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HEMATOLOGY ANALYSIS AS A POTENTIAL TOOL TO PREDICT BONE FRACTURE IN ARABIAN RACING CAMELS (*CAMELUS DROMEDARIES*)

N. S. Alshamisi[1], T. S. Ksiksi, and S. Salman Ashraf*

Faculty of Science, UAE University, Alain, U. A. E.
[1]Management of Scientific Center and Presidential Camels, Alain, U. A. E.
*Corresponding author Email: salman.ashraf@uaeu.ac.ae

ABSTRACT

Racing camels (*Camelus dromedaries*) are historically, culturally and financially important to the United Arab Emirates (UAE). In order to develop better predictive approaches to identify at-risk animals amongst racing camels, hematological analyses of healthy (n=60), lame (n=31) and bone-fractured (n=20) camels were carried out in the present study. The hematological parameters analyzed in this study were: white blood cells (WBC, K/µL), red blood cells (RBC, M/µL), hemoglobin (HGB, g/L), mean cell volume (MCV, fL), mean cell hemoglobin (MCH, pg), mean cell hemoglobin concentration (MCHC, g/dL), platelet count (PLT, K/µL), and red blood cell distribution width (RDW, %), as well as differential leukocytic count (DLC, % neutrophil, % lymphocyte, % monocyte, % eosinophils, % basophil). Results revealed that out of the 13 parameters analyzed, 10 of the hematological parameters in females and 9 in male camels showed significant ($p \leq 0.05$) differences between at least one of the groups *viz.* healthy *vs.* lame, lame *vs.* fractured, or healthy *vs.* fractured. Stepwise discriminant analysis approach was used to develop a statistical model that could distinguish healthy racing camels from lame/fractured ones. In addition to reporting baseline reference values for these hematological parameters in male and female *Camelus dromedaries* racing camels, our study suggests routine hematological parameters can be used to identify camels that may be at risk of developing bone fractures later in their life, thus enabling better preventive measures for these animals.

Key words: Arabian Racing Camels, Hematology, Bone, Discriminant Analysis

INTRODUCTION

Racing camels represent a historical, cultural and financial richness to the United Arab Emirates and its society. There are approximately 14,000 active racing camels in the UAE (Nawata 2005). In the emirate of Dubai (UAE) alone, there are approximately 120,000 people involved in camel racing. Racing camels are prized and can be very expensive - twelve years ago, a racing camel in Sudan was sold for about USD 4 million (Ahmad *et al.* 2010).The popularity of this sport is growing and attracting interest in different countries as well. However, due to younger and younger camels being trained for racing, there has been an increase in the incidence of bone fractures in racing camels. In addition, shin soreness (bucked shins) and other bone diseases are also being increasingly reported in young animals during training (Nunamaker 2002). Although not fatal, such bone diseases and fractures are the leading cause of lameness in animals, and causes significant economic loss to racing industry. Surprisingly, very little is known about potential causes of the bone disease in camels, except for some general theories drawn from results from humans and other large animals *(Chaney et al*. 2004; Fewtrell *et al.* 2008; Kim *et al.* 2010; Huang and Ouyang 2012). There could be many factors that may affect camel bone health, including hematological abnormalities (Rubino et al. 2006), lack of essential trace elements and minerals (Harris, *et al*. 2003; Ng, *et al*., 2004; Rude and Gruber 2004; Dahlstrand *et al.* 2009; Zhang *et al*. 2012), and less-than optimum functioning of liver and kidneys(Nakamura, *et al,*. 2011) , and abnormalities in levels of some hormones(Wallach, *et al.* 1999; Fox, 2002; Mukherjee, *et al,*. 2004).

