# Hematological profiles of new-born piglets and sows fed with diet containing grape pomace

## Hematologický profil novonarodených prasiatok a prasníc kŕmených kŕmnou dávkou s obsahom hroznových výliskov

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## ABSTRACT

The aim of this study was to evaluate the effect of feeding pregnant sows with diet supplemented with dried grape pomace on hematological parameters of sows and new-born piglets. Sixteen pregnant crossbred sows Large white x Landrace mated with a Duroc boar, were randomly divided to two groups, control (C) and dried grape pomace (DGP) group. During last seven days of pregnancy the sow's diet in DGP group contained 1% of DGP powder. Sows blood samples were taken before intake of diet containing DGP and during first day post partum. Blood of new-born piglets was taken immediately after birth before colostrum intake. All blood samples were analyzed on hematological parameters. After 7 days of treatment with DGP, sows blood showed significant changes in parameters such as total white blood cell count, lymphocytes count, mean corpuscular volume and mean corpuscular hemoglobin, but within the reference interval for pigs. Feeding pregnant sows with DGP affected the hematological parameters of the new-born piglets in a negative way. Compared to the C group, the new-born piglets in the DGP group had significantly lower lymphocyte counts, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin values. Parameters of white and red blood cells are crucial for new-born piglets from an immunological and anemia point of view. Therefore, the addition of DGP during the last week of pregnancy to the sows' diet cannot be recommended.

Keywords: sows, new-born piglets, nutrition, wine by-products, hematological parameters

## ABSTRAKT

Cieľom experimentu bolo vyhodnotiť vplyv skrmovania sušených hroznových výliskov prasnými prasnicami na hematologické parametre prasníc a ich novonarodených prasiatok. Šestnásť vysoko gravidných kríženiek plemien Biela ušľachtilá x Landrace, spárené s kancom Duroca, bolo náhodne rozdelených do dvoch skupín – kontrolnej (C) a skupiny s konzumáciou sušených hroznových výliskov (DGP). Počas posledných siedmich dní gravidity kŕmna dávka prasníc v skupine DGP obsahovala 1% prášku DGP. Vzorky krvi boli prasniciam odobraté pred prvým príjmom kŕmnej dávky obohatenej o DGP a počas prvého dňa po pôrode. Krv novorodených prasiatok bola odoberaná bezprostredne po narodení pred príjmom mledziva. Všetky vzorky krvi boli analyzované na hematologické parametre. Po 7 dňoch skrmovania DGP krv prasníc vykazovala významné zmeny v parametroch ako je celkový počet bielych krviniek, celkový počet lymfocytov, priemerný korpuskulárny objem a priemerný korpuskulárny hemoglobín. Parametre sa pohybovali v rámci referenčných intervalov pre ošípané. Skrmovanie DGP gravidným prasniciam negatívne ovplyvnilo hematologické parametre novorodencov. V porovnaní s kontrolnou skupinou mali novonarodené prasiatka z pokusnej skupiny signifikantne nižší počet lymfocytov, červených krviniek, hemoglobínu, hematokritu, priemerný korpuskulárny objem a priemerné hodnoty korpuskulárny objem a červených

krviniek kľúčové z imunologického hľadiska a anémie. Pridanie DGP počas posledného týždňa gravidity do kŕmnej dávky prasníc preto nemožno odporučiť.

Kľúčové slová: prasnice, novonarodené prasiatka, výživa, vedľajšie produkty spracovania hrozna, hematologické parametre

## INTRODUCTION

According to the International Organization of Vine and Wine, the global vineyard surface area is nearly 7.45 million ha, and the world grape production is approximately 73.1 million tons. In Europe, vineyard surface area is over 3.69 million ha and grape production is more than 29.56 million tons (OIV, 2018). Viniculture is an important agricultural activity in many countries and produces a huge amount of grape pomace. These wine by-products are most often recovered by composting or free storage in open space, which can lead to environmental problems (Rondeau et al., 2013). The use of grape pomace as a feed or as a source of certain nutrients and biologically active substances has been studied by several authors (Gálik et al., 2018; Kafantaris et al., 2018; Hanušovský et al., 2020; Kolláthová et al., 2020). Various types of fruits, berries and pomace contain a high concentration of polyphenols, which have been shown to have anticancer, antimicrobial, antioxidant, and immunomodulatory effects in vertebrates (Sehm et al., 2011; Brindza et al., 2015). Grape pomace is also a source of minerals such as iron (Šimko et al., 2019) which is important for pregnant sows in reducing the risks of anemia in new-born piglets (Svoboda et al., 2004; Estienne et al., 2019). Hematological parameters are an important diagnostic tool for assessing the health status of experimental subjects (Zapletal et al., 2017; Šimák-Líbalová et al., 2013), but for the correct interpretation of the results, it is necessary to consider the reproductive status and parity of sows and the time from birth of the piglets (Verheyen et al., 2007; Etim et al., 2014). In previous studies with ruminants, grape pomace ingestion at an amount of 1% of the diet increased the platelet, but slightly decreased the red blood cell count and hemoglobin concentration (Bíro et al., 2019). Grape pomace might be used as a nutrient source in sow diets. Ingestion of grape pomace affects the hematological parameters of animals, but the question remains how the hematological profile of new-born piglets are affected when the diet of pregnant sows in the late stage of gravidity is supplemented with DGP at an amount of 1%. Can be grape pomace used as a nutrient source for sows in late stage of pregnancy?

