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USE OF DEHYDRATED SAINFOIN IN RABBIT FEEDING. EFFECTS OF A MODERATE DIETARY INCORPORATION ON PERFORMANCE AND HEALTH OF DOES AND GROWING RABBITS UNDER AN OPTIMAL FARMING ENVIRONMENT

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Abstract: The effects of a moderate incorporation of dehydrated sainfoin Pery cultivar (DS) in rabbit feeds on the performance and health of reproductive and growing rabbits were analysed over two consecutive reproductive cycles in a professional breeding environment. Two groups of 192 does and associated litters were fed isonutritive feeds containing either 0 or 13% dehydrated sainfoin (respectively C (control) vs. S ("sainfoin") groups) in replacement mainly of dehydrated alfalfa. Growing rabbit feeds C and S included 0 and 15.6% sainfoin, respectively. Doe live weight, number of live rabbits at birth and stillborn rate were not affected by dietary DS incorporation. In cycle 1, fertility rate was 10% higher for the S-group, but was similar among the groups in the 2nd cycle (significant interaction). Incorporation of DS had no impact on kit growth before weaning, but improved the post-weaning growth rate by 6% ($P<0.001$) and the feed conversion ratio by 7%. Dietary DS incorporation had no effect on doe mortality, which was very low ($<2\%$, $P=0.07$). Doe culling was half lower with sainfoin incorporation in cycle 1 (25% in group C vs. 12% in group S; $P<0.05$). In cycle 2, doe culling rate was low (3.2%) and similar among diets (significant interaction between diet and cycle effects). Pre-weaning mortality of kits was low and slightly higher for S-group (1.1 vs. 1.5%). Post-weaning mortality was also low and was reduced with sainfoin dietary incorporation (3.0 vs. 1.8%; $P<0.001$). A moderate incorporation of dehydrated sainfoin can be recommended for growing rabbits and for reproducing does feeds.

Key Words: dehydrated sainfoin, performance, growing rabbits, reproductive does, mortality.

INTRODUCTION

Sainfoin (*Onobrychis viciifolia*) is a legume containing phenols including condensed tannins. Tannins have beneficial effects depending on their concentration, animal species and feed composition (Nawab *et al.*, 2020), such as antimicrobial properties (Mueller-Harvey *et al.*, 2019), antiparasitic properties (Hoste *et al.*, 2015) or anti-bloating properties in ruminants. They also reduce greenhouse gas and improve utilisation of nitrogen in ruminants. Two studies on the role of sainfoin in growing rabbits to control parasitic infestations have produced encouraging results (Legendre *et al.*, 2017, 2018). Moreover, sainfoin contains high level of lignin, which helps reduce the incidence of digestive disorders around weaning (Gidenne *et al.*, 2010). Thus, using sainfoin could help to improve health in growing rabbits around weaning. However, tannins are also described as anti-nutritional factors, as they tend

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to decrease protein digestion. Nevertheless, digestion of dehydrated sainfoin was studied by Gayrard *et al.* (2021) who observed relatively high levels of digestible protein and energy. The effects of the incorporation of sainfoin at 13 and 26% in the feed on rabbit performance and health status were studied in a previous trial in an experimental farm (Gayrard *et al.*, 2022). Here, we aimed to analyse the effect of a moderate incorporation (13%) of sainfoin in iso-nutritive diets on performance and health of reproducing and growing rabbits for two consecutive reproductive cycles and in a professional breeding farm with a larger number of reproducing and growing rabbits than in Gayrard *et al.* (2022).

MATERIALS AND METHODS

The experiments were carried out in a French professional farm (Vendee department). The study was conducted 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 given by the farmer to study his animals.

Dehydrated sainfoin and experimental feeds

The Multifolia company (Viapres-le-Petit, France) provided pellets of dehydrated sainfoin (DS) from a first cut of a Peryly cultivar harvested in May 2019. Sainfoin pellets were used to formulate two reproductive “R” feeds: R0 containing 0% of DS and R13 containing 13% of DS. Similarly, fattening “F” feeds were formulated by the incorporation of DS at 0 and 15.6%, respectively, in F0 and F16 feeds (Table 1). R and F feeds were formulated to meet the requirements of reproductive does and growing rabbits, respectively. They were isonutritive for their main nutrients (protein, energy, fibre) and differed essentially in their DS incorporation rate. The four feeds were manufactured in one batch by Arrivé-Bellanné Company (Nueil-les-Aubières, France). Diets did not contain drugs or coccidiostatic supplementation.

