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

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Abstract

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

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

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

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

1. Introduction

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

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

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

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

2. Materials and methods

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

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

2.1. Animals and experimental design

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

Within each trial, four-pelleted (Ø 3.5mm) diets (Table 2) were manufactured in once time (Arrivé-Béllanné, Nueil-les-Aubiers, France) using the same batches of raw materials (Table 1), and formulated identically with each sainfoin cut: a control diet without sainfoin (DS0) or sainfoin diets where 120g/kg (DS120), 240g/kg (DS240), or 360g/kg (DS360) of the basal mixture was substituted by either SC3 or SC1. For the trial 2, a supplemental diet containing 980g/kg (SD98) of SC1 was produced to provide a direct measure of the nutritional value of SC1 to be compared with the regression procedure. Since SC3 and SC1 were added in dilution to a basal mixture, the diets were not isonutritive. Diets did not contain drug or coccidiostatic supplementation. Pelleted diets and fresh water were available *ad libitum*. Live weight and feed intake were checked weekly, while the health status was daily checked.

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

2.2. Digestibility measurements and nutritive value calculation

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

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

2.3. Chemical analyses

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

2.4. Data quality control and statistical analysis

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

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

3. Results

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

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

3.2. Feed intake and growth performance

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

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

3.3. Diets digestibility and nitrogen metabolism according to the dietary incorporation of sainfoin

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

In trial 2, only protein digestibility linearly decreased with SC1 incorporation (P<0.001, table 4, P1). For each 10% increase of SC1 incorporation level the protein digestibility decreased by 2.7%. There is a lesser decrease in protein digestibility with SC1 compared to SC3 incorporation level (P<0.05). Within a range of 360g/kg dietary incorporation, SC1 did not affect the energy (58.9%), organic matter (59.0%) or aNDFom digestibility (26.2%), contrary to SC3 diets (P<0.001, table 4). With a 980 g/kg SC1 incorporation level there was a decrease in energy digestibility by 3 units compared to DS120 to DS360 (P< 0.01).

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

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

3.4. Nutritive value of dehydrated sainfoin calculated by regression procedure

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

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

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

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

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

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

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

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

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

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

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

4. Discussion

4.1. Chemical composition of sainfoin

Sainfoin composition depends on environmental factor and the number of harvest. Tannins are plants metabolites that are produced due to the plant stresses. Tannins increases from May to September due to the temperature increase and because of an increasing number of harvest (Simonnet et al., 2013). Thus, phenols and tannins levels of a third cut are logically higher than in a first cut, and for our Perly sainfoin, the phenols and tannins level of the third cut were 14% and 28% (resp.) higher than for the first cut. However, the level of condensed tannins in a sainfoin hay averaged 30 g/kg (Feedipedia), about 40% higher than a third cut of dehydrated Perly sainfoin (18 g/kg). Also, with age the plant produces more rod and less leafs that contains more proteins (less fibre) than in rods. Feedipedia tables show that sainfoin hay contains a high quantity of lignocellulose (ADFom = 320 g/kg as fed) and lignins (lignins = 81 g/kg as fed). Moreover, as a legume, sainfoin hay contains high level of crude protein (136 g/kg as fed) and quite high level of digestible energy (DE = 7.36 MJ/kg as fed). Compared to sainfoin hay, dehydrated and pelleted sainfoin (Perly cultivar) have a higher protein concentration (200 and 159 g/kg respectively for a first and a third cut), and a much lower ADFom content (208 and 228 g/kg respectively for SC1 and SC3), while the lignins content of the third cut was higher (100g/kg).. Compared to dehydrated sainfoin, a dehydrated alfalfa which is commonly used in rabbit feeding, contains similar protein and lignocellulose, but less lignins (76 g/kg) and no condensed tannins. Since a balance feed for the growing rabbit requires a high level of lignins (Gidenne *et* al., 2020), the sainfoin should be more convenient than alfalfa in such feed formulation, and moreover sainfoin would contribute to protect against parasitism.

4.2. Feed intake and growth performances

Since the dietary fibre level increased according to the incorporation of the third cut sainfoin, we logically observed a linear increase of the feed intake in relation with the lower DE content of the diets. This intake increase corresponds to a classical intake regulation of the growing rabbit (Gidenne et al., 2020) that adjust its DE intake to maintain its growth. In return, increasing the incorporation of the first cut sainfoin did not affect the dietary fibre level and the feed intake, or the energy digestibility.