Hematology and serum/plasma biochemistry are important diagnostic tools to assess metabolic status in racing camels. Hematology is the study of blood and blood-forming organs including the diagnosis, treatment, and prevention of diseases of the blood, bone marrow, and immunologic, haemostatic, and vascular systems. Although, it is routinely used for the diagnosis and treatment of animal diseases (Washington and Hoosier 2012) there are very few published reports on the reference ranges of hematology and chemistry parameters in camels. Even in the few studies that have been published, only 'partial hematology results' have been reported (Alsaad 2009; Aichouni *et al*. 2010; Farooq *et al*. 2011). Furthermore, only a few detailed studies on hematological parameters of 'racing' camels have been published (Mohamed and Hussein 1999; Chaudhary and Iqbal 2000; Eltahir *et al*. 2010). More importantly, to the best of our knowledge, no published reports have attempted to establish a correlation between hematological values and bone diseases in camels.

The objectives of the present study were 1) to establish a reference range of hematological profile in male and female racing camels, 2) to use step-wise multivariate discriminant analysis to study the variability in normal, lame and fractured camels, and lastly 3) to develop a model which could group camels in one of these three groups (normal, lame, and fractured) using only a few of the routine hematological parameters.

MATERIALS AND METHODS

Geo-location of the study: The camels were selected from different racing camps owned by the Presidential Affairs in the Abu Dhabi Emirate of the United Arab Emirates.

Experimental animals: The study was conducted on (*Camelus dromedaries*) racing camels, both females and males, aged between one and four years. The camels were selected from different racing camps owned by Presidential Affairs in Abu Dhabi Emirate, UAE. Animals were fed on a ration of fresh clover, dry clover, barley, camel milk, pre-mix feeds, multi- vitamins and mineral. All camels had free access to drinking water. Three groups of camels were identified: healthy racing camels, racing camels with lameness, and racing camels with bone fractures and referred to as normal, lame and fractured; respectively, thereafter. Bone fracture was diagnosed by a veterinary doctor through a field examination and when necessary an x-ray. Lameness was diagnosed through visual observations on the basis of limp in their walk. Camels in the control groups consisted of apparently healthy animals. All groups were similar in age and gender (95% were between 1-4 years). Camels were split into three gender specific groups with a total of 110 individuals as outlined in Table 1.

Blood collection: Blood samples were collected in the morning hours, except in cases of fractures, when blood samples were collected immediately after the fracture was diagnosed. BD Vacutainer EDTA tubes (Becton Dickinson, USA), were used to collect blood samples.

Hematological analyses: Hematology analysis was performed on a Sysmex XT-2000iV hematology analyzer (Kobe, Japan), fitted with a veterinarian hematology software package. Proficiency testing were carried out using VETQAS reference samples (PT0050) to confirm proper functioning of the analyzer for ruminant blood samples. The parameters that were analyzed included: white blood cells (WBC, K/µL), red blood cells (RBC, M/µL), hemoglobin (HGB, g/L), mean cell volume (MCV, fL), mean cell hemoglobin (MCH, pg), mean cell hemoglobin concentration (MCHC, g/dL), platelet count (PLT, K/µL), and red blood cell distribution width (RDW, %), as well as differential leukocytic count (DLC, % neutrophil, % lymphocyte, % monocyte, % eosinophils, % basophil).

Statistical analyses: The software package IBM SPSS Statistics 19.0.1 (Statistical Package and Service Solutions) was used for all statistical analyses reported here, including unpaired t-test analysis and discriminant analyses. The step-wise multivariate discriminant analysis was performed to assess the significance of contributions from each parameter measured for the three camel groups (i.e. normal, lame and fractured). Testing for normality was performed for all variables included in the analyses. All variables were normally distributed except for monocytes, eosinophils, basophils, hemoglobin and MCHC in normal group.

RESULTS

The mean (±SE) values of the various hematological parameters from the three camel groups are shown in Table 2 (for female camels) and Table 3 (male camels). Also shown are the results from unpaired t-test analysis of significant differences in any of the hematological parameters between any of the three groups. For the female camels, 10 of the 13 parameters showed significant differences between at least one of the groups - healthy vs. lame, lame vs. fractured, or healthy vs. fractured. Likewise, for the male camels 9 of the 13 parameters were significantly different between at least one of the three groups.

