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## Nutritive value of dehydrated sainfoin (*Onobrychis viciifoliae*) for growing rabbits, according to the harvesting stage

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37

## 38 Abstract

39 In rabbit production, the use of dehydrated sainfoin, a Legume containing phenols and  
40 condensed tannins (CT), has received recent attention because of its nutraceutical properties.  
41 Here, we aimed to compare the nutritive value of dehydrated Perly cultivar sainfoin for a first cut  
42 (SC1: crude protein=200g/kg, Acid detergent fiber (ADFom) =208 g/kg, CT=11.8 g/kg), or a  
43 third cut (SC3: crude protein=159 g/kg, ADFom=228 g/kg, CT=17.5 g/kg). The digestibility of  
44 two sets of 4 diets, made either with SC1 or with SC3, and containing either 0, 120, 240, or 360  
45 g/kg of dehydrated sainfoin (DS0, DS120, DS240, DS360) in dilution with a basal mixture was  
46 compared based on a regression statistical procedure. A direct measure of the nutritive value of  
47 SC1 was performed with a supplemental diet containing 980g/kg (DS980) of SC1. In trial 1, four  
48 groups of 12 growing rabbits received SC3 diets. In the trial 2, five groups of 8 to 9 rabbits  
49 received SC1 diets. Rabbits were allotted at weaning (35 days), housed individually in  
50 metabolism cages and fed *ad libitum* during six weeks. Sainfoin digestibility was measured for  
51 both trials in 6 weeks old rabbit, and in 8 weeks old rabbit only for trial 2. The digestibility of  
52 energy and particularly that of protein linearly decreased with the increase in SC3 incorporation  
53 in diets ( $P<0.001$ ). Using a regression procedure, SC3 digestible energy (DE) and protein (DP)  
54 concentrations were respectively  $6.70 \pm 0.15$  MJ DE/kg and  $53 \pm 4$  g DP/kg as fed,  
55 corresponding respectively to a 40% and 33% digestibility coefficient. On the contrary energy  
56 digestibility was relatively stable from 0 to 360 g/kg SC1 incorporation in diets, in both 6 wks.  
57 and 8 wks. old rabbits whereas the digestibility of protein decreased linearly with SC1  
58 incorporation in diets ( $P<0.001$ ) as observed with SC3 incorporation. SC1 DE and DP  
59 concentration were respectively  $8.84 \pm 0.25$  MJ DE/kg, corresponding to an energy digestibility  
60 of 58%, and

61 79 ± 5 g DP/kg corresponding to a protein digestibility coefficient of 40%. Nitrogen retention  
62 coefficients slightly decreased with SC3 incorporation. With SC1 incorporation, nitrogen  
63 retention coefficients were stable. With SC3 incorporation level the protein digestibility  
64 decreased sharper (P<0.05) than for SC1. The DE content of SC1 calculated by regression gave  
65 similar results than a direct measurement (DS980), while the DP content was lower by regression  
66 compared to the direct measure (74 vs 92 g/kg). Thus for future assays of sainfoin nutritive value  
67 the use of a direct measure could be recommended for DE estimation.

68 *Keywords:* Sainfoin, nutritive value, growing rabbits, harvesting stage

69 *Abbreviations:* ADFom, acid detergent fibre; CT, condensed tanins; DE, digestible energy; DP,  
70 digestible protein; DM, dry matter; aNDFom, neutral detergent fibre

71

## 72 **1. Introduction**

73 Some legumes containing condensed tannins (CTs), such as sainfoin (*Onobrychis viciifolia*),  
74 have been described as nutraceutical because combining feeding values and antiparasitic  
75 properties (Hoste et al, 2015) sourcing from its high content in CTs (Mueller-Harvey et al., 2019;  
76 Wang et al., 2014). For instance, CTs anthelmintic properties to control parasitic gastro intestinal  
77 nematodes have been shown in sheep and goats (Hoste et al., 2015). Also, some results suggest  
78 that CTs could contribute to limit coccidian infections in small ruminants (Saratsis et al., 2012;  
79 Dykes et al., 2019). First studies in growing rabbits fed a high level (40%) of a pelleted  
80 dehydrated sainfoin reached to similar conclusions for helminth (Legendre et al., 2017) and for  
81 coccidia control (Legendre et al., 2018).

82 Compared to alfalfa, dehydrated sainfoin has a higher content of fibre and especially of  
83 lignins (Legendre et al., 2018) that would contribute to limit the risk of digestive disorders

84 around weaning in rabbits (Gidenne, 2015). As a legume, dehydrated sainfoin has a high protein  
85 level (over 160g/kg), and would contain a high digestible energy (DE) level (Legendre et al.,  
86 2018). Introducing sainfoin in rabbit feeding would thus contribute to reduce the risk of digestive  
87 disorders, either through the supply of lignins or through the potential anti-coccidian effect of  
88 CT. Thus, sainfoin could be a good candidate as a nutraceutical for rabbit feeding, because of its  
89 good adequacy between its composition and the rabbit nutritional needs and potential favourable  
90 effects on digestive health.

91 However, the nutritive value of sainfoin in rabbits was never measured precisely, and in a large  
92 range of dietary incorporation and using a regression calculation. In addition, the composition of  
93 sainfoin varies according to different factors, such the phenologic or harvesting stage (Delgado et  
94 al., 2008; Aufrère et al., 2013).

95 Thus, we aimed to measure the nutritive values of sainfoin in growing rabbits based on  
96 measurements of digestible energy and protein content (and nitrogen retention) of dehydrated  
97 sainfoin of the same Perly cultivar. We also address the question of the variability according to  
98 the phenologic states, by comparing a first and a third cut of sainfoin (SC1, SC3), using a  
99 procedure of regression calculation using four incorporation level (0 to 360 g/kg) in a basal  
100 mixture. Additionally, we compared a direct measure of the nutritive value of sainfoin (first cut)  
101 with a 980 g/kg incorporation level, with the value obtained by regression.

## 102 **2. Materials and methods**

103 The experiments were carried out at INRAE experimental farm (Castanet-Tolosan, France).  
104 The study was conducted in accordance with the French legislation on animal experimentation  
105 and ethics (agreement number: 2017121918296225), and the researchers were authorized by the

106 French Ministry of Agriculture to conduct experiments on living animals at the INRAE facilities  
107 in Auzeville, France.

