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Associations of Hematological Parameters with Age, Body Weight, Birth Ttype and Season in Lori-Bakhtiari Ewes

Research Article

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ABSTRACT

Blood parameters are important indicators for health conditions in animals, but can be influenced by several factors. This study was conducted to explore reference intervals for hematological parameters and investigate their possible relationships with different factors in Lori-Bakhtiari ewes. A total of 96 blood samples were randomly collected from healthy ewes at a research flock. Different hematological parameters, including red blood cell, white blood cell, neutrophil and lymphocyte counts (RBC, WBC, NP and LC, respectively), neutrophil and lymphocyte percentages (NP and LP, respectively), hematocrit (Hct) and hemoglobin (Hb), mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration (MCV, MCH and MCHC, respectively) were measured at laboratory. Based on the coefficients of variations, NC (44.4%), LC (43.6%) and WBC (39.0%) were the most variable parameters, while the MCV (1.6%) had the lowest variability. Simple correlation coefficients and cluster analysis showed that erythrocyte-related parameters (Hct, MCH, MCV, Hb and RBC) were independent from the leukocyte traits (WBC, NC, NP, LC and LP). Birth type and age significantly (P<0.05) affected NC and LP, respectively. Heavier animals in 6 and 9 months of age had higher levels of Hb, MCH and MCHC in adulthood. Body weight traits also had some correlations with leukocyte parameters in higher ages, indicating that growth rate of the lambs may influence their immunity in the future or vice versa, the animals with stronger immunity may have higher growth rate. More attention to immunological parameters is needed, when breeding programs are planned for growth rate.

KEY WORDS birth type, body weight, breeding, immunology, sheep.

INTRODUCTION

Sheep is an important multipurpose livestock, which plays important roles in world food supply and economics (Mohammadi *et al.* 2022). Therefore, optimal health, metabolism and physiological conditions are vital concerns for sheep production industry. Hematological traits are informative biomarkers of managing and nutritional activities and physiological and health conditions of the animals. Moreover, some ovine haematological parameters may be correlated with productive and reproductive traits (Al-Thuwaini, 2021).

Hemoglobin, is responsible for oxygen transferring in blood and is influenced by genetic factors. Hemoglobin is also correlated with economic traits in farm animals (Al-Khuzai and Al-Khazaji, 2019). Other blood parameters, such as leukocytes, hematocrit, erythrocytes, lymphocyte and neutrophil counts are also hematological traits associated with health conditions and therefore reproduction and production performances.

Hematological parameters may be influenced by different factors, including sex, age, nutrition, diseases, geographical conditions, physiological status, genetic factors and so on (Badawi and Al-Hadity, 2014; Al-Thuwaini *et al.* 2020; Seixas *et al.* 2021; Santarosa *et al.* 2022). Therefore, consideration of the possible nuisance factors is necessary for interpretation of hematological parameters profile. There is little information on the effects of birth season, growth rate and birth type on hematological parameters and little information on Iranian indigenous breeds can be found in literature. The present study was conducted to determine the reference values for hematological parameters in Lori-Bakhtiari ewes and investigation of their associations with some factors, including age, growth rate, birth type and season.

MATERIALS AND METHODS

The studied population

This study was conducted on a research flock of Lori-Bakhtiari sheep, a large meat-type breed, mainly reared in western Iran (Almasi *et al.* 2020), at Shooli Breeding Station in Chaharmahal and Bakhtiari province (32.31362˚ N, 51.05340˚ E). A detailed information about the management and climatic properties of the studied population can be retrieved from Mohammadi *et al.* (2022).

The samples

A total of 96 ewes were randomly selected from the studied flock. The rams were excluded from the sampling process, because only a few adult males were available in the population and their count was not enough for statistical analysis. The sampled ewes were in middle stages (third month of pregnancy) of pregnancy and all were healthy, without any external and internal parasitic infection. Health conditions of the selected animals were evaluated based on several factors, such as body condition and temperature, feed consumption, respiration status, heart rate, faeces appearance and parasitic infection tests in laboratory. The sampled animals also had complete records of birth weight and body weights at 3, 6, 9 and 12 months of age (BW0, BW3, BW6, BW9 and BW12, respectively).

