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A robust rabbit line increases leukocytes counts at weaning and reduces mortality by digestive disorder during fattening

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Abstract

The aim of this study was to determine how genetic type could affect the physiological and immune status of commercial rabbits at weaning, as well as their performance and health during the growing period. The study was conducted on a total of 2904 young rabbits weaned at 30 days, belonging to three different genetic types (line H, founded for litter size at birth and selected for litter size at weaning during 17 generations; line LP, founded for reproductive longevity criteria and selected for litter size at weaning for 7 generations; and line R, founded and selected during 25 generations for average daily gain from the 4^{th} to the 9^{th} week of life). Two different diets were used during lactation. The two diets were both isoenergetic and isoproteic but their main energy source differed, being either animal fat (AF) or cereal starch (CS). Leukocyte subsets were characterised at weaning, and growing performance was studied until 58 days of age (feed intake, live weight, mortality by digestive disorders and morbidity) for both medicated and non-medicated dietary versions. At weaning, young rabbits fed an AF lactating diet evidenced greater B lymphocyte count (+ 46%) than those fed a CS diet. Blood from LP rabbits had higher counts for total B, T $CD5^+$ and $CD8^+$ lymphocytes with respect to H and R (on av. +40, +57, +28, and +27%, respectively; $P<0.05$), and $CD4^+$ lymphocytes, monocytes and granulocytes with respect to R (on av. +24, +32 and +44%, respectively; $P<0.05$) at weaning. LP line rabbits also showed lower mortality by digestive disorders (on av. −8 points of percentage) and morbidity (on av. −4 points) than those from H and R lines during the growing period ($P<0.05$). R animals presented higher feed intake and daily weight gain, and a lower feed conversion ratio than H and LP animals (on av. +16.7±2.7 g dry matter/day, +10.3±0.4 g/day and −0.22±0.04 g dry matter/g, respectively). In conclusion, the foundation of a line for reproductive longevity, which has been previously reported to give greater robustness (low environmental sensitivity) to their reproductive

stock, could have also conferred a greater immunological development at weaning to their offspring, as well as a better ability to confront digestive disorders as compared to other lines founded or selected exclusively for productive criteria.

Keywords: Litter size; Growth rate; Robustness; Leukocytes subsets; Mortality; Rabbit.

Introduction

Maintaining the right balance between health and production rates during the growing period is one of the main targets in rabbit farming. The rise in digestive disorders during this period, such as Epizootic Rabbit Enterophaty (ERE), has increased the dependence on the use of antimicrobials and has reduced farm profitability in recent decades. Several studies have been conducted to define the aetiology of this illness (Licois et al., 2006) and to develop adequate feeding systems to minimize sanitary risk during the fattening period (Falção-e-Cunha et al., 2007; Gidenne et al., 2010). Genetic type could also condition the body condition and health of females, as well as the predisposition of the litter to suffer digestive disorders. In fact, a maternal effect on the development of digestive microbiota (Abecia et al., 2007) and on the rate of digestive disorders in growing rabbits (Quevedo et al., 2003; Carabaño et al., 2006) has previously been described.

Several studies have indicated a line founded by productive longevity has increased ability to overcome productive (Theilgaard et al., 2009), environmental (Savietto et al., 2013) and immunological challenges with LPS (Ferrian et al., 2013). The concept of robustness in farm animals was defined by Knap (2005) as 'the ability to combine a high production potential with resilience to stressors, allowing for unproblematic expression of a high production potential in a wide variety of environmental conditions'. Therefore, this line founded by productive longevity could be considered as robust. This robust line was characterized by greater modulation of their body resources under heat stress conditions, when the immune system is widely affected (Ferrian et al., 2012), and improved ability of the immune system to respond when faced with a specific immunological challenge (Ferrian et al. 2013). Therefore, the use of genetic types characterized by a greater robustness could contribute to improving the general health status of the farm.

Weaning happens when the immune system is still being developed, and the transition to solid feeding frequently leads to increased digestive disorders and mortality (Rosell and De la Fuente, 2009). The adequate performance of young rabbits during the growing period could be also affected by their degree of maturity at this time. The immune system in young rabbits is subjected to several changes during the first weeks of life. Increased levels of red (RBC) and white blood cells (WBC) are related to greater maturity (Jeklova et al., 2009), as well as changes in leukocyte populations in peripheral blood. On the other hand, the type of feed given to rabbits during the lactation period affects the amount of milk ingested, the promotion of solid feed intake and probably the degree of maturity at weaning. Fat enriched diets usually increase milk yield of females and live weight (LW) of kits at weaning (Pascual et al., 2003), but could lead to a less progressive weaning process.

