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Original Contribution

STUDY OF THE EFFECTS OF INBREEDING, BREED AND GENDER ON PHAGOCYTIC ACTIVITY OF LEUCOCYTES IN RABBITS

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SUMMARY

The aim of the present study was to evaluate the effects of various levels of inbreeding, breed and gender on the phagocytic activity of leucocytes in rabbits. A total of 60 rabbits was used for this purpose (30 ♀ + 30 ♂). They were allotted into groups by breed, gender and inbreeding level as determined by Wright's method. The phagocytic reaction was determined by Valchanov's method in which a 24 h microbial suspension of *St. aureus* strain 209 with a density of $2\times 10^9/cm^3$ was used. The phagocytic activity was determined using phagocytosis percentage and phagocytic number. Data were statistically processed using three-factor MANOVA linear additive model with fixed effects. The results showed that the breed (degree of homogeneity and heterogeneity) and the inbreeding level had significant negative effects on phagocytic activity. This correlation was more clearly manifested in purebred California rabbits, whereas the heterogeneous groups presented a locally high phagocytosis activity in most cases, at a level of inbreeding of Fx = 0.25. A clear tendency towards domination of male animals versus female with regard to studied traits was present, although the differences were not significant.

Key words: rabbits, phagocytic activity, phagocytosis percentage, phagocytic number, inbreeding.

INTRODUCTION

Phagocytosis is the earliest form of cellular natural immunity.

As a function of polymorphonuclear granulocytes and the cells of mononuclear-phagocytic system in vertebrates and humans, phagocytosis reflects the status of systemic defence and accommodation (1, 2, 3).

During the last years, phagocytosis had been used extensively as a test characterising the systemic reactivity and the influence of various factors because it alters under the influence of physical, chemical and biological effects of various agents (4, 5, 6, 7, 8, 9, 10, 11, 12).

It is established that in the different animal species there are age-, breed-, gender- and genetically-related differences in phagocytic activity (13, 5, 6, 7, 14, 15, 16, 17, 18, 19).

The use of inbreeding in domestic animals has been studied predominantly from the point of view of its effect on various production and biological traits such as reproductive, growth and fattening traits. The breeding of relatives is successfully used for creation of highly inbred lines of laboratory animals (mice, rats, guinea pigs, miniature pigs, rabbits etc.), used as experimental models in both human and veterinary medicine as well as in experimental biology. The studies on the factors affecting the immune response phagocytic system in inbred animals are few (20, 7, 19, 21, 22, 23, 24, 25, 26).

The aim of the present investigation was to evaluate the effect of various degrees of inbreeding, the breed and the gender on the phagocytic activity of leukocytes in rabbits.

MATERIAL AND METHODS

A total of 60 rabbits was investigated. Their allotment into groups, depending on their breed, level of inbreeding and gender, is presented on **Table 1**.

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Table 1. *Rabbits included in the study*

Group	***Breed and origin***	***Level of inbreeding (Fx), gender and number***					
		Fx = 0		***Fx = 0,25***		***Fx = 0,375***	
		♀	♂	♀	♂	♀	♂
I	"Homogeneous" California rabbits	5	5	5	5	5	5
	Total	*10*		*10*		*10*	
II	"Heterogeneous" crosses	5	5	5	5	5	5
	Total	***10***		***10***		***10***	

All animals were of same age (7-8 months), live body weight (3.5-4 kg) and were placed under equal conditions of feeding and housing.

The division of rabbits into "homogeneous" and "heterogeneous" groups was conditional. Thus, the higher theoretical similarity among genotypes in purebred California rabbits and the expected higher heterogeneity in mixed-breed crosses was expressed.

The original parental group of heterogeneous rabbits included male and female crosses reared in two small private farms without records of the used crossbreeding. Both farms were located at 50 km distance from one another and therefore we assumed that the animals were not relatives. For purebred California rabbits, a strict control of mating was performed, that guaranteed the lack of a previously accumulated inbreeding.

The phagocytic reaction was determined by the method of Valchanov (27). For this purpose, a 24 h microbial suspension of *St. aureus* strain 209 with a density of $2\times 10^9/cm^3$ was used. The phagocytic activity was determined via two parameters – phagocytosis percentage (% phagocytosis) and phagocytic number (PN – the number of bacteria, phagocytised by one leukocyte). The phagocytosis percentage was determined as a ratio of phagocytised leukocytes and the total leukocyte counts, multiplied by 100.

