IN VIVO ASSESSMENT OF THE ANTHELMINTIC EFFECTS OF BY-PRODUCTS (PEELS) FROM THE CHESTNUT INDUSTRY.

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GASTROINTESTINAL NEMATODES (GINs) of SMALL RUMINANTS

Haemonchus

Teladorsagia

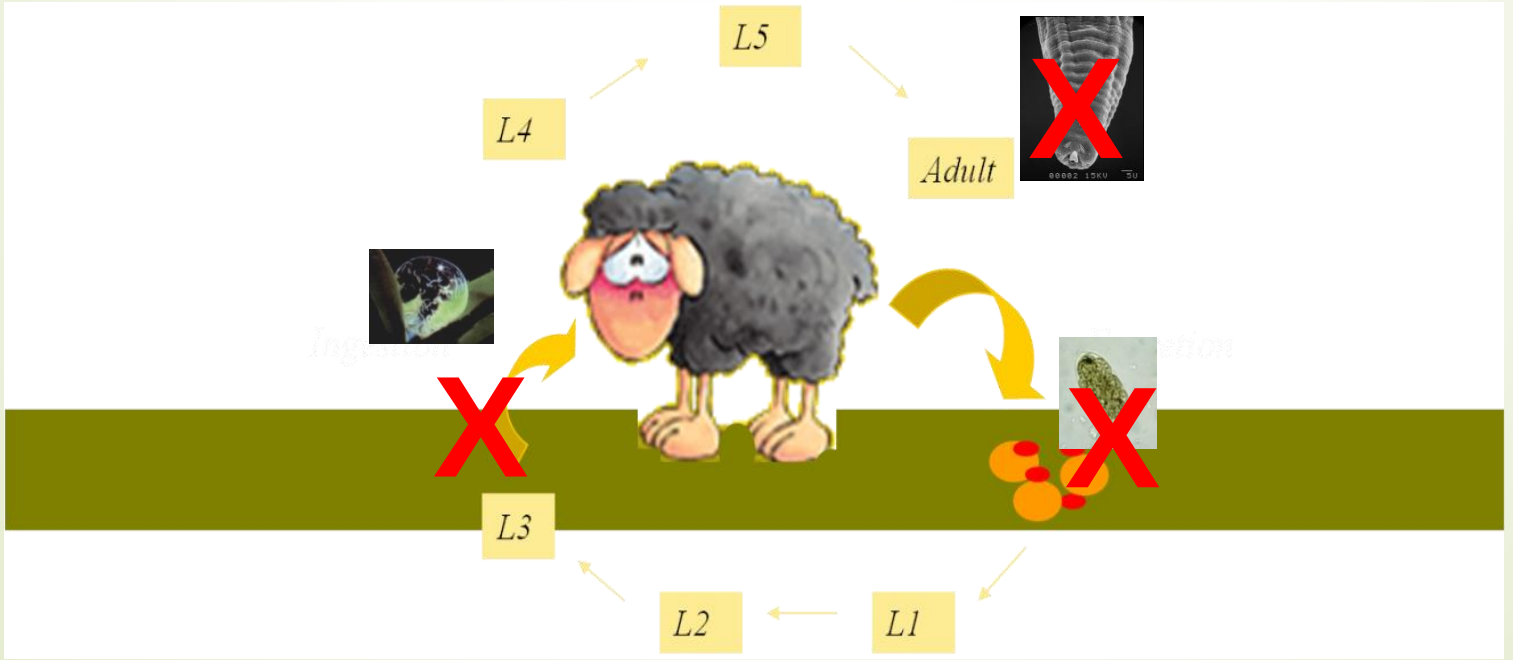
Trichostrongylus

- Grazing = GINs
- Ubiquitous
- Main pathological consequences
  - Production losses
  - Clinical signs and possible deaths
- According to the FAO, the main parasitic infections in Ruminants, worldwide.

CONTROL of GINs = SYNTHETIC ANTHELMINTIC DRUGS

ANTHELMINTIC (AHs) TREATMENTS

=



INCREASED LIMITS on the USE of AHs and NEED for ALTERNATIVE SOLUTIONS of CONTROL.

