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The influence of sex, age and season on the haematological profile of alpacas (*Vicugna pacos*) in Central Europe

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ABSTRACT: The aim of this study was to establish reference intervals for the haematological profile of alpacas on the basis of a large population of clinically healthy animals, and to determine the influence of sex, age and season on these indicators. Blood samples were collected from 243 alpacas (53 males and 156 females over six months of age and 34 crias – 12 males and 22 females – under six months of age). The selected farms were located in Central Europe (Czech Republic and Germany). We determined 13 haematological indicators. Comparison of the results was performed with respect to the sex of animals and for the older group also with regard to the season and to the feeding period. We found no highly significant ($P > 0.001$) differences between males and females. We did find highly significant differences ($P < 0.001$) between the group of crias under six months of age and the older alpacas (mean corpuscular volume – MCV, mean corpuscular haemoglobin concentration – MCHC, red cell distribution width – RDW, white blood cell count – WBC, neutrophil count). Based on our findings we suggest that for some indicators different reference intervals (esp. WBC and differential cell counts) be used for the two above mentioned age groups. We found some highly significant differences ($P < 0.001$) in haematological indicators in the older group of alpacas between the summer and winter feeding period (haemoglobin concentration, MCHC). Clinical laboratory diagnosis may be improved by the use of age-based and season-based haematological reference values.

Keywords: clinical pathology; reference ranges; camelids; seasonal differences; blood

The alpaca (*Vicugna pacos*) is a member of the camelid family, genus *Vicugna*. The species originates from South America, where it evolved at altitudes of 4000 meters or more and therefore alpacas possess some unique features. More recently, alpacas have been growing in numbers and popularity also in European countries. Breeders and veterinarians need to be aware of the characteristics of these animals. Haematological tests are very important for assessment of animal health status and reference values are essential for diagnosis. There are studies on some haematological features of alpacas conducted in North America (Dawson et al. 2011), Australia (Hajduk 1992) and New Zealand (Ellison et al. 2006) as well as in Europe (Burri et al. 2005; Foster et al. 2009). However, most of the studies were performed on relatively small numbers of animals or did not take into consideration the age and sex of animals. Currently, to the author's knowledge, the only work considering the need of different reference intervals

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for different age groups of alpacas was performed by Burri et al. (2005). Furthermore, as nutrition, physiology and environmental conditions influence clinical pathology profiles (Husakova et al. 2014a, Husakova et al. 2014b), it is necessary to establish normal reference values for each animal species under defined environmental and nutritional conditions (Bogin 2000).

In our study we took into consideration the specific management conditions of alpacas in Central Europe such as the higher amount of nutrients that may influence haematological parameters. Furthermore, we took into account the seasonal effect (different feeding patterns in summer and winter periods) to examine the possible influence on haematological values (Zapata et al. 2003). It was our wish that this study be useful for breeders and veterinary practitioners, and, therefore, we considered and statistically evaluated the influence of age, sex and season on haematological parameters in the blood of alpacas. The objective of the study was to establish a reference interval for haematological parameters in alpacas bred in Central Europe on the basis of a large population of clinically healthy animals with regard to all the above factors.

MATERIAL AND METHODS

Animals. Blood samples were collected from 209 alpacas over six months of age (53 males and 156 females) and 34 crias under six months of age (12 males and 22 females) over the course of the period from 2010 to 2012 as part of research work (approved by the committee of the project grant) that was carried out in the course of preventive field work. All the conditions of animal welfare were ensured. Animals ranged from a two day old cria to a 16.5 year old female. The farms were located in Central Europe (15 in the Czech Republic and two in Germany). The owners were requested to complete a questionnaire regarding details of the farm (number of animals, feeding pattern in winter period vs. summer period, the age, sex, medical history, and origin of individual animals). All animals included in our study had either been born on their farm or had been in the herd for at least three months. All farms had similar management systems, i.e. animals were kept outside all year round with freely accessible shelter (wooden barn). The feeding pattern was also similar: green pasture and some hay in the summer period, mainly hay and some supplements (grain, mineral and vitamin mixture) in the winter period.