#### MATERIAL AND METHODS

#### Animals and feeding

All the animals were under regular veterinary supervision by the veterinarian at the farm for the duration of the entire experiment. Animal care was carried out in compliance with Directive 2010/63/EU of the European Parliament and of the Council of 22. September 2010 on the Protection of Animals Used for Scientific Purposes. The experiment was carried out at Pig Farm in Dubovany (SPD Veselé, Slovakia, GPS 48°32'07.6"N 17°43'45.3"E). Sixteen crossbreed pregnant sows (Large White x Landrace) mated with a Duroc boar were used. The animals included in the experiment were housed under standard conditions. The experiment started seven days before the expected parturition (7 d a. p.), which was also the day of the sows' removal to the farrowing stall, where the sows were placed into farrowing pens. The animals were examined by a veterinarian at the beginning of the experiment, and all evaluated as clinically healthy. All 16 sows were housed in one farrowing room with a controlled environment. The experimental unit was the sow and their litter. Sows were randomly divided into two groups. The C group of sows was fed a basal diet for lactating sows. The DGP group of sows was fed the same basal diet with an addition of 1% DGP powder, which equals to 0.035kg per sow and day. The nutritional characteristics of the sow diets are given in Table 1. Water during the whole experiment and diet after farrowing were provided to all sows ad libitum. Before farrowing, all sows were fed twice a day and received 3.5 kg/day of the diet. Grape pomace from the white wine variety (Pinot Gris) gained from the Academic Winery of the Slovak University of Agriculture

JOURNAL Central European Agriculture ISSN 1332-9049 in Nitra contained parts of skins, seeds and pulp. The predried grape pomace (at  $55 \pm 5$  °C) was stored in paper bags until further use. They were ground in a laboratory grinder (particles smaller than 1 mm) before being added to the feed ration of the experimental group. Diets in both group of sows were analyzed in the Laboratory of Quality and Nutritional Value of Feeds (SUA in Nitra, Slovakia). For the objectivity of the experiment, each litter was represented by at least one piglet.

### Feed analysis

Standard laboratory techniques were used to determine the basic nutrient concentrations (AOAC, 2000). Samples were pre-dried at 55  $\pm$  5 °C for three days and milled by a laboratory mill (Fritsch, Germany) to pass through a 1 mm sieve. The dry matter concentration was determined gravimetrically at 103 ± 2 °C. Crude protein, represented by the total nitrogen concentration, was determined by the Kjeldahl method (N x 6.25). Ether extracts, representing crude fat, were determined by the extraction and gravimetric method according to the Soxhlet principle, without previous acid treatment. The crude fiber concentration was determined gravimetrically as the difference between the residue after hydrolysis and after combustion. The ash was determined by the resulting inorganic residue weight after ignition in a Muffle furnace at 550 ± 25 °C. Mineral concentrations (Ca, Mg, Na, K, Mn, Fe, Cu, Zn) were determined by a ContrAA®700 (Analytik Jena, Germany), and P was determined using a 6400 Spectrophotometer (Jenway, United Kingdom). The nitrogen-free extract was calculated according to the concentration of dry matter and other nutrients. Starch was determined polarimetrically after the clarification and filtration of the optical rotation of the solution (Automatic Digital Polarimeter, P3002RS, Krüss, Germany). Total sugar was determined by extraction of samples in an ethanol solution followed by Carrez solution treatment and titration by the Luff-Schoorl method. Amino acid concentrations were analyzed using an AAA 400 amino acid analyzer (Ingos Prague, Czech Republic). Metabolisable energy (ME) was calculated according to the method outlined by Noblet and Perez (Noblet and Perez, 1993). Both diets were analyzed in duplicate, and the nutritional values as well as diets composition are given in Table 1.

