PRESENCE OF IMMUNOGLOBULINS IN THE SMALL INTESTINE OF NEONATAL PIGLETS

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Abstract

The purpose of this study was to investigate the presence of immunoglobulins (IgG, IgA, IgM) in the small intestine of piglets during the first 12 hours of postnatal life. A total of 20 crossbred Large White x Landrace sows (180-250 kg body weight) were used, 10 sows with confirmed infection of PRRS virus (Experimental Group) and 10 healthy, PRRS-negative sows (Control Group). Three piglets from each sow were tested at defined time intervals: 3, 6 and 12 hours after the first colostrum intake. Individual intestinal section of duodenum and jejunum were taken at specified intervals and the tissue was subjected to histological and immunohistochemical evaluation. Immunoglobulins were detected by using the primary pig antibodies (Bethyl Laboratories, Montgomery, USA). The colostrum samples from all sows were collected 3, 6 and 12 hours after the onset of labour. The content of the immunoglobulins in the skimmed colostrum was determined by the ELISA method (Bethyl Laboratories, Inc). Subsequently, the comparison between the content of immunoglobulins in colostrum of sows and the presence of immunoglobulins in the small intestine of piglets was performed. The presence of immunoglobulins was significantly higher in the control group, IgM ($P<0.05$), IgG and IgA ($P<0.01$), with IgG predominance. The highest mean values of IgG were observed in duodenum and jejunum in interval from 3 to 6 hours after the first colostrum intake. The content of immunoglobulins in colostrum significantly influenced the presence of immunoglobulins in the small intestine of neonatal piglets.

Key Words: Immunoglobulins, colostrum, small intestine, piglet

Pig breeding has been centred on production of healthy and resistant individuals with maximal growth properties (Bučko et al., 2009). Health of pigs is largely limited by breeding system and by their environment (Václavková et al., 2008). Piglets need a necessary care immediately after birth (Mlynek et al., 2011). The most critical periods of piglet life are periods shortly after birth and after weaning. Piglets are often affected by the organ, infectious and metabolic diseases in these 2 critical periods (Vavrišínová et al., 2007). Presence of immunoglobulins from colostrum plays an important role in piglet small intestine (Walser and Bostedt, 1990). Colostrum of sows provides a primary protection for piglets against bacterial and viral diseases (Wagstrom et al., 2000). Level of natural (adaptive) immunity is considerably participated in a body reaction to the possible contact with pathogens (Opletal et al., 2007). We had considered above facts in connection to studies of our workplace about immunoglobulins content in colostrum and in blood serum of sows; and we decided to look through the microstructure of small intestine (*duodenum* and *jejunum*) of piglets in relation to creating of passive immunity. Experimental task was centred on passive immunity expressed by transport of immunoglobulins (IgG, IgM, IgA) from wall of the small intestine into the blood of piglets; on demonstration of transport and on detecting the presence of immunoglobulins in the *jejunum* and *duodenum* of neonatal piglets after the colostrum intake. Under the experiment, we compared the presence of immunoglobulins in *duodenum* and *jejunum* of neonatal piglets originated from the breeding with confirmation of PRRS infection and from the breeding without this infection.

Material and Methods

In the experiment, we placed 20 Large White × Landrace sows of F1 generation. Their weighs ranged from 180 kg to 250 kg. Experimental group (E) contained sows from the breeding with PRRS infection (n=10) and control group (C) contained sows from the breeding without PRRS infection (n=10). Under the experiment, 3 piglets were taken from every sow and these piglets were registered by marks 3, 6, 12 indicating the interval (hours) after the first colostrums intake. Euthanasia of piglets was performed by diethyl-ether. Samples were taken from the small intestine (*duodenum* and *jejunum*). Then, samples were washed, taken of 5×5 mm^2 size and fixed by 10% formaldehyde. Then, following operations were performed: eluting, dehydrating, submerging in paraffin and cutting to slices of 7-8 μm by Microtom. Samples were mounted: 2 samples, each of 5 glass slides; 5 slices per slide. Preparation for histochemical analysis consisted of the following steps: deparaffining of slices, dehydrating, incubation of control slices and staining of IgG, IgM and IgA by primary pig antibodies (Bethyl

Laboratories, Montgomery, USA). Statistical and graphical evaluation were performed using the light microscope (Olympus Provis AX) and by the program linked for the evaluation of individual morphological structures - Statgraphics ver.7, Image ProPlus (Spectra Services Inc,NY) and MS Excel 2000. Statistical significance was assessed by Duncan test in Statgraphic program (STATPOINT TECHNOLOGIES, INC. USA).