Animals and experimental design

The trial was carried out on two consecutive reproductive cycles, on 384 multiparous does (Hycole® line, Hycole, Marcoing, France) inseminated with Hyplus® semen (Groupe Grimaud, Sèvremoine, France). The parity order of does varied between parity 4 and 11 (Table 2).

Ten days (D-10) before parturition (D0, Figure 1), does were allotted to two equal groups according to the diet: C-group (control, n=192) and S-group (sainfoin, n=192) and fed freely reproductive feeds, R0 for does from the C-group and R13 for does allotted to the S-group. At D4, litters were equalised to 10 kits and had access to the “R” feed until they were 25 d-old. All animals (does and their litters) were housed in conventional cages (68×62×48 cm, length×width×height). At 25 d of age (D25), litters and doe switched to a fattening “F” feed containing either 0 or 16% DS

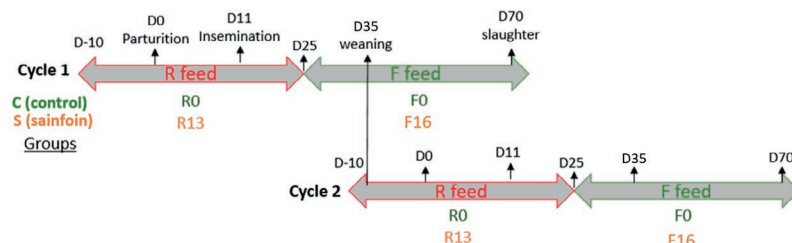


Figure 1: Experimental design. D=days of age of kits. Reproductive R feed were given to does and litter from D-10 to D25. From D25 to D32, doe and litter were fed fattening F feeds. From D35 to D70, only growing rabbits remained on F feed. At D35, does were moved to another unit to begin cycle 2 and were fed again with R feed. Doe measurements: live weight and feed intake measured at D-7, D4, D15, D21 and D35. Litter measurement: live weight measured at D4, D15, D21 and D35. Growing rabbits' measurements: live weight measured at D33, D48 and D70. Beginning of 1st cycle: 08/22/2019. Beginning of 2nd cycle: 10/04/2019.

Table 1: Ingredient and chemical composition of diets and of DS pellets

Ingredients, g/kg	Diets of reproducing does		Diets of growing rabbits		Dehydrated sainfoin first cut
	R0	R13	F0	F16	1st cut 2019
Dehydrated sainfoin, 1 st cut	0	130	0	156	
Wheat bran	217	249	110	223	
Soft wheat	110	114	24	50	
Beet pulp	106	94	242	196	
Rapeseed meal	80	76	70	74	
Sunflower meal	190	170	162	114	
Dehydrated alfalfa meal	60	0	100	0	
Peas	56	44	30	0	
Maize	30	30	30	0	
Corn sprouts meal	30	20	20	0	
Sugar cane molasses	22	10	15	10	
Grape pulp	0	0	46	20	
Lapilest® ^a	58	24	114	136	
Mineral and amino acids ^b	41	39	37	21	
Chemical composition, g/kg as fed					
Dry matter	887	890	886	891	894
Crude ash	152	148	148	151	149
Crude protein	172	180	142	154	158
aNDFom	400	370	466	460	421
ADFom	158	167	227	223	294
Lignin	45	57	85	87	124
Starch ^c	169	170	84	82	-
Digestible energy, kcal ^d	2618	2620	2332	2335	1912
Total phenols ^e	10.2	9.8	9.0	11.8	29.4
Total tannins ^e	<5	<5	<5	<5	17.4

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

^bMineral and amino acids provided per diet: lysine (0.71%), methionine (0.23%), threonine (0.64%), tryptophan (0.22%), isoleucine (0.74%), valine (0.85%), arginine (0.68%), phosphorus (0.28%), calcium (2.6%), sodium (0.03%) and potassium (2.42%).

^cAnalysed by Arrivé-Bellanné, France.

^dDigestible energy of the feeds were calculated from DE concentration of each ingredient. ^eAnalysed by Inovalys Nantes, Folin-Ciocalteu method.

(feeds F0, F16: Table 1) and were freely fed till weaning (D35). At weaning, the litters remained in their cage and two growing rabbits were removed from each cage to equalise cages to 8 rabbits per fattening cage. Removed animals were under or over the mean live weight. Growing rabbits were fed the same F feeds (according to their group) until 70 d of age, but under a restriction programme (using an automatic feeding machine): 80 g per rabbit at weaning that was daily increased by 2 g. At weaning, the does were moved to another breeding room (within the same building arranged for “all in-all out®”, system, Sanders, France), and fed again with R0 and R13 feeds to start their 2nd reproductive cycle (cycle 2, Figure 1).

