World Rabbit Science

World Rabbit Sci. 2017, 25: 51-62
doi:10.4995/wrs.2017.6486

INTAKE OF DIFFERENT HAYS WITH PRESENCE OF *COLCHICUM AUTUMNALE* BY RABBITS

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Abstract: The ingestion of 3 hay types by growing rabbits and the consequences on animal performance were studied in a first part. Two kinds of hay from high nature value grasslands [CFP (*Colchico-Festucetum pratensis*) and SBR (*Senecioni-Brometum racemosi*)] differentiated by their botanical composition were compared to a classical agricultural hay (control). The 3 hays, offered *ad libitum*, were ingested at 30.6, 34.8 and 36.1 g/kg body weight (BW) respectively for SBR, CFP and the control, where the consumption of SBR hay was significantly lower. (P<0.01). The type of hay distributed did not affect the consumption of commercial feed or growth (44 g/kg BW and 36 g/d, respectively). The effect of meadow saffron concentration (*Colchicum autumnale*) in CFP hay was tested at 3 doses (absence, 2.75 and 15% of the distributed hay mass) with respect to their effect on ingestion and animal health. Again, no effect of the presence of meadow saffron (MeS) in the hay on these parameters was observed, even at the highest dose. However, during this second part, rabbits preferred to consume meadow saffron instead of the rest of the distributed hay (hardly any MeS was refused by rabbits at the representative dose, whereas 12% of the rest of the hay was refused, and 3.4 and 20% respectively at the maximal dose). The intake of the toxic alkaloid colchicine corresponded to 30 to 44% of the median lethal doses (LD_{50}) at the representative and maximal doses respectively. The real exposure of the rabbits was probably lower, as the absorption of ingested colchicine is reported to be around 50% of the LD_{50} values which are established after an intravenous injection. Nevertheless, the absence of any effect should be interpreted with caution. The colchicine content of MeS varied greatly depending on the time of harvest and the parts of the plant taken into consideration. A late harvest (mid-June or later) would ensure (very) low concentrations of colchicine in the aerial parts of MeS. The presence of cloves on this plant should be completely avoided, due to the very high concentration of colchicine in the seeds. Moreover, long term exposure of the animals to this toxic plant may increase the risk of adverse effects. The study shows that hay distribution can aid digestion without altering the animal's performance. Moreover, the valorisation of hay from high nature value grasslands would not only add real value to the commercialisation of rabbit meat by commercial breeders, but also to pet owners on the condition that hay quality would be guaranteed.

Key Words: colchicine, hay intake, meadow saffron, rabbit, toxicity.

INTRODUCTION

Rabbits need to ingest nutritional fibre to ensure digestive comfort, even though in commercial breeding the nutritional supply of roughage is moderate (Gidenne, 2015). Very little is known concerning the amount of daily hay intake and sensitivity to its quality. Moreover, interactions between hay quality and the intake of commercial feed can be expected, at least in terms of possible competition for the available volume in the digestive tract. Various types of hay are available to commercial breeders as well as to private owners of rabbits and other herbivorous pets. Hay is mainly destined for ruminants or horses and should be harvested quite early to ensure these species can digest the fibre content correctly (Korva and Tuori, 1986). Nevertheless, a later harvest would not only favour a richer botanic composition of grass and

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doi:10.4995/wrs.2017.6486

especially a greater presence of more plant species (Gaujour *et al.*, 2012), but would also promote the development of wildlife such as ground-nesting birds (Grüebler *et al.*, 2012; Humbert *et al.*, 2012). However, a higher fibre content and lignification, consequences of a late harvest, would reduce the nutritional value of such hay for ruminants (Korva and Tuori, 1986). The organoleptic properties of hay from grasslands with high environmental values represent a high-value hay appreciated by herbivorous pet owners (Stainmesse, 2015). Moreover, absence of hay in the diet could increase overweight as well as dental diseases, highlighting the need for hay in a rabbit's diet on health grounds (Lord, 2014; Prebble *et al.*, 2015). This is especially apparent in the owners of dwarf rabbits, where the availability of such very different hays can be an important argument for their economic valorisation.

One issue with hay from multi-species meadows is the possible presence of toxic plants in varying proportions (Cortinovis *et al.*, 2015). If eaten as part of their daily intake of hay, toxicological thresholds may be exceeded and lead to adverse effects on animal health.

Thus, neither real intake of hay from different grassland systems by rabbits nor their ability to select specific plants present in hay is known.

The National Park of Lorraine (PNRL, East France) includes 3200 hectares of high nature value grassland, composed of specific botanic species and classified by 2 major associations: CFP (*Colchico-Festucetum pratensis*) and SBR (*Senecioni-Brometum racemosi*) (Jager and Muller, 1999). The economic valuation of hay from these highly valuable grasslands would be a way of ensuring their preservation at an ecological level. However, CFP grasslands can include significant proportions of *Colchicum autumnale* (Plantureux and Amiaud, 2010), commonly known as meadow saffron (MeS), which is highly toxic for numerous animal species (Scherrmann *et al.,* 1989) due to the presence of the alkaloid colchicine in the plant (Poutareau and Girardin, 2002). Moreover, feed preferences of rabbits concerning rich hay is unknown and their willingness to ingest these plants or not needs to be studied.

