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Source / Izvornik: **Poljoprivreda, 2017, 23, 46 - 52**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

https://doi.org/10.18047/poljo.23.2.7

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:151:722538

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Hematološki pokazatelji ovaca tijekom laktacije u ekološkom uzgoju

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Poljoprivreda/Agriculture

ISSN: 1848-8080 (Online)
ISSN: 1330-7142 (Print)

http://dx.doi.org/10.18047/poljo.23.2.7

Poljoprivredni fakultet u Osijeku, Poljoprivredni institut Osijek

Faculty of Agriculture in Osijek, Agricultural Institute Osijek

ISSN 1330-7142
UDK: 636.32/.38:636.082
DOI: 10.18047/poljo.23.2.7

HEMATOLOGICAL PARAMETERS IN EWES DURING LACTATION IN ORGANIC FARMING

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Original scientific paper
Izvorni znanstveni članak

SUMMARY

The aim of this investigation is to determine haematological parameters of ewes during lactation in organic farming. Research was carried out with 32 Merinolandschaf ewes in age of 4 years and in third lactation. Sampling of blood from the same ewes was conducted on 20th, 60th and 100th day of lactation. Feeding was based on feed mixture and meadow hay from organic farming. Haematological parameters (number of leukocyte-WBC, erythrocytes-RBC, and platelet-PLT, as well as content of haemoglobin-HGB, haematocrit-HCT, mean corpuscular volume-MCV, the average haemoglobin content in erythrocytes-MCH and mean haemoglobin concentration in erythrocytes-MCHC) in whole blood of ewes and differential blood test (segmented neutrophils-SEG, band cells-NESEG, lymphocytes-LYM, eosinophils-EOS, monocytes-MON and basophils-BAS) were determined. During the lactation significant decrease of RBC (9.36-8.62×10^{12} L^{-1}), HGB (92.59-86.25 g L^{-1}) and number of BAS (0.41-0.03%) was determined, while MON (0.53-0.06%) decreased until 60th day of lactation. Significant correlations were determined between most of the haematological parameters in blood of ewes in lactation. Significant positive total correlation was determined between RBC and HGB (0.96), very strong correlation between HCT:HGB, RBC:HCT and MON:MCH (0.82, 0.76 and 0.80), as well as strong negative correlation between MCH:MCHC (-0.63). The abovementioned indicated significant interdependence of the most of haematological parameters in ewes during lactation in organic farming.

Key-words: ewes, organic farming, haematological parameters, blood, lactation

INTRODUCTION

The organic farming sector in the EU has been rapidly developing during past decades (Sauer, 2015). Accordingly, organic farming of livestock is becoming more important. With an increasing interest in organic sheep production, the number of sheep is increasing in Europe. Based on the data from EUROSTAT (2017), in the year 2014 there were 4.5 million sheep in Europe in organic farming, 2.9% of the total number of sheep. In the EU 4 156 884 sheep present 4.2% of the total number of sheep. In the Republic of Croatia 23 774 sheep were reared in organic farming in the year 2015, and in the recent years, a significant increase of 50.90% was recorded (Antunović et al., 2016).

The importance of organic sheep farming in the Republic of Croatia is also indicated by the fact that from the total number of domestic animals on certified organic farms, sheep farming is 78% (CAA, 2017). Therefore, control over quality and production is necessary. Along with monitoring of production, it is also necessary to monitor the health status of animals in

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organic production. Lactation is a very demanding period for ewes, especially the first half of lactation, when it is difficult to satisfy the nutritional requirements of ewes because of high milk production (Antunović et al., 2011). Due to that, significant changes in the ewes in early lactation period lead to the occurrence of metabolic disorders (Karapehlivan et al., 2007). The blood parameters profile has become as essential tool that can be used for evaluating and monitoring the health as well as nutritional and metabolic conditions of the animals (Carlos et al., 2015). Within that, haematological parameters are included, whose interpretation with clinical findings may suggest a specific differential diagnosis or prognosis (Braun et al., 2010; Polizopoulous, 2010). Investigation of PCV, RBC and WBC values during the lactation period could be explained as signs of anaemia, infections, digestive disorders, and reproductive and metabolic diseases in animals (Nozad et al., 2014). Numerous factors may influence haematological parameters in sheep as follows: living conditions, region, rearing, location, diet, age, season, as well as organic farming (Kaneko et al., 2008; Tschuor et al., 2008; Lepherd et al., 2009; Šoch et al., 2010a and b; Vojta et al., 2011; Shek Vugrovečki et al., 2017). Determination of haematological parameters is important for evaluation of physiological and health status of farm animals and especially in organic farming, where permitted veterinary interventions are strictly regulated (Arfuso et al., 2016). Monitoring of the haematological parameters in the blood of small ruminants gives us a clearer picture of their health status (Dias et al., 2010; Šoch et al., 2011; Antunović et al., 2011 and 2013). However, only several research studies have been carried out recently in sheep organic farming (Antunović et al., 2009; Vojta et al., 2011), especially during lactation.

