

1 **Residual feed intake in dairy ewes: an evidence of intraflock variability**

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9 Abstract

10 This study examined the intraflock variability of feed efficiency in dairy ewes, through
11 monitoring residual feed intakes (RFI). Primiparous lactating ewes ($n=43$; 57.7 ± 0.91 kg body
12 weight [BW] at lambing), representative of a French Lacaune dairy flock, were allocated in an
13 equilibrated 2×2 factorial design experiment, lasting for 63 days during mid-lactation and
14 combining 2 litter sizes (singletons, SING or twins, TWIN) and 2 daily milking frequencies (once,
15 ONE or twice, TWO). Ewes were individually fed a diet based on ryegrass silage, local hay and
16 supplements. Individual DMI was recorded daily and further used to evaluate (and compare)
17 differences in RFI between ewes at 35, 42, 49, 56, 63, 70, 77, 84, 91 and 98 days relative to
18 lambing (DIM). Total (BW) and metabolic ($BW^{0.75}$) body weight, BCS, milk yield and plasma
19 NEFA were monitored weekly. Differences in DMI were mainly due to the lactation stage and
20 litter size and were 11% higher in ewes with TWIN compared to SING. This was positively
21 correlated to milk yield and consistent with differences in RFI which varied due to litter size and
22 to the milking frequency \times lactation stage interaction. Ewes that lambed SING showed higher feed
23 efficiency ($\square 0.13\pm 0.020$ vs. 0.08 ± 0.015 kg DM/ewe/d of RFI in SING vs. TWIN, respectively),
24 whereas there was no differences in BW or BCS. Milking frequency did not affect DMI but milk
25 yields were higher in TWO, which was related to a higher feed efficiency in this group
26 (0.04 ± 0.017 vs. $\square 0.10\pm 0.018$ kg DM/ewe/d of RFI in ONE vs. TWO, respectively). Average RFI
27 was affected ($P < 0.0001$) by the ewe, thus allowing a ranking among individuals to be established.
28 High ($n=22$) or low ($n=21$) feed efficiency ewes averaged $\square 0.17\pm 0.09$ or 0.18 ± 0.09 kg DM/d RFI,
29 respectively. Estimates of RFI were not correlated to the individual milk production potential.
30 Even if no differences in BW, $BW^{0.75}$ or BCS were detected, high efficiency ewes mobilised
31 almost two-fold their body reserves when compared to the low efficiency group. The observed
32 intraflock variability in feed efficiency of this dairy ewes flock was affected by litter size and
33 milking frequency but also by evident differences between individuals' physiologies.
34 **Keywords:** feed efficiency; residual feed intake; lactating dairy ewes; intraflock variability

35 **Introduction**

36 It is well known that feed accounts for most of the total farm expenses in animal production
37 systems and that a possible solution to lessen overall feed costs and alleviate the associated
38 negative environmental impacts is to select for feed efficiency traits. In the past, producers have
39 primarily focused on feed conversion ratios; however, animals with similar ratios differ in feed
40 intake and productivity. As an alternative, Koch *et al.* (1963) proposed selecting residual feed
41 intake (RFI), sometimes referred to as net feed intake. Considered the deviation of actual intake
42 from the predicted intake for a given measure of growth (ADG) and body weight, RFI can be used
43 to compare individuals with the same or differing levels of production during the period of
44 measurement.

45 In contrast to feed conversion, selection based on RFI seems to select for lower rates of
46 consumption and animal maintenance requirements than contemporaries to yield the same amount
47 of product without changing adult weight or rate of gain, so theoretically these animals should cost
48 less to feed on a daily basis when the costs of all other maintenance factors for these animals
49 (breeding, health, etc.) are held constant (Bezerra *et al.*, 2013; Potts *et al.*, 2015). Heritability of
50 RFI has been reported to be moderate, e.g. 0.06 to 0.24 depending on lactation stage (Tempelman
51 *et al.*, 2015) in dairy cattle e.g. 0.32–0.33 (Gonzalez-Recio *et al.*, 2014; Veerkamp *et al.*, 1995).
52 Nevertheless, five major physiological processes are likely to contribute to variations in RFI, with
53 these processes being associated with the intake and digestion of feed, metabolism (anabolism and
54 catabolism associated with and including variation in body composition), physical activity, and
55 thermoregulation (Herd and Arthur, 2009).

56 Thus, there is growing interest among producers with respect to using RFI as a tool for
57 genetic improvement, with a greater experience in swine (Patience *et al.*, 2015) and poultry;
58 (Aggrey and Rekaya, 2013), numerous research efforts have investigated the effectiveness of
59 selecting for feed efficiency using RFI in beef cattle (Fitzsimons *et al.*, 2014; Gomes *et al.*, 2012),
60 dairy cattle (Green *et al.*, 2013; Potts *et al.*, 2015; Pryce *et al.*, 2014) or sheep (Cockrum *et al.*,

61 2013; Meyer et al., 2015; Redden et al., 2013). The sheep industry, however, has yet to fully
62 investigate the potential impacts associated with selecting for RFI on carcass merit, growth traits,
63 reproduction traits, and fleece characteristics (Cockrum et al., 2013). In the particular case of the
64 dairy sheep sector, to our knowledge, there is no available information on RFI. Beyond its
65 economic attractiveness, evaluating factors affecting intraflock variability in feed efficiency, also
66 contributes to increasing our knowledge regarding the available spectrum of adaptive capacities
67 that can be found at the intraflock level, when the interpretation of individual RFI is combined
68 with other physiological processes like body reserves mobilisation-accretion.

