IMMUNOGLOBULINS IN COLOSTRUM OF SOWS WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME - PRRS

IMUNOGLOBULÍNY V KOLOSTRE PRASNÍC S REPRODUKČNÝM A RESPIRAČNÝM SYNDRÓMOM OŠÍPANÝCH - PRRS

Michal ROLINEC, Daniel BÍRO, Pavel ŠŤASTNÝ, Branislav GÁLIK, Milan ŠIMKO, Miroslav JURÁČEK

Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976 Nitra, Slovak Republic

*Corresponding author: Michal Rolinec, Department of Animal Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic, tel.:-421-37-6414326, michal.rolinec@uniag.sk

ABSTRACT

The aim of this study was to examine the effect of PRRS occurrence on sow colostrum immunological quality. We realised the experiment on 20 sows (breed: Large white). From farm without presences of PRRS were 10 sows and other 10 sows were from farm with presence of PRRS. We took the samples of sows colostrums during sucking. We detected concentration of immunoglobulins (IgG, IgA, IgM) in sows colostrum in time of 0 hours to 12 hours after beginning of farrowing with pig Ig ELISA quantitation kits. We determined statistically significant ($P<0.01$) higher concentration of IgG at the beginning of farrowing, 3 hours, 6 hours and 12 hours from beginning of farrowing in favour of farm without presence of PRRS. We determined statistically significant ($P<0.01$) higher concentration of IgA at 6 and 12 hours from beginning of farrowing in favour of farm without presence of PRRS. We determined statistically significant ($P<0.01$) higher concentration of IgM at 6 and 12 hours from beginning of farrowing in favour of farm without presence of PRRS. Lower concentrations of colostral immunoglobulins in group with PRRS can be caused of presence of PRRS. Virus PRRS can evocate synthesis of cytokine IL-10, which inhibited the function of macrophages and lymphocytes and so PRRS decrease the production of immunoglobulins and their concentration in blood of sows and consequently also concentration of immunoglobulins in sows colostrum.

Keywords: colostrum, IgA, IgG, IgM, PRRS, sow

DETAILED ABSTRACT IN NATIVE LANGUAGE

Cieľom tejto práce bolo zistiť vplyv výskytu reprodukčného a respiračného syndrómu ošípaných - PRRS na obsah imunoglobulínov v kolostre prasníc. Do sledovania sme zaradili 20 prasníc plemena biela ušľachtilá. 10 prasníc bolo z chovu bez výskytu

PRRS a 10 prasníc bolo z chovu s výskytom PRRS. Vzorky kolostra prasníc sme odoberali ručným oddojením počas cicania prasiatok v priebehu prvých 12 hodín od začiatku pôrodu. Vo vzorkách kolostra sme sledovali obsah imunoglobulínov triedy IgG, IgA, a IgM. Na stanovenie imunoglobulínov sme použili voľne dostupnú sadu na stanovenie imunoglobulínov ošípaných od firmy Bethyl. Štatistickým spracovaním vzoriek sme dospeli k nasledovným výsledkom. Signifikantne (P<0,01) vyšší obsah IgG v kolostre prasníc sme zistili v chove bez výskytu PRRS na začiatku pôrodu (55,12 mg.ml^{-1}), 3 hodiny od začiatku pôrodu (48,88 mg.ml^{-1}), 6 hodín od začiatku pôrodu (45,59 mg.ml^{-1}) a 12 hodinu od začiatku pôrodu (34,02 mg.ml^{-1}). Signifikantne (P<0,01) vyšší obsah IgA v kolostre prasníc sme zistili v chove bez výskytu PRRS na 6 hodinu od začiatku pôrodu (8,12 mg.ml^{-1}) a 12 hodinu od začiatku pôrodu (7,91 mg.ml^{-1}). Signifikantne (P<0,01) vyšší obsah IgM sme zistili v kolostre prasníc v chove bez výskytu PRRS na 6 hodinu od začiatku pôrodu (1,36 mg.ml^{-1}) a 12 hodinu od začiatku pôrodu (1,02 mg.ml^{-1}). Nižšia koncentrácia kolostrálnych imunoglobulínov v skupine prasníc s PRRS môže byť zapríčinená výskytom PRRS. Vírus PRRS evokuje syntézu cytokínu IL-10, ktorý inhibuje funkciu makrofágov a lymfocytov a tým PRRS znižuje produkciu imunoglobulínov a teda aj ich koncentráciu v krvi prasníc a v dôsledku toho aj v kolostre prasníc.

