

EFFECT OF DRIED GRAPE POMACE INGESTION ON ERYTHROCYTES AND THROMBOCYTES OF SHEEP

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Abstract

The aim of this study was to determine the parameters of erythrocytes and thrombocytes in blood of wethers which were used for in vivo determination of nutrients digestibility after the introduction of dried grape pomace meal into feed the ration. Three different feed ration were fed to eight wethers of the Ile de France breed with an average weight 34.50 ± 1.97 kg. First feed ration consisted from meadow hay, soybean meal, wheat meal and mineral supplement. Second and third feed rations were the same like the first feed ration but with an addition of dried grape pomace meal in an amount of 1.0 and 2.0 % of feed ration dry matter respectively. The time of feed rations ingestion was as follows, first 19 days, second 12 days and third 12 days. During the last day of ingestion of each feed ration the blood samples were collected into K3 EDTA tube and analysed using hematology analyzer Abacus Junior Vet (Diatron, Austria). With the increased ingestion of dried grape pomace (0 %, 1.0 % and 2.0 %) the values of red blood cells count (RBC), hemoglobin (HGB), hematocrit (HCT), red cell distribution width (RDWc), platelets count (PLT) and mean platelet volume (MPV) decreased. Significant effect of feed ration composition on erythrocyte and thrombocyte indices was detected for RBC ($P < 0.05$), HGB ($P < 0.01$), HCT ($P < 0.01$) and MPV ($P < 0.05$). After the beginning of ingestion of dried grape pomace by wethers the parameters of erythrocytes and platelets decreased, but still stayed in the physiological range for sheep blood. Ingestion of dried grape pomaces in an amount tested in this study did not have negative effect on erythrocyte and thrombocyte parameters of sheep blood.

Keywords: dried grape pomace meal, sheep blood, erythrocytes, thrombocytes

1. INTRODUCTION

Erythrocytes from all mammals are anucleated and most are in the shape of biconcave discs called discocytes [1]. Mammalian erythrocytes normally circulate for 2 to 5 months in blood despite limited synthetic capacities and repeated exposure to mechanical and metabolic insults. Erythrocyte have three functions: transport of oxygen to tissue, transport of carbon dioxide to the lungs, and buffering of hydrogen ions H^+ [2]. Hemoglobin is formed from hem, where is bivalent iron and from protein (globin) part [3]. Hemoglobin tetramer is capable of binding four molecules of O_2 when fully oxygenated. Also hemoglobin is the major protein buffer in blood. Hematocrit is characterized as a proportion between blood cells and blood volume. To next erythrocyte indices belong the mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and red cell distribution width (RDWc) [2]. MCV represent the average volume of a single erythrocyte in femtoliter in a population of erythrocytes [4]. MCH is calculated dividing the hemoglobin value (in gram per deciliter) by the RBC count (in millions of cells per microliter) and multiplying by 10. MCHC represents the average hemoglobin concentration within erythrocytes. RDWc is an electronic measure of anisocytosis or erythrocyte volume heterogeneity. Thrombocytes, blood platelets in mammals are small round-to-oval anucleated cell fragments, thin discs when unstimulated, that form from proplatelet cylinders of megakaryocyte cytoplasm [2]. To thrombocytes indices belong platelet count (PLT) and mean platelet volume (MPV). MPV is the average volume of a single platelet recorded in femtoliters. Within the normal range of platelet count and MPV, there is an inverse correlation between platelet counts and MPV [5]. Normal physiological values of different blood parameters of animals are influenced by a number of factors such age, sex breed, season, altitude, climatic conditions, life habits of the species and nutrition [6, 7, 8]. Evaluation of blood parameters in animals used for experiment is essential for

determination the positive, or negative effect. During whole realisation of experiment experimental animals have to have its blood parameters within the physiological range [9]. It is know that some components of plants or by-products obtained for example by grape processing have potential to influence the health status [10, 11]. Therefore the aim of this study was to determine the effect of feed ration supplementation with dried grape pomace meal on sheep erythrocyte and thrombocyte indices.

2. MATERIALS AND METHODS

Blood sampling was done during realization of experiment aimed to determine the effect of dried grape pomace meal feeding on *in vivo* nutrients digestibility of sheep. Three feed rations were tested: first, without an addition of dried grape pomace meal, second and third, with an addition of 1% and 2% of dried grape pomace meal into feed ration respectively.