69 The objective of this study was to evaluate factors affecting the intraflock variability in the feed
70 efficiency of individually fed primiparous Lacaune dairy ewes, by focusing in the analysis of
71 individual differences in RFI during several weeks in mid-lactation. We evaluated the hypothesis
72 that variability in feed efficiency of individuals belonging to the same breed, cohort, productive
73 purpose, with similar age, and reared under identical conditions, could be due to non-genetic
74 factors but also to differences between the individuals. A second hypothesis was that mechanisms
75 responsible for differences in RFI among individuals would probably be related to those linked to
76 the use of body reserves.

77

78 **Materials and methods**

79 *2.1. Animals, management, treatments, and measurements*

80 The experiment was carried out with a representative flock of primiparous Lacaune dairy ewes
81 belonging to the INRA Experimental Farm La Fage, Causse du Larzac (43°54'54.52"N;
82 3°05'38.11"E; ~800 m above sea level), Aveyron, France, following the procedures approved by
83 the Regional Ethics Committee on Animal Experimentation, Languedoc-Roussillon (France),
84 Agreement 752056/00.

85 A detailed description of the animals, management and experimental design used for collecting
86 the data employed in this study can be obtained from González-García et al. (2015). We used the

87 data belonging to the primiparous (PRIM) group, considering that they were individually fed.
88 Briefly, forty-three Lacaune dairy ewes (PRIM; two-tooth ewes) were chosen from the main flock
89 at the end of pregnancy. Lambing took place in January (mean lambing date was 12 January) with
90 a mean body weight (BW) of 57.7 ± 0.91 kg. Litter size was determined about 2 months before
91 lambing, by obtaining an ultrasound image for each ewe in the flock. The effects of two
92 contrasting daily milking frequencies (once, ONE vs. twice, TWO) were evaluated.
93 Approximately one half of the experimental flock was submitted to the ONE milking regime
94 whereas their performance was compared to the other group, which was submitted to the
95 conventional milking frequency (TWO).

96 The experimental ewes were thus distributed in homogeneous groups according to BW, BCS,
97 and litter size and were allocated to a 2×2 factorial design according to litter size (lambing
98 singletons [SING], $n = 16$ or twins [TWIN], $n = 32$) and daily milking frequency (FREQ; ONE,
99 $n=24$; or TWO, $n=24$). Thus, the design finally comprised 4 randomly assigned balanced groups
100 for which the mean BW at lambing was as follows: (1) SING×ONE ($n=8$; 57.2 ± 2.46 kg); (2)
101 SING×TWO ($n=8$; 59.7 ± 2.47 kg BW); (3) TWIN×ONE ($n= 16$; 58.5 ± 1.62 kg BW); (4)
102 TWIN×TWO ($n=16$; 56.1 ± 1.44 kg).

103 Ewes were housed in confinement in straw-bedded pens and had access to an individual
104 feeding post controlled by an electronic device that allowed each animal to get into its correct
105 place using individual electronic identification (IDE). Each ewe-lamb was equipped with an IDE
106 ear tag that recorded its presence at the feed bunk and allowed (or not) access to the individual
107 feeder. When a ewe approached the feed bin, the unique passive ear transponder was identified,
108 the barrier was unlocked, and the animal was allowed access to the feed.

109 Ewes were thus individually fed with a standard *ad libitum* lactating total mixed ration for dairy
110 ewes composed of a 55% dry matter (DM) ryegrass silage, 18% hay (28% second cut alfalfa-
111 cocksfoot and 72% of a third harvest local hay called *Foin de Crau*, composed of a multiple
112 mixture of grasses, legumes, and other species), 13% barley grain, 9% dehydrated alfalfa

113 (*Luzapro*, 26.5% crude protein), and 6% commercial concentrate (*Brebitane*, 46% crude protein).
114 The total mixed ration was offered twice daily, one-third in the morning and two-thirds in the
115 afternoon, at about 9 AM and 6 PM, respectively. Its distribution was adjusted to an allowance
116 rate of 115% of the previous day's voluntary intake. In addition, 90 g DM of *Brebitane* was
117 offered at each milking in the milking parlour, or twice this amount in the morning for the group
118 that was milked once a day. Ewes had free and continuous access to fresh water and salt block.

119 2.2 Determination of residual feed intake (RFI) and monitoring related zootechnical and 120 metabolic parameters

121 The quantities of feed offered and refused were recorded daily in order to determine the
122 individual daily actual feed intake and thus the daily dry matter intake (DMI) per ewe. Average
123 DMI was thus individually calculated weekly, and further used to evaluate (and compare)
124 individual RFI per ewe. Expected feed intake was calculated based on the INRA table
125 recommendations for dairy sheep, according to their lactation stage, BW and overall physiological
126 requirements (Hassoun and Bocquier, 2014). The RFI was calculated as the difference between the
127 expected feed intake (Hassoun and Bocquier, 2014) and the actual, individually measured feed
128 intake in the experiment.

