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STABILIZING SELECTION FOR LOWER PHENOTYPE VARIABILITY OF RABBITS

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ABSTRACT

Stabilizing selection for lower variability of liveborn kits in a litter resulted in higher vitality and significantly ($P < 0.05$) higher mean number of weaned kits at 35 days of age. In the group of kits from selected mothers with higher variability per litter and coefficient of variation > 11 %, the mortality to weaning (35 days) and post-weaning (42 days) was 27.27 %. In the group from mothers with lower variability between litters and coefficient of variation < 11 %, the mortality within this period was zero. Females with lower variability of liveborn kits per litter and their kits at the 42nd day of age showed lower level of C-reactive protein (CRP) in blood plasma compared to the second group. Strong negative correlation (r = -0.795) was confirmed between the coefficient of variation of liveborn kits and the number of weaned kits with coefficient of determination $r^2 = 0.633$. In practice it means that higher balance or lower variability of the number of liveborn kits between the individual litters has positive influence on the number of weaned kits and this parameter was influenced on up to 63.3 %, while the rest of the influence (36.7 %) was entirely random. Strong negative correlation (r = -0.94) was noted also between the markers of milk yield in females up to the 21st day and the variation coefficient of liveborn kits. The determination coefficient in this case was $r^2 = 0.884$. These data suggest that higher stability of liveborn kits numbers between litters has a positive effect on the number of weaned kits of rabbits and also that targeted selection for lower levels of C-reactive protein in blood plasma can help to improve effective production through more effectively innate immunity against pathogens and therefore lower mortality of growing rabbits.

Key words: stabilizing selection; number of liveborn kits; milk yield; determination coefficient; vitality

INTRODUCTION

Selection at an early age for breeding and intensive production is an unavoidable prerequisite of a successful system for intensive animal production all around the world (Niranjan *et al*., 2010). Studies of multiple authors determined that direct as well as maternal influences are especially important for the intensity of growth of animals to weaning (Ferraz *et al*., 1992; Lukefahr *et al*., 1993; 1996) and affect the phenotype expression of young animals through the genotype of their mother for maternal behaviour and intensity of growth. On the other hand, in multiparity species such as rabbit, it was proven that the influence of the mother, which is expressed in the size of the litter and the birth weight, also influences the mortality in the period since milk nutrition to weaning of kits (Poigner *et al*., 2000). The presence of maternal antigens in the blood serum of rabbit kits is notable and detectable up to 6 – 9 weeks after birth, at a later age its levels are low with negligible effect (Blasco *et al*., 1983; Szendrö *et al*., 1984). Basing on the aforementioned, the number of weaned kits correlates with the genetic

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predisposition of the mother, her health status and the total breeding condition of the mother (Ondruska *et al*., 2016a; 2016b). The first factor usually affecting the impact of various diseases is the immunity of the given individual – host. One of the main characteristics of a health profile, immunologic and genetic predispositions is the C-reactive protein (CRP). The main characteristics of the biological action of CRP is its ability to bind phosphocholine, which allows the immune system to recognize foreign pathogens, as well as damaged or otherwise impacted cells of its own body. From the immunological standpoint, CRP acts as opsonin. CRP binds itself to the surface of the antigen and activates its phagocytose through immune cells. Opsonised can be, for example, bacteria or other pathogen organisms and afterwards, opsonins are recognized by Fc receptors, which are located on the surface of phagocytes (macrophages, neutrophiles) and they are phagocytosed and neutralised. CRP is, therefore, a ligand directly bound to neutrophiles, macrophages and other phagocyte cells, which stimulates inflammatory reaction and production of cytokines. The quick increase of CRP levels, as a result of induced stimulus, shows that CRP is a component of the inherent immunity response (Black *et al*., 2004).

Subject of the selection is always the individual's phenotype but not the genes. It is important to realize that selection for genes is always mediated by the phenotype, because biological fitness is a complex phenomenon. Fitness, as such, cannot be considered a trait; instead, it comprises a number of morphological and physiological traits, of which each can have its own hereditary basis. Selection, due to its impact on division of any trait affecting biological fitness, can therefore affect several genes at the same time. In stabilizing selection, the higher chance for survival and reproduction belongs to the individuals with values of the trait close to the population mean. Alleles that cause extreme values of the trait (high or low) are removed from the population, what leads to decreased variability from one generation to the next, while the population mean remains unchanged. For the evolutionary genetics, phenotypic variance is one of the selection key issue for several animal models.