Measurement of hematological parameters

Blood samples were separately taken from jugular vein, by Ethylenediaminetetraacetic acid (EDTA) containing vacutainer tubes. The sample were immediately transferred to a laboratory to measure various hematological parameters, including red blood cell, white blood cell, neutrophil and lymphocyte counts (RBC, WBC, NC and LC, respectively), neutrophil and lymphocyte percentages (NP and LP, respectively), hemoglobin (Hb) and hematocrit (Hct). The cell-count parameters, including RBC, WBC, NC and LC were manually measured, by dilution ratio of 1:10, using Neubauer counting chambers. Hct was measured as proportion of the packed cells to total blood volume.

Mean corpuscular volume (mean red blood cells volume) was calculated as follows:

$$MCV\ (fL) = (Hct \times 10) / RBC$$

Where:
MCV, Hct and RBC: mean corpuscular volume (fL), hematocrit (%) and red blood cell count ($10^6/\mu L$), respectively.

Mean corpuscular hemoglobin or average hemoglobin mass in erythrocytes, was calculated as proportion of total hemoglobin mass to RBC:

$$MCH = (Hb \times 10) / RBC$$

Where:
MCH, Hb and RBC: mean corpuscular hemoglobin (pg), total hemoglobin concentration (g/dL) and red blood cell count ($10^6/\mu L$), respectively.

Mean corpuscular hemoglobin concentration, or average concentration of hemoglobin in red blood cells was calculated as follows:

$$MCHC = (Hb \times 100) / Hct$$

In this equation, MCHC, Hb and Hct were mean corpuscular hemoglobin concentration (g/dL), whole blood hemoglobin concentration (g/dL) and hematocrit (%), respectively.

Statistical analysis

The data were analyzed by different methods. In the first step, descriptive statistics and Pearson correlation coefficients for the studied hematological parameters and body weight traits were calculated using the MEANS and CORR procedures of SAS 9.4 (SAS, 2013), respectively. Reference intervals for the studied parameters were calculated as the ranges comprising 95% of the observations (Geffré *et al.* 2009). Relationships between different hematological parameters were also investigated by a cluster analysis, based on absolute correlation coefficients, using Minitab 17 (Minitab Inc., 2013). The data were also analyzed by the following generalized linear model:

$$Y_{ijkl} = \mu + A_i + BT_j + S_k + \beta(BW_{ijkl}) + e_{ijkl}$$

Where:

μ: overall mean.

$A_i + BT_j + S_k$: i[th] year of age (2-3, 4, 5, 6 and +7 years), the j[th] birth type (singleton or twin) and the k[th] birth season (spring or winter) effects, respectively.

B: regression coefficient of the observed hematological parameter (y) on estimates of additive genetic effects for body weight (BW).

e_{ijkl}: residual effects.

Estimates of additive genetic effects for body weight were obtained by whole population data analysis (15859 body weight records of 4402 animals), using an animal mixed model, fitting contemporary group (birth year-season), birth type (litter size), sex and dam age as the fixed factors and direct additive genetic, permanent environment, and maternal genetic and environmental effects, as the random factors. Random factors of the model, including direct additive genetic effects were predicted by Average Information algorithm of Restricted Maximum Likelihood (AI-REML), using the Wombat software (Meyer, 2007). Least square means for different levels of the fixed factors were estimated and compared by Tukey-Kramer test at P= 0.05 level. The generalized linear model (GLM) and least square means were analyzed using the GLM procedure of SAS 9.4 (SAS, 2013).

RESULTS AND DISCUSSION

Descriptive statistics of the hematological parameters are summarized in Table 1, but simple statistics of body weight traits (birth weight and body weights at 3, 6, 9 and 12 years of age) in the studied samples can be retrieved from Mohammadi *et al.* (2022). Based on the estimates of coefficients of variation, MCV (average volume of erythrocytes) was the most uniform (CV=1.62%), while NC and LC and WBC were the most variable hematological parameters, with 44.36%, 43.61% and 39.03% CVs, respectively (Table 1). Hb, Hct, RBC. MCH and MCHC had intermediate variations, with 14.05-18.34 % CVs. Lymphocyte and neutrophil percentages had almost similar averages (49.33% *vs.* 46.33) and totally comprised 95.66% of whole white blood cells (Table 1). Reference intervals, calculated for the studied parameters are also presented in Table 1.

