

1 **Feed efficiency of lactating Holstein cows was not as repeatable across diets**  
2 **as within diet over subsequent lactation stages**

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**ABSTRACT**

10 **Background:** Improving feed efficiency has become a common target for dairy farmers to  
11 meet the requirement of producing more milk with fewer resources. To improve feed  
12 efficiency, a prerequisite is to ensure that the cows identified as most or least efficient will  
13 remain as such, independently of diet composition. Therefore, the current research analysed  
14 the ability of lactating dairy cows to maintain their feed efficiency while changing the energy  
15 density of the diet by changing its concentration in starch and fibre. A total of 60 lactating  
16 Holstein cows, including 33 primiparous cows, were first fed a high starch diet-low fibre (diet  
17 S<sup>+</sup>F<sup>-</sup>), then switched over to a low starch diet-high fibre (diet S<sup>-</sup>F<sup>+</sup>). To know if diet affect feed  
18 efficiency, we compared the ability of feed efficiency to be maintained within a diet over  
19 subsequent lactation stages, known as repeatability of feed efficiency, with its ability to be  
20 maintained across diets, known as reproducibility of feed efficiency. To do so we used two  
21 indicators: the estimation of the error of repeatability/reproducibility, which is commonly  
22 used in metrology, and the coefficient of correlation of concordance (CCC), which is used in  
23 biology. The effect of diet change could also lead to a change in cows sorting behaviour  
24 which could potentially affect feed efficiency if for example the most efficient cows select  
25 more concentrate than the least efficient. We therefore analysed the relationship between the

26 differences in individual feed refusals composition and the differences in feed efficiency. To  
27 do so, the composition of each feed refusal was described with its near infra-red (NIR)  
28 spectroscopy and was performed on each individual feed ingredient, diet and refusals and  
29 used as composition variable. The variability of the NIR spectra of the refusals was described  
30 with its principal components thanks to a principal component analysis (PCA). The Pearson  
31 correlation was estimated to check the relationship between feed efficiency and refusals  
32 composition, i.e. sorting behaviour.

33 **Results:** The error of reproducibility of feed efficiency across diets was 2.95 MJ/d. This error  
34 was significantly larger than the errors of repeatability estimated within diet, which were 2.01  
35 MJ/d within diet S<sup>-</sup>F<sup>+</sup> and 2.40 MJ/d within diet S<sup>+</sup>F<sup>-</sup>. The CCC was 0.64 between feed  
36 efficiency estimated within diet S<sup>+</sup>F<sup>-</sup> and feed efficiency estimated within diet S<sup>-</sup>F<sup>+</sup>. This CCC  
37 was smaller than the one observed for feed efficiency estimated within diet between two  
38 subsequent lactation stages (CCC = 0.72 within diet S<sup>+</sup>F<sup>-</sup> and 0.85 within diet S<sup>-</sup>F<sup>+</sup>). Feed  
39 efficiency was poorly correlated to the first two principal components, which explained 90%  
40 of the total variability of the NIR spectra of the individual refusals. This suggests that feed  
41 sorting behaviour did not explain differences in feed efficiency.

42 **Conclusions:** Feed efficiency was significantly less reproducible across diets than repeatable  
43 within the same diet over subsequent lactation stages, but cow's ranking for feed efficiency  
44 was not significantly affected by diet change. This loss in repeatability across diets could be  
45 due to a more pronounced feed sorting subsequent to the change in diet composition.  
46 However, the differences in sorting behaviour between cows were not associated to feed  
47 efficiency differences in this trial neither with the S<sup>+</sup>F<sup>-</sup> diet nor with the S<sup>-</sup>F<sup>+</sup> diet. Those  
48 results have to be confirmed on diets having different forage to concentrate ratios to ensure  
49 that the least and most efficient cows will not change.

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51 Key words: residual feed intake, repeatability, diet, dairy cattle, sorting behaviour

## 52 **BACKGROUND**

53 To be more competitive, dairy farmers have to increase the efficiency of resources use while  
54 reducing their environmental footprint. With an expected increase of world population  
55 (United Nations, 2017), feeds for dairy cow may shift towards more non-human edible feeds.  
56 The challenge for dairy farmers will be to improve feed efficiency, while facing more volatile  
57 feed prices (HLPE, 2011) and lower availability of feeds, which are directly edible by human.  
58 Dairy cows' diets will therefore become more variable in the future. Feed efficiency can be  
59 improved either by selecting the most efficient cows, thanks to an index including feed  
60 efficiency, or by improving feed efficiency of the least efficient cows with precision feeding.  
61 If feed efficiency is included as a genomic selection trait, feed efficiency has to be  
62 reproducible independently of diet and environment. The sensitivity of dairy cow's ranking  
63 for feed efficiency to diet composition, also called interaction between genetic and  
64 environment (Hill and Mackay, 2004), needs to be evaluated to know if some cows perform  
65 better and some worse when changing diet composition.  
66 Repeatability is defined as the capacity of a method to give the same results when using the  
67 same sample and repeating measurements in the same experimental conditions (JCGM, 2012),  
68 that is within diet when applied to efficiency. Reproducibility is a repeatability done while  
69 changing one specific characteristic in the experimental conditions (temperature, diet,  
70 operator; (JCGM, 2012)), that is for example by changing diet's composition. When  
71 estimating reproducibility of an indicator under different environmental conditions, it is  
72 therefore essential to compare it with its repeatability under similar conditions to isolate errors  
73 associated with diet change from errors associated with the method. In literature, the  
74 reproducibility of feed efficiency when changing the diet was lower compared to the  
75 repeatability within a given diet, as estimated with the correlations of residual feed intake

76 (RFI) within and across diets ( $r = 0.33$  vs  $0.42$  in steers, (Durunna et al., 2011);  $r = 0.44$ - $0.64$   
77 vs  $0.53$ - $0.70$  in dairy cows, (Potts et al., 2015)). Animals will thus not necessarily rank the  
78 same for feed efficiency when changing diet composition. Repeatability estimation, as defined  
79 by the proportion of genetic and permanent environment variances within and across  
80 lactations in the total variance of RFI, is highly variable across studies and countries with  
81 values between  $0.47$  and  $0.90$  (Connor et al., 2013; Tempelman et al., 2015). This variability  
82 across studies is associated with differences in diet composition and in period length between  
83 studies. Indeed, in lactating dairy cows, the correlation of short-term feed efficiency with full  
84 lactation feed efficiency increases with later lactation stages and longer periods (Connor et al.,  
85 2019). The comparison between repeatability and reproducibility needs therefore feed  
86 efficiency to be estimated over a long enough period to get a robust estimation within diet.  
87 Feed efficiency is generally estimated with diets offered ad libitum with a minimum amount  
88 of refusals (in general 5 – 10% of offered). Cows can therefore potentially perform feed  
89 sorting, which may lead to a consumed diet that differs from the distributed diet both in  
90 composition and in nutritive value. This difference between refusals composition and  
91 distributed diet composition has therefore to be considered when analysing the change in feed  
92 efficiency while changing diet's composition because feed sorting behaviour could affect feed  
93 efficiency. For instance, Dykier et al. (2020) observed that RFI was negatively correlated to  
94 the intake of long particles ( $r = -0.30$ ,  $p < 0.05$ ) and positively correlated to intake of short  
95 particles ( $r = 0.22$ ,  $p < 0.1$ ) in beef steers fed ad libitum. This difference in sorting behaviour  
96 resulted in a consumed diet which concentration in crude protein increased with RFI ( $r = 0.25$ ,  
97  $p < 0.05$ ). Differences in feed composition were characterized by particle size differences in  
98 Dykier et al. (2020). However the method for particle size composition (Lammers et al., 1996;  
99 Kononoff et al., 2003) is burdensome and time consuming. The advent of near-infrared (NIR)  
100 spectroscopy opens new ways to determine diet or feed compositions at high throughput.

101 Indeed the NIR spectrum is sensitive to physical and chemical characteristics of the sample,  
102 and has therefore been used to determine nutritive value of feed, but also to discriminate  
103 samples according to their composition (Pérez-Marín et al., 2004; De la Roza-Delgado et al.,  
104 2007; Li et al., 2007).