## Blood sampling and analysis

Blood samples from the sows were taken from the vena cava cranialis. The first blood samples were taken 7 days before the expected farrowing, before the start of DGP intake (7 d a.p.), and the second blood samples were taken during the first day after the end of farrowing (1 d p.p.). Blood samples of the new-born piglets were taken from the umbilical cord immediately after birth before colostrum intake. Uncoagulated blood samples were collected into EDTA K3 tubes (Sarstedt, Germany). Collection of blood samples was performed by the veterinarian from the Pig Farm in Dubovany. Blood sampling from new-born piglets was very difficult. As we did not want to expose the new-born piglets to excessive stress, we managed to take samples only with a few pieces, those in which the blood in the umbilical cord has not been coagulated. Blood samples from new-born piglets were successfully taken from 12 piglets from the C group and 24 piglets from the DGP group. On the day of collection, uncoagulated blood samples were transported to the laboratory and analyzed using the hematological analyzer Abacus Junior Vet (Diatron, Austria). The analyzer determined 18 hematology parameters, including a three-part white blood cell differential. The parameters assessed were total white blood cell count (WBC, 10<sup>9</sup>/L), lymphocyte count (LYM, 10<sup>9</sup>/L), medium-sized cell count (MID, 10<sup>9</sup>/L), granulocyte count (GRA, 10<sup>9</sup>/L), lymphocyte percentage (LY%, %), medium-sized cell percentage (MI%, %), granulocyte percentage (GR%, %), red blood cell count (RBC, 10<sup>12</sup>/L), hemoglobin (HGB, g/L), hematocrit (HCT, %), mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/L), red cell distribution width (RDWc, %), platelet count (PLT, 10<sup>9</sup>/L), platelet percentage (PCT, %), mean platelet volume (MPV, fl), and platelet distribution width (PDWc, %). The hematological profile of all blood samples was analyzed in duplicate.

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 Table 1. Nutritional characteristics of the sow diets

	Control group	Dried grape pomace group
Ingredients of the diet (%)		
Barley grain	33	32.6
Maize grain	33	32.6
Soy bean meal	15.8	15.6
Wheat grain	7	6.93
Rape seed meal	3	2.97
Mineral premix <sup>1</sup>	3	2.97
Sunflower oil	2.6	2.57
PKK energy <sup>2</sup>	1.5	1.49
Abrocel RC <sup>3</sup>	1	0.99
Neutox <sup>4</sup>	0.1	0.1
Dried grape pomace powder	0	1
Nutritional characteristic		
Dry matter (g/kg)	893	892
Crude protein (g/kg)	174	173
Ether extract (g/kg)	20.8	21.3
Crude fibre (g/kg)	46.7	48
Ash (g/kg)	56.2	56
Nitrogen free extract (g/kg)	595	594
Starch (g/kg)	408	404
Total sugar (g/kg)	41.3	42.6
Non-fibre saccharides (g/kg)	508	505
Metabolisable energy (MJ/kg)	12.8	12.9
Ca (g/kg)	8.78	8.73
P (g/kg)	6.24	6.21
Mg (g/kg)	2.59	2.58
Na (g/kg)	2.97	2.94
K (g/kg)	9.37	9.4
Cu (mg/kg)	25.9	25.7
Fe (mg/kg)	354	351
Mn (mg/kg)	84.7	84
Zn (mg/kg)	182	180
Threonine (g/kg)	6.18	6.15
Lysine (g/kg)	9.37	9.31
Cysteine (g/kg)	2.01	1.99
Methionine (g/kg)	1.74	1.72

<sup>1</sup> Tekromix PKK,Tekro Nitra s.r.o., Slovakia; one kilogram of mineral premix contained: Ca 193 g; P 60 g; Mg 9 g; Na 50 g, Vitamin A 312000U.I.; Vitamin D3 65000 U.I.; Vitamin E 3200 mg; Vitamin B1 65 mg; Vitamin B2 170 mg; Vitamin B6 135 mg; Vitamin B12 1 mg; Vitamin K3 100 mg; Biotine 10 mg; Folic acid (3a316) 145 mg; Niacinamide (3a315) 1000 mg; Calcium pantothenate 685 mg; Choline chloride (3a890) 5000 mg; Lysine 50 g; Fe 4000 mg; Cu 450 mg; Zn 3900 mg; Mn 2000 mg; I 45 mg; Se 10 mg

<sup>2</sup> Tekro Nitra s.r.o., Slovakia: one kilogram contained: E 551a silicid acid 9 g; Crude protein 20 g; Ether extract 279 g; Crude fiber 2 g; Ash 102 g; Ca 2 g; P 0.50 g; Na 0.1 g; ME 11.5 MJ