Pairwise pearson correlation coefficients between the studied variables are presented in Table 2. Based on the estimates of correlation coefficients, erythrocyte-related parameters (RBC, Hct, Hb, MCV, MCH and MCHC) were independent from the leukocyte-related traits, including WBC, NC, LC, NP and LP (P>0.05). Neutrophil and lymphocyte counts (NC and LC) were also independent from their proportional frequencies in WBC (NP and LP), (P>0.05). On the other hand, highly significant correlations (P<0.01) were observed between red blood cell and hemoglobin traits (Hb, Hct, RBC, MCH and MCHC). Specially, Hct had high correlations with RBC (1.00) and MCV (0.97). High correlation coefficients were also observed between RBC - MCV (0.97), and MCH - MCHC (1.00). RBC had significant negative correlations with MCHC (-0.56) and MCH (-0.50). WBC obviously had high significant correlations with NC (0.84) and LC (0.83), (Table 2). The independencies of erythrocyte and leukocyte-related parameters, as well as intragroup correlations were also confirmed by the cluster analysis (Figure 1).

Some hematological parameters in mature animals, including Hb, MCH, MCHC, NC, LC and LP had significant correlations (P<0.05) with body weights at different ages (Table 2). BW0 had a significant positive correlation with LC. BW6 had significant positive correlations with Hb, MCH and MCHC and a negative correlation with NC. BW9 had significant positive correlations with MCH, MCHC and LP and a significant positive correlation was observed between BW12 and LP. Body weight traits had some close to significant correlations (P<0.10) with blood parameters at higher ages, as presented in Table 2.

In the general linear model analysis, age, birth type, birth season and body weight's additive genetic effects did not have any significant effect on the erythrocyte-related parameters, *i.e.*, Hct, Hb, RBC, MCV, MCH and MCHC (P>0.05). Similarly, most of the leukocyte-related parameters, including WBC, LC and NP were not significantly affected by the studied factors. However, NC and LP were significantly (P<0.05) affected by birth type and age, respectively. In comparison of means, average of neutrophil count in singletons was significantly higher than the twins (P<0.05). Lymphocyte percentage in 2-3-year-old ewes was also lower than the 4- and 5-year-old ewes (P<0.05). The additive genetic effects, estimated for body weight had a close to significant correlation (P<0.10) with WBC, whereby the animals with higher growth rates tended to have a lower WBC (β=-354.1±190.23 10^9/L/kg). Birth season had also close to significant effects (P<0.1) on some erythrocyte-related traits, including RBC, Hct, MCH and MCHC. Results of the general linear model analysis, including p-values and estimates of least square means for the studied parameters are presented in Table 3.

Determination of reference values for hematological parameters is considered as a powerful tool for investigation of health status of farm animals. References ranges for the hematological parameters have been reported for several sheep breeds, such as Merino (Lepherd *et al.* 2009), Awassi (Badawi and Al-Hadity, 2014. Al-Thuwaini *et al.* 2020) and crossbreds (Greguła-Kania *et al.* 2020). Averages of the observed parameters were in the range reported by the previous studies (Table 4).

Table 1 Descriptive statistics for the studied hematological parameters variables (N=96)

Parameters	Unit	Descriptive statistics							
		Average	SD	SE	CV (%)	95% CIM	Min	Max	RI
Hct	%	33.06	5.20	0.53	15.75	32.00-34.11	21.00	49.00	27.00-46.00
Hb	g/dL	8.41	1.36	0.14	16.18	8.14-8.96	5.99	14.42	6.40-10.85
MCV	fL	35.60	0.57	0.06	1.62	35.48-35.72	33.6	39.98	34.83-36.80
MCH	pg	9.19	1.61	0.16	17.56	8.86-9.52	4.71	16.96	6.83-11.46
MCHC	g/dL	25.87	4.74	0.48	18.34	24.90-26.83	12.78	48.06	19.02-33.42
RBC	10^6/μL	9.26	1.30	0.13	14.05	9.00-9.52	6.25	13.25	7.75-12.50
WBC	10^9/L	6.75	2.63	0.27	39.03	6.21-7.28	0.26	17.80	3.50-10.40
NC	10^9/L	3.11	1.38	0.14	44.36	2.83-3.39	0.16	8.19	1.18-5.62
LC	10^9/L	3.32	1.44	0.15	43.61	3.02-3.91	0.10	8.11	1.42-6.18
NP	%	46.33	10.91	1.11	23.55	44.12-48.54	28.00	71.00	29.00-65.00
LP	%	49.33	11.49	1.17	23.29	47.00-51.66	14.00	68.00	28.00-67.00

SD: standard deviation; SE: standard errors of means; CV: coefficient of variation; 95% CIM: 95% confidence interval of means; Min: minimum; Max: maximum; RI: reference interval;
Hct: hematocrit; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RBC: red blood cells count; WBC: white blood cells count; NC: neutrophil count; LC: lymphocyte count; NP: neutrophil percentage; LP: lymphocyte percentage.

