Effects of different supplement amounts on dry matter intake, milk production and milk composition of high-producing Lacaune dairy ewes

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▶ To cite this version:

HAL Id: hal-03338822

https://hal.inrae.fr/hal-03338822v1

Submitted on 22 Aug 2023

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12   **HIGHLIGHTS**

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

14   Milk composition is not modified when energy requirements are exceeded

15   Increasing concentrate decreases forage intake

16   Current INRA equations predicting intake capacity and substitution rate remain valid

17

18   **ABSTRACT**

19   In general, dairy sheep are fed in large groups that receive the same diet based on the average group milk yield (MY), which is

20   increased by approximately 15% and 25% to cover the energy and protein requirements respectively of part of the high-producing

21   ewes, according to the French INRA system recommendations. Despite this, some of the highest producing ewes are still

22   underfed, especially in terms of energy. This experiment was designed to measure the effect of increasing the amount of energy

23   supplement on milk yield, milk composition and forage intake. Three groups of 16 multiparous Lacaune dairy ewes (DIM mean

24   ± 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)

25   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

26   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

27   constant level (0.34 ± 0.034 kg DM/d/ewe) to cover initial protein requirements. Barley grain was offered at three average levels

28   (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

1

29 the corresponding amount of barley in the three successive periods: in P1, 0.485 for the three groups, in P2, 0.470 for MMH,

30 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

31 measured each week over five days; milk yield and composition (total fat, protein and urea content) were measured once a week;

32 and BW and BCS were measured every two weeks. Modifying the amount of concentrate (P2, P3) gave no significant ($P<0.05$)

33 difference between groups for MY, milk composition, BW or BCS. In P2, increasing the level of barley led to a high substitution

34 effect with a decrease of the forage dry matter intake. In P3, increasing or decreasing the amount of barley moderately decreased

35 or increased the forage. Taking into account the whole experimental period (P1-P3), increasing the level of barley did not improve

36 ($P>0.05$) MY, but slightly increased ($P<0.05$) BW and BCS. In conclusion, increasing the amount of energy of high-producing

37 dairy ewes fed high-quality forage *ad libitum*, reduces the forage intake, did not improve MY or change milk composition, but

38 increases BW and BCS.

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

40

41 **1. Introduction**

42 In South of France as well as in most Mediterranean regions, dairy ewes are reared in large groups,

43 leading to a wide range of variability in individual milk yield (**MY**). Animals are fed a common diet

44 regardless of their requirements, making it necessary for the ewes to cope with this feeding system by

45 adapting their feed intake. In the Roquefort area, Lacaune dairy ewes are fed according to INRA

46 recommendations (Hassoun and Bocquier, 2010). Energy (expressed as forage unit for lactation, **UFL**)

47 and protein (protein truly digestible in the small intestine, **PDI**) requirements are based on average group

48 values, taking body weight (**BW**) and standard MY (**sMY**) corrected for fat (**FC**) and protein (**PC**) content

49 into account (Bocquier et al., 1993). It has been shown (Hassoun and Bocquier, 2010) that the mean Intake

50 Capacity (**IC**) of the group can be predicted with both BW and sMY (Bocquier et al., 1997). Intake

51 Capacity is a concept that expresses an animal's ability to consume different amounts of forages according

52 to their bulkiness (or Fill Unit, **UE**). In this system, the fill value (Jarrige et al., 1986) is obtained from

53 equations that link the forage species to its chemical composition (INRA, 1989). This fill value is

54 calculated for sheep (UEM), dairy cattle, goats (UEL) and beef cattle (UEB).

55 When concentrate is added to the formulated diets, the forage intake of ewes is reduced. This reduction is

56 referred to as the substitution rate (**S**). S varies according to several factors, as reported by Jarrige et al.