The aim of the present research was to monitor the blood haematological parameters of ewes during lactation in organic farming.

MATERIAL AND METHODS

Animals, locations of research and diets

Biological research was carried out on 32 clinically healthy Merinolandschaf ewes during lactation and kept on one farm during winter season. Rearing and feeding of ewes were performed according to Regulation on Ecological Production of Animal Products (EZ, 834/2007). Selection of ewes was carried out according to the registers from the flock of 200 sheep of Merinolandschaf breed. The criterion for selection was the age of ewes, lactation, ewes´ origin and litter size. The ewes were an average age of 4 years in third lactation with one lamb in litter. This study was conducted from February to May 2016 at the family farm Ursic (Croatia, located 35 km south-east of Osijek, TX 42.150^0 N; long 52.647^0 E). This area is located within the Baranja region with mean annual temperature of 11.4°C, mean annual humidity 87% and sum of annual rainfall of 704.6 L, and calculated THI was 51.4. The THI values were calculated using the equation by Kibler (1964). Sampling of blood from the same ewes was conducted on 20^{th}, 60^{th} and 100^{th} day of lactation ($\pm$ 5 days). Ewes were fed a feeding mixture (0.6 kg/day: 71% corn, 10.5% barley, 16.5% soybean meal, 2% mineral-vitamin premix) and meadow hay (*Lolium perenne, Lolium italicum, Phleum phleoides, Trifolium repens and Dactylis glomerata*) from organic farming *ad libitum*. Water was provided *ad libitum* to all ewes during the whole period of research.

Sampling and analysis

Blood was collected from the jugular vein (10 ml) into Vacutainer tubes (Venoject®, Sterile Terumo Europe, Leuven, Belgium) with ethylenediamine tetra-acetic acid (EDTA) after morning feeding. The EDTA tubes were inverted several times to ensure adequate mixing of the blood with anticoagulant. Determination of haematological parameters (number of leukocyte-WBC, erythrocytes-RBC, and platelet-PLT, as well as the content of haemoglobin, haematocrit, mean corpuscular volume-MCV, the average haemoglobin content in erythrocytes-MCH and mean haemoglobin concentration in erythrocytes-MCHC) in whole blood of ewes was carried out on an automatic three differential haematology analyser Sysmex PocH-100iV. A differential blood test was carried out by microscope using the prepared blood smears coloured by Pappenheim.

Statistical analysis

Data were analysed with the statistical software SAS 9.3® (SAS Inst. Inc., Cary NC). The results are presented as arithmetic mean and standard error of mean estimated with MEANS procedure, while the Pearson´s correlation between blood haematological parameters was estimated with CORR procedure. The strength of correlation was determined according to scale: 0.0-0.10 none of correlation; 0.10-0.25 very weak correlation; 0.25-0.40 weak correlation; 0.40-0.50 moderate correlation; 0.50-0.75 strong correlation; 0.75-0.90 very strong correlation and 0.90-0.999 total correlation. Significant differences were determined with GLM repeated measures procedure, and differences between groups were analysed with Fisher LSD test, at the level $P<0.05$ or lower.

RESULTS AND DISCUSSION

Tables 1 and 2 present haematological parameters and differential blood picture in ewes during lactation in organic farming. During lactation RBC and HGB significantly decreased. Similarly, a decrease in WBC was found, although without significant difference. The content of HCT, MCV, MCH and MCHC as well as PLT number in the blood of ewes during lactation did not differ. No significant differences were observed in leukocyte distribution during lactation except for the number of BAS which significantly decreased during lactation and the number of MON which significantly decreased on 60^{th} day of lactation.