Data were statistically processed by three-factor MANOVA linear additive model with fixed effects.

The inbred offspring were obtained from breeding full sibs, and the coefficient of inbreeding was calculated by the method of Wright:

$$Fx= \sum[(1/2)^{n1+n2+1}. (1 + Fa)]$$

The results were analysed by three - factor MANOVA linear additive model with fixed effects as follows:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \delta_k + \left(\alpha_i\beta_j\right) + \left(\alpha_i\delta_k\right) + \left(\beta_j\delta_k\right) + \left(\alpha_i\beta_j\delta_k\right) + \varepsilon_{ijkl},$$

where:

y_{ijkl} - the values of the k^{th} observation of phagocytosis percentage and the phagocytic number

μ - population mean ;

α_i - the differential effect of the breed;

β_j - the differential effect of the level of inbreeding;

δ_k - the differential effect of gender;

$\alpha_i\beta_j$ - the combined effect of breed and level of inbreeding;

$\alpha_i\delta_k$ - the combined effect of breed and gender;

$\beta_j\delta_k$ - the combined effect of level of inbreeding and gender;

$\alpha_i\beta_j\delta_k$ - the combined effect of all three factors;

ε_{ijkl} - random error.

RESULTS AND DISCUSSION

The data from the analysis of the effect of all factors on the phagocytic activity – phagocytosis percentage and phagocytic number, are shown on **Table 2.** The breed of rabbits and the level of inbreeding had a significant effect on studied traits.

Table 2. *Effect of studied factors on phagocytic activity as a whole*

Source of effect	***Wilks' Lambda***	***Rao's R***	***df 1***	***df 2***	***p-level***
Breed (Breed affiliation)- α_i	0,861168*	3,788515*	2*	47*	0,029824*
Level of inbreeding - β_j	0,780490*	3,100157*	4*	94*	0,019145*
Gender - δ_k	0,951581	1,195745	2	47	0,311512
Breed + Level of inbreeding - $\alpha_i\beta_j$	0,896649	1,317419	4	94	0,269198
Breed + Gender - $\alpha_i\delta_k$	0,975167	0,598427	2	47	0,553809
Level of inbreeding + Gender - $\beta_j\delta_k$	0,976527	0,280764	4	94	0,889772
Breed + Level of inbreeding + Gender - $\alpha_i\beta_j\delta_k$	0,991916	0,095569	4	94	0,983644

