

1 **Litter Survival Differences between Divergently Selected Lines for**
2 **Environmental Sensitivity in Rabbits**

3 Ivan Agea¹, María-Luz García^{1*}, Agustín Blasco², María-José Argente¹

4 ¹*Departamento de Tecnología Agroalimentaria. Universidad Miguel Hernández*
5 *de Elche, Ctra de Beniel Km 3.2, 03312 Orihuela, Spain*

6 ²*Institute for Animal Science and Technology. Universitat Politècnica de València,*
7 *P.O. Box 22012. 46022 Valencia, Spain*

8 * Correspondence: mariluz.garcia@umh.es; Tel.: +34-966749707

9

10 **Simple summary:** Two rabbit lines are divergently selected for increasing or
11 decreasing the variability of litter size at birth. Decreasing litter size variability
12 produces more resilient females with less sensitivity to diseases, being an indirect
13 selection way for improving environmental sensitivity. Kits' survival at weaning
14 was higher in the homogeneous line. Moreover, this line lead to greater uniformity
15 of kits' weight at weaning, although the variability of weight at birth was higher,
16 which could be due to a higher lactation capacity of the homogeneous line.

17

18 **Abstract:** A divergent selection experiment on environmental sensitivity was
19 performed in rabbits. The aim of this study was to estimate the correlated
20 response in kits' weight and its survival, and weight distance from birth to
21 weaning. Weight distance was calculated as the absolute values of the
22 differences between the individual value and the mean value of its litter. Also,
23 relationship between probability of survival at 4 d of age and weight at birth was
24 studied. Environmental sensitivity was measured as litter size variability. A total
25 of 2484 kits from 127 does of the low line (selected for reducing litter size

26 variability) and 1916 kits of 114 does of the high line (selected for increasing litter
27 size variability) of the 12th generation were weighed. Bayesian methodology was
28 used to estimate the correlated response to selection, and LOGISTIC procedure
29 of SAS was used to estimate the relationship between weight and probability of
30 survival. Both lines showed similar individual weight at birth and at weaning, and
31 similar survival at birth and at 4 d of age. Survival at weaning was higher in the
32 low line than in the high line (0.67 and 0.62; P= 0.93). Weight distance was higher
33 at birth but lower at weaning in the low line (47.8 g and 54.1 g; P=0.98). Kit's
34 weight at birth affected its survival. In conclusion, selection for environmental
35 sensitivity showed correlated response in kits survival and in homogeneity of litter
36 weight at weaning.

37

38 **Keywords:** correlated response; pre-weaning; survival; weight; welfare

39

40 1. Introduction

41 The aim of genetic selection in maternal rabbit lines has traditionally been to
42 improve the mean of productive traits: litter size [1] or length of does' productive
43 life [2,3]. Overall, this intensive selection for increasing productivity has had
44 success but it has also had negative consequences on animal welfare, increasing
45 culling at early ages [4,5]. Consequently, resistance to disease and stress are
46 current priorities in rabbit breeding, also leading to better does resilience and
47 welfare.

48

49 Selection for environmental sensitivity, measured as litter size variability, is an
50 indirect selection methodology for improving resilience and robustness [6,7]. A

51 divergent selection experiment for this trait has been performed with success [6],
52 leading to lines with high and low litter size variability. Higher litter size variability
53 affects the heterogeneity of littermates, which can produce lower pre-weaning
54 survival [8,9]. The aim of this work is to study the correlated response in pre-
55 weaning survival in two rabbit lines divergently selected for environmental
56 sensitivity.

57

58 **2. Material and methods**

59 All experimental procedures involving animals were approved by the Miguel
60 Hernández University of Elche Research Ethics Committee (Reference number
61 2019/VSC/PEA/0017), in accordance with Council Directives 98/58/EC and
62 2010/63/EU.

63

64 *2.1. Animals*

65 A divergent selection experiment for litter size variability was carried out over
66 twelve generations. Selection was based on the phenotypic variance of litter size
67 within the doe, after correcting litter size for both year-season and parity-lactation
68 status [6].

