EFFECT OF CT DIVERSITY, SYNERGY AND PELLETING TREATMENT ON *IN VITRO* ANTHELMINTIC ACTIVITY AGAINST *HAEMONCHUS CONTORTUS*

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ABSTRACT

Condensed tannins are known to have anthelmintic properties and can be used in an integrated approach to control gastrointestinal induced pathologies of small ruminants.
Pelleting condensed tannins-rich plants allows their availability during the whole year and facilitates their use by farmers. Several authors have shown that condensed tannin profiles differ between plant species and anthelminthic activity can be influenced by the chemical structure of the condensed tannins (Mueller-Harvey *et al*., 2019). To evaluate the effect of condensed tannin diversity and pelleting process on anthelmintic properties, this study was conducted on condensed tannins extracts of three tropical plants (*Manihot esculenta, Leucaena leucocephala*, *Cajanus cajan)* and their mix.

Two *in vitro* assays: the larval development inhibition assay and the larval exsheathment inhibition assay were used to evaluate anthelmintic activities of extracts of both plants and pellets against *Haemonchus contortus*. Half maximum effective concentration (EC_{50}) was calculated for each extract. HPLC profiles and free flavan-3-ol determination were performed on extracts of condensed tannins from plants and pellets**.**

Results showed that anthelmintic properties were dose-dependent and varied according to condensed tannin profiles (Tables 1 and 2). Regarding development inhibition, EC_{50} values confirmed the important anthelmintic potential of *M. esculenta* extract (Marie-Magdeleine *et al.*, 2010), that was more active than the two other plants and the mix. Concerning exsheathment inhibition, condensed tannins of *L. leucocephala* and *C. Cajan* were the most active. Pelleting decreased the efficacy of the extracts but did not cancel the anthelmintic activity. A synergistic effect was found only for larval exsheathment.

The differences observed between plants could be explained by the differences in condensed tannins composition reflected by the chromatographic profiles. HPLC profiles showed that *M. esculenta* was different from *C. cajan* and *L. leucocephala* due to a larger amount of one compound (more than 70% of the condensed tannins concentration). All extracts contained free flavan-3-ols, mainly Prodelphinidins, that are differentiated from Procyanidins by the presence of an additional hydroxyl on the B-ring. Prodelphinidins are reported to be more active on the exsheathment of *H. contortus*, *in vitro* (Brunet and Hoste, 2006). *C. cajan* and *L. leucocephala* had greater amounts of Prodelphinidin monomers in their composition compared to *M. esculenta*. The pelleting process increased the amounts of free flavan-3-ols in the pellets. All pellet extracts contained a greater amount of Prodelphinidins than plant extracts. Our results also confirmed the synergistic effect of condensed tannins and flavonoid monomers on larval exsheathment of *H. Contortus* found *in vitro* by Klongsiriwet et al., (2015).

Keywords: Condensed tannins, larval development, larval exsheathment, pelleting process, synergistic effect, *Haemonchus contortus*.

References

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Table 1. Effect of plant pelleting on larval development inhibition of *Haemonchus contortus*.

		Larval development (%)							
Plant	Species	*C cajan*		*L. leucocephala*		*M. esculenta*		Mix	
	Sample	Leaves	Pellets	Leaves	Pellets	Leaves	Pellets	Leaves	Pellets
CT Dosis (mg/ml)	**0**	76,5	76,5	76,5	76,5	76,5	76,5	76,5	76,5
	0,1	72,1a	56,5b	25,1d	58,8b	25,0d	56,1b	Nd	51,6c
	0,5	73,4a	41,2b	20,9d	42,3b	13,9e	14,0e	36,7c	37,5c
	1,25	56,8a	18,4b	0,0	8,8d	0,0	3,7e	0,4f	12,7c
	2,5	26,0a	0,0b	0,0b	0,0b	0,0b	0,0b	0,0b	0,0b
	5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	SEM	1,34	1,34	1,34	1,34	1,34	1,34	1,32	1,04
EC_{50} (mg/ml)		0,934	0,868	0,489	0,729	0,195	0,182	0,51	0,752

[a–c] Means within a row with different superscript letters differ significantly (p<0.05).

EC_{50} : half maximum effective concentration

Table 2. Effect of plant pelleting on larval exsheathment inhibition of *Haemonchus contortus*

		Larval exsheathment (%)							
Plant	Species	*C cajan*		*L. leucocephala*		*M. esculenta*		Mix	
	Sample	Leaves	Pellets	Leaves	Pellets	Leaves	Pellets	Leaves	Pellets
CT Dosis (mg/ml)	**0**	98,2	98,2	98,2	98,2	98,2	98,2	98,2	98,2
	0,05	99,6a	98,3ab	100a	98,7ab	99,4ab	99,3a	98,6b	99,4a
	0,25	96,8a	86,5b	99,2a	99,3a	95,7a	99,4a	95,7a	98,9a
	0,5	2,8a	0,3b	0,3b	0,26b	3,4a	89,1c	0,8b	0,2b
	1,25	0,5a	0,2a	0,15a	0,13a	0,2a	0,2a	1,4a	0,3a
	2,5	0a	0,2a	0a	0,2a	0a	0,5a	0,2a	0,5a
	SEM	0,72	0,74	0,72	0,7	0,74	0,7	0,7	0,7
EC 50 (mg/ml)		0,364	0,342	0,3	0,364	0,684	0,758	0,36	0,348

[a–c] Means within a row with different superscript letters differ significantly (p<0.05).

EC_{50} : half maximum effective concentration.