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GENETIC FACTORS OF FUNCTIONAL TRAITS

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ABSTRACT

Selection of functional traits is a challenge for researchers, but an increasingly necessary objective due to the growing concern regarding animal welfare and overcoming the problems of reducing antibiotic use in rabbit production without undermining the animals’ productivity. The aim of this review is to discuss the genetic control of resistance to diseases, longevity and variability of birth weight within a litter, or litter size variability at birth within doe, and describing the selection programmes and the first results from a multi-omics analysis of resistance/susceptibility to diseases. The heritability is around 0.13 for longevity, 0.01 for uniformity in birth weight, 0.09 for litter size variability and around 0.11 for disease resistance. Genetic correlations between functional traits and production traits are mostly no different from zero, or are moderately favourable in some cases. Six selection programmes developed in three countries are reviewed. Line foundation with high pressure for selection or divergent selection experiments are different methodologies used, and favourable responses to selection have been obtained. Genomics studies have revealed associations in regions related to immune system functionality and stress in lines selected for litter size variability. Knowledge of gut microbiota role on the rabbit’s immune response is very limited. A multi-omics approach can help to know microbial mechanisms on regulation immunity genes of the host.

Keywords: Genetic, longevity, omics, resilience, resistance to diseases, selection.

INTRODUCTION

Breeding programmes have played an important role in improving efficiency in meat rabbit production. Traditionally, maternal lines are selected for litter size at birth or at weaning (Baselga, 2004) and paternal lines are selected for post-weaning growth rate or body weight at a point close to market age (Rochambeau et al., 1989; Lukefahr et al., 1996; Piles and Blasco, 2003; Larzul et al., 2005). Other traits have been studied as criteria in breeding programmes, either in maternal lines, such as ovulation rate and kit survival (Piles et al. 2006; Ziadi et al., 2013), or in paternal, such as carcass dressing percentage, thigh muscle volume, intramuscular fat, food efficiency and heat tolerance (Zomeño et al., 2013; Matics et al., 2014; Piles et al., 2014, Piles and Sánchez, 2019). Nowadays, priorities in rabbit breeding are related to improving animal welfare and disease resistance, which leads to better adaptation of females to changing environmental conditions.

Functional traits are used to summarized those characters of an animal that increase efficiency by reduced costs of input. Major groups of breeding goal traits belonging to this category are health, fertility or longevity (Groen et al., 1997). Functional traits determine the response to environmental factors (Reiss et al., 2009). Therefore, robustness, rusticity, resilience, plasticity and resistance to diseases are concepts related to them.
The notion of robustness refers to the combination of a high production potential and a low sensitivity to environmental perturbations. The importance of robustness-related traits in breeding objectives is progressively increasing towards the production of animals with a high production level in a wide range of climatic conditions and production systems (Knap, 2005), together with a high level of animal welfare (Mormede and Terenina, 2012). When an animal has the ability to adapt to an unfavourable environment, but without the requirement of maintaining a high production level, rusticity is defined (Sauvant and Martin, 2010). Colditz and Hine (2016) defined resilience in animal production as the animal’s capacity to be minimally affected by disturbances or to rapidly return to the state it was in before exposure to a disturbance.

Both robustness and resilience refer to the ability of an animal to survive disruptions. However, robustness is considered a static concept where the animal can resist disruptions and retain its previous stable situation, whereas resilience is more of a dynamic concept incorporating adaptation, where an animal can return to a new stable situation after surviving a threat. Therefore, resilience is also related to plasticity.

Genetic selection of functional traits has been used to increase robustness in pigs (Knap, 2005), poultry (Star et al., 2008) and rabbits (Sánchez et al., 2008; Garreau et al., 2017). Recently, environmental variance has been proposed as a measure of resilience (Berghof et al., 2019). Many studies have provided statistical evidence that environmental variance is partly under genetic control (mice, Ibáñez-Escriche et al., 2008a; pigs, Ibáñez-Escriche et al., 2008b; chickens, Mulder et al., 2009) and selection experiments support these findings in rabbits (for birth weight variability, Bolet et al., 2007; for litter size variability, Blasco et al., 2017) and mouse (for birth weight variability, Formoso-Rafferty et al., 2016).

The main objective of this review is to discuss the genetic control of longevity, diseases resistance traits and variability of birth weight and litter size, presenting the selection programmes with inclusion of these traits and describing the first results from a multi-omics analysis of resistance/susceptibility to diseases.

GENETIC CONTROL OF FUNCTIONAL TRAITS

Genetic variability is the prerequisite for any breeding programme. In rabbits, the first studies to determine if disease resistance were heritable started in 1969 in Australia, then in 1988 in Europe (Sobey, 1969; Baselga et al., 1988). Analyses of longevity began in the 2000s, while the study of the genetic parameters of homogeneity traits was initiated in 2008. Longevity and homogeneity of birth weight or litter size were studied in maternal rabbit lines, while disease resistance traits were studied in both paternal and maternal lines.

The heritability of longevity is around 0.13, varying from 0.02 to 0.24 (Table 1). Heritability of the homogeneity traits is low, 0.01 for the uniformity in birth weight within a litter and 0.08 for the litter size variability at birth within a doe (Table 3). Heritability of the resistance traits varies from 0.02 to 0.64 depending on the disease, its prevalence and the model used (Table 4). On average, the heritability of disease resistance is around 0.11 on the observed scale and 0.15 on the underlying scale. In summary, the heritability of these traits tends to be low to moderate.
### Table 1: Heritability for longevity

| Trait definition | Heritability | Model   | Country  | Line/breed          | Authors                  |
|------------------|--------------|---------|----------|---------------------|--------------------------|
| Length of lifetime | 0.13         | Linear model | Germany | New Zealand white   | Youssef et al, 2000      |
| Number of AI | 0.10         | Weibull   | France   | INRA 1077           | Garreau et al, 2001      |
| Number of AI | 0.05         | Discrete model | France  | INRA 1077           | Garreau et al, 2001      |
| Length of lifetime | 0.05         | Cox model | Spain    | V line              | Sánchez et al, 2004      |
| Length of lifetime | 0.10         | Cox model | Spain    | V line              | Sánchez et al, 2006      |
| Length of lifetime | 0.16 to 0.24 | Cox model | Spain    | Prat                | Piles et al, 2006        |
| Number of AI | 0.17 to 0.19 | Discrete model | France  | INRA 1077           | Piles et al, 2006        |
| Number of AI | 0.12         | Discrete model | France  | Hycole line D       | Lenoir et al, 2013       |
| Functional longevity | 0.07         | Cox model | Spain    | A line              | El Nagar et al, 2020     |
| Functional longevity | 0.03         | Cox model | Spain    | V line              | El Nagar et al, 2020     |
| Functional longevity | 0.14         | Cox model | Spain    | H line              | El Nagar et al, 2020     |
| Functional longevity | 0.05         | Cox model | Spain    | LP line             | El Nagar et al, 2020     |
| Functional longevity | 0.02         | Cox model | Spain    | R line              | El Nagar et al, 2020     |

Longevity is defined as: the length of lifetime production in months, the total number of artificial inseminations (AI) performed after the first kindling or death, date of the first presentation to a male and the data of death or culling, the time in days between date of the first positive pregnancy diagnosis and date of culling, or difference between the date of the first positive palpation test and the date of death or culling due to involuntary cause.

### Table 2: Pseudo-genetic correlations (standard error) between longevity and production traits

| Trait                        | Pseudo-Genetic correlation | Authors                  |
|------------------------------|----------------------------|--------------------------|
| Number of kits born alive    | 0.16 (0.10)                | Sánchez et al, 2006     |
| Number of kits born alive    | -0.72 (0.11)               | Lenoir et al, 2013      |
| Number of kits at weaning    | -0.17 (0.11)               | Sánchez et al, 2006     |
| Litter weight at weaning     | -0.7 (0.11)                | Lenoir et al, 2013      |
| Teat number                  | -0.39 (0.11)               | Lenoir et al, 2013      |
| Adult weight                 | -0.2 (0.11)                | Lenoir et al, 2013      |

Longevity is defined as: the time in days between date of the first positive pregnancy diagnosis and date or culling, as the number of inseminations completed before culling.