Supplementary feeding used by breeders (Supplementary feeding for alpacas and llamas, Sehnoutek and Sons v.o.s., Czech Republic) contained dried alfalfa stuff, oat, corn, sunflower meal, linseed, toasted soybean meal, calcium carbonate, sodium chloride, dicalcium phosphate monohydrate, Vitamin A (40 000 IU/kg), D3 (10 000 IU/kg), E (404 mg/kg) and copper sulphate pentahydrate $CuSO_4 \times 5\ H_2O$ (36 mg/kg). The recommended daily dose for alpacas is 300 g/50 kg body weight. We included only clinically healthy animals (an individual physical examination was performed on each animal prior to sampling) in the study with known parasitic burden. At the time of blood collection we also took faecal samples to test for gastrointestinal parasites. As all the farms included in our study were on regular deworming programs, the results of testing were mostly 0 to + (negligible) with only a few having ++ (moderate) parasitic burden. Only a few animals harboured highly pathogenic parasites of the genera *Trichuris* and *Nematodirus* and all of these had only negligible amounts of eggs (less than three) in the whole sample.

Sample collection and analysis. Blood samples were collected all year round. No sedation was used and only minimum restraint was required. To correctly determine the seasonal variation we divided the samples into two groups based on the feeding pattern. Group S (summer period) consisted of alpacas from which blood samples were collected in the period April 26th to October 5th and Group W (winter period) included animals from which samples were taken from October 25th to April 13th. Blood samples were collected via jugular venipuncture using a 20 G needle (Henry Schein, U.K.) into evacuated sample tubes (Aquisel K3E/EDTA 3K) containing dipotassium ethylene-diamine tetraacetate. From each sample one blood smear was also made (drop of blood from collecting needle immediately after sample collection). Samples were placed in a Thermo Cooler box for transportation to the laboratory. All laboratory analyses were performed at the Department of Laboratory Diagnostics for Large Animals (University of Veterinary and Pharmaceutical Sciences Brno). The haematological tests were performed on the BC-2800 Vet auto haematology analyser (Mindray Medical International Limited, China) with the camelid setting using anticoagulated blood and included white blood cell count (WBC), red blood cell count (RBC), haemo-

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globin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RDW). Blood smears stained with May-Grunwald-Giemsa stain were manually evaluated by an experienced medical technologist, who performed a differential count of 100 leukocytes visually under a microscope. In order to minimise sources of error, the differential cell counts were all performed by the same experienced person.

Statistical analysis. The basic statistical parameters of our set of results (means for each haematological variable and group of animals, pooled standard deviation) were calculated. Reference intervals were established from the lower 2.5th and upper 97.5th percentiles, after exclusions of obvious outliers detected manually. The data set was divided by sex, age and feeding pattern into the following groups: males and females over six months of age in summer/winter feeding period, and male and female crias less than six months of age in summer.

The set of haematological variables was statistically tested in a factorial design within two different subsets. To find out if males over six months of age significantly differed from females, and if both sexes differed between the summer and winter periods, we applied factorial ANOVA to obtain a response to both factors (sex and season) and their interaction. Because no data from winter crias were available, we had to test the difference between summer data for adult and cria males and females by factorial ANOVA separately. Scripts for statistical tests were written in the R program (version 3.0.1; www.r-project.org).

RESULTS

Mean and pooled standard deviation for all haematological indicators within alpaca groups differing in sex, age and season and statistical significance of factorial ANOVA for subsets (sex and season for the older group of alpacas; sex and age in summer season for both groups) are presented in Tables 1 and 2. Calculated reference ranges of haematological variables of different groups of alpacas are presented in Table 3.