### 108 *2.1. Animals and experimental design*

109 Two trials were carried out to measure the nutritive value of a sainfoin third cut (harvested in  
110 august 2017, SC3, trial 1) and of a sainfoin first cut (harvested in May 2018, SC1, trial 2). Both  
111 sainfoin cuts were dehydrated and pelleted, and were provided by Multifolia Company (Viapres-  
112 le-petit, France). Trial 1 took place in spring 2018 (May/June) and use SC3 sainfoin, while trial 2  
113 in winter 2019 (January/February) and use SC1 sainfoin. Each trial was carried out on 48 rabbits  
114 (Hypharm PS19 X HypharmPS59 line) housed individually in metabolism cages (500 mm  
115 length, 400 mm width and 310 mm height) allowing collection of faeces separately from the  
116 urine, from weaning (35-days old) to slaughter (70-days old). Rabbits were allotted according to  
117 gender (homogeneous parity), weaning weight ( $997\pm 68$ g trial 1;  $1051 \pm 125$ g, trial 2) and litter  
118 origin into 4 groups of 12 rabbits for the trial 1, and into 5 groups of 9 to 10 rabbits for the trial  
119 2.

120 Within each trial, four-pelleted ( $\emptyset$  3.5mm) diets (Table 2) were manufactured in once time  
121 (Arrivé-Béllanné, Nueil-les-Aubiers, France) using the same batches of raw materials (Table 1),  
122 and formulated identically with each sainfoin cut: a control diet without sainfoin (DS0) or  
123 sainfoin diets where 120g/kg (DS120), 240g/kg (DS240), or 360g/kg (DS360) of the basal  
124 mixture was substituted by either SC3 or SC1. For the trial 2, a supplemental diet containing  
125 980g/kg (SD98) of SC1 was produced to provide a direct measure of the nutritional value of SC1  
126 to be compared with the regression procedure. Since SC3 and SC1 were added in dilution to a  
127 basal mixture, the diets were not isonutritive. Diets did not contain drug or coccidiostatic

128 supplementation. Pelleted diets and fresh water were available *ad libitum*. Live weight and feed  
129 intake were checked weekly, while the health status was daily checked.

130 The trials lasted for 5 weeks (from weaning to slaughter): one week for adaptation to cage  
131 and diet (35 to 42 days of age), a 4-days period of faecal and urine collections (42 to 46 days of  
132 age) and three weeks of growth and ingestion monitoring. A supplementary faeces collection  
133 period was performed from 56 to 60 days of age in trial 2 to check the age effect on diet  
134 digestion particularly when the SC1 incorporation was very high (980g/kg).

## 135 2.2. *Digestibility measurements and nutritive value calculation*

136 Individual and total faecal collections started after one week adaptation period (35 to 42 day  
137 of age) on young rabbits (42 to 46 days old, P1) and on older rabbits (56 to 60 days old, P2, for  
138 SC1 trial), according to the 'European' reference method (Perez et al., 1995). Urines were only  
139 collected on young rabbits (42 to 46 days old, P1). Faeces and urines were collected separately  
140 (between 8 am and 11am) every day on the 48 rabbits. Faeces were collected using a 1mm mesh,  
141 whereas urine was collected using a stainless funnel hooked up under the cage, in a beaker  
142 containing 40 mL of sulfuric acid (10%, vol/vol) to avoid ammonia volatilization (Udert et al.,  
143 2003). After weighing, 10% of the urine sample collected was frozen ( $-18^{\circ}\text{C}$ ) for subsequent N  
144 analysis. The whole faecal collection from each rabbit was frozen at  $-18^{\circ}\text{C}$  for further analysis.  
145 The N retention coefficient was calculated by dividing N intake on N excretion in faeces and  
146 urine.

147 The nutritive value of the dehydrated sainfoin (SC1 and SC3) was calculated by regression  
148 and according to the procedure described by (Villamide et al., 2001), using a control diet and  
149 three levels of SC3 incorporation (120, 240 and 360 g/kg). The nutritive value of SC1 directly  
150 measured with DS980 diet was compared to the values obtained by regression.

### 151 2.3. Chemical analyses

152 Chemical analyses were performed at INRAE on dehydrated sainfoin pellets (SC3 and SC1),  
153 diets and faeces from SC3 and SC1 trial. Dry matter (DM) content were determined at 103°C for  
154 24 h and ash at 550°C for 5 h. Nitrogen was analysed in diet, faeces and urine according to  
155 Dumas combustion method (Marcó et al., 2002). The nitrogen retention coefficient was  
156 calculated as: intake – total excretion (faeces + urine). An adiabatic combustion was used to  
157 measure energy (Calorimeter Parr instrument) in feed and faeces. Detergent "Van Soest"  
158 sequential procedure was used to analyse fibre fractions (aNDFom, ADFom and Lignins)  
159 (EGRAN, 2001) in faeces samples. The fibre content of the dehydrated sainfoin (SC1 and SC3)  
160 and of diets was analysed according to the modified procedure of Van Soest, using bisulphite for  
161 tannins rich samples (Van Soest et al., 1991). We calculated hemicelluloses as aNDFom-  
162 ADFom. Total phenols and tannins were analysed in diets and dehydrated sainfoin SC3 and SC1  
163 by the Folin-Ciocalteu method (Council of Europe, 2007; Makkar, 2000) (Inovalys, Nantes,  
164 France). In sainfoin, total tannins correspond almost totally to condensed tannins (Wang et al.,  
165 2014; Mueller-Harvey et al., 2019).

### 166 2.4. Data quality control and statistical analysis

167 Data were first screened to detect outliers. No outlier was found for animal performance  
168 measurements. Dry matter digestibility was first calculated on all animals (48 per trial). Some  
169 outliers were found for dry matter digestibility results (trial 2: 2outliers for SD980, 1 for SD240,  
170 1 for SD120, 1 for SD0; trial 1: 2outliers for SD360, 2 for SD240, 2 for SD120, 1 for SD0). In  
171 trial 1, 8 rabbits (on 12) per diet were finally selected for the chemical analyses of their faeces  
172 and further calculations; while in trial 2, 8 to 9 rabbits per groups were selected.



173 All data were analysed using R Core Team (2020). Shapiro-Wilk test was used to check  
174 normality. A single factor (diet) variance analysis was used to estimate the effect of the diet on  
175 performance traits and digestibility coefficients in both trial 1 and 2. Additionally, in trial 2, a  
176 bifactorial model was used to analyse the effects of the diet and the age on digestibility  
177 measurements. Tukey multiple mean comparison test was used to compare the means between  
178 groups (within a trial). A regression analysis was used to calculate the digestible energy and  
179 protein concentration of the dehydrated sainfoin (1st and 3rd cut). Linear effect of sainfoin  
180 inclusion was studied on growth traits.

