Determination of some blood parameters in the African lion (*Panthera leo*)

by

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Declaration

I hereby declare that the dissertation submitted by me to the University of the Free State for the degree, **Magister Scientiae Agriculturae,** is my own independent work and has not previously been submitted for a degree to any other university. I furthermore cede copyright of the dissertation in favour of the University of the Free State.

Heidi Louise Erasmus
Bloemfontein
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For my husband Charles Erasmus and parents Des and Elza Bothma

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1. Introduction

The South African wildlife ranching industry has grown rapidly over the past few decades (National Agricultural Marketing Council, 2006). According to this report the total land area of South Africa is 122.2 million ha, of which 7.5 million ha are protected by different levels of government. Furthermore, the report estimated that almost three times that area of land, namely 20.2 million ha are protected through private initiative, mostly as wildlife ranches.

According to De Waal (2007) most wildlife ranchers prefer to restrict their ranching activities to herbivorous mammal species. These animals range from the small and medium-sized species (e.g. duiker, springbuck, impala and blesbuck) to the large (e.g. eland and giraffe) and mega herbivores (e.g. elephant) and only a small number of operators also keep carnivorous wildlife such as the lion (*P. leo*), leopard (*P. pardus*), cheetah (*Acinonyx jubatus*) and African wild dog (*Lycaon pictus*).

The African lion (*P. leo*) is the largest terrestrial African predator species. Being regarded as a charismatic icon species and one of the "Big Five", it is a major tourist attraction. Unlike the situation in most other African countries, most of the African lions are kept behind special wildlife and lion proof fences (De Waal, 2007). Several different views are held about lions in captivity on ranches, but lions form an important part of the wildlife industry and are successfully bred and reared in captivity on many South African wildlife ranches.

Although not unique to this species only, lions are dangerous animals and therefore special facilities are required to safely keep captive lions (De Waal, 2007). Despite their high intrinsic value as an eco-tourist attraction, the management practices employed on wildlife ranches often seem to be based on a trial and error approach. Therefore, there is a need to develop a more scientific approach to lion ranching.

According to Bauer and Van der Merwe (2004) the African lion is currently listed as vulnerable on the IUCN Red List. In this regard a lesson can be taken from the literature. The black wildebeest (*Connochaetes gnou*) is endemic to the open plains

of central South Africa and, through concerted conservation activity by conservation-minded farmers and landowners in the Free State and North West Provinces, the black wildebeest has been prevented from going extinct (Friedmann, 2003). Similarly, ranching with captive lions can play an important role in preserving the species.

As with livestock farming or wildlife ranching practices, sound practical knowledge on the physiology, nutrition and diseases of the relevant species is required to manage the animals appropriately and to farm and breed them successfully. One of the major constraints to lion ranching is the monitoring of their health status as part of disease control. This is particularly important as the wildlife ranching system intensifies and larger numbers of animals are kept in smaller areas. According to Labuschagne (1955), the mortality rate among lion cubs and juveniles can be as high as 50% on some ranches and in natural parks. However, no data are currently available to indicate that the situation has changed since for the African lion.

Blood constituents (cells, plasma and chemical composition) can be used to monitor the health status and diagnose diseases, nutritional deficiencies and the reproductive status (*i.e*. pregnancy) of animals. Techniques to perform complete blood analysis are available for humans and most livestock species. The same procedures can be used for wildlife species to identify deviations from normal values in certain blood parameters (cytological or biochemical). However, before such deviations can be detected in an animal, the reference values regarded as normal for the species must be well established. Once the reference values have been established, any deviations, their causes, and possible interventions can be investigated. This information is therefore very valuable for both ranchers of captive lions and veterinarians involved in wildlife ranching.

To the author's knowledge, very little research on reference values for blood constituents has been done on lions. Values for some blood variables have been published by ISIS (International Species Information System, 1999), Wallach and Boever (1983) and Pospísil *et al.* (1987). The ISIS values were obtained from 36 member institutions all over the world with no indication of the different countries or whether there were any differences between regions. The ISIS list contains 16

haematological and 35 biochemistry parameters. The number of lions used to determine these reference values varies from 2 to 91, regardless of sex (both sexes combined) and age (varies from 8 days to 3 years). No information is provided on the health status of the animals. In the case of the reference values published by Wallach and Boever (1983), no information is provided on the number, age, sex, or health status of the animals. According to Wallach and Boever (1983), the biological values of the wild Felidae are similar to the basic values of the domestic cat (*Felis sylvestris*), suggesting that the reference values for the domestic cat could be used for lions. However, no evidence to support this statement could be found. Some haematological and biochemical reference values in the same species (*i.e*. in humans) vary between sexes and/or between different age groups (Bain, 1995). It is therefore important to determine if this is the case for lions as well.

Reference values being regarded as normal for cytological and biochemical blood variables in lions could be associated with certain physiological status such as age, sex, and pregnancy. It is therefore important to establish reference values and then determine which of the physiological factors affect the cytological and biochemical profiles of lion blood. Usually when a differential white cell count is done as part of a haematology analysis, it is done by manually counting white blood cells on a blood smear under the microscope (Undritz, 1973; Rosenberger, 1979). This method has two important disadvantages, namely time and accuracy (Rosenberger, 1979). Applying the conventional visual manual method to assess blood smears by counting cells is time consuming. Thin smears have to be made, fixed, stained, and dried before the counting can be done which by itself is time consuming. To do an accurate differential count manually, at least three blood smears of good quality have to be counted and the means calculated for each sample. If the smears are too thick or too thin, or if the staining period is too short or too long, it is very difficult to obtain an accurate result. Sitting behind a microscope for hours on end is a tiresome and strenuous process and the human error is always present.

Against this background and with these challenges in mind when conducting the manual differential count on blood, it was realised that it would be of great help if an automated analyzer (cell counter) could be used in the field. Therefore, the

opportunity was welcomed to compare these two procedures, to determine if an automated Haematology Analyzer could be used for this purpose.

In addition to haematological and biochemical blood parameters, De Waal and Combrinck (2004) proposed that accurate information on the growth and development of an animal, namely its body size can be used as an indicator of its wellbeing. Body size of an animal is a function of genetics and environment, mostly nutrition and health, and may serve as an indicator of normal growth and development (De Waal *et al*., 2004a). Collection of data on body measurements is a basic activity when studying a species. The morphometric data provide a characterization of the species and describes, among others, also the differences between males and females (Roth & Mercer, 2000; De Waal *et al*., 2004b; De Waal & Combrinck, 2004). Many wildlife species exhibit sexual dimorphism, which are the visual differences between the sexes, for example the larger body sizes and manes in male lions (Smuts *et al*., 1980).

Various body measurements are associated with age and sex and can therefore be used to estimate both the age and the growth rate of the animals. Factors such as the nutrition, climate, and health of the animals also affect the growth rate of animals. Therefore, as is the case with other species (*e.g*. livestock species), morphometric data are valuable when assessing the overall management as well as the health status of individuals and groups of animals. Furthermore, if a high correlation exists between body weight and a particular body measurement, this body measurement could be used in field work to accurately estimate the weight of these animals. This may be of great practical value to the management on lion ranches. If for instance a lion on a ranch needs to be immobilised for physical handling and treatment, its body mass and therefore the correct dose of a drug required for treatment or chemical immobilisation can be estimated quickly from known body measurements instead of having to weigh the lion. By collecting a set of practical and accurate body measurements from chemically immobilized or hunted lions (De Waal *et al.,* 2004a), the information base could be improved to assist in monitoring and managing both wild and captive lion populations (De Waal & Combrinck, 2004).

According to Roth and Mercer (2000), morphometric measurements are not a concept, or a particular set of phenomena, but rather a tool; a means of extracting information about biological material and biological processes. These authors further define morphometric measurements as the quantitative characterization, analysis, and comparison of biological form.

Measuring the growth and development in live animals by indirect and non-invasive methods is very important (De Waal *et al*., 2004a; De Waal & Combrinck, 2004). The attributes can be monitored also in ongoing experiments or in commercial agriculture (Swatland, 1984). Brody (1964) published growth curves for horses and four dairy breeds (namely Holstein, Guernsey, Ayrshire and Jersey), while Lawrence (1980) published growth curves linking age to live weight for Holstein calves and Suffolk lambs. Farmers could use these reference values to assess their management programs, particularly nutrition or feeding practices. In wild species in general and in large predators such as the lion in particular, body weight is often considered difficult or impractical to determine in the field. Therefore, researchers (Brody, 1964; Keep, 1973; Lawrence, 1980) have considered variables that are well correlated to body weight and are easy to measure. Research done in this regard on lions is scarce. Research done on livestock (Brody, 1964; Lawrence, 1980) and other wildlife species (Keep, 1973) can serve as guidelines for similar work on lions.

Smuts *et al.* (1980) reported that the majority of growth in lions takes place during the first three years of life. These authors plotted scatter diagrams of age against body weight, chest girth, shoulder height, and vertebral column length. The best correlations between body weight and age were recorded for animals younger than three years of age, in both sexes.

Keep (1973) studied the Nyala (*Tragelaphus angasii*) population of the Ndumu Game Reserve in Kwa-Zulu Natal and, among others, measured head length, body length, shoulder height, heart girth, hind foot length and body weight, while the age of the animals was estimated. An objective was to identify the body measurement that can be measured the easiest and with the smallest operator or interpretation errors to correlate with body weight (Keep, 1973). Among the measurements considered were the hind foot length (the distance from the point of the hock to the

tip of the hoof). This measurement was the most accurate, as it was the least affected by operator error. The latter was attributed to the fact that the points between which the measurements were taken could be located easily and accurately and were in a straight line without curves. Keep (1973) suggested that a possible reason for the other measurements showing poor correlations with body weight could be due to operator errors resulting from various recorders interpreting the exact points to measure differently.

However, in studies by De Waal *et al*. (2004a) and De Waal and Combrinck (2004) the repeatability was high, suggesting that operator error was negligible when measuring the bodies of several African predator species, namely African lion, leopard (*Panthera pardus*), cheetah (*Acinonyx jubatus*), caracal (*Caracal caracal*), black-backed jackal (*Canis mesomelas*) and Cape fox (*Vulpes chama*).

Against this background this study was initiated with the following four objectives:

1.1 to determine reference values for haematological and biochemical blood parameters for lions bred in captivity, as a function of age and sex;

1.2 to evaluate the possibility to use the Beckman Coulter Ac•T 5diff Haematology Analyzer for lion differential white blood cell analyses;

1.3 to determine morphometric measurements and establish growth curves for lions bred in captivity as a function of age and sex;

1.4 to determine reference values for some practical and meaningful body measurements and their correlations.

2. Materials and Methods

2.1 Study material and general management of the lions

In this study data of lions were obtained from four different sites in the Free State Province of South Africa. Three groups of the captive African lions were located at three different private lion ranches in the following districts: Bothaville, Brandfort, and Reddersburg. A fourth group of captive lions belonged to the Bloemfontein Zoological Gardens (Bloemfontein Zoo), in the Bloemfontein district.

The general facilities and management procedures to which the lions were subjected at each of the four sites are briefly described in the following sections.

2.1.1 Bothaville district

The lions were kept in several large camps (about 5 to 10 ha each) in the veld, fenced in by high predator proof fences. Unlike the social structure of lions living *in situ* in prides, the lions in this *ex situ* environment were grouped by age and/or sex (HO de Waal, 2008; personal communication). The lions in different camps were mostly within sight of at least one other group of lions.

The lions were moved regularly between the camps to allow the mowing of the natural grass component with a tractor drawn mower in the vacant camps. The programme to control ecto-parasites (ticks) depended on the season of the year. During the spring and summer rainy seasons, the animals required more frequent treatments.

The lions were fed once a week a diet consisting mainly of the carcasses of larger ruminants (about 30 kg per adult lion per week). The carcasses were obtained from cattle being slaughtered on the farm or carcasses bought elsewhere. Based on the number of lions grouped in a camp, the number of carcasses offered to each lion was calculated. The total amount of food for the group of lions was deposited as large chunks of the carcasses in the camps.

2.1.2 Brandfort district

The lions were kept in relatively small camps (about 1-2 ha each) in the veld, fenced in by high predator proof fences. Again, unlike the social structure of lions living *in situ* in prides, the lions in this *ex situ* environment were grouped according to age and/or sex groups within sight of at least another lion or group of lions (HO de Waal, 2008; personal communication).

The lions were fed twice a week a diet consisting mainly of whole chicken carcasses with the feathers still attached (about 12 kg per feeding per adult lion). The farmer was also a commercial producer of fresh eggs. Layers that were too old for egg production or that were poor egg producers were culled, and fed to the lions.

2.1.3 Reddersburg district

The lions were kept in relatively small camps (about 1-2 ha each) in the veld, fenced in by high predator proof fences. Again, the lions in this *ex situ* environment were grouped according to age and/or sex within sight of at least another lion or group of lions (HO de Waal, 2008; personal communication).

The lions were fed twice a week a diet consisting mainly of the carcasses of large ruminants (about 12 kg per feeding per adult lion). The carcasses were cattle or game hunted on the same wildlife ranch or carcasses bought elsewhere.

2.1.4 Bloemfontein Zoological Gardens (Bloemfontein Zoo)

The lions were kept in small open leisure areas with separate enclosed sleeping quarters. The facilities in which the lions are housed have been described by Borstlap (2002). The facilities consist of two brick and concrete enclosed night chambers (2.35 m x 2.6 m and 5.65 m x 2.6 m), separated by steel grate trapdoors from an open air leisure yard. The leisure yards measure about 729 m^2 and planted with Kikuyu grass (*Pennisetum clandestinum*) as ground cover and further naturalized or landscaped with large rocks and tree trunks. The steel trapdoors are remotely controlled by a system of pulleys and cables to protect the operators from the lions.

The lions in the Bloemfontein Zoo are accustomed to a strict feeding routine, namely being fed on Sunday and Wednesday afternoons at about 14h30 (Borstlap, 2002). The fresh food allowances per lion were about 14 to 16 kg of animal carcass. The diets consisted mainly of donkey and horse carcasses, whole chickens, chicken tripe and meat from unborn calves or culled game and livestock.

2.2 Experimental animals

The data of the lions used in this study were collected during an extensive field study conducted under the auspices of ALPRU (African Large Predator Research Unit) (De Waal *et al*., 2004a,b; De Waal & Combrinck, 2004). The morphometric data of the lions were collected according to the comprehensive procedures described by De Waal *et al.* (2004) to measure the body dimensions of large African predators.

The data of 72 lions of both sexes, with ages ranging from 3 months to 9 years, were collected from all the lions present on the lion ranches and at the Bloemfontein Zoo at the time when the study was conducted. The distribution of lions in the different classes (age groups and sex), at the four sites is shown in Table 2.1.

The age of males varied between 3 months and 5 years, while the females varied between 3 months and 9 years. Record keeping is an important feature of the lion ranches and the Bloemfontein Zoo and accurate records on the birth dates of the lions were provided by the operators. The four age groups (categories) for analysis of the data were determined taking in consideration the guidelines proposed by Smuts *et al.* (1980). The age groups considered in this study were the following:

Age group 1: 0 – 1 years (Young cubs)
Age group 2: 1 – 2 years (Cubs)
Age group 3: 2 – 4 years (Juveniles and sub-adults)
Age group 4: >4 years (Adults)

2.3 Chemical Immobilization of the lions

The older and larger lions were darted for chemical immobilization (De Waal *et al*., 2004a,b). An intra-muscular dose of 4 to 5 mg/kg Zoletil® 100 (50 mg Tiletamine HCl and 50 mg Zolazepam HCl/ml; VIRBAC) was administered for the chemical

immobilization of the lions (De Waal *et al*., 2004b). Before administering the chemical immobilisation the body weight of each lion was visually estimated, with the caretaker/farmer's assistance, to calculate the correct dosage required for immobilization. An initial doses of 4 mg/kg was used to immobilize the animals. If the animals started to wake up before all the samples and measurements were collected, a top-up dose of a maximum of 1 mg/kg was used to allow the operators to safely complete the collection of the necessary samples and body measurements.

Table 2.1 The distribution of 72 lions of different age and sex classes at the four sites in the Free State Province

Location	Age groups								Total	
	Group 1 < 1 year (young cubs)		Group 2 1 – 2 years (cubs)		Group 3 2 – 4 years (juveniles, sub-adults)		Group 4 4 years (adults)			
	M	F	M	F	M	F	M	F	M	F
Bothaville	7	10	9	8	10	2	2	5	28	25
Bloemfontein Zoo	1	2	0	0	1	2	0	1	2	5
Brandfort	0	0	0	0	1	2	0	0	1	2
Reddersburg	0	0	3	2	0	0	1	3	4	5
Sub Total	8	12	12	10	12	6	3	9	35	37
Total	72									

The 7 male young cubs (Table 2.1; Group 1) at Bothaville were about three months old. These cubs were caught and easily restrained by hand because they were hand reared and still relatively tame. The Zoletil® 100 dose was injected intra-muscularly with the aid of a syringe and a hypodermic needle in the large muscles of the femural region of the hind leg. The remaining lion cubs in Group 1 (10 females from Bothaville as well as the one male and two females from the Bloemfontein Zoo) were too old to be handled and restrained by hand. These cubs were therefore darted with the aid of a dart gun and a dart containing the estimated required dose of Zoletil® 100. The 52 older animals (Table 2.1; Groups 2, 3 and 4) were immobilized

with the aid of a darting gun and darts containing the estimated required doses of Zoletil® 100. The darts were projected at the lateral region of the neck, or alternatively at the muscles of the femural region of a hind leg.

At all four sites, the lions aged 8 months and older were usually fed twice a week. The protocol of this study was therefore planned accordingly and in such a way that the lions were darted 5 to 7 days after their last meal for the following reasons:

- Safety of the animals: Chemical immobilization of animals with Zoletil® 100 is risky while digestion is still in progress, because the stomach may still contain appreciable amounts of food which may often cause vomiting that could be aspirated into the lungs (VIRBAC).
- Facilitating darting: Older animals are kept in relatively large camps and the effect of hand rearing had worn off. Therefore they had to be lured into smaller enclosures or closer to a fence to ensure accurate and safe darting. If the animals are hungry, they are more readily attracted closer to the fence with food where they can be easily darted.
- Accurate weighing: Weighing animals 5 days after the last meal provides a more accurate bodyweight because the ingesta is almost completely digested and most of the indigestible waste products were excreted in faeces by the time that the animals were weighed (Borstlap, 2002).
- Metabolites: Blood glucose and urea values can be ascertained with a higher degree of accuracy because both these parameters are higher in non-fasting monogastric animals (Latimer *et al.*, 2003).

After darting or administering the Zoletil® 100 for chemical immobilisation by intra muscular injection/darting, it took between 10 and 20 minutes for the lions to be fully immobilized. Variation was due to individuals reacting differently to the chemical compound. As soon as the lions were deemed fully immobilized (*i.e*. lying down and unable to lift its head and/or consciously bite), they were transported to a shaded place for protection against heat exhaustion. Their eyes were covered with a blindfold to keep them calm, prevent damage to the eye (Young, 1975), and limit unnecessary excitement. The lions living in the same camp were all immobilized rapidly in succession and then transported as a group, weighed, measured and

blood sampled as quickly as possible, before being returned to their camps. This procedure was necessary to ensure that the immobilizing effect of the compound was still in effect.

Once back in their camps, the lions were allowed to recover under close observation from the effect of the immobilization compound and wake up at more or less the same time. These procedures were followed not only to ensure the safety of the operators, but also to ensure that the lions still affected by the immobilisation were not attacked by those that may not have been immobilized. If animals are not fully recovered at the same time, the lions that have not been immobilized or may have recovered earlier from the compound may sometimes viciously attack those that are still partially sedated and more vulnerable. This aggressive behaviour has been described by Pienaar *et al*. (1969) and Young (1975) and has also been observed by several of the lion ranchers involved in this study. It must be stressed that despite the fact that these lions are bred in captivity, they remain wild and dangerous.

2.4 Collection and preservation of blood

Once the immobilised lions arrived at the shaded working area, the procedure to collect blood commenced. Simultaneously two operators started measuring the different body parameters (Section 2.6).

Blood was collected from the *vena saphena lateralis* (ramus caudalis) into three different types of vacutainer tubes (as instructed by the laboratory where the blood analyses were conducted): two plain (red top) vacutainer tubes; two EDTA anti-coagulant (purple top) vacutainer tubes; and one sodium fluoride potassium oxalate anti-coagulant (grey top) vacutainer tube. The two red top tubes and the one grey top tube were centrifuged at room temperature for 10 minutes at 3000 rpm. The two red top tubes were left at ambient temperature for approximately 30 minutes to coagulate before centrifugation to obtain serum. The grey top tubes were centrifuged as soon as possible after collection to obtain plasma. The serum from one plain tube (red top) and the blood from one vacutainer tube containing EDTA (purple top) were used for analysis and the other tubes stored as backup specimens. The plasma obtained from the tube containing sodium fluoride potassium oxalate

(grey top) was split into two aliquots (approximately 1 ml each), one aliquot to be analyzed and the other to be stored as a backup. In some cases the second backup aliquot was used for analyses by the laboratory.

2.4.1 Plain vacutainer tubes (red tops, 2 x 10 ml per animal)

The blood in these tubes was left to coagulate for 30 minutes at room temperature before being centrifuged for 10 minutes at 3 000 rpm. The serum was then removed with the aid of a glass pipette fitted with a sucking rubber top and placed into small plastic tubes (two tubes per lion) containing approximately 3 to 4 ml serum each and preserved at -20ºC until analyzed. These serum samples were used to perform a full biochemical analysis (serological profile), using the Beckman Coulter® LX20. The list of biochemical parameters that were analyzed is shown in Table 2.2.

Table 2.2 Biochemical parameters assayed in lion blood

Variable	Unit
Sodium (Na)	mmol/L
Chlorine (Cl)	mmol/L
Urea (BUNm)	mmol/L
Calcium (CALC)	mmol/L
Total Bilirubin (TBIL)	μmol/L
Phosphorus (PHOSm)	mmol/L
Total carbon dioxide (CO_2)	mmol/L
Cholesterol (CHOL)	mmol/L
Potassium (K)	mmol/L
Creatinine (CREm)	μmol/L
Uric Acid (URIC)	mmol/L
Albumin (ALBm)	g/L
Total Direct Bilirubin (DBIL)	μmol/L
Total Protein (TPm)	g/L
Magnesium (Mg)	mmol/L
Glucose (GLU)	mmol/L
Gama Glutamil Transpherase (GGT)	IU/L
Lactate Dehydrogenase (LDH)	IU/L
Alanin Transpherase (ALT)	IU/L
Aspartate Transaminase (AST)	IU/L
Alkaline Phosphatase (ALP)	IU/L

2.4.2 Vacutainer tubes containing sodium fluoride potassium oxalate anti-coagulant (grey tops, 1 x 5 ml per animal)

The tubes were centrifuged for 10 minutes at 3 000 rpm, immediately after blood was collected from each lion. The plasma was separated with the aid of a glass pipette fitted with a sucking rubber top and placed into 5 ml plastic tubes (two tubes per lion, each containing approximately 1 ml plasma) and preserved at -20ºC until analyzed. The plasma in these tubes was used for glucose analysis.

