PROCEEDINGS OF THE 11th WORLD RABBIT CONGRESS

Qingdao (China) - June 15-18, 2016

ISSN 2308-1910

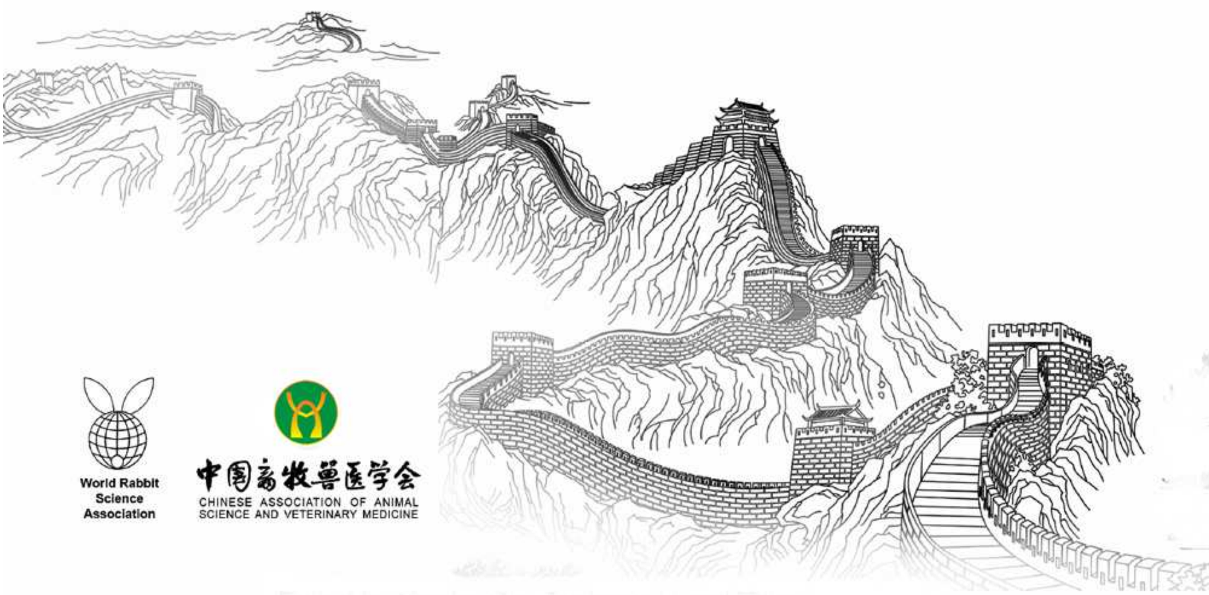
Session
Pathology and Hygiene

Legendre H., Hoste H., Gidenne T.

SAINFOIN IN RABBIT DIET: IMPACT ON PERFORMANCES AND ON A NEMATODE CHALLENGE.

Full text of the communication
+
Slides of the oral presentation

How to cite this paper :
Legendre H., Hoste H., Gidenne T., 2016 - Sainfoin in rabbit diet: impact on performances and on a nematode challenge. *Proceedings 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China, 549-552 + Presentation*



SAINFOIN IN RABBIT DIET: IMPACT ON PERFORMANCES AND ON A NEMATODE CHALLENGE.

Legendre H.[1,2], Hoste H.[2], Gidenne T.[1]

[1]INRA Toulouse, GenPhySE, 31326 Castanet-Tolosan, France;
[2]INRA Toulouse, IHAP, 23 Chemin des Capelles, 31076 Toulouse, France
[*]Corresponding author: thierry.gidenne@toulouse.inra.fr

ABSTRACT

This study aimed to examine the effect of partial substitution of alfalfa (*Medicago sativa*) with a tannin containing Legume sainfoin (*Onobrychis viciifolia*) on rabbit performance, and on a *Trichonstrongylus colubriformis* (Tc) challenge. Sixty-four young rabbits were infected (I) or not (NI) with Tc , and fed *ad libitum* either a control diet (alfalfa base, C) or a sainfoin-enhanced diet (S) to form 4 groups (IC, IS, NIC, NIS). Diets were isoproteic, isoenergetic, isofiber, and anthelmintic free, but the sainfoin diet contained condensed tannins (CT), potentially having an anthelmintic activity. At day 0, sixteen rabbits from each group were infected (group IC and IS) by third-stage larvae of *T. colubriformis.* Fecal egg count (FEC) was measured throughout the experiment, as well as rabbit growth and intake. At the end of the experiment (D45) rabbits were sacrificed and adult worms counted in the small intestine. Weight gain were similar among the four groups (meanly 38.9 g/d), while the feed conversion ratio was better for C than for S groups (2.65 vs 2.88, P<0.001). No significant difference was detected between IS and IC animals, neither in FEC nor in worm numbers and female fecundity (eggs *in utero*). Egg hatching tended to be lower for IS than for IC groups (85.2 vs 58.3%, P=0.077).