Kľúčové slová: IgA, IgG, IgM, kolostrum, prasnica, PRRS,

INTRODUCTION

PRRS is now redoubtable pathogen almost in all countries with pig farming in Europe, North America and Asia. Vaccine programs, sanitations strategy and new diagnostics process are the main subject of international symposiums and in scientific letters [18]. Increasing request for food quality and safety, by definition “from feed to food” [33], compels us to deal with problematic of pig meat production [14, 16, 17, 20, 21]. Pajtáš, et al., (2009) characterizes globulins as full-value proteins classified among dissoluble proteins. They are mostly in colostrum and in animal feeds. Immunoglobulins consist of four chains (two „heavy“ and two „light“), that creating „Y“ form. Each chain consists of sundry domains that are coupled with disulfide bridge [22, 31]. Dominating classes of immunoglobulins in pigs are: IgG, IgA and IgM [6, 26]. IgG is in body the dominating immunoglobulin and is the most important factor of secondary immune response, also IgG participate on opsonisation. IgA has general function in gastrointestinal tract by local safety. IgA couple on mucin and make the safety layer of the inner *surface* of the *intestine*. IgA is resistant for enzymes of proteolysis. IgM is active in primary immune response. The synthesis of IgM continues also by secondary immune response, but here is not in the fore. IgM acts as agglutinating, cytolytic and *activate* the *complement* [28, 29]. Concentration of immunoglobulins is related with resistance of pigs versus virus, bacterium and parasite, therefore guarantee sow colostrum basic prevention for the pathogens [18, 23]. Colostrum is secret of mammary gland during the parturition and 36 hours after the parturition [10, 25]. Stokes and Bourne (1989) reported that 40% of IgA, 85% of IgM and 100% of IgG in colostrum is from sow blood. Just minimum of colostral immunoglobulins is synthesized by B-lymphocytes directly in mammary

gland [24]. Beside infection with PRRS virus can be often observad decrease of immune response. Halbur, et al. (1996) has found on pigs with PRRS virus severity of clinical respiratory disease, rectal temperatures, gross lung lesions and microscopic lung lesions. Microscopic lung lesions were characterized by hyperplastic and hypertrophied type 2 pneumocytes, septal infiltration by mononuclear cells, and accumulation of necrotic alveolar exudate. Lymph node follicular hyperplasia and focal necrosis was seen with all isolates. Sutherland, et al. (2007) has found that total number of white blood cells, natural killers cytotoxicity, macrophage numbers, macrophage subpopulations and performance were all significantly affected by social rank, heat stress, and infection status of the pig. Heat stress and PRRS status also significantly influenced the amount of time that pigs spent lying with or without contacting another animal. Cortisol and various immune measures were also affected by PRRS status. However Shimizu, et al. (1996) say that, PRRS virus caused neither alteration of T-cell subpopulation nor cell proliferation. Ficek, et al. (2010) detected PRRS virus as parallel viral infection with the presence of porcine circovirus 2. In agreement with actual knowledge can PRRS virus mark up producing *of cytokine IL-10, and that result in a inhibition of function of macrophages and T-lymphocytes* [7]. The aim of this study was to examine the effect of PRRS occurrence on sow colostrum immunological quality.