Table 2 Estimates of pairwise Pearson correlation coefficients for the studied hematological parameters and body weight traits (P-values are presented in parentheses)

Variable	Hct	Hb	MCV	MCH	MCHC	RBC	WBC	NC	LC	NP	LP
Hb	0.26										
	(0.011)										
MCV	0.97	0.28									
	(<.001)	(0.007)									
MCH	-0.50	0.70	-0.49								
	(<.001)	(<.001)	(<.001)								
MCHC	-0.56	0.64	-0.55	1.00							
	(<.001)	(<.001)	(<.001)	(<.001)							
RBC	1.00	0.26	0.97	-0.49	-0.55						
	(<.001)	(0.011)	(<.001)	(<.001)	(<.001)						
WBC	0.13	0.13	0.12	0.01	0.00	0.13					
	(0.205)	(0.200)	(0.243)	(0.892)	(0.976)	(0.205)					
NC	0.12	0.08	0.12	-0.03	-0.04	0.12	0.84				
	(0.256)	(0.440)	(0.237)	(0.788)	(0.716)	(0.256)	(<.001)				
LC	0.06	0.14	0.04	0.08	0.08	0.06	0.83	0.44			
	(0.581)	(0.178)	(0.723)	(0.421)	(0.456)	(0.581)	(<.001)	(<.001)			
NP	-0.03	-0.13	-0.01	-0.10	-0.09	-0.03	-0.06	0.46	-0.50		
	(0.795)	(0.224)	(0.946)	(0.338)	(0.360)	(0.795)	(0.566)	(<.001)	(<.001)		
LP	-0.02	0.11	-0.04	0.12	0.12	-0.02	-0.04	-0.49	0.49	-0.88	
	(0.838)	(0.299)	(0.724)	(0.255)	(0.261)	(0.838)	(0.686)	(<.001)	(<.001)	(<.001)	
BW0	0.03	0.14	0.04	0.08	0.08	0.03	0.19	0.14	0.20	-0.08	0.03
	(0.798)	(0.184)	(0.680)	(0.411)	(0.464)	(0.798)	(0.055)	(0.173)	(0.049)	(0.448)	(0.774)
BW3	0.07	0.07	0.05	0.01	0.01	0.07	-0.05	-0.05	-0.01	-0.10	0.12
	(0.498)	(0.510)	(0.619)	(0.919)	(0.950)	(0.498)	(0.646)	(0.626)	(0.935)	(0.310)	(0.234)
BW6	0.04	0.31	0.03	0.24	0.22	0.04	-0.18	-0.20	-0.07	-0.15	0.19
	(0.697)	(0.002)	(0.775)	(0.020)	(0.030)	(0.697)	(0.086)	(0.046)	(0.513)	(0.136)	(0.065)
BW9	-0.06	0.18	-0.09	0.20	0.20	-0.06	-0.11	-0.15	0.00	-0.19	0.22
	(0.574)	(0.087)	(0.384)	(0.046)	(0.047)	(0.574)	(0.269)	(0.151)	(0.979)	(0.059)	(0.034)
BW12	-0.03	0.10	-0.04	0.11	0.11	-0.03	-0.18	-0.19	-0.05	-0.16	0.24
	(0.775)	(0.331)	(0.671)	(0.281)	(0.286)	(0.775)	(0.076)	(0.070)	(0.629)	(0.121)	(0.018)

Hct: hematocrit; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RBC: red blood cells count; WBC: white blood cells count; NC: neutrophil count; LC: lymphocyte count; NP: neutrophil percentage; LP: lymphocyte percentage; BW0, BW3, BW6, BW9 and BW12: birth weight and body weights at 3, 6, 9 and 12 months of age, respectively.

