

## Polymorphism of Prealbumins and Transferrins in Blood Serum of Pigs of Polish Large White Breed

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**Summary.** Determinations of prealbumin and transferrin phenotypes were made on 171 Large White Polish pigs. Family investigations proved that the prealbumins are determined by two alleles, Pa<sup>A</sup> and Pa<sup>B</sup>, which are inherited on the basis of co-dominance. The frequency of alleles Pa<sup>A</sup>, Pa<sup>B</sup>, and Tf<sup>A</sup>, Tf<sup>B</sup> and Tf<sup>C</sup> amounts to 0,6637, 0,3363 and 0,4941, 0,5020 and 0,0039, respectively. No essential differences were noted between the breeding groups of animals of various phenotypes such as Pa AA, Pa AB, Pa BB and Tf AA, Tf AB when considering such factors as average number of piglets, average weights of litters after farrowing and at 21 days in age, average milking capacity of sows and evaluation of sows on a 100-point scale.

Investigations on the determination of polymorphism in particular protein fractions of farm animals' blood, and on the eventual connection of such characteristics with useable predispositions, are not numerous in this country.

The authors decided to attempt the determination of polymorphism of the prealbumin and transferrin fractions in pigs of the Polish White Large breed and to utilize these features for hog raising.

### Introduction

The application, by Smithies in 1955, of electrophoresis in starch gel for investigations on human serum proteins (inherited proteins) caused a rapid development of studies on the genetic protein sub-fraction of all species of animals.

Ashton (1960), while investigating beta-globulins in the bloodserum of Large White and Essex Saddleback pigs, ascertained various migration rates of proteins from the transferrin group and determined three phenotypes of transferrins, A, AB and B, determined by two codominant alleles Tf<sup>A</sup> and Tf<sup>B</sup>.

These results were also supported by parallel investigations carried out by Kristjansson (1960).

Further investigations on polymorphism of pig transferrins, carried out by King (1962), Kristjansson (1963) and Imlah (1964), led to the discovery of transferrins C and D, which are controlled by the alleles Tf<sup>C</sup> and Tf<sup>D</sup>.

Schröffel (1966), when determining the transferrin polymorphism of Czechoslovakian pigs, noted the occurrence of all four siderophilous alleles Tf<sup>A</sup>, Tf<sup>B</sup>, Tf<sup>C</sup> and Tf<sup>D</sup>, with the last one appearing as heterozygotes Tf<sup>A</sup>/Tf<sup>D</sup> and Tf<sup>B</sup>/Tf<sup>D</sup>.

Hristič *et al.* (1967) noted three transferrin alleles, Tf<sup>A</sup>, Tf<sup>B</sup> and Tf<sup>D</sup>, in pigs of Yugoslavian breeds.

Baker (1968) discovered in Hampshire pigs a new type of transferrin resulting from mutation and denoted it Tf<sup>E</sup><sub>Ames</sub> controlled by the allele Tf<sup>E</sup><sub>Ames</sub>. Only the alleles Tf<sup>A</sup> and Tf<sup>B</sup> were noted in the parents.

Willer and Neuffer (1970), investigating the polymorphism of transferrins in German Landschwein and German Edelschwein pigs, noted four alleles, Tf<sup>A</sup>, Tf<sup>B</sup>, Tf<sup>C</sup> and Tf<sup>D</sup>. Madejska-Lewandowska (1971) determined the frequency of occurrence of transferrin types in pig breeds raised in Poland. In pigs of the Large White, Polish White Zwiśloucha, Puławska and Złotnicka White breeds, there were three transferrin alleles, Tf<sup>A</sup>, Tf<sup>B</sup> and Tf<sup>C</sup>, and in Spotted Złotnicka, four transferrin alleles, Tf<sup>A</sup>, Tf<sup>B</sup>, Tf<sup>C</sup> and Tf<sup>D</sup>.

Kristjansson (1963) found polymorphism of prealbumins, which occur before the albumins in rate of electrophoretic migration. In pigs of the Yorkshire and Landrace breeds, there were three phenotypes of prealbumins, Pa AA, Pa AB and Pa BB, which on the basis of family investigation proved to be determined by two alleles, Pa<sup>A</sup> and Pa<sup>B</sup>.

Kristjansson, having examined the serum of 358 pigs, noted Pa AA in 26,8%, Pa AB in 56,5% and Pa BB in 16,7%. Smith *et al.* (1968) quantified the polymorphism of prealbumins and transferrins on 2887 pigs of the Duroc and 2351 pigs of the Hampshire breeds. The frequency of alleles Pa<sup>A</sup> and Pa<sup>B</sup> in the Duroc breed amounted to 0,51 and 0,49, respectively, and in the Hampshire breed, 0,31 and 0,69 respectively.

