Development of leukocytes countin blood of neonatal pigs after colostrum intake

Vývoj počtuleukocytov v krvi neonatálnych ošípaných po príjme kolostra

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Abstract

The aim of this study was to analyze the development of white blood cells in blood of neonatal piglets immediately after birth and colostrum intake. Together 8 newborn piglets (Large white) were included in this study. Piglets were during whole experiment under his mother and the colostrum intake was ad libitum. Blood samples (1.0 mL) were collected before colostrum intake (0 hour) and on 3rd, 6th and 12th hour after first colostrum intake. Heparinized blood samples were used for total white blood cells, lymphocytes, mid-sized cells and granulocytes determinations. Blood samples were analyzed using haematological analyser Abacus Junior Vet (Diatron, Austria). The results were statistically analyzed by a one-way ANOVA, the differences in average means of blood cells between different sampling times were tested with T-test. The content of white blood cells ($P<0.01$), lymphocytes ($P<0.001$), mid-sized cells ($P<0.05$) as well as granulocytes ($P<0.05$) changed statistically during the first 12 hours of piglets life. Only in the samples collected on 6th and 12th hour of piglets life, the white blood cells (11.68 resp. 10.82 G*l^{-1}) and lymphocytes (8.13 resp. 9.63 G*l^{-1}) reached the lower physiological range for pigs. Other white blood cells indices of neonatal piglets did not reach the lower limit of the reference range. However in all white blood cells indices, we detected very high differences between minimal and maximal values. It points out, that some newborn piglets had very low, whereas some newborn piglets had very high content of white blood indices and it suggest, that some piglets of the litter had better protection, whereas another piglets not.

Keywords: neonatal pigs, white blood cells

Abstrakt

Cieľom tejto práce bolo analyzovať vývoj obsahu bielych krviniek v krvi neonatálnych ošípaných po narodení a príjme kolostra. V experimente bolo použitých 8 novonarodených prasiatok plemena Biela ušľachtilá. Počas experimentu všetky prasiatka prijímali kolostrum matky ad libitum. Vzorky krvi (1 ml) sme odoberali ihneď po narodení a následne 3, 6 a 12 hodín po prvom príjme kolostra. V heparinizovaných vzorkách krvi sme stanovili obsah bielych krviniek, lymfocytov, buniek strednej veľkosti a granulocytov. Tieto krvné elementy boli stanovené na prístroji Abacus Junior Vet (Diatron, Austria). Výsledky boli štatisticky spracované jednofaktorovou analýzou rozptylu ANOVA a rozdiely v priemerných hodnotách krvných buniek medzi rozličnými časmi odberu vzoriek boli realizované pomocou T-testu. Počas prvých dvanástich hodín života prasiatok sa v ich krvi štatisticky preukazne zmenil obsah bielych krviniek ($P<0,01$), lymfocytov ($P<0,001$), buniek strednej veľkosti ($P<0,05$) a aj granulocytov ($P<0,05$). Spodnú hranicu fyziologického optima pre ukazovatele krvného obrazu pre ošípané dosiahli iba biele krvinky na 6. hodinu života (11,68 $G*l^{-1}$) a 12. hodinu života (10,82 $G*l^{-1}$) a lymfocyty na 6. hodinu života (8,13 $G*l^{-1}$) a 12. hodinu života (9,63 $G*l^{-1}$). Ostatné sledované ukazovatele spektra bielych krviniek v krvi neonatálnych ošípaných nedosiahli ani hodnoty spodnej hranice referenčného rozhrania. Pri všetkých parametroch bielych krviniek sme zaznamenali veľmi veľké rozdiely medzi minimálnou a maximálnou hodnotou, čo poukazuje na to, že niektoré novonarodené prasiatka majú veľmi nízky obsah bielych krviniek, zatiaľ čo niektoré prasiatka majú vyšší obsah bielych krviniek a teda niektoré prasiatka v rámci vrhu majú lepšiu imunitnú ochranu, zatiaľ čo iné nie.