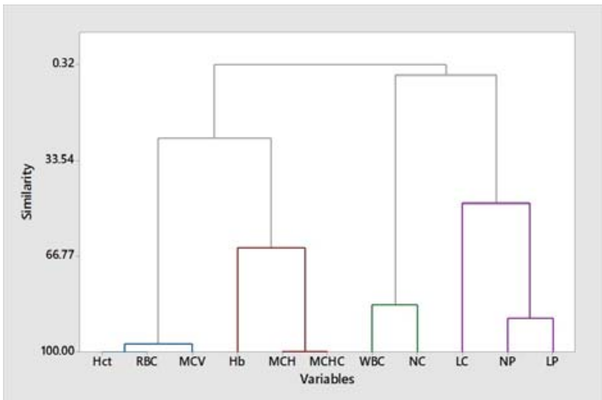

Figure 1 Dendrogram of the cluster analysis, based on the absolute correlation coefficients. Hct: hematocrit; Hb: hemoglobin; MCV, MCH and MCHC: mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, respectively; RBC, WBC, NC and LC: red blood cell, white blood cell, neutrophil and lymphocyte counts, respectively; NP and LP: neutrophil and lymphocyte percentages, respectively

However, a huge variation was observed between the reported values in literature. Based on the information presented in Table 4, different hematological parameters in sheep are highly dependent on various factors, such as breed, sex and pregnancy stage (Sharma *et al.* 2015; Islam *et al.* 2018; Al-Thuwaini *et al.* 2020; Greguła-Kania *et al.* 2020; Abdel-Lattif and Al-Muhja, 2021; Seixas *et al.* 2021; Yenilmez *et al.* 2021). In addition of genetic factors and physiological status, several none-genetic factors, such as climatic conditions, geographical location, season, day length, nutritional status and stress can alter the hematological parameters (Etim *et al.* 2014). Based in the information presented in the Table 4, slight differences, especially in Hb, MCH, MCHC, WBC and NP were found between the present study and another study on Lori-Bakhtiari sheep (Nikbakht Broojeni and Talebi, 2000). These differences can be attributed to several factors, such as season, climatic conditions, management, health, nutrition and physiological status, especially all sampled ewes in the present study were pregnant, thus the found reference values in this study (Table 1) are rather specific to pregnant ewes.

Based on estimates of coefficients of variations (Table 1), neutrophil, lymphocyte and total white blood cell counts had the highest variabilities, while mean corpuscular volume had the lowest variation among the studied parameters, which means higher sensitivity of leukocyte-related parameters to genetic or individual factors.

Breed effect is one of the main factors affecting hematological parameters. This effect can be attributed to different genetic structures and body's hemostatic parameters of the breeds (Alonso *et al.* 1997).

As it was mentioned previously, in addition to genetic factors, the hematological system is also sensitive to several none-genetic factors, e.g., age, sex, physiological status and management, which may affect the blood parameters variations (Ribeiro *et al.* 2018).

As it was expected previously, based on the estimates of pairwise correlation coefficients (Table 2) and the result of cluster analysis (Figure 1), the red cell-related parameters (Hb, Hct, RBC. MCH and MCHC) were highly correlated together. Significant correlations between the red cell parameters agrees with the previous reports such as the Hrković-Porobija *et al.* (2019) study on Pramenka sheep. Moreover, it must be noticed that some of the haematological parameters are calculated as ratio traits and are not independently measured, thus their high correlations is due to the calculation method.

High and positive correlations of Hct with RBC and MCV (Table 2) indicates that a high level of Hct is probably due to a high RBC, and a higher MCV would result in an increased Hct. In other words, this result shows that the hematocrit value is determined by both number and size of the red cells. Negative correlations of RBC with MCHC and MCH (Table 2), indicates that a higher RBC would probably result in lower levels of hemoglobin mass and concentration in red blood cells.

On the other hand, based on both correlation coefficients (Table 2) and cluster analysis (Figure 1), the red cell parameters (Hb, Hct, RBC, MCH and MCHC) were independent from the white cell parameters (WBC, NC, LC, NP and LP). This study showed that the RBC, Hct and Hb levels do not have any significant correlation with the white blood cell parameters. However, due to several functions of the red blood cells, such as oxygen carrying and binding and scavenging chemokines, nucleic acids, and pathogens, the erythrocytes may promote the innate immunity system (Anderson *et al.* 2018).

In the present study, body weight traits had significant correlations with some hematological parameters in higher ages, whereby heavier animals in 6 and 9 months of age tended to have higher levels of Hb, MCH and MCHC. On the other hand, some leukocyte-related parameters had significant ($P<0.05$) or close to significant ($P<0.10$) correlations with body weight traits. WBC and NC had negative correlations with 6 and 12 month-weights (Table 2), in accordance with the results of the general linear model analysis, which estimates of additive genetic effects for body weight had a nearly significant association ($P<0.10$) with WBC. On the other hand, body weight traits in 6, 9 and 12 months of age, had some significant ($P<0.05$) or close to significant ($P<0.010$) negative correlations with NP and positive correlations with LP (Table 2).

