dl) concentrations. The apparently healthy RRP had the highest phosphorus concentration (1.05 ± 0.03 mg/dl) and lowest globulin fraction (2.89 ± 0.05 g/dl). The overall range values for PCV, Hb, RBC, TWBC, heterophil, lymphocyte, basophil, eosinophil, monocyte counts, H/L ratio and thrombocyte count for all the free-living wild birds used in this study were 31.98 ± 1.67 to $46.25 \pm 1.43\%$, 11.81 ± 0.56 to 17.75 ± 5.31 g/dl, 2.86 ± 0.14 to $5.24 \pm 0.3 \times 10^{12}/l$, 0.63 ± 0.08 to $3.36 \pm 0.44 \times 10^9/l$, 0.04 ± 0.02 to $0.62 \pm 0.11 \times 10^9/l$, 0.54 ± 0.08 to

2.47±0.23 x10⁹/l, 0.01±0.00 to 0.08±0.02 x10⁹/l, 0.01±0.00 to 0.15±0.04 x10⁹/l, 0.01±0.00 to 0.13±0.03 x10⁹/l, 0.08±0.01 to 1.95±1.90 and 0.06±0.01 to 0.92±0.17 x10⁹/l, respectively. The overall range values for serum glucose, urea, creatinine, TP, albumin, globulin, calcium and phosphorus concentration for all the free-living wild birds used in this study were 139.20±4.64 to 154.70±5.72 mg/dl, 4.24±0.15 to 5.53±0.28 mg/dl, 0.43±0.01 to 0.49±0.02 mg/dl, 6.65±0.10 to 6.93±0.10 g/dl, 3.77±0.10 to 3.99±0.10 g/dl, 2.89±0.05 to 2.97±0.04 g/dl, 2.45±0.02 to 2.64±0.05 mg/dl and 0.83±0.06 to 1.05±0.03 mg/dl, respectively. It was concluded that there were interspecies differences in both haematological and serum biochemical parameters of the apparently healthy free-living wild birds studied.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	– per cent
µl	– microlitres
°C	– degree centigrade
A:G	– albumin/globulin ratio
ALT	– alanine aminotransferase
AP	– alkaline phosphatase
ASB	- African-silver billed
AST	– aspartate aminotransferase
BGP	- Bruce's green pigeon
BUN	– blood urea nitrogen
CE	- Cattle egret
CK	– creatine kinase
cm	– centimetre
CPK	– creatine phosphokinase
DIC	– disseminated intravascular coagulopathy
dl	– decilitre
E	– east
EDTA	– ethylene diamine tetra acetic acid
ELISA	– enzyme-linked immunosorbent assay
fl	– femtolitres
ft	– feet
g	– grammes

GGT – gamma glutamyltransferase
GI – gastrointerstinal
GLDH – glutamate dehydrogenase
GOT – glutamate oxalate transaminase
GPT – glutamate pyruvate transaminase
H/L – heterophils/lymphocytes ratio
Hb – haemoglobin
in – inch(es)
kg – kilogrammes
km – kilometre
l – litres
LD - Laughing dove
LDH – lactate dehydrogenase
LDH – lactate dehydrogenase
LW - Little weaver
m – metres
MCD - Mourning collar dove
MCH – mean corpuscular haemoglobin
MCHC – mean corpuscular haemoglobin concentration
MCV – mean corpuscular volume
mg – milligrammes
ml – millilitre
mm – millimetre
N – North

N/C – nucleus/cytoplasm ratio
NRB - Northern red bishop
PCV – packed cell volume
pg – picogrammes
r – Rotation radius in millimeter
RBC – red blood cells
RBQ - Red billed quelea
RCF – centrifugal force
RIA – radio-immunoassay
RPM – rotation per minute
RRP - Rose-ringed parakeet
SE – standard error
SNG - Senegal parrot
SP – Speckled pigeon
TWBC – total white blood cells
WBC – white blood cells
WRS - White-rumped swift
 $x g$ – gravitation force

CHAPTER ONE

INTRODUCTION

1.1 Background Knowledge

Birds live and breed in the terrestrial (aboreal) habitats on all the seven continents (Brooke, 2004). It is believed that the highest bird diversity occurs in tropical regions. It was earlier thought that this high diversity was the result of higher speciation rates in the tropics, however, studies have found higher speciation rates in the high latitudes that were offset by greater extinction rates than in the tropics (Weir and Schluter, 2007).

Many bird species migrate to take advantage of global differences of seasonal temperatures, therefore, optimising availability of food sources and breeding habitat. These migrations vary among the different groups of bird (Klaassen, 1996; Battley *et al.*, 2000).

Since birds are highly visible and common, animals and humans have had relationships with them since the dawn of man (Bonney and Rohrbaugh, 2004). Sometimes, these relationships are mutualistic, like the cooperative honey-gathering among honeyguides of Borana in Africa (Dean *et al.*, 1990). Other times, the relationship may be commensal, as when species, such as the House Sparrows have benefited from human activities (Singer and Yom-Tov, 1988). Several bird species have become commercially significant as agricultural pests (Dolbeer, 1990). Human activities, such as hunting, lead and pesticides poisoning, road kill can also be detrimental, and have threatened numerous bird species with extinction. Predation by pet cats and dogs are common sources of death for birds (Beyer *et al.*, 2000; Wight, 2002; Fisher *et al.*, 2006).

Birds can act as vectors for spreading diseases, such as psittacosis, salmonellosis, campylobacteriosis, mycobacteriosis (avian tuberculosis), avian influenza (bird flu),

giardiasis, cryptosporidiosis and Newcastle disease over long distances. Some of these diseases are zoonoses that can also be transmitted to humans (Reed *et al.*, 2003; Oladele *et al.*, 2011).

Investigations into the baseline haematological and serum biochemical parameters of animals have received increasing attention in recent years in both the temperate and tropical countries. There are scientific and economic reasons for attempting to establish normal haematological and serum biochemical parameters of healthy animals in the tropics. For example, in biological research where many species of animals are involved, it is important to establish normal values for purposes of comparison with naturally or experimentally induced changes in the body of animals (Oladele, 2009).

The values of haematological and biochemical indices in domestic and wild birds could be important sources of information with valuable diagnostic significance. They could provide an objective assessment of the health status, and support the correct diagnosis in different pathological states (Miller *et al.*, 2001; Hauptmanová *et al.*, 2002; Scope *et al.*, 2002; Pavlak *et al.*, 2005). Considering the significantly extended spectrum of animal species under veterinary care, the data on haematological and biochemical indices of wild bird species are particularly important in a given geographical area. This is because these data could be a signal for an impaired ecological equilibrium influenced by different factors, such as age, season, stress and illnesses (Piersma *et al.*, 2000; Seiser *et al.*, 2000; Nava *et al.*, 2001; Scope *et al.*, 2002; Villegas *et al.*, 2004).

1.1 Statement of Research Problems

In Veterinary Medicine, blood analyses have been performed much less often in avian medicine in comparison to its routine use in mammalian species (Oladele, 2009). Haematological and serum biochemistry determinations during diagnosis are among the methods, which are used to detect some subclinical changes in health status which affect the fitness of the birds (Cooper, 1998; Oladele, 2000; Harr, 2002; Naidoo *et al.*, 2008).

It is established that the haematological and serum biochemical parameters of animals vary depending on factors, such as prevailing environmental condition, sex and nutrition (Oladele, 2009). Despite the growing number of haematological studies in birds (Moreno *et al.*, 1998; Hauptmanová *et al.*, 2002; Uhart *et al.*, 2003; Davis *et al.*, 2004; Sergent *et al.*, 2004; Friedl and Edler, 2005; Mercurio *et al.*, 2008), data on blood cellular composition are still only available for a limited number of bird species, and the majority of such data on wild avian blood parameters are obtained for waterfowl, raptors or wildlife maintained in zoos or aquaria came from developed countries of the world (Clubb *et al.*, 1991b).

Health has a major effect on body condition and vigour, which consequently determines individual fitness. Adoption of appropriate methods that enable a reliable estimation of health is therefore of crucial importance to most ecological and evolutionary research. Although there are various ways to investigate health, the most widely used method for health assessment is the basic haematological survey (Ardia and Schat, 2008). Therefore, application of this conventional veterinary medical method to wild bird species in Zaria will provide additional tool for wildlife management practice in Nigeria.

1.2 Justification of the Research

Blood analyses, in combination with other diagnostic methods, enable veterinarians to assess the health status of animals, and identify organ or systems affected by infectious diseases (bacterial, viral, fungal or protozoan) (Oladele *et al.*, 2005), toxins (heavy metals, petroleum products, pesticides), biotoxins (saitoxin) and metabolic disease or nutritional deficiencies (Oladele *et al.*, 2012). Serum or plasma biochemical profile is known to complement haemogram. It provides information about internal organs (liver, kidneys), electrolytes (sodium, chloride, potassium, calcium phosphate), proteins (immunoglobulins, albumin) and nutritional or metabolic parameters (cholesterol, triglycerides and glucose) of the avian species (Woerpel and Roskopf, 1984; Gylstorff and Grimm, 1987; Hochleithner, 1989b,c; Hochleithner, 1991a,b).

Haematological analyses provide information about the immunological status of an individual and can serve as diagnostic adjuncts in the development of a presumptive diagnosis (Campbell, 1995; Harr, 2002; Campbell and Ellis, 2007). Therefore, complete blood analyses from a bird can provide a thorough evaluation of the health status of that bird, while examination of blood from many birds from the same colony can provide an assessment of colony health (Newman *et al.*, 1997).

The ability to distinguish between normal and abnormal changes in blood, and especially when the normal values are determined for birds in a particular region in relation to the locally prevailing factor is very important. This is because without definite knowledge of what is normal, it is difficult to differentiate the abnormal conditions of birds (Oladele, 2009).

Since avian medicine is undergoing a new turn in the use of haematological and biochemical indices as tools for making diagnosis of diseases (Oladele, 2009), it is

pertinent to establish reference data that will be used as aids to diagnosis in our geographical locations, hence this study was designed. From the available literature and to the best of our knowledge there are no published data on the baseline haematological and biochemical parameters of wild birds in Zaria, Nigeria.

1.2 Aim of the Study

The aim of the study was to establish baseline haematological and serum biochemical values of some species of apparently healthy free-living wild birds in Zaria, Nigeria.

1.3 Objectives of the Study

The objectives of this study were to establish baseline:

- haematological parameters for some species of apparently healthy free-living wild birds in Zaria, Nigeria.
- serum biochemical values for some species of apparently healthy free-living wild birds in Zaria, Nigeria.

1.4 Research Questions

1. What are the haematological values for some species of apparently healthy free-living wild birds in Zaria, Nigeria?
2. What are the serum biochemical values for some species of apparently healthy free-living wild birds in Zaria, Nigeria?

CHAPTER TWO LITERATURE REVIEW

2.1 Birds

Birds (class Aves) are winged, bipedal, endothermic (warm-blooded), vertebrate animals that lay eggs. There are about 10,000 living species of birds, making them the most numerous tetrapod vertebrates. They inhabit ecosystems across the globe, from the Arctic to the Antarctic. Birds range in size from 5 cm (2 in), Bee Hummingbird to 3 m (10 ft) Ostrich (Cowen, 2000).

Modern birds are characterised by feathers, a beak with no teeth, the laying of hard-shelled eggs, a high metabolic rate, a four-chambered heart, and a lightweight but strong skeleton (Panger *et al.*, 2002). All birds have forelimbs modified as wings and most can fly, with some exceptions including ratites, penguins, and a number of diverse endemic island species. Birds also have unique digestive and respiratory systems that are highly adapted for flight. Some birds, especially corvids and parrots, are among the most intelligent animal species; a number of bird species have been observed manufacturing and using tools, and many social species exhibit cultural transmission of knowledge across generations (Panger *et al.*, 2002).

Many species undertake long distance annual migrations, and many more perform shorter irregular movements. Birds are social; they communicate using visual signals and through calls and songs, and participate in social behaviours including cooperative breeding and hunting, flocking, and mobbing of predators (Hernández-C, 2013). The vast majority of bird species are socially monogamous, usually for one breeding season at a time, sometimes for years, but rarely for life. Other species have breeding systems that are polygynous ("many females") or, rarely, polyandrous ("many males"). Eggs are usually laid in a nest and incubated by the parents. Most birds have an extended period of parental care after hatching (Berthold *et al.*, 2001).

Many species of birds are of economic importance, mostly as sources of food acquired through hunting or farming. Some species, particularly songbirds and parrots, are popular as pets. Other uses include the harvesting of guano (droppings) for use as fertiliser. Birds figure prominently in all aspects of human culture from religion, poetry to popular music. About 120 – 130 species have become extinct as a result of human activity since the Seventeenth Century, and hundreds more before then. Currently, about 1,200 species of birds are threatened with extinction by human activities, though efforts are underway to protect them (del Hoyo *et al.*, 1992).

2.1.1 Taxonomy of birds

In the 1750's, Carl Linnaeus, a Swedish scientist who described many North American birds, established a system or hierarchy of living organisms so that all scientist over the world could understand each other. The science of naming things, such as birds, is called "Taxonomy" and it can become quite complicated, but the basics are easy to understand (Linnaeus, 1758).

Kingdom - Animalia

Phylum - Chordata

Class - Aves

At the Order level (which is 23 in number) the birds begin to diverge. Some of the Order includes:

Order - Columbiformes (e.g. Laughing Dove, Mourning Collared Dove, Speckled Dove, Bruce's Green Pigeon)

Order - Psittaciformes (e.g. Senegal Parrot, Rose-Ringed Parakeet)

Order - Ciconiiformes (e.g. Cattle Egret)

Order - Passeriformes (e.g. African Silverbill, Little Weaver, Northern Red Bishop, Red-Billed Quelea)

Order - Apodiformes (e.g. White-Rumped Swift)

2.1.1.1 Order - Columbiformes

In general, the terms "dove" and "pigeon" are used somewhat interchangeably. Pigeon derives from the Latin *pipio*, for a "peeping" chick, while dove is a Germanic word that refers to the bird's diving flight (Harper, 2014). Pigeons and doves constitute in the order Columbiformes and family Columbidae that includes 308 species. They are easily recognizable and have a world-wide distribution (although they are not found in Antarctica and the high arctic). They live in almost all types of terrestrial habitats from desert to dense forest and large urban areas. Pigeons and doves are rocky birds that range from 15 to 75 cm long and weigh from 30 g to over 2,000 g (Baptista *et al.*, 1992). Many of the seed-eating columbids (e.g. laughing dove, collar dove, speckled dove) are buff, grey and brown colours, while the fruit-eaters (e.g. Bruce's green pigeon) are often more bright coloured. Many have ornamentation and iridescent feathers on the neck, breast, back, wings and face. They range from solitary to extremely social; the now extinct Passenger Pigeons (*Ectopistes migratorius*) are reported to have occurred in flocks of up to two million birds that were so dense that they block out the sun (Baptista *et al.*, 1992; Wells and Wells, 2001; Dickinson, 2003; Lack, 2003).

In ornithological practice, "dove" tends to be used for smaller species and "pigeon" for larger ones, but this is in no way consistently applied, and historically, the common names for these birds involve a great deal of variation between the terms. The species most commonly referred to as "pigeon" is the feral rock pigeon, common in many cities (Harper, 2014).

Doves and pigeons are monogamous. They build relatively flimsy nests – often using sticks and other debris – which may be placed in trees, ledges, or, on the ground,

depending on species. They lay one or two eggs, and both parents care for the young, which leave the nest after seven to 28 days (Crome, 1991). Unlike most birds, both sexes of doves and pigeons produce "crop milk" to feed their young, secreted by a sloughing of fluid-filled cells from the lining of the crop. Young doves and pigeons are called "squabs" (Baptista *et al.*, 1992; Wells and Wells, 2001; Dickinson, 2003; Lack, 2003).

Pigeons and doves are known for their navigation abilities and have been used by humans as messengers. Some species are sedentary, and others are migratory. Some are nomadic moving as their food supply changes, and some make altitudinal movements as season change. Some fly up to 40 km each day from their roosting sites to their foraging sites. Many species have high nest-site fidelity and during breeding they are aggressive and defend small territories around their nest (Baptista *et al.*, 1992; Wells and Wells, 2001; Dickinson, 2003; Lack, 2003).

Columbids are often thought of as crop pests because they feed on cultivated grains. They are also pests in urban areas where they nest in man-made structures and their droppings can be a nuisance. They are also known to carry human disease (Baptista *et al.*, 1992; Lack, 2003).

2.1.1.2 Order - *Psittaciformes*

Psittaciformes are birds of roughly 372 species in 86 genera found in most tropical and subtropical regions ranging from South America, Central America to Asia, Australia and Africa (ZNR, 2008). They range in size from 10 – 16 cm and are thick, hooked bills, zygodactyls feet with two toes pointing forward and two backward; and muscular tongues. The upper mandible of their bill is hinged to their skull and their lower mandible fits under the upper mandible. Several parrots inhabit the cool, temperate regions of South America and New Zealand. One, the Carolina parakeet, lived in

temperate North America, but was hunted to extinction in the early Twentieth Century (Butler, 2005; Steve, 2013). Few parrots are wholly sedentary or fully migratory. Most falls somewhere between the two extremes, making poorly understood regional movements, with some adopting an entirely nomadic lifestyle (Collar, 1997).

There are numerous challenges in studying wild parrots, as they are difficult to mark. Most wild bird studies rely on banding or wing tagging, but parrots chew off such attachments (Collar, 1997). Parrots also tend to range widely, and consequently, there are many gaps in knowledge of their behaviour. Some parrots have strong, direct flight. Most species spend much of their time perched on climbing in tree canopies. They often use their bills for climbing by gripping or hooking on branches and other supports. On the ground, parrots often walk with a rolling gait. The diet of parrots consists of seeds, fruit, nectar, pollen buds, and sometimes arthropods and other animal preys. The most important of these for most true parrots and cockatoos are seeds. The evolution of the large and powerful bill can be explained primarily as an adaptation to opening and consuming seeds. All true parrots except the Pesquet's parrot employ the same method to obtain the seed from husk; the seed is held between the mandibles and the lower mandible crushes the husk, whereupon the seed is rotated in the bill and the remaining husk is removed (Collar, 1997). Although there are a few exceptions, parrots are monogamous breeders which nest in cavities and hold no territories other than their nesting sites (Collar, 1997; Rowley, 1997).

2.1.1.3 Order - Ciconiiformes

Cattle Egret (*Bubulcus ibis*), a well-known example belong to the order Ciconiiformes from the family Ardeidae has a worldwide distribution. They are chiefly tropical marsh-dwelling and a variety of large, long-legged wading birds with large bills: storks, herons, egrets, ibises, spoonbills and several others (Encyclopaedia Britannica, 2014).

Most of the species in the Ciconiiformes are communal breeders though with few exceptions (Telfair II, 2006).

Cattle Egrets are often found associated with cattle and occasionally with pigs, goats, and horses, and also with moving vehicles such as tractors. The birds appear to exploit their beating effect whereby insects and other prey are disturbed by the larger animal and hence are easier to detect and capture. The most preferable habitats are as the following, grass fields followed by shallow water were the most frequently used habitats throughout the season (Seedikkoya *et al.*, 2005).

2.1.1.4 Order - Passeriformes

A passerine is any bird of the order Passariformes. They consist more than half of all bird species. A notable feature of passerines is the arrangement of their toes (three pointing forward and one back) which facilitates perching. Sometimes they are known as perching birds or, less accurately, as songbirds, the passerines form one of the most diverse terrestrial vertebrate orders. Of the world's approximately 9,600 species of birds, nearly 60% belong to the passerine clade, Passeriformes (Sibley and Monroe, 1990) with over 6,000 identified species (Mayr, 1946). It has roughly twice as many species as the largest of the mammal orders, the Rodentia. Compared with other avian groups of comparative age, no other clade has evolved such great species richness and range of ecological diversification as the passerines (Ericson *et al.*, 2006).

Most passerines are smaller than typical members of other avian orders. The heaviest and almost largest passerines are the thick-billed raven and the larger races of common raven, each exceeding 1.5 kg and 70 cm. the superb lyrebird and some birds-of-paradise, due to very long tails or tail coverts, are longer overall. The smallest passerine is short-tailed pygmy tyrant, at 6.5 cm and 4.2 g. Their diets are mostly grass seeds and grains. Most lay coloured eggs, in contrast with non-passerines, most of whose eggs are

white except in some ground-nesting groups such as Charadriiformes and nightjars, where camouflage is necessary, and in some parasitic cuckoos, which match the passerine host's egg (Dickinson, 2003).

The red-billed quelea is the world's most abundant passerine and wild bird species with an estimated adult breeding population of 1.5 billion pairs. Some estimates of the overall population have been as large as 10 billion (Birdlife International, 2012).

2.1.1.5 Order - Apodiformes

The name Apodiformes is based on the Greek word "*a pous*" meaning "*without foot.*" Traditionally, the bird order Apodiformes contained three living families: swift, the tree swifts and the hummingbirds. With nearly 450 species identified to date, they are the most diverse order of bird after the passerines (Other Free Encyclopaedias, 2014).

Swifts are found throughout most of the world. They live on every continent except Antarctica and do not live in Polar Regions. Members of all three families live in trees. Swifts sometimes make nests in chimneys and on cliffs. Some hummingbirds and swiftlets live in caves. Some swifts and hummingbirds migrate, travelling to another area where food is more plentiful (Other Free Encyclopaedias, 2014).

Swifts and tree swifts are insectivores, birds that eat insects. Swifts catch most prey while flying with their mouths open. The type of insects eaten depends on where the swifts are and the weather. On warm days, there are more insects in the air. Swifts' prey includes mayflies, termites, and ants, and sometimes even spider. Swifts are sociable and live in large colonies. Tree swifts are usually found alone or in pairs. They may, however, form groups of 10 to 12 individuals. The swifts and tree swifts are noisy birds and may create quite a bit of noise when congregating (Other Free Encyclopaedias, 2014). When food is scarce, swifts and hummingbirds may hibernate. Swifts and hummingbirds are active during the day. The tree swifts are active at twilight or just

before sunrise. The Apodiformes use various materials for their nests. The birds “glue” their nests together with saliva, a watery solution in their mouths, thereby hardening and holding the nest. Swifts and tree swifts are monogamous while the hummingbird is polygamous. Swifts spend so much time in the air that they are usually safe from mammal predators. The birds fly rapidly, but sometimes are caught by hawks (Other Free Encyclopaedias, 2014).

2.2 Haematology of Birds

Current scientific knowledge regarding early haematopoiesis in vertebrates came from experiments first performed on the avian embryo. Primitive haematopoiesis in the chicken embryo begins in the blood islands of the yolk sac during the second day of incubation. At that time, only nucleated erythrocytes and thrombocytes are produced (Dieterlen-Lievre, 1988; Baumann and Dragon, 2005). Haemoglobin can be detected as early as 24 hours after fertilization (2 - 4 somite stage). The yolk sac in avian embryos will remain haematopoietic for most of the embryonic development, until bone marrow haematopoiesis is established (Zon, 1995; Baumann and Dragon, 2005). Definitive haematopoiesis begins in the dorsal mesenchyma (aortic and para-aortic foci) from days 5 to 8 of incubation. New stem cells from these areas colonize the spleen, which is haematopoietic from days 9 to 18, and finally the bone marrow from day 12 of incubation into adult life. The foetal liver is not a major haematopoietic organ in birds (Dieterlen-Lievre, 1988).

Birds were an early model for immunology studies, leading to the identification of the B- and T-lymphocyte lineages. Maturation of T-lymphocytes occurs in the thymus. Birds have a specific organ, the bursa of Fabricius, which is the site of differentiation for B-lymphocytes (Dieterlen-Lievre, 1988).

Many insights into avian biology have been gained with techniques involving laboratory analysis of blood samples taken from wild birds. Avian blood can yield information for a wide variety of disciplines, including endocrinology (Oring *et al.*, 1988), energetics (Utter and Le-Febvre 1970) genealogy (Sherman, 1981; Burke, 1989), haematology (Puerta *et al.*, 1990), pathology (Seegar, 1979), population genetics (Evans, 1980; Barrowclough *et al.*, 1985; Burson, 1990), and taxonomy (Sibley and Ahlquist, 1983).

In evaluating the health of a bird, the physiological and biological parameters commonly used include haematological examinations that check the haemoglobin and haematocrit (for the evaluation of anaemia), the rate of leucocytes or white blood cells (infection indicators) and the fraction of heterophils, lymphocytes (stress indicator), as well as weight and morphometric measures (Débora *et al.*, 2011). The afore mentioned haematological parameters are widely used to monitor the health of various avian species and are predictive of physiological changes caused by various stress factors (Moreno *et al.*, 2002; Kilgas *et al.*, 2006).

The main procedure for a haematological survey is to determine the cellular composition of blood (Vinkler *et al.*, 2010). In avian species, the normal values of cellular proportions may differ among species and may also vary between free-living and captive birds (Ewenson *et al.*, 2001; Campbell and Ellis, 2007; Davis, 2009). Blood components may also be influenced by physiological factors, such as age and species, and by pathological factors (Szabo *et al.*, 2005; Lloyd and Gibson, 2006).

2.2.1 Blood collection

Blood can be collected from the brachial or ulnar veins, jugular veins, metatarsal or leg veins, toenails, or the heart. The choice of site for the collection of blood depends on the size of bird and the volume of blood to be collected (Owen, 2011). Taking into

cognisance the restraint method is paramount to efficient blood sampling (Proctor and Lynch, 1998).

Table 2.1: Advantages and disadvantages of the different blood collection techniques in wild birds

Blood collection technique	Advantages	Disadvantages
Brachial vein	<ul style="list-style-type: none"> • Easier and less intimidating to learn for a novice bleeder • When done poorly, has less severe consequences than some other methods (e.g., jugular vein) • Well-suited for obtaining small samples or when sampling small birds • Allows multiple attempts from the same bird (because there is a vein in each wing) 	<p>Increased risk of hematomas</p> <p>More time required to obtain large blood volumes, relative to collecting blood via a syringe</p> <p>Some species/individuals may clot quickly and may be better candidates for jugular bleeds (e.g., doves)</p>
Jugular vein	<ul style="list-style-type: none"> • Allows more control in volume of blood collected • Quicker and does not require use of multiple capillary tubes • Easier to collect more blood from smaller birds because it is larger than the brachial vein • Less likely to result in a hematoma than with the brachial vein 	<ul style="list-style-type: none"> • More difficult and intimidating to learn for novice bleeders • If measuring haematocrit or making a blood smear, blood must be dispensed from the syringe into a capillary tube or on glass slide, increasing the likelihood of lysing red blood cells • Increased risk of mortality if done incorrectly, particularly for small birds (<20 g)
Toenail clip	<ul style="list-style-type: none"> • Low risk of mortality • Potentially greater ease in obtaining permission when working with sensitive species compared to other, more invasive techniques • Simple procedure that requires little experience 	<ul style="list-style-type: none"> • Possible interference with perching ability • Only a small volume of blood (5-10 μl) can be obtained • Not considered acceptable by the Ornithological Council for species other than hummingbirds
Metatarsal vein	<ul style="list-style-type: none"> • Easier to perform on large birds • Leads to fewer hematomas than jugular or brachial vein methods due to the location of muscles and tightness of skin around the leg vein • Large vein from which to obtain large blood samples 	<ul style="list-style-type: none"> • Vein constriction is common when bird has been exposed to cold ambient or water temperatures, reducing blood flow to the extremities • Need for a second person to hold the bird, but this is true for most methods associated with large birds
Cardiac puncture	<ul style="list-style-type: none"> • Efficient method for obtaining blood from a recently deceased or bird near certain death • Technique used on large birds (e.g. raptors and waterfowl) 	<ul style="list-style-type: none"> • Difficult to locate heart without opening up the bird • High risk of mortality on live birds when done incorrectly • Requires substantial experience to do correctly

Source: Owen, 2011.

For collecting blood from the brachial veins, 28 to 25 gauge needles work well for passerine birds ranging in mass from 8 to 100 g. For large birds of about 900 to 1,200 g, blood can be collected from brachial veins using a 22 to 25 gauge needle and a 3 ml syringe. Obtaining blood from the jugular vein of passerines is best done with insulin syringes rather than capillary tubes and a 28 gauge needle. Larger birds can be bled via leg or jugular veins using a 22 to 25 gauge needle and 3 ml syringe (Owen, 2011).

2.2.2 Amount of blood that can be collected

Based on current guidelines, no more than 1% of a bird's body mass should be collected per blood collection event and no more than 2% of a bird's body mass should be collected over a 14-day period (McGuill and Rowan 1989; Fair *et al.*, 2010). A bird can be sampled multiple times over a 14-day period as long as the total amount of blood collected does not exceed 2% of the bird's mass. To determine acceptable sample volumes, multiply a bird's mass in grams by 10 and the resulting number is the volume of blood in microliters that can be collected at one time (e.g. 250 μ l of blood can be collected at one time from a 25 grams bird). Voss *et al.* (2010) cautioned that investigators should be conservative about a bird's mass if they do not weigh a bird at the time of blood collection because overestimating a bird's mass may result in collecting too much blood (i.e. >1% body mass). Investigators should also be aware that hematomas and bleeding into extravascular space can lead to additional blood loss. However, studies have shown that birds are fairly resilient to blood loss since they do not exhibit acidosis and thus do not go into shock when blood is lost (Sturkie and Griminger, 1986). Therefore, they can survive relatively greater blood loss than mammals (Kovach *et al.*, 1969).

Collecting more blood than required for a particular assay (within guidelines) is generally a good idea for several reasons, including:

- 1) the need to replicate assays due to failure of initial assays or to verify results,
- 2) some blood always adheres to collection vials so a small volume is lost with every transfer of samples to other containers, and
- 3) <50% of the total volume of blood collected may be plasma or serum due to high packed cell volumes (Fair *et al.*, 2007).

However, if information from blood samples is to be linked with other life history events, such as reproductive success or survival, investigators should minimize the amount of blood collected to avoid possibly compromising other aspects of their project (Brown and Brown, 2009; Voss *et al.*, 2010).

2.2.3 Post collection blood processing

Methods of blood collection, processing, and storage must be compatible with the assay(s) of interest and should be determined prior to blood sampling. Requirements for collecting, processing, and storing samples for several assays:

- 1) haematocrit (packed red blood cell volume or PCV),
- 2) leucocyte and parasite counts from blood smears,
- 3) hormones,
- 4) genetics,
- 5) plasma metabolites, and
- 6) serology (e.g. antibody titres).

In general, blood can be stored for 1 - 9 hours after collection in a refrigerator or cooler with ice. Except for genetic-based assays where whole blood is preserved and leucocyte and parasite counts from blood smears, blood needs to be centrifuged for 5 – 20 minutes at $10,000 - 15,000 \times g$ (centrifugal force, or RCF) to achieve maximum packing of red blood cells. Centrifuge speed is often reported as rpm (revolutions per minute), but this has no meaning if the rotating radius of the centrifuge is unknown. Reporting speed

using centrifugal force (RCF) or g-force ($\times g$) is more accurate. Use the equation, $RCF = 1.12 r (\text{RPM}/1,000)^2$ to calculate RCF (r = rotation radius in mm) (Ehret *et al.*, 2002).

Centrifuging separates the blood into an upper plasma/serum layer, a bottom layer of red blood cells, and a layer of white blood cells in between (i.e., buffy coat) (Wardlaw and Levine, 1983).

2.2.3.1 Haematocrit (or packed cell volume)

To determine the haematocrit, either collect blood directly or transfer blood at time of collection into heparinised micro-haematocrit capillary tubes. Only the first tube collected should be used to determine PCV and this tube should be as full as possible to obtain the most accurate value (Dufty, 1988). To achieve maximum packing of red blood cells, spin tubes in a centrifuge with a micro-haematocrit rotor for 15 minutes at a minimum of $3,000 \times g$ (Ehert *et al.*, 2002). To measure the haematocrit, either a microcapillary haematocrit tube reader or digital callipers is used.

2.2.3.2 Leucocyte, erythrocyte counts and differential leucocyte count

In contrast to mammals where reliable complete blood count is obtained routinely using automatic haematology analyzers (Knoll, 2000), reliable automatic methods to perform the complete blood count are unavailable because avian erythrocytes and thrombocytes contain nuclei. In such species, complete blood count is almost exclusively performed manually, although an automated total erythrocyte count can be made (Campbell, 2004b; Wakenel, 2010).

Anticoagulants (EDTA, heparin, or sodium citrate) may be used when collecting blood for blood smears (Fudge, 2000; Ballard and Cheek, 2010). Without anticoagulant, blood will clot making the smear difficult to read or interpret. However, when collecting blood to count leucocytes and erythrocyte using a haemocytometer, blood should be analyzed within 24 hours to prevent decline in cell quality due to anticoagulant (Fudge, 2000).

Heparin is the most common anticoagulant used in avian studies and prolonged exposure of blood to heparin may interfere with staining and reduce cell quality (Ballard and Cheek, 2010). However, this is rarely an issue because blood should be placed on a glass slide and smears made within seconds after blood collection (Ballard and Cheek, 2010).

For accurate erythrocyte and leucocyte counts, blood smears must have few lysed cells, little clumping and pushing of cells to the periphery, and should be even monolayers. For differential leucocyte count, staining slides within 2 hours of collection is best (Walberg, 2001), staining within 2 weeks post-collection does not affect slide quality after the slide has been fixed (Owen, 2011). For best results, a Giemsa–Wright combination stain should be used (Woronzoff- Dashkoff, 2002).

2.3 Erythrocytes

Avian erythrocytes are oval in shape with elliptic and centrally placed nuclei, and with the erythrocyte maturity the nuclear chromatin becomes increasingly condensed. The mature avian erythrocyte is larger than that of most mammals but smaller than those of reptiles and amphibians (Campbell, 2004a) and has been reported to vary in size with species (Sturkie and Griminger, 1986). With Romanowsky stains, the cytoplasm of the mature erythrocyte stains orange-pink, except for a thin, pale perinuclear band (Claver and Quaglia, 2009), and the nucleus stains dark purple (Campbell, 2004a; Campbell and Ellis, 2007; Mitchell and Johns, 2008). Haemoglobin is found in both the cytoplasm and nucleus (Claver and Quaglia, 2009).

Total erythrocyte concentration, packed cell volume (PCV), and haemoglobin concentration (Hb) may be influenced by age, gender, hormones, and other factors. Packed cell volume and total erythrocyte count tend to be higher in male birds than in

female birds and also tend to increase with age (Herbert *et al.*, 1989). The normal PCV for many bird species ranges approximately between 35% and 55% (Thrall, 2004).

