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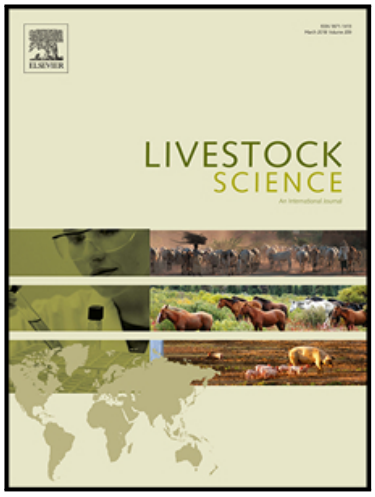

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Highlights

- No differences in intake and nutrient digestibility between ewes belonging to two contrasting feed efficiency genetic lines FE lines were observed.
- Selecting rams tested with one diet (concentrate-based diet) whereas their daughters (ewes) are fed with another (forage-based diet) may change the expected impacts of genetic selection programs for feed efficiency in sheep.

Short title: Genetics and feed efficiency in ewes

Intake and digestibility of meat ewes belonging to two contrasting feed efficiency genetic lines, during their two first production cycles

Azizi A.,§ H. Nascimento,¶ F. Tortereau,* D. Hazard,* S. Douls,† C. Durand,† L. Bonnal, ‡ P. Hassoun,‡ S. Parisot,† M. Tlidjane,§ and E. González-García‡,#

§ *Veterinary Department, Veterinary Sciences and Agricultural Sciences Institute, University of Batna-1, 05000 Batna, Algeria*

¶*Animal Science Faculty, Universidade Federal Rural de Pernambuco, 52171-900 Recife, Pernambuco, Brazil*

**INRAE UMR1388 GENPHYSE Université de Toulouse, ENVT, 31326 Castanet-Tolosan, France*

†*INRAE UE321 La Fage, 12250 Roquefort-sur-Soulzon, France*

‡*SELMET, INRAE, CIRAD, Montpellier SupAgro, Univ Montpellier, 34060 Montpellier, France*

#*Correspondance*: eliel.gonzalez-garcia@inrae.fr; abdennour.azizi@univ-batna.dz

Abstract

Feed is the largest cost in animal production, therefore improving feed efficiency (**FE**) is one of the main priorities when redesigning farming systems. The objective of this work was to evaluate the effects of a divergent genetic selection for FE in Romane rams [i.e. efficient (**RFI-**) or inefficient (**RFI+**)] on the feed intake and nutrient digestibility of their daughters (ewes), reared in different conditions. Different to their sires, which were fed high concentrate-diets, the ewes were fed *ad libitum* total mixed ration (**TMR**) composed of silage (65%), hay (25%) and barley (10%). A series of *in vivo* digestibility trials were conducted using Romane ewes (n =20; 10 RFI- and 10 RFI+) during their 2 first production cycles. The **BW**, metabolic BW (**MBW**), body condition score (**BCS**), DM intake (**DMI**) and nutrient intake and digestibility were individually monitored. All parameters were significantly affected ($P < 0.05$) by the production cycle. The BW, MBW, BCS as well as the feed intake and nutrient digestibility were higher in the cycle 2 compared to cycle 1. The exception was the CP digestibility, which was higher in younger ewes (cycle 1). The BW and MBW was, as expected, higher in older ewes thus increasing the feed intake capacity in cycle 2 compared with cycle 1. Slight differences in TMR nutritive composition between the years of the study were also probably responsible of differences in CP digestibility. The RFI+ ewes showed a better BCS than RFI- ewes (3.0 vs. 2.7; $P < 0.0001$). In the conditions used in this study, we could not demonstrate any differences in intake and digestive efficiency between the two ewe FE lines, probably because the selected rams were tested with one diet (concentrate-based diet) whereas their daughters (ewes) with another (forage-based diet). Further research is warranted to evaluate RFI testing in animals fed a more fibrous diet closer to that of ewes under range conditions whereas also assessing responses to multiple-generation divergent selection on RFI.

Keywords

Intake; digestibility; ruminants; feed efficiency; divergent selection

Implications

Selecting for feed efficiency is a current priority in ruminant production. In this study we demonstrated that conducting feed efficiency selection programs in actively growing Romane lambs fed concentrate-based diets, may not impact the voluntary feed intake and the digestive efficiency of their daughters, fed roughage-based diets when reared in rangelands. This factor must be taken into consideration in future breeding programs, which would imply to test animals in a larger and more representative spectrum of farming and feeding conditions.

Introduction

To face current and future challenges, improving feed efficiency (**FE**) is one of the main priorities when redesigning farming systems (Dumont et al., 2014; Phocas et al., 2016). Feed is the largest single cost item in livestock production systems (accounting for up to 70% of total costs), and the way the feed resource base is managed contributes significantly to the environmental footprint and consumer criticism of livestock production systems (overgrazing, arable land occupation, GHG emissions, soil pollution, etc.). Two main strategies are implemented to achieve FE improvement goals. First, by searching for alternative feed resources, thus reducing food-feed competition while mitigating environmental impacts by replacing edible feed crops with human-inedible biomass in animal diets, such as plant by-products (Salami et al., 2019). Second, by focusing on the animal component of the farming system by considering FE as a key trait in selection programs (Pryce et al., 2013). Regarding this second animal focused strategy, various reports have demonstrated significant heritabilities for FE in ruminants by conducting tests on residual feed intake (**RFI**). Many authors have suggested that potential

improvements in cattle should begin by focusing in the considerable between-animal variation in RFI at the breed and herd levels (Berry and Crowley, 2013; Cantalapiedra-Hijar et al., 2018; Crowley et al., 2011a, b; Herd and Bishop, 2000; Herd et al., 2019). However, in small ruminants, only limited data is available, even if some reports confirm a promising role for the RFI trait in sheep (Rajaei et al., 2016; Redden et al., 2011; 2013; 2014; Tortereau et al., 2020).

