



***In vivo* anthelmintic activity of *Acacia cochliacantha* leaves against
Haemonchus contortus in Boer goat kids**



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Abstract:

This study aimed to evaluate the effect of supplementing the maintenance diet of Boer goat kids with *Acacia cochliacantha* leaves. The endpoints evaluated were *Haemonchus contortus* fecal egg count (FEC) and water and dry matter intake. Two experimental treatments were evaluated on ten recently weaned goat kids (16.850 ± 1.630 kg of initial live weight and three months of age) experimentally infested with *H. contortus* larvae (L3) (350 larvae per live weight kilogram). Treatment 1 (T1) served as the control and consisted of infested animals without diet supplementation with *A. cochliacantha* leaves. Treatment 2 (T2) consisted of infested animals fed diets supplemented with 5% of *A. cochliacantha* leaves. Animals were grouped from highest to lowest based on their FEC. The two animals groups with the highest values were randomly assigned to T1 or T2; this was repeated until completing five repetitions per treatment. The evaluated variables were: FEC (per gram of feces), water intake, and dry matter intake (DMI). The results show that goat kids fed diets with 5% of *A. cochliacantha* leaves have lower ($P < 0.05$) FEC than the control. There were no significant differences in water intake and DMI (g d^{-1}) between treatments. This study demonstrates the anthelmintic activity of diets supplemented with *A. cochliacantha* leaves in goat kids. Thus, this arboreal legume could represent a viable option for the comprehensive management of the nematodiasis of growing Boer goat kids.

Key words: Goat kids, Anthelmintic, *Acacia cochliacantha*, *Haemonchus contortus*.

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Introduction

The diseases caused by gastrointestinal nematodes (GIN) affect the health and productivity of small ruminants, representing an important problem worldwide. *Haemonchus contortus* is the main GIN responsible for the greatest economic losses in the livestock industry in Mexico; this problem can represent up to 445.1 million dollars annually⁽¹⁻³⁾. In the last decades, the indiscriminate use of antiparasitic agents against GIN has generated anthelmintic resistance, in addition to the residual effects of some drugs on the organisms and the environment^(4,5). This problem has prompted research on sustainable control alternatives focused on studying the anthelmintic properties of the secondary metabolites in legume plants⁽⁶⁻⁹⁾. Among these compounds are condensed tannins, terpenes, saponins, and flavonoids⁽¹⁰⁾. Specifically, the synergistic nematicide activity of

flavonoids and free condensed tannins against GIN has been demonstrated^(11,12). *Acacia cochliacantha* is a legume known as “cubata,” used in traditional medicine to treat gastrointestinal diseases⁽¹³⁾. Various studies have reported the *in vitro* nematicide activity of leaf extracts⁽¹³⁾ or specific compounds like tannins, quercetin, and caffeic, coumaric, and ferulic acids⁽¹²⁾. This study aimed to evaluate the effect of a diet supplemented with *A. cochliacantha* leaves on egg shedding, dry matter and water intakes in growing Boer goat kids experimentally infested with *H. contortus*.

Material and methods

Area of study

This study was carried out in the Experimental unit for small ruminants of the UAEM, Temascaltepec. This area has a mean altitude of 1,740 m asl, with a tropical savanna climate with summer precipitations (Aw)⁽¹⁴⁾.

Plant material

Fresh leaves (young and mature) from *A. cochliacantha* were collected in Salitre Palmarillos, municipality of Amatepec, Mexico State, Mexico; located at 18°43'28" N and 100°17'03" W. The plant material was collected between March and April 2016 during the early hours of the morning. Leaves were stored in a freezer to inhibit changes in their chemical structure due to photooxidation and were then transported to the Animal Nutrition laboratory at the UAEM, Temascaltepec; here, a specimen was selected for its identification in the Herbario Nacional de México of the Universidad Nacional Autónoma de México (UNAM). The plant material was identified by Prof. Rafael Torres-Colín and deposited in the Herbario Nacional under the collection code OD07042016. The fresh leaves were shadow-dried at room temperature until reaching constant weight; after drying, leaves were ground to a particle size of 4 to 6 mm using an electrical Wiley® mill (Mod. TS3375E15).