The first aim of this study is to clarify the amount of hay intake as well as its effect on the intake of commercial feed and growth. A second aim focuses on the behaviour of rabbits concerning the presence of MeS in hay, their ability to select this plant before ingestion and the possible adverse effects on animal health. This knowledge is necessary before considering a commercial use of these hays for commercial breeders or owners of pet rabbits.

MATERIALS AND METHODS

The experiment was authorised by the French Higher Research Ministry (APAFIS#1688-2015090917378531) after an ethical evaluation.

Experimental hays

Three hay types were distributed to the animals: 2 different hays from the PNRL and a control hay. The latter corresponded to a classical agricultural hay harvested from permanent grasslands (fertilised at 80 N-units per ha) in the village of Xirocourt (E 6°10'19", N 48°25'53") near Nancy (France), in mid-June 2015 and dried for 3 d. The 2 other hays came from 2 extensive grassland types: the first type is the CFP hay and the second one is the SBR hay. To preserve grassland biodiversity, both hays were harvested late (mid-July) on non-fertilised permanent grasslands in the village of Trondes (E 5°46'21", N 48°42'57") near Toul (France). The CFP hay included MeS, which was manually separated from the rest of the sampled CFP hay for the first part of the experiment, and added again in the second part in controlled portions.

Experimental design

The study was divided into 2 parts: firstly, the impact of hay intake on commercial feed intake and animal growth, and then the impact of the presence of MeS in CFP hay on feedstuff intake and growth as well as animal health. After an adaptation period of 1 wk to get the rabbits accustomed to the experimental facilities, hay intake was studied in a 3×3 Latin square design using 3 groups of 5 rabbits, each group being offered one of the test hays as a complement to the commercial feed (Table 1). Each experimental period lasted 7 d and measurements were taken during the last 5 d of each period, leaving 2 d for the animals to become accustomed to the new modality. Then, the different effects of the presence of MeS in the PNRL grasslands were studied by distributing 3 different proportions of dry MeS in the

Table 1: Latin square design of the first part of the experiment studying the effect of hay type on intake of hay and commercial feed as well as on growth.

Experimental week	1	2	3
Age of the rabbits on the first day of the corresponding week	53 d	60 d	67 d
Group 1 (5 rabbits)	Control	CFP	SBR
Group 2 (5 rabbits)	SBR	Control	CFP
Group 3 (5 rabbits)	CFP	SBR	Control

CFP: *Colchico-Festucetum pratensis*, SBR: *Senecioni-Brometum racemosi*.

hay (in accordance with Plantureux and Amiaud, 2010, absence: 0%, representative: 2.75%, and maximal: 15% on the fresh weight basis of the CFP hay) during 7 d (Table 2). In the first part of the experiment, rabbits were allocated to the treatments on a body weight (BW) basis from the previous week. In the second part, rabbits were allocated on their level of hay intake recorded during the first part, in order to balance the groups using this parameter.

Animals and housing

Sixteen young male rabbits of the commercial breed ZIKA® (Zimmermann GbR, Abtsgmünd-Untergröningen, Germany) were purchased. One rabbit was excluded from the first part of the study after weighing, as only 15 rabbits were needed for a Latin square (Table 1), and another one was removed from the second part as it ingested less hay than the others. The second part of the study also used 15 animals, not in a Latin square design but by using 5 rabbits per studied treatment (Table 2). The rabbits were weaned at the age of 32 d, and were 46 d old and weighed 1402 g on average (standard deviation=0.16) when they arrived at the animal housing facility. The animals were housed on sawdust litter in individual 1 m^2 pens with a hutch and were fed with hay and pellets of a commercial feed for growing rabbits (Lapicroq, Lorial, Laxou, France). The quantity of commercial feed was limited to 50 g/kg BW, which corresponded to the lower value of the range indicated in the recommendations (INRA, 1989) to favour hay intake and avoid the rabbits' preferences for higher protein components (Prebble and Meredith, 2014). The amount of offered feed was calculated weekly using the BW from the previous week throughout the study. Fresh water was available *ad libitum* throughout the study. A preventive treatment against coccidiosis (Sulfadiméthoxine® 23%, Qalian, Segre, France; 120 mL/100 L of water) was administered for 5 d *via* the water supply during the adaptation period.

Characterisation of feedstuffs

All feedstuffs used, i.e. commercial feed and hays, were characterised by fibre fractions (van Soest method), ash (incineration at 550°C during 6 h), crude protein (N by method of Dumas) and starch content (enzymatic method, only for the commercial feed).

