Use of dehydrated sainfoin (Onobrychis viciifolia) in rabbit feeding. 2 - Effects of a high dietary incorporation on performance and health of does and growing rabbits, under a sub-optimal breeding commercial environment

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USE OF DEHYDRATED SAINFOIN (*ONOBRYCHIS VICIIFOLIA*) IN RABBIT FEEDING. 2 - EFFECTS OF A HIGH DIETARY INCORPORATION ON PERFORMANCE AND HEALTH OF DOES AND GROWING RABBITS, UNDER A SUB-OPTIMAL BREEDING COMMERCIAL ENVIRONMENT

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Abstract: The effects of a high dietary (26%) incorporation of dehydrated sainfoin (DS) (Perly cultivar) on the performance and health of reproductive does and growing rabbits were analysed in a sub-optimal professional breeding environment (presenting previous coccidiosis), and over two non-consecutive reproductive cycles (2 replicates). Performance and health of does and growing rabbits were compared for 2 groups of 194 does and associated litters, when fed isonutritive feeds containing either 0 or 26% DS (DS0 and DS26 groups). Dietary DS incorporation had no effect on doe live weight, fertility rate, mortality, culling rate and doe coccidia excretion levels (P>0.05). In replicate 1, kit growth before weaning was similar among the two groups, but was 12% lower for the DS26 group in the 2[nd] replicate (significant interaction). Similarly, a significant interaction was detected between the effect of the diet and the replicate for the mortality rate of kits before weaning, i.e. a higher mortality was detected for DS26 (3.3 *vs.* 1.8%) in replicate 1, while in replicate 2 it was lower (2.1 *vs.* 4.4%). After weaning, the post-weaning growth rate was improved by 4% (P=0.02) for the DS26 group, while the mortality rate decreased (7.1 *vs.* 4.5%, P<0.001). Coccidia excretions of growing rabbits were not affected by dietary sainfoin or by replicates. A high incorporation of DS (26%) should be recommended after weaning to improve the performance of growing rabbits without impact on reproducing does.

Key Words: dehydrated sainfoin, performance, growing rabbits, reproducing does, health status, coccidiosis, mortality.

INTRODUCTION

In rabbit farming, digestive disorders around weaning occur frequently, and their incidence is reduced by supplying sufficient dietary fibres and particularly lignins (Gidenne *et al.*, 2020). Moreover, coccidiosis is one of the main constraints impairing the health status and performance of growing rabbit (Licois, 2004; Marlier *et al.*, 2003). Sainfoin (*Onobrychis viciifolia*) is a legume characterised by high fibre and lignin contents that could help reduce the incidence of digestive disorders. It also contains condensed tannins, which have antimicrobial effects (Mueller-Harvey *et al.*, 2019) and antiparasitic properties (Hoste *et al.*, 2015). The nutritive value of dehydrated sainfoin Perly cultivar (DS) was recently studied by Gayrard *et al.* (2021), who reported a relatively high level of digestible proteins and energy. Two recent studies on the effect of sainfoin in the feeding of growing rabbits have produced encouraging results in the control of parasite infestations (Legendre *et al.*, 2017, 2018). Thus, using sainfoin in feeds could improve health in growing rabbits.

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Sainfoin incorporation levels and their effects on performance were recently studied both in an experimental farm (Gayrard *et al.*, 2022) and on a commercial farm with a large number of rabbits and does (Gayrard *et al.*, 2023) and under optimal sanitary status. However, the effect of diets containing sainfoin on performance as well as its "health value" has never been studied at a high incorporation level and in a commercial farm with sub-optimal health status. Thus, the current study aimed at measuring the effect of a high DS incorporation rate (26%) in isonutritive diets on reproductive and growth performance and on health of a large number of does and growing rabbits, in a commercial farm that had several previous coccidiosis episodes (i.e. sub-optimal health status).