The aim of the present study was to evaluate how three genetic types founded and selected for different criteria (reproduction, robustness or growth rate) and the dietary energy source of the lactation feed (fat or starch) could have affected the maturity and leukocyte populations in peripheral blood at weaning, as well as their subsequent performance and health status during the growing period.

Material and methods

Animals

The experimental procedure was approved by the animal welfare ethics committee of the Universitat Politècnica de València (UPV) and carried out following the European Union (2003) recommendations on care and protection of animals used for experimental purposes and the advice for applied nutrition research in rabbits according to the European Group on Rabbit Nutrition (Fernández-Carmona et al ., 2005). The experiment involved a total of 325 litters from 196 female rabbits studied at the most until third parity (from January to August of 2012). A total of 2904 young rabbits weaned at 30 d were used. Weaned rabbits belonged to three different genetic types from the Institute for Animal Science and Technology of the Universitat Politècnica de Valencia: line H, founded for litter size at birth and selected for litter size at weaning during 17 generations (n=807); line LP, founded for reproductive longevity criteria by selecting females from commercial farms that had a minimum of 25 parturitions with more than 7.5 kits born alive per parity (more details of LP line constitution are given in Sánchez et al., 2008) and then selected for litter size at weaning for 7 generations (n=1311), characterized by a high robustness (Theilgaard et al., 2009; Ferrian et al., 2012, 2013); and line R, founded and selected during 25 generations for average daily gain from the 4^{th} to the 9^{th} week of life (n=786).

Diets

Two experimental diets were formulated according to the recommendations of De Blas and Mateos (2010) for reproductive rabbit does. The two diets were meant to be both isoenergetic and isoproteic [approx. 11.6 MJ of digestible energy (DE) and 117 g of digestible protein per kg of dry matter (DM)], but were to have a differing main energy source. Diet CS was prepared using cereal starch [247 g of starch and 21 g of ether extract (EE) per kg DM], whereas in diet AF, part of starch was replaced by animal fat (104 g of starch and 85 g of EE per kg DM). After weaning, two versions of the same commercial diet were used: non-medicated (NM) and medicated [M; 40 ppm of tiamulin fumarate A (Caliermutin 2%, Laboratorios Calier S.A., Barcelona, Spain), 120 ppm of neomycin sulphate (Hipramix 14%, Hipra S.A., Girona, Spain), 29 ppm of lincomycin

hydrochloride and 29 ppm of spectinomycine sulphate (Linco-spectin 880, Pfizer, Madrid, Spain)]. Both diets included 66 ppm of robenidine as coccidiostat.

Chemical analyses of diets were performed according to the methods of the Association of Official Analytical Chemists (2000): 934.01 for DM, 942.05 for ash, 976.06 for crude protein and 920.39 for EE, with acid-hydrolysis of samples prior to the extraction. Starch content was determined according to Batey (1982), by means of a two-step enzymatic procedure with solubilisation and hydrolysis to maltodextrins with thermostable α-amylase followed by complete hydrolysis with amyloglucosidase (both enzymes from Sigma-Aldrich, Steinheim, Germany), and the resulting glucose being measured using the hexokinase/glucose-6 phosphate dehydrogenase/ NADP system (R-Biopharm, Darmstadt, Germany). Neutral detergent fibre (NDF), ADF and acid detergent lignin (ADL) fractions were analysed sequentially (Van Soest et al., 1991) with a thermo-stable α-amylase pre-treatment and expressed exclusive of residual ash, using a nylon filter bag system (Ankom, Macedon, NY, USA). The ingredients of the reproduction diets and chemical composition of all the diets are shown in the Table 1.

Experimental design

At first parturition, females of each genetic type were randomly allocated to both reproduction diets, which were provided ad libitum throughout the whole experiment. Females and their litters received the same reproduction diet. After weaning at 30 days of age, young rabbits from the same litter were identified by tattoo and housed together in collective cages until 58 days of age. During this growing period, two thirds of the litters received the M diet and one third the NM diet, provided ad libitum and randomly allocated from within genetic type and reproduction diet. Blood samples of 3 kits per litter from 136 females in the first reproductive cycle were extracted, mixed and processed as a unique sample at weaning for subsequent flow cytometry analysis (n=408). Samples were drawn from the median artery of the ear using vacuum EDTA tubes. Diurnal variations in haematological parameters were minimized by collecting blood at approximately the same time (8:00-9:00 h). Mortality by digestive disorders was studied daily (n=2904). Individual LW and litter feed intake were studied at 30, 44 and 58 days of age for 1231 growing rabbits. An animal was considered as morbid when there were sings of illness and/or