2.4.3 Vacutainer tubes containing EDTA anti-coagulant (purple tops, 2 x 5 ml per animal)

One of the tubes was used for the field haematological analysis done with the Ac•T 5diff Haematology Analyzer (Beckman Coulter®) within about 5-15 minutes from collecting the blood. Due to logistics at the Reddersburg ranch, the analysis with the Ac•T 5diff Haematology Analyzer was only done about 30 minutes after collection. Since the EDTA anti-coagulant effect on the blood lasts approximately 24 hours, no problem with the time delay was foreseen or experienced.

Additionally, three thin blood smears were prepared from each animal using the EDTA blood. The smears were prepared according to the method described by Undritz (1973), namely a small drop of blood was placed on a glass microscope slide. Using a second slide as spreader, it was placed at an angle of 45° against the drop of blood and the drop was then dragged or spread across the slide in one single movement to obtain an even, thin smear. The blood smears were allowed to dry and fixed in methanol before being wrapped in tissue paper to protect them from sunlight and dust and then taken to the laboratory for staining and analysis (Archer, 1965). The second blood tube was cryopreserved at -20ºC for future studies on lion DNA.

The Ac•T 5diff Haematology Analyzer (Beckman Coulter®) was used at the ranches to perform a full blood count and a differential white blood cell count on the EDTA preserved blood specimen of each animal.

At the laboratory all blood smears (3 smears per animal) were stained with the Wright's stain (Undritz, 1973; Hookey *et al*., 2001). A differential cell count was

performed on each blood smear, using the classical visual 100-cell manual method with 1 000 x magnification (under immersion oil). The average results of the three blood smears was then calculated and used as the descriptive statistical values for the respective variable from that animal. These average values were used as descriptive statistics in the rest of the study and later compared with the differential counts obtained by the Ac•T 5diff Haematology Analyzer.

2.5 The Ac•T 5diff Haematology Analyzer

The Ac•T 5diff Haematology Analyzer is a self-contained bench top analyzer designed for use in small human laboratories (O'Neil *et al*., 2001). Due to the nature of this study and the field circumstances under which the work was done, the possibility to use the Ac•T 5diff Haematology Analyzer in a field laboratory for lion blood was also evaluated. The Ac•T 5diff Haematology Analyzer is capable of determining a complete human blood cell count (CBC) with comprehensive red blood cell, white blood cell and platelet (PLT) counts - including a five part differential leukocyte count (DLC). The system uses a 30 µL blood sample in CBC mode and a 53 µL sample in CBC/DLC mode (Kern, 2001). In this study, the CBC/DLC mode was used.

The Ac•T 5diff system uses the following five reagents, with these specific functions:

- *Ac•T 5diff Diluent*, to dilute the whole blood and stabilize the cell membranes.
- *Ac•T 5diff Fix,* for the lyses of red blood cells, preserve leukocytes in their natural state and stain the granules of the monocytes, neutrophils and eosinophils with vital stain Chorazole Black E.
- *Ac•T 5diff WBC Lyse,* for the lyses of white blood cells for the leukocyte count and specifically differentiate basophils from other leukocytes.
- *Ac•T 5diff HGB Lyse,* for the lyses of blood cells and determine haemoglobin (HGB) content.
- *Ac•T 5diff Rinse,* a rinsing agent.

These reagents are stable at room temperature and automatically pre-heated to 35°C, before being mixed with the blood sample in the reaction baths (O'Neil *et al.*, 2001).

The Ac•T 5diff system uses the principles of absorbance cytochemistry and volume to provide a complete five part white blood cell differential count. Lysis of the red blood cells and cytochemical staining of the granular components of the monocytes, basophils, neutrophils and eosinophils by the fixative reagent prepares the white blood cells for the automated differential analysis. Diluents are added to stabilize the reactions and the sample is analyzed in the flow cell using dual focused flow technology. This technology focuses cells in a stream of diluent and aligns them to pass individually through the flow cytometer (O'Neil *et al.,* 2001).

The differential leukocyte count is performed in two different channels:

- Flow analyses by means of two measurements realized on each cell independently: the volume by impedance method and light scatter after contact of WBC and Chlorazol Black E stain.
- A basophil channel in which the total leukocyte count is performed after mixing with a specific reagent. This reagent gently strips the leukocyte membranes and preserves the basophil membranes to be able to count the basophils, if they are present (Kern, 2001).

The Ac•T 5diff system compares the white blood cell differential parameters, neutrophils, lymphocytes, monocytes and eosinophils by using regression analysis (O'Neil *et al.*, 2001). The haematological parameters measured on lion blood samples in this study with the aid of the Ac•T 5diff Haematological Analyzer (Beckman Coulter®) are presented in Table 2.3.

The Ac•T 5diff Haematology Analyzer also presents a differential plot (DiffPlot) for each differential count. The DiffPlot is a graph, distinguishing between the volume and absorbency of the leukocyte populations as illustrated by O'Neil *et al.* (2001). See Appendix 1 for illustrations/examples.

Table 2.3 Haematological parameters measured on African lion blood with the aid of the Ac•T 5diff Haematological Analyzer (Beckman Coulter®)

Variable	Unit
Red Blood Cell Count (RBC)	$10^6/\mu L$
Haemoglobin (HGB)	g/dL
Haematocrit (HCT)	%
Platelets (PLT)	$10^3/\mu L$
Mean Cellular Volume (MCV)	FL
Mean Cellular Haemoglobin (MCH)	Pg
Red Cell Distribution Width (RDW)	%
Mean Cellular Haemoglobin Concentration (MCHC)	g/dL
Mean Platelet Volume (MPV)	FL
White Blood Cell Count (WBC)	$10^3/\mu L$
Neutrophils (NE)	% and $10^3/\mu L$
Lymphocytes (LY)	% and $10^3/\mu L$
Monocytes (MO)	% and $10^3/\mu L$
Eosinophils (EO)	% and $10^3/\mu L$
Basophils (BA)	% and $10^3/\mu L$

2.6 Body measurements

The body weight and the body measurements of lions were recorded, according to the procedures described by De Waal *et al.* (2004a). The complete procedure to measure an immobilised adult male lion was performed in about 10 minutes by two persons, recording the respective body measurements and ascribe recording of the data (De Waal *et al.,* 2004a, b).

2.6.1 Body weight

The lions were weighed on an electronic scale to the nearest 0.5 kg. The scale consisted of a metal platform (designed to weigh cattle) rested on two electronic pressure cells connected to an electronic head, powered by a 12 volt battery.

2.6.2 Chest girth

A cloth measuring tape was used to measure the circumference of the chest, immediately caudally to the front limbs just behind the *margo tricipilatis*.

2.6.3 Body length

Body length was measured with a cloth measuring tape from the base of the incisors (prosthion; most anterior point of skull), over the nose, following a central line between the eyes over the head - along the contours of the body (*linea mediana dorsalis*) to the tip of the last caudal vertebra of the tail (bony tip of the tail, excluding the tail tuft).

2.6.4 Tail length

Tail length was measured from the proximal base of the tail, to the tip of the last caudal vertebra (tail), as described for body length.

2.6.5 Tail circumference

Tail circumference was measured at the proximal base of the tail, with a cloth measuring tape.

2.6.6 Head length

Measured in a straight line from the base of the incisor teeth (prosthion: most anterior point of the skull) to the inion (most posterior point of the skull), with a large vernier calliper.

2.6.7 Head width

Measured in a straight line between the *zygions* (most outer points of the zygomatic arches), using a large vernier calliper.

2.6.8 Abdominal girth

The circumference of the abdomen immediately cranially to the hind limbs was measured, using a cloth measuring tape.

2.6.9 Front leg length

A metal measuring tape was used to measure the front leg length from the elbow (tip of the *olecranon process*) to the tip of the longest (third) digit, without the claw (*sine unguis*).

2.6.10 Front leg circumference

The widest proximal part of the front leg (*regio antebrachi*, just below the elbow joint) was measured, using a metal measuring tape.

2.6.11 Front and hind paw width

The widest parts across the outer digits (second and fourth) of the *manus* and the *pes* were measured, using a metal measuring tape.

2.6.12 Hind foot length

The hind foot length was measured from the heel (tip of the *calcaneus)* to the tip of the longest (third) digit, without claw (*sine unguis*), using a metal measuring tape.

2.6.13 Front and hind paw length

The distance from the posterior part of the sole pad to the tip of the longest digit (third), without the claw (*sine unguis*), was measured using a metal measuring tape.

2.6.14 Upper and lower canine teeth length

A small sliding vernier calliper was used to measure the canine length or height from the unbroken tip to its base or insertion in the gum.

2.7 Statistical Analysis

Morphometric and laboratory data were statistically analyzed using the procedures of the Statistical Analysis Software (SAS) (version 8.2, Cary, NC, USA).

Quantitative variables were summarised using descriptive statistics (including the number of animals with available data [n], mean, standard deviation [SD], median, minimum [min] and maximum [max]) values. Qualitative variables were summarised using absolute [n] and relative [%] frequencies. Percentages were calculated from the total number of animals and were rounded off to one decimal place.

For the reporting of descriptive statistics, the minimum and maximum values were presented to the same precision, as the source data. Means and medians were reported to one decimal place more than the source data. Standard deviations were presented to two decimal places more than the source data. Data were analyzed according to age, sex and location (as independent variables). As it was found that location was not a significant factor ($p>0.05$) influencing the data, the data were separated and presented by age group and/or sex, where applicable.

Where applicable, the animals were stratified by age group similar to that presented by Smuts *et al.* (1980), as follows:

Age group 1: 0 – 1 years (Young cubs)
Age group 2: 1 – 2 years (Cubs)
Age group 3: 2 – 4 years (Juveniles and sub-adults)
Age group 4: >4 years (Adults)

2.7.1 Morphometric measurements

The morphometric measurements (*i.e*. body weight, chest girth, body length, tail circumference, tail length, head length, head width, abdominal girth, front leg length

left and right, front leg circumference left and right, front paw width left and right, front paw length left and right, hind foot length left and right, hind paw length left and right, hind paw width left and right and canine length upper and lower left and right) were summarized and tabulated by age group and sex, where applicable. Descriptive statistics were presented for age and all the body measurements.

Most morphometric measurements are related to age and sex (Lawrence, 1980). Therefore growth curves, as a function of age, as well as the reference ranges for the morphometric measurements, were calculated using the Emax model (Gabrielsson & Weiner, 2000). Details on the Emax model are provided in section 2.7.3. For all morphometric measurements considered, growth curves and references ranges were calculated for both sexes separately.

2.7.2 Dependent parameters determined in the laboratory from the blood samples

All laboratory parameters measured were summarized and tabulated by age group and sex, where applicable. Descriptive statistics were presented for all laboratory determined parameters.

All variables were subjected to an analysis of variance (ANOVA) with age group and sex as main effects, to detect potentially significant ($p<0.05$) differences between age groups and sex, regarding each variable.

For those laboratory parameters that did not show significant differences between age groups and/or sex, the reference ranges were calculated according to the following formula (Harris & Boyd, 1995; Burtis & Ashwood, 1999):

Lower reference limit = μ - 1.96 x SD

Upper reference limit = μ + 1.96 x SD

where μ are the estimates of the mean and SD for the dependent variable in question, for the respective group of animals (all, per sex or age group).

For those dependent variables which were significantly affected by sex of the animal, the reference ranges were calculated with the same formulas as described above. In this case μ was the estimate of the mean and SD the estimate for the dependent variable in question, from the two separate sex samples of animals, but pooling data from all age groups.

For those laboratory parameters which did show significant differences between age groups, the reference ranges were calculated at the 95% confidence interval for the predicted value calculated from a fit of the Emax model (Section 2.7.3) - by pooling data from both sexes, but keeping age groups separately.

2.7.3 The Emax model

2.7.3.1 Introduction

If the variable y represents an observed morphometric measurement, such as weight, height or length, the expected value of y was modelled as a function of age $x \geq 0$, thus $E(y) = \mu = f(x)$. In order to identify a suitable model, or class of models, the plots the data were taken into account as a first step.

The first assumption to be made is that the growth curve $f(x)$ is a monotonically increasing function of age: lions, like all animals, grow with age. Certainly, for morphometric measurements that are essentially determined by the size of the skeleton, such as body length or shoulder height, $f(x)$ is a monotonically increasing function of age. Furthermore, under customary feeding conditions, such as those practiced on a wildlife ranch, even measurements that are influenced by soft tissue mass, such as weight, can, in the mean of the values, be modelled as monotonically increasing functions of age; possible short term decreases in weight due to variations in feed intake or health status of an individual can be viewed as random deviations from the mean. Finally, the age range of the experimental animals was limited, so that phenomena like a potentially decreasing body weight in very old animals did not need to be modelled.

At birth (x =0), f(x) is close to zero, relative to the magnitude of f(x) reached at older (or mature) ages. After the lions reach maturity, that is after about 4 years of age, (Furstenburg, 1999; Smuts *et al*. 1980), f(x) approaches an asymptote, the maximum average weight, height or length of a lion. In the case of body weight of females, average maximum weight appears to be around 160 kg and in males around 200 kg. Since the average size of female lions is smaller than that of males, separate growth curves for males and females need to be determined.

In summary, the growth curve f(x) to be modelled is a monotonically increasing function of age, and is bounded both below and above. Therefore, f(x) is necessarily a non-linear function of x. It seems reasonable to assume this for all other morphometric measurements considered in this study.

2.7.3.2 Calculation of the Emax values

Let the variable y be a morphometric measurement and x be the age of the animal. The author attempted to model the expected value of y as a function of x, that is E(y)=f(x).

The simplest form of the Emax model can be written as:

$$\mathrm{E}(y) = \mu = f(x) = \frac{\mathrm{E}_{\max} \cdot x}{\xi_{50} + x}$$

where Emax =lim$_{x\to\infty}$ f(x) is the maximum value and ξ_{50} is the value of x at which 50% of the maximum value (f(ξ_{50}) =Emax/2) is reached.

A variation on the Emax model is the Sigmoid Emax model which can be used as an increasing or decreasing function

$$f(x) = \frac{\mathrm{E}_0 \cdot \xi_{50}^{\gamma} + \mathrm{E}_{\infty} \cdot x^{\gamma}}{\xi_{50}^{\gamma} + x^{\gamma}}$$

where E$_0$ = f(0), E$_\infty$ =lim$_{x\to\infty}$ f(x), and f(ξ_{50}) =(E$_0$ + Emax)/2.

The Sigmoid Emax Model was fitted to the available data using standard non-linear regression software (Proc NLIN of SAS).

Three parameters of the Sigmoid Emax Model are easy to interpret, namely:

- the intercept (E_0; for example, the expected value of morphometric measurement at birth),
- the asymptote/maximum response (E_∞; expected value of morphometric measurement after having reached maturity),
- and the value of x to achieve 50% response (ξ_{50}; age at which 50% of growth was achieved).

Consequently, fairly accurate starting values for those parameters for the non-linear regression could be obtained simply by inspection of a plot of the data against x. Furthermore, a starting value of γ =1 for the sigmoid parameter γ worked well in all applications.

2.7.3.3 Application to lions' morphometric measurements: growth curves

Firstly the Sigmoid Emax Model with additive error was fitted to the morphometric measurement data, using the NLIN procedure of SAS (version 8.2).

The observed data, the fitted mean curve and the 95% confidence interval for the predicted values were then plotted. The 95% confidence interval for the predicted values can be used as a reference range for the data.

The graphs for morphometric measurements suggested a good fit for the mean response, but that the variance of the data increased with the mean. This observation was confirmed by plots of the residuals against the fitted values. In order to account for the apparent heteroscedasticity, the model with multiplicative error was fitted [log(y) was regressed against log f(x)]. The fitted curve and 95% confidence interval for the predicted value on the logarithmic scale were back-transformed to the original scale by taking the anti-log. Under the additive model, the prediction intervals for the morphometric measurements of young lions were

clearly too wide, while they were too narrow for older lions. Using the multiplicative model, the prediction intervals seem to be adequate over the whole age range.

Under the multiplicative model the fitted curve represents an estimate of the geometric mean response as a function of age. The boundaries of the prediction interval are symmetric around the fitted curve on the multiplicative scale, and the prediction interval has a constant width on the multiplicative scale.

2.7.3.4 Application of the Emax model to laboratory parameters

The Emax Model (additive or multiplicative model, where relevant) was fitted to those blood biochemistry and cytology parameters for which significant differences between age and/or sex groups were detected. Depending on the association between the fitted values and the variance of the data, an additive or multiplicative model was fitted.

For those laboratory parameters which did not show significant differences between age groups and sex, the reference ranges were calculated as described in Section 2.7.2.

3. Reference values for blood serological biochemistry in the African lion *(Panthera leo)*

3.1 Introduction

The wildlife ranching industry in South Africa has grown rapidly over the last few years (National Agricultural Marketing Council, 2006) and the African lion is an important attraction for eco-tourism. In both livestock farming and wildlife ranching, a sound knowledge of the physiology, nutrition and health of the species in question is required, in order to manage the animals appropriately and to farm and breed them successfully. One of the major constraints on lion ranches is disease control and health monitoring. Therefore, the important role of reference values for lion blood biochemistry should not be underestimated.

Blood is a liquid tissue and its constituents can be used to monitor the health status, diagnose diseases, nutritional deficiencies and the metabolic status (i.e. pregnancy) of animals. Techniques to perform complete blood biochemistry analyses have been developed and established for humans and livestock species. The same procedures can in principle be used for any wild species to identify deviations from normal values in certain blood parameters (cytological or biochemical). However, before deviations can be detected in an animal, the normal reference values for the different blood variables of the species in question must be well established. Once this has been done, deviations, their causes and possible corrections can be investigated. This information is therefore valuable for lion breeders and veterinarians.

Very little information on African lion blood biochemistry is available in the literature (Wallach & Boever, 1983; Pospísil *et al.,* 1987; International Species Information System, 1999). The objective of this study was to determine the normal reference values for the most important biochemical blood parameters for African lions bred in captivity.

3.2 Materials and methods

Seventy-two lions (between the ages of 3 months and 9 years of both sexes) from three private ranches and the Bloemfontein Zoo were used in this study. An intra-muscular administration (4-5 mg/kg of Zoletil® 100; 50 mg Tiletamine HCL and 50 mg Zolazepam HCL/ml; VIRBAC) was used for the chemical immobilization of the animals. Body weights were visually estimated to calculate the appropriate immobilization dose for every lion.

The 7 male cubs aged 3 months from the 0 – 1 year old group from the ranch in Bothaville (See Table 2.1) were caught by hand, since they were hand raised and still relatively tame. The Zoletil® 100 dose was injected IM with the aid of a syringe and needle in the hind leg. The other 65 older animals (8 months and older) were darted using a darting gun and a dart containing the estimated required dose of Zoletil® 100. For more details on the study area, management practices and the immobilization procedures, please refer to Chapter 2 – General Materials and Methods.

Blood specimens were collected for analysis from the *vena saphena lateralis* (ramus caudalis) into the following tubes:

Two plain vacutainer tubes (red tops, 10 ml):

The blood in these blood collection tubes was left to coagulate for 30 minutes at room temperature before being centrifuged for 10 minutes at 3000 rpm/min. The serum was then removed with the aid of a glass Pasteur pipette fitted with a sucking rubber top and placed into small 5 ml plastic tubes (two for each lion) containing approximately 3-4 ml of serum each and preserved at -20ºC, until analyzed in the laboratory. These sera samples were used to perform a full biochemical analysis. See Table 2.2 for the different parameters determined from the serum.

One vacutainer tube containing sodium fluoride potassium oxalate anti-coagulant (grey top, 5 ml):

These tubes were centrifuged immediately after blood collection, the plasma separated and placed into small 5 ml plastic tubes (two for each lion, containing

approximately 1 ml of plasma each) and preserved at -20ºC until analyzed later in the laboratory. The plasma from these tubes was used for glucose (Glu) analysis.

3.2.1 Biochemical analysis

The serum and plasma obtained from both the red and grey top tubes were analyzed at the laboratory using those from Beckman Coulter® LX20.

3.3 Statistical analysis

The following independent variables were considered: location, age and sex of lions. As mentioned in paragraph 3.7, it was found that location did not significantly affect any of the parameters considered, therefore it was removed from the model. A two-way ANOVA model with sex and age group (0 −1 years, 1−2 years, 2−4 years, > 4 years) as the independent variables was fitted to the various biochemistry variables considered.

For those biochemistry parameters that were not significantly affected by age or sex, the normal reference range was calculated as follows (Harris & Boyd, 1995; Burtis & Ashwood, 1999):

Lower reference limit = $\hat{\mu} - 1.96 * \hat{SD}$

Upper reference limit = $\hat{\mu} + 1.96 * \hat{SD}$

where $\hat{\mu}$ and $\hat{SD}$ are respectively the estimates of the mean and SD for the variable in question, from the total number of animals (age groups and two sexes combined).

For those laboratory parameters which showed only significant differences between the two sexes, the normal reference ranges were calculated with the same formulas as described above. However $\hat{\mu}$ and $\hat{SD}$ are respectively the estimates of the mean and SD for the variables in question, for male and female animals of all age groups combined.

For those variables which where significantly affected by age, the normal reference ranges were calculated as the 95% confidence interval for the predicted value calculated from a fit of the Emax model (see paragraph 3.7.3 for more detail on the Emax model), pooling data from both sexes. For some biochemistry parameters the additive model was used and for other variables the multiplicative model was used.

3.4 Results and discussion

A summary of the results from the ANOVA model on the biochemistry variables for data from all locations is presented in Table 3.1. As it can be seen from this table, sex had no significant effect on the biochemistry parameters considered in this study ($p>0.05$). Due to the success of the E_{max} model when applied to lions' body measurements (Chapter 5), the model was also applied to the biochemistry parameters that appeared age-dependent. Excellent fits were obtained in all cases. As opposed to the body measurements, which typically had a constant coefficient of variation and therefore were analyzed using a multiplicative error model, the biochemistry data typically exhibited constant variation and were analyzed using an additive error model.

The normal reference ranges for the biochemistry parameters that were not significantly affected by neither age, nor sex or the interaction between these two independent variables. These are summarized in Table 3.2, while those significantly affected by age are summarized in Table 3.3.

The normal reference ranges for the biochemistry parameters affected by the age of the animal are summarized in Table 3.3.

For those age groups for which the blood biochemistry parameter presented similar results ($P>0.05$), the reference values (range) are presented for the combined age groups (Table 3.3).

