



# World Rabbit Science

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JOURNAL OF THE WORLD RABBIT SCIENCE ASSOCIATION

**April - June 2022**

**World Rabbit Sci. 30 (2) 107 - 186**



World Rabbit Science  
Association



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POLITÈCNICA  
DE VALÈNCIA



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## FEED INCORPORATION OF DEHYDRATED SAINFOIN: EFFECTS ON HEALTH AND PERFORMANCES OF DOES AND GROWING RABBITS

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**Abstract:** The performance and health of does and growing rabbits were compared over three consecutive reproductive cycles for three groups of 20 nulliparous does and their litters (DS0, DS13, DS26) fed isonutritive feeds containing 0, 13 or 26% dehydrated sainfoin (DS, Perly cultivar). Feed intake, live weight and fertility of does were not affected by DS feed incorporation. The number of live kits at birth increased linearly with increasing DS incorporation (+1.5 from DS0 to DS26,  $P=0.042$ ) and the stillborn rate tended to linearly decrease in groups fed DS (16.6 vs. 10.4%,  $P=0.086$ ). Increasing the level of DS in feeds had no impact on the growth of the kits before weaning, but led to a linear reduction in the post-weaning growth rate ( $P<0.01$ , -2 for 26% DS), whereas the feed conversion ratio increased linearly with DS incorporation ( $P<0.01$ , 2.91 vs. 2.98, resp. for DS0 and DS26). No effect of DS feed incorporation was detectable on doe and kit mortality rates. Excretion of coccidia by both does and growing rabbits was not affected by DS incorporation. For 70 d old rabbits, the levels of immunoglobulins A and G and of white blood cells were not significantly different between groups and high levels of IgG (average: 8.1 mg/mL) were recorded, suggesting a coccidia infestation. Overall doe mortality remained under 5% and was not affected by the reproductive cycle ( $P=0.24$ ). The stillborn rate decreased from 18 to 6%, ( $P<0.01$ ) from cycle 1 to 2, and the number of live rabbits at birth increased from 8.0 to 10.7 ( $P<0.01$ ). Kit mortality remained low before weaning (under 2.5%), and very low after weaning (<1%). Excretion of coccidia by does decreased from cycle 1 to cycle 3, whereas excretions by growing rabbits remained stable.

**Key Words:** dehydrated sainfoin, rabbit feeding, growth, reproductive performance, coccidiosis, immunity.

## INTRODUCTION

To cover the nutritional needs of rabbits, the feed must contain not only energy and protein, but also fibres, particularly lignins, which are required for their digestive health (Gidenne *et al.*, 2020). Sainfoin (*Onobrychis viciifolia*) is a legume traditionally planted in pasture, and used as hay or silage for livestock (Aufrère *et al.*, 2013). Sainfoin seems a good candidate for rabbit feed, as it contains relatively high levels of digestible proteins and energy (Gayrard *et al.*, 2021), as well as a high concentration of fibre and especially of lignins (Legendre *et al.*, 2018). Today, sainfoin is available as dehydrated pellets (Multifolia company, Perly cultivar) and its nutritive value for growing rabbits was recently studied according to the cut (Gayrard *et al.*, 2021). However, no information is available on the optimal incorporation level of dehydrated sainfoin (DS) pellets in balanced feed and its effect on the performance and health of growing rabbits and does.

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Moreover, sainfoin could be an alternative to the alfalfa classically used as a fibre source. Sainfoin also contains phenols, including condensed tannins (CTs), which could contribute to the control of digestive parasitism and thus help reduce the use of anthelmintic and anticoccidials (Hoste *et al.*, 2015). Two studies on the role of DS (Perly cultivar) in controlling parasitic infestations in growing rabbits have shown encouraging results (Legendre *et al.*, 2017, 2018). Tannins also have antimicrobial properties and could influence immune responses (Mueller-Harvey *et al.*, 2019).

Thus, our study aimed to assess the effect of the DS incorporation rate (0, 13, 26%) in balanced diets on performance and health of does and growing rabbits over three consecutive reproductive cycles.