Mortality and performance measurements

Mortality rates of does and growing rabbits and reproductive performance of does (fertility, live rabbits at birth and stillborn rates) were measured for each cycle and on all animals. The live weights of does and growing rabbits were measured on 36 cages per group (Table 3) for doe at D-7, D4, D15, D21 and D35) and for their litter at the same dates. Live weight of growing rabbits was measured for 36 cages (8 rabbits/cage) at 35, 48 and 70 d of age. After weaning, rabbits were restricted, and no refusals were detected. Thus, feed conversion ratio was calculated

Table 2: The parity order of females in the control (C) and sainfoin (DS) groups during cycle 1 and cycle 2.

Parity	The parity number distribution in each diet group	
	group C	group S
4	27	28
5	31	30
6	32	32
7	31	30
8	17	18
9	24	23
10	21	22
11	9	9

as feed intake/daily weight gain. Mortality was checked daily. The number of does used (384 reproductive does) was similar for each cycle, as dead does were replaced. If reproductive does belonging to the 36 cages selected for live weight measures died, they were replaced by a doe from the same dietary group and with the same reproductive parity. Feeds were distributed daily by an automated device for all animals, resulting in a single measure of the quantity of R and F diets distributed.

Chemical analyses of diets

Chemical analyses on DS and feeds were performed at INRAE. Dry matter (DM) content was determined at 103°C for 24 h and ash at 550°C for 5 h. Crude protein (CP) was analysed according to Dumas's combustion method (Buckee, 1994). Detergent "Van Soest" sequential

procedure was used to analyse fibre fractions (aNDFom, ADFom and Lignin) (Van Soest *et al.*, 1991) in diets and DS. Total phenols and tannins were analysed in diets and sainfoin pellets (DSp) using Folin-Ciocalteu method (Council of Europe, 2007; Makkar, 2000) (Inovalys, Nantes, France). For the 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 (diet×cycle and interaction) model was used to estimate the effect of the diet group (C-group, DS-group) and that of the cycle (cycle 1 or 2) for performance traits (growth) and reproductive traits (doe live weight, number of live kits). Tukey's multiple mean comparison test was used to compare the means between the different groups and cycles. A Chi-Square test was used on mortality rates and fertility rates. A generalised linear model with the two above mentioned factors was used to analyse stillborn rates.

Table 3: Number of replicates per treatment.

	Groups	
	group C	group S
Mortality		
Doe	192	192
Kits (10 kits per doe)	1920	1920
Growing rabbits (8 per litter from weaning)	1536	1536
Reproductive performance		
Fertility	192	192
Live rabbits at birth	192	192
Stillborn rate	192	192
Live weight		
Doe	36	36
Kits ^a	36	36
Growing rabbit ^a	36	36
Growth performance		
Kits (pre-weaning) ^a	36	36
Growing rabbits (post-weaning) ^a	36	36

^a36 cages, containing either 10 kits per cage, or 8 growing rabbits per cage, were weighed to calculate a mean live weight and mean growth rate. Does were individually weighed.

RESULTS

Diets and sainfoin chemical composition

Dehydrated sainfoin from a 1st cut of a Perly cultivar (Table 1) contained 158 g/kg CP and 294 g/kg acid detergent fibre (ADFom). R and F diets were formulated to fit the requirements of reproductive doe and growing rabbits, respectively. Within F or R feeds, the composition was isonutritive, with similar levels of CP, fibre and digestible energy. Indeed, R diets only differed by 3 and 1.2 percentage units in NDF and ADL, while F diets differed by 1.2 percentage units in CP. For R diets, incorporation of dehydrated sainfoin was achieved mainly at the expense of alfalfa, and Lapilest®; while for F diets, DS replaced mainly alfalfa, beet pulp and sunflower meal (Table 1). Following rabbit nutrition recommendations (Gidenne *et al.*, 2015), F diets had a higher content in ADFom (+17%) and in lignin (+41%), and a lower content of CP (-16%) and digestible energy (-11%) compared to R diets. With increasing sainfoin incorporation rates, phenol levels ranged from 9 to 12 g/kg in feeds.