A step-wise discriminant analysis was also carried out using the hematological parameters of the male and female camels (separately) to determine if any of these hematological parameters could be used to categorize camels into one of the three groups (normal, lame or fractured). The results showed that in female racing camels, neutrophils, eosinophils and MCHC were the main parameters in differentiating between normal, lame and fractured animals. While in males, basophils, neutrophils, MCH and WBCs were the most important variables in differentiating between normal, lame and fractured racing camels. Neutrophils were found to be an important parameter in differentiating normal, lame and fractured groups in both male and female groups. This is shown in Figures 1 and 2. This data (from the multi-variate step-wise discriminant analysis) was subsequently used to develop linear models representing the contribution of each of the important variables to be able to discriminate between the three groups. Two different functions (for each gender) were the outcome of this statistical analysis, and are shown below:

Female camels:

*Y1 = -5.338 – (0.332*__Eosinophils__) – (0.02*__MCHC__) + (0.122 *__Neutrophils__)*

*Y2 = -24.325 + (0.272*__Eosinophils__) + (0.469*__MCHC__) + (0.037 *__Neutrophils__)*

Male camels:

*Y1 = -9.398 – (3.797****Basophils****) + (0.453****MCH****) + (0.083****Neutrophils****) + (0.095****WBC****)*

*Y2 = 7.49 + (1.343****Basophils****) – (1.17****MCH****) + (0.052****Neutrophils****) + (0.38****WBC****)*

The contributions of each of these functions for discriminating between the three animal groups are shown in Table 4. Function 1 was found to discriminate fractured camels from the other two groups (i.e. normal and lame), while function 2 was important in differentiating normal from lame camels. This can be seen in the canonical discriminant function plots for all the male and female camels (Fig. 3 and Fig. 4).

The effectiveness of this model (and functions) to be able to distinguish between the three animal groups (normal, lame and fractured) using only three hematological parameters for female camels and only four hematological parameters for male camels was also tested. The result of this analysis is shown in Table 5, where, it can be seen that the male animals were more accurately differentiated than the female camels - 96% normal camels, 83% lame camels, and 100% fractured camels were correctly predicted and grouped. For the female camels, the model was also able to predict 100% of the fractured camels correctly, but due to the weakness of female camel Function 2 (which was to differentiate between normal and lame camels), 69% and 79% of the normal and lame female camels were correctly grouped.

Table 1. Number, gender, and physiological status of the camels used in the present study.

Group	Number of animals	Gender	Group name
Normal	35	Females	Control female camels
	25	Males	Control male camels
Lame	19	Females	Lame female camels
	12	Males	Lame male camels
Fractured	14	Females	Fractured female camels
	5	Males	Fractured male camels

Table 2: Averages, standard deviations, and statistical significance in the various hematological parameters in the three female camel groups.

Parameters	Mean ±SD			Significance		
	Normal (N)	Lame (L)	Fractured (F)	N vs. L	N vs. F	F vs. L
WBC (K/µL)	12.7±2.2	10.9±5.4	15.2±3.5	NS	*	**
%Neutrophils	51.9±8.4	57.1±8.5	81.4±5.2	*	**	**
%Lymphocyte	38.2±8.0	32.1±9.9	11.8±5.3	*	**	**
%Monocyte	5.5±1.2	5.7±1.3	5.7±2.5	NS	NS	NS
%Eosinophils	3.5±1.9	4.2±2.3	0.7±0.9	NS	**	**
%Basophils	1.0±0.3	0.9±0.3	0.3±0.3	NS	**	**
RBCs (M/µL)	10.3±0.9	9.8±0.7	9.9±2.1	NS	NS	NS
HGB(g/L)	134.3±12.4	131.6±10.7	136.9±22.8	NS	NS	NS
MCV(fL)	28.7±2.2	28.9±2.2	31.0±2.8	NS	**	**
MCH(pg)	12.9±0.8	13.4±1.1	13.9±1.2	NS	**	NS
MCHC(g/dL)	45.1±1.8	46.6±1.3	45.0±2.5	**	NS	*
%RDW	30.2±2.3	27.4±3.2	27.9±4.5	**	*	NS
PLT(K/µL)	353.5±102.1	273.3±94.1	276.2±85.0	**	*	NS

* ($p<0.05$) ** * ($p<0.01$) NS = Non significantly different

Table 3. Averages, standard deviations, and statistical significance in the various hematological parameters in the three male camel groups.