Results and Discussion

Active components of *duodenum* and *jejunum* (mucous membrane and epithelium) were evaluated in terms of presence and absorption of colostral immunoglobulins in small intestine. The presence of immunoglobulins (%) in evaluated time intervals (3, 6 and 12 hours after the first colostrum intake) is recorded in Table 1. Evident differences in the presence of immunoglobulins IgG, IgA and IgM were found between the control and experimental group in the analysed parts of *duodenum* and *jejunum*. From physiological point of view, sow has an epithelio-chorial type of placenta, which is characterised by very little or no ability to transfer of immunoglobulins from blood of mother to blood of fetus in the prenatal period (Herman and Schleierbach, 2000). Wicherin (1993) stated that resorption of colostral immunoglobulins by the small intestine is possible to 36 hours after birth and has a decreasing tendency. Immunoglobulins of all classes are characterised as secreted proteins (Löffler and Petrides, 1999). According to Bourne and Curtis (1973), presence of immunoglobulins in the body relates with breed, number of lactations, age of animal, stage of lactation, feed, season and breeding system. Piglets are born as agamaglobulinemic individuals and they need colostrum as a source of antibodies (Toman et al., 2000). Neonate obtains the antibodies (mainly IgG) through the colostrum in the first 24-36 hours after birth. IgG is responsible for a humoral immunity. According to Wille and Wincler (1999), immunoglobulins, obtained from colostrum in the first days after birth, are used 20-25 days in the immunological defence of piglets and 30-50 days in the immunological defence of calves. IgG is typical for secondary immune response and is dominant immunoglobulin of the body (Trautwein, 1990). Wagstrom et al. (2000) stated that the highest values of IgG immunoglobulin class in *duodenum* and *jejunum* have been achieved to 12 hours after colostrum intake. After colostrum intake, we found gradual increase of IgG immunoglobulin class in *duodenum* from 3 to 12 hours in control group (Table 1). Significant differences of IgG in *duodenum* were recorded for the benefit of control group: 6 hours after colostrum intake ($P<0.05$) and 12 hours after colostrum intake ($P<0.01$). Ferenčík et al. (1999) found the highest values of IgG in *duodenum* within 12 hours following the colostrum intake. Klobasa et al. (1981) detected the highest values of IgG in *jejunum* in the interval of 12-24 hours after colostrums intake. Presence of IgG was relatively equal in *jejunum* of control group (Table 2). The highest value was recorded in the 6th hour after colostrum intake. Significant differences ($P<0.05$) were found in the 3rd and 12th hour after colostrum intake for the benefit of control group. IgA is bound to mucin and thus creates a protective layer (intestine mucosa) on the surface of the intestine. This protective layer of intestinal mucosa inhibits the adhesion of microorganisms on the intestinal epithelium (Goddeeris, 2002). Roitt et al. (2001) found a minimal value of IgA in pre-colostral piglets. Roitt et al. (2001) stated that IgA presence had an increased tendency after the first intake of colostrum and the highest values were recorded in *jejunum*. In the immunoglobulin class of IgA, increasing tendency was found from 3 to 6 hours after colostrum intake with significant differences ($P<0.01$) for the benefit of control group. Immunoglobulin class of IgA has a decreasing tendency from 6 to 12 hours after colostrum intake in both groups. The highest value for immunoglobulin class of IgA in *jejunum* was detected in the 3rd hour after colostrum intake (Table 2). Slight decrease was observed in the interval from 6 to 12 hours after colostrum intake. In the experimental group, slight increase of IgA was found in the interval from 3 to 12 hours after colostrum intake. Significant difference was not identified between the control and experimental group. Presence of IgM is usually limited only for plasma (Buc, 1997). Wagstrom et al. (2000) found that antibodies of IgM type occured as the first during the defensive reaction and they recorded an increasing tendency after colostrum intake in *duodenum* as well as in *jejunum*. Roitt et al. (2001) detected higher values of IgM in *duodenum* with the maximum at 24th hour after colostrum intake and followed decreasing tendency. The highest value for immunoglobulin class of IgM in *duodenum* of control group was recorded in the 3rd hour after colostrum intake (Table 1). The value markedly decreased by almost 50% in the 6th hour after colostrum intake, then, it has an increased tendency, but the value was lower as the value detected in the 3rd hour. Significant differences were found for the benefit of control group in the 3rd and 6th hour after colostrum intake ($P<0.05$) as well as in the 12th hour after colostrum intake ($P<0.01$). The highest value of IgM in *jejunum* of control group was found in the 3rd hour after colostrum intake with slight decrease in the 6th hour after colostrum intake. In the 12th hour after colostrum intake, considerable decrease of IgM was recorded, this decrease was 75% compared with the 3rd hour after colostrum intake. In the experimental group, we found a gradual decrease from the 3rd to the 12th hour after colostrum intake. Significant difference ($P<0.05$) was for the benefit of control group. Similar to results of *duodenum* and *jejunum*, Rolinec et al. (2012) detected lower concentration of immunoglobulins in the colostrum of sows with PRRS infection compared to colostrums of healthy sows.