129 Measurements of RFI were scheduled at 35, 42, 49, 56, 63, 70, 77, 84, 91 and 98 days relative
130 to lambing (or days in lactation, DIM). Around each sampling date, ewes were individually
131 weighed, BCS was assessed by trained observers using the 5-point scale proposed by Russel et al.
132 (1969) and a blood sample was taken before the first meal distribution for metabolic profile (i.e.
133 plasma non-esterified fatty acids, NEFA; González-García et al., 2015). Here, we use data
134 referring to the plasma concentration of NEFA, as an indicator of body reserves mobilisation.

135 Ewes were milked twice daily at 8 AM and 5 PM. Machine milking was performed in a double-
136 24 stall parallel milking parlour. Milk yield and milk composition (fat and protein content) were
137 monitored and standardised milk yield (SMY) calculated (Bocquier et al., 1993).

138

139 2.3 Data processing and statistical analyses

140 In the first step, the effects of major sources of variation i.e. lactation stage (35–98 DIM), litter
141 size, milk frequency, and their first-order interactions on the main variables of interest i.e. RFI
142 (kg/ewe/day) and other zootechnical parameters (i.e. dry matter intake –DMI-, RFI, and actual or
143 standardised milk yields –SMY-), linked to the feed efficiency of these primiparous Lacaune dairy
144 ewes, were determined by using the PROC MIXED procedure of SAS (SAS; v. 9.1.3.,
145 2002–2003 by SAS Institute Inc., Cary, NC, USA) with the following statistical model:

$$146 Y_{ijk} = \mu + LitSi_i + Ewe_{ij} + DIM_k + Freq_l + (LitSi \times DIM)_{ik} + (LitSi \times Freq)_{il} + \varepsilon_{ijk}$$

147 where Y_{ijk} is the response at time k on ewe j with litter size i , μ is the overall mean, $LitSi_i$ is a
148 fixed effect of litter size i ($i = 1-2$), Ewe_{ij} is a random effect of ewe j with litter size i , DIM_k is a
149 fixed effect of time or days relative to lambing (DIM; 35–98) k , $Freq_l$ is a fixed effect of daily
150 milking frequency l ($l = 1-2$), $(LitSi \times DIM)_{ik}$ is a fixed interaction effect of litter size i with time k ,
151 $(LitSi \times Freq)_{il}$ is a fixed interaction effect of litter size i with daily milking frequency l and ε_{ijk} is
152 random error at time k on ewe j with litter size i .

153 In a second step, and after the determination of RFI, ewes were classified into high or low feed
154 efficiency individuals based on their distribution in this experimental population, when
155 considering their average RFI values determined for the whole experimental period (i.e. from 35
156 to 98 DIM). The analysis of variance (developed in the first step), allowed the level of variation at
157 each significant intra-factor level to be analysed in detail with regard to the main variable of
158 interest i.e. feed efficiency through RFI. Using the PROC RANK of SAS, the average ranking of
159 the individual ewes for the RFI variable was established. The same procedure allowed the
160 experimental ewes to be classified as high, medium and low milk producers. The last allowed a
161 relationship between the individual RFI and the SMY potential of each ewe to be established
162 using the PROC REG of SAS. The dependency of feed efficiency from milk yield potential was
163 thus analysed.

164 In the final step, once the ewes were classified as tending to belong to the high or low feed
165 efficiency groups, the relationships between the RFI and the average total (BW) or metabolic body
166 weight (BW^{0.75}), BCS and plasma NEFA profile were evaluated using the PROC GLM procedure
167 of SAS. The general statistical model used for this was as follows:

$$168 \quad Y_{ij} = \mu + FEffic_i + \varepsilon_{ij}$$

169 where Y_{ij} is the observation, μ , the population mean, $FEffic_i$, the feed efficiency rank effect ($i =$
170 1–2; low or high) and ε_{ij} is the residual error.

171 For all traits, the experimental unit was considered the ewe, as they were individually fed and
172 included in the model as a random effect. Significance was declared at probability levels of $\leq 5\%$
173 and comparisons between means were tested with the least squares means (LSMeans) separation
174 procedure using the PDIF option of SAS.

175

176 **Results**

177 The statistical significance of the lactation stage, litter size, milking frequency and first-order
178 interactions on DMI, milk yield and RFI are presented in Table 1. Observed differences in DMI
179 were mainly due to the lactation stage (DIM) and litter size effects, but not because of changes in
180 milking frequency *per se*. The effects of milking frequency on DMI depended on its interaction
181 with litter size. Similarly to milk yields, RFI was strongly affected ($P < 0.0001$) by the three major
182 sources of variation evaluated here (i.e. DIM, litter size and milking frequency), and by the
183 interaction milking frequency \times lactation stage. A similar tendency was observed for SMY (Table
184 1).