69

70 All animals were reared in the farm of the Miguel Hernández University of Elche
71 (Spain). Rabbits were fed a standard commercial diet (17% crude protein, 16%
72 fiber, 3.5% fat, Nutricun Elite Gra ®, De Heus Nutrición Animal). Food and water
73 were provided *ad libitum*. Does were housed in individual cages (37.5 x 33 x 90
74 cm) under a constant photoperiod of 16 h continuous light: 8 h continuous
75 darkness and controlled ventilation throughout the experiment. They were first

76 mated at 18 wk of age and at 10 d after parturition thereafter. Matings took place
77 every week. The nest was made with textile by-products and the doe had free
78 access to the nest from 2 days before delivery until 21 days after delivery, when
79 the nest was removed. Litters were not standardised and kits were weaned at 28
80 days of age.

81

82 Data come from the 12th generation of selection. Litter size at birth (LS), number
83 of born alive (NBA), number of born dead (NBD), number of rabbits at 4 days of
84 age (N4), and number of rabbits at weaning (NW) were recorded. Rabbits were
85 individually weighed and sexed within 24 h after birth. Some kits had suckled
86 before being weighed. The milk intake was verified by recording a white mark in
87 the abdominal area. Kits were also weighed at weaning. A total of 2484 kits from
88 127 does of the low line and 1916 kits of 114 does of the high line were weighed.

89

90 2.2. *Traits*

91 The following traits were analysed: LS; survival at birth (NBA/LS); survival at 4
92 days of age (N4/NBA); survival at weaning (NW/N4); individual weight at birth of
93 live and dead kits; individual weight at weaning; weight distance of live, dead and
94 weaned rabbits. Weight distance was calculated as the absolute values of the
95 differences between the individual value and the mean value of its litter.

96

97 2.3. *Statistical analysis*

98 The model used for analysing LS and litter survivals were:

$$99 y_{ijkl} = \mu + L_i + S_j + LP_k + p_{ijkl} + e_{ijkl}$$

100 where L_i is the line effect with two levels (the high and the low lines); S_j is the
101 season effect with three levels (winter, spring and summer), LP_k is the lactation-
102 parity effect with five levels (nulliparous, lactating and non-lactating primiparous
103 doe, and lactating and no-lactating multiparous doe), p_{ijkl} is the dam permanent
104 effect with 241 levels, and e_{ijkl} is the residual term.

105

106 Individual weight at birth for live and dead kits, and their correspondence distance
107 were analysed using the following model:

$$y_{ijklmnop} = LK_i + S_j + LP_k + IM_l + SE_m + p_{ijklmn} + C_{ijklmn} + b^* LS_{ijklmn} + e_{ijklmnop}$$

109 where LK_i is the line-survival effect (live kits of the high line, dead kits of the high
110 line, live kits of the low line, and dead kits of the low line), IM_l is the intake of milk
111 effect (whether the kit suckled or not before being weighed), SE_m is the sex effect
112 (male and female), p_{ijklmn} is the dam permanent effect with 241 levels, C_{ijklmn} is
113 the common litter effect with 541 levels, b is the regression coefficient of the
114 covariate, LS_{ijklmn} is the covariate litter size and $e_{ijklmnop}$ is the residual term.

115

116 Individual weight at weaning and its distance were analysed with the same model,
117 but line effect with two levels (high and low lines) was used instead of line-survival
118 effect.

119

120 All analyses were performed using Bayesian methodology [10]. Bounded uniform
121 priors were used for all effects. The joint prior distribution for the permanent
122 environmental effect of the doe and the common litter effect was $N(0, I \otimes G_p)$,
123 where G_p was the (co)variance matrix between these effects. Residuals priori
124 distribution was $N(0, I \otimes \sigma^2_e)$. Residuals, permanent environmental effects and

125 common litter effects are uncorrelated. The priors for the variances were also
126 bounded uniform. Features of the marginal posterior distributions for all
127 unknowns were estimated using Gibbs sampling. The program TM was used [11].
128 We used a chain of 250,000 samples, with a burn-in period of 50,000. Only one
129 out of every 100 samples was saved for inferences. Convergence was tested
130 using the Z criterion of Geweke [12] and Monte Carlo sampling errors were
131 computed using time-series procedures [13].