<sup>3</sup> JRS GmbH + Co KG, Germany: Crude fiber concentrate for sows. Effects: optimized faeces consistency and intestinal peristalsis, prevents constipation, better digestibility of nutrients, higher water absorption, better milk production, reduced MMA risk, faster farrowing

<sup>4</sup> Bioferm, Czech Republic, Is a broad-spectrum toxin scavenger with additional mold control



#### Statistical analysis

The results were statistically processed by IBM SPSS v 26.0. (IBM Corp., 2019) Descriptive statistics (mean, residual standard deviation) were generated using a multivariate general linear model (the dependents were hematological parameters, and the fixed factors were the experimental group and sampling time). The differences in blood cell parameter mean values between groups at blood sampling time, as well as between sampling times within a group, were tested with T-tests. A P value less than 0.05 were considered significant.

## RESULTS

The results of the determined hematological parameters of the sows' are shown in Table 2. At the beginning of the experiment (7d a.p.), non-significant differences between the parameters were determined. Differences between the groups at 1d p.p. were determined in WBC, LYM, LY%, GR%, MCV and MCH.

Higher values of WBC, LYM and LY% were detected in the C group, whereas the GR%, MCV and MCH were higher in the DGP group of sows. After comparison of values at 7d a.p. to 1 d p.p., in both groups, a non-

Table 2. Hematological parameters of sows									
	7d <i>a.p</i> .		1d <i>p.p</i> .			<i>P</i> -value			
						C x DGP		7d <i>a.p.</i> x 1d <i>p.p</i> .	
	С	DGP	С	DGP	RSD	7d <i>a.p</i> .	1d p.p.	С	DGP
WBC	12.8	11.3	14.4	12.1	1.28	0.32	0.021	0.25	0.44
LYM	5.43	5.15	7.06	5.07	1.08	0.68	<0.001	0.008	0.88
MID	0.49	0.29	0.46	0.12	1.34	0.45	0.089	0.9	0.25
GRA	6.9	5.83	6.96	6.88	1.31	0.43	0.93	0.97	0.15
LY%	45.4	46.4	50.1	42	0.7	0.89	0.047	0.46	0.22
MI%	3.83	2.26	3.14	1.02	1	0.41	0.11	0.72	0.22
GR%	50.8	51.4	46.8	57	0.59	0.92	0.005	0.47	0.06
RBC	6.1	5.77	5.36	5.14	0.81	0.31	0.36	0.003	0.054
HGB	109	109	98.8	101	0.72	0.91	0.66	0.017	0.14
НСТ	34	33.9	32.4	33	0.7	0.99	0.68	0.2	0.58
MCV	55.6	59	60.7	64.3	0.78	0.1	0.013	0.002	0.009
MCH	17.8	19	18.4	19.6	0.75	0.064	0.014	0.16	0.33
MCHC	320	322	304	305	0.68	0.41	0.93	<0.001	<0.001
RDWc	17.7	17.6	18.6	18.4	0.61	0.95	0.65	0.018	0.034
PLT	147	145	227	248	1.1	0.96	0.49	0.013	0.005
PCT	0.15	0.15	0.21	0.24	1.16	0.84	0.39	0.042	0.033
MPV	9.97	10.5	9.27	9.49	0.88	0.26	0.61	0.11	0.054
PDWc	41.3	42.5	39.8	40.6	0.67	0.26	0.38	0.12	0.075

7d. a.p. - 7<sup>th</sup> day ante partum

1d. p.p. -1<sup>st</sup> day post partum

C-control group of sows (n=8)

DGP- group of sows fed with an addition of dried grape pomace (n=8); RSD - residual standard deviation; WBC - total white blood cell count ( $10^{9}/L$ ), LYM - lymphocyte count ( $10^{9}/L$ ), MID - medium size cells count ( $10^{9}/L$ ), GRA - granulocyte count ( $10^{9}/L$ ), LY% - lymphocyte percentage (%), MI% - medium size cells percentage (%), GR% - granulocyte percentage (%), RBC - red blood cell count ( $10^{12}/L$ ), HGB - haemoglobin (g/L), HCT - haematocrit (%), MCV - mean corpuscular volume (fl), MCH - mean corpuscular haemoglobin (pg), MCHC - mean corpuscular haemoglobin concentration (g/L), RDWc - red cells distribution width (%), PLT - platelet count ( $10^{9}/L$ ), PCT - platelet percentage (%), MPV - mean platelet volume (fl), PDWc - platelet distribution width (%)