Recently, Tortereau et al. (2020) reported genetic gains for FE after assessing responses to single-generation divergent selection on RFI with 951 male Romane lambs in France. The authors concluded that the efficient rams selected (with low RFI breeding values) will produce progeny that will require less concentrate during the growing or fattening period compared with progeny from less efficient rams, without affecting their growth performance. Considering these results, the next issue is to determine whether the improved FE of these sires will continue to be transmitted in other farming systems (e.g. from confinement to rangeland) and feeding systems (e.g. from concentrate to roughage) where their progeny will be raised and whether it will persist throughout their progenies' entire productive life (e.g. young growing animals to adult productive animals).

Based on the statement that most RFI sheep testing has been conducted post-weaning on medium-to-high energy (but not in forage) based diets, Redden et al. (2011) assessed the relationship between RFI measurements performed with different feeding systems using the same ewes during two successive experimental phases or physiological stages (as actively growing ewe-lambs and yearlings at maintenance). They found a lack of relationship between the RFI calculated for the two stages, which led the authors to suggest that determining RFI by means of a post-weaning growth test may not increase the efficiency of ewes in rangelands.

Therefore, to continue advancing the state of the art on this subject, the objective of this study was to evaluate the voluntary feed intake and nutrient digestibility of ewes of different FE lines (efficient vs. inefficient, according to the RFI genotype of their sires), during the first two production cycles.

Material and Methods

All experiments were conducted at the INRAE Experimental Farm *La Fage*, Causse du Larzac (43°54'54.52"N; 3°05'38.11"E), Aveyron, France, following the procedures approved by the Regional Ethics Committee on Animal Experimentation number 115, Languedoc-Roussillon (France; file number 2016031819254696; Agreement with reference APAFIS_4597).

Animals

Twenty Romane ewes (initial BW±SD, 49.8±5.8 kg), selected from the main flock of the *La Fage* farm and reared on extensive rangeland, were included in this study which consisted of 4 consecutive *in vivo* digestibility trials (10 ewes each time) over a period of 3 consecutive years. The number of experimental animals per digestibility assay (n= 10) was limited by the number of metabolic crates available in the experimental facility (n= 10), which unanimously accepted in the conventional protocols of digestibility trials with sheep. Ewes belonged to 2 cohorts: 10 born in April 2017 (**Coh2017)** and 10 born in April 2018 (**Coh2018**). Equal numbers of ewes from each cohort and from each FE genetic line were used for the trials [i.e. based on their RFI; Tortereau et al., 2020; 5 efficient (**RFI-**) and 5 inefficient (**RFI+**) ewes per trial]. Rams were selected based on their breeding values for RFI, calculated as the difference between the actual and predicted feed intakes. Irrespective of the cohort, the phenotypic RFI being the average RFI index of efficient sires was -60.5 g/d (ranging from -83.0 g/d to -32.4 g/d) whereas for inefficient sires the average was -69.6 g/d (ranging from -113.8 g/d to -19.2 g/d; Table 1). Dams

from the studied ewes were not evaluated because they had no genetic link with the population evaluated for RFI. These dams were thus expected to have an average breeding value of 0.00 g/d. On average, the expected breeding value for RFI of the studied progeny was 30.25 g/d and -34.8 g/d for the ewes from RFI+ and RFI- sires, respectively. This difference corresponds to 0.85 genetic standard deviation of RFI (Tortereau et al., 2020). Ewes belonging to each cohort and genetically divergent RFI line were randomly selected at an early stage of their first pregnancy (first mating at age 7 months in November for both cohorts) based on their **BW**, body condition score (**BCS**) and litter size. The number of lambs (litter size) was determined by ultrasonography and ewes with two fetuses were selected preferentially for the experiment (see Figure 1 for further details). Finally, in 2017, the 10 selected ewes were born from 6 rams, (x1 rams having 1 ewe in the trial and x2 rams having 2 ewes in the trial), and in 2018, the 10 selected ewes were born from 5 rams (y1 rams having 1 ewe in the trial and y2 rams having 2 ewes in the trial). Ewes subsequently underwent a 3-wk digestibility trial at the same physiological stage (mid-pregnancy) during their two first production cycles [respectively primiparous (**PRIM**) and multiparous (**MULT**)]. Table 2 reports some of the main characteristics of the experimental ewe population assessed in this study.

In vivo *digestibility assays and experimental design*

Four consecutive *in vivo* digestibility assays were carried out over three years and included ewes from the 2 above-described cohorts. The ewes were evaluated during their 2 first production cycles. Figure 1 is a schematic representation illustrating the sequence of the 4 digestibility assays performed, and show the dates, experimental design and main measurements carried out. Each assay included 10 ewes with 5 replicates for each RFI line (n = 2; efficient RFI-, inefficient RFI+; 5 ewes/line). For each cohort, the ewes first evaluated as PRIM were then

evaluated as MULT during the following pregnancy. The digestibility assays (trials) were conducted indoors and organized as follows: *Trial 1*: **PRIM2017×RFI-** vs. **PRIM2017×RFI+**; *Trial 2*: **MULT-2017×RFI-** vs. **MULT2017×RFI+**, *Trial 3*: **PRIM2018×RFI-** vs. **PRIM2018×RFI+**; and *Trial 4*: **MULT-2018×RFI-** vs. **MULT2018×RFI+**. They took place, respectively, from February 12 to March 5, 2018 (Trial 1); from January 14 to February 2, 2019 (Trial 2); from February 11 to March 4, 2019 (Trial 3), and from January 13 to February 1, 2020 (Trial 4).