## MATERIALS AND METHODS

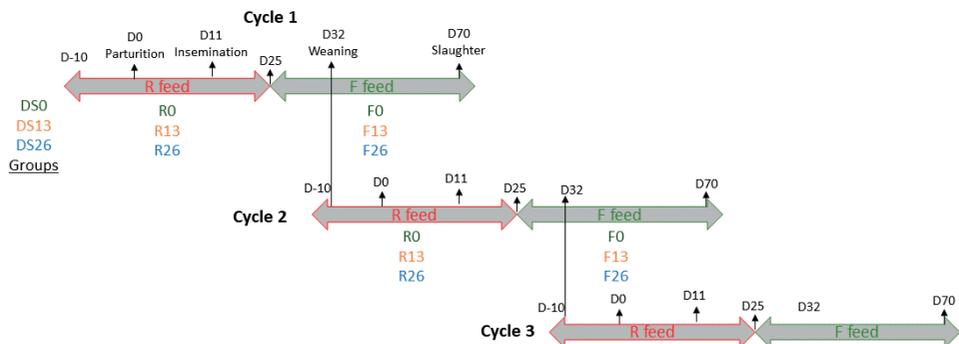
The experiments were conducted at the INRAE experimental farm (Pectoul, INRAE, Castanet-Tolosan, France) in accordance with French legislation on animal experimentation and ethics (authorisation number: SSA\_2019\_008), and the researchers were authorised by the French Ministry of Agriculture to conduct experiments on living animals.

### Dehydrated sainfoin and experimental feed

DS was obtained from a first cut (Perly cultivar) harvested in May 2019, and provided as pellets by Multifolia company (Viapres-le-Petit, France). Three reproductive "R" diets and three fattening "F" diets (Table 1) were formulated to meet the requirements of reproductive does and growing rabbits, respectively (Gidenne *et al.*, 2015). Within a category (R or F), diets differed mainly in their DS incorporation rate (0, 13 or 26%, in R0 and F0, R13 and F13 or R26 and F26, respectively) but were isonutritive (crude protein, fibres and digestible energy). The six feeds were manufactured in one batch by Arrivé-Bellanné Company (Nueil-les-Aubières, France). The diets contained no drugs or coccidiostat supplementation.

### Animals and experimental design

The trial was carried out in three consecutive reproductive cycles (Figure 1), starting with 70 nulliparous does (genotype INRAE 0171) of similar weight at the start of the first cycle (insemination 1 at 20 weeks old). All the



**Figure 1:** Experimental design. R feed was given to does and their litters from D-10 to D25. Does and litters were fed F feed from D25 to D32. From D32 to D70, growing rabbits were kept in fattening pens and fed F feed. At D32, does began cycle 2 and were again fed R feed. Measurements made of does: live weight and feed intake measured at D-10, D4, D14, D24 and D32; faecal samples collected at D-5, D2, D9, D16, D23 and D30. Measurement of litters: live weight measured at D4, D14, D24 and D32. Measurements of growing rabbits: live weight measured at D32, D49 and D70; intake refusal checked daily; faecal collections at D38, D45, D52, D59, D66. Cycle 1 started on 09/27/2019 (D-10); cycle 2 started on 11/08/2019; cycle 3 started on 12/20/2019. DS incorporation rate in feed (0, 13 or 26%, in R0 and F0, R13 and F13 or R26 and F26, respectively) .

