



## **PROCEEDINGS OF THE 12<sup>th</sup> WORLD RABBIT CONGRESS**

Nantes (France) - November 3-5, 2021

ISSN 2308-1910

### Session **PATHOLOGY & HYGIENE**

***Belloumi D., Argente M.J., García M.L., Blasco A., Santacreu M.A.***

**STUDY OF BIOMARKERS OF DISEASE SENSITIVITY  
IN A ROBUST AND A STANDARD MATERNAL LINE**

**Full text of the communication**

+

**Poster**

#### *How to cite this paper*

Belloumi D., Argente M.J., García M.L., Blasco A., Santacreu M.A., 2021. Study of biomarkers of disease sensitivity in a robust and a standard maternal line. Proceedings 12th World Rabbit Congress - November 3-5 2021 - Nantes, France, Communication P-07, 4 pp. + presentation

## STUDY OF BIOMARKERS OF DISEASE SENSITIVITY IN A ROBUST AND A STANDARD MATERNAL LINE

Belloumi D.<sup>1</sup>, Argente M.J.<sup>2</sup>, García M.L.<sup>2</sup>, Blasco A.<sup>1</sup>, Santacreu M.A.<sup>1\*</sup>

<sup>1</sup>Institute for Animal Science and Technology. Universitat Politècnica de València, P.O. Box 22012. 46022 València, Spain

<sup>2</sup>Departamento de Tecnología Agroalimentaria. Universidad Miguel Hernández de Elche, Ctra de Beniel Km 3.2, 03312 Orihuela, Spain

\*Corresponding author: msantacr@dca.upv.es

### ABSTRACT

Robustness is related to less sensitiveness to stress and diseases. The objective of this paper is to study biomarkers of resilience to disease in two commercial maternal lines, a robust line founded using longevity criteria (line LP) and a standard line (line A) after stimulation by vaccination against rabbit haemorrhagic disease (RHD). No clear difference in white blood cells (WBC) was found between lines before and after vaccination ( $P=0.80$ ). The A line showed lower percentage of lymphocytes ( $-7.13\%$ ,  $P=0.99$ ), lower percentage of monocytes ( $-2.32\%$ ,  $P=0.98$ ) and higher percentage of granulocytes ( $+9.26\%$ ,  $P=1.00$ ) than the LP line. These differences were maintained after vaccination. Lower percentage of lymphocytes in line A suggests a higher disease sensitivity of this line, it is known that lymphocytes are involved in the adaptive immune system. A higher basal C-reactive protein (CRP) concentration was observed in the line A ( $+41.40 \mu\text{g/ml}$ ,  $P=1.00$ ). After vaccination, the A line still shows higher CRP concentration ( $+23.81 \mu\text{g/ml}$ ,  $P=0.97$ ), but it should be noticed that the LP line showed a higher response to stimulation by vaccination than the A line ( $+14.6 \mu\text{g/ml}$ ,  $P=0.93$ ) and this is related to a good functionality of the inflammatory system (McDade et al., 2005). There is not enough evidence of having differences between lines in bilirubin before vaccination ( $P=0.83$ ) but line A showed lower bilirubin than the LP line after vaccination ( $-0.14 \mu\text{mol/l}$ ,  $P=0.93$ ). This weak response to stimulation in line A could be explained by a high susceptibility to diseases of this line. Both lines showed similar concentrations of triglycerides and cholesterol before and after vaccination, but the response to vaccination was higher in the LP line than in the A line for triglycerides ( $+0.05 \mu\text{mol/l}$ ,  $P=0.90$ ), which is related to a good functionality of the liver metabolism in combating infections and reducing inflammations. Rabbit females from line LP showed better disease biomarkers results than line A, which could be related to a higher resistance to diseases.