Each digestibility assay was conducted over 3 consecutive weeks. The first week allowed the ewes to adapt to the changes in housing conditions (managed in groups in 2 × 6 m pens), overall environment and diet. During the second week of adaptation, ewes were housed in the same building and were fed the same total mixed ration (**TMR**), but were moved to individual metabolic crates and the routine of distribution and measurements remained the same until the end of each trial. Fresh intake was monitored beginning on the second week. Data on feeding, refusals and feces used to assess DM and nutrient intake and digestibility were collected during the third week. The BW and BCS were monitored once weekly for the three weeks.

Diet composition and feeding. NIRS predictions and nutritive value

The experimental diet was composed of *ad libitum* TMR containing 65% silage [mix of ryegrass (*Lolium perenne*) and alfalfa (*Medicago sativa*)], 25% hay (mix of *Dactylis glomerata* and alfalfa) and 10% barley. Animals had continuous free access to water and a salt mineral block. The TMR was distributed twice daily, one third in the morning and two thirds in the afternoon, at about 8 AM and 4 PM, respectively. The amount distributed each day was adjusted to 115% of the animal's voluntary intake on the previous day.

Table 3 shows the chemical composition and nutritive value of the ingredients and TMR provided during the 4 trials. Samples of TMR were taken twice daily from the rations provided morning and evening, respectively. Feces and refusals were weighed every morning before the first meal was distributed. Samples (10%) were collected and dried at 60 °C during 48 hours to determine DM content. At the end of each trial, all samples were milled through a 1-mm screen in a hammer mill and stored for further analysis in the laboratory.

The chemical composition of the dried and ground feed and feces samples were determined by monochromatic NIRS (NIRS 6500, Foss NIRSystems, Silver Spring, MD, USA). Samples were placed in a 50 mm diameter ring cup with quartz window and scanned in the reflectance mode (wavelengths ranging from 400 to 2500 nm in 2 nm steps). Spectral data acquisition was performed in duplicate (with two different cup fillings) and the average spectrum was used for chemometric analysis.

The chemical composition of each sample was predicted on the basis of its NIRS spectrum using reference data (CIRAD, Montpellier) derived from a large sample population collected over multiple years in two databases. The quality of NIRS predictions were verified by analyzing a small number of samples with conventional chemical procedures. The parameters considered were CP (Kjeldahl method), fiber fractions (NDF, ADF, ADL; method number 973.18; Van Soest et al., 1991), and *in vitro* OM digestibility (Aufrère et al., 2007).

The net energy forage unit for lactation (**FUL**), digestible proteins in the intestine when nitrogen (**DPIN**) or energy (**DPIE**) are limiting, and the forage fill value for sheep (**FFV**) were calculated using INRAE's PrevAlim software (Baumont et al., 1999). Although measures were taken to maintain the quality of TMR ingredients over the experimental period, natural interannual climatic variability caused slight differences in the silage and hay qualities (e.g.

lower CP and higher NDF of hay in Trials 2 and 3), that somewhat affected the nutritive value of TMR used in the different trials (Table 3).

Experimental variables

The BW and BCS were recorded three times, at the beginning, in the middle and at the end of each trial (Figure 1). The BCS was assessed by the same trainer operator according to an adaptation of the original grid described by Russel et al. (1969) which was further divided into a 1/10 scale, i.e., from 1 to 5 with 0.1 increments.

During the first week of each trial, feed intake was monitored on a fresh matter basis. During the second week, TMR DM was determined (oven dried samples at 65°C during 48 h) and feed refusals were measured, so daily individual DM intake (**DMI**) was recorded. During the third and last week, both fresh matter intake and DMI continued to be recorded, and the daily individual *in vivo* digestibility of nutrients was determined daily using the total feces collection method.

The daily feed was distributed, the refusals and the feces of the animals were weighed and sampled individually in order to determine feed intake and digestibility. The DMI was calculated as the difference between the quantity of DM provided and DM refusals. The OM content (%) was calculated as the difference between 100 and the percentage of ash in each sample. The apparent DM digestibility (**DMD**) was calculated using the equation: DMD (g/kg) = [(DMI – DM excreted in feces (**DMF**)) / DMI] × 1000.

Design and statistical analyses

A pooled database was built by grouping the outputs from the 4 trials. Data were analyzed by using a split plot design with the PROC MIXED of SAS (ver. 9.4; SAS Institute, Cary, NC, USA). Indeed, in the experimental design of this study there are 20 experimental units (ewes 1 to

20), which corresponds somehow to two experiments - cohorts 2017 and 2018 being in some way practically detached. Each cohort participates in the two sub-experiments, but the experimental units are the same in the two cycles for each cohort. The 10 units (ewes) belonging to the two Lines (representing two populations) are selected in this study, rather than randomized. However, we may consider it as an experiment with two blocks (blocks representing Lines or RFI genotypes) but split for Cycles (i.e. production cycles, PRIM and MULT; 1 and 2). Though such experiments can be described as repeated measures, in the case of two samples (Cycles) a split-plot analysis is then performed. The corresponding analysis of variance would ensure that the differences between Lines are tested at the between Ewe stratum, and then Cycle and Line × Cycle interaction are tested at the within Ewe stratum. Clearly, this model is expressed as:

$Y_{ijk} = \mu + \text{Line}_i + \text{delta}_{ij} + \text{Cycle}_k + (\text{Line} \times \text{Cycle})_{ik} + \varepsilon_{ijk}$

where i= 1, 2; j= 1…5 (ewes/Line) and k= 1, 2; represents the 'between ewe' error term, and the residual (or 'within ewe') error.