Parameters	Mean ±SD			Significance (p<0.05)		
	Normal (N)	Lame (L)	Fractured (F)	N vs. L	N vs. F	F vs. L
WBC (K/µL)	11.2±1.2	9.1±1.9	156±3.9	**	**	**
%Neutrophils	54.6±6.1	57.7±11.4	82.5±5.9	NS	**	**
%Lymphocyte	35.4±5.7	30.0±12.5	12.2±5.9	NS	**	**
%Monocyte	5.2±1.4	6.2±1.4	4.6±3.7	NS	NS	NS
%Eosinophils	3.9±2.3	5.5±2.8	0.5±0.4	NS	**	**
%Basophils	0.9±0.2	0.6±0.2	0.2±0.2	**	**	**
RBCs (M/µL)	9.7±0.9	9.2±0.9	8.8±1.6	NS	NS	NS
HGB (g/L)	126±11.2	130±12.8	125±18.7	NS	NS	NS
MCV (fL)	29.4±2.1	31.2±1.9	31.4±3.6	*	NS	NS
MCH (pg)	12.9±0.4	14.2±0.7	14.5±0.9	**	**	NS
MCHC (g/dL)	44.2±2.2	45.5±2.2	46.5±4.1	NS	NS	NS
%RDW	29.1±1.3	25.7±2	25.45±1.5	**	**	NS
PLT (K/µL)	314.0±45.3	233.9±73.4	265.5±73.5	**	NS	NS

