

## The development of platelets indices in blood of neonatal pigs in early postnatal period

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The aim of the study was to evaluate the platelet count (PLT), platelet percentage (PCT), mean platelet volume (MPV) and platelet distribution width (PDWc) in different sampling time during the first 12 hours of piglets' life. The total of 8 new-born piglets was included in this study. The blood samples were collected before colostrum intake (0 hour) and at 3<sup>rd</sup>, 6<sup>th</sup> and 12<sup>th</sup> hour after first colostrum intake by newborn piglets. The heparinized blood samples were used for PLT, PCT, MPV and PDWc determinations. The blood samples were analysed, using haematological analyser Abacus Junior Vet. The results were statistically analysed by a one-way ANOVA, the differences in average means of blood cells between different sampling times were tested with *T*-test. No significant differences ( $P > 0.05$ ) were found in platelet count, platelet percentage as well as platelet mean volume between different sampling times. We found significant difference ( $P < 0.05$ ) in platelet distribution width between time of birth and the other sampling times. We can conclude that some newborn piglets as well as some piglets at 6<sup>th</sup> and 12<sup>th</sup> hour of their life suffer from thrombocytopenia.

**Keywords:** newborn piglets, blood, platelets

### 1. Introduction

The performance of the animals is determined by many factors such as health status (Podkowka et al., 2005), genetics (Trakovická et al., 2006), nutrition (Humer and Schedle, 2012; Mlynek et al., 2009; Grabowicz et al., 2002), breeding (Cebulska et al., 2012; Kolesárová et al., 2004) etc. Blood cells count of healthy pigs should be in the reference values (Rolinec et al., 2010). Hemostasis depends on vascular integrity, platelet numbers, and function and coagulation. Vascular integrity is determined in large measure by the health of endothelial cells and their extracellular matrix. The damage to vessel walls can result in hemorrhage and – or activation of platelets and coagulation. When arteries are severed, there is a transient reflex vasoconstriction that slows the loss of blood and allows some time for the platelet plug to begin forming and coagulation to commence, which eventually result in the formation of a stable thrombus (Harvey, 2012). Platelets have several functions in hemostasis. The first is the formation of a platelet plug at the site of vessel injury. Formation of a platelet plug alone is sufficient to stop bleeding from an injury to a small vessel. Second, platelet activation results in the translocation of negatively charged phospholipids from the inner surfaces to the outer surfaces of platelets. These aminophospholipids bind certain coagulation factors in a close proximity on

platelet surfaces, thereby accelerating coagulation (Jurk and Kehrel, 2005). Third, activated platelets secrete PF4 (CXCL4) from their  $\alpha$ -granules, which bind to heparin-like molecules on endothelial surfaces near the activated platelets. This binding displaces antithrombin, which also binds to these heparin-like molecules, thereby inhibiting local antithrombin activity and promoting coagulation (Arepally and Ortel, 2010). Reference interval for platelets in pigs' blood marked the value of 211 to 887 G l<sup>-1</sup> (Harvey, 2012). A few works dealing with pig blood cells count describe the platelet count. The mean platelet volume (MPV) is the average volume of a single platelet recorded in femtoliters (fl). Within the normal ranges of platelets count and MPVs, there is an inverse correlation between platelet count and MPV and a direct correlation between MPV and megacaryocyte ploidy in healthy humans (Bessman et al., 1981; Bessman, 1984). An inverse correlation between platelet counts and MPVs has also been reported in cats and dogs, but not in horses, cattle or goats (Boudreaux and Ebbe, 1998). From among the domestic animals species, cats had the highest MPVs (mean 11fl), followed by dogs (mean 7.2 fl), horses (mean 5.0 fl), cattle (mean 4.8fl) and goats (mean 4.2 fl) when blood was collected with sodium citrate and assayed using the same automated analyser – Series 810, Baker Instruments Corp., Allentown, PA (Boudreaux and Ebbe, 1998).

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The aim of this study was to evaluate the platelet count, platelet percentage, mean platelet volume and platelet distribution width in different sampling time during the first 12 hours of piglets' life.

## 2. Material and methods

The eight sows (Large white breed) used in this study were obtained from the Sheep and Pig Farm Žirany (University Farm, Ltd., Kolířany, Slovak University of Agriculture in Nitra). The sows were mated with boars of their own breed. Immediately after birth, one piglet of similar body weight was selected from each sow. Total of the 8 newborn piglets was included in this study. The blood samples (1.5 ml) were collected before colostrum intake (0 hour) and at 3<sup>rd</sup>, 6<sup>th</sup> and 12<sup>th</sup> hour after first colostrum intake by newborn piglets. The first blood samples, before colostrum intake by newborn piglets, were taken from umbilical cord. The other blood samples were taken from *vena jugularis externa*. The heparinized blood samples were used for platelet count (PLT), platelet percentage (PCT), mean platelet volume (MPV) and platelet distribution width (PDWc) determinations. The blood samples were analyzed using haematological analyser Abacus Junior Vet (Diatron Ltd, Vienna, Austria). The results were statistically analysed by a one-way ANOVA, the differences in average means of blood cells between different sampling times were tested with *T*-test (SAS system 9.1, SAS Institute Inc.)