MATERIALS AND METHODS

The experiment was realised on 20 sows (breed: Large white) body weight 193 ± 11 kg. Each sow was stabled in farrowing crate and with the same nutrition. In experimental group was 10 sows from farm with presence of PRRS (acknowledged by State Veterinary and Food Administration of the Slovak Republic). In control group was 10 sows without presence of PRRS also acknowledged by State Veterinary and Food Administration of the Slovak Republic. First sample of colostrum we took directly at the beginning of farrowing (0. hour). The next three samples we took in time 3rd hour, 6st hour, 12st hour after the taking the first sample. The colostrum from sows was taken with hand in quantity of circa 5 – 7 ml to the sterile test-tubes. Samples of colostrum were centrifuged for 15 minutes at 2500 g (Labofuge 300, Heraeus). Milk serum was stored in eppendorf test-tubes at minus 20 °C (freezing box, PDF 370S, Evermed, Italy). In samples of colostrum we determined contents of immunoglobulins types IgG, IgA, IgM (Pig IgG, IgA, IgM ELISA Quantitation sets, Bethyl Laboratories Inc., USA). The addition of the stop reagent resulted in a colour change from blue to yellow. Absorbance was determined using the microwell strip reader (Neogen, USA) at 450 nm. Statistical analysis was carried with a one-way analysis of variance (ANOVA). The differences between experimental and control group was determined by Duncan´s test (Statgraphics centurion XV.I.).

RESULTS AND DISCUSSION

The highest concentration of colostral immunoglobulins was at the beginning of farrowing as in group without PRRS (IgG 55.12 mg.ml^{-1}; IgA 8.77 mg.ml^{-1}; IgM 1.32 mg.ml^{-1}) so in group whit PRRS (IgG 42.03 mg.ml^{-1}; IgA 7.94 mg.ml^{-1}; IgM 1.22 mg.

ml^{-1}). In the time of 3rd, 6th and 12th hour after beginning of farrowing is the concentration of immunoglobulins lower than in the time of beginning of farrowing (Table 1.). Development of concentration of immunoglobulins has decreasing tendency, this result explains Hiss and Sauerwein [13], that colostrum and milk has different composition therefore also different function. Colostrum in which is dominant IgG has a role of passive immunization, which is important for the newborn piglets, they are almost without immunoglobulins [11]. Milk in which is dominant IgA has a role of defence of intestinal mucosa [18]. In publications we can find big differences in concentration of IgG in sows colostrum. Concentration of IgG in sows colostrum can be variable from 12 to 183 $mg.ml^{-1}$ [1, 2, 15, 32]. Similarly as we [1, 3] detected big individual difference in concentration of immunoglobulins in sows colostrum. This detection confirmed the high coefficient of variation, that we find by statistically analysis of results. IgG was the dominant type of immunoglobulins in all samples of colostrum. Concentration of IgG in colostrum at the beginning of farrowing was in control group (without PRRS) 55.12 $mg.ml^{-1}$ and in experimental group (with PRRS) 42.03 $mg.ml^{-1}$ ($P<0.01$). Concentration of IgG is getting in both groups in first 12 hour after farrowing dramatically down. Concentration of IgG in colostrum at 12st hour after beginning of farrowing was in control group (without PRRS) 34.20 $mg.ml^{-1}$ and in experimental group (with PRRS) 22.85 $mg.ml^{-1}$ ($P<0.01$). In concentration of IgG in sows colostrum was in all samples statistically ($P<0.01$) higher value in group without PRRS (Table 1). Klobasa and Butler (1987) detected concentration of IgG in sows colostrum at the beginning of farrowing 95.6 $mg.ml^{-1}$, after 12 hours was the concentration of IgG 32.1 $mg.ml^{-1}$ and 24 hours after farrowing was the concentration of IgG in sows colostrum just 14.2 $mg.ml^{-1}$. Concentration of IgA was the second highest after IgG in sows colostrum in both groups.

Table 1. Concentration of immunoglobulins in sows colostrum ($mg.ml^{-1}$)

n=10	Group without PRRS			
	Time of sample	Mean	S.D.	CV
IgG	0th hour	55.12^{A}	4.05	7.34
	3rd hour	48.88^{B}	4.29	8.77
	6th hour	45.59^{C}	2.22	4.86
	12th hour	34.20^{D}	2.91	8.55
IgA	0th hour	8.77	0.94	10.77
	3rd hour	8.28	0.98	11.81
	6th hour	8.12^{E}	1.50	18.49
	12th hour	7.91^{F}	1.12	14.13

