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Long-term implications of feed energy source in different genetic types of reproductive rabbit females. I. Resource acquisition and allocation

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Long-term implications of feed energy source in different genetic types of reproductive rabbit females. III. Fitness and productivity

Abstract

To achieve functional but also productive females, we hypothesised that it is possible to modulate acquisition and allocation of animals from different genetic types by varying the main energy source of the diet. To test this hypothesis, we used 203 rabbit females belonging to 3 genetic types: H (n=66), a maternal line characterised by hyper-prolificacy; LP (n=67), a maternal line characterised by functional hyper-longevity; R (n=79), a paternal line characterised by growth rate. Females were fed with 2 isoenergetic and isoprotein diets differing in energy source: animal fat (AF) enhancing milk yield; cereal starch (CS) promoting body reserves recovery. Feed intake, weight, perirenal fat thickness (PFT), milk yield and blood traits were controlled during 5 consecutive reproductive cycles. Females fed with CS presented higher PFT (+0.2mm, $P<0.05$) and those fed AF had higher milk yield (+11.7%, $P<0.05$). However, the effect of energy source varied with the genetic type and time. For example, R females presented a decrease in PFT at late lactation (−4.3%; $P<0.05$) significantly higher than that observed for H and LP lines (on av. −0.1%; $P>0.05$), particularly for those fed with AF. Moreover, LP females fed with AF progressively increased PFT across the RC, whereas those fed with CS increased PFT during early lactation (+7.3%; $P<0.05$), but partially mobilised it during late lactation (-2.8%; $P<0.05$). Independently of the diet offered, LP females reached weaning with similar PFT. H females fed with either of the two diets followed a similar trajectory throughout the RC. For milk yield, the effect of energy source was almost constant during the whole experiment, except for the first reproductive cycle of females from the maternal lines (H and LP). These females yielded +34.1% ($P<0.05$) when fed with CS during this period. Results from this work indicate that the resource acquisition capacity and allocation pattern of rabbit females is different for each

genetic type. Moreover, it seems that by varying the main energy source of the diet it is possible to modulate acquisition and allocation of resources of the different genetic types. However, the response of each one depends on its priorities over time.

Keywords: Strategy, energy partitioning, life trajectory, animal fat, cereal starch.

Implications

In a context in which productive but also balanced and functional animals are demanded, understanding the way animals acquire and allocate resources is becoming highly relevant. Acquisition and allocation over the lifetime of reproductive females is defined by their priorities at all times and could condition their performance and health in the long term. In this work, we have evaluated the way three genetic types of reproductive females acquire and allocate resources in the long term and how energy source of the diet modulates them. This information could be used to develop specific nutritional strategies for each genetic type in order to maximise their productivity while maintaining their functionality.

Introduction

In the last 40 years, there has been a huge phenotypic improvement in most productive traits of domestic animal species (Hill, 2008). Long-term selection exclusively for productive criteria tends to generate specialised animals (Poggenpoel *et al.*, 1996) that prioritise functions related to the global context in which they were selected (Savietto, 2014). As a result of this specialisation, selection exclusively for production criteria could be accompanied by undesired side effects (Rauw *et al.*, 1998). Therefore, one of the main challenges in current animal science consists of developing strategies that provide productive but also balanced animals in their breeding context. In these circumstances, the importance of the way animals acquire and allocate resources among life functions is becoming highly relevant (Rauw,

2009). Acquisition and allocation of resources are affected by the animal's priorities throughout its life and could condition performance and health in the long term, as they define the investment in each function at each moment of its life trajectory.

The rabbit represents a good zootechnical model to investigate these relationships in the long term, as they have a relatively short reproductive cycle and there are genetic lines founded and selected for a wide range of goals (Baselga, 2004) with different priorities among life functions. For instance, females coming from selection programmes aiming to improve daily gain during the growing period tend to be bigger and gain more fat, but have lower maternal abilities (Gómez *et al.*, 1999). Furthermore, females coming from selection programmes aiming to improve litter size tend to yield more milk; some genetic types base reproduction on body fat utilisation and others on feed intake ability (Savietto *et al.*, 2013). Consequently, it would be interesting to evaluate the way each genetic type acquires and allocate resources, as well as to provide tools to modulate them. In this sense, energy source of the diet could be a good modulator for energy allocation. It has been reported that fat-enriched diets favour milk yield, whereas starch-enriched diets favour body reserve gain (Pascual *et al.*, 2003).

This is the first of three consecutive scientific papers that aim to evaluate the hypothesis that the effect of energy source of the diet varies with the genetic type, having implications in the way each genetic type acquires and allocates resources over time, their immunological status or their fitness and productivity (see companion papers Arnau-Bonachera *et al.*, 2017; Penadés *et al.*, 2017). Specifically, in the present work we studied: (i) the way three genetic types, widely differing in their genetic background, acquire and allocate resources. (ii) The dynamics of resource

acquisition and allocation of each genetic type. (iii) How feed energy source could modulate acquisition and allocation of each genetic type over time.

Material and methods

The experimental procedure was approved by the Animal Welfare Ethics Committee of the Universitat Politècnica de València and carried out following the recommendations of the European Group on Rabbit Nutrition (Fernández-Carmona *et al.*, 2005) and Spanish Royal Decree 53/2013 on the protection of animals used for scientific purposes.

Animals

A total of 203 female rabbits were used from their first artificial insemination until their sixth parturition (from December 2011 to April 2013). Rabbit females belonged to three genetic types (H, LP, R) developed at the Institute of Animal Science and Technology of the Universitat Politècnica de València, differing greatly in their genetic background (foundation, referring to the criteria used to select animals for the generation 0, and criteria used during the genetic selection). Animals from generation 0 of Line H were obtained following hyper-prolific criteria at birth (more than 17 young born alive in any parity or cumulative number of young born alive in all recorded parities equal or higher to the threshold corresponding to the best 0.01 in a population with a mean of nine young born alive, a standard deviation of 2.65 and a repeatability of 0.2.; Cifre *et al.*, 1998). Generations 1 and 2 were obtained without selection. H females used in this experiment belonged to the 17^{th} generation of selection by litter size at weaning (n = 66; survival rate at 6^{th} parturition=42%; av. fertility=63%; av. born alive=9.3); Animals from generation 0 of line LP were obtained following functional hyper-longevity criteria (females with at least 25 parturitions in commercial farms and an average live litter size of 8.8; more details in Sánchez *et*

123 *al.*, 2008). Generations 1 and 2 were obtained without selection. LP females used in
124 the experiment belonged to the 7th generation of selection by litter size at weaning
125 (n=67; survival rate at 6th parturition=72%; av. fertility=79%; av. born alive=9.5). In
126 previous experiments, females from this line have shown less environmental
127 sensitivity to environmental constrains, indicating greater robustness than other
128 commercial lines (Savietto *et al.*, 2015); Animals from generation 0 of line R were
129 obtained after 2 generations of randomly mating from a pool of animals of 3
130 commercial sire lines (Estany *et al.*, 1992). R females from this experiment belonged
131 to the 38th generation of selection by average daily gain during the growing period (n
132 =70; survival rate at 6th parturition=28%; av. fertility=78%; av. born alive=5.6).
133 *Diets*
134 Two experimental diets were formulated and pelleted (Table 1), following the
135 recommendations of De Blas and Mateos (2010) for reproductive rabbit does,
136 enhancing major differences in energy source. The CS diet was prepared promoting
137 cereal starch (237 g of starch and 21 g of ether extract per kg of DM), whereas in AF
138 diet part of the starch was replaced by animal fat (105 g of starch and 86 g of ether
139 extract per kg of DM). Nevertheless, both diets were designed to be isoenergetic and
140 isoprotein (on av. 11.3 MJ of digestible energy and 126 g of digestible protein per kg
141 of DM). Chemical analyses of diets were performed according to the methods from
142 the Association of Official Analytical of Chemists (AOAC, 2000).
143 *Experimental procedure*
144 Females were housed under conventional environmental conditions (average daily
145 temperatures varying from 13.3 to 26.1 ºC), with an alternating cycle of 16 h of light
146 and 8 h of darkness. Although not all the females began the experiment at the same
147 time (231 days between the first and the last female), most of them did so during the