* (p<0.05) ** * (p<0.01) NS = Non significantly different

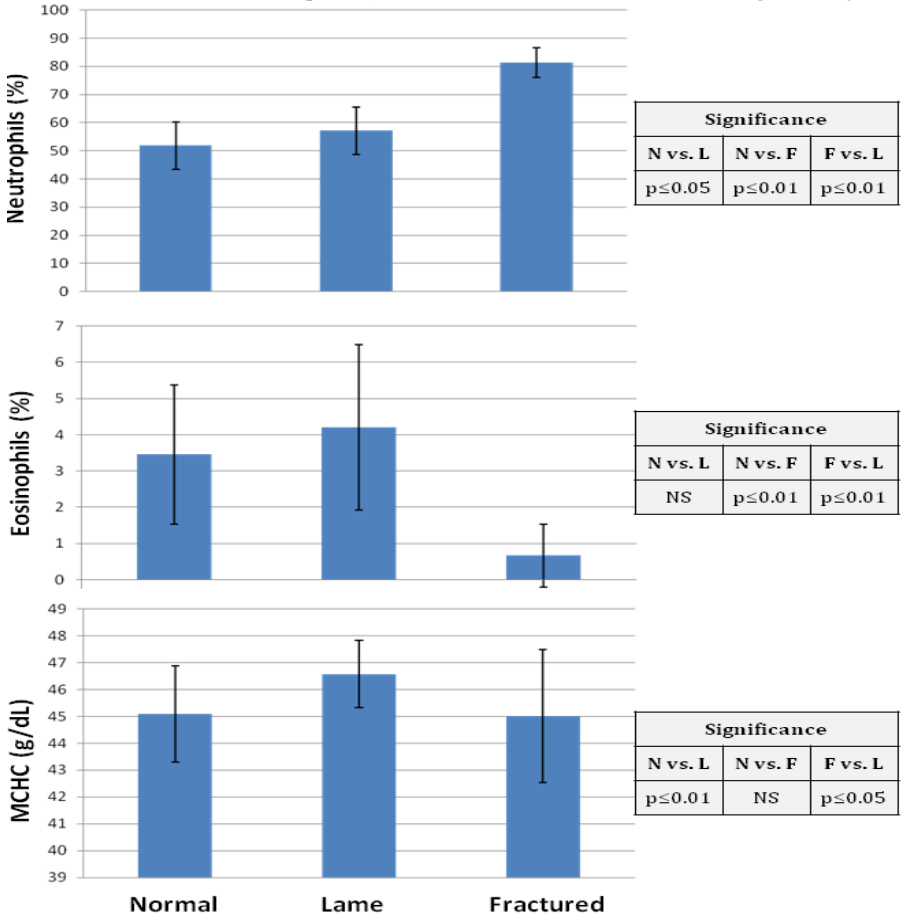
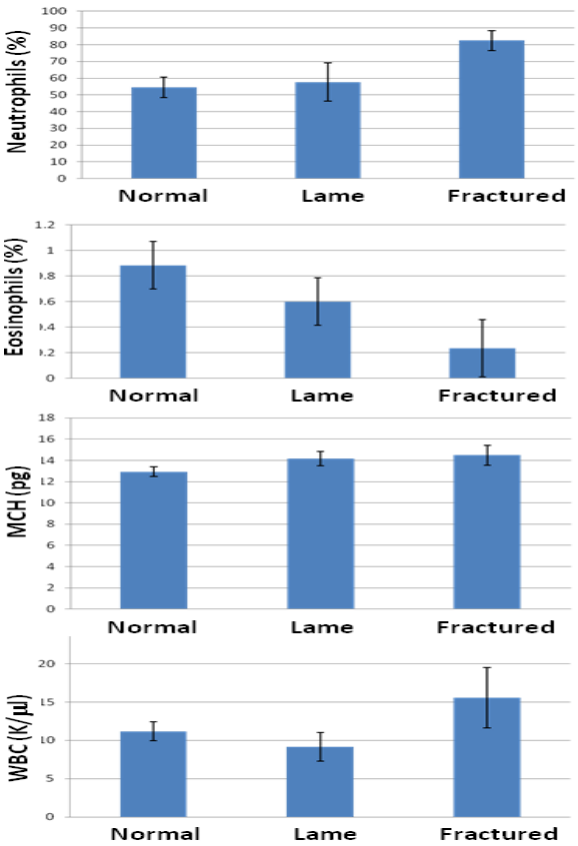


Figure 1. Averages and standard deviations of neutrophils, eosinophils, and MCHC values of female camels.



Significance		
N vs. L	N vs. F	F vs. L
NS	p≤0.01	p≤0.01

Significance		
N vs. L	N vs. F	F vs. L
p≤0.01	p≤0.01	p≤0.01

Significance		
N vs. L	N vs. F	F vs. L
p≤0.01	p≤0.01	NS

Significance		
N vs. L	N vs. F	F vs. L
p≤0.01	p≤0.01	p≤0.01

Figure 2. Averages and standard deviations of neutrophils, eosinophils, MCH, and WBC values of male camels.

Table 4. Percentage contribution of each of the functions (for male and female camels) in the model for discriminating between the three animal groups.