Table 1. Haematological parameters in ewes during lactation in organic farming

Tablica 1. Hematološki pokazatelji u ovaca tijekom laktacije u ekološkom uzgoju

Parameter *Pokazatelj*	Stage of lactation (days) *Stadij laktacije (dani)*			SEM	P-values *P-vrijednosti*	Reference values* *Referentne vrijednosti**
	20th Mean	60th Mean	100th Mean			
WBC, $x10^9$ L^{-1}	8.39	7.43	7.46	0.226	0.068	4-12
RBC, $x10^{12}$ L^{-1}	9.36^{Aa}	8.50^{B}	8.62^{b}	0.229	0.009	9-15
HGB, g L^{-1}	92.59^{a}	85.16^{b}	86.25^{b}	1.412	0.027	90-150
HCT, g/L	0.33	0.30	0.32	0.006	0.162	0.27-0.45
MCV, fL	45.51	46.95	46.64	0.368	0.133	28-40
MCH, pg	11.29	11.63	11.59	0.076	0.051	8-12
MCHC, g L^{-1}	247.00	241.06	250.00	2.637	0.346	310-340
PLT, $x10^9L^{-1}$	397.88	357.77	402.09	9.527	0.005	250-750

Mean=mean value - *srednja vrijednost*; SEM = mean standard error - *standardna pogreška srednje vrijednosti*; WBC - white blood cells - *leukociti*; RBC - red blood cells - *eritrociti;* HGB - haemoglobin - *hemoglobin;* HCT - haematocrit - *hematokrit;* MCV - mean corpuscular volume - *srednji volumen eritrocita;* MCH - mean corpuscular haemoglobin - *prosječan sadržaj hemoglobina u eritrocitima;* MCHC - mean corpuscular haemoglobin concentration - *srednja koncentracija hemoglobina u eritrocitima*; PLT - platelet number - *trombociti*; $^{a, b}P<0.05$, $^{A,B}P<0.01$; *Moris et al. (2002a,b)

Table 2. Differential blood picture in ewes during lactation in organic farming

Tablica 2. Diferencijalna krvna slika u ovaca tijekom laktacije u ekološkom uzgoju

Distribution of leukocytes (%) *Distribucija leukocita* (%)	Stage of lactation (days) *Stadij laktacije (dani)*			SEM	P-values *P-vrijednosti*	Reference values* *Referentne vrijednosti**
	20th Mean	60th Mean	100th Mean			
Segmented neutrophils *Segmentirani neutrofili*	41.59	40.58	43.34	1.19	0.602	10-50
Band cells *Nesegmentirani neutrofili*	0.21	0.06	0.09	0.04	0.343	0
Lymphocytes Limfociti	53.56	55.55	52.00	1.21	0.414	50-75
Eosinophils *Eozinofili*	3.71	3.68	4.19	0.32	0.671	1-8
Monocytes *Monociti*	0.53^{a}	0.06^{b}	0.28^{ab}	0.147	0.018	0-4
Basophils *Bazofili*	0.41^{A}	0.03^{B}	0.03^{B}	0.052	<0.01	0-1

Mean = mean value - *srednja vrijednost*; SEM = mean standard error - *standardna pogreška srednje vrijednosti*; *Latimer et al. (2003); $^{a, b}P<0.05$; $^{A,B}P<0.01$

Compared with reference values (Moris et al., 2002a, b) RBC, HGB and MCHC were slightly below, and content of MCV above reference values. Polizopoulous (2010) concluded that increased MCV (macrocytosis) is an indicator of regenerative response, while decreased MCHC can indicate reticulocytosis or iron deficiency anemia. However, the same author stated that in sheep, anemia is considered when PCV values fall below 24%, which is not the case in the present study where the HCT values were 0.30 to 0.33 g/L. The abovementioned indicates an increased load on the ewes´ organism in lactation due to milk secretion, as indicated by the established changes in haematological parameters. The decline in WBC count, as well as the number of basophils and monocytes during lactation in ewes´ blood, indicated their migration from blood into milk for more efficient phagocytosis and mammary gland defence against pathogens (Paape et al., 1992). In studies with lactating goats a similar decrease of blood WBC, monocytes and basophils was also found with lactation progress (Antunović et al., 2013). The RBC and HGB decreased during lactation, particularly during early lactation which might be attributed to the hemodilution effect resulting from an increase in plasma volume and/or the increasing water mobilization to mammary gland through the vascular system (El-Sherif and Assad, 2001). Similar changes in count of RBC and HGB in the blood of ewes and goats in early lactation were determined by Sharma