Key words: Rabbit, Sainfoin, Condensed Tannins, Gastrointestinal nematodes, growth.

INTRODUCTION

The French specifications for organic breeding requires that rabbits have the possibility to graze. In such conditions, rabbits could be exposed to free living stages of helminthes on pasture. In addition, in organic farming the use of anthelmintics and anticoccidians is restricted. Therefore, it is necessary to use alternative strategies, such the use of condensed tannin-containing plants (Hoste et al., 2012), and in particular sainfoin (*Onobrychis viciifolia*). This legume was historically used to feed rabbits and empirically to purge them of parasites. However to our knowledge, no data are available about the potential effect of sainfoin on rabbit performances or on resistance to helminth. *Trichostrongylus colubriformis* is a ubiquitous parasite, infecting ruminants naturally but has also been found to infest the European rabbit (Audebert *et al.*, 2003; Sommerville, 1963). (Molan et al., 1999)) suggested that sainfoin condensed tannins (CT) extract could disrupt the life cycle of *T. colubriformis*. Our objectives were to determine the effect of CT from sainfoin during a controlled *in vivo* infestation of rabbits by *T. colubriformis,* and the effect of alfalfa replacement by sainfoin in a pelleted diet on performances.

MATERIALS AND METHODS

Animals and experimental design

Sixty-four rabbits (strain INRA 1777) were housed, from weaning (32d old) to slaughter (77d), 2 per cage in an enclosed building, and randomly divided into four groups (balanced according to weight): Infected Control (IC), Infected Sainfoin (IS), Non Infected Control (NIC), and Non Infected Sainfoin (NIS). On the first day of the trial (D-4) animals were fed *ad libitum* either a pelleted experimental diet containing 40% dehydrated sainfoin (Perli variety, containing 4.92 g tannic acid eq/g dry matter and with a protein precipitation activities of 2.21cm/g dry matter) or 40% alfalfa (control diet, table 1). Diets were similar in crude protein and NDF content, and were free of anthelminthic or coccidiostatic. Weight and feed intake (DI) were weekly controlled.

Table 1: Ingredients and chemical composition of the experimental diets

Ingredients (%)	Control "C"	Sainfoin "S"	Chemical composition	C	S
Sainfoin dehydrated	-	39.6	% as fed basis		
Alfalfa dehydrated	40.0	24.0	Crude ash*	8.0	8.0
Wheat	16.0	9.6	Starch*	11.0	6.6
Wheat bran	7.0	4.2	Crude protein*	17.3	17.2
Beet pulp	10.0	6.0	ADF*	21.9	25.2
Soybean meal	6.0	3.6	NDF*	34.0	35.1
Sunflower meal	11.9	7.1	Digestible energy, MJ/kg*	8.97	8.86
Wheat straw	8.0	4.8	At 40% of diet		
Mineral and vitamin premix	1.1	1.1	Total tannins [1]	NA	4.92
			Biological activity [2]	NA	2.21

* calculated values from tabulated data [1]g tannic acid eq/kgDM [2]protein precipitation activities (PPA, cm/gDM) NA=not analyzed

Parasitological techniques

At day 0, rabbits of groups IC and IS were orally infested with 2,125 infective larvae of *Trichonstrongylus colubriformis*. Weekly from D-4 to D17, fecal samples were collected to count fecal worms egg concentration (FEC, in g per gram of fresh feces "EPG") based on the modified McMaster technique (Raynaud, 1970), and twice a week after D21. Total fecal excretion was weighed once a week to determine the total fecal egg output. Fecal dry matter was determined at D22 and D25, during a digestibility trial. At D39, 75g of feces were stored for 10 days at 23°C in humid conditions. Larvae extracted with a Baermann apparatus for 6 hours, were counted and the number of larvae was extrapolated to the total amount of cultured feces and divided by the number of fecal eggs, in order to calculate an egg hatching ratio. Rabbits were sacrificed on day 45. The small intestine was entirely and individually collected, as well feces in the distal colon. The small intestine was opened and washed to collect digesta on a sieve. In a 10% aliquot, adult worms were counted and sex identification was performed. The survival rate (% of initial dose) was estimated by the number of adult worm on the number of infective larvae. The number of eggs contained *in utero* of 10 female worms was also determined (Fecundity).