Avian erythrocytes have a shorter half-life, ranging from roughly 25 to 45 days in various species, than that of many mammals. Because erythrocyte turnover is more rapid, birds tend to have higher percentages of polychromatophils in health than mammals. Avian erythropoietin is a glycoprotein that is synthesized in the kidneys and stimulates bone marrow erythropoiesis. There is apparently no cross-reactivity between avian and mammalian erythropoietin (Herbert *et al.*, 1989).

Several erythrocyte parameters, including RBC count, may be measured quantitatively on impedance-based or flow cytometric haematology analyzers with appropriate adjustments for other nucleated cells. Manual methods of counting avian erythrocytes are well described, and are often implemented in practice, given the paucity of automated instrumentation appropriate for use in avian haematology. The PCV can easily be obtained by means of centrifugation of a filled microhaematocrit tube. Haemocytometer counting of erythrocytes can be accomplished using the Unopette (Becton-Dickinson, Rutherford, New Jersey) method or using Natt and Herrick's solution (Natt and Herrick, 1952).

The total erythrocyte count can be obtained using either a standard manual method or an automated method, such as those used for mammalian blood. The packed cell volume is determined using the standard microhaematocrit method with centrifugation at 12,000 *g* for 5 minutes. The haemoglobin concentration is best determined using the cyanmethemoglobin method following proper removal of free red cell nuclei by centrifugation.

The mean corpuscular values (or the RBC indices are blood tests that provide information about the haemoglobin content and size of RBC) which include the average

red blood cell size (MCV), haemoglobin amount per red blood cell (MCH), and the amount of haemoglobin relative to the size of the cell (Hb) per red blood cell (MCHC) can be calculated once the red blood cell count, PCV, and Hb have been obtained using the standard formulae. These RBC measures are used to diagnose the presence of anaemia and the types of anaemia (Campbell, 2004a).

Anucleated erythrocytes (erythroplastids) and immature erythrocytes are occasionally present in the peripheral blood of birds and other lower vertebrates. The degree of their occurrence is also based upon the average number found in a 1,000X monolayer field. A 1+, 2+, 3+, and 4+ degree of erythroplastids seen is based upon 1 – 2, 3 – 5, 6 – 10, and greater than 10 erythroplastids found in an average 1,000X monolayer field (Samour *et al.*, 1984). More immature avian erythrocytes are round cells with basophilic cytoplasm, round nuclei, and more open nuclear chromatin; elliptic erythrocytes develop approximately at the reticulocyte or polychromatophil stage (Mitchell and Johns, 2008).

Immature erythrocytes, especially the rubricyte stages often appear smaller than mature erythrocytes and are round to slightly oval. The cytoplasm of immature erythrocytes have an irregular cytoplasmic polychromasia and stains more basophilic than mature erythrocytes and a more rounded, pale nucleus (Campbell and Dein, 1984; Campbell, 1988; Campbell, 2004b). Early immature erythrocytes (rubriblasts and prorubricytes) are rarely seen in peripheral blood films of birds and their presence may indicate a marked erythropoietic response or erythrocyte dyscrasia such as erythroblastosis. Birds suffering from heavy metal toxicosis, especially lead poisoning, often reveal an inappropriate release of immature erythrocytes in a non-anaemic patient. This is reflected in the peripheral blood film by two distinct populations of erythrocytes, immature cells (i.e. metarubricytes and polychromatic erythrocytes) and old mature

cells with pyknotic nuclei in the blood films from lower vertebrate patients, it is relevant to take note of the number of immature erythrocytes. Animals that are responding to anaemia may exhibit an increase in the number of immature erythrocytes in the peripheral blood film (Campbell, 2004a).

The erythrocyte morphology, including size variation (anisocytosis), shape abnormalities (poikilocytosis), and abnormalities in hemoglobinization, can be evaluated in the monolayer region of a well made smear. Semi-quantitative estimates of polychromasia, anisocytosis, poikilocytosis, and degree of erythrocyte parasitism (if present) can be performed (Mitchell and Johns, 2008).

2.3.1 Erythrocyte abnormalities

Haematologic abnormalities consistent with immune-mediated haemolytic anaemia have been rarely reported in birds. Erythrocyte agglutination can be visualized microscopically in a stained blood smear or by performing a saline dispersion test, supporting cross-linking of erythrocytes by surface immunoglobulin. In addition, two clinical reports of avian patients that have disease resembling immune-mediated haemolytic anaemia describe small round erythrocytes, potentially spherocytes, in blood smears from the patients (Johnston *et al.*, 2007).

Heinz bodies, aggregates of precipitating denatured haemoglobin, have been documented in avian erythrocytes. In birds, Heinz bodies are small, round to irregular, and often multiple inclusions that stain light blue with new methylene blue staining and can occur in the cytoplasm and the nucleus (Leighton, 1985). They may also be seen as refractile inclusions in unstained blood smears and appear as more densely staining haemoglobin aggregates in Wright's-stained smears. Heinz bodies form as a result of oxidative damage to the haemoglobin molecule and cause increased rigidity of the erythrocyte membrane. Decreased deformability may lead to intravascular lysis of the

erythrocyte or phagocytosis within the splenic microcirculation. Haemolytic anaemia and Heinz bodies have been documented in marine birds exposed to petroleum products (Leighton, 1985; Troisi *et al.*, 2007). Geese fed green onions in one study infrequently developed erythrocyte Heinz bodies compatible with evidence implicating several thiosulfate compounds present in onions and onion products as causes of Heinz body haemolytic anaemia in cats and other domestic animal species (Robertson *et al.*, 1998; Crespo and Chin, 2004).

2.3.2 Polychromasia

Polychromasia (polychromatophilia) is defined as increased affinity for acidic and basic stains. In avian erythrocytes, it is seen as increased cytoplasmic basophilia resulting in lavender- to gray-staining cytoplasm with Wright's staining (Thrall, 2004; Mitchell and Johns, 2008). Polychromatic erythrocytes are often seen in the peripheral blood films of normal birds and these cells usually represent five per cent or less of the erythrocyte population. The degree of polychromasia is a good indicator of the erythrocytic regenerative response. In birds, the degree of polychromasia can be evaluated based upon the average number of polychromatic erythrocytes per 1,000X monolayer field (Campbell, 2004a; Thrall, 2004). A slight degree of polychromasia (1+) is represented by 2 - 10 polychromatic cells per 1,000X monolayer field which is a common finding in healthy birds; one reference interval for healthy psittacines reports that polychromatophils comprised 0.41% to 6.78% of all erythrocytes (Johns *et al.*, 2008). A mild polychromasia (2+) is indicated by 11 - 14 polychromatic cells per 1,000X monolayer field. A moderate (3+) and marked (4+) polychromasia are represented by 15 - 30 and greater than 30 polychromatic erythrocytes per 1,000X monolayer field, respectively. For example, anaemic birds showing ten per cent or greater polychromasia (3+ or 4+ polychromasia) are demonstrating a good regenerative response to their

anaemia. Polychromatic erythrocytes have nuclei that are less pyknotic than mature erythrocytes and have cytoplasmic basophilia (Campbell, 2004a).

In Wright-stained smears, polychromatophils smears are considered to be roughly equivalent to reticulocytes in supravitaly stained smears. Avian reticulocytes are defined by means of specific morphologic criteria, because it has been shown that a high percentage of avian erythrocytes contains basophilic granular material (“*reticulum*”) when supravitaly stained (Gurd, 1935; Thrall, 2004). This granular material is present in a perinuclear ring in immature erythroid cells; as cells mature, the reticulum first disperses into scattered cytoplasmic aggregates and then diminishes and becomes punctate in appearance (Lucas and Jamroz, 1961). When avian reticulocytes (defined as having bands of aggregated reticulum forming a ring around at least half of the erythrocyte nucleus in total) were counted in new methylene blue-stained blood smears, the percentage of reticulocytes strongly correlated with percentage of polychromatophils in samples from a variety of avian species (Campbell, 1995; Johns *et al.*, 2008). In the same comparison, reticulocyte percentage also proved to be a more precise value than polychromatophil percentage. Either measurement may therefore be used as an index of erythroid regenerative capacity in birds. Erythroid cells more immature than reticulocytes are round and smaller than reticulocytes or mature erythrocytes, with deeply basophilic cytoplasm. Increases in more immature erythrocytes have been seen with marked regenerative responses in birds. Lead poisoning of birds can cause an increase in more immature erythrocytes without evidence of anaemia (Campbell, 2004a; Campbell and Ellis, 2007).

2.3.3 Hypochromasia

Hypochromasia, or decreased amount of staining haemoglobin in an erythrocyte, is associated with several disease conditions in birds, including acute blood loss and

inflammation (Christopher *et al.*, 2004). Hypochromasia in mammalian erythrocytes is a finding commonly linked to iron deficiency and poorly regenerative anaemia, and similar findings have been reported in birds (Campbell and Ellis, 2007). Inflammation causes redistribution of body iron stores, reducing iron available for erythropoiesis and resulting in functional iron deficiency. Acute blood loss and nutritional iron deficiency can cause hypochromic, poorly regenerative, or non-regenerative anaemia in birds because of absolute iron deficiency. Hypochromasia is also reported with lead and zinc toxicosis in birds. Experimentally, induced haemolytic anaemia attributable to zinc toxicosis in mallards resulted in poorly regenerative anaemia with high percentages of hypochromic cells in birds that died or were euthanized as a result of severe clinical disease (Christopher *et al.*, 2004). The degree of hypochromasia can be rated based upon the average number of hypochromatic erythrocytes per 1,000X monolayer field where a 1+, 2+, 3+, and 4+ hypochromasia is represented by 1 - 2, 3 - 5, 6 - 10, and greater than 10 hypochromatic cells, respectively (Campbell, 2004a).

2.3.4 Poikilocytosis

Poikilocytosis, particularly fusiform erythrocytes, and erythrocyte nuclear abnormalities have been reported in mallards that experimentally suffered lead and zinc toxicosis. However, surviving birds from the experiment had an increased percentage of polychromatophils, indicating a regenerative anaemia, and significantly lower levels of poikilocytosis. The impaired regenerative response in the more severely affected birds suggests functional iron deficiency as a cause of decreased erythropoiesis and is compatible with evidence in birds and mammals that excess ingested zinc impairs iron absorption and use (Storey and Greger, 1987; Pimental *et al.*, 1992). Zinc and lead

toxicosis can cause regenerative haemolytic anaemia, impaired haeme synthesis, hypochromasia, and a shortened erythrocyte life span in birds, although one clinical report suggests that haemolysis does not occur in zinc-intoxicated birds (Romagnano *et al.*, 1995; Christopher *et al.*, 2004). In early lead toxicosis, hypochromic erythrocytes are described as having cytoplasmic ballooning, sometimes described as “D cells” when eccentric (Fudge, 2000; Christopher *et al.*, 2004). The degree of poikilocytosis is also based upon the average number of abnormal cells in a 1,000X monolayer field. A 1+, 2+, 3+, and 4+ poikilocytosis is indicated by 5 - 10, 11 - 20, 21 - 50, and greater than 50 abnormal erythrocytes, respectively per 1,000X monolayer field (Campbell, 2004a).

2.3.5 Anisocytosis

Slight anisocytosis is also considered an unremarkable finding in birds (Campbell and Ellis, 2007). Prominent anisocytosis may be seen with regenerative anaemia or with dyserythropoiesis, however, and was seen in blood smears from marine birds exposed to crude oil (Leighton, 1985). The degree of anisocytosis in birds can also be rated on a similar scale. A 1+ and 2+ anisocytosis are indicated by an average of 5 - 10 and 11 - 20 erythrocytes that vary in size per 1,000X monolayer field, respectively. A 3+ and 4+ anisocytosis is based upon an average of 21 - 30 and greater than 30 erythrocytes that vary in size per 1,000X monolayer field (Campbell, 2004a).

2.3.6 Nuclear abnormalities

Nuclear abnormalities in avian erythrocytes are usually attributable to dyserythropoiesis but occasionally occur because of markedly accelerated erythrocyte production (Christopher *et al.*, 2004). Nuclear abnormalities can include nuclear fragmentation or pyknosis, Howell-Jolly bodies, nuclear shape changes, and binucleation (Mitchell and Johns, 2008).

Binucleated erythrocytes are rare in blood smears from normal birds, but the presence of higher numbers of binucleated cells is considered abnormal (Campbell and Ellis, 2007), which could be due to neoplastic, viral, or genetic disease (Caxton-Martin and Nganwuchu, 1978). Binucleated erythrocytes, anisokaryosis, or mitotic activity can be associated with marked erythrocytic regenerative responses, severe inflammation, malnutrition, and starvation (Bessis, 1977). Chronic lead toxicosis can also result in nuclear abnormalities, including pyknosis within smaller senescent erythrocytes (Campbell and Ellis, 2007).

Basophilic stippling of the cytoplasm can be seen in avian erythrocytes stained with Wright's stain. As is the case with mammalian erythrocytes, basophilic stippling in avian erythrocytes is considered evidence of degrading ribonucleoproteins and can be seen in regenerative anaemia as a result of accelerated erythrocyte production or, rarely, because of lead toxicosis (Campbell and Ellis, 2007).

2.3.7 Avian anaemia

In evaluating an avian patient that has anaemia clinicians are faced with challenges some of which include recognizing clinically that anaemia is present, determining the severity and chronicity of the anaemia, determining the underlying cause, treating the underlying cause, and deciding whether treatment for the anaemia itself is warranted. Some classical clinical signs of anaemia obvious in birds include weakness, lethargy, collapse, and respiratory signs. In addition, on physical examination findings that lead to a suspicion of anaemia include pale oral or cloacal mucous membranes, decreased cutaneous ulnar vein size, poor peripheral arterial pulses, tachycardia, and a physiologic heart murmur. There may be obvious signs of blood loss, such as trauma, broken blood feathers, bruising, melena, or haematochezia. There may not be an obvious cause for the anaemia, however, and additional diagnostics must be performed (Mitchell and Johns, 2008).

In situations where anaemia is suspected, a blood sample should be collected and the PCV and RBC morphology should be evaluated. In general, anaemic birds are defined as having a PCV of less than 35% (Thrall, 2004). According to the work done by Polo *et al.* (1998), many psittacines, however, normal PCV is greater than 45%; therefore reference ranges for individual species should be consulted. A PCV of 25% to 35% in the psittacines indicate mild to moderate anaemia, whereas a PCV of less than 20% is a severe anaemia (Bos *et al.*, 1990).

It is important to determine whether the anaemia is regenerative or non-regenerative. As mentioned previously, a reticulocyte count is the best method for determining regeneration (Johns *et al.*, 2008). In the absence of this technique regeneration can be estimated using the degree of polychromasia because a small amount of polychromasia is normal in the absence of anaemia, moderate (2p) or higher polychromasia would be

expected with a regenerative anaemia. Differentials for regenerative anaemia include acute blood loss or haemolysis. Acute blood loss, such as is caused by trauma, gastrointestinal (GI) bleeding (e.g. parasitism, GI ulceration, GI neoplasia), or coagulopathy (e.g. rodenticides, erythremic myelosis syndrome, secondary to aflatoxicosis), is the most common cause of regenerative anaemia in birds (Campbell, 1994; Goodman, 1996).

In birds, haemolytic anaemia has also been reported. Some common causes include haemoparasites, septicaemia (i.e. salmonellosis), toxins (e.g. lead, zinc, petroleum products), and immune-mediated haemolytic anaemia (Leighton, 1985; Ochiai *et al.*, 1993; Campbell, 1994; Jones *et al.*, 2002; Johnston *et al.*, 2007). Slide agglutination test, radiographs, liver aspirate, liver biopsy, splenic biopsy, and bone marrow aspirate, are additional diagnostic test that can be used to confirm haemolysis and to evaluate for an underlying cause (Johnston *et al.*, 2007; Jones *et al.*, 2002). The differential diagnoses for non-regenerative anaemia include anaemia of chronic disease (especially chlamydophilosis, mycobacteriosis, aspergillosis, West Nile virus, or neoplasia), toxicity (e.g. lead toxicosis, aflatoxicosis), iron deficiency, hypothyroidism, and leukemia (Newell *et al.*, 1991; Campbell, 1994; Tell *et al.*, 2001; Joyner *et al.*, 2006; Campbell and Ellis, 2007). In attempts to establish the cause of a non-regenerative anaemia, additional diagnosis such as infectious disease testing, toxicology, radiology, ultrasound, and bone marrow aspiration, may be required. Bone marrow aspirates may be collected from the proximal tibio-tarsus, sternum, or long bones that are not pneumatized. Birds should be anesthetized before collection of bone marrow because of pain and stress associated with the procedure. Details on the collection, preparation, and interpretation of bone marrow aspirates have been previously described (Murray, 1997; Campbell and Ellis, 2007).

For a successful treatment of anaemia in birds, identifying the underlying cause, treating or removing the underlying cause, providing supportive care, and, in some cases, providing blood transfusions are the best approach. Supportive care includes administration of crystalloid fluids, colloidal fluids, iron dextran, and vitamin B (Bos *et al.*, 1990; Rupley, 1997; Lichtenberger *et al.*, 2001). Oxyglobin (a purified polymerized bovine haemoglobin product in lactated Ringer's solution) (Biopure Corporation, Cambridge, Massachusetts) may also be used for support of anaemic patients. It is approved for use in dogs but has been successfully used in exotic species, including birds, when whole blood is not available for transfusion (Lichtenberger *et al.*, 2001; Lichtenberger, 2004).

As a result of its vasoconstrictive effect that decreases the volume necessary for resuscitation, oxyglobin is also useful in the treatment of haemorrhagic shock (Lichtenberger, 2004). However, oxyglobin should be used in birds that are normovolemic or hypervolemic with caution owing to the fact that it is a colloid (Lichtenberger, 2004).

Judging whether or not to administer a blood transfusion to an anaemic bird depends on several variables. With few exceptional cases, transfusion is recommended for birds with a PCV of less than 20%. Some exceptional cases, for instance, birds with chronic anaemia may acclimatize to a lower PCV and fail to demonstrate clinical signs of anaemia even with a PCV less than 20%. Blood transfusion is not indicated in these birds unless they are undergoing a major procedure, such as surgery. Transfusion is also contraindicated in some cases of acute blood loss. Pigeons have been demonstrated experimentally to survive acute blood loss of up to 70% of the blood volume (Bos *et al.*, 1990). Recovery in birds that are otherwise healthy can normalise its PCV in 3 to 6 days after acute blood loss (Ploucha *et al.*, 1981; Bos *et al.*, 1990). Therefore, birds in good

health that experience acute blood loss, such as from a broken blood feather, may only require fluid therapy, supportive care, and time to recover from the event. The species of donor and recipient birds is another striking factor that determines whether to perform a transfusion (Mitchell and Johns, 2008). There is no substantial information available regarding blood groups in avian species. Chickens have been demonstrated to have at least 28 different blood group antigens, and blood groups have also been studied in other Galliformes and waterfowl (Gilmour, 1984; Morrisey, 2004). It has been shown that birds do not have preformed antigens to blood groups, however; therefore, a single blood transfusion, even from a donor from a different species or genus (heterologous blood transfusion), may be safely administered (Bos *et al.*, 1990; Sandmeier *et al.*, 1994; Degernes *et al.*, 1999a,b). After the initial transfusion, birds become sensitized to donor antigens; therefore, multiple transfusions carry the risk for fatal reactions (Hoefler, 1992).

Red blood cells from donors of the same species as the recipient (homologous blood transfusions) have been shown to survive significantly longer than RBC from heterologous transfusions (Sandmeier *et al.*, 1994, Degernes *et al.*, 1999a,b). Although the RBC provided by a heterologous transfusion can provide support in the short term, the added physiologic stress of haemolysis and removal of cellular breakdown products by the recipient may negate the benefits of the transfusion. Therefore, whenever possible, a homologous transfusion should be performed. In the absence of a donor of the same species, oxyglobin may be a superior choice to a heterologous transfusion. The exception is birds with significant ongoing bleeding; in such a case, the transfusion may be lifesaving. A cross-match should be performed before administration of a blood transfusion if time and sample volume allow, and birds should be monitored for transfusion reactions, although transfusion reactions have not been reported in birds

(Lichtenberger, 2004). Blood transfusions and oxyglobin may be administered by means of intravenous or intra-osseous catheters (Hoefler, 1992; Lichtenberger, 2004). Blood for transfusion must be collected fresh because none of the storage media currently available preserves avian blood without the development of dangerously high levels of potassium (Morrisey and Giger, 1997).

2.3.8 Avian polycythaemia

Polycythaemia refers to an increase in the PCV and RBC count. It is an uncommon finding in birds. Generally, polycythaemia in birds is defined as a PCV greater than 70% (Campbell and Ellis, 2007). Polycythaemia is divided into two categories: absolute and relative.

Relative polycythaemia results from dehydration and a loss of plasma volume. Relative polycythaemia can be corrected by rehydrating the bird and treating the cause of the dehydration. Absolute polycythaemia on the other hand can be further divided into two distinct categories: primary polycythaemia and secondary polycythaemia (Campbell and Ellis, 2007).

Primary polycythaemia, or polycythaemia vera, is a rare finding in birds but can occur (Campbell and Ellis, 2007). This condition is caused by a myeloproliferative disorder that results in an increase in erythrocytosis. Diagnosis of polycythaemia vera requires ruling out secondary causes of polycythaemia. Secondary polycythaemia occurs as a result of an increased need for tissue oxygenation or because of an increase in the production of erythropoietin. Some common diseases that lead to secondary polycythaemia include chronic pulmonary disease, adaptation to high altitude, cardiac disease, iron storage disease, rickets, or renal disease or neoplasia leading to increased production of erythropoietin (Samour, 2006; Campbell and Ellis, 2007). Treatment of polycythaemia involves treatment of the underlying cause and periodic phlebotomy.

The major function of erythrocytes is oxygen transport, but it has been reported that bird erythrocytes, as non-immune cells, are able to participate in some immune responses that contribute to host defence (Passantino *et al.*, 2007).

2.4 Leucocytes

Total leucocyte count with differential leucocyte count is part of the minimum data base for evaluation of ill animals, and of routine evaluation of healthy animals, and influence their diagnostic work up and treatment (Aroch *et al.*, 2013). The leucocyte consists of granulocytes (named for their conspicuous cytoplasmic granules which are the heterophils, eosinophils and basophils) and the agranulocytes (lymphocytes and monocytes) which are the mononuclear leucocytes (Lucas and Jamroz, 1961; Mitchell and Johns, 2008). In non-mammalian species generally differentiating between eosinophils and heterophils, small lymphocytes and thrombocytes, and large lymphocytes and monocytes is often difficult (Latimer and Bienzle, 2010). In birds species with published values of haematological traits, however, only lymphocytes and heterophils are detected in sufficient numbers to enable reliable inter-individual comparison (the combined number of lymphocytes and heterophils typically accounts for 85- 95% of all leucocytes in a blood smear) (Davis, 2009).

The differential leucocyte count in non-mammalian species is performed by counting 100-200 leucocytes in stained blood smears, and has been studied mostly in the domestic chicken (*Gallus gallus*) (Campbell, 2004b).

The total leucocyte count in non-mammalian vertebrates can be counted using three methods;

- 1) direct haemocytometer counting with Natt and Herrick's or toluidine blue stain solutions (Natt and Herrick, 1952; Lane, 1991; Robertson and Maxwell, 1993; Campbell, 2004b);

- 2) semi-direct haemocytometer count with phloxine-B dye or the (now discontinued) commercial eosinophil Unopette 5877 (Becton - Dickinson, Rutherford, NJ) (Natt and Herrick, 1952; Costello, 1970; Robertson and Maxwell, 1990; Lane, 1991; Campbell, 2004b; Campbell, 2010; Wakenel, 2010), combined with manual differential leucocyte count in Romanowsky-stained blood smears (Ferris and Bacha, 1984; Zinkl, 1986; Latimer and Bienzle, 2010);
- 3) Semi-quantitative total leucocyte count evaluation in Romanowsky stained blood smears, which is considered less accurate (Campbell, 2004b; Wakenel, 2010).

The latter can be done by averaging leucocyte number in 10 monolayer, X400 fields and multiplying it by 2,000, yielding the total leucocyte count in cell/ μ l. Alternatively, one can determine the leucocyte average number in five X1,000 monolayer fields, multiply it by 3,500,000 (the approximate number of erythrocytes in 1 μ l of blood in birds when the PCV is 35-55%), and dividing the result by 1,000 (the average number of erythrocytes in 5 X1,000 monolayer fields). The result should be corrected if PCV is abnormally high or low (Zinkl, 1986; Campbell, 2004b; Wakenel, 2010).

Direct leucocyte count is a complex, time-consuming procedure, involving preparation of the stain solution, and is complicated by the need to differentiate lymphocytes from thrombocytes, and with presence of stained erythrocytes in the haemocytometer (Zinkl, 1986; Campbell, 2004b; Latimer and Bienzle, 2010). The semi-direct counting method relies on the positive staining of heterophils and eosinophils by the dye, allowing their counting in the haemocytometer. Calculating their total number per microlitre, and then the total leucocyte count is achieved by performing a differential leucocyte count using a Romanowsky-stained blood smear. This method is more precise and less time

consuming than the direct haemocytometer count (Zinkl, 1986; Campbell, 2004b; Campbell, 2010; Wakenel, 2010); however, it becomes progressively less accurate for estimation of the total leucocyte count with increasing proportion of mononuclears to granulocytes (Campbell, 2004b). Currently, the Unopette 5877 kit has been discontinued, while the other staining solutions mentioned are not readily available in most veterinary clinics. In contrast, Romanowsky-based quick staining solutions are readily available and are used in many veterinary clinics for blood smear staining. Based on the staining affinity of heterophils and eosinophils, eosin-based staining solutions are expected to stain these cells similarly as phloxine-B (Aroch *et al.*, 2013).

It is worth noting that the direct haemocytometer counts are more accurate than WBC estimates determined by the Unopette system method because of the variation in differential counts (Russo *et al.*, 1986). Both are subject to the technical error of the haemocytometer method, and changes in the WBC count may be caused by the variability inherent in the method of enumeration (Russo *et al.*, 1986). Part of this error may be decreased by standardizing the technique (using the same chamber, cover slip and pipette and having the same person perform the counts) (Russo *et al.*, 1986).

2.4.1 Basophils

Basophils are slightly smaller than heterophils and are recognised by a rounded nucleus and characteristic violet to reddish purple granules which are much smaller than those of eosinophils although the granules may dissolve, coalesce, or appear abnormal when stained with alcohol-based stains, such as Wright stain (Mitchell and Johns, 2008; Claver and Quaglia, 2009). Basophils are much more common in avian peripheral blood smears than in mammalian blood though little is known about basophilic function in birds (Mitchell and Johns, 2008, Brandon *et al.*, 2011).

Basophils appear to play important role in the initial phases of acute inflammation and immediate hypersensitivity reactions, but differ from those in mammals by not contributing to delayed hypersensitivity. This, however, does not always result in peripheral basophilia (Montali, 1988; D'Aloia *et al.*, 1994; Maxwell and Robertson, 1995; Campbell and Ellis, 2007).

The granules of avian basophils contain histamine, as in mammals (Campbell and Ellis, 2007). Therefore, it is suggested that they function in acute inflammatory and type IV hypersensitivity reactions, similar to mammalian basophils and mast cells (Mitchell and Johns, 2008). Severe stress has also been proposed as an underlying cause for an increased basophilic response in birds (Maxwell, 1993; Altan *et al.*, 2003; Campbell and Ellis, 2007; Bedáňová *et al.*, 2007).

As avian basophil granules are extremely water soluble, they may be easily damaged during the staining process (Lucas and Jamroz, 1961), which impairs the detection of these cells. This might be the reason why precise data on the variability of basophil levels in peripheral blood are limited in free-living birds. Nevertheless, there is some clear evidence suggesting that the proportion of basophils among blood-borne leucocytes is much higher in some avian species than the normal physiological values of most mammals (Maxwell and Robertson, 1995). A good example of natural variability in basophil counts was given by Friedl and Edler (2005), who found that the percentage of basophils ranges from 0 to 24% in the Red Bishop (*Euplectes orix*). High levels of basophils in peripheral blood were also reported in some other passerine species (e.g. *Pine siskin*, *Carduelis pinus*, or *Pied flycatcher*, *Ficedula hypoleuca*) (Davis, 2009). Even in non-passerines such as *Puna ibis* (*Plegadis ridgewayi*) (Coke *et al.*, 2004), the Common Pheasant (*Phasianus colchicus*) (Lucas and Jamroz 1961), and some strains of the domestic chicken (Maxwell and Robertson, 1995), normal basophil levels in adults

may exceed 10%. Much lower basophil counts (0–5%) were detected in most finches, other than Scarlet Rose finches (Campbell and Ellis, 2007; Davis, 2009). Nevertheless, in the House Finch (*Carpodacus mexicanus*), a species that is closely related to the Scarlet Rose finch, the basophil granulocytes are more frequent than heterophils in the peripheral blood of free-living individuals (Davis *et al.*, 2004; Davis, 2005).

2.4.2 Eosinophils

Morphologically, the eosinophils are round granulocytes that are similar in size to the heterophils (Campbell, 2004c). They contain eosinophilic granules that tend to be round as opposed to needle-shaped granules of heterophils. The cytoplasmic granules of avian eosinophils lack the central refractile body that is observed in avian heterophils (Campbell and Ellis, 2007). Variation among species and staining artifacts can lead to granules that are colourless or blue. Comparison with other cells on the slide must be made to identify these cells as eosinophils (Mitchell and Johns, 2008). In avian, the exact function of the eosinophils is poorly understood (Montali, 1988; Latimer and Bienzle, 2000; Cherry, 2008).

Cytochemically, the avian eosinophil granules, similar to mammalian counterparts contain peroxidase and high concentrations of arginine (Montali, 1988; Andreasen and Latimer, 1990; Campbell and Ellis, 2007). Additionally, certain hydrolytic enzymes (e.g. acid phosphatase and aryl sulphatase) have been detected in avian eosinophilic granules, supporting the theory that these structures are lysosomal in nature (Andreasen and Latimer, 1990; Montali, 1998; Latimer and Bienzle, 2000; Campbell and Ellis, 2007).

In mammals, eosinophils are modulators of immediate hypersensitivity and suppress parasitic infection. Some studies have shown a limited association between eosinophils

and nematode infection in grouse and other water fowls (Maxwell and Burns, 1985). Eosinophilia has been observed after foreign antigen administration and possibly in association with alimentary tract parasitism (Montali, 1988; Latimer and Bienzle, 2000), though parasite antigens do not generally induce eosinophilia in birds (Montali, 1988). The parasitic response by this cell type in birds has not been scientifically confirmed (Montali, 1988; Latimer and Bienzle, 2000; Cherry, 2008).

Other studies have shown eosinophilia with generalised inflammation in birds. It is possible that avian eosinophils play a role in delayed hypersensitivity, but they have been shown to be related to anaphylaxis or other acute hypersensitivity reactions (Montali, 1988). Severe eosinophilia have also been observed in cases of poxvirus infection in red-tailed hawks, although the mechanism of this response is unknown (Mitchell and Johns, 2008).

In some bird groups, the granules may actually be rod shaped (e.g. Anatidae, Tinamidae, Falconidae), whereas in others, they may have bluish colouration (e.g. in psittacine birds) (Campbell, 2000). Crystalline cores have been described in specific granules of eosinophils in some birds such as geese and ducks, but these structures are not commonly found in other species (Maxwell and Siller, 1972). The nucleus of the eosinophil is lobed and basophilic, whereas the cytoplasm stains clear or pale blue (Campbell, 1988).

2.4.3 Heterophils

In Wright stained preparations of the avian blood smears, mature heterophils are medium sized round shaped cells with a lobed basophilic nucleus (usually 2 to 3 lobes) with densely clumped chromatin and prominent eosinophilic (orange red to red brown or pale blue) cigar to rod shaped granules. However, there is significant variation among

bird species in the shape of the cytoplasmic granules (Mitchell and Johns, 2008). Specific granules are elliptical, although in some species they may be oval or rounded and have a distinct central body that appears refractile (Campbell, 2004b). The cytoplasm of heterophils is generally colourless (Mitchell and Johns, 2008). Heterophils are the most common granulocytes in circulation in the majority of birds (Mitchell and Johns, 2008; Claver and Quaglia, 2009). The response of heterophils to infections is similar to that of the mammalian neutrophil, migrating to the sites of inflammation caused by any offending pathogen, hence, killing it (Vegad and Katyar, 1995). Owing to their highly phagolytic activities, they are capable of a broad spectrum antimicrobial activity (Claver and Quaglia, 2009).

The avian heterophil contains many lysosomal and non-lysosomal enzymes (Maxwell and Robertson, 1998) that function in phagocytosis and destruction of bacterial organisms (Mitchell and Johns, 2008) and they are devoid of myeloperoxidase (unlike the mammalian neutrophils) which function in phagolysosomal killing and depend primarily on non-oxidative mechanisms, lysosome, and acid phosphatase for antimicrobial activity (Andreasen and Latimer, 1990).

The B-cell defences have a broad spectrum of activity against the binding abilities of infectious agents. Spherical granules contain acid hydrolases, whereas a third, smaller, round vacuole granule has been identified by electron microscopy (Maxwell and Robertson, 1998).

There are 2 types of changes observed in heterophils during the course of disease processes in birds. One change is the presence of immature cells in the peripheral blood, representing recruitment of cells from the bone marrow in response to cytokines and other inflammatory mediators. These immature cells (usually myelocytes and metamyelocytes) have more basophilic cytoplasm than mature heterophils. They have

segmented nuclei and fewer specific granules that are occasionally immature (i.e. primary granules) (Campbell and Ellis, 2007).

Band heterophils are similar to mature heterophils, except the nucleus is horseshoe shaped with parallel sided and lacks lobules. Often the nucleus is obscured by the cytoplasmic granules. Metamyelocytes and myelocytes are less mature cells and are larger than the band heterophils. The nucleus of these cells is round to oval, and the cytoplasm is basophilic. Myelocytes and metamyelocytes have spiculate cytoplasmic granules; however, in myelocytes, the granules take up less than one-half of the cytoplasm, whereas in metamyelocytes, the granules take up more than half of the cytoplasm (Campbell and Ellis, 2007).

Band heterophils are identified in peripheral blood smears in the first 12 – 24 hours after the initial insult with persistence of leukocytosis for 7 days (Latimer *et al.*, 1988). However, the presence of immature cells in avian blood smears indicates acute inflammation. A degenerative left shift, in which the number of immature heterophils exceeds the number of mature heterophils indicates intense tissue demand for cells and carries a poor prognosis (Mitchell and Johns, 2008). Typically, when immature heterophils are found in the peripheral blood, normal appearing mature heterophils can be found (Campbell, 2004b).