Taking into account obvious differences in the initial BW of ewes during the first productive cycle between the two cohorts, a further analysis of BW on intake was carried out. The BW was analyzed as a covariate using the ANCOVA procedure of SAS for determining its effect on feed intake with the key parameters total DMI and DMI per kg of MBW, according with the cohort of the ewe, the genetic line and the production cycle. An additional ANOVA was also carried out in order to compare the chemical composition of each ingredient used in the TMR across the three years of the experiment (Table 3). All statistical procedures were performed using SAS. Data are shown as the LSmean ± SEM and were considered to differ significantly when $P < 0.05$. Trends were discussed for P-values between 0.05 and 0.1.

Results

Overall results (LSmean ± SEM) are provided in Tables 4. The results for the Line × Cycle interaction was not significant ($P > 0.05$) and are not shown.

Bodyweight and body condition

A significant effect ($P < 0.001$) of the production cycle was observed for BW, MBW and BCS (Table 4). An expected effect of the production cycle on the progression of BW and MBW was observed (MULT>PRIM; ewes in cycle 2 were heavier than those in cycle 1), irrespective of their genotype. Regarding BCS, ewes displayed a higher BCS during their second cycle compared with their first cycle (3.0 vs. 2.7 ±0.02, respectively). A significant effect ($P < 0.001$) of the line (RFI genotype) on BCS was also found (Table 4) with inefficient RFI+ ewes showing a higher BCS than efficient RFI- ewes (2.9 ± 0.01 vs. 2.8 ± 0.01, respectively).

Dry matter and nutrient intake

In agreement with the increase in BW and MBW, and therefore the increase in feed intake capacity, the highest ($P < 0.001$) DMI values were observed during the second cycle, either for total (1541 vs. 1156 g DM/ kg TMR) or DMI depending ewe's MBW (77 vs. 63 g/kg $BW^{.75}$; Table 4). No significant differences between RFI genetic lines were observed for these parameters (71 g/kg $BW^{.75}$).

Findings similar to those described above for total DMI were observed for nutrients intake (i.e., OMI, CPI, NDFI, ADFI and ADLI; Table 4). The highest nutrient intake values were observed during the second cycle. Ewes ate more OMI, CPI, NDFI, ADFI and ADLI during their second cycle than during their first cycle (Table 4).

Differences in DMI (g/d) between cohorts, cycles and RFI lines, after controlling for the covariate (BW), are illustrated in Figure 2a, b and c, respectively. The model fitted well for

intake parameters (R^2 of DMI = 0.72; 0.80 and 0.67 for cohorts, cycles and RFI line, respectively; and R^2 of DMI_MBW = 0.51; 0.68; 0.44 for cohorts, cycles and RFI line, respectively).

Even though Coh2017 ewes ate more than Coh2018 ewes, both cohorts increased their DMI (total and per kg of MBW) at the same rate and in a linear manner as their BW increased (Figure 2a). The increase in DMI (total and per kg of MBW) as the BW increased was greater during the first production cycle (as the young ewes were still growing). During the second cycle, the trend was similar but the slope of DMI/kg BW was less for cycle 2 compared with cycle 1 (Figure 2b). The DMI (total and per kg of MBW) increased linearly, similarly and at the same rate for the two RFI lines, as their BW increased (Figure 2c).

Nutrient digestibility

Except the CP digestibility (CPD; cycle 1 < cycle 2; 626 vs. 654 g CP/ kg CP), ewes displayed higher nutrient digestibility during their first production cycle compared with the second cycle. On the contrary, no effects were detected of the RFI genotype for diet digestibility (Table 4).

Discussion

There is increasing interest in selection for improved FE. However, before steps are taken towards selecting for FE, correlations with other traits of economic importance (Crowley et al., 2011a, b) and practical relevance must first be quantified and verified.

Recently, Tortereau et al. (2020) reported heritability values for RFI and feed conversion ratio traits of 0.45 and 0.30, respectively, with good results in RFI tests in actively growing Romane lambs. The present study evaluated the offspring of these previously selected Romane males, but failed to demonstrate differences in the voluntary intake and digestive efficiency in

the Romane ewes, daughters of the sires from different FE genotypes. Our ewes were fed with a forage/roughage diet, whereas the sires that underwent FE testing, with subsequent ranking and selection, were fed a concentrate-based diet. We speculate that this discrepancy in diet composition could explain the lack relationship between the rams' RFI and the digestive efficiency of their daughters. Moreover, the ewes used in the present study received only half of the genetic merit of their sires for FE since their dams did not come from divergent lines for FE. This would inevitably reduce the genetic divergence between ewes in the two FE genotype groups and could reduce genotype differences between the ewes for the investigated traits.

Consistently with the digestive physiology of ruminants, it is reasonable to presume that an animal determined to be feed efficient during its active growth period, reared in confinement and fed a concentrate-based ration, could become an animal determined to be feed inefficient during adulthood if its rearing conditions are significantly changed. The same phenomenon could occur in offspring. Our results are consistent with findings reported by Redden et al. (2011) who observed a lack of relationship between RFI determined by means of a post-weaning growth test and RFI and FE in yearling ewes on range.