105 The main objective of the current study was therefore to check the ability of feed efficiency to  
106 be maintained across different diets. To achieve those objectives a trial was set up with  
107 lactating dairy cows that were fed with two diets. These diets differed in energy density, by  
108 lowering the starch concentration and increasing the fibre concentration of the diet. The feed  
109 efficiency was estimated within diet using the method developed in a previous paper (Fischer  
110 et al., 2018). The novelty of the current paper is to estimate feed efficiency reproducibility  
111 across diets by combining two methods: the commonly used CCC in biology and the  
112 comparison of the error of reproducibility across diets with the error of repeatability within  
113 diet, as commonly used in metrology (JCGM, 2012). Indeed, to estimate if FE is maintained  
114 across diets, its reproducibility across diets has to be compared to its repeatability within diet.  
115 If the reproducibility results are as good as the repeatability results within diet, then one can  
116 conclude that FE is as repeatable across diets as it is within diet. Opposedly if the  
117 reproducibility results are worse than repeatability within diet, then one can conclude that the  
118 ability of FE to be maintained across diets is not as good as within diet. As highlighted in the  
119 previous paragraph, a diet change could also lead to a change in cows sorting behaviour  
120 which could potentially affect feed efficiency. A second objective was therefore to check that  
121 the change in feed efficiency associated with diet change was not explained by differences in  
122 sorting behaviour. We therefore checked that feed efficiency was not associated with feed  
123 sorting behaviour by analysing the feed composition of each cow's diet refusals with NIR  
124 spectroscopy.

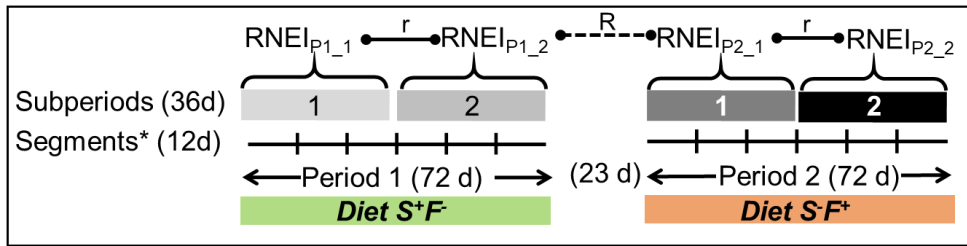
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## MATERIAL & METHODS

### 127 **Experimental Design**

128 The experimentation was performed at the INRAE-Institut Agro UMR PEGASE research  
129 facility of Mejusseaume (Le Rheu, France). An initial group of 68 Holstein cows were housed  
130 in a free-stall barn with free access to water. These cows were monitored for feed efficiency  
131 from calving (grouped between end of August and middle of October 2017) to end of June  
132 2018, near the end of the lactation. A sequential design was adopted to feed the cows with a  
133 first diet that was high in starch and low in Fibre (diet  $S^+F^-$ ) and a second diet that was low in  
134 starch and high in fibre (diet  $S^-F^+$ ). The protein content was adjusted in diet  $S^-F^+$  to have a  
135 ratio between energy and protein that was similar to the ratio in diet  $S^+F^-$ . The diet  $S^+F^-$  was  
136 fed from calving to March 18<sup>th</sup> 2018, then all cows switched over to diet  $S^-F^+$  until end of  
137 June 2018. To make the feed efficiency estimated over the two feeding periods comparable,  
138 the same period length was chosen for both periods. Each diet was fed for at least 95 days.  
139 The first 23 days subsequent to the change in diet from  $S^+F^-$  to diet  $S^-F^+$  were considered as an  
140 adaptation period to the new diet and were therefore removed from the dataset. Each period  
141 included the last 72 days of data. 62 cows had data over both 72-day periods and were  
142 therefore kept for further analysis. Each period has been split in two sub-periods of 36 days  
143 (Figure 1) to be able to estimate a repeatability within diet and to reach a supposedly  
144 correlation with full lactation RFI of at least 0.8 according to Connor et al. (2019).  
145 Reproducibility was estimated using the last 36-days of the first period and the first 36-days  
146 of the second period to be comparable and compared with repeatability estimation over the  
147 two 36-days segments within diet (Figure 1).



\*Segments = time unit used to average the variables NEI, NEMilk, metabolic BW, and BW loss and gain to fit the models for RNEI.

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150 **Figure 1** Diagram of the design used to characterize the repeatability ( $r$ ) and reproducibility  
151 ( $R$ ) of residual net energy intake (RNEI). The repeatability compares RNEI within diet; it is  
152 shown with the solid line (●—●). The reproducibility compares RNEI across diets; it is  
153 shown with the dashed line (●---●). The dairy cows started first with the diet high in starch  
154 and low in fibre ( $S^+F^-$ ) and switched over to the diet low in starch and high in fibre ( $S^-F^+$ ).

155 **Table 1** Composition of both experimental diets ( $S^+F^-$  and  $S^-F^+$ ) and their chemical analysis.

	Diet $S^+F^-$	Diet $S^-F^+$
Feed composition (% DM $\pm$ SD <sup>1</sup> )		
Maize silage	65.0 $\pm$ 1.6	59.1 $\pm$ 0.9
Soybean meal	17.8 $\pm$ 0.9	13.3 $\pm$ 0.3
Dehydrated lucerne	8.1 $\pm$ 1.6	14.4 $\pm$ 0.2
Energy concentrate <sup>2</sup> + Minerals and vitamin complement <sup>3</sup>	9.1 $\pm$ 1	1.6 $\pm$ 0.7
Wheat straw	0	11.6 $\pm$ 0.3
Diet analysis ( $\pm$ SD <sup>1</sup> )		
DM (%)	42.9 $\pm$ 0.7	47.1 $\pm$ 0.3
OM (%)	94.0 $\pm$ 0.15	94.1 $\pm$ 0.04
Crude protein (g/kg)	167 $\pm$ 4.9	145 $\pm$ 1.1
ADF (g/kg)	198 $\pm$ 2.9	249 $\pm$ 1.2
NDF (g/kg)	351 $\pm$ 3.6	420 $\pm$ 1.6
Starch (g/kg)	226 $\pm$ 3	183 $\pm$ 2.2
Net energy for milk (MJ/kg)	6.62 $\pm$ 0.107	5.98 $\pm$ 0.014
Metabolizable protein (g/kg)	105 $\pm$ 1.8	93 $\pm$ 0.4

156 <sup>1</sup>SD were calculated using the day-to-day change in offered diet composition on an individual cow

157 basis. <sup>2</sup>The part of energy concentrate in the diet, as described here, includes the part of energy

158 concentrates distributed at the Greenfeed® station. <sup>3</sup>Concentrates included 17.8% wheat, 17.8%

159 maize, 17.8% sugar beet pulp, 17.8% barley, 13% wheat bran, 3% beet molasses, 0.9% oil, 0.9% salt,

160 11% minerals and vitamin complement (including 6% phosphorus, 24% calcium, 5% magnesium, and

161 other minerals and vitamins).

162 DM = dry matter; OM = organic matter; ADF = acid detergent fibre; NDF = neutral detergent fibre

## 163 **Phenotypic Measurements**

164 *Individual feed intake and feed nutrient analysis.* Cows were fed individually twice a  
165 day after each milking (7:00 and 16:00). Daily intake was estimated individually as the  
166 difference between daily offered feed and next morning's refusals. The diet was offered ad  
167 libitum to maintain an average of 10% refusals per cow. Each cow was fed in her own  
168 manger, only accessible by one cow thanks to the identification chip on her neck collar. Each  
169 feed has been sampled once a week for concentrates, and once a day for the forages to  
170 estimate dry matter, which was used to estimate individual feed dry matter intake (DMI). A  
171 bulk sample was taken for each ingredient and silo for nutrient value analysis. Diet S<sup>+</sup>F<sup>-</sup>  
172 included maize silage, soybean meal, dehydrated alfalfa and a mix of energy concentrates,  
173 minerals and vitamins (Table 1). Diet S<sup>+</sup>F<sup>+</sup> was formulated to have a lower starch  
174 concentration by replacing starch with fibre while keeping a similar ratio between  
175 metabolizable protein and net energy for lactation than in diet S<sup>+</sup>F<sup>-</sup>. To do so, wheat straw was  
176 added to the ingredients included in diet S<sup>+</sup>F<sup>+</sup> to replace the energy concentrate and part of  
177 soybean meal and maize silage (Table 1). In addition to both diets, cows had access to a gas  
178 emissions monitoring system, the Greenfeed® (see "Individual performance: milk, body  
179 weight and body condition, methane and carbon dioxide emissions" section), which  
180 distributes small drops of energy concentrates to maintain the cow in the gas recording  
181 system. The amount of energy concentrates distributed per cow per day in the Greenfeed®  
182 station was added to the daily intake at the manger. All feed samples were freeze-dried and  
183 ground with a 3-blade knife mill through a 0.8-mm screen. The organic matter content was  
184 determined by ashing for 5h at 550°C in a muffle Furnace (Association Française de  
185 Normalisation, 1997). The concentrations of neutral and acid detergent fibre were measured  
186 according to Van Soest et al. (1991) using a Fibersac extraction unit (Ankon Technology,  
187 Fairport, NY, USA). Fat content was measured by ether extraction and starch analyses were



188 performed by polarimetry (LABOCEA, Ploufragan, France). Nitrogen concentration for all  
189 samples was determined by the Dumas method (Association Française de Normalisation,  
190 1997) with a LECO Nitrogen Determinator (Leco, St Joseph, MI, USA). Nutritive values of  
191 feeds were calculated from their chemical composition according to equations in INRA  
192 (2010). Diet S<sup>+</sup>F<sup>-</sup> had 226 g starch/kg DM, 351 g neutral detergent fibre/kg DM for a net  
193 energy concentration of 6.62 MJ/kg DM and 105 g metabolizable protein/kg DM. Diet S<sup>-</sup>F<sup>+</sup>  
194 was 19% lower in starch concentration and 16.4% higher in neutral detergent fibre  
195 concentration than diet S<sup>+</sup>F<sup>-</sup> for a net energy concentration of 5.98 MJ/kg DM and 93 g  
196 metabolizable protein/kg DM (Table 1).