Kľúčové slová: biele krvinky, neonatálne ošípané

Detailný abstract

Počet odstavených prasiatok je ovplyvnených hlavne ich zdravotným stavom, ktorý je vo veľkej miere ovplyvnený pasívnou imunizáciou kolostrom. Zisk pasívnej imunity od matky zahŕňa minimálne tri oblasti: zisk systémovej humorálnej imunity zabezpečenej prostredníctvom IgG v kolostre; zisk lokálnej humorálnej imunity zabezpečenej prostredníctvom IgA v mlieku; zisk bunkovej imunity zabezpečenej prestupom materských imuno-kompetentných buniek, hlavne lymfocytov, vyskytujúcich sa v kolostre. Viacerí autori publikovali hodnoty bielych krviniek a lymfocytov v krvi prasiatok v rozličných dňoch ich života. Publikované hodnoty bielych krviniek a lymfocytov v krvi novonarodených prasiatok sú však iba vo veľmi nízkom počte. Cieľom tejto práce bolo analyzovať vývoj obsahu bielych krviniek v krvi neonatálnych ošípaných po narodení a príjme kolostra. V experimente bolo použitých 8 novonarodených prasiatok plemena Biela ušľachtilá. Počas experimentu všetky prasiatka prijímali kolostrum matky ad libitum. Vzorky krvi (1 ml) sme odoberali ihneď po narodení a následne 3, 6 a 12 hodín po prvom príjme kolostra. V heparinizovaných vzorkách krvi sme stanovili obsah bielych krviniek, lymfocytov,

buniek strednej veľkosti a granulocytov. Tieto krvné elementy boli stanovené na prístroji Abacus Junior Vet (Diatron Austria). Počas sledovania sme so zvieratami zaobchádzali podľa nariadenia vlády Slovenskej republiky 377/2012 zo 14. novembra, ktorým sa ustanovujú požiadavky na ochranu zvierat používaných na vedecké účely alebo vzdelávacie účely. Výsledky boli štatisticky spracované jednofaktorovou analýzou rozptylu ANOVA a rozdiely v priemerných hodnotách krvných buniek medzi rozličnými časmi odberu vzoriek boli realizované pomocou T-testu. Počas prvých dvanástich hodín života prasiatok sa v ich krvi štatisticky preukazne zmenil obsah bielych krviniek ($P<0,01$), lymfocytov ($P<0,001$), buniek strednej veľkosti ($P<0,05$) a aj granulocytov ($P<0,05$). Spodnú hranicu fyziologického optima pre ukazovatele krvného obrazu pre ošípané dosiahli iba biele krvinky na 6. hodinu života (11,68 $G*l^{-1}$) a 12. hodinu života (10,82 $G*l^{-1}$) a lymfocyty na 6. hodinu života (8,13 $G*l^{-1}$) a 12. hodinu života (9,63 $G*l^{-1}$). Ostatné sledované ukazovatele spektra bielych krviniek v krvi neonatálnych ošípaných nedosiahli ani hodnoty spodnej hranice referenčného rozhrania. Pri všetkých parametroch bielych krviniek sme zaznamenali veľmi veľké rozdiely medzi minimálnou a maximálnou hodnotou, čo poukazuje na to, že niektoré novonarodené prasiatka majú veľmi nízky obsah bielych krviniek, zatiaľ čo niektoré prasiatka majú vyšší obsah bielych krviniek. Niektoré prasiatka v rámci vrhu majú lepšiu imunitnú ochranu, zatiaľ čo iné nie.