** significant effect*

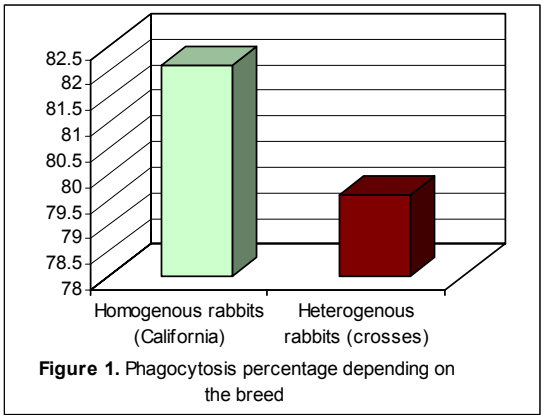

Figure 1. Phagocytosis percentage depending on the breed

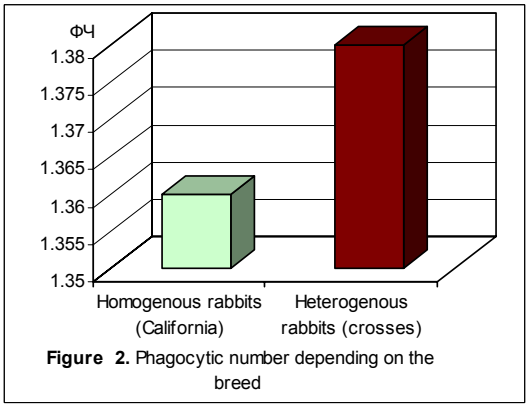

Figure 2. Phagocytic number depending on the breed

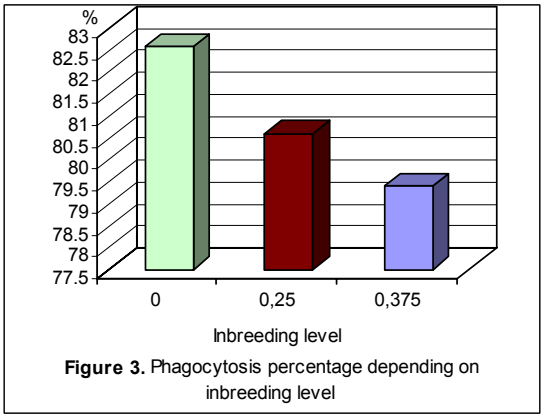

Figure 3. Phagocytosis percentage depending on inbreeding level

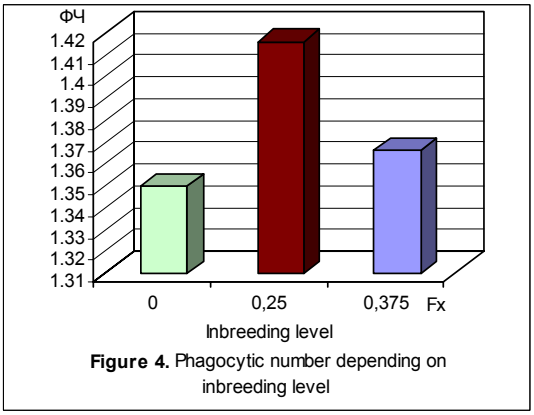

Figure 4. Phagocytic number depending on inbreeding level

Figure 1 shows the dynamics in phagocytosis percentage depending on rabbits' origin and **Figure 2** – the phagocytic number values. The trends were completely different for both parameters. Purebred homogeneous rabbits had higher phagocytosis percentages and lower phagocytic numbers. This cannot be explained in spite of the fact that the traits are related. In both cases however, the observed inter-group differences were not significant.

Figures 3 and **4** show the phagocytosis percentage and the phagocytic number depending on the level of inbreeding of rabbits. Again, the observed tendencies were not unidirectional. While the phagocytosis percentage exhibited a clear but insignificant

reduction with increase of the level of inbreeding for phagocytic numbers, the lowest values were determined in outbred animals whereas the highest – in inbred with Fx = 0,25. The values in rabbits with level of inbreeding Fx = 0,375 were intermediate. In this case again, the differences between groups were not significant.

Figures 5 and **6** showed the studied traits in female and male rabbits. There was a clear trend of dominance of males, albeit with no observed significance.

In **Table 3**, the values of studied traits are given separately depending on the breed and level of inbreeding at a time. The shown tendencies are the same as those observed during the analysis of inbreeding level on traits and so we assume that the effect of this factor was more essential for values' dynamics. The presence of significant differences between groups was probably a consequence of the combined effect of both factors.

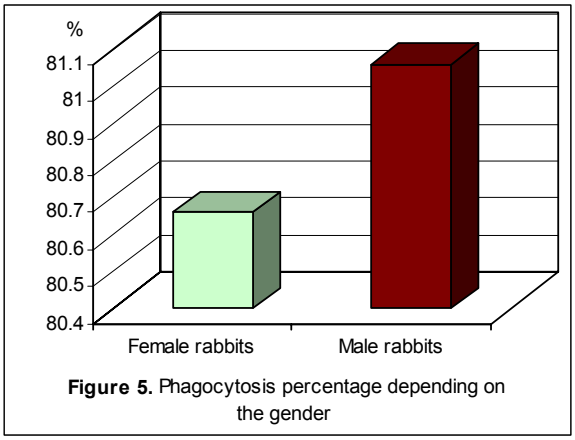

Figure 5. Phagocytosis percentage depending on the gender

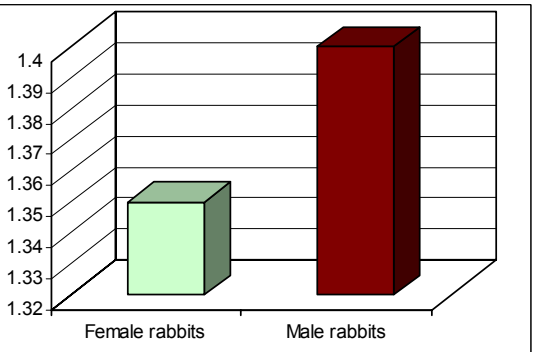

Figure 6. Phagocytic number depending on the gender

Table 3. *Effect of the breed and level of inbreeding*

Level of inbreeding	***Homogeneous rabbits (California)***		***Heterogeneous rabbits (crosses)***	
	Phagocytosis percentage	*Phagocytic number*	*Phagocytosis percentage*	*Phagocytic number*
Fx = 0	$84,8^{ABC}$	1,392	80,4	$1,308^{E}$
Fx = 0,25	81,6	1,376	$79,6^{B}$	$1,456^{DE}$
Fx = 0,375	80^{A}	$1,34^{D}$	$78,8^{C}$	1,392