197 ***Individual performance: milk, body weight and body condition, methane and carbon***  
198 ***dioxide emissions.*** Milk yield was recorded at each milking with milk meters (DeLaval,  
199 Tumba, Sweden). Milk fat and protein concentrations were analysed by mid infrared  
200 spectrometers (Lillab, Chateaugiron, France) from morning and afternoon milk samples of  
201 two days per week. Milk fat and milk protein concentrations were calculated as weighted  
202 averages relatively to the morning and afternoon milk production of the day of sampling.  
203 Cows were weighed automatically after each milking (W-2000, DeLaval, Tumba, Sweden) to  
204 get an empty udder body weight (**BW**). All cows were scored for body condition once a  
205 month by 3 trained persons according to the scale developed by Bazin (1984), going from 0  
206 for an emaciated cow to 5 for a fat cow with 0.25 unit increments.  
207 Methane emissions were recorded with two Greenfeed<sup>®</sup> units (C-Lock Inc., Rapid City, SD,  
208 USA). A Greenfeed<sup>®</sup> unit is designed as a dispenser of concentrates to measure methane and  
209 carbon dioxide emissions each time a cow visits the feeder, therefore both methane and  
210 carbon dioxide were also monitored. A maximum of 720 g (30g/drop) concentrates was  
211 offered daily at the Greenfeed<sup>®</sup> to attract cows in the Greenfeed. Each Greenfeed<sup>®</sup> unit  
212 records methane emissions for up to 23 cows. Given the barn configuration and the two

213 Greenfeed<sup>®</sup> units, only 42 cows could therefore be monitored for methane emissions during  
214 the study. On average the cows visited the Greenfeed 2.2 /d/cow (+/- 0.9). Energy  
215 concentrates distributed at the Greenfeed<sup>®</sup> units were included in the estimation of individual  
216 daily feed intake.

### 217 **Checking for feed sorting behaviour**

218 Individual refusals were sampled by collecting about 0.5 to 1 kg of fresh weight refusals per  
219 cow once a week for 6 weeks during each of the two experimental periods to check if the  
220 change in diet's feed ingredients between diet S<sup>+</sup>F<sup>-</sup> and diet S<sup>-</sup>F<sup>+</sup> modified cow's feed sorting  
221 behaviour. The samples were dried in a forced-air oven at 60°C for 48 h. The refusals samples  
222 of each cow were pooled within period to end up with one sample per cow and per period, and  
223 ground through a 3-blade mill (0.8 mm screen). The same process was applied for each feed  
224 ingredient and each diet to estimate the difference in composition between feeds and refusals.  
225 Instead of determining each sample's feed composition, we used an indirect approach based  
226 on near-infra red (NIR) spectroscopy. Indeed, we have seen in the background section of this  
227 paper that NIR spectroscopy can be used to differentiate samples differing in ingredients  
228 proportion. By definition, if the samples differ on a physical or chemical basis, their spectra  
229 will also be different. In this study, the differences in refusals composition and diets  
230 composition will be analysed through their NIR spectra, without estimating or analysing their  
231 chemical or physical characteristics. A Fourier transform near-infrared (FT-NIR)  
232 spectroscopy with MPA (Bruker Optik GmbH, Ettlingen, Germany) was used to characterize  
233 the spectra (from 3595 to 12490 cm<sup>-1</sup>, resolution 16 cm<sup>-1</sup>) of each individual feed, diet and  
234 refusal sample. A principal component analysis (PCA) was fitted on the spectra of the feeds,  
235 both diets and all refusals to summarize the dataset into principal components. A second PCA  
236 was performed on the spectra of the refusals only, with randomRNEI as a supplementary  
237 variable, to summarize the spectra of the refusals into fewer variables. The relationship

238 between refusals composition and feed efficiency was estimated with the coefficient of  
239 correlation between randomRNEI and each of the principal component of this second PCA.  
240 Both PCA were performed with the FactomineR (Lê et al., 2008) and Factoshiny (Vaissie et  
241 al., 2020) packages of R (R Core Team, 2018).

## 242 **Outlier Detection**

243 Among the 62 cows, 2 cows had issues with their manger and were therefore removed from  
244 the dataset because their intake data were not reliable enough, to end up with a group of 60  
245 cows, including 33 primiparous cows. For methane and carbon dioxide data, a least rectangles  
246 regression of carbon dioxide emission against methane emission was fitted to detect methane  
247 outliers using the least.rect function of package RVAideMemoire (Hervé, 2018) in R (R Core  
248 Team, 2018). The data outside the range of three standard deviations of the residuals around  
249 the regression line were considered as outliers, and were removed. On average 0.9% (SD =  
250 1.3%) of the initial methane data were removed per cow for being outliers. Methane data were  
251 then averaged per experimental period and per cow.

## 252 **Variables Calculation to Estimate Feed Efficiency**

253 Estimation of feed efficiency requires DMI data, as well as all energy outputs or energy inputs  
254 to be considered for a lactating dairy cow. Energy outputs gather net energy in milk, energy  
255 required for maintenance, energy gained as adipose tissue and energy required for gestation.  
256 Other energy inputs include adipose tissue mobilization.

257 Net energy in milk was calculated according to the following equation (Faverdin et al., 2010):

$$258 \text{ NEMilk (MJ / d)} = 7.12 \times \text{MilkProd} \times (0.44 + 0.0055 \times (\text{MFC} - 40) + 0.0033 \times (\text{MPC} - 31))$$

259 Where MilkProd is the milk production in kg/d, MFC is the milk fat concentration in g/kg and  
260 MPC is the milk protein concentration in g/kg.

261 Gestation requirement were estimated with the following equation defined by Faverdin et al.  
262 (Faverdin et al., 2010):

263 
$$\text{Gestation (MJ / d)} = 7.12 \times 0.00072 \times \text{BW}_{\text{birth}} \times \exp^{0.116 \times \text{GestWeek}}$$

264 Where  $\text{BW}_{\text{birth}}$  is calf's weight at birth and assumed to be 40 kg, GestWeek is the week of  
265 gestation.

266 Morning BW data used were smoothed with a moving average of the 15% neighbouring data,  
267 to better reflect change in maintenance and to be less sensitive to daily gutfill change.

268 Monthly body condition score (**BCS**) data were filled to get daily BCS using a cubic Spline  
269 with the function `smooth.spline` in R (R Core Team, 2018) using each scoring day as a knot.

270 Maintenance requirements were estimated with the metabolic BW, using the smoothed BW  
271 data, and calculated as  $\text{BW}^{0.75}$ . Energy gained and energy mobilized as body reserves were

272 estimated as the day-to-day change in smoothed BW. If the change was positive, it was

273 attributed to body reserves gain, and body reserves loss was null. Conversely, if the change

274 was negative, it was attributed to body reserves mobilization, and body reserves gain was null.