Table 3.1 ANOVA results (P- values) for blood serum biochemistry parameters of the 72 African lions

Serological Parameters (Unit)	n	Factor	Degrees of Freedom for the Error	Degrees of Freedom	p-value*
Sodium (Na) (mMol/L)	72	Age Sex	67 67	3 1	0.245 0.340
Chlorine (Cl) (mMol/L)	72	Age Sex	67 67	3 1	0.071 0.243
Urea (BUNm) (mMol/L)	72	Age Sex	67 67	3 1	**0.000** 0.042
Calcium (CALC) (mMol/L)	72	Age Sex	67 67	3 1	**<0.000** 0.972
Total Bilirubin (TBIL) (uMol/L)	72	Age Sex	67 67	3 1	0.670 0.556
Phosphorous (PHOSm) (mMol/L)	72	Age Sex	67 67	3 1	**<0.000** 0.076
Total carbon dioxide ($C0_2$) (mMol/L)	72	Age Sex	67 67	3 1	0.046 0.830
Cholesterol (CHOL) (mMOL/L)	72	Age Sex	67 67	3 1	**0.015** 0.432
Glucose (Glu) (mMol/L)	72	Age Sex	67 67	3 1	**0.004** 0.787
Gamma-glutamyl transferase (GGT) (IU/L)	72	Age Sex	67 67	3 1	0.443 0.484
Alanine transpherase (ALT) (IU/L)	72	Age Sex	67 67	3 1	**0.007** 0.637
Potassium (K) (mMol/L)	72	Age Sex	67 67	3 1	**0.003** 0.028
Creatinine (CREm) (uMol/L)	72	Age Sex	67 67	3 1	**<0.000** 0.700
Uric Acid (Uric) (mMol/L)	72	Age Sex	67 67	3 1	0.200 0.949
Albumin (ALBm) (g/L)	72	Age Sex	67 67	3 1	0.031 0.519
Total Direct Bilirubin (uMol/L)	72	Age Sex	67 67	3 1	0.788 0.115
Total Protein (TPm) (g/L)	72	Age Sex	67 67	3 1	**<0.000** 0.161
Magnesium (MG) (mMOL/L)	72	Age Sex	67 67	3 1	**0.000** 0.135
Lactate dehydrogenase (LD) (IU/L)	72	Age Sex	67 67	3 1	**<0.000** 0.728
Aspartate transaminase (AST) (IU/L)	72	Age Sex	67 67	3 1	0.724 0.824
Alkaline Phosphatase (ALP) (IU/L)	72	Age Sex	67 67	3 1	**<0.000** 0.281

*p-values in bold are <0.05

Table 3.2 The lower and upper limits for the reference range values for blood serum biochemistry parameters of African lions independent of sex and age

Serological Parameters (Unit)	Reference Range	
	Lower Limit	Upper Limit
Sodium (mmol/L)	133.0	161.0
Chlorine (mmol/L)	108.8	133.3
Total Bilirubin (µmol/L)	2.0	8.0
Total carbon dioxide (mmol/L)	7.7	18.6
Gamma-glutamyl transferase (IU/L)	0.0	4.0
Uric acid (mmol/L)	0.0	0.1
Albumin (g/L)	9.0	14.0
Total Direct Bilirubin (µmol/L)	0.0	2.0
Aspartate transaminase (IU/L)	12.0	40.0

For the additive models, both the mean and variance model(s) seem to fit the data in the decreasing functions, showing that the variance of the specific biochemistry parameters (for example alkaline phosphatase) levels in young cubs (<1 year) are much higher than in lions older than 2 years of age, when most lions are considered as juveniles, sub-adults or adults.

For the multiplicative model, both the mean and variance model(s) seems to fit the data in the increasing functions, showing that the variance of the specific biochemistry parameters (for example Creatinine levels) in young lion cubs (< 1 year) are much lower than in lions older than 1 year of age.

In Table 3.4 a comparison between average values determined in this study and three other available sources is given.

Table 3.3 The lower and upper limits for the reference range values for blood serum biochemistry parameters of the African lion in which age has a significant effect

Serological Parameters (Unit)	Age group*	Reference Ranges	
		Lower Limit	Upper Limit
Urea (BUNm) (mmol/L)	1	7.10	23.00
	2	6.40	16.10
	3-4	5.00	12.60
Calcium (CALC) (mmol/L)	1	2.47	2.95
	2	1.87	2.67
	3-4	2.27	2.65
Phosphorous (PHOSm) (mmol/L)	1	2.12	3.00
	2	1.70	2.94
	3	1.51	2.66
	4	1.18	2.16
Cholesterol (CHOL) (mmol/L)	1	2.23	4.64
	2	2.26	3.91
	3	1.96	3.80
	4	1.80	3.54
Glucose (Glu) (mmol/L)	1	5.10	8.40
	2	4.40	7.60
	3	4.80	6.00
	4	4.50	6.80
Alanine transpherase (ALT) (IU/L)	1	25.00	47.00
	2	28.00	70.00
	3	29.00	138.00
	4	19.00	72.00
Potassium (K) (mmol/L)	1	3.70	6.60
	2	3.40	5.10
	3-4	3.70	5.40
Creatinine (CREm) (µmol/L)	1	45.50	173.30
	2	98.80	284.40
	3	143.80	297.60
	4	140.40	292.90
Total Protein (TPm) (g/L)	1	53.00	75.00
	2	56.00	71.00
	3	58.00	80.00
	4	65.00	86.00
Magnesium (MG) (mmol/L)	1	0.83	1.49
	2	0.65	0.96
	3-4	0.82	1.06
Lactate dehydrogenase (LD) (IU/)	1	7.00	266.00
	2	48.00	168.00
	3	11.00	165.00
	4	16.00	100.00
Alkaline Phosphatase (ALP) (IU/L)	1	41.00	199.00
	2	21.00	98.00
	3	8.00	64.00
	4	5.00	19.00

Age group 1: <1 year old; Age group 2: 1 – 2 years old; Age group 3: 2 – 4 years old and Age group 4: >4 years old

Table 3.4 Comparison between the mean reference values for lion blood serum biochemistry results from this study and those of three other published sources (lions) and of the domestic cat

Parameter	This study	ISIS* (1999)	Wallach & Boever (1983)#	Pospísil *et al.* (1987)#	Domestic Cat (Latimer *et al.*, 2003)#
Glucose (mmol/L)	5.63	0.38	0.21	9.36	5.00
Na (mmol/L)	147.08	151.00	5.60	145.45	151.00
K (mmol/L)	5.23	4.40	-	4.04	4.90
Cl (mmol/L)	121.02	119.00	-	105.80	122.50
CO_2 (mmol/L)	13.07	15.80	-	-	20.50
Urea (mmol/L)	10.31	-	2.93	-	9.46
Creatinine(µmol/L)	168.63	229.84	103.16	293.40	137.02
Calcium (mmol/L)	2.54	2.48	2.58	2.48	2.55
Phosphorus (mmol/L)	2.25	1.78	1.63	1.67	1.47
Total protein (g/L)	66.64	74.00	50-84	88.75	69.50
Albumin (g/L)	10.73	33.00	28.00	-	33.50
Total Bilirubin (µmol/L)	5.07	3.42	34.20	1.73	0.89
Direct Bilirubin (µmol/L)	1.06	1.71	-	-	0.89
ALP (IU/L)	54.99	33.00	17.00	-	22.50
GGT (IU/L)	1.78	3.00	-	-	-
AST (IU/L)	25.79	-	-	-	22.50
ALT (IU/L)	42.58	-	-	20.24	61.00
LDH (IU/L)	81.98	142.00	-	-	89.00
Cholesterol (mmol/L)	3.10	4.43	3.91	4.77	2.94
Magnesium (mmol/L)	0.93	0.68	-	0.97	0.89

*International Species Information System
Units were conversed according to Burtis & Ashwood (1999) to be able to compare with values from this study

Only two of the biochemistry parameters considered in this study, namely calcium and cholesterol are in line with the published values. For the values that do not compare well, more than one reason could be applicable. The two most important reasons could be the differences in group sizes, and the health status of the animals. Since the health status of the lions used for the published values in the literature, as well as the management practices are unknown, it would be difficult to speculate about the reasons for these differences.

According to Wallach and Boever (1983), blood biochemistry reference values for wild felidae compare well with those for the domestic cat - suggesting that the reference values for the domestic cat can be used when interpreting biochemical profiles obtained from lions. However the data from Table 3.4 clearly demonstrate that this could only be true in the case of calcium and phosphorous. It is quite clear that the reference values for most of the blood biochemistry parameters are quite different between the two species. The data from this study further highlights the importance of a complete set of reference values for biochemistry parameters for the African lion (*P. leo*).

3.5 Conclusions

This study generated reasonably accurate reference range values for the most important blood biochemistry parameters, for the different age groups of African lions (*P. leo*). From the data presented in this study it is clear that substantially more information and research is required in order to achieve meaningful reference values for biochemistry parameters of the African lion. The larger the number of blood biochemical profiles from lions bred in captivity that can be added to this data base (generated in this study), the more accurate the reference ranges will become. In addition, if lions from a larger area across more geographical regions and kept under different management conditions, including those in the wild could be used, it would be possible to better evaluate the effect of different factors on the blood biochemistry profiles.

4. Haematological reference values for the African lion *(Panthera leo)*

4.1 Introduction

Besides its economic value as a tourist attraction (including for hunting purposes), captive lion breeding has also contributed to the preservation of this endangered species. Most large predators, including the African lion, are considered an endangered or threatened species in most parts of Africa. Therefore captive lion breeding plays an important role in preserving the species in South Africa.

As with any other ranching practices, knowledge on the physiology, nutrition and diseases of the species in question is required - in order to manage the animals appropriately and to farm and breed them successfully. Although lions have been kept and bred in captivity for many years, very little is known on their physiological reference values in general, and their haematologic profile in particular. Blood is a liquid tissue and its constituents (cells, plasma and chemical elements) may be used to monitor nutritional deficiencies and evaluate the metabolic (i.e. pregnancy, hypocalcaemia), health status and assist in the diagnosis of diseases in animals.

One of the major constraints in lion ranching, is disease control and health monitoring. Knowledge of the normal blood composition or the haematologic reference values of lion blood is of great value in this regard. The differential white blood cell count can be used effectively to monitor the health status and assist in the diagnosis of different diseases. The white blood cell count is traditionally done using the manual-visual method under the light microscope. Although this method is accurate, it has three major constraints. It requires a skilled and experienced operator, takes time and it is strenuous for the operator in order to be accurate using the classical visual manual methods described by Archer (1965), Undritz (1973) and Rosenberger (1979).

There are automated systems, like the Ac•T 5diff Haematology Analyzer, that can be used to analyze blood from humans and may also be calibrated to analyze blood samples from other species, capable of performing a complete haematological analysis and producing a report within seconds (Kern, 2001). This author considered

that the disadvantages of the manual-visual method can be overcome by the use of automated blood analyzers such as the Ac•T 5diff Haematology Analyzer. It would be of value to veterinarians involved in the care of lions, if an automated blood cell count could be performed by an accurate and portable blood analyzer which could be used in the field.

The main objective of this part of the study was to determine the normal reference values (ranges) for the most important haematological parameters (blood cells) for the African lion bred in captivity. A secondary objective was to evaluate the potential use of the Ac•T 5diff Haematology Analyzer from Coulter® to analyze African lion blood in the field.

4.2 Materials and Methods

Seventy-two lions from both sexes and different age groups, from four different sites were chemically immobilized using an intra-muscular administration (4-5 mg/kg of Zoletil® 100; 50 mg Tiletamine HCL and 50 mg Zolazepam HCL/ml; VIRBAC). Body weights were visually estimated to calculate the immobilization dose to be used. For more details on the study area, management practices and the immobilization procedures, please refer to Chapter 2 – General Materials and Methods.

As soon as the animals were fully immobilized (it took between 10 and 20 minutes after darting, i.e. being unable to move or consciously being able to bite), they were transported on a canvas stretcher in the back of a truck to a shaded place for protection against heat exhaustion. Their eyes were covered with a blindfold to calm them and prevent damage to the eye (Young, 1975). The animals were then handled as soon as possible so they could be returned to their camps, before the immobilizing effect of the drug started to wear off and the animals regained consciousness.

Blood was sampled from each animal from the *vena saphena lateralis* (ramus caudalis) into two 5 ml vacutainer tubes containing EDTA anti-coagulant (purple top). One tube was used for analyses and the other was stored as a back up specimen.

Three thin blood smears were prepared from each animal using the blood in the EDTA tube. This was done according to the method described by Undritz (1973). A drop of blood was placed on a microscope slide near one end of the slide. Another slide was held against the drop at an angle of 45° and then smeared across the first slide, dragging the blood along and smearing it across the slide. The smears were allowed to dry and fixed in methanol before being wrapped in tissue paper to protect them from sunlight and dust and taken to the laboratory, for staining and analyses (Archer, 1965). A Wright's stain was used, as recommended by Undritz (1973) and Hookey *et al*., (2001). In the laboratory, the smears were examined under the microscope using an immersion oil lens (1000x magnification). A total of 100 white blood cells were then counted from different areas of each slide. From these, the total number of each type of white blood cell (namely lymphocytes, neutrophils, monocytes, eosinophils and basophils) was determined and expressed as a percentage of the total white blood cell population (Houwen, 2001). An average of the three smears made from a blood sample from each lion was calculated and used as the descriptive statistics for the visual differential count used in all further statistical analysis done.

Before using the Ac•T 5diff Haematology Analyzer, this equipment was set up according to the instruction's manual and all its reagents added to the analyzer. Immediately after the blood in the EDTA tubes (purple tops) was collected, it was thoroughly mixed by gently turning the tubes upside down and back for at least five times. One tube was then inserted in the Ac•T 5diff Haematology Analyzer, which automatically drew the correct volume of blood (53 µL) for analysis (Kern, 2001). The analyses could then be completed within a few seconds and the results printed directly from the machine.

The list of haematological parameters analyzed in this study is presented in Table 4.1.

Table 4.1 Haematological parameters analyzed from the blood samples collected from 72 lions

Parameter	Unit
White blood cell count	10^9/L
Neutrophil (NE)	%
Lymphocyte (LY)	%
Monocyte (MO)	%
Eosinophil (EO)	%
Basophil (BA)	%
Red blood cell count	10^6/uL
Haemoglobin	g/dL
Haematocrit	%
Mean cellular volume	fL
Mean cellular haemoglobin	pg
Mean cellular haemoglobin concentrate	g/dL
Platelet count	10^3/µL
Mean platelet volume	fL

4.3 Statistical analysis

For the differential counts done on the blood smears, the average of the values of the tree smears from each animal was calculated and these values were used as the descriptive statistics in all further calculations.

The following independent variables were considered: location, age and sex of lions. As stated in paragraph 3.7, it was found that location did not significantly affect any of the haematological parameters considered and was therefore removed from the model.

In order to investigate the potential sex and age effects on these values, a two-way ANOVA model with sex (male and female) and age group (0 −1 years, 1−2 years, 2−4 years and > 4 years) was fitted to the various haematological parameters. See Table 2.1 for age and sex distribution.

From each animal, the haematological parameters were determined by both methods (manual-visual and the Ac•T 5diff Haematology Analyzer). The two sets of results were then compared by the ANOVA procedures of the SAS statistical program (Statistical Analysis Software (SAS), version 8.2, Cary, NC, USA).

4.4 Results and discussion

4.4.1 Normal reference ranges for haematological parameters in the African lion (*P. leo*)

The statistical analysis identified several haematological parameters, which were significantly ($P<0.05$) affected by age, sex or the interaction between age and sex. Table 4.2 summarizes the results from the ANOVA model on the haematological parameters for data at all the locations.

The majority of the haematological parameters considered were not significantly affected by neither age or sex of the animal, nor by the age or sex interaction. However, some (mainly individual types of white blood cells, but not the total white blood cell count) were significantly affected ($p<0.05$) by age, sex or both - although the causes of such differences are not clear. The relatively low numbers of animals in some categories may distort the means. Irrespective of the cause, it seems logic that the normal reference values (ranges) will have to take these two factors into consideration. Therefore in Table 4.3 the suggested normal reference values for each group of animals (age and sex) are summarized.

The reference ranges for the differential white cell counts are summarized in Table 4.3.

Table 4.2 ANOVA results (P-values) for haematological parameters in the African lion

Haematological Parameters (Unit)	Factor	Degrees of Freedom for the Error	Degrees of Freedom for independent variables	p-value
Neutrophil (%)	Age Group	58	3	**0.007**
	Sex	58	1	**0.000**
Lymphocyte (%)	Age Group	58	3	**0.003**
	Sex	58	1	**0.005**
Monocyte (%)	Age Group	58	3	0.528
	Sex	58	1	**0.003**
Eosinophil (%)	Age Group	58	3	**0.039**
	Sex	58	1	0.805
Basophil (%)	Age Group	58	3	0.430
	Sex	58	1	0.525
White blood cell count (10^9/L)	Age Group	58	3	0.174
	Sex	58	1	0.377
Red blood cell count (10^6/uL)	Age Group	58	3	0.478
	Sex	58	1	0.154
Haemoglobin (g/dL)	Age Group	58	3	0.788
	Sex	58	1	0.361
Haematocrit (%)	Age Group	58	3	0.241
	Sex	58	1	0.104
Mean cellular volume (fL)	Age Group	58	3	0.107
	Sex	58	1	0.695
Mean cellular haemoglobin (pg)	Age Group	58	3	0.438
	Sex	58	1	0.824
Mean cellular haemoglobin Concentrate (g/dL)	Age Group	58	3	0.204
	Sex	58	1	0.934
Platelets (10^3/µL)	Age Group	56	3	**0.007**
	Sex	56	1	0.707
Mean platelet volume (fL)	Age Group	56	3	0.051
	Sex	56	1	0.556

*Indicated in bold are all the p-values <0.05

Table 4.3 Reference value ranges for the differential white blood cell counts of 72 African lions (*P. leo*) of different ages and both sexes

Laboratory Parameter (Unit)	Age group	Sex	Reference Range	
			Lower Limit	Upper Limit
Neutrophil (%)	1	Male	65.0	86.3
		Female	64.7	80.0
	2	Males	62.3	85.7
		Females	57.3	79.0
	3	Males	67.0	80.0
		Females	62.3	76.0
	4	Males	79.0	88.0
		Females	76.0	84.0
Lymphocyte (%)	1	Male	9.7	28.7
		Female	11.7	27.7
	2	Males	7.3	32.7
		Females	17.3	69.7
	3	Males	10.0	19.7
		Females	15.7	21.0
	4	Males	5.7	12.3
		Females	7.0	16.3
Monocyte (%)	1-4	Male	1.3	6.3
		Female	1.6	9.3
Eosinophil (%)	1	Males	0.7	4.3
		Females	1.0	6.3
	2	Males	1.7	7.7
		Females	1.0	5.3
	3	Males + Females	1.3	9.3
	4	Males + Females	0.7	9.7

Age group 1: <1 year old; Age group 2: 1 – 2 years old; Age group 3: 2 – 4 years old and Age group 4: >4 years old

For those age groups for which the differential white blood cell count presented similar results (P>0.05), the reference values (range) are presented for the combined age groups in Table 4.3.

Table 4.4 Reference ranges for haematology parameters of African lion (*P. leo*) independent of age and/or sex

Haematological Parameters (Unit)	Reference Range	
	Lower Limit	Upper Limit
White blood cell count (10^9/L)	7.9	17.6
Basophil (%)	0.0	2.0
Red blood cell count (10^6/µL)	6.3	9.8
Haemoglobin (g/dL)	5.4	20.5
Haematocrit (%)	29.2	45.6
Mean cellular volume (fL)	43.0	50.0
Mean cellular haemoglobin (pg)	9.6	22.4
Mean cellular haemoglobin concentrate (g/dL)	20.5	48.1
Mean platelet volume (fL)	7.1	11.2

The reference values (ranges) for the haematology parameters for the African lion (*P. leo*) for which neither ages nor sex or their interaction (age X sex) have a significant effect on the range values are summarized in Table 4.4.

The reference range values (upper and lower limits) for blood platelets for different age groups of lion, regardless of sex are shown in Table 4.5.

Table 4.5 Reference ranges for platelet counts for both sexes, but different age groups of the African lion (irrespective of sex)

Haematological Parameters (Unit)	Age group	Reference Range	
		Lower Limit	Upper Limit
Platelets (10^3/µL)	Age 1: Less than 1 year	311	627
	Age 2: between 1 and 2 years	155	593
	Age 3: between 2 and 4 years	182	421
	Age 4: older than 4 years	244	457

A comparison between the mean values obtained in this study and in two other available sources from the literature for lions and one for domestic cats, is depicted in Table 4.6.

Table 4.6 A comparison between the mean haematological values obtained from this study with those of two other available sources from the literature for lions and one for domestic cats

Haematological Parameters	This study	ISIS* (1999) #	Wallach & Boever (1983)#	Domestic Cat (Latimer *et al.*, 2003) #
WBC ($x10^3$/µL)	12.71	13.37	12.50	12.50
NE	74.51%	10.000$x10^3$/µL	63.00%	7.50 $x10^3$/µL
LY	17.81%	1.998$x10^3$/µL	30.00%	4.25 $x10^3$/µL
MO	4.29%	0.482$x10^3$/µL	5.00%	0.45 x 10^3/µL
EO	3.60%	0.464$x10^3$/µL	2.00%	0.40 $x10^3$/µL
BA	0.17%	0.088$x10^3$/µL	0.00%	0.10 $x10^3$/µL
RBC ($x10^6$/µL)	8.029	7.86	7.50	7.50
HGB (g/dL)	12.95	13.00	10.00	12.60
HCT (%)	37.37	38.80	-	37.50
MCV (fL)	46.61	49.80	-	47.00
MCH (pg)	15.98	16.70	-	15.00
MCHC (g/dL)	34.29	33.50	-	33.00
PLT ($x10^3$/µL)	355.41	280.00	-	550.00
MPV (fL)	9.11	-	-	15.00

*International Species Information System
Units were conversed according to Duncan & Prasse (2003) to be able to compare with values from this study

From Table 4.6, the means of 8 of the haematological parameters measured in this study, namely white blood cell count (WBC), monocyte count (MO), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean cellular volume (MCV), mean cellular haemoglobin (MCH) and mean cellular haemoglobin concentrate (MCHC), compares favourably with the published values. For the values that do not compare well, more than one reason could be applicable. The single most important one could be the differences in group sizes. Another reason might be the health status of the lions used for the values published in the literature, as well as the management practices under which they are kept, which are both unknown. It would be very difficult to speculate regarding the reasons for the observed differences.

More research with a large number of specimens (lions) is needed to clarify these differences.

Wallach and Boever (1983) claimed that haematology reference values for the domestic cat can be used to interpret blood results for the African lion. However from Table 4.6 this assumption could only be partially confirmed. There is a need for further research and reliable statistical proof before this claim could be accepted.

The results of the manual-visual cell count method can be seen in Table 4.7.

Table 4.7 Mean (±SD) values for the differential white blood cell counts of African lions (*Panthera leo*) determined by the manual-visual method

Laboratory parameter	Number of animals	Mean ± SD (%)
Neutrophils	72	74.5 ±15.4
Lymphocytes	72	17.8 ±32.0
Monocytes	72	4.3 ±4.0
Eosinophils	72	3.6 ±7.5
Basophils	72	0.2 ±0.9

The results of the Ac•T 5diff Haematology Analyzer are presented in Table 4.8.

When the manual method is evaluated, it is a very time consuming method, since the operator has to sit for hours on end, counting the individual white blood cells. On the other hand, it is very accurate. All the different kinds of white blood cells can be accounted for and differentiated from one another. This is an important aspect, especially in the light of the fact that the Ac•T 5diff Haematology Analyzer has not yet been properly calibrated for any other blood than those of humans. This may cause to identify individual cells incorrectly for the differential cell count. If the Ac•T 5diff Haematology Analyzer could be calibrated, it would make the differential cell count easier and more accurate, as the present human error would no longer have an effect on the results.

