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HIGHLIGHTS

Milk yield is not increased in high-producing dairy ewes fed above energy requirements

Milk composition is not modified when energy requirements are exceeded

Increasing concentrate decreases forage intake

Current INRA equations predicting intake capacity and substitution rate remain valid

ABSTRACT

In general, dairy sheep are fed in large groups that receive the same diet based on the average group milk yield (MY), which is increased by approximately 15% and 25% to cover the energy and protein requirements respectively of part of the high-producing ewes, according to the French INRA system recommendations. Despite this, some of the highest producing ewes are still underfed, especially in terms of energy. This experiment was designed to measure the effect of increasing the amount of energy supplement on milk yield, milk composition and forage intake. Three groups of 16 multiparous Lacaune dairy ewes (DIM mean ± SD: 40 ± 1.5) producing 3.4 ± 0.29 L/d, were balanced in terms of MY, milk composition, body weight (BW: 74.7 ± 8.43 kg) and body condition score (BCS: 2.7 ± 0.26). During three periods of three weeks (P1, P2, P3), groups were fed a basal diet based on a mixture of herbage silages and good quality hay (173 g CP/kg DM) offered *ad libitum*. A protein concentrate was offered at a constant level (0.34 ± 0.034 kg DM/d/ewe) to cover initial protein requirements. Barley grain was offered at three average levels (kg DM/d/ewe): medium (M, 0.478), high (H, 0.667) or very high (vH, 0.883). The three groups (MMH, MHH, MvHH) received

the corresponding amount of barley in the three successive periods: in P1, 0.485 for the three groups, in P2, 0.470 for MMH, 0.672 for MHH and 0.883 for MvHH, and in P3, 0.661 for the three groups. Forage and concentrate dry matter intake were measured each week over five days; milk yield and composition (total fat, protein and urea content) were measured once a week; and BW and BCS were measured every two weeks. Modifying the amount of concentrate (P2, P3) gave no significant ($P<0.05$) difference between groups for MY, milk composition, BW or BCS. In P2, increasing the level of barley led to a high substitution effect with a decrease of the forage dry matter intake. In P3, increasing or decreasing the amount of barley moderately decreased or increased the forage. Taking into account the whole experimental period (P1-P3), increasing the level of barley did not improve ($P>0.05$) MY, but slightly increased ($P<0.05$) BW and BCS. In conclusion, increasing the amount of energy of high-producing dairy ewes fed high-quality forage *ad libitum*, reduces the forage intake, did not improve MY or change milk composition, but increases BW and BCS.

Keywords: Intake capacity; substitution rate; dairy ewe; milk yield; milk composition

1. Introduction

In South of France as well as in most Mediterranean regions, dairy ewes are reared in large groups, leading to a wide range of variability in individual milk yield (**MY**). Animals are fed a common diet regardless of their requirements, making it necessary for the ewes to cope with this feeding system by adapting their feed intake. In the Roquefort area, Lacaune dairy ewes are fed according to INRA recommendations (Hassoun and Bocquier, 2010). Energy (expressed as forage unit for lactation, **UFL**) and protein (protein truly digestible in the small intestine, **PDI**) requirements are based on average group values, taking body weight (**BW**) and standard MY (**sMY**) corrected for fat (**FC**) and protein (**PC**) content into account (Bocquier et al., 1993). It has been shown (Hassoun and Bocquier, 2010) that the mean Intake Capacity (**IC**) of the group can be predicted with both BW and sMY (Bocquier et al., 1997). Intake Capacity is a concept that expresses an animal's ability to consume different amounts of forages according to their bulkiness (or Fill Unit, **UE**). In this system, the fill value (Jarrige et al., 1986) is obtained from equations that link the forage species to its chemical composition (INRA, 1989). This fill value is calculated for sheep (UEM), dairy cattle, goats (UEL) and beef cattle (UEB).

When concentrate is added to the formulated diets, the forage intake of ewes is reduced. This reduction is referred to as the substitution rate (**S**). S varies according to several factors, as reported by Jarrige et al.

(1986). It can depend on the animal species (Michalet-Doreau et al., 1997), the fill value of the forage or its voluntary intake (Berge and Dulphy, 1985), the percentage of concentrate (Michalet-Doreau et al., 1997; Berge and Dulphy, 1985), the energy balance, the lactation or gestation stage, etc. Other factors such as concentrate composition (starch or fiber) may interfere but have not yet been taken into account in the equation. The estimation of S and consequently the concentrate fill value is specific to each type of ruminant (Faverdin et al., 2018), and it is not possible to compare S between species and productions. As an example, sheep are more sensitive to the increase in concentrate than cattle (Michalet-Doreau et al., 1997). In order to formulate diets based on forage provided *ad libitum* and fixed amounts of concentrates in dairy ewes, both the IC and S of the animals must be known.

The traditional way of feeding large groups of ewes with *ad libitum* forages and a fixed amount of concentrate is beginning to change. In the dairy sheep system, farmers more and more frequently use automatic concentrate feeders (ACF). Associated with the electronic individual identification, ACF allows farmers to create virtual groups of ewes having less milk yield variability (e.g. from 0.9 to 1.5 L and so on up to the highest milk yields). Consequently, they can feed concentrates according to different milk yield levels and manage BCS to reach target levels (increasing or decreasing it). However, we do not know how the animals will respond in terms of milk yield and composition and if the substitution rate (S) and IC previously established are still valid in these prevailing conditions. The objective of this experiment was to measure the effect of various levels of concentrate on forage dry matter intake, S, milk yield and milk composition on high producing dairy ewes with low milk yield variability, as if they were managed as a virtual group using an ACF. We hypothesized that modulating the amount of energy fed to the ewes will provoke forage dry matter intake variations, and consequently milk yield and composition modifications.

2. Materials and methods

Experimental site

The experiment was conducted from 18 January to 18 March 2016 at INRAE's (Institut National de la Recherche pour l'Agriculture, l'Alimentation et l'Environnement) La Fage Experimental Farm, Causse

du Larzac (43°54'54.52"N; 3°05'38.11"E), Roquefort-sur-Soulzon, Aveyron, France. The experiment took place within the framework of the Regional Languedoc-Roussillon (France) Ethical Committee on Animal Experimentation - Agreement N°752056/00