### Table 3: Heritability (diagonal) and genetic correlation (above) of variability and mean of birth weight and litter size

| Trait           | Variability | Mean         | Country | Line   | Authors                  |
|-----------------|-------------|--------------|---------|--------|--------------------------|
| Birth weight    | 0.012 (0.004) | 0.085 (0.066) | France  | AGP22  | Garreau et al, 2008a, Bodin et al., 2010b |
|                 |             | 0.060 (0.011) |         |        |                          |
| Litter size     | 0.08 (0.05; 0.11) | -0.06 (-0.31; 0.21) | Spain  | Maternal | Blasco et al., 2017     |
|                 |             | 0.10 (0.08; 0.13) |         |        |                          |

1 Within-litter standard deviation, 2 Environmental variability, 3 Standard error, 4 High density posterior interval at 95%

The genetic correlations between the mean and the variability for birth weight and litter size were no different from zero (Table 3). Genetic correlations between resistance to diseases and production traits are either favourable or not different from zero (Table 5). There is evidence that genetic correlation between resistance to different illnesses and production traits decreases over time, so the estimates are higher for daily gain before weaning (Shrestha et al., 2019) than for direct weaning weight (Gunia et al., 2018) and daily gain during the fattening period (Ragab et al., 2015). Finally, genetic correlation is no different from zero for weight at the end of the fattening period (Gunia et al., 2015). The resistance to digestive disorders is favourably correlated with the carcass yield and no different from zero for litter size (Gunia et al., 2015; 2018).

To summarise, the heritabilities tend to be low to moderate. The genetic correlations between functional traits and production traits are mostly not significantly different from zero, or are favourable in some cases. The possible independence of functional and production traits means that functional traits can be included in a breeding programme without trade-offs.
Table 4: Heritability (standard errors) for disease resistance traits

| Disease or syndromes                          | Trait description                          | Variable type | Heritability | Country          | Line/breed | Type of line                  | Authors                  |
|-----------------------------------------------|--------------------------------------------|---------------|--------------|-----------------|------------|--------------------------------|--------------------------|
| Myxomatosis after experimental infection      | Survival time (days)                       | continuous    | 0.33 to 0.64 | Australia       | domestic   rabbits        | -                        | Sobey, 1969              |
|                                               | Survival to myxomatosis                    | 0-1           | 0.36         |                 |            |                                |                          |
| Respiratory infection caused by *Pasteurella multocida* and *Bordetella bronchiseptica* | Extension of lesions on lung lobes         | 0-5           | 0.07 (0.03) to 0.18 (0.09) | Spain        | A.V, R.B  | maternal and paternal          | Baselga *et al.*, 1988   |
|                                               | Average score of lung lobe lesions         | 0-5           | 0.12 (0.05) to 0.28 (0.14) |             |            |                                |                          |
| Bacterial infection caused by *Pasteurella multocida* or *Staphylococcus aureus* | Incidence of infection                     | 0-1           | 0.03 (0.01) to 0.04 (0.01) | France       | 2 commercial populations | paternal                 | Eady *et al.*, 2004       |
|                                               | Weekly incidence of infection              | 0-1           | 0.02 (0.02) to 0.06 (0.02) | Australia    | composite strain     | -                        | Eady *et al.*, 2007       |
|                                               | Overall incidence of infection             | 0-1           | 0.06 (0.02) |                 |            |                                |                          |
|                                               | Overall Mortality from infection           | 0-1           | 0.02 (0.05) |                 |            |                                |                          |
| Pasturellosis after experimental infection    | Extent of abscess dissemination           | 0-5           | 0.11 (0.06) | France         | INRA 1777   and 6 commercial populations | maternal                 | Shrestha *et al.*, 2018   |
|                                               | Extent of bacteria dissemination           | 0-5           | 0.09 (0.05) |                 |            |                                |                          |
|                                               | Resistance: combination of survival, abscess and bacteria scores | 0-5           | 0.14 (0.05) |                 |            |                                |                          |
| ERE2 after experimental infection             | Mortality                                  | 0-1           | 0.05 (0.05) | France         | INRA 1777   | maternal                      | Garreau *et al.*, 2006   |
|                                               | Resilience (alive and normal growth)       | 0-1           | 0.38 (0.21) |                 |            |                                |                          |
|                                               | Diarrhoea                                  | 0-1           | 0.21 (0.16) |                 |            |                                |                          |
|                                               | Abnormal growth                            | 0-1           | 0.08 (0.07) |                 |            |                                |                          |
| Non-specific syndromes                       | Non-specific mortality                     | 0-1           | 0.07 (0.02) to 0.10 (0.02) | Spain        | Caldes     | paternal                      | Ragab *et al.*, 2015      |
|                                               | Morbidity and mortality from ERE2          | 0-1           | 0.05 (0.02) to 0.06 (0.02) |             |            |                                |                          |
|                                               | Respiratory syndromes                      | 0-1           | 0.03 (0.01) |                 |            |                                |                          |
|                                               | Poor body condition score                  | 0-1           | 0.03 (0.02) to 0.06 (0.02) |             |            |                                |                          |
|                                               | Digestive disorders                        | 0-1           | 0.03 (0.00) to 0.11 (0.03) | France       | AGP39,      | paternal and maternal          | Garreau *et al.*, 2008b, Gunia *et al.*, 2015, 2018 |
|                                               | Respiratory disorders                      | 0-1           | 0.04 (0.00) to 0.09 (0.02) |             | AGP59,      |                                |                          |
|                                               | Infectious disease                         | 0-1           | 0.03 (0.00) to 0.08 (0.02) |             | AGP77       |                                |                          |

1Traits are recorded under natural infection; unless otherwise stated: 2Epizootic Rabbit Enteropathy; 3Linear model (or results of threshold models expressed on the observed scales)
## Table 5: Genetic correlations (standard errors) between disease resistance traits and production traits

| Health trait                                                      | ADG\(^1\) before weaning | Weaning weight | ADG during the fattening period | Live weight at the end of the fattening period | Carcass Yield | Number of kits born alive | Authors |
|------------------------------------------------------------------|---------------------------|----------------|---------------------------------|-----------------------------------------------|---------------|----------------------------|---------|
| Incidence of bacterial infection                                 |                           |                |                                 |                                               |               |                            | Eady et al. 2004 |
| Extent of abscess dissemination (pasteurellosis)                 | -0.95 (0.46)              |                |                                 |                                               |               |                            | Shrestha et al., 2019 |
| Extent of bacteria dissemination (pasteurellosis)                | -0.62 (0.43)              |                |                                 |                                               |               |                            | Shrestha et al., 2019 |
| Resistance to pasteurellosis score                               | 0.79 (0.36)               |                |                                 |                                               |               |                            | Shrestha et al., 2019 |
| Non-specific mortality                                           |                           |                |                                 |                                               |               |                            | Ragab et al., 2015 |
| Morbidity and mortality from ERE\(^2\)                          |                           |                |                                 |                                               |               |                            | Ragab et al., 2015 |
| Respiratory syndromes                                           |                           |                |                                 |                                               |               |                            | Ragab et al., 2015 |
| Poor body condition score                                        |                           |                |                                 |                                               |               |                            | Ragab et al., 2015 |
| Digestive disorders                                              |                           |                |                                 |                                               |               |                            | Gunia et al., 2015 |
| Respiratory disorders                                            |                           |                |                                 |                                               |               |                            | Gunia et al., 2015 |
| Infectious disease                                               | -0.34 (0.12) to 0.05 (0.14) | -0.06 (0.20) to -0.04 (0.22) | 0.11 (0.07) to -0.11 (0.06) to -0.40 (0.07) | 0.01 (0.06) to -0.06 (0.06) to -0.10 (0.08) | 0.06 (0.07) to -0.25 (0.06) to -0.35 (0.08) to -0.08 (0.14) to -0.06 (0.16) | Gunia et al., 2015, 2018 |

\(^1\)Average Daily Gain. \(^2\)Epizootic Rabbit Enteropathy
SELECTION PROGRAMMES AND RESPONSE TO SELECTION

The first approach to selection for disease resistance was a programme of mass selection for resistance to myxomatosis, conducted from 1955 to 1967 in Australia (Sobey, 1969). Four virus strains were used and rabbits were infected with the appropriate virus after 16 weeks of age, to obviate the effects of maternal antibodies. Percentage of recovery increased from 50 to 80% for the least virulent strain and from 10 to 20% for the most virulent virus strain.