Limits of AHs

- Worlwide development and diffusion of AH resistance in GINs
- Increasing number of multiresistant isolates in small ruminants
- **Exclusive reliance on AH is not a sustainable option**

- Increased expectations to reduce chemicals in agriculture (e.g OF products)
- Increased constraints in the regulation to use commercial AHs

Seek for alternative solutions

- Biological control, vaccine, genetic selection ……..
- interaction host nutrition
- **Natural bioactive compounds of plants**

Sainfoin and other tannin rich Legumes as nutraceuticals

5

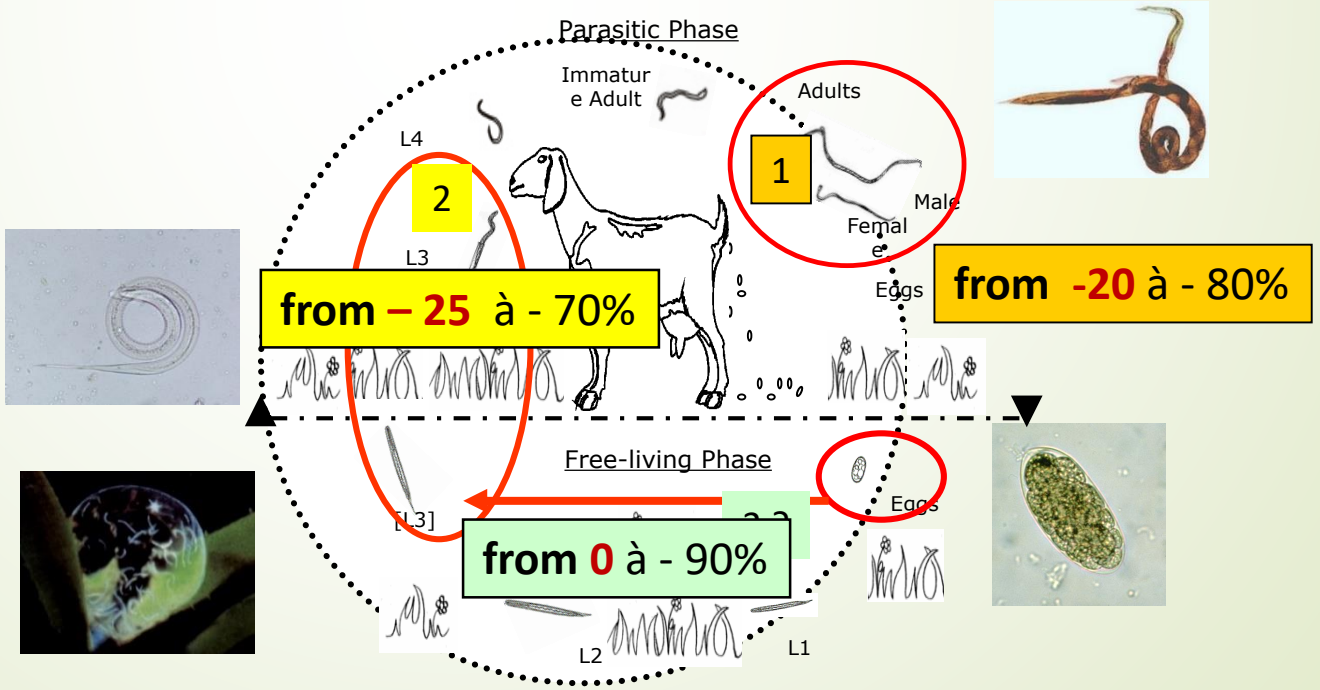
NUTRACEUTICALS in VETERINARY SCIENCES

A feed (a forage) which combined effects on animal **nutrition + health** (Andlauer & Furst, 2002).

