

Effects of *Azadirachta indica* on Sheep Infected Naturally with Helminthes

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Abstract: The objective of this study was to evaluate the effects of neem (*Azadirachta indica*) on natural helminthes infection in lambs. Forty, four-month-old entire Santa Ines lambs were grazed on pasture, over a 20 week period. They were divided into 4 treatments: Without drenching (ND), 3 g *A. indica/animal* (A₃), 6 g *A. indica/animal* (A₆) and 9 g *A. indica/animal* (A₉) over 5 consecutive days, with an interval of 25 days between drenchings. Faeces were collected weekly and lamb weight and blood collection were carried out fortnightly. Four weeks after the last drenching all lambs were slaughtered, and worm burdens calculated. No significant differences were observed for lamb performance between treatments. Blood parameters highlighted the progress of the worm infections, with sheep on treatments A₆ and A₉ ending the experiment showing anemia. There was an increase in the total number of worms associated with increasing levels of neem, especially due to the increase in number of *H. contortus* in detriment to the number of *T. colubriformis* for highest levels of neem (A₆ and A₉). The increasing doses of neem did not improve the control of endoparasites in sheep naturally infected.

Key words: Lambs, neem, nematode, ovine, parasite

INTRODUCTION

Meat consumers are becoming more demanding in terms of product quality. Sheep production systems are therefore looking for technical and economical viable management systems to meet this demand. Products used to control parasite infections in ruminants, in general, leave residues in meat and milk, as well as are eliminated in the faces into the environment. The use of natural products may be an alternative to reduce the use of chemical drugs for the control of parasites. According to Shaalana *et al.*,^[1] botanical insecticides are relatively safe and easily degradable in the environment, thereby becoming possible sources of biopesticides.

Neem (*Azadirachta indica* A. Juss) is a plant from the Meliaceae family, of Asiatic origin, natural of Burma and the arid regions of the Indian sub-continent. It has been indicated for use as a possible alternative phytochemical within the nutritional and sanitary management strategies being used in Brazil. Other species with potential phytochemical pesticide

properties include *A. Juss*, *A. excelsa* Jack, *A. siamensis* Valetton, *Melia azedarach* L., *Melia toosedan sie* and *Melia volkensii* Gurke^[2].

Products obtained from the processing of Indian neem leaves and seeds have been frequently used in this way, especially against ticks, worms and other parasites in cattle and horses^[3]. An *in vitro* study carried out by Pessoa^[4] showed that 1% Azadirachtin inhibited the hatching of 68% of *H. contortus* eggs. Nevertheless there is little information on its use in the field. This study aimed to evaluate the effects of using neem leaves on helminthes in naturally infected sheep.

MATERIALS AND METHODS

The trial was carried out on the Agua Limpa Farm of the University of Brasilia, with approval from the Animal Care Committee. The climate has a tropical (Aw) Koppen classification. The experiment started in October 2005 and lasted 20 weeks, during the rainy season. Forty entire male, 4-month-old, Santa Ines hair lambs, with an initial mean live weight of 11±2.73 kg,

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were divided in four treatments: without drenching (ND), drenching with 3 g *A. indica*/animal (A₃), 6 g *A. indica*/animal (A₆) and 9 g *A. indica*/animal (A₉) during five consecutive days with interval of 25 days between drenching and with a total of four drenchings. The leaves of the *A. indica* were dry, chopped and stored protected of the light, before to offer to the sheep.

The animals were kept together on a 4 hectare *Andropogon gayanus* pasture, at a density of 10 animals/ha. Once a day they received 150 g/animal/day of the concentrate mixture composed of 30 % soybean meal, 20% wheat meal, 50% corn and mineral salt *ad libitum*. Pasture samples were collected monthly. All feed samples were analysed for dry matter (DM), crude protein (CP), ether extract (EE) and ash (A) using Association of Official Agricultural Chemists^[5] procedures and neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest *et al.*,^[6].