### 181 **3. Results**

#### 182 *3.1. Chemical composition of a third sainfoin cut (SC3) and a first sainfoin cut (SC1)*

183 Compared to a first cut (SC1), the third cut of sainfoin (SC3) had a higher content in  
184 ADFom (208 vs 228 g/kg as fed) and especially in lignins (64 vs 106 g/kg as fed, table 1). Total  
185 phenols and tannins concentration were also 15% higher in SC3 compared to SC1. The crude  
186 protein concentration was 20% higher in SC1 than in SC3 (200 vs 159 g/kg as fed, table 1), and  
187 accordingly the crude protein level was similar among the diets, averaging 156 and 178 g/kg  
188 respectively for diets in trial 1 and 2. The fibre content (aNDFom, ADFom and lignins) of the  
189 diets (Table 2) moderately increased with the SC3 incorporation, while only ADFom and lignins  
190 moderately increased with SC1.

#### 191 *3.2. Feed intake and growth performance*

192 From weaning (35 days old) to slaughter (70 days old), all rabbits were in perfect health as  
193 shown by the high feed intake and growth rate (table 3), and no signs of diarrhoea or digestive  
194 troubles were observed during our two trials.

195 When SC3 dietary level increased (trial 1) we observed a parallel linear increase of feed  
196 intake (whole period) of 0.55g/d per % SC3 added ( $P < 0.01$ , table 3), that could be associated  
197 with the increase in fibre (aNDFom and ADFom) concentrations. Thus, feed intake reached 184  
198 g/d for DS360 diet (+ 20 g/d compared to DS0). This increase of feed intake was observed for  
199 the post weaning and for finishing period (49-70days old). In return for the trial 2, the feed intake  
200 remain similar with the increase of SC1 (except for SD98,  $P = 0.57$ , table 3) and averaged 184g/d  
201 (DS0 to DS360). With SC3 incorporation level, the rabbit's growth was similar among the four  
202 diets and averaged 53.3 g/d over the 5 weeks of the trial. With 360 and 980 g/kg of  
203 incorporation, we observed a decrease in growth rate (Trial 2,  $P = 0.01$ , table 3), and particularly  
204 during the post-weaning period. Accordingly, very high SC1 (980) incorporation reduced the  
205 post-weaning feed intake by 15%, whereas no effect was detected from 49 to 70 day old.

### 206 *3.3. Diets digestibility and nitrogen metabolism according to the dietary incorporation of* 207 *sainfoin*

208 Protein, organic matter and energy digestibility linearly decreased with SC3  
209 incorporation level (trial 1,  $P < 0.001$ , table 4). For each 10% increase of SC3 incorporation level  
210 the protein digestibility decreased by 3.8%, while energy digestibility decreased by 2.3% and  
211 organic matter by 2.2%. The fibre (aNDFom and ADFom) digestibility decreased moderately  
212 and linearly with SC3 incorporation level (respectively  $P < 0.001$  and  $P = 0.02$ , table 4).

213 In trial 2, only protein digestibility linearly decreased with SC1 incorporation ( $P < 0.001$ ,  
214 table 4, P1). For each 10% increase of SC1 incorporation level the protein digestibility decreased  
215 by 2.7%. There is a lesser decrease in protein digestibility with SC1 compared to SC3  
216 incorporation level ( $P < 0.05$ ). Within a range of 360g/kg dietary incorporation, SC1 did not affect  
217 the energy (58.9%), organic matter (59.0%) or aNDFom digestibility (26.2%), contrary to SC3

218 diets ( $P < 0.001$ , table 4). With a 980 g/kg SC1 incorporation level there was a decrease in energy  
219 digestibility by 3 units compared to DS120 to DS360 ( $P < 0.01$ ).

220 With SC3 incorporation level, nitrogen intake linearly increased ( $P < 0.01$ , table 5), and  
221 the N faecal excretion almost doubled from DS0 to DS360, while N excreted in urine remained  
222 similar between diets (0.20 g N/day,  $P = 0.18$ ). This increase in N total excretion was mainly due  
223 to the increase in faecal N excretion, which represents 85% of total excretion. Thus N retention  
224 coefficient linearly decreased ( $P < 0.001$ ) due to SC3 incorporation level, ranging from 54.9%  
225 (DS0) down to 39.0% (DS360).

226 Higher nitrogen intake and excretions (faeces or urine) were registered with the  
227 incorporation of SC1 (trial 2) due to a higher nitrogen concentration of SC1. Within a range of  
228 360 g/kg SC1 incorporation, N intake remained stable (4.74,  $P = 0.20$ ,) as for N total excretion  
229 (1.82,  $P = 0.16$ ), contrary to SC3 incorporation level. As with SC3, N urine excretion remained  
230 stable according to SC1 incorporation level (0.43 g N/day, + 0.23 compared to SC3) while that  
231 of N faecal excretion linearly increased ( $P < 0.01$ ). Surprisingly, the linear increase in N faecal  
232 excretion did not influence N total excretion which remained stable according to SC1  
233 incorporation level ( $P = 0.16$ , table 5). N retention coefficient was also stable (36.3%,  $P = 0.93$ )  
234 whatever the SC1 incorporation level. For a 980g/kg SC1 incorporation level, N retention  
235 coefficient, sharply decreased to 12%, due to a high N total excretion (2.36 g N/d) respect to a  
236 relatively low N intake.

#### 237 *3.4. Nutritive value of dehydrated sainfoin calculated by regression procedure*

238 The nutritive value of SC1 and SC3 was calculated by regression with an extrapolation  
239 to 100% sainfoin incorporation. The regression (A) was calculated on the four levels of SC  
240 incorporation levels, i.e. using the usual range of SC in feeds (0 to 360g/kg, table 6). For the trial

241 2, a second regression (B) was also calculated with the five levels of SC1 incorporation levels in  
242 diets (0 to 980g/kg).

243 For each 10% SC3 incorporation level in diets, the dietary digestible protein, energy  
244 and organic matter content decreased by 6.3 g/kg, 0.38 MJ/kg and 20.7g/kg respectively.  
245 Accordingly, the DP content of Sainfoin 3rd cut was  $53 \pm 3.5$  g/kg as fed ( $P < 0.001$ ,  $R^2 = 0.86$ ,  
246 table 6, table 7), corresponding to a protein digestibility of 33.1%. The digestible energy content  
247 of Sainfoin (3rd cut) was  $6.70 \pm 0.15$  MJ/kg corresponding to a digestibility of 40% ( $P < 0.001$ ,  $R^2$   
248  $= 0.92$ , table 6, table 7). Finally, the DOM content of Sainfoin (3rd cut) was  $334 \pm 7.5$  g/kg,  
249 corresponding to a digestibility of 57% ( $P < 0.001$ ,  $R^2 = 0.93$ , table 6).