**Table 1:** Ingredients and chemical composition of the diets and of dehydrated sainfoin.

Ingredients (g/kg)	Diets of reproducing does			Diets of growing rabbits			Dehydrated sainfoin 1st cut 2019
	R0	R13	R26	F0	F13	F26	
Dehydrated sainfoin	0	130	259	0	156	260	
Wheat bran	217	249	250	110	223	269	
Soft wheat	110	114	90	24	50	32	
Beet pulp	106	94	88	242	196	158	
Rapeseed meal	80	76	80	70	74	90	
Sunflower meal	190	170	76	162	114	36	
Alfalfa meal	60	0	0	100	0	0	
Peas	56	44	58	30	0	0	
Maize	30	30	30	30	0	0	
Corn sprouts	30	20	30	20	0	0	
Treacle	22	10	10	15	10	10	
Grapeseed pulp	0	0	0	46	20	0	
Lapilest <sup>a</sup>	58	24	0	114	136	132	
Mineral and amino acids <sup>b</sup>	41	39	29	37	21	13	
Chemical composition (g/kg as fed)							
Dry matter	887	890	891	886	891	893	894
Organic matter	735	742	723	738	740	747	745
Crude ash	152	148	168	148	151	146	149
Crude protein	172	180	171	142	154	155	158
Neutral detergent fibre	400	370	374	466	460	452	421
Acid detergent fibre	158	167	167	227	223	227	294
Lignins	45	57	59	85	87	95	124
Digestible energy, kcal <sup>c</sup>	2618	2620	2621	2332	2335	2335	–
Total phenols <sup>d</sup>	10.2	9.8	11.8	9.0	11.8	12.0	29.4
Total tannins <sup>d</sup>	<5	<5	<5	<5	<5	<5	17.4

<sup>a</sup>Lapilest® 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.

<sup>b</sup>Mineral 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%).

<sup>c</sup>Digestible energy in each feed was calculated from the concentration of digestible energy in each ingredient.

<sup>d</sup>Analysed by Inovalys, Nantes, France.

animals (does and their litters) were housed in wire net cages (length: 68 cm, width: 62 cm, height: 48 cm). Ten extra does were kept for cycle 2 or 3 to replace does that died or were infertile. Ten days (D-10) before parturition (D0, Figure 1), 60 does were allotted to three equal groups based on diets: DS0 (control group), DS13 and DS26, with *ad libitum* access respectively to the feeds R0, R13 and R26. Litters were equalised at 8 kits at D4 and had access to “R” feed until they were 25 d old. At 25 d old (D25), litters and does of groups DS0, DS13 and DS26 fed R0, R13 and R26 respectively were switched to a fattening “F” feed F0, F13 and F26, with *ad libitum* access until weaning (D32). At weaning, the three groups of litters were moved to a fattening unit. They were housed collectively (6 rabbits per cage: length: 68 cm, width: 62 cm, height: 48 cm) and were fed the same F feeds (according to their group) until 70 d old, but under a restriction programme: 90 g per rabbit during the week after weaning, then increased feed distribution at a rate of +20 g/wk. At weaning, the does remained in their cage and were again fed R feed (with respect to their group) to start their 2nd cycle (Figure 1). The third cycle was performed in the same conditions. At the end of the third cycle, 20 growing rabbits per group (DS0, DS13 and DS26) were slaughtered at 70 d old.

### **Mortality and performance measurements**

Mortality was checked daily. When a doe died, her entire litter was removed from the experiment. In each cycle, the live weight of each doe was recorded at D-10, D4, D14, D24 and D32, while the litters (8 kits/doe=160 kits/group) were weighed at D4, D14 and D24. Feed intake of doe and litters was checked on the same dates as

doe live weight. The live weight of each growing rabbit (120 rabbits/group) was recorded at weaning (D32), and at 49 and 70 d old. The feed intake of the growing rabbit was checked weekly in each cage. Reproductive performances including insemination rate, the number of kits alive at birth, and the number of stillborn were measured at each cycle.

### ***Faecal sampling for coccidia concentration measurement***

Faeces were collected once a week from a 1 cm mesh attached under the cages with 6 collections (at D-5, D2, D9, D16, D23 and D30) from cages containing does, and 5 collections (at D38, D45, D52, D59, D66) from cages containing growing rabbits. Faeces were collected from does (1 doe per cage=1 sample of faeces per doe) and for 10 does per group. Faeces from growing rabbits were collected from 10 cages, each cage containing 6 growing rabbits (1 sample corresponded to faeces collected from 6 growing rabbits). Faeces were stored at 4°C for one day prior to oocyst counting at the Toulouse National Veterinary School (ENVT, France). The concentration of faecal oocysts (CFO) was assessed using the modified McMaster technique (Gibbons *et al.*, 2005) and is 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 (5 collections).