Introduction

The numbers of pigs achieve a stage fattening depends on number of live born and weaned piglets. The number of weaned piglets depends mainly on their health, which is connect with passive immunisation by colostrum. During delivery, the neonates come from a sterile environment of the uterus to a world full of antigens and are thus exposed to high antigenic pressure (Nechvatalova et al., 2011). The placenta of pregnant sows is impermeable to immunoglobulin passage, the neonates are born agammaglobulinemic; although immunocompetent, they are unable to develop rapidly an immune response which will protect their systemic and mucosal compartments; thus their survival depend upon the passive acquisition of maternal immunity including at least 3 components: i) a systemic humoral immunity, transmitted through colostrum conveying mainly by IgG; ii) a local humoral immunity, especially secretory IgA, transmitted mainly by milk; iii) a cellular immunity transmitted via maternal immunocompetent cells present in mammary secretions (Salmon, 2000). Le Jan (1996) published, that mammary secretions mainly colostrum besides the antibodies contain viable maternal cells that are mainly lymphocytes, macrophages, neutrophils and epithelial cells. Nechvatalova et al. (2011) published, that not only humoral immunity, but also cell-mediated immunity could be transferred by colostrum. Although the importance of feeding colostrum to provide protective antibody has long been recognized, it is becoming increasingly evident that colostrum also helps to constitute the cellular arm of the neonatal immune system by providing maternal colostral leukocytes. An effective cellular immune response requires antigen specific effector cells as well as antigen presenting capacity (Reber et al., 2008). Brown et al. (2006) published, that numbers and percentages of neutrophils, lymphocytes, monocytes and eosinophils did not change with age. However Becker and Misfeldt (1993) observed that the numbers of leukocytes and

lymphocytes obtained from pigs at day 1; 18 to 19; and 27 to 30 increased with age. Siddiqui et al. (2006) published, that the peripheral blood absolute lymphocyte count is an independent prognostic factor for survival. Few other studies have been published, however data about leukocyte count at different hours of the neonatal pigs are quite limited. Our data was obtained in newborn pigs to study the progress of white blood cells in first hours of their life.

Materials and Methods

The eight sows (Large white breed) used in this study were obtained from the Sheep and Pig Farm Žirany (VPP Kolíňany, Slovak University of Agriculture in Nitra). The sows were mated with the boars of their own breed. Immediately after birth, one piglet of similar body weight from each sow was selected. Together 8 newborn piglets were included in this study. Before colostrum intake (0 hour) and on 3rd, 6th and 12th hour after first colostrum intake, blood samples were collected (1.0 mL). All piglets were in the farrowing pen with their mother, under the sow till weaning, suckling naturally colostrum over the entire period of the experiment. First blood samples, before colostrum intake by newborn piglets, were taken from umbilical cord. Other blood samples were taken by *cava cranialis* puncture. Heparinized blood samples were used for total white blood cells count (WBC), lymphocytes count (LYM), mid-sized cells count (MID) and granulocytes count (GRA) determinations. Blood samples were analyzed using haematological analyser Abacus Junior Vet (Diatron Ltd, Vienna, Austria). The results were statistically analyzed 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.). The animal care protocol for the experiment followed the Slovak guidelines for animal experimentation (Directive of the government of Slovak Republic no. 377/2012).

Results and Discussion

Efficiency in pig breeding depends on many factors, as genetic (Trakovická et al., 2006; Gábor et al., 2008), housing (Kolesárová et al., 2004), nutrition (Grabowicz et al., 2002; Mlynek et al., 2013; Šamanc et al., 2013; Budimir et al., 2014), as well as health (Podkowka et al., 2005). Monitoring of blood parameters is important in preventing of diseases. Many authors research blood cells count of piglets in early postnatal period, but only with the aim protect from anaemia (Svoboda et al., 2004), whereas the other cellular components of blood are important as well, but are studied less. Mammalian leukocytes or white blood cells have been classified as either polymorphonuclear leukocytes or mononuclear leukocytes (Harvey, 2012). The total number of leukocytes varies considerably by species. Numbers of leukocytes change with age after birth. This may have important consequences on the ability of piglets resist an infection (Lessard et al., 2012). Harvey (2012) published reference interval for total white blood cells from 15.6 to 38.9 G*l^{-1} for healthy adult pigs of both sexes and various breeds. Total leukocyte concentration increased through gestation. The count rose from 1.43 G*l^{-1} on 51th day of gestation to 6.26 G*l^{-1} on birth (Waddill et al., 1962). Piglets in our experiment had on birth 5.26 ± 1.35 G*l^{-1} leukocytes in blood and increased to 10.82 G*l^{-}1 on 12th hour ($P<0.001$) (Table 1.). Leukocytes in blood