Faeces collection for faecal egg counts (FEC) by the Ueno and Gonçalves^[7] method were collected weekly. Lamb weights and blood were collected fortnightly. Blood samples were taken from the jugular vein in vacutainer tubes with EDTA to carry out hemograms. The number of erythrocytes, leukocytes and the concentration of hemoglobin were determined in an automatic cell counter (CC 550-CELMTM, Brazil). The hematocrit was determined using a microcentrifuge. The identification of leukocyte types was carried out by observation of 100 cells in a blood smear stained with Giemsa. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were obtained by calculation. The blood was centrifuged and the plasma was taken and stored at -20°C for later analysis of total protein (Labtest®, Brazil). Four weeks after the final drenching all lambs were slaughtered. The abomasum and intestines from each animal were processed to recover the adult worm burden as described by Cenci^[8]. The experiment was in a totally random design, with four treatments and ten repetitions. Data were analysed using SAS^[9]. Traits measured weekly and fortnightly were analysed as repeated measurements (FEC and hematological parameters) by PROC MIXED. For traits measured a single time (initial LW, final LW, total gain, daily gain and worm count) linear and quadratic regressions were carried out using PROC REG. The FEC and worm count were transformed (Log x+10).

RESULTS AND DISCUSSION

The means of the chemical analyses (DM, CP, FDN, FDA, EE and A) carried out on the

Table 1: Mean values for live weight (LW), total gain and daily gain in Santa Ines sheep under natural infection with different levels of Azaradactin drenching

Variables	Not Drenched	Azadirachta indica (neem)			Regression
		3g	6g	9g	
LW initial (kg)	10.8	12.6	10.6	10.6	NS*
LW final (kg)	25.0	27.9	23.6	26.8	NS
Total gain (kg)	14.0	14.2	13.5	15.7	NS
Daily gain (g/day)	123	124	118	137	NS

*NS not significant

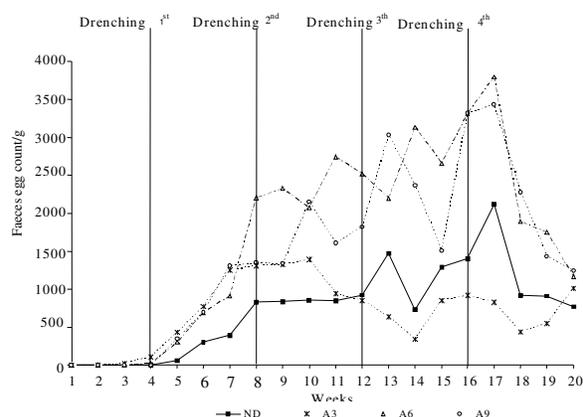


Fig. 1: Faecal egg counts of Santa Ines sheep under natural infection, without drenching (ND), and receiving 3g *A. indica*/animal (A₃), 6g *A. indica*/animal (A₆) and 9g *A. indica*/animal (A₉) during five days with four weeks intervals

Andropogon gayanus grass were 315, 115, 668, 334, 29 and 47 g kg⁻¹ DM and concentrate mixture were 874, 158, 129, 56, 41 and 23 g kg⁻¹ DM, respectively.

Production performance data of the sheep are shown in Table 1. No significant differences (p>0.05) were found between neem treatments for the traits evaluated (initial and final live weight, total gain and daily gain).

Only sheep on treatment A₃ showed FEC values lower than the control (ND) from the 12th week (Fig. 1), but differences were not significant (p>0.05). In weeks 11 to 14, groups ND and A₃ showed a lower egg count than for the A₆ and A₉ treatments (p<0.10). No other significant differences were noted. A positive linear regression was found when increasing levels of Neem were plotted against overall FEC ($y = 133X + 747$, $R^2 = 0.15$, $p = 0.016$).

Data for red cell and total plasma protein are in Table 2. There was a progressive reduction in the hematocrit, number of erythrocytes and hemoglobin along the experiment, associated with the evolution of the infection in all treatments. Below normal and more

Table 2: Mean hematocrit, erythrocytic and hemoglobin values, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and total protein findings in Santa Ines sheep under natural infection with different levels of Azaradactin drenching