A maternal line (LP line) was constituted following a longevity criterion at the Polytechnic University of Valencia (Spain, Sánchez et al., 2008). Then, the selection has been carried out by litter size at weaning. The foundation process of the LP line was inspired by the hyperprolific selection experiments proposed and carried out by the same research group (Cifre et al., 1998). Thus, the LP line was founded by selecting females from commercial farms that showed extremely high productive lives (between 25 and 41 parities) and whose prolificacy ranged from 7.5 to 11.9 young born alive (Sánchez et al., 2008). When the LP line was compared to the V line selected for litter size at weaning for 31 non-overlapping generations, the LP line was 1.3 times less likely to leave the herd than the V line, demonstrating the longer productive life of the LP line (Sánchez et al., 2008) and similar productivity from the fourth parity onwards in both lines (Theilgaard et al., 2007).

The ability of the LP line to sustain reproduction in the different environments without presenting great mobilisation of body reserves and its ability to use reserves at the onset of feed constraints seems to be a safeguarding factor to ensure longevity (Theilgaard et al., 2009; Savietto et al., 2013, 2015). Moreover, the LP line presented higher lymphocyte counts under heat stress conditions than the V line (Ferrian et al., 2012), and before and after haemorrhagic virus vaccination than the maternal A line (Belloumi et al., 2020). This haematological profile contributes to a greater ability to confront infectious challenges and to confer animals a more robust nature (Ferrian et al., 2013).

In France, INRA has developed three divergent selection experiments for longevity, resistance to digestive disorders and weight at birth within litter size. In the first experiment, the objective was to assess the feasibility of selecting for functional longevity, defined as an ability to delay involuntary culling. Functional longevity was measured as the total number of artificial inseminations performed after the first kindling (Larzul et al., 2014). After one generation of selection, the lines differed by 0.75 inseminations over an observation period of 8 artificial inseminations. The number of litters per female was higher in the High longevity line than the Low longevity line. Because of this difference between the lines, the total numbers born alive and weaned per female were higher in the High line. Nevertheless, reproductive performance was similar between lines at the 2nd generation (Garreau et al., 2017). In this experiment, the High longevity line accreted more body reserves at the onset of reproductive life than the Low line and thereafter maintained higher body reserves until third delivery.

The second divergent selection experiment is focused on improving the resistance to enteropathies and digestive disorders. A binary score based on the observed signs of enteropathy during the growing period was the selection criterion. The resistance animals showed similar mortality and growth rate to those of sensitivity animals, but cumulative mortality was lower in resistant than sensitivity animals, when animals were inoculated with an enteropathogenic E. Coli 0103 strain (Garreau et al., 2012).
When the selection criterion of the lines is to increase or reduce the variability around an optimum, a canalising selection is applied. This is the criterion used for within-litter standard deviation of birth weight (Garreau et al., 2008a; Bodin et al., 2010a). The model assumes that environmental variability of residual variance is also partially controlled by genes. This heteroscedastic model was developed by San Cristobal-Gaudy et al. (1998). The within-litter birth weight standard deviations were 7.34 g in the Homogenous line and 11.26 g in the Heterogeneous line after 10 generations of selection (Bodin et al., 2010b). Moreover, the Homogeneous line showed higher litter size at weaning and lower mortality at birth and at weaning than Heterogeneous line. There was no correlated response for the individual weight at birth or the standard deviation and individual weight at weaning (Garreau et al., 2008a). A higher homogeneity in weight birth within litter was related to higher length and capacity of the uterine horn, thus the divergence between the lines could be at least partly due to their characteristics of the reproductive tract (Bolet et al., 2007).

A divergent selection experiment for environmental sensitivity is being carried out at the Miguel Hernández University in Elche (Spain). The selection was based on environmental variance of litter size at birth. This is the first experiment in which selection has been directly performed on environmental variance, treating it as an observed trait. Selection has been successful after 10 generations. The Heterogeneous line showed a greater variability of litter size (4.4 kits) than the Homogeneous line (2.7 kits, Blasco et al., 2017). The lines differed in the inflammatory response and the corticotropic response to stress, which were two important components of physiological adaptation to environmental challenges such as infections, suggesting that the Homogeneous line was more resilient (Argente et al., 2019; Beloumi et al., 2020). Moreover, a correlated response in the plasma fatty acids profile that modulated the immune cell function was observed (Agea et al., 2020b). Homogeneous line showed higher body reserves at delivery and lactation, so the line would be able to better deal with situations of high energy demand than Heterogeneous line (García et al., 2018; Agea et al., 2020a). These results agree with the lower mortality at delivery of the does, lower percentage of litter mortality at birth and at weaning, and higher homogeneity of litter weight at weaning found in the Homogeneous line (Argente et al., 2019; Agea et al., 2019). Therefore, decreasing litter size variability can favour the dam’s survival in the farm.

Furthermore, selection for homogeneity does not seem to reduce litter size, as the Homogenous line resulted in larger litter size than the Heterogeneous line in all generations (Blasco et al., 2017). Studies concerning litter size components have determined that the lines had a similar ovulation rate, but at 48 hours after mating, the embryos of the Homogeneous line were more developed than in the Heterogeneous line and at 72 hours also had greater survival (García et al., 2016; Calle et al., 2017). Thus, when a laparoscopy was performed at 12 days of gestation, the number of implanted embryos was higher in the Homogeneous line than in the Heterogeneous line (Argente et al., 2017).

In summary, selection programs of longevity, resistance to diseases and birth weight or litter size variability using complex models or simple observations seem to be feasible. Taking into account that improving the health of breeding rabbits is becoming a crucial issue due to the decreasing antibiotic use in farms, these programmes could have a great impact on the improvement of animal welfare and disease resistance (Gunia et al., 2018). However, the biological mechanisms underlying environmental sensitivity are not yet fully understood. Recently, gut microbiota has become a key regulator of immunity. So, studies using multi-omics approaches are needed to unravel the mechanisms in play.
Multi-omics approach to study resistance/susceptibility to diseases

Sensitivity to most diseases is caused by a complex combination of genomic, biological and environment factors (Mangino et al., 2017). Therefore, a multi-omics approach can help us gain in-depth knowledge about the host immune genes and the role of the microbiome in expression of the host genes to resistance/susceptibility to diseases. This knowledge could be applied in breeding programmes, contributing to the improvement of disease resistance in commercial rabbit lines.

Genomics

Over the past few years, transcriptomics technologies have helped us to characterize a large number of functional genes involving in the innate immune response. Transcriptome studies in rabbits after exposition to virus and bacteria have showed the up-regulation of the major histocompatibility complex (MHC or RLA) class II genes (e.g., HLA-DMA, HLA-DQB2, HLA-DRA, RLA-DMB, RLA-DRB1, SLA-DQ1), and those encoding cytokines/chemokines/chemokine receptors (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-17, IL-18, IL-36, IL-37, TGFα, TRAF3, IFNα, IFN-γ, IFIT5, CCL4, CCL20, CXCL10, CXCL11 and CXCR3), toll-like receptors/interferon regulation factors (e.g., TLR3, TLR4, TLR6, TLR10, IRF7 and IRF9), immunoglobulins (e.g., 15 subclasses for IgA), T-cell activation (e.g., CD2, CD4, CD27, CD28, CD74, CD80, CD86, and CTLA4), and oxidative stress and apoptosis (e.g., COX-2 and iNOS) (Hou et al., 2016; Jacquier et al., 2015; Neave et al., 2018; Pinheiro et al., 2018; Schnup and Sansonetti, 2012, Schwensow et al., 2017; Subbian et al., 2013; Suen et al., 2016; Uddin et al., 2015). Recently a genome-wide association (GWAS) study has performed in the two lines selected divergently for environmental variance of litter size. Casto-Rebloo et al. (2021a) identified in this study 65 genes related to the immune response, 5 to the stress response, and 50 to energy, carbohydrate and lipid metabolism; among those highlight the genes of C3orf20, GRN, EPCAM, ENSOCUG00000017494, ENSOCUG00000023926, ENSOCUG00000026560, MYLK, HECA, and NMNAT3 because they are fixed in both lines. These findings agree with different sensitivity to infections and stress conditions between the homogeneous and the heterogeneous lines for environmental variance of litter size (Argente et al., 2019; Beloumi et al., 2020), corroborating the immune system’s decisive role in modulation of the animal’s resilience.