The other change observed in avian heterophils during disease is toxic change which is similar to those observed in mammalian neutrophils. Generally, when toxic heterophils are seen, all the heterophils in the film appear toxic and usually to the same degree unless the condition is caught in the per acute stage or is resolving (Campbell, 2004a). Toxic heterophils are classified on a scale of +1, +2, +3 and +4 depending upon the degree of toxicity. A +1 toxic heterophil shows increased cytoplasmic basophilia. A +2 toxic heterophil shows increased cytoplasmic basophilia, slightly abnormal granulation

(i.e. partial degranulation, coalescing granules, or abnormal appearing granules or vacuolation). A +3 toxic heterophil will show changes that are more severe than +2 toxicity and the nucleus may show slight karyorrhexis or karyolysis. A +4 toxic heterophil will show marked changes in the cytoplasm and nucleus. Toxic heterophils are uncommon and usually seen in birds that are critically ill (Campbell, 2004a).

Avian heterophils are involved in controlling bacterial, viral and parasitic infections. One striking difference between birds and mammals is the process of pus formation. In mammals, neutrophils accumulate, leading to liquefaction and abscess formation. This liquid pus can spread along tissue planes or can form exudates that are removed by way of clearance pathways such as mucociliary apparatus (Harmon, 1998). In birds, however, heterophils accumulate and are resolved by means of inspissations of necrotic heterophils into a caseous mass rather than liquefaction. The necrotic heterophils are walled to form heterophilic granulomas (Montali, 1988; Harmon, 1998). The process is advantageous except when the caseous masses that are formed interfere with organ functions, such as in granulomas in the lungs or air sacs. In certain locations, these caseous masses could persist indefinitely. The exact mechanism of pus formation in birds has not been completely understood, though proposed mechanisms include variances in hydrolytic enzyme activity, such as lack of myeloperoxidase in heterophil, or the lack of as yet-to-be identified proteases in birds (Harmon, 1998).

Conditions that cause an increase in the peripheral blood include infection (e.g. bacterial, fungal, viral, parasitic), inflammation, stress, certain toxicities, trauma, and leukemia (Gildersleeve *et al.*, 1987; Andreasen *et al.*, 1993; Harmon, 1998; Bienzle and Smith, 1999). Infectious agents that commonly lead to heterophilia include *Mycobacterium* spp, *Chlamydophila psittaci*, *Aspergillus* spp and birds acutely or

chronically infected with *Mycoplasma* spp. Heterophilia associated with infections with these organisms is commonly accompanied by monocytosis (Branton *et al.*, 1997).

Heterophils have been demonstrated to be the dominant component of exudates in birds, although lymphocytes and basophils may also be present early in inflammation (Harmon, 1998). Heterophilia with toxic change is indicative of severe systemic illness such as septicaemia, chlamydophilosis, fungal infection, or viraemia. The development of toxic change may indicate lack of control of an infectious process and often carries a poor prognosis (Campbell and Ellis, 2007).

Some toxins (e.g. organophosphate) can lead to heterophilia (Heatley and Jowett, 2000). In addition, heterophilia has been observed in cases of zinc toxicosis, presumably as a result of gastrointestinal inflammation, stress, and decreased resistance to pathogens (Campbell and Ellis, 2007).

Similar stress response as seen in mammals has been reported in birds. For example, Macaws may demonstrate marked leukocytosis with heterophilia as a result of transport and handling. Corticosteroid administration results in an increase in circulating heterophils and lymphopaenia in birds (Harmon, 1998).

A decrease in heterophil number can be seen with increased use of the heterophils or its decreased production. Utilization of mature cells and resultant left shift can be seen 12 to 24 hours after the initiation of acute inflammation (Latimer *et al.*, 1988). Overwhelming infection such as septicaemia can lead to a degenerative left shift which indicates bone marrow depletion. Psittacine circovirus can lead to leukopaenia with pancytopenia in African Gray Parrot (Schoemaker *et al.*, 2000).

2.4.4 Lymphocytes

Lymphocytes may be the major circulating leucocyte in the blood of some species of birds (Campbell and Dein, 1984). Avian lymphocytes are similar in morphology to

mammalian lymphocytes. They are round cells with centrally positioned or slightly eccentric nuclei. The nucleus chromatin is densely aggregated and there is a high nuclear-to-cytoplasm (N/C) ratio. The cytoplasm is basophilic and homogenous, with no vacuolation. Generally, there are no granules in lymphocytes, although occasionally, rare azurophilic granules may be present. There is significant variation in the sizes of the avian lymphocytes. Small lymphocytes often resemble thrombocytes (Mitchell and Johns, 2008). Small lymphocytes predominate in most birds, with irregular projections or blebs frequently observed on this cell type (Campbell, 1994). The large lymphocytes may be difficult to distinguish from monocytes. Large B lymphocytes, that is, plasma cells are sometimes seen in avian blood smears. These cells are large with eccentrically located nuclei, an intensely basophilic cytoplasm, and a distinct Golgi zone (Mitchell and Johns, 2008).

Birds have been used as pioneer model to study immunology, which resulted in the identification of a B- and T-lymphocyte lineage. Just like their human counterparts, the avian lymphocytes appear to function in the same manner with the B-cells (bursa dependent) having immunoglobulin receptors for antigens, while T-cells (thymus dependent) involved in cell mediated immunity (Campbell and Dein, 1984; Dieterlen-Lievre, 1988).

Reactive lymphocytes are small to medium sized with densely clumped chromatin and intensely basophilic cytoplasm. Nucleoli are usually absent, and a pale Golgi zone and vacuolation may be present. Reactive lymphocytes are usually seen in small numbers in peripheral blood smears of healthy birds (Mitchell and Johns, 2008). However, an increased presence of reactive lymphocytes is commonly observed in birds that have infectious disease (Mitchell and Johns, 2008).

Blast transformed lymphocytes which are large lymphocytes with smooth dispersed chromatin may occasionally be observed in avian blood smears. There is abundant blue cytoplasm, and there is often a prominent Golgi zone, these cells are neoplastic, indicating lymphoid leukaemia or a leukaemic phase of lymphoma, but can also be seen as a result of immunologic stimulation (Mitchell and Johns, 2008).

Lymphocytosis usually occurs as a result of antigenic stimulation. This can occur in psittacine birds with viral disease, such as herpesvirus or psittacine circovirus (Fudge and Joseph, 2000). This result is inconsistent as the same viral diseases may instead result in heterophilia or leukaemia (Fudge and Joseph, 2000; Schoemaker *et al.*, 2000). Lymphocytosis also occurs with wound healing, inflammatory diseases, parasitic infections, and viral diseases (Campbell, 2004a), and with lymphoid leukaemia (Latimer, 1994). Anaemia and thrombocytopenia may also be present in birds with lymphocytic leukaemia (Latimer, 1994). Lymphocytic leukaemias are rare in birds in comparison to lymphosarcoma (Newell *et al.*, 1991).

Some species of birds are normally lymphocytic, with lymphocytes representing up to 70% of the total leucocytes. Examples of such lymphocytic species include the Amazon parrots and canaries (Mitchell and Johns, 2008). Lymphopenia may be seen as a result of excess endogenous or exogenous corticosteroids (Harmon, 1998) as observed in stressful conditions and malnourished birds (Campbell, 2004b).

2.4.5 Monocytes

Monocytes are the largest leucocytes in normal peripheral blood smears, and they resemble their mammalian counterparts. The typical monocyte is round or amorphous in shape with a kidney-shaped, rounded, oval or lobed nucleus. The chromatin is lace-like and not as densely clumped as in lymphocytes. The cytoplasm is generally deep blue or grayish blue often presenting a fine pink- or purple-staining granular area near the

nucleus and contains discrete vacuoles (Mitchell and Johns, 2008; Claver and Quaglia, 2009). High vacuolated monocyte suggests increased phagocytic activity and may indicate a response to a systemic antigen (Campbell, 2004a). Monocytes have more cytoplasm and a paler nucleus than large lymphocytes (Campbell and Dein, 1984).

Monocytes have phagocytic activity and transform into macrophages after they migrate into tissues. Their cytoplasmic granules contain lysozymes that are involved in the destruction of invading organisms and chemicals involved in mediating inflammation (Latimer and Bienzle, 2000; Mitchell and Johns, 2008).

Increased monocytes in peripheral circulation (monocytosis) is often seen with infectious and/or inflammatory disease, especially with granulomatous diseases such as aspergillosis or mycobacteriosis. *Chlamydophila psittaci* infections in birds also often results in monocytosis usually due to the production of chemotactic agents that attract monocytes (Campbell, 1994). Although monocytosis is common with chronic inflammation, acute infections, such as *Mycoplasma* spp infections, may lead to monocytosis in addition to heterophilia and lymphopaenia (Branton *et al.*, 1997). Monocytosis has also been observed in birds fed a zinc-deficient diet (Wight *et al.*, 1980).

2.4.6 Heterophil-to-lymphocyte (H/L) ratio

In birds, the heterophil-to-lymphocyte (H/L) ratio is a useful tool for monitoring stress response (El Lethey *et al.*, 2003; Post *et al.*, 2003; Davis *et al.*, 2008) as well as infection status for some diseases (Davis *et al.*, 2004; Chakarov *et al.*, 2008; Fokidis *et al.*, 2008; Norte *et al.*, 2009). Acute stress is known to increase the H/L ratio (Lazarevic *et al.*, 2000; Ewenson *et al.*, 2001; Ruiz *et al.*, 2002; Scope *et al.*, 2002; El Lethey *et al.*, 2003; Bedáňová *et al.*, 2007; Davis *et al.*, 2008). These findings have led avian

ecologists to use the H/L ratio as a general measurement of health status, which is quick, repeatable, and easy to obtain (Ots *et al.*, 1998; Ardia and Schat, 2008).

2.5 Thrombocytes

Thrombocytes are the functional equivalent of mammalian platelets (Claver and Quaglia, 2009). The mature thrombocytes are small oval- to rectangular-shaped cells with a round nucleus that contains densely clumped purple-red chromatin. They appear more rounded than erythrocytes. There is a high nucleus/cytoplasm ratio, and the nucleus is rounded than the nucleus of the red cell (Mitchell and Johns, 2008). Distinguishing thrombocytes from small lymphocytes can be challenging. However, the cytoplasm of thrombocytes is colourless to pale gray or blue and may be reticulated. It may contain large vacuoles, or may contain eosinophilic granules (Campbell and Dein, 1984; Campbell, 2004a; Campbell and Ellis, 2007). Thrombocytes often clump together in blood smears particularly when processed without the use of an anticoagulant making them easier to identify (Campbell, 2004a; Claver and Quaglia, 2009). When clumping occurs, they may show degranulation of specific granules, cellular degeneration, and nuclear pyknosis (Latimer and Bienzle, 2000). The avian thrombocytes are derived from the stem cell precursors rather than from megakaryocytes as in mammals (Mitchell and Johns, 2008).

Immature thrombocytes are round cells with slight cytoplasmic basophilia (depending upon the stage of maturity) and round nuclei that appear less pyknotic than mature cells. An increased number of immature thrombocytes are seen in birds responding to thrombocytopaenia (Campbell, 2004a). Thrombocytosis and an increase in the size of thrombocytes may be seen with chronic inflammation in birds (D'Aloia *et al.*, 1994).

Similar to the mammalian platelets, avian thrombocytes produce thromboplastin and they aggregate in a site of vascular injury, forming a haemostatic plug (Mitchell and Johns, 2008; Claver and Quaglia, 2009).

Avian thrombocytes have phagocytic abilities. They play an important role in removing foreign materials from the blood and probably have some functions in non-specific immunity (Edmonds, 1968; Grecchi *et al.*, 1980; Bounous and Stedman, 2000). The importance of thrombocytes in fighting infection in avian species is unknown (Mitchell and Johns, 2008).

Increased destruction or use of thrombocytes in conditions such as septicaemia or possibly disseminated intravascular coagulopathy (DIC) and thrombocytopenia can be seen. Thrombocytopenia can be seen as a component of pancytopenia in some viral diseases, such as psittacine circovirus or polyomavirus infections (Fudge, 1997) and also in cases of lymphoid leukaemia (Latimer, 1994).

Thrombocyte abnormalities and dysfunction syndromes are rarely diagnosed and not described in birds respectively (Claver and Quaglia, 2009).

2.6 Serum or Plasma Biochemistry

The relevance of established reference for biochemical parameters of free ranging populations is not only important for veterinarians dealing with captive and rehabilitating animals, but also for studying animals in their natural habitats. In recent times, few studies have attempted to establish reference values of biochemical parameters for some birds (Ferrer, 1994; Balbontín and Ferrer, 2002; Casado *et al.*, 2002) and most refer to captive individuals under controlled conditions and non-natural diets (Balasch *et al.*, 1976; García-Rodríguez *et al.*, 1987a,b; Ferrer *et al.*, 1987; Polo *et al.*, 1992; Dobado-Berrios *et al.*, 1998). The vast majority of these biochemical profiles are usually modifications of those used to evaluate the health of domestic mammals and

sometimes humans (Campbell, 1998). Depending on the sample volume, the number of tests that can be performed can be limited. However, modern blood biochemical analyzers make use of microtechniques that allow chemistry panels to be performed on small sample volumes (Campbell, 1998).

The age, gender, environment, nutritional status, and physiologic status of the bird have great influence on the establishment of normal reference values for a given species of bird (Rodgers and Gass, 1983; Kaneko, 1997; Campbell, 1998; Bowerman *et al.*, 2000; Artacho *et al.*, 2007; Zaahkouk *et al.*, 2013). Also, it is often difficult to compare results from one laboratory with those obtained from another because of the variations in methodology. Test values will vary depending on the substrate, buffer and incubation temperature used by the laboratory (Hochleithner, 1994). Consequently, most avian practitioners make use of decision levels because it is difficult to guarantee that a given population of birds represents healthy birds of that species and published reference values may vary among laboratories. Decision levels are threshold values that help the clinician decide whether to treat the patient or conduct further diagnostic test (Campbell, 1998). The decision is said to be based on these levels for each individual test; the clinician may either treat or retest if the value obtained is outside the threshold limit (Campbell, 1998). These decision levels are obtained with reference to published values and the personal experience of the clinician, hence, resulting in variations with clinicians. To further forestall this variation, it is usually best to obtain normal set of reference values for the individual bird during health for comparison during illness (Campbell, 1998). In serum or plasma biochemical analysis for the purpose of establishing the health status of an individual, the types of testing carried out include enzymology, metabolites, electrolytes and hormones.

2.6.1 Metabolites and nutrients

Metabolites (which include plasma ammonia, bile acids, some enzymes i.e. amylase and lipase, bilirubin, cholesterol, creatinine, glucose, inorganic phosphate, iron, total protein, urea, uric acid and triglycerides) can be measured to provide information about the functional capacity of the organs that are involved in a particular metabolic pathway. Tests are usually designed to provide measurements of end-point metabolites (Hochleithner, 1994).

2.6.1.1 Glucose

Glucose is continuously required as an energy source by all the body cells and must be maintained at adequate levels in plasma (Hochleithner, 1994). Glucose levels are maintained principally through the conversion of liver glycogen, with some being derived from non-carbohydrate sources (hepatic gluconeogenesis). In periods of starvation, glucose is increasingly derived from the breakdown of fats and proteins, primarily from muscle tissue, through gluconeogenesis in the liver and the kidneys. All plasma glucose is filtered from the blood through the renal glomeruli and then totally reabsorbed in the tubules (Hochleithner, 1994). The normal plasma glucose concentration of birds ranges between 200 and 500 mg/dl. Plasma glucose concentration remains stable during 1 – 5 days of fasting in birds (Campbell, 1998). This however, induces hyperglycaemia rather than starvation hypoglycaemia (Lumeij, 1987a). This finding has important consequences for avian anaesthesia and gastrointestinal surgery, as pre-surgical fasting varying from four hours (emptying of crop) to 24 hours (emptying of the entire gastrointestinal tract) can be advantageous. Prolonged fasting is not recommended in birds that weigh less than 100 grams (Hochleithner, 1994).

Hypoglycaemia is extremely rare in birds and when present, is almost never associated with starvation (Harris, 2000). Hypoglycaemia, however, occurs with septicaemia, severe liver disease, enterotoxaemia and prolonged starvation (Campbell, 1998; Harris,

2000). It is worth noting that glucose concentrations can be artificially decreased during storage if the blood sample is contaminated with bacteria (Hochleithner, 1990).

Plasma glucose levels are higher in juvenile than adult budgerigars (Hochleithner, 1989b). Variations also occur due to time of day and amount of environmental stress (Lewandowski *et al.*, 1986). Hyperglycaemia occurs commonly due to catecholamine release (as seen in exertion and excitement) or after meals, glucocorticosteroid excess, and occasionally diabetes mellitus (Woerpel and Roskopf, 1984; Amand, 1985; Glystorff and Grimm, 1987; Lumeij, 1987a; Campbell, 1998; Harris, 2000). Diabetes mellitus in birds has a variable pathophysiology and is associated with glucose concentration greater than 800 mg/dl (often resulting from glucagon excess) (Campbell, 1998). Diabetes mellitus has been confirmed in budgerigars, cockatiels, Amazon parrots, Scarlet Macaws, Umbrella Cockatoos and a Toco Toucan (Lewandowski *et al.*, 1986). Transient elevations in glucose have been reported in cockatiels with egg-related peritonitis (Woerpel and Roskopf, 1984). Decreases in plasma glucose levels can be due to hepatic dysfunction (e.g. Pacheco's disease virus), impaired glucose production or its excessive utilisation (e.g. septicemia, neoplasia, aspergillosis) (Roskopf, 1982; Woerpel and Roskopf, 1984).

Care should however be ensured in the diagnosis of diabetes mellitus in birds because of the frequency with which hyperglycaemia is caused by stress. Such diagnosis must be supported by other clinical evidence (Harris, 2000). A visibly normal hyperglycaemic patient displaying no polydipsia, no polyuria and no weight loss should not automatically be considered diabetic. To confirm a diagnosis, repeat testing, other diagnostic tests and observations are necessary (Harris, 2000). It is of paramount importance to evaluate for glucose in convulsing birds (especially in young birds of prey) or those with glucosuria (Hochleithner, 1991b).

2.6.1.2 Total protein and electrophoresis

Total protein concentration is a significant indicator of the health condition and production features of the organism because of its numerous roles in the physiology. It is the most frequently examined blood parameter for diagnostic purposes in veterinary clinical laboratories (Georgieva *et al.*, 2009).

The term serum (Altman, 1979) and plasma (Campbell and Dein, 1984) have been used interchangeably. However, refractometry in plasma yields consistently higher values when compared to the Biuret method in both serum and plasma which implies that substances other than protein substantially add to the refractive index (Lumeij, 1987c). In avian patients where only small amounts of blood can be collected, it may be advantageous to determine total protein in plasma instead of serum (Hochleithner, 1994). The concentration of total protein in plasma is about 1.5 g/dl than serum because the former includes fibrinogen (Lumeij, 1987c; Hochleithner, 1994). Total protein concentration by Biuret method range between 2.5 and 4.5 g/dl in normal birds (Campbell, 1998). Campbell and Dein (1984) reported that the total plasma protein values lower than 3.0 g/dl are hypoproteinaemic and birds with values less than 2.5 g/dl have poor prognosis. Amand (1985) reported that birds with total plasma proteins below 3.5 g/dl appear to have less of a chance to recover from their illness than patients with values in the normal range, and that bird with a total plasma protein value of 2.0 g/dl or less have a grave prognosis. However, these statements are invalidated by the fact that reference values for total serum protein in a number of healthy bird species are lower than the above stated values (Lumeij, 1987c). Although determination of plasma proteins seldom leads to a specific diagnosis (e.g. in the case of monoclonal gammopathies), it will help clinicians to evaluate the severity and progress of disease (Lumeij and Westerhof, 1987).

Changes in total protein must be interpreted with respect to physiologic influences disassociated with disease. Age and stage of development will influence the concentration of total protein in birds. Advancing age has been associated with increases in total protein in several species (Glystorff and Grimm, 1987; Clubb *et al.*, 1991a,b).

Hypoproteinaemia can reflect reduced synthesis caused by chronic hepatopathies, malabsorption caused by chronic enteropathies (enteritis, tumours, parasitism), increased loss caused by proteinuria due to renal disease, blood loss and malignant tumours (rarely seen in birds) or starvation and malnutrition (Hochleithner, 1994). Hyperproteinaemia may be induced by chronic infectious diseases that stimulate the synthesis of gamma globulin. It also has been seen with chronic lymphoproliferative disease that resembles leukosis in chicken (Lewandowski *et al.*, 1986) and myelosis in budgerigars (Hochleithner, 1991b). However, dehydration should always be determined and ruled out as a cause of hyperproteinaemia (Hochleithner, 1994).

Most frequently used electrophoresis methods identify five main protein fractions in birds:

- albumin
- globulin, further divided into
 - alpha (α) -1 and -2,
 - beta (β) -1 and -2
 - gamma (γ)
- Pre-albumin fraction has been described in pigeons and some parrot species (Lumeij, 1987c; Lumeij and Overduin, 1990; Clubb *et al.*, 1991a,b; Filipović *et al.*, 2007).

Pre-albumin and albumin fraction:

The diagnostic significance of pre-albumin fraction of the serum protein in birds is not fully known (Harris, 2000). However, similar to albumin, it may function as transport protein. There does not appear to be a comparable component in the mammalian blood. Of the total serum protein in avian samples, pre-albumin may comprise as much as 40%. It appears that pre-albumin values may have the same significance in some species as low albumin values in other species (Harris, 2000).

In healthy birds, the albumin fraction is the largest protein fraction. It typically comprises 45 – 75 of avian serum protein in species that have high pre-albumin values, and tend to be lower in species with low pre-albumin values (Lumeij, 1987d; Harris, 2000).

Albumin in birds, as it does in mammalian species functions primarily in the maintenance of colloid osmotic pressure, as a rapid substitute for indispensable amino acids, assuring glucose through gluconeogenesis in transport of minerals and hormones, in build up of enzymes and immune system in the organism (Harris, 2000; Filipović *et al.*, 2007). Decreases in albumin concentration can occur from decreased synthesis due to chronic liver disease or chronic inflammation, increased albumin loss due to renal disease, parasitism or over-hydration (Quesenberry and Moroff, 1991). A decrease in albumin causes oedema because of a decrease in oncotic pressure (Hochleithner, 1994).

Globulin fraction:

The globulin fraction comprises of α , β , and γ components. High electrophoresis further divides the globulin into subgroups (Werner and Reavill, 1999). Each of the three primary fractions contains proteins active in different physiological and pathophysiological conditions (Harris, 2000; Filipović *et al.*, 2007). In either acute or chronic inflammatory conditions, an increase in total protein caused by elevated α , β ,

and γ globulin concentrations may occur though albumin concentrations are decreased in these situations (Lumeij, 1987c; Hochleithner, 1994).

Alpha globulins:

This consists of 2 principal fractions: α_1 and α_2 . Acute phase inflammatory proteins such as α -lipoprotein, α_1 -antitrypsin and α_2 -macroglobin and haptoglobin are contained within this group of globulins (Harris, 2000). The α_2 -macroglobin sometimes migrates into the β range. Increased α globulin levels in birds have been associated with parasitism. Other consistent correlations have yet to be identified. However, elevations in α globulins are somewhat uncommon (Harris, 2000).

Beta globulins:

Other acute phase inflammatory proteins (β_2 -macroglobin, fibronectin, transferrin and β -lipoprotein, constitute the β globulins. In some bird species, e.g. African grey parrot, the β component of the electrophoresis consists of two primary components: β_1 and β_2 (Harris, 2000).

Increase in β globulin levels may indicate chronic liver or kidney disease, or chronic inflammatory diseases such as aspergillosis or avian chlamydiosis (Harris, 2000). Egg production has been shown to cause an increase in β globulins in birds which is attributed to the transferrin component. This significant elevation combined with a 1.5 to 2 fold increase in the blood calcium (Ca) level in birds of unknown sex, is highly suggestive that the bird is an ovulating female (Campbell, 1998; Harris, 2000).

Gamma globulins:

Contrary to the mammal γ globulins which appear to have two primary fractions, γ_1 and γ_2 , in the avian species only one fraction was demonstrated (Harris, 2000). Antibodies complement and complement degradation products are principally the components

of the gamma globulins. Elevated γ globulins are a common finding in birds suffering from acute *Chlamydomphila psittaci* infection (Harris, 2000).

Albumin:Globulin (A:G) ratio:

This is an important aspect of protein that should always be observed. The total protein is not considered normal until the A:G ratio is known to be normal. The normal A:G ratio ranges from 1.6 to 4.5 (Harris, 2000). Disease conditions such as egg peritonitis and chronic infectious diseases such as aspergillosis, psittacosis and tuberculosis have shown to cause a decrease in A:G ratio (Lumeij, 1987c; Hochleithner, 1994).

When birds respond favourably to treatment, an increase in the albumin concentration and a decrease in the globulin concentration can be observed, which leads to normalisation of the A:G ratio. In liver failure, extremely low serum protein concentrations can occur in combination with a decreased A:G ratio (Lumeij, 1987c; Hochleithner, 1994). In dehydrated birds, elevated total protein concentrations with a normal A:G ratio can be expected (Lumeij, 1987c; Campbell, 1998).

2.6.1.3 Blood urea nitrogen

The diagnostic use of blood urea nitrogen (BUN) in avian medicine is limited because of its low measurable levels. Moreso, the avian kidney appears to excrete most urea via glomerular filtration as long as the patient's hydration is adequate (Lumeij, 1987b; Harris, 2000). The kidney excretes urea by glomerular filtration. Tubular reabsorption can occur and is dependent on the state of hydration. During hydration and dehydration almost all of the filtered urea is excreted and nearly all of the filtered urea is absorbed, respectively (Lumeij, 1987b). The tubular reabsorption of urea in conditions of renal failure accompanied by a low urine flow (e.g. dehydration), causes a disproportionate increase in plasma urea concentrations which consequently results in elevated urea:uric acid ratio (Lumeij, 1987b).

Blood urea may be a better indicator of hydration than renal function, and an increased urea would imply dehydration (Harris, 2000). According to a research done by Lumeij (1987b), reference values for plasma urea concentration in racing pigeons were found to be 7.2 – 12.6 mg/dl. But after water deprivation for 3 days, 6.5 to 15.3 fold increases in plasma concentration were observed.

An increased in the BUN concentration suggests prerenal azotaemia in some avian species, such as racing pigeons (Lumeij, 1987b; Campbell, 1998). Normal plasma urea nitrogen concentrations of most non-carnivorous birds are less than 5 mg/dl, while the carnivores tend to have slightly higher normal values (Balasch *et al.*, 1976; Gee *et al.*, 1981; Ferrer *et al.*, 1987, Campbell, 1998). Urea concentration rises during periods of fast as a consequence of the catabolism of tissue proteins as an energy source (Okumura and Tasaki, 1969; García-Rodríguez *et al.*, 1987b; Ferrer and Dobado-Berrios, 1998; Totzke *et al.*, 1999).

2.6.1.4 Creatinine

Creatinine originates from muscle metabolism and is formed from creatine phosphate. The plasma concentration of creatinine and excretion of creatinine in the urine is remarkably constant per individual and there is a direct relation with muscle mass (i.e. body weight) (Lumeij, 1987b,c). Excretion of creatinine is solely via kidneys. It is freely filtered and reabsorbed in the tubules (Glystorff and Grimm, 1987). In all avian species that have been investigated, the reference intervals for creatinine have been between 0.1 to 0.4 mg/dl, with no significant differences between species (Lumeij, 1987b,c; Hochleithner, 1994). It was well known that creatinine levels in avian serum samples typically fall below a measurable range and are rarely useful in avian clinical pathology especially in evaluating renal function because in birds the amount of creatinine formed is negligible in relation to the amount of creatine (Lewandowski *et*

al., 1986; Lumeij, 1987b,c; Harris, 2000). More also, certain technical factors contribute to a high incidence of artefactual change which may include pseudocreatinines such as glucose, protein, ascorbic acid and pyruvic acid (Lewandowski *et al.*, 1986; Harris, 2000) making its determination of less importance.

Elevated values of creatinine have been recorded in cases of dehydration in racing pigeons (Lumeij, 1987b) and kidney disease, but creatinine is not considered to be a reliable indicator of renal function (Harris, 2000). Elevations have also been described in connection with egg-related peritonitis, septicaemia (e.g. chlamydiosis) and nephrotoxic drugs (Lewandowski *et al.*, 1986).

In dogs and man, the urea:creatinine ratio is used to differentiate between renal and pre-renal causes of azotaemia (Leaf and Cotran, 1976). Prerenal azotaemia can be expected to be a common complication of many disease conditions in birds (i.e. heart failure, dehydration) (Lumeij, 1987b).

2.6.2 Electrolytes

Electrolytes may be positively charged (cations) or negatively charged (anions). Balances of these electrolytes are essential for all living matter. The commonly measured electrolytes include potassium, chloride and sodium. Trace elements including magnesium may also be determined. The major electrolytes occur primarily as free ions while the trace elements exist primarily in combination with proteins (Hochleithner, 1994).

2.6.2.1 Calcium

As a major constituent of bone, calcium plays a vital role in the structure of the body. It also has important physiologic functions involving the transmission of nerve impulses, the permeability and excitability of all membranes, the activation of enzyme systems

(e.g. blood clotting), calcification of egg shells and contraction of the uterus during oviposition (Hochleithner, 1994). Calcium levels are profoundly influenced by a number of normal as well as pathological conditions, therefore, great care should be exercised in the interpretation of abnormal findings (Harris, 2000). Almost all pathological changes are secondarily to conditions not associated with dietary levels. The effectiveness of the parathyroid gland and dietary deficiencies of calcium will rarely cause subnormal blood levels (Harris, 2000). Heparinised plasma or serum can be used for calcium concentration analysis. Some calcium-binding anticoagulants, like EDTA, citrate and oxalate (fluoride oxalate is used for determining glucose levels in mammals) will cause falsely low values of calcium (Hochleithner, 1994).

Hypoalbuminaemia will result in artefactual depression of measured calcium levels. Other causes of lowered blood calcium levels include hypocalcaemic syndrome in African grey parrots, glucocorticoid administration, hypomagnesaemia and insufficient exposure to full spectrum lighting (Hochleithner, 1989b,c; Lumeij, 1990; Hochleithner, 1991b; Harris, 2000).

Elevated calcium levels have been reported in conjunction with elevated albumin (Ivins *et al.*, 1985; Harris, 2000). Two fold or greater elevations typically occur with ovulation. This rise in calcium is caused by an increase in the protein bound calcium, due to oestrogen-induced transport of yolk proteins to the ovary as calcium complexes, whereby the concentration of ionized calcium which is the physically important fraction, remains constant. In most laboratories, for technical reasons, only total calcium is measured (Lumeij, 1990). Young birds generally have lower calcium concentrations than adults (Hannon, 1979; Hochleithner, 1989a). Elevated levels of calcium have been associated with vitamin D₃ toxicity, osteolytic bone tumours, renal adenocarcinoma and dehydration. Even in cases of severe dietary calcium deficiency, parahormone will

normally mobilize bone to maintain calcium blood concentrations within physiologic limits (Hochleithner, 1994; Harris, 2000).

2.6.2.2 Phosphorus

Inorganic phosphorus is derived from the diet. It is a major constituent of bone and a vital cellular component, playing important roles in the storage, release and transfer of energy and in acid-base metabolism (Hochleithner, 1994). Changes in inorganic phosphorus concentration can occur with several diseases, but not on a consistent basis. The diagnostic value of the inorganic phosphorus is poor (Hochleithner, 1994).

Diets that consist mostly of seeds may lead to increased phosphorus levels. Juvenile budgerigars were found to have higher concentrations than adults (Hochleithner, 1989a). Elevated plasma phosphorus is occasionally observed in avian renal failure which suggests chronicity and offers a guarded prognosis (Woerpel and Roskopf, 1984; Amand, 1985; Hochleithner, 1991b; Harris, 2000). Increase in phosphorus has also been reported in hypoparathyroidism (Woerpel and Roskopf, 1984; Lewandowski *et al.*, 1986) and nutritional secondary hyperparathyroidism (Hochleithner, 1989b,c; Harris, 2000).

Haemolysis may result in a false increase in phosphorus concentration (Hochleithner, 1994). Decreased phosphorus values may be associated with malabsorption and vitamin D deficiency (calcium levels also decreased), malabsorption because of phosphate binding agents in the diet (calcium normal) and long term glucocorticoid therapy (Hochleithner, 1994; Harris, 2000).

2.6.3 Enzymology

Each cell within an organ has a specific function and contains enzymes designed to perform those functions. In some situations, enzymes are unique to specific cells within an organ, and in other cases, enzymes are found in numerous cells from various organs. When the integrity of a cell is disrupted, enzymes escape into the surrounding fluid compartment, where their activities can be measured as an index of cellular integrity (Hochleithner, 1994).

An enzyme that is released into the serum/plasma must be easy to assay in order to be of diagnostic value. In addition, the assay must be economically feasible and indicate pathologic changes in a specific organ, or a defined small group of organs. The enzyme must also be stable in the serum/plasma for a sufficient time to permit its detection (Hochleithner, 1994).

It is important to realise that cells must be damaged before they release enzymes into the serum/plasma. Therefore, enzymatic-based tests are a measure of cell damage, and not necessarily a measure of organ function. Anorexia causes the cell membranes to lose its integrity so that soluble enzymes from cytosol can leak into the serum/plasma (Hochleithner, 1994). This loss of integrity may be observed histologically as a swelling of the cell. Anoxic RBCs, for example, leak cytosolic lactate dehydrogenase (LDH) into serum/plasma, causing an increase of LDH activity in a sample. Combining the values obtained for several enzymatic assays will increase the diagnostic values of the biochemical evaluation of a patient (Hochleithner, 1994).

Enzyme activities in tissues or serum/plasma are usually in such low concentrations that it is not practical to quantitate the enzyme directly. Therefore, enzymes are measured indirectly based on their *in vitro* activity under controlled or specific conditions at which their activity is proportional to enzyme concentration (Hochleithner, 1994). Some

of the enzymes assayed include alanine aminotransferase (ALT or GPT), alkaline phosphatase (AP), aspartate aminotransferase (AST or GOT), creatine kinase (CK or CPK), creatinine, gamma glutamyl transferase (GGT), glutamate dehydrogenase (GLDH), and lactate dehydrogenase (LDH) (Hochleithner, 1994).

2.6.4 Hormones

It has been suggested that hormone concentrations may be good indicators of disease in humans or mammals, but their analytic accuracy and precision are difficult to evaluate in birds (Roskopf *et al.*, 1982; Lumeij, 1987b). Hormones are usually detected using a radio-immunoassay (RIA) or an ELISA, both of which require an antigen/antibody reaction. Non-specific cross-reactions that occur when tests designed for mammalian hormones are used for bird plasma can lead to questionable results (Hochleithner, 1994).