2.1. Animals, diets and experimental design

The experiment took place indoors. A total of 48 multiparous high-producing Lacaune dairy ewes in their 2nd to 5th lactation (3 ± 1.0 mean ± SD), and lambed within one week of each other, were selected from among the farm flock. They were separated into three homogenous groups of 16 ewes. Each group was balanced in terms of milk yield (**MY**: 3.4 ± 0.29 l/d), milk fat content (**FC**: 59 ± 8.5 g/l), milk protein content (**PC**: 46 ± 2.8 g/l), body weight (**BW**: 74.7 ± 8.43 kg), body condition score (**BCS:** 2.7 ± 0.26), days in milk (**DIM**: 40 ± 1.5), and average number of lambs reared (1.7 ± 0.47). During the whole experiment, the ewes in each group were reared and group fed. Previously, the animals were fed the total mixed ration of the flock based on the same forages and concentrates as for the experiment. During the experiment, the three groups received a basal diet of mixed forages (**TMF**, Table 1) prepared twice daily with a steady composition (mean ± SD g/kg DM) based on Italian ray grass silage (572 ± 19.2), first cut alfalfa-cocksfoot hay (231 ± 9.0), second cut alfalfa-cocksfoot hay (116 ± 2.0) and alfalfa-cocksfoot round bale silage (81 ± 7.5). The TMF was offered twice daily at about 0900 and 1600 h after milking and adjusted to a daily minimum of 20% refusal. In addition, they received 14 g DM/d mineral vitamin mixture (Turbomix Oviplus A ™, Néolait, Yffiniac, France; with (g/kg) 130 Ca, 110 P, 60 Mg, 20 Na). Each group received a commercial protein concentrate (**CPC**, Brebitanne™, RAGT, Albi, France) and different amounts of barley grain, both split into two equal amounts. Barley was distributed in feed troughs before the TMF, and half of the CPC was provided in feed troughs at the same time as the barley and the other half in the milking parlor. The composition of the TMF and the chemical and nutritive values of the feed are presented in Table 1. The experiment was divided into three periods of three weeks, initially preceded by a one-week adaptation period to accustom the animals to their new flock mates. In the first period (**P1**, control period), each group received the same diet formulated according to INRA recommendations for dairy sheep (Hassoun and Bocquier, 2010) that provided 1.15 and 1.25 times the net

energy requirements (UFL) and protein requirements (PDI), respectively. They received 485 g DM of barley and 337 g DM of CPC per day and per ewe. In the second period (**P2**), one group (MMH) received almost the same level of barley (470 g DM/d) and CPC (399 g DM/d) as in P1. Ewes in the second group (MHH) received 672 g DM/d of barley and 351 g DM/d of CPC, and those of the third group (MvHH) 883 g DM/d of barley and 303 g DM/d of CPC. In the third period (**P3**), the three groups received the same amount of barley (661 g DM/d) and CPC (361 g DM/d). The general scheme of the experimental design is presented in figure 1. The general scheme was set up in order to compare the effect of increasing (P2, MHH and MvHH groups, P3 MMH group) and decreasing (P3, MvHH group) the amount of barley (starch source) on forage DM intake (DMI), substitution rate (S), MY and milk composition, BW and BCS. Indeed, farmer may want to increase or decrease energy for milk or body condition score objectives.

2.2. Intake measurements and substitution rate calculation

Offerings and refusals of TMF and concentrates were weighed every day throughout the experiment. The TMF dry matter intake was measured four to five days a week. Refusals of TMF were weighed and removed before each new TMF distribution. For each group, offerings and refusals were sampled every day in order to determine the DM content (48 h, 60°C in a dry forced-air oven). Forages of TMF were sampled twice a week and concentrates five times during the experiment in order to determine the DM content, as reported above. Samples were gathered per week and kept until laboratory analysis. Total refusals of CPC offered in the milking parlor were gathered and weighed so as to calculate the exact intake for each group. There was no refusal of concentrates provided in troughs.

The French nutritional system for dairy sheep (Hassoun and Bocquier, 2010) makes it possible to predict dry matter intake using the intake capacity (IC) of the animal, forage composition and the substitution rate (S). The IC of the Lacaune breed expressed in fill unit for sheep (UEM), as defined by INRA (1989), is calculated with the following equation:

$$IC = 0.024 \times BW + 0.9 \times sMY \quad [3]$$

where BW is the body weight (kg) and sMY (L/d) is the standard milk yield calculated as follow:

sMY = MY x (0.0071 x milk fat content + 0.0043 x milk protein content + 0.2224) (Bocquier et al., 1993).

135 The total diet intake (TDI), also expressed in UEM, is calculated with the following equation:

136 $$TDI = TFI \times FV + TCI \times S \times FV, \text{ with } S = 3.55 - 2.3 \times FV \quad [4]$$

137 where TFI is the total forage intake (kg DM), TCI is the total concentrate intake (kg DM) and FV is the
138 average fill value of the forages expressed in UEM. Consequently, in sheep fed forage ad libitum, the
139 results of both equations [3] and [4] must be equal.
140 For each group, TMF dry matter intake (TMFI) was calculated by averaging weekly measurements. The
141 substitution rate (S) was calculated and expressed in absolute value as the change in TMFI measured the
142 week before the concentrate change and the week after per unit of concentrate variation.
143 The equation [4] for calculating S, was established with a multiple regression model including several
144 measures of forage and concentrate intake in various experimental situations (Bocquier et al., 1997). For
145 that reason, we decided to calculate S as follows:
146 Period 2 for the MHH and MvHH groups:
147 S = absolute (TMFI in P2 – TMFI in P1)/ (TCI in P2 – TCI in P1) [1]
148 Period 3 for the MMH and MvHH groups:
149 S = absolute (TMFI in P3 – TMFI in P2)/ (TCI in P3 – TCI in P2) [2]
150 where TMFI and TCI are the total mixed forage and the total concentrate dry matter intake, respectively.
151 *2.3. Body weight, body condition score, milk yield and its composition*
152 Before and at the end of the experiment and every two weeks, all animals were weighed and body
153 condition scored (Russel et al., 1969) by the same trained person.
154 Individual milk yield was recorded once a week (afternoon and next morning) and sampled for total fat
155 (**FC**), protein (**PC**), urea (**UC**) and somatic cell (**SCC**) content performed with the medium infrared
156 spectrometry method, applied by the Laboratoire Interprofessionnel d'Analyses Laitières, Aurillac,
157 France.
158 *2.4. Feed chemical analysis*
159 After drying, all feeds (forages and concentrates) were ground through a 1-mm sieve before analysis.
160 Organic matter, crude protein, NDF, ADF and *in vitro* dry matter digestibility of forages were determined