It has to be consumed at **a certain level of concentration for several days**

A nutraceutical is proposed but not imposed to the animals. The effects depend on the animal **Voluntary Feed Intake (VFI)**

The **bioactivity** is related to plant secondary metabolites = **PSMs** (e.g. Tannins)



AGRO INDUSTRIAL BY-PRODUCTS : *in vitro* and *in vivo* Results

- Extracts of tannin rich barks of different trees

- Quebracho (*Athanasiadou et al, 2000, 2001, Paolini et al 2003 a,b)*
- Acacia sp (*Cenci et al, 2007, Minho et al 2008; Max et al, 2010, Zabre et al, 2018*)

- By products of carob industry
  (*Silanikove et 2006, Manolaraki et al, 2010, Sotiraki et al, 2013*)

- By products of Nuts : Hazel nut peels (*Desrues et al, 2012)*
  Use of Chest nuts « skins » ?

in vitro RESULTS

ID	Plant	Type	Tannin Content (TT)	Phenol Content (TP)	EC 50
1	Skin of Chestnut	BIO	5.13%	5.71%	67.4
2	Skin of Chestnut	BIO	2.46 %	2.80%	63.2
3	Skin of Chestnut	Conventional	8.12%	8.96%	82.19
4	Skin of Chestnut	Conventional	4.27%	4.81%	75
5	Skin of Chestnut	OF	4.60%	5.05 %	**30.6**
6	Skin of Chestnut	OF	3.99%	4.57%	75
7	Skin of Chestnut	Conventional	4.43%	4.91%	95.5
8	Skin of Chestnut	Conventional	6.78%	7.53%	80
9	Bugs of Chestnut	Conventional	1.70%	1.84%	140
10	Chips of Chestnut	France	5.19%	5.65%	90.2
11	Chips of Chestnut	Chile	2.50%	2.68%	> 150
12	Leaves of Chestnut	Conventional	NA	NA	124
13	Skin of Chestnut	Spain	6.13%	6.54%	90, 6
14	Skin of Chestnut	Spain	5.11%	5.45%	70

Sainfoin (LEIA method): Mean EC50 = 364 µg/ml (from 84 µg/ml to 630 µg/ml)

OBJECTIVES = To confirm these preliminary data in *in vivo* conditions

In vivo STUDY = MATERIALS and METHODS

- 4 groups of 7 experimentally Infected lambs (on D0)

  5000 L3 *Haemonchus contortus* + 2300 L3 *T colubriformis*

- Distribution of diets starting on D-5. Necropsies on D56
- 4 isoproteic pellets

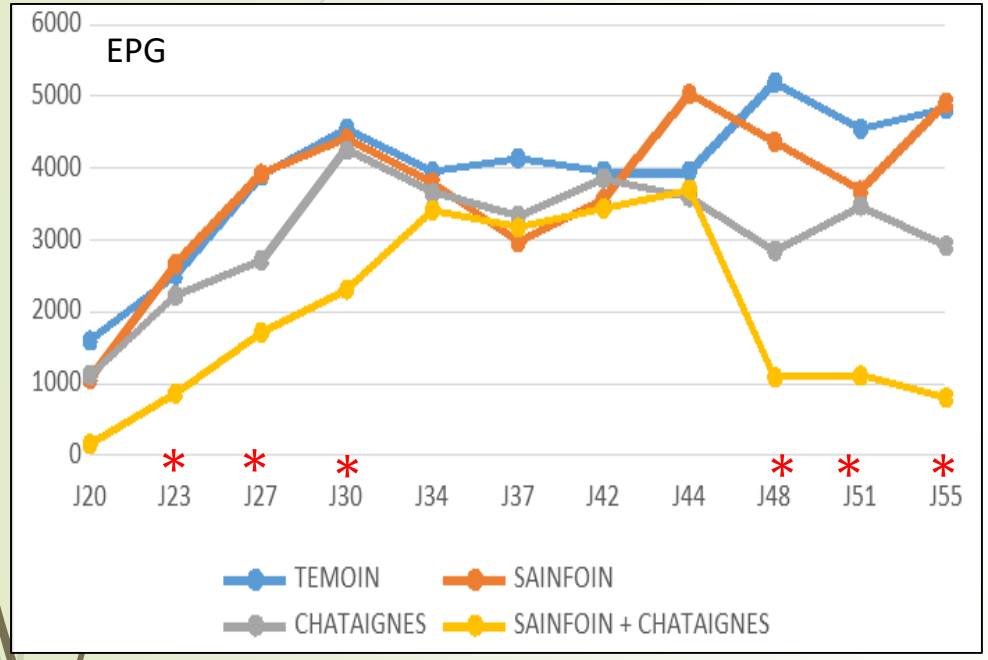
Control / Sainfoin / Chestnut « skins » / Sainfoin + Chestnut « skins »