Table 4.8 Mean (±SD) values for the differential white blood cell counts determined by the Ac•T 5diff Haematology Analyzer

Haematological parameters	Number of animals	Mean ± SD
WBC ($10^3/\mu L$)	72	12.71 ±6.60
Neutrophils (%)	47	33.34 ±17.90
Neutrophils ($10^3/\mu L$)	47	3.99 ±2.91
Lymphocytes (%)	72	53.57 ±30.91
Lymphocytes ($10^3/\mu L$)	72	6.64 ±4.80
Monocytes (%)	68	4.29 ±3.20
Monocytes ($10^3/\mu L$)	68	0.53 ±0.98
Eosinophils (%)	50	3.08 ±10.38
Eosinophils ($10^3/\mu L$)	50	0.38 ±1.45
Basophils (%)	72	0.24 ±0.32
Basophils ($10^3/\mu L$)	72	0.03 ±0.03

Table 4.9 A comparison between the differential white blood cell counts were determined manually and the differential counts using the Ac•T 5diff Haematology Analyzer

Laboratory parameter	Ac•T 5diff Haematology Analyzer		Manual differential count	
	N	Mean (%)	N	Mean (%)
Neutrophils	47	33.343	72	74.509
Lymphocytes	72	53.567	72	17.806
Monocytes	68	4.293	72	4.292
Eosinophils	50	3.084	72	3.602
Basophils	72	0.244	72	0.167

The Ac•T 5diff Haematology Analyzer from Beckman Coulter was introduced in 2000 and designed to do a full leukocyte 5 – part differential cell count (Simón-López,

2001). This was done as an improvement to the other analyzers available at that time, since they could only do a complete blood count. It uses a very small blood volume (30 μl for a complete blood count and 53 μL blood for a complete blood count and differential cell count).

According to the instruction manual, the Ac•T 5diff Haematology Analyzer uses the size of the cells and the number and size of the granules inside the cells to differentiate between the different types of white blood cells. As the machine has only been calibrated for human blood, and the size and granule content of lion blood cells is still unknown, this was an anticipated problem. If the results of the differential count done by the Ac•T 5diff Haematology Analyzer are compared with the results of the visual-manual count (Table 5.3), the difference is obvious for certain cell types. In an attempt to explain this phenomenon, blood smears of human blood were compared to those of lions' blood. It seemed as if the lion red blood cells are smaller than those of humans. That could cause the Ac•T 5diff Haematology Analyzer not to be able to distinguish clearly between the lion red blood cells and platelets. It also seemed as if there is only a small difference in the number of granules in neutrophils and lymphocytes. This could once again be a problem when the Ac•T 5diff Haematology Analyzer has to distinguish between the neutrophils and the lymphocytes to be able to count them properly. This would contribute to an inaccurate result, as the Ac•T 5diff Haematology Analyzer cannot clearly differentiate between specific types of white blood cells in the African lion (*P. leo*).

According to Olsen and Burns (1972), the neutrophils should always be approximately 70-75% of the total white cell count, with the lymphocytes approximately 20-25%. With that in mind, it seems as if the Ac•T 5diff Haematology Analyzer just had it the wrong way round. That could be as a result of the number of granules inside the cells being different from those in humans. As previously mentioned, the Ac•T 5diff Haematology Analyzer is only calibrated for human blood and it could be expected that it would read, interpret and count the lion cells as if they were human. When Table 4.9 is studied, it is easy to see that there is still some work in terms of calibration needed before the Ac•T 5diff Haematology Analyzer could be used to accurately analyze lion blood.

4.5 Conclusions

This study generated reasonably accurate reference range haematological values for different parameters in African lions (*P. leo*) of different age groups and sexes. In general the results obtained are in line with those previously reported in the literature. Due to the number of specimens (subjects) involved, it can be considered that this study contributed substantially towards confirming and establishing a reliable set of reference values for the different haematological parameters for the African lion. Nevertheless there is still substantial work to be done before a final set of haematological reference values for the African lion could be established. Many more lions will have to be bled and their blood cell profiles analyzed to increase the data base, before a more reliable set of reference values can be established for the African lion (*P. leo*).

5. Reference values for morphometrical parameters in the African lion *(Panthera leo)* bred in captivity

5.1 Introduction

Captive lion breeding in South Africa has grown rapidly over the past few decades. However, most husbandry practices used have evolved empirically with little scientific support. Available literature regarding lion breeding or ranching is scanty. It is therefore important to gain as much information as possible regarding the normal growth performance of lions in captivity, in order to monitor and evaluate the management (particularly nutrition) under which they are reared.

In order to monitor the nutritional management of a particular group of lions, it is important to have reference growth curves and reference values for several morphological variables. If for instance it is known what a lion of a certain age and sex is supposed to weigh or measure, it is possible to evaluate the effectiveness of the overall management provided to the animals by comparing its performance (weight and particular measurements) with the set of reference values in question. If high correlations exist between body weight and certain morphometrical variables, it is possible to use easier and more accurate measurements to estimate body weight under field conditions. Practical interventions such as medication and nutritional supplementation needed can be calculated more accurately.

The aim of this part of the study was to determine a set of morphometrical reference values (for age and sex) of lions and to analyze possible correlations between certain relevant measurements (*i.e*. with body weight). Furthermore, to explore the possibility to use easier measurable variables to estimate body weight accurately.

5.2 Materials and Methods

Seventy two African lions of different ages and of both sexes were immobilized with Zoletil® 100 (50 mg Tiletamine HCl and 50 mg Zolazepam HCl / ml; VIRBAC) at a dose of 4 to 5 mg/kg body weight by means of an intramuscular injection administered with the aid of a darting gun or a syringe, depending on the age of the

animal. It took approximately 10-20 min for the animals to be fully immobilized. As soon as it was safe to handle the animals, their eyes were covered, and they were moved on the back of a pickup truck, to a nearby place in the shade. While the animals were immobilized under the effect of the Zoletil® 100, the full range of morphometrical measurements were taken as described by De Waal *et al*. (2004). Details on the management conditions of the lions at the different sites and the detailed information regarding the chemical immobilization and the methodology used to measure or determine the body measurements considered in this study are presented in Chapter 2 (Materials and Methods).

5.3 Results and Discussion

5.3.1 Body measurements

When the data for body measurements were analysed, sex and location of the lion did not show any significant effect on body measurements, but age yielded significant differences ($P<0.05$). Therefore, the results (mean ± standard deviation) for males and females from all four sites were combined and presented in Table 5.1, taking into account the four age groups considered in this study (Chapter 2, Materials and Methods).

5.3.2 Growth curves for male and female African lions

A set of 47 different body measurements were taken from each of the 72 African lions used in this study and analysed statistically. Growth curves for both sexes were established, but for the purpose of this study, only the most relevant and practical body measurements (26) are reported (Table 5.1). The growth curves for body weight and length, as well as their limits (maximum and minimum) for female and male lions, respectively, fitted with the Emax model are presented in Figures 5.1 and 5.2. In Figures 5.3 and 5.4 the growth curves for head length as well as their limits (maximum and minimum) for female and male lions, respectively, fitted with the Emax model are presented. The individual non-linear least squares estimates of Emax model parameters for the body measurements including body weight and head length can be found in Table 5.1 and all other data and predicted intervals are presented in figures of Appendix 2.

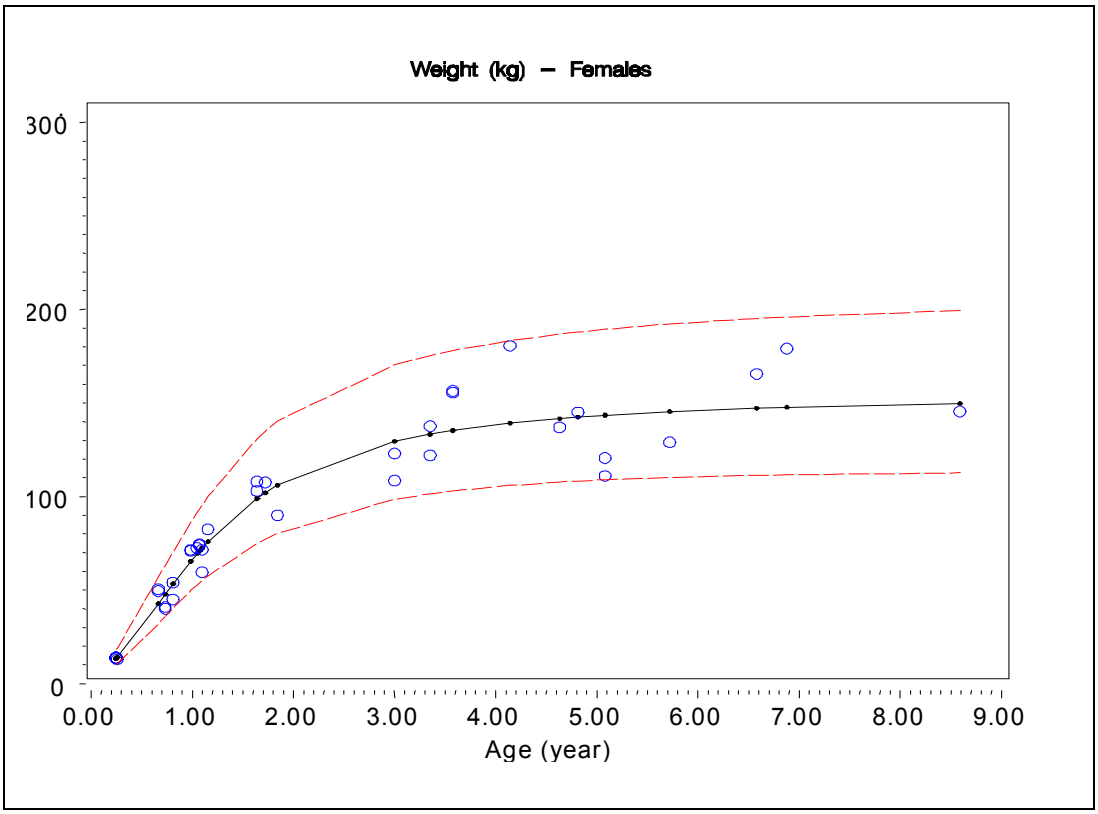

Figure 5.1 Body weight growth curve and normal reference range values for female African lions from 3 months to 9 years of age

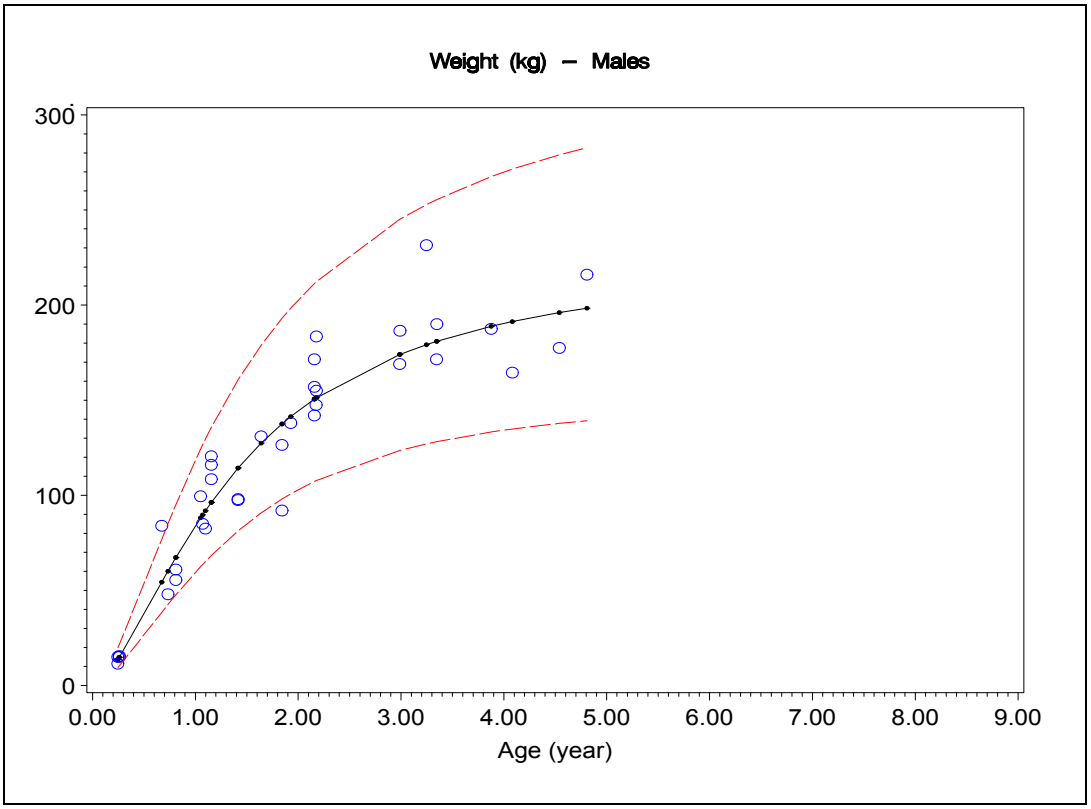

Figure 5.2 Body weight growth curve and normal reference range values for male African lions from 3 months to 5 years of age

Table 5.1 Body measurements (Mean ±SD) recorded of 72 African lions (*P. leo*) of both sexes and different age groups

Measurement	Unit	n	< 1 year	1 – 2 years	2 – 4 years	> 4 years
Body weight	kg	72	39.10±23.50fs	97.10±23.50	160.90±29.60	155.90±30.10
Tail circumference	mm	70	168.30±35.90	225.40±23.40	251.80±16.60	245.00±21.60
Tail length	mm	72	536.70±175.70	784.60±74.30	878.90±70.30	806.40±65.50
Chest girth	mm	71	701.20±150.10	992.60±88.20	1189.60±101.50	1201.80±105.50
Abdominal girth	mm	69	706.40±141.90	991.50±92.20	1141.60±134.30	1186.20±116.80
Body length	mm	72	1608.30±443.20	2316.50±169.50	2728.30±192.10	2619.90±162.40
Head length	mm	72	202.00±44.20	285.90±27.60	357.40±23.60	346.80±28.00
Head width	mm	72	139.70±32.40	197.20±19.10	246.70±14.70	235.90±18.60
Front leg length: Left	mm	72	359.20±99.93	519.50±42.62	591.40±41.31	566.80±30.77
Front leg circumference: Left	mm	70	257.10±61.87	344.70±31.10	400.10±36.97	382.10±38.40
Front leg length: Right	mm	72	360.80±98.89	517.70±38.12	596.50±35.79	573.00±33.68
Front leg circumference: Right	mm	69	257.20±61.05	339.40±29.40	390.60±35.57	379.20±38.77
Front paw length: Left	mm	72	80.60±17.94	106.50±9.15	114.40±9.99	110.90±8.35
Front paw width: Left	mm	72	77.10±16.11	97.30±9.76	106.70±7.44	102.90±7.57
Front paw length: Right	mm	72	80.40±16.60	105.60±8.07	113.10±6.79	114.70±11.75
Front paw width: Right	mm	72	77.50±15.67	98.30±6.99	109.70±6.15	103.40±8.45
Hind foot length: Left	mm	72	253.00±65.89	342.20±20.26	367.80±20.11	347.30±15.54
Hind foot length: Right	mm	72	257.80±70.91	337.90±20.10	366.20±18.20	346.90±15.98
Hind paw length: Left	mm	72	82.30±18.41	101.80±7.64	110.40±5.24	109.60±7.06
Hind paw width: Left	mm	72	69.20±13.90	88.60±6.94	95.50±8.39	86.80±6.70
Hind paw length: Right	mm	72	80.60±18.34	104.30±6.45	109.10±5.30	107.90±6.58
Hind paw width: Right	mm	72	69.10±12.05	86.40±6.72	93.90±5.73	87.30±8.18
Canine length: Left upper	mm	51*/19$^{\#}$/2$^{@}$	13.50±2.14	24.60±11.58	44.10±6.06	43.10±6.57
Canine length: Right upper	mm	50*/20$^{\#}$/2$^{@}$	13.10±2.28	25.00±10.82	44.50±5.39	45.10±5.43
Canine length: Left lower	mm	55*/17$^{\#}$/0$^{@}$	11.20±1.67	21.30±10.32	36.90±4.06	34.00±7.45
Canine length: Right lower	mm	52*/19$^{\#}$/1$^{@}$	11.40±1.40	22.00±10.88	35.70±5.45	35.10±4.74

* Permanent teeth; $^{\#}$Shedding teeth; $^{@}$Shed teeth not yet replaced

The weight for females and males are plotted separately according to age with the results of the Sigmoid Emax model, using a multiplicative error, given as the mean and both the upper and lower 95% confidence intervals for the predicted value (which serve as the reference ranges).

Figures 5.3 and 5.4 show the growth curves for head length for male and female African lions, respectively. It is clear that the head length (Figure 5.3) and body weight (Figure 5.1) of females stop growing earlier (between 4 and 5 years) than in males (Figures 5.2 and 5.4), reaching a plateau at about five to six years of age.

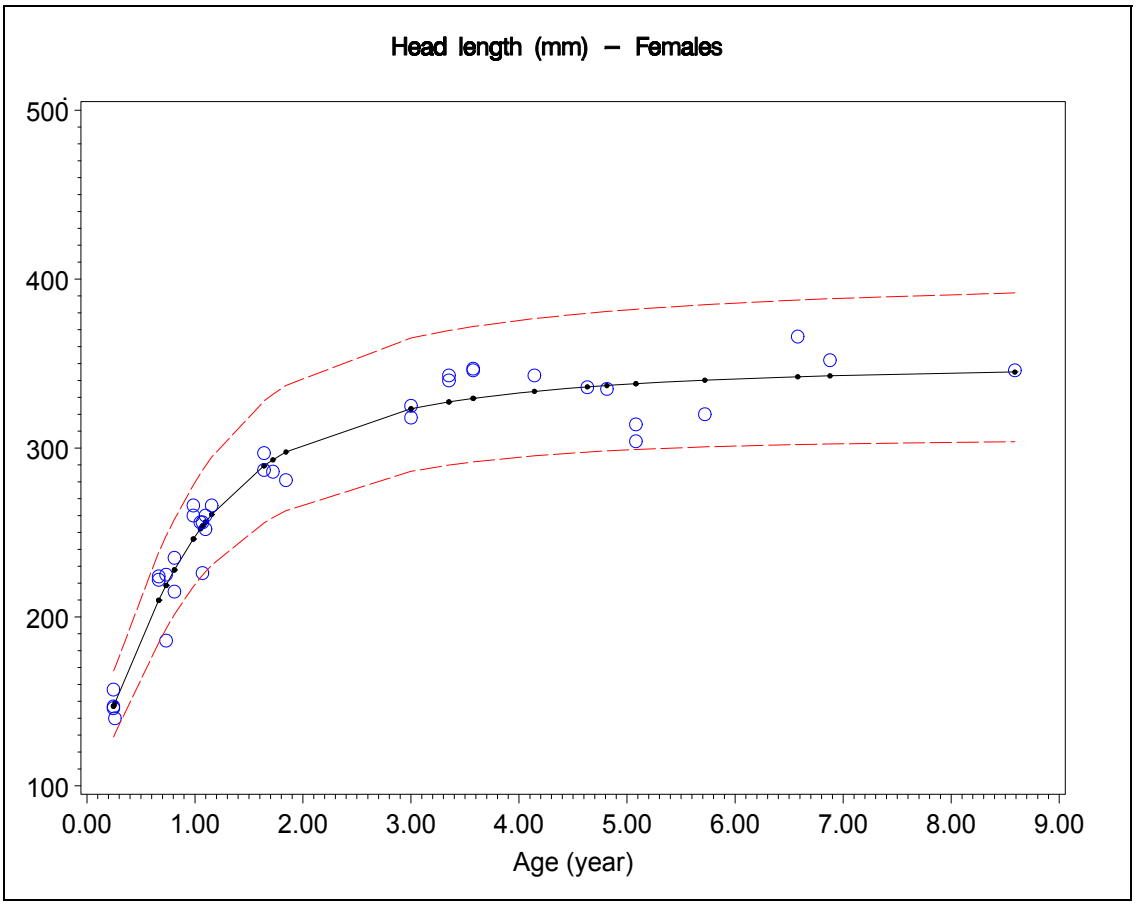

Figure 5.3 Head length growth curve and normal reference range values for female African lion from 3 months to 9 years of age

The same general trend was observed for most parameters considered in this study. The African lion shows strong dimorphism, with males being much larger than females. It is clear from Figure 5.1 that during the first year of life, the female lion grows faster. From the second year the growth rate starts to slow down and from the third year, there is almost no growth reported. Since female lions are the

dominant hunters, they cannot afford to be too heavy and bulky, but they need to be streamline and built for the chase. Males on the other hand (Figure 5.2) grow faster until two years of age and then slower until they are fully grown at about 4 to 5 years of age, compared to female animals. Opposed to the females, they need to be big and strong in order to defend the pride against possible enemies - which are mainly other male lions wanting to take over the control of the pride (Bertram, 1975). Lions are not good parents and the mortality rates among cubs are relatively high (Schaller, 1969). Cubs are mostly dependent on adults for food until at least the age of 16 months, but usually for the first 2½ years of life (Eloff, 1980). It is therefore important that they grow fast enough in order to be sufficiently physically developed, to defend themselves when they have to leave the pride (males) at 2½ to 3 years of age.

In general it was relatively easy to take the set of 47 body measurements considered in this study. The same measurements were taken several times from the same animal and the results revealed a high repeatability, indicating a good accuracy of the techniques suggested by De Waal *et al*. (2004a).

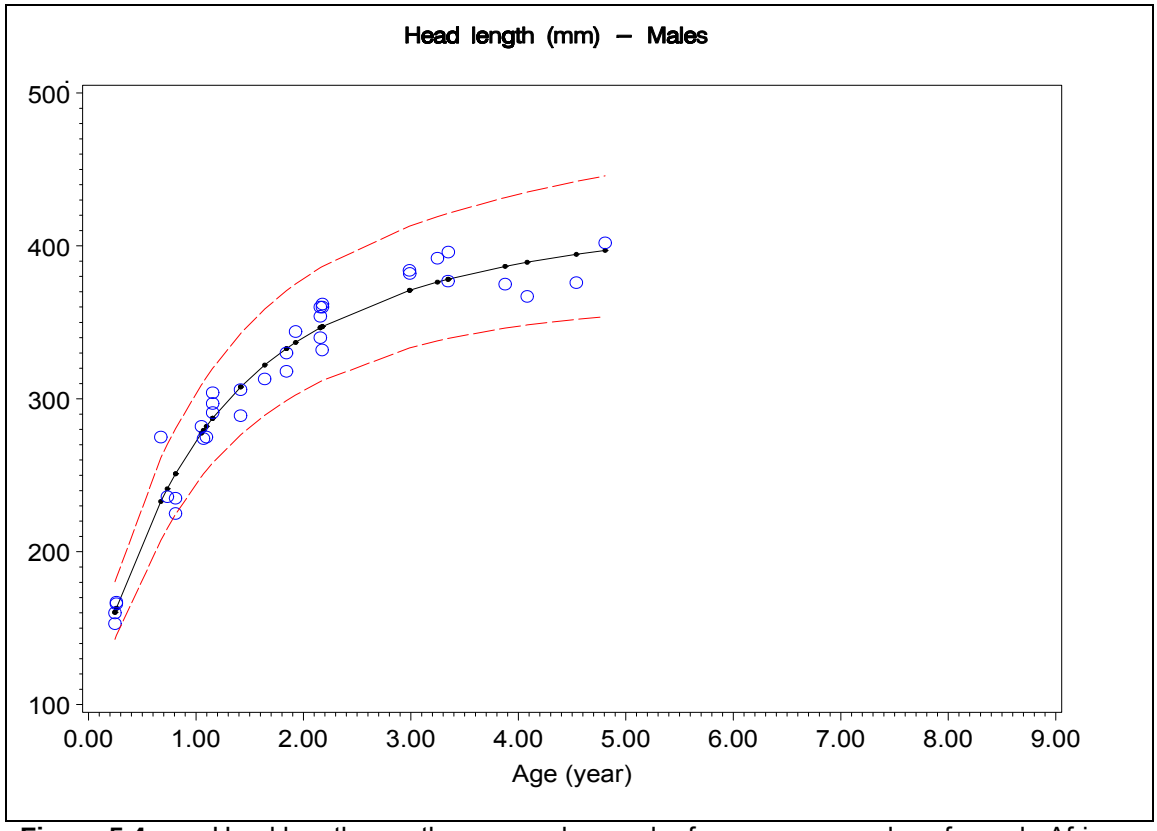

Figure 5.4 Head length growth curve and normal reference range values for male African lion from 3 months to 5 years of age

5.3.3. Correlations between body measurements of African lions (*P. leo*)

The correlation coefficients for the most relevant body measurements measured on 72 African lions (*P. leo*) of different ages and both sexes are summarized in Table 5.2.