132

133 The relationship between probability of survival from birth to 4 d of age and
134 individual weight at birth was analysed by logistic regression. The model included
135 line, season, parity-lactation (with 3 levels: nulliparous, lactating and no-lactating
136 does), milk intake, and sex effects. Table 1 shows the number of kits that
137 survived, classified by weight at birth and line. The LOGISTIC procedure of the
138 statistical package SAS was used [14].

139

140 **3. Results**

141 *3.1. Correlated response to selection in litter survival and preweaning weight*

142 Table 2 shows the features of the estimated marginal posterior distributions of
143 the differences between lines for litter survival, individual weight and weight
144 distances at birth and weaning. Litter size at birth was higher in the low line (H-L
145 = -0.6 kits; P = 1.0). Survival at birth and at 4 d of age were similar between lines,
146 but survival at weaning was 5% higher in the low line (P = 0.93). Both lines
147 showed similar individual weights of kits from birth to weaning. Weight distance
148 for live kits at birth was higher in the low line (H-L = -0.5 g; P = 0.97); however,
149 weight distance at weaning was lower in the low line (H-L = 6.3 g; P = 0.98).

150

151 **3.2. Survival at 4 d of age and individual weight at birth**

152 Probability of survival at 4 d of age and weight at birth were not affected by sex.

153 Both lines showed similar probabilities of survival at 4 d of age with the same

154 weight at birth (Figure 1). Probabilities of survival asymptotically increased with

155 individual birth weight, and raised more than a 90% from 60 g onwards.

156

157 Kits born in winter had less probability of survival than those born in summer or

158 spring (Figure 2). When the weight of kits was higher than 60 g at birth, the

159 probability of survival was maximum, no matter parity-lactation status of the doe

160 (Figure 3). The minimum survival took place in lactating does when weights

161 ranged from 30 to 60 g; non-lactating does showed the highest probability of

162 survival.

163

164 Kits that suckled had always a higher probability of survival than kits that did not

165 suckle (Figure 4). Kits with the minimum weight had a survival probability of 65%

166 when the rabbits suckled but only 35% if they did not suckle.

167

168 **4. Discussion**169 **4.1. Correlated response to selection in litter survival and preweaning weight**

170 Our divergent selection experiment for environmental sensitivity has showed that

171 this trait is genetically determined [6]. This has implications for animal welfare,

172 since animals that cope better with their environment have better welfare than

173 more sensitive animals [7]. After correcting for litter size, both lines had similar

174 individual weight at birth, and survival at birth and survival at 4 days of age were

175 not modified. Moreover, the relationship between probability of survival at 4 d of
176 age and the weight at birth was not affected by the line.

177

178 Weight distance has been used as dispersion measure instead of standard
179 deviation of weight of litter because it provides one record per individual instead
180 of one per litter. It seems that there is a correlated response on both, weight
181 distance at birth and at weaning, but with opposite sign; the kits' weight is more
182 variable at birth in the low line, but less variable at weaning. Up to now, there is
183 no information about weight distance at birth in rabbits, but similar value of weight
184 distance at weaning has been shown by Peiró *et al.* [15].

185

186 Maternal care in the first days after parturition is clearly related to the ingestion of
187 energy by the kits, which is directly related to survival [16]. So the higher survival
188 at weaning of the low line could indicate higher milk production and better
189 maternal behaviour during lactation. In spite of the greater variability of weight at
190 birth of the low line, the lactation capacity of the does produces a greater
191 uniformity of weight at weaning than in the high line. The homogeneity in weight
192 within litter is an important trait in prolific species like rabbits [17], because
193 increasing weight homogeneity within the litter reduces the competition between
194 littermates and increases the viability of them [18].