Table 3 The results of the general linear model and least square means analyses for the studied parameters

Variable	Hct (%)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RBC (10^6/µL)	WBC (10^9/L)	NC (10^9/L)	LC (10^9/L)	NP (%)	LP (%)
Age											
2-3	33.55	8.09	35.60	8.79	24.78	9.39	7600.35	3490.20	3199.89	47.64	42.41[b]
4	33.16	8.19	35.59	8.97	25.28	9.29	6368.06	2589.08	3477.73	41.57	54.02[a]
5	34.20	8.79	35.70	9.30	26.10	9.55	5871.04	2457.59	3178.18	42.40	54.23[a]
6	35.82	8.04	35.83	8.22	23.01	9.95	9642.58	3246.78	3352.73	47.20	48.49[ab]
7+	33.41	7.91	35.60	8.67	24.43	9.35	6480.06	2882.32	3345.03	44.42	51.66[ab]
SEM	1.214	0.317	0.135	0.371	1.092	0.303	590.299	301.168	336.590	2.501	2.527
Birth season											
Spring	35.56	8.07	35.80	8.30	23.26	9.89	6893.08	2982.60	3461.86	44.00	50.50
Winter	32.49	8.33	35.53	9.28	26.18	9.12	6411.75	2883.79	3159.57	45.29	49.82
SEM	1.104	0.288	0.123	0.337	0.993	0.275	536.582	273.701	305.960	2.273	2.297
Birth type											
Singleton	34.22	8.34	35.71	8.83	24.76	9.55	7221.38	3361.92[a]	3488.23	47.09	48.20
Twin	33.84	8.06	35.62	8.76	24.68	9.46	6083.46	2504.47[b]	3133.20	42.20	52.12
SEM	0.967	0.253	0.108	0.269	0.87	0.241	470.394	239.993	268.220	1.993	2.014
P-value											
Age	0.549	0.349	0.681	0.339	0.366	0.549	0.437	0.156	0.967	0.392	0.029
Birth season	0.084	0.575	0.181	0.070	0.068	0.084	0.574	0.821	0.536	0.721	0.853
Birth type	0.817	0.504	0.589	0.886	0.956	0.817	0.148	0.034	0.427	0.142	0.244
BW	0.620	0.227	0.496	0.139	0.139	0.620	0.066	0.244	0.111	0.822	0.846

Hct: hematocrit; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RBC: red blood cells count; WBC: white blood cells count; NC: neutrophil count; LC: lymphocyte count; NP: neutrophil percentage; LP: lymphocyte percentage and BW: linear regression on estimate of additive genetic effects for body weight.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The correlations between body weight traits and leukocyte parameters in higher ages indicate that growth rate of the lambs may influence their immunity in the future. Thus, it seems that more attention should be paid to immunological traits when breeding programs are planned for growth rate in sheep and probably other domestic animals.

Effects of body weight on some hematological parameters have been reported in some studies. In a study, body weight had a significant effect on WBC in Awassi ewes (Abdel-Lattif and Al-Muhja, 2021). However, no report on correlation of body weight and hematological parameters in different ages was found in literature.

In the present study there was a significant association between age and lymphocyte percentage (Table 3). This observation can be attributed to noticeable effects of environmental factors and physiological changes by increase of age. In a meta-analysis of hematological parameters of various breeds of sheep in different regions of Brazil, young animals (under 1 year old) had higher RBC (12.08 *vs.* 9.84 10^6/µL), lower MCV (23.45 *vs.* 32.96 fL) and lower monocyte count (1238.4 *vs.* 2047.3 10^6/µL) compared to the adult animals. In that study, the young animals also and lower Hb, MCHC and WBC than the adult animals (Seixas *et al.* 2021).

Significant effect of physiological status on lymphocyte percentage is supported by Greguła-Kania *et al.* (2020), who reported an increase in LP as a result of the parturition.