Table 1. Effect of sex and season on haematological profile in alpacas older than six months of age

Item	Summer feeding		Winter feeding		Pooled s.d.	Significance		
	females	males	females	males		sex^A	season	sex × season
RBC (×10^{12}/l)	13.10	13.80	13.80	14.50	2.00	*	*	ns
HGB (g/l)	127.00	132.00	139.00	140.00	18.00	ns	***	ns
HCT (%)	29.90	31.00	31.00	32.00	4.40	ns	ns	ns
MCV (fL)	23.00	22.80	22.50	22.50	2.00	ns	ns	ns
MCH (pg)	9.70	9.70	10.10	9.80	0.80	ns	**	ns
MCHC (g/l)	424.00	430.00	452.00	439.00	24.00	ns	***	*
RDW (%)	19.10	18.80	18.00	20.00	1.40	ns	*	***
WBC (×10^9/l)	15.40	14.30	14.30	12.10	4.20	*	*	ns
Neutr (×10^9/l)	8.70	9.10	7.80	6.10	3.20	ns	*	ns
Eos (×10^9/l)	0.33	0.74	0.21	0.31	0.69	**	ns	ns
Mono (×10^9/l)	0.10	0.15	0.10	0.02	0.22	ns	*	ns
Lym (×10^9/l)	6.30	4.90	5.20	4.60	2.60	*	ns	ns
Baso (×10^9/l)	0.01	0.00	0.00	0.00	0.08	ns	ns	ns

RBC = red blood cell count; HGB = haemoglobin concentration; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; WBC = white blood cell count; Neutr = neutrophil count; Eos = eosinophil count; Mono = monocyte count; Lym = lymphocyte count; Baso = basophil count; sex^A = categories of males and females over six months of age; season = the year period of feeding (winter and summer feeding pattern); sex × season = an interaction between sex^A and season; ns = not significant

*P < 0.05, **P < 0.01, ***P < 0.001

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Table 2. Effect of sex and age on haematological profile in all alpacas (younger and older than six months)

Item	Alpacas over six months		Crias		Pooled s.d.	Significance		
	females	males	females	males		sex^{AC}	age	sex × age
RBC (×10^{12}/l)	13.10	13.80	14.20	14.10	1.90	ns	*	ns
HGB (g/l)	127.00	132.00	131.00	131.00	17.00	ns	ns	ns
HCT (%)	29.90	31.00	29.60	29.30	4.00	ns	ns	ns
MCV (fL)	23.00	22.80	21.10	20.90	2.10	ns	***	ns
MCH (pg)	9.70	9.70	9.20	9.30	0.80	ns	**	ns
MCHC (g/l)	424.00	430.00	443.00	447.00	23.00	ns	***	ns
RDW (%)	19.10	18.80	20.30	19.30	1.50	ns	***	ns
WBC (×10^{9}/l)	15.40	14.30	11.20	11.90	4.10	ns	***	ns
Neutr (×10^{9}/l)	8.70	9.10	4.60	5.60	3.10	ns	***	ns
Eos (×10^{9}/l)	0.33	0.74	0.09	0.13	0.70	*	*	ns
Mono (×10^{9}/l)	0.10	0.15	0.10	0.02	0.22	ns	ns	ns
Lym (×10^{9}/l)	6.30	4.90	4.40	4.70	2.50	*	*	ns
Baso (×10^{9}/l)	0.01	0.00	0.01	0.00	0.08	ns	ns	ns

For abbreviations of haematological indicators see Table 1

sex^{AC} = categories of all alpaca males and females; age = alpacas (older than six months) and crias (under six months); sex × age = an interaction between sex^{AC} and age; ns = not significant

*P < 0.05, **P < 0.01, ***P < 0.001

The influence of sex

The differences between all males and females alpacas were relatively weak or non-significant in most variables (Table 2). The only variables significantly influenced by sex were eosinophil counts and lymphocyte counts. We obtained significantly different results between males and females over