195

196 *4.2. Survival at 4 d of age and individual weight at birth*

197 Probability of individual survival at 4 d of age is related to birth weight, since the
198 kits with lower birth weight have lower probability of survival. Neonates require a
199 protective environment, adequate nutrition, and special maternal care, in order to

200 survive [19]. So the season of birth, the intake of milk and the parity-lactation
201 status of the doe affect the probability of survival. The probability of survival at 4
202 d of age was lower in winter than in spring and summer when weight at birth is
203 less than 50 g. If birth weight is less than the optimum weight, energy reserves
204 and thermoregulatory capacity are reduced and perinatal mortality increases [20].
205 If the temperature in the nest is low during their first 5 days of age, the
206 instantaneous energy production capacity of young rabbits is insufficient to
207 compensate for thermal losses through the skin and the probability of survival
208 decreases [21].

209

210 Fat tissue is high at birth and decreases thereafter [22]. Ingestion of milk
211 immediately after birth, allows the rabbit to save fat tissue and thus significantly
212 increase its chances of survival [23,24]. The lack of milk spot at birth increases
213 mortality of kits at 4 d of age, no matter the weight at birth. Similar results were
214 obtained at first week of age [24,25].

215

216 When lactation and gestation were overlapping, the probability of survival was
217 lower than in nulliparous and non-lactating does. It is well known the does
218 undergo a nutritional deficit when lactation and pregnancy overlap [26,27] that
219 affect the probability of kits' survival.

220

221 **5. Conclusions**

222 In conclusion, in spite of the greater variability of weight at birth of the low line,
223 the lactation capacity of these does produced a greater uniformity of weight at

224 weaning. Thus, selection for litter size variability shows a correlated response in
225 survival and uniformity in weights at weaning, without affecting individual weight.

226

227 **Author Contributions:** Conceptualization, M.L.G., M.J.A., A.B.; data curation,
228 I.A.; formal analysis: M.L.G., M.J.A.; funding acquisition, A.B., M.J.A.;
229 methodology, I.A., M.L.G., M.J.A.; writing and editing, I.A., M.L.G., A.B.

230

231 **Funding:** This study is supported by the Spanish Ministry of Economy and
232 Competitiveness (MINECO) with the Projects AGL2017- 86083, C2-1-P and C2-
233 2-P.

234

235 **Conflicts of interest:** The authors declare no conflict of interest.

236

237 **References**

- 238 1. García, M.L.; Baselga, M. Estimation of genetic response to selection in
239 litter size of rabbits using a cryopreserved control population. *Livest. Prod. Sci.* **2002**, *74*, 45-53.
- 241 2. Sánchez, J.P.; Theilgaard, P.; Mínguez, C.; Baselga, M. Constitution and
242 evaluation of a long-lived productive rabbit line. *J. Anim. Sci.* **2008**, *86*,
243 515-525.
- 244 3. Larzul, C.; Ducrocq, V.; Tudela, F.; Juin, H.; Garreau, H. The length of
245 productive life can be modified through selection: An experimental
246 demonstration in the rabbit. *J. Anim. Sci.* **2014**, *92*, 2395-2401.

247 4. Rauw W.M.; Kanis E.; Noordhuizen-Stassen E.N.; Grommers F.J.
248 Undesirable side effects of selection for high production efficiency in farm
249 animals: A review. *Livest. Prod. Sci.* **1998**, *56*, 15–33.

250 5. Rosell, J.M.; de la Fuente, L.F. Culling and mortality in breeding
251 rabbits. *Prev. Vet. Med.* **2009**, *88*, 120-127.

252 6. Blasco, A.; Martínez-Álvaro, M.; García, M.L.; Ibáñez-Escriche, N.;
253 Argente, M.J. Selection for genetic environmental sensitivity of litter size
254 in rabbits. *Genet. Sel. Evol.* **2017**, *49*, 48-55.

255 7. Argente, M.J.; García, M.L.; Zbynovska, K.; Petruska, P.; Capcarová, M.;
256 Blasco, A. Correlated response to selection for litter size environmental
257 variability in rabbits' resilience. *Animal* **2019**, DOI:
258 10.1017/S1751731119000302.