148 first three months (See Supplementary Figure S1). The entry of animals from each of
149 the three genetic types was distributed over time similarly. Animals were housed in
150 individual cages (700 x 500 x 320 mm) at 12 weeks of age, inseminated at 19 weeks
151 of age (with pooled semen from their respective lines) and provided with a nest for
152 litters from day 28th of gestation. Females from each group (within genetic type and
153 experimental diet) were homogeneously distributed across the experimental farm.
154 After the first parturition, all females were randomly assigned to one of the
155 experimental diets. Until this moment, all the females received the same commercial
156 diet for reproductive rabbit does (11.3 MJ of digestible energy, 141 g of digestible
157 protein, 170 g of starch and 34 g of ether extract per kg of DM). Experimental diets
158 were provided *ad libitum*.
159 Litters were standardised to 8-9 kits at first parturition and 9-11 onwards. This
160 procedure was performed to equalise the energetic effort during lactation among
161 females, in order to compare each genetic type under similar lactational effort. This
162 procedure also allows us to decrease the data coefficient of variation which increases
163 the statistical accuracy of the estimates (Fernández-Carmona *et al.*, 2005). Females
164 were inseminated at 11 days postpartum and litters were weaned at day 30 of
165 lactation. Non-pregnant females were re-inseminated 21 days after the insemination
166 attempt for a maximum of three attempts.
167 *Traits*
168 To study the dynamics of acquisition and use of resources, all the traits were
169 recorded at different stages of the reproductive cycle (RC), from the first to the fifth
170 RC.
171 *Performance traits.* Within RC, milk yield was recorded four days a week during the
172 first three weeks of lactation. To record it, nests were closed and once a day were

opened to let the females suckle their kits. Milk yield was measured by weighing the females before and after suckling. From day 18 of lactation (18d), nests were kept permanently open to allow kits to leave the nest and begin solid intake. Finally, data coming from the same week were averaged to obtain one unique value per week and female. Within RC feed intake was recorded during early lactation (EL, from parturition to 18d) and from weaning to parturition (WPI). Body weight (BW) and perirenal fat thickness (PFT) were recorded at parturition, 18d and weaning according to Pascual *et al.* (2000).

Blood plasma traits. Blood samples were collected at parturition of the 1st, 2nd and 5th RC, and at 18d in lactation and at weaning of the 1st and 5th RC. Blood was drawn from the central artery of the ear using tubes with EDTA, always at 11:00 a.m. after a fasting period of 3 h. Samples were immediately centrifuged (3 000 x **g** during 10 min at 4°C). Plasma samples from 11 females per group [3 genetic types (H, LP and R) x 2 diets (AF, CS)] with complete records (from 1st artificial insemination to 5th weaning) were analysed for glucose, β-OH-butyrate, non-esterified fatty acids (NEFA) and leptin. Glucose was determined according to standard procedures (Siemens Diagnostics® Clinical Methods for ADVIA 1 650). β-OH-butyrate was determined as an increase in absorbance at 340 nm due to the production of NADH, at slightly alkaline pH in the presence of β-OH-butyrate dehydrogenase; sample blanks were included and the method involved oxamic acid in the media to inhibit lactate dehydrogenase, as proposed by Harano *et al.* (1985). NEFA were determined using the NEFA C ACS-ACOD assay method (Wako Chemicals GmbH, Neuss, Germany). Analyses of glucose, β-OH-butyrate, and NEFA were performed using an auto-analyser, ADVIA 1650® Chemistry 53 System (Siemens Medical Solutions, Tarrytown, NY 10 591, USA); in all instances the intra- and inter-assay coefficients of

198 variation were below 2.0 and 4.0%, respectively. Leptin was analysed by Multi-
199 Species Leptin assays (RIA, XL-85K) (Millipore Corporation, Billerica, MA, USA),
200 according to the manufacturer's guidelines. Intra- and inter-assay coefficients of
201 variation were 9.1 and 9.3%, respectively.

202 *Statistical analysis*

203 For daily feed intake, BW, PFT and milk yield, all data from each trait was studied
204 using the following model:

205 $y_{gdsrlgki} = GT_g|Diet_d|Stage_s|RC_r + OG_g \cdot Stage_s + OL_l \cdot Stage_s + \beta T_k \cdot Stage_s + p_{is} + e_{gdsrlgki}$

206 , where $y_{gdsrlgki}$ represents one record of a given trait; GT_g was the effect of genetic
207 type (3 levels; H, LP, R); D_d was the diet effect (2 levels: AF, CS); S_s was the stage
208 effect (2 levels for feed intake and intake per metabolic weight: early lactation,
209 weaning to parturition interval; 3 levels for body weight and PFT: parturition, 18d,
210 weaning; 3 levels for milk yield: week 1, week 2, week 3); RC_r was the reproductive
211 cycle fixed effect (5 levels; 1st, 2nd, 3rd, 4th, 5th). For the analysis of each trait we
212 considered all the previous simple effects, as well as all their interaction; OL_l was
213 considered as fixed effect to take into account the effect of getting pregnant during
214 lactation (2 levels: pregnant or not during lactation). OG_g was considered as fixed
215 effect to take into account the effect of being lactating during gestation (2 levels:
216 lactating or not lactating females at the beginning of gestation). By using OL_l and
217 OG_g we intended to take into account the effects of simultaneously gestating and
218 lactating on energy acquisition and allocation at the different stages of the
219 reproductive cycle. T_k was the average ambient temperature of the farm during the
220 reproductive cycle as covariate and β its regression coefficient; As random effects we
221 considered p_{is} and $e_{dgsrlgki}$, where p_{is} was the permanent effect of the i^{th} female at the
222 s^{th} stage and $e_{dgsrlgki}$ represented the random residuals of the records. This analysis

223 was performed using the proc MIXED of SAS (2009), where variance components
224 were estimated by the restricted maximum likelihood (REML) method. The model
225 defining the (co)variance matrix was selected from 16 candidate models with the
226 lowest AIC for most of the traits (or very close to it) and good biological interpretation
227 of its (co)variance components (Arnau-Bonachera, 2017). To perform it, we
228 considered that records within a RC represented different stages of the RC.
229 Consequently, variance was allowed to vary within a RC, remaining constant
230 throughout RCs for a given stage. We included the permanent effect of the animal,
231 which could be different depending on the stage within a RC. These different
232 permanent effects were assumed to be differently correlated among them. Regarding
233 the residuals, we also considered that they could be different at different stages
234 within an RC, being differently correlated among them and correlated in a decreasing
235 way among reproductive cycles (the more distant two measures were, the lower was
236 their correlation).
237 For blood plasma parameters, the model was:

238 $$y_{gdck} = GT_g|Diet_d|C_c + \beta T_k + e_{gdck}$$

239 , where y_{gdck} represents one record of a given trait; GT_g was the effect of genetic type
240 (3 levels; H, LP, R); D_d was the diet effect (2 levels: AF, CS); C_c was the time control
241 (7 levels; parturition, 18d and weaning for the 1st, parturition for the 2nd, and
242 parturition, 18d and weaning for the 5th RC); T_k was the average ambient
243 temperature of the farm during the reproductive cycle as covariate and β its
244 regression coefficient. The model defining the (co)variance matrix described above
245 did not present the best statistical fitting (in terms of AIC). (Co)variance matrix was
246 modelled without assuming any defined structure (unstructured matrix; SAS, 2009)
247 with the REML method.

248 **Results**

249 A proper understanding of mechanisms governing the links between resource
250 acquisition and allocation requires the control of a large number of traits, and could
251 complicate the presentation of results. Table 2 presents the main effects on
252 acquisition and allocation traits. However, numerous interactions were also observed
253 (see *P-values* for all the effects in Supplementary Tables S1 and S2). Consequently,
254 only relevant interactions have been presented to promote understanding.

255 *Resource acquisition capacity*

256 Table 2 shows the results for the main effects on feed intake as absolute value and
257 compared to the metabolic weight of females. As absolute value, feed intake was
258 higher in lactation (+67%; $P<0.05$). It increased over reproductive cycles, reaching
259 the maximum between third and fourth RC (+21.1% compared to primiparous does;
260 $P<0.05$). It was also affected by diet, as females fed with AF had an intake 5.1%
261 greater than those fed with CS ($P<0.05$). R females had greater average feed intake
262 than females from the maternal lines (H and LP females; on av. +10.9%; $P<0.05$). LP
263 females presented higher intake than H females (+6.3%; $P<0.05$). On the other hand,
264 with respect to the metabolic weight of females, results from the effects of the stage,
265 reproductive cycle and diet were comparable to those reported for the absolute
266 value. For the effect of genetic type, LP females presented the highest average
267 intake (+5 and +16% compared to H and R females; $P<0.05$), whereas the intake per
268 metabolic weight of H females was higher than R females (+11%; $P<0.05$).