IgM	0th hour	1.32	0.21	16.00
	3rd hour	1.07	0.14	13.20
	6th hour	1.36^{G}	0.21	15.22
	12th hour	1.02^{H}	0.18	17.64
n=10	Group with PRRS			
IgG	0th hour	42.03^{A}	9.66	22.99
	3rd hour	38.38^{B}	4.27	11.11
	6th hour	31.47^{C}	3.29	10.44
	12th hour	22.85^{D}	2.49	10.91
IgA	0th hour	7.94	0.74	9.27
	3rd hour	8.50	0.88	10.32
	6th hour	5.17^{E}	0.98	18.88
	12th hour	3.79^{F}	0.64	16.88
IgM	0th hour	1.22	0.23	18.49
	3rd hour	1.04	0.21	20.47
	6th hour	0.55^{G}	0.07	13.47
	12th hour	0.59^{H}	0.17	28.03

$^{A\,B\,...}$ The values with identical superscript in the column are significant at the level P<0,01; PRRS: Porcine Reproductive and Respiratory Syndrome; IgG: immunoglobulin G; IgA: immunoglobulin A; IgM: immunoglobulin M; n:number of samples; S.D.: standard deviation; CV: coefficient of variation

The value of concentration of IgA reported in publications is between 12.26 ± 3.30 mg.ml^{-1}, and 14.5 ± 2.28 mg.ml^{-1} [32]. At the beginning of farrowing was the concentration of IgA in sows colostrum in control group 8.77 mg.ml^{-1} and in experimental group 7.94 mg.ml^{-1}. Dunkelberg (2006) detected in his experiments concentration of IgA in sows colostrum during the farrowing from 6.21 to 17.39 mg. ml^{-1}. We detected in experimental group big reduction of IgA concentration in colostrum from the beginning of the farrowing to the 12st hour after beginning of the farrowing. Compared with experimental group the reduction of IgA concentration was in first 12 hours in control group soft (Table 1). These soft reduction of IgA in sows colostrum indicate, that IgA in sows milk will be later the dominant class of immunoglobulins. Statistically (P<0.01) higher concentration of IgA was determined on 6 and 12 hours after the beginning of farrowing in favours of sows without PRRS. The lowest concentration of immunoglobulins in colostrum had the class IgM. Curtis and Bourne (1971) reported that concentration of IgM in sows colostrum is at the height of 3.19 – 9.10 mg.ml^{-1}. Concentration of IgM in this experiment was in both groups at a very low level. Brown, et al. (1975) studied Ig-containing cells in sow mammary gland over the period of lactation. They found a higher presence of IgA cells, but fewer IgG and IgM cells, in the pre-farrowing animal would support the

finding of Bourne and Curtis, (1973) that nearly all colostral IgG, and a high proportion of colostral IgM, are derived from serum of sow. Concentration of IgM in serum of sows was low [26]. At the beginning of farrowing was the concentration of IgM in sows colostrum in control group 1.32 $mg.ml^{-1}$ and in experimental group 1.22 $mg.ml^{-1}$. We detected bigger reduction of concentration of IgM in sows colostrum in experimental group than in control group (Table 1). Statistically ($P<0.01$) higher content of IgM was determined on 6 and 12 hours after the beginning of farrowing in favours of sows without PRRS (control group). For confrontation of immunoglobulins concentration in colostrum from different sows is necessarily identical time of colostrum sampling, because the composition of sows colostrum is in each hour different. If is the definition of colostrum: „secret of mammary gland in firs 24 hour after the farrowing“, that about this time is the biggest change of concentration of immunoglobulins in colostrum.

CONCLUSION

The concentrations of immunoglobulins, which were determined in samples of sows colostrum in control group and also in experimental group are sufficient for passive immunization for the sucking piglets providing that the infectious pressure of environs is low. Almost all colostral immunoglobulins are from sows blood, just minimum of colostral immunoglobulins are synthesised in lymphocytes that are directly in mammary gland [5, 24,]. Lower concentrations of colostral immunoglobulins in group with PRRS can be the cause of presence of PRRS. Virus PRRS can evocate synthesis of cytokine IL-10, which inhibites the function of macrophages and lymphocytes [7]. PRRS decreases the production of immunoglobulins and their concentration in blood of sows and consequently also concentration of immunoglobulins in sows colostrum.