259 8. Bolet, G.; Esparbié, J.; Falieres, J. Relations entre le nombre de foetus par
260 corne utérine, la taille de portée à la naissance et la croissance pondérale
261 des lapereaux. *Ann. Zootech.* **1996**, *45*, 185-200.

262 9. Poignier, J.; Szendrő, Z.S.; Levai, A.; Radnai, I.; Biro-Nemeth, E. Effect of
263 birth weight and litter size on growth and mortality in rabbit. *World Rabbit
264 Sci.* **2000**, *8*, 103-109.

265 10. Blasco, A. *Bayesian data analysis for animal scientists*; Springer
266 International Publishing: New York, 2017.

267 11. Legarra, A.; Varona, L.; López de Maturana, E. TM. Threshold Model.
268 A variable online: <http://snp.toulouse.inra.fr/~alegarra/manualtm.pdf>
269 (accessed on 5 July 2019).

270 12. Sorensen, D.; Gianola, D. *Likelihood, bayesian, and MCMC methods.*
271 *Quantitative genetics*. 1st ed.; Springer-Verlag, New York, USA, 2002.

272 13. Geyer, C.M. Practical markow chain Monte Carlo (with discussion). *Stat.*
273 *Sci.* **1992**, *7*, 467-511.

274 14. SAS. *SAS/STAT User's Guide 9.4*. SAS Inst., Cary, NC, 2017.

275 15. Peiró, R.; Badawy, A.; Blasco, A.; Santacreu, M. Correlated responses on
276 growth traits after two-stage selection for ovulation rate and litter size in
277 rabbits. *Animal*, **2019**, doi:10.1017/S1751731119001423

278 16. Pascual, J.J.; Savietto, D.; Cervera, C.; Baselga, M. Resources allocation
279 in reproductive rabbit does: A review of feeding and genetic strategies for
280 suitable performance. *World Rabbit Sci.* **2013**, *21*, 123-144.

281 17. Bolet, G.; Garreau, H.; Joly, T.; Theau-Clement, M.; Falieres, J.; Hurtaud,
282 J.; Bodin, L. Genetic homogenisation of birth weight in rabbits: Indirect
283 selection response for uterine horn. *Livest. Sci.* **2007**, *111*, 28-32.

284 18. Garreau, H.; Bolet, G.; Larzul, C.; Robert-Granié, C.; Saleil, G.;
285 SanCristobal, M.; Bodin, L. Results of four generations of a canalising
286 selection for rabbit birth weight. *Livest. Sci.* **2008**, *119*, 55-62.

287 19. Hamilton, H.H.; Lukefahr, S.D.; McNitt, J.I. Maternal nest quality and its
288 influence on litter survival and weaning performance in commercial
289 rabbits. *J. Anim. Sci.*, **1997**, *75*, 926-933.

290 20. Vicente, J.S.; García-Ximénez, F.; Viudes de Castro, M.P. Neonatal
291 performance in 3 lines of rabbit (litter sizes, litter and individual weights). *Ann.*
292 *Zootech.* **1995**, *44*, 255-261.

293 21. Hull, D.; Segall, M.M. The contribution of brown adipose tissue to heat
294 production in the new-born rabbit. *J. Physiolo.* **1965**, *181*, 449-457.

295 22. Spencer, S.A.; Hull, D. The effect of over-feeding newborn rabbits on somatic
296 and visceral growth, body composition and long-term growth potential. *Br. J.*
297 *Nutr.* **1984**, *51*, 389-402.

298 23. Venge, O. The influence of nursing behaviour and milk production n early
299 growth in rabbits. *Anim. Behav.* **1963**, *11*, 500-506.

300 24. Coureauaud, G.; Schaal, B.; Coudert, P.; Rideaud, P.; Fortun-Lamothe, L.;
301 Hudson, R.; Orgeur, P. Immediate postnatal sucking in the rabbit: Its
302 influence on pup survival and growth. *Reprod. Nutr. Dev.* **2000**, *40*, 19-32.