142 abnormally low growth compared with animals to the same group based on a subgroup analysis
143 (SAS, 2002).
144 *Flow cytometry analysis*
145 Blood was processed 1 h after sampling. Before performing flow cytometry, white blood cell
146 (WBC) count and percentage of total lymphocytes were determined using a haematology analyzer
147 (MEK-6410, Nihon Kohden, Japan). A millilitre of whole blood was pipetted into a 50 mL tube.
148 WBC were isolated lysing erythrocytes by adding 40 mL of ammonium chloride lysing solution
149 (8.02 g NH4 Cl, 0.84 g NaHCO3, and 0.37 g EDTA per liter of Millipore water) at 4°C. After 6
150 min incubation in the dark, samples were centrifuged at 400g for 5 min at room temperature. The
151 supernatant was carefully discarded and the pellet was resuspended in 1 mL of phosphate-buffered
152 saline (PBS). The cells number of the suspension was adjusted by counting with Neubauer
153 Chamber to 10^6 cells per mL. Primary monoclonal antibodies were added (Table 2), and incubated
154 for 25 min at room temperature in the dark. Then, the pellet was washed with 1 mL of PBS, and
155 centrifuged again under the same conditions mentioned above. Afterwards, secondary antibodies
156 (Rat anti-mouse IgG2a+b Phycoerythrin [VMRD, Inc. α-exalpha] and Goat anti-mouse IgM: R-
157 Phycoerythrin [AbD Serotec]) were added, and incubated for 20 min at room temperature in the
158 dark. Finally, 1 mL of PBS was added before running the flow cytometer. The outcome WBC
159 suspensions were analysed in a Cytomics FC500 flow cytometer (Beckman Coulter, Brea, CA).
160 The common leukocyte antigen CD14 and CD45 expression was used for the "lymphogate" setup
161 as previously described (Jeklova et al., 2007; Guerrero et al., 2010). Total lymphocyte count was
162 calculated from WBC count and lymphocyte percentage, and lymphocyte subset counts as
163 described by Guerrero et al. (2010).
164 *Statistical analysis*
165 Maturity Index (MI) at weaning was calculated as the ratio between LW at weaning and mature
166 weight. Mature weight for each line (4082, 4105 and 5645 g for H, LP and R line, respectively)
167 was obtained from the Gompertz modelling of the LW of reproductive rabbit does until 12 months
168 of age (Arnau, personnel communication). Data from leukocyte subset counts averaged per litter
169 and from individual MI at weaning were analysed using a general linear model (SAS, 2002),

including the genetic line (H, LP, R), the reproduction diet (CS, AF) and their interaction as fixed effects, as well as the parity order (primiparous, multiparous) for only MI at weaning.

Data on growing performance were analysed using a mixed procedure (SAS, 2002), in a repeated measure design which allows variance among animals and the intra-animal covariance to be considered, modelled with a compound symmetry function. Random terms included the permanent effect of each animal or litter (p) and the error term (e), both assumed to have an average of zero and a variance of σ_p^2 and σ_e^2, respectively. Analysis of LW and daily gain data was performed excluding morbid animals (to separate the effect of feed and genetic type from the effect of illness), with a model including as fixed effects the genetic line (H, LP, R), the reproduction diet (CS, AF), the parity order (primiparous, multiparous), the growing diet (NM, M), the control day (30, 44, 58 for LW and 44, 58 for daily gain) and their interactions. Feed intake and feed conversion ratio (FCR) were only analysed from 186 litters receiving M diet excluding morbid or dead animals, with a model similar to that used for daily gain data excluding the growing diet as a fixed effect.

Orthogonal contrasts were computed to test the differences between lines (H−LP, H−R and LP−R) and reproduction diets (AF−CS).

Mortality by digestive disorders and morbidity data were analysed using the genmod procedure (SAS, 2002), with a binomial probability distribution and a logit transformation [ln (μ/1−μ)] as link function. The model included the genetic line (H, LP, R), the reproduction diet (CS, AF), the growing diet (M, NM), the control day (35, 42, 49, 58) and their interactions.

Results

Table 3 shows the LW, MI and blood leukocyte counts of young weaned rabbits at 30 days of age. Young rabbits in the AF dietary group had greater LW and MI (+8%; $P<0.001$) at weaning than those in the CS group, especially in the case of animals from H and LP lines. Dietary group did not affect main blood leukocyte counts at weaning, but AF animals showed a greater count of B lymphocytes (+46%; $P<0.05$). Regarding genetic line, H weaning rabbits had lower LW than those from the LP and R lines (on av. −12%; $P<0.001$). The highest MI at weaning corresponded to LP animals (15.3 % of the adult weight), followed by H (13.6%; $P<0.001$), R showing the lowest MI (11.1%; $P<0.001$). Blood from LP weaning rabbits showed higher counts for total, B, T $CD5^+$ and $CD8^+$ lymphocytes in comparison to H and R rabbits (on av. +40, +57, +28, and +27%, respectively; $P<0.05$), and higher $CD4^+$ lymphocytes, monocytes and granulocytes with respect to R (on av. +24, +32 and +44%, respectively; $P<0.05$). In addition, blood from H weaning rabbits had a higher $CD4^+/CD8^+$ ratio than the LP and R lines (on av. +0.40±0.12; $P<0.01$), and higher $CD25^+$ lymphocyte and monocyte counts than the R line (on av. +93 and +33%, respectively; $P<0.05$).