Kenny et al. (2018) conducted a meta-analysis on growing beef cattle fed an energy-dense high-concentrate diet, and showed that high-RFI individuals spent more time eating than their low-RFI counterparts, which could be related to more DMI in inefficient animals. Similarly, low-RFI animals have been reported to show lower DMI compared with their high-RFI counterparts in ewes (Redden et al., 2011; 2013), rams (Rajaei Sharifabadi et al., 2016; Lima et al., 2019) and dairy heifers (De Assis Lage et al., 2019). In our study, we failed to demonstrate such differences in DMI and nutrient intakes between the two RFI genotypes, even considering the slight variation in the nutritive value of the hay used in the 2019 trials (Trials 2 and 3), which contained

less CP and more fiber compared with the 2018 and 2020 trials (Trial 1 and Trial 4, respectively; Table 3).

Reducing DMI is known to increase nutrient efficiency due to improved nutrient digestion associated with a reduction in metabolic expenditure reserved for nutrient absorption and oxidative metabolism for maintenance (Colucci et al., 1982; Pino et al., 2018). When the proportion of forage is increased in the diet, the rumen retention time increases, thus reducing the flow rate or rate of passage of the ruminal bolus, and increasing microbial growth and feedstuff degradation.

When comparing low-RFI and high-RFI groups, Redden et al. (2011, 2014) reported that the reduction in DMI was greater in yearling ewes than in ewe lambs. A recent study confirmed that feed intake is directly associated with maintenance and BW gain in heifers and cows, and the metabolic rate and BW gain were considered as the main primary drivers (Freetly et al., 2020). This is in agreement with our finding that the RFI genotypes had no effects on DMI total or per kg of MBW, and leads us to conclude that, beyond the differences in the nutritive value of the TMR, the variations in DMI were a consequence of differences in BW between cycles.

In accordance with the present results, previous studies reported no effects of RFI genotypes on BW and metabolic BW in rams (Rajaei Sharifabadi et al., 2016; Lima et al., 2019) and ewes (Redden et al., 2013). We found however significant differences in BCS between the RFI genotypes, which is consistent with other available reports. Crowley et al. (2011a, b) found a positive correlation between RFI and carcass fat when analyzing genetic relationships between FE in growing males and beef cow performances in a large population of beef cattle in Ireland. A meta-analysis in growing cattle reported positive and negative correlations, respectively, between RFI and body fat and lean body mass in live animals and carcass (Berry and Crowley, 2013).

Similarly, Redden et al. (2014) reported that high RFI (inefficient) yearling ewes tended to deposit more fat than low RFI (efficient) yearlings. Regarding the higher BCS observed in RFI+ ewes, we speculate that this group of ewes probably deposited more subcutaneous fat as a consequence of a genetic inheritance from their sires, which could be also related to a more selfish behavior in this group of animals.

We found a lack of effect of the RFI line on digestibility of nutrients. Increased DMD is known to be a consequence of or directly related to decreased DMI, and results from prolonged ruminal residency or retention time. Therefore, efficient ruminants could be expected to show higher apparent digestibility than inefficient ruminants, which was not the case in our study. The literature available on the relationship between nutrient digestibility and RFI-classed phenotypes is somewhat contradictory. Kenny et al. (2018) did not find consistent evidence of any such association in the articles they reviewed. Some authors have described a negative correlation between DM digestibility and RFI in cattle (Nkrumah et al., 2006; Rius et al., 2012). But recent studies did not succeed in demonstrating this correlation in sheep and found no differences in the apparent digestibility of nutrients among RFI classes in ewes (Redden et al., 2011) and rams (Rajaei Sharifabadi et al., 2016; Lima et al., 2019). In other reports in cattle, no differences in digestibility were evidenced between groups of phenotypically efficient and less efficient individuals (Olijhoek et al., 2018; Russel et al., 2016). Cantalapiedra-Hijar et al. (2018) suggested that overall higher DMD in low RFI cattle might be mostly the consequence of lower DMI but likely not the opposite.

We observed an overall higher nutrient digestibility during the first production cycle compared to second. Primiparous ewes, however, were found to better digest CP compared with multiparous. We speculate that such differences are probably related to the quality of the ration

composition. Some authors have reported that efficient pre-weaning dairy heifers exhibit increased CPD, a tendency for improved DMD and OMD and no differences in NDFD (De Assis Lage et al., 2019). Similarly, De la Torre et al. (2019) found a significant effect of RFI divergence in beef cattle on DMD and OMD but not NDFD.

It is unclear in the literature whether the improved apparent digestibility in efficient animals is inherent, or simply due to the longer ruminal retention time related to the lower DMI and higher fiber content (bulk, fill effect) in the diet. According to Kenny et al. (2018), the lack of effect of RFI lines on DMD may be related to the nature of the diets provided, as the effect of feed intake on digestion is lower with forage than concentrate-based diets. This argument fully supports our findings.

Conclusion

Differences in intake were mainly determined by natural differences in BW between the two cycles. As expected, and irrespective of the cohort or RFI genotype, ewes naturally increased their BW, MBW and intake during their second production cycle. However, differences in nutrient digestibility were mostly triggered by slight variations in the quality of diet ingredients from year to year. In the conditions used in this study (forage and not concentrate-based diet), we could not demonstrate the expected differences in feed intake and digestibility between the female offspring of two genotypes of rams divergently selected for feed efficiency. Further research must be carried out however to assess responses to multiple-generation divergent selection on RFI and verify if the lack of intake differences and thus in digestibility are due to not enough divergent RFI lines after just one single generation.