- Measurements of Total Phénols (TP) and Total Tannins (TT)
- Analyses of the structure of tannins

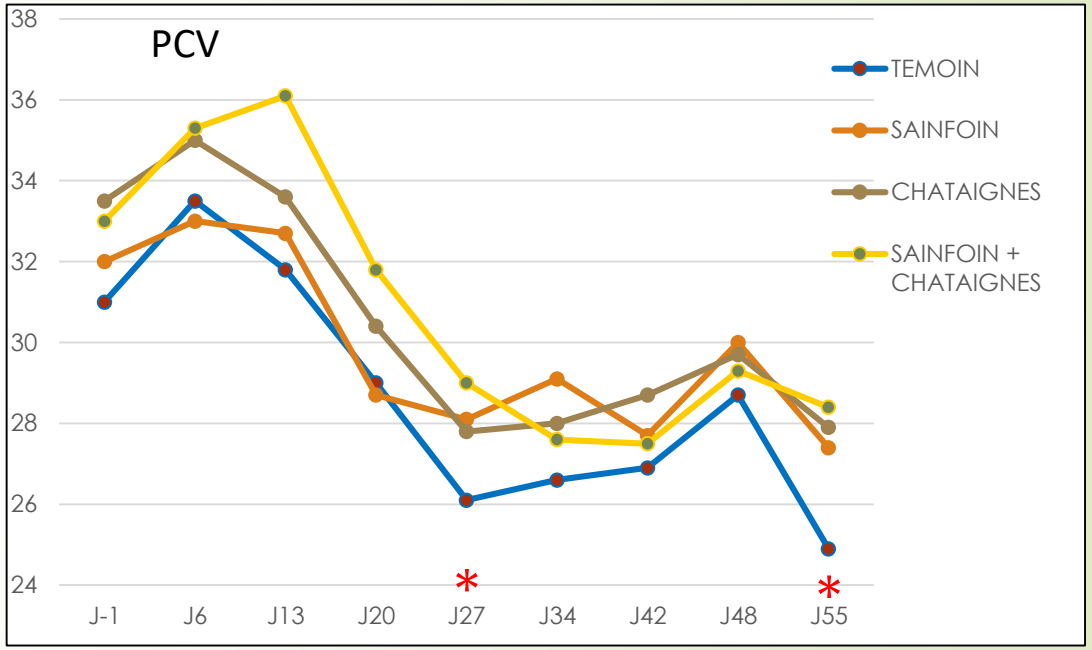

TEMOIN
SAINFOIN
CHATAIGNES
SAINFOIN + CHATAIGNES

In vivo RESULTS

Fecal Egg Counts



Packed Cell Volume



In vivo RESULTS

	Total Phenols	Total Tannins	*Haemonchus contortus*	*Trichostrongylus colubriformis*
Control	0	0	1716	372
Sainfoin	2.2	2.08	2155	340
Chetsnut	4.1	3.9	2300	537
Sainfoin + Chestnut	1.6	1.4	864 *	432
			P < 0,05	NS