- *equal letters designate significant differences between groups*

Table 4. *Effect of level of inbreeding and gender*

Level of inbreeding	***Phagocytosis percentage***		***Phagocytic number***	
	Female rabbits	*Male rabbits*	*Female rabbits*	*Male rabbits*
Fx = 0	83,2	82	$1,332^{A}$	1,368
Fx = 0,25	79,6	81,6	1,388	$1,444^{A}$
Fx = 0,375	79,2	79,6	1,344	1,388

** equal letters designate significant differences between groups*

Table 4 presents the values of studied traits depending at a time on the level of inbreeding and gender. The observed trends are similar to those obtained from the analysis of inbreeding levels. Except for outbred males, higher values of traits were present in male animals compared to females.

The simultaneous effect of origin and gender did not contribute to the dominance of males over females that was already observed in both homogeneous and heterogeneous animals. The differences between the groups were not significant.

Figure 7 shows the phagocytosis percentages depending on all three factors. In homogeneous animals there was a stable but insignificant tendency towards decrease with increasing the inbreeding coefficient in both genders; in heterogeneous rabbits it was not present. In heterogeneous females there was a local minimum in the group with inbreeding level Fx = 0,25, whereas the males from the same group showed a local maximum. The same trend was observed for the effects of

inbreeding level and gender upon this trait. The differences between groups were not significant in any case.

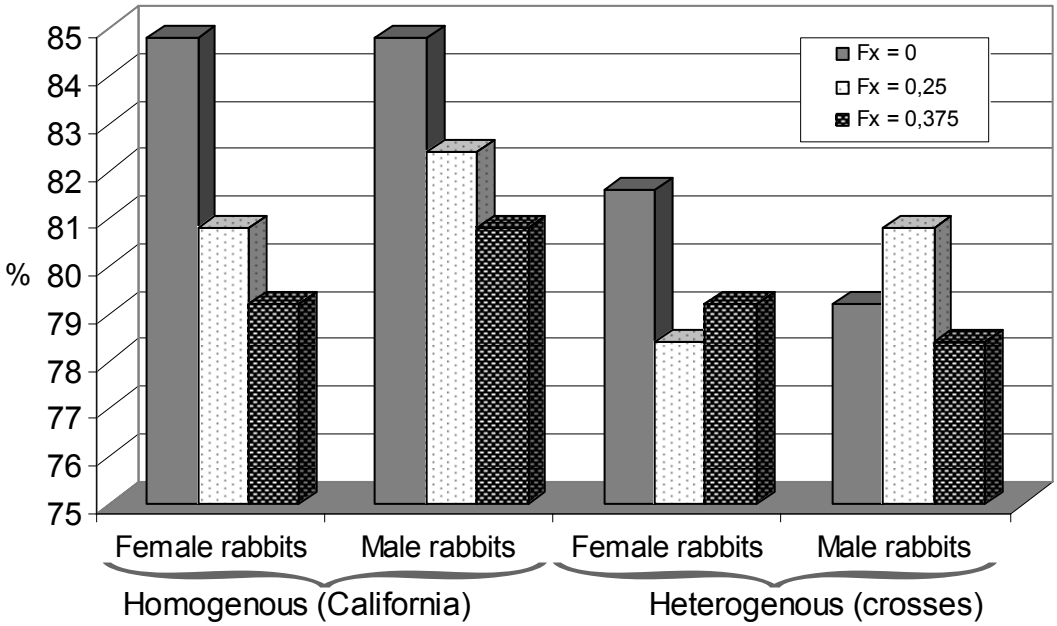

Figure 7. Phagocytosis percentage depending on the origin, level of inbreeding and gender