2.7 Biochemical Assay Method

Historically, wet chemistry systems have been used for evaluation of blood parameters. Wet chemistry means that liquid reagents act with a certain volume of sample under strictly defined constant conditions (e.g. temperature, pH, time) and produce a change of colour that is proportional to the concentration of substances or the activity of enzymes. As the indicator dye changes colour, the reaction is read spectrophotometrically. The reagents can be prepared in the laboratory, hence, the cost for frequently used tests is inexpensive on a per test basis. The minimum sample size often depends on whether reagents are added by hand (older systems that may require 100 to 200 µl/parameter) or automatically (Autoanalyzer only requires about 20 µl/parameter) (Hochleithner, 1994).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The experiment was conducted in Zaria, which is located in the Northern Guinea Savannah zone of Nigeria (11⁰10'N, 07⁰38'E). There are three seasons in this zone, namely harmattan (November to February), hot (March to early June) and rainy (late June to October). Trees and grasses characterise the vegetation of this zone with average rainfall, ranging from 1,000 mm to 1,250 mm and temperature of 17⁰C to 33⁰C (Oladele *et al.*, 2003). Different species of wild birds have been identified in this geographical zone. Some of these include Laughing Dove (*Streptopelia senegalensis*), Bruce's Green Pigeon (*Treron waalia*), Speckled Pigeon (*Columba guinea*), Mourning Collar-Dove (*Streptopelia decipiens*), Senegal Parrot (*Poicephalus senegalus*), Rose-Ringed Parakeet (*Psittacula krameri*), Little Weaver Bird (*Ploceus luteolus*), Cattle Egret (*Bubulcus ibis*), White-Rumped Swift (*Apus caffer*), Red-Billed Quelea (*Quelea quelea*), African Silver-Billed (*Euodice cantans*) and Northern Red Bishop (*Euplectes francisca*) (Oladele *et al.*, 2012).

3.2 Experimental Birds

A total of two hundred and thirty three (233) apparently free-living wild birds from twelve (12) different species were used for the study. The birds included the twenty (20) Laughing Dove (*Streptopelia senegalensis*), twenty (20) Bruce's Green Pigeon (*Treron waalia*), nineteen (19) Speckled Pigeon (*Columba guinea*), sixteen (16) Mourning Collar-Dove (*Streptopelia decipiens*), twenty (20) Senegal Parrot (*Poicephalus senegalus*), twenty (20) Rose-Ringed Parakeet (*Psittacula krameri*), twenty (20) Little Weaver Bird (*Ploceus luteolus*), twenty (20) Cattle Egret (*Bubulcus ibis*), twenty (20) White-Rumped Swift (*Apus caffer*), twenty (20) Red-Billed Quelea (*Quelea quelea*),

twenty (20) African Silver-Billed (*Euodice cantans*) and twenty (20) Northern Red Bishop (*Euplectes francisca*). The birds were trapped around Area C (Ahmadu Bello University staff quarters), Shika and Bomo. They were captured between July 2013 to May 2014 by the use of net, wooden or metal traps.

Sampling of birds was by convenient sampling. After capturing the birds, they were taken to the Department of Biological Sciences, Faculty of Sciences, Ahmadu Bello University, Zaria for identification of species by an ornithologist. Thereafter, the photograph of each bird was taken using the Samsung 10.1 Megapixel Digital Camera, Model: NV10.

3.3 Sample Collection and Processing

3.3.1 Collection of blood and serum samples

Five millilitres of blood were collected from each large bird (doves, pigeons, parakeet, parrot and egret) using a plastic disposable 5 ml syringe and 25 x 7 mm gauge needle via brachial or jugular venipuncture after the birds were properly restrained by an assistant. In cases of the smaller birds (finches and swifts) an insulin syringe was used to collect 1 ml of the blood using the jugular vein and then emptied into a commercially available sample bottle containing ethylene diamine tetra acetic acid (EDTA) for haematology. Prior to this, the area around the right jugular vein (and the brachial vein for the larger birds) was swabbed with 70% methanol to allow for easy access to the vein and for collection of blood.

From the larger birds, the blood sample from each bird was divided into two parts for the purpose of haematological and biochemical analyses. One part was emptied into a sample bottle containing EDTA. The other part was allowed to coagulate, then

centrifuged at 2,054 x g for 15 minutes to obtain serum which was each collected into a commercially available clean plastic sample bottles, labelled and stored at -20°C until required (10 µl each of the serum was immediately used to assay for glucose). Each of the sample bottles were properly labelled using a permanent marker.

Also, thin blood smears were prepared from the collected blood samples and used for total and differential white blood cell and thrombocyte count. Direct contact with blood was avoided by the use of hand gloves and laboratory coat. The birds were however, marked with an indelible marker and a short twine was tied on the left leg so as to avoid re-sampling a bird more than once before they were subsequently released into the wild. The blood samples of the clinically sick wild birds were also collected and used for haematological and serum biochemical analyses. Subsequently, the birds were sacrificed by injecting air directly to the heart using a 21 gauge needle and 5 ml syringe. The sacrificed birds were necropsized and all the organs were examined for any gross lesions. Lesions observed were recorded and the affected organs were photographed. Thereafter, relevant internal organs were obtained and fixed in sample bottles containing 10% neutral buffered formalin, properly labelled and processed in the Histopathology Laboratory of the Department of Veterinary Pathology, Ahmadu Bello University, Zaria.

3.3.2 Packed cell volume determination

The packed cell volume (PCV) was determined using standard technique as described by Rehman *et al.* (2003). Non-heparinized capillary tube was filled up to about $\frac{3}{4}$ of its length from one end and the second end was heat-sealed using Bunsen burner. The blood in the sealed capillary tube was then centrifuged for 5 minutes at 4,383 x g using the Saitexiangyi TG12MX® Micro-haematocrit centrifuge machine. Then the

proportion of cells in the total volume of blood was measured and recorded as a percentage using the Hawksley[®] Micro-haematocrit Reader.

3.3.3 Haemoglobin estimation

Blood haemoglobin concentration was assayed colorimetrically as cyanomethhaemoglobin (Drabkin, 1945). Five millilitres of HICN (Drabkin) solution were measured using a 5 ml syringe into plastic test tubes. Twenty microlitres (20 µl) of blood were measured using a micropipette and added to the Drabkin solution in the test tube and properly mixed by gently shaking the test tube. It was centrifuged at 1,509 x g for 15 minutes to separate the empty RBC from interfering with the reading. The supernatant was separated into a sample bottle. The mixture was absorbed into the haemoglobin meter (XF-C, China). After the wiggling pump stops working, the value displayed on the screen was recorded in g/dl as the haemoglobin concentration.

3.3.4 Red blood cells and total white blood cell count

Red blood cells (RBC) and total WBC (or TWBC) counts were determined with the Natt-Herrick solution (1:200 dilution) and the Improved Neubauer haemocytometer (Campbell and Ellis, 2007) as both counts can be prepared directly from the same sample placed in the haemocytometer.

The heparinised blood samples were slightly agitated and the RBC diluting pipette was used to pipette the blood to the 0.5 marking. The tip of the pipette was cleaned properly using a tissue paper without touching the distal opening of the pipette tip with tissue, as this will cause capillary shift of blood into the tissue. The diluting solution (Natt-Herrick) was also pipette to the 101 marking (1:200) without entirely immersing the pipette tip into the diluting fluid. The mixture was well shaken for 1 minute to obtain equal distribution then emptied into a clean sample bottle. The Neubauer haemocytometer and cover slip were cleaned using a dry, lint free cloth. The cover slip

was properly placed on the haemocytometer. The mixture was then agitated a little and a capillary tube was used to withdraw a small aliquot. Both sides of the haemocytometer were filled up (charged) by gently touching the intersection between the cover slip and haemocytometer with the loaded capillary tube avoiding air bubbles and under-filling or over-filling, then left for 5 minutes for cells to settle down.

The light microscope (Olympus-XSZ-107BN), at low power magnification (X40) was used to view the cells and counting was done using the tally counter.

For TWBC count, the WBC in the four outer large squares of the haemocytometer were counted and calculated using the formula below:

$$N/20 = \text{WBC} \times 10^9 / l$$

Where N = Number of WBC counted in the four outer large squares (or in 64 small squares)

For RBC count, the cells contained in the four corner and central squares in the mid section of the haemocytometer were counted. Following the “L” rule: cells that touch the centre triple lines of the ruling on the left and the bottom sides were counted but cells that touch the centre triple lines of the ruling on the right and the top sides were not counted. The RBC count was calculated using the formula below:

$$N/100 = \text{RBC} \times 10^{12} / l$$

Where N = Number of RBC counted in the 5 squares in the mid section of the haemocytometer (or in 160 squares)

Note that both charged sides of the haemocytometer were counted for both the RBC and TWBC and the average calculated.

3.3.5 Preparation of smears for differential leucocyte count and thrombocytes estimation

In all the birds, a pair of smears for each blood sample was made. A small drop (about 2 μ l) of blood was immediately used for the preparation of blood smears each using the

standard slide-to-slide technique. The air-dried smears were properly labelled using a pencil on the frosted end of the slide and then fixed in a fixing jar containing methanol for 3 minutes and air-dried.

Staining was done by flooding the smears with Wright-Giemsa stain for 3 minutes. An equal amount of Sørensen's buffer (pH of 6.8) was added then mixed gently by blowing using a pipette until green metallic sheen forms on the surface. This was allowed to stand for further 6 minutes. The smears were rinsed with the Sørensen's buffer and allowed to stand for a minute for differentiation. The stained slides were then washed copiously with the Sørensen's buffer and the back of the smears were wiped with tissue paper to remove the excess stain and allowed to air dry. These were neatly packed into a slide box until viewing.

Examination of the blood smears was done using a light microscope (Olympus-XSZ-107BN) under high-power magnification with oil immersion (X1,000). One hundred WBC were counted and classified based on their morphologic features (Campbell, 1988; Hawkey and Dennet, 1989; Campbell and Ellis, 2007). The counting was done using the Marble[®] Blood Cell Calculator. The differential WBC count was then expressed as a percentage of the individual cell group. The percentage of each cell was then converted into absolute numbers by reference to the total WBC using the formula below:

$$\frac{\text{Percentage of WBC counted} \times \text{TWBC}}{100} = \text{Absolute Number} \times 10^9 / \text{l}$$

An estimated thrombocyte count was obtained from the stained blood film using the same formula for the indirect estimation of total WBC (Campbell and Ellis, 2007). Valid and reliable results were not obtained where there were evidence of thrombocytes clumping. The absolute number of thrombocytes was estimated by using the formula below:

$$\frac{\text{Number of thrombocytes counted}}{100} \times \text{TWBC} = \text{Absolute Thrombocytes} \times 10^9 / \text{l}$$

3.3.6 Biochemical analyses

Once the serum was unfrozen, the blood urea nitrogen, creatinine, total protein, albumin, calcium and phosphorus were assayed using the Audiocomb Serum Auto-analyser (Bayer Express Plus, Bayer Germany, Serial Number 15950) in the Chemical Pathology Laboratory, Ahmadu Bello University Teaching Hospital (ABUTH) Shika. The globulin fraction was calculated by subtracting the albumin fraction from the total protein.

3.4 Mean Corpuscular Values (Red Cell Absolute Values)

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were calculated using the following standard formulae (Campbell and Ellis, 2007):

$$\begin{aligned} \text{MCV} &= (\text{PCV} \times 10) / \text{RBC} = \text{MCV femto litres (fl)} \\ \text{MCH} &= (\text{Hb} \times 10) / \text{RBC} = \text{MCH picogram (pg)} \\ \text{MCHC} &= (\text{Hb} \times 100) / \text{PCV} = \text{MCHC (g/l)} \end{aligned}$$

3.5 Statistical Analyses

Descriptive statistics was performed for all the data obtained. One-way Analysis of Variance (ANOVA) with Tukey's multiple comparison test was performed using GraphPad Prism Version 4.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. A value of $p < 0.05$ was considered significant.

CHAPTER FOUR

RESULTS

4.1 Results of Haematological Parameters

The highest mean values for PCV ($46.25 \pm 1.43\%$), Hb concentration (17.75 ± 5.31 g/dl) and RBC count ($5.24 \pm 0.32 \times 10^{12}/l$) were obtained from the apparently healthy White-Rumped Swift, Cattle Egret and the African Silver Billed, respectively. The lowest mean values for PCV ($31.98 \pm 1.67\%$) and Hb concentration (11.81 ± 0.56 g/dl) were recorded from the apparently healthy Bruce's Green Pigeon, while the apparently healthy Cattle Egret recorded the lowest mean value for RBC count, $2.86 \pm 0.14 \times 10^{12}/l$ (Table 4.1).

The apparently healthy Senegal Parrot had the highest mean values for TWBC, $3.36 \pm 0.44 \times 10^9/l$, and also had similar value for the apparently healthy Bruce's Green Pigeon for monocyte count, $0.13 \pm 0.03 \times 10^9/l$. The apparently healthy White-Rumped Swift recorded the highest values for mean eosinophil and basophil counts, $0.15 \pm 0.04 \times 10^9/l$ and $0.08 \pm 0.02 \times 10^9/l$, respectively. The apparently healthy Rose-Ringed Parakeet and Cattle Egret had the highest mean values for lymphocyte ($2.47 \pm 0.23 \times 10^9/l$) and heterophil ($0.62 \pm 0.11 \times 10^9/l$) counts, respectively. The apparently healthy African Silver Billed recorded the lowest mean values for TWBC ($0.63 \pm 0.08 \times 10^9/l$), lymphocyte ($0.54 \pm 0.08 \times 10^9/l$), heterophil ($0.04 \pm 0.02 \times 10^9/l$), basophil ($0.01 \pm 0.00 \times 10^9/l$) and monocyte ($0.06 \pm 0.00 \times 10^9/l$) counts, and also recorded similar lowest mean values with the apparently healthy Northern Red Bishop and Red Billed Quelea for eosinophil ($0.01 \pm 0.00 \times 10^9/l$) count. The H/L ratio was highest in the apparently healthy African Silver Billed (1.95 ± 1.90) and lowest in the Morning Collar Dove (0.08 ± 0.01). Apparently healthy Senegal Parrot had the highest mean estimated thrombocyte count ($0.92 \pm 0.17 \times 10^9/l$), while the apparently healthy African Silver Billed recorded

the lowest mean estimated value for thrombocyte count ($0.06 \pm 0.01 \times 10^9/l$) (Tables 4.1 and 4.2).

The highest values for MCV (142.00 ± 9.65 fl), MCH (73.50 ± 2.13 pg) and MCHC (37.18 ± 0.64 g/l) were obtained from the apparently healthy Speckled Pigeon, Laughing Dove and Bruce's Green Pigeon, respectively. The lowest values for MCV (87.90 ± 5.68 fl) and MCH (29.88 ± 1.88 pg) were obtained from the apparently healthy African Silver Billed, and the MCHC (32.14 ± 0.80 g/l) was recorded from the apparently healthy Red-Billed Quelea. With the exception of the Hb concentration, there were significant differences ($p < 0.05$) in haematological parameters between the species of wild birds studied (Tables 4.1 and 4.2).

4.2 Results of Biochemical Parameters

The highest mean values for glucose, creatinine and urea concentration were obtained from apparently healthy Mourning Collar Dove, Bruce's Green Pigeon and Speckled Pigeon with values of 154.70 ± 5.72 mg/dl, 0.49 ± 0.02 mg/dl and 5.53 ± 0.28 mg/dl, respectively (Table 4.3). Except for the serum calcium concentration which was highest in the apparently healthy Cattle Egret, with mean value of 2.64 ± 0.05 mg/dl, the mean values obtained for the glucose concentration, urea, total protein and albumin were lowest in this species of bird with the values of 139.20 ± 4.64 mg/dl, 4.24 ± 0.15 mg/dl, 6.65 ± 0.10 g/dl and 3.77 ± 0.10 g/dl, respectively. The apparently healthy Laughing Dove and the Senegal Parrot had the lowest creatinine concentration of 0.43 ± 0.01 mg/dl, but the latter recorded the highest mean albumin concentration of 3.99 ± 0.10 g/dl (Table 4.3).

The mean total protein and globulin fraction were highest in the apparently healthy Laughing Dove which was 6.93 ± 0.10 g/dl and 2.97 ± 0.04 g/dl, respectively although it also recorded the lowest calcium and phosphorus concentrations of 2.45 ± 0.02 mg/dl and 0.83 ± 0.06 mg/dl, respectively. The apparently healthy Rose-Ringed Parakeet had

the highest mean phosphorus concentration of 1.05 ± 0.03 mg/dl and lowest mean globulin fraction of 2.89 ± 0.05 g/dl. Similarly, there was significant difference ($p < 0.05$) in most of the biochemical parameters between all the species of birds studied except for the glucose, total protein, albumin and globulin concentrations (Table 4.3).

Table 4.1: Mean (\pm SE) haematological of some species of apparently healthy free-living wild birds found in Zaria, Nigeria.

Parameter	Laughing Dove (<i>Streptopelia senegalensis</i>) (n = 20)	Mourning Collar Dove (<i>Streptopelia decipiens</i>) (n = 16)	Bruce's Green Pigeon (<i>Treron waalia</i>) (n = 20)	Speckled Pigeon (<i>Columba guinea</i>) (n = 19)	Rose-Ringed Parakeet (<i>Psittacula krameri</i>) (n = 20)	Senegal Parrot (<i>Poicephalus senegalus</i>) (n = 20)	Cattle Egret (<i>Bubulcus ibis</i>) (n = 18)	Red-Billed Quelea (<i>Quelea quelea</i>) (n = 20)	Northern Red Bishop (<i>Euplectes francisca</i>) (n = 20)	African Silver-Billed (<i>Euodice cantans</i>) (n = 20)	Little Weaver (<i>Ploceus luteolus</i>) (n = 20)	White-Rumped Swift (<i>Apus caffer</i>) (n = 20)
PCV (%)	38.50 ± 1.62	35.91 ^a ± 1.32	31.98 ^b ± 1.67	38.47 ± 1.88	43.65 ^b ± 1.72	40.33 ± 2.48	35.64 ^c ± 2.98	38.70 ± 2.70	39.60 ± 2.00	43.80 ^{bd} ± 1.73	34.45 ^d ± 1.73	46.25 ^{abcd} ± 1.43
Hb (g/dl)	13.36 ± 0.55	12.63 ± 0.48	11.81 ± 0.56	13.25 ± 0.72	15.71 ± 0.59	13.95 ± 0.79	17.75 ± 5.31	12.44 ± 0.90	13.25 ± 0.61	15.02 ± 0.68	12.15 ± 0.59	15.87 ± 0.58
RBC (x 10 ¹² /l)	3.29 ^g ± 0.21	4.16 ± 0.26	3.50 ^a ± 0.26	2.87 ± 0.13	3.86 ^c ± 0.28	3.54 ^{bd} ± 0.23	2.86 ^e ± 0.14	4.08 ^{bef} ± 0.22	4.40 ^{be} ± 0.25	5.24 ^{abcdefg} ± 0.32	3.71 ± 0.15	4.52 ^{be} ± 0.15
TWBC (x 10 ⁹ /l)	2.28 ^a ± 0.27	1.33 ^b ± 0.25	2.56 ^c ± 0.42	2.07 ^d ± 0.23	3.18 ^{be} ± 0.32	3.36 ^{bf} ± 0.44	2.91 ^b ± 0.31	1.45 ^{efg} ± 0.21	1.14 ^{cefg} ± 0.14	0.63 ^{acdefgh} ± 0.08	1.10 ^{cefgi} ± 0.17	2.62 ^{hi} ± 0.31
MCV (fl)	124.04 ± 8.61	89.15 ^a ± 2.91	95.74 ^b ± 5.47	142.00 ^{ab} ± 9.65	130.01 ^{ad} ± 13.91	116.47 ^c ± 6.24	128.40 ^e ± 12.54	96.42 ^c ± 6.36	94.28 ^c ± 5.44	87.90 ^{cde} ± 5.68	94.82 ^c ± 5.22	104.28 ^c ± 4.50
MCH (pg)	73.50 ^a ± 2.13	31.32 ^{ab} ± 1.14	35.08 ^{ac} ± 1.59	47.56 ^{abc} ± 3.32	46.97 ^{abce} ± 4.93	40.62 ^{ad} ± 2.01	44.70 ^{abf} ± 3.40	30.79 ^{adef} ± 2.00	31.52 ^{adef} ± 1.72	29.88 ^{adef} ± 1.88	33.47 ^{ade} ± 1.62	34.74 ^{ade} ± 1.40
MCHC (g/l)	34.79 ± 0.54	35.30 ± 0.72	37.18 ^a ± 0.64	33.89 ± 0.57	36.17 ^b ± 0.68	35.16 ± 0.86	36.02 ^c ± 1.11	32.14 ^{abcd} ± 0.80	33.62 ^a ± 0.35	34.26 ± 0.67	35.41 ^d ± 0.51	33.29 ^a ± 0.22

- n = total number of birds sampled, Mean \pm SE = standard error of the mean
- values with the same superscript alphabets along the same row are significantly different with p<0.05

Table 4.2: Mean (\pm SE) of differential leucocyte count, heterophil/lymphocyte ratio and estimated absolute thrombocyte count for some species of apparently healthy free-living wild birds found in Zaria, Nigeria.

Parameters	Laughing Dove (<i>Streptopelia senegalensis</i>) (n = 20)	Mourning Collar Dove (<i>Streptopelia decipiens</i>) (n = 16)	Bruce's Green Pigeon (<i>Treron waalia</i>) (n = 20)	Speckled Pigeon (<i>Columba guinea</i>) (n = 19)	Rose-Ringed Parakeet (<i>Psittacula krameri</i>) (n = 20)	Senegal Parrot (<i>Poicephalus senegalus</i>) (n = 20)	Cattle Egret (<i>Bubulcus ibis</i>) (n = 18)	Red-Billed Quelea (<i>Quelea quelea</i>) (n = 20)	Northern Red Bishop (<i>Euplectes francisca</i>) (n = 20)	African Silver-Billed (<i>Euodice cantans</i>) (n = 20)	Little Weaver (<i>Ploceus luteolus</i>) (n = 20)	White-Rumped Swift (<i>Apus caffer</i>) (n = 20)
Heterophils (x 10 ⁹ /l)	0.20 ^a ±0.03	0.08 ^{bc} ±0.02	0.46 ^b ±0.11	0.30 ±0.05	0.44 ^d ±0.09	0.62 ^{abc} ±0.14	0.62 ^{abf} ±0.11	0.17 ^{ef} ±0.06	0.12 ^{ef} ±0.03	0.04 ^{cdef} ±0.02	0.13 ^{ef} ±0.03	0.31 ±0.06
Lymphocytes (x 10 ⁹ /l)	1.93 ^a ±0.24	1.12 ^b ±0.20	2.30 ^c ±0.52	1.56 ±0.16	2.47 ^{bd} ±0.23	2.46 ^{be} ±0.35	2.05 ^f ±0.21	1.19 ^{de} ±0.16	0.96 ^{cde} ±0.10	0.54 ^{acdefg} ±0.08	0.89 ^{cde} ±0.14	2.01 ^g ±0.23
Basophils (x 10 ⁹ /l)	0.05 ±0.01	0.02 ^a ±0.01	0.04 ±0.02	0.02 ^b ±0.01	0.06 ±0.01	0.04 ±0.02	0.04 ±0.01	0.02 ^c ±0.01	0.02 ^d ±0.01	0.01 ^e ±0.00	0.02 ^f ±0.02	0.08 ^{abcdef} ±0.02
Eosinophils (x 10 ⁹ /l)	0.07 ±0.02	0.06 ±0.03	0.08 ±0.03	0.07 ±0.02	0.13 ^a ±0.03	0.13 ^b ±0.03	0.14 ^c ±0.03	0.01 ^{abcd} ±0.00	0.01 ^{abce} ±0.00	0.01 ^{abcf} ±0.00	0.04 ^g ±0.01	0.15 ^{defg} ±0.04
Monocytes (x 10 ⁹ /l)	0.04 ^a ±0.01	0.05 ±0.02	0.13 ^{ab} ±0.03	0.11 ^c ±0.02	0.10 ^d ±0.02	0.13 ^{ae} ±0.03	0.07 ±0.02	0.02 ^{be} ±0.01	0.03 ^{be} ±0.01	0.01 ^{bcde} ±0.00	0.03 ^{be} ±0.01	0.07 ±0.02
H/L Ratio	0.13 ±0.03	0.08 ±0.01	0.24 ±0.04	0.19 ±0.03	0.18 ±0.03	0.29 ±0.07	0.29 ±0.04	0.13 ±0.03	0.12 ±0.02	1.95 ±1.90	0.15 ±0.03	0.14 ±0.02
Thrombocytes (x 10 ⁹ /l)	0.44 ^a ±0.07	0.28 ^b ±0.07	0.49 ^{cd} ±0.11	0.72 ^e ±0.15	0.39 ^f ±0.07	0.92 ^{abcfg} ±0.17	0.42 ^g ±0.06	0.19 ^{eg} ±0.04	0.19 ^{eg} ±0.03	0.06 ^{deg} ±0.01	0.15 ^{eg} ±0.03	0.45 ^g ±0.08

- n = total number of birds sampled, Mean \pm SE = standard error of the mean
- values with the same superscript alphabets along the same row are significantly different with p<0.05

Table 4.3: Mean (\pm SE) serum biochemical parameters for some species of apparently healthy free-living wild birds found in Zaria, Nigeria.

Parameter	Laughing Dove (<i>Streptopelia senegalensis</i>) (n = 20)	Mourning Collar Dove (<i>Streptopelia decipiens</i>) (n = 16)	Bruce's Green Pigeon (<i>Treron waalia</i>) (n = 20)	Speckled Pigeon (<i>Columba guinea</i>) (n = 19)	Rose-Ringed Parakeet (<i>Psittacula krameri</i>) (n = 20)	Senegal Parrot (<i>Poicephalus senegalus</i>) (n = 20)	Cattle Egret (<i>Bubulcus ibis</i>) (n = 18)
Glucose (mg/dl)	143.55 \pm 4.74	154.70 \pm 5.72	147.40 \pm 5.15	149.50 \pm 5.01	154.15 \pm 3.74	151.60 \pm 3.95	139.20 \pm 4.64
Urea (mg/dl)	4.61 \pm 0.19 ^a	4.74 \pm 0.18	4.88 \pm 0.20	5.53 \pm 0.28 ^{ab}	4.39 \pm 0.14 ^b	4.61 \pm 0.18	4.24 \pm 0.15 ^b
Creatinine (mg/dl)	0.43 \pm 0.01 ^a	0.45 \pm 0.01	0.49 \pm 0.02 ^{ab}	0.45 \pm 0.01	0.45 \pm 0.01	0.43 \pm 0.01 ^b	0.46 \pm 0.01
Total Protein (g/dl)	6.93 \pm 0.10	6.84 \pm 0.13	6.74 \pm 0.10	6.73 \pm 0.14	6.73 \pm 0.12	6.91 \pm 0.10	6.65 \pm 0.10
Albumin (g/dl)	3.97 \pm 0.10	3.90 \pm 0.10	3.83 \pm 0.10	3.80 \pm 0.13	3.85 \pm 0.11	3.99 \pm 0.10	3.77 \pm 0.10
Globulin (g/dl)	2.97 \pm 0.04	2.94 \pm 0.05	2.91 \pm 0.06	2.93 \pm 0.07	2.89 \pm 0.05	2.93 \pm 0.08	2.94 \pm 0.07
Calcium (mg/dl)	2.45 \pm 0.02 ^a	2.59 \pm 0.03	2.54 \pm 0.03	2.61 \pm 0.03 ^a	2.52 \pm 0.04	2.50 \pm 0.04	2.64 \pm 0.05 ^a
Phosphorus (mg/dl)	0.83 \pm 0.06 ^a	0.94 \pm 0.04	0.98 \pm 0.03	1.02 \pm 0.06 ^a	1.05 \pm 0.03 ^a	0.93 \pm 0.03	0.95 \pm 0.02

- n = total number of birds sampled, Mean \pm SE = standard error of the mean
- values with the same superscript alphabets along the same row are significantly different with p<0.05

Table 4.4: Range values of packed cell volume, haemoglobin concentration, red blood cell count, total white blood cell count and erythrocyte indices for some species of apparently healthy free-living wild birds found in Zaria, Nigeria.

Parameters	Laughing Dove (<i>Streptopelia senegalensis</i>) (n = 20)	Mourning Collar Dove (<i>Streptopelia decipiens</i>) (n = 16)	Bruce's Green Pigeon (<i>Treron waalia</i>) (n = 20)	Speckled Pigeon (<i>Columba guinea</i>) (n = 19)	Rose-Ringed Parakeet (<i>Psittacula krameri</i>) (n = 20)	Senegal Parrot (<i>Poicephalus senegalus</i>) (n = 20)	Cattle Egret (<i>Bubulcus ibis</i>) (n = 18)	Red-Billed Quelea (<i>Quelea quelea</i>) (n = 20)	Northern Red Bishop (<i>Euplectes francisca</i>) (n = 20)	African Silver-Billed (<i>Euodice cantans</i>) (n = 20)	Little Weaver (<i>Ploceus luteolus</i>) (n = 20)	White-Rumped Swift (<i>Apus caffer</i>) (n = 20)
PCV (%)	48-24	41-19.5	43.5-19.5	57-18	54-27.5	56-37.0	68-22.5	63-29	59-27	61-32	49-21	60-37
Hb (g/dl)	16.8-8.7	16.2-7.3	15.5-7.2	21.4-5.3	19.8-11.3	18.3-12.9	107-9.0	19.9-9.4	18.7-9.4	20.8-10.7	17.2-7.6	23-12.1
RBC (x 10 ¹² /l)	5.10-1.71	6.3-2.12	7.78-1.91	3.9-1.53	6.49-1.75	5.96-2.63	4.15-2.40	5.87-3.12	7.33-2.53	10.45-3.79	5.03-2.51	5.81-3.11
TWBC (x 10 ⁹ /l)	5.0-0.9	3.51-0.25	7.35-0.55	3.7-0.5	6.25-0.95	8.15-1.55	5.75-2.10	4.1-0.8	2.95-0.21	1.35-0.15	2.75-0.2	5.00-0.35
MCV (fl)	226.6-76.47	113.33-58.73	137.55-48.2	258.17-87.18	274.29-53.16	168.67-86.33	288.14-90.91	152.03-68.14	128.95-43.66	158.63-38.28	161.07-43.57	145.78-70.57
MCH (pg)	79.31-30.0	38.06-23.12	45.04-19.92	87.58-29.12	97.71-19.11	55.84-31.25	87.29-34.58	50.95-22.93	42.86-14.60	53.63-15.41	53.69-15.77	46.80-22.38
MCHC (g/l)	39.23-30.0	43.78-32.0	42.96-32.7	37.63-27.14	43.0-32.14	50.83-33.81	50.0-32.86	39.17-31.58	36.43-31.00	40.25-27.91	41.29-31.71	35.00-31.45

Table 4.5: Range values of differential absolute leucocyte count, heterophil/lymphocyte ratio and estimated absolute thrombocyte count for some species of apparently healthy free-living wild birds found in Zaria, Nigeria.

Parameters	Laughing Dove (<i>Streptopelia senegalensis</i>) (n = 20)	Mourning Collar Dove (<i>Streptopelia decipiens</i>) (n = 16)	Bruce's Green Pigeon (<i>Treron waalia</i>) (n = 20)	Speckled Pigeon (<i>Columba guinea</i>) (n = 19)	Rose-Ringed Parakeet (<i>Psittacula krameri</i>) (n = 20)	Senegal Parrot (<i>Poicephalus senegalus</i>) (n = 20)	Cattle Egret (<i>Bubulcus ibis</i>) (n = 18)	Red-Billed Quelea (<i>Quelea quelea</i>) (n = 20)	Northern Red Bishop (<i>Euplectes francisca</i>) (n = 20)	African Silver-Billed (<i>Euodice cantans</i>) (n = 20)	Little Weaver (<i>Ploceus luteolus</i>) (n = 20)	White-Rumped Swift (<i>Apus caffer</i>) (n = 20)
Heterophils (x 10 ⁹ /l)	0.42-0.02	0.28-0.01	1.84-0.07	0.75-0.11	1.25-0.12	2.21-0.08	1.67-0.15	0.98-0.04	0.71-0.01	0.03-0.01	0.55-0.02	0.99-0.10
Lymphocytes (x 10 ⁹ /l)	4.28-0.55	2.77-0.22	10.34-0.07	2.88-0.47	4.44-0.87	5.95-0.5	3.93-1.12	3.03-0.39	1.76-0.18	1.22-0.05	2.48-0.25	4.15-1.29
Basophils (x 10 ⁹ /l)	0.23-0.00	0.11-0.00	0.36-0.00	0.07-0.00	0.24-0.00	0.28-0.00	0.13-0.00	0.10-0.00	0.12-0.00	0.03-0.00	0.12-0.00	0.44-0.00
Eosinophils (x 10 ⁹ /l)	0.25-0.00	0.46-0.00	0.37-0.00	0.37-0.00	0.51-0.00	0.48-0.00	0.42-0.00	0.04-0.00	0.03-0.00	0.07-0.00	0.12-0.00	0.61-0.00
Monocytes (x 10 ⁹ /l)	0.20-0.00	0.35-0.00	0.57-0.00	0.29-0.00	0.29-0.00	0.57-0.00	0.23-0.00	0.08-0.00	0.27-0.00	0.03-0.00	0.14-0.00	0.24-0.00
H/L Ratio	0.67-0.01	0.18-0.00	0.61-0.02	0.59-0.01	0.43-0.00	1.00-0.03	0.63-0.08	0.57-0.01	0.46-0.00	0.13-0.02	0.46-0.01	0.34-0.01
Thrombocytes (x 10 ⁹ /l)	1.36-0.12	0.98-0.02	1.83-0.06	2.74-0.03	0.96-0.07	2.45-0.06	1.02-0.10	0.74-0.01	0.59-0.02	0.21-0.01	0.66-0.02	1.35-0.05

Table 4.6: Range values of some selected blood serum biochemical parameters for some species of apparently healthy free-living wild birds found in Zaria, Nigeria.