161 by NIRS based on a calibration equation established for 100 forage samples (Laboratoire des Aliments,
162 CIRAD, Baillarguet, France).
163 For concentrates, ash content was determined by ashing in a muffle furnace for 5 hours at 550°C. Total
164 nitrogen was determined using the Kjeldahl procedure, and crude protein was calculated as total nitrogen
165 x 6.25. Cell wall fractions (NDF, ADF) were determined sequentially according to the method of Goering
166 and Van Soest (1970) with an amylases and protease pre-treatment. The cell wall fraction is expressed
167 exclusive of residual ash. The *in vitro* dry matter digestibility was determined according to the pepsin-
168 cellulase method (Aufrère et al., 2007).
169 Feeding value data were: net energy forage unit for lactation (UFL), digestible proteins in the intestine
170 when nitrogen (PDIN) or energy (PDIE) is limited, and the fill value of forages for sheep (FV, expressed
171 in UEM) were calculated using PrevAlim 3.23 software (2006), as described by Baumont et al. (1999).
172 Average energy (EB) and protein (PB) balances were calculated per period for each group based on the
173 average requirements (BW, sMY and BW change) (Hassoun and Bocquier, 2010) and TMFI, TCI and
174 their respective energy (UFL) and protein (PDI) values. The PB was calculated with the PDIE values of
175 the diet, which was the limiting factor (PDIE < PDIN).
176 *2.5. Statistical analysis*
177 All comparisons between groups were performed for each period.
178 The MY, sMY, FC, PC, urea content (UC), fat (FY), protein (PY) and urea (UY) yield were averaged per
179 ewe per period. The results were compared between groups with a one-way analysis of variance using the
180 model:
181 $$Yi = \mu + \alpha i + \varepsilon ij$$
182 where μ is the mean of MY, FC, PC, UC, FY, PY or UY, αi is the main effect of group (MMH, MHH or
183 MvHH), and εij is the term of error.
184 The somatic cell count (SCC), expressed as log 10, was compared with the non-parametric Kruskal-Wallis
185 test for independent samples because data were not normally distributed (Sprent, 1992).
186 Body weight was compared between groups with the one-way analysis of variance using the model:

187 $$Yi = \mu + \alpha i + \varepsilon ij$$

188 where μ is the mean of BW, αi is the main group effect (MMH, MHH or MvHH), and εij is the term of
189 error.

190 The BCS was compared with the non-parametric Kurskal-Wallis test for independent samples because
191 data were not normally distributed and several attempts to transform the data did not succeed.

192 The comparisons of TMFI, TCI and total diet dry mater intake (TDI) between groups were performed with
193 the non-parametric Kurskal-Wallis test for independent samples using the average values per week as the
194 unique measurement.

195 We calculated the difference between the milk yield (dMY), standard milk yield (dsMY), body weight
196 (dBW) and BCS (dBCS) before and after concentrate changed (between periods P1 and P2 and P2 and P3,
197 and for the total experiment P1 and P3) for each ewe, in order to verify whether concentrate change had a
198 different effect on BW and BCS change.

199 The differences observed for dMY and dsMY were compared between groups with a one-way analysis of
200 variance using the model:

201 $$Yi = \mu + \alpha i + \varepsilon ij$$

202 where μ is the mean of dMY or dsMY, αi is the main effect of group (MMH, MHH or MvHH), and εij is
203 the term of error.

204 For dBW or dBCS, they were compared with the non-parametric Kurskal-Wallis test for independent
205 samples because data were not normally distributed and several attempts to transform the data did not
206 succeed.

207 For each group we also compared the BW, BCS, MY and sMY between P1 and P3 (P1 *vs* P3) with the
208 non-parametric Wilcoxon test for paired samples.

209 All statistical analyses were performed using STATISTICA v10 for Windows (Statsoft 2010,
210 www.statsoft.fr).

211

212 **3. Results**

213 One ewe in group MHH was discarded after 22 days because of severe udder injury. Data concerning milk
214 urea content for the first milk recordings were lost by accident.
215 *3.1. Milk yield and milk composition within period*
216 The average values per period and group are presented in Table 2. Milk yield, sMY, milk FC, PC and UC
217 were not different (P > 0.05) between groups at any period. Standard MY (Figure 2) regularly decreased
218 as the lactation stage advanced. Increasing (MvHH and MHH groups in P2, and MMH group in P3) or
219 decreasing (MvHH group, P3) barley amounts did not modify MY or milk composition (P>0.05). No
220 mastitis was observed in the three groups. Only one ewe showed high SCC values for three consecutive
221 weeks with no impact on milk yield or milk composition. Except for this one animal, SCC was always
222 lower than 600,000/mL. Fat, protein and urea yields did not differ (P>0.05) between groups.
223 *3.2 Milk yield, standard milk yield and variations of milk yield and standard milk yield*
224 The average values of MY and sMY measured at the end of each period for each group and the differences
225 between periods P2 and P1 (P2-P1), P3 and P2 (P3-P2) and P3 and P1 (P3-P1) are presented in Table 3.
226 The MY or sMY were never different (P>0.05) between groups at any period.
227 Within the three groups, MY or sMY (figure 2) decreased (P<0.001) by about the same value (0.77-0.79
228 and 0.45-0.53 L respectively, P>0.05). The differences of individual MY between two periods were not
229 different (P>0.05) whatever the group and the periods.
230 *3.3. Body weight, body condition score, variations of body weight and body condition score*
231 The body weight and BCS of the three groups were not different (P > 0.05, Table 3), regardless of the
232 periods P1, P2 or P3. Within groups, the BW slightly increased (P<0.05) in MHH and MvHH groups and
233 tended to increase (P=0.098) in MMH group from P1 to P3 (Table 3). However, across the whole period,
234 the BW changes (dBW) were not different (P>0.05) between groups. In the same period, the BCS slightly
235 increased (P<0.05) in MMH group or decreased (P<0.05) in MHH and MvHH groups, and BCS changes
236 (dBCS) were different (P < 0.01) between groups (Table 3).
237 *3.4. Dry matter intake and substitution rate*

238 In P1, TMFI, TCI and TDI were not different (P > 0.05) between the three groups (Table 4). In period 2,
239 when concentrate amounts increased for the MHH and MvHH groups, the TMFI was different (P =
240 0.0001, Table 4). The TMFI decreased with increasing amounts of concentrate, with the ewes in the MMH
241 group having the highest TMFI values (2.75 kg DM/d/ewe), those in the MvHH group the lowest (2.32 kg
242 DM/d/ewe), and the ewes in the MHH groups with intermediate values (2.50 kg DM/d/ewe). The TDI was
243 higher (P = 0.025) for the MMH group and not different between the MHH and MvHH groups. In P3, the
244 TMFI of the MvHH (2.32 kg DM/d/ewe) and MHH (2.50 kg DM/d/ewe) groups were lower (P=0.0001)
245 compared to the MMH group (2.64 kg DM/d/ewe) and did not change when compared to P2 (Table 4).
246 Considering the three periods, the TDI did not markedly change between the beginning and the end of the
247 experiment for MMH (3.57 and 3.65 kg DM/d/ewe) and MHH (3.59 and 3.51 kg DM/d/ewe). However,
248 for the MvHH group, the TDI markedly decreased between P1 and P2 when the amount of concentrate
249 increased, but then did not change until the end of the experiment (Table 4). In P3, when the total
250 concentrate increased by 0.15 kg DM/d/ewe for the MMH group or decreased by 0.16 kg DM/d/ewe for
251 the MvHH group, there was a slight decrease (-0.11 kg DM/d/ewe) or almost no change (+0.06 kg
252 DM/d/ewe) in TMFI, respectively, the week after. In period 2 (Table 4), S values calculated from equation
253 [1] for MHH (0.86) and MvHH (0.84) are similar to the theoretical value (0.88) calculated with the current
254 equation (Hassoun and Bocquier, 2010). In P3, S values calculated from equation [2] for the MMH (0.73)
255 and MvHH (0.40) groups are lower than the theoretical value (0.88), but the TMFI slightly decreased
256 (0.11 kg DM) for MMH or did not change for MvHH, which means that, theoretically, no substitution
257 occurred in the MvHH group when the amount of concentrate decreased by 0.15 kg DM.
258 *3.5. Energy and protein balance*
259 Energy and protein balance are reported in Table 4. In P1, the energy balance was negative for the MMH
260 (-0.34 UFL/d/ewe) and MvHH (-0.51 UFL/d/ewe) groups and null for the MHH group. The protein
261 balance was positive with 30 to 53 g PDI/d/ewe. In P2, the energy balance increased for the three groups
262 (0.48 to 0.71 UFL/d/ewe) with increasing amounts of concentrate (MMH and MvHH groups). The protein
263 balance also increased for the three groups (Table 4). During P3, all groups had a positive energy balance