A main objective of this study was to determine the correlation coefficients between the most relevant and practical body measurements in lions. The idea was to determine if there are one or more practical and easy methods to take measurements that could be used to help estimate the weight of a lion should a scale not be available to be used in the field. The need also arises to estimate the weight of lions accurately in order to treat or dose it with a medicine or drug, which has to be administered according to body weight. From the data presented in Table 5.2 the head length has the highest correlation (r=0.969) with body weight. Moreover high correlations (r>0.9) were also established between body weight and other body measurements (chest girt, head width and body length). Chest girth is a commonly used measurement to estimate body weight in several livestock species, including cattle (Lundborg *et al.*, 2003) goats (Nsoso *et al*., 2003) and pigs (Iwasawa *et al*., 2004). A high correlation (r=0.88) was also established between body weight and tail circumference (at the base of the tail), in this study.

With these high correlations it is possible to accurately estimate the body weight of lions, without a scale, using practical and easy measurements (i.e. head length) from the lions. Tables can be easily drawn to allow accurate body weight estimations from all these variables mentioned. The reference growth curves obtained in this study provide useful benchmarks to evaluate the nutritional management of lions bred in captivity and certain reference values that can be used to evaluate the availability of prey in the wild.

There was considerable variation in the morphometric variables considered in this study, particularly in those presented in this report. In general, the variation recorded in male lions was greater than in females. Again, this seems to be a result of the sexual dimorphism. It is well known that bigger and stronger males conquer the

rights to mate females (Schaller, 1969). So it seems logic that greater variation in body size is expressed in males to aid in the natural selection of the species. Nevertheless there is also a considerable amount of variation in the morphological variables considered in this study amongst female lions at all ages. The relatively low number of individuals in certain age groups has probably exacerbated this finding.

Table 5.2 Correlation coefficients between the most relevant body measurements of African lions (*P. leo*; N=72)

Measurement 1	Measurement 2	Correlation coefficient
Weight	Head length	0.969
	Chest Girth	0.966
	Head width	0.958
	Body length	0.929
	Tail Circumference	0.880
Tail Circumference	Body length	0.906
	Head width	0.901
	Chest Girth	0.889
	Head length	0.889
Chest Girth	Head length	0.967
	Head width	0.957
	Body length	0.954
Body length	Head width	0.968
	Head length	0.962
Head length	Head width	0.978

Part of this variance should be the result of the nutritional management of the animals. As no significant differences were recorded between sites/ranches, it can be considered that despite the different types of feeding diets and regimens between the four sites used, all seem to be equally effective.

5.4 Conclusions

The African lion (*P. leo*) displays strong sexual dimorphism in its body measurements. Males grow faster and are in general larger and heavier than their female counterparts at all ages. Females seem to grow and reach adult size at 4 to 5 years of age, while males seem to keep growing for about one more year than females (5-6 years of age). High correlations were determined between body weight and a few easy and practical to obtain measurements. Head length showed the highest correlation with body weight, but heart girth, head width and tail base circumference also demonstrated high correlations with body weight in lions of both sexes. With such high correlations it is possible to accurately estimate the body weight of lions without a scale, using practical and easy measurements obtainable with a simple measuring tape from immobilised lions. The reference growth curves obtained in this study provide useful benchmarks to evaluate the nutritional management of lions bred in captivity.

Further research is warranted and more individuals should be measured, whenever an opportunity occurs (as part of routine examination of lions or even after the animals have been hunted) to gather more morphometric data from a larger population and to refine the reference growth range values for African lions bred under different management conditions.

DETERMINATION OF SOME BLOOD PARAMETERS IN THE AFRICAN LION (*Panthera leo*)

by

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Abstract

The goal of this study was to generate a database of laboratory results for African lion (*Panthera leo*) blood to obtain reliable reference ranges to augment what is currently available in literature. Also to investigate the possibility of age and sex having an influence on these reference ranges.

The specific objectives of this study were:

- to determine reference values for haematological and biochemical blood variables for lions bred in captivity, as a function of age and sex;
- to evaluate the Beckman Coulter Ac•T 5diff Haematology Analyzer for lion differential white blood cell analyses;
- to determine morphometric measurements and establish reference growth curves (and range reference values) for lions bred and reared in captivity as a function of age and sex;

- to determine reference values for some practical and meaningful body measurements and their correlations.

This study was conducted on three lion ranches in the Free State province and at the Bloemfontein Zoological Gardens (Bloemfontein Zoo) with captive lions (*Panthera leo*) of both sexes and ages ranging from three months to nine years. Lions were divided into four age groups according to published literature.

Animals were chemically immobilized (darted) with Zoletil® 100 at 4 to 5mg/kg in their holding camps and moved to a shaded place as soon as the drug had taken its full effect. Blood was collected into three different types of blood collection tubes and body measurements were taken. This was all done as fast as possible before the effect of the immobilizing drug could wear off. In some cases it was necessary to give an animal a top-up dose to prevent it from waking up too quickly. Animals were moved back to their holding camps to fully recover from the immobilization.

Blood analyses done with the Ac•T 5diff Haematology Analyzer from Beckman Coulter® for haematological parameters was conducted within 30 minutes after blood collection. Blood for biochemistry parameters was centrifuged, serum collected and cryo preserved at -20°C until it could be taken to the laboratory for analyses. Blood smears were made on the lion ranches and Bloemfontein zoo immediately after the analysis with the Ac•T 5diff Haematology Analyzer, fixed and packed for transport to the laboratory.

At the laboratory the serum was used for biochemistry analyses, using standard laboratory techniques. Blood smears were stained and examined under a light microscope for the differential white blood cell count by means of the manual-visual method.

Results were statistically analyzed to determine reference ranges and the influence of age and sex on these reference range values for the different parameters, were considered.

Body measurement were also statistically analyzed to determine correlations between body weight and different other measurements. These correlations were then used to determine if it will be possible in a field situation to use the age and sex of an animal together with a certain body measurement to estimate body weight accurately, if actual weighing was not possible.

From these analyses it was concluded that age and sex do have an influence on blood analysis and blood reference ranges for the African lion (*Panthera leo*). Unfortunately, it differs between parameters and there is not one rule to apply. The conclusion could also be made that body weight could be determined by measuring the head length of an animal. More research is warranted to obtain more data set and establish range reference values that can be validated and used with a high degree of confidence in the lion breeding industry.

References

Archer, R.K., 1965. Haematological techniques for use on animals, Blackwell Scientific Publications, Oxford.

Bertram, B.R., 1975. The social system of lions. *Scientific American* 232, 54-65.

Badylak, S.F. & Van Vleet, J.F., 1981. Sequential morphologic and clinicopathology alteration in dogs with experimentally induced glucocorticoid heptopathy. *American Journal of Veterinary Research* 42, 1310-1318.

Bain, B.J., 1995. Blood cells, a practical guide, 2nd ed, (edited by Bain, B.J.). Blackwell Scientific Publications, Oxford.

Bauer, H. & Van der Merwe, S., 2004. Inventory of free-ranging lions *Panthera leo* in Africa. *Oryx* 38(1), 26-31.

Blood, D.C. & Radostis, O.M. 1989. Veterinary Medicine. A textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 7th ed. p501.

Borstlap, D.G., 2002. Intake and digestibility studies with captive African lions (*Panthera leo*), leopards (*Panthera pardus*) and cheetahs (*Acinonyx jubatus*). M.Sc. (Agric.) thesis, University of the Free State, Bloemfontein, South Africa.

Bothma, J. du P., 2000. Wildplaasbestuur, 3rd ed. Van Schaik Publishers, Pretoria, p159-161; pp288-316.

Brody, S., 1964. Bioenergetics and growth, Hafner Publishing Company Inc., New York.

Burtis, C.A. & Ashwood, E.R., 1999. Tietz Textbook of Clinical Chemistry, 3rd ed. Burtis, C.A. & Ashwood, E.R., Saunders, St. Louis, Elsevier.

Bush, B.M., 1991. Interpretation of laboratory results for small animal clinicians, Blackwell Science, Marston Book Services Ltd., Oxon.

Campbell, H. & Harthoorn, A.M., 1963. The capture and anesthesia of the African Lion in his natural environment. *The Veterinary Record* 75(10), 275-276.

Clampitt, R.B. & Hart, R.J., 1978. The tissue activities of some diagnostic enzymes in ten mammalian species, pp607-621.

D'Agostino, V., 1974. Bronx Zoo, N.Y., In: Altman, P.L. & Dittmer, D.S. Biology Data Book, 2nd ed. Volume III. *Federation of the American Society for Experimental Biology*. Bethesda, Maryland, pp1751-2041.

Davson, H. & Segal, M.B., 1975. Introduction to physiology, Vol. 2. Academic Press Inc. (London) Ltd. and Grune & Stratton Inc., New York.

De Waal, H.O., 2007. Conservation and utilization of large predators. Member of the Executive Council Game Industry Indaba. Department of Tourism, Environmental and Economic Affairs. Vista Campus, University of the Free State, Bloemfontein, 15 February 2007.

De Waal, H.O., Combrinck, W.J. & Borstlap, D.G., 2004a. A comprehensive procedure to measure the body dimensions of large African predators with comments on the repeatability of measurements taken from an immobilized African lion (*Panthera leo). J. Zool., Lond*. 262, 393-398.

De Waal, H.O. & Combrinck, W.J., 2004. Application of a comprehensive procedure to measure the body dimensions of large African predators. *Game and Wildlife Science* 21(4), 797-810.

De Waal, H.O., Osthoff, G., Hugo, A., Myburgh, J. & Botes, P., 2004b. The composition of African lion (*Panthera leo*) milk collected a few days postpartum. *Zeitschrift für Säugetierkunde* 69(6), 375-383.

Elof, F.C., 1980. Cub mortality in the Kalahari lion (*Panthera leo vernayi*) *Kudu* 3*,* pp163-170

Eulenberger, K., 1979. Zur Bedeutung der Bestimmung des Serumeisenspiegels für die klinisch-chemische Krankheitsdiagnostik und zur Kontrolle der Aufzucht bei Grosskatxen. *XXI Internationales Symposium über die Erkrankungen der Zootiere, Malhouse,* 1979. Akademie Verlag, Berlin, DDR*.,* pp151-157.

Eulenberger, K., 1981. Zur Bedeutung der Bestimmung der Kreatininkonzentration in Blutserum für Frühdiagnostik der Kreatinin-funktionsstörungen bei Zootieren unter besonderer Berücksichtigung der Feliden. XIII *Internationales Symposium über die Erkrankungen der Zootiere. Halle/Saale* 1981. Akademie Verlag, Berlin, DDR., pp327-335.

Fowler, M.E., 1978. Zoo and wild animal medicine. W.B. Saunders, Philadelphia.

Friedmann, Y. (editor), 2003. Black Wildebeest (*Connochaetes gnou*) Hybridisation Action Plan. *Final Workshop Report.* Conservation Breeding Specialist Group (SSC/IUCN) / CBSG Southern Africa, Endangered Wildlife Trust.

Furstenburg, D., 1999. Wild en Jag Vol. 5, p11.

Gabrielsson, J. & Weiner, D., 2000. Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts & Applications, 3rd ed. Gabrielsson, J. and Weiner, D., Swedish Pharmaceutical Press, Stockholm.

Harris, E.K. & Boyd, J.C., 1995. Statistical basis of reference values in laboratory medicine statistics: textbooks and monographs, New York. Marcel Dekker Inc. (146).

Hookey, L., Dexter, D. & Lee, D.H., 2001. The use and interpretation of quantitative terminology in reporting of red blood cell morphology. *Laboratory Haematology* 7, 85-88.

Houwen, B., 2001. The differential cell count. *Laboratory Haematology* 7, 89-100.

Immelman, A. (editor), 1996. Index of Veterinary Specialities Desk Reference (IVS) Vol. 3, 7-11.

ISIS, 1999. Physiological Reference Values. International Species Information System www.worldzoo.org.

Iwasawa, T., Young, M.G., Keegan, T.P., Todach, M.D., Dritz, S.S., Goodband, R.D., DeRouchey, J.M. & Nelssen, J.L., 2004. Comparisson of heart girt or flank-to-flank measurements for predicting sow weight. *Swine Day* 2004.

Keep, M.E., 1973. Factors contributing to a populations crash of Nyala in Ndumu Game Reserve. *The Lammergeyer* 19, 16-23.

Kern, C., 2001. An evaluation of the Beckman Coulter Compact 5-part Differential Haematology Analyzer Ac•T5diff. *Laboratory Haematology* 7, 125-129.

Kritchevsky, D., 1958. Cholesterol. John Wiley and Sons, New York.

Kuntze, A. & Hunssdorff, P., 1976. Hämatologische Befunde und Serum-elektrolytwerte (Na, K, Ca, Mg, P) bei kranken und gesunden Grosskatzen (Löwe, Tiger, Leopard, Puma). *XVIII Internationales Symposiums über die Erkrankungen der Zootiere. Innsbruck,* 1976, Akademie Verlag, Berlin, DDR, pp353-359.

Labuschagne, R.J., 1955. Our National Parks, Hayne & Gibson, Dagbreekpers Ltd., 28 Height Street, Johannesburg

Latimer, K.S., Mahaffey, E.A. & Prasse, K.W., 2003. Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology, 4th ed. Iowa State Press, Iowa, pp1-193; 331-342.

Lawrence, T.L.J., 1980. Growth in animals, Butterworths, London, Boston.

Lundborg, G.K., Oltenacu, A., Maizon, D.O., Svensson, E.C. & Liberg, P.G.A., 2003. Dam-related effects on heart girt at birth, morbidity and growth rate from birth to 90 days of age in Swedish dairy calves. *Preventive veterinary medicine* 60, 175-190.

Loeb, W.F. & Quimby, F., 1989. The clinical chemistry of Laboratory animals, 1st ed. Pergamon Press, New York, pp179-260.

McKee, T. & McKee J.R., 1996. Biochemistry, an introduction. Wm.C. Brown Publishers.

Meissner, H.H., 1982. Classification of farm and game species to predict carrying capacity. *Farming in South Africa, Wool production,* Vol. 3, 138-145.

Melton, D.A., Berry, H.H., Berry C.U. & Joubert, S.M., 1987. Aspects of the blood chemistry of wild lions, *Panthera leo*. *Suid-Afrikaanse Tydskrif vir Dierkunde*, 22(1), 40-44.

Morse, R.W. & Follis, T.B., 1974. Physiologic normals – research in lions. In: The world's cats, Vol. 2. Eds. Eaton, R.L., Feline Research Group, Seattle.

Nason, A., 1968. Essentials of modern biology. John Wiley & Sons, INC, New York.

National Agricultural Marketing Council, 2006. Report on the investigation to identify opportunities and address problems for sustainable growth and development in South African wildlife ranching. *Research Report* 2006-03. 8 August 2006. National Agricultural Marketing Council, Pretoria.

Needham, A.E., 1964. The growth process in animals. Sir Isaac Pitman & Sons Ltd, London.

Nsoso, S.J., Aganga, A.A., Moganetsi, B.P. & Tshwenyane, S.O., 2003. Body weight, body condition score and heart girt in indigenous Tswana goats during the dry and wet seasons in southeast Botswana. *Livestock Research for rural development* (15) 4, article 5.

O'Neil, P., Vital, E., Betancourt-Loria, N. & Montes, D., 2001. Performance evaluation of the complete blood count and white blood cell differential parameters on the Ac•T 5diff Haematology Analyzer. *Laboratory Haematology* 7, 116-124.

Olsen, R.E. & Burns, K.F., 1972. Federation of American Society for Experimental Biology. In: Altman, P.L. & Dittmer, D.S., Biology Data Book, 2nd ed., Volume III. Bethesda, Maryland, pp1751-2041.

Pienaar, U. de V., Le Rich, E. & Le Roux, C.S., 1969. The use of drugs in the management and control of large carnivorous mammals. *Koedoe* 12, 177-183.

Pospíšil, J., Špála, P., Váhala, J. & Kaše, F., 1987. Biochemical values in the blood serum of some felids kept in the East-Bohemian Zoological Garden at Dvθr Králové Nad Labem. *Acta Vft. Brno* 56, 343-360.

Ross, J.S. & Wilson, K.J.W., 1981. Foundations of anatomy and physiology, 5th ed. Churchill Livingstone, London, England.

Roth, V.L. & Mercer, J.M., 2000. Morphometrics in development and evolution. *American Zoologist* 40(5), 801-810.

Schaller, G.B., 1969. Life with the king of the beasts. *National Geografic Magazine* 135(4), 494-519.

Seal, U.S., Plotka, E.D. & Gray, C.W., 1978. Baseline haematology, serum chemistry and hormone data for captive tigers (*Panthera tigris*) and lions (*Panthera leo*). *International Tiger Studbook Congress. Proceedings of the 1st International*

Symposium on the Management and Breeding of the tiger. Zoologischer Garten Leipzig, pp174-191.

Simón-López, R. 2001. Introducing 5-part differential instruments in low- to moderate-volume laboratories. *Laboratory Haematology* 7, 113-115.

Smuts, G.L., Robinson, G.A. & Whyte, I.J., 1980. Comparative growth of wild male and female lions (*Panthera leo). J. Zool. Soc., London*, pp365-373.

Swatland, H.J., 1984. Structure and development of meat animals, Prentice-Hall, INC., Englewood Cliffs, New Jersey.

Undritz, E., 1973. Sandoz Atlas of Haematology, 2nd ed. SANDOZ, Sandoz, Basle.

Wallach, J.D. & Boever, W.J., 1983. Diseases of exotic animals, medical and surgical management. Blackwell Science, Marston Book Services Ltd., Boston, pp345-403.

Ward, E.E., 2002. Feline Anemia, www.allergicpet.com/articles/feline_anemia.html

Yamaguchi, Nobuyuki, 2002. Practical demonstration: measuring the skull of a lion. *In:* De Waal, H.O. (ed.). *Proceedings of the 2nd Meeting of the African Lion Working Group.* 9 to 10 May 2002. Willem Pretorius Game Reserve, Free State Province, South Africa. Brandhof South Africa, pp157-161.

Appendix 1

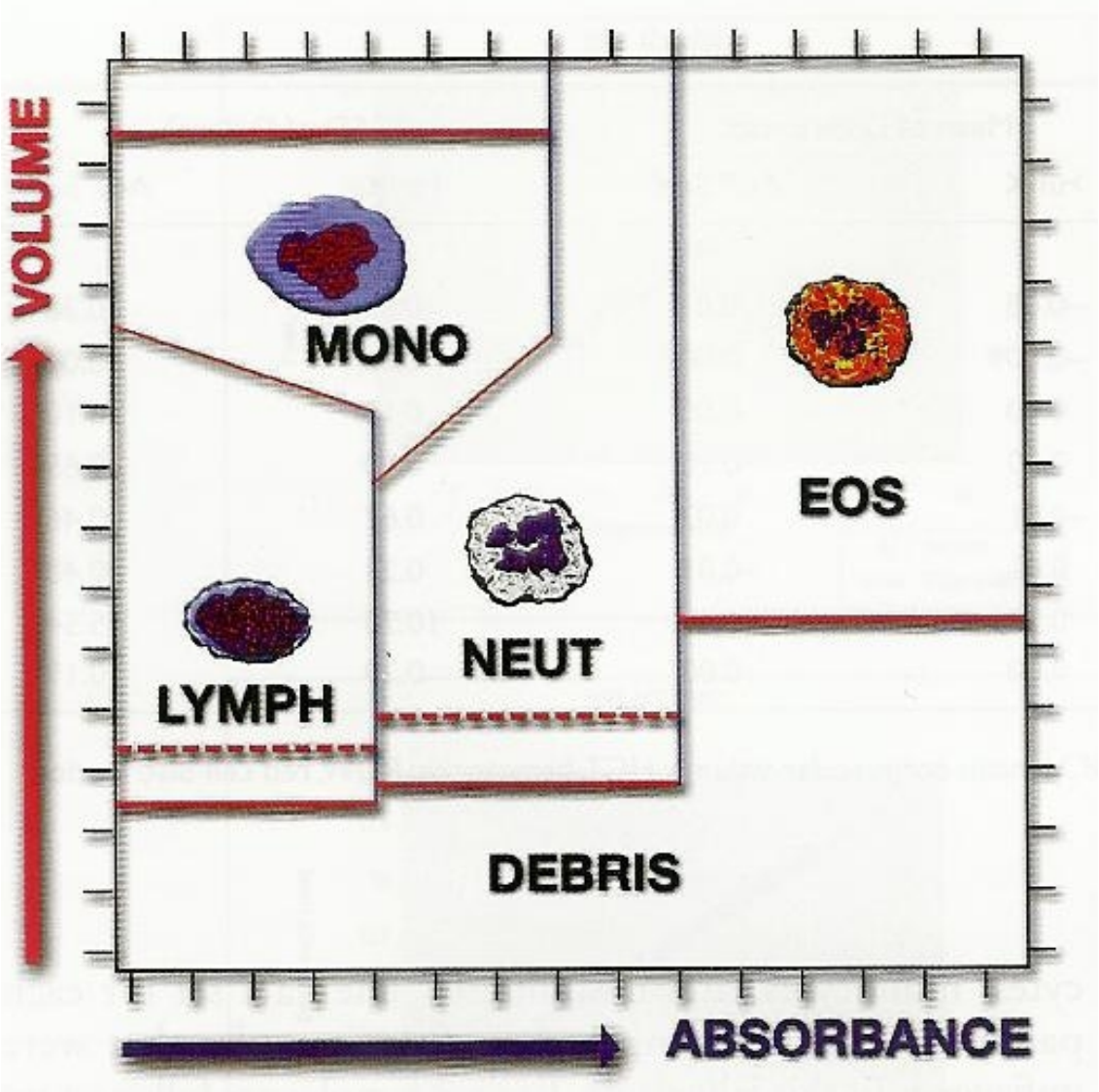

Figure 1a A schematic representation of the sites of accumulation of different types of human blood cells origin in a DiffPlot (O'Neil *et al*., 2001)