275 Both BW gain and BW loss were constructed to be positive variables. Both, BW gain and BW

276 loss, were multiplied by daily BCS to account for body reserves differences within a given

277 BW change, resulting in the variables **BWlossBCS** and **BWgainBCS**.

## 278 **Estimation of feed efficiency**

279 Feed efficiency was estimated as the residual feed intake with the method developed in a

280 previous paper (Fischer et al., 2018). Briefly, instead of being estimated as the residual of the

281 multiple linear regression that estimates the observed DMI with the main energy outputs and

282 inputs, RFI was defined with a mixed model as the repeatable animal effect. Applied to our

283 study, each sub-period of 36 days was subdivided in segments of 12 days to end up with three

284 repeated measures for each cow within each sub-period (Figure 1). The initial model

285 explained net energy intake (NEI) with the fixed effects of net energy in milk, metabolic BW,

286 BWlossBCS, BWgainBCS, gestation requirement, BCS, their interaction with parity and sub-  
287 period, and the fixed effect of parity, sub-period, and 12-day segment nested within sub-  
288 period. This model included the repeated effect of cow across the 12-day segments within  
289 sub-period and the random effect of cow within sub-period, and were grouped within sub-  
290 period. Only significant ( $p \leq 0.05$ ) interactions and variables were kept in the model. As in  
291 our previous paper (Fischer et al., 2018), feed efficiency was defined as the random part of the  
292 intercept of the mixed model 1 below, that was performed using PROC MIXED in SAS  
293 (Version 9.4 of the SAS System for Linux. 2017. SAS Institute Inc., Cary, NC, USA) with a  
294 heterogeneous autoregressive variance covariance matrix for the repeated statement. The  
295 variables were averaged per 12-day segment.

$$\begin{aligned} \text{NEI (MJ / d)} = & (\mu + \mu_{\text{cow}}) + \text{NEMilk} + \text{BW}^{0.75} + \text{BWlossBCS} + \text{BWgainBCS} \\ 296 & + \text{gestation} + \text{parity} + \text{sub - period} + \text{parity} \times \text{BW}^{0.75} + \text{sub - period} \times \text{NEMilk} \quad (\text{model 1}) \\ & + \text{sub - period} \times \text{BW}^{0.75} + \text{segment} | \text{sub - period} + \varepsilon \end{aligned}$$

297 where NEMilk is the net energy in milk in MJ/d,  $\text{BW}^{0.75}$  is the metabolic BW in  $\text{kg}^{0.75}$ ,  
298 gestation is the gestation requirement in MJ/d, parity is the fixed effect of parity, sub-period is  
299 the fixed effect of sub-period, segment|sub-period is the fixed effect of 12-day segment nested  
300 within sub-period,  $\text{parity} \times \text{BW}^{0.75}$  is the interaction between parity and  $\text{BW}^{0.75}$ , sub-  
301 period  $\times$  NEMilk and sub-period  $\times$   $\text{BW}^{0.75}$  are the interactions between sub-period and NEMilk,  
302 and sub-period and  $\text{BW}^{0.75}$ ,  $\mu$  is the fixed intercept and  $\mu_{\text{cow}}$  is the random part of the intercept  
303 and  $\varepsilon$  is the error. Feed efficiency was defined as  $\mu_{\text{cow}}$  in model 1 and will be called random  
304 residual net energy intake (**RandomRNEI**).

### 305 **Repeatability and Reproducibility of Feed Efficiency**

306 Repeatability and reproducibility were estimated with 2 methods: the standard deviation of  
307 repeatability and standard deviation of reproducibility as defined by ICAR (JCGM, 2012),  
308 and Lin's coefficient of correlation of concordance (CCC) (Lin, 1989).

309           ***Estimating Error of Repeatability and Reproducibility.*** Repeatability was estimated  
310 within diet with an analysis of variance. For repeatability the model 3 below of analysis of  
311 variance was fitted once with the data of feed efficiency within diet S<sup>+</sup>F<sup>-</sup> to get the  
312 repeatability within diet S<sup>+</sup>F<sup>-</sup>, and once with the data of diet S<sup>-</sup>F<sup>+</sup> to get the repeatability  
313 within diet S<sup>-</sup>F<sup>+</sup>. Reproducibility was estimated with an analysis of variance with the data of  
314 the second sub-period when cows were fed diet S<sup>+</sup>F<sup>-</sup> and the data of the first sub-period when  
315 cows were fed the diet S<sup>-</sup>F<sup>+</sup>, to be able to estimate the variance associated with diet change.  
316 Both analysis of variance were performed using the Anova function of car package (Fox and  
317 Weisberg, 2011) in R (R Core Team, 2018) as follows:

$$318 \quad NEI = \mu + \text{Cow} + \varepsilon \quad (\text{model 3})$$

319 Where Cow stands for the fixed effect of cow,  $\mu$  is the intercept and  $\varepsilon$  is the error. Standard  
320 deviations of repeatability and of reproducibility were defined as the standard deviation of  $\varepsilon$  in  
321 model 3. An F-test was performed with the var.test function in R (R Core Team, 2018) to test  
322 if the error of repeatability in diet S<sup>+</sup>F<sup>-</sup> was similar to the error of reproducibility across diets,  
323 and similarly by comparing error of repeatability in diet S<sup>-</sup>F<sup>+</sup> with the error of reproducibility  
324 across diets.

325           ***Estimation of Repeatability and Reproducibility Correlations.*** The Lin's coefficient  
326 of correlation of concordance was estimated between the feed efficiency estimated at two  
327 different sub-periods within diet for repeatability, and between feed efficiency estimated  
328 during the second sub-period in diet S<sup>+</sup>F<sup>-</sup> and first sub-period in diet S<sup>-</sup>F<sup>+</sup> for reproducibility.  
329 The CCC were estimated with the epi.ccc function of epiR package (Stevenson et al., 2020) in  
330 R (R Core Team, 2018).

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332 All statistical analysis were done with the significance level of 0.05 ( $p \leq 0.05$ ).

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## RESULTS

### Period Effect on Cows Performance

The decrease in dietary net energy for lactation and in metabolizable protein was confounded with the increase of lactation stage as the experimentation was based on a sequential design. Therefore when effect of diet is mentioned here, it is confounded with the effect of lactation stage.

The diet change decreased net energy intake, without significantly changing dry matter intake ( $p = 0.26$ ). Indeed, Cows ate on average 23.4 kg DM/d over both periods. They ate 156 MJ/d when they were fed with diet  $S^+F^-$ , which was 13.9 % (19 MJ/d) more net energy than when they were fed with diet  $S^-F^+$  ( $p < 0.01$ ; Table 2).

Cows partitioned 92.1 MJ/d in milk on diet  $S^-F^+$ , which was 17.3% (19.3 MJ/d) less than with diet  $S^+F^-$  ( $p < 0.01$ , Table 2). This difference in net energy exported in milk between diets was also observed for milk production. Indeed, cows produced 29.9 kg milk/d with diet  $S^-F^+$ , which was 17.9% (6.5 kg/d) less than with diet  $S^+F^-$  ( $p < 0.01$ , Table 2). Change in dietary net energy and metabolizable protein concentrations did neither significantly affect milk protein concentration ( $p = 0.88$ ) nor milk fat concentration ( $p = 0.56$ ), with average concentrations of 31.4 g protein/kg milk and 38.8 g fat/kg milk (Table 2). Given the steady milk content and a decreasing milk production, the decrease in net energy and metabolizable protein in the diet decreased milk protein production ( $p < 0.01$ ) and milk fat production ( $p < 0.01$ ).

Maintenance related variables, known as BW and BCS, were lower when cows were fed diet  $S^+F^-$  ( $p = 0.03$  for BW and  $p < 0.01$  for BCS), which was also at earlier lactation stages, with averages of 651 kg and 1.96 BCS with diet  $S^+F^-$ , and 680 kg and 2.08 BCS with diet  $S^-F^+$  (Table 2).

358 **Table 2** Dry matter intake, performance, methane emissions and efficiency of the 60 Holstein  
 359 cows fed S<sup>+</sup>F<sup>-</sup> diet first and then S<sup>-</sup>F<sup>+</sup> diet three months later.