Table 3. Calculated reference ranges for the haematological profile of alpacas

Item	Alpacas older than six months					Crias (under six months of age) effect of age crias (F + M)
	effect of sex		effect of season (F + M)		effect of age	
	F	M	summer feeding	winter feeding	F + M	
RBC (×10^{12}/l)	10.0–17.9	10.3–20.0	10.0–18.3	10.0–18.4	9.6–18.4	11.8–16.1
HGB (g/l)	100.0–160.0	96.0–192.0	96.0–160.0	111.0–179.0	96.0–179.0	114.0–151.0
HCT (%)	23.5–40.0	23.2–42.0	23.6–39.3	23.2–42.0	22.9–41.6	23.9–34.3
MCV (fL)	19.7–27.2	18.6–27.0	19.2–27.2	18.9–25.7	19.0–27.2	18.2–25.9
MCH (pg)	8.6–11.3	8.2–11.3	8.2–11.2	8.6–11.8	8.2–11.3	8.1–10.9
MCHC (g/l)	389.0–497.0	387.0–479.0	389.0–462.0	399.0–507.0	387.0–482.0	403.0–508.0
RDW (%)	16.7–21.2	16.7–22.4	16.7–21.4	16.7–22.4	16.7–21.7	16.7–22.5
WBC (×10^{9}/l)	7.6–21.8	4.6–21.0	7.6–21.9	4.9–21.0	5.1–21.9	4.2–22.8
Neutr (×10^{9}/l)	2.6–14.8	1.3–13.8	2.6–14.8	1.3–12.0	1.7–14.8	0.7–8.1
Eos (×10^{9}/l)	0–1.6	0–2.1	0–2.7	0–1.2	0–2.1	0–0.2
Mono (×10^{9}/l)	0–0.7	0–0.6	0–0.8	0–0.5	0–0.7	0–0.4
Lym (×10^{9}/l)	1.7–11.6	2.4–9.6	2.2–11.6	1.6–10.1	1.6–10.9	2.0–6.4
Baso (×10^{9}/l)	0–0.1	0	0	0	0–0.01	0

For abbreviations of haematological indicators see Table 1

F = female; M = male

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Table 4. Reference ranges of the haematological profile of alpacas in the literature

Item	Hajduk (1992)	Bogin (2000)	Burri et al. (2005)			Foster et al. (2009)	Fowler (2010)		Dawson et al. (2011)
			C	M	F		C	A	
RBC ($\times10^{12}$/l)	11.5–14.1	8.3–12.5	12.9–15.4	12.8–15.6	10.5–15.0	9.1–13.8	9.6–17.2	10.5–17.2	9.4–18.1
HGB (g/l)	131–185	116–145	134–166	127–166	110–161	104–170	101–181	119–194	102–193
HCT (%)	30–41		29–37	29–37	26–37	24–36	24–28.5	27–45	22–45
MCV (fL)	23–32		21.5–25.4	22–25	22.8–26.3	21.8–28.9	21.5–29.9	22–29.9	21–28
MCH (pg)			9.7–11	10–10.8	9.8–11	10.6–12.7	9–11.9	10.1–12.7	9–12
MCHC (g/l)	382–557		419–464	414–459	411–454	418–496	394–449	393–468	420–490
WBC ($\times10^9$/l)	6.4–21.6	8.9–22.0	7.3–16.0	9.8–15.8	8–16	5.7–32.9	7.1–22.9	8–21.4	7.1–18.6
Neutr ($\times10^9$/l)	3.4–16.2		2.9–8	4.5–9.3	3.4–9.1	2.6–24.9			3.5–12
Neutr (%)	42–75	22–48	31–63	44–71	39–70		35–63	42–73	
Eos ($\times10^9$/l)	0.2–2.7		0.1–3.1	1–3.6	0.8–3.4	0–2.2			0.1–4.3
Eos (%)	2–20	3.5–6.0	1–18	8–27	7–27		0–9.5	2.2–21.4	
Mono ($\times10^9$/l)	0–0.8		0.3–0.9	0.1–0.7	0.2–0.9	0–2.1			0–1.0
Mono (%)	0–4	0.5–2.0	3.5–7	2–5.5	1.5–7.7		0–5.2	0–4.6	
Lym ($\times10^9$/l)	2.3–7.3		1.4–5.9	1.7–4.5	1.1–5.2	1.2–8.9			1.1–5.5
Lym (%)	19–53	15–59	15.7–54	15.2–31	11–39		18.3–41.9	9.2–25.2	
Baso ($\times10^9$/l)			0–0.1	0–0.3	0–0.2	0			0–0.4
Baso (%)		0.5–3.0	0–1	0–2	0–1.5		0–1	0–14	

For abbreviations of haematological values see Table 1

C = cria; M = male; F = female; A = adult alpaca

six months of age (Table 1). When we compared the obtained results between older males and females, one indicator exhibited a highly significant ($P < 0.01$) difference (eosinophil count) and three indicators were found to exhibit significant ($P < 0.05$) differences (RBC, WBC, and lymphocyte count). However, when we compared the calculated ranges (Table 3), there were only minimal differences between males and females. The most obvious difference in calculated ranges between males and females was observed in white blood cell count (the lower limit was significantly lower in males).