269 However, absolute feed intake from each genetic type varied with energy source and
270 time. The results from this interaction are presented in Figure 1. Females from the
271 maternal lines increased their intake during early lactation over reproductive cycles,
272 reaching a maximum around third lactation (on av. 257, 288 and 303 g DM/day for

273 primiparous, secundiparous and multiparous, respectively; $P<0.05$), whereas for R
274 females, feed intake did not increase with age. R primiparous females fed with AF
275 ate 8.0% more than those fed with CS ($P<0.05$), whereas primiparous females from
276 the maternal lines did not differ in feed intake independently of the offered diet. In
277 multiparous, LP females had a feed intake during early lactation as high as R
278 females (on av. +6.9% compared to H; $P<0.05$). On the other hand, the greatest
279 intake of R females was observed especially between weaning and the next
280 parturition (+16.3% compared to H and LP; $P<0.05$; Figure 1B). Moreover, between
281 weaning and next parturition, greater feed intake with AF was observed in R females
282 (+7.1%; $P<0.05$) and H multiparous females (+11.8%; $P<0.05$).
283 *Resource allocation*
284 *Weight and perirenal fat thickness.* In Table 2 we can observe that within the RC
285 body weight increased as lactation progress. It also increased over reproductive
286 cycles, reaching a plateau around fourth RC (on av. +9% compared to primiparous
287 females; $P<0.05$). No effect of energy source on body weight was observed. R
288 females presented the highest values for BW (+36.0%; $P<0.05$). Regarding PFT, the
289 lowest value was observed at parturition (Table 2); it increased until 18d (+6%;
290 $P<0.05$), and subsequently decreased until weaning (-2%; $P<0.05$). PFT decreased
291 over reproductive cycles. Females fed with CS presented higher PFT (+0.2mm,
292 $P<0.05$). R females presented the highest PFT (on av. +27.5%; $P<0.05$) and LP the
293 lowest (-0.19 and -1.79 mm compared to H and R females, respectively; $P<0.05$).
294 However, the effect of energy source on the PFT pattern within the RC was different
295 depending on the genetic type. Figure 2 shows that the PFT decrease in R females
296 at late lactation (–4.3%; $P<0.05$) was significantly higher than that observed for H and
297 LP lines (on av. –0.1%; $P>0.05$), particularly for those fed with AF. Moreover, LP

females fed with AF progressively increased PFT across the RC, whereas those fed with CS increased PFT during early lactation (+7.3%; $P<0.05$), but partially mobilised it during late lactation (-2.8%; $P<0.05$). H females followed a similar trajectory throughout the RC independently of the offered diet.

Milk yield. In Table 2 we can observe that milk yield increased as lactation progress. The lowest average value was presented at first RC (on av. -33%; $P<0.05$). Females fed AF diet presented on average higher milk yield than those fed with CS (+11.7%, $P<0.05$). Regarding the effect of genetic type, LP females presented the highest average value for milk yield (on av. +19%; $P<0.05$).

However, milk yield from each genetic type varied with energy source and time. Figure 3 represents this interaction. In general, females fed with AF diet had higher milk yield compared to CS from second parturition, regardless of genetic type. This occurred particularly in the 2nd and 3rd week of lactation, although differences were not significant in the second RC of H females. However, although primiparous R females yielded more milk with AF diet (+26.2%; $P<0.05$), primiparous H and LP females yielded more with CS (+34.1%; $P<0.05$), especially from second week of lactation on. R females yielded less milk at first week of lactation than H, and especially LP females (on av. 87.5, 98.5 and 119.5 g/day, respectively; $P<0.05$).

Blood plasma parameters

Average glucose plasma concentration was always higher for LP and R than for H females (on av. +4.6%; $P<0.05$; Table 3), but differences were mainly due to the higher glucose concentration of LP females at 18d of first RC and at second parturition, and of R females at fifth weaning (Figure 4a). There were no significant differences in average NEFA plasma concentration between genetic types (Table 3). However, R females presented lower NEFA values at 18d and weaning of the first

323 RC than LP and H, whereas LP females had lower values at second and fifth
324 parturitions compared to R and H (Figure 4b). Average β-OH-butyrate concentration
325 was significantly lower in the plasma of LP compared to H females (–27.6%; $P<0.05$;
326 Table 3). However, although β-OH-butyrate plasma concentration decreased as the
327 first lactation progressed independently of genetic type and diet (on av. –81.8% from
328 parturition to weaning, Figure 5; $P<0.05$), the evolution of β-OH-butyrate during the
329 fifth RC depended on genetic type and diet (Figure 5). In contrast to that observed
330 during the first RC, females fed with AF and LP females fed with CS had no relevant
331 variations in BOBH plasma concentration throughout the fifth lactation (Figure 5).
332 However, β-OH-butyrate concentration of R and H females fed with CS decreased
333 significantly throughout the fifth lactation (on av. –79.8% from parturition to weaning,
334 Figure 5; $P<0.05$) as in the first RC.

335 **Discussion**

336 *Resource acquisition capacity*

337 Information regarding the effect of dietary energy source on dry matter intake is
338 highly controversial. However, in agreement with our results it seems that fat
339 enriched diets tend to increase feed intake (reviewed by Pascual *et al.*, 2003),
340 especially during early lactation (Lebas and Fortun-Lamothe, 1996). The increase in
341 feed intake of females fed on AF diet during early lactation could be related to the
342 increase in their nutritional requirements (due to the higher milk yield of females fed
343 with this diet; Pascual *et al.*, 2003). Between weaning and the next parturition, it
344 seems that feed intake is more closely related to the utilisation of body reserves
345 during lactation (Pascual *et al.,* 2002 and 2003). Consequently, during this period,
346 differences between diets for each genetic type and RC could be related to different
347 degrees of body reserve utilisation during lactation of each genetic type at each

348 reproductive cycle (Figure 1).

349 R females presented the highest energy acquisition (Table 2). Nevertheless, it was
350 the lowest when considering intake per metabolic weight. This indicates that their
351 great average acquisition capacity is mainly due to their heavy body weight. Contrary
352 to what was expected (Xiccato, 1996), results reported in the present experiment
353 suggest that the acquisition capacity of R females during early lactation could be
354 almost fully developed when primiparous, as there was no difference in feed intake
355 between primiparous and multiparous does (Figure 1A). On the contrary, the intake
356 of primiparous females from the maternal lines was lower than that observed later
357 (multiparous), indicating a limited acquisition capacity of these females during their
358 early reproductive career (Xiccato, 1996). Nevertheless, the average acquisition
359 capacity observed in LP females was much higher than that expected for their size
360 (Table 2). Specifically, LP females were characterised by a high acquisition capacity
361 during lactation (Figure 1). This great acquisition capacity agrees with the results
362 reported by Theilgaard *et al.* (2009) and Savietto *et al.* (2015). Finally, H females
363 presented an intermediate acquisition capacity between R and LP females, similar to
364 that of LP females between weaning and parturition and to that of R females during
365 lactation.

366 *Resource allocation*

367 Regarding the energy source effect, females fed on CS diet presented higher PFT
368 whereas females fed with AF yielded more milk. These results agree with those
369 reported by Xiccato *et al.* (1995), Fortun-Lamothe and Lebas (1996) and Pascual *et*
370 *al.* (2002). Consequently, it seems that by selecting the energy source of the diet we
371 could impose a shift of energy partitioning between milk and body reserves of
372 females (Pascual *et al.*, 2003).