303 25. Argente, M.J.; Santacreu, M.A.; Climent, A.; Blasco, A. Phenotypic and
304 genetic parameters of birth weight and weaning weight of rabbits born from
305 unilaterally ovariectomized and intact does. *Livest. Prod. Sci.* **1999**,
306 *57*, 159-167.

307 26. Xiccato, G.; Trocino, A.; Sartori, A.; Queaque, P.I. Effect of parity order and
308 litter weaning age on the performance and body energy balance of rabbit dos.
309 *Livest. Prod. Sci.* **2004**, *16*, 239-251.

310 27. Rebollar, P.G.; Pérez-Cabal, M.A.; Pereda, N.; Lorenzo, P.I.; Arias-
311 Álvarez, M.; García-Rebollar, P.G. Effects of parity order and reproductive
312 management on the efficiency of rabbit productive systems. *Livest. Sci.*
313 **2009**, *121*: 227-233.

314 Table 1. Number of kits by line effect and individual birth weight (g).

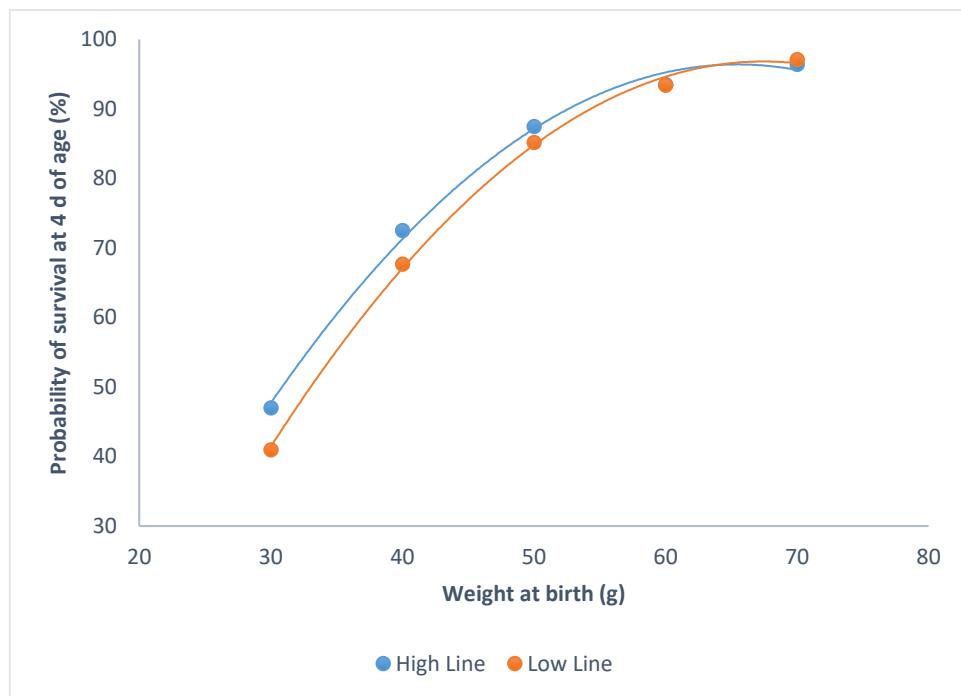
	<30	40	50	60	>70
Line H	73	316	644	494	234
Line L	128	239	756	661	338

315

316 Table 2. Features of the marginal posterior distribution of the differences between the
 317 high and the low litter size variability lines for litter size at birth, survival, individual
 318 weight and weight distance before weaning.

	H	L	H-L	HPD _{95%}	P
Litter size at birth	7.7	8.3	-0.6	-1.1; -0.2	1.0
Survival					
At birth	0.89	0.87	0.02	-0.03; 0.06	0.79
At 4 days of age	0.88	0.87	0.01	-0.04; 0.05	0.67
At weaning	0.62	0.67	-0.05	-0.12; 0.01	0.93
Individual weight					
Live at birth (g)	53.5	54.1	-0.4	-1.7; 0.8	0.75
Dead at birth (g)	46.3	46.1	-0.2	-2.4; 1.9	0.60
At weaning (g)	495	480	15	-17; 47	0.82
Weight distance					
Live at birth (g)	4.9	5.4	-0.5	-0.9; 0.0	0.97
Dead at birth (g)	7.0	6.8	0.2	-0.9; 1.3	0.68
Weaned (g)	54.1	47.8	6.3	0.2; 12.3	0.98