Zhang and Hill (2005) found that the optimum phenotypes are selected for reproduction and less variable genotypes are favoured. Bodin *et al*. (2010) described the useful genetic uniformity in production traits in rabbits. They found correlations between the homogeneity of litter's birth weight and higher viability of the kits. This is a trait directly related to fitness; environmental variance can be related to the capacity of animals to cope with new environmental conditions (Blasco *et al*., 2017). Animals with less adaptable genotypes can be a more sensitive to diseases and stress and show a higher degree of variability in litter size (García *et al*., 2012; Argente *et al*. 2014; García *et al*. 2016).

For this reason, the main aim of the research programme was the application of standard selection procedures (1st stage of research) supplemented by results from immunologic testing by the ELISA method (2nd stage of research) in order to create suitable selection criteria to improve rabbits' vitality. The aim of the suggested selection procedures was to increase the vitality of young animals and the efficacy of rabbit production in extensive and intensive breeding systems.

MATERIAL AND METHODS

Experimental design, animals and management

All experiments were performed in accordance with relevant institutional and national guidelines for the care and use of animals, and all experimental procedures involving animals were approved by an ethical committee. The trial was performed at the accredited facility of experimental rabbit farm SK CH 17016 at the National Agricultural and Food Centre, Nitra, Slovak Republic.

The trials performed on clinically healthy rabbits were divided into two stages. In the first stage a total of 24 bucks (four different populations of rabbit meat lines; 6 bucks from each one) were involved. Totally, 164 pregnant does and 1513 kits from six different populations of rabbit meat lines were evaluated (Table 1). The production parameters (the number of liveborn kits, still-born kits and milk production until the 21st day of lactation) and the number of weaned kits at the age of 35 days were monitored.

For the milk yield determination, the indirect method of predicting milk production using formula based on a high correlation between milk production

and litter live weight gain at 21 days was used. Equation for the calculation of milk production of female rabbits is as follows:

Milk yield at 21 days (g) = 2 . (m21 – m0)
m21 – weight of the litter on the 21st day
m0 – weight of the litter after birth

During the second stage, stabilizing selection of females from the population with the best reproduction parameters from the first stage (P91 and M91) was performed in order to arrange two groups, which were then immunologically tested (ELISA) for CRP levels in blood plasma. The basic criterion to form the two groups of females from the original meat lines (M91 and P91) was the number of kits per litter in 3 – 9 litters, where females with 7 – 10 kits per litter were included into the CRP-1 group and females with higher variability of the number of kits per litter (1–15 kits) were in the group CRP-2. ELISA analyses were performed on blood plasma samples collected from 24 females of original meat lines of the rabbit bred. The females of the parental generation were divided into two groups: 1. Experimental group of females (12 animals) was subjected to strict stabilizing selection: minimum number of liveborn kits 7–10 per litter, in a minimum of three litters. 2. Control group (12 animals) had large variations in the number of liveborn kits per litter among at least three litters (1–15 kits per litter). The experimental females of all genotypes were at the age between 11 and 18 months. The does with kits were housed in cages made of spot-welded wire mesh of 560 × 760 mm in size (width × length) and with a resting area (560 × 310 mm) arranged in flat decks on one level. The nest (560 × 260 mm) was lined with sterile wood shavings. The nest area and cage were separated by a sheet metal wall with a door. The rabbits were fed a commercial diet (crude protein 177.25 $g.kg^{-1}$; crude fibre 168.28 $g.kg^{-1}$; fat 34.21 $g.kg^{-1}$; acid detergent fibre 185.21 $g.kg^{-1}$; neutral detergent fibre 316.19 $g.kg^{-1}$ and metabolizable energy 11.08 $MJ.kg^{-1}$). All animals had *ad libitum* access to feed. Drinking water was provided with nipple drinkers *ad libitum*. A cycle of 16 h of light and 8 h of dark was used throughout the trial. Temperature and humidity in the building were recorded continuously with a digital thermograph. Heating and forced ventilation systems allowed the building temperature to be maintained within 18 ± 4 °C throughout the trial. Relative humidity was in the interval of 70 ± 5 %.

Sample collection and ELISA analysis

Samples of peripheral blood for testing were taken from the *vena auricularis centralis* into heparinized tubes. Afterwards, 30 minutes after collection the samples were centrifuged at 1000 x g for 15 minutes at 4 °C to produce blood plasma for immunologic testing using ELISA (Enzyme Linked Immuno Sorbent Assay) for antigen detection. A commercial rabbit C-reactive ELISA kit (SunRed Bio, Shanghai, China; cat. No. 201-09-0003) with a detection range 50 $\mu g.L^{-1}$ – 1000 $\mu g.L^{-1}$ was used for CRP level determination. Values of CRP in rabbit blood plasma were measured using a PowerWave XS microplate spectrophotometer (Biotek) at the wave length of 450 nm.