Parameters	Laughing Dove (<i>Streptopelia senegalensis</i>) (n = 20)	Mourning Collar Dove (<i>Streptopelia decipiens</i>) (n = 16)	Bruce's Green Pigeon (<i>Treron waalia</i>) (n = 20)	Speckled Pigeon (<i>Columba guinea</i>) (n = 19)	Rose-Ringed Parakeet (<i>Psittacula krameri</i>) (n = 20)	Senegal Parrot (<i>Poicephalus senegalus</i>) (n = 20)	Cattle Egret (<i>Bubulcus ibis</i>) (n = 18)
Glucose (mg/dl)	172-120	173-128	186-116	200-122	180-108	190-118	168-115
Urea (mg/dl)	7.1-3.3	6.1-3.7	6.6-3.3	7.2-2.8	5.1-2.6	5.5-2.7	5.5-3.2
Creatinine (mg/dl)	0.51-0.33	0.55-0.38	0.59-0.35	0.55-0.37	0.52-0.32	0.51-0.32	0.54-0.33
Total Protein (g/dl)	7.7-6.0	7.7-5.8	7.4-6.0	7.7-5.8	7.7-5.5	7.7-5.9	7.2-5.9
Albumin (g/dl)	4.7-3.0	4.8-3.1	4.7-3.2	4.8-2.8	4.6-2.8	4.7-3.1	4.7-3.0
Globulin (g/dl)	3.6-2.7	3.3-2.7	3.6-2.5	3.5-2.4	3.3-2.5	3.8-2.3	3.8-2.4
Calcium (mg/dl)	2.64-2.26	2.8-2.3	2.72-2.33	3.01-2.33	2.81-2.09	2.85-2.06	2.9-2.21
Phosphorus (mg/dl)	1.21-0.06	1.15-0.67	1.16-0.69	1.96-0.77	1.31-0.69	1.21-0.67	1.15-0.82

CHAPTER FIVE

DISCUSSION

The different species of free-living wild birds examined in this study had fairly similar mean values for PCV, RBC count and Hb concentration. This is in line with the findings in previous studies (Sturkie, 2000; Olayemi *et al.*, 2006; Saleem *et al.*, 2008; Lashev *et al.*, 2009; Vinkler *et al.*, 2010; Oladele *et al.*, 2012; Azeez *et al.*, 2013).

In the studies carried out by Lashev *et al.* (2009) in Bulgaria, and Olayemi *et al.* (2006) and Azeez *et al.* (2013) both in Ibadan, Southern Nigeria on the Laughing Dove, the values reported for the PCV, Hb concentration and RBC count were relatively similar to the values for Laughing Dove, Mourning Collar Dove, Bruce's Green Pigeon and Speckled Pigeon reported in this study. However, the values (PCV, Hb concentration, RBC count) for the Bruce's Green Pigeon were similar to the values reported for the lower limit for Laughing Dove by afore mentioned authors. Abdel-Rachied *et al.* (2014) in Cairo, Egypt, reported the values for RBC count, Hb concentration and PCV for the apparently healthy Cattle Egret. These values were similar to the values reported in this study for the apparently healthy Cattle Egret.

The high mean PCV and mean RBC count obtained from the apparently healthy White-Rumped Swift and the African Silver Billed, respectively cannot be associated with stress, dehydration or other disease conditions, such as chronic pulmonary disease, cardiac diseases, iron storage disease, rickets, renal disease and neoplasia leading to increased production of erythropoietin. This is because stress as the causative factor for the increased RBC count can be ruled out as the avian spleen lack both storage capacity and a muscular capsule, thus making it physiologically impossible for the avian spleen to inject red cells into circulation under stressful conditions such as during blood sampling as seen in mammals (John, 1994; Latimer *et al.*, 2003). Dehydration and

diseased conditions were also ruled out as possible causes of the increased PCV and RBC count because on physical examinations obvious abnormalities were not observed. Therefore, the species with higher values of PCV and RBC count in this study may be considered as those in the upper limits of the avian range. The significantly high mean PCV value observed in the White-Rumped Swift could be associated to the fact that the bird flies rapidly and spends so much time in the air, hence the physiological increased need for adequate gaseous exchange. However, the exact cause of interspecies variability in the PCV and RBC count in this study and the slight differences in the Hb concentrations was not elucidated. These differences could probably be influenced by the species differences and nutrition.

The TWBC value obtained for apparently healthy Laughing Dove in this study was similar to the value reported for the Laughing Dove in Ibadan, Southern Nigeria (Olayemi *et al.*, 2006). The predominant leucocytes of the apparently healthy birds sampled in this study were the lymphocytes and heterophils, accounting on the average for up to 95% or more of the TWBC. The numbers of monocytes, eosinophils and basophils were low, though variable. The variability is most likely indicative of individual response to different immunological challenges and different stages of response to any pathology (Stein *et al.*, 1998).

It is known that basophils and monocytes usually have a very low range of variation in most species (Mitchell and Johns, 2008). This might be an additional explanation for the existence of slight significant differences in basophils, eosinophils and monocytes between species of wild birds in this study.

The heterophil/lymphocyte (H/L) ratio, considered by several authors as providing important information for immune system tension following prolonged stress factors (Moreno *et al.*, 2002; Scope *et al.*, 2002; Clinchy *et al.*, 2004; Williams, 2005) was

highest in the apparently healthy African Silver Billed and lowest in the apparently healthy Mourning Collar Dove. Scope *et al.* (2002) observed a considerable change in H/L proportion and increased H/L ratio following stress associated with transporting and handling of Racing Pigeon (*Columba livia domestica*). With respect to heterophils and lymphocytes, the data obtained from this study for apparently healthy Mourning Collar Dove was lower than that obtained by Lashev *et al.* (2009) and Azeez *et al.* (2013). The high value was assumed in their work to be associated with possible stress during restraint and rearing in captivity. Therefore, the low H/L ratio reported in this study could possibly be as a result of the adequate restrain method employed and the short time (about an hour or less) used for collecting the blood samples.

In this study, the mean thrombocyte values were significantly low. This may be due to the fact that the thrombocytes were observed to clump during the differential leucocyte count (Campbell and Ellis, 2007).

The wide range MCV and MCH values found in this study could be due to the significant wide range of values recorded in RBC count, Hb concentration and PCV values. This is because the red cell indices (MCV, MCH and MCHC) were calculated from the PCV, Hb concentration and RBC count, their validity is influenced by the values of the RBC count, the Hb concentration and PCV (Amand, 1985).

The mean serum urea and creatinine levels obtained from the apparently healthy wild bird species in this study were similar to the values obtained by Lumeij (1987b,c) who established reference values for urea and creatinine for the Racing Pigeon (*Columba livia domestica*). The slightly significant difference between species observed in urea and creatinine concentration maybe due to species variation and diet rather than stress or water deprivation. This is because 6.5 to 15.3 fold increase in plasma urea and 1.2 to 1.5 fold increase in creatinine have been found in Racing Pigeon deprived of water

(Lumeij, 1987b). Similarly, extremely high values of serum urea and creatinine were observed in stressed Cattle Egret (Zaahkoug *et al.*, 2013).

In this study, the total protein, albumin and globulin concentrations obtained were not significantly different between all the birds examined. However, the values obtained for total protein, albumin and globulin concentration were approximately twice as high as those reported by Zaahkoug *et al.* (2013) for the Cattle Egret in Cairo, Egypt. The values obtained in this study were also relatively higher when compared to the values obtained by Oladele *et al.* (2012). Differences in results obtained by these authors and those of this study might be due to different methods employed in analyzing for total protein concentration.

The mean serum glucose levels for the apparently healthy large wild bird species (doves, pigeons, parakeet, parrot and egret) showed no statistical significant interspecies differences. In a study carried out by Hernández and Margalida (2010) with the Bearded Vultures of different ages. The immature (3 – 6 years old) and the free-living Bearded Vultures showed almost similar values with those reported in this study. However, the slight differences observed in the serum glucose levels in this study and those from other species of birds could be associated with differences in the diets of these birds.

Hernández *et al.* (1990) established a range value for phosphorus concentration for the Common Buzzard. In another work by Hernández and Margalida (2010), they established levels for phosphorus concentration in nestling, immature, adult and free-living Bearded Vultures where they recorded a significant decreasing trend with increasing age in inorganic phosphorus concentration. The lower limits in both studies of the afore mentioned authors correspond with the values obtained in this study. This could be associated to the fact that the wild birds sampled in this work were adults.

These values reported for calcium concentration were about two- to three-fold lower than the values obtained for the Common Buzzard (Hernández *et al.*, 1990), wild Bearded Vultures (Hernández and Margalida, 2010), Spanish Imperial Eagle (Ferrer *et al.*, 1998), magpies, crows and pigeons (Pop *et al.*, 2010). The low values obtained in this study could be attributed to the method employed for the analysis of calcium concentration.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- To the best of our knowledge, this is the first time the baseline values of haematological and serum biochemistry of Laughing Dove (*Streptopelia senegalensis*), Bruce's Green Pigeon (*Treron waalia*), Speckled Pigeon (*Columba guinea*), Mourning Collar-Dove (*Streptopelia decipiens*), Senegal Parrot (*Poicephalus senegalus*), Rose-Ringed Parakeet (*Psittacula krameri*), Little Weaver Bird (*Ploceus luteolus*), Cattle Egret (*Bubulcus ibis*), White-Rumped Swift (*Apus caffer*), Red-Billed Quelea (*Quelea quelea*), African Silver-Billed (*Euodice cantans*) and Northern Red Bishop (*Euplectes francisca*) were determined in Zaria, Nigeria.
- Haematological values (with exception of haemoglobin concentration) and serum biochemical values (with exception of glucose, total protein, albumin and globulin concentrations) showed significant interspecies differences ($p < 0.05$) in this study.
- The White-Rumped Swift, Cattle Egret, African Silver Billed, Senegal Parrot, Speckled Pigeon, Laughing Dove and the Bruce's Green Pigeon had the highest values for PCV ($46.25 \pm 1.43\%$), Hb (17.75 ± 5.31 g/dl), RBC count ($5.24 \pm 0.32 \times 10^{12}/l$), TWBC count ($3.36 \pm 0.44 \times 10^9/l$), MCV (142.00 ± 9.65 fl), MCH (73.50 ± 2.13 pg) and MCHC (37.18 ± 0.64).
- The Bruce's Green Pigeon recorded the lowest values for PCV ($31.98 \pm 1.67\%$) and Hb (11.81 ± 0.56 g/dl), while the African Silver Billed recorded the lowest values for TWBC count ($0.63 \pm 0.08 \times 10^9/l$), MCV (87.90 ± 5.68 fl) and MCH (29.88 ± 1.88 pg). The Cattle Egret and the Red Billed Quelea had the lowest

values for RBC count ($2.86 \pm 0.14 \times 10^{12}/l$) and MCHC (32.14 ± 0.80 g/l), respectively.

- The heterophil/ lymphocyte (H/L) ratio was highest in the African Silver Billed with a value of 1.95 ± 1.90 and lowest in the Mourning Collar Dove with 0.08 ± 0.01 .
- The Mourning Collar Dove, Speckled Pigeon, Bruce's Green Pigeon, Senegal Parrot, Cattle Egret and the Rose-Ringed Parakeet recorded the highest values for glucose (154.70 ± 5.72 mg/dl), urea (5.53 ± 0.28 mg/dl), creatinine (0.49 ± 0.02 mg/dl), albumin (3.99 ± 0.10 g/dl), calcium (2.64 ± 0.05 mg/dl) and 1.05 ± 0.03 mg/dl), respectively. The Laughing Dove recorded the highest values for total protein (6.93 ± 0.10 g/dl) and globulin (2.97 ± 0.04 g/dl).
- The Cattle Egret had the lowest values for glucose (139.20 ± 4.46 mg/dl), urea (4.24 ± 0.15 mg/dl), total protein (6.65 ± 0.10 g/dl) and albumin (3.77 ± 0.10 g/dl). The Rose-Ringed Parakeet had the lowest value for globulin as 2.89 ± 0.05 g/dl. The Senegal Parrot and the Laughing Dove had the lowest value for creatinine concentration as 0.43 ± 0.01 mg/dl though the latter had the lowest values for calcium (2.45 ± 0.02 mg/dl) and phosphorus (0.83 ± 0.06 mg/dl) concentrations.

6.2 Recommendations

- More sensitive species-specific equipment (e.g. Veterinary Haematology and Biochemistry Auto-analyzers) should be employed to explain the variability in reference range values observed and elucidate ecological factors which may influence haematological and serum biochemical values.

- Further studies should be carried out to establish the baseline values for other biochemical parameters that were not determined in this study for the purposes of obtaining more information.
- Further studies should be carried out to include the effects of age, nutrition and weather on haematological and serum biochemical parameters of wild birds in Zaria, Nigeria.

REFERENCES

- Abdel-Rachied, H. G., Zaahkouk, S. A., El-Zawhry, E. I. and Elfeky, Kh. Sh. (2014). Flying or running stress effect on some haematological and biochemical parameters in some birds and mammals. *Journal and Entomology and Zoology Studies*, 2(2): 153 – 158.
- Altan, O., Pabuccuoglu, A., Altan, A., Konyalioglu, S. and Bayraktar, H. (2003). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *British Poultry Science*, 44: 545 – 550.
- Altman, R. B. (1979). Avian clinical pathology, radiology, parasitic and infectious diseases. *American Animal Hospital Association Annual Proceedings*, pp. 15 – 26.
- Amand W. B. (1985). Avian clinical haematology and blood chemistry. In: Fowler, M. E. (Ed). *Zoo and Wild Animal Medicine*. W. B. Saunders Co., Philadelphia, pp. 263 – 276.
- Andreasen, C. B. and Latimer, K. S. (1990). Cytochemical characteristics of chicken heterophils and eosinophils. *Veterinary Clinical Pathology*, 19: 51 – 54.
- Andreasen, J. R. Jr., Andreasen, C. B., Anwer, M. and Sonn, A. E. (1993). Heterophil chemotaxis in chickens with natural Staphylococcal infections. *Avian Diseases*, 37(2): 284 – 289.
- Ardia, D. R. and Schat, K. A. (2008). Eco-immunology. In: Davison, F., Kaspers, B. and Schat, K. A. (Eds). *Avian Immunology*. Academic/Elsevier, London, pp. 421 – 441.
- Aroch, I., Targan, N. and Gancz. A. Y. (2013). A novel modified semi-direct method for total leucocyte count in birds. *Israel Journal of Veterinary Medicine*, 68(2): 111 – 118.
- Artacho, P., Soto Gamboa, M., Verdugo, C. and Nespolo, F. R. (2007). Blood chemistry reveals malnutrition in black necked swans (*Cygnusm elanocoryphus*) living in a conservation priority area. *Comparative Biochemistry and Physiology*, 146: 283 – 290.
- Azeez, O. I, Oyagbemi, A. A., Olawuwo, O. S. and Oyewale J. O. (2013). Changes in haematology, plasma biochemistry and erythrocyte osmotic fragility of the Nigerian laughing dove (*Streptopelia senegalensis*) in captivity. *Nigerian Journal of Physiological Sciences*, 28: 63 – 68.
- Balasz, J., Musquera, S., Palacios, L., Jimenez, M. and Palomeque, J. (1976). Comparative haematology of some falconiforms. *Condor*, 78: 258 – 273.
- Balbontín, J. and Ferrer, M. (2002). Plasma chemistry reference values in free-living Bonelli's Eagle (*Hieraaetus fasciatus*) nestlings. *Journal of Raptor Research*, 36: 231 – 235.
- Ballard, B. M. and Cheek, R. (2010). Exotic animal medicine for the veterinary technician (Second edition). Blackwell Publishing, Ames, Iowa, United States, pp. 484.
- Baptista, L., Trail, P. and Horblit, H. (1992). Family Columbidae (pigeons and doves). In: del Hoyo, J., Elliot, A. and Sargatal, J. (Eds). *Handbook of the Birds of World* (vol. 4). Barcelona, Lynx Edicions, pp. 60 – 243.
- Barrowclough, G. F., Johnson, N. K. and Zink, R. M. (1985). On the nature of genic variation in birds. In: Johnson, R. F. (Ed). *Current Ornithology*, (vol. 2). Plenum Press, New York, pp. 135 – 154.
- Battley, P. F., Piersma, T., Dietz, M. W., Tang, S., Dekinga, A. and Hulsman, K. (2000). Empirical evidence for differential organ reductions during trans-oceanic bird flight. *Proceedings of the Royal Society B*, 1439: 191 – 195.

- Baumann, R. and Dragon, S. (2005). Erythropoiesis and red cell function in vertebrate embryos. *European Journal of Clinical Investigation*, 35(3): 2 – 12.
- Bedáňová, I., Voslářová, E., Večerek, V., Pištěková, V. and Chloupek, P. (2007). Haematological profile of broiler chickens under acute stress due to shackling. *Acta Veterinaria Brno*, 76: 129 – 135.
- Berthold, P., Hans-Günther, B. and Valerie, W. (2001). *Bird Migration: A General Survey*. Oxford: Oxford University Press. ISBN 0198507879.
- Bessis, M. (1977). Blood smears reinterpreted. Springer-Verlag, Berlin, pp. 25 – 63.
- Beyer, W. N., Audet, D. J., Heinz, G. H., Hoffman, D. J. and Day, D. (2000). Relation of waterfowl poisoning to sediment lead concentrations in the Coeur d'Alene River Basin. *Ecotoxicology*, 9: 207 – 218.
- Bienzle, D. and Smith, D. A. (1999). Heterophilic leucocytosis and granulocyte hyperplasia associated with infection in a cockatoo. *Comparative Haematology International*, 9(4):193 – 197.
- BirdLife International (2012). *Quelea quelea*. IUCN Red List of Threatened Species. Version 2013.2. International Union for Conservation of Nature. Retrieved 26 November 2013.
- Bonney, R. and Rohrbaugh, J. R. (2004). *Handbook of Bird Biology* (Second edition). Princeton, N. J., Princeton University Press. ISBN 0-938027-62-X.
- Bos, J. H., Todd, B., Tell, L. A., Ramsay, E. C. and Fowler, M. E. (1990). Treatment of anaemic birds with iron dextran therapy: homologous and heterologous blood transfusions. *Tijdschr Diergeneeskde*, 117(1): 22S – 23S.
- Bounous, D. I. and Stedman, N. L. (2000). Normal avian haematology. Chicken and turkey. In: Feldman, B. F., Zinkl, J. G. and Jain, N. C. (Eds). *Schalm's Veterinary Haematology*. Lippincott Williams and Wilkins, Philadelphia, pp. 1145 – 1154.
- Bowerman, W. W., Stickle, E. J., Sikarskie, G. J. and Giesy, P. J. (2000). Haematology and serum chemistries of nestling bald eagles (*Haliaeetus leucocephalus*) in the lower peninsula of USA. *Chemosphere*, 41: 1575 – 1579.
- Brandon, M. S., Hong-Erh, L., Jennifer, K. B., Davina, W., Laurence, E. C., James, K. M., Christopher, D. C. A. and Richard, M. L. (2011). Genetic analysis of basophil functions *in vivo*. *Nature Immunology*, 12: 527 – 535.
- Branton, S. L., May, J. D., Lott, B. D. and Mashin, W. R. (1997). Various blood parameters in commercial hens acutely and chronically infected with *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. *Avian Diseases*, 41(3): 540 – 547.
- Brooke, M. (2004). *Albatrosses and Petrels Across the World*. Oxford, Oxford University Press. ISBN 0-19-850125-0.
- Brown, M. B. and Brown, C. R. (2009). Blood sampling reduces annual survival in Cliff Swallows (*Petrochelidon pyrrhonota*). *Auk*, 126: 853 – 861.
- Burke, T. (1989). DNA fingerprinting and other methods for the study of mating success. *Trends in Ecology and Evolution*, 4: 139 – 144.
- Burson III, S. L. (1990). Population genetics and gene flow of the Common Tern. *Condor*, 92: 182 – 192.
- Butler, C. (2005). Feral Parrots in the Continental United States and United Kingdom: Past, Present and Future. *Journal of Avian Medicine and Surgery*, 19(2): 142 – 149.
- Campbell, T. W. (1988). *Avian Haematology and Cytology*. Iowa State University Press, Ames, IA, pp. 101.
- Campbell, T. W. (1994). Haematology. In: Ritchie, B. W., Harrison, G. J. and Harrison, L. R., (Eds), *Avian Medicine: Principles and Application*. Wingers Publishing, Lake Worth (FL), pp. 176 – 198.

- Campbell, T. W. (1995). Avian haematology. In: *Avian Haematology and Cytology*, (Second edition). Iowa State University Press, Ames, Iowa, pp. 1 – 19.
- Campbell, T. W. (1998). Routine avian plasma chemistry. *Exotic Pet Practice*, 3(1): 1 – 7.
- Campbell, T. W. (2000). Normal haematology of psittacine birds. In: Feldman, B. F., Zinkl, J. G. and Jain, N. C. (Eds). *Schalm's Veterinary Haematology*. Lippincott Williams and Wilkins, Philadelphia, pp. 1155 – 1160.
- Campbell, T. W. (2004a). Haematology of lower vertebrates. In: *55th Annual Meeting of the American College of Veterinary Pathologists (ACVP) and 39th Annual Meeting of the American Society of Clinical Pathology (ASVCP), ACVP and ASVCP* (Eds). American College of Veterinary Pathologists and American Society for Veterinary Clinical Pathology, Middleton, USA. Internet Publisher: International Veterinary Information Service, Ithaca, New York, available at: <http://www.ivis.org/proceedings/ACVP/2004/Campbell1/ivis.pdf>. Accessed at 28.08.13. 16.12.01.
- Campbell, T. W. (2004b). Haematology of birds. In: Thrall, A. T., Baker, D., C., Campbell, T. W., DeNicola, D., Fettman, M., Duane L., E., Rebar, A. and Weiser, G. (Eds). *Veterinary Haematology and Clinical Chemistry*. Lippincott, Williams and Wilkins, Baltimore, pp. 225 – 258.
- Campbell, T. W. (2004c). Haematology of common non-domestic animals. In: Thrall, M. A. (Ed). *Veterinary Haematology and Clinical Biochemistry*. Lippincott Williams and Wilkins, Philadelphia, pp. 225 – 276.
- Campbell, T. W. (2010). Haematology of psittacines. In: Weiss, D. J. and Wardrop, K. J. (Eds) *Schalm's Veterinary Haematology* (Sixth edition). Wiley-Blackwell, Ames, pp. 968 – 976.
- Campbell, T. W. and Dein, F. J. (1984). Avian haematology. In: Harrison, G. J. (Ed). Symposium on caged bird medicine. *Veterinary Clinics of North America: Small Animal Practice*, 14: 223 – 248.
- Campbell, T. W. and Ellis, C. K. (2007). Haematology of birds. In: Campbell, T. W. and Ellis, C. K. (Eds). *Avian and Exotic Animal Haematology and Cytology* (Third edition.). Blackwell Publishing Professional, Ames (IA), pp. 3–50.
- Casado, E., Balbontín, J. and Ferrer, M. (2002). Plasma chemistry in Booted Eagle (*Hieraetus fasciatus*) during breeding season. *Comparative Biochemistry and Physiology A*, 131: 233 – 241.
- Caxton-Martins A. E. and Nganwuchu, A. M. (1978). A cytochemical study of the blood of the rainbow lizard (*Agama agama*). *Journal of Anatomy*, 125: 477 – 480.
- Chakarov, N., Boerner, M. and Krüger, O. (2008). Fitness in common buzzards at the cross-point of opposite melanin-parasite interactions. *Functional Ecology*, 22: 1062 – 1069.
- Cherry, H. N. J. (2008). ABCs of a CBC: an introduction to avian and mammalian complete blood count, *Wildlife Rehabilitation*, 26: 133 – 142.
- Christopher, M. M., Shooshtari, M. P. and Levengood, J. M. (2004). Assessment of erythrocyte morphologic abnormalities in mallards with experimentally induced zinc toxicosis. *American Journal of Veterinary Research*, 65(4): 440 – 446.
- Claver, J. A. and Quaglia, A. I. E. (2009). Comparative morphology, development, and function of blood cells in non-mammalian vertebrates. *Journal of Exotic Pet Medicine*, 18 (2): 87–97
- Clinchy, M., Zanette, L., Boonstra, R., Wingfield, J. C. and Smith, J. N. M. (2004). Balancing food and predator pressure induces chronic stress in songbirds. In;

- Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271: 2473 – 2479.
- Clubb, S. L., Schubot, R. M. and Joiner, K. (1991a). Haematology and serum biochemical reference intervals in juvenile electus parrots (*Electus roratus*). *Association of Avian Veterinarians Journal*, 4(4): 218 – 225.
- Clubb, S. L., Schubot, R. M., Joyner, K., Zinkl, J. G. S., Wolf, Esobar, J. and Kabbur, M. B. (1991b). Haematological and serum biochemical reference intervals in juvenile Cockatoos. *Journal of the Association of Avian Veterinarians*, 5(1): 16 – 26.
- Coke, R. L., West, G. D. and Hoover, J. P. (2004). Haematology and plasma biochemistry of captive puna ibis (*Plegadis ridgewayi*). *Journal of Wildlife Diseases*, 40: 141 – 144.
- Collar, N. (1997). Family Psittacidae (Parrot). In: Sandgrouse to cuckoos. del Hoyo, J., Elliot, A. and Sargatel, J. (Eds). *Handbook of the Birds of the World* (vol.4). Barcelona, Lynx Edicions, pp. 296 – 339.
- Cooper, J. E. (1998). Minimally invasive health monitoring of wildlife. *Animal Welfare*, 7: 35 – 44.
- Costello, R. T. (1970). A Unopette for eosinophil counts. *American Journal of Clinical Pathology*, 54: 249 – 250.
- Cowen, R. (2000). History of life (Third edition). Blackwell Science, Iowa, pp. 11. ISBN 063204444-6.
- Crespo, R. and Chin, R. P. (2004). Effect of feeding green onions (*Allium ascalonicum*) to white Chinese geese (*Threskiornis spinicollis*). *Journal of Veterinary Diagnostic Investigation*, 16(4): 321 – 325.
- Crome, F. H. J. (1991). Birds. In: Forshaw, J. (Ed). *Encyclopaedia of Animals*. Merehurst Press, London, pp. 115 – 116.
- D'Aloia, M-A. E., Samour, J. H., Howlett, J. C., Bailey, T. A. and Naldo, J. (1994). Haemopathologic responses to chronic inflammation in the Houbara bustard (*Chlamydotis undulata macqueenii*). *Comparative Haematology International*, 4(4): 203 – 206.
- Davis, A. K. (2005). Effect of handling time and repeated sampling on avian white blood cell counts. *Journal of Field Ornithology*, 76: 334 – 338.
- Davis, A. K. (2009). The wildlife leucocytes webpage: the ecologist's source for information about leucocytes of wildlife species. www.wildlifehematology.uga.edu. Last accessed on 20 November, 2013. 11.09.56.
- Davis, A. K., Cook, K. C. and Altizer, S. (2004). Leucocyte profiles of house finches with and without mycoplasmal conjunctivitis, a recently emerged bacterial disease. *EcoHealth*, 1: 362 – 373.
- Davis, A. K., Maney, D. L. and Maerz, J. C. (2008). The use of leucocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology*, 22: 760 – 772.
- Dean, W., Siegfried, R. and MacDonald, I. (1990). The fallacy, fact, and fate of guiding behaviour in the greater honey guide. *Conservation Biology*, 4(1): 99 – 101.
- Débora, N. C. L., Érika, M. Braga., Nayara, de O. B. and Yasmine, A. (2011). Haematological and parasitological health conditions of the Pale-breasted Thrush (*Turdus leucomelas*) (Passeriformes: Turdidae) in southeastern Brazil. *Zoologia*, 28 (6): 771 – 776.
- Degernes, L. A., Crosier, M. L., Harrison, L. D., Dennis, P. M. and Diaz, D. E. (1999a). Autologous, homologous, and heterologous red blood cell transfusions in

- cockatiels (*Nymphicus hollandicus*). *Journal of Avian Medicine and Surgery*, 13(1): 2 – 9.
- Degernes, L. A., Harrison, L. D., Smith, D. W., Newton, H., Ross, C. E. and Diaz, D. E. (1999b). Autologous, homologous, and heterologous red blood cell transfusions in conures of the genus *Aratinga*. *Journal of Avian Medicine and Surgery*, 13(1): 10 – 14.
- del Hoyo, J., Elliott, A. and Sargatal, J. (1992). *Handbook of Birds of the World* (vol. 1). *Ostrich to Ducks*. Barcelona, Lynx Edicions. ISBN 84-87334-10-5.
- Dickinson, E. C. (2003). *The Howard and Moore Complete Checklist of the Birds in the World*. (Third edition). Princeton University Press, Princeton, New Jersey, pp. 1039. ISBN 0-691-11701-2.
- Dieterlen-Lievre, F. (1988). Birds. In: Rowley, A. F. and Ratcliffe, N. A. (Eds). *Vertebrate Blood Cells*. Cambridge University Press, Cambridge, pp. 257 – 336.
- Dobado-Berrios, P. M., Tella, J. L., Ceballos, O. and Donazar, J. A. (1998). Effects of age and captivity on plasma chemistry values of the Egyptian Vulture. *Condor*, 100: 719 – 725.
- Dolbeer, R. (1990). Ornithology and integrated pest management: Red-winged blackbirds *Agleaius phoeniceus* and corn. *Ibis*, 132(2): 309 – 322.
- Drabkin, D. R. (1945). Crystallographic and optical properties of human haemoglobin. A proposal for standardization of haemoglobin. *American Journal of Medicine and Surgery*, 209: 268 – 270.
- Dufty, A. M., Jr. (1988). The effects of repeated blood sampling on survival in Brown-headed Cowbirds. *Condor*, 90: 939 – 941.
- Edmonds, R. H. (1968). Electron microscope studies on the haemostatic process in bird embryos I: the initial plug. *Journal Ultrastructure Research*, 24: 295 – 310.
- Ehret, W., Heil, W., Schmitt, Y., Töpfer, G. T., Wisser, H. and Zawta, B. (2002). Use of anticoagulants in diagnostic laboratory investigations and stability of blood, plasma and serum samples. *World Health Organization*, Geneva, Switzerland, pp. 1 – 62.
- El Lethey, H., Huber-Eicher, B. and Jungi, T. W. (2003). Exploration of stress-induced immune-suppression in chickens reveals both stress-resistant and stress-susceptible antigen responses. *Veterinary Immunology and Immunopathology*, 95: 91 – 101.
- Encyclopaedia Britannica, Inc. (2014). Ciconiiform. www.global.britannica.com/EBchecked/topic/117649/ciconiiform/48983/Reproduction-and-nesting. Retrieved 30 November 2014. 23.59.13.
- Ericson, P. G. P., Anderson, C. L., Britton, T., Elzanowski, A., Johansson, U. S., Källersjö, M., Ohlson, J. I., Parsons, T. J., Zuccon, D. and Mayr, G. (2006). Diversification of Neoaves: integration of molecular sequence data and fossils. *Biology Letters*, 2: 543 – 547.
- Evans, P. G. H. (1980). Population genetics of the European Starling, *Sturnus vulgaris*. *Ph. D. Dissertations*, University of Oxford, Oxford, England.
- Ewenson, E. L., Zann, R. A. and Flannery, G. R. (2001). Body condition and immune response in wild zebra finches: effects of capture, confinement and captive-rearing. *Naturwissenschaften*, 88:391 – 394.
- Fair, J. M., Paul, E. and Jones, J. (2010). Guidelines to the use of wild birds in research (Third edition). *Ornithological Council*, Washington, DC. www.nmnh.si.edu/birdnet/guide/index.html. Retrieved on 28 June 2012.
- Fair, J., Whitaker, S. and Pearson, B. (2007). Sources of variation in haematocrit in birds. *Ibis*, 149: 535 – 552.

- Ferrer, M. (1994). Nutritional condition of Spanish Imperial Eagle nestlings, *Aquila adalberti*. *Bird Study*, 41: 120 – 123.
- Ferrer, M. and Dobado-Berrios, P. (1998). Factors affecting plasma chemistry values of the Spanish Imperial Eagle, *Aquila adalberti*. *Comparative Biochemistry and Physiology-A: Molecular and Integrative Physiology*, 120(2): 209 – 217.
- Ferrer, M., García-Rodríguez, T., Carrillo, J.C. and Castroviejo, J. (1987). Haematocrit and blood chemistry values in captive raptors (*Gyps fulvus*, *Buteo buteo*, *Milvus migrans*, *Aquila heliaca*). *Comparative Biochemistry and Physiology A*, 87: 1123 – 1127.
- Ferris, M. and Bacha, W. J. (1984). A new method for the identification and enumeration of chicken heterophils and eosinophils. *Avian Diseases*, 28: 179 – 182.
- Filipović, N., Stojević, Z., Milinković-Tur, S., Ljubić, B. B. and Zdelar-Tuk, M. (2007). Changes in concentration and fractions of blood serum proteins of chickens during fattening. *Veterinarski Arhiv*, 77 (4): 319 – 326.
- Fisher, I. J., Pain, D. J. and Thomas, V. G. (2006). A review of lead poisoning from ammunition sources in terrestrial birds. *Biological Conservation*, 131: 421 - 432.
- Fokidis, H. B., Greiner, E. C. and Deviche, P. (2008). Inter-specific variation in avian blood parasites and haematology associated with urbanization in a desert habitat. *Journal of Avian Biology*, 39: 300 – 310.
- Friedl, T. P. and Edler, R. (2005). Stress-dependent trade-off between immunological condition and reproductive performance in the polygynous red bishop (*Euplectes orix*). *Evolution Ecology*, 19: 221 – 239.
- Fudge, A. M. (1997). Avian clinical pathology haematology and chemistry. In: Altman, R. B., Clubb, S. L. and Dorrestein, G. M. (Eds). *Avian Medicine and Surgery*. Saunders, Philadelphia, WB, pp. 142–57.
- Fudge, A. M. (2000). Disorders of avian erythrocytes. In: Fudge, A. M. (Ed). *Laboratory Medicine: Avian and Exotic Pets*. Saunders, Philadelphia, pp. 28 – 34.
- Fudge, A. M. and Joseph, V. (2000). Disorders of avian leucocytes. In: Fudge, A. M. (Ed). *Laboratory Medicine: Avian and Exotic Pets*. Saunders, Philadelphia, pp. 19–27.
- García-Rodríguez, T., Ferrer, M., Carrillo, J.C. and Castroviejo, J. (1987a). Metabolic responses of *Buteo buteo* to long-term fasting and re-feeding. *Comparative Biochemistry and Physiology A*, 87: 381 – 386.
- García-Rodríguez, T., Ferrer, M., Recio, F. and Castroviejo, J. (1987b). Circadian rhythms of determined blood chemistry values in Buzzards and Eagle owls. *Comparative Biochemistry Physiology A*, 88: 663 – 669.
- Gee, G. F., Carpenter, J. W. and Hensler, B. L. (1981). Species differences in haematological values of captive cranes, geese, raptors and quails. *Journal of Wildlife Management*, 45: 463 – 483.
- Georgieva, T. M., Zapryanova, D. S., Dishlyanova, E. V., Tanev, S. I., Georgiev, I. P., Andonova, M. I., Kanelov, I. N., Lazarov, L. V. and Koleva, P. I. (2009). Comparison of the results of serum total protein concentration measured by 3 methods: preliminary results. *Turkish Journal of Veterinary and Animal Sciences*, 33(1): 67 – 70.
- Gildersleeve, R. P., Satterlee, D. G., Scott, T. R., McRee, D. I., Parkhurst, C. R. and Cook, M. E. (1987). Haematology of Japanese quail selected for high or low serum corticosterone responses to complex stressors. *Comparative Biochemistry and Physiology A*, 86(3): 569 – 573.