264 according to the amount of concentrate and actual sMY. The PB continued to increase, with close values
265 (97 to 108 g PDI/d/ewe) for the three groups. The PB calculated with the PDIN values gave higher values
266 (results not presented): 83 to 106 (P1), 133 to 152 (P2) and 144 to 157 g/d/ewe (P3).

267 **4. Discussion**

268 Changing concentrate levels during the 40 to 104 DIM period of high producing Lacaune dairy ewes had
269 limited effects on group mean milk yields and no significant effects on milk composition (FC and PC).
270 This is due to the adaptive capabilities (Blanc et al., 2006) of highly selected dairy animals (Barillet et al.,
271 2016) that maintained milk yields and controlled body weight changes through regulation of *ad libitum*
272 feed intake. Cannas et al., (2013) observed in an experiment with dairy ewes in mid lactation (89 DIM)
273 that increasing the amount of non-fiber carbohydrates (starch) from 23 to 36 % in the diet did not increase
274 milk yield, but increased milk protein content and decreased milk urea content. In the present experiment,
275 the starch content of the diets ranged from 8.7 to 16.4 % on the DM basis and despite the total decrease of
276 forage intake, no effect was observed on milk or milk composition, probably due to the low starch levels.
277 Before considering the effects of MY and BW on predicted feed intake (TDI), the metabolic status of each
278 group of ewes must be established. The energy balance calculated for each period shows that during P1,
279 energy intake does not completely fulfill the animals' total requirements (Hassoun and Bocquier, 2010)
280 based on actual sMY, BW and BW change. During this period, the fixed ingredients of the diet were
281 formulated to provide 115 and 125% of the energy and protein requirements based on the initial BW and
282 sMY, respectively, ignoring the possible weight gain. Since these lactating ewes were observed after peak
283 milk yield, it has often been observed (Bocquier et al., 1999) that excesses in energy intake (i.e., positive
284 energy balance) are diverted into BW changes rather than into milk yield. This was previously reported for
285 other dairy sheep breeds fed good quality forage and supplemented with starchy concentrate (Avondo et
286 al., 1995; Cannas et al., 2002) when concentrate allowances were increased from 40 to 45% and 25 to
287 40%, respectively. Similarly, both of them observed a body weight increase. Such results were also
288 reported for different breeds of dairy cows (Prendiville et al., 2011). Gonzalez-Garcia et al. (2015)
289 observed in dairy sheep that after weaning, the mobilization of body reserves rapidly decreased and the

290 BCS simultaneously increased with an increase in plasma concentration of leptin, which was higher when
291 the energy balance was higher without increasing milk yield. After an increase in the amount of barley in
292 P2 for the MHH and MvHH groups, and in P3 for the MMH group, energy and protein balances became
293 positive for all groups, mainly because sMY declined similarly in all groups and BW change was almost
294 null or slightly positive (Table 3). Consequently, excess energy would be converted into body reserves and
295 body weight gain. However, the BCS slightly increased for MMH (+ 0.1, $P<0.05$) or decreased for MHH
296 and MvHH (-0.1, $P<0.05$) throughout the whole experiment, although weight gain did. It is possible that
297 the BCS could not precisely estimate fat deposition because according to Termatzidou et al. (2020), milk
298 sheep breeds have more perirenal and omental fat deposition than subcutaneous, compared to meat breeds.
299 Gonzalez-Garcia et al. (2015) observed a low increase in BSC when measuring the effects of a high
300 positive energy balance of overfed dairy Lacaune dairy sheep whereas plasmatic leptin increased
301 considerably. Hence, we must consider that short durations between P1 and P2 and P2 and P3 (2 or 3
302 weeks) may not be sufficient for obtaining significant BW or BCS changes related to the positive energy
303 balance.
304 Conversely, increasing positive nitrogen balance leads to increasing milk urea content, which is known as
305 a good indicator of nitrogen utilization and efficiency in dairy ruminants (Cannas et al., 1998; Nousiainen
306 et al., 2004). In the present study, milk urea content increased markedly from period 1 to periods 2 and 3
307 because total crude protein intake did not change (684 ± 20.1 g CP), whereas milk protein yield decreased
308 (from 153 to 130 g protein/d). The high milk urea content measured in the three groups over the whole
309 experiment (0.576 g/L) is related to the protein balance (+ 100 g PDI/d/ewe). Cannas (2004) suggests that
310 milk urea content of more than 0.4-0.5 g/L is associated with an excess of protein in the diet, which is the
311 present situation and even more so when PB is calculated with PDIN values. However, it has been shown
312 that excessive protein supply has no detrimental effect and is accompanied by a marginal positive
313 response on protein milk yield (Cannas et al., 1998; Gonzalez et al., 1982; INRA, 1989; Lagriffoul et al.,
314 1999). It is worth noting that milk composition was unaffected by the level of concentrates throughout the
315 experimental periods. It has been shown (Bocquier and Caja, 1999) that milk fat and protein content may