Statistical analysis

The collected data were statistically analysed by a t-test using an Excel software and the commercial SAS 9.1 statistics package (SAS Institute Inc, USA). Statistical significance was indicated by *P*-values lower than 0.01 or 0.05. Correlations of production parameters were evaluated by Pearson Correlation Coefficient Calculator (https://www.socscistatistics.com/tests/pearson/Default2.aspx).

Coefficient of variance was calculated using the following formula:

$$v = \frac{sd}{\bar{x}} \cdot 100\ (\%)$$

v = coefficient of variance; sd = standard deviation; $\bar{x}$ = mean

RESULTS AND DISCUSSION

The results from the first stage of the experiment with long-term monitoring of production parameters (Table 1) showed strong negative correlation (r = -0.795) between the variation coefficients of liveborn kits and the number of weaned kits with the coefficient of determination $r^2 = 0.633$. In practice this means, that higher stability of litters or lower variability of liveborn kit numbers between litters has a positive effect on the number of weaned kits, and this parameter affects as much as 63.3 %

with the remaining influence (36.7 %) being entirely random. Strong negative correlation (r = -0.94) was determined also between the markers of milk yield of females before the 21st day and the coefficient of variation of liveborn kits. Coefficient of determination in this case was $r^2 = 0.884$.

Our results are in accordance with Blasco *et al*. (2017), who monitored litter size in two different rabbit lines: one line of rabbits for litter-size homogeneity and one line for litter-size heterogeneity by measuring intra-female phenotypic variance. Litter size was consistently larger in the low variance of litter size line than in the high variance of litter size line throughout the experiment. They found that the selection for reduced rabbit litter size variability does not decrease litter size.

In our study we determined strong positive correlation (r = 0.815) between the parameters of milk yield and the number of weaned kits with coefficient of determination $r^2 = 0.664$. These results are in line with several studies described the importance of milk as the essential and the only source of nutrition for kits in a first days after birth (Fortun-Lamothe and Gidenne 2000; Maertens *et al*. 2006). According to the study of Bonachera *et al*. (2017) the milk production in the first 21 days of lactation has a significant impact on the growth and health of the kits and is very important and the limiting factor for successful rearing during the pre-weaning period.

Table 1. Selected production parameters of the evaluated meat lines and breeds of rabbits

Parameter	Genotype (♂ x ♀)					
	Hy x B1	M91 x M91	P91 x P91	Hy x Hy	Ch x Ch	M91 x Bls
Number of kindled females	36	33	30	34	15	16
Total number of liveborn kits	360	304	247	330	128	144
Mean number of liveborn kits per litter	10.00 ± 1.96	9.21 ± 1.41	8.23 ± 1.38	9.71 ± 1.96	8.53 ± 1.96	9.00 ± 1.55
Mean number of weaned kits per litter	5.11 ± 1.78	6.3 ± 1.49	6.4 ± 1.58	4.79 ± 2.15	5.30 ± 1.77	6.20 ± 1.40
Coefficient of variation of the number of liveborn kits (%)	19.6	15.31	16.77	20.19	22.98	17.22
Milk yield (g)	4 190	5 030	5 170	4 340	3 810	4 660

Hy – Hycole rabbit hybrid; B1 – F2 generation of animals created during hybridization of standard local synthetic broiler line (M91) with sires of Belgian giant white; M91 and P91 – local synthetic broiler rabbit lines; Ch – Chinchilla giganta; Bls – Big light silver.

During the second stage, using stabilizing selection two groups were formed from the population's females (P91 and M91) with the best reproduction parameters from the 1st stage. They were then immunologically tested (ELISA) for CRP levels in blood plasma. Stabilizing selection of females was performed in relation to variability of the number of liveborn kits. Additionally, we also evaluated other selected reproduction parameters (conception rate, mean number of liveborn kits, vitality of kits after weaning (42 days of age) and the levels of CRP in blood plasma of females.

C-reactive protein (CRP), as one of the main components of the inherited immunity mechanism, is in the hierarchy of proteins the first to be produced in the liver during the acute phase of the immune response or in the case of any invasion by a pathogen or immunostimulation (Figure 1). Targeted selection for lower levels of C-reactive protein (CRP) in blood plasma will help to improve effective production of rabbits due to innate immunity against pathogens and, therefore, lower levels of mortality especially after weaning (Table 2).