MATERIALS AND METHODS

The experiment was carried out on a French commercial farm (western part of France), in accordance with the French legislation on animal experimentation and ethics (decree N°2013-118), and researchers were authorised by the French Ministry of Agriculture to conduct experiments on living animals in this farm. Informed consent was obtained from the farmer to perform the study on his animals. The farm was chosen for this experiment because it was described as having a sub-optimal health status, as several coccidia infections had occurred for 6 mo, in contrast to a previous experiment done in another commercial farm described as providing an optimal breeding environment (Gayrard *et al.*, 2023) without specific pathological problems.

Dehydrated sainfoin and experimental feeds

Dehydrated sainfoin (DS) from a first cut of a Perly cultivar (harvested in May 2019) was provided in pelleted form by the company Multifolia (Viapres-le-Petit, France). After milling sainfoin, a total of four pelleted diets were manufactured: two formulated to cover reproducing does' requirements (R) and two adapted for fattening rabbits (F), in one batch from the company Arrivé-Bellanné (Nueil-les-Aubiers, France). R and F diets were prepared either without incorporating DS (R0 and F0) or with a 26% incorporation of DS (R26 and F26). Within feed type (R or F), diets were isonutritive for their main nutrients (protein, energy, fibres) and differed essentially in their DS incorporation rate (Table 1). Diets did not contain drugs or coccidiostatic supplementation.

Animals and experimental design

The experimental design was carried out on two replicates (Figure 1), corresponding to a first reproductive cycle (replicate 1). Rabbit does (Hycole® line) with a number of parturitions ranging from 1 to 10 were used (Table 2). This

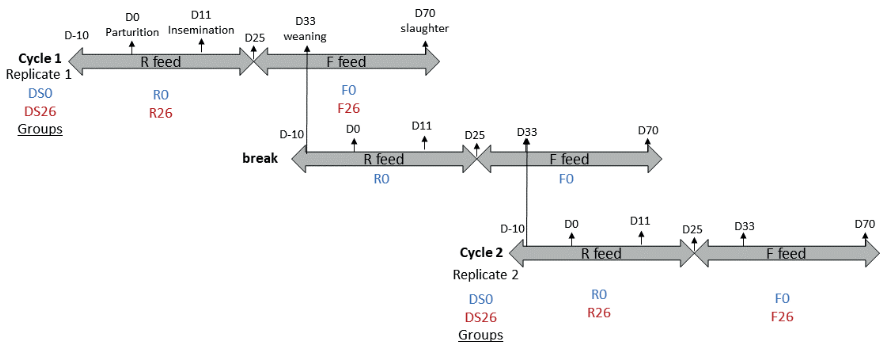

Figure 1: Experimental design. D0=day of parturition. Reproductive R feed were given to does and litters from D-10 to D25. From D25 to D33, doe and litter were fed Fattening F feeds. At replicate 1 (cycle 1), from D33 to D70, only growing rabbits remained on F feeds, while all does were fed R0 during the reproductive "break" cycle. At replicate 2 (cycle 3) the does were fed R0 and R26 feeds from the weaning of the previous batch (cycle 2). Doe measurements: live weight and feed intake measured at D-9, D4, D21, D28 and D33; faecal collection at D-7, D0, D8, D15, D21 and D34. Litter measurement: live weight measured at D4, D21, D28 and D33. Growing rabbit measurements: live weight measured at D33, D47 and D70; faecal collection at D34, D42, D49, D56, D70. Beginning replicate 1: 9/16/2019 (corresponds to Cycle 1). Beginning replicate 2: 12/9/2019 (corresponds to Cycle 3).

Table 1: Ingredient and chemical composition of diets and dehydrated sainfoin (DS).

	Diets of reproducing does		Diets of growing rabbits		Dehydrated sainfoin
Ingredients, g/kg	R0	R26	F0	F26	1st cut 2019
DS 1st cut	0	259	0	260	
Wheat bran	217	250	110	269	
Wheat	110	90	24	32	
Beet pulp	106	88	242	158	
Rapeseed meal	80	80	70	90	
Sunflower meal	190	76	162	36	
Dehydrated alfalfa meal	60	0	100	0	
Peas	56	58	30	0	
Corn	30	30	30	0	
Corn sprouts meal	30	30	20	0	
Sugar cane molasses	22	10	15	10	
Grape pulp	0	0	46	0	
Lapilest® [a]	58	0	114	132	
Minerals and amino acids[b]	41	29	37	13	
Chemical composition, g/kg as fed					
Dry matter	887	891	886	893	894
Organic matter	735	723	738	747	745
Crude ash	152	168	148	146	149
Crude protein	172	171	142	155	158
aNDFom	400	374	466	452	421
ADFom	158	167	227	227	294
Lignins	45	59	85	95	124
Digestible energy, kcal/kg[c]	2618	2621	2332	2335	-
Total phenols[d]	10.2	11.8	9.0	12.0	29.4
Total tannins[d]	<5	<5	<5	<5	17.4