Mean establishment rate for *Haemonchus* larvae = 35 %; for *T colubriformis* = 18,3 %

in vivo Results = larval cultures

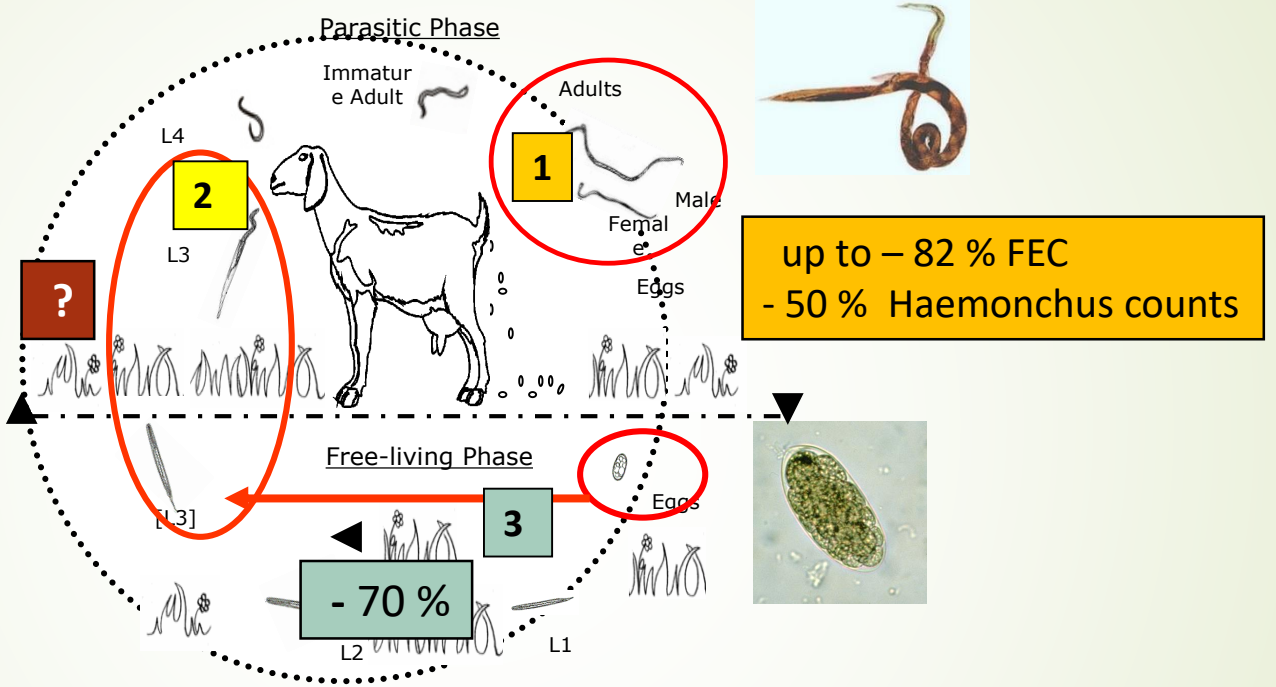
- Ratio *Haemonchus / Trichostrongylus* = 70- 80 % vs 30 -20 %
  Difference NS entre les groupes

- Yield of group larval culture

	J27	J34	J41	J48	mean
Group C	15	10,4	10	11	11,6 %
Group S	16	20,6	16	15	16,9 %
Group Ch	8	9,7	6	8	7,9 %
Group S+Ch	**4**	**1,2**	**6**	**3**	**3,6 %**

Discussion / Conclusions

- **Parasites populations :** No effects of Sainfoin and chestnut pellets
  Statistical effects with Sainfoin + Chestnut pellets



- **Resilience** (PCV) some effects on PCV for tannin containing pellets vs controls

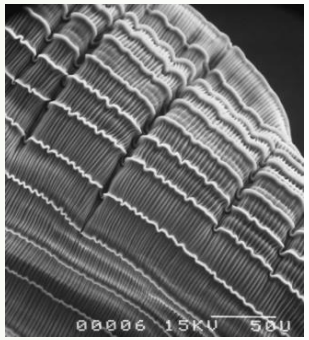
- **Total tannins:** S + C= 1,4; S= 2,1; C = 3,9
- Possible interactions between tannins ???

Thank you for your attention !
Any Questions ?

THANK YOU FOR YOUR ATTENTION

MODE of ACTION against the different STAGES of GINs ?

