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Expression of immunoglobulin genes in piglets*

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Expressions of IgG genes were analysed in the sera of piglets of Line 990 and crosses of Line 990 with Polish Large White and Polish Landrace. The analysis involved the course of synthesis of immunoglobulins – carriers of 14 heavy-chain markers and one light-chain marker in 143 piglets from birth to the fifth month of age. The antigenic markers were determined in agar gel using specific alloantibodies against individual epitopes. From the presence (or absence) of an epitope (antigen) the presence (or absence) was concluded of the respective protein, and indirectly the expression of a given gene. Although in some piglets the expression of immunoglobulin genes was found as early as by day 14 of age, in most animals it first appeared at the age of 1-2 months, and in some even later, *i.e.* at the age of 4 months.

KEY WORDS: gene expression / immunoglobulin / piglets / synthesis

First observations on the use of antigenic markers of protein genes for analysing physiological processes such as immunoglobulin synthesis, or absorption and catabolism in young animals were described by Węgrzyn [1975] and Skiba [2002] in cattle, by Rapacz and Hasler-Rapacz [1983] in pigs, and by Węgrzyn *et al*. [2001] and Krzyścin *et al*. [2002] in sheep, providing important information on the development of antibody immunity in the first period of animals´ life.

In this study we determined and analysed the presence of 15 epitopes of immunoglobulins encoded by fourteen heavy-chain genes and one light-chain gene in growing

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piglets. Determining the expression of immunoglobulin genes can give insights into genetic control of specific humoral immunity and thus make it possible to exert a targeted influence on the immunological system of an organism through its genotype.

Material and methods

The material was collected at the National Institute of Animal Production, Experimental Station Mełno, Ltd., during the years 2001-2002. The piglets belonged to Line 990, or were crosses of Line 990 with Polish Large White and Polish Landrace. Blood samples were taken without preservatives, once from parents, and eight times from the offspring – at birth (from the umbilical cord) and then at the age of 7, 14, 30, 60, 90, 120 and 150 days. A total of 143 piglets were analysed, being the progeny of 17 sows and six boars.

In the blood sera 14 antigenic IgH markers were determined, designed as A2, B1, B2, C1, D2, E2, F1, G1, SI, SII, SIII, SIV, X2, and Y1 encoded by IgG heavy-chain genes $\boldsymbol{a^2}$, $\boldsymbol{b^1}$, $\boldsymbol{b^2}$, $\boldsymbol{c^1}$, $\boldsymbol{d^2}$, $\boldsymbol{e^2}$, $\boldsymbol{f^1}$, $\boldsymbol{g^1}$, $\boldsymbol{sI^1}$, $\boldsymbol{sII^1}$, $\boldsymbol{sIII^1}$, $\boldsymbol{sIV^1}$, $\boldsymbol{x^2}$ and $\boldsymbol{y^1}$, respectively [Węgrzyn and Skiba 1999]. Moreover, determined was the epitope Ig(l/k)A1 encoded by the immunoglobulin light-chain gene ***IG(L/K)A1*** [Węgrzyn and Skiba 2000].

The intensity of precipitation lines was assessed in a double immunodiffusion test [Krzyścin *et al.* 2002] using a four-grade scale**:** weak ("+"), medium ("++"), normal ("+++") and strong ("++++"). Absence of a reaction was designated as "–". In evaluating the reactions we also accounted for the situation of precipitate in relation to the pores with antibody (constant amount) and antigen (different amounts). Based on the reactions obtained, concluded was the presence (or absence) of a corresponding marker, and then indirectly the presence (or absence) of its carrier-protein. Both the level of antigen (protein) and the expression of genes determining the epitopes were appraised based on the strength and situation of precipitation reactions.

Results and discussion

In studying the expression of 15 immunoglobulin genes in piglets we used antigenic markers determined by these genes. These markers are associated with IgG heavy chains A2, B1, B2, C1, D2, E2, F1, G1, SI, SII, SIII, SIV, X2 and Y1, and light chain Ig(l/k)A1. The expression of a gene was confirmed by the presence of a corresponding antigenic marker in the serum of the offspring.

The studies included serum samples of 143 piglets obtained from mating of 17 sows to six boars. The number of informative piglets (gilts and barrows) for individual markers ranged from 1 to 34 (Tab. 1). These animals were from the matings in which sires were homozygous or heterozygous for each of the genes analysed, while dams did not carry these genes. In piglets from such matings, their own immunoglobulins were not masked by the antibodies absorbed from the colostrum.