185 Average DMI during the evaluated mid-lactation period was 11% higher in ewes that lambed
186 twins when compared to those lambing singletons, and was positively correlated (data not shown)
187 to the total or SMY milk yields (Table 2). Differences ($P < 0.0001$) in RFI were also found
188 between ewes that lambed SING and TWIN ($\square 0.13 \pm 0.020$ vs. 0.08 ± 0.015 , respectively; Table 2).
189 Thus, despite a lower milk yield, ewes that lambed singletons converted feeds more efficiently.

190 Milking frequency did not affect DMI (Table 1) but, as expected, the actual or SMY milk
191 yields were higher in ewes being milked twice (Table 2) which was related to a higher overall feed
192 efficiency in this group for the whole experimental period, as interpreted by differences in RFI
193 (0.04 ± 0.017 vs. 0.10 ± 0.018 in ewes milked once vs. twice a day, respectively; Table 2).

194 Those effects of milking frequency on feed efficiency were dependent on the lactation week
195 (DIM; Figure 1) (see also significant interactions between milking frequency and DIM on RFI;
196 Table 1). Only at 35 DIM, RFI was lower in ewes milked once daily when compared to those
197 milked twice. This tendency changed from the second week (42 DIM) until the end of the
198 experiment (98 DIM), the period during which ewes belonging to the group TWO were always
199 more efficient, as interpreted by their lower RFI, with regard to ewes from the group ONE (Figure
200 1).

201 The average RFI for the whole experimental period was significantly ($P < 0.0001$) affected by
202 the ewe itself. As a consequence, ewes were ranked as having a tendency to high or low feed
203 efficiency as a function of their average RFI (Figure 2). Ewes classified as high feed efficiency
204 ewes ($n=22$) averaged 0.17 ± 0.09 kg DM/d of RFI; whereas, on the other hand, ewes classified
205 as low feed efficiency ewes ($n=21$) showed an average RFI value of 0.18 ± 0.09 kg DM/d.

206 The expected RFI was independent of the individual milk production potential (Figure 3). In
207 more than half of the cases, ewes classified as tending to be high feed efficiency ewes (left side
208 panel of Figure 2) corresponded to ewes submitted to two milking per day (13 ewes in TWO and 9
209 in ONE vs. 6 in TWO and 15 in ONE in high vs. low efficiency groups, respectively; Table 3).

210 Interestingly, and even if no differences in BW, $BW^{0.75}$ or BCS were detected, high efficiency
211 ewes mobilised almost two-fold their body reserves when compared to the low efficiency group
212 (see and compare plasma NEFA values in Table 3). The latter probably supported a higher energy
213 requirement for milk production, considering the larger proportion of ewes being milked twice
214 (TWO) in the high efficiency group. However, three of the four most efficient ewes that lambed
215 singletons and were milked once a day, which illustrate the fact that intraflock variability in feed

216 efficiency is also affected by differences in individual natures among animals belonging to the
217 same breed, cohort and receiving the same management, and their implicit, not well known related
218 mechanisms.

219

220 **Discussion**

221 Evaluating factors affecting intraflock variability of feed efficiency, through RFI, increases our
222 knowledge regarding the available spectrum of adaptive capacities which can be found at the
223 intraflock level; this becomes more interesting when the interpretation of RFI is combined with
224 other physiological processes like body reserves mobilisation-accretion. However, the exercise is
225 also interesting from an economic point of view for the industry in question since the
226 identification of animals that require less feed for normal production would clearly increase
227 overall farm productivity, thus leading to the argument that feed conversion efficiency of farm
228 animals could be considered an important component of the profitability of farming systems
229 (Cockrum et al., 2013; Pryce et al., 2014; Redden et al., 2013; Williams et al., 2011).

230 The RFI quantifies efficiency within a production level and allows for the identification of
231 animals that convert gross energy into net energy more efficiently by reducing energetic losses in
232 faeces, urine, gas, and nonmaintenance heat; thus, this is independent of the dilution of
233 maintenance when multiple of maintenance is calculated based on requirements for observed
234 production (Potts et al., 2015).

235 Currently, there are several reports arguing the interest, pertinence and possibilities of using
236 RFI as a selection characteristic to increase feed efficiency and farm profitability in non-ruminants
237 (Patience et al., 2015), but also in ruminants (beef: Fitzsimons et al., 2014; Gomes et al., 2012;
238 dairy: Green et al., 2013; Potts et al., 2015; Pryce et al., 2014). There is a lack of information,
239 however, in small ruminants, although some works have been developed mainly during the growth
240 phase in sheep (Cockrum et al., 2013; Meyer et al., 2015; Redden et al., 2013) and the sheep

241 industry has yet to fully investigate the potential impacts associated with selecting for RFI on
242 carcass merit, growth traits, reproduction traits, and fleece characteristics (Cockrum et al., 2013).

243 In the dairy sheep industry, to our knowledge there is no available information on RFI studies.
244 Our work and results could thus be considered original in that sense. The question of using RFI as
245 a tool for increasing feed efficiency in the future dairy ewe' industry remains.