Scopes (1963), examining the protein serum of pigs, obtained three prealbumin sub-fractions.

Jensen *et al.* (1968) were the first workers to investigate the relationship between polymorphism of prealbumins and transferrins and hog-raising characteristics.

Kraeling *et al.* (1971) investigated the polymorphism of prealbumins and transferrins in Duroc and Yorkshire pigs, which were selected for thin and thick backfat over many generations. The results of this investigation did not reveal any essential differences in backfat thickness of the Duroc pigs with various phenotypes of prealbumins and of Yorkshire with various phenotypes of transferrins.

Kuźmienko and Dziecina (1972) indicated that the highest mortality for various farrowing periods prevailed for the embryos with homozygotic type Tf AA (48%), and the lowest for those with heterozygotic type Tf AB. The authors ascertained also that the prealbumin fractions in the serum of the embryo differed from those of the adult.

### Material and Method

The present investigations used pigs of the Large White breed originating from Experimental Farm Lipki of the Agriculture Academy Szczecin. A total of 171 pigs were examined, of which 121 were complete families (mother, father, progeny). In parents and young dams blood was taken from the zygomatic vein, and in young sires from the tails.

The fractions of prealbumin and transferrin were determined by horizontal electrophoresis in starch gel prepared at Connaught Laboratory. The analysis was performed by the method of Smithies (1955) using the modification of Kristjansson (1963) with some modifications by the authors. The authors, therefore, consider it necessary to present the full analytical method.

### Preparation of Gel

The buffer for gel preparation comprised 0,014 M Trisu and 0,004 M citric acid dissolved in 1000 ml of distilled water for the pH value of buffer solution 7,5. The gel was prepared by adding 200 ml of buffer solution to 22 g of starch. The suspension obtained was brought to boiling point, kept there for about 3–4 min. and then de-aired. The gel suspension was placed on a plexiglas plate of dimensions 20 × 30 × 0,6 cm.

The gel was cooled under room temperature for 100 to 120 min. before applying the samples of serum. For the electrode chambers a buffer solution was used comprising 0,3 M of boric acid and 0,1 M NaOH dissolved in 1000 ml of distilled water to give pH 8,7.

### Process of Electrophoresis

After cooling, the gel was split at 50 mm from the plate edge and then carefully separated; the strips of Whatman 3 MM filter paper of dimensions 1 × 0,6 cm, soaked with the examined serum, were inserted into the gaps. The cut opening was carefully set together. Both ends of the gel were joined by filter paper Whatman 1 with the buffer solution in trays. The surface of the gel was covered with foil. A 165 V 40 mA current was applied during the first phase and was switched off after 30 min. and the strips of filter paper removed from the gel. A current of 350 V 80 mA was applied for about 2,5 hrs. When the borate line migrated a distance of 11,2 cm from the starting line, electrophoresis was terminated. A faster or longer time of electrolysis influenced the weaker or stronger separation of prealbumins. The gel was horizontally sliced into two parts and the lower part was subjected to staining.

Staining of gel. The gel was stained for 30 minutes in Amido Black 10B solution composed of methanol, distilled water and glacial acetic acid in the ratio 5:5:1. The gel was de-stained for 20 hours in a similar solution, but without Amido Black. The preparations obtained after de-staining possessed distinctly marked sub-fractions of prealbumins and beta-globulins of serum.

The relationships between the breeding features of the sows and the phenotypes of prealbumin were investigated on 31 specimens, and of transferrin phenotypes, on 26 sows. The following breeding parameters of the sows were taken into consideration:

1. average number of progenies of one litter raised up to 21 days;
2. average weight of litters after farrow;
3. average weight of litters at the age of 21 days;
4. average milking capacity of sows.

The breeding features were calculated on the first three litters. Additionally, the sows were qualitatively evaluated on a 100-point scale.

### Results and Discussion

The investigations permitted the determination of polymorphism of prealbumins and transferrins in the serum of the Polish Large White Breed. The polymorphism of prealbumins appeared under 3 forms of phenotype, determined by 2 fractions (fig. 1, 2) which possess different migration rates in an electric field

Table 1. *Distribution of prealbumin phenotypes in progenies in relation to the phenotypes of parents*

Phenotypes of mated parent pairs	Number of progenies	Number of progenies with given phenotype						χ <sup>2</sup>
		AA		AB		BB		
		observed	expected	observed	expected	observed	expected	
AA × AA	18	18	18					
AA × BB	16			16	16			
AA × AB	20	11	12,01	9	6,97			0,67
AB × AB	20	10	9,80	8	8,40	2	1,80	0,04
AB × BB	9			7	3,96	2	1,01	1,85
AB × AA	21	15	15,42	6	5,14			0,07
BB × BB	2					2	2	
BB × AB	3			1	0,83	2	2,08	1,74
BB × AA	12			12	12			
Global I	121	54	55,23	59	53,30	8	6,89	0,73