Microbiomics

The gut microbiota has a pivotal responsibility in susceptibility to diseases and to stress conditions in the host (review by Pickard et al., 2017; review by Kraimi et al., 2019). In this regard, studies with germ-free animals have provided clear evidence that gut microbiota composition plays an essential role in full intestinal blood vessel development, in promoting development of B and T cells in Peyer’s Patches of gut-associated lymphoid tissue (GALT), and in driving production of mucosal Ig (review by Martin et al., 2010). Breakthroughs in high-throughput sequencing technology in recent years have made it possible to investigate the rabbit microbiota composition throughout the digestive tract (Arrazuria et al., 2016; Arrazuria et al., 2018; Bäuerl et al., 2014; Beaumont et al., 2020; Combes et al., 2017; Cotozzolo et al., 2021; Crowley et al., 2017; Jin et al., 2018; Mattioli et al., 2019; Massip et al., 2012; Paës et al., 2020; North et al., 2019; Read et al., 2019; Velasco-Galilea et al., 2018; Zhu et al., 2015). In accordance with the different functions in each section of the digestive tract, microbial community composition is different. For example, the stomach and small intestine show a similar composition, Firmicutes being the most abundant phylum (~48%), followed by Bacteroidetes (~18%), Proteobacteria (~22%), and Actinobacteria (~5%). Meanwhile, in the distal segment of the digestive tract (sacculus rotundus, caecum and vermiform appendix), Firmicutes doubles its presence (~76%), whereas Bacteroidetes (~12%), Proteobacteria (~2%) and Actinobacteria (~1%) are reduced (see Table 6). Bibliography has reported that factors such
as age, sex, food composition and texture, feed intake levels and drinking water temperature can remodel the composition of the gut microbiota (Beaumont et al., 2020; Combes et al., 2013 and 2017; Cotozzolo et al., 2021; Jin et al., 2018; Paës et al., 2020; Mattioli et al., 2019; Read et al., 2019; North et al., 2019; Wang et al., 2019a; Wu et al., 2018; Zhu et al., 2015).

The gut microbiota synthesises and releases a large number of metabolites to gut lumen and epithelial surface, such as short-chain fatty acids (SCFAs), mainly acetate, propionate and butyrate, folic acid, indole and indole derivatives, polyamines, histamine, retinoic acid, secondary bile acids, taurine and tryptophan metabolites, which stimulate development of the host immune system (review by Wang et al., 2019b in humans; Table 7). Few studies in rabbits have examined the relationship between the microbiota composition and the production of SCFAs (review by Combes et al., 2013; Jin et al., 2018; Wang et al., 2019a; Wu et al., 2018), and between the microbiota composition and the immune gene expression in intestinal mucosa (Bäuerl et al., 2014; Wang et al., 2019a). These studies have identified a positive correlation between some members of the families Bifidobacterium, Ruminococcaceae and Coprococcus and concentration of butyric acid. Beaumont et al. (2020) noted that butyric acid might be involved in the gut barrier maturation through the up-regulation of genes associated with the intestinal absorption (ALPI, CA2 and MCT1), the transcytosis of the immunoglobulin A (PIGR), and the antibacterial activity (CCL20, GPX2 and NOS2). Regarding relationships between the gut microbiota and immune genes, Bäuerl et al. (2014) found in caecum a large association between the families Verrucomicrobiaceae, Enterobacteriaceae and Bacteroidaceae, with expression of genes coding for pro-inflammatory cytokines such as IL-8, IL-6 and TNF-α, but association was low with families Lachnospiraceae and Ruminococcaceae. Likewise, Wang et al. (2019a) reported negative correlations between several members of Ruminococcaceae and Coprococcus and expression of genes coding for pro-inflammatory cytokines such as TGF-β1 and IL-1β, and positive correlation between Ruminococcaceae and expression of gene coding for anti-inflammatory cytokines such as IL-10.

It is important to note that all studies in rabbit gut microbiota to date have been based on the use of 16S rRNA sequencing. However, this technique has several limitations that metagenomics tries to resolve, such as how to provide a higher taxonomic resolution at the level of species and strain, and to reveal the entire gene repertoire of the community. In order to figure out the effect of the microbiota on host immune gene expression and its susceptibility to diseases, a metagenomic study is being performed on the divergent selection experiment for litter size environmental variance by the UMH team. A preliminary analysis with PLS-DA has allowed to separate the two divergent lines according to the microbial genes (Belloumi et al., 2021b) and the gut microbiota metabolites (Casto-Rebollo et al., 2021b). From all relevant metabolites identified, the glycerophosphoglycerol, N6-acetyllysine, behenoylcarnitine, ethyl betaglucopyranoside and equol had the largest contribution to the classification between rabbit lines. These metabolites are involved in the xenobiotics, amino acids and lipids metabolisms. However, further studies are needed to understand the role of these metabolites and the bacteria that produce them. This study can help us better understand how the selection for litter size environmental variance can modify the gut microbiota and the mechanisms underlying the microbial role in regulation of host resilience.
Table 6. Average percentages of bacteria phyla in the stomach, small intestine, *sacculus rotundus* (SR) caecum and vermiform appendix content (VA).

|                | Stomach | Small Intestine | SR | Caecum | VA |
|----------------|---------|-----------------|----|--------|----|
|                | Jin *et al.*, 2018a | Cotozzo lo *et al.*, 2021b | Jin *et al.*, 2018c | Arrazuri *et al.*, 2018c | Massip *et al.*, 2012d | Baeuerl *et al.*, 2014e | Zhu *et al.*, 2015f | Arrazuri *et al.*, 2016g | Combes *et al.*, 2017b | Crowley *et al.*, 2017i | Velasco-Galilea *et al.*, 2018j | Jin *et al.*, 2018a | Mattioli *et al.*, 2019k | North *et al.*, 2019l | Read *et al.*, 2019m | Beaumont *et al.*, 2020n | Paes *et al.*, 2020o | Cotozzo lo *et al.*, 2021b | Arrazuri *et al.*, 2018c |
| Firmicutes     | 44.6    | 68              | 41.7 | 40.5   | 87.1 | 90 | 78.3 | 77.0 | 71.0 | 83 | 53 | 76.5 | 72.1 | 78.9 | 72.0 | 91 | 60 | 89 | 43 | 87.0 |
| Bacteroidetes  | 18.9    | 16              | 32.3 | 1.5    | 2.10 | 4.6 | 15.7 | 7.9  | 13.7 | 5.8 | 42 | 7.46 | 13.2 | 14.1 | 9.82 | 6  | 30 | 9  | 40 | 1.63 |
| Proteobacteria | 27.5    | 18.3            | 0.83 | 0.7    | 1.4  | 0.16 | 0.58 | 3.74 | 1.61 | 5.09 | 3.02 | 11.1 | 2   | 1   | 0.40 |
| Tenericutes    | 1.42    | 2.39            | <1   | 0.43   | 0.15 | 7.48 | 3.02 | 1.4  | 1.67 | 5   | 0.3 | 1.18 |
| Actinobacteria | 5.10    | 2               | 4.05 | 11     | 0.81 | 0.9 | 0.39 | 0.37 | 0.79 | 0.73 | 2.81 | 0.85 | 3.62 | 0.5 | 0   | 0.78 |
| Cyanobacteria  | 1       | 0.5             | 1.25 | 4.23   | 0.87 |      |      |      |      |      |      |      |      |      | 0   | 1.35 |
| Verrucomicrobia| 5       | 3               | 2.40 | 7.9    | 1.81 | 1.28 |      |      |      |      |      |      |      |      |      | 15  |
| Euryarchaeota  | 6       | 30.5            |      | 0.06   |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Saccharibacteria| 3.39  | 0.16            |      |        |      |      |      |      |      |      |      |      |      |      |      |      |      | 3.85 |
| Spirochaetae   | <1      |                 |      | 0.09   |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Patescibacteria| 3       | 13.5            |      |        |      |      |      |      |      |      |      |      |      |      |      |      | 1   |
| Epsilonbacteraeota| 1     |                 |      | 0.2    |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Unknown        | 3.10    | 9.89            | 3.43 |        |      |      |      |      |      |      |      |      |      |      |      |      |      |