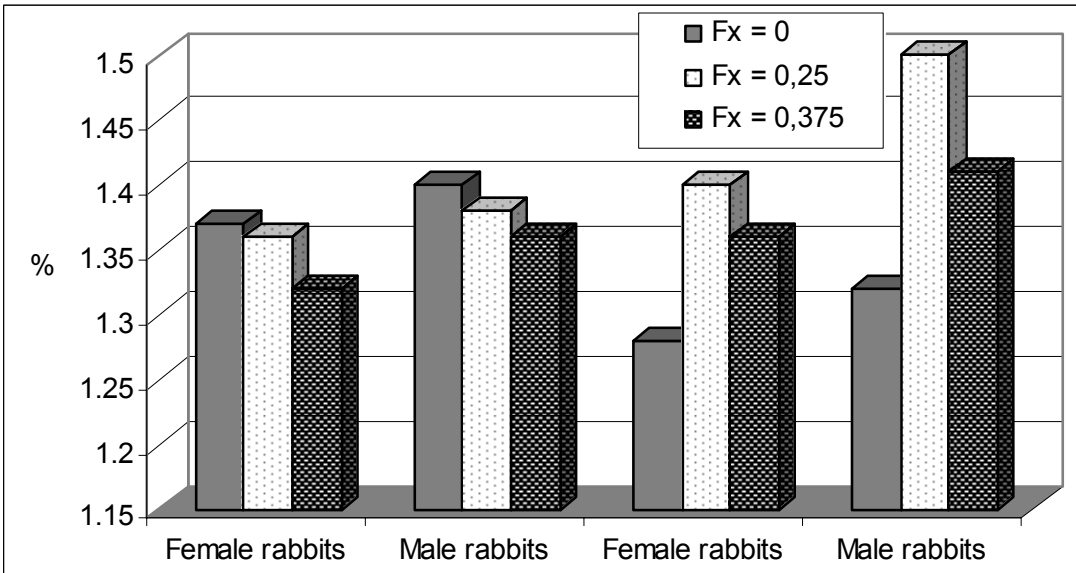

Figure 8. Phagocytic number depending on the breed, levels of inbreeding and the gender

Nearly the same variation was observed with regard to phagocytic number. **Figure 8** presents the values of these parameters depending on all three factors. Again, in homogeneous rabbits the increased level of inbreeding resulted in decreased phagocytic numbers, although the variation was within narrower limits. A parallel dynamics in values was noticed in heterogeneous rabbits too. They showed local minima in outbred females and males and peaks in groups with inbreeding level Fx = 0,25. The rabbits with inbreeding level Fx = 0,375 had also higher values than their homogeneous analogues. In this case, the males inbred rabbits with Fx = 0,25 dominated considerably over the outbred heterogeneous animals of the same age.

The differences with regard to the effect

of studied factors in homogeneous and heterogeneous groups on one part, and the observed stable tendency towards reduction of phagocytic activity with increasing the inbreeding level on the other, allowed us to assume that the similarity and divergence in original parental genotypes determined the pattern of variation of studied traits in the offspring.

Thus, it is logical to anticipate a higher variability and even contradictory results for studied traits in heterogeneous groups. Consequently, some conflicting and insignificant results in outbred and inbred heterogeneous rabbits could be explained to a certain extent.

Moreover, it must be stated that the observed significant effect of the breed (homogeneity and heterogeneity) and the level of inbreeding, evaluated separately (**Table 2**), as well as most of the significant differences when assessed simultaneously (**Table 3**), allowed us to suggest that both factors were important for the phagocytic activity in rabbits.

A similar reasoning was proposed by Vagonis and Schveistis (20), which was to the effect that increase in inbreeding level in pigs produced a significant lowering of natural resistance manifested via decreased agglutinins, globulins and blood phagocytic activity.

The same view was held by Tanchev et al., 2005, despite the lack of significance obtained in this study.

A higher mortality rate in young inbred rabbits compared to outbreds of the same age was reported for Farghaly (21). This indirectly pointed to a lower natural resistance.

In summary, it could be said that our results and those of other authors would make us conclude that the increase in the level of inbreeding resulted in a definite significant negative effect upon the phagocytic activity in rabbits. This is valid especially for more homogeneous populations with higher degree of homozygosity, while in heterogeneous groups the results were less regular and in most cases - not in agreement. There was also a clear tendency towards dominance of male individuals over females with regard to phagocytic activity, although the differences were not significant.

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