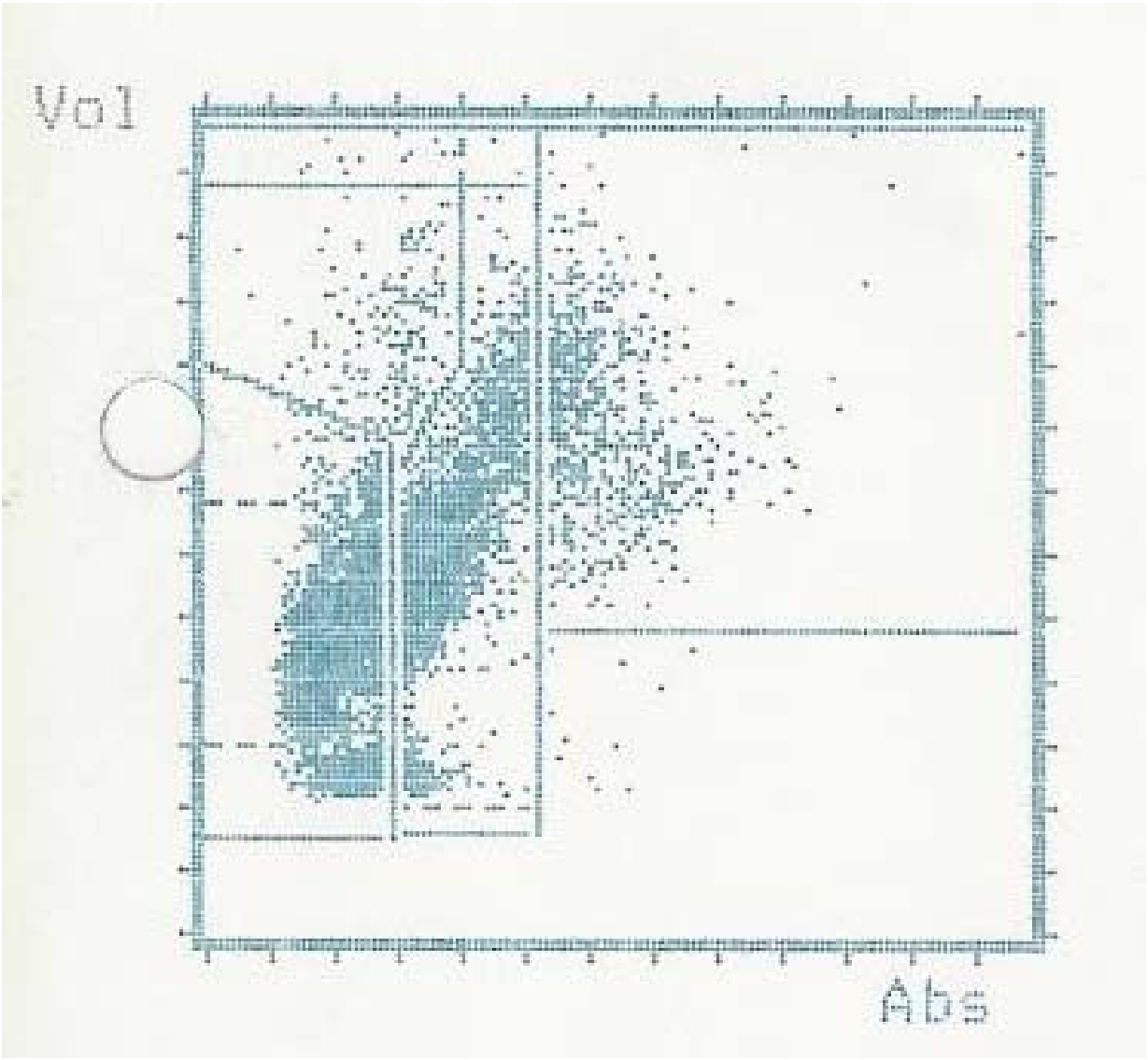

Figure 1b The DiffPlot for one of the lions as seen on the computer printout from the Ac•T 5diff Haematology Analyzer in this study

Appendix 2

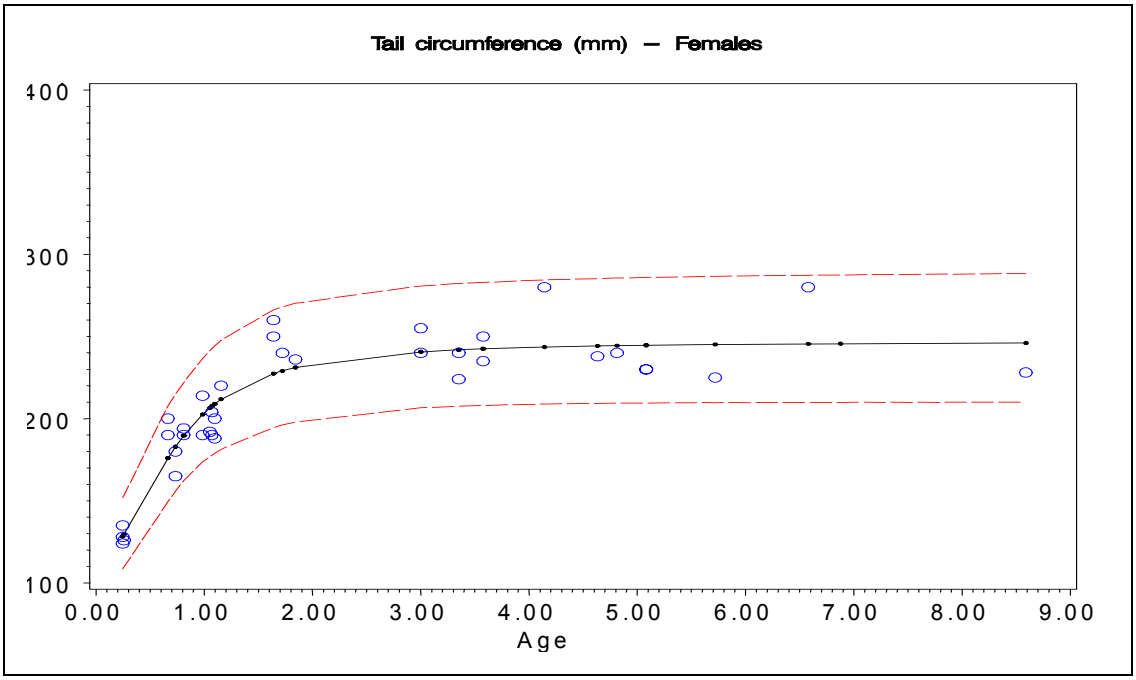

Figure 2a Tail circumference growth curve and normal reference range values for female African lions from 3 months to 9 years of age

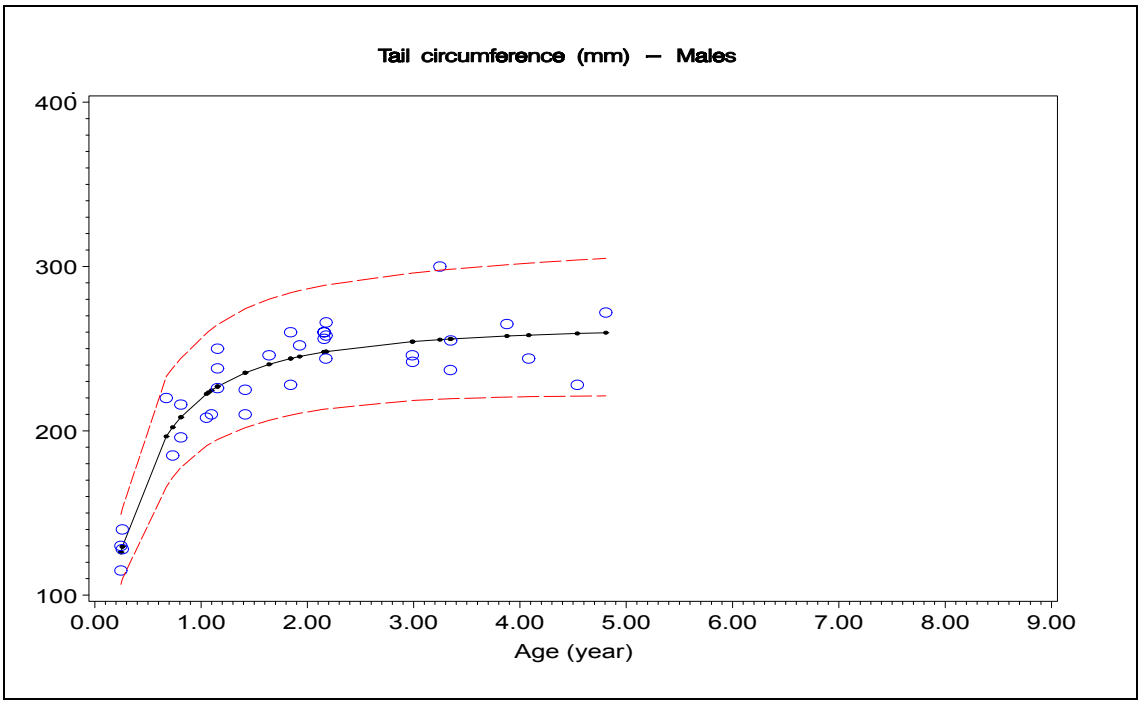

Figure 2b Tail circumference growth curve and normal reference range values for male African lions from 3 months to 5 years of age

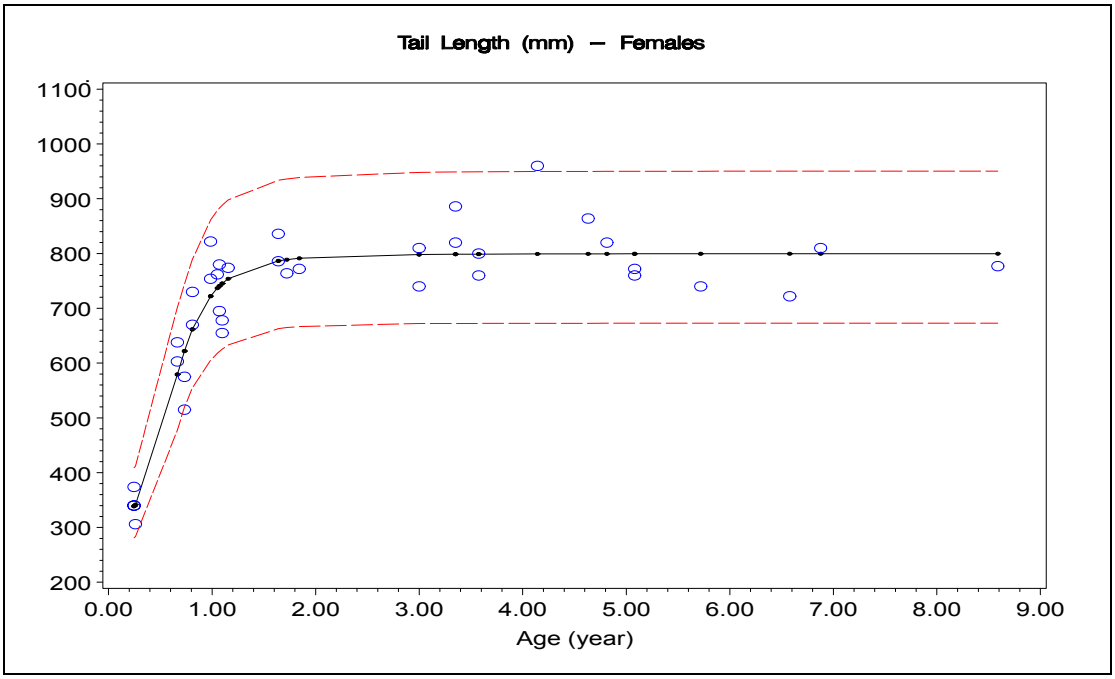

Figure 2c Tail length growth curve and normal reference range values for female African lions from 3 months to 9 years of age

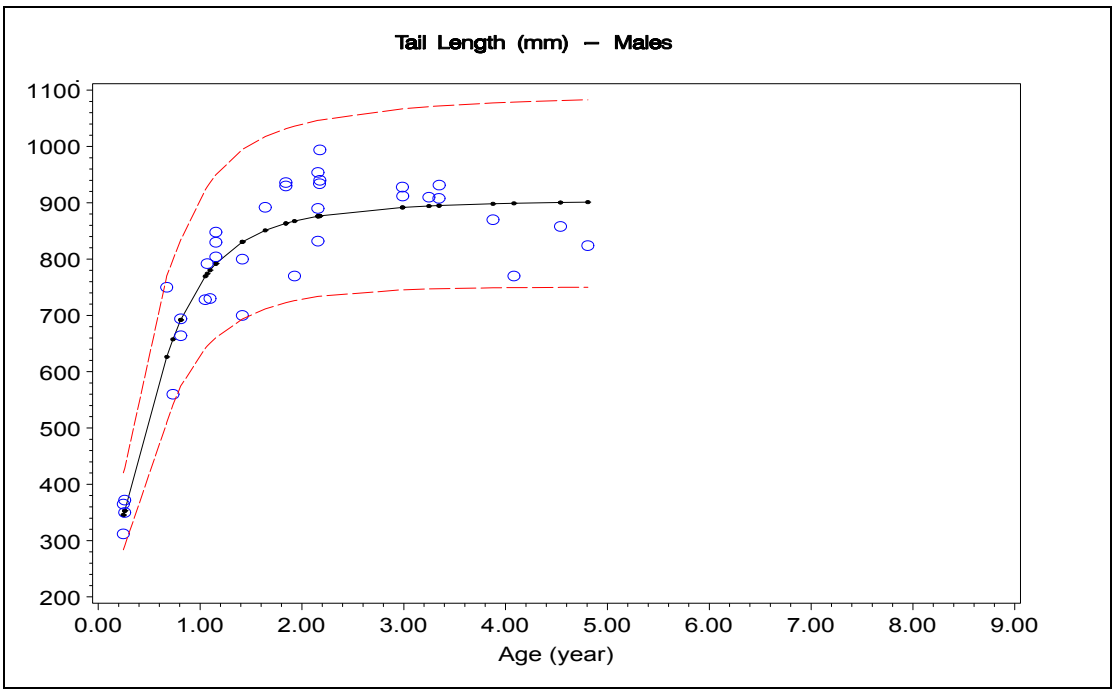

Figure 2d Tail length growth curve and normal reference range values for male African lions from 3 months to 5 years of age

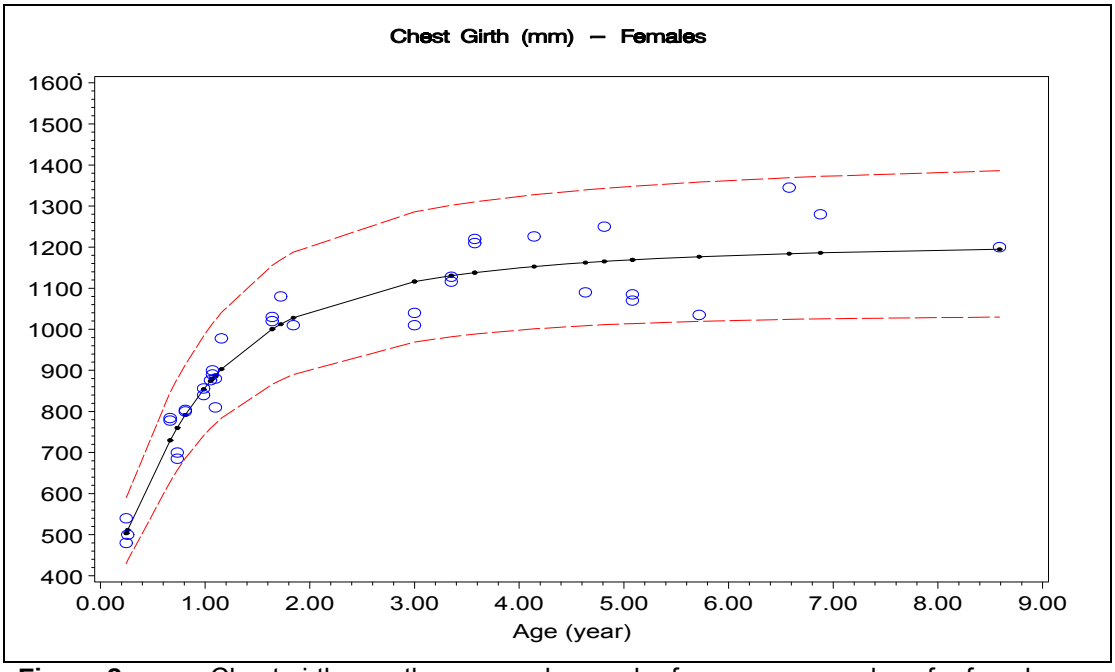

Figure 2e Chest girth growth curve and normal reference range values for female African lions from 3 months to 9 years of age

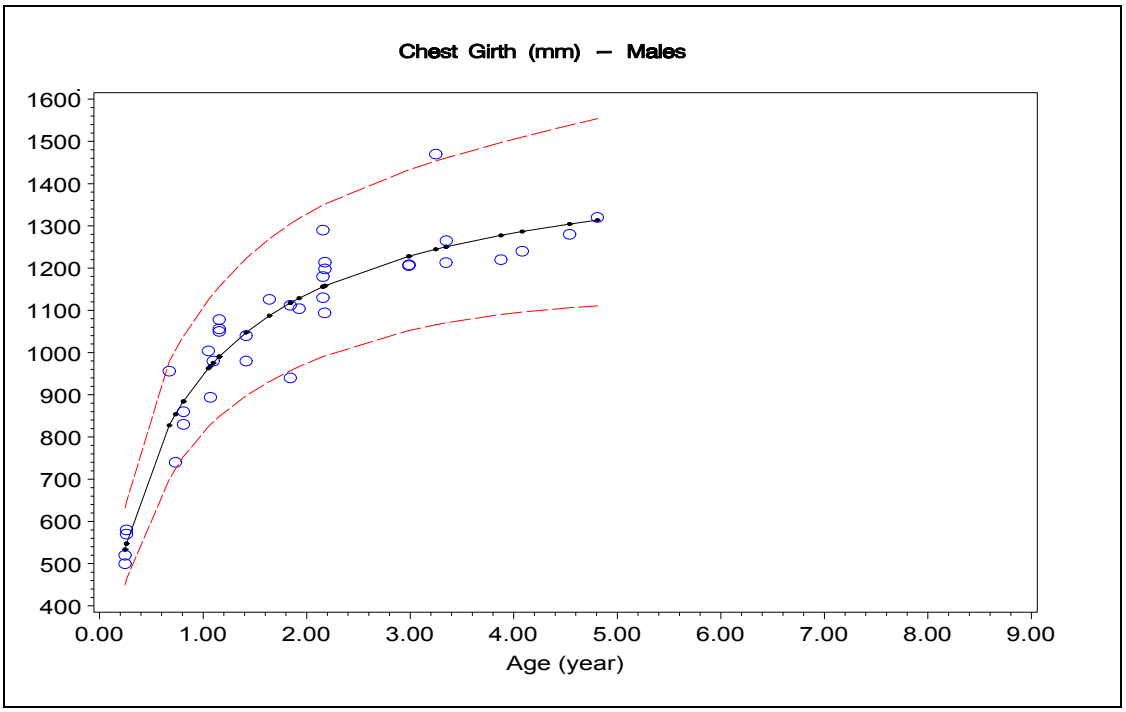

Figure 2f Chest girth growth curve and normal reference range values for male African lions from 3 months to 5 years of age

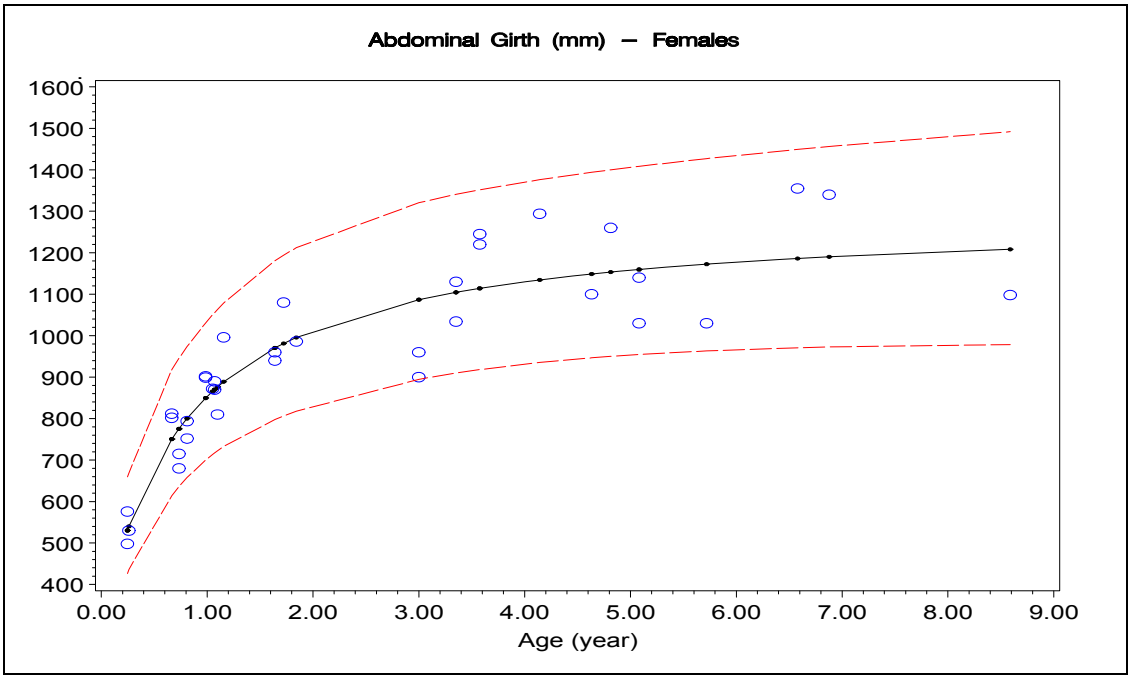

Figure 2g Abdominal girth growth curve and normal reference range values for female African lions from 3 months to 9 years of age

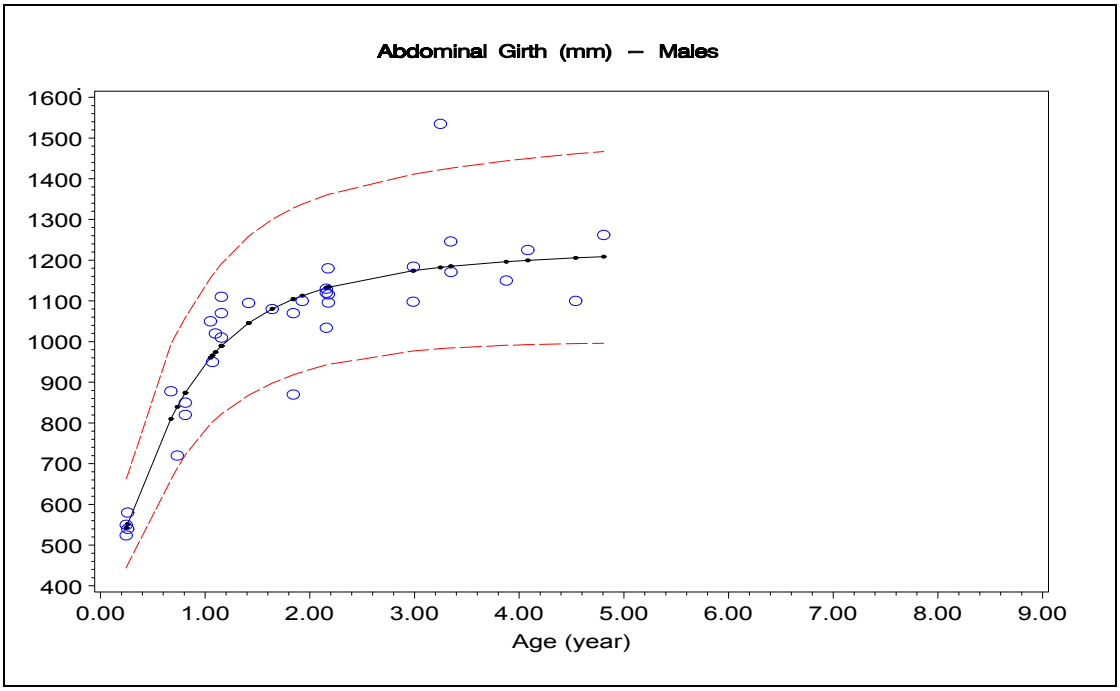

Figure 2h Abdominal girth growth curve and normal reference range values for male African lions from 3 months to 5 years of age