significant increase in WBC was detected. However, in the same time interval, the LYM in the blood of control sows increased (P=0.008), while in the DGP group, the LYM remained nearly the same (P=0.882). In both groups of sows between 7d a.p. and 1d p.p. an increase was detected for MCV, RDWc, PLT and PCT, and a decrease was detected for MCHC. Between 7d a.p. and 1d p.p., the decrease in RBCs and HGB was only in the control group. Hematological parameters of the new-born piglets from sows of both groups are shown in Table 3. Lower lymphocyte counts were detected in piglets from the DGP group (P=0.025), and similar results for lymphocytes were also detected in sows of the DGP group at 1 d p.p. New-born piglets from the DGP group had lower values of RBCs (P=0.002), HGB (P<0.001), HCT (P<0.001), MCV (P<0.001), and MCH (P=0.008) compared to the piglets from the control group.

#### Table 3. Hematological parameters of the new-born piglets

#### DISCUSSION

The scope of this research was to investigate the hematological parameters of new-born piglets born from sows fed a diet supplemented with dried grape pomace during the last seven days of pregnancy. During these days, sows received 3.5 kg of diet per day. Accordingly, sows in the DGP group ingested 0.035 kg of DGP powder daily. A positive effect of grape pomace feeding on hematological, health and performance parameters has been reported by several authors in various animal species (Sehm et al., 2011; Kafantaris et al., 2018; Bíro et al., 2019; Nuda et al., 2019; Kolláthová et al., 2020). In this study, the determined hematological parameters of sows were within the reference values of pigs published by Thorn (2010). Sipos et al. (2011) and Bhattarai et al. (2019) specified the reference intervals of hematological

	С	DGP	RSD	P-value C x DGP
WBC	3.34	2.51	0.57	0.088
LYM	2.25	1.51	0.77	0.025
MID	0.12	0.07	0.34	0.348
GRA	0.97	0.93	0.49	0.897
LY%	71.38	62.9	1.26	0.264
MI%	3.6	2.72	1.03	0.519
GR%	25.03	34.38	1.32	0.257
RBC	5.36	4.57	1.06	0.002
HGB	97.58	76.79	1.15	<0.001
HCT	34.38	26.92	1.16	<0.001
MCV	64.08	58.83	1.02	<0.001
MCH	18.18	16.76	1.09	0.008
MCHC	283.6	284.4	1.11	0.824
RDWc	17.03	17.86	1.09	0.062
PLT	44.17	62.48	0.7	0.179
РСТ	0.04	0.05	0.62	0.115
MPV	7.82	8.14	0.92	0.33
PDWc	37.78	38.28	1.23	0.696

C - new-born piglets from control group of sows (represented 8 litter of which 12 piglets); DGP -new-born piglets from sows fed with an addition of dried grape pomace powder (represented 8 litter of which 24 piglets); RSD - residual standard deviation; WBC - total white blood cell count (10<sup>9</sup>/L), LYM - lymphocyte count (10<sup>9</sup>/L), MID - medium size cells count (10<sup>9</sup>/L), GRA - granulocyte count (10<sup>9</sup>/L), LY% - lymphocyte percentage (%), MI% - medium size cells percentage (%), GR% - granulocyte percentage (%), RBC - red blood cell count (10<sup>12</sup>/L), HGB - haemoglobin (g/L), HCT - haematocrit (%), MCV - mean corpuscular volume (fl), MCH - mean corpuscular haemoglobin (pg), MCHC - mean corpuscular haemoglobin (g/L), PCT - platelet percentage (%), MPV - mean platelet volume (fl), PDWc - platelet distribution width (%)