ACKNOWLEDGEMENTS

0.5 This work was financially supported by Grant Agency of the Slovak Ministry of Education and Slovak Academy of Sciences. Project No. 1/0662/11 – Increasing of Nutrients Transformation to Suitable Production of Safety Animal Food by Effectively Utilization of Origin Natural Resources.

0.5 This work has been supported by the “Excellence Center for Agrobiodiversity Conservation and Benefit Plus” projected implemented under the Operational Programme Research and Development financed by European Fund for Regional Development.

REFERENCES

[1] Bland I.M., Rooke J.A., Bland, V.C., Sinclair A.G., Edwards S.A., (2003) Appearance of immunoglobulin G in the plasma of Piglets following intake of colostrum, with or without a delay in sucking. Journal of Animal Science, 77, 277-286.

[2] Bland I.M., Rooke J.A., Bland, V.C., Sinclair A.G., Edwards S.A., (2000) Effect of delayed sucking on piglet plasma IgG concentration. Proceedings of the Nutrition Society, 59, 40A

[3] Bontempo V., Sciannimanico D., Patorelli G., Rossi R., Rossi F., Corino C., (2004) Dietary conjugated linoleic acid positively affects immunologic variables in lactating sows and piglets. The Journal of Nutrition, 134, 817-824.

[4] Bourne F.J., Curtis J., (1973) The transfer of immunoglobulins IgG, IgA and IgM from serum to colostrum and milk in the sow Immunology, 24, 157-162.

[5] Brown P.J., Bourne F.J., Denny H.R., (1975) Immunoglobulin-containing cells in pig mammary gland. Journal of Anatomy, 120, 329-335.

[6] Curtis J., Bourne F.J., (1971) Immunoglobulin quantitation in sow serum, colostrum and milk and the serum of young pigs. Biochimica et Biophysica Acta, 236, 319-332.

[7] Drábek J., Bernady J., (2004) Reprodukční a respirační syndrom prasat (PRRS) – rewiev. Veterinářství, 54, 160-170.

[8] Dunkelberg S., (2006) Haptoglobin in Milch und Blut von Sauen und ihren Ferkeln. JLU, Gießen,

[9] Ficek R., Pšikal I., Fictum P., Bendová J., Kosinová E., Smítalová R., Škorič M., (2010) Exploratory Epidemiological Study on Porcine Circovirus Type 2 Infection and Postweaning Multisystemic Wasting Syndrome in the Czech Republic. Acta Veterinaria Brno, 79, 81-90.

[10] Foisnet A., Farmer C., Etienne M., Le Dividich J., Lippert E., Westphal G., (2008) Endocrine regulation of colostrum production in primiparous sows. Proc. Joint. ADSA-ASAS Annual Meeting, and 8th International Workshop on the Biology of Lactation in Farm Animal, Indianapolis, USA, July 7-11, p.168.

[11] Grieshop C.M., Stahly T.S., Ewan R.C., Nonnecke B.J., Cunnick J.E., (2002) Effect of gestational folic acid supplementation on offspring immune organ development and postnatal immune response. The Journal of Nutrition, 132, 8-10.

[12] Halbur P.G., Paul P.S., Meng XJ., et al. (1996) Comarative pathogenicity of nine US porcine reproductive and respiratory syndrome virus (PRRSV) isolates in a five-week-old cesarean-derived, colostrum-deprived pig model. Journal of Veterinary Diagnostic Investigation, 8, 11-20.

[13] Hiss S., Sauerwein H., (2002) Influence of dietary β-glucan on growth performance, lymphocyte proliferation, specific immune response and haptoglobin plasma concentrations in pigs, Journal of Animal Physiology and Animal Nutrition, 86, 1-10.

[14] Imrich I., Mlynek J., Mlyneková E., Moravcová ľ., (2011) Vplyv geneticky modifikovaných plodín na produkčné parametre a kvalitu mäsa ošípaných. Slovak University of Agriculture, Nitra.

[15] Inoue T., Kitano K., Inoue K., (1980) Possible factors influencing immunoglobulin G concentrations in swine colostrums. American Journal of Veterinary Research, 41, 1134-1136.

