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Original Contribution

PHAGOCYTIC ACTIVITY OF LEUKOCYTES IN PIGS, PRODUCT OF NARROW INBREEDING

Svetlin Tanchev[1]*, Valentin Semerdjiev[2], Nikolay Sandev[3], Lilian Sotirov[2], Evgeniy Zhelyazkov[1], Svetlana Georgieva[1]

[1]Department of Genetics, Breeding and Reproduction, Faculty of Agriculture
[2]Department of General Animal Breeding, Faculty of Veterinary Medicine
[3]Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine
Trakia University, 6000 Stara Zagora, Bulgaria

ABSTRACT

The study aimed to determine the effect of inbreeding on phagocytic activity in pigs from the Danube White breed.
Thirty-three pigs, each weighing 80 kg, divided into 4 groups were studied: female and male outbred and female and male inbred pigs with inbreeding coefficient Fx=0.25.
The phagocytosis was performed via mixing equal volumes of 0.1 mL heparinized blood and 0.1 mL suspension of 24-hour microbial culture of S*taphylococcus aureus* (strain 209) with a density of 2×10^9 cfu /mL. After a 30-min incubation in a thermostat at 37°C and vigorous ahaking at 5 min intervals, blood smears were prepared on clean defatted glass slide and stained according to Giemsa-Romanovsky. In each blood smear, 100 leukocytes were counted under oil immersion on a light microscope. Two parameters of phagocytic activity were determined: the phagocytosis percentage (the ratio of leukocytes that phagocytized bacteria and the total leukocyte counts) and phagocytic number (the average number of bacteria , phagocytized by one leukocyte).
A trend towards a higher phagocytic activity in female pigs vs males was present.
The phagocytic numbers and the % phagocytosis tended to decrease in the animals, product of inbreeding, compared to outbred animals at the same age, but not significantly.
The coefficients of phenotypic correlations between the phagocytosis percentage and the phagocytic number were determined. They were all high, positive and significant. The correlation coefficients in inbred animals were considerably higher.

Key words: pigs, phagocytic activity; phagocyte percentage, phagocyte number, inbreeding.

INTRODUCTION

The earliest form of natural cellular immunity in animals is phagocytosis. As a function of polymorphonuclear granulocytes and of cells of the mononuclear-phagocytic system in vertebrates and men, phagocytosis reflects the status of systemic resistance and adaptation (1, 15, 19).

Recently, phagocytosis is commonly used as a test characterizing systemic reactivity and the effect of various factors as phagocytic activity changes under the influence of physical, chemical and biological effects of the various agents (2, 4, 5, 6, 11, 12, 13, 25).

It is also established that in different animal species, there are age-, breed-, gender- and genetic-related differences in phagocytic activity (3, 4, 5, 7, 11, 16, 17, 18, 20, 21).
The use of inbreeding in domestic animals is studied especially from the point of view of its effect under the productive and biological traits such as reproductive parameters, growth and fattening abilities etc. The mating of relating individuals is successfully used for creating highly inbred lines of laboratory animals species (mice, rats, guinea pigs, miniature pigs, rabbits etc.) used as models in both human and veterinary medicine as well as in experimental biology. The studies upon the phagocytic activity in inbred animals are relatively few. The reports on influencing the immune (respectively, the phagocytic) system in inbred animals are very scarce (7, 8, 9, 10, 11, 14, 22, 23)

The aim of the present study was to determine the effect of inbreeding on

phagocytic activity in pigs from the Danube White breed.

MATERIALS AND METHODS

Thirty-three pigs weighing 80 kg, divided into 4 groups were used: female and male outbred and female and male inbred pigs with inbreeding coefficient Fx=0.25 (progeny of breeding one brother and 4 sisters – full sibs).

The phagocytic reaction was performed by the method of Valchanov (1956). For the purpose, 24-hour microbial suspension of *St. aureus* (strain 209) with a density of 2×10^9 /ml was used. The phagocytic activity of pigs was determined via two parameters – phagocytosis percentage (% phagocytosis) and phagocytic number (PN – the number of bacteria, phagocytized by one leukocyte). The phagocytosis percentage was determined as a ratio of phagocytized leukocytes and the total leukocyte counts, multiplied by 100.

Blood was obtained aseptically from the median ophthalmic sinus of pigs.

The effect of inbreeding and gender upon studied traits was determined by two-factor MANOVA linear additive model with fixed effects as followed:

$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha_i . \beta_j) + \varepsilon_{ijk}$, where

y_{ijk} - the values of the k^{th} observation of the respective levels of studied traits
μ - population mean
α_i - the differential effect of inbreeding
β_j - the differential effect of gender
$(\alpha_i . \beta_j)$ – the combined effect of both factors
ε_{ijk} – random error

RESULTS AND DISCUSSION

The effects of inbreeding and gender are shown in **Table 1**. They were not statistically significant.

The mean values of percentages of phagocytized leukocytes (phagocytosis percentages) and phagocytic numbers (PN) are shown in **Table 2**. It could be noticed that in outbred female pigs, phagocytosis percentages were higher (84.67%) vs those in males (80.00%). The same trend was present in PN values – 1.47 and 1.40 in females and males, respectively.