Statistical Analysis

All data were analyzed using R and checked for normality with Shapiro-Wilk test or D'Agostino-Pearson test (in presence of ex-aequo). FEC values were log(n+1)-transformed, and analyzed using T-tests. Wilcoxon and Kruskal-Wallis tests were performed on FEC values when normality could not be obtained. Due to problems at infestation, some rabbits were not infested (five for each group) and were thus excluded from the trial. In the case of unequal samples, an equality of variance test was used.

RESULTS AND DISCUSSION

During the experiment, health status of rabbits was good, and no mortality was observed. Live weight at trial start and end, and growth was similar between all groups, regardless if they were infected or not, although the growth tended (P=0.06, table 2) to be 2% lower for S groups (IS & S). The feed intake was 6% lower for C compared to S groups (-5.2g DM/d; P =0.005), and was not affected by worm infection. Accordingly, the feed conversion ratio was lower for the control groups (IC & C) compared to S groups (2.65 vs 2.88, P<0.001). As diets were calculated to isoproteic, isoenergetic and isoNDF but differed in ADF, these results suggested that either ADF impact the feed efficiency or also the CT impaired the protein digestion. Further digestibility and CT measurements will be performed to clear this point.

Table 2: Effect of infection and diet on growth performance in young rabbits.

		Groups					*P* value		
	n	IC	IS	NIC	NIS	*RMSE*	*I * Diet*	*Inoc.*	*Diet*
Live weight D0 (g)	4 x16	625	641	624	635	*68.8*	*0.89*	*0.84*	*0.43*
Live weight D45 (g)	4 x 16	2419	2391	2492	2392	*149.5*	*0.34*	*0.34*	*0.099*
Weight gain from D0 to D42 (g/d)	4 x 16	38.8	38.2	40.2	38.2	*2.6*	*0.30*	*0.27*	*0.06*
Feed intake from D0 to D42 (g DM/d)	4 x 8	103.1 [a]	110.1[b]	106.0 [ab]	109.5 [b]	*7.1*	*0.33*	*0.51*	*0.005*
Feed conversion ratio	4 x 8	2.66 [a]	2.89 [b]	2.64 [a]	2.87 [b]	*0.2*	*0.92*	*0.71*	*<0.001*

Means with different letters on the same row differ significantly (Bonferroni test). RMSE: root mean square error

Throughout the experiment, egg output remained quite low and no differences of FEC were observed (table 3), although variability between samples was high (± 200). Same observations on total fecal output could be done (20558 eggs, *P* value = 0.25), and on fecal output expressed in egg per gram in dry matter (168 eggs, *P* value = 0.75). Considering the kinetic of FEC (figure 1), a difference was solely observed on D32, as FEC of IC group was lower than IS group (-198 EPG, *P* value = 0.026). FEC from feces collected directly in distal colon did not differ between treatments (*P* = 0.20).

Table 3: Effect of diet on daily excretion of fecal eggs, egg hatching, survival rate, sex ratio and fecundity of *T. colubriformis*.

	n	IC	IS	RMSE	*P* value
Fecal eggs (eggs/g) [1]	15	253	267	*213.8*	*0.57*
Egg hatch (%) [2]	10	85.2	58.3	*28.9*	*0.077*
Fecal eggs at slaughter (eggs/g)	22	473	327	*245.3*	*0.20*
Survival rate (% of initial dose)	22	47.	43.7	*1.3*	*0.50*
Fecundity (eggs *in utero* per female)	28	13.6	13.9	*2.9*	*0.83*

[1] Average of samples day. Means are back transformed from log-transformation for statistical analysis ; [2] Percentage of GIN's eggs in feces hatching and developing to L-3 larvae after 10 days of incubation

Survival rate was similar in both groups (P= 0.50), and after D45 more than 40% of the infective larvae were able to settle. Rabbits received experimental diets 5 days before infestation, yet feed intake was low (49 g DM/d and 59g DM/d for the groups IC and IS, resp.), and therefore CT concentrations in the digestive tract could be too low to impair larvae implantation, as seen in previous studies (Hoste *et al.*, 2012). But Min *et al.* (2004) suggested that CT acts to reduce helminthes fecundity rather than survival in the digestive tract.