250 In trial 2, for each 10% increase of SC1 incorporation level the dietary digestible  
251 protein level reduced by 5.4 g/kg. The DP content of sainfoin (1<sup>st</sup> cut) was  $79 \pm 4.6$  g/kg (+26  
252 g/kg DP than with SC3), corresponding to a protein digestibility of 40%. Within a range of 360  
253 g/kg SC1 incorporation, the energy and organic matter digestibility coefficients were stable  
254 ( $P > 0.05$ , table 3). Thus, for each 10% SC1 incorporation level, the dietary digestible energy and  
255 organic matter content respectively decreased by 0.03 MJ/kg and increased by 1.9 g/kg.  
256 Consequently, the DE content of Sainfoin 1<sup>st</sup> cut was  $8.84 \pm 0.25$  MJ/kg (+2.14 MJ/kg than  
257 SC3), corresponding to an energy digestibility of 58%. The DOM content of Sainfoin (1<sup>st</sup> cut)  
258 was  $484 \pm 14.4$  g/kg as fed (+150 g/kg DOM than SC3, table 6, P1), corresponding to an organic  
259 matter digestibility of 64%.

260 Using the regression procedure "B" (trial 2), the DP, DE and DOM content of the  
261 Sainfoin 1<sup>st</sup> cut was  $96 \pm 3.0$  g/kg,  $8.60 \pm 0.15$  MJ/kg and  $438 \pm 3.7$  g/kg respectively (table 5,  
262 table 6). Digestible protein were higher (+17 g/kg), because for each 10% increase of SC1 the  
263 dietary digestible protein level reduced less (- 3.4 g/kg) than with regression procedure "A" (5.4

264 g/kg, table 6). On the contrary, with regression procedure "B", the DOM and DE content  
265 decreased (table 6 and 8). These deviations in nutritive value prediction according to the  
266 regression A or B suggest to recommend to use the predicted values obtained in the real range of  
267 sainfoin dietary incorporation, therefore with A regression for most situations in rabbit feeding.

### 268 *3.5 Comparison between the regression procedures and the direct measure DS980 in SC1*

269 In 7 weeks old rabbits (P1), the direct measurement provided by DS980 diet gave a DP,  
270 DE and DOM content of respectively  $98 \pm 6.1$  g/kg,  $8.58 \pm 0.38$  MJ/kg and  $434 \pm 19.9$  g/kg  
271 (table 7) corresponding respectively to 53%, 56% and 57% digestibility coefficients. DE and  
272 DOM were respectively 3% and 10% higher when calculated with "A" regression. On the  
273 contrary, DP was 19% lower with "A" regression. In 9 weeks old rabbit (P2), the direct  
274 measurement of SC3 nutritive value (diet DS980) gave a DP, DE and DOM content of  
275 respectively  $92 \pm 2.9$  g/kg,  $8.55 \pm 0.13$  MJ DE/kg and  $415 \pm 8.1$  g DP/kg (table 7) corresponding  
276 respectively to 49%, 56% and 55% digestibility coefficients. Likewise, DP content was lower  
277 when calculated by regression method A, but dropped more in 9 weeks old rabbit (23% vs 19%).  
278 Surprisingly, DE content was 2% lower while DOM was 6% higher with regression method A.  
279 With regression method B, we logically observed similar DP, DE and DOM than with the direct  
280 measure, in both ages.

281 Globally, the DE content of SC1 calculated by regression A gave similar results than a direct  
282 measurement (DS980), while the DP content was 20% lower by regression compared to the  
283 direct measure (meanly for both age: 74 vs 92 g/kg).

### 284 *3.6. Age effect on SC1 nutrient digestibility (trial 2)*

285 The same nutrient digestibility trends were observed between 7 weeks old and 9 weeks  
286 old rabbit. Indeed, protein digestibility linearly decreased with SC1 incorporation in young and

287 older rabbits (table 4, trial 2). However protein digestibility coefficients were lower with age  
288 ( $P < 0.001$ ), and accordingly the dietary digestible protein concentration decreased by 10% (79 vs  
289 71 g/kg, regression A, table 7) and by 7% (96 vs 89 g/kg, regression B, table 7) corresponding to  
290 a protein digestibility of 35% (vs 40%, regression A), and of 45% (vs 48%, regression B). In 9  
291 weeks old rabbit (P2, table 4) and within a range of 360 g/kg SC1 incorporation, energy (mean  
292 59.7%), organic matter (mean 57.2%) and fibre digestion (mean 24.1% aNDFom, 12.8%  
293 ADFom) were stable, as observed with younger rabbits. With age organic matter digestibility  
294 decrease from 59% vs 57.0% ( $P < 0.05$ ). DOM content decreased by 9% with regression method  
295 A (484 vs 439 g/kg as fed) and decreased by 5% using regression method B (438 vs 417 g/kg as  
296 fed) or by 4% using direct measurement. Thus corresponding OM digestibility coefficient  
297 decreased by 6 units (58% vs 64%, regression A) and by 3 units (55% vs 58%, regression B).  
298 Surprisingly energy digestibility was not affected by age ( $P > 0.05$ ). Likewise aNDFom  
299 digestibility were similar between young and old rabbits ( $P > 0.05$ ) with SC1 incorporation. Only  
300 ADFom digestibility coefficients reduced with age ( $P < 0.05$ ). In both age, SC1 high incorporation  
301 level (980 g/kg) decreased energy and aNDFom digestibility and increased ADFom digestibility  
302 coefficient. Only a slight decrease in OM digestion was observed in 8 weeks old rabbits ( $P = 0.02$ )  
303 compared to 6 weeks old rabbits.