373 R females were heavier and fatter than those from the maternal lines (Table 2;
374 Naturil-Alfonso *et al.* 2016). Milk yield was not low (similar to H females), but lower
375 than expected for their metabolic weight. Apart from the particular average allocation,
376 energy was differently allocated across time compared to females from the maternal
377 lines; on the one hand, lactation effort was low at the beginning but increased as
378 lactation progressed (Figure 3); on the other, females recovered a great amount of
379 body reserves during early lactation (+0.2 mm than females from maternal lines;
380 $P<0.05$), which was used afterwards during late lactation (Figure 2). Both facts
381 suggest that, at the onset of lactation, R females seem to prioritise their body
382 recovery more than current litter interests in comparison to maternal lines, whereas
383 as lactation progressed these priorities could have been inverted. Consequently, the
384 greater acquisition capacity of R females seems to be mainly addressed to
385 maintaining their heavier body size rather than litter development. Moreover, they
386 were highly dependent on body reserves to cope with the reproductive requirements
387 of the current reproductive cycle, especially at the end of lactation.

388 Regarding the shift imposed by energy source, milk yield was higher during the
389 whole controlled lactation for R females fed with AF (Figure 3), but the effects on
390 body condition (Figure 2) and feed intake (figure 1) were more evident from mid
391 lactation onward. R females fed with AF presented higher mobilisation during late
392 lactation (Figure 2) and, in response to this higher mobilisation, had higher feed
393 intake between weaning and the next parturition (Figure 1B). So, it seems that
394 females fed with AF made a greater effort in the current litter at the end of lactation
395 than those fed with CS.

396 Regarding the LP females, they presented the lowest amount of body reserves, and
397 the highest milk yield (Table 2). Moreover, as previously discussed, they presented

398 the highest average intake per kg of metabolic weight. This was especially evident
399 during early lactation of multiparous females, when their acquisition capacity was
400 fully developed. In this sense, it has been proposed that LP females base production
401 on energy acquisition, whereas they use body reserves as a safety factor (Savietto *et*
402 *al.*, 2015). According to this idea, it seems that LP females fed with AF tended to gain
403 body reserves during the whole lactation, whereas those fed with a diet promoting
404 body reserve gain (CS) accumulated a large amount of reserves during early
405 lactation, but they mobilised later (Figure 2), resulting in a similar value of PFT at
406 weaning independently of the offered diet. Consequently, it seems that LP females
407 were able to adapt their allocation across time (Savietto *et al.*, 2013). This strategy
408 would have allowed them to reach second parturition in suitable metabolic conditions
409 (higher glucose, lower NEFAs and lower β-OH-butyrate levels compared to females
410 from the other genetic lines). Thus, LP females could be characterised by an
411 acquisition capacity and an allocation pattern adapted to changing requirements
412 (imposed by physiological stage or diet) that allow them to confront high reproductive
413 efforts, but safeguarding body reserves (Savietto *et al.*, 2015).

414 The pattern of H females could be located between the patterns of R and LP
415 females. As previously reported for females specialised in prolificacy (Rauw *et al.*,
416 1999), lower values of average feed intake and glucose but higher values of β-OH-
417 butyrate and PFT (Tables 2 and 3) would indicate that H females were more
418 dependent on body reserves than LP females. However, H females tended to
419 accumulate reserves in early lactation and maintain them during late lactation (Figure
420 2). As H females accumulated reserves during early lactation that were not used in
421 late lactation and this pattern was observed for females fed with either diet, these
422 results suggest that H females tend to store body reserves for the next reproductive

423 cycle. Moreover, CS diet could be encouraging the storing skills of H females at the
424 end of lactation even more. The higher values of PFT, and lower values for β-OH-
425 butyrate and milk yield than when fed with AF, support this statement. However,
426 similarly to R females fed with CS, this strategy would lead H females fed with CS to
427 high mobilisation at parturition (high values of β-OH-butyrate) and high β-OH-
428 butyrate changes during the reproductive cycle compared to LP females (Figure 5).
429 Therefore, H females were dependent on body reserves but, in contrast to R
430 females, it seems that they accumulated them to cope with future reproduction.
431 Finally, the effect of diet on milk yield of primiparous females from the maternal lines
432 was different to that reported in other experiments (Pascual *et al.*, 2002) and to that
433 observed for R females, LP and H average values. This lack of agreement could be
434 the consequence of confused effects, as most of the females had their first parturition
435 during winter (Supplementary Figure S1). In fact, a low-temperature challenge could
436 be the underlying cause of these results. Although we cannot properly elucidate
437 whether the observed results were the consequence of the temperature or an
438 interaction between temperature and the RC, we hypothesised that they could be the
439 consequence of an interaction. In this sense, the effects of a low-temperature
440 challenge on performance depend on food availability (Manning and Bronson, 1990)
441 and the moment it takes place (Bronson and Marsteller, 1985), but can be attenuated
442 or exaggerated by body reserves (Schneider and Wade, 1991). In contrast to R
443 females, females from the maternal lines seemed to be physically limited, as their
444 feed intake when primiparous was similar independently of the offered diet and lower
445 than when multiparous (Figure 1). This different development of the acquisition
446 capacity could have affected food availability, conditioning the response to the
447 challenge for each genetic type. As the acquisition capacity of the R females was

448 almost fully developed, they were able to respond to diets as expected because they
449 could have sufficient intake to ensure adequate body condition and milk yield. In fact,
450 R females fed with AF were able to increase their intake during early lactation to
451 confront that situation (Figure 1A). On the contrary, females from the maternal lines,
452 with a limited acquisition capacity, were not able to increase feed intake when they
453 were fed a diet that did not ensure body condition (AF; Figure 1A). In that situation,
454 instead of yielding more milk, it seems that they accelerated the weaning process
455 (Figure 3; Martin and Sauvant, 2010) to give priority to maintenance and to
456 safeguarding body condition under this low temperature challenge.
457

458 **Conclusions**

459 The resource acquisition capacity and allocation pattern of rabbit females is different
460 for each genetic type. Each pattern would be differently modulated by energy source
461 according to the priorities of the females, given by their genetic background. R
462 females were characterised by a high dependence on their body reserves to cope
463 with the reproductive requirements of the current reproductive cycle, being more
464 evident when females were fed with diets promoting milk yield (AF). Similarly, H
465 females were also highly dependent on body reserves, but with a different goal. The
466 criteria used to obtain females from generation 0 of this line (hyper-prolificacy) would
467 have promoted a pattern based on body reserve accretion during lactation to cope
468 with future reproduction, magnified when fed with diets promoting body condition
469 (CS). Finally, LP females were characterised by an acquisition capacity better fitted
470 to changing requirements. The criteria used to obtain females from generation 0 of
471 this line (functional longevity) would have promoted body reserve safeguards to
472 ensure performance in the long term.

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Table 1 *Ingredients and chemical composition of experimental diets for rabbit females*

Ingredients	Diets[1]		Chemical composition	Diets[1]	
(g/kg)	AF	CS	(g/kg DM)	AF	CS
Barley grain	129	91.5	DM (g/kg)	911	909
Maize starch	0	180	Organic matter	901	911
Soybean meal	142.5	180	Ether extract	86	21
Lard	60	0	Starch	105	237
Wheat bran	100	100	CP	173	172
Alfalfa hay	400	350	NDF	364	286
Sugar beet pulp	100	40	ADF	195	162
Defatted grape seed	30	30	ADL	40	31
Sugarcane molasses	10	0	Gross energy (MJ/kg DM)	18.1	17.2
DL-Methionine	2.5	2.5	Digestible energy (DE; MJ/kg DM)[3]	11.4	11.1
Dicalcium phosphate	18	18	Digestible protein (DP)[3]	126	126
Sodium chloride	3	3	DP/DE (g/MJ)	11.1	11.3
Vitamin/mineral mixture[2]	5	5			
Robenidine (ppm)	66	66			

[1] AF: diet enhancing animal fat inclusion as main energy source; CS: diet enhancing cereal starch as main energy source.

[2]Contains (g/kg): thiamine, 0.25; riboflavin, 1.5; calcium pantothenate, 5; pyridoxine, 0.1; nicotinic acid, 12.5; retinol, 2; cholecalciferol, 0.1; α-tocopherol, 15; phytylmenaquinone, 0.5; cyanobalamin 0.0006; choline chloride, 100; $MgSO_4$ H_2O, 7.5; ZnO, 30; $FeSO_4$ $7H_2O$, 20; $CuSO_4$ $5H_2O$, 3; KI, 0.5; $CoCl_2$ $6H_2O$, 0.2; Na_2SeO_3, 0.03.

[3]Experimentally determined according to Perez *et al.* (1995). Using 24 healthy growing rabbits of 42 days of live per diet weaned at 30 days. Faces were collected during 4 days after a period of 7 days of adaptation to diets.