However, in another study, lambing resulted in decrease of LP and increase of NP and WBC, whereby the observed leukocytosis and neutrophilia were attributed to the parturition injuries, and decrease of lymphocytopenia (LP) was ascribed to the lambing stress (Khan *et al.* 2002). In a previous study on Lori-Bakhtiari sheep, RBC, Hb and Hct increased while WBC decreased in higher ages sheep (Nikbakht Broojeni and Talebi, 2000). The difference between current study and the values reported by Nikbakht Broojeni and Talebi (2000) can be attributed to population structures, environmental conditions, parameter measurement methods and physiological status of the sampled animals.

It has been found that, various hematological parameters, such as Hb, Hct, MCV, MCHC, RBC, WBC, NC, LC and neutrophil to lymphocyte ratio can be influenced by the reproductive stage (Bezerra *et al.* 2017).

In a study on Awassi ewes, LP of 2-6-year-old ewes varied from 69-77%, however because of a small sample size (15 animals), these differences were not significant (Abdel-Lattif and Al-Muhja, 2021).

Table 4 Comparison of the observed means of the studied hematological parameters in recent study and some reports in literature

Source	Hematological parameters										
	Hct (%)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RBC (10^6/µL)	WBC (10^9/L)	NC (10^9/L)	LC (10^9/L)	NP (%)	LP (%)
Current study	**33.1**	**8.4**	**35.6**	**9.2**	**25.9**	**9.3**	**6.8**	**3.1**	**3.3**	**46.3**	**49.3**
Abdel-Lattif and Al-Muhja, (2021)[1]	19.1	7.99	-	-	-	4.29	17.3	-	-	16.5	73.1
Al-Thuwaini *et al*. (2020)[2]	-	8.1	34.6	8.4	23.3	6.7	8.7	3.9	4.9	32.9	60.2
Al-Thuwaini *et al*. (2020)[3]	-	9.9	35.4	8.2	22.4	8.1	9.2	3.1	6.8	30.5	87.8
Badawi and Al-Hadity, (2014)[4]	34.9	11.5	32.1	10.6	33.0	10.9	9.5	3.7	5.5	-	-
Badawi and Al-Hadity, (2014)[5]	32.1	10.3	31.3	10.1	32.2	10.3	10.4	4.5	5.4	-	-
Dias *et al*. (2010)[6]	40.8	11.8	41.6	12.2	29.2	9.8	5.7	1.8	3.3	33.1	58.5
Greguła-Kania *et al*. (2020)[7]	34.0	12.3	26.5	9.6	-	12.6	11.3	3.7	7.3	32.5	64.5
Greguła-Kania *et al*. (2020)[8]	30.0	11.5	33.3	12.8	-	9.0	7.1	3.8	3.2	54.0	45.5
Greguła-Kania *et al*. (2020)[9]	27.0	11.8	30.3	13.3	-	8.9	8.6	4.0	4.5	46.7	52.7
Islam *et al*. (2018)[10]	34.2	9.9	-	-	-	4.4	6.9	2.4	3.7	34.6	54.0
Islam *et al*. (2018)[11]	33.3	9.8	-	-	-	4.4	5.9	2.1	3.2	34.7	54.4
Khan *et al*. (2002)[12]	25.0	6.0	-	-	-	9.0	6.0	-	-	43.0	43.0
Khan *et al*. (2002)[13]	24.0	6.0	-	-	-	9.0	7.0	-	-	55.0	34.0
Lepherd *et al*. (2009)[14]	33.0	12.1	32.0	11.0	36.2	10.6	9.0	2.2	6.1	-	-
Nikbakht Broojeni and Talebi, (2000)[15]	32.9	9.8	36.7	11.0	30.1	9.0	14.0	-	-	34.1	47.8
Nikbakht Broojeni and Talebi, (2000)[16]	36.4	10.7	36.1	10.7	29.6	10.1	12.4	-	-	29.7	53.4
Nikbakht Broojeni and Talebi, (2000)[17]	36.8	10.9	36.3	10.8	29.8	10.2	13.0	-	-	29.3	50.0
Nikbakht Broojeni and Talebi, (2000)[18]	36.8	11.1	36.2	10.9	30.1	10.2	11.5	-	-	30.7	49.0
Rahman *et al*. (2018)[19]	32.2	10.5	-	-	-	6.8	7.3	-	-	33.7	56.8
Rahman *et al*. (2018)[20]	29.6	9.9	-	-	-	7.5	6.9	-	-	35.2	55.4
Seixas *et al*. (2021)[21]	28.9	8.5	23.7	-	29.5	12.2	9.3	5.0	3.5	-	-
Seixas *et al*. (2021)[22]	31.5	9.0	23.9	-	28.8	13.0	8.5	4.6	3.3	-	-
Sharma *et al*. (2015)[23]	30.0	9.5	32.8	9.7	32.7	9.8	10.4	-	-	-	-
Sharma *et al*. (2015)[24]	34.5	11.0	30.3	9.5	31.6	11.4	9.6	-	-	-	-
Sharma *et al*. (2015)[25]	28.9	10.9	31.7	12.2	36.7	9.2	7.7	-	-	-	-
Yenilmez *et al*. (2021)[26]	21.4	10.9	-	-	-	8.0	11.8	-	6.8	-	-
Yenilmez *et al*. (2021)[27]	20.3	10.5	-	-	-	7.8	12.2	-	7.1	-	-