Reproductive performance

Independently of group-diet, doe feed intake (and of litters before 25 d of age, considered small) was globally estimated to 317 and 402 g/d, respectively, in cycle 1 and cycle 2 (unique measure for all does of each group). The higher feed intake during cycle 2 could be explained by the colder weather. Indeed, doe feed intake was measured from August to October and from October to November, respectively, for cycle 1 and cycle 2. Doe mean live weight at D35 increased by 4%, from cycle 1 to cycle 2 (Table 4; $P<0.001$), suggesting a good body state. Throughout the study, mean live weight of does was not affected by the dietary DS incorporation rate. A significant interaction between the cycles and the diet was detected for the fertility rate. Does of the S-group had a similar fertility rate from cycle 1 to cycle 2 (95% and 93% respectively, Figure 2). The fertility rate of the control group (86%) was lower than in the S-group (95%) at cycle 1 only ($P=0.002$). Stillborn rate and the number of live kits at birth were similar between groups (6.5% and 10 live kits per doe). A significant interaction between cycle and diet (Figure 3) was observed for litter growth. In cycle 1, both groups had a similar growth rate (28.7 g/d; $P=0.28$), whereas in cycle 2, the C-group had a 5% lower growth rate compared to the S-group (26.8 vs. 28.2 g/d; $P=0.024$).

Performance of growing rabbits after weaning.

No interaction between cycle and diet was found for growing rabbit performance (Table 5). Daily weight gain and feed conversion ratio were higher in the 2nd cycle (respectively +9 and -5%; $P<0.001$). A 6% higher growth rate ($P<0.001$) was found for the S-group compared to the C-group (Table 5), and accordingly the feed conversion ratio was improved by 7% for the S-group.

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

	Cycles			Groups			P-value		
	1	2	RSD	C	S	RSD	Cycle	groups	Cycle×groups
Doe live weight (g) ^a	4818	4881	26.8	4821	4878	26.8	0.24	0.29	0.71
D4	4710	4849	28.8	4753	4806	29.3	0.016	0.37	0.61
D35	4838	5060	28.7	4891	5005	29.8	<0.001	0.055	0.96
Fertility rate, (%) ^b	90.6	94.0	0.96	90.6	94.0	0.96	0.08	0.08	<0.01
Live rabbits at birth ^b	9.8	10.3	0.13	10.0	10.1	0.13	0.054	0.83	0.58
Stillborn rate, (%) ^b	8.21	4.72	0.58	6.10	6.82	0.59	<0.01	0.54	0.24
Litter daily weight gain, (g/d) (D4-D35)	28.6	27.5	0.2	27.8	28.3	0.2	<0.01	0.26	0.01

^aN=36 does/diet. D-7: 7 d before parturition; D4: 4 d after parturition; D35: 35 d after parturition.

^bcalculated on all does (192 does per diet).

RSD: Residual standard deviation. Means within a row with different superscript letters differ ($P<0.05$).

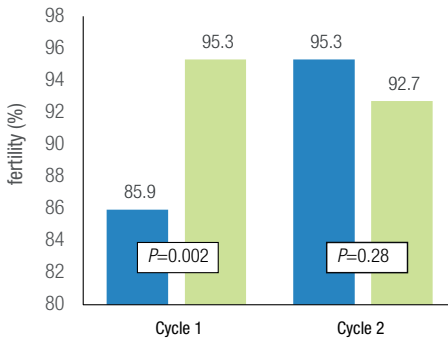


Figure 2: Doe fertility rate by cycle and groups. ■ C-group, ■ S-group.

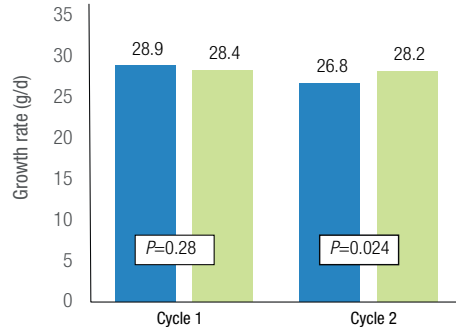


Figure 3: Litter growth rate before weaning (D2 to D33), according to groups in cycles 1 and 2. ■ C-group, ■ S-group.

Mortality of does and growing rabbits

Doe mortality was globally low (2.0%) and was not affected by cycle or diet (Table 6). Mortality occurred near parturition and between artificial insemination (D11) and D21. A significant interaction between cycle and diet was detected ($P < 0.01$; Table 6) for the culling rate, as it was very low in the second cycle. In cycle 1, the culling rate was half lower in the S-group (12 vs. 25%; $P < 0.01$; Figure 4), whereas in cycle 2, the culling rate of both groups did not differ (meanly 3.1%; $P = 0.77$). The apparent causes of culling rate were abscesses, torticollis, mastitis and lower prolificity (old age, abortion).