[a]Lapilest® is a commercial product containing a mix of fibrous materials such as grape pulp, beet pulp, sunflower hulls, grape seeds, dehydrated apple pomace and grape seed meal.

[b]Minerals and amino acids provided through the premix, per diet: lysine (0.71%), methionine (0.23%), threonine (0.64%), tryptophan (0.22%), isoleucine (0.74%), valine (0.85%), arginine (0.68%), phosphorus (0.60%), calcium (1.2%), sodium (0.2%) and potassium (1.2%).

[c]Digestible energy of the feeds were calculated from DE concentration of each ingredient (Gidenne *et al.*, 2015; Gayrard *et al.*, 2021 for DS nutritive values).

[d]Analysed by Inovalys, Nantes, France, using the Folin-Ciocalteu method.

was followed by a reproductive cycle without involving the animals in the experiment. Consecutively, a third cycle was engaged that corresponded to replicate 2 of the trial. Overall, a total of 387 multiparous does (Hycole® line) were used for each replicate (Table 3), with a parity order ranging from 1 to 10 (Table 2). The first replicate started with insemination of does with Hyplus® semen. Taking the day of parturition as D0 (Figure 1), on D-10 does were allotted to two equal groups according to parity order and to the diet, DS0 (N=193) and DS26 (N=193) groups, freely fed a reproductive R feed (Table 1): R0 for DS0 group and R26 for DS26 group. On D4, litters were equalised to 10 kits which had access to the respective R feed. On D25, litters and does switched to a fattening F feed (Table 1): F0 for DS0 group and F26 for DS26 and were freely fed till weaning (D33). All animals (does and their litters) were housed in conventional cages (length: 68 cm - width: 62 cm - height: 48 cm) and fed with an automatic feeding chain. At 25-d old (D25), litters and does switched to a fattening "F" feed containing either 0 or 26% DS (feeds F0, F26: Table 1) and were freely fed till weaning (D33). At weaning, the litters were moved to a fattening unit and some growing rabbits were removed from each cage to equalise cages to 7 rabbits per fattening cage (length: 68 cm - width: 62 cm - height: 48 cm), choosing the animals removed in such a way as to alter the average live weight as little as possible. Growing rabbits were fed the same F feeds (according to their group) until 70 d old, but under a restriction programme: 65 g per rabbit at weaning, which was increased daily by 2 g. At weaning, does remained in their cage and all groups were fed with only R0 feed during the following reproductive cycle (without experiment). Then, for the

Table 2: Parturition number of females in DS0 and DS26 groups of both replicates.

	Number of females per parity in DS0 group		Number of females per parity in DS26 group	
Parity number	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	53	-	53	-
2	34	36	35	36
3	37	37	36	37
4	40	26	41	26
5	24	32	24	32
6	5	33	4	34
7	-	9	-	9
10	-	21	-	20
Total	193	194	193	194

DS0 group: females and kits fed R0 (without dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F0 (without dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F0 until slaughter. DS26 group: females and kits fed R26 (26% dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F26 (26% dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F26 until slaughter.

third reproductive cycle (replicate 2, 194 does), the experimental design of replicate 1 was applied to does and litters. During cycle 2, all does were fed an R0 feed, while fattening rabbit had the F0 feed. All animals were fed with an automatic feeding chain and had free access to water.