- The main hypothesis on the mode of action is a **direct (pharmacological-like) effect**

	CONTROL	SAINFOIN	TZALAM
CUTICLE			
CEPHALIC REGION			

FUNCTIONAL CONSEQUENCES

1) Infective larvae (L3)

Reduced establishment

2) Adult worms

Motility

Nutrition and Reproduction

Viability

Martinez Ortiz de Montellano et al, 2013, Kommmuru et al, 2014

MODE of ACTION against the GINs ?

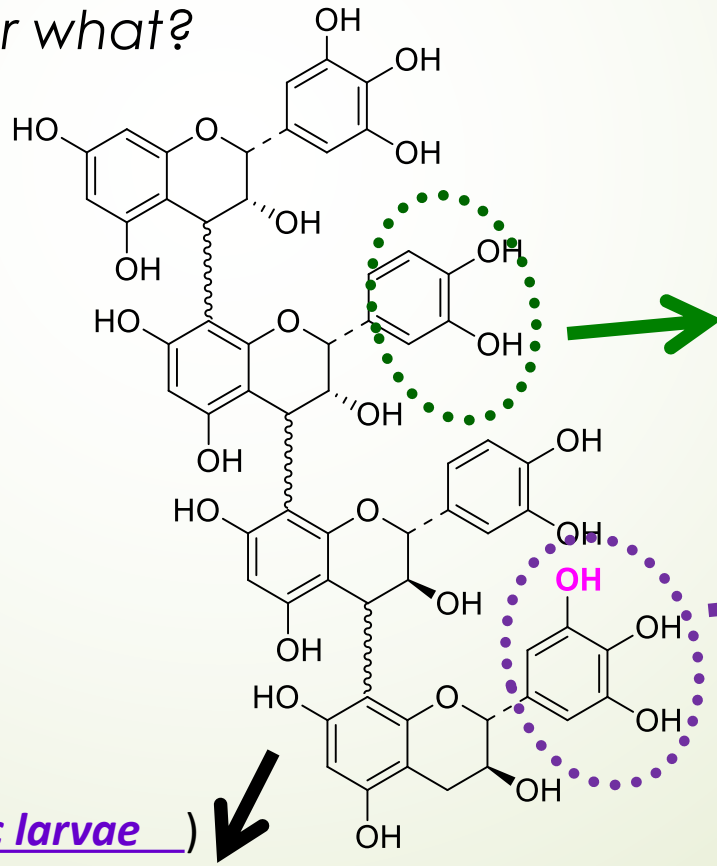
1 CT and also some flavonoids are responsible for the AH properties

(Molan et al, 2003, (Barrau et al 2006,Brunet et Hoste, 2006, Novolbisky et al, 2013, Quijada et al, 2015)

2 *in vitro* and *in vivo* results : Dose dependent activity – Threshold

(Terrill et al, 2007, Brunet el, 2007)

3 *Which tannins are best for what?*



Procyanidin tannins
(***amino acid absorption***)