57 (1986). It can depend on the animal species (Michalet-Doreau et al., 1997), the fill value of the forage or

58 its voluntary intake (Berge and Dulphy, 1985), the percentage of concentrate (Michalet-Doreau et al.,

59 1997; Berge and Dulphy, 1985), the energy balance, the lactation or gestation stage, etc. Other factors

60 such as concentrate composition (starch or fiber) may interfere but have not yet been taken into account in

61 the equation. The estimation of S and consequently the concentrate fill value is specific to each type of

62 ruminant (Faverdin et al., 2018), and it is not possible to compare S between species and productions. As

63 an example, sheep are more sensitive to the increase in concentrate than cattle (Michalet-Doreau et al.,

64 1997). In order to formulate diets based on forage provided *ad libitum* and fixed amounts of concentrates

65 in dairy ewes, both the IC and S of the animals must be known.

66 The traditional way of feeding large groups of ewes with *ad libitum* forages and a fixed amount of

67 concentrate is beginning to change. In the dairy sheep system, farmers more and more frequently use

68 automatic concentrate feeders (ACF). Associated with the electronic individual identification, ACF allows

69 farmers to create virtual groups of ewes having less milk yield variability (e.g. from 0.9 to 1.5 L and so on

70 up to the highest milk yields). Consequently, they can feed concentrates according to different milk yield

71 levels and manage BCS to reach target levels (increasing or decreasing it). However, we do not know how

72 the animals will respond in terms of milk yield and composition and if the substitution rate (S) and IC

73 previously established are still valid in these prevailing conditions. The objective of this experiment was

74 to measure the effect of various levels of concentrate on forage dry matter intake, S, milk yield and milk

75 composition on high producing dairy ewes with low milk yield variability, as if they were managed as a

76 virtual group using an ACF. We hypothesized that modulating the amount of energy fed to the ewes will

77 provoke forage dry matter intake variations, and consequently milk yield and composition modifications.

78

79 **2. Materials and methods**

80 *Experimental site*

81 The experiment was conducted from 18 January to 18 March 2016 at INRAE's (Institut National de la

82 Recherche pour l'Agriculture, l'Alimentation et l'Environnement) La Fage Experimental Farm, Causse

83 du Larzac (43°54'54.52"N; 3°05'38.11"E), Roquefort-sur-Soulzon, Aveyron, France. The experiment took

84 place within the framework of the Regional Languedoc-Roussillon (France) Ethical Committee on Animal

85 Experimentation - Agreement N°752056/00

86 *2.1. Animals, diets and experimental design*

87 The experiment took place indoors. A total of 48 multiparous high-producing Lacaune dairy ewes in their

88 2nd to 5th lactation (3 ± 1.0 mean $\pm$ SD), and lambed within one week of each other, were selected from

89 among the farm flock. They were separated into three homogenous groups of 16 ewes. Each group was

90 balanced in terms of milk yield (**MY**: 3.4 ± 0.29 l/d), milk fat content (**FC**: 59 ± 8.5 g/l), milk protein

91 content (**PC**: 46 ± 2.8 g/l), body weight (**BW**: 74.7 ± 8.43 kg), body condition score (**BCS:** 2.7 ± 0.26),

92 days in milk (**DIM**: 40 ± 1.5), and average number of lambs reared (1.7 ± 0.47). During the whole

93 experiment, the ewes in each group were reared and group fed. Previously, the animals were fed the total

94 mixed ration of the flock based on the same forages and concentrates as for the experiment. During the

95 experiment, the three groups received a basal diet of mixed forages (**TMF**, Table 1) prepared twice daily

96 with a steady composition (mean $\pm$ SD g/kg DM) based on Italian ray grass silage (572 ± 19.2), first cut

97 alfalfa-cocksfoot hay (231 ± 9.0), second cut alfalfa-cocksfoot hay (116 ± 2.0) and alfalfa-cocksfoot round

98 bale silage (81 ± 7.5). The TMF was offered twice daily at about 0900 and 1600 h after milking and

99 adjusted to a daily minimum of 20% refusal. In addition, they received 14 g DM/d mineral vitamin

100 mixture (Turbomix Oviplus A ™, Néolait, Yffiniac, France; with (g/kg) 130 Ca, 110 P, 60 Mg, 20 Na).