parameters for a specific category of pigs, namely sow. In experiment, all sow blood samples met these reference intervals. Similar to this experiment, a higher WBC count in lactating sows in comparison to gestating sows was detected by Ježek et al. (2018). Between 7d a.p. and 1d p.p. RBC decreased in both group of sows. As published by Thorn (2010), a decrease in RBCs is detectable throughout lactation and lasts until weaning. Joksimović-Todorović et al. (2010) also found a decrease in the values of RBCs, HGB and HCT during lactation, which is comparable to the results of this study. The PLT of sows in the DGP group after grape pomace intake (1 d p.p.) was slightly higher than that of the C group (Table 2). Sehm et al. (2011) concluded that polyphenol-rich feed, such as grape pomace, has the potential to increase the PLT. This conclusion is applicable also in this study. However, the addition of DGP powder to the diet of gestating sows had a negative effect on the WBC of new-born piglets. The determined WBC count of new-born piglets was very low, below the reference interval for pigs (Thorn, 2010), lower than that detected in a previous study of new-born piglets 5.26 10<sup>9</sup>/L (Rolinec et al., 2015) and much lower than that published by Egeli et al. (1998) 9.89 10<sup>9</sup>/L for one-day-old piglets. Compared to the control group, new-born piglets in the DGP group had a lower WBC count. Similar results were found for the LYM in new-born piglets. In previous study, LYM 2.69 10<sup>9</sup>/L in the blood of new-born piglets were detected (Rolinec et al., 2015). Egeli et al. (1998) determined only 1.64 10<sup>9</sup>/L of LYM in the blood of one-day-old pigs. In contrast, the LY% was higher than the published reference value for young Duroc-Jersey pigs (Thorn, 2010) and one-day-old pigs (Egeli et al., 1998). During the first day, the WBC value rises, which may be influenced by the transfer of colostral WBCs into the blood of neonates (Reber et al., 2008). The WBC count, mainly LYM, of new-born piglets must be as high as possible, which is crucial within the frame of new-born piglets' own immunity connected with passive immunization, which starts after the first colostrum intake. Therefore, the starting count of WBCs, mainly LYM in the blood of new-born piglets, is very important. The alarming finding was the concentration of

HGB in new-born piglets in the DGP group, which was lower compared to the C group and lower than 80 g/L, which is considered as evidence of anemia. As published by Svoboda et al. (2004), the concentration of HGB decreased with time after birth, which increased the risk of death of piglets born with anemia. The decrease in HGB concentration after birth can be caused by the increase in plasma volume, and the lower HGB concentration at birth can be attributed to grape pomace ingestion by sows. In a previous study, 95.6 g/L HGB in the blood of new-born piglets was detected by Rolinec et al. (2010). Additionally, Egeli et al. (1998) published a similar concentration of HGB, 89 g/L, for one-day-old piglets. However, these two values were comparable only to the concentration of HGB in the new-born piglets from the C group. Despite significantly higher values of MCV and MCH in the blood of sows from the DGP group at 1d p.p. (Table 2), the blood of new-born piglets from the DGP group had significantly lower values of MCV and MCH compared to the control group (Table 3). Palova et al. (2019) published lower values of the MCV and MCH physiological intervals at 51 fl and 17 pg, respectively. These red blood cell parameters (MCV and MCH) confirm the risk of anemia in the new-born pigs after the addition of grape pomace to the sows diet in the last week of pregnancy. In the DGP group of new-born piglets, a slightly higher PLT was detected. At any rate, the PLT in both groups of piglets is below the physiological optimum for pigs published by Harvey (2012), as well as below the PLT of newborn piglets published by Rolinec et al. (2013). A lower concentration of HGB and a higher PLT in the blood of piglets fed with the addition of red grape pomace were attributed by Sehm et al. (2011) to the higher polyphenol intake in this group of piglets. It can be concluded that supplementation of the sows' diet during the last week of pregnancy with DGP significantly decreased the LYM and RBC and the concentration of HGB in the blood of new-born piglets. Low values of these parameters are undesirable in relation to the immature immune system of new-born piglets and to the risk of anemia in newborn piglets. Therefore, according to these findings, feeding pregnant sows with grape pomace cannot be

recommended, particularly in the last stage of pregnancy. By-products of the wine industry could find application in swine nutrition, but further research is needed in this regard. They should focus on the amount of grape pomace fed and other categories of swine.

## CONCLUSION

The blood of new-born piglets of sows that ingested grape pomace had lower red blood cell, hemoglobin which are related to the presence of anemia and lymphocyte counts which participate on the passive immunization. Therefore, the addition of dried grape pomace during the last week of pregnancy to the sows' diet cannot be recommended.

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#### REFERENCES

- AOAC. (2000) Official Methods of Analysis AOAC, 17<sup>th</sup> ed.; Rockville, MD: AOAC International.
- Bhattarai, S., Framstad, T., Nielsen, J.P. (2019) Hematologic reference intervals of Danish sows at mid-gestation. Acta Veterinaria Scandinavica, 61 (1), 1-5.

DOI: https://doi.org/10.1186/s13028-019-0451-7

- Bíro, D., Gálik, B., Juráček, M., Šimko, M., Hanušovský, O., Vašeková, P., Rolinec, M. (2019) Effect of dried grape pomace ingestion on erythrocytes and thrombocytes of sheep. Journal of International Scientific Publications: Agriculture & Food 7,179-184.
- Brindza, J., Ivanišová, E., Grygorieva, O., Abrahamová, V., Schubertová, Z. (2015) Antioxidants from plants in human nutrition and improving of health. Acupuncture and Natural Medicine, 2, 40-51.
- Council Directive 2010/63/EU of 22 September 2010 on the Protection of Animals Used for Scientific Purposes.
- Egeli, A.K., Framstad, T., Morberg, H., Tverdal, A. (1998) Evaluation of piglet blood utilizing the Technicon H. Veterinary clinical pathology, 27 (4), 123-128.