Author Statement

Conception and design of study: E. González-García; acquisition of data: A. Azizi, H. Nascimento, S. Douls, C. Durand; analysis and/or interpretation of data: A. Azizi, E. González-García; Drafting the manuscript: A. Azizi, E. González-García; revising the manuscript critically for important intellectual content: F. Tortereau, D. Hazard. Approval of the version of the manuscript to be published: A. Azizi, H. Nascimento, F. Tortereau, D. Hazard, S. Douls, C. Durand, L. Bonnal, P. Hassoun, S. Parisot, M. Tlidjane, and E. González-García.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Table 1. Average, minimum and maximum of genetic value (RFI index) of the sires, belonging to the two feed efficiency genotypes (RFI+, inefficient; RFI-, efficient), and used to produce the ewes from each cohort (2017; 2018) participating in the study.

Cohort	Genotype	Sires, *n* =	RFI index			Daughters per sire		
			Average	Minimum	Maximum	Average	Minimum	Maximum
2017	RFI+	3	62.1	50.0	73.5	1.67	1	2
	RFI-	3	-89.2	-45.2	-113.8	1.67	1	2
2018	RFI+	3	59.0	32.4	83.0	2	2	2
	RFI-	2	-40.3	-19.2	-61.3	3	2	4

[1]RFI-: low residual feed intake (efficient animal).

[2]RFI+: high residual feed intake (inefficient animal).

Table 2. Main features of the experimental Romane ewes (n = 10 per trial) evaluated in 4 consecutive *in vivo* digestibility trials at the early-mid pregnancy stage, including 2 cohorts (2 consecutive years of birth, in April) during their 2 first production cycles.

		Cohort 2017								Cohort 2018						
		The average estimated breeding values [3]	Cycle 1			Cycle 2				The average estimated breeding values [3]	Cycle 1			Cycle 2		
Ewe	RFI Genotype of the sire		BW, kg	BCS[4] (1-5)	n° of fetus [§]	BW, kg	BCS (1-5)	n° of fetus	Ewe		BW, kg	BCS (1-5)	n° of fetus	BW, kg	BCS (1-5)	n° of fetus
1	RFI[1] +	-72,5	48.4	3.2	2	54.3	2.9	1	*11*	22,4	40.7	2.8	2	41.4	2.7	2
2	RFI[2] -	93,6	45.0	2.9	2	49.7	2.8	2	*12*	-30,9	39.1	2.7	2	45.9	2.8	2
3	RFI +	-91,1	47.1	2.8	2	55.3	2.8	1	*13*	66,9	45.9	2.5	2	53.4	3.0	2
4	RFI -	93,6	47.5	2.8	2	49.	2.5	2	*14*	-30,9	44.	2.6	2	47.	2.5	2

						5					6			6		
5	RFI +	-46,6	47.0	3.1	2	54.5	2.6	2	*15*	22,4	43.6	2.8	2	54.1	3.2	3
6	RFI -	110,9	48.0	3.0	2	54.6	2.9	2	*16*	-81,1	42.3	2.6	2	50.2	3.0	2
7	RFI +	-46,6	46.5	3.2	2	51.8	3.0	1	*17*	66,9	43.8	2.9	2	56.4	3.1	2
8	RFI -	39,1	47.5	3.1	2	48.5	2.6	1	*18*	-57,2	39.5	2.5	2	59.8	2.7	3
9	RFI +	-72,5	50.2	2.9	2	54.0	3.1	2	*19*	22,4	42.8	2.7	2	56.2	3.0	2
10	RFI -	39,1	50.5	2.9	2	53.3	2.6	1	*20*	-81,1	44.9	2.5	2	60.3	3.1	2

[1]RFI -: low residual feed intake (efficient ewe); [2]RFI +: high residual feed intake (inefficient ewe); [3]indexRFI: The average estimated breeding values; [4]BCS: Body Condition Score; [§]: Diagnosed by ultrasonography during the first month of pregnancy

Table 3. Chemical composition and nutritive value of the ingredients composing the total mixed ration distributed *ad libitum* (115% of the previous day intake), and evaluated in Romane ewes during 4 consecutive *in vivo* digestibility trials at the early pregnancy stage, including 2 cohorts (2 consecutive years of birth, in April) during their 2 first production cycles.