- Gilmour, D. G. (1984). Blood groups. In: Freeman, B. M. (Ed). *Physiology and Biochemistry of the Domestic Fowl* (vol. 5). Academic Press, New York, pp. 263 – 276.
- Goodman, G. J. (1996). Metabolic disorders. In: Roskopf, W. J. and Woerpel, R. W. (Eds) *Diseases of Cage and Aviary Birds*. Williams and Wilkins, Baltimore (MD), pp. 470–479.
- Grecchi, R., Saliba, A. M. and Mariano, M. (1980). Morphological changes, surface receptors and phagocytic potential of fowl mono-nuclear phagocytes and thrombocytes *in vivo* and *in vitro*. *Journal of Pathology*, 130(1): 23 – 31.
- Gurd, M. R. (1935). The use of grain-fed pigeons in the biological assay of liver preparations. *Quarterly Journal of Pharmacy and Pharmacology*, 8: 39 – 53.
- Gylstorff, I. and Grimm, F. (1987). Vogelkrankheiten. Stuttgart, Eugen Ulmer, pp. 133 – 146.
- Hannon, S. J. (1979). Plasma calcium as an indicator of reproductive condition in female Blue Grouse. *Canadian Journal of Zoology*, 57: 463 – 465.
- Harmon, B. G. (1998). Avian heterophils in inflammation and disease resistance. *Poultry Science*, 77(7): 972 – 977.
- Harper, D. (2014). Dove. *Online Etymology Dictionary*. www.etymonline.com. Assessed 9 December 2014 at 11:54.
- Harr, K. E. (2002). Clinical chemistry of companion avian species: A review. *Veterinary Clinical Pathology*, 31: 140 – 151.
- Harris, D. J. (2000). Clinical tests. In: Tully, T. N., Lawton, M. P. C. and Dorrestein, G. M. (Eds). *Handbook of Avian Medicine*. Butterworth Heinemann, Oxford, pp. 43 – 51.
- Hauptmanová, K., Literák, I. and Bártová, E. (2002). Haematology and leucocytozoonosis of Great tits (*Parus major* L.) during winter. *Acta Veterinaria Brno*, 71: 199 – 204.
- Hawkey, C. M. and Dennet, T. B. (1989). *A Colour Atlas of Comparative Veterinary Haematology*. Wolfe Publishing, London, UK, pp. 192.
- Heatley, J. and Jowett, P. (2000). What is your diagnosis? *Journal of Avian Medicine and Surgery*, 14(4): 283 – 284.
- Herbert, R., Nanney, J., Spano, J. S., Pedersoli, W. M. and Krista, L. M. (1989). Erythrocyte distribution in ducks. *American Journal of Veterinary Research*, 50(6): 958 – 960.
- Hernández, M. and Margalida, A. (2010). Haematology and blood chemistry reference values and age-related changes in wild bearded vultures (*Gypaetus barbatus*). *Journal of Wildlife Diseases*, 46(2): 390 – 400.
- Hernández, M., Martin, S. and Fores, P. (1990). Clinical haematology and blood chemistry values for the common buzzard (*Buteo buteo*). *Journal of Raptor Research*, 24(4): 113 – 119.
- Hernández-C, O. (2013). Cooperative mobbing of three passerines species on red squirrel (*Sciurus granatensis*) (rodentia, sciuridae). *Revista Colombiana Ciencia Animal*, 5(1): 154 – 157.
- Hochleithner, M. (1989a). Blutchemische Untersuchungen beim adulten und juvenilen Wellensittich (*Melopsittacus undulates*). (Blood chemistry in adult and juvenile budgerigars). *In-aug Diss Wein*.
- Hochleithner, M. (1989b). Convulsion in African grey parrots (*Psittacus erythacus*) in connection with hypocalcaemia. Five selected cases. In: *Proceedings of Second European Symposium of Avian Medicine and Surgery*, pp. 44 – 52.

- Hochleithner, M. (1989c). Reference values for selected psittacine species using a dry chemistry system. *Journal of the Association of Avian Veterinarians*, 3(4): 207 – 209.
- Hochleithner, M. (1990). Verwertbarkeit von Vogelvollblut- und Plasmaproben nach unterschiedlicher Lagerung zur Bestimmung blutchemischer Parameter. (On the serviceability of avian blood and plasma samples for the determination of various blood-chemical parameters following different forms of storage). *Verh ber VII Tagung über Vogelkrankheiten*, pp. 25 – 33.
- Hochleithner, M. (1991a). Use of Reflotron® in pet birds, Tagungsbericht WSAVA Kongreß, Wien, pp. 585 – 587.
- Hochleithner, M. (1991b). Möglichkeiten der chemischen Blutuntersuchung beim Wild- und Ziervogel. (Possible approaches to haematochemical investigation in wild and pet birds). *Verhandlungsbericht des 33. Internationalen Symposiums über die Erkrankungen der Zoo- und Wildtiere*, pp. 153 – 160.
- Hochleithner, M. (1994). Avian medicine: principles and application. In: Ritchie, B. W., Harrison, G. J. and Harrison, L. R. (Eds). *Biochemistries*. Wingers Publishing Inc., Lake Worth, FL, pp. 223 – 245.
- Hoefer, H. L. (1992). Transfusions in exotic species. *Problems in Veterinary Medicine*, 4(4): 625 – 635.
- Ivins, G. K., Weedle, G. D. and Halliwell, W. H. (1985). Haematology and serum chemistry in birds of prey. In: Fowler, M. P. (Ed). *Zoo and Wild Animal Medicine*. W. B. Saunders, Philadelphia, pp. 434 – 437.
- John, J. L. (1994). The avian spleen: a neglected organ. *The Quarterly Review of Biology*, 69: 327 – 351.
- Johns, J. L., Shoostari, M. P. and Christopher, M. M. (2008). Development of a technique for quantification of avian reticulocytes and assessment of erythrocyte regenerative capacity in birds. *American Journal of Veterinary Research*, 69(8): 1067 – 1072.
- Johnston, M. S., Son, T. T. and Rosenthal, K. L. (2007). Immune-mediated haemolytic anaemia in an Eclectus parrot. *Journal of American Veterinary Medical Association*, 230(7): 1028 – 1031.
- Jones, J. S., Thomas, J. S., Bahr, A. and Phalen, D. N. (2002). Presumed immune-mediated haemolytic anaemia in a Blue-crowned conure (*Aratinga acuticaudata*). *Journal of Avian Medicine and Surgery*, 16(3): 223 – 229.
- Joyner, P. H., Kelly, S., Shreve, A. A., Snead, S. E., Sleeman, J. M. and Pettit, D. A. (2006). West Nile virus in raptors from Virginia during 2003: clinical, diagnostic, and epidemiologic findings. *Journal of Wildlife Diseases*, 42(2): 335 – 344.
- Kaneko, J. J. (1997). Serum proteins and the dysproteinemias. In: Kaneko, J. J., Harvey, J. W. and Bruss, M. L. (Eds). *Clinical Biochemistry of Domestic Animals* (Fifth edition) Academic Press, San Diego, London, Boston, New York, Sydney, Tokyo, Toronto, pp. 117 – 138.
- Kilgas, P., Mand, R., Magi, M. and Tilgar, V. (2006). Haematological parameters in brood rearing great tits in relation to habitat, multiple breeding and sex. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 144: 224 – 231.
- Klaassen, M. (1996). Metabolic constraints on long-distance migration in birds. *Journal of Experimental Biology*, 199(1): 57 – 64.
- Knoll, J. S. (2000). Clinical automated haematology systems. In: Feldman, B. F., Zinkl, J. G. and Jain, N. C. (Eds). *Schalm's Veterinary Haematology* (Fifth edition). Lippincott Williams and Wilkins, Philadelphia, pp. 3 – 12.

- Kovach, A. G. B., Szasz, E. and Pilmayer, N. (1969). The mortality of various avian and mammalian species following blood loss. *Acta Physiologica Academiae Scientiarum Hungaricae*, 35: 109 – 126.
- Lack, P. (2003). Pigeons and doves. In: Perrins, C. (Ed). *The New Encyclopaedia of Birds*, Oxford University Press, Oxford, pp. 288 – 295.
- Lane, R. (1991). Basic techniques in pet avian clinical pathology. *Veterinary Clinics of North America: Small Animal Practice*, 21:1157 – 1179.
- Lashev, L., Hubenov, H., Nikolov, Y., Lasheva, V. and Mihailov, R. (2009). Comparison of some haematological parameters between three bird species from the *Columbidae* family - short communication. *Veterinarski Arhiv*, 79(4): 409 – 414.
- Latimer, K. S. (1994). Oncology. In: Ritchie, B. W., Harrison, G. J. and Harrison, L. R. (Eds) *Avian Medicine: Principles and Application*. Wings Publishing, Lake Worth (FL), pp. 640 – 672.
- Latimer, K. S. and Bienzle, D. (2000). Determination and interpretation of the avian leukogram. In: Feldman, B. F., Zinkl, J. G. and Jain, N. C. (Eds). *Schalm's Veterinary Haematology*. Lippincott Williams and Wilkins, Philadelphia, pp. 417 – 432.
- Latimer, K. S. and Bienzle, D. (2010). Determination and interpretation of the avian leukogram. In: Weiss, D. J. and Wardrop, K. J. (Eds). *Schalm's Veterinary Haematology* (Sixth edition). Wiley-Blackwell, Ames, pp. 345 – 357.
- Latimer, K. S., Mahaffey, E. A., Prasse, K. W. and Duncan, J. R. (2003). Duncan and Prasse's Veterinary Laboratory Medicine. *Clinical Pathology*. Iowa State University Press, Iowa, pp. 46 – 80.
- Latimer, K. S., Tang, K. N., Goodwin, M. A., Steffens, W. L. and Brown J. (1988). Leucocyte changes associated with acute inflammation in chickens. *Avian Diseases*, (4): 760 – 772.
- Lazarevic, M., Zikic, D. and Uscebrka, G. (2000). The influence of long term sound stress on the blood leucocyte count, heterophil cells/lymphocyte ratio and cutaneous basophil hypersensitive reaction to phytohemagglutinin in broiler chickens. *Acta Veterinaria Beograd*, 50: 63 – 75.
- Leaf, A. and Cotran, R. (1976). Renal pathophysiology. Oxford University Press, New York, pp. 164 – 165.
- Leighton, F. A. (1985). Morphological lesions in red blood cells from herring gulls and Atlantic puffins ingesting Prudhoe Bay crude oil. *Veterinary Pathology*, 22(4): 393 – 402.
- Lewandowski, A. H., Campbell, T. W. and Harrison, G. J. (1986). Clinical chemistries. In: Harrison, G. J. and Harrison, L. R. (Eds). *Clinical Avian Medicine and Surgery*. W. B. Saunders Co., Philadelphia, pp. 192 – 200.
- Lichtenberger, M. (2004). Transfusion medicine in exotic pets. *Clinical Technique in Small Animal Practice*, 19(2): 88 – 95.
- Lichtenberger, M., Rosenthal, K., Brue, R. and Kirby, R. (2001). Administration of oxyglobin and 6% hetastarch after acute blood loss in psittacine birds. In: *Proceedings of the Association of Avian Veterinarians*. Orlando (FL), pp. 15 – 18.
- Linnaeus, Carolus (1758). Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis (Tenth edition). In: Tomus I. (Ed). Holmiae, (Laurentii Salvii), pp. 824.
- Lloyd, S. and Gibson, J. S. (2006). Haematology and biochemistry in healthy young pheasants and red-legged partridges and effects of spironucleosis on these parameters. *Avian Pathology*, 35(4): 335 – 340.

- Lucas, A. M. and Jamroz, C. (1961). *Atlas of Avian Haematology*. Agriculture Monography 25. United States Department of Agriculture, Washington, DC, pp. 271.
- Lumeij, J. T. (1987a). The influence of blood samples treatment, feeding and starvation on plasma glucose concentrations in racing pigeons. In: Lumeij, J. T. (Ed). *A Contribution to Clinical Investigation Methods for Birds with Special Reference to Racing Pigeon. Ph.D. Thesis*, Utrecht University, pp. 26 – 30.
- Lumeij, J. T. (1987b). Plasma urea, creatinine and uric acid concentrations in response to dehydration in racing pigeons (*Columba livia domestica*). *Avian Pathology*, 16(3): 377 – 382.
- Lumeij, J. T. (1987c). The diagnostic value of plasma proteins and non-protein nitrogen substances in birds. *Veterinary Quarterly*, 9(3): 262 – 268.
- Lumeij, J. T. (1987d). A contribution to clinical investigative methods for birds, with special reference to the Racing Pigeon (*Columbia livia domestica*). *Ph.D. Thesis*, Utrecht University, Utrecht, Proefschrift, pp. 151 – 166.
- Lumeij, J. T. (1990). Relation of plasma calcium to total protein and albumin in African grey parrot (*Psittacus erythacus*) and Amazon (*Amazon spp.*) parrots. *Avian Pathology*, 19: 661 – 667.
- Lumeij, J. T. and Overduin, L. M. (1990). Plasma chemistry reference values in psittaciformes. *Avian Pathology*, 19: 235 – 244.
- Lumeij, J. T. and Westerhof, I. (1987). Blood chemistry for the diagnosis of hepatobiliary disease in birds. *Veterinary Quarterly*, 9: 255 – 261.
- Maxwell, M. and Burns, R. (1985). Blood eosinophilia in adult bantams naturally infected with *Trichostrongylus tenuis*. *Research in Veterinary Science*, 39(1): 122 – 123.
- Maxwell, M. H. (1993). Avian blood leucocyte response to stress. *World's Poultry Science Journal*, 49: 34 – 43.
- Maxwell, M. H. and Robertson, G. W. (1995). The avian basophilic leucocyte: a review. *World's Poultry Science Journal*, 51: 307 – 325.
- Maxwell, M. H. and Robertson, G. W. (1998). The avian heterophil leucocyte: a review. *World's Poultry Science Journal*, 54: 155 – 178.
- Maxwell, M. H. and Siller, W. G. (1972). The ultra-structural characteristics of the eosinophil granules in six species of domestic birds. *Journal of Anatomy*, 112: 289 – 303.
- Mayr, E. (1946). The number of species of birds. *Auk*, 63(1): 64 – 69.
- McGuill, M. W. and Rowan, A. N. (1989). Biological effects of blood loss: Implications for sampling volumes and techniques. *Institute for Laboratory Animal Research Journal*, 31: 5 – 18.
- Mercurio, D. D. G., Marte, B. R. G. and Cruzana, B. C. (2008). Haematological values of Chestnut mannikin (*Lonchura malacca*) caught in Laguna. *Philippine Journal of Veterinary Medicine*, 45: 63 – 66.
- Miller, M. J., Wayland, M. E. and Bortolotti, G. R. (2001). Haemograms for and nutritional condition of migrant bald eagles tested for exposure to lead. *Journal of Wildlife Diseases*, 37: 481 – 488.
- Mitchell, E. B. and Johns, J. (2008). Avian haematology and related disorders. *Veterinary Clinics of Exotic Animals Practice*, 11: 501 – 522.
- Montali, R. J. (1988). Comparative pathology of inflammation in the higher vertebrates (reptiles, birds and mammals). *Journal of Comparative Pathology*, 99(1): 1 – 26.

- Moreno, J., de Leon, A., Fargallo, J. A. and Moreno, E. (1998). Breeding time, health and immune response in the chinstrap penguin (*Pygoscelis antarctica*). *Oecologia*, 115: 312 – 319.
- Moreno, J., Merino, S., Martinez, J., Sanz, J. J. and Arriero, E. (2002). Heterophil/lymphocyte ratios and heat-shock protein levels are related to growth in nestling birds. *Ecoscience*, 9: 434 – 439.
- Morrissey, J. K. (2004). Transfusion medicine in birds (VET-598). In: *Proceedings of the Western Veterinary Conference*. Las Vegas (NV).
- Morrissey, J. K. and Giger, U. (1997). Comparison of three media for the storage of avian whole blood. In: *Proceedings of the Association of Avian Veterinarians*. Reno (NV), pp. 279 – 280.
- Murray, M. J. (1997). Diagnostic techniques in avian medicine. *Seminar in Avian and Exotic Pet Medicine*, 6(2): 48 – 54.
- Naidoo, V., Diekmann, M., Wolters, K. and Swan, G. E. (2008). Establishment of selected baseline blood chemistry and hematologic parameters in captive and wild-caught African White-Backed Vultures (*Gyps africanus*). *Journal of Wildlife Diseases*, 44: 649 – 654.
- Natt, M. P. and Herrick, C. A. (1952). A new blood diluent for counting erythrocytes and leucocytes of the chicken. *Poultry Science*, 31: 735 – 738.
- Nava, M. P., Veiga, J. P. and Puerta, M. (2001). White blood cell counts in house sparrows (*Passer domesticus*) before and after moult and after testosterone treatment. *Canadian Journal of Zoology*, 79: 145 – 148.
- Newell, S., McMillan, M. and Moore, F. (1991). Diagnosis and treatment of lymphocytic leukaemia and malignant lymphoma in a Pekin duck (*Anas platyrhynchos domesticus*). *Journal of the Association of Avian Veterinarians*, 5(2): 83 – 86.
- Newman, S. H., Piatt, J. F. and White, J. (1997). Haematological and plasma biochemical reference ranges of Alaska seabirds: their ecological significance and clinical importance. *Colonial Waterbirds*, 20(3): 492 – 504.
- Norte, A. C., Araujo, P. M., Sampaio, H. L., Sousa, J. P. and Ramos, J. A. (2009). Haematozoa infections in a great tit *Parus* major population in Central Portugal: relationships with breeding effort and health. *Ibis*, 151: 677 – 688.
- Ochiai, K., Jin, K., Goryo, M., Tsuzuki, T. and Itakura, C. (1993). Pathomorphologic findings of lead poisoning in white-fronted geese (*Anser albifrons*). *Veterinary Pathology*, 30(6): 522 – 528.
- Okumura, J. and Tasaki, I. (1969). Effect of fasting, re-feeding and dietary protein level on uric acid and ammonia content of blood liver and kidney in chickens. *Journal of Nutrition*, 97: 316 – 320.
- Oladele, S. B. (2000). Haematological parameters of some apparently healthy and some clinically sick indigenous poultry species in Zaria. *MSc. Thesis*, Ahmadu Bello University, Zaria, pp. 116.
- Oladele, S. B. (2009). The significance of haematology in the diagnosis and therapy of avian disease: a review. *Nigerian Veterinary Journal*, 30(2): 24 – 39.
- Oladele, S. B., Ayo, J. O., Ogundipe, S. O. and Esievo, K. A. N. (2003). Seasonal and species variations in erythrocytes osmotic fragility of indigenous poultry species in Zaria, Northern Guinea Savannah Zone of Nigeria. *Bulletin of Animal Health and Production in Africa*, 51: 204 – 214.
- Oladele, S. B., Nok, A. J., Esievo, K. A. N., Abdu, P. A. and Useh, N. M. (2005). Haemagglutination inhibition antibodies, rectal temperature and total protein of

- chickens infected with local Nigerian isolate of velogenic Newcastle disease virus. *Veterinary Research Communications*, 29: 171 – 179.
- Oladele, S. B., Obaje, O. S. and Ogunbodede, M. A. (2011). Newcastle disease virus antibody in apparently healthy free-living birds in Zaria, Nigeria. *Sahel Journal of Veterinary Science*, 10: 71 – 76.
- Oladele, S. B., Enam, S. J. and Okubanjo, O. O. (2012). Pathogenic haemoparasites and antibody to Newcastle disease virus from apparently healthy wild birds in Zaria, Nigeria. *Veterinary World*, 5: 13 – 18.
- Olayemi, F. O., Ojo, E. O. and Fagbohun, O. A. (2006). Haematological and plasma biochemical parameters of the Nigerian laughing dove (*Streptopelia senegalensis*) and the Nigerian duck (*Anas platyrhynchos*). *Veterinarski Arhiv*, 76(2): 145 – 151.
- Oring, L. W., Fivizzani, A. J., Colwell, M. A. and Halawani, M. E. (1988). Hormonal changes associated with natural and manipulated incubation in the sex-role reversed Wilson's Phalarope. *General Comparative Endocrinology*, 72: 247 – 256.
- Other Free Encyclopaedias (2014). Apodiformes Behaviour and Reproduction. *Animal Life Resource*. www.animals.jrank.org/pages/838/Swifts-Hummingbirds-Apodiformes-PHYSICAL-CHARACTERISTICS.html. Retrieved 29 September 2014.
- Ots, I., Murumagi, A. and Hõrak, P. (1998). Haematological health state indices of reproducing great tits: methodology and sources of natural variation. *Functional Ecology*, 12: 700 – 707.
- Owen, J. C. (2011). Collecting, processing and storing avian blood: a review. *Journal Field Ornithology*, 82(4): 339 – 354.
- Panger, M. A., Brooks, A. S., Richmond, B. G. and Wood, B. (2002). Older than the Oldowan? Rethinking the emergence of hominin tool use. *Evolutionary Anthropology: Issues, News, and Reviews*, 11:235-245.
- Passantino, L., Massaro, M. A., Jirillo, F., Di Modugno, D., Ribaud, M. R., Modugno, G. D., Passantino, G. F. and Jirillo, E. (2007). Antigenically activated avian erythrocytes release cytokine-like factors: a conserved phylogenetic function discovered in fish. *Immunopharmacology and Immunotoxicology*, 29: 141 – 152.
- Pavlak, M., Vlahović, K., Jerčić, J., Dovc, A. and Župančić, Z. (2005). Age, sexual and seasonal differences of haematological values and antibody status to *Chlamydophila* sp. In: Feral and racing pigeons (*Columba livia* forma domestica) from an urban environment (Zagreb, Croatia). *European Journal of Wildlife Research*, 51: 271 – 276.
- Piersma, T., Koolhaas, A., Decunga, A. and Gwinner, E. (2000). Red blood cell and white blood cell counts in sandpipers (*Philomachus pugnax*, *Calidris canntus*): Effects of captivity, season, nutritional status and frequent bleedings. *Canadian Journal of Zoology*, 78: 1349 – 1355.
- Pimental, J. L., Greger, J. L., Cook, M. F. and Stahl, J L. (1992). Iron metabolism in chicks fed various levels of zinc and copper. *Journal of Nutrition and Biochemistry*, 13: 140 – 145.
- Ploucha, J. M., Scott, J. B. and Ringer, R. K. (1981). Vascular and haematologic effects of haemorrhage in the chicken. *American Journal of Physiology*, 240(1): H9 – H17.
- Polo, F. J., Celdrán, J. F., Peinado, V. I., Viscor, G. and Palomeque, J. (1992). Haematological values for four species of birds of prey. *Condor*, 94: 1007 – 1013.
- Polo, F. J., Peinado, V. I., Viscor, G. and Palomeque, J. (1998). Haematologic and plasma chemistry values in captive psittacine birds. *Avian Diseases*, 42(3): 523 – 535.

- Pop, A., Lazar, O. G., Fafaneata, C. and Predoi, G. (2010). Evaluation of serum biochemical parameters in wild birds around Bucharest International Airport. *Bulletin of University of Agricultural Sciences and Veterinary Medicine, Veterinary Medicine*, 67(1): 217 – 221.
- Post, J., Rebel, J. and ter Huurne, A. (2003). Automated blood cell count: a sensitive and reliable method to study corticosterone-related stress in broilers. *Poultry Science*, 82(4): 591 – 595.
- Proctor, N. S. and Lynch, P. J. (1998). *Manual of Ornithology: Avian Structure and Function*. Yale University Press, New Haven, CT, ISBN 0300076193.
- Puerta, M. L., Alonso, J. C., Huecas, V., Alonso, J. A., Abelenda M. and Munoz-Pulido, R. (1990). Haematology and blood chemistry of wintering Common Cranes. *Condor*, 92: 210 – 214.
- Quesenberry, K. and Moroff, S. (1991). Plasma electrophoresis in psittacine birds. In: *Proceedings from Association of Avian Veterinarians*, Chicago, Illinois, pp. 112 – 117.
- Reed, K. D., Meece, J. K., Henkel, J. S. and Shukla, S. K. (2003). Birds, migration and emerging zoonoses: West Nile Virus, Lyme Disease, Influenza A and Enteropathogens. *Clinical Medicine and Research*, 1(1): 5 – 12.
- Rehman, H., Abbas, S. and Lohahet, N. (2003). *Laboratory Manual of Physiology*, (vol. 1). Society of Veterinary Physiology, Lahore, Pakistan.
- Robertson, G. W. and Maxwell, M. H. (1990). Modified staining techniques for avian blood cells. *British Poultry Science*, 31: 881 – 886.
- Robertson, G. W. and Maxwell, M. H. (1993). Importance of optimal mixtures of EDTA anticoagulant blood for the preparation of well-stained avian blood smears. *British Poultry Science*, 34: 615 – 617.
- Robertson, J. E., Christopher, M. M. and Rogers, Q. R. (1998). Heinz body formation in cats fed baby food containing onion powder. *Journal of American Veterinary Medical Association*, 212: 1260 – 1266.
- Rodgers, J. D. and Gass, G. H. (1983). The effect of age on serum proteins in mice. *Experimental Gerontology*, 18: 39 – 45.
- Romagnano, A., Grindem, C. B., Degernes, L. A. and Mautino, M. (1995). Treatment of a hyacinth macaw with zinc toxicity. *Journal of Avian Medicine and Surgery*, 9: 185 – 189.
- Roskopf, W. R., (1982). Pacheco's disease and aspergillosis in a parrot. *Modern Veterinary Practice*, 63: 300 – 301.
- Roskopf, W. R., Woerpel, R. W., Roskopf, G. and Van De Water, D. (1982). Normal thyroid values for common pet birds. *Veterinary Medical and Small Animals Clinics*, 77(3): 409 – 412.
- Rowley, I. (1997). Family Cacutuidae (Cockatoos). In: del Hoyo, J., Elliot, A. and Sargatel, J. (Eds). *Handbook of the Birds of the World* (vol. 4). Lynx Edicions, Barcelona, pp. 246 – 279.
- Ruiz, G., Rosenmann, M., Novoa, F. F. and Sabat, P. (2002). Haematological parameters and stress index in rufous-collared sparrows dwelling in urban environments. *Condor*, 104: 162 – 166.
- Rupley, A. E. (1997). Emergency procedures: recovering from disaster. In: *Proceedings of the Association of Avian Veterinarians*. Reno (NV), pp. 249 – 257.
- Russo, E. A., McEntee, L., Applegate, L. and Baker, J. S. (1986). Comparison of two methods for determination of white blood cell counts in macaws. *Journal of American Veterinary Medical Association*, 189: 1013 – 1016.

- Saleem, M. H., Khan, M. S., Chaudry, A. S. and Samad, H. A. (2008). Prevalence of trichomoniasis in domestic and wild pigeons and its effects on haematological parameters. *Pakistan Veterinary Journal*, 28(2): 89 – 91.
- Samour, H. J., Riskey, D., March, T., Savage, B., Nieva, O. and Jones, D. M. (1984). Blood sampling techniques in reptiles. *Veterinary Records*, 114: 472 – 476.
- Samour, J. (2006). Diagnostic value of haematology. In: Harrison, G. J. and Lightfoot, T. L. (Eds). *Clinical Avian Medicine* (vol. 2). Spix Publishing, Palm Beach (FL), pp. 587 – 610.
- Sandmeier, P., Stauber, E. H., Wardrop, K. J. and Washizuka, A. (1994). Survival of pigeon red blood cells after transfusion into selected raptors. *Journal of American Veterinary Medical Association*, 204(3): 427 – 429.
- Schoemaker, N. J., Dorrestein, G. M., Latimer, K. S., Lumeij, J. T., Kik, M. J., van der Hage, M. H. and Campagnoli, R. P. (2000). Severe leukopenia and liver necrosis in young African grey parrots (*Psittacus erithacus erithacus*) infected with psittacine circovirus. *Avian Diseases*, 44(2): 470 – 478.
- Scope, A., Filip T., Gabler, C. and Resch, F. (2002). The influence of stress from transport and handling on haematologic and clinical chemistry blood parameters of racing pigeons (*Columba livia domestica*). *Avian Diseases*, 46: 224 – 229.
- Seedikkoya, K., Azeez, P. and Shukkur, E. (2005). Cattle Egret, *Bubulcus ibis*, habitat use and association with cattle. *Forktail*, 21: 174 – 176.
- Seegar, W. S. (1979). Comparison of four blood survey techniques for detecting microfilariae in avian blood. *Ibis*, 121: 104 – 106.
- Seiser, P., Duffy, L., Mcguire, A., Roby, D., Golet, G. and Litzow, M. (2000). Comparison of pigeon guillemot, *Cephus columba*, blood parameters from oiled and unoled areas of Alaska eight years after the Exxon Valdez oil spill. *Marine Pollution Bulletin*, 40: 152 – 164.
- Sergent, N., Rogers, T. and Cunningham, M. (2004). Influence of biological and ecological factors on haematological values in wild little penguins (*Eudyptula minor*). *Comparative Biochemistry and Physiology A - Molecular Integrative Physiology*, 138: 333 – 339.
- Sherman, P. W. (1981). Electrophoresis and avian genealogical analyses. *Auk*, 98: 419 – 422.
- Sibley, C. G. and Ahlqist, J. E. (1983). Phylogeny and classification of birds based on the data of DNA-DNA hybridization. In: Johnston, R. F. (Ed). *Current Ornithology*, (vol. 1). Plenum Press, New York, pp. 245 – 292.
- Sibley, C. G. and Monroe Jr., B. L. (1990). *Distribution and taxonomy of birds of the world*. Yale University Press, New Haven, London, CT. ISBN 0-300-04969-2, pp. 1111.
- Singer, R. and Yom-Tov, Y. (1988). The breeding biology of the house sparrow *Passer domesticus* in Israel. *Ornis Scandinavica*, 19(2): 139 – 44.
- Steve, B. (2013). About the Wild Parrots of Brooklyn. BrooklynParrots.com. Retrieved 2013-02-27. 08.20.12.
- Stein, R. W., Yamamoto, J. T., Fry, D. M. and Wilson, B. W. (1998). Comparative haematology and plasma biochemistry of Red-Tailed Hawks and American Kestrels wintering in California. *Journal of Raptor Research*, 32(2): 163 – 169.
- Storey, M. L. and Greger, J. L. (1987). Iron, zinc and copper interactions: chronic versus acute responses of rats. *Journal of Nutrition*, 117: 1434 – 1442.
- Sturkie, P. D. (2000). The cardiovascular system. In: Whittow, G. C. (Ed). *Avian Physiology* (Fifth edition). Academic, San Diego, pp. 176 – 178.

- Sturkie, P. D. and Griminger, P. (1986). Body fluids: Blood. In: Sturkie, P. (Ed). *Avian Physiology* (Fourth edition). Springer-Verlag, New York, pp. 102 – 129.
- Szabo, A., Mezes, M., Horn, P., Suto, Z., Bazar, G. and Romvari, R. (2005). Developmental dynamics of some blood biochemical parameters in the growing turkey (*Meleagris gallopavo*). *Acta Veterinaria Hungary*, 53(4): 397 – 409.
- Telfair II, R. C. (2006). Cattle egret (*Bubulcus ibis*). In: Poole, A. (Ed). *The Birds of North America Online*. Cornell Laboratory of Ornithology, Ithaca, New York, USA. Available at <<http://bna.birds.cornell.edu/bna/species/113>>[Accessed 10 March 2012].
- Tell, L. A., Woods, L. and Cromie, R. L. (2001). Mycobacteriosis in birds. *Revue Scientifique et Technique*, 20(1): 180–203.
- Thrall, M. A. (2004). Haematology of birds. In: Thrall, M. A., Baker, D. C. and Campbell T. W. (Eds), *Veterinary Haematology and Clinical Chemistry*. Lippincott, Williams and Wilkins, Baltimore (MD), pp. 225 – 258.
- Totzke, U., Fenske, M., Huppopp, O., Raabe, H. and Schach, N. (1999). The influence of fasting on blood and plasma composition of Herring Gulls (*Larus argentatus*). *Physiological and Biochemistry Zoology*, 72: 426 – 437.
- Troisi, G., Borjesson, L. and Bexton, S. (2007). Biomarkers of polycyclic aromatic hydrocarbon (PAH)-associated haemolytic anaemia in oiled wildlife. *Environmental Research*, 105(3): 324 – 329.
- Uhart, M. M., Quintana, F., Karesh, W. B. and Braselton, W. E. (2003). Haematology, plasma biochemistry, and serosurvey for selected infectious agents in southern giant petrels from Patagonia, Argentina. *Journal of Wildlife Diseases*, 39: 359 – 365.
- Utter, J. M. and Le-Febvre, E. A. (1970.) Energy expenditure for free flight by the Purple Martin (*Progne subis*). *Comparative Biochemistry and Physiology*, 35: 713 – 719.
- Vegad, J. L. and Katyar, A. K. (1995). The acute inflammatory response in the chicken. *Veterinary Bulletin*, 65: 309 – 409.
- Villegas, A., Saucher, J. M., Corbacho, C., Corbacho, P. and Vargas, J. M. (2004). Blood values of bald ibis (*Geronticus eremita*) in captivity: comparative ranges and variability with age, sex and physical condition. *Journal of Ornithology*, 5: 98 – 104.
- Vinkler, M., Schnitzer, J., Munclinger, P., Votýpka, J. and Albrecht, T. (2010). Haematological health assessment in a passerine with extremely high proportion of basophils in peripheral blood. *Journal of Ornithology*, 151 (4): 841 – 849.
- Voss, M., Shutler, D. and Werner, J. (2010). A hard look at blood sampling of birds. *Auk*, 127: 704 – 708.
- Wakenel, P. S. (2010). Haematology of chickens and turkeys. In: Weiss, D. J. and Wardrop, K. J. (Eds). *Schalm's Veterinary Haematology* (Sixth edition). Wiley-Blackwell, Ames, pp. 958 – 967.
- Walberg, J. (2001). White blood cell counting techniques in birds. *Seminars in Avian and Exotic Pet Medicine*, 10: 72 – 76.
- Wardlaw, M. S. C. and Levine, M. R. A. (1983). Quantitative buffy coat analysis: a new laboratory tool functioning as a screening complete blood cell count. *Journal of the American Medical Association*, 249: 617 – 620.
- Weir, J. T. and Schluter, D. (2007). The latitudinal gradient in recent speciation and extinction rates of birds and mammals. *Science*, 315(5818): 1574 – 1576.