a: Samples taken at 55 days. b: samples taken at 110 days. c: Samples taken at 39 weeks. d: Samples taken at 63 days. e: Samples taken at 40 days. f: Samples taken at 82 days. g: Samples taken at 36 weeks. h: Samples taken at 64 days. i: No specific data obtained in wild animal. j: Samples taken at 66 days. k: Samples taken at 45 days. l: Samples taken at 13 weeks. m: Samples taken at 49 days. n: Samples taken at 30 days. o: Samples taken at 57 days.
| Metabolite                          | Molecular mechanisms                                                                 | Effects on immune function                                                                 |
|-----------------------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| Folic acid                        | Increased expression of the antiapoptotic factor BCL-2                                 | Promotes activation of regulatory T cells                                                  |
| Histamine                         | Activation of H1R and H2R                                                             | Regulates Th1 and Th2 polarisation                                                          |
|                                   | Inhibits expression of pro-inflammatory cytokines and the MAPK pathway                 | Inhibits expression of pro-inflammatory cytokines and the MAPK pathway                     |
| Indole and indole derivatives     | Activation of AhR                                                                      | Promotes production of IL-22                                                                |
| Polyamines                        | Inhibition of pro-inflammatory cytokines expression                                    | Increases production of occludin, zonula                                                   |
| Retinoic acid                     | Activation of RAR and RXR heterodimer                                                 | Activates the TGFβ-3-SMAD pathway                                                          |
| Secondary bile acids              | Activation of GPBAR1 and FXR                                                          | Inhibits NF-κB                                                                             |
| Short-chain fatty acids           | Activation of GPR41 and GPR43                                                         | Promotes production of IL-10                                                                |
| (acetate, propionate, butyrate)   | Inhibition of histone deacetylase                                                     | Promotes chemotaxis                                                                        |
| Taurine                           | Activation of NLRP3 inflammasome (butyrate only)                                       | Promotes production of IL-18                                                                |
| Tryptophan metabolites            | Activation of GPR35, GPR109A and AhR                                                  | Promotes production of IL-18                                                                |

BCL-2: B-cell lymphoma 2. H1R: Histamine H1 Receptor. H2R: Histamine H2 Receptor. Th1: Linfoctos T helper 1. Th2: Linfoctos T helper 2. MAPK: Mitogen-activated protein kinases. IL: Interleukin. AhR: Aryl hydrocarbon receptor. RAR: Retinoid acid receptor. RXR: Retinoid X receptor. TGF-β: Transforming growth factor-β. GPBAR1: G-Protein Coupled Bile Acid Receptor 1. FXR: Farnesoid X receptor. NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells. GPR41: G-Protein Coupled Receptor 41, also called free fatty acid receptor 3 or FFAR3. GPR43: G-Protein Coupled Receptor 43, also called free fatty acid receptor 2 or FFAR2. NO: nitric oxide. ROS: reactive oxygen species. NLRP3: NLR Family Pyrin Domain Containing 3. TNF-α: tumor necrosis factor. INF-γ: Interferon gamma. NLRP6: NLR Family Pyrin Domain Containing 6. GPR35: G-Protein Coupled Receptor 35. GPR109A: G-Protein Coupled Receptor 109A, also called Hydroxycarboxylic Acid Receptor 2 or HCAR2.
CONCLUSIONS

Selection programs based on longevity, resistance to diseases or variability of weight at birth and litter size has been carried out successfully, without decreasing the production traits. Moreover, multi-omics studies are being carried out to gain in-depth knowledge of the host immune genes and the microbiome’s role in expression of the host genes for resistance/susceptibility to diseases. This knowledge could be used in breeding programmes, contributing to improving the response to selection for disease resistance in commercial rabbit lines.

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REFERENCES

Agea I., García M.L., Blasco A., Argente M.J. 2019. Litter survival differences between divergently selected lines for environmental sensitivity in rabbits. Animals, 9, 603. https://doi.org/10.3390/ani9090603.
Agea I., García M.L., Blasco A., Massányi P., Capcarová M., Argente M.J. 2020a. Correlated response to selection for litter size residual variability in rabbits’ body condition. Animals, 10(12):2447. https://doi.org/10.3390/ani10122447.
Agea I., Muelas R., García ML., Hernández P., Santacreu M.A., Armero E., Blasco A., Argente M.J. 2020b. Correlated response in plasma fatty acids profile in rabbits selected for environmental sensitivity. In Proc. 12th World Rabbits Congress, July, Nantes, France.

Argente M.J., Calle E.W., García M.L., Blasco A. 2017. Correlated response in litter size components in rabbits selected for litter size variability. J. Anim. Breed. Genet., 134, 505-511. doi: 10.1111/jbg.12283.
Argente M.J., García M.L., Zbyňovská K., Petruška P., Capcarová M., Blasco A. 2019. Correlated response to selection for litter size environmental variability in rabbits’ resilience. Animal, 13, 2349-2355. doi: 10.1017/S1751731119000302.
Arrazuria R., Elguezabal N., Juste R. A., Derakhshani H., Khaipour E. 2016. Mycobacterium avium Subspecies paratuberculosis Infection Modifies Gut Microbiota under Different Dietary Conditions in a Rabbit Model. Front. Microbiol., 7, 446. doi: 10.3389/fmicb.2016.00446.
Arrazuria R., Pérez V., Molina E., Juste R.A., Khaipour E., Elguezabal N. 2018. Diet induced changes in the microbiota and cell composition of rabbit gut associated lymphoid tissue (GALT). Sci. Rep., 8(1), 141031. doi: 10.1038/s41598-018-32484-1.
Baselga M., Deltoro J., Camacho I., Blasco A. 1988. Genetic analysis on lung injury in four strains of meat rabbit. In: Proc. 4th World Rabbit Congress, Budapest, Hungary, Vol. 1, 120–127.

Baselga M. 2004. Genetic improvement of meat rabbits. Programmes and diffusion. In Proc. 8th World Rabbit Congress, 2004 September, Puebla, México, Vol. 1, 1-13.

Bäuerl C., Collado M. C., Zúñiga M., Blasco A., Pérez Martínez G. 2014. Changes in cecal microbiota and mucosal gene expression revealed new aspects of epizootic rabbit enteropathy. PloS one, 9(8), e105707. doi:10.1371/journal.pone.0105707.

Beaumont M., Paix C., Mussard E., Knudsen C., Cauquil L., Aymard P., Barilly C., Gabinou B., Zemb O., Foure S., Gautier R., Lencina C., Eutamène H., Theodorou V., Canlet C., Combes S. 2020. Gut microbiota derived metabolites contribute to intestinal barrier maturation at the suckling-to-weaning transition. Gut Microbes.,11(5), 1268-1286. doi: 10.1080/19490976.2020.1747335.

Beloumi D., Blasco A., Muelas R., Santacreu M.A., García M.L., Argente M.J. 2020. Inflammatory correlated response in two lines of rabbit selected divergently for litter size environmental variability. Animals, 10(9):1540. https://doi.org/10.3390/ani10091540

Belloumi D., Argente M.J., García M.L., Blasco A., Santacreu M.A. 2021a. Study of biomarkers of disease sensitivity in a robust and standard maternal line. In Proc. 12th World Rabbits Congress, July, Nantes, France.

Belloumi D., Casto-Rebollo C., Blasco A., García M.L., Ibáñez-Escriche N., Argente M.J. 2021b. Análisis Metagenómico de la microbiota cecal en dos líneas de conejo seleccionadas divergentemente por varianza ambiental del tamaño de camada. XIX Jornadas de Producción Animal, June, Zaragoza, Spain.

Berghof T.V.L., Poppe M., Mulder H.A. 2019. Opportunities to improve resilience in animal breeding programs. Front. Genet. 9, 692. doi: 10.3389/fgene.2018.00692
Garreau H., Eady S.J., Hurtaud J., Legarra A. 2008b. Genetic parameters of production traits and resistance to digestive disorders in a commercial rabbit population. In Proc. 9th World Rabbit Congress, June, Verona, Italy. Vol. I, 103-107.

Garreau H., Brand S., Hurtaud J., Guittton E., Caquiol L., Licois D., Schwartz B., Combes S., Gidenne T. 2012. Divergent selection for digestive disorders in two commercial rabbit lines: response of crossbred young rabbits to an experimental inoculation of *Escherichia coli* 0-103. In Proc. 10th World Rabbit Congress, September, Sharm El-Sheikh, Egypt. Vol. I, 153-157.