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

4.3. Dehydrated sainfoin digestion and nutritive value

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

Protein digestion sharply decreased with sainfoin incorporation, ranging from 74 to 61% for a 0 to 360 g/kg incorporation of a third cut. Tannins are antinutritional factors that impaired digestion of proteins in the small intestine (Naumann et al., 2017). For sainfoin, the total tannins are condensed tannins that explain the decrease in protein digestion more particularly with a third cut higher in CTs. Legendre et al. (2017) also observed a loss of 6 units for protein digestion with 40% of dehydrated sainfoin (third cut). Besides, since the dietary fibre level slightly changed according to the sainfoin incorporation, the proportion of protein linked to fibre cannot be ascribed to contribute to a decrease in protein digestion.

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

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

Prediction of the nutritional value of raw materials is one of the main goals in animal nutrition in order to improve the nutritive value calculation of compounds diets by formulation. Two main ways are available to measure the nutritive value (i.e. digestible energy and protein content), by comparing the digestion of several diets with increasing incorporation of the raw material in a basal mixture (Villamide et al., 2001; Villamide et al., 2003), or by a direct measurement when the feed is sufficiently balanced for the rabbit such for fibrous legumes (alfalfa, Perez et al., 1998; alfalfa meal, Maertens and De Groote, 1981; lucerne hay, Garcia et al., 1995; Villamide et al., 2003; sulla, Kadi et al., 2012). Perez *et* al. (1998) reached similar DE values for dehydrated alfalfa estimated by regression or measured directly (with 995g/kg incorporation). Here, for a dehydrated first cut sainfoin we also reached a similar DE content calculated either by regression or directly. Thus for future assays of sainfoin nutritive value the use of a direct measure (more simple and less costly) would be recommended for DE estimation. However, the DP content of sainfoin was 20% lower by regression compared to the direct measure. Thus for future assays of sainfoin nutritive value the use of a direct method is recommended for DE but not for DP estimation.

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

5. Conclusions

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

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

Chemical composition, g/kg as fed	Dehydrated sainfoin	Alfalfa [b]
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 [c]	334	373 [d]
ADFom [c]	228	291 [d]
Lignins (sa) [e]	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 [c]	268	
ADFom [c]	208	
Lignins (sa) [e]	64	
Energy matter (MJ)	15.24	
Total Phenols	24.1	
Total Tannins	11.8	

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

Table 2
Ingredient and chemical composition of the diets.

	Experimental diets				
	DS0	DS120	DS240	DS360	DS980 [b]
Ingredients, g/kg					
Dehydrated sainfoin [a]	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[c]	11	11	11	11	11
Chemical composition, g/kg as fed					
Trial 1 (using SC3, 3rd cut 2017)					
Organic matter	832	825	819	819	-
Crude Protein	157	157	154	155	-
aNDFom [d]	295	295	307	301	-
ADFom [d]	167	182	197	201	-
Lignins (sa) [e]	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 [d]	321	313	305	319	262
ADFom [d]	188	193	200	191	210
Lignins [e]	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

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

[d] aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; ADFom, acid detergent fibre expressed exclusive of residual ash; according to the sequential procedure of Van Soest method AFNOR, NF-18-122 + bisulfite

[e] lignin (sa), acid detergent lignin determined by solubilisation of cellulose with sulphuric acid; according to the sequential procedure of Van Soest method NF-EN-ISO 13906, direct lignin analysis.

Table 3
Feed intake and growth of rabbits [a] according to sainfoin (3rd or 1st cut) incorporation rate

	Experimental diets						Diet	Linear
	DS0	DS120	DS240	DS360	DS980	SEM [b]	Effect	Effect.[c]
<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

[a] Feed intake, weight gain and Feed conversion ratio were calculated from weaning to 70 days old for 12 rabbits per diet for trial 1/ for 9 rabbits per diet (except DS120 with 8 rabbits) in trial 2. Results are presented for 3 periods: from 35 to 49 days old, from 49 to 70 days old and for total trial period from 35 to 70 days old.
Feed intake = distributed feed– refused feed
Weight gain = highest Live Weight – lowest Live Weight (LW49 – LW35, LW70-LW49, LW70-LW35 for each period)
Feed conversion ratio = feed intake / weight gain
[b] SEM: standard error mean.
[c] linear effect was calculated without DS980 diet
Means having the same superscript letter did not significantly differ.