### ***Sampling of blood and digesta from growing rabbits***

Twenty healthy growing rabbits of similar weight per group (DS0, DS13 and DS26) in cycle 3 were sacrificed at 70 d old by electrical stunning followed by exsanguination. Blood was collected from the aorta in two heparinised tubes (Vacurette, 9ml NH Sodium Heparin) and immediately stored on ice. One tube was used to measure total white blood cell counts and the diversity of white blood cell types with a haemocytometer (MS9-5 VET, Malet Schloesing, Osny, France). The second tube was centrifuged, and plasmas were stored at -20°C until analysis of immunoglobulins. Faeces were collected from the rectum of euthanised rabbits and stored at -20°C until analysis. Caeca were isolated, weighed and sampled and then stored at -20°C for analysis.

### ***Analysis of immunoglobulins: extraction and ELISA measurements***

ELISA was used to quantify IgG in plasma and the concentration of IgA in caecum digesta and in faeces. In a preliminary step, caecal content was diluted to 50 mg/mL in TBS buffer while a small amount of faecal matter (200-600 mg) was diluted at 25 g/L in PBS buffer. After thorough shaking, the samples were centrifuged at 3000 g for 10 min at 4°C. The supernatants were collected and stored at -20°C until analysis. Total IgG or IgA contents were determined in triplicate by sandwich ELISA in 96-well plates coated with specific polyclonal goat anti-rabbit IgG or IgA antibody (Bethyl Laboratories, Montgomery, Texas, USA). Appropriate sample dilutions were performed: from 1:64 000 to 1:128 000 for IgG, 1:10 for caecal IgA and from 1:7 to 1:50 for faecal IgA determination. Plates were read at 450 nm using a GloMax® Discover plate reader (Promega, Madison, WI) to construct the standard curve based on a four-parameter model. IgG in plasma was quantified using a reference IgG serum (Bethyl Laboratories, Montgomery, Texas, USA). Regarding caecal and faecal IgA, respectively sixty caecal and faecal samples were pooled to build a reference sample to construct standard curves. Relative IgA concentrations were then interpolated from this curve. Only results with intra-assay coefficients of variation <10% were kept (Wong *et al.*, 2004).

### ***Chemical analyses of diets***

Chemical analyses of dehydrated sainfoin and of the different feeds were performed at INRAE. Dry matter (DM) content was determined after drying at 103°C for 24 h and ash content after heating at 550°C for 5 h. Crude proteins were analysed using the Dumas combustion method. The "Van Soest" detergent procedure was used to analyse fibre fractions (aNDFom, ADfom and lignins) (Van Soest *et al.*, 1991) in the feed and in the dehydrated sainfoin. Total phenols and tannins were analysed in the feed and in dehydrated sainfoin using the Folin-Ciocalteu method (Council of Europe, 2007; Makkar, 2000) (Inovalys, Nantes, France). In sainfoin, total tannins correspond almost entirely to condensed tannins (Wang *et al.*, 2014; Mueller-Harvey *et al.*, 2019).

### **Statistical analyses**

Data were first screened to detect outliers. No outlier was found for animal growth and feed intake. All data were analysed using R (R Core Team, 2020). A Shapiro-Wilk test was used to check normality. A two-factor (group and cycle, and interaction) variance analysis model was used to estimate the effect of the group (DS0, DS13, DS26) and that of the cycle (cycle 1, 2 or 3) on performance (live weight and growth) and reproductive traits (number of live rabbits). For each cycle, a single factor variance analysis was used to estimate the effect of diet on health traits (proportion of blood cells and concentrations of immunoglobulins). A Tukey's multiple means comparison test was used to compare means between the different groups and cycles. A Chi-Square test was used on mortality and fertility rates. A generalised linear model with the two above mentioned factors was used to analyse stillborn rates and faecal oocyst concentrations. A linear regression model was used to check the effect of the DS incorporation rate (0, 13, 26%) on the number of live rabbits at birth, on the post-weaning daily weight gain, and on the feed conversion ratio. Finally, another linear regression model was used to check the evolution of oocyst excretion in growing rabbits from D38 to D66.