Table 1. Presence of the Ig positive areas in duodenum (%) (n =10)

Type	Sampling time	Group	Average value	Standard deviation	Coefficient of variation	Minimum	Maximum
IgG	3rd hour	C	**19.86**	21.41	107.83	25.13	46.80
		E	**5.22**	4.62	88.65	10.64	19.52
	6th hour	C	**28.73**[a]	9.79	34.10	33.49	49.10
		E	**16.07**	4.01	24.98	14.39	19.57
	12th hour	C	**37.17**[b]	6.65	17.89	7.10	49.35
		E	**10.77**	3.77	35.09	9.81	15.77
IgA	3rd hour	C	**34.63**[b]	9.08	26.24	2.14	46.70
		E	**14.68**	3.52	23.99	1.93	12.83
	6th hour	C	**43.29**[b]	6.88	15.89	21.15	42.80
		E	**16.87**	2.08	12.36	10.99	21.62
	12th hour	C	**26.92**[a]	17.86	66.34	30.19	44.81
		E	**13.34**	2.41	18.07	5.72	15.95
IgM	3rd hour	C	**30.94**[a]	20.6	66.64	7.90	58.90
		E	**4.04**	1.20	30.23	2.35	5.17
	6th hour	C	**18.55**[a]	7.08	38.18	11.23	26.52
		E	**9.94**	1.54	15.48	8.24	12.37
	12th hour	C	**25.09**[b]	8.40	33.49	16.23	34.50
		E	**8.43**	1.98	23.46	6.17	11.12

C – control group, E – experimental group; [a,b] - Average values of Ig presence between the control and experimental group are statistically different at the level of importance [a] $P< 0.05$, [b] $P< 0.01$

Table 2. Presence of the Ig positive areas in jejunum (%) (n=10)

Type	Sampling time	Group	Average value	Standard deviation	Coefficient of variation	Minimum	Maximum
IgG	3rd hour	C	**22.51**[b]	2.43	10.83	19.97	26.11
		E	**11.64**	1.65	14.13	9.61	13.28
	6th hour	C	**25.29**[a]	8.57	33.88	15.44	33.37
		E	**12.31**	2.09	16.99	10.60	15.62
	12th hour	C	**23.91**[b]	6.38	26.68	14.53	30.41
		E	**9.75**	1.84	18.96	6.79	11.67
IgA	3rd hour	C	**13.11**	5.79	44.20	5.39	19.51
		E	**8.37**	2.15	25.76	6.28	11.59
	6th hour	C	**11.09**	2.92	26.31	8.27	15.27
		E	**8.48**	1.78	21.04	6.07	10.91
	12th hour	C	**11.63**	10.22	87.84	2.63	27.47
		E	**9.91**	1.92	19.35	6.98	12.14
IgM	3rd hour	C	**21.84**[b]	3.66	16.79	17.21	26.52
		E	**10.05**	1.89	18.82	7.28	12.40
	6th hour	C	**21.07**[b]	2.59	12.36	17.35	24.58
		E	**9.27**	3.34	36.01	5.17	13.86
	12th hour	C	**5.50**	2.92	53.11	2.46	8.65
		E	**4.31**	1.11	25.82	2.53	5.24

C – control group, E – experimental group; [a,b] - Average values of Ig presence between the control and experimental group are statistically different at the level of importance [a] $P< 0.05$, [b] $P< 0.01$

Conclusion

The aim of the experiment was to find a presence of colostral immunoglobulins in the *duodenum* and *jejunum* of neonatal piglets in the time interval of the 3, 6 and 12 hours after colostrum intake. Presence of immunoglobulin classes (IgG, IgA and IgM) was confirmed. We detected a positive dependence between the immunoglobulin content in the colostrum of sows and their presence in the small intestine of piglets. In the breeding without PRRS infection, higher presence of colostral immunoglobulins was recorded in the *duodenum* and *jejunum*. Significant differences at the levels of $P<0.01$ and $P<0.05$ were found for the benefit of control group in almost all evaluated time periods. Immunoglobulin class of IgA in the *jejunum* was an exception, because no significant differences were found in all time periods. Dominant immunoglobulin in *duodenum* of control group was IgA and IgG was dominant in *jejunum*. In the breeding with PRRS infection, presence of immunoglobulins was relatively equal, but it was markedly lower compared to the control group. In conclusion, presence of colostral immunoglobulins in the small intestine has been largely influenced by the development of PRRS infection in pig breeding.

References

BOURNE, F.J., CURTIS, J. The transfer of immunoglobulins IgG, IgA and IgM from serum to colostrum and milk in the sow. In: Immunology 24, 1973, p. 157-162

BUC, M., BISS, B., ROVENSKÝ, J. Klinická farmakológia imunofarmák. In: Memorix klinickej farmakológie, Bratislava, SAP 2002, 879 p.