Table 4 shows the performance of young rabbits during the growing period. The type of diet offered during lactation did not affect main growth performance traits. Only daily weight gain from 30 to 44 days of age was higher for animals from the CS group (+6%; $P<0.001$). As expected, R rabbits showed higher feed intake, daily weight gain and lower FCR than H and LP throughout the whole growing period (on av. +16.7±2.7 g DM/day, +10.3±0.4 g/day and −0.22±0.04 g DM/g, respectively; $P<0.001$). The difference in LW at weaning between LP and H lines was maintained at slaughter time (+81±12 g in favour of LP animals; $P<0.05$).

All dead and morbid animals presented digestive disorders compatible with ERE. As can be seen in Table 5, animals from the LP line showed lower mortality (on av. −8 points of percentage) and morbidity (on av. −4 points) than those from H and R lines during the growing period ($P<0.05$). Type of diet offered during lactation did not affect subsequent mortality and morbidity, but R animals from the AF group had higher mortality (+7 points of percentage; $P<0.05$) than those from CS (Figure 1). The inclusion of antimicrobials in the growing diet significantly reduced the mortality registered during the growing period (−19 points of percentage; $P<0.05$). However, this effect was different depending on the genetic line (Figure 2). Although the LP line showed the lowest cumulated mortality regardless of the diet, the highest cumulated mortality was registered in H animals when fed the M diet (3.6, 7.9 and 14.6 for LP, R and H, respectively; $P<0.05$), but on R animals when fed the NM diet (20.8, 27.8 and 37.1 for LP, H and R, respectively; $P<0.05$).

Discussion

As was expected, an animal fat enriched diet (AF) led to a higher milk yield in females, resulting in better and greater development of kits during the lactation period (Pascual et al., 2002). In fact, these young rabbits reached weaning with higher LW and increased B lymphocyte counts. Milk could be exerting a protecting effect, as several compounds in the milk could promote the proliferation of B lymphocytes in lactating animals (Juto, 1985; Orlando, 1995; Tuaillon et al., 2009). In this regard, Bienertova-Vasku et al. (2012) described a direct link between the B-cell activating factor (BAFF) secretion and leptin plasma level, concluding that BAFF expression was tightly related to adipose tissue. In fact, BAFF plasma level was also significantly correlated with the energy derived from

238 the diet, therefore the higher milk energy output could be responsible for the greater B
239 lymphocytes counts in AF weaned rabbits.

240 However, higher LW and increased B lymphocyte counts at weaning do not seem to give
241 any additional advantage in the subsequent performance during the fattening period. Due
242 to the compensatory growth observed in the CS group there was no difference in LW
243 between diets at slaughter time, mortality by digestive disorders and morbidity rates being
244 similar in both dietary groups. In this respect, Quevedo et al. (2006) suggested that
245 lactating kits with higher milk availability, which usually lead to lower feed intake at late
246 lactation, could result in more sudden weaning (less progressive transition from milk to
247 solid feeding). This fact might be related to the gut maturity of each animal, as the lower
248 the milk and the more the feed intake before weaning, the more the digestive tract
249 develops. Frequently, lactating rabbits showing earlier solid intake present a higher food
250 intake and growth during fattening, as well as lower digestive incidences (Maertens and
251 De Groote, 1990; Fortun-Lamothe et al., 2001; Pascual et al., 2001). In fact, animals from
252 the R line, which showed the lowest MI, had higher mortality by digestive disorders
253 during the fattening period when they had access to a lactation diet promoting milk yield.

254 As expected, and previously reported by Gómez et al. (1992), animals from the genetic
255 lines selected for daily gain showed better growth performance traits than those from the
256 lines founded and/or selected for reproductive criteria. Otherwise, a line characterized by
257 high robustness (LP), but also reproductive performance, showed traits which were
258 similar to the other maternal line (H). These results concur with those obtained in a large
259 scale trial (n=344,608; Mínguez et al., 2011), where similar or even better growing
260 performance in LP rabbits was reported in comparison to other maternal lines (A, V and
261 H).