**Key words:** Resilience, Longevity, Robustness, Disease Biomarkers

### INTRODUCTION

Intensive rabbit producers are interested in having robust reproductive females; i.e., as defined Berghof et al., (2019), does with high productivity and resilience to external stressors. Robust females are less sensitive to stress and diseases. A way of measuring robustness is using biomarkers. Acute phase proteins and haematologic parameters are classical biomarkers to measure sensitivity to inflammatory or infectious processes (Ferrián et al., 2013; García-Quirós et al., 2014; Argente et al., 2019; Gunia et al., 2019). Moreover, some lipids and proteins in blood related to fat metabolism are also used as biomarkers of resilience because some studies show changes in the liver fat metabolism as part of the immune response process (Argente et al., 2019).

The objective of this paper is to study biomarkers for disease resilience in two commercial maternal lines, a robust line founded using a longevity criterion (line LP) and a standard line (line A), after stimulation by vaccination against rabbit haemorrhagic disease (RHD).

## MATERIALS AND METHODS

All experimental procedures involving animals were approved by the Universitat Politècnica de València Research Ethics Committee.

### Experiment animals

A total of forty-four females from two different rabbit lines were used in this experiment. Twenty-one females from the 15<sup>th</sup> generation of a robust line founded using longevity criteria selected for litter size at weaning (line LP; Sánchez et al., 2008) and twenty-three females from the 50<sup>th</sup> generation of a standard maternal line selected for litter size (line A; Ragab and Baselga, 2011). Line LP was founded with females with a minimum of 25 parities and having an average prolificacy per parity close to the average of the Spanish commercial population in 2002 (9 kits per parity).

### Blood sample collection

Blood samples were taken at hour zero from the central ear artery from each doe at eighteen weeks of age, and they were collected into two tubes, one contains tripotassium ethylenediaminetetraacetic acid (K3-EDTA) and the other contains Lítio-Heparin. The tube with Lítio-Heparin was centrifuged at 4000 rpm for 15 min for the biochemical analysis (bilirubin, cholesterol and triglycerides). Each K3-EDTA sample was divided into two aliquots, one aliquot was used to haematological analyses, and the other one was centrifuged at 4000 rpm for 15 min for the determination of the concentration of C-reactive protein (CRP). After blood collection, females were vaccinated subcutaneously against rabbit haemorrhagic disease (ERAVAC, Hipra Laboratory, Girona, Spain) in order to stimulate the immune system. After 72 hours of vaccination, two blood samples were taken to quantify the same parameters. The plasma obtained by centrifugation was stored at -80° C for posterior analysis.

### Haemogram

White blood leukocyte count (WBC), and the percentage of lymphocytes, granulocytes and monocytes were measured in 21 females of the A line and in 23 females of the LP line, before and after the vaccination. These parameters were assessed by ADVIA 120 Hematology Analyzer (Siemens Healthcare Diagnostics, Erlangen, Germany).

### Assessment of C-reactive protein and biochemical parameters

Twenty females of each line were used, before and after the vaccination to perform these analyses. C-reactive protein concentration was quantified in 20 females of each line, using a commercially available ELISA kit for rabbits (catalogue number 2210-5; Life Diagnostics Inc., West Chester, PA, USA). Plasma concentrations of bilirubin, cholesterol and triglycerides were analysed using a standardized and automatic method Spin model 200E using reagents from Spinreact (Girona, Spain).

### Statistical Analysis

A bayesian analysis was made using the following model:

$$y_{ijk} = \mu + p_i + LH_j + L_k + b X_{ik} + e_{ijk}$$

where  $p_i$  is the dam permanent effect,  $LH_j$  is the effect of Line-Hour (four levels: the A line before vaccination, the LP line before vaccination, the A line at 72 hours after vaccination and the LP line at 72 hours after vaccination),  $L_k$  is the effect of batch (three levels),  $b$  is the regression coefficient,  $X_{ik}$  is the covariate weight of female at hour zero and  $e_{ijk}$  is the residual term. The dam permanent effect was normally distributed with mean  $\mathbf{0}$  and variance  $\mathbf{I}\sigma_p^2$ . Residuals were normally distributed with mean  $\mathbf{0}$  and variance  $\mathbf{I}\sigma_e^2$ . Residuals and dam permanent effects are uncorrelated. Bounded flat priors were used for the rest of the effects and the variances. Marginal posterior distributions of the differences between lines were estimated for all unknowns using Gibbs sampling with the program Rabbit developed by the Institute for Animal Science and Technology (Valencia, Spain). The following parameters were obtained: the median of the difference (D), the highest posterior density region at 95% (HPD95%) and the probability of the difference being higher than zero when  $D > 0$  or lower than zero when  $D < 0$  (P).