Table 1 [illegible]

[illegible]	[illegible]	[illegible]							
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
				[illegible]					
[illegible]	[illegible]	—	I	I	II	III	IIII	IIII	IIII
	[illegible]	—	—	I	III	IIII	IIII	IIII	IIII
[illegible]	[illegible]	—	—	—	II	IIII	IIII	IIII	IIII
	[illegible]	—	—	—	—	IIII	IIII	IIII	IIII
[illegible]	[illegible]								
				[illegible]					
[illegible]	[illegible]	—	—	II	II	III	IIII	IIII	IIII
	[illegible]	—	—	—	—	II	IIII	IIII	IIII
[illegible]	[illegible]	—	—	—	—	—	III	IIII	IIII
[illegible]	[illegible]								
				[illegible]					
	[illegible]	I	III	III	III	IIII	IIII	IIII	IIII
[illegible]	[illegible]	—	II	III	IIII	IIII	IIII	IIII	IIII
	[illegible]	—	—	II	III	IIII	IIII	IIII	IIII
[illegible]	[illegible]	—	—	—	III	III	IIII	IIII	IIII
	[illegible]	—	—	—	—	—	IIII	IIII	IIII
[illegible]	[illegible]								
				[illegible]					
	[illegible]	—	I	I	III	IIII	IIII	IIII	IIII
	[illegible]	—	—	I	II	III	IIII	IIII	IIII
[illegible]	[illegible]	—	—	—	II	III	IIII	IIII	IIII
[illegible]	[illegible]	—	—	—	—	III	IIII	IIII	IIII
	[illegible]	—	—	—	—	—	I	I	I
	[illegible]	—	—	—	—	—	—	I	I
[illegible]	[illegible]								
				[illegible]					
[illegible]	[illegible]	—	—	I	II	III	IIII	IIII	IIII
	[illegible]	—	—	—	II	III	IIII	IIII	IIII
[illegible]	[illegible]	—	—	—	—	II	III	IIII	IIII
	[illegible]	—	—	—	—	—	III	III	IIII
[illegible]	[illegible]								
				[illegible]					
[illegible]	[illegible]	—	—	—	—	I	II	III	III
	[illegible]	—	—	—	—	II	III	III	IIII
[illegible]	[illegible]	—	—	—	—	—	II	II	III
[illegible]	[illegible]								
				[illegible]					
[illegible]	[illegible]	—	I	I	II	III	III	IIII	IIII
[illegible]	[illegible]	—	—	I	I	I	II	II	III
[illegible]	[illegible]								
				[illegible]					
[illegible]	[illegible]	—	I	II	IIII	IIII	IIII	IIII	IIII
[illegible]	[illegible]	—	—	I	IIII	IIII	IIII	IIII	IIII
[illegible]	[illegible]								

Table 1 Continued

Number of genotype of parents	Number and sex of offspring	Response of Drosophila melanogaster individuals to ammonia							
		at birth	on day 1	on day 1+	on month 1	on month 2	on month 3	on month 4	on month 5
						sna-C1			
	1 male, 1 female	–	–	+	++	++	++	++	++
1 male z^1v^1	4 male, 4 female	–	–	–	+	+++	+++	+++	+++
×	1 male, 1 female	–	–	–	–	++	+++	+++	+++
1 female z^1w^1	1 male	–	–	–	–	–	+++	+++	+++
	1 female	–	–	–	–	–	–	++	nb
sum:	11 male, 10 female								
						sna-B1			
1 male z^1w^1	1 male, 1 female	–	–	–	+++	+++	++++	++++	++++
×	1 male	–	–	–	–	+++	+++	++++	++++
1 female z^1w^1	1 male	–	–	–	–	–	++	+++	+++
sum:	3 male, 1 female								
						sna-J1			
1 male z^1v^1									
×	1 male	–	+	++	++	++	+++	+++	+++
1 female v^1w^1									
sum:	1 male								
						sna-P1			
1 male f^1y^1	1 male, 1 female	–	–	–	+	++	++	+++	+++
×	1 male, 1 female	–	–	–	–	++	++	+++	+++
1 female f^1w^1	1 male, 1 female	–	–	–	–	–	++	+++	+++
sum:	6 male, 5 female								
						sna-G1			
1 male g^1z^1	1 male, 1 female	–	–	–	+	++	++	+++	+++
×	1 male, 1 female	–	–	–	–	++	++	+++	+++
1 female g^1w^1	1 male, 1 female	–	–	–	–	–	++	+++	+++
sum:	4 male, 3 female								
						sna-Y1			
1 male y^1y^1	1 female	–	+	+	++	+++	++++	++++	++++
×	1 male, 1 female	–	–	+	++	+++	++++	++++	++++
1 female y^1w^1	11 male, 10 female	–	–	–	+	++	+++	++++	++++
	1 male	–	–	–	–	–	++++	++++	++++
sum:	13 male, 11 female								
						sna-g(18)x1			
1 male [illegible]									
×	1 male, 1 female	–	–	+	+	++	+++	+++	+++
[illegible]	4 male, 4 female	–	–	–	+	++	+++	+++	++++
[illegible]	7 male, 9 female	–	–	–	–	++	+++	+++	++++
sum:	11 male, 14 female								