101 Each group received a commercial protein concentrate (**CPC**, Brebitanne™, RAGT, Albi, France) and

102 different amounts of barley grain, both split into two equal amounts. Barley was distributed in feed

103 troughs before the TMF, and half of the CPC was provided in feed troughs at the same time as the barley

104 and the other half in the milking parlor. The composition of the TMF and the chemical and nutritive

105 values of the feed are presented in Table 1. The experiment was divided into three periods of three weeks,

106 initially preceded by a one-week adaptation period to accustom the animals to their new flock mates. In

107 the first period (**P1**, control period), each group received the same diet formulated according to INRA

108 recommendations for dairy sheep (Hassoun and Bocquier, 2010) that provided 1.15 and 1.25 times the net

4

109 energy requirements (UFL) and protein requirements (PDI), respectively. They received 485 g DM of

110 barley and 337 g DM of CPC per day and per ewe. In the second period (**P2**), one group (MMH) received

111 almost the same level of barley (470 g DM/d) and CPC (399 g DM/d) as in P1. Ewes in the second group

112 (MHH) received 672 g DM/d of barley and 351 g DM/d of CPC, and those of the third group (MvHH) 883

113 g DM/d of barley and 303 g DM/d of CPC. In the third period (**P3**), the three groups received the same

114 amount of barley (661 g DM/d) and CPC (361 g DM/d). The general scheme of the experimental design is

115 presented in figure 1. The general scheme was set up in order to compare the effect of increasing (P2,

116 MHH and MvHH groups, P3 MMH group) and decreasing (P3, MvHH group) the amount of barley

117 (starch source) on forage DM intake (DMI), substitution rate (S), MY and milk composition, BW and

118 BCS. Indeed, farmer may want to increase or decrease energy for milk or body condition score objectives.

119 *2.2. Intake measurements and substitution rate calculation*

120 Offerings and refusals of TMF and concentrates were weighed every day throughout the experiment. The

121 TMF dry matter intake was measured four to five days a week. Refusals of TMF were weighed and

122 removed before each new TMF distribution. For each group, offerings and refusals were sampled every

123 day in order to determine the DM content (48 h, 60°C in a dry forced-air oven). Forages of TMF were

124 sampled twice a week and concentrates five times during the experiment in order to determine the DM

125 content, as reported above. Samples were gathered per week and kept until laboratory analysis. Total

126 refusals of CPC offered in the milking parlor were gathered and weighed so as to calculate the exact

127 intake for each group. There was no refusal of concentrates provided in troughs.

128 The French nutritional system for dairy sheep (Hassoun and Bocquier, 2010) makes it possible to predict

129 dry matter intake using the intake capacity (IC) of the animal, forage composition and the substitution rate

130 (S). The IC of the Lacaune breed expressed in fill unit for sheep (UEM), as defined by INRA (1989), is

131 calculated with the following equation:

132 $$IC = 0.024 \times BW + 0.9 \times sMY \qquad [3]$$

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

134 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:

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

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　$$Y_i = \mu + \alpha_i + \varepsilon_{ij}$$

182　where μ is the mean of MY, FC, PC, UC, FY, PY or UY, α_i is the main effect of group (MMH, MHH or

183　MvHH), and ε_{ij} 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:

7

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

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

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

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

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

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

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

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

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

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

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

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*

9

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

11

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.

13

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 (6th 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

416 related to milk yield. This is due to the fact that feed intake does not decrease as quickly as milk yield

417 decreases. This is not a problem when ewes are in positive energy balance because extra energy is

418 diverted into replenishment of body reserves. Finally, due to the adaptive capacities of high-yielding

419 Lacaune dairy ewes, rationing of group-fed ewes is feasible thanks to the Fill Unit System. The group

420 feeding strategy suggested by INRA (mean energy requirements x 1.15 and protein requirements x 1.25) is

421 still valid. However, further studies are needed to assess the effect of individual feeding based on

422 automatic concentrate feeder in sheep kept in virtual groups.