[16] Jankowiak H., Kapelanski W., Wilkanowska A., Cebulska A., Biegnewska M., (2011) The effect of CLPS and RYR1 gene polymorphism on meat quality of złotnicka spotted pigs. Journal of Central European Agricultuture. 1, 93-98.

[17] Jankowiak H., Sielska N., Kapelanski W., Bocian M., Zmudzinska A., (2011) The Effect of H-FABP gene polymorphism on carcass and meat quality in the polisch native złotnicka spotted pig. Journal of Central European Agricultuture. 4, 459-464.

[18] Kanka T., (2010) Imunohistochemické charakteristiky čreva neonatálnych prasiat. Slovak University of Agriculture, Nitra.

[19] Klobasa F., Butler J.E., (1987) Absolute and relative concentrations of immunoglobulins G, M and A, and albumin in the lacteal secretion of sows of different lactation numbers. American Journal of Veterinary Research, 48, 176-182.

[20] Kolesárová A., Sirotkin A., Kováčik J., (2008) Endokrinné a vnútrobunkové mechanizmy pohlavného dospievania prasničiek. Slovak University of Agriculture, Nitra.

[21] Kolesárová A., Capcarová M., Roychoudhury S., (2010) Metal induced ovarial signalling. Slovak University of Agriculture, Nitra.

[22] Lukáč N., (2000) Determinácia stavu pasívnej humorálnej imunity teliat v peripartálnom období. Slovak University of Agriculture, Nitra.

[23] Pajtáš M., Bíro D., Horniaková E., Beňuška N., Šimko M., Juráček M. (2009) Výživa a kŕmenie zvierat: terminologický náučný slovník (Nutrition and Feeding of Animals: terminology vocabulary). Slovak University of Agriculture, Nitra.

[24] Riedel-Caspari G., Schmidt F.W., (1990) Kolostralleukozyten und ihre Bedeutung für das Immunsystem der neugeborenen. Deutsche Tierärztliche Wochenschrift, 97, 180-186.

[25] Rolinec M., Mindek S., Kanka T., Čanakyová Z., Danková Z., Schubertová Z., (2009) Effect of Farrowing fortification on the concentration of proteins in sows colostrums. Konference mladých vědeckých pracovníků, Brno, Česká republika, Juny 3.

[26] Rolinec M., bíro d., šťastný p., gálik b., šimko m., juráček m., (2011) Obsah imunoglobulínov v sére prasníc s reprodukčným a respiračným syndrómom ošípaných – PRRS. Proteiny 2011, Zlín, Česká republika, May 3-4.

[27] Shimizu M., Yamada S., Kawashima K., et al. (1996) Change of lymphocyte subpopulations in pigs infected with porcine reproductive and respiratory syndrome (PRRS) virus. Veterinary Immunology and Immunopathology, 50, 19-27.

[28] Stokes C.R., Bourne F.J. (1989) Mucosal immunity. In: Halliwell R.E.W., Gorman N.T. (1989) Veterinary Clinical Immunology. W B Saunders, Philadelphia.

[29] Stone S.S., Phillips M., Kemeny L.J., (1979) Stability of porcine colostral immunoglobulins IgA, IgG 2 a IgM to proteolytic enzymes. American Journal of Veterinary Research, 40, 607-612.

[30] Sutherland M.A., Niekamp S.R., Johnson R.W., et al. (2007) Heat and social rank impact behaviour and physiology of PRRS-virus-infected pigs. Physiology & Behavior, 90, 73-81.

[31] Toman M., Bárta O., Dostál J., Faldyna M., Holáň V., Hořín P., et al. (2000) Veterinární imunologie. Grada, Praha.

[32] Walser K., Bostedt H. (1990) Neugeborenen- und Säuglichskunde der Tiere. Ferdinand Enke Verlag, Stuttgart.

[33] Wanner M. (2010) Forschung und Lehre in der tierärztlichen Tierernährung. In: Kreuz M., Lanzini T., Wanner M., Bruckmaier R., Bee G. (2010) Landwirtschaftliche und veterinärmedizinische Tierernährungsforschung im Verbund. Uni Zurich, Zurich.