Gender	Function	% Contribution
FEMALE	1	93.6
	2	6.4
MALE	1	75.8
	2	24.2

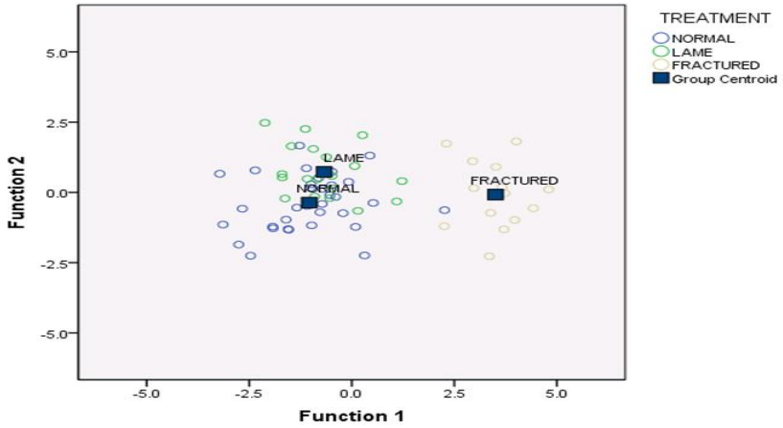


Figure 3. Canonical discriminant function plot of functions discriminating between normal, lame and fractured female camels.

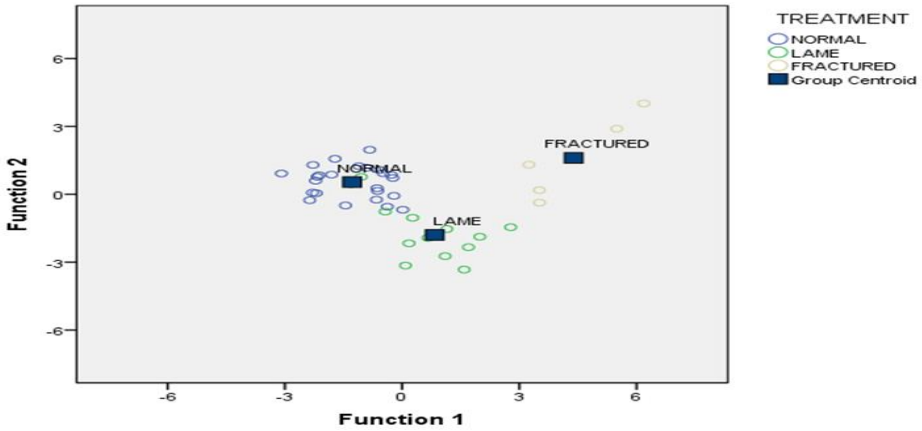


Figure 4. Canonical discriminant function plot of functions discriminating between normal, lame and fractured male camels.

Table 5: Grouping of camels based on the discriminant model using functions 1 and 2 for males and females.

Gender		Animal Group	Predicted Group Membership		
			NORMAL	LAME	FRACTURED
FEMALES	Count	NORMAL	24	10	1
		LAME	4	15	0
		FRACTURED	0	0	14
	%	NORMAL	69	39	3
		LAME	21	79	0
		FRACTURED	0	0	100
MALES	Count	NORMAL	24	1	0
		LAME	2	10	0
		FRACTURED	0	0	5
	%	NORMAL	96	4	0
		LAME	17	83	0
		FRACTURED	0	0	100

DISCUSSION

Although camels are economically and socially important animals in many parts of the world, only a very few detailed hematological analyses of Arabian racing camels have been published (Mohamed and Hussein 1999; Chaudhary and Iqbal 2000). Our hematological reference values for healthy (normal) racing camels reported here are in very good agreement with previously published studies on UAE racing camels (Chaudhary and Iqbal, 2000) as well as healthy Kuwaiti and Omani racing camels (Mohamed and Hussein, 1999; Eltahir *et al*., 2010). The only differences were in MCV and MCH values, which were lower in UAE racing camels as compared to Kuwaiti camels - the average MCV for UAE healthy females and male racing camels were 28.7±2.2 fL and 29.4±2.1 fL, respectively, while in Kuwaiti racing camels the average MCV value was 42.8±4.68fL. In addition, the average MCH in Kuwaiti racing camels were 17.78±1.97 pg while in UAE racing camels it was 12.9±0.8 pg and 12.9±0.4 pg in females and males, respectively. These differences could be due to the differences in the age and diet of the two different camel groups.