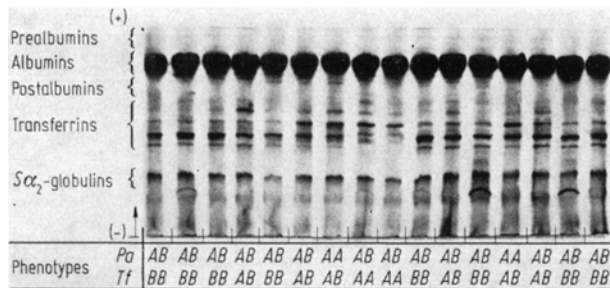


Fig. 1. Starch gel stained with Amido Black 10 B showing prealbumins and transferrins after electrophoresis

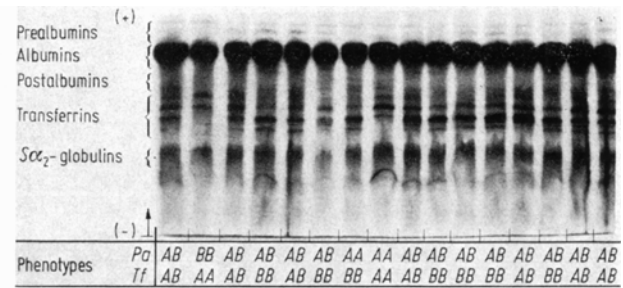


Fig. 2. Starch gel stained with Amido Black 10 B showing prealbumins and transferrins after electrophoresis

The fractions migrating at the highest rate were denoted as A and those migrating at the lowest rate as B. The investigations of family (tab. 1) show that these fractions are determined by 2 co-dominant alleles, Pa<sup>A</sup> and Pa<sup>B</sup>, which give three phenotypes Pa AA, Pa AB and Pa BB ( $\chi^2 = 0,67$ ;  $\chi^2 = 0,04$ ;  $\chi^2 = 1,85$ ;  $\chi^2 = 0,07$ ;  $\chi^2 = 1,74$ ).

Of the examined population of 172 pigs (tab. 2), the phenotype of prealbumin Pa AA was noted in 73 individuals, the phenotype Pa AB in 81 individuals, and the phenotype Pa BB in 17 individuals.

The transferrins of the phenotypes Tf AA, Tf AB, Tf BB and Tf BC occurred in 37, 95, 38 and 1 individuals, respectively. The observed and expected distribution of prealbumin phenotypes and transferrins shows that the population is in genetical balance (tab. 3).

The frequency of prealbumin genes is  $q_A = 0,6637$ ,  $q_B = 0,3363$ , while the frequency of transferrin alleles is  $q_A = 0,4941$ ,  $q_B = 0,5020$ ,  $q_C = 0,0039$ . For frequency of transferrin alleles our results are nearly the same as the results obtained by Madejska-Lewandowska (1971) in the same breed of pig (tab. 4).

The frequency of prealbumin alleles in Large White pigs differs from that in other breeds (tab. 5).

Table 2. Types of prealbumin and transferrin in pigs of Large White breed

Number of animals	Phenotypes of prealbumin							
	AA		AB		BB			
	Number of animals	%	Number of animals	%	Number of animals	%		
171	73	42,70	81	47,37	17	9,93		

Number of animals	Phenotypes of transferrin							
	AA		AB		BB		BC	
	Number of animals	%	Number of animals	%	Number of animals	%	Number of animals	%
171	37	21,63	95	55,55	38	22,22	1	0,58

Table 6 presents the breeding features of sows possessing the phenotypes of prealbumins Pa AA, Pa BB and of transferrin phenotypes Tf AA, Tf AB. The analysis of variance did not demonstrate any essential differences between the average number of progenies raised up to 21 days of life, average weights of litters after farrowing, average weights of litters at 21 days, average milking capacity of sows, qualitative evaluation of sows and phenotypes of prealbumins Pa AA, Pa AB, Pa BB and of transferrins Tf AA and Tf AB.

However, it must be emphasized that the investigations were made on a relatively small population and require further and wider investigations before final conclusions can be drawn.

### Conclusion

The investigations proved that:

1. The method applied in our investigation may be used for simultaneous determination of prealbumin and transferrin fractions in the blood serum of pigs.
2. The prealbumins of Polish Large White pigs are determined by two alleles Pa<sup>A</sup> and Pa<sup>B</sup> which are inherited on the basis of co-dominance.