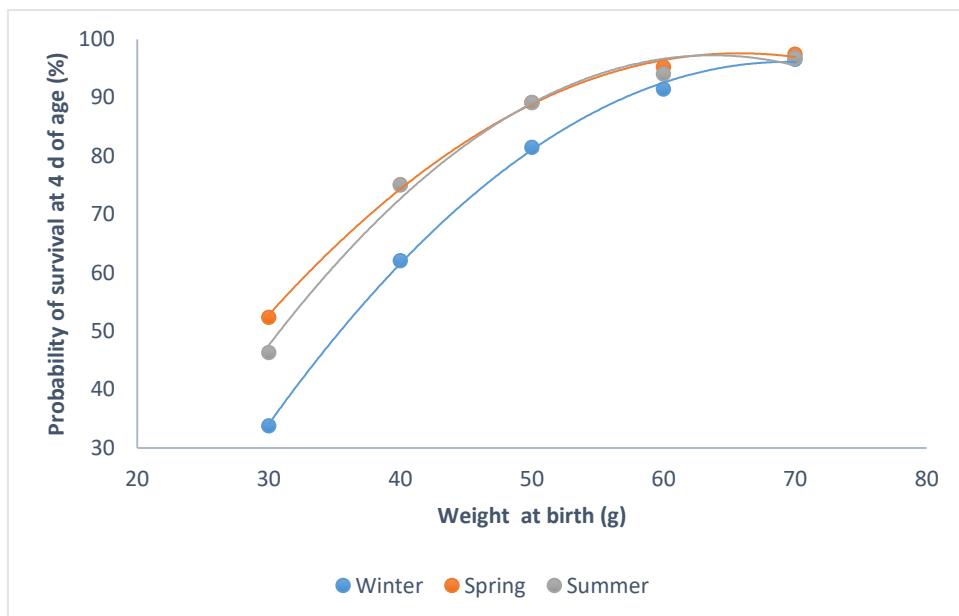
319 H = median of the high line; L = median of the low line; H-L = median of the difference between
 320 the high and the low lines; HPD_{95%} = Highest posterior density region at 95%; P = probability of
 321 the difference being >0 when H-L > 0 and probability of the difference being < 0 when H-L < 0



322

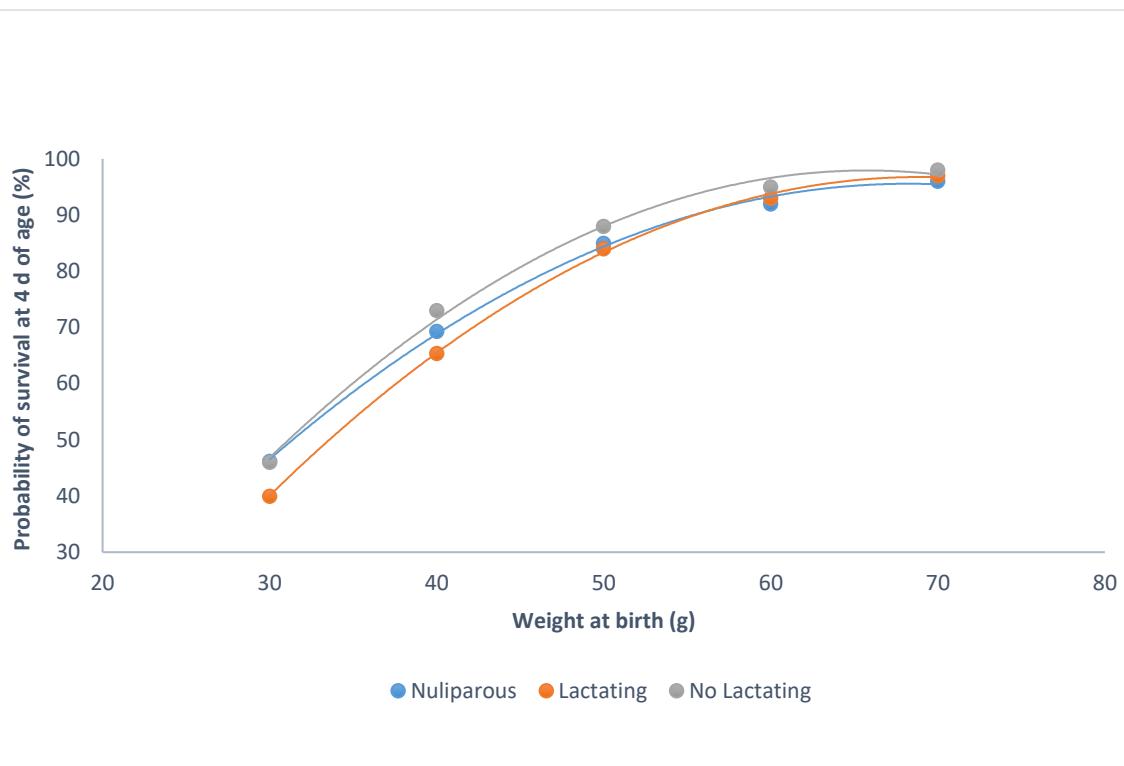
323 Figure 1. Relationship between survival at 4 d of age and individual birth weight