	Least squares means		Residual SD	p-value
	Diet S <sup>+</sup> F <sup>-</sup>	Diet S <sup>-</sup> F <sup>+</sup>		
n (primiparous)	60 (33)	60 (33)		
<b>Intake</b>				
DMI (kg/d)	23.7	23.1	2.94	0.26
NEI (MJ/d)	156	137	18.58	<0.01
<b>Performance</b>				
Milk production (kg/d)	36.4	29.9	4.93	<0.01
Milk Fat production (g/d)	1,387	1,154	194.0	<0.01
Milk Protein production (g/d)	1133	933	145.6	<0.01
Milk Protein concentration (g/kg)	31.4	31.4	2.44	0.88
Milk Fat concentration (g/kg)	38.5	39.0	4.27	0.56
NE in Milk (MJ/d)	111.4	92.1	14.17	<0.01
BW (kg/d)	651	679	68.2	0.03
BCS	1.96	2.08	0.23	<0.01
BW Gain (kg/d)	0.40	0.37	0.16	0.28
BW Loss (kg/d)	0.14	0.32	0.12	<0.01
<b>Methane</b>				
Methane production (g/d)	510	523	66.2	0.41
Methane yield (g/kg DMI)	21.4	22.6	2.39	0.03
Methane yield (g/kg Milk)	14.0	17.6	2.58	<0.01

360 *DMI: dry matter intake; NEI: net energy intake estimated using equation in Faverdin et al. [12]; NE*  
 361 *in milk: net energy in milk estimated using equation in Faverdin et al. [12]; BW: body weight; BCS:*  
 362 *body condition score; CH<sub>4</sub>: methane emission. Diet S<sup>+</sup>F<sup>-</sup>: high in starch and low in fibre; diet S<sup>-</sup>F<sup>+</sup>:*  
 363 *low in starch and high in fibre.*

364 Variables associated with body reserves change, identified as BW loss and BW gain in table  
 365 2, were differently affected by diet. Cows mobilized more BW when they were fed with S<sup>+</sup>F<sup>-</sup>  
 366 diet, also known as period 2, with a loss of 0.32 kg/d, than when fed with S<sup>+</sup>F<sup>-</sup> diet, also  
 367 known as period 1, with a loss of 0.14 kg/d (p < 0.01, Table 2). Gain in BW did not  
 368 significantly differ between both diets, with an average gain of 0.39 kg/d.  
 369 Dietary decrease in starch, replaced with fibre, confounded with the effect of lactation stage,  
 370 did not significantly change methane emissions, as cows emitted on average 517 g methane/d



371 (Table 2). Nevertheless, this dietary change increased methane yield from 21.4 g methane/kg  
372 DMI up to 22.6 g methane/kg DMI ( $p = 0.03$ ) and from 14.0 g methane/kg milk up to  
373 17.6 g methane/kg milk when switching from diet S<sup>+</sup>F<sup>-</sup> to diet S<sup>-</sup>F<sup>+</sup> ( $p < 0.01$ , Table 2).

#### 374 **Effect of Diet change on Feed Efficiency**

375 Feed efficiency was more variable when cows were fed the S<sup>-</sup>F<sup>+</sup> diet than when fed the S<sup>+</sup>F<sup>-</sup>  
376 diet. Feed efficiency, as estimated with randomRNEI, had a standard deviation of 4.49 MJ/d  
377 in sub-period 1 and 4.61 MJ/d in sub-period 2 when cows were fed S<sup>+</sup>F<sup>-</sup> diet, and 5.18 MJ/d in  
378 sub-period 1 and 5.21 MJ/d in sub-period 2 when cows were fed the S<sup>-</sup>F<sup>+</sup> diet. The change in  
379 randomRNEI induced by diet change (randomRNEI diet S<sup>+</sup>F<sup>-</sup> - randomRNEI diet S<sup>-</sup>F<sup>+</sup>) was  
380 negatively correlated with the change in methane yield, as per kg DMI, ( $\text{CH}_4/\text{DMI}$  diet S<sup>+</sup>F<sup>-</sup>  
381 -  $\text{CH}_4/\text{DMI}$  diet S<sup>-</sup>F<sup>+</sup>) with a Pearson correlation of - 0.31 ( $p = 0.05$ ), but was neither  
382 significantly correlated with the change in methane production per day ( $p = 0.12$ ) nor with the  
383 change in methane yield, as per kg milk ( $p = 0.98$ ). This means that a cow that had a lower  
384 randomRNEI (higher feed efficiency) in diet S<sup>-</sup>F<sup>+</sup>, also had a higher methane yield per kg  
385 DMI in diet S<sup>-</sup>F<sup>+</sup> than when fed with the S<sup>+</sup>F<sup>-</sup> diet, and conversely.

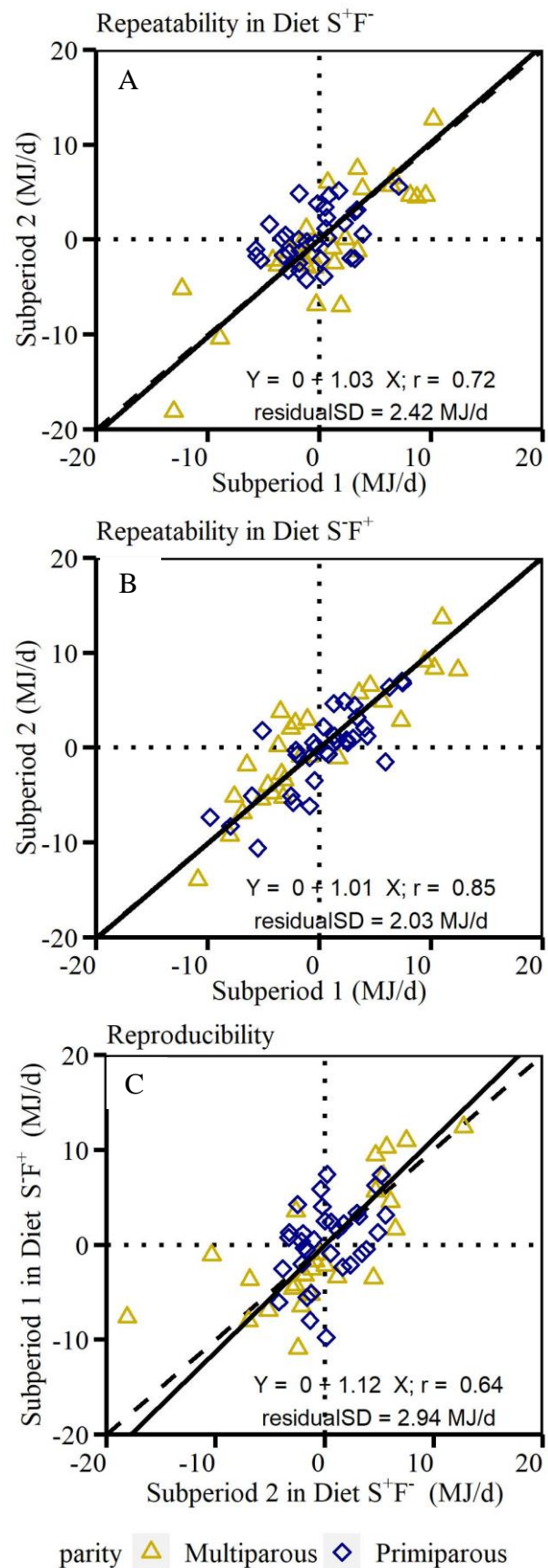
#### 386 **Feed Efficiency Reproducibility across Diets**

387 Cows' feed efficiency was not as reproducible across diets than repeatable within diet (Table  
388 3 and Figure 2). Errors of reproducibility, when comparing efficiency across diets, were larger  
389 than the errors of repeatability within diet, regardless of diet (Table 3). Indeed, the  
390 reproducibility error of randomRNEI that was 2.95 MJ/d was significantly larger ( $p < 0.05$ ,  
391 Table 3) than the repeatability errors for diet S<sup>+</sup>F<sup>-</sup> and for diet S<sup>-</sup>F<sup>+</sup> that were 2.40 and

392 2.01 MJ/d, respectively (Table 3). This  
393 lower reproducibility across diets  
394 compared with repeatability within diet  
395 tended to be observed with the CCC when  
396 comparing reproducibility across diets with  
397 repeatability in diet S<sup>-</sup>F<sup>+</sup>, but not when  
398 compared with repeatability within diet  
399 S<sup>+</sup>F<sup>-</sup> (Table 3). Indeed, the CCC between  
400 randomRNEI estimated in diet S<sup>+</sup>F<sup>-</sup> and  
401 randomRNEI estimated in diet S<sup>-</sup>F<sup>+</sup> was  
402 0.64, which was smaller compared with the  
403 correlations of 0.72 (p = 0.55) within diet  
404 S<sup>+</sup>F<sup>-</sup> and 0.85 (p = 0.055) within diet S<sup>-</sup>F<sup>+</sup>  
405 (Table 3).

406  
407 **Figure 2** Relationship between feed  
408 efficiency estimated within the same diet  
409 for repeatability estimation within diet S<sup>+</sup>F<sup>-</sup>  
410 (A), S<sup>-</sup>F<sup>+</sup> (B) or across diets for  
411 reproducibility estimation (C). The dashed  
412 black line stands for the first bisector, and  
413 the solid black line stands for the least  
414 rectangles regression.