Hay fibres were characterised on the basis of the particle size and length of the hay stalks. Both parameters were determined on the basis of 3 individual samples taken at 3 different heights within the hay bound (high, middle and low) for each hay type.

The length of 100 individual stalks was randomly taken from each sample and measured manually. Measuring the particle size was carried out with a Vibratory Sieve Shaker (AS 200 basic, Retsch®, Haan, Germany): for each sample, 15 g were placed in the largest sieve and shaken for 5 min over sieves of sizes: 5, 2, 1, 0.1 and 0.05 mm, to separate and weigh each fraction. Moreover, the colour was measured using finely ground samples of each hay which were put

Table 2: Experimental design of the second part of the experiment studying the impact of the presence of meadow saffron (MeS) in CFP hay on intake and growth as well as animal health.

Experimental week	4
Age of the rabbits on the first day	74 d
Group a (5 rabbits)	Absence (no MeS in the hay)
Group b (5 rabbits)	Representative (2.75% of MeS in the hay)
Group c (5 rabbits)	Maximal (15% of MeS in the hay)

CFP: *Colchico-Festucetum pratensis.*

on a Petri dish and then analysed using a colorimeter (Chromacal, Datacolour international, Dietlekon, Switzerland). The colorimeter used 3 parameters to describe the colour: brightness (L) running from the darkest black (L=0) to the brightest white (L=100), and colour-opponent dimensions on the scale "a" from green (–127) to red (+128) and on the scale "b" from blue (–127) to yellow (+128).

Measurements

After the 2-d adaptation at the beginning of each measurement period, the rabbits were weighed 3 times a week throughout the study. In order to control daily variations over short periods of 7 d, the recorded BWs were fitted to a linear model at individual level. As these individual growth models fitted well with measured BW points (r^2>0.96), the BW used for a given period corresponded to the average modelled BW.

The intake of commercial feed and hay was determined daily at an individual level by weighing the amount distributed and the amount refused. During the first part of the study, hay was distributed *ad libitum* to allow animals to regulate the intake themselves. During the second part of the study, only CFP hay was used, as MeS was only present in this hay. The amount of CFP hay, containing MeS, distributed to each rabbit, was estimated by calculating the median consumption of the last 7 d of the previous period, on an individual basis. This aimed to provide hay and MeS at the proportion each rabbit was used to ingesting, and not *ad libitum* as it was in the first part of the study. The amount of MeS distributed was calculated to the same proportions found in PNRL grasslands: i.e. without MeS for "absence", 2.75% of the offered CFP hay for "representative" and 15% for "maximal". The amount of MeS was weighed and then made up using CFP hay to reach the amount previously calculated. Commercial feed was still given in proportion to the animal's weight. The next day, the refusals of hay and MeS were weighed separately to determine the intake.

Health effects were noted by qualitatively monitoring each animal and the following factors were studied. A score between 0 (normal) and 2 (very degraded) was given:

Vitality: mobile/curious (0) – calm/attentive (1) – motionless/prostrate (2)

Diarrhoea: solid faeces (0) – soft faeces (1) – liquid faeces (2)

Anal cleanliness: clean (0) – some trace of faecal material (1) – rump very dirty (2)

Deaths were noted as well as any health issue which arose, which could not be analysed using the aforementioned scoring, during the experiment.

To estimate the amount of colchicine ingested by the animals, an analysis of this alkaloid was carried out prior to the experiment, according to the method described by Poutaraud and Girardin (2002). This liquid chromatography based method showed an average concentration of 0.02% of colchicine in dried leaves. Then, the distributed dose of colchicine, depending on the proportion of MeS in the distributed hay, was compared to the median lethal dose (LD_{50}) reported at 2.75 mg/kg of live weight (Scherrmann *et al.,* 1989).

Statistical Analysis

Firstly, the particle size did not follow a normal distribution. This variable was therefore categorised in 4 classes: dust (<100 µm, ATMO federation, Paris, France), fine (100 mm to 2 mm), small (2 to 5 mm) and large particles (>5 mm). Then, means and standard deviations were calculated to compare the distribution of each hay within these 4 classes. Secondly, hay stalk length and colour (a*, b* and L* parameters) and intake variables (of commercial feed and hay) were analysed with a one-factor (type of hay) Anova. The means were compared by a multiple t-test (Tukey correction) and signification was decleared at P<0.05.

Thirdly, the second part of the study considered the proportion of MeS in hay as a factor in an ANOVA on intake and growth variables. The ingestion behaviour of the rabbits was evaluated by calculating 2 ratios. Firstly, the intake ratio (IR) of rabbits for MeS in comparison to hay was calculated as the ratio between intakes and calculated as follows:

$$\frac{\text{MeS ingested} / \text{MeS given}}{\text{Hay ingested} / \text{Hay given}}$$

An IR value >1 would mean that rabbits would ingest more MeS in comparison to the rest of the hay. The refusal proportion ratio (RR) was calculated as the ratio between refusals and offered as follows:

$$\frac{\text{MeS refused} / \text{MeS given}}{\text{Hay refused} / \text{Hay given}}$$

An RR<1 would mean that MeS was rejected less by the rabbits than the rest of the hay. These 2 ratios give similar information, as a rabbit ingesting proportionally more MeS will refuse proportionally less of it. Nevertheless, each ratio provides quick and visual access to the corresponding information.