Table 3. Observed and expected distribution of prealbumin and transferrin phenotypes in examined pigs of Large White Breed

Number of animals	Distribution	Phenotypes of prealbumin				$\chi^2$
		AA	AB	BB		
		171	observed	73	81	
	expected	75,32	76,33	19,34		

Number of animals	Distribution	Phenotypes of transferrin				$\chi^2$
		AA	AB	BB	BC	
			observed	37	95	
	expected	41,79	84,81	43,09	0,65	

frequency of genes  
 prealbumin pa<sup>A</sup> = 0,6637 Pa<sup>B</sup> = 0,3363  
 transferrin Tf<sup>A</sup> = 0,4941 Tf<sup>B</sup> = 0,5020 Tf<sup>C</sup> = 0,0039

Table 4. *The frequency of transferrin alleles in serum of various breed pigs*

Author	Breed	Quantity pcs	Frequency of transferrin genes			
			Tf <sup>A</sup>	Tf <sup>B</sup>	Tf <sup>C</sup>	Tf <sup>D</sup>
Kraeling et al. (1971)	Duroc	—	—	0,996	0,004	—
	Yorkshire	766	0,45	0,55	—	—
Smith et al. (1968)	Duroc	2877	0,23	0,77	—	—
	Hampshire	2351	0,20	0,80	—	—
Imlah (1964)	Large White	161	0,36	0,64	—	—
	Landrace	95	—	0,98	0,02	—
Willer and Neuffer (1970)	German Landschwein	873	0,03	0,94	0,02	0,01
	German Edelschwein	157	0,11	0,87	0,01	0,01
Hristić et al. (1967)	Resavka	60	0,30	0,70	—	—
	Mangalica	73	—	1,00	—	—
	Pfajfer	105	0,11	0,88	—	0,01
Madejska-Lewandowska (1971)	Large white	211	0,4668	0,5308	0,0024	—
	Polish white zwisloucha	85	0,1823	0,7941	0,0235	—
	Pulawska	86	0,2732	0,7200	0,0058	—
	Zlotnicka white	167	0,0718	0,9101	0,0179	—
	Zlotnicka spotted	269	0,2640	0,6858	0,0204	0,0287
Authors investigation	Polish large white	171	0,4941	0,5020	0,0039	—

Table 5. *The frequency of prealbumin alleles in serum of various breed pigs*

Author	Breed	Quantity pcs	Frequency of prealbumin genes	
			Pa <sup>A</sup>	Pa <sup>B</sup>
Smith et al. (1968)	Duroc	2877	0,51	0,49
	Hampshire	2351	0,31	0,69
Kraeling et al. (1971)	Duroc	766	0,49	0,51
	Yorkshire		0,98	0,02
Authors investigation	Large White	171	0,6637	0,3363

3. The frequency of prealbumin and transferrin alleles in pigs of the White Large breed are, respectively: for prealbumins —  $q_A = 0,6637$ ,  $q_B = 0,3363$ ; for transferrins —  $q_A = 0,4941$ ,  $q_B = 0,5020$ ,  $q_C = 0,0039$ .

4. The results obtained did not show any essential differences between average number of progenies, average weights of litters after farrowing, average weights of litters at age 21 days, average milking capacity of sows, average evaluation by points of the sows of various phenotypes of prealbumins Pa AA, Pa AB and Pa BB and phenotypes of transferrins Tf AA and Tf AB.

Table 6. *Average value (x) of features in sows in relation to prealbumin phenotypes Pa and transferrins (Tf)*

Protein phenotype	I	II	III	IV	V	VI
Pa AA	12	9,34	14,37	57,87	176,39	76,75
Pa AB	11	8,88	14,08	53,61	159,96	74,90
Pa BB	8	9,40	13,21	54,20	170,33	74,14
Analysis of variance of value F		a > 0,445 < b	a > 0,878 < b	a > 0,8337 < b	a > 1,506 < b	a > 2,52 < b
Tf AA	9	9,30	14,19	56,07	171,61	76,00
Tf AB	17	8,88	13,68	54,46	167,25	76,00
Analysis of variance of value F		a > 0,6296 < b	a > 0,425 < b	a > 4,18 < b	a > 0,80 < b	a > 0,000 < b

a = P<sub>(0,01)</sub>, b = P<sub>(0,05)</sub>

I Quantity of sows [pcs]

II Average number of progenies of one litter raised upto 21 days [pcs]

III Average weight of litters after farrowing [kg]

IV Average weight of litters at age 21 days [kg]

V Average milking capacity of sows [kg]

VI Evaluation of sows by 100-points scale.

x Average values are considered from first three litters and lactation.

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