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ORGANIC RABBIT FARMING: SHOULD WE BE AFRAID OF GASTRO-INTESTINAL PARASITES?

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ABSTRACT

Our trial aimed to study the gastro-intestinal parasitism in pasture raised rabbits, during three seasons: winter 2014/2015, summer 2015 and spring 2016. For every season, at weaning two groups of five movable cages (three rabbits per cage) were disposed either in a sainfoin or a grass pasture. Nematodes eggs and *Eimeria* oocysts were counted on a weekly basis in faeces, oocysts were identified at species level every two weeks. At slaughter (100-day old), the liver was examined and the digestive tract was sampled to count the number of nematodes. The type of pasture had no significant effect on egg excretion or on nematode prevalence and intensity, or oocyst excretion of all species combined. Spring 2016 was characterized by a high prevalence of *Trichostrongylus* sp. (93% of rabbits) and a 50% increase in the total excretion of oocysts, particularly in sainfoin pasture (6.5 M.OPG) where the rotation times was shorter. No diarrhoea was observed during the trial, neither were intestinal macro-lesions in the slaughtered rabbits. However, 64% of the livers presented white nodules due to *Eimeria stiedai*. A negative correlation between the intensity of infection by *Trichostrongylus* sp. and daily gain was observed. The mean excretion of *E. flavescens* may explain a part of the lower daily weight gain (-5 g/d) observed, whatever the pasture type, at spring 2016 compared to the two other seasons. Our first results suggested increasing the pasture rotation time over the two months requested by current organic rabbit farming regulation in France to reduce worm infections.

Key words: Organic rabbit farming, Gastro-intestinal parasites, *Eimeria*, *Trichostrongylus,* Sainfoin.

INTRODUCTION

The management of parasitism is one of the major obstacle to the development of organic rabbit farming (Roinsard et al., 2013) which relies on grazing. Rabbits with access to grazing are a priori subject to a greater risk of parasitic infection compared to indoor conventional rabbit farming (wire mesh cages), and in particular with strongyles of stomach (*Graphidium strigosum*) or small intestine (*Trichostrongylus retortaeformis, Trichostrongylus* sp.), with possible consequences on the digestive physiology and growth of the host. Coccidiosis (genus *Eimeria*) is also an important parasitosis in grazing and indoor rabbit breeding systems, but their characterization remains to be determined for outdoor systems. Therefore, our goal was to study gastrointestinal parasitism in grazing rabbits, to improve the knowledge of the parasitic risk of grazing and to consider recommendations for reducing it. In particular, we test the interest of introducing sainfoin (*Onobrychis viciifolia*), a legume rich in condensed tannins, that could limit the parasitic risk in rabbits (Legendre et al., 2017, 2018), as has been shown in small ruminants (Hoste et al., 2015).

MATERIALS AND METHODS

Experimental protocol

The trial was conducted on the experimental domain of the University of Perpignan, in accordance with the specifications for organic rabbit farming (Ministère de l'Alimentation, de l'Agriculture et de la Pêche [MAAP], 2010), during winter 2014/2015, summer 2015 and spring 2016. At each season, 30 rabbits were alloted (according to weaning weight and litter origin) at weaning (between 41 and 48 days of age) in two groups of 15 and housed in movable cages (3 rabbits per cage) from weaning and for 9 weeks, disposed either on "sainfoin" or a "grass" plot. The grazing area of was 0.4 m² per rabbit per day (cages were moved daily). During the winter of 2014/2015, plots of sainfoin (70% of aboveground DM) and pure tall fescue (*Festuca arundinacea*) were grazed for the first time by rabbits. A Mediterranean natural meadow was grazed in the summer 2015 (dominated by grass species) and had been grazed more than one year ago by rabbits. The pure tall fescue plot grazed during the spring of 2016 was the same as those used in winter 2014/2015. It has not been grazed in the meantime. The same plot A of sainfoin was used during the summer of 2015 and winter 2014/2015, with a free period superior to three months between them (56% sainfoin). In spring 2016, rabbits had been grazing plot B of sainfoin which who was grazed less than three months ago (70% sainfoin). Rabbits come from the same breeding unit (reared up to weaning in cage-mobile or in the semi-open building).