In addition to the lack of detailed hematological studies on racing camels, we were unable to find any published studies on the hematological parameters of racing camels with lameness or bone fractures. Our results show the hematological profiles of normal racing camels were significantly different than those of lame or fractured camels. As shown in Table 2, of the 13 majority of the hematological parameters analyzed, 10 of them

showed significant differences ($p \leq 0.05$) between at least one of the female camel groups *viz.* healthy *vs.* lame, lame *vs.* fractured, or healthy *vs.* fractured. Similarly, Table 3 shows 9 of the hematological parameters were significantly different ($p \leq 0.05$) amongst the three male camel groups (*viz.* healthy *vs.* lame, lame *vs.* fractured, or healthy *vs.* fractured). This is a very significant finding, as it has never before been shown that such significant differences exist in the hematological profiles of normal healthy racing camels and lame/fractured camels. Furthermore, our data implies that factors leading to bone diseases (lameness or eventual fracture) may also cause significant changes in other physiological parameters, such as hematology, which may be used as surrogate markers for bone diseases.

Our second objective of the present study was to explore the possibility of using advanced statistical tools to analyze the differences in the hematological profiles of the three grouped animals, and to come up with a short-list of hematological parameters that may be important in differentiating between normal and lame/fractured camels. This is a novel approach, as to the best of our knowledge; no systematic effort has been reported that attempts to use routine laboratory analyses and statistical tools to find any diagnostic or predictive biomarkers for camel bone fractures. Therefore, in the present study, multi-variate discriminant analysis tool was used, which showed that three of the hematological parameters in female camels (neutrophils, eosinophils and MCHC) and four parameters in male camels (basophils, neutrophils, MCH and WBCs) appeared to be important in successfully differentiating between normal, lame and fractured racing camels (Fig. 1 and Fig. 2). Neutrophils were found to be an important parameter in differentiating normal, lame and fractured groups in both male and female groups. These parameters were further used to develop a statistical models composed of two functions capable of differentiating between the three animal groups. As can be seen from Fig. 3, for the female camels, function 1 was very effective in differentiating the fractured camels from the other two groups, but unfortunately function 2 showed significant overlap between normal and lame camels. Similarly, as can be seen from Fig. 4, function 1 was able to differentiate male fractured camels from the normal and lame camel groups. However, in contrast to the female camel function 2, which was not as effective in differentiating normal and lame female camels, the male camel function 2 was quite effective in being able to differentiate between normal and lame male camels.

It is worth noting that in both males and females, the fractured camels had significantly higher neutrophil count as compared to normal camels. Neutrophils are known to play a very critical role in inflammation processes, specifically Rheumatoid Arthritis, as they can damage bone and surrounding tissues by secreting proteases and reactive oxygen species (Wright, *et al*., 2010). It is our hypothesis that higher neutrophil count in the lame/fractured camels is one of the reasons for their poor bone condition. In addition to elevated levels of neutrophils in fractured camels, our results show significantly lower levels of eosinophils in male and female fractured camels. It is well-established that eosinophils, in addition to other functions, are important in fighting viral and parasitic infections. It is possible that due to decreased levels of eosinophils, the fractured camels may end up with viral/parasitic infections, leading to inflammatory responses and eventually leading to bone and surrounding tissue damage (along with other systemic effects). Additionally, it appears that hemoglobin concentrations (MCHC and MCH) may also hold indicative potential for differentiating between normal and fractured animals. Again, this and other hypotheses needs to be further investigated, but our initial data presented here appear to show a very strong correlation between neutrophils, eosinophils, and hemoglobin concentration (MCHC and MCH) and camel bone health.

Lastly, we tested our statistical model (which relied on only these limited hematological parameters) to see if fractured camels could be successfully differentiated from the normal camels. Our initial results are very encouraging which show that 100% of the male and female fractured camels could be successfully differentiated from the normal/lame groups, using only three hematological parameters for female camels and four for the male group. Our statistical model was also to successfully differentiate between normal and lame male camels. These preliminary results are very exciting and appear to suggest that routine hematological analyses may have value in possibly diagnosing and/or predicting camel lameness and fractures. We plan to test this hypothesis in our future work on this topic.

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