Parameters	Treat. ¹	Weeks							
		5	7	9	11	13	15	17	19
Hematocrit % (27 - 45)*	ND	37.0	33.0	30.0	30.3 ^{ab}	28.5 ^{ab}	27.2 ^{ab}	26.0 ^b	28.9 ^{ab}
	A ₃	37.3	30.4	27.8	33.6 ^b	29.3 ^b	28.3 ^b	28.6 ^b	30.8 ^b
	A ₆	36.1	30.6	28.3	29.3 ^a	24.9 ^a	25.0 ^a	21.9 ^a	26.9 ^a
	A ₉	36.3	30.0	28.2	31.2 ^{ab}	26.0 ^{ab}	26.5 ^{ab}	25.9 ^b	26.7 ^a
Erythrocytes x10 ⁶ /μL (9 - 15 x10 ⁶)*	ND	13.2 ^a	11.5 ^c	9.1	9.5 ^{ab}	7.9 ^b	8.7	8.4 ^c	9.9 ^{ab}
	A ₃	14.6 ^{ab}	10.2 ^{ab}	8.2	9.9 ^b	7.6 ^b	9.3	8.3 ^c	10.6 ^b
	A ₆	14.9 ^b	11.1 ^{bc}	8.1	8.5 ^a	6.2 ^a	8.7	5.8 ^a	8.7 ^a
	A ₉	13.0 ^a	9.9 ^a	8.7	9.2 ^{ab}	6.6 ^{ab}	9.8	6.9 ^b	8.8 ^a
Hemoglobin (g/100mL) (9 - 15)*	ND	9.9	9.8 ^b	9.4 ^b	9.2 ^{ab}	6.9 ^{ab}	6.7 ^b	8.2 ^b	9.2 ^b
	A ₃	9.3	9.0 ^{ab}	8.3 ^a	9.6 ^b	7.3 ^b	6.4 ^b	8.5 ^b	9.7 ^b
	A ₆	9.5	8.9 ^a	8.0 ^a	8.4 ^a	6.4 ^{ab}	6.1 ^a	6.2 ^a	8.5 ^{ab}
	A ₉	9.3	8.8 ^a	8.2 ^a	8.7 ^{ab}	6.3 ^a	6.3 ^a	7.1 ^{ab}	7.9 ^a
MCV (fL) (28 - 40)*	ND	29.3 ^a	28.7 ^a	33.0 ^{ab}	32.8	36.7 ^a	31.4	31.8 ^a	29.1
	A ₃	31.3 ^b	29.7 ^{ab}	33.8 ^{ab}	33.8	38.6 ^{ab}	30.7	34.7 ^{ab}	29.3
	A ₆	31.1 ^b	27.9 ^{ab}	35.5 ^b	34.8	41.0 ^b	30.1	38.6 ^c	31.4
	A ₉	30.5 ^{ab}	30.1 ^b	32.7 ^a	34.7	41.7 ^b	27.7	37.6 ^{bc}	30.5
MCHC (%) (31 - 34)*	ND	27.0	29.5	31.3 ^b	31.3 ^b	24.8	22.8	31.2 ^b	32.2
	A ₃	25.54	29.8	28.9	28.7 ^a	25.5	23.7	29.4 ^{ab}	31.6
	A ₆	26.3	29.0	28.1 ^a	28.7 ^a	25.5	24.3	29.0 ^{ab}	32.0
	A ₉	25.6	29.5	28.7	27.8 ^a	23.9	23.4	28.6 ^a	31.2
Total Protein (g/100mL) (6,0 - 7,5)*	ND	6.8	6.6 ^b	6.5	6.5 ^a	6.4	6.7 ^{ab}	6.4 ^{ab}	6.9 ^b
	A ₃	6.8	6.4 ^{ab}	6.5	6.9 ^b	6.5	6.8 ^b	6.6 ^b	6.9 ^b
	A ₆	6.7	6.3 ^a	6.3	6.5 ^a	6.1	6.3 ^{ab}	5.9 ^a	6.6 ^a
	A ₉	6.6	6.6 ^b	6.4	6.9 ^b	6.3	6.2 ^a	6.2 ^{ab}	6.7 ^a

¹ND (not drenched), A₃ (3g *A. indica*/animal), A₆ (6g *A. indica*/animal) and A₉ (9g *A. indica*/animal). * Reference values from Jain, (1993). ^{ab,c} Mean followed by different letters in the same column are significantly different (P≤0.05)

Table 3: Mean leukocytic values findings in Santa Ines sheep under natural infection with different levels of Azaradactin drenching