246 Here, we evaluated different factors with the potential for affecting feed intake i.e. litter size at
247 lambing and during the suckling period, milking frequency and lactation stage. However, we were
248 also able to confirm evidence for individually intrinsic factors leading to differences in feed
249 efficiencies at the intraflock level in ewes belonging to the same dairy breed, cohort, with the
250 same age and reared under identical conditions, using the same day to day management and
251 feeding.

252 Differences between the energy requirements of ONE and TWO milking frequencies and ewes
253 lambing SING or TWIN were great enough to cause significant differences in energy partitioning,
254 which were translated into differences in milk yield and feed intake. Thus, our findings provide
255 support for the use of RFI as a tool to identify animals that eat less than others within a production
256 level, independent of the energy balance or the related management practice.

257 Although some re-ranking occurred, this was minor, so that within a level of production, ewes
258 that eat less than their contemporaries when receiving a particular management (e.g. milked once
259 daily) should also consume less than their contemporaries when returning to the average
260 management of the flock (e.g. being milked twice a day).

261 We also verified that RFI was independent of the individual milk production potential (Figure
262 3). Thus, we could assume that ewes with low RFI required less feed to produce the same amount
263 of product as their contemporaries, independently of their milk production potential. Consistent
264 with these results, the most feed-efficient ewes ($n = 22$) ate ~ 350 g of DM/d less than the least
265 efficient ewes in our study (i.e. 0.17 ± 0.09 vs. 0.18 ± 0.09 kg DM/d of RFI in high and low feed
266 efficiency ewes, respectively).

267 Potts et al. (2015) reported that RFI was moderately repeatable across 2 consecutive feeding
268 periods for replacement beef heifers classified as high (>0.5 SD), medium (± 0.5 SD), and low
269 (<-0.5 SD) RFI. These authors argued that the estimation of RFI across different periods may be
270 more repeatable if measurements are obtained from periods when animals were in similar
271 physiological states. There is little to no information available on those effects in cattle or sheep,
272 but species may differ in their response to RFI selection (Cockrum et al. (2013).

273 Here, we focused on the mid-lactation of this Lacaune dairy flock, an important period on
274 which a relative stabilisation is achieved; thus, comparison among individuals becomes feasible.
275 The weekly estimates of RFI were also repeatable within and across group of ewes in the design,
276 which suggests that we were able to account for many of the production and BW changes that
277 occurred from one week to the next, affecting the calculation of RFI. However, genotype \times
278 environment interactions may be an important factor to take into account.

279 While upwards of 60 d of feed intake measurements are needed to accurately estimate RFI in
280 beef cattle (Sainz and Paulino, 2004), the necessary duration in sheep is unknown (Cockrum et al.,
281 2013). In our study, we chose a period (63 days) similar to that recommended by Sainz and
282 Paulino (2004) which also fits well in the range indicated by Cockrum et al. (2013) (42–63d) for
283 determining RFI in sheep. The last authors confirmed that both the variance and the R^2 of their
284 results in rams provide support that a period of 40–63 d is needed to accurately determine
285 individual RFI values in sheep.

286 The current study contributes to the literature on the relationship between RFI and productive
287 performance in dairy ewes offered a forage diet. To date, the majority of studies examining this
288 area in sheep have focused on growing or finishing animals offered energy-dense diets.

289 Similar to reports in beef cattle, our RFI results varied widely in dairy ewes (see standard errors
290 in Figure 2). This variation may be attributed to individual differences in heat production from
291 metabolic processes, body composition, and physical activity; these factors account for around
292 70% of the variation in RFI (Herd and Arthur, 2009), but were not measured in this study.

293 Results indicated that BW, BCS and milk production potential are phenotypically independent
294 of RFI estimates but further research is necessary to determine the relative weighting value for
295 RFI in successive physiological states before it can successfully be considered a potential
296 selection tool.

297 Based on their results, Cockrum et al. (2013) recommended that, ideally, selection decisions for
298 RFI should take place at weaning, and feed efficiency status should be applicable over an animal's
299 lifetime.

300 Fitzsimons et al. (2014) found changes in backfat thickness, which were negative for low-RFI
301 cows and positive for high-RFI cows. The reduction in backfat thickness in low-RFI cows
302 suggested that these cows were mobilising more body fat to meet their nutritional requirements
303 during pregnancy than high-RFI cows. These authors also suggested that the calculation of RFI in
304 beef cows should include body composition traits such as subcutaneous body fat and BCS.

305 Potts et al. (2015) also argued that because body energy changes are accounted for in the
306 prediction of RFI, it is expected that cows with low RFI will not be any more likely to mobilise
307 body tissue to support production than cows with high RFI. The independence of RFI from BW
308 loss is important because excessive tissue mobilisation can lead to negative energy balance, which
309 is related to metabolic diseases and poor fertility.