However, the number of eggs *in utero* (13.8) did not differ between IC and IS group. In contrast, the eggs hatching tended to differ according to the diet: with a lower value for IS compared to the IC group (58.3 % *vs* 85.2%, *P* = 0.077). Collas et *al.* (2013) reported a similar effect of sainfoin CT in horses. For Molan *et al.* (1999) this ability of CT was thought to be linked to the exposure of eggs to CT during their development, as CT are not absorbed from the digestive tract but remained in the faeces.

Rabbits and horses are both monogastric herbivores and therefore the action of CT could be different in their digestive tracts compared to ruminants, which could explain the absence of differences of FEC but also the percentage of egg hatching difference outside digestive tract. Another possible may be the effect of pelleting and dehydrating the sainfoin on their CT properties. But Terrill *et al*. (2007) found that pelleting S*ericea lespedeza* at a maximum of 70°C does not impact anthelmintic properties, and even improve them.

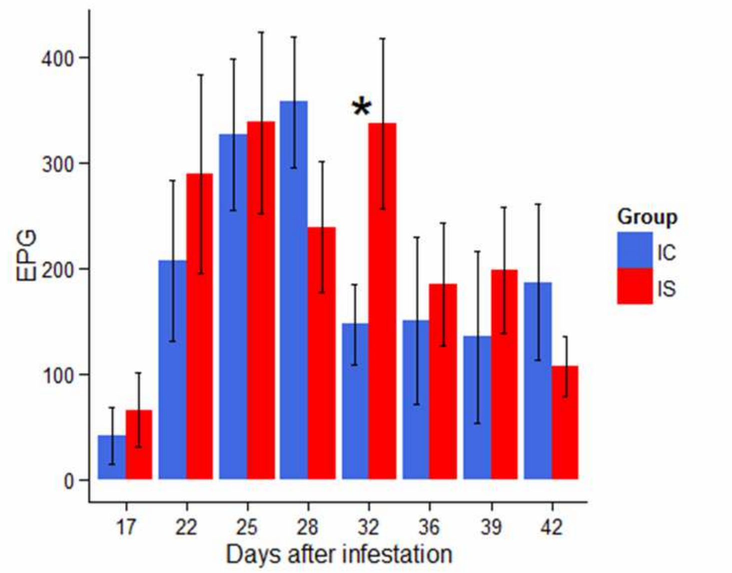


Figure 1: Comparison of back transformed means of log fecal egg counts on eggs per gram (EPG) in groups receiving control diet (blue) or sainfoin diet (red). Error bars indicates standard error. **P<0.05*.

CONCLUSIONS

Substitution of alfalfa by sainfoin (40%) did not affect rabbit growth, but improved the intake and impaired the feed conversion. The worm infection did not affect rabbit performances. Expected anthelmintic properties of CT from sainfoin were only detected for the worm's egg hatching, while fecal egg excretion, worm number and worm fertility remained unaffected.

ACKNOWLEDGEMENTS

This work was funded by the INRA metaprogram GISA (project PROF). The authors thank all the colleagues involved in data collection and analysis, especially animal keepers from ENVT and Dr Sotiraki of Veterinary Research Institute of Thessaloniki for providing larvae of *T. colubriformis*. We also thank MG2Mix society (Rennes, France, www.mg2mix.fr) for the furniture of dehydrated pellet of sainfoin.

REFERENCES

Audebert, F., J. Cassone, D. Kerboeuf, and M. C. Durette-Desset, 2003, Development of Trichostrongylus colubriformis and Trichostrongylus vitrinus, parasites of ruminants in the rabbit and comparison with Trichostrongylus retortaeformis: Parasitol Res, v. 90, p. 57-63.

Collas, C., G. Fleurance, W. Martin-Rosset, J. Cabaret, L. Wimel, and B. Dumont, 2013, Alimentation à l'herbe de la jument de selle en lactation : quels effets d'une complémentation énergétique sur l'ingestion, les performances zootechniques et l'état parasitaire ?, 39ème Journée de la Recherche Equine, Paris, FRA, IFCE, p. 131-134.

Hoste, H., C. Martinez-Ortiz-De-Montellano, F. Manolaraki, S. Brunet, N. Ojeda-Robertos, I. Fourquaux, J. F. J. Torres-Acosta, and C. A. Sandoval-Castro, 2012, Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infections: Veterinary Parasitology, v. 186, p. 18-27.