Garreau H., Larzul C., Tudela F., Ruesche J., Ducrocq V., Fortun-Lamothe L. 2017. Energy balance and body reserves in rabbit females selected for longevity. *World Rabbit Sci.*, 25, 205-213. https://doi.org/10.4995/wrs.2017.5216

Groen A.F., Steine T., Colleau J.J., Pedersen J., Pribyl J., Reinsch N. 1997. Economic values in dairy cattle breeding, with special reference to functional traits. Report of an EAAP-working group. *Livest. Prod. Sci.*, 49, 1-21. https://doi.org/10.1016/S0301-6226(97)00041-9.

Gunia M., David I., Hurtaud J., Maupin M., Gilbert H. Garreau H. 2015. Resistance to infectious diseases is a heritable trait in rabbits. *J. Anim. Sci.*, 93, 5631–5638. doi: 10.2527/jas.2015-9377

Gunia M., David I., Hurtaud J., Maupin M., Gilbert H., Garreau H. 2018. Genetic parameters for resistance to non-specific diseases and production traits measured in challenging and selection environments; application to a rabbit case. *Front. Genet.*, 9, 467. https://doi.org/10.3389/fgene.2018.00467

Hou Y., Zhao D., Liu G., He F., Liu B., Fu S., Hao Y., Zhang W. 2016. Transcriptome analysis of rabbit spleen with hog cholera lapinized virus infection based on high-throughput sequencing technology. *Bing Da Xue Bao*. 32(3):316-23.

Ibáñez-Escriche N., Moreno A., Nieto B., Piquerás P., Salgado C., Gutiérrez J.P. 2008a. Genetic parameters related to environmental variability of weight traits in a selection experiment for weight gain in mice; signs of correlated canalised response. *Genet. Sel. Evol.*, 40, 279–293. DOI: 10.1051/gse:2008003

Ibáñez-Escriche N., Varona L., Sorensen D., Noguera J.L. 2008b. A study of heterogeneity of environmental variance for slaughter weight in pigs. *Animal*, 2, 19–26. doi: 10.1017/S1751731107001000

Jacquier V., Estellé J., Schmaltz-Panneau B., Lecardonnel J., Moroldo M., Lemonnier G., Turner-Maier J., Duranthon V., Oswald J.P., Gidenne T., Rogel-Guillard C. 2015. Genome-wide immunity studies in the rabbit: transcriptome variations in peripheral blood mononuclear cells after in vitro stimulation by LPS or PMA-Ionomycin. *BMC Genomics*, 16, 26-44. doi: 10.1186/s12864-015-1218-9

Jin D.X., Zou H.W., Liu S.Q., Wang L.Z., Xue B., Wu D., Tian G., Cai J., Yan T.H., Wang Z.S., Peng Q.H. 2018. The underlying microbial mechanism of epizootic rabbit enteropathy triggered by a low fiber diet. *Sci. Rep.*, 8(1), 12489. DOI: 10.1038/s41598-018-30178-2

Knap P.W. 2005. Breeding robust pigs. *Aust. J. Exp. Agric.*, 45, 763-773. https://doi.org/10.1071/EA05041

Kraimi N., Dawkins M., Gebhardt- Henrich SG., Gebhardt-Maier J., Rychlik I., Volf J., Leterrier C. 2019. Influence of the microbiota-gut-brain axis on behavior and welfare in farm animals: A review. *Physiol. Behav.*, 210, 112658. doi: 10.1016/j.physbeh.2019.112658

Larzul C., Gondret F., Combes S., Rochambeau H. de. 2005. Divergent selection on 63 day body weight in rabbits. *Rev. Sci. Tech.*, 24, 489-499. doi: 10.2527/jas.2013-7216

Larzul C., Ducrocq V., Tudela F., Juin H., Garreau H. 2014. The length of productive life can be modified through selection: an experimental demonstration in the rabbit. *J. Anim. Sci.*, 92, 2395-2401. doi: 10.2527/jas.2013-7216

Lenoir G., Maupin M., Leloire C., Garreau H. 2013. Analysis of the longevity of the lapine d’une lignée commerciale. In Proc. 15èmes Journées de la Recherche Canicole. Le Mans, France, 181-184.

Lukefahr S.D., Odi H.B., Atakora J.K.A. 1996. Mass selection for 70-day body weight in rabbits. *J. Anim. Sci.*, 74, 1481-1489. https://doi.org/10.2527/1996.7417481x

Mangino M., Roederer M., Beddall M., Nestle K.O., Spector T.D. 2017. Innate and adaptive immune traits are differentially affected by genetic and environmental factors. *Nat. Commun.*, 8, 13850-13858.

Martin R., Nauta A.J., Ben Amor K., Knippels L.M.J.,Knoj L., Garssen J. 2010. Early Life: Gut microbiota and immune development in infancy. *Benef. Microbes*, 1(4), 367–82. doi: 10.3920/BM2010.0027

Massip K., Combes S., Caquiol L., Zemb O., Gidenne T. 2012. High throughput 16SDNA sequencing for phylogenetic affiliation of the caecal bacterial community in the rabbit - Impact of the hygiene of housing and of the intake level. In Proc. 10th World Rabbit Congress, September, Sharm El-Sheikh, Egypt. Vol. I, 1003-1005.

Matics Z.S., Nagy I., Gerencsér Z.S., Radnai I., Gyövai P., Donkó T., Dalle Zotte A., Carik I., Szendrö Z.S. 2014. Pannon rabbitized virus infection based on high-throughput sequencing technology. *World Rabbit Sci.*, 22, 287-300. https://doi.org/10.4995/wrs.2014.1511

Mattioi S., Dal Bosco A., Combes S., Moscati L., Crotti S., Cartoni Mancinelli A., Cotozzolo E., Castellini C. 2019. Dehydrated alfalfa and fresh grass supply in young rabbits: Effect on performance and caecal microbiota biodiversity. *Animals*, 9(6):341. https://doi.org/10.3390/ani9060341

Mormede P., Terenina E. 2012. Molecular genetics of the adrenocortical axis and breeding for robustness. *Domest. Anim. Endocrinol.*, 43, 116-131. doi: 10.1016/j.domani.2012.05.002

Mulder H., Hill W., Vereijken A., Veerkamp R. 2009. Estimation of genetic variation in residual variance in female and male broiler chickens. *Animal*, 3:1673–1680. doi: 10.1017/S1751731109900668
Neave M.J., Hall R.N., Huang N., McColl K.A., Kerr P., Hoehn M., Taylor J., Strive T. 2018. Robust innate immunity of young rabbits mediates resistance to rabbit hemorrhagic disease caused by Lagovirus Europaeus GI.1 But Not GI.2. *Viruses, 10*, 512-534. doi: 10.3390/v10090512

Nielsen H., Amer P. 2007. An approach to derive economic weights in breeding objectives using partial profile choice experiments. *Animal, 1*, 1254-1262. doi:10.1017/S1751731107000729

North M.K., Dalle Zotte, A., Hoffman, L.C. 2019. Composition of rabbit caecal microbiota and the effects of dietary quercetin supplementation and sex thereupon. *World Rabbit Sci., 27*(4), 185-198. https://doi.org/10.4995/wws.2019.11905

Paës C., Gidenne T., Bébin K., Duperrat J., Gohier C., Guéné-Grand E., Rebows G., Bouchez O., Barilley C., Aymard P., Combels S. 2020. Early introduction of solid feeds: Ingestion level matters more than prebiotic supplementation for shaping gut microbiota. *Front. Vet. Sci., 7*, 261. https://doi.org/10.3389/fvets.2020.00261

Pickard J.M., Zeng M.Y., Caruso R., Núñez G. 2017. Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease. *Immunol. Rev., 279*(1), 70-89. doi: 10.1111/imr.12567

Piles M., Blasco A. 2003. Response to selection for growth rate in rabbits. *World Rabbit Sci., 11*, 53-62. https://doi.org/10.4995/wws.2003.497

Piles M., Garreau H., Rafel O., Larzul C., Ramon J., Ducrocq V. 2006. Survival analysis in two lines selected for reproductive traits. *J. Anim. Sci., 84*, 1658-1665. 10.2527/jas.2005-678

Piles M., Baselga M., Sánchez J.P. 2014. Expected response to different strategies of selection to increase heat tolerance assessed in litter size in rabbit. *J. Anim. Sci., 92*, 4306-4312. https://doi.org/10.2527/jas.2014-7616

Piles M., Sánchez J.P. 2019. Use of group records of feed intake to select for feed efficiency in rabbit. *J. Anim. Breed. Genet., 136*, 474-483. doi: 10.1101/jbg.12395

Pinheiro A., de Sousa-Pereira P., Strive T., Knight K.L., Wool J.M., Esteves P.J., Abrantes J. 2018. Identification of a new European rabbit IgA with a serine-rich hinge region. *PLoS ONE 13*(8), e0201567. https://doi.org/10.1371/journal.pone.0201567

Ragab M., Ramon J., Rafel O., Quintanilla R., Piles M., Sanchez J.P. 2015. Paramètres génétiques des phénotypes liés aux maladies chez le lapin en engraissement nourri avec deux régimes alimentaires différent. In *Proc. 16ème Journées de la Recherche Cunicole. Le Mans, France.*, 69-72.