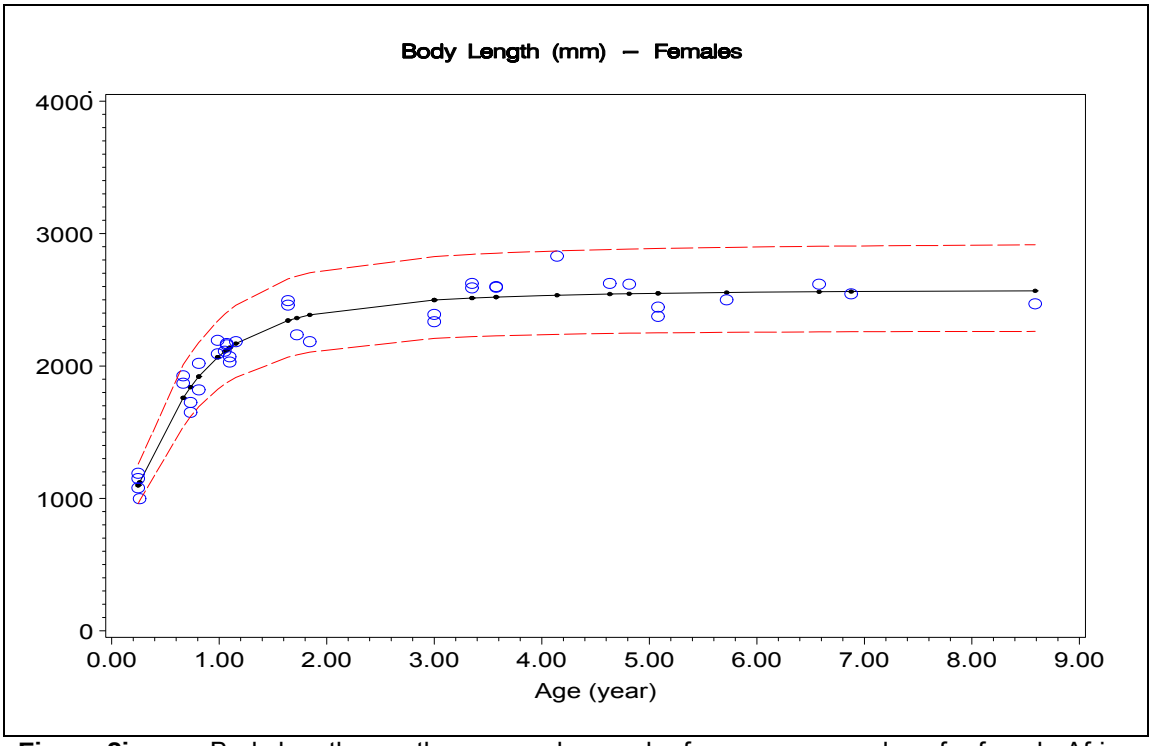

Figure 2i Body length growth curve and normal reference range values for female African lions from 3 months to 9 years of age

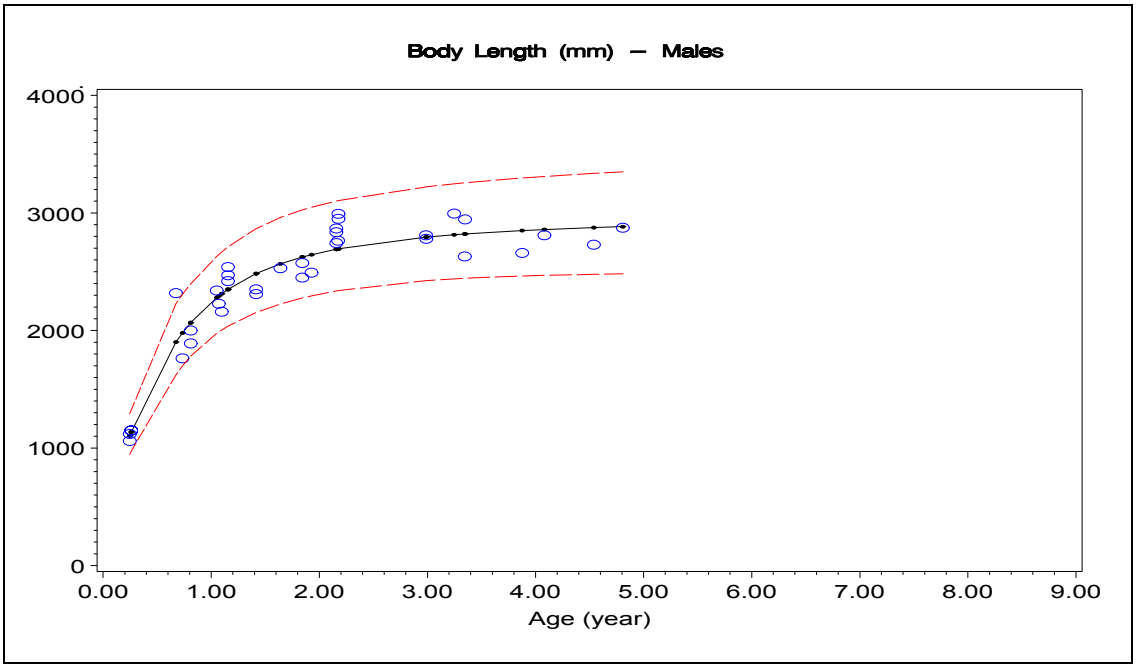

Figure 2j Body length growth curve and normal reference range values for male African lions from 3 months to 5 years of age

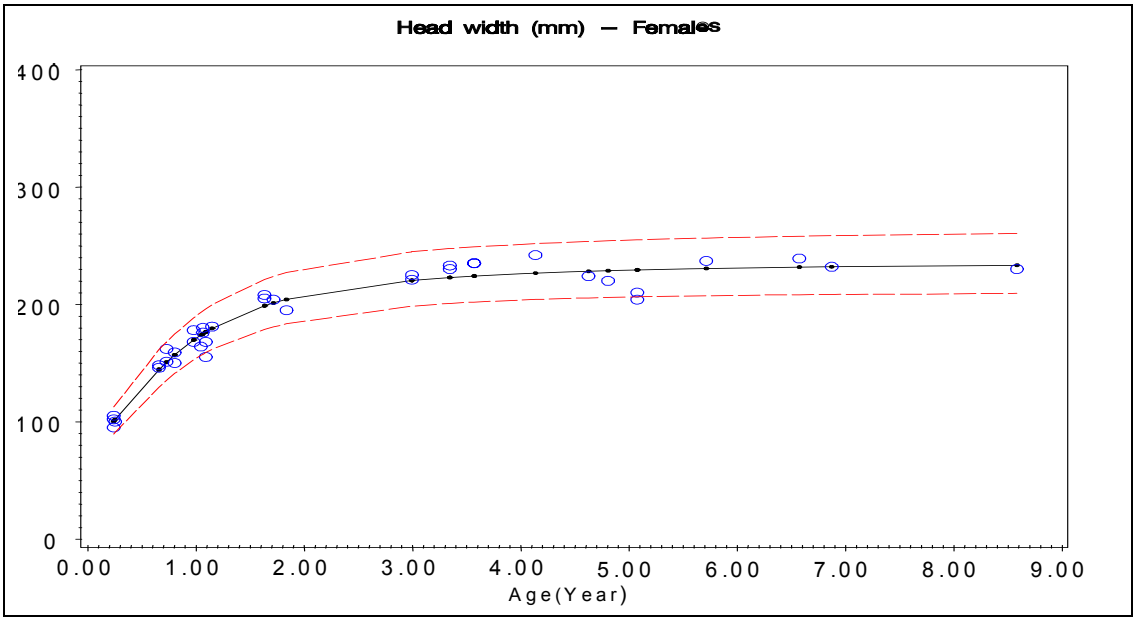

Figure 2k Head width growth curve and normal reference range values for female African lions from 3 months to 9 years of age

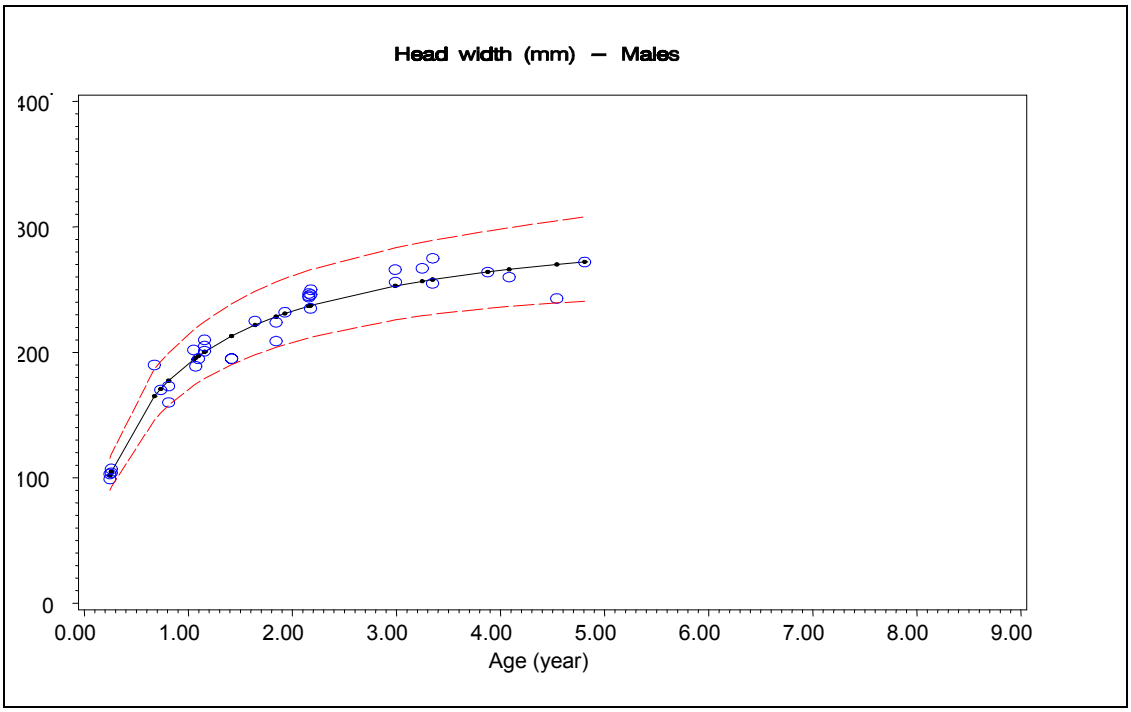

Figure 2l Head width growth curve and normal reference range values for male African lions from 3 months to 5 years of age

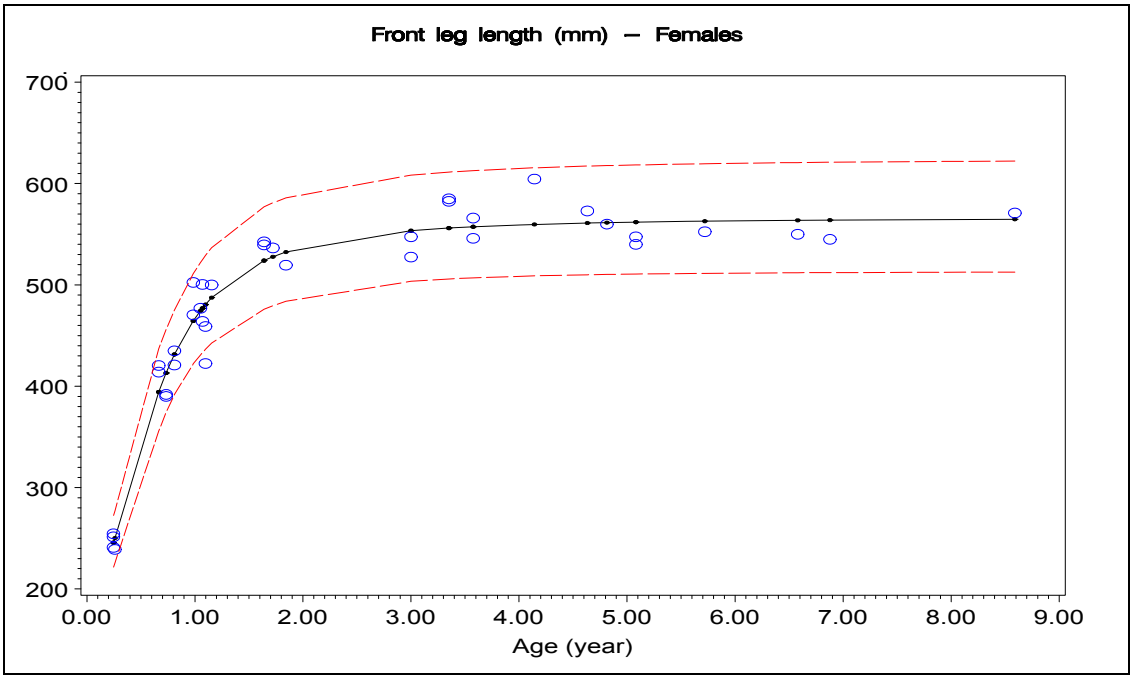

Figure 2m Front leg length growth curve and normal reference range values for female African lions from 3 months to 9 years of age

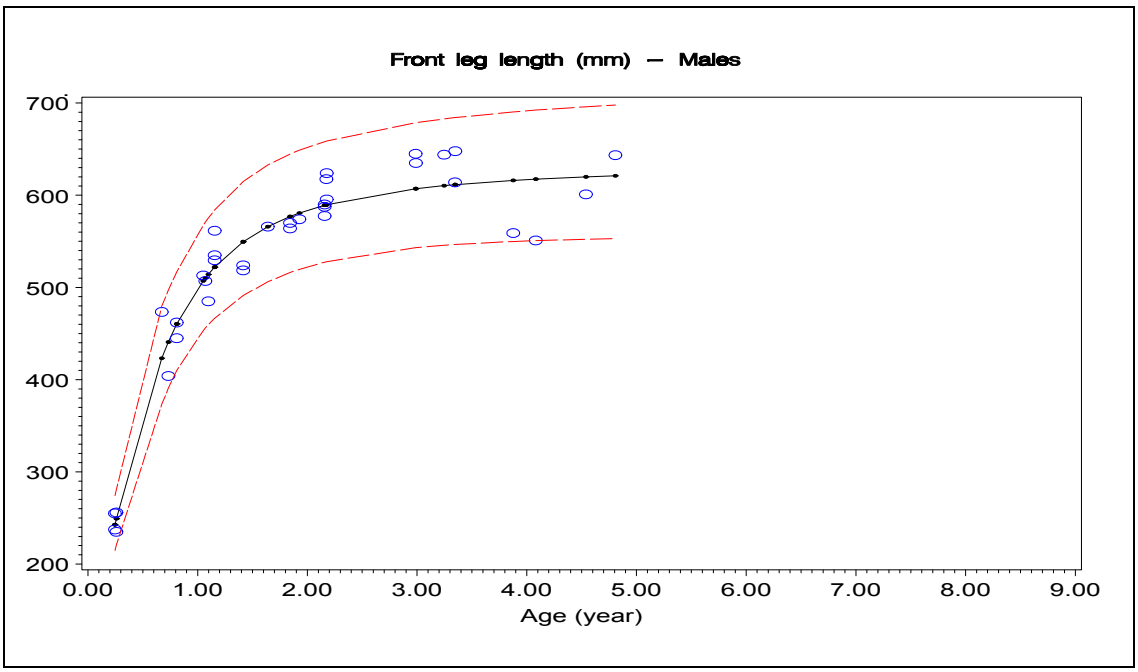

Figure 2n Front leg length growth curve and normal reference range values for male African lions from 3 months to 5 years of age

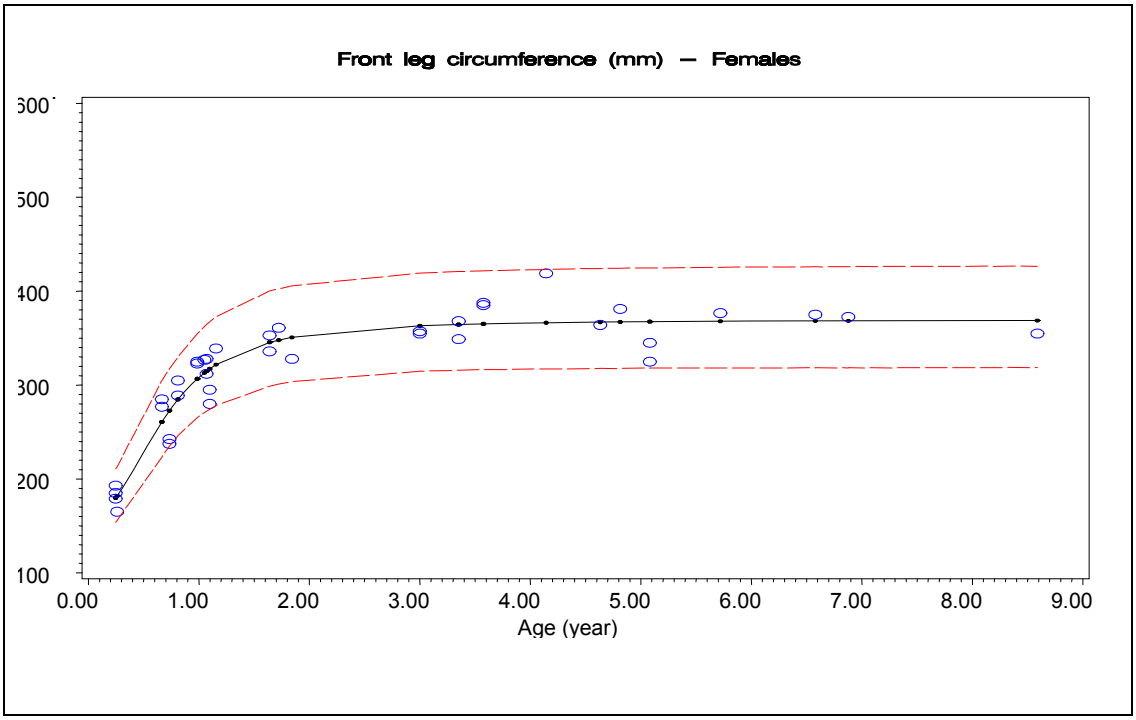

Figure 2o Front leg circumference growth curve and normal reference range values for female African lions from 3 months to 9 years of age

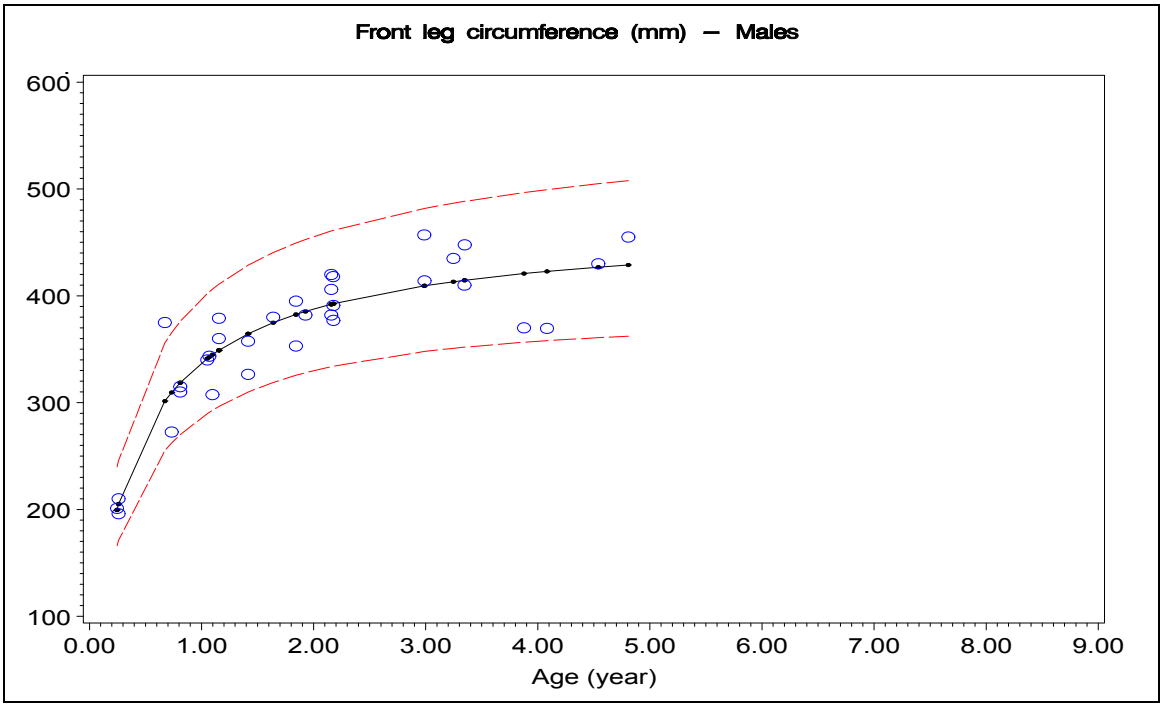

Figure 2p Front leg circumference growth curve and normal reference range values for male African lions from 3 months to 5 years of age

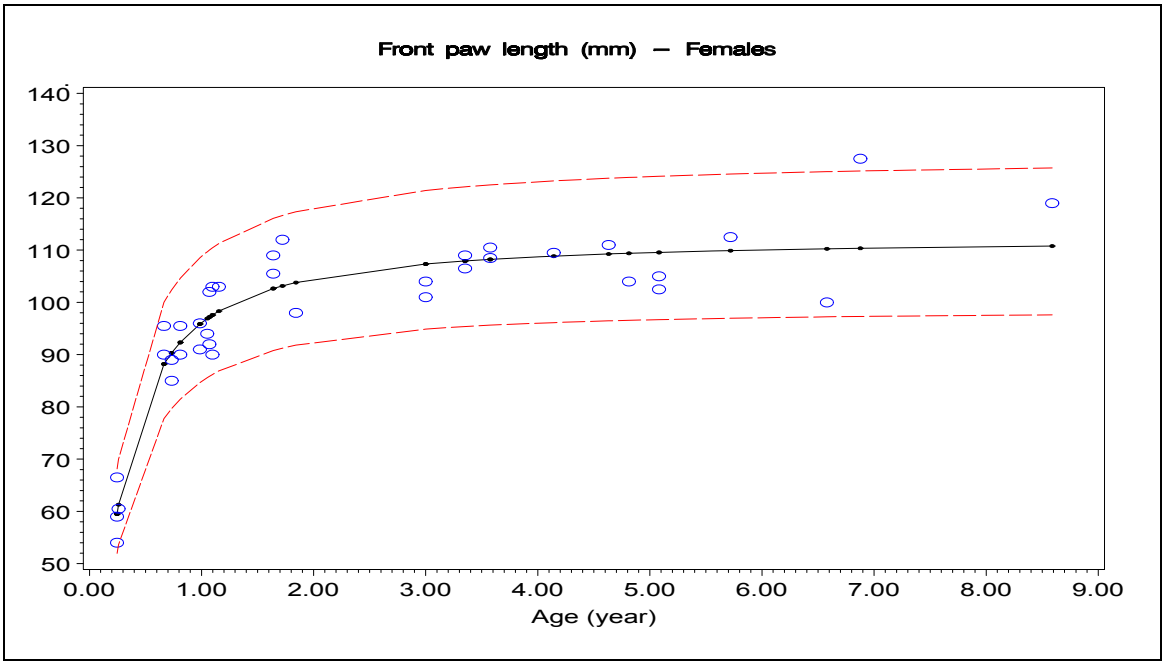

Figure 2q Front paw length growth curve and normal reference range values for female African lions from 3 months to 9 years of age