Min, B. R., W. E. Pomroy, S. P. Hart, and T. Sahlu, 2004, The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing wether goats: Small Ruminant Research, v. 51, p. 279-283.

Molan, A., G. Waghorn, and W. McNabb, 1999, Condensed tannins and gastro-intestinal parasites in sheep: Proceedings Of The Conference-New Zealand Grassland Association, p. 57-62.

Raynaud, J. P., 1970, [Study of the efficiency of a quantitative coproscopic technic for the routine diagnosis and control of parasitic infestations of cattle, sheep, horses and swine]: Ann Parasitol Hum Comp, v. 45, p. 321-42.

Sommerville, R. I., 1963, Distribution of Some Parasitic Nematodes in the Alimentary Tract of Sheep, Cattle, and Rabbits on JSTOR: The Journal of Parasitology, v. 49.

Terrill, T. H., J. A. Mosjidis, D. A. Moore, S. A. Shaik, J. E. Miller, J. M. Burke, J. P. Muir, and R. Wolfe, 2007, Effect of pelleting on efficacy of sericea lespedeza hay as a natural dewormer in goats: Veterinary Parasitology, v. 146, p. 117-122.

11th WRC - 17th June 2016

Sainfoin in rabbit diet: impact on performances and on a nematode challenge

Héloïse LEGENDRE,
Hervé HOSTE, and Thierry GIDENNE

Background

French specifications for organic rabbit farming:

- Rabbit = Herbivore → **Grazing**

↗ **Gastro-intestinal parasite infestation**

↘ **Impairs health and growth**

- ~~Synthetic chemical drugs~~

= **Need for alternative strategies**

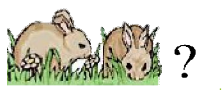
- Plant rich in Condensed Tannins : Sainfoin

.02

Condensed Tannins

- Plant Secondary Metabolites (under stress);
- Positive effects on **Ruminants**:
  - Biology disruption of **Nematodes** (and Coccidia),
  - Preservation performances.

Ruminants



Monogastrics

Protocol

Effects of sainfoin in dehydrated pellet feed, and ***Trichostrongylus colubriformis*** **challenge:**

64 growing rabbits, indoor

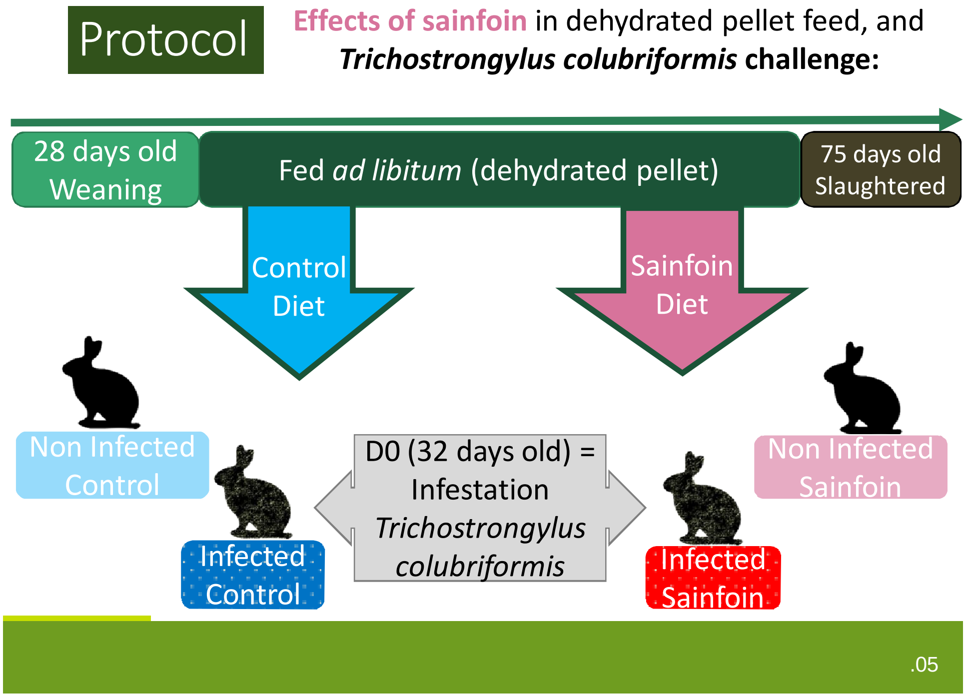
28 days old Weaning → Fed *ad libitum* (dehydrated pellet) → 75 days old Slaughtered