of neonatal pigs consist also from colostral leukocytes, which are absorbed via the digestive tract and have immunomodulating effect on neonatal pigs. The peak appearance of colostral leukocytes in blood occurred between 5th and 7th hour after colostrum intake (Williams, 1993). This increase in WBC is similar to our found (Table 1.). Brown et al. (2006) published, that the development of the porcine immune system based on immunoglobulins concentration, cytokine concentrations, as well as determining cell numbers function and phenotypic expression of surface antigens of lymphocytes isolated from blood at different ages in the postnatal period. They found following concentrations of the white blood cells: on 7th day of life 7.4 $G*l^{-1}$; on 14th day of life 9.0 $G*l^{-1}$ and on 18th day of life 6.7 $G*l^{-1}$.

Table 1. Development of the white blood cells in blood of neonatal piglets (n=8)

Hours of life	Mean ($G*l^{-1}$)	SD	V	Min	Max
0. hour	$5.26^{**6h,**12h}$	1.35	1.81	3.22	7.60
3rd hour	8.09	3.64	13.24	2.64	14.30
6th hour	11.68	4.59	21.04	5.95	18.72
12th hour	10.82	3.54	12.54	6.36	17.74

The differences in average means of blood cells between different sampling times: ** P<0.01; SD-standard deviation; V-variation

In the current study, the concentration of white blood cells changed (P<0.01) with the hours of the life (Table 1.). Total peripheral WBC count in the newborn piglets is 4.2 $G*l^{-1}$ (Mandel and Travnicek 1982). Newborn piglets, in our experiment, had 5.26 ± 1.35 $G*l^{-1}$ WBC in peripheral blood. The highest concentration was determined on 6th and 12th hour after first colostrum intake (Table 1.), which corresponded with findings of Williams (1993). This increase in white blood cells is necessary for obtaining the passive immunity. The reference values for WBC specified by the manufacturer of the haematological analyser Abacus Junior Vet, are 11 to 22 $G*l^{-1}$. It is clear, that WBC in blood is affected by many factors. Šperanda et al. (2006) published content of leukocytes in blood of weaned healthy piglets 17.63 ± 2.77 $G*l^{-1}$. Blood of growing – finishing pigs fed with varied amounts of conjugated linoleic acid (0.12 to 1.0% of the diet) contained 15.77 to 17.61 $G*l^{-1}$ WBC (Wiegand et al., 2011). White blood cells are the primary effector cells against infection and tissue damage, hence is good, when the piglets have more than 11 $G*l^{-1}$ of WBC in peripheral blood. This WBC level reached neonatal pigs in our experiment on 6th hour after first colostrum intake. We observed big differences between minimum and maximum value of WBC at each sampling time. It points out that some piglets had very low, whereas some piglets had very high concentration of WBC in blood (Table 1.). Similar results published Wlliams (1993); mean leukocyte total cell count in treated blood of neonatal pigs was 12.5 ± 13 $G*l^{-1}$.