All health scores were categorised as frequencies for a given observation factor.

RESULTS

Hay characteristics

The chemical composition of the different hays showed the expected generally high proportions of fibres (i.e. neutral detergent fibre [NDF]>58%), especially in the control hay (Table 3). SBR hay had slightly more ash and CFP hay was poorer in crude protein.

Large particles represent the large majority in all three hays even if there were differences. These particles account for over 94% of the mass of SBR and the control hay, but less than 63% in CFP hay (Table 4). On the other hand, CFP hay showed more dust and small particles (i.e. 37% smaller than 2 mm) whereas these classes covered only 2% of the mass of SBR hay and 5.5% of the control hay (Table 4). Concerning the length of the hay stalks, CFP hay had shorter stalks than SBR and the control hays, respectively 15.1, 20.4 and 18.3 cm and there was no significant difference between SBR and the control hays (P<0.05; Table 4).

The brightness parameter L* was very similar between the 3 hays (P>0.05; Table 5). However, small but significant (P<0.05) differences showed on the colour scales (Table 5): both alternative hays (SBR and CFP) were slightly more yellow (+1 point) compared to the control hay. Additionally, SBR hay was slightly redder than (+0.5 point) CFP and the control hay. Nevertheless, these differences on a scale of 256 points would hardly be perceptible to the human eye. To our knowledge, scientific literature does not mention the sensitivity of rabbits' eyes and we consider it unlikely that the animals would perceive this small difference.

Intakes

Rabbits ingested on average 150 g (±6g) of total dry matter (DM; pellets and hay) per day during the first part of the experiment when they were around 9 wk old (Table 6). The total intake capacity could reach 80 g/kg BW per day, but the relative DM intake was lower for the SBR hay, (74.5 g/kg BW per day against 79 g/kg BW and 80.2 g/kg BW, respectively, for CFP and control hays; P<0.01). Thus, the total DM intake comprised 41-45% hay to 55-59% feed pellets. So, we estimate that fibre proportions from hay ingestion were 58% for SBR hay diet, 60% for the CFP hay diet and 64% for the control hay diet, respectively.

Intake of commercial feed was not affected either by the type of distributed hay (P>0.05; Table 6) or by the proportion of MeS in the hay (P>0.05; Table 7), independently of their expression as absolute amounts (g/animal and day) or

Table 3: Results of chemical analysis (g/100 g) of used feedstuffs.

	Hay SBR	Hay CFP	Control hay	Commercial Feed
Ash	6.6	5.6	5.6	7.9
Crude Protein	6.2	5.7	6.2	15.5
Neutral detergent fibre	61.4	58.6	68.8	31.1
Acid detergent fibre	35.3	38.4	38.5	16.0
Acid detergent lignin	5.8	5.6	5.8	3.5
Starch	nd	nd	nd	16.4

CFP: *Colchico-Festucetum pratensis*, SBR: *Senecioni-Brometum racemosi*. nd: not determined.

Table 4: Physical properties of used hays.

	Hay SBR	Hay CFP	Control hay
Proportion of particle size (mm), means±standard deviation			
Dust (<0.1)	0.01±0.0	0.06±0.04	0.05±0.05
fine particles (0.1 to 2)	0.49±0.3	8.39±3.5	1.04±1.1
small particles (2 to 5)	1.44±0.8	29.19±10.9	4.65±3.4
large particles (>5)	98.06±1.9	62.36±14.1	94.26±4.8
Mean blade lengths (cm)	20.4[a]	15.1[b]	18.3[a]
Effect of hay type	*P*<0.001		
Root MSE	14.3		

CFP: *Colchico-Festucetum pratensis*; SBR: *Senecioni-Brometum racemosi*; MSE: mean square error.
[a,b]Means with a different superscript differ at *P*<0.05.

relative to the BW. At the average age of 9 wk for this first part of the study, the animals ingested 85.5 g of pelleted feed per day, which corresponded to a relative intake of 44 g/kg BW. The total amount ingested was higher (*i.e.* 112 g/d) during the second part of the study, as rabbits were 2 wk older (Table 7), but without any significant change in the relative intake (46 g/kg BW).