Susceptibility to ammonium acetate: "–" no reaction, "+" weak, "++" medium, "+++" normal, "++++" strong

Epitope B1. Found in 23 piglets. In 20 of them it first appeared at the age of one month. In the remaining piglets B1 was already present on day 7 (one piglet) and 14 (one piglet), or as late as on month 2 (one piglet).

Epitope C1. Found in 32 piglets. Carried by four piglets already on day 14. In the remaining animals C1 appeared as late as at the age of two and three months (13 and 15 piglets, respectively).

Epitope S1. Found in 34 piglets, in one of them already at birth. On day 7 and 14 first appeared in 21 and 9 piglets, respectively. In two animals first detected at the age of one, and in one at the age of three months.

Epitope SII. Found in 34 piglets. On day 7 and 14 first appeared in one piglet and then in 13 at the age of one, and in 14 at the age of two months. In the remaining piglets, SII first appeared on month 3 or 4, in one and four animals, respectively.

Epitope SIII. Found in 29 piglets, first appearing on day 14. In six animals appeared first on month 1, in 21 on month 2, and in one on month 3.

Epitope SIV. Found in 34 piglets. First determined on month 2 in 32, and on month 3 in the remaining two piglets.

Epitopes A2 and B2. Both found in two piglets only. In one animal immunoglobulins with both markers appeared already on day 7, while in the other on day 14.

Epitope D2. Found in 21 piglets. First detected on day 14 in three, and on month one in 10 piglets. In five piglets D2 appeared on month 2, and in the other three even later – on month 3 (two piglets) and month 4 (one piglet).

Epitope E2. Found in four piglets only. In two of them, E2 was already present at the age of one month, in the third at two months, and in the fourth at three months of age.

Epitope X2. Found in one piglet only, first determined on day 7.

Epitope F1. Found in 11 piglets. In five animals first appearing on month 1, in four at month 2, and in two as late as month 3.

Epitope G1. Found in 10 piglets. On month 1 of age it first appeared in five piglets, then in three on month 2 and in two on month 3.

Epitope Y1. Found in 29 piglets. In three animals already determined on day 7 and in another two on day 14. On month 1 already present in remaining 23 piglets. On month 2 determined in one animal only.

Epitope Ig(l/k)A1. Found in 28 piglets. On day 14 of age carried by four animals, and on month 1 and 2 by 8 and 16 piglets, respectively.

The results presented here show a marked individual variation in the activity of the genes analysed, as evidenced by the appearance in piglets of immunoglobulins with different epitopes in the first period of life. The earliest determined immunoglo-bulins (with SI epitope) were found in one piglet already at birth. By the first week of life, the synthesis of immunoglobulins with determinants B1, B2, A2, X2, SI, SII, and Y1 began in 27 piglets. The expression of genes determining markers C1, D2, SIII and Ig(l/k)A1 took place as late as between day 7 and 14 of age. The synthesis of immunoglobulin carriers of determinants D2 and SII occurred in some piglets the latest, *i.e.*on month 3 and 4 of age. However, in most of animals the immunoglobulin synthesis began at

the age of 1-2 months.