Colostral lymphocytes absorbed from the digestive tract confer an active cellular immunity on the newborn piglets (Tuboly et al., 1988). In newborn piglets, the total WBC count is made of about 60% neutrophils and 38% lymphocytes. This ratio is

inverted by day 10 after birth: 38% neutrophils and 60% lymphocytes (Mandel and Travnicek, 1982). Our studies indicate, that concentration of lymphocytes in peripheral blood at birth is 2.69 G*l^{-1} and rose every hour to 9.63 G*l^{-1} on 12th hour after first colostrum intake. This increase in lymphocytes count during first 12 hours of piglets life was statistically significant (Table 2.). The same trend had also the percentage of lymphocytes from WBC. Waddill et al. (1962) determined that, the percentage of lymphocytes from WBC of newborn pigs is 38.1%. In our experiment, the percentage of lymphocytes from WBC at birth was 56.2% and this value increased to 84.50% on 12th hour of piglets life. Gardiner et al. (1953) reported that the percentage of neutrophils exceeds the percentage of lymphocytes at birth, but in our study during the first 12 hours after colostrum intake, the percentage of lymphocytes exceeded the percentage of neutrophils. However the content of lymphocytes in blood of newborn piglets was very low 2.69 ± 0.86 (0. hour) and 4.77 ± 3.92 G*l^{-1} (3rd hour) (Table 2.). It is very important to reach maximum values of WBC especially of lymphocytes concentration in blood of the newborn piglets. Brown et al. (2006) observed following decrease in lymphocytes count: 4.6 G*l^{-1} on 7th day of age; 3.7 G*l^{-1} on 14th day of age and 3.1 G*l^{-1} on 18th day of age. Lymphocytes count can be affected by many factors as diseases (Polaček et al., 2007); nutrition (Suchý et al., 2010; Havlík, et al. 2011); stress and social status (Sutherland et al., 2006). Svoboda et al. (2005) published content of lymphocytes in 2 weak old minipigs: 7.94 ± 2.87 G*l^{-1}. Šperanda et al. (2006) evaluated percentage of lymphocyte of weaned piglets 29.16%, and in piglets, which were for 14 days fed with zearalenone contaminated feed in addition of clinoptilolite: 40.20 to 42.75%. Harvey (2012) published the reference interval for lymphocytes in pigs 7.7 to 20.4 G*l^{-1}. Ellersieck et al. (1979) examined effect of electrical stress on lymphocytes. The pre-stress values of lymphocytes were 8.87 to 13.78 G*l^{-1}, whilst post-stress overall mean values of lymphocytes were 10.62 to 12.68 G*l^{-1}. In our study, the content of lymphocytes after colostrum intake on 6th and 12th hour was in reference interval (Table 2.). The content of lymphocytes increased (P<0.001) with the time after first colostrum intake. But we observed very high differences between minimal and maximal values in each sampling time. It is clear, that some piglets had very low, whereas some piglets had very high concentration of lymphocytes in peripheral blood.

Table 2. Development of the lymphocytes in blood of neonatal piglets (n=8)

Hours of life	Mean (G*l^{-1})	SD	V	Min	Max
0. hour	2.69^{*6h,***12h}	0.86	0.74	0.83	3.47
3rd hour	4.77^{*12h}	3.92	15.33	1.38	3.47
6t^{h} hour	8.13	6.24	38.92	1.08	18.62
12th hour	9.63	4.32	18.70	2.39	17.47

The differences in average means of blood cells between different sampling times: * P<0.05; *** P<0.001; SD-standard deviation; V-variation

Mid-sized cells (MID) consist primarily from monocytes. Reference interval for monocytes in pigs is 0.6 to 3.4 $G*l^{-1}$ (Harvey, 2012). Reference value for monocytes specified by the manufacturer of the haematological analyser Abacus Junior Vet is 0.66 to 1.32 $G*l^{-1}$. It is clear, that the count of monocytes in blood of newborn piglets till 12th hour of their life (Table 3.) is at very low level compared to the reference values. Brown et al. (2006) published the content of monocytes of pigs on 7th day of age 0.04 $G*l^{-1}$; on 14^{th} day of age 0.04 $G*l^{-1}$ and on 18^{th} day of age 0.02 $G*l^{-1}$, which is markedly less than in our study. In our study, the percentage of MID from WBC at time of birth and on 12^{th} hour after first colostrum intake was within the reference value for monocytes percentage specified by the manufacturer of the haematological analyser Abacus Junior Vet. Count and percentage of monocytes changed ($P<0.05$) with the hours of life (Table 3.), however Brown et al. (2006) published, that this indicators did not change with age. We found that the percentage of monocytes tended, between newborn and 12 hours old piglet, to decrease. We observed very high differences between minimal and maximal values of MID count in each sampling time.