Parameters	Treatments ¹	Weeks			
		5	9	13	19
Leukocytes/μL (4,000-12,000)*	No Drenched	11,300 ^b	10,500	8,500 ^a	12,100
	3 g	9,600 ^{ab}	9,500	9,300 ^{ab}	12,200
	6 g	10,600 ^{ab}	10,000	8,900 ^a	10,900
	9 g	9,000 ^a	10,700	11,000 ^b	11,600
Monocyte/μL (0-750)*	No Drenched	709 ^b	257	463	382 ^{ab}
	3 g	308 ^a	205	271	587 ^b
	6 g	296 ^a	371	293	223 ^a
	9 g	506 ^{ab}	272	250	338 ^a
Lymphocyte/μL (2,000-9,000)*	No Drenched	5,378 ^{bc}	3,743	3,539	5,591
	3 g	3,881 ^a	4,108	4,739	5,459
	6 g	5,780 ^c	4,220	4,537	4,502
	9 g	4,498 ^{ab}	4,673	4,779	4,786
Neutrophil/μL (700-6,000)*	No Drenched	5,339	6,018 ^b	3,327	5,474
	3 g	5,078	3,851 ^a	3,006	5,285
	6 g	4,393	5,591 ^b	4,199	5,628
	9 g	5,390	5,629 ^b	5,781	4,125
Eosinophil/μL (0-1,000)*	No Drenched	308 ^b	844 ^{ab}	619 ^a	703
	3 g	195 ^{ab}	823 ^{ab}	822 ^a	521
	6 g	287 ^{ab}	600 ^a	1,023 ^a	586
	9 g	132 ^a	1,475 ^b	2,346 ^b	750
Basophil/μL (0-300)*	No Drenched	56	22	33	87
	3 g	38	35	61	109
	6 g	49	22	58	76
	9 g	34	0	33	70

¹ND (no drenched), A₃ (3 g *A. indica*/animal), A₆ (6 g *A. indica*/animal) and A₉ (9 g *A. indica*/animal). * Reference values from Jain, (1993). ^{ab,c} Mean followed by different letters in the same column are significantly different (p≤0.05).

significant values for these traits were seen from the 13th week on which coincides with the third development cycle of adult worms. Values for total protein were within normal ranges. In general, these parameters were higher for the ND and A₃ treatments than A₆ e A₉ in the last four weeks. This was associated with a lower infection with *H. contortus* and higher predominance of *T. colubriformis*.

The white cell analyses are in Table 3, with values within normal values for most of them. There was a progressive increase in the number of eosinophils with the advance of parasite infection in all treatments, with higher than normal values for treatment A₆ (13th week) and A₉ (9th and 13th weeks).

The adult worm results are shown in Table 4. Predominant species were *T. colubriformis* and

Table 4: Number of worms per species and sex in Santa Ines sheep under natural infection with different levels of Azaradactin drenching

Species	No Drenched	Azadirachta indica (neem)			Regression
		3 g	6 g	9 g	
Total geral	3539	5356	4855	6353	Y=525.81X + 517.55
Female: male	2093: 1286	2679: 2387	2554: 2301	3538: 2815	R ² =0.31 P=0.0009
<i>T. colubriformis</i>	2822 (79.74)*	4099 (73.53)	1966(40.51)	1436(22.60)	NS ¹
Female: male	(1642 : 1180)	2264 : 1835	1089 : 878	797 : 639	
<i>H. contortus</i>	439* (12.40)	979 (18.27)	2619 (53.93)	4647 (73.14)	Y=474X +56.74
Female: male	289 : 150	533 : 446	1306 : 1313	2566 : 2081	R ² =0.28 P=0.0011
<i>O. columbianum</i>	248 (7.0)	214 (3.9)	230 (4.73)	223 (3.51)	NS
Female: male	152 : 96	115 : 99	124 : 106	134 : 89	
<i>Cooperia sp</i>	7 (0.19)	3 (0.05)	0	0	NS
Female: male	7 : 0	3 : 0	0 : 0	0 : 0	
<i>S. papillosus</i>	16(0.45)	38(0.7)	28(0.57)	5(0.07)	NS
<i>T. globulosa</i>	5(0.14)	19(0.35)	6(0.12)	9(0.14)	NS
Female: male	3 : 2	12 : 7	2 : 4	3 : 6	
<i>M. expansa</i>	2 (0.056)	4 (0.07)	5 (0.10)	33 (0.52)	NS

* % total geral species as reference. ¹Not significant

H. contortus, the sum of these being higher than 92% of the total worm count over all treatments. For the total count a positive linear regression was found with increasing levels of neem in the treatments ($Y = 525.81X + 517.55$, $R = 0.31$, $P = 0.0009$). This was mainly influenced by a positive linear regression for number of *H. contortus* ($Y = 474X + 56.74$, $R = 0.28$, $P = 0.00011$). Although there was a numerical decrease for *T. colubriformis* as neem level rose, the negative regression ($Y = -20X + 347$, $R = 0.06$) was not significant ($P = 0.1667$). Nevertheless the proportion of the *T. colubriformis* population decreased from 74% in treatment ND to 22.60% in treatment A₉. The inverse was seen with *H. contortus* which was 12.40% in treatment ND and rose to 73.14% in treatment A₉. No other worm species showed treatment effects in this experiment.