The analysis of the occurrence of all markers in the tested sera indicates that in most piglets, the immunoglobulin synthesis increased with age. Genes that determine these proteins usually reached their full expression by 3-4 months of age. In some piglets, full activity of the genes determining epitopes Ig(l/k)A1 and SIV was noted even later, *i.e*. at the age of 5 months. There were only five animals in which the exceptionally late (age 3 and 4 months) and weak expression of the gene determining epitope SII persisted until the end of the period studied. Family analysis showed that these piglets were derived from the same pair of parents.

Similar results for the expression of four immunoglobulin genes in piglets were also obtained by Rapacz and Hasler-Rapacz [1983].

Concluding, the expression of IgG heavy-chain genes – ***a*^{2}**, ***b*^{1}**, ***b*^{2}**, ***c*^{1}**, ***d*^{2}**, ***e*^{2}**, ***f*^{1}**, ***g*^{1}**, ***sI*^{1}**, ***sII*^{1}**, ***sIII*^{1}**, ***sIV*^{1}**, ***x*^{2}** and ***y*^{1}**, and of the light-chain gene – ***IG(L/K)A1*** – of these proteins, began between birth and month 4 of age with wide inter-piglet differences. In most animals, the expression of genes and the synthesis of immunoglobulins started by 1-2 months of age.

REFERENCES

1. KRZYŚCIN P., SKIBA E., WĘGRZYN J., 2002 – Expression of IgG immunoglobulin heavy chain IGHGA1 and IGHGA2 genes and absorption and catabolism of these proteins in lambs. ***Annals of Animal Science*** 2 (1), 101-111.
2. RAPACZ J., HASLER-RAPACZ J., 1982 – Immunogenetic studies on polymorphism, postnatal passive acquisition and development of immunoglobulin gamma (IgG) in swine. Proceedings of the 2nd World Congress on Genetics Applied to Livestock Production, Madrid, 4-8 October, VIII, 601-606.
3. SKIBA E., 2002 – Ekspresja genów alfa-globulin, beta-globulin i łancuchów ciężkich immunoglobulin klasy IgG oraz synteza, absorpcja i katabolizm tych białek u cieląt (Expression of alpha-globulin, beta-globulin and IgG immunoglobulin heavy chain genes and synthesis, absorption and catabolism of these proteins in calves). In Polish with English summary. Thesis. ***Roczniki Naukowe Zootechniki*** 16.
4. WĘGRZYN J., 1975 – Preliminary investigations of the occurrence and development of IgG in calves, on application of the allotype BA3. ***Genetica Polonica*** 16 (3-4), 331-342.
5. WĘGRZYN J., SKIBA E., 1999 – Classification of porcine IgG based on heavy chain epitopes and column chromatography. ***Roczniki Naukowe Zootechniki*** 25 (4), 25-32.
6. WĘGRZYN J., SKIBA E., 2000 – Ig(l/k)A1 – antigenic marker of light-chain immunoglobulins in pigs. ***Annals of Animal Science*** 27 (2), 37-44.
7. WĘGRZYN J., SKIBA E., KACZOR U., 2001 – Expression of IGM immunoglobulin heavy chain IGHMA2 gene and absorption and catabolism of these proteins in lambs. ***Annals of Animal Science*** 1 (2), 45-52.

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Ekspresja genów immunoglobulin u prosiąt

Streszczenie

Analizami objęto zwierzęta pochodzące z wybranych kojarzeń świń linii 990 oraz mieszańce linii 990 z wielką białą polską i polską białą zwisłouchą. Prześledzono przebieg syntezy immunoglobulin – nośników 14 markerów łańcuchów ciężkich i jednego markera łańcucha lekkiego – w surowicy krwi 143 prosiąt, od urodzenia do piątego miesiąca życia. Markery antygenowe oznaczano w żelu agarowym stosując specyficzne alloprzeciwciała przeciw poszczególnym epitopom. Z obecności (bądź braku) epitopu (antygenu) wnioskowano pośrednio o obecności (bądź braku) odpowiadającego mu białka, a tym samym o ekspresji danego genu. Uzyskane wyniki wskazują, że synteza immunoglobulin w organizmach niektórych prosiąt rozpoczyna się jeszcze przed ukończeniem przez nie 14 dnia życia. W przypadku większości osobników początek syntezy obserwowano przed upływem drugiego miesiąca życia. Zidentyfikowano również prosięta, u których ekspresja genów immunoglobulin rozpoczęła się jeszcze później – dopiero w 4 miesiącu życia.