## RESULTS AND DISCUSSION

Features of the marginal posterior distributions of the differences between the A and the LP lines for immunological parameters, before and after stimulation by vaccination, are given in Table 1. Before and after vaccination, there is not enough evidence of having differences between lines (A and LP) in WBC ( $P=0.80$ ). The A line showed a lower percentage of lymphocytes (-7.13%,  $P=0.99$ ), a lower percentage of monocytes (-2.32%,  $P=0.98$ ) and a higher percentage of granulocytes (+9.26%,  $P=1.00$ ) than the LP line. These differences were maintained after vaccination (-5.38 %,  $P=0.96$  for percentages of lymphocytes; -3.93 %,  $P=1.00$  for percentages of monocytes and +9.70 %,  $P=1.00$  for percentage of granulocytes).

Gunia et al. (2019) showed lower percentages of lymphocytes in susceptible rabbits than control rabbits after inoculation with *Pasteurella multocida*. It is known that lymphocytes are involved in the adaptive immune system. Thus, these results suggests a higher disease sensitivity of the A line. Higher basal CRP concentration was observed in the line A (+41.40  $\mu\text{g/ml}$ ,  $P=1.00$ ).

After vaccination, the A line still shows higher CRP concentration (+23.81  $\mu\text{g/ml}$ ,  $P=0.97$ ) but it should be noticed that the LP line shows a higher response to stimulation by vaccination than the A line (+14.6  $\mu\text{g/ml}$ ,  $P=0.93$ ). Low basal CRP concentration is related to a lower sensitivity to disease (Markanday, 2015) and a higher increase in response to stimulation by vaccination is related to good functionality of the inflammatory system (McDade et al., 2005). Similar results were obtained by Argente et al (2019) in two lines divergently selected by environmental sensitivity. Thus, it could be expected higher resistance to disease in the LP line than in the A line.

**Table1:** Features of the marginal posterior distribution of the differences between the A line and the LP line for immune parameters.

		A	LP	$D_{A-LP}$	HPD <sub>95%</sub>	P
WBC ( $\times 10^3/\mu\text{l}$ )	Before vaccine	11.1	12.1	-1.0	-3.4, 1.3	0.80
	After vaccine	11.7	12.8	-1.1	-3.6, 1.4	0.81
Lymphocytes (%)	Before vaccine	35.7	42.8	-7.1	-13, -1	0.99
	After vaccine	36.3	41.7	-5.4	-11.7, 0.5	0.96
Monocytes (%)	Before vaccine	15.9	18.2	-2.3	-4.5, -0.2	0.98
	After vaccine	16.0	20	-4	-6.2, -1.8	1.00
Granulocytes (%)	Before vaccine	48.2	38.9	9.3	3.2, 14.8	1.00
	After vaccine	47.7	38.0	9.70	4.1, 15.6	1.00
CRP ( $\mu\text{g/ml}$ )	Before vaccine	66.4	25.0	41.4	16.5, 65.8	1.00
	After vaccine	63.3	39.6	23.7	0.3, 48.9	0.97

A= median of the line A; LP= median of the line LP;  $D_{A-LP}$ = differences between line A and line LP; HPD<sub>95%</sub> = highest posterior density region at 95%; P = probability of the difference being >0 when  $D_{A-LP}>0$  or being <0 when  $D_{A-LP}<0$ ; WBC = white blood cells; CRP= C-reactive protein.

Table 2 displays the medians of the differences between the A and the LP lines for biochemical parameters before and after stimulation by vaccination. There is not enough evidence of having differences between A and LP lines in bilirubin before vaccination ( $P=0.83$ ), but line A showed lower bilirubin than the LP line after vaccination (-0.14  $\mu\text{mol/l}$ ,  $P=0.93$ ). This weak response to stimulation in the A line could be explained by a high susceptibility to diseases of this line (Argente et al., 2019).