Table 3. Development of the mid-sized cells in blood of neonatal piglets (n=8)

Hours of life	Mean ($G*l^{-1}$)	SD	V	Min	Max
0.hour	0.26^{*6h}	0.16	0.03	0.04	0.48
3^{rd} hour	0.13	0.12	0.01	0.02	0.34
6^{th} hour	0.09	0.05	0.002	0.03	0.18
12^{th} hour	0.33	0.39	0.15	0.05	1.22

The differences in average means of blood cells between different sampling times: * $P<0.05$; SD-standard deviation; V-variation

Reference value for granulocytes specified by the manufacturer of the haematological analyser Abacus Junior Vet is 5 to 10 $G*l^{-1}$. Wiegand et al. (2011) determined granulocytes count in growing pigs during 56 day long feeding experiment with conjugated linoleic acid in interval from 4.81 to 5.99 $G*l^{-1}$. Sutherland et al. (2006) did not find differences in neutrophils and eosinophils between stressed and control pigs. We found statistically significant ($P<0.05$) decrease in granulocytes count as well as percentage between 6th and 12^{th} hour of piglets life (Table 4.). The highest count of granulocytes was determined on 6^{th} hour of piglets life.

Table 4. Development of the granulocytes in blood of neonatal piglets (n=8)

Hours of life	Mean ($G*l^{-1}$)	SD	V	Min	Max
0. hour	2.33	1.78	3.19	0.07	4.91
3rd hour	3.16	2.52	6.34	0.04	6.17
6th hour	4.14^{*12h}	3.64	13.22	0.08	11.86
12th hour	1.06	1.30	1.70	0.11	4.10

The differences in average means of blood cells between different sampling times: * P<0.05; SD-standard deviation; V-variation

Brown et al. (2006) explain, the differences between studies could be due to variation in age in which each study isolated lymphocytes from the piglet. Another possible reason for the variation could be due to the different levels of antigenic exposure within the surrounding environment of the piglet and its effects on the maturation or activation of lymphocytes. If the young pigs are at a greater level of antigenic exposure, there may be a greater proportion of immune cells in an activated state as well as a greater proportion of immature immune cells being produced to mature into functional T helper or cytotoxic T cells that can facilitate antigen elimination. These immune cell populations (activated and immature T cells) seem to have an inherently greater spontaneous proliferation rate, which could lead to differences observed between the studies.

Increase in WBC and LYM in blood of neonatal piglets is very important and confirm passive immunization per maternal colostral leukocytes. If we accept that colostrum derived lymphocytes should protect piglets from pathogens, one can expect that memory-effector cells should be transferred preferentially (Nechvatalova et al., 2011), and then the piglets should have better immunological protection against pathogens, which come into contact with the sow.

On the base of the results, content of white blood cells, lymphocytes, mid-sized cells as well as granulocytes statistically changed during colostrum intake in first 12 hours of piglets life. In the blood samples collected on 6th and 12th hour of piglets life, the WBC and LYM counts reached the lower range of the reference values for pigs. Other white blood cells indices did not reach the lower range of the reference values. But in all studied white blood cells indices, we observed very high differences between minimal and maximal values. It points out, that some newborn piglets had very low, whereas some newborn piglets had very high content of white blood indices. Hence it is very important that all piglets take sufficient quantity of colostrum. These results give yet unpublished values of WBC in blood of neonatal pigs and will be used as a comparison to similar researches in the future.

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