304

## 305 **4. Discussion**

### 306 *4.1. Chemical composition of sainfoin*

307 Sainfoin composition depends on environmental factor and the number of harvest.  
308 Tannins are plants metabolites that are produced due to the plant stresses. Tannins increases from  
309 May to September due to the temperature increase and because of an increasing number of

310 harvest (Simonnet et al., 2013). Thus, phenols and tannins levels of a third cut are logically  
311 higher than in a first cut, and for our Perly sainfoin, the phenols and tannins level of the third cut  
312 were 14% and 28% (resp.) higher than for the first cut. However, the level of condensed tannins  
313 in a sainfoin hay averaged 30 g/kg (Feedipedia), about 40% higher than a third cut of dehydrated  
314 Perly sainfoin (18 g/kg). Also, with age the plant produces more rod and less leaf that contains  
315 more proteins (less fibre) than in rods. Feedipedia tables show that sainfoin hay contains a high  
316 quantity of lignocellulose (ADFom = 320 g/kg as fed) and lignins (lignins = 81 g/kg as fed).  
317 Moreover, as a legume, sainfoin hay contains high level of crude protein (136 g/kg as fed) and  
318 quite high level of digestible energy (DE = 7.36 MJ/kg as fed). Compared to sainfoin hay,  
319 dehydrated and pelleted sainfoin (Perly cultivar) have a higher protein concentration (200 and  
320 159 g/kg respectively for a first and a third cut), and a much lower ADFom content (208 and 228  
321 g/kg respectively for SC1 and SC3), while the lignins content of the third cut was higher  
322 (100g/kg).. Compared to dehydrated sainfoin, a dehydrated alfalfa which is commonly used in  
323 rabbit feeding, contains similar protein and lignocellulose, but less lignins (76 g/kg) and no  
324 condensed tannins. Since a balance feed for the growing rabbit requires a high level of lignins  
325 (Gidenne *et al.*, 2020), the sainfoin should be more convenient than alfalfa in such feed  
326 formulation, and moreover sainfoin would contribute to protect against parasitism.

#### 327 *4.2. Feed intake and growth performances*

328 Since the dietary fibre level increased according to the incorporation of the third cut sainfoin, we  
329 logically observed a linear increase of the feed intake in relation with the lower DE content of  
330 the diets. This intake increase corresponds to a classical intake regulation of the growing rabbit  
331 (Gidenne et al., 2020) that adjust its DE intake to maintain its growth. In return, increasing the

332 incorporation of the first cut sainfoin did not affect the dietary fibre level and the feed intake, or  
333 the energy digestibility.

334 For a very high sainfoin (1<sup>st</sup> cut) incorporation (980 g/kg) the feed intake was reduced by 20%  
335 only during the two weeks after weaning (Table 3), while from 7 weeks old it was not affected.  
336 This suggests a period of adaptation after weaning to reach a sufficiently high intake for very  
337 high sainfoin incorporation, possibly in relation to a high condensed tannins dietary level and a  
338 lower appetency. Tannins have an astringent taste, which classically leads to a reduction in the  
339 voluntary feed intake (Soares et al., 2020). Despite having tannins, sainfoin is an appetizing feed  
340 in ruminants, as shown for lambs (Fraser et al., 2000). In pastured rabbits, a sainfoin plot is more  
341 consumed than tall fescue, and led to a 20% higher growth rate (Legendre et al., 2019).

#### 342 *4.3. Dehydrated sainfoin digestion and nutritive value*

343 With procedures using only one level of sainfoin hay incorporation, a rather high  
344 digestible energy content respect to fibre content of the hay is referenced (8.20 MJ/kg) in  
345 Feedipedia tables (Fernandez Carmona et al., 1996; Feedipedia, 2019). Also with one level of  
346 incorporation Legendre *et al.* (2017) estimated a much higher DE (11.12 MJ/kg) and DP content  
347 (110 g/kg) for a dehydrated sainfoin (third cut & Perly cultivar). Using a regression procedure  
348 with four incorporation levels we obtained a nutritive value more reliable with the chemical  
349 composition of this plant, and higher for a first cut compared to a third cut: 79 vs 53 g DP/kg,  
350 8.84 vs 6.70 MJ DE/kg. Therefore the DE content of our dehydrated first cut sainfoin was 0.5 MJ  
351 higher than dehydrated alfalfa (8.30 MJ/kg) commonly used in rabbit feeding (Feedipedia).

352 Protein digestion sharply decreased with sainfoin incorporation, ranging from 74 to  
353 61% for a 0 to 360 g/kg incorporation of a third cut. Tannins are antinutritional factors that  
354 impaired digestion of proteins in the small intestine (Naumann et al., 2017). For sainfoin, the



355 total tannins are condensed tannins that explain the decrease in protein digestion more  
356 particularly with a third cut higher in CTs. Legendre et al. (2017) also observed a loss of 6 units  
357 for protein digestion with 40% of dehydrated sainfoin (third cut). Besides, since the dietary fibre  
358 level slightly changed according to the sainfoin incorporation, the proportion of protein linked to  
359 fibre cannot be ascribed to contribute to a decrease in protein digestion.

360 N intake and excretion increased with sainfoin incorporation. We found that urine contributed to  
361 only 15 to 22% of the N excretion (78–85% for faeces) in agreement with previous studies  
362 (Calvet et al, 2008; Gidenne et al., 2013). For growing rabbit, the N retention generally ranges  
363 between 35 and 45% (Gidenne et al., 2013; Villamide et al., 2020) and decreases when the  
364 dietary N level increases. The N retention coefficient here decreased with the increasing N intake  
365 in SC3 diets. With a first cut, the nitrogen retention remained stable and was reduced only for a  
366 very high (980 g/kg) sainfoin incorporation. Besides, we confirmed a decrease in digestion  
367 during the post-weaning period, as already described (Debray et al., 2000; Gallois et al., 2008).

#### 368 *4.4. Prediction of the nutritive value by regression or by direct measurement.*

369 Prediction of the nutritional value of raw materials is one of the main goals in animal nutrition in  
370 order to improve the nutritive value calculation of compounds diets by formulation. Two main  
371 ways are available to measure the nutritive value (i.e. digestible energy and protein content), by  
372 comparing the digestion of several diets with increasing incorporation of the raw material in a  
373 basal mixture (Villamide et al., 2001; Villamide et al., 2003), or by a direct measurement when  
374 the feed is sufficiently balanced for the rabbit such for fibrous legumes (alfalfa, Perez et al.,  
375 1998; alfalfa meal, Maertens and De Groote, 1981; lucerne hay, Garcia et al., 1995; Villamide et  
376 al., 2003; sulla, Kadi et al., 2012). Perez *et al.* (1998) reached similar DE values for dehydrated  
377 alfalfa estimated by regression or measured directly (with 995g/kg incorporation). Here, for a

378 dehydrated first cut sainfoin we also reached a similar DE content calculated either by regression  
379 or directly. Thus for future assays of sainfoin nutritive value the use of a direct measure (more  
380 simple and less costly) would be recommended for DE estimation. However, the DP content of  
381 sainfoin was 20% lower by regression compared to the direct measure. Thus for future assays of  
382 sainfoin nutritive value the use of a direct method is recommended for DE but not for DP  
383 estimation.