## 3. Results and discussion

The platelet life span reaches 4 to 6 days in dogs, cats, horses and cattle (Snyder et al., 2002; Crawford et al., 1996; Heilmann et al., 1993; Jacobs et al., 1986). Normal platelet count depends on the species, with maximal reference values as high as 800 G l<sup>-1</sup> in several domestic animal species and minimal reference values as low as 100 G l<sup>-1</sup> in horses (Jain, 1993). The results of platelet indices of the blood of neonatal pigs are presented in Table 1.

The highest count of platelet in blood was detected at 3<sup>rd</sup> hour of the piglets' life. From 3<sup>rd</sup> hour to 12<sup>th</sup> hour the platelet count decreased from 358.13 to 240.25 G l<sup>-1</sup>. This decrease was not statistically significant ( $P > 0.05$ ). Platelet count detected at all sampling times were in reference values from 211 to 887 G l<sup>-1</sup> as published by Harvey (2012). The blood of healthy weaned piglets contains 739.16 ± 68.54 G l<sup>-1</sup> platelets (Šperanda et al., 2006). The same authors published that the content of platelets in blood decreased from 330.80 to 239.75 G l<sup>-1</sup>, when the weaned piglets were 8 days fed by zearalenone contaminated feed in addition to clinoptilolite. Jaros (2007) determined, the platelets count from 138.4 to 612.2 G l<sup>-1</sup> in the PCV2 and PMWS positive as well as negative pigs. Ellegaard et al. (1995) published that normal value for platelets count in adult male Göttingen minipigs is 349 G l<sup>-1</sup>, what corresponded with our blood sample collected at 3<sup>rd</sup> hour of piglets' life. In our results, high differences were found between minimum and maximum values of the platelets count in piglets' blood. This is similar with results of Jaros (2007). Platelet percentages

**Table 1** Platelet indices in the piglets blood during the first 12 hours of life ( $n = 8$ )

Index	Hours of life	Mean	Standard deviation	Variance	Minimum	Maximum
PLT in G l <sup>-1</sup>	0.hour	308.63	164.03	26906.27	21.00	518.00
	3 <sup>rd</sup> hour	358.13	149.20	22259.27	22.00	516.00
	6 <sup>th</sup> hour	282.13	78.16	6108.98	166.00	387.00
	12 <sup>th</sup> hour	240.25	103.04	10617.36	74.00	397.00
PCT in %	0.hour	0.35	0.19	0.04	0.02	0.56
	3 <sup>rd</sup> hour	0.46	0.19	0.04	0.02	0.69
	6 <sup>th</sup> hour	0.34	0.09	0.01	0.22	0.50
	12 <sup>th</sup> hour	0.30	0.15	0.02	0.07	0.46
MPV in f l <sup>-1</sup>	0.hour	10.91	1.45	2.09	8.40	13.20
	3 <sup>rd</sup> hour	12.17	1.73	2.98	8.20	13.50
	6 <sup>th</sup> hour	12.21	0.86	0.74	10.70	13.50
	12 <sup>th</sup> hour	12.25	1.24	1.53	10.10	14.20
PDWc in %	0.hour	40.24 <sup>*3 h, 6 h, 12 h</sup>	2.30	5.28	37.70	44.60
	3 <sup>rd</sup> hour	43.01	2.38	5.68	38.00	46.30
	6 <sup>th</sup> hour	42.50	1.07	1.14	41.00	43.90
	12 <sup>th</sup> hour	42.50	1.132	1.73	40.70	44.60

The differences in average means of blood cells between different sampling times: \*  $P < 0.05$ .

PLT: platelet count; PCT: platelet percentage; MPV: mean platelet volume; PDWc: platelet distribution width

copied the values of platelet count. Mean platelet volume increased slowly during the first 12 hours of the piglets' life, from  $10.91 \text{ f l}^{-1}$  at time of birth to  $12.25 \text{ f l}^{-1}$  at 12<sup>th</sup> hour of life. No significant differences ( $P > 0.05$ ) were found in platelet percentage as well as platelet mean volume between different sampling times. De et al. (2013) published MPV of different pig breeds of Andaman and Nicobar Islands. They estimated the MPV in Andaman wild pig, Nicobari pig and Andaman desi pig in range from  $6.66 \pm 0.34$  to  $6.83 \pm 0.35 \text{ f l}^{-1}$ . We found significant difference in platelet distribution width between the time of birth and other sampling times (Table 1). De et al. (2013) published PDWc in Andaman wild pig, Nicobari pig and Andaman desi pig in range from  $11.88 \pm 0.59$  to  $12.08 \pm 0.29 \%$ . Olsen et al. (2001) found that the effects of anticoagulant, storage time and temperature on platelets count are significant. The stress in relation to blood sampling may also be a reason for PLT variations.

#### 4. Conclusions

A healthy pig must have blood cells count as well as platelet indices within the reference values. We found the highest content of platelets in blood of pigs, which were alive for 3 hours. We did not find significant difference ( $P > 0.05$ ) in platelet count, platelet percentage as well as platelet mean volume between different sampling times. The significant difference ( $P < 0.05$ ) was found in platelet distribution width between time of birth and other sampling times.

#### 5. Acknowledgements

This work has been supported by the "Excellence Center for Agrobiodiversity Conservation and Benefit" project implemented under the Operational Programme Research and Development financed by the European Regional Development Fund.

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