316 be altered by energy balance and a high level of starchy concentrates that decrease milk fat content. In the
317 same way, increasing the energy tends to increase the milk protein content. In the present experiment, the
318 level of concentrate was relatively low in period 2 for MHH (29%) and MvHH (34%), and 28 to 31% in
319 the third period. Possibly, because of the high nutritive value of the diet and the moderate concentrate
320 level, we did not observe a negative effect on milk fat content. Similarly, Lawrence et al. (2015) observed
321 no effect on the milk composition of dairy cows when the amount of concentrate was increased up to 37%,
322 and Ferris et al. (2001) observed that increasing the concentrate level in high genetic merit dairy cows has
323 a negative effect on milk fat content at 50% and beyond. They also observed that increasing concentrate
324 has a moderate effect on milk yield when associated with good nutritive value forage. Consequently, we
325 may assume that in the present experiment, the level of concentrate was not high enough to negatively
326 affect milk composition.
327 Based on equation [3] and animal characteristics, the theoretical IC of the three groups in period 1 would
328 be 4.21 to 4.28 UEM. Taking the IC into account, S (0.882, from equation [4]), TCI (0.81 kg DM) and the
329 fill value of TMFI (1.16 UEM), the predicted TMFI from equations [3] and [4] would be 2.92 to 2.97 kg
330 DM/d/ewe in P1. In this period, when the three groups were fed the same diet, the TMFI intake observed
331 for the three groups was not different (Table 4), but lower (2.74 to 2.77 kg DM/d/ewe) than the expected
332 value. There is no explanation for such a difference. The fill value of TMFI over the total experiment does
333 not greatly vary (1.16 ± 0.01 UEM/kg DM) and, consequently, cannot explain the difference observed.
334 Conversely, the differences for MMH, MHH and MvHH were much lower in P2 (0.01, -0.12 and -0.07,
335 respectively) and P3 (0.14, 0.02 and -0.07, respectively). When considering the three periods, the average
336 total diet DM intake of the three groups did not considerably change (Table 4) from P1 (3.56 kg
337 DM/d/ewe) to P3 (3.50 kg DM/d/ewe), while average sMY decreased from 2.71 to 2.22 L/d/ewe. Several
338 authors (Avondo et al., 1995; Bizelis et al., 2000; Molina et al., 2001), with different dairy sheep breeds
339 and diets, observed that from the third to the sixth week of lactation for 5 to 12 weeks, the DMI did not
340 change, whereas the MY decreased by at least two-fold.

341 The present experiment was also designed to explore amplitudes in substitution rates of forage by
342 concentrate. Increasing concentrate amounts for the MvHH and MHH groups in P2 and for the MMH
343 group in P3 significantly decreased the TMFI, indicative of a substitution phenomenon. The S values
344 calculated in P2 for the MvHH (0.84) and MHH (0.86) groups were close to those previously estimated
345 though equation [4] (S= 0.88) but lower (0.73) for MMH in P3. Indeed, the difference between S = 0.73
346 and S = 0.88 corresponds to a small variation of TMFI (+ 0.020 kg DM), which means that if TMFI would
347 be 1.13 kg DM instead of actual 1.11 value, S would be 0.87. The result of S calculation is very sensitive
348 to small variations of forage DMI. Consequently, we should rounded the forage value to ± 0.05 or 0.10 kg
349 DM, which is in the range of precision generally observed or used for diet calculation, otherwise, we
350 could find different S values due to excessive forage intake precision. Hence, we can consider that there is
351 no difference between the three S values obtained in this experiment and the S predicted with equation [4]
352 proposed by Bocquier et al. (1997). This also suggests that the equation can still be used in a relatively
353 wide range of lactation stages accompanying MY evolution. In addition, the present results are in
354 agreement with those of Molle et al. (1997) who observed comparable S values (0.85 - 0.95) with
355 Sardinian dairy sheep in early lactation (58 to 88 DIM) when grazing a good quality pasture (213 g CP/kg
356 DM) and supplemented with 0.5 kg of corn grain but with somewhat lower MY (1.07 L/d). In another
357 experiment, Gomez-Cortes et al. (2011) measured the effect of increasing concentrate (0.6 to 1.2 kg
358 DM/d) in a total mixed diet based on dehydrated alfalfa and concentrate. The diet was offered *ad libitum*
359 to Assaf dairy ewes in early lactation (6^{th} week) with high initial MY (3.2 kg/d). The S value calculated
360 was higher by 1.18 than previous results. It is well established that S increases with higher proportions of
361 concentrate in the diet (Michalet-Doreau et al., 1997; Berge and Dulphy, 1985). In the experiment of
362 Gomez-Cortes et al. (2011), concentrate represented 50 and 70% of the diet compared to the initial value
363 of 30%. In the present experiment, the average percentage of concentrate in the total intake was 22% in
364 P1, and increased up to 34% (MvHH group in P2), and in the experiment of Molle et al. (1997), the
365 percentage of concentrate varied within the same range. Altogether, such differences in concentrate

366 proportions agree with the lower S values observed in our experiment when compared to very high
367 concentrate proportions (Gomez-Cortes et al 2011).
368 In other experiments (D'Urso et al., 1993; Avondo et al., 1995), lower calculated S values were observed
369 (0.35 to 0.43) but experimental conditions were very different. In these experiments, dairy sheep in the
370 early lactation stage (35 to 112 DIM) were grazed on good quality pastures in daylight from 10.00 to
371 15.00 and supplemented with a small amount of medium quality hay and various amounts of concentrate
372 after grazing. The percentage of concentrates ranged from 18 to 37%. The low values of S observed in
373 these two experiments, could be explained because the ewes were fed the concentrate in the afternoon,
374 more than 12 hours before the grazing period, when the rumen environment (pH) is more favorable to the
375 cellulolytic bacteria. As reported by Lamb et al., (1979), the reduction of forage intake when supplements
376 are fed, is associated with a lowering of rumen fluid pH, which reduce the rate of digestion of the fibers.
377 We may also consider that the equation [4] used for calculating S for Lacaune breed, is not appropriate for
378 Mediterranean sheep breeds. Indeed, Caja et al., (2002) determined a specific equation for calculating S
379 for Mediterranean breeds (Manchega and Latxa) with lower milk yield than the Lacaune or Assaf breeds.
380 Consequently, we must be careful when comparing such a result because breeds and experimental
381 conditions must be taken into consideration. Faverdin et al., (1991), studying the S values in different
382 conditions pointed out that several aspects must be considered (breeding, housing, milk potential etc.)
383 before drawing any conclusions. Forages studied were different, but they were also measured in different
384 situations that may modulate the results.
385 In P3, the effect of decreasing concentrate in the MvHH group (-0.16 kg DM/d) on TMFI was negligible
386 (+0.06 kg DM/d). It was below the standard deviation for the MvHH group (±0.11 kg DM/d). Decreasing
387 (MvHH group) or increasing (MMH group) concentrate with the same amplitude (-0.16 and +0.15 kg
388 DM/d, respectively) gave a similar response in terms of TMFI, with +0.06 and -0.10 kg DM/d
389 respectively, but below their respective standard deviation (0.11 and 0.14). Since the effects are not
390 measurable with precision, we have no reason to modify the calculated S with equation [4].