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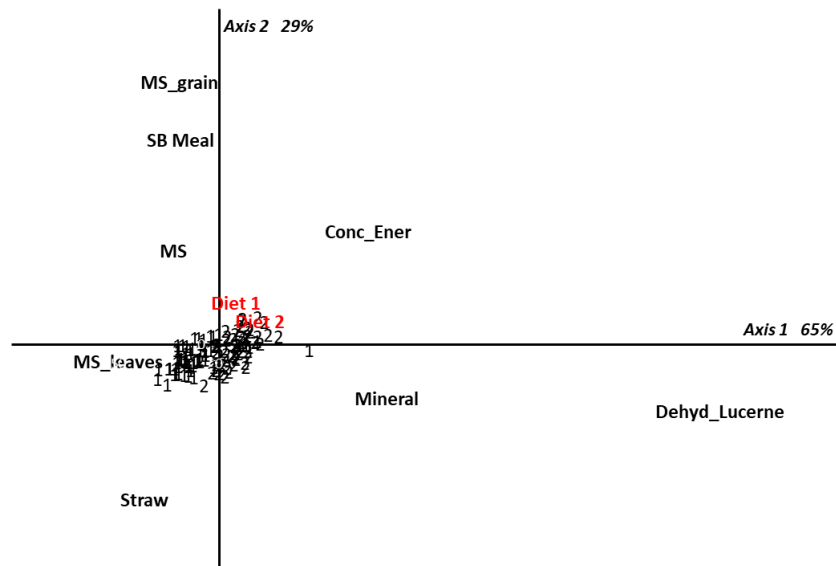
417 **Table 3** Error of repeatability and reproducibility, and Lin's concordance correlation  
 418 coefficient within diet for repeatability and across diets for reproducibility (Repro.) for feed  
 419 efficiency.

	Repeatability <sup>1</sup>		Repro. <sup>2</sup>	P-value		
	Diet S <sup>+</sup> F <sup>-</sup>	Diet S <sup>-</sup> F <sup>+</sup>		S <sup>-</sup> F <sup>+</sup> vs S <sup>+</sup> F <sup>-</sup>	S <sup>-</sup> F <sup>+</sup> vs Repro.	S <sup>+</sup> F <sup>-</sup> vs Repro.
SD (MJ/d)	2.401	2.014	2.953	0.06	<0.01	0.02
CCC	0.72	0.85	0.64	0.19	0.05	0.55

420 *All cows were first fed with the S<sup>+</sup>F<sup>-</sup> diet (S<sup>+</sup>F<sup>-</sup>: high in starch and low in fibre) and then with the S<sup>-</sup>F<sup>+</sup>*  
 421 *diet (S<sup>-</sup>F<sup>+</sup>: low in starch and high in fibre). CCC: Lin's coefficient of correlation of concordance; SD:*  
 422 *standard deviation of the residuals of the analysis of variance defined in model 3. <sup>1</sup>Repeatability was*  
 423 *estimated within diet using two repetitions of 36 days within diet. Repeatability was defined as the*  
 424 *standard deviation of the residuals of model 3. The lower the error of repeatability, the more*  
 425 *repeatable it is. <sup>2</sup>Reproducibility was estimated using two repetitions of 36 days (1 repetition/diet): the*  
 426 *last 36 days of diet S<sup>+</sup>F<sup>-</sup> and the first 36 days of diet S<sup>-</sup>F<sup>+</sup>. Reproducibility was defined as the standard*  
 427 *deviation of the residuals of model 3. The lower the error of reproducibility, the more reproducible it*  
 428 *is.*

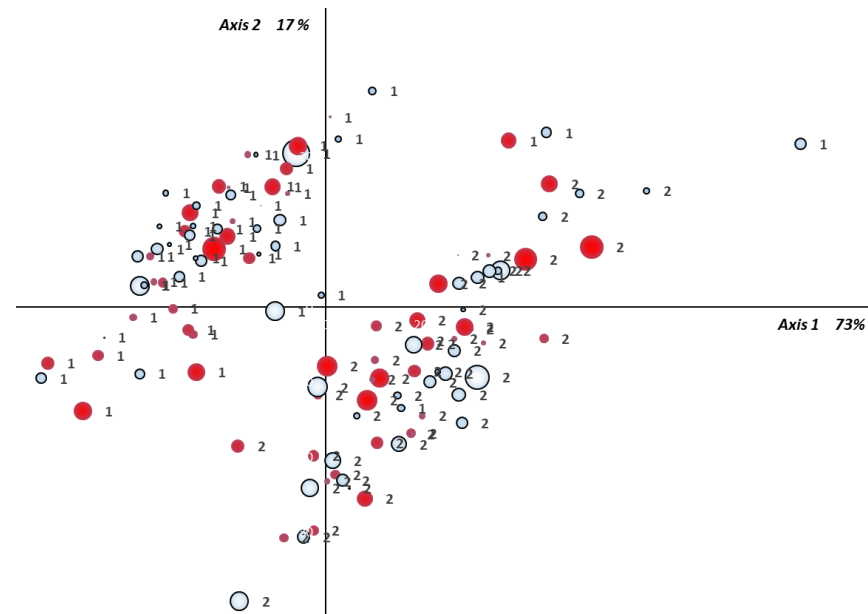
### 429 **Effect on feed selection and feed efficiency**

430 The change in diet composition was associated with a change in cows' feed sorting behaviour.  
 431 Indeed, the first plan of the PCA (Figure 3) showed that the individual refusals were clustered  
 432 around the origin of this first plan, close to the two diets samples, and slightly shifted to the  
 433 more fibrous feed ingredients of the diets (leaves part of maize silage, and straw). This  
 434 suggests that the cows may have left more fibrous ingredients than concentrates or grains in  
 435 the refusals, and that individual refusals seem to be different to any particular feed ingredient  
 436 in the diet. Despite the high number of spectra length waves used to describe the spectrum of  
 437 each sample, the two first principal components of the PCA explained 94% of the total  
 438 variability of the spectra of the refusals and the feed samples (Figure 3). When focusing only  
 439 on the refusals samples (second PCA), the first plan explained 90% of the total variability of



440

441 **Figure 3** Principal Component Analysis of NIR spectra of the feed  
 442 ingredients (MS=Maize Silage, MS\_leaves = leaves and cane of Maize  
 443 Silage, MS\_grain=Grain of maize silage, Conc\_Ener=concentrate  
 444 energy, SB Meal = soybean meal, Dehyd\_Lucerne = dehydrated  
 445 Lucerne, Mineral), diets (Diet 1 = diet S<sup>+</sup>F<sup>-</sup> and Diet 2 = diet S<sup>-</sup>F<sup>+</sup>) and  
 446 refusals of diet S<sup>+</sup>F<sup>-</sup> (1) and diet S<sup>-</sup>F<sup>+</sup> (2). The 2 first components  
 447 explain 94% of the total variance.  
 448 Diet S<sup>+</sup>F<sup>-</sup> : diet high in starch and low in fibre; Diet S<sup>-</sup>F<sup>+</sup>: diet low in  
 449 starch and high in fibre.



450

451 **Figure 4** Principal Component Analysis of NIR spectra of the refusals  
 452 of diet S<sup>+</sup>F<sup>-</sup> (1) and diet S<sup>-</sup>F<sup>+</sup> (2). The 2 first components explain 90%  
 453 of the total variance. The size of the points is proportional to the  
 454 absolute value of the residual net energy intake and the color is red if  
 455 randomRNEI is positive (inefficient cows) and blue if randomRNEI is  
 456 negative (efficient cows).  
 457 Diet S<sup>+</sup>F<sup>-</sup> : diet high in starch and low in fibre; Diet S<sup>-</sup>F<sup>+</sup>: diet low in  
 458 starch and high in fibre.

459 the refusals' spectra (Figure 4). This focus on refusals' spectra only showed that the refusals  
460 were clustered in 2 groups identifiable as the 2 diets (Figure 4). This suggests that the refusals  
461 reflect the composition difference between both diets. Within each diet, the refusals spectra  
462 differed across cows, but this difference was not associated with feed efficiency differences.  
463 Indeed, the randomRNEI was evenly distributed within diet with no clear clustering within  
464 diet that was associated with randomRNEI. Moreover, the principal components were poorly  
465 correlated with randomRNEI with a correlation of  $-0.05$  between randomRNEI and principal  
466 component 1, and of  $0.009$  between randomRNEI and principal component 2. If some  
467 variability exists in the composition of refusals due to feed sorting, this selection was not  
468 associated to feed efficiency differences in this trial neither when cows were fed with the S<sup>+</sup>F<sup>-</sup>  
469 diet nor when fed with the S<sup>-</sup>F<sup>+</sup> diet.

## 470 **DISCUSSION**

### 471 **Feed Efficiency was less reproducible across diets than within diet**

472 Feed efficiency was less reproducible across diets than within diet when using the  
473 method defined by ICAR (JCGM, 2012) based on the estimation of errors. However, as  
474 shown with the CCC and the cow's ranking (see section "Availability of data and material"  
475 for this supplementary material), the change in cow's ranking was similar when comparing  
476 between the two diets than when comparing within diet over subsequent lactation stages. This  
477 shows that the change in diet affected the absolute value of feed efficiency but not cow's  
478 ranking.