2.1. Animals and housing

This study was realized at Experimental center for farm animals (Slovak University of Agriculture in Nitra). Eight wethers, Ile de France breed with an average weight of 34.50 ± 1.97 kg included this study. Wethers were obtained from University farm in Žirany (VPPSPU s.r.o. Kolíňany). This study consisted of three time periods. Each time periods had preparatory time period when wethers were housed in two pens per four animals and experimental period, which lasted always 5 days and wethers were housed in the individual balance cage. During housing in the individual balance cage the individual intake and excretion of nutrients were monitored for another experiment. Pens had concrete floor. During whole study the wethers were housed without bedding. Herewith was assured that wethers ingested only feed ration made for this study.

2.2. Feeding and blood sampling

According to time period of this study a three different feed rations were fed. Nutritional value of feeds used in this study is shown in Table 1. Composition of feed rations belonging to time period of this study is shown in Table 2. The composition of feed rations was calculated so that meet the requirements of wethers. Water was during whole study *ad libitum*. Minerals were provided *ad libitum* during each preparatory time period in form of mineral block lick (Ján Valášek, Slovakia). During last day of each time period a sample of uncoagulated blood was taken. The K3 EDTA tube (Sarstedt, Germany) were used. Blood samples were taken after fixation of wethers from *vena jugularis externa*. Together 24 blood samples were gained - 3 times (1st, 2nd and 3rd sampling) from 8 wethers.

Table 1. Nutritional characteristic of feeds used in feed rations

	Meadow hay	Wheat meal	Soy bean meal	Dried grape pomace meal
Dry matter (%)	84.5	91.1	89.9	94.2
Crude protein (g.kg⁻¹ OM)	55.6	112.3	437.2	99.8
Ether extract (g.kg⁻¹ OM)	11.9	14.4	13.4	98.9
Crude fibre (g.kg⁻¹ OM)	303.4	28.7	46.9	184.5
Ash (g.kg⁻¹ OM)	45.2	17.1	61.7	34.8
NFE (g.kg⁻¹ OM)	429.2	738.9	339.5	616.3
Organic matter (g.kg⁻¹ OM)	800.1	894.2	836.5	907.3

OM – original matter; NFE – nitrogen free extract.

Table 2. Feed ration composition according to study time period

	Meadow hay	Wheat meal	Soy bean meal	Dried grape pomace meal
1st time period (19 days)*	66%	23%	11%	-
2nd time period (12 days)*	66%	22%	11%	1%
3rd time period (12 days)*	65%	22%	11%	2%

* blood samples were collected during last day of each time period.

2.3. Analyses and statistic

All 24 blood samples were analysed within two hours after collecting. Red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDWc), platelet count (PLT), platelet percentage (PCT), mean platelet volume (MPV) and platelet distribution width (PDWc) were determined by hematology analyzer Abacus Junior Vet (Diatron, Austria). Gained results were statistically processed by one-way ANOVA. Description statistic, SEM – standard error of mean and P – the effect of feed ration composition on erythrocyte and thrombocyte indices was calculated using IBM SPSS v. 20.0. Differences of means between sampling periods were tested by Tukey HSD test. $P < 0.05$ was considered significant.

3. RESULTS

The highest erythrocyte indices were detected at the end of the first period of this study (1st sampling) except MCH and MCHC. The lowest erythrocyte indices were detected at the end of the third period of this study (3rd sampling) except MCV and MCHC. Significant effect of feed ration composition was detected on RBC ($P < 0.05$), HGB ($P < 0.01$) and HCT ($P < 0.01$), however significant differences of means between samplings were not detected ($P > 0.05$). During the whole study all values RBC, HCT and MCH were within the reference interval. Concentration of hemoglobin was in the first two samplings on the upper value of reference interval and all three values of MCHC were above the reference interval. On the other hand, MCV was under the reference interval for sheep.

Table 3. Red blood cell indices of the wether's blood during study

	1 st blood sampling	2 nd blood sampling	3 rd blood sampling	SEM	P
RBC (x 10¹²/L)	14.91	14.65	13.45	0.199	*
HGB (g/L)	152.6	150.4	135.9	1.850	**
HCT (%)	35.82	33.67	31.56	0.425	**
MCV (fL)	24.13	22.88	23.63	0.277	
MCH (pg)	10.25	10.29	10.10	0.053	
MCHC (g/L)	426.6	447.3	431.4	4.354	
RDWc (%)	24.59	23.91	23.36	0.278	

SEM – standard error of the mean; P – effect of feed ration composition on erythrocyte parameters was significant * at $P < 0.05$ or ** at $P < 0.01$; Significant difference of means between samplings was not detected ($P > 0.05$).