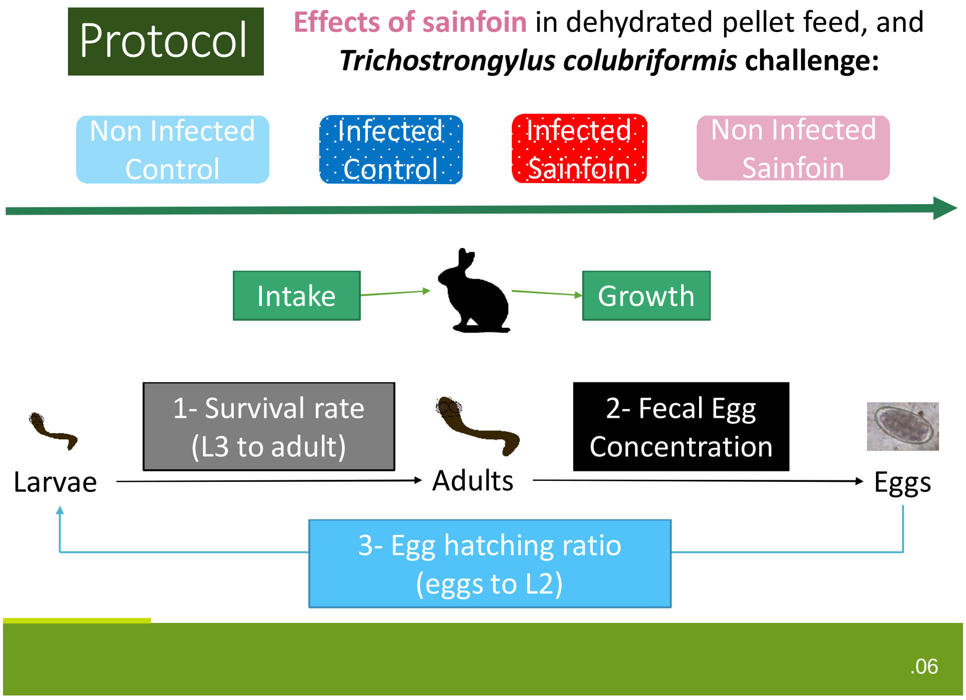
Control Diet

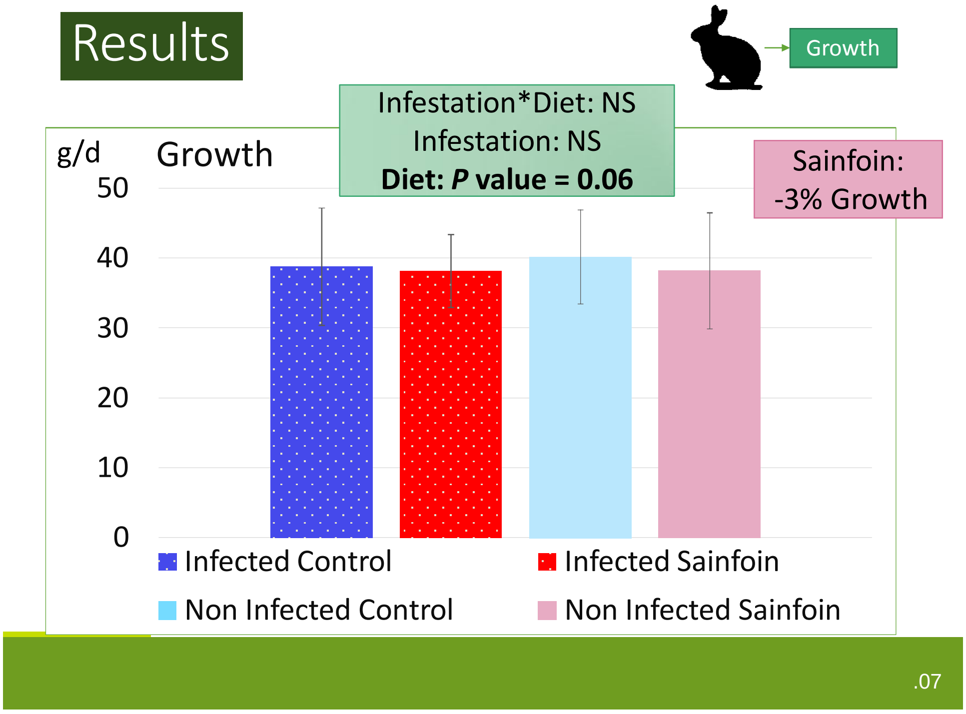
Alfalfa base
1% TA eq.