- Wells, J. and Wells, A. (2001). Pigeons and doves. In: Elphick, C., Dunning, J. and Sidley, D. (Eds), *The Sibley Guide to Birds Life and Behaviour*, New York, pp. 319 – 325.
- Werner, L. L. and Reavill, D. R. (1999). The diagnostic utility of serum protein electrophoresis. *Veterinary Clinics of North America: Exotic Animal Practice*, 2: 651 – 662.
- Wight, P. (2002). Supporting the principles of sustainable development in tourism and ecotourism: governments' potential role. *Current Issues in Tourism*, 5: 222 – 243.
- Wight, P. A. L., Dewar, W. A. and Mackenzie, G. M. (1980). Monocytosis in experimental zinc deficiency of domestic birds. *Avian Pathology*, 9(1): 61 – 66.
- Williams, T. D. (2005). Immune defence and host life history. *American Naturalist*, 160: 9 – 22.
- Woerpel, W. R. and Roskopf, W. (1984). Clinical experiences with avian laboratory diagnostics. *Veterinary Clinics of North America*, 14(2): 249 – 286.
- Woronzoff-Dashkoff, K. K. (2002). The Wright-Giemsa stain: secrets revealed. *Clinics in Laboratory Medicine*, 22: 15 – 23.
- Zaahkouk, S. A., El Zawhry, I. E., Abdel-Rached, H. G. and El Feky, K. S. (2013). Physiological study on some biochemical parameters in two types of birds. *International Journal of Biology, Pharmacy and Allied Sciences*, 2(7): 1379 – 1387.
- Zinkl, J. G. (1986). Avian haematology. In: Jain, N.C. (Ed). *Schalm's Veterinary Haematology* (Fourth edition). Lea and Febiger, Philadelphia, pp. 256 – 273.
- Zon, L. I. (1995). Developmental biology of haematopoiesis. *Blood*, 86: 2876 – 2891.
- Zoological Nomenclature Resources (ZNR). (2008). Psittaciformes (version 9.013). www.zoonomen.net.2008-12-29.11.01.12

APPENDICES

Appendix I Photograph of Free-living Wild Birds used for the Study



Laughing Dove (*Streptopelia senegalensis*)



Mourning collar dove (*Streptopelia decipiens*)



Bruce's Green Pigeon (*Treron waalia*)



Speckled Pigeon (*Columba guinea*)



Rose-Ringed Parakeet (*Psittacula krameri*)



Senegal Parrot (*Poicephalus senegalus*)



Red-Billed Quelea (*Quelea quelea*)



Northern Red Bishop (*Euplectes francisca*)



African silver billed (*Euodice cantans*)



Little Weaver (*Ploceus luteolus*)



White-Rumped Swift (*Apus caffer*)



Cattle Egret (*Bubulcus ibis*)

Appendix II

Preparation of Drabkin's Solution

Potassium cyanide (KCN)	-	0.05 g
Potassium ferricyanide [$K_3Fe(CN)_6$]	-	0.2 g
Sodium bicarbonate [$Na_2(CO_3)_2$]	-	1 g

Dissolved in 1 litre of distilled water and store in amber bottle at room temperature.

Procedure

- Measure 5 ml of HICN (Drabkin) reagent into test tube
- Measure 20 μ L of blood using a micro pipette
- Properly mix with Drabkin reagent.
- Centrifuged at 3000 rpm for 15 minutes to separate the empty RBC from interfering with the reading.
- Decant supernatant into sample bottle.
- Absorb supernatant into the haemoglobin meter
- Take reading after wiggling pump stops working

Appendix III

Preparation of Natt-Herrick Solution

Sodium Chloride (NaCl)	-	3.88 g
Sodium sulphate (NaSO ₄)	-	2.50 g
Sodium phosphate (Na ₂ HPO ₄)	-	2.91 g
Potassium phosphate (KH ₂ PO ₄)	-	0.25 g
Formaline (37%)	-	7.50 ml
Methyl violet	-	0.10g

+ 1,000 ml with distilled water + filter and store in amber bottle at room temperature.

Procedure

- Use a standard RBC diluting pipette
- Dilute whole anticoagulated blood with Natt-Herrick's solution at the rate of 1:200.
- Allow diluted blood to mix for a minute or two
- Discharge into the haemocytometer counting chamber (using a capillary tube).
After charging haemocytometer, allow contents to settle for approximately 3 minutes.

Performing the Total Erythrocyte Count

- Use high dry (40X) objective of the microscope.
- Count total number of RBC (easily recognizable by their nuclei) in the four corner and central squares of the central large square of the counting chamber.
- Count all cells that overlap the top and left border.

NB: Do not count any cells that overlap the bottom or right borders.

The computation for RBC count is as follows:

$$N/100 = \text{RBC} \times 10^{12} / \text{l}$$

Where N = Number of cells counted in the 5 squares in the mid section of the haemocytometer (or in 160 squares)

Performing the Total Leucocyte Count

- (As previously noted, the same 1:200 dilution is used for counting white cells.)
WBC tends to stain dark blue to purple and may exhibit some granularity.
- The total leucocyte count is obtained by counting all leucocytes present in the nine large ruled squares of the haemocytometer.
- Count all cells that overlap the top and left border.

NB: Do not count any cells that overlap the bottom or right borders.

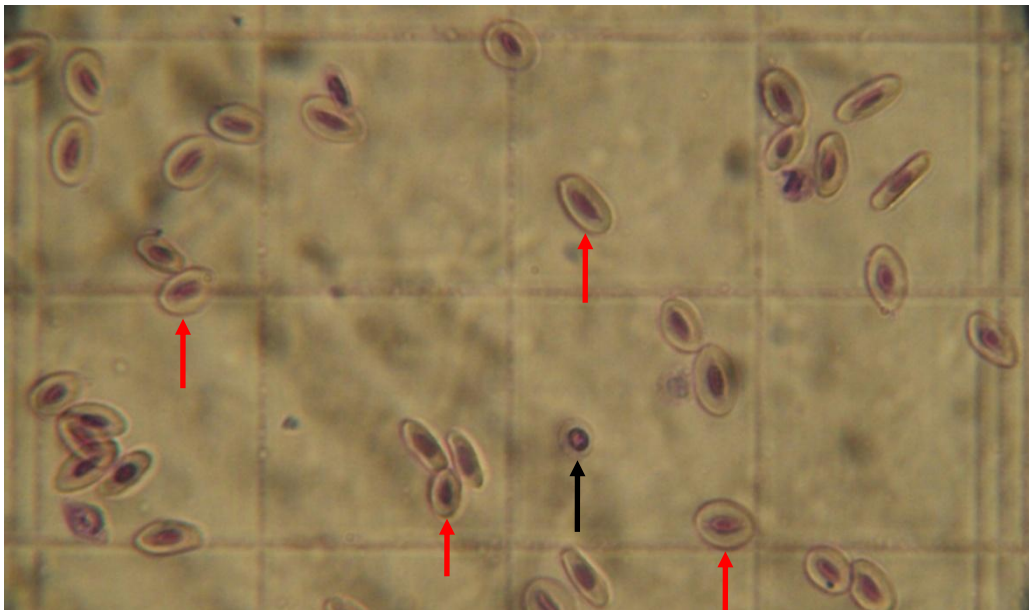
The computation for total leucocyte count is as follows:

$$N/20 = \text{WBC} \times 10^9 / \text{l}$$

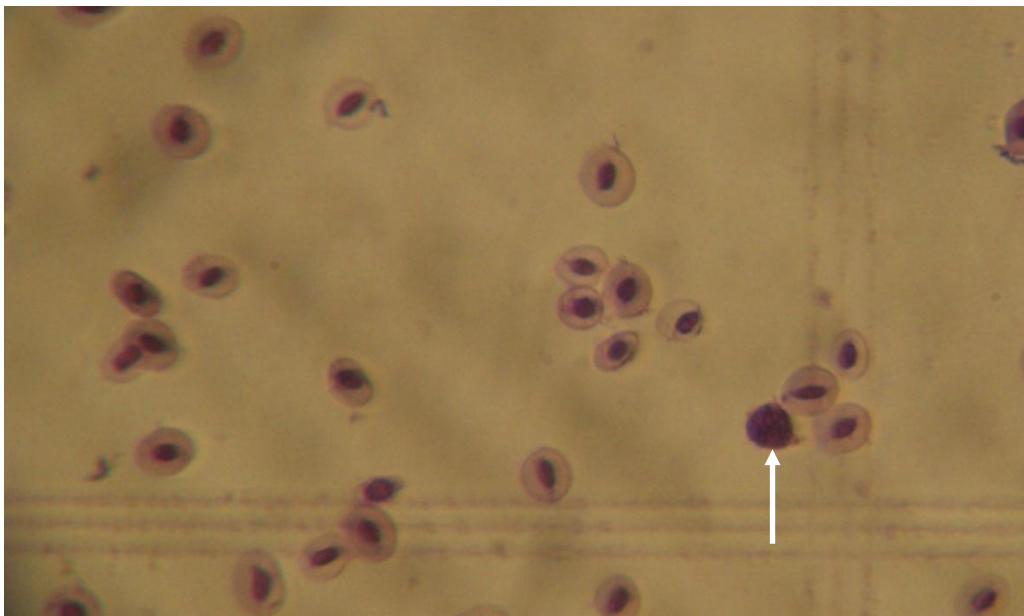
Where N = Number of cells counted in the four outer large squares (or in 64 small squares)

Appendix IV

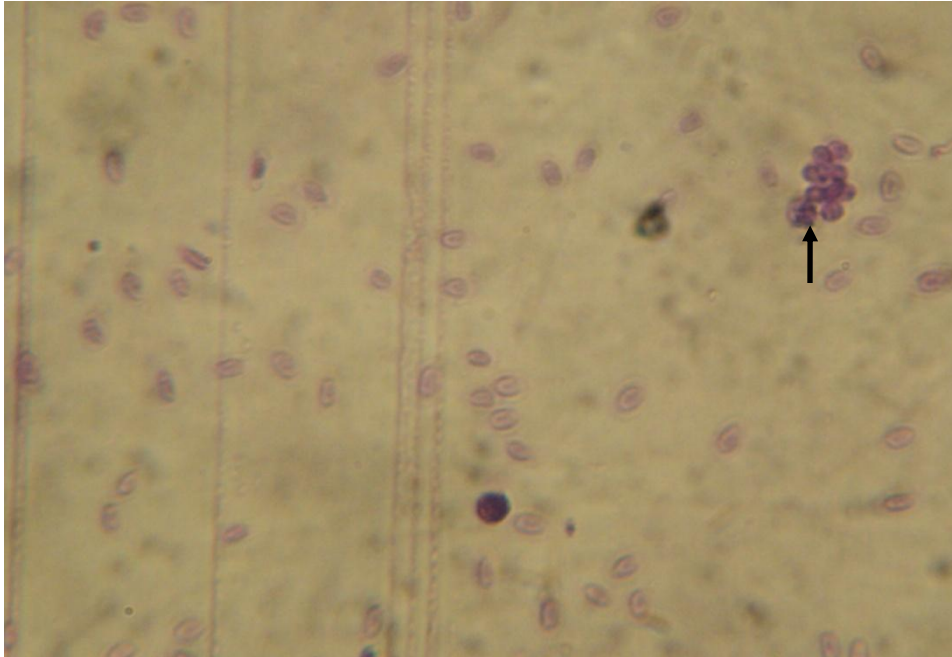
Photograph of Blood Cells Stained with Natt-Herrick



Blood sample from the Cattle Egret. Characteristic appearance of the RBC (red arrows) and thrombocyte (black arrow) using the Natt-Herrick Solution with haemocytomer (X40)



Blood sample from the Cattle Egret. Characteristic appearance of the WBC (white arrow) using the Natt-Herrick Solution with haemocytomer (X40)



Blood sample from the Laughing Dove. Thrombocyte showing clumping of the cells (black arrow) using the Natt-Herrick Solution with haemocytomer (X40)

Appendix V

Procedure for Modified Wright-Giemsa Stain

Working Stain

- 3 g Wright stain powder
- 0.3 g Giemsa stain powder
- 5 ml glycerol
- To 1,000 ml absolute methanol (acetone free)
- Filter and store at room temperature in amber bottle

Preparation of Sørensen's Buffer

Stock Solution A: 0.1 M potassium dihydrogen phosphate. Dissolve 13.61 g KH_2PO_4 in 1,000 ml distilled water.

Stock Solution B: 0.1 M disodium hydrogen phosphate. Dissolve 17.8 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 1,000 ml distilled water.

50 ml Stock Solution A + 50 ml Stock Solution B = 100 ml Sørensen's Buffer (pH 6.81)

Refrigerate until required.

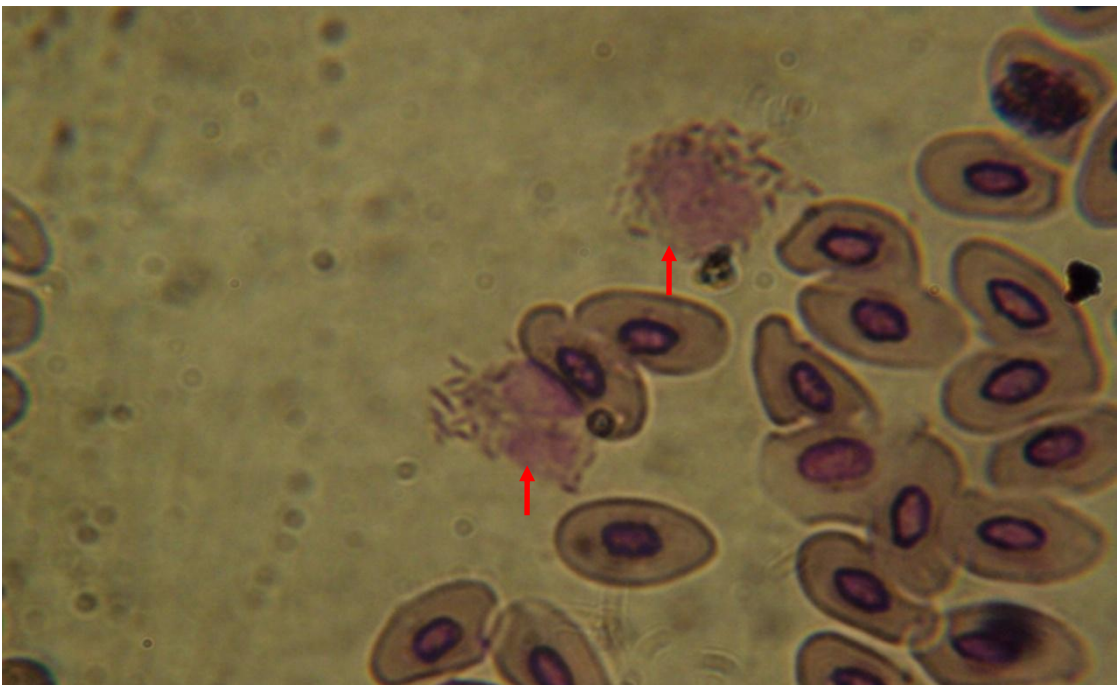
Procedure

- Prepare thin blood smears.
- Place blood smear on staining rack.
- Flood smear with Wright-Giemsa stain, allow to stand for 3 minutes.
- Add equal amount of Sørensen's pH 6.5-6.8 buffer, depending on batch stain.
- Mix gently by blowing using a pipette until metallic green sheen forms on the surface, allow to stand for 6 minutes.
- Rinse with buffer, allowing to stand for 1 minute for differentiation.
- Wash copiously with buffer.
- Wipe the back of smear with tissue to remove excess stain and drop in rack until dry.

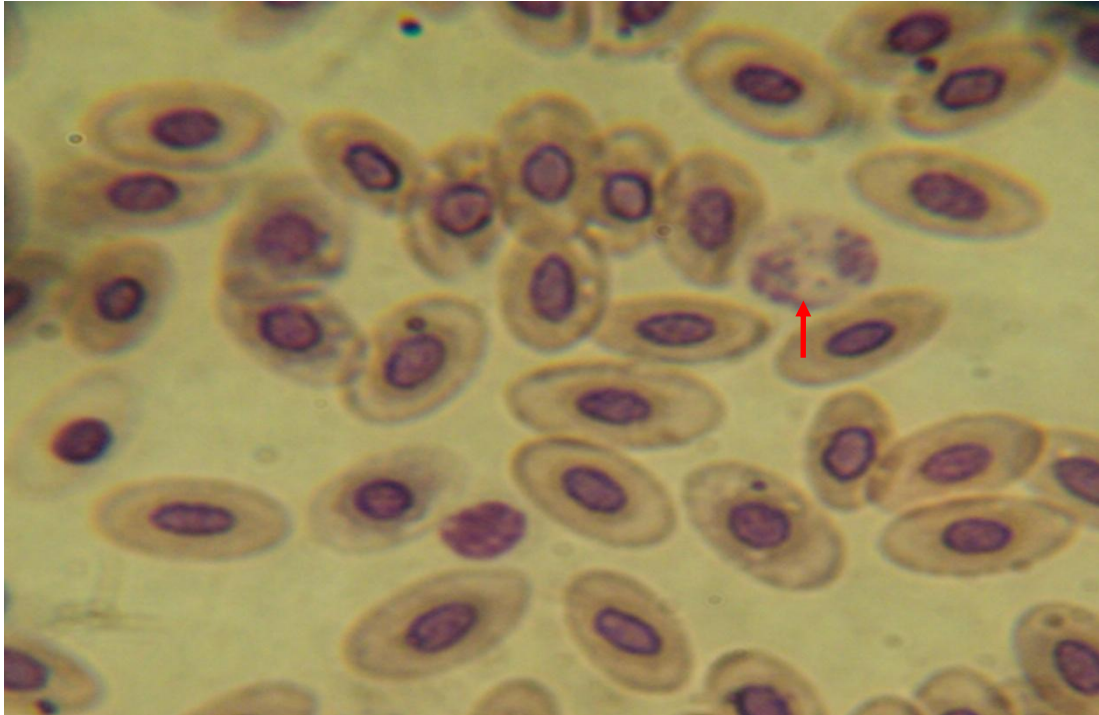
Appendix VI
Photograph of Blood Cells Stained with Modified Wright-Giemsa



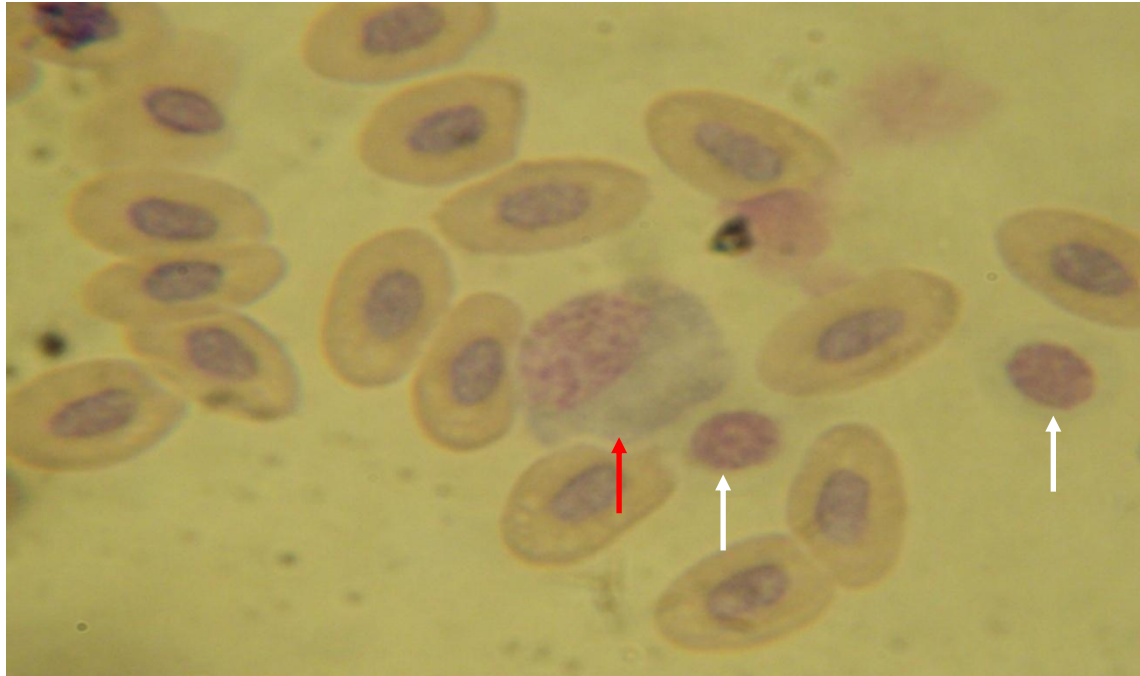
Eosinophil of the Cattle Egret (arrow). Note the medium size, round shape cell with bilobed nucleus and round to oval brick red granules. Modified Wright-Giemsa stain, x1,000



Heterophils of Mourning Collar Dove. Note bi-lobed basophilic nucleus with densely clumped chromatin and prominent eosinophilic (orange red to red brown or pale blue) cigar to rod shaped granules. Modified Wright-Giemsa stain, x1,000



Basophil (red arrow) from Speckled Pigeon. Note characteristic violet to reddish purple granules (much smaller than those of eosinophils) are partly dissolved and coalesced when stained with alcohol-based stains. Modified Wright-Giemsa stain. x1,000



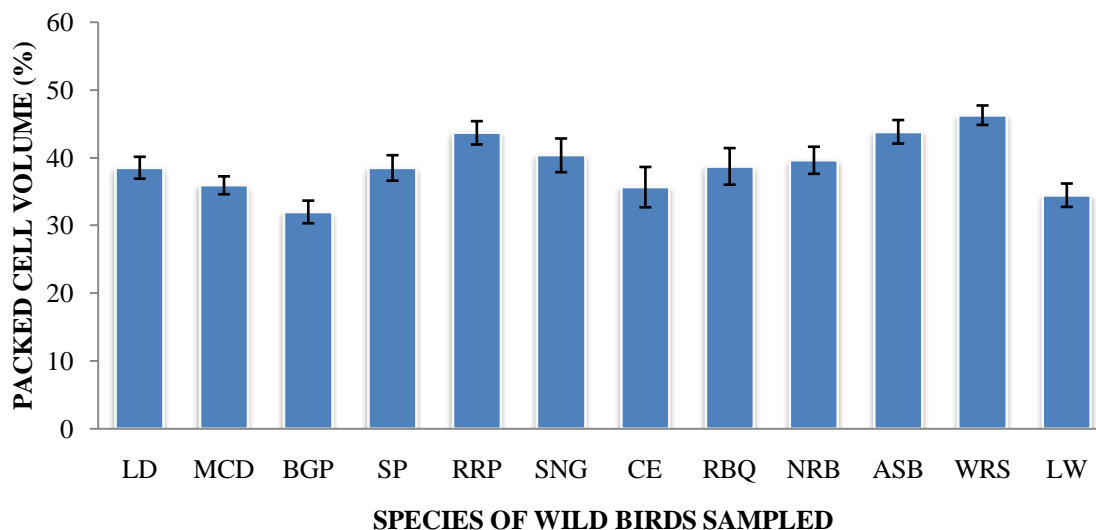
Monocyte (red arrow) and thrombocytes (white arrows) from the Laughing Dove. Note: **Monocyte**, the large amorphous-shaped cell with oval-shaped nucleus, lace-like chromatin and deep blue to grayish blue cytoplasm. **Thrombocyte**, small oval-cells with a round nucleus that contains densely clumped purple-red chromatin appearing more rounded than erythrocytes with colourless cytoplasm. Modified Wright-Giemsa x1,000



Lymphocyte of African Silver Billed (red arrow). Note round cells with centrally positioned nucleus that shows densely aggregated chromatin and a high nuclear-to-cytoplasm (N/C) ratio with basophilic and homogenous cytoplasm. Modified Wright-Giemsa stain, x1,000

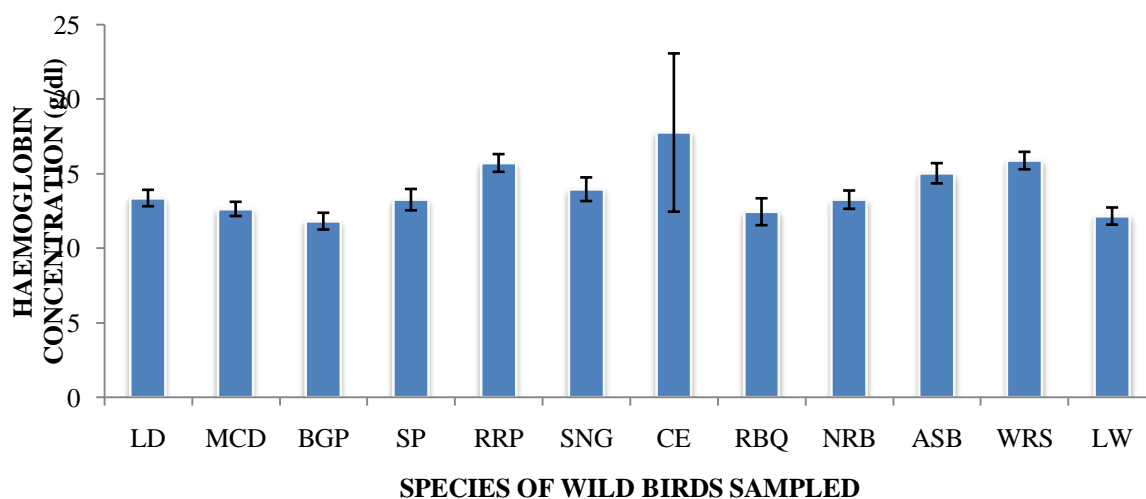
Appendix VII

Charts for Haematological Parameters



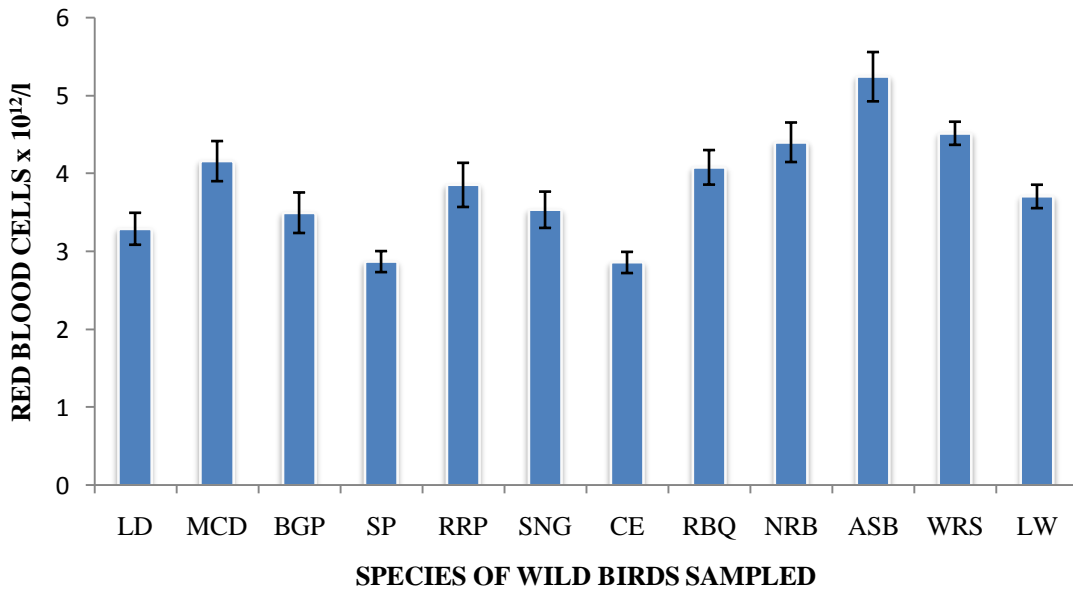
Packed cell volume for apparently healthy free-living wild birds (Mean±SE)

Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce's Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE), Red Billed Quelea (RBQ), Northern Red Bishop (NRB), African Silver Billed (ASB), White-Rumped Swift (WRS), Little Weaver (LW)



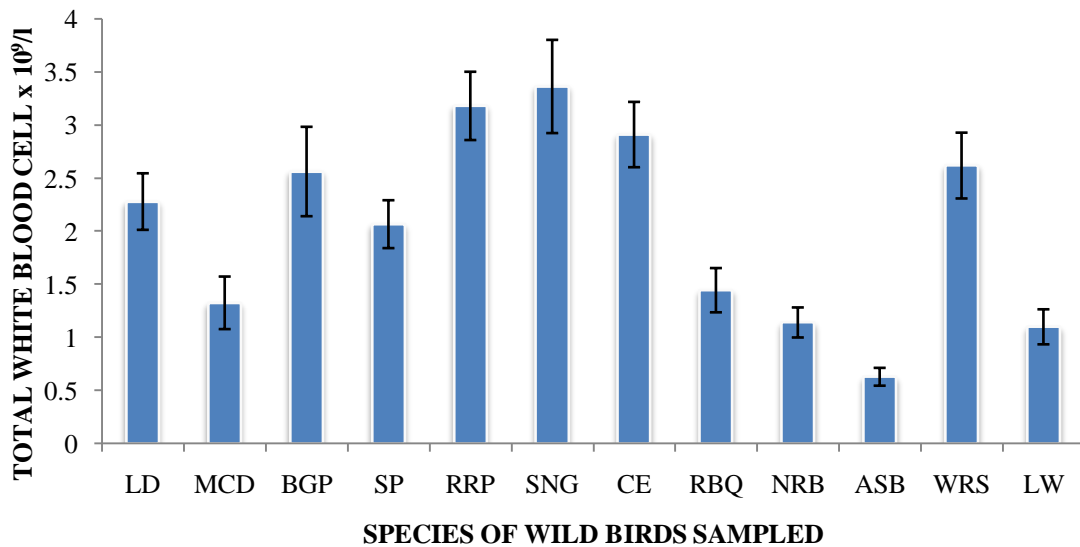
Haemoglobin concentration for apparently healthy free-living wild birds (Mean±SE)

Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce's Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE), Red Billed Quelea (RBQ), Northern Red Bishop (NRB), African Silver Billed (ASB), White-Rumped Swift (WRS), Little Weaver (LW)



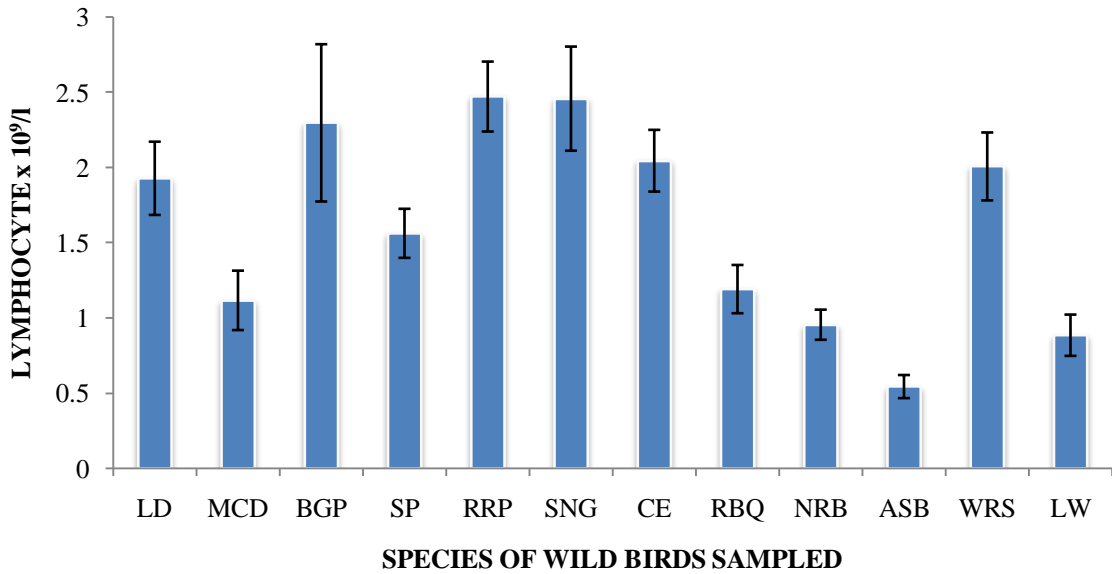
Red blood cell count for apparently healthy free-living wild bird species (Mean±SE)

Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce’s Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE), Red Billed Quelea (RBQ), Northern Red Bishop (NRB), African Silver Billed (ASB), White-Rumped Swift (WRS), Little Weaver (LW)



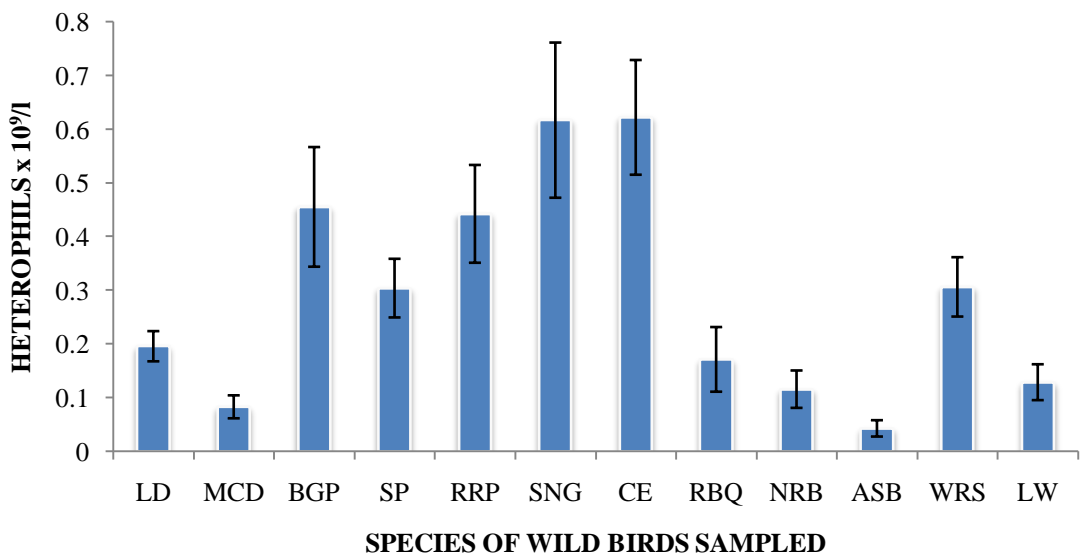
Total white blood cell count for apparently healthy free-living wild birds (Mean±SE)

Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce’s Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE), Red Billed Quelea (RBQ), Northern Red Bishop (NRB), African Silver Billed (ASB), White-Rumped Swift (WRS), Little Weaver (LW)