384 As described in dairy cows (Parker et Moss, 1981) or in sheep (Aufrère et al., 2013), a first cut  
385 dehydrated sainfoin have a similar nutritive value (DE and DP) than a dehydrated alfalfa. A third  
386 cut of sainfoin, had a moderately lower DE and DP content compared to alfalfa, however it  
387 supplies more lignins and condensed tannins that could positively contribute to the digestive  
388 health of the growing rabbit. Since a balance feed for the growing rabbit requires a high level of  
389 lignins (Gidenne et al., 2020), the sainfoin should be more convenient than alfalfa in such feed  
390 formulation, and moreover sainfoin would contribute to protect against parasitism according to  
391 its phenols and condensed tannins content.

392

## 393 **5. Conclusions**

394 A higher digestible protein and energy concentration was logically found for a first cut, than for  
395 a third cut, higher in fibre (acid detergent fibre, lignins) and condensed tannins. A dehydrated  
396 sainfoin (first cut) has a similar nutritive value than dehydrated alfalfa. The digestible energy  
397 content of the sainfoin could be estimated directly with a full (980 g/kg) incorporation, while the  
398 digestible protein content would require a measure by regression. The high lignins and  
399 condensed tannins content of the sainfoin could give a high "health value" regarding its potential  
400 positive role on digestive health. In perspective, further studies will be conducted to analyse the

401 effect of incorporating dehydrated sainfoin in a balanced diet on the growth and reproductive  
402 performances and health (coccidian infection) of the rabbit.

403

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408

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- 512

513 Table 1  
 514 Chemical composition of dehydrated sainfoin (3rd and 1st cut) and of Alfalfa.  
 515

<u>Chemical composition, g/kg as fed</u>		
	<u>Dehydrated sainfoin</u>	<u>Alfalfa<sup>b</sup></u>
Trial 1 (using SC3, 3rd cut 2017)		
Dry matter	890	900
Organic matter	812	801
Ash	78	99
Crude Protein	159	157
Crude fat	31	32
aNDFom <sup>c</sup>	334	373 <sup>d</sup>
ADFom <sup>c</sup>	228	291 <sup>d</sup>
Lignins (sa) <sup>e</sup>	106	65
Energy matter (MJ)	16.82	-
Total Phenols	27.9	0
Total Tannins	17.5	0
Trial 2 (using SC1, 1st cut 2018)		
		Similar as trial 1
Dry matter	888	
Organic matter	760	
Ash	128	
Crude Protein	200	
Crude fat	-	
aNDFom <sup>c</sup>	268	
ADFom <sup>c</sup>	208	
Lignins (sa) <sup>e</sup>	64	
Energy matter (MJ)	15.24	
Total Phenols	24.1	
Total Tannins	11.8	

516  
 517 <sup>a</sup> dehydrated sainfoin (Perly cultivar), either from a first cut (SC1) or from a third cut (SC3).  
 518 <sup>b</sup> The alfalfa used for trial 1 and 2 was harvested in 2015. The same harvest batch was used for both  
 519 trials 1 and 2.  
 520 <sup>c</sup> aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of  
 521 residual ash; ADFom, acid detergent fibre expressed exclusive of residual ash; according to the  
 522 sequential procedure of Van Soest method AFNOR, NF-18-122 + bisulfite to correct tannins high  
 523 level.  
 524 <sup>d</sup> aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of  
 525 residual ash; ADFom, acid detergent fibre expressed exclusive of residual ash; according to the  
 526 sequential procedure of Van Soest method without addition of bisulfite.  
 527 <sup>e</sup> lignin (sa), acid detergent lignin determined by solubilisation of cellulose with sulphuric acid;  
 528 according to the sequential procedure of Van Soest method NF-EN-ISO 13906, direct lignin  
 529 analysis.  
 530



531 Table 2  
 532 Ingredient and chemical composition of the diets.  
 533

	Experimental diets				
	DS0	DS120	DS240	DS360	DS980 <sup>b</sup>
<u>Ingredients, g/kg</u>					
Dehydrated sainfoin <sup>a</sup>	0	119	237	356	989
Dehydrated alfalfa	290	255	220	186	0
Barley	80	70	61	51	0
Wheat	120	106	91	77	0
Sunflower meal	169	149	128	108	0
Grape pulp	50	44	38	32	0
Beet pulp	135	119	103	86	0
Wheat bran	111	97	84	70	0
Wheat straw	30	26	23	19	0
Soya oil	5	4	4	3	0
Mineral and vitamin premix <sup>c</sup>	11	11	11	11	11
<u>Chemical composition, g/kg as fed</u>					
Trial 1 (using SC3, 3rd cut 2017)					
Organic matter	832	825	819	819	-
Crude Protein	157	157	154	155	-
aNDFom <sup>d</sup>	295	295	307	301	-
ADFom <sup>d</sup>	167	182	197	201	-
Lignins (sa) <sup>e</sup>	49	66	73	82	-
Energy (MJ)	16.46	16.45	16.39	16.46	-
Total Phenols	7.9	9.2	10.3	13.1	-
Total Tannins	<5.0	<5.0	<5.0	6.3	-
Trial 2 (using SC1, 1st cut 2018)					
Organic matter	788	803	787	794	762
Crude Protein	177	179	178	173	185
aNDFom <sup>d</sup>	321	313	305	319	262
ADFom <sup>d</sup>	188	193	200	191	210
Lignins <sup>e</sup>	51	56	57	63	63
Energy (MJ)	15.38	15.50	15.32	15.43	15.37
Total Phenols	8.3	9.4	11.2	14.0	24.4
Total Tannins	<5.0	<5.0	5.1	6.5	11.5

534  
 535 <sup>a</sup> dehydrated sainfoin (Perly cultivar), either from a first cut (SC1) or from a third cut (SC3).  
 536 <sup>b</sup> diet DS980 processed only with first cut of sainfoin (SC1, trial 2).  
 537 <sup>c</sup> Premix provided per kg diet: vit. A, 12,000 IU; vit. D3, 1,000 IU; vit. E acetate, 50 mg; vit. K3, 2  
 538 mg; biotin, 0.1 mg; Iron, 100 mg; Copper, 20 mg; Manganese, 50 mg; Cobalt, 2 mg; Iodine, 1 mg;  
 539 Zinc, 100 mg; Selenium, 0.1 mg; Robenidine, 66 mg.  
 540

541 <sup>d</sup> aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of  
542 residual ash; ADFom, acid detergent fibre expressed exclusive of residual ash; according to the  
543 sequential procedure of Van Soest method AFNOR, NF-18-122 + bisulfite  
544 <sup>e</sup> lignin (sa), acid detergent lignin determined by solubilisation of cellulose with sulphuric acid;  
545 according to the sequential procedure of Van Soest method NF-EN-ISO 13906, direct lignin  
546 analysis.  
547  
548  
549