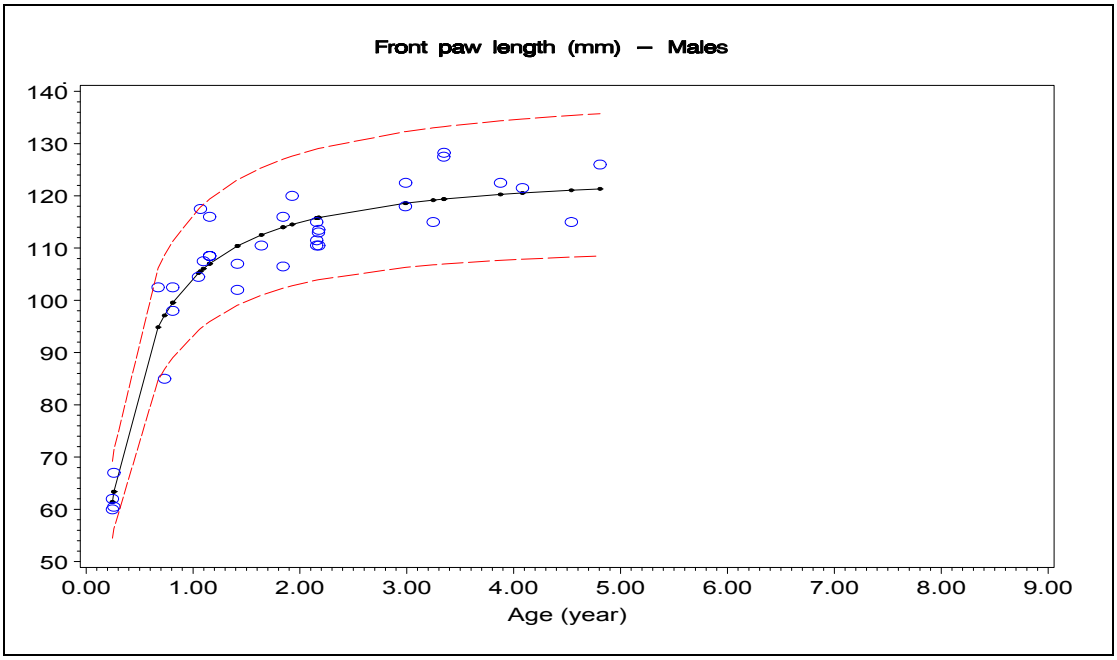

Figure 2r Front paw length growth curve and normal reference range values for male African lions from 3 months to 5 years of age

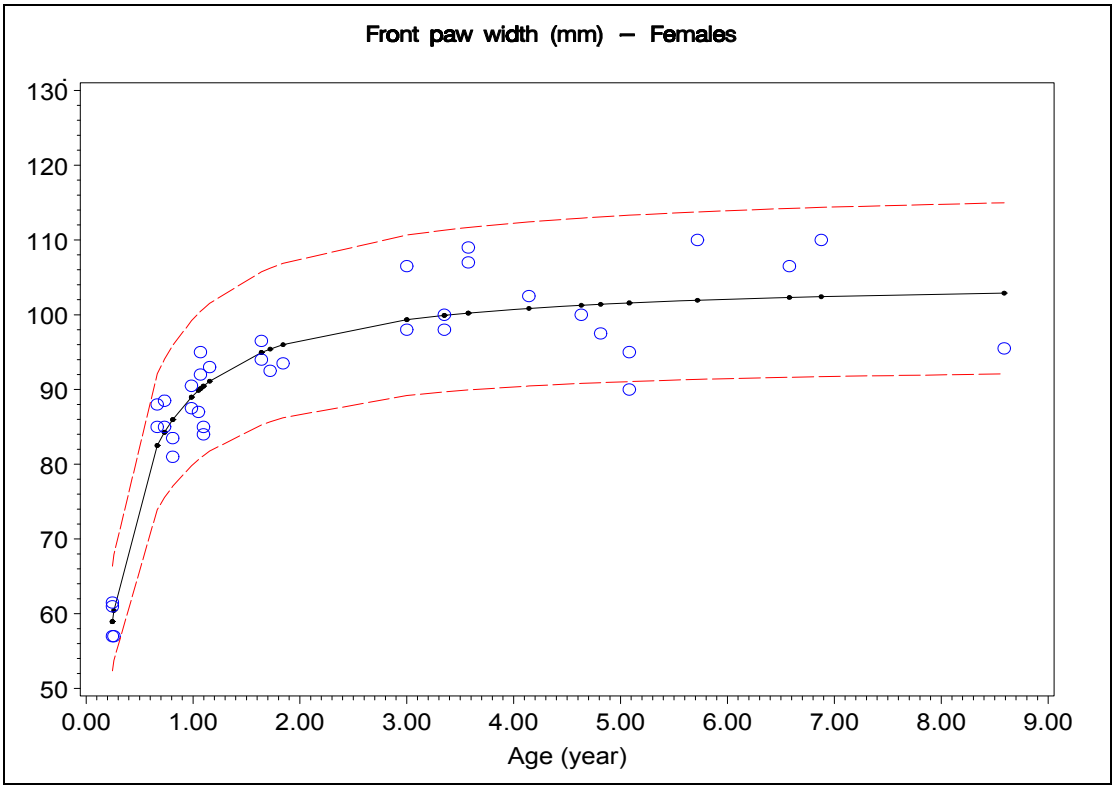

Figure 2s Front paw width growth curve and normal reference range values for female African lions from 3 months to 9 years of age

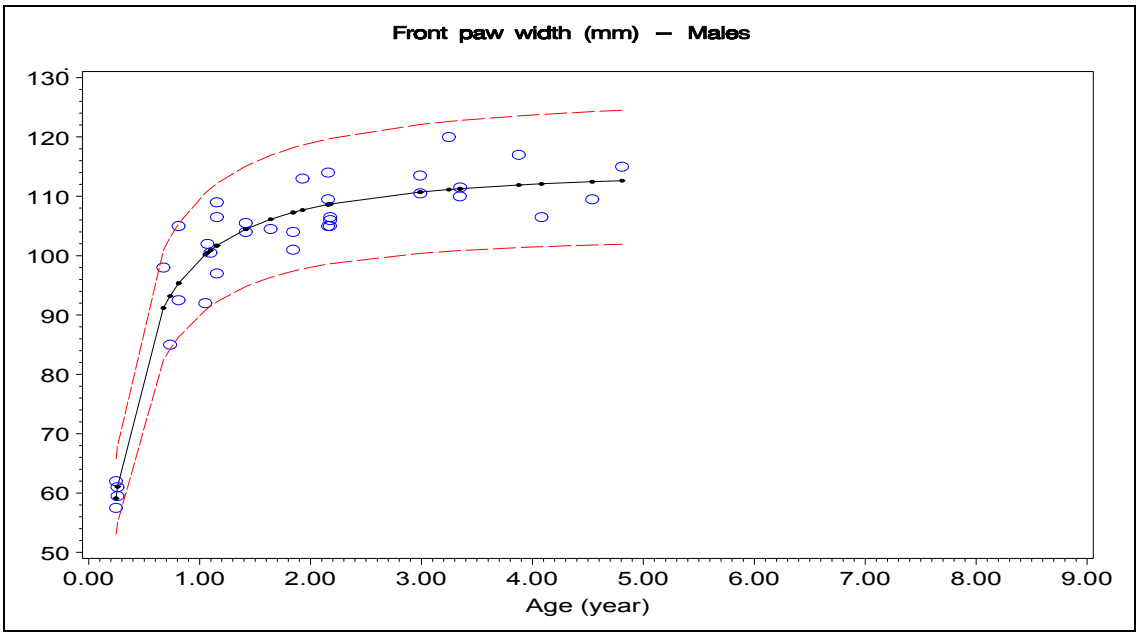

Figure 2t Front paw width growth curve and normal reference range values for male African lions from 3 months to 5 years of age

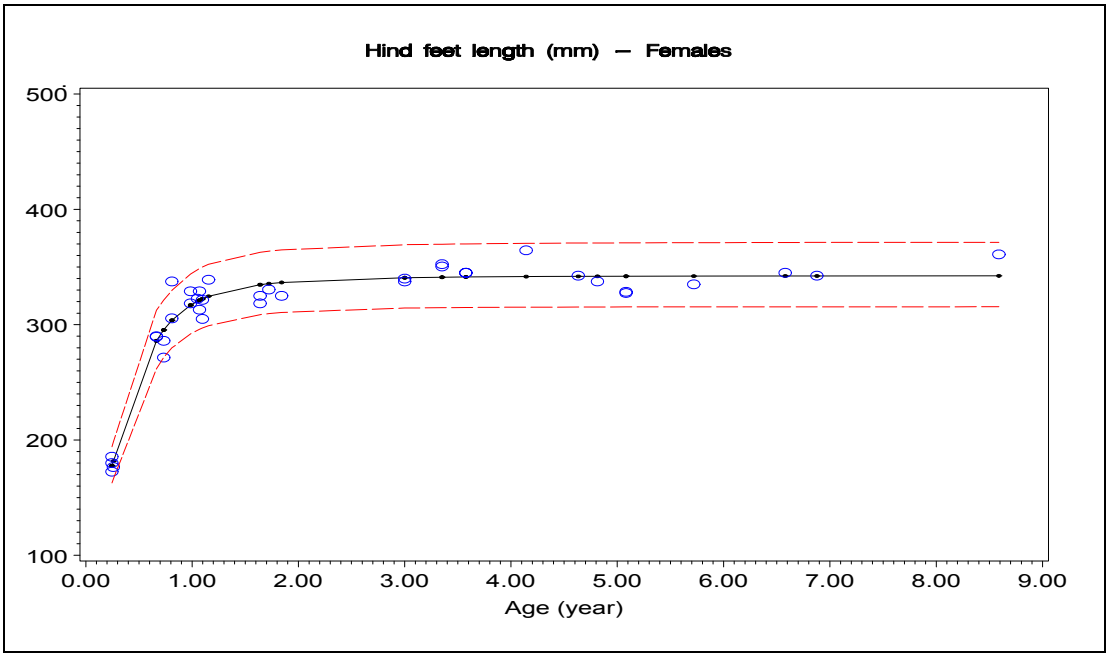

Figure 2u Hind feet length growth curve and normal reference range values for female African lions from 3 months to 9 years of age

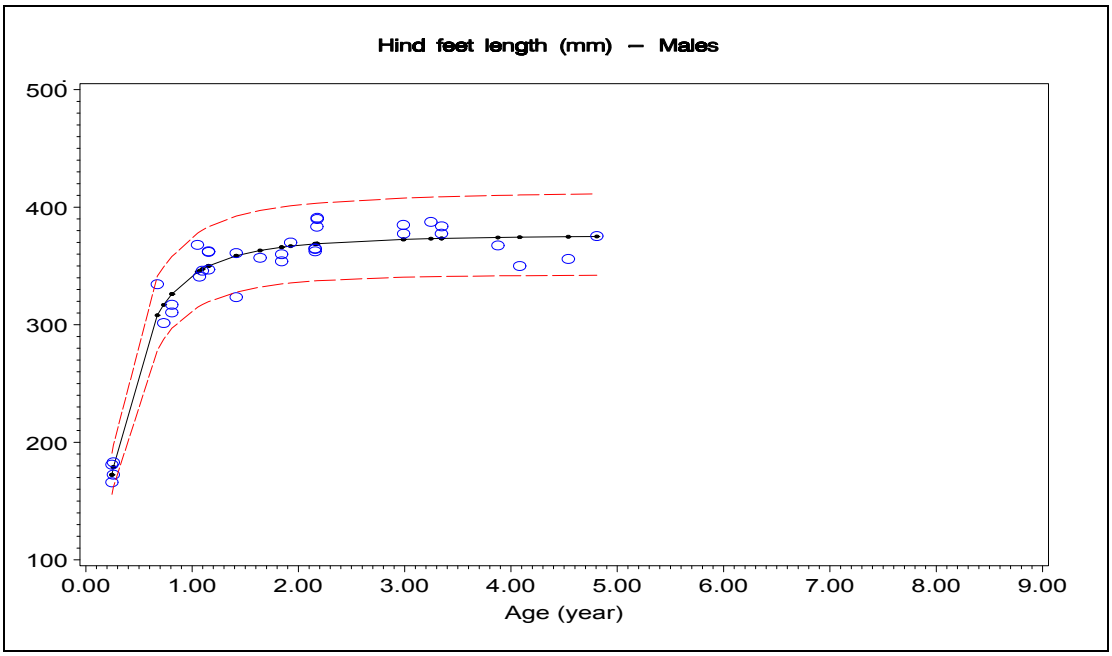

Figure 2v Hind feet length growth curve and normal reference range values for male African lions from 3 months to 5 years of age

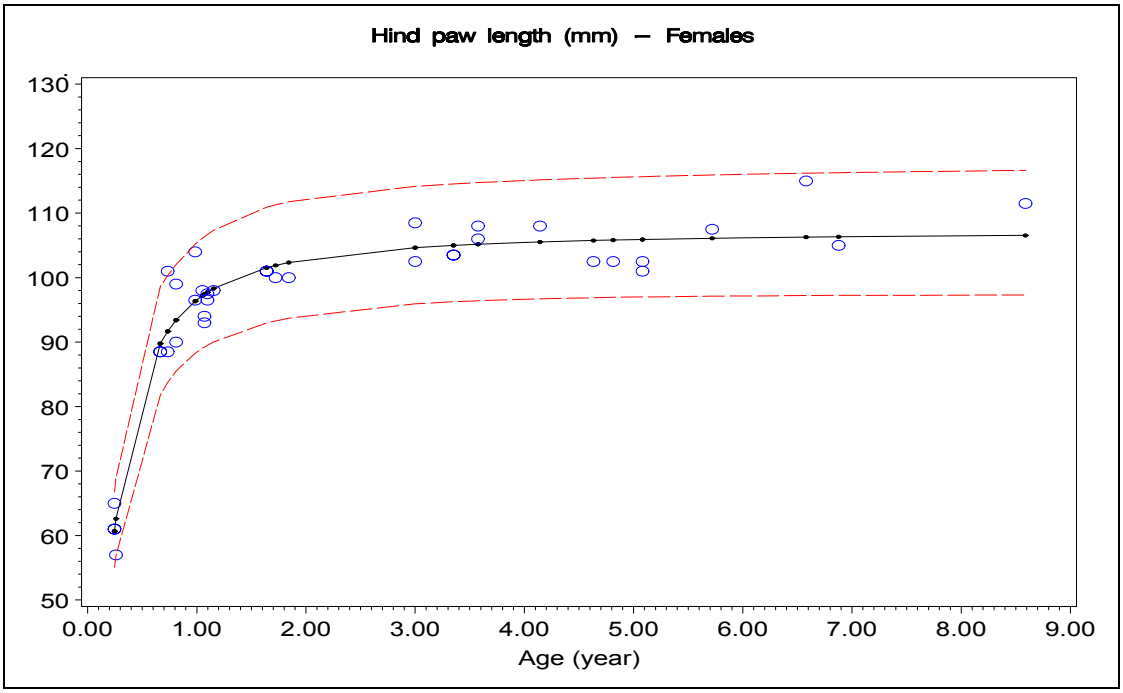

Figure 2w Hind paw length growth curve and normal reference range values for female African lions from 3 months to 9 years of age

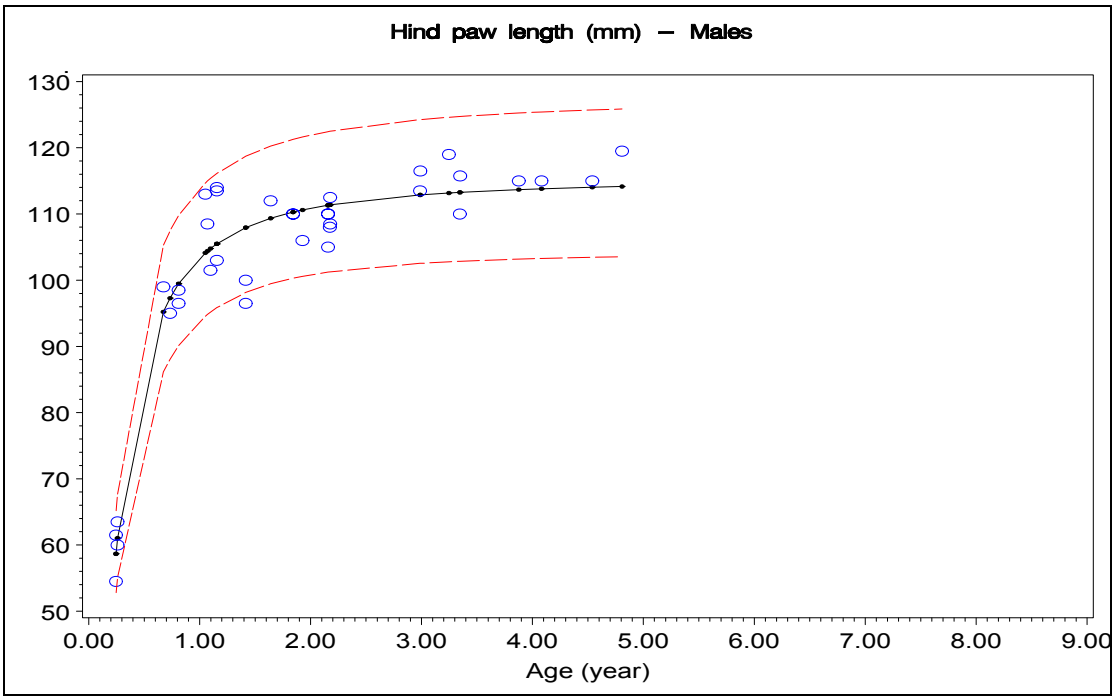

Figure 2x Hind paw length growth curve and normal reference range values for male African lions from 3 months to 5 years of age

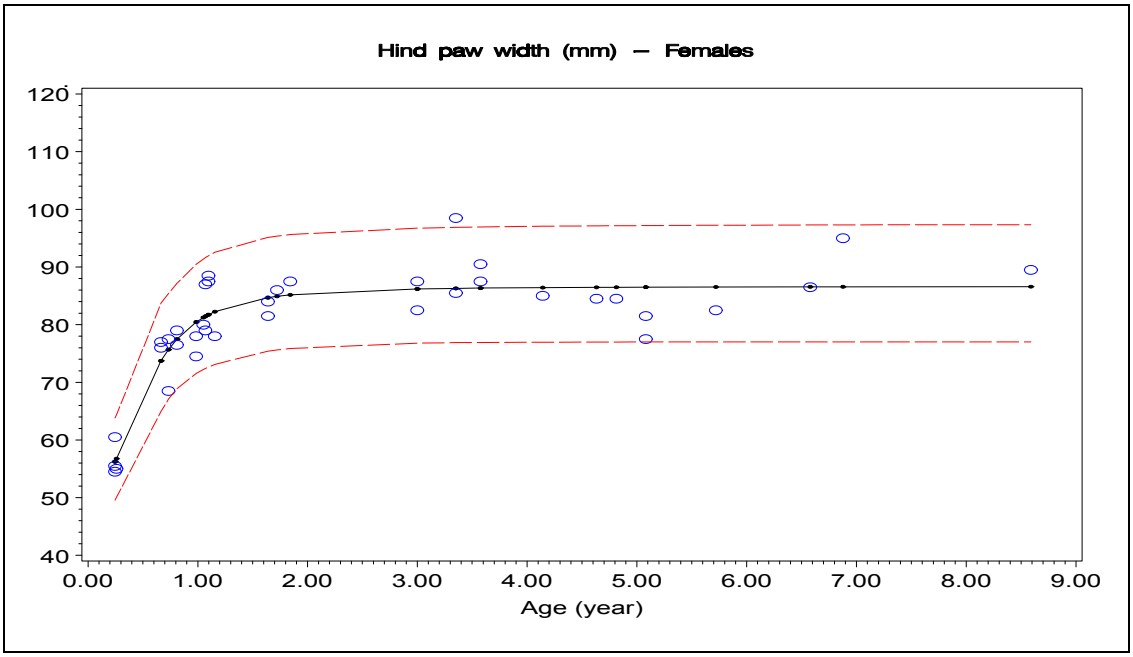

Figure 2y Hind paw width growth curve and normal reference range values for female African lions from 3 months to 9 years of age

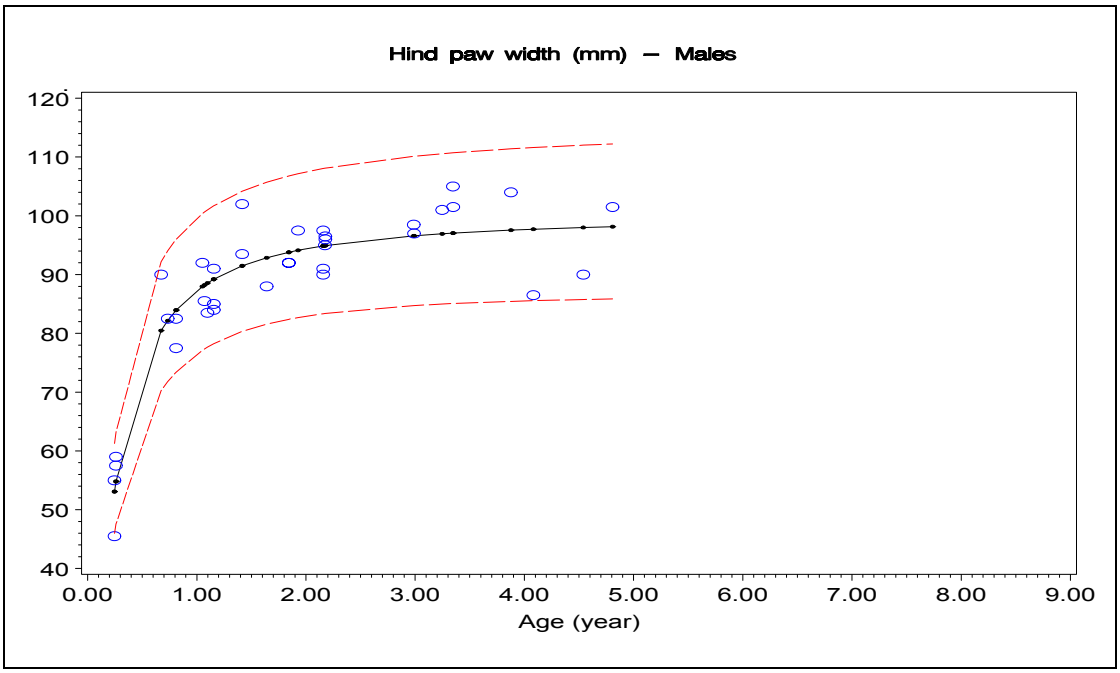

Figure 2z Hind paw width growth curve and normal reference range values for male African lions from 3 months to 5 years of age