The influence of age

When we compared the data between the group of crias under six months of age and alpacas older than six months (Table 2) we found that from 13 variables evaluated in our study nine significantly differed between the two groups (five at $P < 0.001$, one at $P < 0.01$ and three at $P < 0.05$).

When, however, we compared the calculated ranges for observed variables (Table 3), most of the range values were similar for both groups. The most marked differences influenced by age were found in means of WBC counts and neutrophils which were significantly higher in the older group of alpacas. This group also exhibited a much larger calculated range for neutrophil counts (both lower and upper range limits were markedly lower in the crias group).

When we expressed the differential cell counts in percentages then for alpacas older than six months vs. crias under six months our reference ranges were as following: neutrophils, 26 to 80% vs. 12 to 64%; lymphocytes, 16 to 72% vs. 34 to 84%; eosinophils, 0 to 18% vs. 0 to 4%; monocytes, 0 to 5% vs. 0 to 4%; and basophils, 0 to 1% vs. 0 to 2% from the total WBC, respectively. These findings demonstrate higher levels lymphocytes, lower levels of neutrophils and lower levels of eosinophils in crias when compared with older alpacas.

The influence of season

Comparisons of the observed indicators in alpacas over six months of age sampled in the summer feeding period with the winter feeding period

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is presented in Tables 1 and 3. When we compared the means of tested indicators in different periods, we found higher values for most of the red blood cell parameters in the winter feeding period while the opposite was true for white blood cell indicators (higher mean values in the summer feeding period). The calculated range values are, however, similar for most indicators with the exception of HGB and MCHC (higher values) and WBC (lower values) in the winter feeding period.

DISCUSSION

Our study establishes comprehensive reference intervals for the haematological profile of alpacas bred in Central Europe with consideration of age, sex and seasonal differences. In Table 4 we present other relevant published data for comparison with our results (Table 3). The red blood cell indicator range values in our study are comparable to most previously published values for alpacas. Our lower limits for RBC counts, haemoglobin concentrations and haematocrit in adult alpacas are correspond with the values reported by Dawson et al. (2011), Foster et al. (2009) and by Ellison et al. (2006), while studies on alpacas in Switzerland (Burri et al. 2005) and in Australia (Hajduk 1992) reported narrower range limits of these indicators for male and female adult alpacas. In an alpaca study from Italy very low range limits for HGB (80 to 157 g/l) were reported (Morgante et al. 2001). However, the authors suspected nutritional deficiency in those animals. The lower limits for RBC count, HGB concentration and HCT are lower in the crias tested here than in those from a previous study conducted in Switzerland (Burri et al. 2005). These differences may be attributed to the lower numbers of animals in previous studies and also, in the case of crias, to the wide range of age of crias in our study.

Other, more common, causes of lower RBC counts and/or lower HCT may be subclinical disease (gastrointestinal parasitism or subclinical infection with *Mycoplasma haemolamae*) or differences in diet, e.g. iron and cooper deficiency (Morgante et al. 2001; Jones and Allison 2007). We examined all alpacas in our study for gastrointestinal parasites but a test for *M. haemolamae* was not performed as at the time of our study we did not consider this possibility. Over the course of the last year a few alpacas in the Czech Republic were confirmed to be positive for *M. haemolamae* by PCR testing. Therefore, alpacas, which are apparently healthy but have slightly lower packed cell volumes might be subclinical carriers of that disease (Tornquist et al. 2010).

Our range values for leucocytes are similar to these reported by most previous researchers, but wider than values reported for alpacas in Switzerland (Burri et al. 2005). There are many possible causes for an increased number of leucocytes such as bacterial infection, nutritional reasons – e.g., forage from industrial contaminated sites (Szakova et al. 2012). The most common cause, also suspected in our case, would be as a result of a stress response. On the other hand, a decreased number of neutrophils can be also caused by acute severe inflammatory diseases (e.g. gram-negative sepsis, pneumonia, and many others) or bone marrow injury. Lymphopenia may be observed in response to stress or corticosteroid administration, acute viral infections, and rare immunodeficiencies (Jones and Allison 2007). Viral infections, such as bovine viral diarrhoea virus (BVDV), usually cause leucopenia, which is well documented in the study of Johnson et al. (2010) on alpacas that were experimentally infected by BVD virus.