550 Table 3

551 Feed intake and growth of rabbits <sup>a</sup> according to sainfoin (3rd or 1st cut) incorporation rate

552

	Experimental diets					SEM <sup>b</sup>	Diet Effect	Linear Effect. <sup>c</sup>
	DS0	DS120	DS240	DS360	DS980			
<u>Trial 1 - third cut sainfoin SC3</u>								
From 35 to 49 days								
Feed intake, g/day	127a	136ab	144b	145b	-	11.1	0.003	<0.001
Weight gain, g/day	61.2	62.5	61.4	58.7	-	4.13	0.422	0.235
Feed conversion ratio	2.08a	2.18a	2.34b	2.47b	-	0.151	<0.001	<0.001
From 49 to 70 days								
Feed intake, g/day	194a	202a	204a	210b	-	11.0	0.035	0.004
Weight gain, g/day	47.7	47.7	47.3	49.8	-	4.57	0.675	0.403
Feed conversion ratio	4.13	4.30	4.38	4.23	-	0.408	0.676	0.552
From 35 to 70 days								
Feed intake, g/day	167a	176a	180a	184b	-	10.8	0.012	0.001
Weight gain, g/day	53.1	53.6	52.9	53.4	-	3.56	0.983	0.975
Feed conversion ratio	3.16a	3.29ab	3.41b	3.45b	-	0.210	0.016	0.002
<u>Trial 2 - first cut sainfoin SC1</u>								
From 35 to 49 days								
Feed intake, g/day	148a	161a	161a	153a	125b	16.0	<0.001	0.569
Weight gain, g/day	64.6a	67.1a	65.2a	56.2b	38.5c	9.55	<0.001	0.004
Feed conversion ratio	2.29a	2.41ab	2.47ab	2.71b	3.30c	0.339	<0.001	<0.001
From 49 to 70 days								
Feed intake, g/day	207	204	207	194	192	14.3	0.214	0.184
Weight gain, g/day	53.4a	52.5a	52.2a	49.2ab	43.6b	4.50	<0.001	0.076
Feed conversion ratio	3.88a	3.89a	3.98a	3.93a	4.43b	0.260	0.001	0.517
From 35 to 70 days								
Feed intake, g/day	183ab	187ab	189a	178ab	165b	13.7	0.027	0.571
Weight gain, g/day	57.9a	58.3a	57.4ab	51.9b	40.9c	6.12	<0.001	0.011
Feed conversion ratio	3.16a	3.20a	3.29a	3.43a	4.04b	0.308	<0.001	0.007

553

554 <sup>a</sup> Feed intake, weight gain and Feed conversion ratio were calculated from weaning to 70 days old  
555 for 12 rabbits per diet for trial 1/ for 9 rabbits per diet (except DS120 with 8 rabbits) in trial 2.  
556 Results are presented for 3 periods: from 35 to 49 days old, from 49 to 70 days old and for total trial  
557 period from 35 to 70 days old.

558 Feed intake = distributed feed– refused feed

559 Weight gain = highest Live Weight – lowest Live Weight (LW49 – LW35, LW70-LW49, LW70-  
560 LW35 for each period)

561 Feed conversion ratio = feed intake / weight gain

562 <sup>b</sup> SEM: standard error mean.563 <sup>c</sup> linear effect was calculated without DS980 diet

564 Means having the same superscript letter did not significantly differ.

565

566  
567  
568  
569

Table 4  
Dietary nutrients digestibility <sup>a</sup> according to sainfoin (3rd or 1st cut) incorporation level

%	Experimental diets					SEM <sup>e</sup>	Diet Effect.	Linear Effect <sup>b</sup>
	DS0	DS120	DS240	DS360	DS980			
<u>Trial 1 (SC3<sup>d</sup>) Digestibility coefficient (P1<sup>c</sup> = 43-47 day old)</u>								
Crude protein	73.7a	70.0b	64.3c	60.6d	-	4.70	<0.001	<0.001
Energy	63.9a	60.7b	58.2c	55.6d	-	2.69	<0.001	<0.001
Organic matter	64.8a	61.5b	59.6c	56.7d	-	2.56	<0.001	<0.001
aNDFom <sup>f</sup>	30.9a	27.0b	23.4c	24.7bc	-	3.12	<0.001	<0.001
ADFom <sup>f</sup>	23.0a	19.8ab	20.8ab	18.0b	-	3.05	0.061	0.019
<u>Trial 2 (SC1<sup>d</sup>) Digestibility coefficient (P1<sup>c</sup> = 43-47 day old)</u>								
Crude protein	74.0a	72.4a	68.6b	64.4c	53.1d	6.63	<0.001	<0.001
Energy	59.2a	59.0a	58.5ab	58.7a	55.9b	1.68	0.008	0.435
Organic matter	58.6	59.0	58.7	59.7	57.0	1.68	0.088	0.306
aNDFom <sup>f</sup>	27.0	25.6	25.5	26.8	22.8	3.27	0.136	0.918
ADFom <sup>f</sup>	18.9a	17.3a	19.2a	18.2a	-	4.32	<0.001	0.955
<u>Trial 2 (SC1<sup>d</sup>) Digestibility coefficient (P2<sup>c</sup> = 56-60 day old)</u>								
Crude protein	71.5a	69.9a	65.1b	61.2c	49.5d	6.46	<0.001	<0.001
Energy	60.9a	60.4a	58.0ab	59.3a	55.7b	2.18	<0.001	0.054
Organic matter	57.3ab	58.3a	56.1ab	57.2ab	54.5b	2.03	0.024	0.505
aNDFom <sup>f</sup>	25.6a	24.9a	22.4ab	23.6ab	17.9b	3.94	0.013	0.215
ADFom <sup>f</sup>	11.0a	14.2ab	12.7a	13.3a	-	4.79	0.005	0.534

570

571 <sup>a</sup> faecal digestibility for Crude protein, energy, organic matter, aNDFom and ADFom was  
572 calculated for 8 rabbits per diet in trial 1 and for 9 rabbits per diet in trial 2. Faecal digestibility  
573 results are given for animals from 43 to 47 days old (P1). In trial 2 a second period of measurement  
574 was done on rabbits from 56 to 60 days old.

575 <sup>b</sup> linear effect was calculated without DS980 diet

576 <sup>c</sup> P1= digestibility measured from 43 to 47 days old ; P2= digestibility measured from 56 to 60  
577 days old.