Rabbits ingested significantly less SBR hay than CFP hay (–4.2 g/d) or control hay (–5.5 g/d; *P*<0.01; Table 6). The same difference was shown for the relative hay intake (SBR intake: 30.6 g/kg BW; CFP intake: 34.8 g/kg BW; control intake: 36.1 g/kg BW). An increasing proportion of MeS in the hay enhanced a slight (but not significant) increase in the intake of hay independently of the expression as absolute or relative intake (*P*>0.05; Table 7). As less hay was distributed in the second part of the experiment, the rabbits ingested less hay per kg BW (–10.5 g/kg BW/d; *P*<0.01; Table 9) in comparison to the first part of the study.

The daily weight gain of individual rabbits averaged 35.1 (±2.6) g/d, ranging between 29.7 and 39.7 g/day. BWs were modelled for a given rabbit throughout the experiment, based on the different starting weights. Therefore, the determined growth could not vary at an individual level between the experimental periods. This absence of variations did not allow us to test the effect of hay types on growth statistically.

The analysis of intake behaviour (Table 8) illustrated that hardly any MeS was refused by the rabbits at the representative dose and 3.4% at the maximal dose. On the other hand, 12 to 20% of the offered hay was refused by the animals depending on the quantity of MeS (0, 2.75 and 15%). This result was confirmed by intake ratios systematically >1 for all animals receiving MeS. Refusal ratios were not only <1 but very close to 0, meaning very few refused MeS (Table 8). This MeS intake corresponded to 0.13 and 0.95 mg of colchicine per kg BW for the representative and maximal doses respectively (Table 8). These concentrations of colchicine correspond, for the MeS levels of representative and maximal respectively, to 4.5 and 34.6% on average of the median lethal doses (LD_{50}) reported for this species. The highest dose reached was 43.9% of the LD_{50} (Table 8) for one rabbit.

No health effects were monitored during exposure to the tested doses of MeS in the hay. Indeed, every rabbit scored 0 throughout the observation period of 7 d and no other health issues were observed.

Table 5: Effect of hay type on colour parameters.

	Hay type				
Colour parameter	SBR	CFP	Control	Root MSE	*P*-value
Brightness L[1]	29	30	31	3.7	NS
Red-green scale a[2]	+0.1[b]	–0.3[a]	–0.4[a]	0.22	<0.001
Yellow-blue scale b[3]	+13.0[a]	+13.3[a]	+14.2[b]	0.75	<0.001

CFP: *Colchico-Festucetum pratensis*, SBR: *Senecioni-Brometum racemosi.* MSE: mean square error. NS: not significative.
[a,b]Means with a different superscript differ at *P*<0.05.
[1]Measured on a scale from 0 (darkness) to 100 (brightest white).
[2]Colour scale from red (+127) to green (-128).
[3]Colour scale from yellow (+127) to blue (-128).

Table 6: Effect of the offered hay type on intakes of hay and commercial feed (g).

	Hay SBR	Hay CFP	Control hay	Root MSE	*P*-value
Number of rabbits	15	15	15		
Feed intake					
absolute (g/d)	85.3	85.3	85.7	15.37	NS
relative (g/kg BW)	43.9	44.1	44.2	2.31	NS
Hay intake					
absolute (g/d)	58.1[a]	65.9[b]	69.7[b]	8.15	<0.05
relative (g/kg BW)	30.6[a]	34.8[b]	36.1[b]	4.16	<0.05
Dry matter intake					
absolute (g/d)	143.3	151.2	155.4	20.42	NS
relative (g/kg BW)	74.5[a]	79.0[b]	80.2[b]	3.72	<0.01

CFP: *Colchico-Festucetum pratensis*; SBR: *Senecioni-Brometum racemosi*; MSE: mean square error; BW: body weight; NS: not significative.
[a,b]Means with a different superscript differ at $P<0.05$.

DISCUSSION

Hay characteristics

As expected, the NDF level was twice as high in the hays than in the pelleted balanced feed. This fraction was lower in PNRL hays compared to the control hay. This could be due to the higher proportion of dicotyledonous plants in the botanic composition (Semler, 2014). Gidenne (2015) recommended acid detergent fibre (ADF) concentrations of at least 17% for growing rabbits without any indication of an upper limit. The pelleted feed did not reach this level, but once the hay was included in the total diet, the minimum level would be exceeded. Indeed, thanks to the chemical analysis (Table 3), we estimate the daily fibre ingestion (ADF) at 24.5 g /kg BW for the SBR hay diet, 27.1 g /kg BW for the CFP hay diet and 27.6 g/kg BW for the control hay diet. This corresponds to a fibre concentration in the whole diet of 33% for the SBR hay diet, 34% for the CFP hay diet and 34% for the control hay diet. The recommended acid detergent lignin concentration of more than 5% was met in the hays, but not when considering all the ingested diet. Thus, all 3 distributed hays (SBR, CFP and control) provided a good fibre supply to complement the commercial feed, thereby preventing digestive disorders in our growing rabbits (Gidenne, 2015).

Table 7: Effect of the proportion of meadow saffron (MeS) in the offered hay on intakes of CFP hay and commercial feed as well as growth.