DOI: https://doi.org/10.1111/j.1939-165X.1998.tb01031.x

Estienne, M. J., Clark-Deener, S. G., Williams, K. A. (2019) Growth performance and hematology characteristics in pigs treated with iron at birth and weaning and fed a nursery diet supplemented with a pharmacological level of zinc oxide. Journal of Swine Health and Production, 27 (2), 64-75. Etim, N. N., Williams, M. E., Akpabio, U., Offiong, E. E. (2014) Haematological parameters and factors affecting their values. Agricultural Science, 2 (1), 37-47.

DOI: https://doi.org/10.12735/as.v2i1p37

- Gálik, B., Kolláthová, R., Bíro, D., Juráček, M., Šimko, M., Rolinec, M., Hanušovský, O., Balušíková, Ľ., Vašeková, P., Barantal, S. (2018) Grape pomace as a possible bioactive compounds source for animal nutrition: a preliminary result. In: Book of Abstracts of the 69<sup>th</sup> Annual Meeting of the European Federation of Animal Science. Dubrovnik, Croatia, 27-31 August 2018, Wageningen Academic Publishers, 635 p.
- Hanušovský, O., B. Gálik, D. Bíro, M. Šimko, M. Juráček, M. Rolinec, L. Zábranský, C. Philipp, R. Puntigam, J. A. Slama, M. Gierus. (2020) The nutritional potential of grape by-products from the area of Slovakia and Austria. Emirates Journal of Food and Agriculture, 32 (1), 1-10. DOI: <a href="https://doi.org/10.9755/ejfa.2020.v32.il.2051">https://doi.org/10.9755/ejfa.2020.v32.il.2051</a>
- Harvey, J. W. (2012) Veterinary Hematology: A Diagnostic Guide and Color Atlas. Missouri: Elsevier Saunders.
- IBM Released (2019) IBM SPSS Statistics for Windows, Version 26.0. [Software]. Armonk, NY: IBM Corp.
- International Organisation of Vine and Wine. (2018) [Online] Available at: <u>https://www.oiv.int/en/statistiques/recherche?year=</u> <u>2018&countryCode=oiv</u> [Accessed 05 September 2021].
- Ježek, J., Starič, J., Nemec, M., Plut, J., Oven, I. G., Klinkon, M., Štukelj, M. (2018) The influence of age, farm, and physiological status on pig hematological profiles. Journal of Swine Health and Production, 26 (2), 72-78.
- Joksimović-Todorović, M., Davidovic, V., Bokan, L. (2010) Red blood cell counts in sows during lactation Veterinarski glasnik, 64, 5-6, 359-365. DOI: https://doi.org/10.2298/VETGL1006359J
- Kafantaris, I., Stagos, D., Kotsampasi, B., Hatzis, A., Kypriotakis, A., Gerasopoulos, K., Makri, S., Goutzourelas, N., Mitsagga, C., Giavasis, I., Petrotos, K., Kokkas, S., Goulas, P., Christodoulou, V., Kouretas, D. (2018) Grape pomace improves performance, antioxidant status, fecal microbiota and meat quality of piglets. Animal, 12 (2), 246-255. DOI: https://doi.org/10.1017/S1751731117001604
- Kolláthová R, Gálik B, Halo M, Kováčik A, Hanušovský O, Bíro D, Rolinec M, Juráček M, Šimko M. (2020) The effects of dried grape pomace supplementation on biochemical blood serum indicators and digestibility of nutrients in horses. Czech Journal of Animal Science, 65 (2), 58-65. DOI: <u>https://doi.org/10.17221/181/2019-CJAS</u>
- Noblet, J., Perez, J. M. (1993) Prediction of digestibility of nutrients and energy values of pig diets from chemical analysis. Journal of animal science, 71 (12), 3389-3398. DOI: https://doi.org/10.2527/1993.71123299y

DOI: https://doi.org/10.2527/1993.71123389x

- Nudda, A., Buffa, G., Atzori, A. S., Cappai, M. G., Caboni, P., Fais, G., Pulina, G. (2019) Small amounts of agro-industrial byproducts in dairy ewes diets affects milk production traits and hematological parameters. Animal Feed Science and Technology, 251, 76-85. DOI: https://doi.org/10.1016/j.anifeedsci.2019.02.007
- Palova, N., Marchev, Y., Nedeva, R., Nakev, J., Krusheva, D., Slavov, T., Nedeva, I., Popova, T. (2019) Hematological and serum biochemical profile in East Balkan pigs at different age and seasons. Agrofor International Journal, 4, 164-175.