568

Table 2 *LSmeans and standard errors of the main effects on feed intake, perirenal fat thickness and milk yield of rabbit females*

	n	Feed intake (g DM/day)	Feed intake (g DM/kg$^{0.75}$ per day)	Weight (g)	PFT (mm)	Milk yield (g/day)
Records per trait		1 316	1 256	2 079	2 071	2 068
Genetic type[1]						
H	66	223(2.9)^{a}	79.5(0.92)^{b}	4 016(43)^{a}	6.96(0.08)^{b}	159.7(4.4)^{a}
LP	67	237(2.7)^{b}	83.1(0.83)^{c}	4 063(42)^{a}	6.77(0.07)^{a}	192.0(4.0)^{b}
R	70	255(3.0)^{c}	71.6(0.96)^{a}	5 493(43)^{b}	8.75(0.08)^{c}	165.3(4.5)^{a}
P-Value		<0.001	<0.001	<0.001	<0.001	<0.001
Energy source[2]						
AF	103	245(2.3)^{b}	79.8(0.74)^{b}	4 524(34)	7.39(0.06)^{a}	181.8(3.5)^{b}
CS	100	233(2.4)^{a}	76.4(0.74)^{a}	4 525(35)	7.59(0.06)^{b}	162.9(3.5)^{a}
P-Value		<0.001	0.001	0.990	0.024	<0.001
Reproductive cycle						
1	203	219(2.7)^{a}	75.9(0.94)^{a}	4 249(28)^{a}	7.62(0.06)^{b}	123.6(4.2)^{a}
2	167	234(2.3)^{b}	76.6(0.74)^{b}	4 526(26)^{b}	7.51(0.06)^{ab}	179.9(3.4)^{b}
3	149	245(2.6)^{c}	78.4(0.87)^{c}	4 593(28)^{c}	7.47(0.06)^{a}	186.3(4.0)^{b}
4	130	249(2.6)^{c}	79.9(0.87)^{c}	4 610(28)^{cd}	7.43(0.06)^{a}	186.5(4.0)^{b}
5	110	248(2.9)^{c}	79.7(0.99)^{c}	4 642(29)^{d}	7.43(0.06)^{a}	185.2(4.3)^{b}
P-Value		<0.001	0.003	<0.001	0.097	<0.001
Stage[3]						
First (i)	203	299(2.3)^{b}	99.9(0.76)^{b}	4 358(26)^{a}	7.25(0.05)^{a}	106.1(1.7)^{a}
Second (ii)	203	179(1.8)^{a}	56.3(0.59)^{a}	4 512(26)^{b}	7.70(0.05)^{c}	187.3(2.8)^{b}
Third (iii)	203	-	-	4 702(28)^{c}	7.53(0.05)^{b}	223.6(3.3)^{c}
P-Value		<0.001	<0.001	<0.001	<0.001	<0.001

n: Number of animals per treatment. PFT: Perirenal Fat Thickness. RC: Reproductive cycle. [1] Genetic Type: H characterised by hyper-prolificacy; LP characterised by functional hyper-longevity; R characterised by high average daily gain. [2] Energy source: AF animal fat; CS cereal starch (Table 1 for details). [3]Stage within RC according to the trait were: [For feed intake: early lactation (i) and weaning to parturition interval (ii)]; [For weight and PFT: parturition (i), day 18th of lactation (ii) and weaning (iii)]; [For milk yield: first week of lactation (i), second week (ii) and third week (iii)]. ^{a,b,c,d} Means in the same effect and column not sharing superscripts significantly differ at $P<0.05$.

569

Table 3 *LSmeans and standard errors of the main effects on plasma concentration of glucose, β-OH-butyrate, non-esterified fatty acids (NEFA) and leptin of rabbit females*

	n	Glucose (mM)	β-OH-butyrate (log_{10} mM)	NEFA (log_{10} µekv/l)	Leptin (log_{10} ng/ml)
Records per trait		462	462	462	462
Genetic type[1]					
H	22	6.51(0.07)^{a}	-1.09(0.04)^{b}	2.55(0.02)	-0.40(0.03)
LP	22	6.82(0.07)^{b}	-1.23(0.04)^{a}	2.54(0.02)	-0.40(0.03)
R	22	6.79(0.07)^{b}	-1.16(0.04)^{ab}	2.55(0.02)	-0.32(0.03)
P-Value		0.003	0.037	0.923	0.093
Energy source[2]					
AF	33	6.67(0.06)	-0.96(0.03)^{b}	2.56(0.02)	-0.39(0.02)
CS	33	6.75(0.06)	-1.36(0.03)^{a}	2.53(0.02)	-0.35(0.02)
P-Value		0.312	<0.001	0.187	0.178
Time control					
RC1:Parturition	66	6.70(0.13)^{cd}	-0.73(0.04)^{d}	2.57(0.02)^{b}	-0.50(0.04)^{a}
RC1:18d	66	6.58(0.07)^{bc}	-1.17(0.04)^{b}	2.59(0.02)^{bc}	-0.42(0.04)^{ab}
RC1:Weaning	66	6.33(0.08)^{a}	-1.51(0.03)^{a}	2.47(0.02)^{a}	-0.39(0.04)^{bc}
RC2:Parturition	66	7.07(0.19)^{de}	-0.95(0.07)^{c}	2.63(0.02)^{bc}	-0.42(0.04)^{ab}
RC5:Parturition	66	7.10(0.08)^{e}	-1.13(0.06)^{b}	2.48(0.02)^{a}	-0.19(0.02)^{d}
RC5:18d	66	6.65(0.05)^{c}	-1.18(0.04)^{b}	2.64(0.02)^{c}	-0.32(0.03)^{c}
RC5: Weaning	66	6.51(0.05)^{b}	-1.46(0.03)^{a}	2.44(0.02)^{a}	-0.39(0.04)^{bc}
P-Value		<0.001	<0.001	<0.001	<0.001

n: Number of animals per treatment.[1] Genetic Type: H characterised by hyper-prolificacy; LP characterised by functional hyper-longevity; R characterised by daily gain. [2] Energy source: AF animal fat; CS cereal starch (Table 1 for details). RC: Reproductive cycle. $^{a.b.c.d,e}$ Means in the same effect and column not sharing superscripts significantly differ at $P<0.05$.

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Figure 1 Evolution of feed intake of rabbit females over reproductive cycles [RC, primiparous, secundiparous and multiparous (av. of 3rd, 4th and 5th cycles)] for the different stages within the RC (Early lactation, Weaning to parturition) depending on genetic type (H, characterised by hyper-prolificacy; LP, characterised by functional hyper-longevity; R, characterised by daily gain) and the energy source [AF(□);CS (■)]. LSmeans and standard errors. [a,b,c,d,e,f,g,h] Means within a stage not sharing superscripts significantly differ at $P<0.05$.

Figure 2 Evolution within a reproductive cycle of perirenal fat thickness of rabbit females depending on genetic type [H (— ■ —), characterised by hyper-prolificacy; LP (—▲—), characterised by functional hyper-longevity; R (—◆—), characterised by daily gain] and energy source [AF (□△◇), animal fat; CS (■▲◆), cereal starch]. P: Parturition; 18d: day 18th of lactation; W: Weaning. LSmeans and standard errors. [a,b,c,d] Means within a genetic type not sharing superscripts significantly differ at $P<0.05$.

Figure 3 Evolution of the lactation curve over reproductive cycles of rabbit females [primiparous, secundiparous and multiparous (av. of 3rd, 4th and 5th cycles)] depending on genetic type: [H (—■—), characterised by hyper-prolificacy; LP (—▲—), characterised by functional hyper-longevity; R (—◆—), characterised by daily gain] and energy source [AF(□△◇), animal fat; CS (■▲◆), cereal starch]. LSmeans of average yield at 1st, 2nd and 3rd week of lactation and standard error. [a,b,c,d,e,f,g] Means within a genetic type not sharing superscripts significantly differ at $P<0.05$.

Figure 4 Plasma glucose [a)] and non-esterified-fatty-acids [b), NEFA] concentration of rabbit females over time depending on genetic type. Least squared means and standard error for H (- -■- -), characterised by hyper-prolificacy; LP (—▲—), characterised by functional hyper-longevity; R (–·–◆–·–), characterised by daily gain. RC: Reproductive cycle; P: Parturition; 18d: day 18th of lactation; W: Weaning. LSmeans and standard errors. [a,b] Means in a time control and cycle not sharing superscripts significantly differ at $P<0.05$.