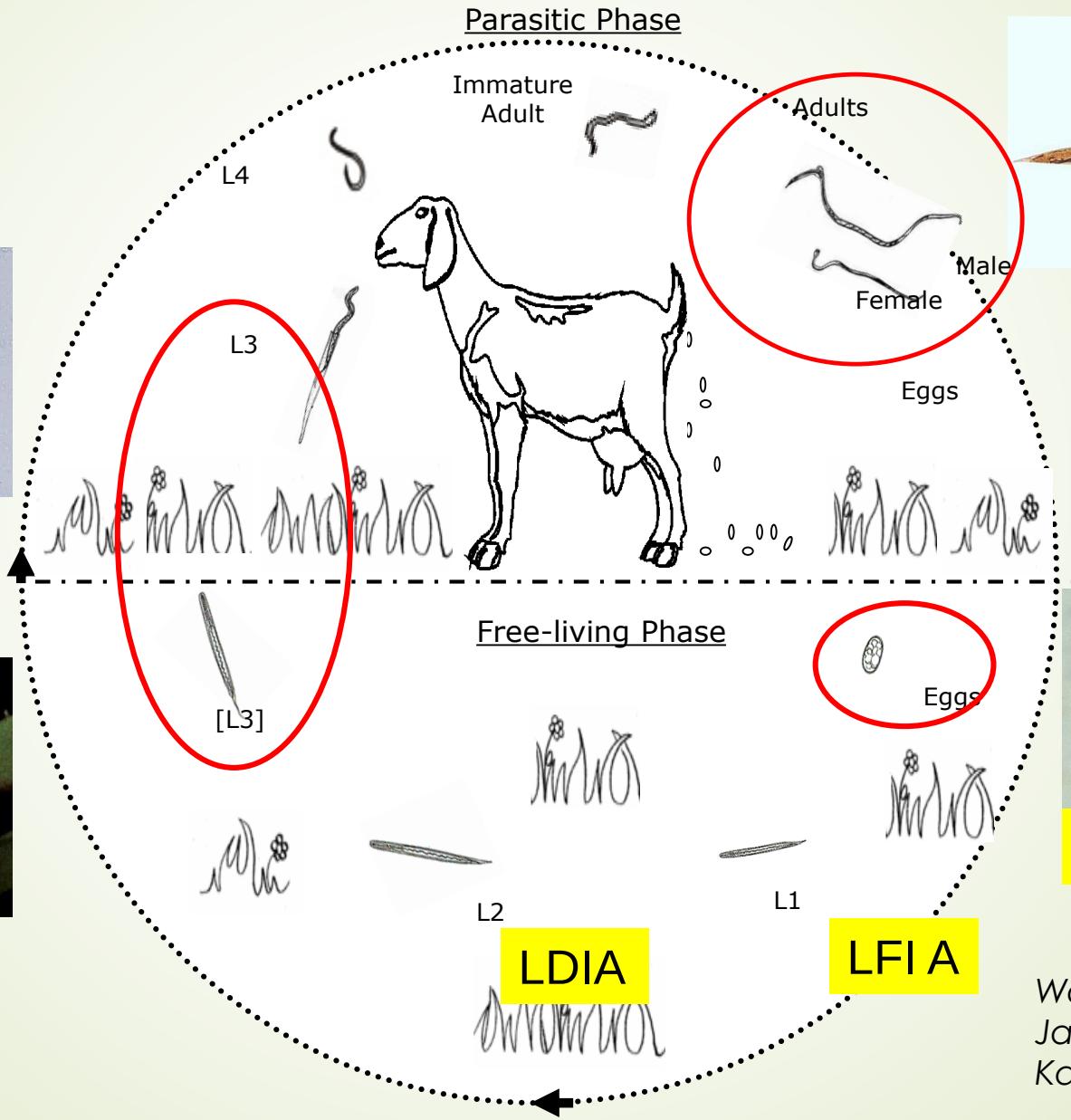
Prodelphinidin tannins
(***parasitic worms***)

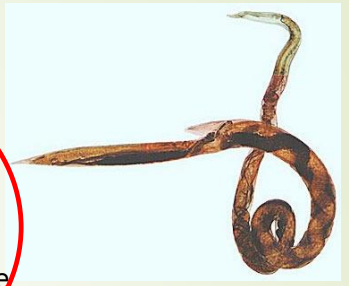
Larger tannins
(***parasitic larvae***)

(Mueller-Harvey 2006; Waghorn 2008)

IN VITRO METHODS TO SCREEN AH PROPERTIES

LMIA
LEIA





AMIA
C. elegans

EHA

Wood et al, WAAVP guiidelines 1995
Jackson and Hoste, 2010
Katiki et al., 2013

WHAT ELSE ?

Other RESSOURCES ? Other LIVESTOCK SPECIES ? Other PARASITES ?

1. **Cultivated Legume forages**
   - Sulla (*Hedysarum coronarium*),
   - Sainfoin (*Onobrychis viciifoliae*)
   - Sericea lespedeza (*Lespedeza cuneata)*



2. **Browsing tropical Legumes**

3 **Exploiting agro industrial by-products (Caroob, nuts, cocoa..)**


4 GIN in cattle and pigs (Williams et al, 2014; Desrues et al, 2015)

5 Coccidia in small ruminants and rabbits