Mortality and performance measurements

In each replicate, mortality rates of does, kits and growing rabbits (checked daily) and fertility rate of does were measured on all animals, while doe and litter live weight were measured on a sample of 18 cages per group, at D-9, D4, D21, D28 and D33. Similarly, live weight of growing rabbits was measured for 18 cages of 7 rabbits per group, at D33, D47 and D70. Mortality was checked daily. As feed was distributed daily using an automated device, single estimates of feed intake were obtained for feeds F and R.

Faecal sampling and measurement of coccidia oocyst excretion

In each replicate, faeces from does (6 samplings at D-7, D0, D8, D15, D21 and D34) and growing rabbits (5 samplings at D34, D42, D49, D56, D70) were collected once every week using a wire net to retrieve faeces under the cages. For each day of collection (24 h collection), 6 faecal samples from each group were obtained from 6 different cages. Faeces were maintained at 4 °C during the 30-min transport to the laboratory (Reseau Cristal-RESALAB laboratory, Les Herbiers, France). The concentration of faecal oocysts (CFOs) was assessed using the modified McMaster technique (Gibbons *et al.*, 2004) and expressed as the number of oocysts per gram (OPG) of fresh faeces. A mean CFO value was calculated for each cage for the entire faecal collection period.

Chemical analyses of diets

INRAE laboratory (Toulouse, France) performed chemical analyses on DS and feeds. Dry matter contents were determined at 103 °C for 24 h and ash at 550 °C for 5 h. Dumas combustion method was used to analyse crude protein. Detergent “Van Soest” sequential procedure was used to analyse fibre fractions (aNDFom, ADFom and Lignins) (Van Soest *et al.*, 1991). Total phenols and tannins were analysed in diets and sainfoin pellets by the Folin-Ciocalteu method (Council of Europe, 2007; Wang *et al.*, 2014) (Inovalys, Nantes, France). In sainfoin, total tannins correspond almost totally to condensed tannins (Wang *et al.*, 2014; Mueller-Harvey *et al.*, 2019).

Statistical analyses

All data were analysed using R Core Team (2020). Shapiro-Wilk test was used to check normality. A two-factor analysis of variance was used to estimate the effects of the group (DS0 or DS26), the replicate (1 or 2) and their interaction on live weight of does and daily weight gain of litters and growing rabbits.

In the case of a significant interaction, a Tukey multiple mean comparison test was used to compare the 4 means between diet×groups. Chi-square test was used on mortality rates and fertility rates. Statistical signification was determined when *P*<0.05. A generalised linear model was used to analyse faecal oocyst concentration measures.

Table 3: Workforce per group and per replicate.

	Groups	
	DS0	DS26
Mortality		
Doe	194	194
Kits (10 kits per doe)	1940	1940
Growing rabbits (7 per cage)	1358	1358
Reproductive performance		
Fertility	194	194
Live weight		
Doe	18	18
Kits[a]	18	18
Growing rabbits[a]	18	18
Growing performance		
Kits (pre-weaning)[a]	18	18
Growing rabbits (post-weaning)[a]	18	18

[a]18 cages, containing either 10 kits per cage or 7 growing rabbits per cage, were weighed to calculate a mean live weight and mean growth rate. Does were individually weighed.
DS0 group: females and kits fed R0 (without dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F0 (without dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F0 until slaughter. DS26 group: females and kits fed R26 (26% dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F26 (26% dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F26 until slaughter.

RESULTS

Diets and sainfoin chemical composition

The composition of the DS (Perly cultivar, first cut, Table 1) was within the usual range, showing a crude protein level of 158 g/kg, while acid detergent fibre (ADFom) reached 294 g/kg. Within F or R feeds, the composition was isonutritive, with similar levels of crude proteins, fibres and digestible energy. For R diets, incorporation of dehydrated sainfoin was achieved mainly at the expense of sunflower meal, alfalfa and Lapilest®, while for F diets DS replaced mainly sunflower meal, alfalfa and beet pulp (Table 1). Following nutritional recommendations (Gidenne *et al.*, 2015), F diets had a higher content in ADFom (+40%) and in lignins (+73%) compared to R diets. Diets for growing rabbits were 13% and 11% poorer in crude protein and digestible energy, respectively, than R diets. With DS inclusion, phenols levels in feeds were near to 12 g/kg.