391 We think that it is more correct to calculate S within a group instead of between groups because when
392 concentrate amount is changed in a diet it will apply to the same animals. If we had calculated S in P2
393 between groups, the values would be very different: 1.06 between MvHH and MHH, 1.38 between MvHH
394 and MMH and 1.73 between MHH and MMH. This experiment addressed only high producing dairy
395 ewes, and not a group with wide MY variability as it was the case in the studies reported by Bocquier et
396 al., (1997). Consequently, conclusions on S value must be considered with caution. Finally, although
397 cereal with rapidly degraded starch in the rumen (oat, wheat, barley) have high impact on cell wall
398 degradation in the rumen compared to corn or sorghum with slowly degraded starch, such an effect is less
399 pronounced when concentrate levels in the diets are below 30% (Michalet-Doreau et al., 1997; Nozière et
400 al., 1996) like in the present study.
401
402 **5. Conclusions**
403 Feeding dairy ewes in large groups is considered general practice. In order to maximize forage
404 consumption, it is important to predict forage intake at a given level of concentrate. INRA's Fill Unit
405 system (INRA, 1989) was quite accurate in predicting forage intake when the substitution rate of forage to
406 concentrate is known. The present study confirms that in high producing dairy ewes, the previous equation
407 of intake capacity is still valid. When the energy and protein requirements of high producing dairy ewes
408 are fully covered with a diet based on good quality forages, increasing the energy level through cereals
409 lead to a high substitution effect with a decrease of forage intake without milk yield or milk composition
410 change. Only BW and BCS slightly increase or decrease within the short duration of the experiment. At
411 this lactation stage (2 months and more) when MY linearly decreases, the excess of energy intake is not
412 used for increasing milk production in high producing ewes but will serve for body reserve reconstitution,
413 although in this short duration experiment we observed only slight changes of BCS and BW (Table 3).
414 Fortunately, an accurate prediction of feed intake is possible at an early stage of the milking period when
415 nutritional requirements for milk production are high. By the middle of lactation, feed intake is poorly

related to milk yield. This is due to the fact that feed intake does not decrease as quickly as milk yield decreases. This is not a problem when ewes are in positive energy balance because extra energy is diverted into replenishment of body reserves. Finally, due to the adaptive capacities of high-yielding Lacaune dairy ewes, rationing of group-fed ewes is feasible thanks to the Fill Unit System. The group feeding strategy suggested by INRA (mean energy requirements x 1.15 and protein requirements x 1.25) is still valid. However, further studies are needed to assess the effect of individual feeding based on automatic concentrate feeder in sheep kept in virtual groups.

Credit authorship statement

P. Hassoun and F. Bocquier contributed to the design and implementation of the research, to the analysis of the results and the writing. S. Parisot contributed to the implementation of the research, management of the staff and all the animal procedure in agreement with animal welfare. M.A. Cordoba contributed to the management of the experiment, recording the results, following all animal measurements, and writing part of the first draft of the paper. D. Portes and J. Pradel, were in charge of the animal management, participated in the execution of the experiment and collection of samples.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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565

566 Table 1. Chemical and nutritive composition (mean ± SD of the feed offered during the experiment, expressed in g/kg DM, if not
567 stated otherwise)

	Silage	Round bale	Hay 1	Hay 2	TMF	Barley	CPC
DM (g/kg fresh)	317 ± 8.5	579 ± 65.6	864 ± 12.5	870 ± 11.9	426 ± 8.1	900 ± 25.4	885 ± 7.5
OM	898 ± 8.3	883 ± 7	930 ± 5.3	921 ± 11	907 ± 5.1	976 ± 0.6	924 ± 1.7
CP	-	-	-	-	-	644	-
Starch	166 ± 19.4	241 ± 6.8	137 ± 12	217 ± 7.3	171 ± 5.8	134 ± 5.2	451 ± 8.8
NDF	454 ± 24.2	364 ± 24.2	558 ± 13.4	431 ± 15.1	467 ± 15.7	173 ± 19.8	193 ± 15.7
ADF	282 ± 21.8	200 ± 5.5	354 ± 13.3	273 ± 16.4	291 ± 6.4	51 ± 2	112 ± 5.3
DMD	687 ± 2.6	748 ± 8.3	584 ± 1.7	720 ± 2.4	666 ± 9.2	872 ± 1.2	902 ± 2.5
PDIN	96 ± 11.2	137 ± 3.9	88 ± 7.6	139 ± 4.6	103 ± 3.51	91 ± 3.7	329 ± 6.2
PDIE	81 ± 3.9	76 ± 1.6	83 ± 3.3	111 ± 2.5	85 ± 1.8	104.5 ± 2.2	221 ± 3.1
UFL (/kg DM)	0.92 ± 0.02	0.81 ± 0.01	0.65 ± 0.02	0.81 ± 0.02	0.84 ± 0.01	1.05 ± 0.02	0.92 ± 0.3
UEM (/kg DM)	1.17 ± 0.06	0.78 ± 0.01	1.34 ± 0.05	1.01 ± 0.02	1.16 ± 0.01	NA	NA

568 Hay 1 and Hay 2 = alfalfa cocksfoot mixture, first and second cut, respectively; TMF = total mixed forages; CPC = commercial
569 protein concentrate (Brebitanne ™); DM = dry matter; OM = organic matter; CP = crude proteins; NDF = neutral detergent fiber;
570 ADF = acid detergent fiber; DMD = *in vitro* DM digestibility; PDIN = protein truly digestible in the small intestine when
571 degradable nitrogen in the rumen is limiting; PDIE = protein truly digestible in the small intestine when degradable energy in the
572 rumen is limiting; UFL = net energy expressed as forage unit for lactation; UEM = fill value unit for sheep; NA = not appropriate.

573

574 Table 2 Average individual values of milk production and composition from ewes fed successively with medium, medium and

575 high (MMH) or medium high and high (MHH) or medium, very high and high (MvHH) amounts of barley grain.

		MMH	MHH	MvHH	P	RSME
PERIOD 1	Milk yield (L/d/ewe)	3.21	3.24	3.24	0.9374	0.285
	Total fat content (g/L)	57.4	59.6	57.7	0.5795	6.48
	Total protein content (g/L)	48.1	47.3	47	0.5522	2.89
	Total urea content (g/L)	0.539	0.522	0.535	0.8543	0.085
	Somatic cell count (log10 (n/1000))	6.28	6.31	6.04	0.7417	1.062
	Standard milk yield (L/d/ewe)	2.68	2.75	2.7	0.8265	0.299
	Fat yield (g/d/ ewe)	184.2	193	187.3	0.6807	28.17
	Protein yield (g/d/ ewe)	154	153	152	0.9416	15.64
	Urea yield (g/d/ ewe)	1.69	1.66	1.69	0.9622	0.326
PERIOD 2	Milk yield (L/d/ewe)	2.80	2.80	2.75	0.8804	0.524
	Total fat content (g/L)	60.3	62.2	59.9	0.4331	10.24
	Total protein content (g/L)	52.3	51.6	50.4	0.2700	8.2
	Total urea content (g/L)	0.612	0.582	0.564	0.2356	0.117
	Somatic cell count (log10 (n/1000))	6.59	6.38	6.23	0.5315	1.258
	Standard milk yield (L/d/ewe)	2.45	2.48	2.37	0.6638	0.482
	Fat yield (g/d/ ewe)	169	174	164.6	0.6241	36.01
	Protein yield (g/d/ ewe)	146.3	144	138.1	0.4916	28.59
	Urea yield (g/d/ ewe)	1.71	1.63	1.55	0.3121	0.386
PERIOD 3	Milk yield (L/d/ewe)	2.44	2.35	2.36	0.7533	0.502
	Total fat content (g/L)	66.9	69.3	64.7	0.1969	12.01
	Total protein content (g/L)	55.6	55.4	52.9	0.1334	9.00
	Total urea content (g/L)	0.652	0.608	0.618	0.3606	0.126
	Somatic cell count (log10 (n/1000))	6.59	6.38	6.33	0.6774	1.271
	Standard milk yield (L/d/ewe)	2.28	2.24	2.14	0.5302	0.473
	Fat yield (g/d/ ewe)	162.7	162.7	152.5	0.5010	35.89
	Protein yield (g/d/ ewe)	135.5	129.8	124.5	0.3525	28.41
	Urea yield (g/d/ ewe)	1.58	1.43	1.45	0.2842	0.353