Chemical analysis

The bromatological analysis of the experimental diets and the *A. cochliacantha* leaves was performed using the following methods: dry matter (DM), organic matter (OM), crude protein (CP), and ether extract (EE)⁽¹⁵⁾; cell wall fractions (NDF and ADF) were evaluated using the methodology described by Van Soest *et al*⁽¹⁶⁾. The content of total condensed tannins (TCT) in the percentage of inclusion of *A. cochliacantha* leaves was determined following the technique described by Terrill *et al*⁽¹⁷⁾ and modified by López *et al*⁽¹⁸⁾; the protein- and fiber-bound condensed tannins were determined with the method reported by Porter *et al*⁽¹⁹⁾ and modified by Hagerman⁽²⁰⁾.

Biological material

Infective larvae of *H. contortus* (L3, INIFAP strain) were obtained from a donor sheep (22.3 ± 0.5 kg of live weight) infected with a single oral dose of 350 L3 per kg of live weight. The lamb was cared for according to the NOM-062-ZOO-1999. The infective larvae of *H. contortus* were obtained from cultures following the technique reported by Baermann⁽²¹⁾.

Experimental animals

A total of 14 male Boer goat kids (16.850 ± 1.6 kg of live weight and 3 mo of age) from the Salitre farm, UAEM, Temascaltepec, were used. Before initiating the experiment, all animals were dewormed with a single dose of commercial ivermectin (0.22 mg⁻¹ kg of live weight) to eliminate any natural infection with GIN. After applying the antiparasitic agent, goat kids were housed in individual cages (equipped with shade and drinking and feeding troughs). Then, it was confirmed that animals were negative for GIN infection using the McMaster technique⁽²²⁾, and on d 16, animals were inoculated with a single dose of 350 L3 of *H. contortus* per kilogram of live weight. Once goat kids were diagnosed as positive for *H. contortus* (d 30), they were assigned into two groups (n=7) based on their FEC and their body weight. Before infection, animals received an adaptation diet without *A. cochliacantha* leaves (Table 1). Diets were balanced based on the requirements of growing goat kids using the NRC tables of 2007 for small ruminants⁽²³⁾.

Table 1: Ingredients, protein, and metabolizable energy of the experimental maintenance diets fed to goat kids infected with *H. contortus*

Ingredient (%)	Treatment	
	1	2
<i>Acacia cochliacantha</i>	0.00	5.00
Alfalfa hay	33.00	28.00
Ground corn	5.00	5.00
Ground sorghum	17.50	17.50
Soybean meal	7.00	7.00
Wheat bran	10.00	10.00
Oat straw	25.00	25.00
Molasses	0.00	0.00
Vitamins and minerals premix	2.50	2.50
Total	100.00	100.00
Crude protein	12.78	12.76
Metabolizable energy (MJ/kg DM) ¹	2.84	2.83

T1: control (animals infected, without diet supplementation with *A. cochliacantha* leaves); T2: animals infected, fed diets supplemented with 5% of *A. cochliacantha* leaves.

¹ Calculated based on the individual energy contents of each feed included in the diet⁽²³⁾.

Experimental design

During the experiment, the life of four goat kids was endangered due to post-weaning stress. These animals showed decreased packed-cell volumes (PCV), which indicate a severe anemic state, and high fecal egg counts (FEC > 12,500); therefore, these animals were removed from the experiment, leaving only ten goat kids, which were assigned into the two previously established treatments: T1: control (animals infected with L3 larvae of *H. contortus*, without diet supplementation with *A. cochliacantha* leaves) and T2: treatment (animals infected with L3 larvae of *H. contortus* fed diets supplemented with 5% of *A. cochliacantha* leaves). Response variables were: FEC per gram of feces, dry matter and water intake. Samples were collected weekly (post-infection d 1, 7, 14, 21, and 28) directly from the rectum. The FEC per gram of feces was determined following the McMaster technique⁽²²⁾. Dry matter and water intake were determined daily by subtracting the rejected water or feed from what was offered. The antiparasitic efficacy was estimated using Abbott's formula:

$$\text{Efficacy (reduction of FEC per g of feces, \%)} = [(\text{FEC of control group} - \text{FEC of treated group}) / \text{FEC of control group}] * 100$$

Statistical analysis

Results were processed through an analysis of variance using the GLM procedure of SAS⁽²⁴⁾. The experiment followed a completely randomized design; when there were differences between treatments, means were separated using the PDIFF test, STDERR statement. Statistical difference was declared at a $P \leq 0.05$ and the $0.05 < P \leq 0.10$ trend⁽²⁵⁾.