Reproductive performance

Does' feed intake was estimated to 365 g/d and 444 g/d, respectively, in replicate 1 and replicate 2 (unique measure for all does of both dietary treatments). Throughout the study, the live weight of does was not affected by the dietary DS incorporation (Table 4). Fertility rate of does increased from replicate 1 to 2 (+5%, *P*=0.044) and was similar between the 2 diets (average 88%). A significant interaction between the replicate and the diet (*P*=0.01, Figure 2) was observed for litter growth. In replicate 1, litter growth rate was similar in both groups (24.8 g/d, *P*=0.34), whereas in replicate 2, kits of DS26 group had a 12% lower growth rate (Figure 2, *P*<0.001).

Table 4: Reproductive performance of does according to replicate and dietary sainfoin incorporation group.

	Replicates (R)			Group (G)[1]			*P*-value		
	1	2	SEM	DS0	DS26	SEM	R	G	R×G
Doe live weight (g)[a]									
9 d before parturition	4553	4796	35.3	4690	4659	38.2	<0.001	0.68	0.81
4 d before parturition	4562	4855	38.1	4731	4686	41.9	<0.001	0.58	0.86
33 d before parturition	4608	4873	42.4	4812	4663	44.6	<0.01	0.09	0.90
Fertility rate, (%)[b]	86.0	90.7	1.17	88.6	88.1	1.17	0.044	0.83	0.13
Litter daily weight gain, g/d (4 to 33 d old)	24.8	29.0	0.3	28.0	25.8	0.4	<0.001	<0.01	0.01

[a]n=18 does per replicate and per group.
[b]Calculated on all does (97 does per diet).
SEM: standard error of the mean.
[1]DS0 group: females and kits fed R0 (without dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F0 (without dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F0 until slaughter. DS26 group: females and kits fed R26 (26% dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F26 (26% dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F26 until slaughter.

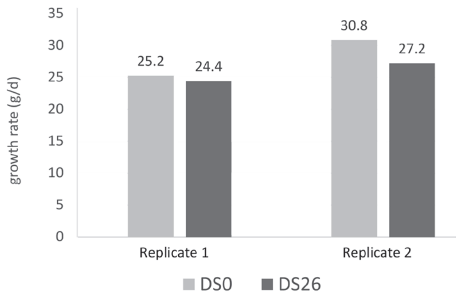

Figure 2: Litter growth rate during lactation (D2 to D33), according to groups and replicates. ■ DS0 group: females and kits fed R0 (without dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F0 (without dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F0 until slaughter. ■ DS26 group: females and kits fed R26 (26% dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F26 (26% dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F26 until slaughter.

Performance of growing rabbit after weaning.

Throughout the study, daily weight gain of growing rabbits was similar between replicates (mean 38.6 g/d, Table 5). A 4% increase in growth rate was obtained for DS26 group (P=0.02) compared to DS0 rabbits. The feed intake was restricted after weaning, and was fixed within a replicate. No feed refusal was detected and we calculated an average feed intake of 97 g/d in replicate 1 and 108 g/d in replicate 2. Thus, it was possible to calculate a feed conversion ratio for the 18 "sample" cages, which did not differ between the two groups (mean 2.65).

Mortality of does and growing rabbits

No interaction between replicate and diet was found for doe mortality and culling rate (Table 6). Doe mortality averaged 6.9% and was neither affected by the replicate nor by the diet. Doe mortality occurred mainly around parturition; outside the parturition period, it was due to digestive disorders. Similarly, doe culling rate averaged 9.8%, and was neither affected by replicate (P=0.33) nor by diet (P=0.75). The main causes of culling were abscesses, torticollis, mastitis and low fertility (old age, abortion).