576 P = value of the statistical test; RMSE= root mean square error.

577 Table 3: Body weight (BW), body condition score (BCS), milk yield (MY) and standard MY (sMY) measured at the end of period
578 1 (P1), 2 (P2) and 3 (P3) and variations of BW (dBW), BCS (dBCS), MY (dMY) and sMY (dMY), between periods P1 and P2
579 (P2-P1), P2 and P3 (P3-P2) and periods P1 and P3 (P3-P1) in groups MMH, MHH and MvHH.

	P1	P2	P3	P3-P1	P2-P1	P3-P2	P3-P1
		BW (kg)		P		**dBW (kg)**	
MMH	80	80	81	0.0980	0.36	0.78	1.13
MHH	77	77	78	0.0409	-0.11	1.82	1.69
MvHH	76	76	77	0.0299	-0.08	1.29	1.21
P	0.3526	0.254	0.3635		0.817	0.453	0.842
RMSE	8.3	8.100	7.900		0.208	0.267	0.317
		BCS				**dBCS**	
MMH	2.7	2.7	2.8	0.0367	0.03	0.06	0.09
MHH	2.9	2.9	2.8	0.0281	-0.01	-0.11	-0.11
MvHH	2.9	2.9	2.8	0.0135	0.05	-0.19	-0.14
P	0.178	0.118	0.791		0.619	0.0001	0.0030
RMSE	0.33	0.27	0.27		0.024	0.025	0.029
		MY (L)				**dMY (L)**	
MMH	3.11	2.62	2.33	0.0004	-0.482	-0.291	-0.772
MHH	3.1	2.61	2.31	0.0007	-0.488	-0.305	-0.793
MvHH	3.09	2.56	2.30	0.0004	-0.528	-0.261	-0.789
P	0.9907	0.8651	0.9686		0.874	0.8481	0.9841
RMSE	1.76	1.67	1.521		0.039	0.031	0.049
		sMY (L)				**dsMY (L)**	
MMH	2.61	2.36	2.16	0.0004	-0.255	-0.193	-0.448
MHH	2.68	2.40	2.21	0.0007	-0.281	-0.189	-0.47
MvHH	2.60	2.26	2.07	0.0004	-0.341	-0.189	-0.529
P	0.8078	0.5055	0.5308		0.533	0.9971	0.6825
RMSE	1.621	1.544	1.465		0.032	0.028	0.039

580 MMH = ewes fed successively with medium, medium and high amounts of barley grain; MHH = ewes fed successively with
581 medium, high and high amounts of barley grain; MvHH = ewes fed successively with medium, very high and high amounts of
582 barley grain; P = value of the statistical test; RMSE= root mean square error

583

584 Table 4. Mean (standard error of the mean in brackets) of dry matter intake of total mixed forage (TMFI), total concentrates (TCI)
585 and total diet (TDI), energy (EB) and protein (PB) balance averaged per period, and differences between average values the week
586 after concentrate changed and the week before, for TMFI (D TMFI) and TCI (D TCI) and substitution rate (S) for groups MMH,
587 MHH and MvHH.

		MMH	MHH	MvHH	P
PERIOD 1	TMFI (kg DM/d/ewe)	2.75 (0.060)	2.77 (0.066)	2.74 (0.050)	0.888
	TCI (kg DM/d/ewe)	0.80	0.81	0.82	0.190
	TDI (kg DM/d/ewe)	3.55 (0.056)	3.58 (0.065)	3.56 (0.048)	0.896
	EB (UFL/d/ewe)	-0.34	0.02	-0.51	
	PB (g PDI/d/ewe)	30	53	34	
PERIOD 2	TMFI (kg DM/d/ewe)	2.75 (0.034)[a]	2.50 (0.013)[b]	2.32 (0.033)[c]	0.0001
	TCI (kg DM/d/ewe)	0.87[a]	1.02[b]	1.19[c]	0.0001
	TDI (kg DM/d/ewe)	3.61 (0.034)[a]	3.52 (0.015)[b]	3.51 (0.032)[b]	0.025
	D TMFI (kg DM/d/ewe)		-0.20	-0.33	
	D TCI (kg DM/d/ewe)		0.22	0.38	
	S		0.86	0.84	
	EB (UFL/d/ewe)	0.48	0.64	0.71	
	PB (g PDI/d/ewe)	97	94	94	
PERIOD 3	TMFI (kg DM/d/ewe)	2.64 (0.044)[a]	2.50 (0.021)[b]	2.32 (0.033)[c]	0.0001
	TCI (kg DM/d/ewe)	1.02[a]	1.02[a]	1.03[b]	0.003
	TDI (kg DM/d/ewe)	3.65 (0.042)[a]	3.51 (0.020)[b]	3.35 (0.033)[c]	0.0001
	D TMFI (kg DM/d/ewe)	-0.11		0.06	
	D TCI (kg DM/d/ewe)	0.15		-0.15	
	S	0.73		0.40	
	EB (UFL/d/ewe)	0.58	0.25	0.33	
	PB (g PDI/d/ewe)	108	97	101	

588 MMH = ewes fed successively with medium, medium and high amounts of barley grain; MHH = ewes fed successively with
589 medium, high and high amounts of barley grain; MvHH = ewes fed successively with medium, very high and high amounts of
590 barley grain; P = probability value. Values with different superscript letters in a row are significantly different.