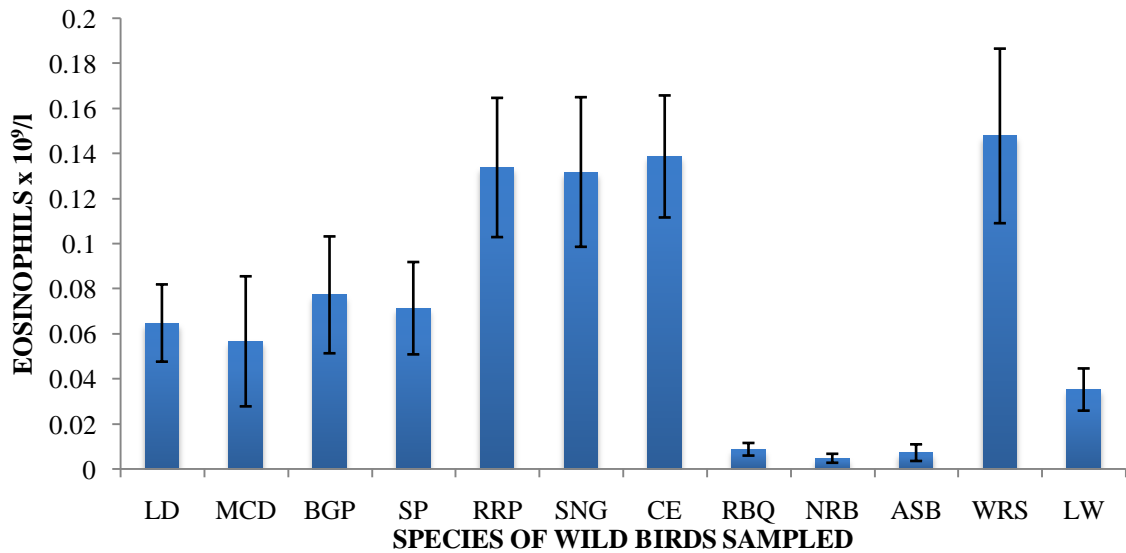
Absolute lymphocyte count for apparently healthy free-living wild birds (Mean±SE)

Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce’s Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE), Red Billed Quelea (RBQ), Northern Red Bishop (NRB), African Silver Billed (ASB), White-Rumped Swift (WRS), Little Weaver (LW)

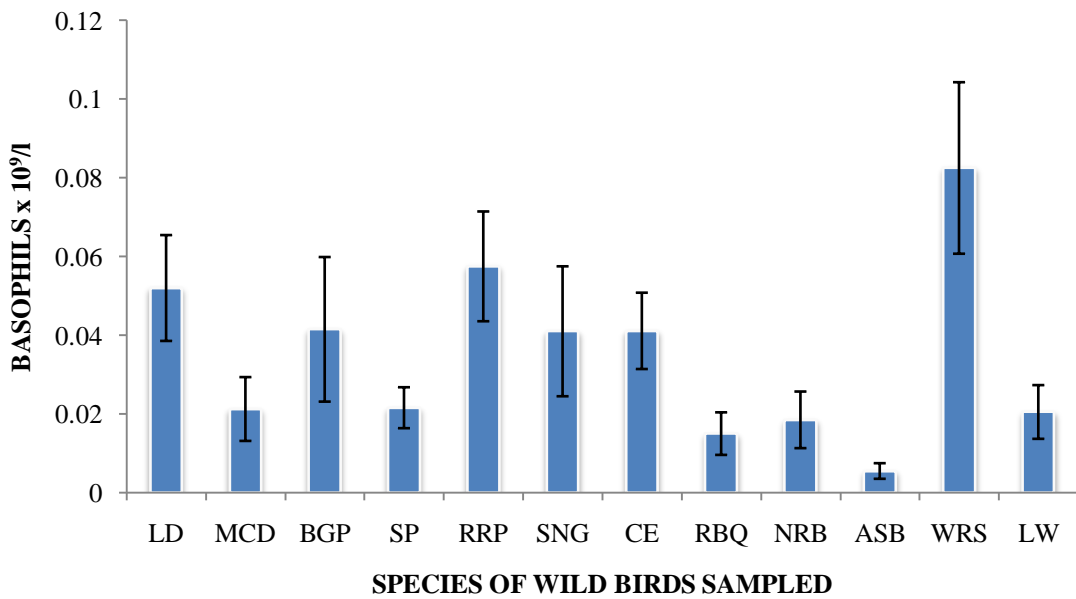


Absolute heterophil count for apparently healthy free-living wild birds (Mean±SE)

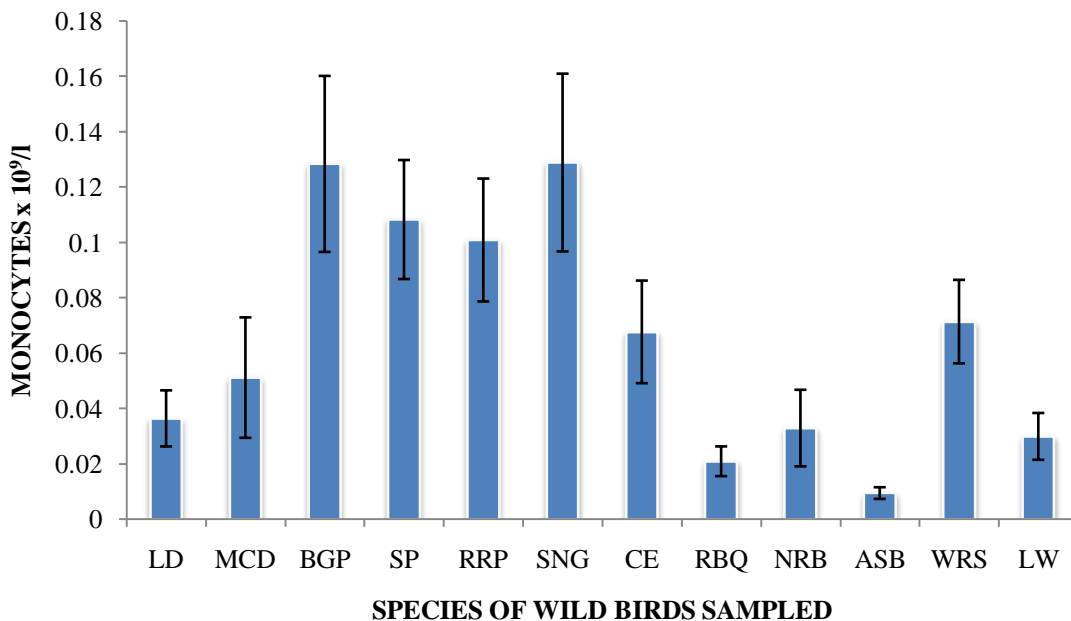
Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce’s Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE), Red Billed Quelea (RBQ), Northern Red Bishop (NRB), African Silver Billed (ASB), White-Rumped Swift (WRS), Little Weaver (LW)



Absolute eosinophil count for apparently healthy free-living wild birds (Mean±SE)
Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce’s Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE), Red Billed Quelea (RBQ), Northern Red Bishop (NRB), African Silver Billed (ASB), White-Rumped Swift (WRS), Little Weaver (LW)

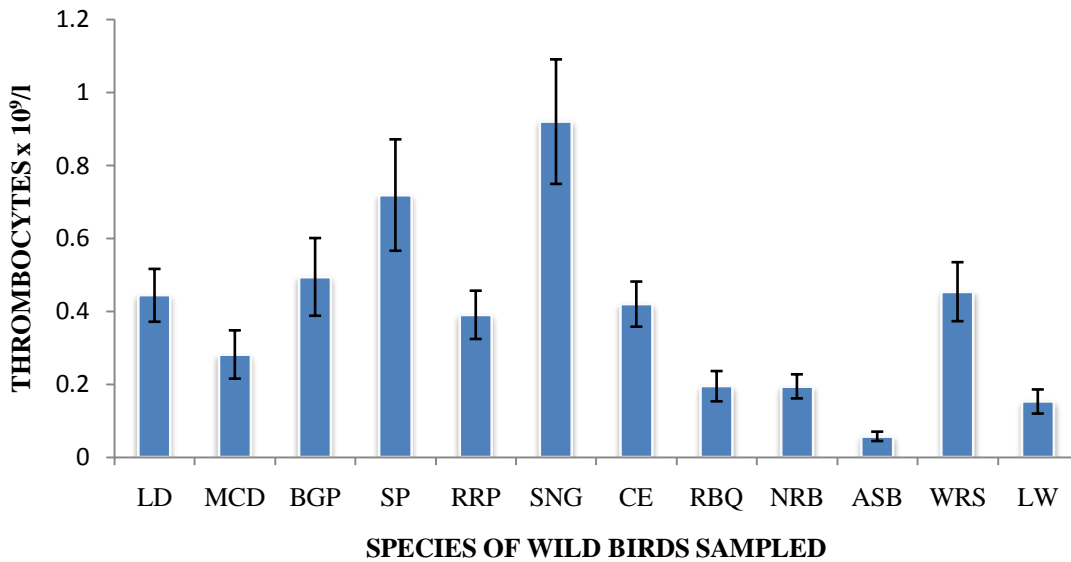


Absolute basophil count for apparently healthy free-living wild birds (Mean±SE)
Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce’s Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE), Red Billed Quelea (RBQ), Northern Red Bishop (NRB), African Silver Billed (ASB), White-Rumped Swift (WRS), Little Weaver (LW)



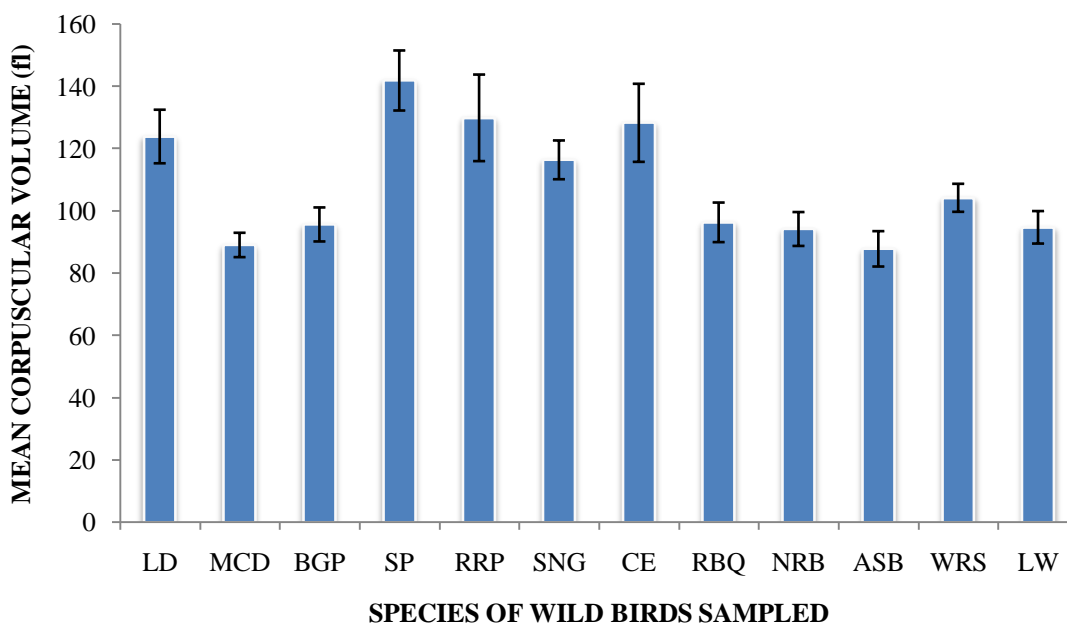
Absolute lymphocyte count for apparently healthy free-living wild birds (Mean±SE)

Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce’s Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE), Red Billed Quelea (RBQ), Northern Red Bishop (NRB), African Silver Billed (ASB), White-Rumped Swift (WRS), Little Weaver (LW)



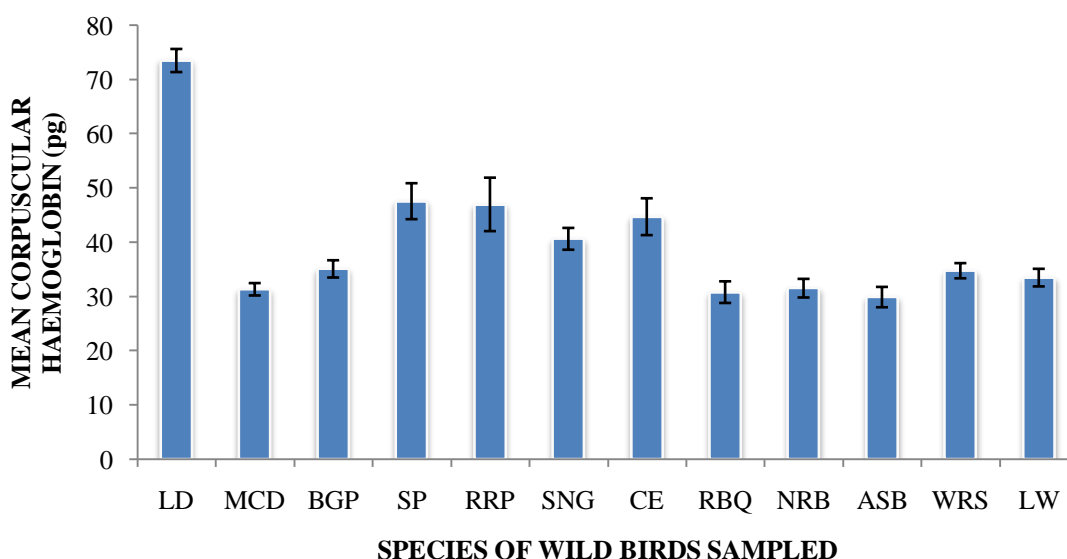
Estimated absolute thrombocyte count for apparently healthy free-living wild birds (Mean±SE)

Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce’s Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE), Red Billed Quelea (RBQ), Northern Red Bishop (NRB), African Silver Billed (ASB), White-Rumped Swift (WRS), Little Weaver (LW)



Mean Corpuscular volume for apparently healthy free-living wild birds (Mean±SE)

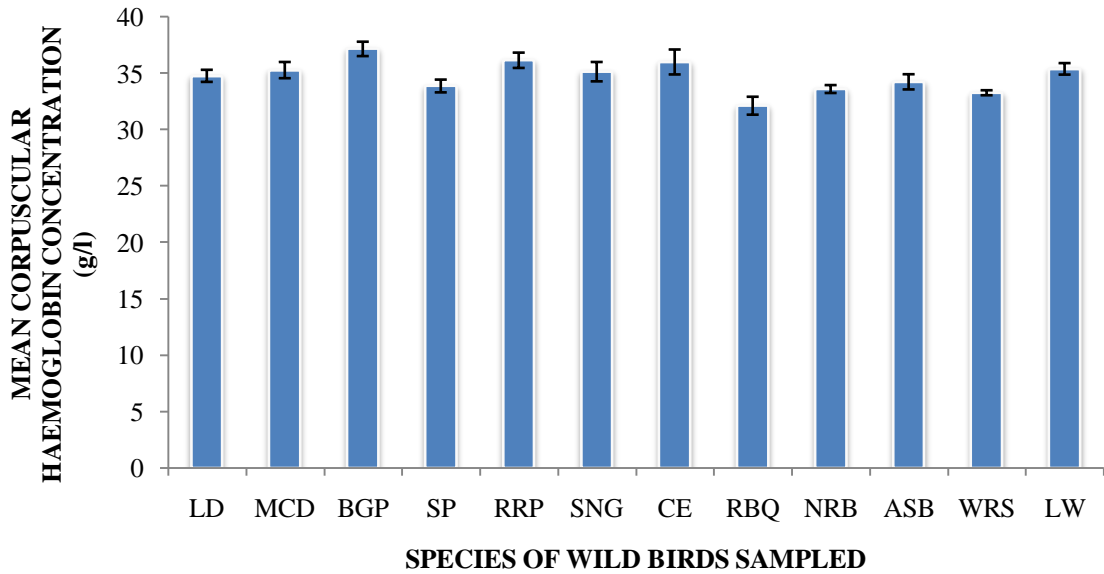
Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce’s Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE), Red Billed Quelea (RBQ), Northern Red Bishop (NRB), African Silver Billed (ASB), White-Rumped Swift (WRS), Little Weaver (LW)



Mean Corpuscular haemoglobin for apparently healthy free-living wild birds (Mean±SE)

Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce’s Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG),

Cattle Egret (CE), Red Billed Quelea (RBQ), Northern Red Bishop (NRB), African Silver Billed (ASB), White-Rumped Swift (WRS), Little Weaver (LW)

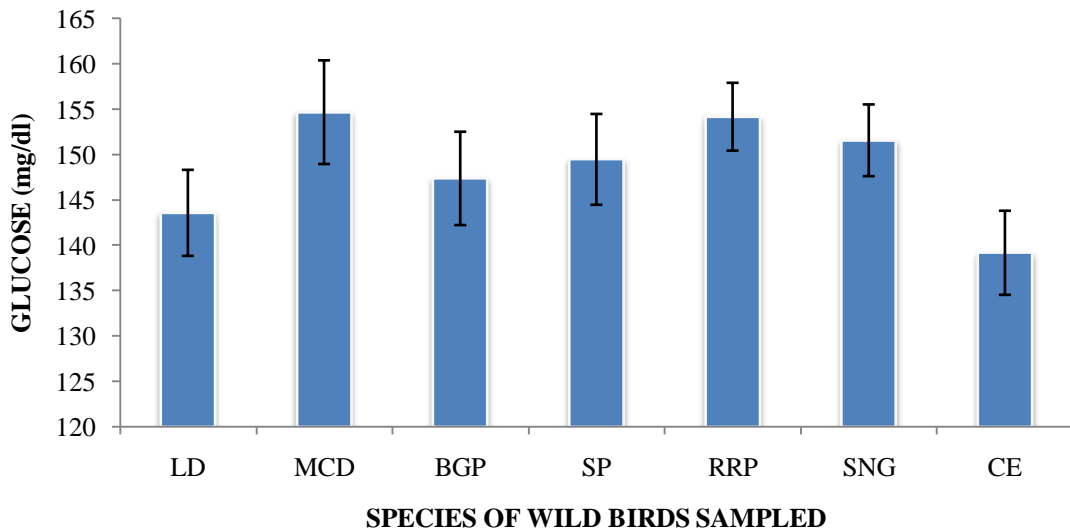


Mean Corpuscular haemoglobin concentration for apparently healthy free-living wild birds (Mean±SE)

Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce’s Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE), Red Billed Quelea (RBQ), Northern Red Bishop (NRB), African Silver Billed (ASB), White-Rumped Swift (WRS), Little Weaver (LW)

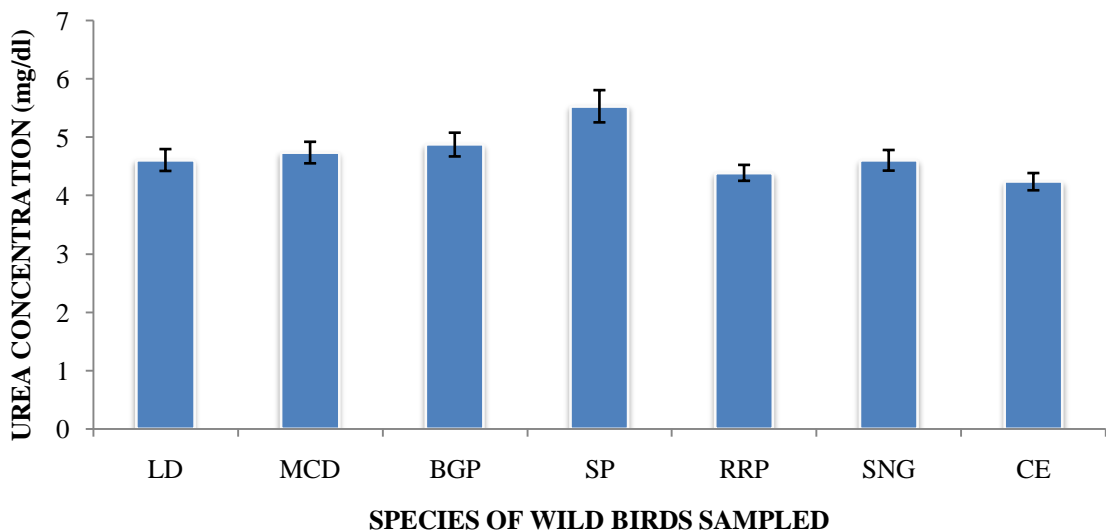
Appendix VIII

Charts for Serum Biochemical Parameters



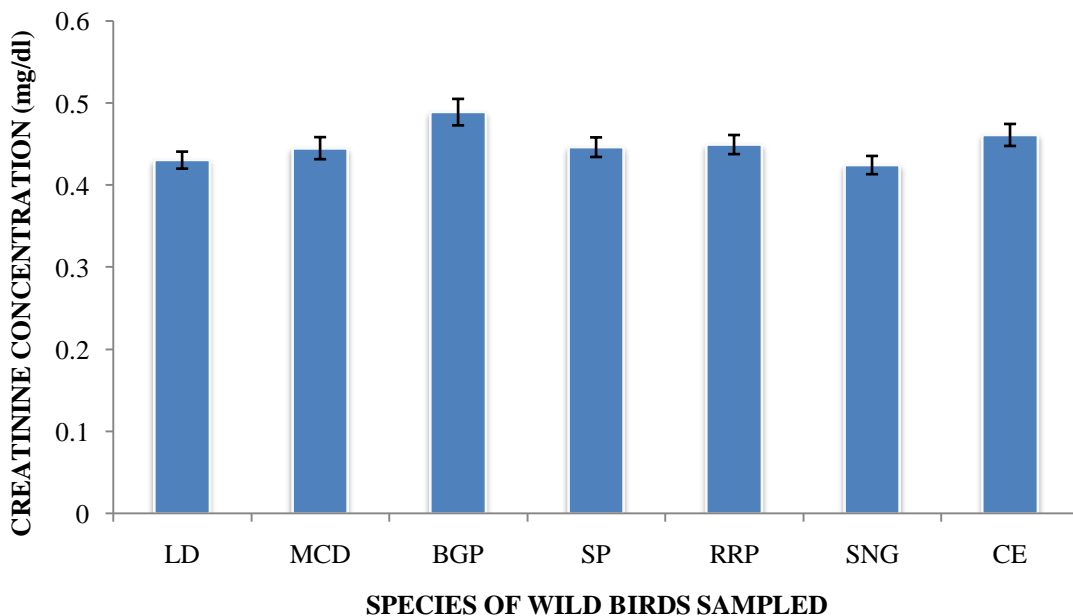
Glucose concentration for apparently healthy free-living wild birds (Mean±SE)

Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce's Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE)

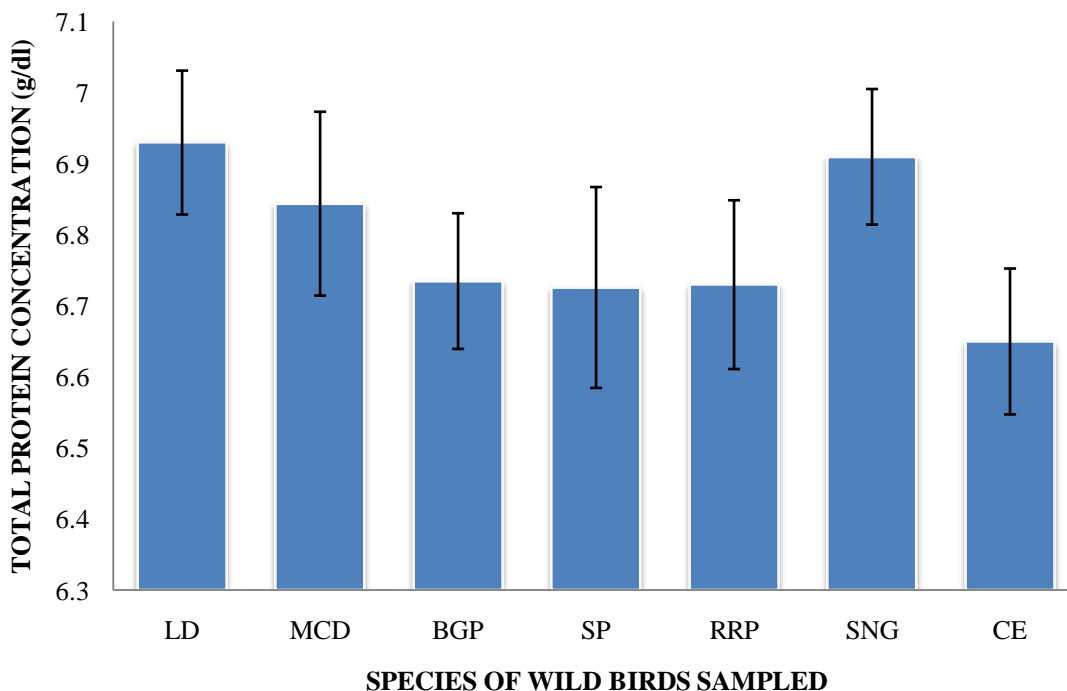


Urea concentration for apparently healthy free-living wild birds (Mean±SE)

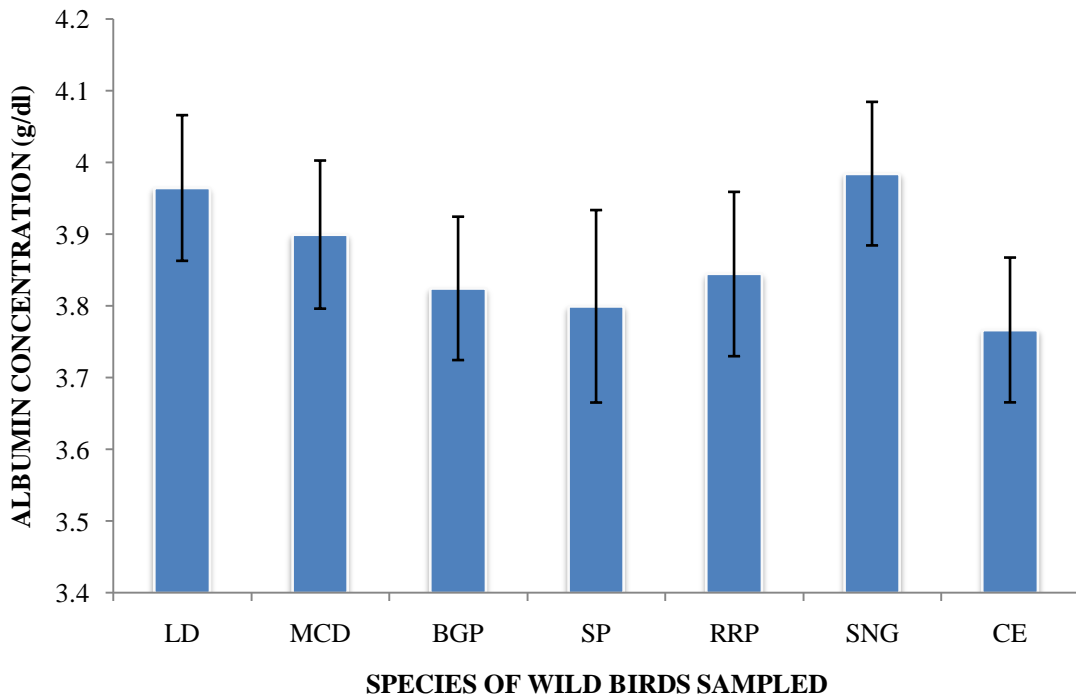
Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce's Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE)



Creatinine concentration for apparently healthy free-living wild birds (Mean±SE)
Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce’s Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE)

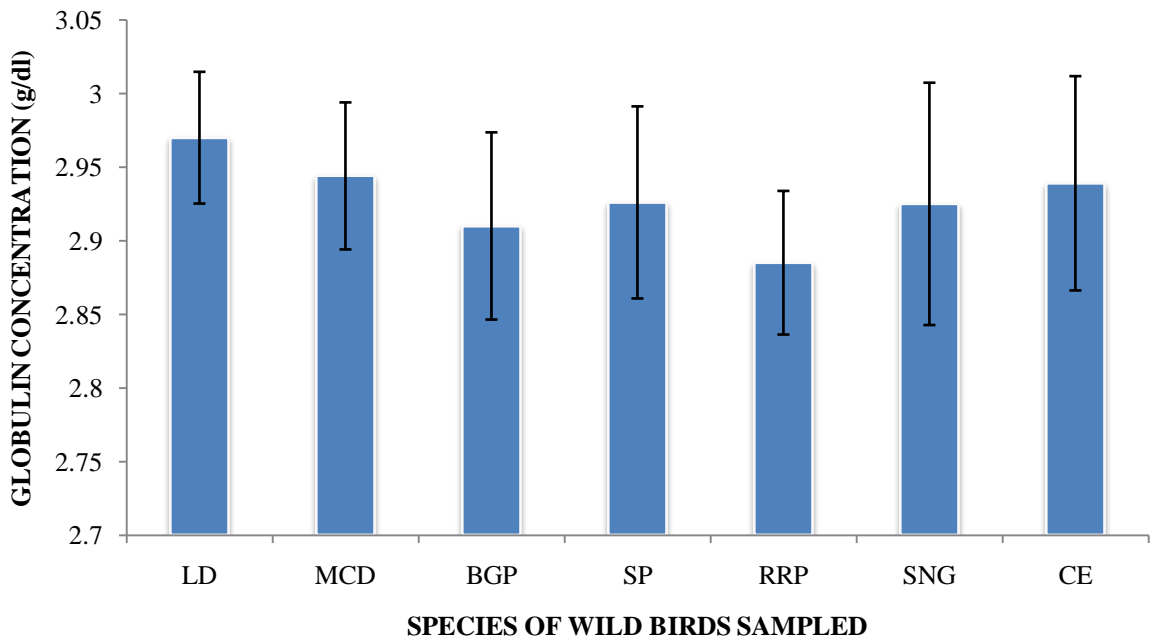


Total protein concentration for apparently healthy free-living wild birds (Mean±SE)
Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce’s Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE)



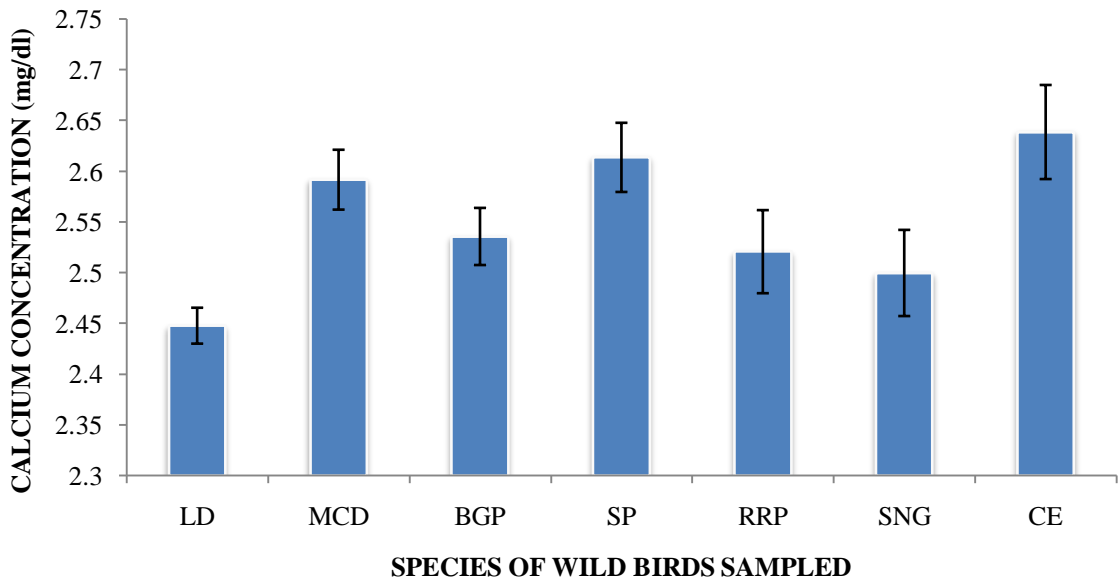
Albumin concentration for apparently healthy free-living wild birds (Mean±SE)

Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce's Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE)



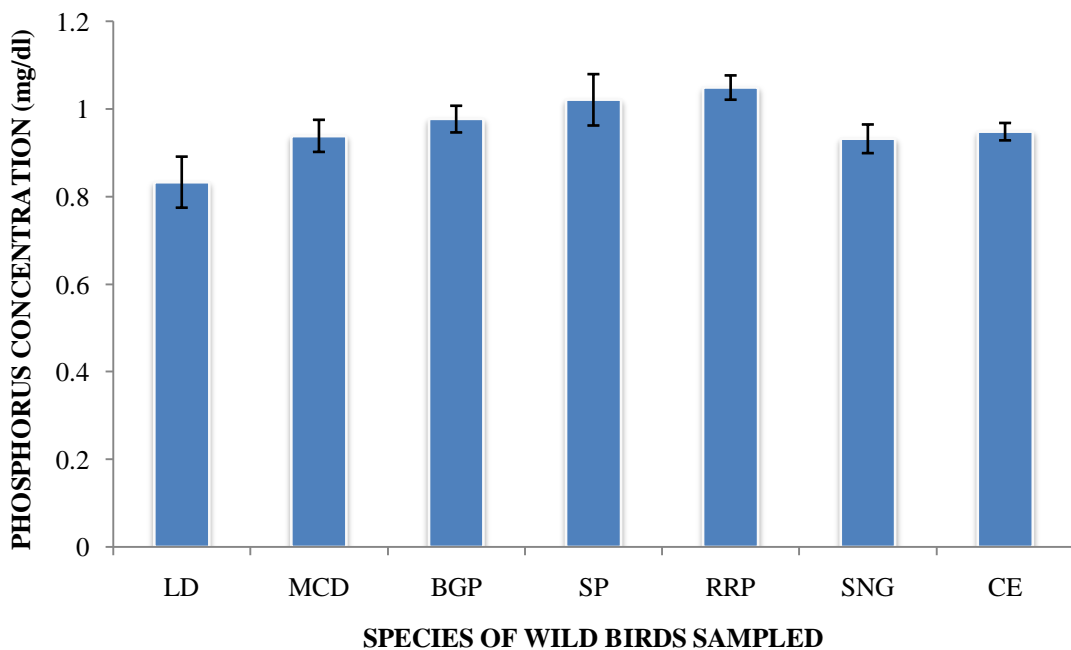
Globulin concentration for apparently healthy free-living wild birds (Mean±SE)

Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce's Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE)



Calcium concentration for apparently healthy free-living wild birds (Mean±SE)

Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce's Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE)



Phosphorus concentration for apparently healthy free-living wild birds (Mean±SE)

Note: Laughing Dove (LD), Mourning Collar Dove (MCD), Bruce's Green Pigeon (BGP), Speckled Pigeon (SP), Rose-Ringed Parakeet (RRP), Senegal Parrot (SNG), Cattle Egret (CE)