479 This difference between the results observed with the error method and those observed  
480 with the CCC shows that it is therefore important to use several indicators when the objective  
481 is to characterize reproducibility and repeatability of a method, here of feed efficiency. In our  
482 study, we showed that the error of reproducibility across diets was significantly higher than  
483 the error of repeatability within diet, while the CCC were similar when comparing across diet

484 and within diet  $S^+F^-$ , but different when comparing across diets and within diet  $S^-F^+$ . With one  
485 indicator we would have concluded that diet has a significant effect on feed efficiency  
486 repeatability, while with the second we would have concluded that the effect of diet does not  
487 seem significant. The two indicators are complimentary: the CCC will give the strength of the  
488 relationship between the two diets feed efficiency without any information about the  
489 dispersion of the residuals of this relationship, while the errors give the dispersion of the  
490 relationship. Most of the studies characterizing the reproducibility of feed efficiency in cattle  
491 used coefficient of correlations and percentages of cows which changed their efficiency group  
492 (Durunna et al., 2011; Potts et al., 2015; Asher et al., 2018). Conclusions based on animals'  
493 re-ranking are highly subjected to the definition of efficiency groups, which is variable across  
494 studies. The conclusions about repeatability may even differ with different definitions of  
495 efficiency group. We therefore preferred not to use re-ranking to characterize reproducibility  
496 of feed efficiency.

497       Upon the objective of cow's selection based on feed efficiency, one will prefer one  
498 indicator or the other. For example for selection purposes, one may especially be interested in  
499 the rate of cows able to maintain their efficiency class, and therefore use the CCC indicator.  
500 With this in mind, our results and the literature show (Durunna et al., 2011; Potts et al., 2015;  
501 Asher et al., 2018) that the ranking is quite similar within diet over time and across diets, and  
502 therefore that the risk to misidentify the most or least efficient cows is minimal. If the  
503 objective is to improve efficiency on an existing herd, one may prefer to use the errors  
504 indicator. With this in mind, our results show that a diet change affects the efficiency of the  
505 herd because the errors are significantly higher when predicting feed efficiency after a diet  
506 change than within the same diet. Indeed if efficiency would be reproducible when changing  
507 diet's composition, all cows should behave the same way and their efficiency would be easily  
508 predictable with their previous efficiency. This is not observed, because when randomRNEI

509 in first subperiod within diet S<sup>-</sup>F<sup>+</sup> was predicted with the randomRNEI in second subperiod  
510 within diet S<sup>+</sup>F<sup>-</sup>, the regression was able to explain 41 % of the total variability of  
511 randomRNEI in first subperiod within diet S<sup>-</sup>F<sup>+</sup>. This low coefficient of determination is  
512 mostly explained by the diversity of adaptation of each cow's DMI to the diet change (Figure  
513 2). Indeed, for similar randomRNEI and similar DMI there were cows which decreased their  
514 DMI and decreased their randomRNEI, while others increased both their DMI and  
515 randomRNEI, and others maintained both their DMI and randomRNEI. This shows that cows  
516 having similar feed efficiency and intake on a specific diet, will not necessarily end up with  
517 similar efficiency and intake on a different diet.

518 To conclude about reproducibility, one should also estimate repeatability and compare  
519 it to reproducibility. If the reproducibility is similar to repeatability, the method or estimation  
520 is highly reproducible. If the reproducibility is significantly worse than repeatability, the  
521 method is less reproducible than repeatable, and therefore the method or estimation is  
522 sensitive to the environment. The estimation of repeatability is therefore essential when  
523 characterizing the reproducibility of a method or estimation. The lack of confidence interval  
524 or statistical test to compare the reproducibility and repeatability indicators in most of the  
525 studies (Durunna et al., 2011; Potts et al., 2015; Asher et al., 2018) limits the possibility to  
526 conclude about the reproducibility of feed efficiency.

### 527 **Similar reproducibility results than in literature**

528 The CCC comparing efficiencies before and after diet change was significantly lower  
529 when compared with the CCC of repeatability within diet S<sup>-</sup>F<sup>+</sup> for feed efficiency ( $p = 0.05$ ),  
530 but was not different when compared with diet S<sup>+</sup>F<sup>-</sup> CCC ( $p > 0.1$ ). The lack of significance  
531 observed when comparing the reproducibility CCC when diet changed with the repeatability  
532 CCC estimated within diet S<sup>+</sup>F<sup>-</sup> can be explained by the lower repeatability of feed efficiency  
533 observed when cows were fed with diet S<sup>+</sup>F<sup>-</sup>. The lower repeatability of randomRNEI within



534 diet S<sup>+</sup>F<sup>-</sup> can be explained by the lower repeatability observed for NEI within diet S<sup>+</sup>F<sup>-</sup> as its  
535 error of repeatability was 4.60 MJ/d within diet S<sup>-</sup>F<sup>+</sup> and 5.82 MJ/d within diet S<sup>+</sup>F<sup>-</sup>. The  
536 observed decrease in correlation for feed efficiency under reproducibility conditions was also  
537 found in literature with Pearson's correlations of 0.54-0.70 in heifers and 0.42 in steers within  
538 diet, that were larger than those observed after diet changed with correlations of 0.40 for  
539 heifers and 0.33 for steers (Durunna et al., 2011; Cassady et al., 2016). In dairy cows,  
540 correlations averaged 0.65 within diet and 0.56 across diets (Potts et al., 2015). The  
541 correlations they observed were lower and closer together compared to the correlations  
542 observed in the current study. The higher correlations observed in our study can be explained  
543 by the longer period used, that is 36 days, compared to Potts et al. (2015) who used 7 days per  
544 sub-period to estimate repeatability correlations within diet. In fact, the longer and the closer  
545 to middle of lactation the period was, the better the RFI approximates a RFI estimated over  
546 the full lactation (Connor et al., 2019). It would be worth to look at the period length required  
547 to reach the maximum repeatability within diet and within lactation for RFI, and then apply  
548 this length within diet and estimate reproducibility of feed efficiency when changing diet.

#### 549 **Diet and Period Effects on Performance**

550 Dietary starch and fibre concentration modified intake and performance in lactating dairy  
551 cows. Diets high in starch and lower in fibre compared to diets low in starch and higher in  
552 fibre do not necessarily increase intake (Boerman et al., 2015; Potts et al., 2015; Karlsson et  
553 al., 2018). Similarly, in the current study, intake, expressed as DM, was not significantly  
554 higher when cows were fed with S<sup>+</sup>F<sup>-</sup> diet than when they were fed with S<sup>-</sup>F<sup>+</sup> diet ( $p = 0.26$ ).  
555 However, when expressed as net energy, cows ate more when fed with the S<sup>+</sup>F<sup>-</sup> diet than  
556 when fed with the S<sup>-</sup>F<sup>+</sup> diet ( $p < 0.01$ ), such as observed in Karlsson et al. (2018). Decrease in  
557 dietary starch and fibre concentrations reduced milk production ( $p < 0.01$ ), as observed in  
558 literature (Boerman et al., 2015; Potts et al., 2015; Karlsson et al., 2018), but in the current



559 study this effect was confounded with the effect of lactation stage. This decrease in milk  
560 production subsequent to a decrease of dietary starch and increase of dietary fibre is usually  
561 observed with an increased milk fat concentration (Boerman et al., 2015; Potts et al., 2015;  
562 Karlsson et al., 2018). This was not observed in the current study because neither milk fat nor  
563 milk protein concentrations were different between diet S<sup>+</sup>F<sup>-</sup> and diet S<sup>-</sup>F<sup>+</sup> ( $p = 0.56$  for milk  
564 fat and 0.88 for milk protein). One could argue that our change in NDF between both diets, of  
565 16%, was too low compared to the 18-38% across Boerman et al. (2015), Potts et al. (2015)  
566 and Karlsson et al. (2018) to see significant changes in milk fat concentrations. However, in  
567 Boerman et al. (2015) only the lowest increase in NDF (18%) had a significant change in milk  
568 fat concentration; the other diets having a higher change in NDF did not significantly increase  
569 the milk fat concentration. Therefore the increase in NDF may not systematically increase  
570 milk fat concentration. This steadiness of milk solids concentrations, combined with the  
571 decrease in milk production, resulted in lower net energy exported in milk ( $p < 0.01$ ) and  
572 lower milk fat and protein productions ( $p < 0.01$ ) on diet S<sup>-</sup>F<sup>+</sup>. Having a diet higher in net  
573 energy concentration should have led to more body reserves replenishment and less body  
574 reserves mobilization, as observed in Boerman et al. (2015) and Potts et al. (2015). In the  
575 current study, a higher dietary starch concentration was associated with lower BW loss  
576 ( $p < 0.01$ ), and similar BW gain ( $p = 0.28$ ). Those conclusions differences for change in milk  
577 solids and BW gain between our study and literature results can be explained by the  
578 difference in experimental design. Indeed, the current study was based on a sequential design  
579 while the other studies were based on a crossover design (Boerman et al., 2015; Potts et al.,  
580 2015; Karlsson et al., 2018). The main limit of a sequential design, as chosen in the current  
581 study, is the confounding between the effect of stage of lactation and the effect of treatment,  
582 which does not exist in a crossover design. However, crossover designs have limits too: it is  
583 impossible to characterize the effect of interaction between treatment and time or to