Increasing levels of neem (0, 3, 6 and 9 g leaves/animal/5 consecutive days with a 25 day interval over 5 months), did not result in a lower infection of endoparasites in sheep at pasture. Only treatment A₃ FEC values were below the control, but no significant differences were noted. This group was clinically characterized as having a light infection with FEC values below 1,000 FEC, after the 12th week. The A₆ and A₉ treatments showed a severe infection from the 10th week, with FEC values above 2,000, and highest values being 3,976 and 3,433 in the 17th week, respectively. These two treatments (A₆ and A₉) showed a predominance of *Haemonchus*, whose females produce a higher number of eggs than *T. colubriformis*^[7].

Although in mice neem leaf extracts were shown to be hematostimulatory and immunostimulatory^[10], the same effects were not found in this experiment, where the parasite infection did not decrease with the addition

of the leaves. The leukocyte count did not change and there was a discrete eosinophilia. It is important to note that there was a greater reduction in the hematocrit and number of red blood cells in the 13th, 15th and 17th weeks, when the higher infections were noted. This was probably due to the complete development of three or four parasite cycles as the cycle for the worms found is generally 4 weeks/cycle. This was reflected by the high blood loss caused by these parasites. During this period all animals showed normocytic-hypochromic anemia, which happens in cases of transitional phase from acute to chronic blood loss in which iron deficiency is setting in with a reduction of erythropoiesis caused by intense parasitism^[11]. In the 19th week the hematocrit and number of erythrocytes returned to within normal reference values, with higher total protein in the plasma for treatments ND and A₃, probably due to the predominance of *T. colubriformis* in these treatments. This is a less pathogenic species than *H. contortus* (hematophage), which was predominant in treatments A₆ and A₉, and whose animals still showed anemia at this stage, but no differences on animal performance was observed between treatments suggesting resilience despite of the more pathogenic infection found.

Haque *et al.*,^[10] did not observe any signs of toxicity using preparations of neem leaf extracts (NLP-1 unit) once weekly for four weeks in mice, as indicated by hepatic enzymes and histopathological exams. Intoxication is associated with the use of a high number of leaves of the plant (100g/animal/day) as an integral part of the diet of sheep^[12]. In the present study, the doses used were lower than this value and although no specific toxicity tests were carried out, the growth and blood parameters of these animals did not indicate that higher neem levels had been hepatotoxic or debilitating

for the sheep which could have justified the higher infection level in treatment A₉.

The quality of the infection changed with increasing levels of neem administrated (A₆ and A₉). There was a greater development of *H. contortus*, which is pathologically more serious than that of *T. colubriformis*. Considering the ND treatment, the proportion of these two species was 79.74% and 12.40% for *T. colubriformis* and *H. contortus* respectively, but as the neem level rose the *T. colubriformis* population fell to 22.60% and the *H. contortus* rose to 73.14% in treatment A₉ at the end of the trial (week 20). Given the complexity of factors involved in a mixed natural infection it is difficult to discover what is happening. The neem could be interfering in the gastrointestinal tract environment, affecting the motility (laxative), microbiotics, improving the availability of certain nutrients, among others and thereby promoting the development of a certain species in detriment of the other. There are studies with tannin rich plants which show that the pH of the gastrointestinal tract interferes in the mode of action of these substances^[13]. This may be happening with the neem as these two worm species inhabit distinct portions of the gastrointestinal tract. The *H. contortus* develops in the abomasum (acid pH) while *T. colubriformis* develops in the small intestine (alkaline pH). All these questions are speculative and need to be further investigated.

Hordegen *et al.*^[14] did not observe any action on parasites of neem seed extract at 3 mg kg⁻¹ live weight in sheep artificially infected with *H. contortus* and *T. colubriformis*. Costa *et al.*^[15] evaluated the daily use of neem leaves in the concentrate of sheep at 0.1g leaves/kg LW and 0.2 g leaves/kg LW to control endoparasites over a three months period. They also concluded that neem was not efficient as to control worm loads.

In plant studies it is important to note that the plant part, quantity utilized should be clearly defined. In published studies all of this information is not always available, which makes comparison between studies more difficult. According to Martinez^[16], as well as azadiractina, which is considered the most potent limonoid present in neem, there are other substances in this plant which may interact or interfere with its action on parasites^[17].

New studies should be carried out with this plant with controlled infections, to attempt to decipher the mode of action, as it is still too early to draw any conclusions about this plant as to its use in parasite control. Its action in increasing resilience of the host animal to the worm infection should not be discarded.

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