On the other hand, foundation criterion seems to affect the maturity and leukocyte peripheral blood counts, being both higher at weaning for the animals from the line characterized by greater robustness (LP). Higher MI was due to their heavier weight at weaning as compared to the other maternal line (H) and to their lighter mature weight with respect to the paternal line (R). In young rabbit females, Theilgaard et al. (2005, 2009) already described a greater LW for LP line animals than in the case of other maternal lines at the beginning of their reproductive life (approx. +5%), and better maturity at first mating has frequently been related to higher life span and robustness in sows (Tarrés et al., 2006) and rabbit females (Sánchez et al., 2008).

The higher blood leukocyte counts observed for LP young rabbits at weaning are consistent with those previously reported for female rabbits by Ferrian et al. (2012), where total lymphocytes were significantly higher for LP animals when compared to another maternal line. Based on the results of this study it can be concluded that the use of a line characterized by higher robustness could improve the ability of the animals to cope with usual farm diseases. In fact, females of this robust line showed more efficient resource allocation and immunological response, allowing them to maintain reproductive performance in the long term even when subjected to different eventual environmental challenges, such as high productive level (Theilgaard et al., 2009), heat stress (Ferrian et al., 2012; Savietto et al., 2014), feed restriction (Savietto et al., 2014), or lipopolysaccharide inoculation (Ferrian et al., 2013).

On the other hand, several studies have hypothesised that some selection criteria based solely on production traits, such as reproduction or growth rate, could have affected the ability of their offspring to face immunological challenges or stress conditions (Clapperton et al., 2005; Pascual et al., 2012). Weaned rabbits of the line selected for growth rate (R) had lower levels of innate immune cells (monocytes) than maternal lines,

287 which have been reported to be more heritable than adaptive immune cells counts (Edfors-
288 Lilja et al., 1994 and 1998). Moreover, apparent negative genetic correlation between
289 growth performance traits and innate immune cell counts has been reported in pigs
290 (Clapperton et al., 2008). These results might suggest that the possible reduction in innate
291 immunity associated with selection for growth performance might negatively affect the
292 ability of the animals to confront pathogens.

293 In addition, weaned rabbits from lines founded and selected for productive criteria (H and
294 R) presented lower adaptive immune cells counts than those from the robust line. In other
295 species, a reduction in lymphocyte peripheral blood counts was reported in lines
296 characterized by high growth rate (in turkeys, Huff et al., 2005), hyper-prolificacy (in
297 Chinese sow lines, Clapperton et al., 2005) or higher milk energy output (in cows, Banos
298 et al., 2013). Higher lymphocytes counts in LP animals could be a characteristic of the
299 robust line, but heritability of adaptive immune cells counts have been described as
300 moderate (Clapperton et al., 2008; Thompson-Crispi et al., 2012). These higher counts
301 could be also related to the previously mentioned greater maturity of LP animals, which
302 might have a more mature immune system than animals from the other lines at the same
303 age. In fact, Jeklova et al. (2009) described how the number of total lymphocytes in the
304 peripheral blood of SPF rabbits rises during the first 10 weeks of life until reaching adult
305 values, while monocytes and neutrophils counts were not affected by age.

306 The inclusion of four antibiotics in the feed leads to a decrease in mortality by digestive
307 disorders of 19 points of percentage in the present study. These results concur with those
308 presented by Chamorro et al. (2007), who evaluated some of the main factors affecting
309 performance and health in growing rabbits, reporting the use of antibiotics as the best
310 option for reducing mortality by digestive disorders (−21 points of percentage).
311 Therefore, these results evidence the dependence on antimicrobials in environments

312 characterized by high rates of digestive disorders, being the most efficient way to reduce
313 the incidence of these disorders that highly affect farm profitability. However, the effect
314 of genetic type on mortality by digestive disorders and morbidity of growing rabbits in
315 this trial should be highlighted. Using animals from a robust line alone led to an average
316 reduction of 13 points of percentage in the sanitary risk index (mortality by digestive
317 disorders plus morbidity). Quevedo et al. (2006) described a higher sanitary risk index
318 (+4 percentage points; $P<0.05$) for the three-way crossbred young rabbits (A×V×R) from
319 current generations with respect to those from previous generations (on av. 12 generations
320 of difference). Thus, selection for exclusively production criteria could have increased
321 the susceptibility of young rabbits to common pathogens (as described in other species,
322 i.e. in pigs, Frank et al., 1997), and the introduction of genetic resources characterized by
323 a greater robustness could contribute to reducing the dependence on the use of
324 antimicrobials against infectious diseases.