Read T., Fortun-Lamothe L., Pascal G., Bouchel M.L., Cauquil L., Gabinaud B., Bannelier C., Balmisse E., Destombes N., Bouchez O., Gidenne T., Combels S. 2019. Diversity and co-occurrence pattern analysis of cecal microbiota establishment at the onset of solid feeding in young rabbits. *Front. Microbiol., 10*, 973. doi: 10.3389/fmicb.2019.00973

Reiss J., Bridle J.R., Montoya J.M., Woodward G. 2009. Emerging horizons in biodiversity and ecosystem functioning research. *Trends Ecol. Evol., 24*, 505-514. doi: 10.1016/j.tree.2009.03.018.

Rochambeau H., de la Fuente L.F., Rouvier R., Ouhayoun J. 1989. Sélection sur la vitesse de croissance post-sevrage chez le lapin. *Genet. Sel. Evol., 21*, 527-546. https://doi.org/10.1186/1297-9686-21-4-527

Sánchez J.P., Baselga M., Peiró R., Silvestre M.A. 2004. Analysis of factors influencing longevity of rabbit does. *Livest. Prod. Sci., 90*, 227-234. https://doi.org/10.1016/j.livprodsci.2004.06.002

Sánchez J.P., Baselga M., Ducrocq V. 2006. Genetic and environmental correlations between longevity and litter size in rabbits. *J. Anim. Breed. Genet., 123*, 180-185. doi: 10.1111/j.1439-0388.2006.00990.x.

Sánchez J.P., Thellgaard P., Mínguez C., Baselga M. 2008. Constitution and evolution of a long-lived productive rabbit line. *J. Anim. Sci., 86*, S15-S25. doi: 10.2527/jas.2007-0217

San Cristobal-Gaudy M., Elsen J.M., Bodin L., Chevallot C. 1998. Prediction of the response to a selection for canalization of a continuous trait in animal breeding. *Genet. Sel. Evol., 30*, 423-451. https://doi.org/10.1023/A:1004489129328

Sauvant D., Martin O. 2010. Robustesse, rusticité, flexibilité, plasticité...les nouveaux critères de qualité des animaux et des systèmes d’élevage: définitions systémique et biologique des différents concepts. *INRA Prod. Anim., 23*, 5-10.

Savietto D., Cervera C., Blas E., Baselga M., Larsen T., Friggens N.C., Pascual J.J. 2013. Environmental sensitivity differs between rabbit lines selected for reproductive intensity and longevity. *Animal, 7*, 1969-1977. doi: 10.1017/S175173111300178X.

Savietto D., Friggens N., Pascual J.J. 2015. Reproductive robustness differs between generalist and specialist maternal rabbit lines: the role of allocation and allocation of resources. *Genet. Sel. Evol., 47*, 2. https://doi.org/10.1186/s12711-014-0073-5

Shrestha M., Garreau H., Balmisse E., Bed’hom B., David I., Fadoua A., Guittot E., Hellon E., Lenoir G., Maupin M., Robert R., Lantier F., Gunia M. 2018. Estimation of Genetic Parameters of Pasteurellosis Resistance in Crossbred Rabbits. In *Proc. 11th World Congress on Genetics Applied to Livestock Production, Auckland, New-Zealand.*

Shrestha M., Garreau H., Balmisse E., Bed’hom B., David I., Guittot E., Lenoir G., Maupin M., Robert R., Lantier F., Gunia M. 2019. Projet RELAPA (génomique pour la RESistance génétique des LApins à la Pasteurellose): paramètres génétiques. In *Proc. 18èmes Journées de la Recherche Cunicole. Nantes, France.*, 77-80.

Schwensow N.I., Detering H., Pederson S., Mazzoni C., Sinclair R., Peacock D., Kovaliski J., Cooke B., Fickel J., Sommer S. 2017. Resistance to RHD virus in wild Australian rabbits: Comparison of susceptible and resistant individuals using a genome wide approach. *Mol Ecol., 26*, 4551-4561. doi: 10.1111/mec.14228.
Schnup P., Sansonetti P.J. 2012. Quantitative RT-PCR profiling of the rabbit immune response: assessment of acute Shigella flexneri infection. PLoS One.;7(6):e36446. https://doi.org/10.1371/journal.pone.0036446

Sobey W.R. 1969. Selection for resistance to myxomatosis in domestic rabbits (Oryctolagus cuniculus). J. Hygiene 67:743-754. doi: 10.1017/s0022172400042194

Star L., Ellen E.D., Uitdehaag K., Brom F.W.A 2008. A plea to implement robustness into a breeding goal: poultry as an example. J. Agric. Environ. Ethics. 21, 109-125. doi:10.1007/s10806-007-9072-7

Subbian S., O'Brien P., Kushner N.L., Yang G., Tsenova L., Peixoto B., Bandyopadhyay N., Bader J.S., Karakousis P.C., Fallows D., Kaplan G. 2013. Molecular immunologic correlates of spontaneous latency in a rabbit model of pulmonary tuberculosis. Cell Commun Signal.11(1):16. https://doi.org/10.1186/1478-811X-11-16

Suen W.W., Uddin M.J., Prow N.A., Bowen R.A., Hall R.A., Bielefeldt-Ohmann H. 2016. Tissue-specific transcription profile of cytokine and chemokine genes associated with flavivirus control and non-lethal neuropathogenesis in rabbits. Virology. 494:1-14. DOI: 10.1016/j.virol.2016.03.026

Theilgaard P., Sánchez J.P., Cervera C., Pascual J.J. 2007. Late reproductive senescence in a rabbit line hyper selected for reproductive longevity, and its association with body reserves. Genet. Sel. Evol., 39, 207-223. DOI: 10.1051/gse:2006043

Theilgaard P., Basela M., Blas, M., Friggens N.C., Pascual J.J. 2009. Differences in productive robustness in rabbits selected for reproductive longevity or litter size. Animal, 3, 637-646. https://doi.org/10.1017/S1751731109003838

Uddin MJ, Suen WW, Prow NA, Hall RA, Bielefeldt-Ohmann H. 2015. West Nile virus challenge alters the transcription profiles of innate immune genes in rabbit peripheral blood mononuclear cells. Front. Vet. Sci., 14, 2:76. https://doi.org/10.3389/fvets.2015.00076

Velasco-Galilea M., Piles M., Viñas M., Rafel O., González-Rodríguez O., Guiverneau M., Sánchez J.P. 2018. Rabbit microbiota changes throughout the intestinal tract. Front Microbiol, 9: 2144. https://doi.org/10.3389/fmicb.2018.02144

Wang Q., Fue W., Guo Y., Tang Y., Du H., Wang M., Liu A., Li Q., An L., Tian J., Li M., Wu, Z. 2019a. Drinking warm water improves growth performance and optimizes the gut microbiota in early postweaning rabbits during winter. Animals, 9(6), 34. doi: 10.3390/ani90600346

Wang G., Huang S., Wang Y., Cai S., Yu H., Liu H., Zeng X., Zhang G., Qiao S. 2019b. Bridging intestinal immunity and gut microbiota by metabolites. Cell Mol Life Sci., 76(20):3917-3937, doi: 10.1007/s00018-019-03190-6

Wu Z., Zhou H., Li F., Zhang N., Zhu Y. 2018. Effect of dietary fiber levels on bacterial composition with age in the cecum of meat rabbits. Microbiologiyopen, 8(5), e00708. doi: 10.1002/mbo3.708

Youssef Y.M.K., Khalil M.H., Afifi E.A., El-Raffa A.M.E., Zaheds M. 2000. Heritability and non-genetic factors for lifetime production traits in New Zealand White rabbits raised in intensive system of production. In Proc. 7th World Rabbit Congress. Valencia, Spain. 497–503.