Table 4

Dietary nutrients digestibility [a] according to sainfoin (3rd or 1st cut) incorporation level

%	Experimental diets DS0	DS120	DS240	DS360	DS980	SEM [e]	Diet Effect.	Linear Effect [b]
Trial 1 (SC3 [d]) Digestibility coefficient (P1 [c] = 43-47 day old)								
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 [f]	30.9a	27.0b	23.4c	24.7bc	-	3.12	<0.001	<0.001
ADFom [f]	23.0a	19.8ab	20.8ab	18.0b	-	3.05	0.061	0.019
Trial 2 (SC1 [d]) Digestibility coefficient (P1 [c] = 43-47 day old)								
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 [f]	27.0	25.6	25.5	26.8	22.8	3.27	0.136	0.918
ADFom [f]	18.9a	17.3a	19.2a	18.2a	-	4.32	<0.001	0.955
Trial 2 (SC1 [d]) Digestibility coefficient (P2 [c] = 56-60 day old)								
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 [f]	25.6a	24.9a	22.4ab	23.6ab	17.9b	3.94	0.013	0.215
ADFom [f]	11.0a	14.2ab	12.7a	13.3a	-	4.79	0.005	0.534

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

[b] linear effect was calculated without DS980 diet

[c] P1= digestibility measured from 43 to 47 days old ; P2= digestibility measured from 56 to 60 days old.

[d] SC3: sainfoin 3rd cut/ SC1: Sainfoin 1st cut

[e] SEM= standard error mean

[f] aNDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; ADFom, acid detergent fibre expressed exclusive of residual ash; according to the sequential procedure of Van Soest method.

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

Table 5

Nitrogen balance [a] according to sainfoin (3rd or 1st cut) incorporation rate level

	Experimental diets							
	DS0	DS120	DS240	DS360	DS980	SEM [b]	Diet effect	Linear effect [d]
Trial 1 - Third cut sainfoin SC3								
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 [c] %	54.9a	47.7ab	35.7c	39.0bc	-	7.583	<0.001	<0.001
Trial 2 - First cut sainfoin SC1								
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 [c] %	35.6a	36.1a	41.1a	32.6a	12.3b	10.411	<0.001	0.930

[a] measured on rabbits from 43 to 47 days old

[b] SEM = standard error mean

[c] (N retained/N intake) x 100

[d] linear effect was calculated without DS980 diet

SC3= sainfoin 3rd cut/ SC1 = sainfoin 1st cut

Table 6

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

	Equation No.	R^2	RSD	P-value	N
Sainfoin, 3rd cut, SC3 [c] : nutrient prediction (A) [a]					
DOM [e] = -2.02x + 536	(1)	0.931	1.37	<0.001	32
DP [e] = -0.63x + 116	(2)	0.857	0.64	<0.001	32
DE [e] = -9.00x + 10.475	(3)	0.920	0.028	<0.001	32
Sainfoin, 1st cut, SC1 [c] : nutrient prediction (A) [a]					
DOM P1 [d] = 0.19x + 465	(4)	-0.032	2.65	0.329	32
DOM P2 [d] = -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
Sainfoin, 1st cut, SC1 : nutrient prediction (B) [b]					
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

x in equations corresponds to sainfoin SC3 or SC1 incorporation rate.

[a] (A) Equation of prediction with 4 levels of sainfoin incorporation.

[b] (B) Equation of prediction with 5 levels of incorporation (including 98%, "DS980")

[c] SC3 = dehydrated sainfoin 3rd cut / SC1 = dehydrated sainfoin 1st cut

[d] P1= measured from 43 to 47 days old ; P2= measured from 56 to 60 days old

[e] DOM: Digestible organic matter, DP: Digestible protein, DE: Digestible energy

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

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

Predicted values and their residual standard deviation (using equation of the table 5)
[a] (A) Equation prediction without DS980
[b] (B) Equation prediction with DS980
[c] S3C = dehydrated sainfoin 3rd cut 2017 / S1C = dehydrated sainfoin 1st cut 2018
[d] P1= measured from 43 to 47 days old ; P2= measured from 56 to 60 days old