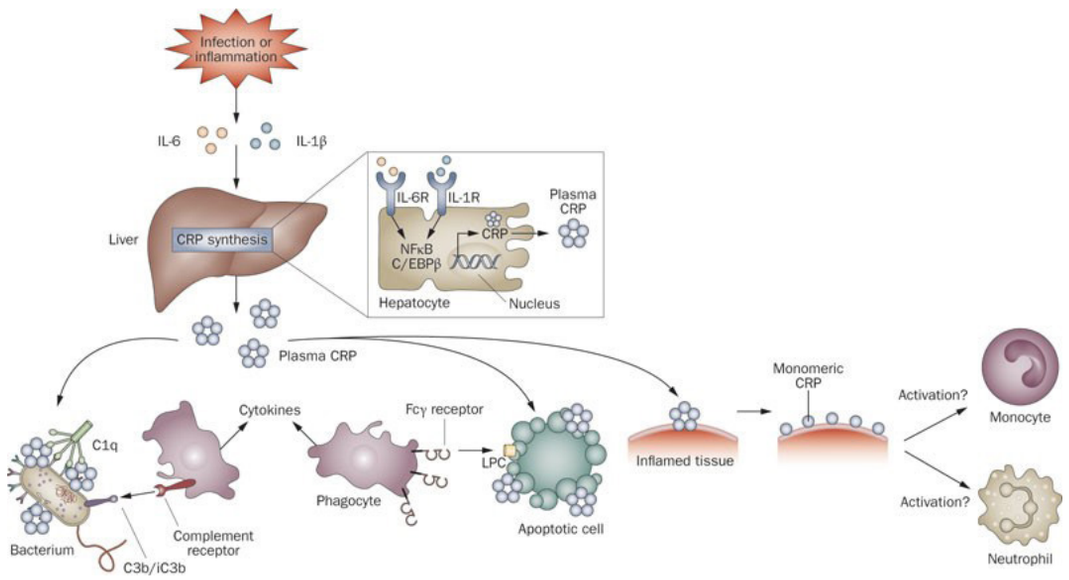

Figure 1. Synthesis and expression of C-reactive protein

From the results presented in Table 2 it is evident that stabilizing selection for lower variability of liveborn kits per litter led to higher vitality and significantly ($P < 0.05$) higher mean numbers of kits weaned at the age of 35 days. In the group of kits from selected mothers with higher variability per litter, with the coefficient of variation > 11 %, the mortality to weaning (35 days) and the post-weaning period (42 days) was 27.27 %, while in the group from mothers with lower variability per litter with coefficient of variation < 11 % the mortality over the same period was zero. Females with lower variability of liveborn kits per litter and their kits on the 42nd day showed lower level of C-reactive protein (CRP) in the blood plasma compared to the other group. These results are in line with Blasco *et al*. (2017), who found higher and more effectively tolerated of line with litter size homogeneity for external stressors than the line selected for litter size heterogeneity. The line with high variance of litter size and a higher subclinical immune response, according to Rauw (2012), is related to a greater sensitivity to diseases or to less tolerance to common microorganisms in the farm microenvironment. On the other hand, García *et al*. (2012) and Argente *et al*. (2014) recorded the faster and stronger response to invading agents in rabbit line with low variance of litter size.

Table 2. Selected production parameters of meat rabbit populations after stabilizing selection

Group	Conception rate (%)	Total number of liveborn kits	Mean number of liveborn kits per litter $\bar{x} \pm sd$	Mean number of weaned kits per litter $\bar{x} \pm sd$	CRP levels in mothers' blood plasma (μg.L^{-1}) $\bar{x} \pm sd$	Coefficient of variation of liveborn kits (%)	CRP levels in plasma of growing rabbits (μg.L^{-1}) $\bar{x} \pm sd$
CRP–1	84.61	78	7.8 ± 0.84	7.8 ± 0.84	129.45 ± 24.75	10.77	56.78 ± 7.6
CRP–2	81.81	67	6.60 ± 3.05	4.80 ± 3.27	143.58 ± 50.82	46.21	120.69 ± 46.93
t-test	-	-	-	*	-		***

*Difference is statistically significant at $P < 0.05$; ***Difference is statistically significant at $P < 0.001$.

CONCLUSION

The obtained results objectively verify the hypothesis of genetic and immunologic research about the application of stabilizing selection of rabbits at the level of variability of the liveborn kit number and concentrations of C-reactive protein in the blood plasma of rabbits, as well as their direct relationship to better vitality of kits before weaning and the reproduction fitness of the selected females (high milk production up to the 21st day). At the same time, positive impact of the given breeding programme, involved in the selection program, on the economy and efficiency is clearly declared, as with the same costs and inputs a higher number of weaned animals is achieved. Based on these results, we recommend breeding of meat line rabbits under strict stabilizing selection of females of the founding herd after a minimum of 2 litters for low variability of liveborn kits (7–10 animals) in the litter with a coefficient of variation less than 11 %.

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