Table 4. Thrombocyte indices of the wether's blood during study

	1 st blood sampling	2 nd blood sampling	3 rd blood sampling	SEM	P
PLT ($\times 10^9/L$)	505.9	597.4	448.4	51.120	
PCT (%)	0.26	0.30	0.21	0.025	
MPV (fL)	5.23	5.09	4.60	0.086	*
PDWc (%)	26.58	26.33	24.78	0.366	

SEM – standard error of the mean; P – effect of feed ration composition on thrombocyte parameters was significant * at $P < 0.05$; Significant difference of means between samplings was not detected ($P > 0.05$).

The highest values of PLT and PCT were detected in wethers on the second blood sampling, whereas MPV and PDWc in the first blood sampling. All thrombocyte indices were the lowest in the third blood sampling. Only MPV was significantly ($P < 0.05$) affected by feed ration composition. By thrombocyte indices the differences of means between blood samplings were not significant ($P > 0.05$). Platelet count in blood of wethers was during the whole study within reference interval.

4. DISCUSSION

Similar results of RBC and HGB were found in the study of Soysal et al. (2011) [12], where they researched the effect of conventional and organic management system and genotype on haematological parameters of sheep. They determined RBC in interval from 12.9 to $15.5 \times 10^{12}/L$ and HGB in interval from 138 to 149 g/L. Other authors published lower count of red blood cells from 7.3 to $10.3 \times 10^{12}/L$ as well as of hemoglobin from 67 to 118 g/L in comparison with results of this study [13, 14, 15, 16, 17]. Hematocrit value from 33.67 to 31.56 % detected after ingestion of dried grape pomace meal (Table 3) was higher than detected Correddu (2014) [14], 30.5 % by dairy ewes fed with addition of 300 g per head/day of grape seeds and also Paolini et al. (2003) [18], 26 % by goats experimentally infected with *Haemonchus contortus* but fed with addition of tannins. On the other hand, Cash et al. (2016) [16] determined by lambs drenched with organic Pinot noir fermented grape extract values of hematocrit similar as in this study 33% . Rolinac et al. (2012) [19] did experiment with *in vivo* nutrients digestibility with Lacaune wethers and detected between start and end of experiment similar decrease of haemoglobin concentration from 154.8 to 143.5 g/L. RBC, HGB and HCT decreased between the first and second blood sampling, as well as between the second and third blood sampling. All three feed rations had enough copper but were slightly deficit on iron (data not shown). This shortage of iron can be the reason of decrease of RBC, HGB and HCT throughout this study. Harvey (2008) published that iron deficiency usually results in anemia in mammals. Vitseva et al. (2005) [20] published, that flavonoids derived from the purple grape have anti-thrombotic and anti-inflammatory properties. Bijak et al. (2013) [21] determined that polyphenol-rich extract from grape seeds acts as prevention of thrombotic states. There is a shortage of experiment determining the thrombocyte count in sheep blood after the ingestion of grape seeds. Correddu (2014) [14] published, that sheep in the group feed with addition of 300 g of grape seed per head/day had compared to control group lower count of thrombocytes 643 vs. $729 \times 10^9/L$. Similar decrease of thrombocyte count determined Cash et al. (2016) [16] after use of organic Pinot noir fermented grape extract, but they also detected decrease in the control group. After comparison of blood after the first and second sampling (Table 4), also occurs decrease of thrombocyte count. On the other hand, the highest count of thrombocytes was detected in wether's blood after 12 days long ingestion of feed ration with an addition of 1.0% of dried grape seed meal.

5. CONCLUSIONS

During the realization of *in vivo* digestibility experiment with feed rations supplemented with 1% or 2% of dried grape pomace meal the erythrocyte and thrombocyte indices of experimental wethers were within the reference interval for sheep. Significant effect of feed ration composition on the erythrocyte and thrombocyte indices was detected for RBC ($P<0.05$), HGB ($P<0.01$), HCT ($P<0.01$) and MPV ($P<0.05$). Ingestion of dried grape pomaces in an amount tested in this study did not have negative effect on erythrocyte and thrombocyte parameters of sheep blood. However, the addition of iron into the feed ration will be during long lasting feeding with grape pomace essential for haemoglobin concentration.

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