423 **Credit authorship statement**

424 P. Hassoun and F. Bocquier contributed to the design and implementation of the research, to the analysis

425 of the results and the writing. S. Parisot contributed to the implementation of the research, management of

426 the staff and all the animal procedure in agreement with animal welfare. M.A. Cordoba contributed to the

427 management of the experiment, recording the results, following all animal measurements, and writing part

428 of the first draft of the paper. D. Portes and J. Pradel, were in charge of the animal management,

429 participated in the execution of the experiment and collection of samples.

433 **Declaration of Competing Interest**

434 The authors declare no conflicts of interest.

438 improved the article through their constructive remarks and comments. The authors would also like to

439 thank Gail Wagman for revising the English.

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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 [TM]); 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.
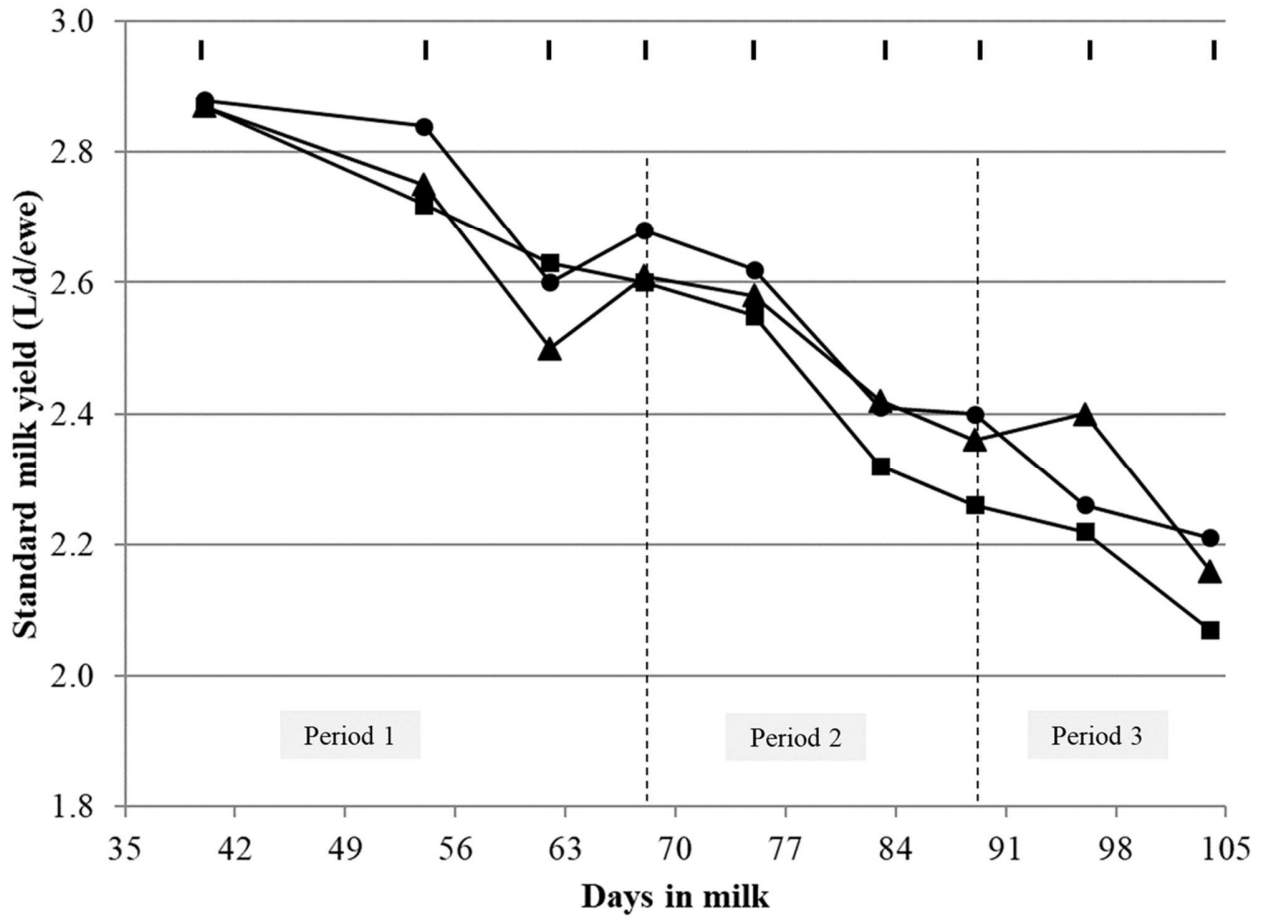
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592   Figure 1. General scheme of the experimental design, for the three groups of ewes fed successively with medium, very high and