584 characterize a possible remnant effect of the first treatment on the following treatments. It is  
585 therefore difficult to conclude if diet change or just lactation stage explained the decrease in  
586 NEI and in milk production in the current study. However, given that cows should replenish  
587 their body reserves more and mobilize them less with increasing lactation stages, we can  
588 hypothesize that the lack of change in BW gain and the increased BW loss is mostly due to  
589 decreased dietary starch concentration. The observed increase in BW and BCS in the current  
590 study may be explained by more advanced lactation stages, and less by diet change.

591 Reducing starch and increasing fibre concentrations of the diet increased methane yield, as  
592 per kg DMI ( $p = 0.03$ ) and as per kg milk produced ( $p < 0.01$ ). This increase of methane per  
593 kg milk produced is partly explained by a lower milk production with similar feed intake. The  
594 increase of methane yield per kg of DM is more associated with the diet composition. By  
595 decreasing starch concentration in the diet and increasing dietary fibre concentration with  
596 increased wheat straw and lucerne, the production of volatile fatty acids in the rumen may  
597 have shifted in favour of acetate or butyrate profile instead of propionate profile. This shift is  
598 usually associated with a friendlier methanogenic environment (Knapp et al., 2014). The  
599 expected higher methane yield with lower dietary starch concentration, as per kg DMI or per  
600 kg milk, as observed in the current study, has also been observed in literature (Bougouin et  
601 al., 2018) but was not consensual. For some research, methane yield per kg DMI did not differ  
602 when changing dietary starch concentration (Hatew et al., 2015; Pirondini et al., 2015). A  
603 decrease in dietary starch concentration has usually been associated with an increase in  
604 methane emitted per day (Pirondini et al., 2015; Bougouin et al., 2018), which was not  
605 significant in the current study ( $p = 0.41$ ). These diverging results about effect of dietary  
606 starch concentration on methane production may reflect differences in the effect of dietary  
607 starch concentration on DMI, milk yield and diet digestibility. Higher methane emissions per  
608 day were observed when the change in dietary starch concentration was associated with

609 significant differences in DMI (Bougouin et al., 2018) or significant differences in diet  
610 digestibility (Pirondini et al., 2015; Bougouin et al., 2018). The significant change in methane  
611 yield per kg DMI was observed when the change in dietary starch concentration was  
612 associated with a change in DMI and a change in diet digestibility (Bougouin et al., 2018).  
613 The conclusions on dietary starch concentration on methane emissions seem to depend on the  
614 effect of diet composition on both, DMI and diet digestibility.

### 615 **Limits of the study**

616         Given that digestibility partly explains feed efficiency differences in lactating dairy  
617 cows (Oliveira et al., 2016; Potts et al., 2017), it would be interesting to evaluate the cows'  
618 ability to maintain their efficiency for diets with decreased DM digestibility or for diets with  
619 greater physically effective fibre affecting rumen fill. For example, a future study could  
620 compare a classic highly digestible fibre, as S<sup>+</sup>F<sup>-</sup> diet in current study, with a diet including  
621 fresh grass or hay.

622         In the current study, the effect of diet was confounded with the effect of lactation stage  
623 because all cows went through the same sequence at the same time. The change in  
624 performance, as well as in intake, could therefore be attributed to the diet, but also to lactation  
625 stage. This experimental design can also lead to remnant effects of the previous treatment to  
626 subsequent periods: results could have been different if diet sequence would have been  
627 reversed between the two periods. Repeatability errors in diet S<sup>+</sup>F<sup>-</sup> were similar to  
628 repeatability errors in diet S<sup>-</sup>F<sup>+</sup> ( $p = 0.06$ ). Feed efficiency was therefore as repeatable in diet  
629 S<sup>+</sup>F<sup>-</sup> as in diet S<sup>-</sup>F<sup>+</sup> (Table 3), which suggests that there was no remnant effect of diet S<sup>+</sup>F<sup>-</sup> on  
630 feed efficiency under diet S<sup>-</sup>F<sup>+</sup> and that lactation stage did not affect feed efficiency  
631 variability in our study. Another way to tackle confusion between lactation stage and  
632 treatment would have been to adopt a crossover design. However, this design can possibly  
633 lead to an interaction between time and treatment, which is not quantifiable, or leads to a

634 remnant effect of the first treatment. Moreover, a similar study was conducted by Fischer et  
635 al. where a cross-over design (paper under review) was used. The results and conclusions  
636 were similar to the current study. This supports the validity of the current paper.

637 A last limit to the study is the length of the adaptation period between diet S<sup>+</sup>F<sup>-</sup> and S<sup>-</sup>  
638 F<sup>+</sup>. When comparing subperiod 2 of diet S<sup>+</sup>F<sup>-</sup> with subperiod 1 of diet S<sup>-</sup>F<sup>+</sup>, we compared two  
639 periods which had different length of adaptation period. Indeed the first had at least subperiod  
640 1 of diet S<sup>+</sup>F<sup>-</sup> (36 days) whereas the second had 23 days. As we commonly consider that 2 to  
641 3 weeks are enough to ensure that the cows are fully adapted to a new diet, we considered that  
642 the adaptation to the diets was achieved for the data used in the current study, and therefore  
643 we considered that the difference in length of the adaptation period did not influence the  
644 results.

## 645 CONCLUSIONS

646 The study estimated the reproducibility of feed efficiency in dairy cows when changing the  
647 diet concentration in starch and fibre, by comparing the reproducibility across diets with its  
648 repeatability within diet. The results showed that feed efficiency was significantly less  
649 reproducible when changing diet's starch and fibre concentration than repeatable within diet  
650 over subsequent lactation stage. However the change in feed efficiency ranking of dairy cows  
651 was not significantly different when comparing the ranking when cows were fed with the two  
652 different diets than when comparing the ranking when cows were fed with the same diet over  
653 subsequent lactation stages. The diet change in starch and fibre concentration did not affect  
654 the ranking of dairy cows more than does the lactation stage. The change in diet composition  
655 affected the feed sorting behaviour of the dairy cows. However this feed sorting was neither  
656 related to feed efficiency differences nor to the change in feed efficiency subsequent to diet  
657 change.

## 658 LIST OF ABBREVIATIONS

659 BCS: body condition score; BW: body weight; CCC: Lin's coefficient of correlation of  
660 concordance; CH<sub>4</sub>: methane; DM: dry matter; DMI: dry matter intake; S<sup>+</sup>F<sup>-</sup>: diet high in  
661 starch and low in fibre; S<sup>-</sup>F<sup>+</sup>: diet low in starch and high in fibre; NEI: net energy intake;  
662 randomRNEI: random residual net energy intake; SD: standard deviation.

## 663 **DECLARATIONS**

### 664 **Ethics approval**

665 The protocol has been approved by the ethical committee and the French Ministry of Higher  
666 Education, Research and Innovation (Authorization of the French Ministry of Higher  
667 Education, Research and Innovation reference APAFIS 3122-2015112718172611).

### 668 **Consent for publication**

669 Not applicable.

### 670 **Availability of data and material**

671 The datasets generated and/or analysed during the current study are available in the  
672 data.INRAE repository: <https://doi.org/10.15454/FHRTWJ>. In this repository you will also  
673 find the supplemental table with the cows' ranking per subperiod (see table "RFI").

### 674 **Competing interests**

675 The authors declare that they have no competing interests.

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### 679 **Author's contributions**

680 PF designed the experiment and monitored it; AF gathered and cleaned the data; PG  
681 contributed to define the methodology used to check the hypothesis of feed sorting, to analyse

682 and interpret NIRS analysis of feeds and refusals; AF and PF analysed the data and  
683 interpreted the results; AF was the major contributor in writing the manuscript; AF and PF  
684 read and approved the final manuscript.

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