Figure 5 Plasma β-OH-butyrate concentration of rabbit females over time depending on genetic type [H (- -■- -), characterised by hyper-prolificacy; LP (—▲—), characterised by functional hyper-longevity; R (–·–◆–·–), characterised by highly daily gain] and energy source [AF(□△◇), animal fat; CS (■▲◆), cereal starch. RC: Reproductive cycle; P: Parturition; 18d: day 18th of lactation; W: Weaning. LSmeans and standard errors. [a,b,c,d] Means in a time control and cycle not sharing superscripts significantly differ at $P<0.05$.

Figure 1

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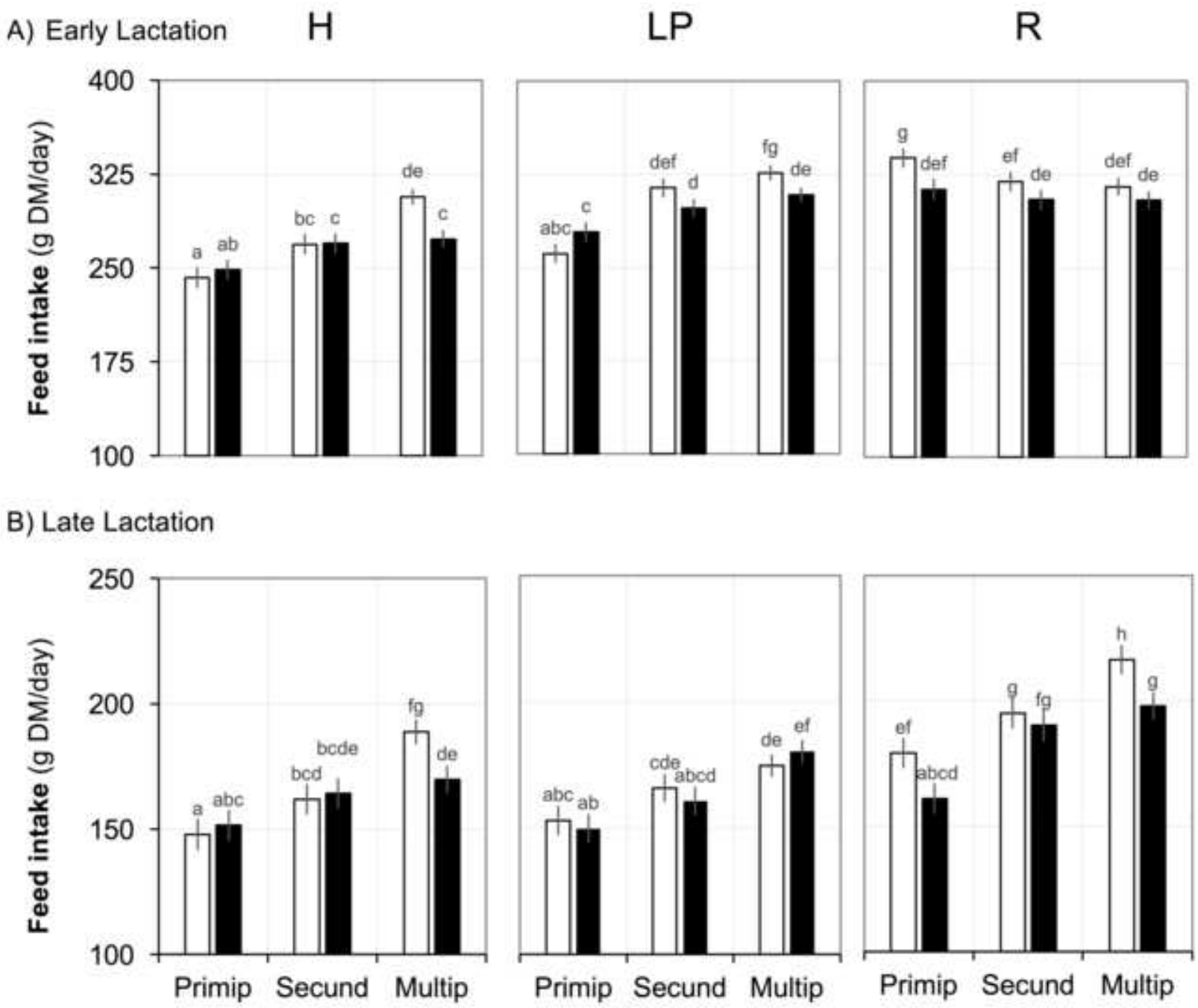

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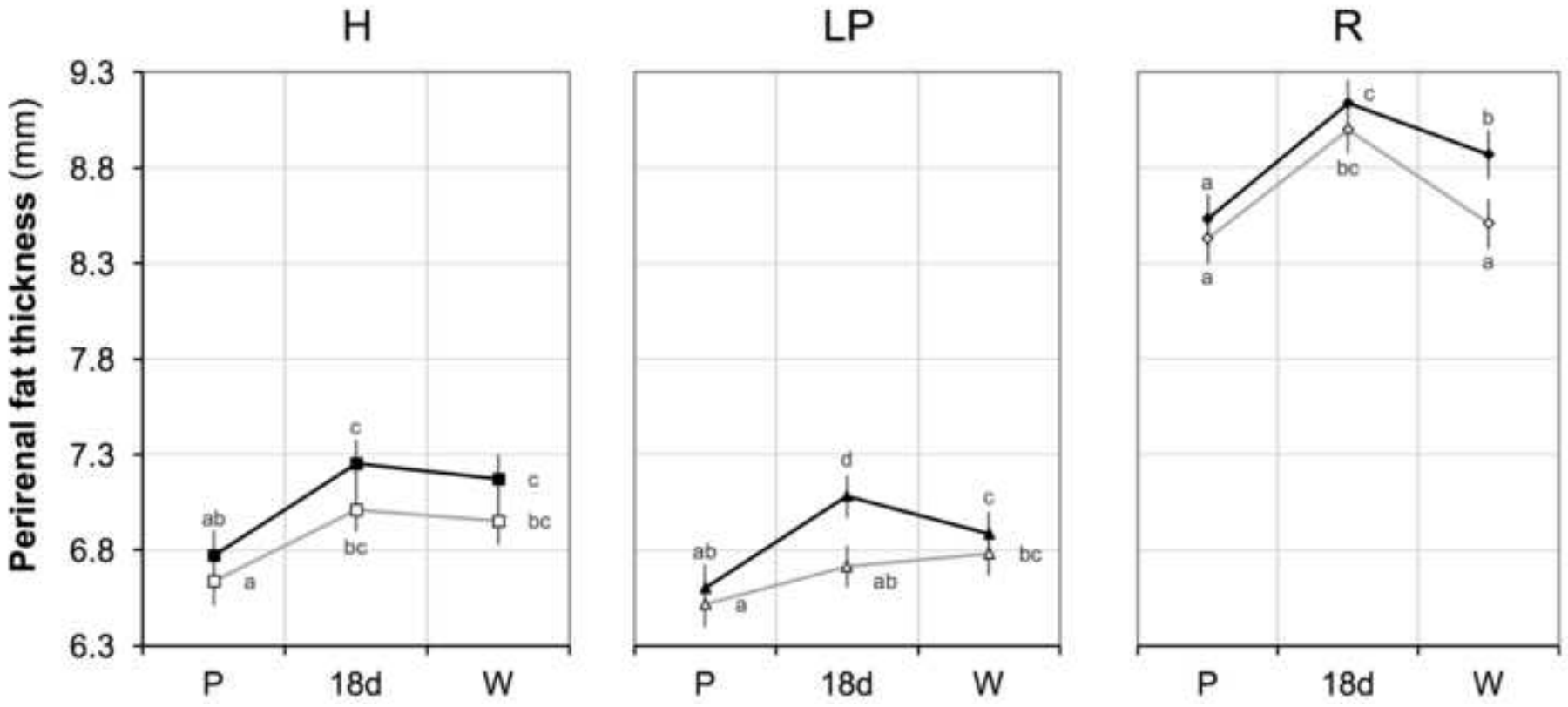