593   high (MvHH), or medium, high and high (MHH) or medium, medium and high (MMH) amounts of barley grain during the three

594   periods.

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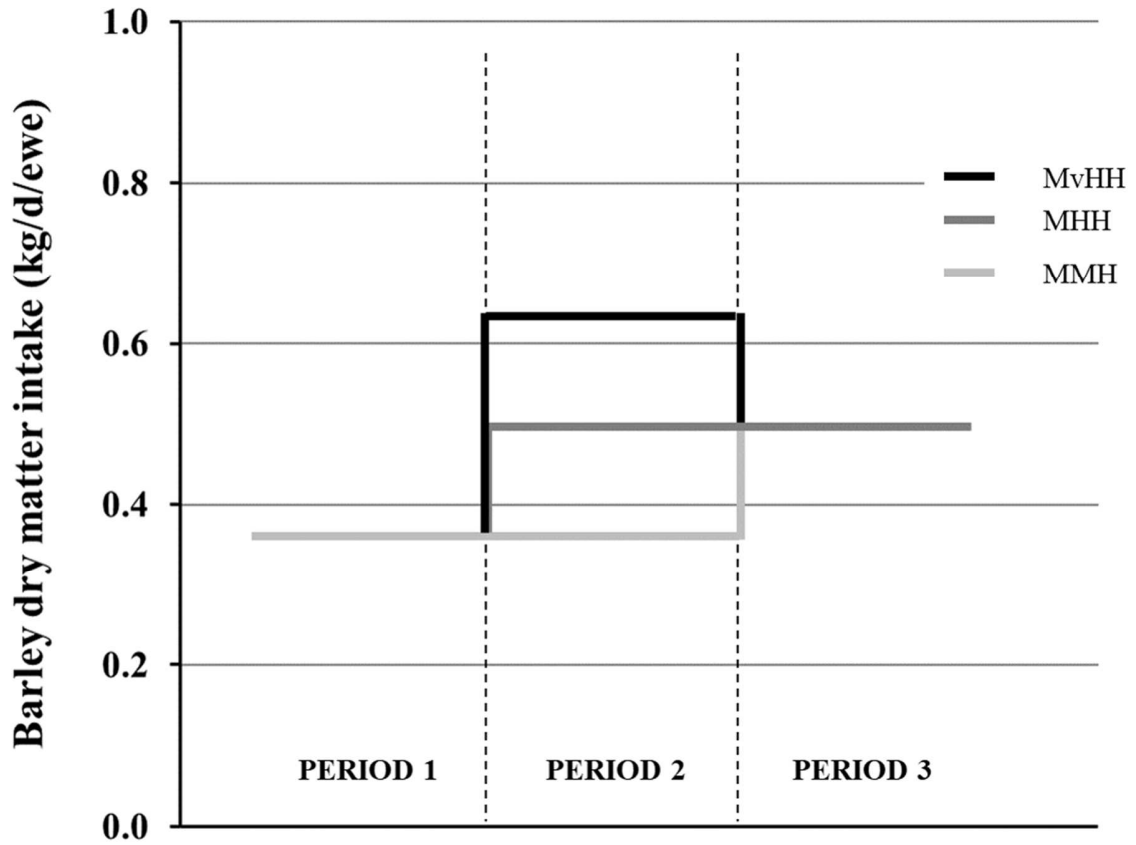
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599 Figure 2 Average daily standard milk yield produced during the experiment of ewes fed successively with medium, very high and

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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