Both lines showed similar concentrations of triglycerides and cholesterol before and after vaccination, but the response to vaccination was higher in the LP line than in the A line for triglycerides (+0.05  $\mu\text{mol/l}$ ,  $P=0.90$ ., data not shown in table 2), which is related to a good functionality of the liver metabolism in fighting infections and decreasing inflammations (Barcia and Harris, 2005).

**Table2:** Features of the marginal posterior distribution of the differences between the A line and the LP line for biochemical parameters related to liver functionality.

		<b>A</b>	<b>LP</b>	<b>D<sub>A-LP</sub></b>	<b>HPD<sub>95%</sub></b>	<b>P</b>
Bilirubin (µmol/l)	Before vaccine	0.79	0.69	0.10	-0.10, 0.28	0.83
	After vaccine	0.48	0.62	-0.14	-0.32, 0.05	0.93
Triglycerides (mmol/l)	Before vaccine	0.61	0.56	0.05	-0.05, 0.17	0.83
	After vaccine	0.6	0.61	-0.01	-0.13, 0.10	0.54
Cholesterol (mmol/l)	Before vaccine	1.36	1.32	0.04	-0.19, 0.26	0.62
	After vaccine	1.36	1.38	-0.02	-0.26, 0.21	0.56

A= median of the line A; LP= median of the line LP; D<sub>A-LP</sub>= differences between line A and line LP; HPD<sub>95%</sub> = highest posterior density region at 95%; P = probability of the difference being >0 when D<sub>A-LP</sub>>0 or being <0 when D<sub>A-LP</sub><0.

## CONCLUSION

Rabbit females from line LP, founded using a longevity criterion, showed better disease biomarkers results than a standard line A, which could be related to a higher resistance to diseases of LP line.

## ACKNOWLEDGEMENTS

This study is supported by the Spanish Ministry of Economy and Competitiveness (MINECO) with the Project AGL2017- 86083. The authors thank Victor Múgica and Paula Bono for their collaboration.

## REFERENCES

- Argente MJ., García ML., Zbyňovská K., Petruska P., Capcaravá M., Blasco A., 2019. Correlated response to selection for litter size environmental variability in rabbits's resilience. *Animal.*, 2348-2355.
- Barcia AM., Harris HW., 2005. Triglyceride-rich lipoproteins as agents of innate immunity. *Clinical Infectious Diseases*41, S498-S503.
- Berghof TVL., Poppe M., Mulder HA., 2019. Opportunities to Improve Resilience in Animal Breeding Programs. *Front. Genet.* 9, 1-7.
- Ferrián S., Blas E., Larsen T., Sánchez JP., Friggens NC., Corpa JM., Baselga M., Pascual JJ., 2013. Comparison of immune response to lipopolysaccharide of rabbit does selected for litter size at weaning or founded for reproductive longevity. *Research in Veterinary Science*, 94, 518-525.
- García-Quirós A., Arnau-Bonachera A., Penadés M., Cervera C., Martínez-Paredes E., Ródenas L., Selva L., Viana D., Corpa J.M., Pascual J.J., 2014. A robust rabbit line increases leukocytes counts at weaning and reduces mortality by digestive disorder during fattening. *Veterinary Immunology and Immunopathology*. 161:123-131.
- Gunia M., Lantier F., Balmisse E., Guitton E., Helloin E., Le Cren D., Lenoir G., Maupin M., Robert R., Riou M., Garreau H., 2019. Projet RELAPA (génomique pour la REsistance génétique des LAPins à la PAsteurellose): statut hématologique de lapins résistants et sensible. *18èmes Journées de la Recherche Cunicole*, 27-28 mai 2019, Nantes, France, 73-76.
- McDade TW., Borja JB., Kuzawa CW., Perez TL , Adair LS., 2005. C-reactive protein response to influenza vaccination as model of mild inflammatory stimulation in the Philippines. *Vaccine* 33, 2004-2008.
- Markanday A 2015. Acute phase reactants in infections: evidence-based review and a guide for clinicians. *Open Forum Infectious Diseases* 2, 1-7.
- Ragab, M., Baselga M. , 2011. A comparison of reproductive traits of four maternal lines of rabbits selected for litter size at weaning and founded on different criteria. *Livest. Sci.* 136 : 201-206.
- Sánchez JP., Theilgaard P., Mínguez C., Baselga M., 2008. Constitution and evaluation of a long-lived productive rabbit line. *J.Anim. Sci.* 86,515-525.