Mortality of kits and growing rabbits was associated with diarrhoea and digestive disorders (paresis, enteropathy, non-specific enteritis). Before weaning (D4-D35), kit mortality was very low ($< 1.7\%$; Table 7) and higher in cycle 1 (1.7 vs. 0.9%; $P < 0.001$). Kits fed the control diet had a slightly lower mortality rate than kits of the S-group (1.1 vs. 1.5%; $P < 0.001$). For growing rabbits, a significant interaction between cycle and diet effect was detected (Figure 5; $P < 0.01$). During cycle 1, the mortality rate of S-group was half lower (2.8 vs. 5.0%; $P = 0.004$), whereas in cycle 2, mortality rate was very low and similar between groups (meanly 0.9%; $P = 0.85$).

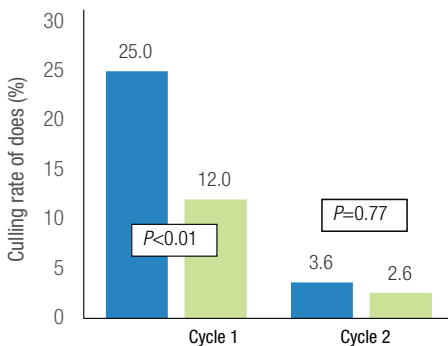


Figure 4: Culling rate of does in cycle 1 and in cycle 2, by groups. ■ C-group, ■ S-group.

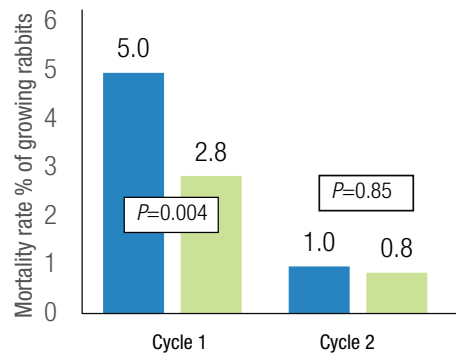


Figure 5: Mortality rate of growing rabbits from weaning (D32) to slaughter (D70), according to groups and cycles. C-group: group fed a F0 control diet without incorporation of DS pellets; S-group: group fed a F16 diet containing 13% DS pellets. ■ C-group, ■ S-group.

Table 5: Performance of growing rabbits after weaning, according to cycle and dietary sainfoin incorporation.

D36-D70	Cycle			Groups			P levels		
	1	2	RSD	C	S	RSD	Cycle	groups	Cycle×groups
Live weight at D36	1033	975	76.5	999	1010	81.6	<0.001	0.42	0.0496
Live weight at D70	2406	2314	133.5	2312	2409	132.4	<0.001	<0.001	0.91
Daily weigh gain, g/d	38.1	41.9	0.3	38.7	41.2	0.3	<0.001	<0.001	0.27
Feed conversion ratio	2.58	2.43		2.59	2.42		-	-	-

Weight gain was calculated on a sample of 36 cages (8 rabbits per cage). After weaning, rabbits were restricted, and no refusals were detected. Thus, feed conversion ratio was calculated as feed intake/daily weight gain.

RSD: Residual standard deviation.