BUČKO, O., Kováč, Ľ. Aktuálne otázky rozvoja chovu ošípaných na Slovensku. In Aktuální poznatky v chovu a šlechtění prasat : sborník z mezinárodní vědecké konference konané při příležitosti 90. výročí založení MZLU v Brně, 4. června 2009. Brno: Mendelova zemědělská a lesnická univerzita v Brně, 2009, p. 13--20. ISBN 978-80-7375-303-0.

FERENČÍK, M., ROVENSKÝ, J., NYULASSY, Š. Imunológia základné termíny a definície, Slovak academic press, Bratislava, 1999, 283 p., ISBN 80-88908-38-8

GODDEERIS, B.M. Where do we go in protection against pathogens? In: Vet. Immunol. Immunopathol. 87, 2002, p. 391-394.

HERRMANN, M.L., SCHLEYERBACH, R., KIRSCHBAUM, B.J. Leflunomide an imunomodulatory drug of rheumatoid arthritis and autoimmune diseases: Immunopharmacol.47, 2000, p. 273 – 289.

KLOBASA, F., WERHAHN, E., BUTLER, J.E. Regulation of humoral imunity in the piglet by immunoglobulins of maternal origin. In: Res. Vet. Sci. 31, 1981, p. 195-206

LÖFFLER, G., PETRIDES, P.E. Fyziologická chémia, 4.prepracované a rozšírené vydanie. Springer, 1990, p.27-32.

MLYNEK, J., LORENCOVÁ, V., STUPKA, R., IMRICH, I., MLYNEKOVÁ, E., ŠPRYSL, M., ČÍTEK, J. Vyhodnotenie vplyvu pôrodnej hmotnosti na produkčné parametre ošípaných. Vedecká monografia., 2011, 74 p., ISBN 987-80-84415-37-5

OPLETAL, L., ROZKOT, M., ŠIMERDA, B. Přírodní látky a strukturované biologické systémy v prevenci a adjuvantní terapii infekčních onemocnění uprasat. Research in Pig Breeding, 1, 2007 (2): 12-21

ROITT, I.M., BROSTOFF, J., MALE, D.K. Kurzes Lehrbuch der Immunologie, 2. Auflag, Thieme Verlag, Stuttgart, New York, 2001, p. 211-216.

ROLINEC, M., BÍRO, D., ŠŤASTNÝ, P., GÁLIK, B., ŠIMKO, M., JURÁČEK, M. Immunoglobulins in colostrum of sows with porcine reproductive and respiratory syndrome – PRRS. In J Central European Agriculture, vol. 13, 2012, p. 303-311.

VAVRIŠÍNOVÁ, K., BUČKO, O., ČUBOŇ, J., MLYNEKOVÁ, L. Hmotnosť prasiatok pri narodení vo vzťahu ku kondícii prasníc pred a po pôrode = : The piglets weight at birth in relation to sows condition before and after delivery method of weaning. In Agri-environment and animal welfare : book of proceedings of 2nd international conference on agricultural and rural development, November 28.- December 1. 2007, Nitra, Slovak Republic. Nitra : Slovak Agricultural University, 2007. p. 680--686. ISBN 978-80-8069-962-8.

VÁCLAVKOVÁ, E., BEČKOVÁ., R. Housing systems and pig performance – review RESEARCH IN PIG BREEDING, 2 , 2008 (1): 35-39

TOMAN, M. ET AL. Veterinární imunologie, Praha, Grada, 2000, 413 p., ISBN 80-7169-727-3

TRAUTWEIN, G. Spezifische Krankheitsbedingungen, in: Schulz, L.-C. (Hrsg.): Lehrbuch der Allgemeine Pathologie. 10. Auflag, Enke Verlag, Stuttgart, 1990, p. 65-71.

WAGSTROM, E.A., YOON, K.J., ZIMMERMANN, J.J. Immune components in porcine mammary secretions. In: Viral Immunology 13, 2000, p. 383-397.

WALSER, K., BOSTEDT, H. Neugeborenen - und Säuglingskunde der Tiere. Ferdinand Enke Verlag, Stuttgart, 1990, p. 185-194.

WICHERIN, B. Beziehungen zwischen den Immunoglobulin-Laktoferin-und Albuminkonzentrationen in der Sauenmilch und deren Einfluss auf die Aufzuchtleistung. Honnover, Vet. Med. Univ., Diss. 1993, p. 147.

WILLE, H., WINKLER, T.G. Suckling induces rapid intestinal growth and changes in brush border digestive functions of newborn pigs. Journal of Nutrition, 131, 1999, p. 512-519.

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