Results

Chemical analysis

The chemical composition of the experimental diets and the tannin content in *A. cochliacantha* leaves are shown in Tables 1 and 2.

Table 2: Chemical proximate analysis and tannin content of dehydrated *A. cochliacantha* leaves

	OM	CP	NDF	ADF	FCT	PBCT	FBCT	TCT
A. <i>cochliacantha</i>	937.0	163.0	698.6	581.7	140.0	26.0	36.0	202.0

OM= organic matter, CP= crude protein, NDF= neutral detergent fiber, ADF= acid detergent fiber, FCT= free condensed tannins, PBCT= protein-bound condensed tannins, FBCT= fiber-bound condensed tannins, TCT= total condensed tannins.

Fecal egg count

Table 3 shows the results for FEC and efficacy percentage of the diet supplemented with *A. cochliacantha* leaves. The reduction percentage caused by this legume ranged between 32.8-70.8 from day 14 to 28, respectively.

Table 3: Fecal egg count of *H. contortus* (FEC, mean ± standard deviation) and efficacy percentage of the diet supplemented with *A. cochliacantha* leaves

Treatment		Days of sampling				
		1	7	14	21	28
T1	FEC (/g of feces)	1100±120	3250±735 ^b	2980±345 ^a	5300±435 ^a	6850±1432.90 ^a
	%	----	----	----	----	----
T2	Efficacy					
	FEC (/g of feces)	1100±133	6850±828 ^a	2000±367 ^b	2050±432 ^b	2000±178 ^b
	%	----	-110.7	32.8	81.1	70.8
	Efficacy					
<i>P</i> value		> 0.15	<0.05	< 0.01	< 0.01	< 0.01

T1: control (animals infected with L3 larvae of *H. contortus*); T2: animals infected with L3 larvae of *H. contortus* supplemented with 5% of *A. cochliacantha* leaves.

Water and dry matter intake

Table 4 shows the results for water and dry matter intake. No effect was observed in both variables ($P>0.05$).

Table 4: Water intake (WI) and dry matter intake (DMI) per day in goat kids infected with *H. contortus* and fed a diet supplemented with *A. cochliacantha* leaves

	Days of sampling									
	1		7		14		21		28	
T	WI	DMI	WI	DMI	WI	DMI	WI	DMI	WI	DMI
1	1.7±0.	436.3±47.	1.6±0.	530.0±80.	1.8±0.	650.4±17	1.7±0.	647.7±20	1.8±0.	651.5±21
3	6	6	1	4	1	3.2	08	6.7	2	1.0
2	1.6±0.	448.7±10	1.5±0.	559.7±14	1.8±0.	625.0±21	1.7±0.	745.1±27	1.7±0.	694.5±19
3	0.0	0.0	4	1.3	5	6.6	4	2.5	4	4.2
P	0.71	0.80	0.69	0.69	0.76	0.84	0.90	0.52	0.75	0.76

T= treatment; 1= control (animals infected with L3 larvae of *H. contortus*); T2= infected animals supplemented with 5% of *A. cochliacantha* leaves. Water intake in liters and dry matter intake in grams per day.

Discussion

This study demonstrated the antiparasitic effect of a diet supplemented with *A. cochliacantha* leaves in goat kids; the prolonged consumption of this legume has benefits on animal health. Recently, this legume was evaluated *in vitro* against sheep and cattle gastrointestinal parasites with positive results^(8,12). These studies demonstrated the ovicidal and larvicidal activities against different GIN, *Haemonchus contortus* among them. With this background, the results confirm the *in vivo* antiparasitic effect against the most important gastrointestinal nematodes in small ruminants.

Similar results were previously reported in hair sheep infected with L3 larvae of *H. contortus* and fed diets supplemented with *Lysiloma acapulcensis* dehydrated leaves; these sheep showed a 67.7 % decrease in fecal egg count per gram of feces⁽⁹⁾. A different study in Brazil reported a decrease of up to 70 % after supplying *Mimosa caesalpinifolia* leaves in sheep infected with *H. contortus*⁽²⁶⁾.