## **RESULTS**

### **Diets and sainfoin chemical composition**

We used a Perly sainfoin from a first cut harvested in 2019 (crude protein=158 g/kg, acid detergent fibre=294 g/kg, crude fibre=17.4 g/kg) for F and R diets. The R and F diets were formulated to meet the requirements of reproductive does and growing rabbits, respectively. Within each feed category (R or F) the diets were isonutritive and contained similar levels of crude proteins, fibres, and digestible energy. Incorporation of dehydrated sainfoin in the R diets was achieved mainly at the expense of sunflower meal, alfalfa meal and Lapilest®; whereas in the F diets, DS mainly replaced beet pulp, sunflower meal, alfalfa meal and rapeseed pulp (Table 1). The F diets had higher concentrations of ADFom (+27%) and lignins content (+39%) than the R diets. Diets for growing rabbits were 14% poorer in crude proteins and 11% poorer in digestible energy than R diets. With increasing rates of sainfoin incorporation, the levels of phenols logically increased from 10 to 12 g/kg and from 9 to 12 g/kg in the R and F feeds, respectively.

### **Reproductive performance**

No interaction between cycles and diets was found for reproductive performance of does (Table 2). As expected, doe live weight increased (except at D32) by 7% from cycle 1 to cycle 3 (Table 2,  $P<0.001$ ), since they reached their adult weight. Throughout the study, the live weight of the does was not affected by the diet. The feed intake of does (and of litters before 25 d of age) increased by 20% ( $P<0.05$ ) from cycle 1 to cycle 3 (Table 2), while it was not affected by the diets and averaged 356 g/d. The fertility rate remained similar between cycles and diets and averaged 78%. The number of live kits at birth was lower in cycle 1 (-2 live rabbits at birth) than in cycle 2 and 3 ( $P<0.001$ ), and was similar in all three groups (9.5 live kits per doe,  $P=0.067$ ). However, the number of liveborn kits increased linearly with the DS incorporation ( $P=0.042$ ), resulting in +1.5 live rabbits at birth in DS26 group. From cycle 1 to cycle 2, the stillborn rate decreased by 67%, then increased again by 13% in cycle 3. The stillborn rate was similar among the three diets ( $P=0.18$ ) and averaged 13%. However, when we compared groups with sainfoin diets (DS13+DS26) with the control group (DS0) in a contrast analysis, the stillborn rate tended to be lower in groups (10.4%,  $P=0.086$ ) compared to DS0 (17%).

Feed intake by litters and does (D25-D33) increased by 15% from cycle 1 to cycle 3, and litter growth was 9% lower in the first cycle than in the two others ( $P<0.05$ ). Feed intake by the litter and the doe was not affected by the DS dietary incorporation rate (mean: 799 g/d). Similarly, between 4 and 33 d of age, litter growth was not affected by the diet and averaged 28.8 g/day.

### **Performance of growing rabbits after weaning.**

No interaction between cycle and diet was found for the performance of the growing rabbits (Table 3). Growing rabbits in all three groups were subject to similar restrictions from weaning (D33) until D70, and the feed intake averaged

**Table 2:** Reproductive performances of does according to cycle and sainfoin dietary incorporation.

Doe	Cycles				Groups				P-value		
	1	2	3	RSD	DS0	DS13	DS26	RSD	Cycle	Groups	Cycle × Groups
Live weight (g)											
D-10 <sup>1</sup>	5168 <sup>a</sup>	5454 <sup>b</sup>	5560 <sup>c</sup>	35.0	5447	5372	5346	37.6	<0.001	0.45	0.85
D4	5031 <sup>a</sup>	5298 <sup>b</sup>	5408 <sup>c</sup>	30.9	5296	5282	5159	33.4	<0.001	0.13	0.74
D32	5225	5383	5348	37.5	5366	5358	5239	37.6	0.18	0.28	0.60
Doe feed intake:											
D-10 to D25 (g/d)	319 <sup>a</sup>	365 <sup>b</sup>	384 <sup>c</sup>	3.83	364	352	351	4.48	<0.001	0.31	0.88
Litter+doe feed intake:											
D25-D32 (g/d)	737 <sup>a</sup>	796 <sup>b</sup>	866 <sup>c</sup>	6.7	816	790	792	8.1	<0.001	0.18	0.44
Fertility rate (%)	80.0	71.9	81.4	3.02	78.9	76.4	77.6	3.03	0.40	0.95	0.99
Live kits at birth	8.0 <sup>a</sup>	10.7 <sup>b</sup>	10.0 <sup>b</sup>	0.3	9.0	9.0	10.5	0.3	<0.01	0.067	0.61
Stillborn rate (%)	17.9 <sup>b</sup>	5.9 <sup>a</sup>	12.7 <sup>ab</sup>	1.8	16.6	11.8	9.0	1.8	<0.01	0.18	0.47
Litter daily weight gain:											
D4-D32 (g/d)	27.2 <sup>a</sup>	29.3 <sup>b</sup>	30.2 <sup>b</sup>	0.2	29.4	28.2	28.9	0.2	<0.001	0.10	0.87