Kit and growing rabbit mortality averaged 2.9% and 6.8%, respectively, (Table 7) and were associated with diarrhoea and digestive disorders (paresis, Epizootic Rabbit Enteropathy, non-specific enteritis). During lactation (D4-D33), a significant interaction between the replicate and the diet was detected (Figure 3; P<0.001) for kit mortality. In replicate 1, kits of DS26 group had a higher mortality rate: 3.3 *vs.* 1.8% (P=0.012), whereas in replicate 2, the kits of DS26 group showed a mortality rate halved compared to DS0, namely: 2.1 *vs.* 4.4% (P<0.001). After weaning, the mortality was stable (5.9%) from replicate 1 to replicate 2 (P=0.60), and was reduced by 2.6 units in the DS26 group (4.5 *vs.* 7.1%, P<0.001).

Oocyst excretion in does and growing rabbits

No interaction between the replicate and the diet was observed for the CFO in both doe and growing rabbits (P=0.47 and P=0.096, respectively, Table 8). Throughout the trial, CFO of both doe and growing rabbits was similar in replicate 1 and 2 and was not affected by the diet. Doe CFO was very low (on average 53 OPG) while it was much higher in growing rabbits (4501 OPG). As shown in Figure 4, the CFO of growing rabbits did not evolve significantly from weaning to slaughter due to a very high oocyst excretion variability (Standard error=3.969).

Table 5: Growth performance of rabbits from weaning to slaughter, according to replicate and dietary sainfoin incorporation group.

	Replicates (R)			Group (G)[1]			*P*-value		
	1	2	SEM	DS0	DS26	SEM	R	G	R×G
Daily weigh gain (g/d)	39.0	38.2	0.3	37.9	39.3	0.3	0.24	0.02	0.11
Feed conversion ratio	2.47	2.84		2.70	2.60				

[1]DS0 group: females and kits fed R0 (without dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F0 (without dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F0 until slaughter. DS26 group: females and kits fed R26 (26% dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F26 (26% dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F26 until slaughter.
Weight gain was calculated on a sample of 18 cages (7 rabbits per cage) per replicate and per group. After weaning, rabbits were restricted and no refusals were detected. Thus, feed conversion ratio was calculated as feed intake/daily weight gain, but no statistics were provided since there is null variability for feed intake.
SEM: standard error of the mean.

Table 6: Mortality and culling of does according to replicate and dietary sainfoin incorporation group.

	Replicate (R)		Group (G)[1]		*P*-value		
	1	2	DS0	DS26	R	G	R×G
Mortality	27/388 (7.0%)	26/388 (6.7%)	20/388 (5.2%)	33/388 (8.5%)	1	0.11	0.42
Culling [a]	43/388 (11.1%)	33/388 (8.5%)	36/388 (9.3%)	40/388 (10.3%)	0.33	0.75	0.23

[1]DS0 group: females and kits fed R0 (without dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F0 (without dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F0 until slaughter. DS26 group: females and kits fed R26 (26% dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F26 (26% dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F26 until slaughter.
[a]Causes of culling were: abscesses, torticollis, mastitis and low fertility (old age, abortion).

DISCUSSION

Compared to a sainfoin first cut harvested in 2018 (Gayrard *et al.*, 2021), the sainfoin first cut harvested in 2019 used in our trial had a much higher content in ADFom (208 *vs.* 294 g/kg as fed) and especially in lignins (64 *vs.* 124 g/kg as fed, Table 1), but a lower content in crude protein (200 *vs.* 158 g/kg as fed). However, compared to a “standard” sainfoin hay (Feedipedia), our DS had +14% of crude protein. Phenols and tannins levels of our sainfoin cut from 2019 were 18 and 32% (resp.) higher than that cut in 2018 (Gayrard *et al.*, 2021). The level of condensed tannins in sainfoin hay is higher than in our first cut 2019 (average 30 g/kg in sainfoin hay, Feedipedia). Logically, with the incorporation of sainfoin in feeds, phenol and tannin levels in diets containing 26% DS (R26 and F26) should be higher than in control feed. Indeed, F26 had 25% more phenols than F0 feed. However, R26 had only 16% more phenols than R0 feed. This lower difference could be due to the higher level of Lapilest® incorporated in R0 than in R26 feed, as Lapilest® contains grape pulp and seeds, which are rich in phenols and tannins.