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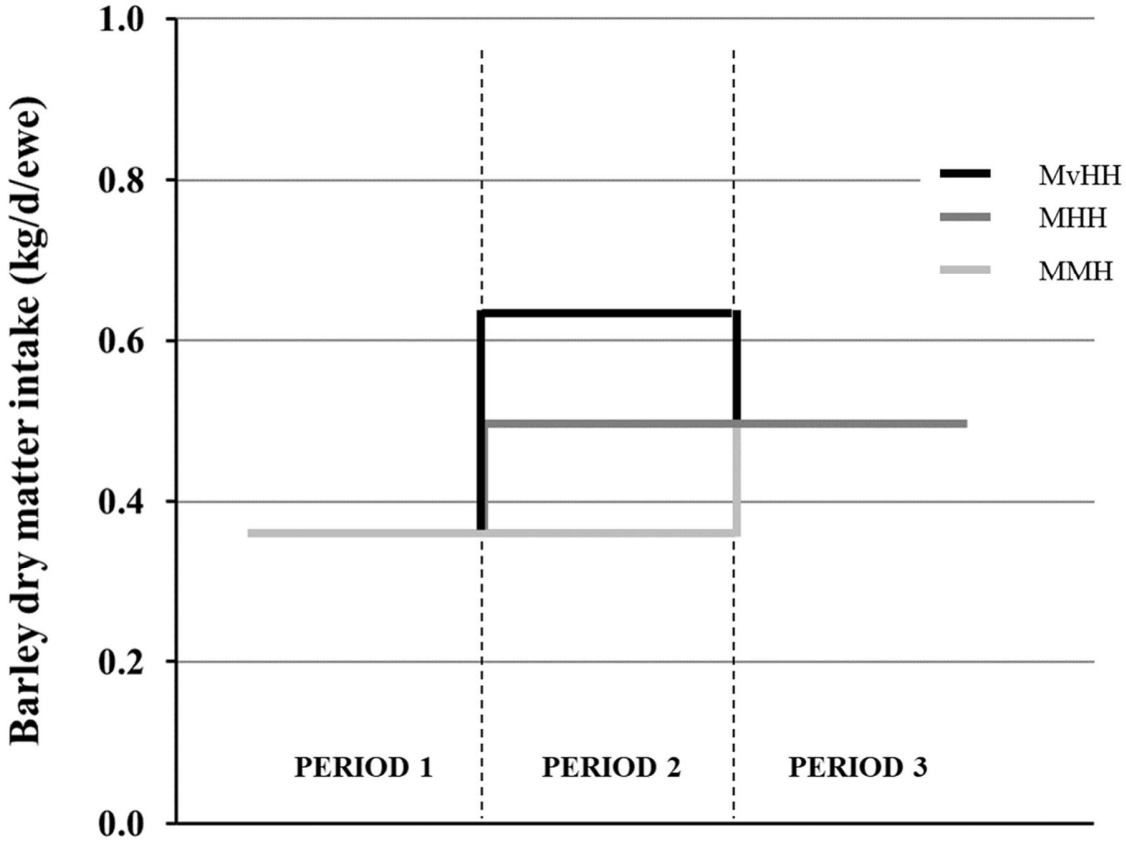
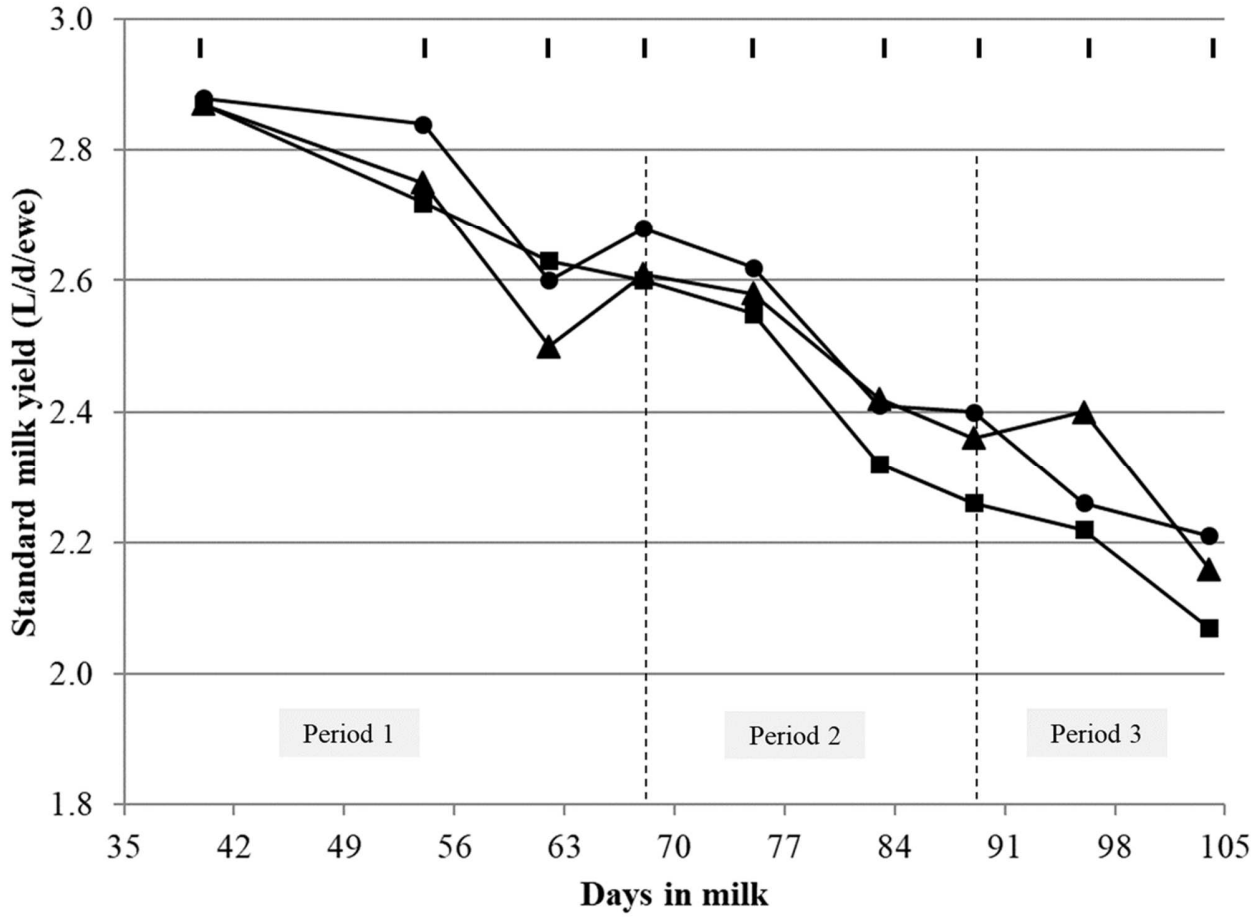
Figure 1. General scheme of the experimental design, for the three groups of ewes fed successively with medium, very high and high (MvHH), or medium, high and high (MHH) or medium, medium and high (MMH) amounts of barley grain during the three periods.



599 Figure 2 Average daily standard milk yield produced during the experiment of ewes fed successively with medium, very high and
600 high (MvHH, square), or medium, medium and high (MMH, triangle) or medium, high and high (MHH, circle) amounts of barley
601 grain during the three periods. Vertical bars represent the standard error of the mean.



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