DOI: https://doi.org/10.7251/AGRENG1903164P

Reber, A. J., Donovan, D. C., Gabbard, J., Galland, K., Aceves-Avila, M., Holbert, K. A., Marshall, L., Hurley, D. J. (2008) Transfer of maternal colostral leukocytes promotes development of the neonatal immune system: Part II. Effects on neonatal lymphocytes. Veterinary immunology and immunopathology, 123 (3-4), 305-313. DOI: <u>https://doi.org/10.1016/j.vetimm.2008.02.009</u>

- Rolinec M, Bíro D, Šimko M, Gálik B, Juráček M, Šťastný P, Šťastná D, Kanka T, Schubertová Z. (2013) The development of platelet indices in blood of neonatal pigs in early postnatal period. Acta Fytotechnica et Zootechnica, 16, 58-60.
- Rolinec, M., Bíro, D., Šťastný, P., Kanka, T. (2010) Analysis of haematological profile of piglets in early postnatal period. Acta Fytotechnica et Zootechnica, 13 (special issue), 40-43.
- Rolinec, M., Bíro, D., Šťastný, P., Kanka, T., Gálik, B., Šimko, M., Juráček, M., Schubertová, Z. (2015) Development of leukocytes count in blood of neonatal pigs after colostrum intake. Journal of Central European Agriculture, 16, 399-409. DOI: https://doi.org/10.5513/JCEA01/16.4.1644
- Rondeau, P., Gambier, F., Jolibert, F., Brosse, N. (2013) Compositions and chemical variability of grape pomaces from French vineyard. Industrial Crops and Products, 43, 251-254.
   DOI: https://doi.org/10.1016/j.indcrop.2012.06.053
- Sehm, J., Treutter, D., Lindermayer, H., Meyer, H.H.D., Pfaffl, M.W. (2011) The Influence of apple- or red-grape pomace enriched piglet diet on blood parameters, bacterial colonisation, and marker gene expression in piglet white blood cells. Food and nutrition sciences, 2, 366–376. DOI: https://doi.org/10.4236/fns.2011.24052
- Sipos, W., Duvigneau, C. J., Hartl, R. T., Schwendenwein, I. (2011) Exploratory reference intervals on hematology and cellular immune system of multiparous Large White sows. Veterinary immunology and immunopathology, 141 (3-4), 307-311. DOI: https://doi.org/10.1016/j.vetimm.2011.03.007

- Svoboda, M., Bouda, J., Drábek, J., Doubek, J. (2004) Effect of per os iron lactate supplement on development of haematological profile of piglets in the early postnatal period. Acta Veterinaria Brno, 73 (4), 431-436. DOI: https://doi.org/10.2754/avb200473040431
- Šimák-Líbalová, K., Šoch, M., Šimková, A., Švejdová, K., Pálka, V., Zábranský, L., Čermák, B. (2013) The influence of parasitic infection on the blood count of the extensively reared sheep. Acta Universitatis Cibiniensis. Series E: Food Technology, 17(2), 145-151. DOI: https://doi.org/10.2478/aucft-2013-0023
- Šimko, M., Bíro, D., Gálik, B., Juráček, M., Rolinec, M., Hanušovský, O. (2019) The effect of locality on the grape pomace mineral content from the different locations of Slovakia. Journal of International Scientific Publications: Agriculture & Food, 7, 185-190.
- Thorn C. (2010) Hematology of the Pig. In: Weis, D.J., Wardrop, K.J., eds. Schalm`s Veterinary Hematology (6<sup>th</sup> edition). Ames, IA: Blackwell Publishing, 843-851.
- Verheyen, A. J., Maes, D. G., Mateusen, B., Deprez, P., Janssens, G. P., de Lange, L., Counotte, G. (2007) Serum biochemical reference values for gestating and lactating sows. The Veterinary Journal, 174 (1), 92-98. DOI: <u>https://doi.org/10.1016/j.tvjl.2006.04.001</u>
- Zapletal, D., Kudělková, L., Šimek, V., Jakešová, P., Macháček, M., Straková, E., Suchý, P. (2017) Haematological indicators in hybrid mallard ducks (Anas platyrhynchos) with regard to the use of meal from whole white lupin seeds in their diet. Acta Veterinaria Brno, 86 (3), 309-315. DOI: <u>https://doi.org/10.2754/avb201786030309</u>