Hct: hematocrit; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RBC: red blood cells count; WBC: white blood cells count; NC: neutrophil count; LC: lymphocyte count; NP: neutrophil percentage and LP: lymphocyte percentage.
1: Awassi ewes; 2 and 3: singleton and twin born Awassi ewes, respectively; 4 and 5: male and female Iraqi Awassi sheep, respectively; 6: Churra-da-Terra-Quente ewes; 7-9: non-pregnant, two weeks before lambing and two weeks after lambing in a crossbred line, respectively; 10 and 11: male and female Dhumba sheep, respectively; 12 and 13: Thalli ewes at pre- and post-lambing stages, respectively; 14: weaned female Merino lambs; 15-18: 2-5 year-old Lori-Bakhtiari ewes, respectively; 19 and 20: Immature (<6 months) and adult (>6 months) Bangladeshi indigenous fat-tailed sheep, respectively; 21 and 22: averages of commercial wool a local hair breeds of sheep in Brazil, respectively; 23-25: Himalayan Gaddi sheep at anestrus, pregnancy and postpartum stages, respectively; 26 and 27: twin- and singleton-pregnant Kıvırcık ewes, respectively.

Alonso *et al*. (1997) did not show any significant difference in LP at different ages in Merino sheep. It seems that the effects of age on LP is largely dependent on environmental conditions and the infections induced by various detectable and undetectable microorganisms (Egbe-Nwiyi *et al*. 2000).

In the present study, birth type of the animals did not have significant effects on most of the studied parameters, however, neutrophil count in singleton-born individuals was significantly higher than the twins (Table 3). Birth type of an adult sheep have not been studied in previous researches. However, it has been found that the reproductive traits, including litter size and physiological condition may induce noticeable changes in hematological parameters. In a study on dorper ewes, the twin-pregnant ewes had higher erythrocyte-related parameters and neutrophil to lymphocyte ratio than the singleton-pregnant individuals (Santarosa *et al*. 2022).

Similarly, twin-pregnant Awassi ewes significantly had higher Hct, MCH, MCHC, RBC, WBC and LP than the singleton-pregnant ewes (Al-Thuwaini *et al*. 2020; Kadhem and AL-Thuwaini, 2022; Khazaal *et al*. 2023). These evidences may indicate a higher hematopoietic adaptation during multiple pregnancy (Santarosa *et al*. 2022), which may result in different hematological parameters profile in singleton and multiple-born infants. In other words, because of different physiological conditions of dams during the pregnancy, the newborn singletons and twins may have different hematological parameters. In a study on Canadian infants, the multiples had 14% lower WBC, 30% lower neutrophils, 31% lower monocytes and 28% lower basophils than the singletons (Shah *et al*. 2015). In the present study, a higher neutrophil count level in adult singletons can be attributed to lower colostrum intake and therefore, lower passive immunity and higher immunological challenges in the twins that the singletons.

CONCLUSION

This study indicated leukocyte-related parameters are possibly more sensitive to genetic or environmental factors, compared to erythrocyte-related parameters. The erythrocyte-related parameters were also independent from the leukocyte parameters. Neutrophil and lymphocyte counts were significantly affected by birth type and age, respectively. Heavier lambs in 6 and 9 months of age tended to have higher levels of Hb, MCH and MCHC in adulthood. Some correlations between body weight traits and leukocyte parameters in higher ages, indicated possible influence of the growth rate on immunity in higher ages or higher growth rate in the animals with strong immunity. Based on the results of this study, growth rate, age and birth type should be considered for interpretation of the hematological parameters in adult animals. Moreover, more attention should be paid to immunological traits when breeding programs are planned for growth rate.

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