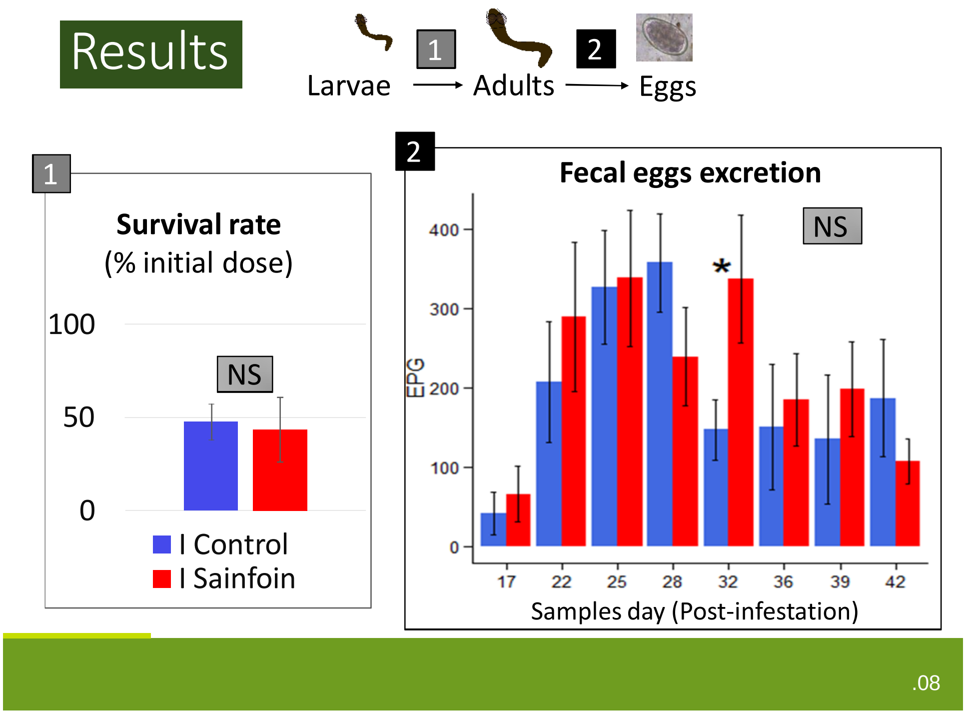
Sainfoin Diet

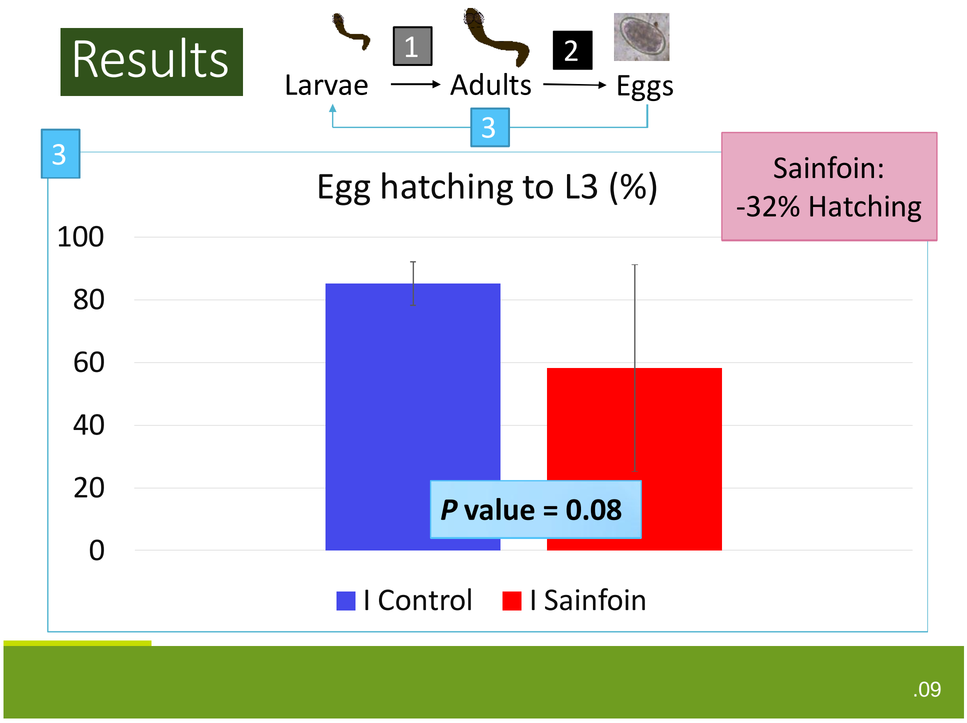
40% Sainfoin
(substitution of Alfalfa base)
1.8% TA eq.