310 Our results are in agreement with statements relating feed efficiency with body reserves
311 utilisation. We found a positive correlation between RFI and the profile of body reserves
312 mobilisation, analysed through regular monitoring of plasma NEFA, with the low-RFI ewes
313 consistently showing higher plasma NEFA. Even if no differences in BW, $BW^{0.75}$ or BCS were
314 detected, high efficiency ewes mobilised almost two-fold their body reserves when compared to
315 the low efficiency group. In the available literature, we did not find any previous report
316 concerning the direct relationships between efficiency in body reserves administration and RFI in
317 small ruminants and particularly in dairy sheep, as evidenced in the current study.

318

319 **Conclusions**

320 Under the conditions of this experiment, low-RFI lactating dairy ewes ate less and produced
321 similar levels when compared to high-RFI cohorts, without changing body weight or BCS; thus,
322 they could be said to use their feed more efficiently. The observed intraflock variability in feed
323 efficiency is probably the consequence of indirect factors affecting metabolism and energy
324 balance of ewes, like litter size and milking frequency, but it is also affected by differences among
325 the individuals' natures. However, entry into different physiological stages may present some
326 challenges and more research will be needed to investigate the long-term applicability of the RFI
327 estimates found here. Finally, this is probably the first report demonstrating a close relationship
328 between RFI and body reserves mobilisation in small ruminants, and particularly in dairy ewes.

329

330 **Acknowledgements**

331 The authors are grateful to the technical staff of La Fage experimental farm unit for assisting
332 with animal care, monitoring tasks and data collection.

333 **Conflict of interest statement**

334 There are no conflicts of interest associated with this publication, and there has been no
335 significant financial support for this work that could have influenced its outcome.

336

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386 **Table 1.** Significance (*P* value) of the fixed effects lactation stage (days in milk, DIM), litter
 387 size (LS), milking frequency (MF) and their interactions on residual feed intake (RFI,
 388 kg/ewe/day) and other zootechnical parameters linked to feed efficiency of primiparous
 389 Lacaune dairy ewes during lactation (35–98 DIM).

	DIM	Litter size	Milking frequency	First-order interactions across major fixed effects			
				LS×MF	LS×DIM	MF×DIM	LS×MF×DIM
DMI	0.0165	<.0001	0.2694	0.0030	0.9926	0.9748	0.8628
MY	<.0001	<.0001	<.0001	0.0428	0.9880	0.2395	0.9814
SMY	<.0001	0.0105	<.0001	0.0052	0.9837	0.0187	0.9413
RFI	<.0001	<.0001	<.0001	0.3539	0.7881	<.0001	0.2485

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392 **Table 2.** Effects of litter size and milking frequency on dry matter intake (DMI, kg/ewe/day),
 393 actual or standardised (SMY) milk yields (l/ewe/day) and residual feed intake (RFI, kg/ewe/day)
 394 of individually fed primiparous Lacaune dairy ewes at mid-lactation (35–98 DIM).

Item	Litter size		Milking frequency	
	SING	TWIN	ONE	TWO
Dry matter				
intake (DMI, kg/ewe/day)	2.14±0.028	2.38±0.021	2.28±0.024	2.24±0.025
Milk yield (kg/ewe/day)	1.59±0.032	1.77±0.024	1.57±0.028	1.80±0.029
Standardised milk (SMY, L/ewe/day)	1.37±0.024	1.45±0.018	1.32±0.021	1.50±0.022
RFI (kg DM/ewe/day)	-0.13±0.020	0.08±0.015	0.04±0.017	-0.10±0.018

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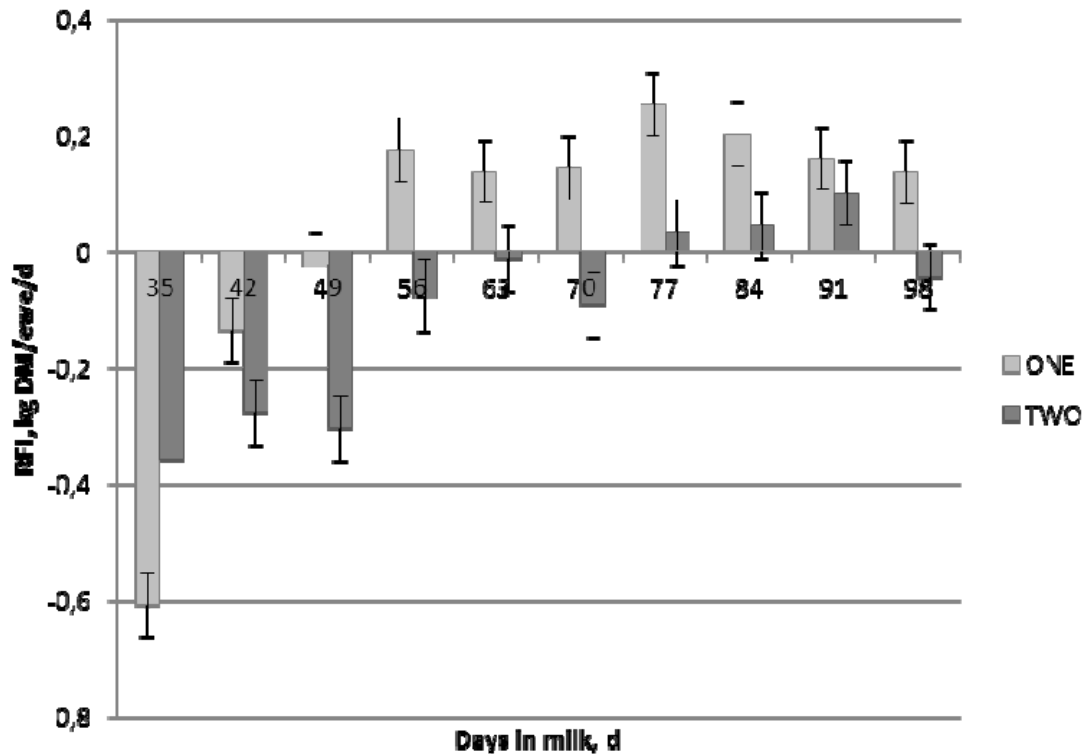
397 **Table 3.** Relationships between residual feed intake (RFI) and average total (BW) or metabolic
 398 body weight ($BW^{.75}$), body condition score (BCS) and plasma non-esterified fatty acids (NEFA)
 399 profile in individually fed primiparous Lacaune dairy ewes at mid-lactation (35–98 DIM). Ewes
 400 were classified as presenting high or low feed efficiency in accordance to their average RFI during
 401 the 8 week period. NS = non-significant; LS = litter size; MF = milking frequency.