DISCUSSION

Compared to sainfoin (Perly cultivar) from a 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 lignin (64 vs. 124 g/kg), and a lower content in CP (200 vs. 158 g/kg). However, compared to a “standard” sainfoin hay (Feedipedia), our sainfoin 1st cut had +14% of CP. Phenol and tannin levels of our sainfoin were higher than for a first cut from 2018 (Gayrard *et al.*, 2021). The level of condensed tannins in sainfoin hay was higher than that of our 1st cut 2019 (30 g/kg in sainfoin hay, Feedipedia). Logically, with the incorporation of sainfoin in feeds, phenol levels should be higher than in control feed. Indeed, F16 had 24% more phenols than F0 feed. However, R0 and R13 feeds have similar phenol contents. This could be due to the higher level of Lapilest® incorporated in R0 than in R13 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. However, despite a high tannin level, sainfoin seemed an appetising feed for rabbits, given the good intake level 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 moderate incorporation of sainfoin in the feed of reproductive does had no impact on reproductive performance of does, as observed by Gayrard *et al.* (2022). The fertility rate (meanly 92%) was over the mean value registered in French rabbit farms (83%, Coutelet, 2015) and 15% over the fertility rate found by Gayrard *et al.* (2022). In the sainfoin group, the fertility rate seemed more stable over time when comparing our two cycles. A moderate incorporation of sainfoin had no effect on the number of live rabbits at birth (10 on average) and ranged within the referenced data for French rabbit farms (Theau-Clément *et al.*, 2016). Min *et al.* (2001) observed a higher lambing percentage (number of lambs born per ewe lambing, +20%) in ewes fed *Lotus corniculatus* (rich in tannins). Here, the incorporation level of sainfoin was moderate and may have been too low to show an increase in the number live rabbits at birth. Indeed, tannins increase ovulation rates (Attia *et al.*, 2016), resulting in more kits born. Similarly, the stillborn rate (6.5%) was not affected by the incorporation of sainfoin in the diet and was half lower than in Gayrard *et al.* (2022), and similar to the results of Elmaghraby and Elkholya (2010), who observed a stillborn rate of 7%.

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

	Cycles		Groups		P levels		
	1	2	C	S	Cycle	Groups	Cycle×Groups
Mortality	8/384 (2.1%)	7/384 (1.8%)	4/384 (1.0%)	11/384 (2.9%)	0.79	0.07	0.79
Culling ^a	71/384 (18.5%)	12/384 (3.1%)	55/384 (14.3%)	28/384 (7.3%)	<0.01	<0.001	<0.01

^aCauses of culling were: torticollis, abscess, bad mother, no milk, high parity, infertility.

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

	Cycles		Groups		P levels		
	1	2	C	S	Cycle	Diets	Cycle×Diet
Birth-weaning ^a	58/3456 (1.7%)	34/3708 (0.9%)	38/3582 (1.1%)	54/3582 (1.5%)	<0.001	<0.001	0.31
Weaning-70d old ^b	121/3108 (3.9%)	28/3108 (0.9%)	92/3108 (3.0%)	57/3108 (1.8%)	<0.01	<0.001	<0.01

C-group: group fed a R0^a or a F0^b control diet without incorporation of DS pellets;

S-group: group fed a R13^a or a F16^b diet with 13% incorporation of DS pellets in the feed.

Before weaning, litter growth rate was not affected by sainfoin incorporation. However, as observed for doe fertility, sainfoin intake seems to stabilise growth rate over time, in contrast to what occurs in rabbits fed the control diet. Moreover, post-weaning growth was improved by the moderate incorporation of sainfoin in feed, whereas on the contrary, Gayrard *et al.* (2022), observed no improvement of performance when animals were fed a moderate sainfoin incorporation. This could be due to a better digestion of nutrients in the feed due to a better health status (lower mortality rate) of growing rabbits fed moderate sainfoin (mostly in cycle 1). Indeed, in Gayrard *et al.* (2022) both groups (control and with DS) had a similar health status.

The incorporation of sainfoin had no effect on doe mortality (2.0%) which was very low. Besides, when the culling rate was high (cycle 1), a moderate sainfoin incorporation can sharply reduce this rate. For a low culling rate situation (such as in cycle 2) adding sainfoin in the feed had no supplemental beneficial effect. Before weaning, kits mortality rate was low, but slightly increased with sainfoin incorporation (+0.4%). After weaning, when the health status is not optimal (such in cycle 1), sainfoin incorporation favoured the health status with a lower mortality rate. Similarly, post-weaned rabbits had a lower mortality rate when fed a tannin rich diet (12%) compared to control diet (17%, Maertens and Struklec, 2006). Tannins prevent or dissociate the colonisation of intestinal parasites, bacteria, protozoa and viruses, and thus may help to reduce the incidence of diarrhoea (Lewis, 2003).

CONCLUSIONS

A moderate dietary incorporation of sainfoin for reproducing does did not affect most of their reproductive performance, but could maintain a low culling rate. In addition, incorporating sainfoin in feeds for the growing rabbit improved growth and health status. However, our first results indicated that sainfoin incorporation in feeds for kits seemed not to be beneficial. Thus, a moderate incorporation of DS in feeds should preferably be recommended for growing rabbits after weaning and for reproducing does.

A future study will measure, still under a professional breeding environment, the impact of a high incorporation rate of sainfoin on reproductive and growing performance of the rabbit, and thus to test the potential favourable effect of sainfoin under a sub-optimal sanitary environment of the farm.

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