<sup>1</sup>D-10: 10 d before parturition (D0); D4: 4 d after parturition; D32: 32 d after parturition.

N=20 does/diet. RSD: Residual standard deviation.

<sup>abc</sup>Means within a row and treatment with different superscript letters differ ( $P<0.05$ ).

127 g/d/rabbit. No feed was refused whatever the diet. The growth rate and feed conversion improved from cycle 1 to cycle 3, respectively +14 and -14%. Increasing the sainfoin incorporation in the diets linearly reduced the growth rate (linear effect:  $P<0.01$ ), resulting in a 2.3% lower growth rate ( $P<0.001$ ) in the DS26 group compared to the DS0 group (Table 3). Accordingly, the feed conversion ratio linearly increased with the DS incorporation (linear effect:  $P<0.01$ ), resulting in 2.3% higher feed conversion in group DS26.

### Mortality of does and growing rabbits

Doe mortality was studied on a mean of 48 does per cycle instead of 60 does per cycle due to a 78% fertility rate and to gestating does dying before beginning the trial (2%). Doe mortality was low (5%) and not affected by the reproductive cycle (Table 4). However, doe mortality differed among groups, with higher mortality in group DS13 (15%) than in DS0 (0%) or DS2p6 (2%). Indeed, when doe losses were cumulated over the 3 cycles, six does out of the seven does that died belonged to group DS13. During cycle 1, four does died (at D0, D7 and D15) and three out of the four belonged to group DS13; veterinary diagnosis identified two pathogenic agents: *Escherichia coli* and *Staphylococcus aureus*. Consequently, from D18 to D24 (cycle 1), all the does were treated with 70 g/d of doxycycline, and from D25 to D30 (cycle 1) does received 0.5 ml/L of colistin in their drinking water. Thereafter, except for three does in group DS13 that died between 23 and 28 d in cycle 2, no more does died. The does that died in cycle 1 died abruptly with no sign of poor sanitary status (normal live weight, intake and body condition) and

**Table 3:** Performances of growing rabbits after weaning, according to the cycle and sainfoin dietary incorporation.

g/d	Cycles				Groups				P-value		
	1	2	3	RSD	DS0	DS13	DS26	RSD	Cycle	Groups	Cycle X Groups
D32-D70											
Feed intake	126.9	127.1	127.2	1.7	127.2	127.1	127.0	1.7	0.997	0.999	1
Daily weight gain	39.8 <sup>a</sup>	44.7 <sup>b</sup>	46.3 <sup>c</sup>	0.1	44.2 <sup>b</sup>	43.4 <sup>ab</sup>	43.2 <sup>a</sup>	0.2	<0.001	<0.001	0.76
Feed conversion ratio	3.22 <sup>c</sup>	2.87 <sup>b</sup>	2.76 <sup>a</sup>	0.01	2.91 <sup>a</sup>	2.97 <sup>ab</sup>	2.98 <sup>b</sup>	0.01	<0.001	<0.01	0.72

RSD: Residual standard deviation.

<sup>abc</sup>Means within a row and treatment with different superscript letters differ ( $P<0.05$ ).

N=104 in DS0, 70 in DS13 and 100 in DS26.

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