Zhu Y., Wang C., Li F. 2015. Impact of dietary fiber/starch ratio in shaping caecal microbiota in rabbits. Can. J. Microbiol., 61: 771-784. doi: 10.1139/cjm-2015-0201

Ziadi C., Mocé M.L., Laborda P., Blasco A., Santacreu M.A. 2013. Genetic selection for ovulation rate and litter in rabbits: Estimation of genetic parameters and direct and correlated response. J. Anim. Sci., 91, 3113-3120. doi: 10.2527/jas.2012-6043

Zomeño C., Hernández P., Blasco A. 2013. Divergent selection for intramuscular fat content in rabbits. I. Direct response to selection. J. Anim. Sci., 91, 4526-4531. doi: 10.2527/jas.2013-6361
GENETIC FACTORS OF FUNCTIONAL TRAITS

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GENETIC FACTORS OF FUNCTIONAL TRAITS

—INTRODUCTION

—GENETIC CONTROL

—SELECTION PROGRAMS AND RESPONSE TO SELECTION

—MULTI-OMICS APPROACH TO STUDY RESISTANCE/SUSCEPTIBILITY TO DISEASES

—CONCLUSIONS
INTRODUCTION

• PRIORITIES IN RABBIT BREEDING
  1. ANIMAL WELFARE
  2. DISEASE RESISTANCE
  3. REDUCE ANTIBIOTICS
  4. PRODUCTIVITY

• TRAITS MEASURED:
  1. LONGEVITY
  2. HEALTH INDICATORS
  3. ENVIRONMENTAL VARIANCE

• CONCEPTS:
  1. ROBUSTNESS
  2. RUSTICITY
  3. PLASTICITY
  4. RESILIENCE
The objectives of this review are:

— to discuss the genetic control of longevity, diseases resistance traits and variability of birth weight and litter size.

— presenting the selection programmes with inclusion of these traits

— describing the first results from a multi-omics analysis of resistance/susceptibility to diseases
GENETIC CONTROL
DISEASE RESISTANCE TRAITS
LONGEVITY
HOMOGENETIY TRAITS

- Main diseases:
  - Pasteurellosis
  - Myxomatosis
  - Epizootic rabbit enteropathy

- Traits description:
  - Survival/Mortality
  - Lesions
  - Abcess
  - Abnormal growth
  - Poor body condition

h^2 = 0.02-0.64
Average (0.11)

Genetic correlations:
- Growth traits: <0
- Litter size, weight at weaning: = 0
GENETIC CONTROL

DISEASE RESISTANCE TRAITS
LONGEVITY
HOMOGENETIY TRAITS

- **Length of lifetime**
  - Date of the first presentation to a male and the date of death or culling
  - $h^2 = 0.05-0.24$

- **Number of AI**
  - Total number of artificial inseminations performed after the first kindling or death
  - $h^2 = 0.05-0.19$

- **Functional longevity**
  - Difference between the date of the first positive palpation test and the date of death or culling due to involuntary causes
  - $h^2 = 0.02-0.14$
GENETIC CONTROL

DISEASE RESISTANCE TRAITS
LONGEVITY
HOMOGENETIY TRAITS

Number of born alive: 0.16; -0.72
At weaning:
  Litter size: -0.17
  Litter weight: -0.7
Number of teats: -0.39
Adult weight: -0.2

Pseudo-genetic correlations
Table. Heritability (diagonal) and genetic correlation (above) of variability and mean of birth weight and litter size

| TRAIT          | VARIABILITY | MEAN  |
|----------------|-------------|-------|
| BIRTH WEIGHT   | 0.01        | 0.09  |
|                |             | 0.06  |
| LITTER SIZE    | 0.08        | -0.06 |
|                |             | 0.10  |
SELECTION PROGRAMMES AND RESPONSE TO SELECTION

SELECTION PROGRAMMES:

AUTRALIA
Resistance to myxomatois

POLITECHNIC UNIVERSITY OF VALENCIA
Longevity

INRA
Divergent selection:
Longevity
Resistance to digestive disorders
Weight at birth within litter size

MIGUEL HERNÁNDEZ UNIVERSITY
Divergent selection:
Litter size variability
SELECTION PROGRAMMES AND RESPONSE TO SELECTION

- Mass selection
- Rabbits were infected with 4 virus strains
- Percentage of recovery increased from 50 to 80% for the least virulent strain and from 10 to 20% for the most virulent virus strain
SELECTION PROGRAMMES AND RESPONSE TO SELECTION

- Line was founded by applying high selection intensity:
  - Females: between 25 and 41 parities
  - Prolificacy ranged from 7.5 to 11.9 young born alive

- Selection criterium: litter size at weaning
  - LP line was 1.3 time less likely to leave the herd than the V line
  - ≠ Body condition
  - ≠ Haematological profile: leukocytes populations
Selection criterium: Functional longevity

After 1 generation:
- Lines differ 0.75 inseminations

After 2 generations:
- Reproductive performance was similar between lines
- More body reserves in the High longevity line.
SELECTION PROGRAMMES AND RESPONSE TO SELECTION

✓ Selection criterion: Binary score based on the observed signs of enterophaty during growing period

✓ Similar mortality and growth rate in both lines.

✓ Cumulative mortality was lower in resistant than sensitivity line, when animals were inoculated with *E. Coli* 0103 strain.
Model of San Cristobal-Gaudi et al (1998)

Criterion of selection: within litter standard deviation of birth weight
SELECTION PROGRAMMES AND RESPONSE TO SELECTION

- Divergent Selection
- Weight at birth within litter size

INRA

✓ Direct response to selection: 1.7% per generation of litter weight standard deviation
✓ Correlated response:
  ✓ Higher litter size at weaning in Homogenous line
  ✓ Lower mortality at birth and at weaning in Homogenous line

Figure. Genetic trends for birth weight variability (Bodin et al., 2010)
SELECTION PROGRAMMES AND RESPONSE TO SELECTION

SIX SELECTION PROGRAMMES:

✓ Criterion of selection: environmental variance of litter size at birth (Ve)

✓ After 10 generations:
  ✓ Direct response to selection:

- Divergent Selection
- Litter size variability
SELECTION PROGRAMMES AND RESPONSE TO SELECTION

SIX SELECTION PROGRAMMES:

✓ Correlated response (I):

✓ Homogenous line showed........

  o Lower sensitivity to stress and diseases,

  o Higher body reserves at delivery and lactation,

  o Lower mortality at delivery of the does,

  o Lower mortality at delivery of the does,

  o Lower percentage of litter mortality at birth and at weaning,

  o and higher homogeneity of litter weight at weaning
SELECTION PROGRAMMES AND RESPONSE TO SELECTION

**SELECTION PROGRAMMES:**

- Correlated response (II):
  - Homogenous line showed........
    - No difference in ovulation rate
    - Higher embryo development from 48 h p.c.
    - Higher number of implanted embryos at 12 d of gestation
    - Higher litter size at birth
MULTI-OMICS APPROACH TO STUDY ENVIRONMENTAL SENSITIVITY

- Genomics
- Candidate genes:
  - Immune response 65
  - Stress response 5
  - Energy, carbohydrate and lipid metabolism 50
  - Reproduction and embryo development 29

Among those highlight the genes:

- TTC23L
- FBXL20
- GHDC
- ENSOCUG00000031631
- SLC18A1
- CD300LG
- ENSOCUG00000006264
- MC2R
SELECTION PROGRAMMES AND RESPONSE TO SELECTION

SIX SELECTION PROGRAMMES:

✓ Gut Microbiota (GI)
  - Two divergent lines separated according to 48 bacteria, 294 microbial genes, and 29 microbiota metabolites
  - Highlighting, 3 species bacteria (*Limosilactobacillus fermentum*, *Alistipes shahii* y *Acetatifactor muris*), 13 genes and 7 metabolites involved in phenylalanine, tyrosine and tryptophan metabolism
  - These metabolites suggest that selection for VE modified GI
CONCLUSIONS

✓ Selection programs for longevity, resistance to diseases and birth weight or litter size variability have been successful.

✓ Knowledge of gut microbiota in these lines can help to design probiotics for increasing resistance to illness.
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THANKS FOR YOUR ATTENTION