325 However, although LP rabbits always had the lowest rates, the mortality by digestive
326 disorders of the other two lines was different depending on antimicrobial use, being
327 higher for the R animals which were not medicated and for H rabbits when feed was
328 medicated. It would be expected that animals of the R line with a higher feed intake had
329 a higher sanitary risk when fed with a non-medicated diet, as weaning could be less
330 progressive and the amount of nutrients reaching cecum would be increased (including N
331 which has been related with higher ERE incidence; Villamide et al., 2010). In fact, feed
332 restriction is a common strategy to reduce the ERE incidence (Gidenne et al., 2009). On
333 the contrary, higher feed intake of R animals when the diet contained antimicrobials led
334 to a higher dosage per animal as compared to the H line (+21%), which could explain the
335 inversion in the mortality rate between both lines.

In conclusion, the results of the present study show how the foundation of a rabbit line for reproductive longevity criteria, which has been previously reported to give a greater robustness to their reproductive stock, might have also conferred positive attributes to their offspring. These young rabbits would have greater maturity and immune status at weaning, showing a better ability to confront the digestive disorders associated with an ERE context as compared to other lines founded or selected exclusively for productive criteria. The implementation of these lines at the commercial farms might be considered of interest for a possible improvement of general farm health and a decreased dependence on antimicrobials.

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Table 2. Monoclonal antibodies used in this study.

Monoclonal antibodies	Isotype	Specificity	Cell labeling	Clone	References	Company
Mouse anti-rabbit T lymphocytes: FITC[a]	IgG1	CD5	T cell	KEN-5	Kotani et al., 1993	Abd Serotec
Mouse anti-rabbit α-pan B	IgM	IgM	B cell	MRB143A	Davis and Hamilton, 2008	VMRD, Inc.
Mouse anti-rabbit CD4	IgG2a	CD4	T cell subset	KEN-4	Kotani et al., 1993	Abd Serotec
Mouse anti-rabbit α- CD8	IgG2a	CD8	T cell subset	ISC27A	Davis and Hamilton, 2008	VMRD, Inc.
Mouse anti-rabbit CD25	IgG2b	CD25	Activated T cells	KEI-ALPHA1	Kotani et al., 1993	Abd Serotec
Mouse anti-human CD14: FITC	IgG2a	CD14	Monocytes and granulocytes	TÜK4	Jacobsen et al., 1993	Abd Serotec
Mouse anti-rabbit α- CD45	IgM	CD45	All leukocytes	ISC76A	Davis and Hamilton, 2008	VMRD, Inc.

[a] Clon KEN-5 recognises rabbit T lymphocytes and immunoprecipitates. This antibody recognises rabbit CD5, but does not bind to rabbit CD5 transfectants. Known rabbit CD5 antibodies also show binding to most B lymphocytes, which are not labelled by this clone (information obtained from datasheet).

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Table 3. Effect of genetic line and reproductive diet on the status of young rabbits at weaning (30 days of age)

Lines[1]		H		LP		R		Contrasts[3]			
Reproduction diet[2]	No.[5]	AF	CS	AF	CS	AF	CS	AF−CS	H−LP	H−R	LP−R
Live weight (g)	1231	593^{b}	515^{a}	661^{d}	606^{bc}	636^{cd}	622^{c}	48 ± 10***	-79 ± 12***	-75 ± 14***	4 ± 12
Maturity index[4]	1231	0.144^{c}	0.127^{b}	0.159^{d}	0.147^{c}	0.114^{a}	0.109^{a}	0.011 ± 0.002***	-0.017 ± 0.002***	0.024 ± 0.002***	0.041 ± 0.002**
Leukocytes counts (10^{6}/L):											
Total lymphocytes	129	1820^{bc}	1368^{ab}	1944^{c}	2175^{c}	1342^{a}	1344^{ab}	73 ±139	-465 ± 168**	251 ±176	716 ±166***
Lymphocytes B	129	25.3^{ab}	15.8^{a}	35.3^{b}	25.3^{ab}	21.1^{a}	14.9^{a}	8.6 ± 3.5*	-9.8 ± 4.2*	2.5 ± 4.4	12.3 ± 4.1**
Lymphocytes T $CD5^{+}$	129	910^{a}	958^{a}	1048^{a}	1293^{b}	922^{a}	881^{a}	-83 ± 69	-236 ± 84**	33 ± 88	296 ±83**
$CD4^{+}$	129	519^{a}	579^{ab}	575^{ab}	667^{b}	490^{a}	510^{a}	-57 ± 39	-72 ± 48	49 ± 50	121 ±47*
$CD8^{+}$	129	246^{a}	273^{ab}	313^{bc}	377^{c}	297^{ab}	274^{ab}	-23 ± 23	-86 ± 28**	-26 ± 29	59 ±28*
$CD25^{+}$	129	15.7	13.6	12.6	12.2	8.0	7.2	1.1 ± 2.4	2.3 ± 3.0	7.1 ± 3.1**	4.9 ±3.0
$CD4^{+}/CD8^{+}$	129	2.23^{c}	2.33^{c}	1.87^{ab}	1.81^{ab}	1.75^{a}	2.09^{bc}	-0.13 ± 0.10	0.44 ± 0.12***	0.36 ± 0.12**	-0.08 ± 0.11
Monocytes	129	278^{ab}	275^{ab}	244^{ab}	304^{b}	188^{a}	228^{ab}	-33 ± 27	23 ± 33	69 ± 34*	66 ±32*
Granulocytes	129	1640^{abc}	1552^{abc}	1705^{bc}	2015^{c}	1209^{a}	1376^{ab}	-129 ± 152	-264 ± 184	303 ± 194	567 ±182**