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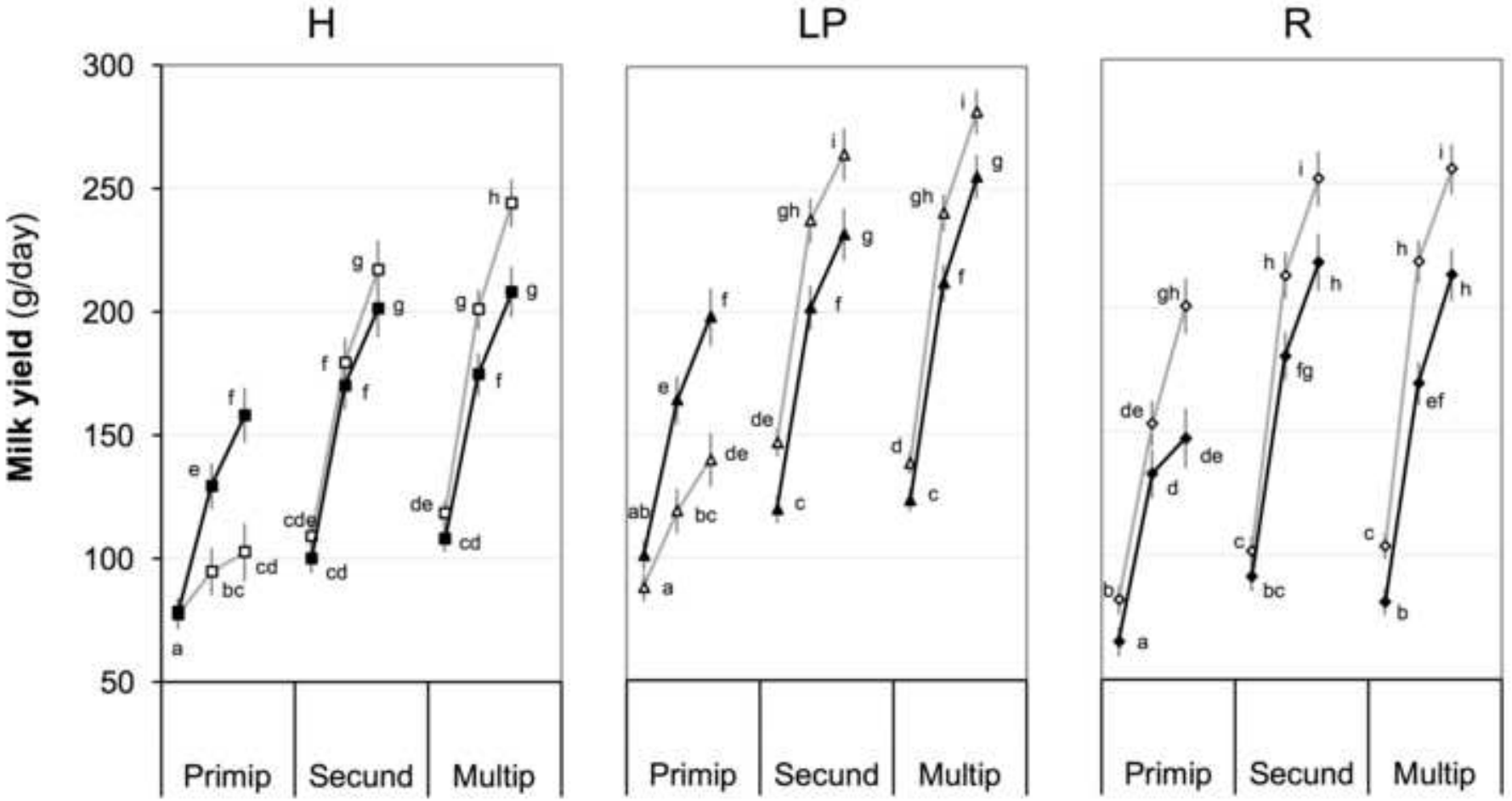

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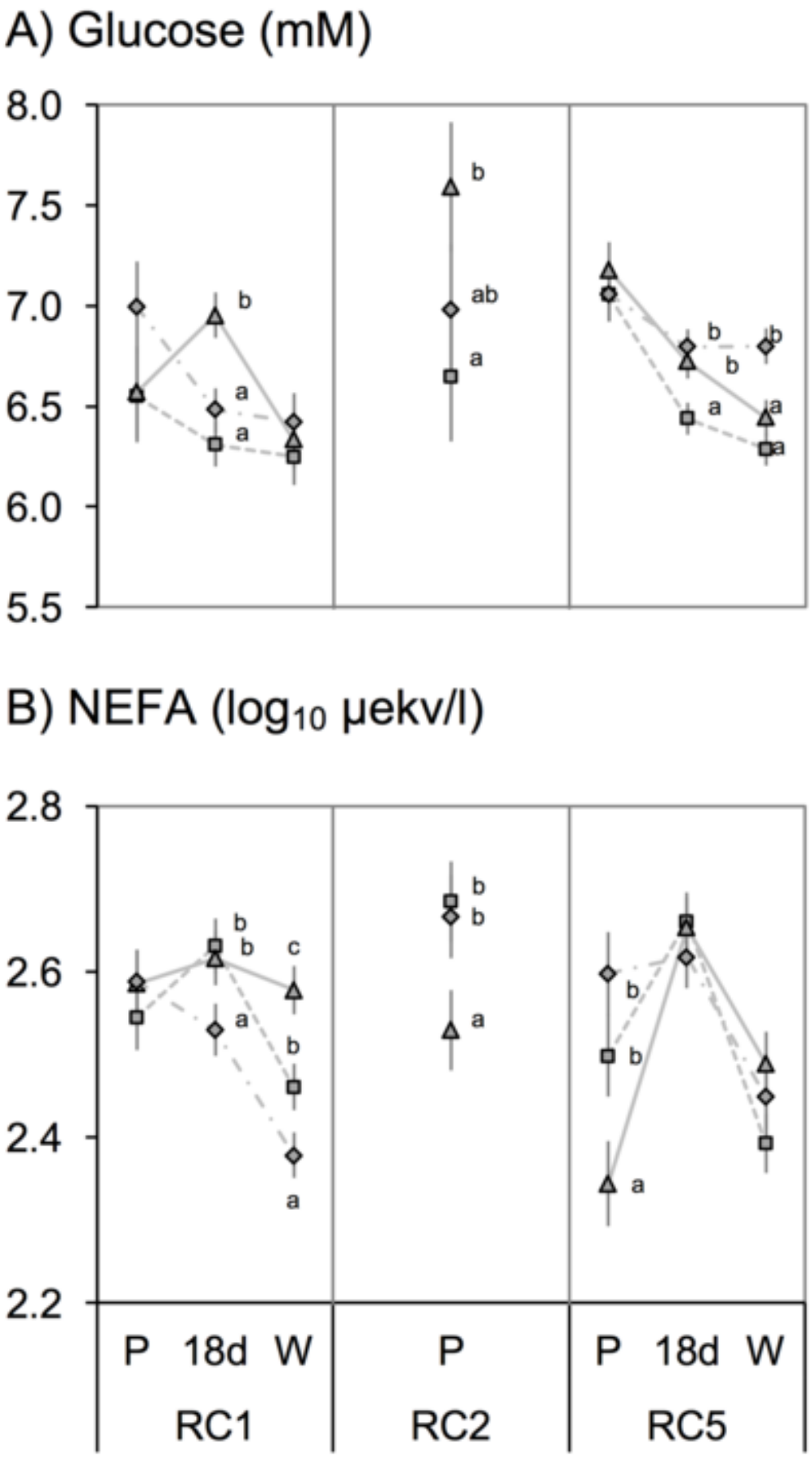