Proportion of MeS	absence	representative (2.75%)	maximal (15%)	Root MSE	*P*-value
Number of rabbits used	5	5	5		
Feed intake					
absolute (g/d)	114.0	113.2	111.2	10.45	NS
relative (g/kg BW)	45.9	46.1	46.1	0.78	NS
Hay intake					
absolute (g/d)	55.3	59.2	66.5	11.23	NS
relative (g/kg BW)	22.1	24.2	27.7	4.34	NS
Dry matter intake					
absolute (g/d)	169.3	172.4	177.7	18.07	NS
relative (g/kg BW)	67.0	69.4	72.7	4.15	NS
Mean BW (g)	2522.0	2486.7	2446.8	209.41	NS
Growth (g/d)	35.5	36.0	35.2	2.61	NS

CFP: *Colchico-Festucetum pratensis*; BW: body weight; MSE: mean square error. NS: not significative.
[a,b]Means with a different superscript differ at $P<0.05$.

Table 8: Intakes of meadow saffron (MeS) and colchicine alkaloid by the animals.

		Intake of plants		MeS preference		Intake of colchicine[1]	
		MeS	Hay	intake	refusal		
MeS level Dose	Rabbit	(% of offered fresh weight)		IR[2]	RR[3]	mg/kg BW	% of LD_{50}
Absence (0%)	B	nd	76.9	nd	nd	0	0
	C	nd	90.7	nd	nd	0	0
	D	nd	80.7	nd	nd	0	0
	E	nd	93.4	nd	nd	0	0
	F	nd	70.0	nd	nd	0	0
Means±SD			82.3±9.7				
Representative (2.75%)	G	100	95.8	1.043	0.0	0.121	4.4
	H	100	89.2	1.121	0.0	0.118	4.3
	I	98.0	84.9	1.155	0.13	0.123	4.4
	J	100	82.4	1.213	0.0	0.147	5.3
	K	100	88.6	1.129	0.0	0.114	4.1
Means±SD		99.6±0.9	88.2±5.1	1.13±0.06	0.03±0.06	0.13±0.01	4.5±0.46
Maximal (15%)	L	99.7	84.0	1.186	0.019	0.883	32.1
	M	95.0	81.9	1.160	0.278	0.884	32.2
	N	98.8	76.5	1.292	0.050	0.964	35.1
	O	93.5	71.5	1.308	0.229	0.813	29.6
	P	95.9	87.5	1.095	0.331	1.207	43.9
Means±SD		96.6±2.6	80.3±6.3	1.21±0.09	0.18±0.14	0.95±0.15	34.6±5.6

SD: standard deviation; nd: not determined; BW: body weight.
[1]calculated on the basis of the analysis of the proportion of colchicine (0.02% in dried leaves).
[2]Intake ratio (IR)=[(MeS ingested/MeS offered)/(hay ingested/hay offered)].
[3]Refusal ratio (RR)=[(MeS refused/MeS offered)/(hay refused/hay offered)].

The CFP hay contained notable proportions of small and fine particles in contrast to the 2 other hays. This can be annoying for customers, especially for pet owners who keep their rabbits in their homes. Although it can be assumed that the majority of small particles would fall to the bottom of the rack and would not be ingested, high proportions of dust and small particles may affect rabbits' health. Indeed, Pozet (2009) reported that the presence of small particles in their environment could facilitate infections as a rabbit's respiratory system is sensitive to dust.

The higher proportions of small and fine particles in CFP hay can be explained by different facts: firstly, the hays came from different grasslands with different botanic compositions. Even though notable variations within the same grassland type were reported by Semler (2015), CFP hay is mainly composed of *Festuca pratensis* and *Colchicum autumnale,* whereas in SBR grasslands/meadows, *Jacobaea aquatica* and *Anthoxanthum odoratum* are the dominant species. *Lotus corniculatus* is more frequent in CFP grasslands (4-5%) compared to the SBR type (2-4%). Such

Table 9: Comparison of the hay intake between the 2 parts of the experiment.

Part	1 Hay type comparison	2 MeS in hay	Root MSE	*P*-value
Number of rabbits used	15	15		
Mean BW (kg)	1.93	2.48		NT
Hay intake				
absolute (g/d)	65.5	58.1	12.9	NS
relative (g/kg BW)	33.9[a]	23.4[b]	6.0	<0.001

MeS: meadow saffron; BW: body weight; NS: not significant; MSE: mean square error; NT: Not tested (see Results, paragraph Intakes).
[a,b]Means with a different superscript differ at $P<0.05$.

botanic differences would modify the drying abilities (e.g. cut plant mass per surface unit, ratio between drying surface and harvested weight). Moreover, each hay was mown and dried at different farms in different conditions resulting in different drying levels. As CFP hay seemed more powdery and more breakable than the other 2 hays, manipulation could have increased the crumbling and the proportions of fine and small particles, especially as MeS was sorted by hand in the CFP hay. As cutting and drying conditions determine these characteristics, a standardisation of harvesting conditions would be helpful to limit the proportions of fine particles and dust.