[1] Genetic line: line H, founded by litter size at birth and selected by litter size at weaning during 17 generations; line LP, founded by reproductive longevity criteria by selecting females from commerci farms that had a minimum of 25 parturitions with more than 7.5 kits born alive per parity and then selected by litter size at weaning for 7 generations; line R, founded and selected during 25 generatio by average daily gain from the 4th to the 9th week of life.

[2] Reproduction diet: CS, mainly based on cereal starch (247 g of starch and 21 g of ether extract (EE) per kg dry matter (DM)); AF, mainly based on animal fat (104 g of starch and 85 g of EE per kg DM

[3] Orthogonal contrasts to test the differences between lines [H−LP, H−R and LP−R] and reproduction diets [AF−CS].

[4] Maturity index at weaning as the quotient between live weight at weaning and mature weight for each line.

[5] No. for leukocytes counts refers to single samples. Each sample was obtained by pulling together blood from three kits from the same litter.

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Table 4. Effect of genetic line and reproductive diet on the performance of growing rabbits

Lines[1]		H		LP		R		Contrasts[3]			
Reproduction diet[2]	No.	AF	CS	AF	CS	AF	CS	AF−CS	H−LP	H−R	LP−R
30 to 44 days:											
Daily weight gain (g)	1231	34.2^{a}	36.1^{b}	34.0^{a}	37.5^{c}	43.6^{d}	45.1^{e}	-2.3 ± 0.4***	-0.6 ± 0.4	-9.2 ± 0.5***	-8.6 ± 0.5***
Daily feed intake (g DM)	186	65.5^{a}	64.4^{a}	67.7^{a}	71.2^{ab}	74.5^{ab}	79.6^{b}	-2.5 ± 3	-4.5 ± 3.5	-12.1 ± 4**	-7.6 ± 3.5*
Feed conversion ratio (g DM/g)	186	1.95^{b}	1.84^{ab}	1.97^{b}	1.93^{b}	1.73^{a}	1.72^{a}	0.06 ± 0.05	-0.05 ± 0.06	0.17 ± 0.07*	0.22 ± 0.06***
44 to 58 days:											
Daily weight gain (g)	1231	34.6^{a}	35.6^{a}	35.2^{a}	35.7^{a}	47.8^{b}	47.4^{b}	-0.4 ± 0.4	-0.3 ± 0.4	-12.5 ± 0.5***	-12.2 ± 0.5***
Daily feed intake (g DM)	186	99.5^{a}	109.6^{a}	105.7^{a}	107.0^{a}	129.9^{b}	128.2^{b}	-3.3 ± 3	-1.8 ± 3.5	-24.5 ± 4***	-22.7 ± 3.5***
Feed conversion ratio (g DM/g)	186	2.94^{b}	2.93^{b}	3.01^{b}	3.00^{b}	2.72^{a}	2.62^{a}	0.04 ± 0.05	-0.07 ± 0.06	0.27 ± 0.07***	0.34 ± 0.06***
Live weight at 58 days (g)	1231	1550^{a}	1524^{a}	1611^{b}	1626^{b}	1931^{c}	1900^{c}	14 ± 10	-81 ± 12***	-378 ± 14***	-297 ± 12***

[1] Genetic line: line H, founded by litter size at birth and selected by litter size at weaning during 17 generations; line LP, founded by reproductive longevity criteria by selecting females from commerci farms that had a minimum of 25 parturitions with more than 7.5 kits born alive per parity and then selected by litter size at weaning for 7 generations; line R, founded and selected during 25 generatio by average daily gain from the 4th to the 9th week of life.

[2] Reproduction diet: CS, mainly based on cereal starch (247 g of starch and 21 g of ether extract (EE) per kg dry matter (DM)); AF, mainly based on animal fat (104 g of starch and 85 g of EE per kg DM

[3] Orthogonal contrasts to test the differences between lines [H−LP, H−R and LP−R] and reproduction diets [AF−CS].

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Table 5. Effect of genetic line, reproductive diet and growing diet on the mortality by digestive disorders and morbidity during the growing period.