Item		Ingredients														
		Silage[§]					Hay[¥]					Barley				
		Trial 1	Trial 2	Trial 3	Trial 4	Effect, *P* value	Trial 1	Trial 2	Trial 3	Trial 4	Effect, *P* value	Trial 1	Trial 2	Trial 3	Trial 4	Effect, *P* value
% of inclusion in TMR		64	65	64	70		24	25	26	20		12	10	10	10	
DM, %	*m*	41.4	29.8	35.4	29.5	***	79.0	79.5	89.7	86.1	ns	87.7	90.9	91.8	93.2	ns
	s	*1.74*	*4.37*	*6.64*	*6.55*		*8.60*	*10.97*	*1.50*	*1.90*		*4.41*	*0.30*	*1.58*	*0.53*	
OM	*m*	900	909	904	893	***	923	941	946	920	***	972	967	971	966	ns
	s	*4.3*	*5.4*	*5.9*	*5.5*		*4.7*	*10.6*	*6.0*	*5.8*		*4.7*	*3.0*	*1.6*	*1.6*	
CP	*m*	149	143	133	143	ns	160	122	102	129	***	120	125	125	126	ns
	s	*8.4*	*14.8*	*5.3*	*14.8*		*10.0*	*19.0*	*12.8*	*10.8*		*15.0*	*1.1*	*2.6*	*0.5*	
NDF	*m*	466	535	530	491	***	520	602	615	549	***	186	289	267	160	***
	s	*20.6*	*34.1*	*21.0*	*19.2*		*33.9*	*28.3*	*31.0*	*19.5*		*7.7*	*14.8*	*7.0*	*19.8*	
ADF	*m*	281	343	333	312	***	328	355	387	351	***	60	84	73	44	***
	s	*12.1*	*18.1*	*12.7*	*16.8*		*25.5*	*21.0*	*12.2*	*18.3*		*6.3*	*2.6*	*2.9*	*9.3*	
ADL	*m*	36	50	53	58	***	81	66	62	67	***	12	15	14	9	ns
	s	*8.5*	*5.1*	*9.8*	*10.4*		*11.4*	*5.6*	*4.8*	*7.6*		*1.7*	*0.2*	*1.1*	*3.0*	
Nutritive value[Ω]																
FUL		0.90	0.80	0.79	0.80	-	0.83	0.76	0.66	0.74	-	1.09	1.12	1.12	1.12	-
PDIA, g/ kg DM		24	22	21	22	-	42	32	27	34	-	29	30	30	30	-
DPIN, g/ kg DM		94	87	85	87	-	100	76	64	80	-	79	80	80	80	-
DPIE, g/ kg DM		67	62	60	62	-	96	83	74	83	-	101	101	101	101	-
FFV		1.49	1.62	1.58	1.56	-	1.30	1.33	1.54	1.32	-	0.72	0.51	0.51	0.51	-

[§]Silage was locally produced during the previous year, resulting from the harvest of a forage field composed by a deliberated mix of ryegrass (*Lolium perenne*) and alfalfa (*Medicago sativa*); [¥]Hay was locally produced during the previous year, resulting from the harvest of a forage field composed of a deliberated mix of *Dactylis glomerata* and alfalfa (*Medicago sativa*); OM: Organic Matter; *m*: Sample mean estimates; *s*: Sample standard deviation estimates; [Ω]Estimated using the INRAE PrevAlim software (Baumont et al., 1999). FUL= forage unit for lactation (1 FUL being equivalent to the average energy produced by 1 kg of standard barley); UDPI= undegraded dietary protein, in the rumen, which is digestible in the intestine; DPIE= UDPI + DDIMN (microbial protein that could be synthesized from the degraded dietary N when energy is not limiting); DPIN= UDPI + DPIME (microbial protein that could be synthesized from the energy available in the rumen when degraded N is not limiting). Final DPI value of the diet is the minimum of DPIN or DPIE (INRA, 1988); FFV= forage fill value for sheep. ns: non-significant; $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$.

Table 4. Least square means (±*SEM*) of bodyweight, body condition, voluntary feed intake and *in vivo* digestibility of nutrients. Effects of the genetic line of the ewe for feed efficiency (RFI+ vs. RFI-), production cycle (cycle 1 vs. cycle 2), cohort (2017 vs. 2018), and their first-order interaction.

Item	RFI[1]-	RFI[2]+	Cycle1	Cycle2	*SEM*	DF	Effect, *P* value		
							Line	Cycle	Line × Cycle
BW, kg	50.6	51.0	47.1	54.3	*0.41*	236	ns	***	ns
MBW[3], kg	18.9	19.0	18.0	20.0	*0.11*	236	ns	***	ns
BCS[4], 1-5	2.8	2.9	2.7	3.0	*0.02*	236	***	***	ns
DMI[5]									
g/d	1340	1357	1156	1541	*26*	236	ns	***	ns
g/kg $BW^{.75}$/d	70	71	63	77	*1*	236	ns	***	ns
Nutrient intake, g/d									
OM[6]	1221	1238	1054	1404	*23*	236	ns	***	ns
CP	206	208	179	235	*5*	236	ns	***	ns
NDF	651	662	559	754	*12*	236	ns	***	ns
ADF	394	401	336	460	*7*	236	ns	***	ns
ADL	67	68	57	78	*1*	236	ns	***	ns
Nutrient digestibility, g/kg DM									
DMD[7]	610	621	624	607	*6*	236	ns	*	ns
OMD[8]	632	639	646	625	*6*	236	ns	**	ns
CPD[9]	633	646	626	654	*8*	236	ns	**	ns
NDFD[10]	630	634	649	616	*6*	236	ns	***	ns
ADFD[11]	633	638	650	621	*6*	236	ns	***	ns

[1]RFI+: high residual feed intake (inefficient ewe); [2]RFI-: low residual feed intake (efficient ewe); [3]MBW: metabolic bodyweight ($BW^{.75}$); [4]BCS: body condition score; [5]DMI: DM intake; [6]OM: organic matter; [7]DMD: DM digestibility; [8]OMD: OM digestibility; [9]CPD: CP digestibility; [10]NDFD: NDF digestibility; [11]ADFD: ADF digestibility. Significance is considered when *P-value* < 0.05 and tendency when the *P-value* lay between 0.05 to 0.1. ns: non-significant; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

Figure 1. Schematic representation of the followed experimental layout illustrating the specific dates per trial, activities and variables measured during each of the 3 weeks (2 for adaptation and 1 for full data collection) comprised on each of the 4 *in vivo* digestibility trials. Evaluation was carried out during the 2 first production cycles of 2 Romane ewes' cohorts, daughters of rams divergently selected for feed efficiency based on their residual feed intake (RFI) genotype (i.e., efficient or inefficient).