Statistical analysis showed that SBR hay was less green in colour than CFP and the control hay, but this difference was very small and probably not perceptible to humans. However, impact of the colour on consumption was not tested in this study, because we chose to treat colour as a factor in the buying decisions of pet owners. Therefore, even if owners (commercial breeders as well as pet owners) were shown to buy hay of an intense green colour rather than a yellow hay (Stainmesse 2015), it should have no impact on the sale of the tested hays.

Animal performance

The interpretation of animal performance should be focussed on the results of the first part of the study. Indeed, the second part (MeS proportion in CFP hay) studied intake behaviour of MeS and health observations and the use of only 5 rabbits per treatment meant low statistical power. Total and relative hay intake were 12 to 17% lower for SBR hay in comparison to the CFP and control hay, respectively. As the nutritional effect of hay on rabbits is assumed to be moderate, no variations in animal performance can be attributed to this difference. Indeed, the hay type and observed hay intake differences did not affect feed intake (45 g/kg BW) or growth (35 g/d), which varied across the range expected by commercial breeders.

This difference can be taken to mean the intake preference of rabbits which should be seen as a link to the hay's characteristics. Firstly, differences in botanic composition specified before may modify organoleptic properties. Secondly, variations in hay gathering, treatment and storage due to technical differences between farms can change the conservation of metabolites, as shown by Farruggia *et al.* (2008). These metabolites would influence the organoleptic properties of hay and therefore the intake by the animals. Finally, most of the hay stalks left in the rack were brown or dark green, which may indicate a rabbit's preference for green hays (Prebble and Meredith, 2013; Prebble *et al.,* 2014). As SBR hay was slightly less green coloured than the control and CFP hays, it could have made a difference to the rabbits' ingestion even if this difference was small and hardly perceptible to humans. Thus, the significantly lower intake of SBR hay in comparison to the other 2 did not affect animal health or performance in any way, but might be noticed by the owner.

During the second part of the study, the intake of (CFP) hay was clearly lower compared to in the first one, particularly when expressed as a relative intake (–7.1 to –12.7 g/kg BW). This is due to the experiment's design: during the first part, hay was distributed *ad libitum* to evaluate the animals' voluntary hay intake. During the second part, CFP hay was given in a limited amount to encourage MeS intake. This choice would at least partly explain the lower hay intake in comparison to the first part of the study.

Although the proportion of MeS in the CFP hay did not significantly affect its intake, there was a slight but not significant tendency of increased consumption of the hay with the highest proportion of MeS. As this second part of the study was not carried out in a Latin square design, individual differences between animals, reflected by an increased root MSE, could enhance such (non-significant) variations and therefore should not be attributed to the dose of MeS.

Effects of MeS presence

These results should be interpreted cautiously, as only 5 rabbits per MeS proportion in hay is very few in order to study the effects on health. Nevertheless, these exploratory results showed some interesting tendencies, as no observable effects were revealed on rabbit health, growth and commercial feed intake by the presence of MeS in hay, even at the highest level of 15% of the ingested hay. These results can be related to the low concentration of colchicine in the dried MeS leaves. Indeed, measurements in samples revealed a concentration of 0.02% of colchicine in dried leaves. This concentration is very low compared to concentrations of 0.1 to 0.6% reported by Brvar *et al.* (2004), or 0.15 to 0.4% by Kupper *et al.* (2010), and were in line with Poutaraud and Giradin (2002), stating that the concentration of colchicine in leaves of MeS decreased sharply between May and June from 0.2 to 0.02%. As hay harvesting is not allowed before mid-July on the PNRL's high nature value grasslands, this result is not surprising and would confirm that a summer harvest is an efficient way of reducing the risk of adverse effects due to MeS.

The amount of colchicine did not have any observable effect on rabbit health, despite one rabbit consuming 43.9% of the LD_{50} reported in this species (Scherrmann *et al.,* 1989). However, the LD_{50} was calculated for an intravenous injection of colchicine. In this experiment, a difference between the amount ingested and the amount that reached the blood circulation should be considered. This bioavailability was calculated at 44% for humans by Kupper *et al.* (2010) meaning, by extrapolation, that less than half the dose of the alkaloid entered the blood stream. Furthermore, the plants were ingested over a period of 24 h, leaving time for the organism to gradually eliminate the alkaloid (Kupper *et al.,* 2010). Nevertheless the intake of MeS seeds, increased by the presence of cloves in the hay, would considerably increase the risk of adverse effects or poisoning.