Table 1. *Effect of inbreeding and gender on phagocytic activity*

Source of influence (factor)	***Traits***			
	Phagocytosis percentage		***Phagocytic number***	
	$F_{(1,29)}$	***p***	$F_{(1,29)}$	***p***
Inbreeding	1,68	0,21	0,21	0,89
Gender	2,72	0,11	2,18	0,15
BOTH INBREEDING + gender	0,17	0,68	0,07	0,80

Table 2. Mean values of studied traits depending on inbreeding- and gender-related effects

Traits	***Source of influence***			
	Inbred– Fx=0,25		***Outbred***	
	♀ ***- n=10***	♂ ***- n=10***	♀ ***- n=6***	♂ ***- n=7***
Phagocytosis percentage	80.80 ± 1.967	78.00 ± 2.373	84.67 ± 2.377	80.00 ± 2.667
Phagocytic number	1.480 ± 0.045	1.370 ± 0.070	1.470 ± 0.071	1.400 ± 0.060

In animals, progeny of breeding relatives with inbreeding coefficient Fx=0.25, the phagocytosis percentages were lower in both genders (80.8 in females and 78.0 in males), but the differences were statistically in significant (P>0.05). There was no difference between PN values in female outbred and inbred pigs whereas male inbred pigs manifested lower PN values.

For both genders as a whole, the values in control animals were higher (82.15% phagocytosis percentage and PN equal to 1.434) than the respective levels in inbred pigs (79.40 and 1.428). Again, the differences were not statistically significant.

Table 3 presents the correlation

coefficients between studied traits in outbred pigs and Table 4 – the same relationships in inbred ones.

Table 3. *Coefficients of phenotypic correlation between the phagocytosis percentage and phagocytic numbers in outbred pigs*

Groups	***r***	***±Sr***
Female (♀)	0.7563	0.3437
Male (♂)	0.6074	0.3553
Total (♀ + ♂)	0.6786[a]	0.2215

Legend:
[a] *statistically significant at p<0.05*
[b] *statistically significant at p<0.01*
[c] *statistically significant at p<0.001*

Table 4. *Coefficients of phenotypic correlation between the phagocytosis percentage and phagocytic numbers in inbred pigs*

Groups	***r***	***±Sr***
Female (♀)	0.752[a]	0.2233
Male (♂)	0.8557[b]	0.1829
Total (♀ + ♂)	0.8309[c]	0.1311

Legend:
[a] *statistically significant at p<0.05*
[b] *statistically significant at p<0.01*
[c] *statistically significant at p<0.001*

The coefficients of phenotypic correlation between phagocytosis percentages and phagocytic numbers were positive and high in all groups and subgroups inbred and outbred animals. In most cases, correlation coefficients were highly significant. The formation of such a relationship seems logical because in this case, the correlating traits were interrelated. Sometimes, however, the magnitude of this relationship between the different groups of studied animals could vary within a specific range depending on the degree of homogeneity and heterogeneity. Similar phenomena were observed in our study too. In the groups of pigs, product of inbreeding, the coefficients of phenotypic correlation were considerably higher and statistically significant. Probably, this resulted from increase in homozygosity and respectively, in genetic similarity.

An analogous relationship between the phagocytosis percentage and phagocytic number is reported by Arsov et al. (3) about age-related features of phagocytic activity in pigs.

Possibly, the breeding of relatives, which is generally resulting in increasing homozygosity of genomes, is the cause for the lower values of both parameters in inbred pigs. This assumption could be however confirmed by additional studies of the main histocompatibility complex in pigs and other immunological traits.

Vagonis and Schveistis (23) determined that the increase in the degree of inbreeding in inbred pigs resulted in statistically significant lowering of immune reactivity and natural resistance due to inbreeding depression, manifested by reduced agglutinins, globulins and blood phagocytic activity.

The results obtained by other authors (3, 13, 23) evidence that the phagocytic activity in pigs changes after the birth under the influence of various reagents as a result of maturation of immune system as a whole. During and after the end of growth and development of pigs, the phagocytic activity is different in the various breeds and is influenced adequately to the different environmental factors.

That is why the difference in phagocytic activity in inbred and outbred Danube White pigs, observed by us, is important. Although statistically insignificant, the low PN and phagocytosis percentage values in inbred pigs are indicative for the lower reactive potential of animals submitted to various environmental influences during their ontogenic development.

On the other hand, the observed low and statistically insignificant differences between outbred and inbred animals could be explained by the fact that the Bulgarian porcine breed Danube White is characterized by a relatively high heterogeneity. This is a new breed created through a complex reproductive breeding with the participation of many other breeds and yet, it undergoing consolidation. It is the higher heterogeneity that does not allow the breeding of relatives to

result in a rapid increase in homozygosity and the manifestation of inbreeding depression. This hypothesis of ours should be verified in future investigations where animals with a higher degree of inbreeding (Fx=0.375) should be included.

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