Item	Feed efficiency of ewes, according to RFI ranking				Effect, <i>P</i> value
	High		Low		
	LSmean	S.E.M. (\pm)	LSmean	S.E.M.	
BW, kg	59.0	1.46	56.3	2.81	NS
$BW^{0.75}$, kg	21.3	0.39	20.5	0.99	NS
BCS, 1–5	2.88	0.035	2.77	0.132	NS
NEFA, $\mu\text{mol/L}$	0.39	0.017	0.22	0.016	<.0001
LS distribution	13 SING; 9 TWIN		3 SING; 18 TWIN		
MF distribution	9 ONE; 13 TWO		15 ONE; 6 TWO		

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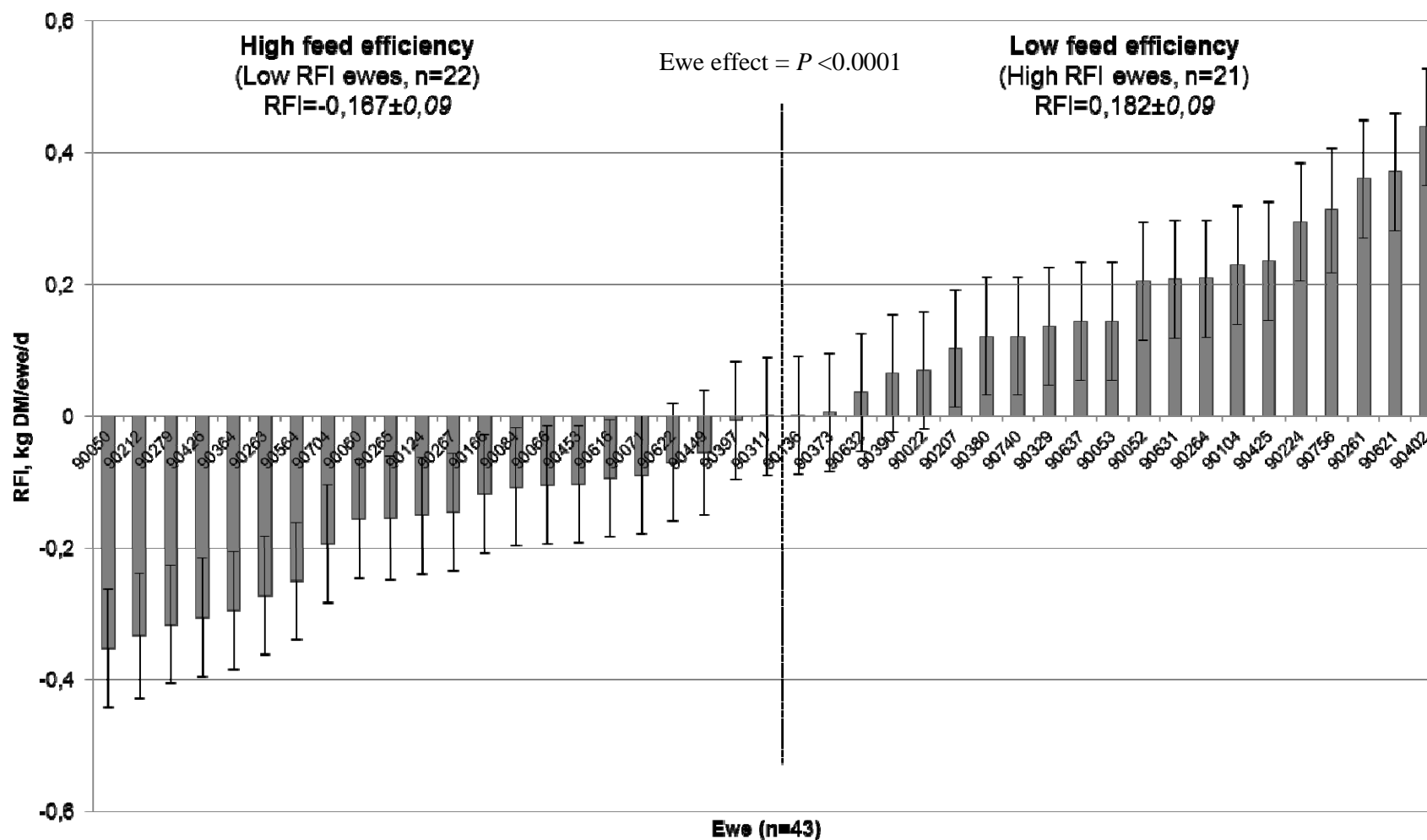
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404 **Figure 1.** Effects of the lactation stage (days in milk, DIM) and its interaction with milk frequency
405 on residual feed intake (RFI) of primiparous Lacaune dairy ewes during mid-lactation (35–98
406 DIM).