Condensed tannins can reduce the palatability of feeds (Frutos *et al.*, 2004) due to tannins having an astringent taste. In the current study, the level of tannins was below the technical detection limit in all feeds, and it does not seem that the inclusion of DS impaired feed palatability, taking into account the intake and performance levels recorded for does or growing rabbits. Similarly, the palatability of a crude pellet of sainfoin was high, since during the first week after weaning the intake reached 79 g/d and was 10% higher than for a pelleted commercial feed (Tudela *et al.*, 2017).

A high incorporation of sainfoin in the feed of does had no major impact on reproductive performance. The fertility rate (mean 88%) was in a normal range, and close to the value registered in French rabbit farms (83%, Coutelet, 2015). However, fertility was higher than our previous results obtained in a similar experiment with does fed 26% sainfoin (78%, Gayrard *et al.*, 2022) but lower than in the case of does fed a moderate sainfoin level in an optimal environment (94%, Gayrard *et al.*, 2023).

During the lactation period, growth rate was similar between diets in replicate 1 but 12% lower in rabbits fed sainfoin in replicate 2. Gayrard *et al.* (2022) observed no effect on growth rate of kits fed a high sainfoin incorporation for three consecutive reproductive cycles, but in an optimal health environment. In turn, post-weaning growth rate was improved by the high level of sainfoin in feed and was associated with a better health status (reduced post-weaning mortality with sainfoin feed). Surprisingly, in optimal health conditions (<1% mortality), the high level of sainfoin in

Table 7: Mortality of kits and growing rabbits according to replicate and dietary sainfoin incorporation group.

	Replicates (R)		Group (G)[1]		*P*-value		
	1	2	DS0	DS26	R	G	R×Diets
4th d old to weaning (33 d old)	89/3492 (2.5%)	114/3492 (3.3%)	109/3492 (3.1%)	94/3492 (2.7%)	0.097	0.33	<0.001
D33-D70	139/2296 (6.1%)	129/2296 (5.6%)	164/2296 (7.1%)	104/2296 (4.5%)	0.60	<0.001	0.059

[1]DS0 group: females and kits fed R0 (without dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F0 (without dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F0 until slaughter. DS26 group: females and kits fed R26 (26% dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F26 (26% dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F26 until slaughter.

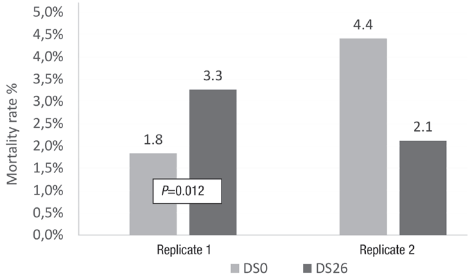

Figure 3: Mortality rate of kits during lactation (D4-D33), according to replicates and groups. DS0 group: females and kits fed R0 (without dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F0 (without dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F0 until slaughter. DS26 group: females and kits fed R26 (26% dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F26 (26% dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F26 until slaughter.

feed impaired growth rate (-2%) and FCR (+2%) (Gayrard *et al.*, 2022), whereas a moderate sainfoin incorporation (13%) enhanced growth and FCR as well as health status (Gayrard *et al.*, 2023). It seems that high sainfoin intake provides an advantage when used in sub-optimal sanitary conditions by improving digestive health of the fattening rabbit after weaning.

The incorporation of sainfoin had no effect on doe mortality (on average 7%) or on culling rate (on av. 10%), and both were close to the values recorded in French rabbit farms (2.4 and 13%, respectively, Coutelet, 2013). Similarly, the high dietary incorporation of sainfoin produced controversial results on the health status of litters. After weaning, a high sainfoin incorporation decreased growing rabbit's mortality. Similarly, weaned rabbits had a lower mortality rate when fed a tannin rich diet (12.2%) compared to a control diet (17.4%) (Maertens and Struklec, 2006). Tannins prevent or dissociate the colonisation of intestinal parasites, bacteria, protozoa, and viruses and thus may contribute to reduce the incidence of diarrhoea (Lewis, 2003). In a sub-optimal farming environment, the incorporation of sainfoin in the feed would improve health, whereas no effects were detectable in an optimal farming environment (Gayrard *et al.*, 2022).