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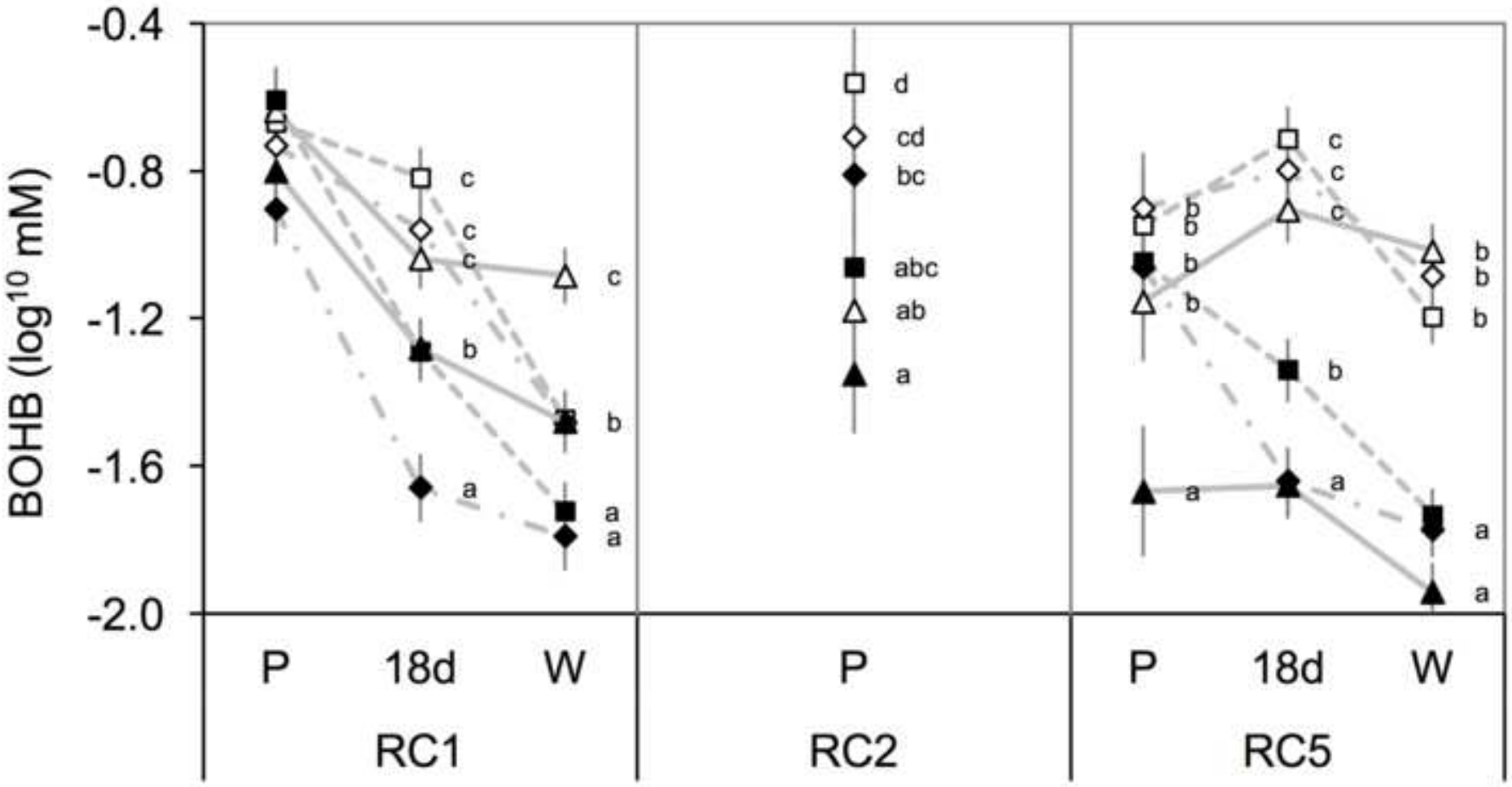

Long-term implications of feed energy source in different genetic types of reproductive rabbit females. I. Resource acquisition and allocation

A. Arnau-Bonachera, C. Cervera, E. Blas, T. Larsen, E. Martínez-Paredes, L. Ródenas and J.J. Pascual.

Table S1 *P-Values for all the effects considered in the models used to analyse acquisition and allocation traits*

Effect	Order[1]	*P-Value*				
		Feed intake[2]	Feed intake[3]	Weight	PFT	Milk Yield
Genetic Type (GT)	1	<.0001	<.0001	<.0001	<.0001	<.0001
Energy source (ES)	1	0.0007	0.0011	0.9904	0.0235	0.0001
Reproductive cycle (RC)	1	<.0001	0.0025	<.0001	0.0972	<.0001
Stage within RC	1	<.0001	<.0001	<.0001	<.0001	<.0001
OG[4]	1	<.0001	<.0001	<.0001	0.1614	0.0149
OL[5]	1	0.0227	0.1923	<.0001	0.3969	0.5118
GTxES	2	0.3084	0.2631	0.6862	0.9964	0.0896
GTxRC	2	<.0001	<.0001	0.0835	0.3493	0.0479
ESxRC	2	0.0348	0.0161	0.1216	0.6842	<.0001
GTxStage	2	<.0001	<.0001	<.0001	0.0087	<.0001
ESxStage	2	0.1870	0.1510	0.9156	0.2949	0.0237
RCxStage	2	0.2445	0.0017	<.0001	<.0001	<.0001
StagexOG	2	<.0001	<.0001	0.0004	0.1651	0.7937
StagexOL	2	0.6298	0.2929	<.0001	0.1638	0.2698
TemperaturexStage	2	<.0001	<.0001	<.0001	<.0001	0.0002
GTxESxRC	3	0.0108	0.0048	0.9075	0.6368	0.0035
GTxESxStage	3	0.4367	0.3205	0.1899	0.1594	0.1083
GTxRCxStage	3	<.0001	<.0001	0.0018	0.2304	0.2486
ESxRCxStage	3	0.4175	0.4515	0.7731	0.0749	<.0001
GTxESxRCxStage	4	0.1229	0.1914	0.0245	0.2818	0.0079

[1] Order 1 for the main effects and higher values for the corresponding order of interactions among effects. [2] Feed intake expressed as g DM/day. [3] Feed intake expressed as g DM/kg$^{0.75}$ per day. [4] OG the fixed effect to take into account the effect of being lactating during gestation. [5] OL the fixed effect to take into account the effect of getting pregnant during lactation.

Table S2 *P-Values for all the effects considered in the models used to analyse blood plasma traits*

Effect	Order[1]	*P-Value*			
		Glucose	BOHB	NEFA's	Leptin
Genetic Type (GT)	1	0.0029	0.0371	0.9234	0.0934
Energy source (ES)	1	0.3118	<.0001	0.1871	0.1778
Time control (R)	1	<.0001	<.0001	<.0001	<.0001
Temperature	1	0.0028	0.9517	0.9433	0.0363
GTxES	2	0.7243	0.5934	0.3868	0.9236
GTxR	2	0.0153	<.0001	<.0001	0.1174
ESxR	2	0.2451	<.0001	0.6879	0.8061
GTxESxR	3	0.4500	0.0073	0.7065	0.9684

[1] Order 1 for the main effects and higher values for the corresponding order of interactions among effects.

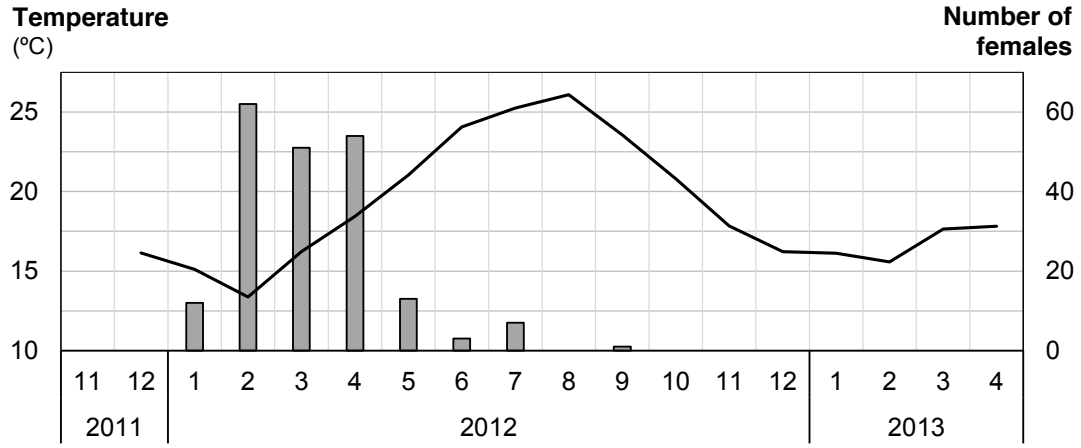

Figure S1 Inner-average temperature per month of the farm (black line) and number of females (grey bars) that had their first parturition in the corresponding month.