Parasitological technique and measurements

Weekly, a sample of at least 10g of feces was collected by hand for each movable cage over the entire grazed area used the day before with a zig-zag pattern. Counts of nematode eggs without distinction of the species and of *Eimeria* oocysts (OPG) by the modified Mc Master method was performed. Every two weeks, *Eimeria* oocysts were identified at the species level according to a list of morphological criteria established by (Coudert et al., 1995). At slaughter (around 100 days of age), stomach, small intestine, cecum and colon of some rabbits (Winter 2014/2015: 10, Summer 2015: 20, Spring 2016: 28) was weighed and stored at -20° C (Hoste, Kerboeuf, and Parodi, 1988) until parasitic assessment carried out on a 50 mL aliquot through sieves (600 μm then 40 μm). The liver was examined for the presence of whitish nodules, characteristic of the presence of *Eimeria stiedai*.

Statistical analyses

Nematode egg counts were normalized by a logarithmic transformation ($\log_{10}(x+1)$), and an analysis of variance for repeated measurements was performed using R software. A linear regression between the number of *Trichostrongylus* sp. (after logarithmic transformation) and average daily gain (ADG), with the type of pasture (grass or sainfoin) as fixed effects was performed using jamovi software. Total faecal oocyst excretion was analysed by the area under the curve method. The correlation table between total and specific oocyst excretion and growth rate was determined using jamovi software. For significant correlations ($P < 0.05$), a linear regression with factors of season and type of pasture was calculated, as well as a logistic regression on the number of oocysts excreted. A Chi² test was set up using the R software to compare the proportions of livers with nodules between treatments.

RESULTS AND DISCUSSION

Parasitological risk

Nematode egg excretion was almost null during winter 2014/2015 and summer 2015. No effect of grazing on egg excretion was detected ($P = 0.70$, Figure 3) during the spring period (2016). In winter 2014/2015 and in summer 2015, the kinetics of oocyst excretion was characterized by a peak (> 50 k.OPG) between 48 and 55 days of age (7 to 14 days post-weaning). At 97 days of age, excretion was reduced (<20 k.OPG). In spring 2016, excretion kinetics were regular on fescue, but remained high at 97 days of age (> 50 k.OPG). For sainfoin, we observed 2 excretion peaks: at 48 days of age, then between 69 and 83 days of age. Mean total oocyst excretion between 48 and 97 days of age, measured during winter 2014/2015 and summer 2015, was 2.6 M.OPG. Nevertheless, we also observed a high individual variability (inter-cages). In spring 2016, the total excretion level doubled (+ 50%) especially on the "sainfoin" grazing (6.5 vs. 4.2 M.OPG) where the return period on the plot was shorter (<3 months), compared to that of fescue grazing (> 1 year). One of the most pathogenic *Eimeria* species (Coudert et al., 2007), *E. flavescens* was identified during the summer of 2015 and the spring of 2016. For the total duration of the fattening in spring 2016, the number of oocysts of *E. flavescens* excreted by rabbits grazing tall fescue was increased by + 72%, compared to those grazing sainfoin (respectively 11682 vs 6796 OPG, P <0.05). This observation on *E. flavescens* could be linked to the higher intake of condensed tannins from the "sainfoin" group, when concentrations of condensed tannins (1.4% tannic acid equivalent) were comparable to those observed in a previous study (Legendre et al., 2018) where a coccidiostatic effect was detected for rabbit reared in a conventional system.

While the prevalence was zero or low in winter 2014/2015 and in summer 2015, spring 2016 was characterized by the high prevalence of *Trichostrongylus* sp. (93% of rabbits). The experimental design did not reveal a significant difference in *Trichostrongylus* sp. intensity of infestation between grazing (P = 0.13) in spring 2016. The prevalence of *Passalurus ambiguus* was still above 70%, but numerically above 90% in summer 2015 (95%) and spring 2016 (90%). *Graphidium strigosum* was only found in two rabbits grazing sainfoin in spring 2016. Rabbits grazing sainfoin in spring 2016 have the highest levels of infestations. The spring season has favorable weather conditions (humidity and temperature) for the Trichostrongyloidea cycle, and presents also favorable climatic conditions for the eimerian cycle. Similarly, Gres et al. (2003) found higher prevalence and intensity of coccidia in the spring for wild rabbit populations. Furthermore, in Spring 2016, rabbits ingested 45% more grass when grazing sainfoin than tall fescue (Legendre et al., 2019), which may have increased the likelihood of ingesting infecting forms and the return time on sainfoin (<three months) was much lower than that for tall fescue (one year). This was probably a determining factor in the increase in *Trichostrongylus* sp. on the sainfoin pasture, and observed pattern of oocyst excretion. It should also be added that, unlike the tall fescue plot, the sainfoin plot was irrigated by sprinkling before the rabbits were placed and thus increasing the moisture. In addition, differences in vegetative structure and plant density could induce differences in the moisture level of the herbaceous layer (Niezen et al., 1998) and exposure to UV rays (Van Dijk et al., 2009), and thus a potentially different number of infesting larvae. Also, high attractiveness of sainfoin to wild rabbits (GNIS, 2013) could lead to more frequent visits, and thus increase the potential transmission of parasites.