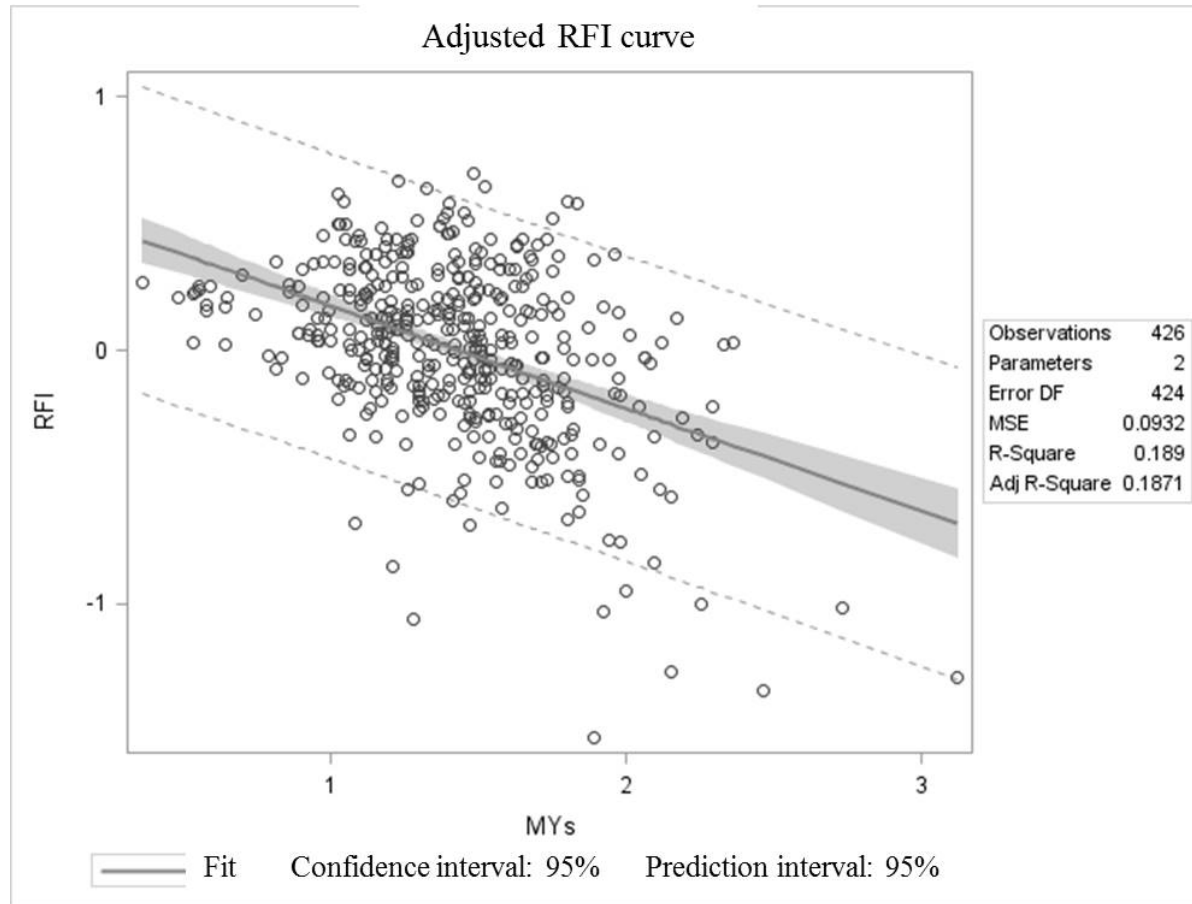
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408 **Figure 2.** Ranking of primiparous Lacaune dairy ewes (n=43) in function of their average residual feed intake (RFI, kg DM/ewe/day) during
 409 mid-lactation (35-98 days in milk). Overall RFI during the whole period was 0.004 ± 0.090 kg DM/ewe/d.



410

411 **Figure 3.** Adjusted curve for average individual residual feed intake (RFI) and fat-corrected milk yield of primiparous Lacaune dairy ewes
412 (n=43) during mid-lactation (35-98 days in milk). MYs= standardise milk yield (SMY).



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