578 <sup>d</sup> SC3: sainfoin 3<sup>rd</sup> cut/ SC1: Sainfoin 1<sup>st</sup> cut

579 <sup>e</sup> SEM= standard error mean

580 <sup>f</sup> aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of  
581 residual ash; ADFom, acid detergent fibre expressed exclusive of residual ash; according to the  
582 sequential procedure of Van Soest method.

583 Means having the same superscript letter (a, b) did not differ at the level P=0.05.

584

585 Table 5  
 586 Nitrogen balance <sup>a</sup> according to sainfoin (3rd or 1st cut) incorporation rate level  
 587

	Experimental diets					SEM <sup>b</sup>	Diet effect	Linear effect <sup>d</sup>
	DS0	DS120	DS240	DS360	DS980			
<u>Trial 1 - Third cut sainfoin SC3</u>								
Nitrogen intake and excretion, g N/day								
N intake	3.36a	3.62ab	3.66ab	4.00b	-	0.326	0.010	0.001
N excreted in faeces	0.89a	1.09ab	1.31b	1.58c	-	0.241	<0.001	<0.001
N excreted in urine	0.15	0.21	0.24	0.21	-	0.053	0.179	0.102
Total N excreted	1.03a	1.29ab	1.54bc	1.74c	-	0.247	<0.001	<0.001
N retention coefficient <sup>c</sup> %	54.9a	47.7ab	35.7c	39.0bc	-	7.583	<0.001	<0.001
<u>Trial 2 - First cut sainfoin SC1</u>								
Nitrogen intake and excretion, g N/day								
N intake	4.67ab	4.98b	4.97b	4.33ab	4.06a	0.487	<0.001	0.201
N excreted in faeces	1.23a	1.35a	1.58ab	1.53a	1.92b	0.258	<0.001	0.006
N excreted in urine	0.46	0.46	0.43	0.34	0.44	0.111	0.340	0.066
Total N excreted	1.66a	1.81a	1.96ab	1.86a	2.36b	0.304	<0.001	0.161
N retention coefficient <sup>c</sup> %	35.6a	36.1a	41.1a	32.6a	12.3b	10.411	<0.001	0.930

588

589 <sup>a</sup> measured on rabbits from 43 to 47 days old

590 <sup>b</sup> SEM = standard error mean

591 <sup>c</sup> (N retained/N intake) x 100

592 <sup>d</sup> linear effect was calculated without DS980 diet

593 SC3= sainfoin 3<sup>rd</sup> cut/ SC1 = sainfoin 1<sup>st</sup> cut

594

595 Table 6  
 596 Nutritive value of dehydrated sainfoin : prediction equation for digestible concentration of organic  
 597 matter, crude protein (g/kg as fed) and energy (MJ/kg as fed) of a third and a first cut of sainfoin.  
 598

	Equation No.	R <sup>2</sup>	RSD	P-value	N
<u>Sainfoin, 3rd cut, SC3<sup>c</sup> : nutrient prediction (A)<sup>a</sup></u>					
DOM <sup>e</sup> = -2.02x + 536	(1)	0.931	1.37	<0.001	32
DP <sup>e</sup> = -0.63x + 116	(2)	0.857	0.64	<0.001	32
DE <sup>e</sup> = -9.00x + 10.475	(3)	0.920	0.028	<0.001	32
<u>Sainfoin, 1st cut, SC1<sup>c</sup> : nutrient prediction (A)<sup>a</sup></u>					
DOM P1 <sup>d</sup> = 0.19x + 465	(4)	-0.032	2.65	0.329	32
DOM P2 <sup>d</sup> = -0.18x + 457	(5)	-0.018	3.68	0.522	34
DP P1 = -0.54x + 133	(6)	0.716	0.84	<0.001	32
DP P2 = -0.59x + 129	(7)	0.772	0.77	<0.001	32
DE P1 = -0.003x + 9.111	(8)	0.299	0.046	0.430	32
DE P2 = -2.35x + 9.367	(9)	0.085	0.067	0.058	32
<u>Sainfoin, 1st cut, SC1 : nutrient prediction (B)<sup>b</sup></u>					
DOM P1 = -0.35x + 473	(10)	0.341	2.74	<0.001	41
DOM P2 = -4.48x + 525	(11)	0.858	9.55	<0.001	41
DP P1 = -0.35x + 130	(12)	0.826	2.89	<0.001	41
DP P2 = -0.37x + 126	(13)	0.868	0.80	<0.001	39
DE P1 = -1.32x + 9.157	(14)	0.304	0.046	<0.001	40
DE P2 = -1.95x + 9.341	(15)	0.389	0.060	<0.001	39

599  
 600 x in equations corresponds to sainfoin SC3 or SC1 incorporation rate.  
 601 <sup>a</sup> (A) Equation of prediction with 4 levels of sainfoin incorporation.  
 602 <sup>b</sup> (B) Equation of prediction with 5 levels of incorporation (including 98%, "DS980")  
 603 <sup>c</sup> SC3 = dehydrated sainfoin 3rd cut / SC1 = dehydrated sainfoin 1st cut  
 604 <sup>d</sup> P1= measured from 43 to 47 days old ; P2= measured from 56 to 60 days old  
 605 <sup>e</sup> DOM: Digestible organic matter, DP: Digestible protein, DE: Digestible energy  
 606

607  
 608 Table 7  
 609 Nutritive value of dehydrated sainfoin calculated by the regression (A) and (B), and by a direct  
 610 measure.  
 611

			Regression value without DS980 (A) <sup>a</sup>		Regression value with DS980 (B) <sup>b</sup>	Direct measure (DS980)
			Third cut (S3C trial 1) <sup>c</sup>	First cut S1C (trial 2) <sup>c</sup>	First cut S1C (trial 2) <sup>c</sup>	First cut S1C (trial 2)
Digestible energy	P1 <sup>d</sup>		6.70±0.15	8.84±0.25	8.60±0.15	8.58±0.38
Digestible energy	P2 <sup>d</sup>		-	8.38±0.37	8.52±0.18	8.55±0.13
Digestible protein as fed)	P1 (g/kg		53±3.5	79±4.6	96±3.0	98±6.1
Digestible protein as fed)	P2(g/kg as		-	71±8.8	89±2.6	92±2.9

612  
 613 Predicted values and their residual standard deviation (using equation of the table 5)  
 614 <sup>a</sup> (A) Equation prediction without DS980  
 615 <sup>b</sup> (B) Equation prediction with DS980  
 616 <sup>c</sup> S3C = dehydrated sainfoin 3rd cut 2017 / S1C = dehydrated sainfoin 1st cut 2018  
 617 <sup>d</sup> P1= measured from 43 to 47 days old ; P2= measured from 56 to 60 days old  
 618  
 619