		Mortality (%)	Morbidity (%)
Genetic line[1]:	H	19.0[b]	6.6[b]
	LP	9.5[a]	3.9[a]
	R	16.5[b]	9.9[b]
Reproductive diet[2]:	AF	13.7	7.2
	CS	14.4	5.3
Growing diet[3]:	M	7.9[a]	6.7
	NM	26.8[b]	5.4

[1] Genetic line: line H, founded by litter size at birth and selected by litter size at weaning during 17 generations; line LP, founded by reproductive longevity criteria by selecting females from commercial farms that had a minimum of 25 parturitions with more than 7.5 kits born alive per parity and then selected by litter size at weaning for 7 generations; line R, founded and selected during 25 generations by average daily gain from the 4th to the 9th week of life.

[2] Reproduction diet: AF, mainly based on animal fat (104 g of starch and 85 g of EE per kg DM); CS, mainly based on cereal starch (247 g of starch and 21 g of ether extract (EE) per kg dry matter (DM));.

[3] Growing diet: M, medicated (40 ppm of tiamulin fumarate A, 120 ppm of neomycin sulfate, 29 ppm of lincomycin hydrochloride and 29 ppm of spectinomycine sulphate); and NM, non-medicated.

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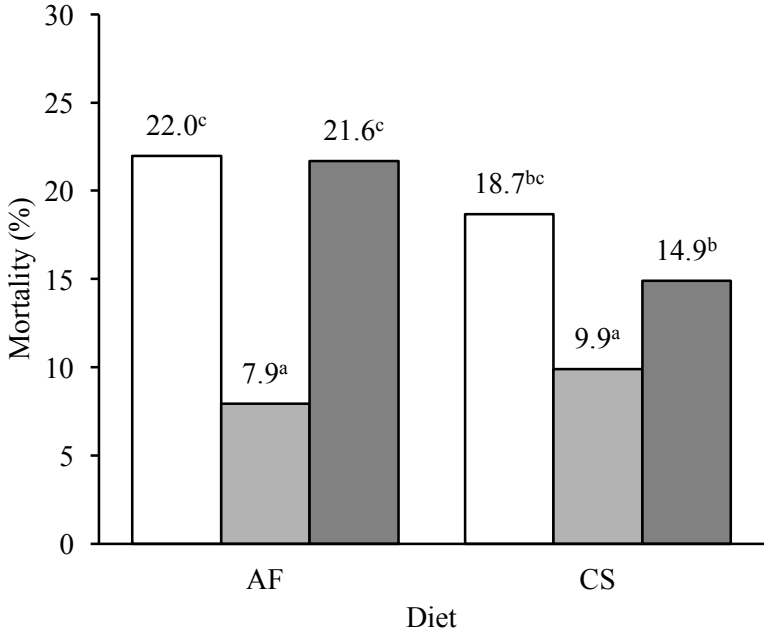

Figure 1. Effect of genetic line (□H, ■LP and ■R) and reproduction diet (AF and CS) on mortality by digestive disorders registered during the growing period (30 to 58 days of age).

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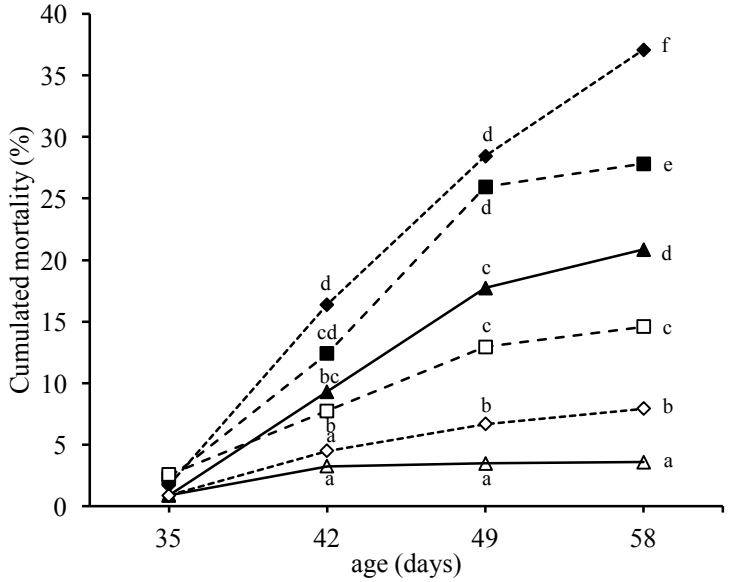

Figure 2. Evolution of cumulated mortality by digestive disorders depending on genetic line [– –■– – H, —▲— LP and ---◆--- R] and the use of antimicrobials in the feed [medicated, (□△◇); non-medicated, (■▲◆).[abcdef] Means at a same age not sharing superscript differ significantly at $P<0.05$.

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