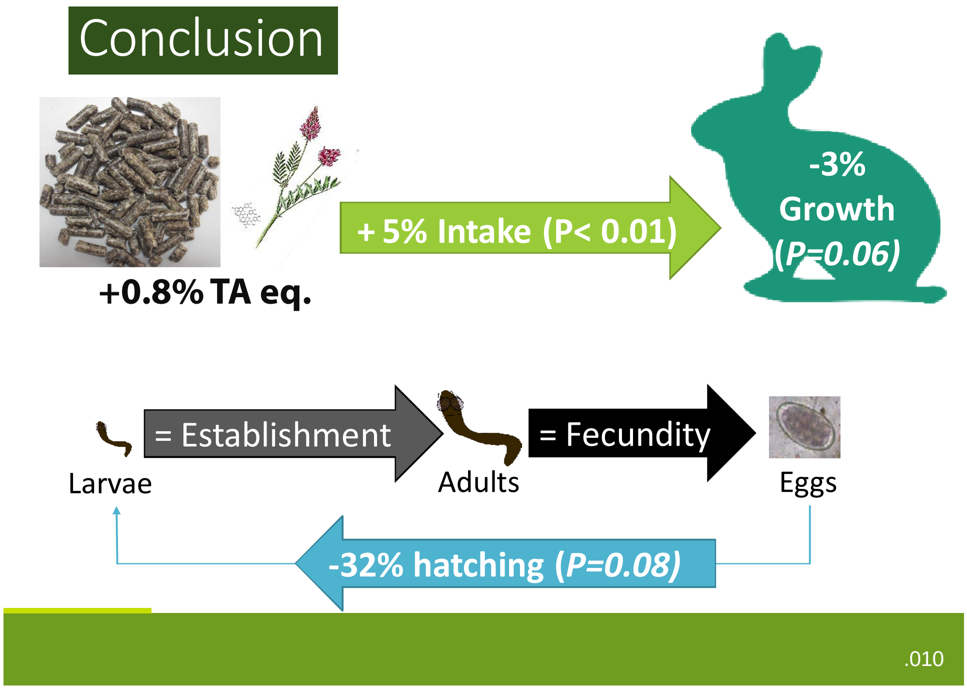

Protocol
Effects of sainfoin in dehydrated pellet feed, and
Trichostrongylus colubriformis challenge:
28 days old
Weaning
Fed *ad libitum* (dehydrated pellet)
75 days old
Slaughtered
Control
Diet
Sainfoin
Diet
Non Infected
Control
Infected
Control
D0 (32 days old) =
Infestation
Trichostrongylus
colubriformis
Infected
Sainfoin
Non Infected
Sainfoin
.05


Protocol
Effects of sainfoin in dehydrated pellet feed, and
Trichostrongylus colubriformis challenge:
Non Infected
Control
Infected
Control
Infected
Sainfoin
Non Infected
Sainfoin
Intake
Growth
1- Survival rate
(L3 to adult)
2- Fecal Egg
Concentration
Larvae
Adults
Eggs
3- Egg hatching ratio
(eggs to L2)
.06


Results
Growth
Infestation*Diet: NS
Infestation: NS
Diet: P value = 0.06
g/d
Growth
Sainfoin:
-3% Growth
50
40
30
20
10
0
Infected Control
Infected Sainfoin
Non Infected Control
Non Infected Sainfoin
.07


Results
Larvae
1
Adults
2
Eggs
1
Survival rate
(% initial dose)
100
50
0
NS
I Control
I Sainfoin
2
Fecal eggs excretion
NS
*
EPG
400
300
200
100
0
17
22
25
28
32
36
39
42
Samples day (Post-infestation)
.08


Results
Larvae
1
Adults
2
Eggs
3
3
Egg hatching to L3 (%)
Sainfoin:
-32% Hatching
100
80
60
40
20
0
P value = 0.08
I Control
I Sainfoin
.09


Conclusion
+0.8% TA eq.
+ 5% Intake (P< 0.01)
-3%
Growth
(P=0.06)
= Establishment
= Fecundity
Larvae
Adults
Eggs
-32% hatching (P=0.08)
.010


Thank you for
your attention