The analysis of intake behaviour shows that hay refused by the rabbits came mainly from the MeS free CFP hay with 12 to 20% of the distributed mass. At the representative dose of 2.75% of MeS in the hay, no countable refusals of MeS were observed. Even at the maximal level of 15% MeS, their refusals were much lower than for the rest of the hay. This could imply a preference of the rabbits for MeS, supposing that their stems and leaves were easier to grab. Spilberg *et al.* (1979) reported the first adverse effects when animals were exposed to 0.2 mg/kg BW of colchicine, which is less than the exposure of 0.8 and 1.2 mg/kg BW in the maximal level group in our study. The question about whether the appearance of MeS refusals in the maximal dose group (on av. 3.4% but up to 6.5% on an individual level) reflects a first negative reaction of the animals to the ingested dose of colchicine could be considered.

The study showed that the presence of MeS in hay, even in the highest proportion of 15%, had no effect on rabbits during an exposure of 7 d. Nevertheless, if commercialised in pet husbandry, this hay would be used daily and animals would be exposed to it for a longer period. Therefore, the absence of risks cannot be generalised, especially when established using only a few animals, and such exposure should be avoided.

Practical implications

The positive effect of fibre intake on the digestive comfort of growing rabbits has been discussed before. Our results showed that growing rabbits can ingest around 34 (±7) g hay/kg BW daily without any effect on the animals' performance. A rabbit's ability to self-regulate its intake of voluminous feedstuffs was shown by the absence of refusals of commercial feed, but approximately 15% of the distributed hay was refused (results not shown).

Another aspect is the possible valuation of these hays by different rabbit owners.

On commercial rabbit farms, the extra work required to store and distribute the hay must be compensated by an added value. In this context, the valuation of local hay made from grasslands with high botanic and environmental values could be a commercial argument if rabbits were sold locally. Under these conditions, consumers are more willing to accept a higher price when buying rabbit meat and therefore support the preservation of these grasslands by doing so.

Besides this, private rabbit owners are also interested in these hays. Indeed, these holders do not expect anything from the animals and the very slight (but significantly) lower intake of SBR hay in comparison to the other 2 hays would hardly be noticed by them. However, these owners are very attentive to the personal perception of the purchased feedstuff. Organoleptic properties, as well as colour and odour and the proportion of dust are sensitive characteristics when consumers are choosing hay . No trace of moisture is tolerated and hay must therefore be dried properly. The elevated proportion of fine and small particles in CFP hay can be a problem and potentially dissuade rabbit owners from selecting this hay. The colour differences should not be an obstacle to sale, as they seem too small to be noticed by humans and the majority of hays are sold in pet shops wrapped in coloured plastic films.

The main objective for private owners is their pet rabbit's health, and adverse effects due to the presence of toxic plants is a very sensitive question, especially in CFP hays. Even though no adverse effects of the presence of up to 15% of MeS in the hay were observed in our study, we need to be cautious when generalising. The concentration of toxic alkaloid in the green parts of the plant vary depending on the age of the plant, as described above. In this context, the prohibition of cutting high nature value grasslands before mid-July (sometimes later) makes the use of such hays safer. Another point is the possible presence of seeds containing 4 times more colchicine than the green aerial parts of the MeS (Poutaraud, 2002). Therefore, the presence of cloves with seeds should be completely avoided. Although we did not observe rabbits to be highly sensitive to up to 40% of the toxic reference value LD_{50}, the sensitivity of owners to the possible appearance of adverse effects would suggest the commercialisation of hays from surfaces containing very few MeS, not cut before mid-July and without bulbs of this plant. The intermediate level of 2.75% MeS in CFP hays used in our study seems to be without risk when harvest date and absence of seeds can be guaranteed. On this point, SBR hay can be considered risk free, as no toxic plants are known in their botanic composition.

A classic agricultural hay such as our control hay can also be used as rabbit feed. It has no special buying points for pet owners – unlike the 2 studied hays from high nature value grasslands. Nevertheless, excellent drying conditions to ensure good organoleptic quality (colour and very few dust particles, absence of moisture) and no adverse effects on animal health are necessary conditions in their valuation.

CONCLUSIONS

Growing rabbits voluntarily ingest daily around 34 g hay/kg BW but small intake differences between hay types did not modify feed intake or growth. These intake preferences seem to be linked to the organoleptic properties of the hays, to which rabbit owners are also very sensitive. No adverse effect of the presence of up to 15% MeS in hay on rabbit health or performance was noted. Nevertheless, the concentration of colchicine can vary widely depending on the age of the plant or the plant organs and long term effects cannot be excluded. Therefore, only a very low level of toxic plants in the botanic composition can be tolerated to ensure the absence of adverse health effects. When toxic harmlessness and high organoleptic quality of hay is guaranteed, their use in rabbit feeding can bring real added value for commercial breeders, as well as for pet owners.

Acknowledgements: The authors thank PNRL for providing the studied hay types and the specialization staff of ENSAIA for their technical support. Furthermore, we warmly thank Thierry Gidenne from INRA Toulouse and Sylvain Plantureux from INRA Nancy for their scientific coaching for rabbit and grassland sciences, respectively.

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