et al. (2015) and Azab and Abdel-Maksoud (1999). Similar values for content of HCT and WBC, as well as higher content of HGB and number of ewes´ blood during lactation, were determined in Brazil by Britto et al. (2006). Antunovic et al. (2011) determined a slight decrease of WBC, RBC and content of HGB in the blood of ewes in lactation, while El-Tarabany et al. (2016) determined similar changes in blood of Baladi goats. Abdelatif et al. (2009) determined decrease ($P<0.05$) of HGB in ewes in the first month of lactation. Das and Singh (2000) also obtained significantly higher number of MON and BAS in blood of ewes at the beginning of lactation in comparison with other stages of lactation. Polizopoulou (2010) concluded that the differential WBC count is more important than the total white blood cell count, because changes in individual cell types may occur simultaneously, leaving total count unchanged. Generally, the changes of all haematological parameters in blood of ewes during lactation in organic farming were very small.

In Table 3, significant correlations were presented between haematological parameters in the blood of ewes during lactation in organic farming. Significant correlations were determined between most of the haematological parameters in the blood of ewes, which indicated that lactation is a very demanding period. Significant positive and total correlation was determined only between RBC and HGB (0.96, $P<0.01$), and very strong positive correlation between HCT:HGB, RBC:HCT and MON:MCH (0.82, 0.76 and 0.80; $P<0.01$), as well as strong negative correlation between MCH:MCHC (-0.63; $P<0.01$). In male calves, Hrković-Porobija et al. (2013) also determined a significant strong positive correlation between RBC and HGB (0.760), as well as Mohri et al. (2005.) in sheep (0.93). Similarly, Nozad et al. (2014) determined a very strong correlation between HGB:HCT (0.86), RBC:HCT (0.88), RBC:HGB (0.83) in cows during lactation. Haemoglobin values change according to haematocrit alterations (Panousis i sur., 2007). The MCV, MCH, and MCHC are characteristics of the RBC, indicating average cell size, average cell HGB content, and average cell HGB concentration, respectively (Jones and Allison, 2007). Aforementioned correlations indicated significant interdependence of haematological parameters in blood of ewes during lactation in organic farming.

Table 3. Significant correlations between haematological parameters in ewes during lactation in organic farming

Tablica 3. Značajne korelacije između hematoloških pokazatelja u ovaca tijekom laktacije u ekološkom uzgoju

Ratio *Omjer*	Correlation with P-value *Korelacija i P-vrijednost*	Ratio *Omjer*	Correlation with P-value *Korelacija i P-vrijednost*	Ratio *Omjer*	Correlation with P-value *Korelacija i P-vrijednost*
RBC:HGB	0.96 (<0.01)	LYM:MCH	-0.41 (<0.01)	EOZ:HCT	0.25 (0.013)
RBC:HCT	0.76 (<0.01)	LYM:MCHC	0.20 (0.046)	EOZ:MCV	-0.24 (0.017)
HCT:HGB	0.82 (<0.01)	SEG:RBC	-0.27 (0.009)	EOZ:MCH	0.35 (<0.01)
WBC:MCV	-0.22 (0.028)	SEG:HGB	-0.26 (0.011)	BAS:WBC	0.21 (0.040)
RBC:MCV	-0.41 (<0.01)	SEG:HCT	-0.29 (0.004)	MON:WBC	0.22 (0.034)
HGB:MCV	-0.21 (0.038)	SEG:MCH	-0.31 (0.002)	MON:MCH	0.80 (<0.01)
RBC:MCHC	-0.22 (0.031)	SEG:MCHC	0.25 (0.012)	MON:MCHC	-0.53 (<.001)
HGB:MCHC	-0.22 (0.034)	SEG:PLT	0.22 (0.033)	EOZ:LYM	-0.29 (0.004)
HCT:MCHC	-0.24 (0.017)	SEG:LYM	-0.69 (<0.01)	EOZ:SEG	-0.21 (0.036)
MCH:MCHC	-0.63 (<0.01)	NESEG:MCV	0.25 (0.016)	BAS:SEG	-0.23 (0.025)
PLT:MCH	-0.29 (0.005)	EOZ:RBC	0.35 (<0.01)	MON:LYM	-0.40 (<0.01)
PLT:MCHC	0.27 (0.008)	EOZ:HGB	0.30 (0.003)	MON:BAS	0.28 (0.005)