Influence on the health and growth of rabbits

Despite significant levels of strongyle intensity (up to 13,000 adults of the genus *Trichostrongylus*), no signs of diarrhea or gross lesions were observed during the study, although these may be difficult to identify. For Barker and Ford (1975) the intensity of *Trichostrongylus retortaeformis* is not necessarily correlated with the intensity of the lesions. Movement of female *Passalurus ambiguus* in the area of the anus can lead to itch and scratches, but we did not observe such problems during our study. Liver examinations revealed a high proportion (64%) of whitish nodules attributable to *E. stiedai*, with no significant differences in distribution according to grazing (P = 1), and between seasons (P = 0.55). After the end of the study and without irrigation, the proportion of liver with nodules decreased (unpublished data) but for Mykytowycz (1962), *E. stiedai* is also subjected to fluctuations in rabbit populations. Macro-lesions due to *Eimeria* spp. were not found within intestines. Post-weaning mortality was less than or equal to 10% for all seasons.

The correlation between oocyst excretion of *E. flavescens* and the growth rate was significant (P <0.05, r = 0, 485, df = 28). Thus, this higher excretion of oocysts in the spring of 2016 would explain 24% (r^2 = 0.24) of the growth rate variations, and in particular the lowest growth observed in the spring of 2016, compared to the other 2 seasons (whatever the grazing). A significant negative correlation between infestation intensity with *Trichostrongylus* sp. and the growth rate [ADG = 1.1 log (*Trichostrongylus* sp. intensity) + 20.8] was detected, but with a low incidence (r = 0.275, i.e. $r^2 = 0.075$, df = 56, P <0, 05). This preliminary result is corroborated by a decrease of 5 g / d between the spring of 2016 and the other observed periods where *Trichostrongylus* sp. had hardly been found. This potential decrease would therefore be related in part to the increase in gastrointestinal nematode populations, without it being possible to rule out a less favorable effect of environmental conditions during the test or during the pre-weaning period at spring on the resilience of the rabbits (Seltmann et al., 2019; Mykytowycz, 1962).

CONCLUSION

Spring presented the higher risk of gastro-intestinal parasitic infestation for rabbits, with reduction of the growth rate but without clinical observations. Our first results suggest to increase the return time of rabbits on the same grazing area, beyond the 2 months recommended in the French specifications of organic rabbit farming but it will be necessary to confirm with larger numbers of animals.

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O5 – P2

Organic rabbit farming: Should we be afraid of gastro-intestinal parasites?

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Organic Rabbit Farming (ORF) and GI Parasites

Photo Gidenne T.

- Link to the soil + « Fresh » Feed
- Restricted used of allopathic antiparasitic drugs

→ Strongyles of stomach (*Graphidium strigosum*) or small intestine (*Trichostrongylus* spp.)

→ Coccidia (*Eimeria* spp.)

Management of parasitism = Major obstacle to the development of ORF (Roinsard et al., 2013)

Experimental Protocol – University of Perpignan

Winter 2014/2015	Summer 2015	Spring 2016

At weaning (41 to 48-day old)

Grass plot

3 rabbits/cage
5 cages

9 weeks

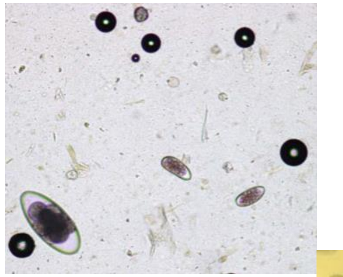
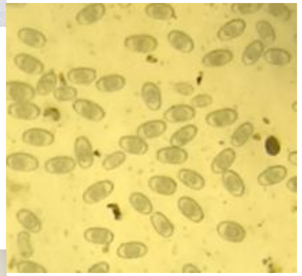
+ 60g/day/rabbit of concentrate pelleted feed

Sainfoin (*Onobrychis viciifolia*) plot

3 rabbits/cage
5 cages

In accordance with French specifications for ORF (MAAP, 2010)

Gastro Intestinal Nematodes (GINs) and Coccidia

Sample 10 g/cage, Once a week

McMaster's method : Fecal eggs count (nematodes - FEC) + Fecal Oocysts counts (coccidian - FOC)