# STUDY OF BIOMARKERS OF DISEASE SENSITIVITY IN A ROBUST AND A STANDARD MATERNAL LINE

Belloumi<sup>1,2</sup>, D., Argente<sup>3</sup>, M.J., García<sup>3</sup>, M.L., Blasco<sup>1</sup>, A. y Santacreu<sup>1\*</sup>, M.A

<sup>1</sup>Institute for Animal Science and Technology, UPV, P.O. Box 22012. 46022 Valencia, Spain,

<sup>2</sup>CITA-IVIA, 12400 Segorbe, Spain,

<sup>3</sup>Departamento de Tecnología Agroalimentaria. UMH de Elche, Ctra de Beniel Km 3.2, 03312 Orihuela. Soain



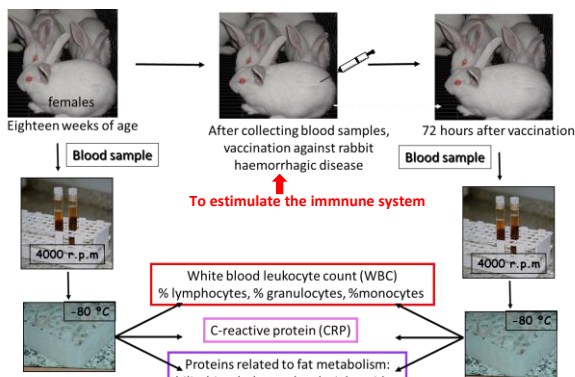
\* photo of attending author

**Context and objectives:** Intensive rabbit producers are interested in having does with high productivity and resilience to external stressors. The objective of this paper is to study biomarkers for resilience in two commercial maternal lines after stimulation by vaccination against rabbit haemorrhagic disease (RHD).

## Animals:

- 21 females from a robust line founded using a longevity criterion (line LP)
- 23 females from a standard line (line A)

## Methods :



## Results :

	Before vaccine		After vaccine	
	D <sub>A-LP</sub>	P	D <sub>A-LP</sub>	P
WBC ( $\times 10^9/\mu\text{l}$ )	-	0.80	-	0.81
Lymphocytes (%)	-	0.99	-	0.96
Monocytes (%)	-	0.98	-	1.00
Granulocytes (%)	+	1.00	+	1.00
CRP ( $\mu\text{g/ml}$ )	+	1.00	+	0.97
Bilirubin ( $\mu\text{mol/l}$ )	+	0.83	-	0.93
Triglycerides (mmol/l)	+	0.83	-	0.54
Cholesterol (mmol/l)	+	0.62	-	0.56

Patron showed for **haematological parameters** suggests a lower disease sensitivity of the **line L**.

Before vaccine D<sub>A-LP</sub>=+23.81  $\mu\text{g/ml}$  (P=0.97)  
 Higher response in L after vaccine (+14.6  $\mu\text{g/ml}$ , P=0.93)  
 These results for **CRP** suggest a lower sensitivity to disease and a good functionality of the inflammatory system, respectively in **line L**.

**Line L** showed higher **bilirubin** concentration after vaccination (D<sub>ALP</sub>=0.14  $\mu\text{mol/l}$ , P=0.93).

D<sub>A-LP</sub>= differences between line A and line LP (Green color when difference is positive, Red color when difference is negative); P = probability of the difference being >0 when D<sub>A-LP</sub>>0 or being <0 when D<sub>A-LP</sub><0; WBC = white blood cells; CRP= C-reactive protein.

**Take home message :** Rabbit females from line LP, founded using a longevity criterion, showed better resilience biomarkers results than the standard line A which could be related to a higher resistance to stress and diseases of LP line.