Figure 2. Differences in dry matter intake [top: total (g/ewe/d); bottom: per kg of metabolic body weight] between cohorts (A), production cycles (B) and RFI genetic lines (C), after controlling the body weight of the ewes as covariate.

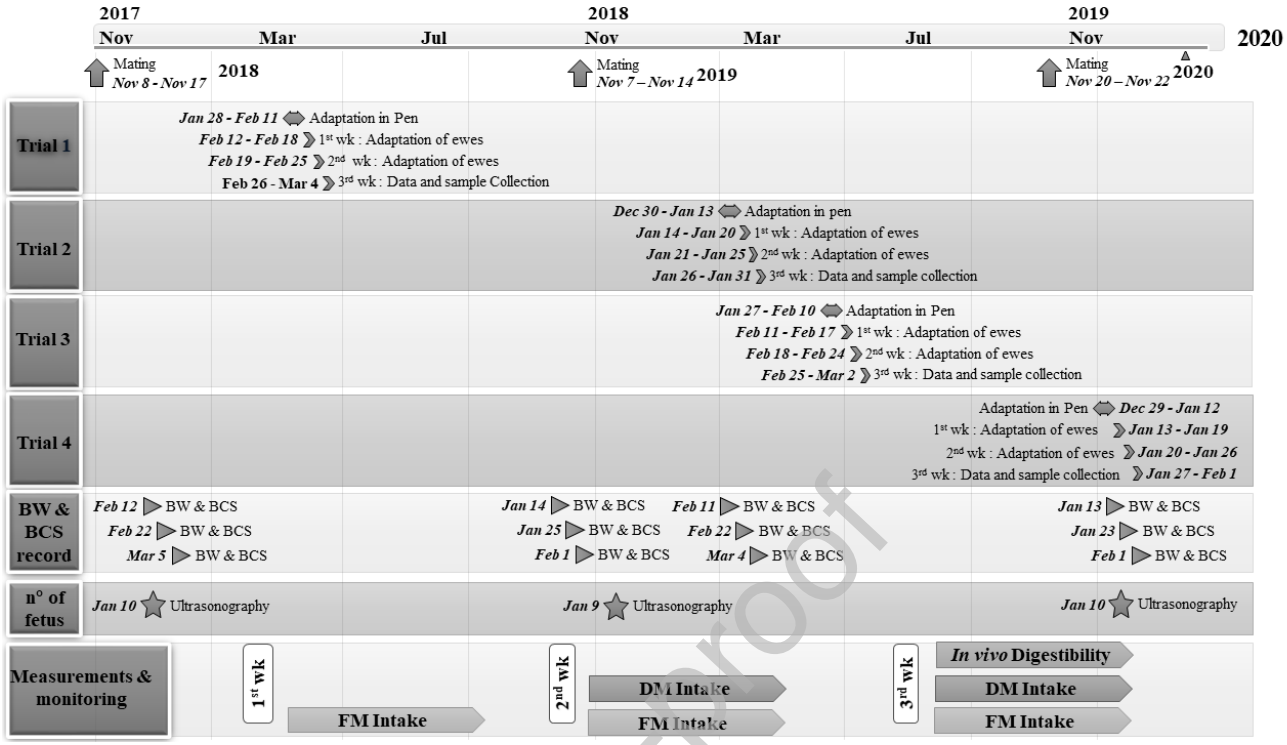

Figure 1

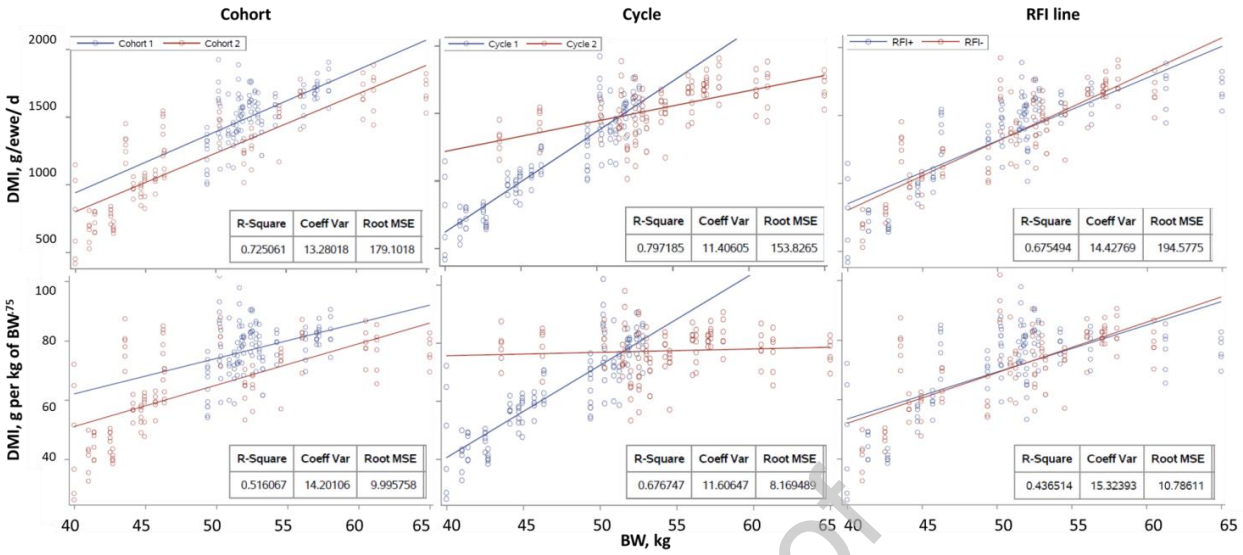

Figure 2