Several species of coccidia (hosted mainly in the digestive tract) can infect specifically rabbits and, according to the infection level, they develop the associated disease. In our trial, does and mostly growing rabbits were infected by coccidia since oocysts were excreted in faeces. In most coccidia species, the maximum total number of oocysts per rabbit that can be excreted ranged between $1\text{-}5\times10^8$ (Pakandl, 2009), 10^7 and 10^5 times more than reproducing and growing rabbit's excretions levels observed here (10^3 and 10^1, respectively). In our study, a 26% sainfoin incorporation had no effect on coccidia excretions levels, as observed in Gayrard *et al.* (2022). Coccidia excretions were very low in does (53 OPG). On the contrary, growing rabbits' oocyst faecal concentration was high (4500 OPG) throughout the trial, on the threshold (4000-5000 OPG) for applying a coccidia prophylaxis (Coudert *et al.*, 2000). Some signs of the disease were visible, such as diarrhoea, although weight losses were not observed (Licois, 2004; Pakandl, 2009). It is likely that higher tannin intake is needed to detect an anticoccidial effect in growing rabbits. The antiparasitic properties of sainfoin (Hoste *et al*, 2015) are due to its condensed tannin contents (CTs) (Mueller-Harvey *et al.*, 2019; Wang *et al.*, 2014). CTs have anthelmintic properties, as already shown in sheep and goats (Hoste *et al.*, 2015), and are known to help limit coccidia infections in small ruminants (Saratsis *et al.*, 2012; Dykes *et al.*, 2019). Studies on

Table 8: Doe and growing rabbits' CFOs [a] according to replicate and dietary sainfoin incorporation group.

	Replicate (R)			Group (G)[1]			*P*-value		
Oocyst count[a]	1	2	RSD	DS0	DS26	RSD	R	G	R×Diets
Does[b]	67	38	20.04	39	67	20.07	0.46	0.45	0.47
Growing rabbits[c]	5254	3747	965.71	4205	4796	977.98	0.40	0.75	0.096

[a] Number of oocysts per gram of faeces (OPG number).
[b] Individual values, n=6 does (6 d of collection, 1 per week)/replicate and group.
[c] Collective values, n=6 cages (7 rabbits per cage, 5 d of collection, 1 per week)/replicate and group.
[1]DS0 group: females and kits fed R0 (without dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F0 (without dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F0 until slaughter. DS26 group: females and kits fed R26 (26% dehydrated sainfoin) from 10 d before parturition to 25th d of lactation and F26 (26% dehydrated sainfoin) from 25th d of lactation to weaning (33rd d of lactation); weaned rabbits fed F26 until slaughter.

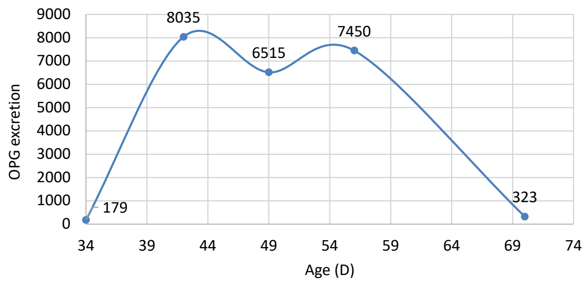

Figure 4: Oocyst excretion (number of oocysts per gram of faeces) in growing rabbits, according to age. Mean standard error=3969 OPG, for a statistical model with the age as single factor (regardless group or replicate).

growing rabbits fed a high percentage (40 and 34%) of DS reached similar conclusions for helminth (Legendre *et al.*, 2017) and coccidia control (Legendre *et al.*, 2018). In does, no anticoccidial effect of sainfoin was detected due to an already low level of infection of oocysts. As in growing rabbits, higher tannin intake may also be needed for detectable antiparasitic levels.

CONCLUSIONS

Incorporation of sainfoin in the feeds for growing rabbits was beneficial for the growth rate and health status. This did not affect the performance of the reproducing does or their health status. However, high sainfoin incorporation in the feed might impair the litter growth rate without health impact. Thus, incorporation of DS in feeds should be recommended in sub-optimal health environments to improve the digestive health and growth of the rabbits.

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