324 for the high and the low litter size variability lines



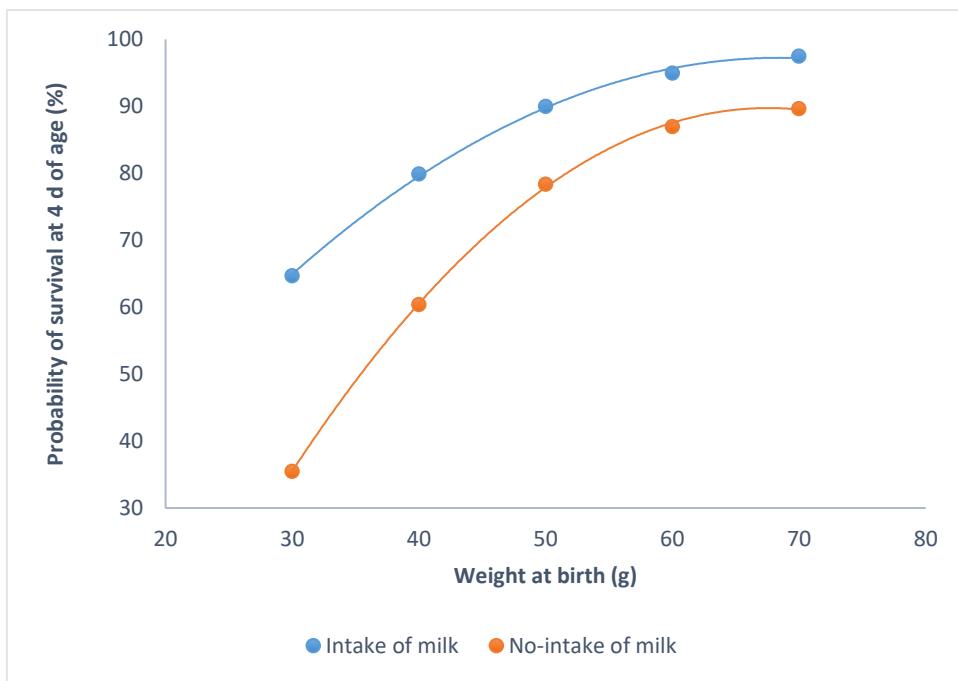
325

326 Figure 2. Relationship between survival at 4 d of age and individual birth weight
327 for the seasons.



328

329 Figure 3. Relationship between survival at 4 d of age and individual birth weight
330 for the parity-lactation status.



331

332 Figure 4. Relationship between survival at 4 d of age and individual birth weight
333 for the milk intake effect.