WBC - white blood cells - *leukociti*; RBC - red blood cells - *eritrociti*; HGB - haemoglobin - *hemoglobin;* HCT - haematocrit - *hematokrit; MCV* - mean corpuscular volume - *srednji volumen eritrocita;* MCH - mean corpuscular haemoglobin - *prosječan sadržaj hemoglobina u eritrocitima;* MCHC - mean corpuscular haemoglobin concentration - *srednja koncentracija hemoglobina u eritrocitima*; PLT - platelet number - *trombociti*; SEG - segmented neutrophils- *segmentirani neutrofili*; LYM - lymphocytes - *limfociti*; EOZ - eosinophils - *eozinofili*; BAS - basophils - *bazofili*; MON - monocytes - *monociti*; NESEG - band cells - *nesegmentirani neutrofili*

CONCLUSION

Significant decrease of RBC number and content of HGB, as well as the number of BAS and MON during the lactation, were determined. In comparison with reference values, content of RBC, HGB and MCHC were slightly below, and content of MCV above reference values. The determined significant correlations between most of the haematological parameters indicate a very demanding period for ewes. The aforementioned changes indicated the need for monitoring the blood haematological parameters during lactation in organic farming.

ACKNOWLEDGEMENT

The research required for the present study was part of the VIP-project financed by the Ministry of Agriculture, Republic of Croatia.

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HEMATOLOŠKI POKAZATELJI OVACA TIJEKOM LAKTACIJE U EKOLOŠKOM UZGOJU

SAŽETAK

Cilj istraživanja bio je utvrditi promjene hematoloških pokazatelja u ovaca tijekom laktacije u ekološkom uzgoju. Istraživanje je provedeno na 32 Merinolandschaf ovce, dobi od 4 godine, u 3. laktaciji. Uzimanje uzoraka krvi provedeno je u istih ovaca 20., 60. i 100. dan laktacije. Ovce su bile hranjene krmnom smjesom i livadnim sijenom ekološkoga podrijetla. Utvrđeni su hematološki pokazatelji (broj leukocita-WBC, broj eritrocita-RBC, broj trombocita-PLT te sadržaj hemoglobina-HGB, hematokrit-HCT, prosječni volumen eritrocita-MCV, prosječni sadržaj hemoglobina u eritrocitima-MCH, prosječna koncentracija hemoglobina u eritrocitima-MCHC) u punoj krvi ovaca te diferencijalna krvna slika (segmentirani neutrofili-SEG, nesegmentirani neutrofili-NESEG, limfociti-LYM, eozinofili-EOZ, monociti-MON i bazofili-BAS). Tijekom laktacije utvrđeno je značajno smanjenje broja RBC-a (9,36-8,62×10^{12} L^{-1}) i sadržaja HGB-a (92,59-86,25 g L^{-1}) te broja BAS-a (0,41-0,03%), dok se broj MON-a (0,53-0,06%) smanjivao do 60. dana laktacije. Utvrđene su značajne korelacije između većega broja hematoloških pokazatelja u krvi ovaca u laktaciji. Značajno pozitivna i potpuna korelacija utvrđena je između sadržaja RBC-a i HGB-a (0,96), a vrlo jaka pozitivna korelacija između HCT:HGB-a, RBC:HCT-a te MON:MCH-a (0,82, 0,76 i 0,80) i jaka negativna između MCH:MCHC-a (-0,63). Navedeno ukazuje na značajnu međuovisnost većine hematoloških pokazatelja u ovaca tijekom laktacije u ekološkom uzgoju.

Ključne riječi: ovce, ekološki uzgoj, hematološki pokazatelji, krv, laktacija

(Received on 26 July 2017; accepted on 20 November 2017 – *Primljeno 26. srpnja 2017.; prihvaćeno 21. studenoga 2017.*)