+ Identification at the species level for oocysts, Every 2 weeks

Liver examination after slaughtering

➡ *(Eimeria stiedai)*

Worm counts in stomach, small intestine + cecum and colon

Winter: 10 animals, Summer: 20 animals, Spring: 28 animals

Results - Results Feces

	Winter 2014/2015		Summer 2015		Spring 2016	
	Grass	Sainfoin	Grass	Sainfoin	Grass	Sainfoin
FEC ($\log_{10}$ [Eggs per gram of feces])	0	0	0	0	1.1	1.3
	Almost null				No effect of grazing	
FOC - Eimeria (millions Oocysts per gram of feces)	2.9	1.3	4.1	2.0	4.2	6.5
	FOC X3 on sainfoin when the return period < 3 months + irrigation					
			Identification of *E. flavescens*			

Results – At slaughter

	Winter 2014/2015		Summer 2015		Spring 2016	
	Grass	Sainfoin	Grass	Sainfoin	Grass	Sainfoin
Liver with nodules attributable to *E. stiedai*	40%	60%	70%	70%	67%	62%
Prevalence of *Trichostrongylus* sp.	0%	0%	10%	20%	93%	92%
Prevalence of *Graphidium strigosum*	0%	0%	0%	0%	0%	15% (2/13)

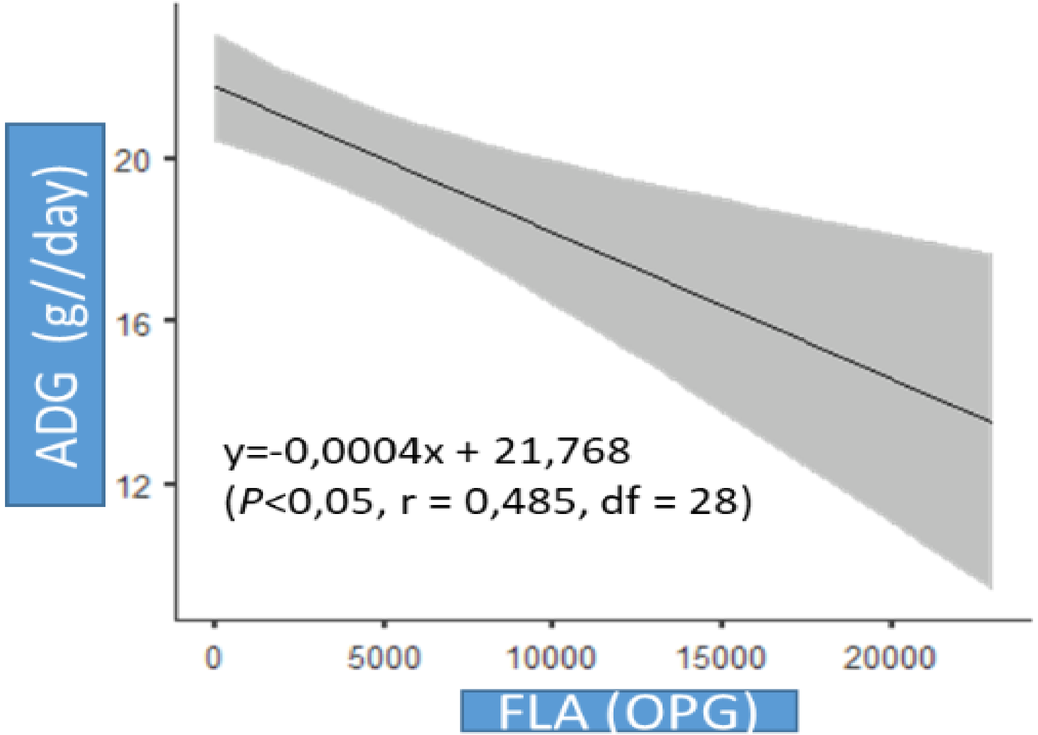
Progressive increased infections by pathogenic species

No signs of diarrhea or macro-lesions (gastro-intestinal tract)

Prevalence = N infected animals/ N sampled animals

Results – Growth

- Significant negative correlation between OPG of *E. flavescens* and growth rate* (see above);
- Significant negative correlation between intensity of *Trichonstrongylus* sp. and growth rate* (r= - 0.275, df =56, P<0.05);
- Growth rate in Spring 2016 decreased by 5g/d
  - Effect of parasitism?
  - Or environmental conditions?



Relation between FOC excretion of *E. flavescens* (OPG) and total average daily gain (ADG, 95% CI)

* Overall ADG from weaning to slaughtering (but animals were weighted weekly)

Conclusions

- Spring = Higher risk of GI parasitic infection for rabbits ?

- Suggestion to prevent parasite infections = to increase return time of rabbits on the same grazing area, beyond 2 months (as recommended in the French specification in 2010).

Mobile cages on sainfoin (Spring 2016)