Moreover, ruminants fed legume trees not only obtain health benefits, but they also increase their daily protein intake as a result of the high CP content, more than 18 %, in the organic matter of these forage resources, which are high concentrations compared to those of grasses that rarely exceed 10 %. Therefore, these plants exert a nutraceutical effect on animals⁽²⁷⁾. Consequently, several studies have hypothesized that in animals fed grasses and legumes, the intake proportion will depend on the protein content of grasses. If it is lower than 8 %, legume intake would increase; legumes can be arboreal or shrubs. Méndez-Ortíz *et al*⁽²⁸⁾ observed that sheep infected with *H. contortus* consumed more arboreal legume (*Havardia albicans*) rich in secondary metabolites. However, these legumes can have positive or negative effects on confined animals, directly affecting the level of dietary intake and metabolizable protein, which is directly related to the amount of condensed tannins per gram of dry matter. A previous report mentioned that to obtain benefits, condensed tannins must not exceed 5 % of the total diet⁽²⁹⁾.

In this study, there were no negative effects on water and dry matter intake in animals fed a diet supplemented with *A. cochliacantha*, which demonstrated that the intake of total condensed tannins did not affect dry matter intake. According to that reported in the literature, high dietary levels of secondary compounds, particularly free condensed tannins (greater than 50 g kg⁻¹ of DM), could inhibit the microbial activity in the rumen and thus affect the digestibility of the potentially digestible fraction of the nutrients, decreasing dry matter intake as a result of the increase in the rumen mean retention time⁽³⁰⁾. Although this phenomenon could have a positive effect on the metabolism of nitrogenous compounds by decreasing ruminal proteolysis, which would increase

the bypass protein and the amino acid flux to the duodenum, it would increase the metabolizable protein for productive purposes in the animals⁽³¹⁾.

The analysis of the condensed tannins in *A. cochliacantha* (Table 2) revealed that this plant could have similar effects to that explained before since it contains 20.2 % of total condensed tannins and 14.0 % of free condensed tannins (FCT), which are the bioactive compounds and can sequester carbohydrates and proteins from the diet. However, since it was only included 5% of *A. cochliacantha* leaves in the total diet, the intake of total condensed tannins (TCT) was 5.70 g, and of these, only 3.9 g were FCT; this concentration was not enough to decrease dry matter intake in the animals fed a diet supplemented with *A. cochliacantha* leaves. These results are similar to those reported by León-Castro *et al*⁽³²⁾; they observed a significant decrease in the FEC per gram of feces in goat kids artificially infected with *H. contortus* and fed diets supplemented with 10 % of *A. cochliacantha* leaves without negative health effects.

Future studies should consider the dose-response on dry and organic matter intake, and the digestibility of basal diets with different amounts of *A. cochliacantha* leaves. Plants rich in secondary metabolites, such as tannins, contain nutraceutical properties⁽⁷⁾, and it has been confirmed in numerous animal nutrition studies that tannins are the polyphenols responsible for improving the nutritional value of livestock diets. Similarly, plant extracts rich in tannins have been reported to have antiparasitic properties⁽³²⁻³⁵⁾. However, although it could be inferred that the high amounts of condensed tannins in *A. cochliacantha* are responsible for the antiparasitic activity, Castillo-Mitre *et al*⁽¹²⁾ reported that the compounds with antiparasitic properties derived from the hydroxycinnamic acid (caffeic, coumaric, ferulic, and methyl caffeate acids). Hence, the antiparasitic activity of this legume could be due to these compounds, and tannins could act as secondary collaborators on the control of *H. contortus* by protecting the dietary protein from the rumen microorganisms. Therefore, this *in vivo* study, in addition to previous *in vitro* studies⁽¹²⁾ with *A. cochliacantha*, could be important for future research on its nutraceutical properties in small ruminants.

Conclusions and implications

These results show that *Acacia cochliacantha* has antiparasitic properties and can function as an important feed source for goat kids infected with GIN since it does not affect water and dry matter intake.

Conflicts of interest

Authors declare no conflicts of interest.

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