When we compared the results of our study with the haematological values of alpacas living at high altitudes in Peru (Fowler 1998), the effect of environment on blood indicators, such as RBC, haemoglobin, haematocrit and also WBC (all markedly higher in Peruvian alpacas) is clearly visible. The above finding supports the theory that haematological reference intervals should be regularly re-established to account for geographical and genetic variations (Passler et al. 2013).

Our conclusion that the influence of sex on most blood indicators is small is in accordance with other reports on alpacas. Hajduk (1992) found no significant differences for any variables except eosinophils, which corresponds with our findings, where eosinophils were the significant variable. For the other three variables in our study that were influenced by sex we found similar results only for erythrocytes (females having lower values than males) with the study of Burri et al. (2005). We found no similar results in the literature for WBC and lymphocytes in alpacas but we consider the level of significance low and from the practical point of view, not important. To the best of our knowledge there is only one other study on

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alpacas that considers the influence of sex on the haematological profile (Dawson et al. 2011) and the authors of this study reported no sex-associated differences for any of the median haematological results with the exception of mean platelet volume. There is, however, one study on farmed guanacos in Central Chile (Zapata et al. 2003) that describes differences between males and females in lymphocyte counts with males having significantly lower values than females. The authors interpreted this as being linked with the social behaviour of males, which consists of permanent hierarchical fights. Despite the fact that the studied guanaco males were castrated, there was still some hierarchical fighting behaviour observed. The alpaca males involved in our study were mostly breeding males; therefore, the above explanation for the reduced lymphocyte count is the most probable.

Our finding that most haematological indicators are significantly influenced by age is difficult to compare with literature as most studies presenting values for these indicators were done on adult animals or did not specify the age. The only work we can compare our findings with is the previously cited Swiss study, in which the lower MCV values in crias than in adult alpacas correspond with our findings. The results of the Swiss study also correspond with our finding that erythrocyte values were lower in adult females than in adult males but, unlike this study, the authors of this previous work did not find that age significantly influenced RBC (higher in crias). For the other RBC values monitored in our study, where the means significantly differed between crias and adult alpacas, we found no similar results in the literature. The same is true for WBC parameters, most of these were significantly influenced by age in our study but there is scarcity of data on this subject for comparison. The study from Switzerland (Burri et al. 2005) found that most WBC values were not influenced by age with the exception of lymphocytes (lower in adults) and eosinophils (lower in crias), which corresponds with our results.

Our finding that most haematological indicators were affected by season is difficult to compare with the literature as to the best of our knowledge no similar study has been carried out on alpacas. Similarly to the results of the study on farmed guanacos (Zapata et al. 2003), we found that haemoglobin was significantly higher in the winter feeding period. This may be caused by haemoconcentration, which probably stemmed from slight dehydration caused by reduced access to water or decreased intake of water. White blood cell parameters were not influenced by season in the previous study, while in our study we found differences in leucocytes, neutrophils and monocytes. However, the level of significance was low and the clinical importance of this finding is debatable.

In this study we have confirmed the theory that camelids are different from true ruminants in a number of important characteristics, one of them being the fact that most of the leucocytes in alpacas were neutrophils, while in other ruminants lymphocytes are the predominant leucocyte (Azwai et al. 2007).

CONCLUSIONS

Our study provides reference intervals for haematological indicators in clinically healthy young and adult alpacas in Central Europe. We found no clinically important differences between males and females. The age factor would appear to be the most important variable evaluated as we found many significant differences between the group of crias under six months of age and the older alpacas. We suggest that for some indicators different reference intervals should be used (esp. WBC, neutrophils and lymphocytes) for the two above mentioned groups, as in the younger group we found lower values of these indicators when compared with the older group of alpacas. We found higher values for most red blood cell indicators in the winter feeding period while the opposite was true for white blood cell indicators.

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