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Propolis in the control of helminths in sheep

Jocélio Dos Santos Araujo*[1], Ivo Claudio Levistki[2]

[1]*Center for Agrarian and Environmental Sciences, Campus of Chapadinha, Federal University of Maranhão, Chapadinha, Maranhão, Brazil*

[2]*Pigs Production Unit at Copacol, Cafelândia, Paraná, Brazil*

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Abstract

The objective of the study was to evaluate the efficacy of the alcoholic solution of propolis in comparison to a commercial vermicide, in the control of gastrointestinal parasites in sheep. Thirty Santa Ines ewes were used, divided in 3 groups of 10 animals. The dose administered was 10mL for propolis and, for the vermicide, the dose recommended by the manufacturer. The samples were taken at intervals of 21 days, and the coprological exams were performed shortly after collection, analyzing light eggs, heavy eggs and egg counts per gram of faecal (FEC). Statistical differences between treatments. The efficacy of propolis in the control of gastrointestinal helminths, thus recommending its use, Emphasizing that it is still necessary to carry out more studies, relation to ideal concentrations, its mode of action and it residual effect.

* **Corresponding Author:** Jocélio dos Santos Araujo ✉ jocelios@yahoo.com.br

Introduction

At present, some studies are being carried out in search of alternatives for the control of gastrointestinal parasites, with products of animal or vegetable origin of low toxicity, both for domestic animals and for the ecosystem. Such is the case of propolis, resinous substance collected by Apis mellifera bees, from different parts of different plant species such as bud, flower buds and resinous exudates.

It has been used in traditional medicine since antiquity due to its broad spectrum of biological activity, such as antioxidant, anti-inflammatory, antibacterial, antiviral, antifungal and even anticancer (Banskota *et al.,* 2000; Lima *et al.,* 2016) besides being used in veterinary medicine (Principal *et al.,* 2002; Bačić *et al.,* 2016).

Ethanolic, hydro-alcoholic and aqueous extracts of propolis have been used and studied in different situations as anti-parasitic agents (Moura *et al.,* 1998), anti-tyrosomal and antimicrobial (Marcucci *et al.,* 2001). In this context, propolis emerges as an alternative in scientific research as a natural product that can be studied to reduce anthelmintic resistance and as a consequence improve the productive performance of small ruminants, as resistance to anti-helminths by gastrointestinal parasites, especially in small ruminants, has been a challenge for the production of goats and sheep. Based on the above, the objective was to evaluate the efficacy of the use of propolis in the control of gastrointestinal parasites compared to a commercial anthelmintic in sheep.

Material and methods

Location and duration

The research was carried out in the sheep industry, located in the Experimental Farm Prof. Dr. Antônio Carlos dos Santos Pessoa, belonging to the Nucleus of Experimental Stations of the State University of the West of Paraná (Unioeste), in the municipality of Marechal Cândido Rondon, Paraná State, Brazil.

Systems and experimental treatments

Thirty-one Santa Inês sheep were used, with a mean live weight of 33.91 ± 0.03kg, with approximately 24 months of age and without anthelmintic treatment for the minimum period of six weeks, necessary for reinfestation of the herd, during the dry season (June to August) of the year 2006, in this period the average temperature was 20.7°C, the average relative humidity was 42.4% and the average rainfall was 3.8 mm, data collected at the Unioeste, Meteorological Station located at the Experimental Farm. The animals were kept in three pickets, consisting of Tifton 85 grass (Cynodon dactilon), in the morning until the end of the afternoon, and were collected at the sheepfold for overnight stay, where they received mineral supplementation.

The selected ewes were individually tagged by numbering earrings and randomly assigned to three groups of ten animals with the following treatments: Group 1 - control group; Group 2 - treated with commercial anthelmintic, Albendathor 10 (albendazole - Oral dosage, 1mL for each 20kg of body weight); Group 3 - treated with propolis alcoholic solution, administered orally single dose of 10mL/animal.

Preparation of the propolis solution

The method of dilution and preparation of propolis was carried out in the proportion of 70% of cereal alcohol at 96ºGL (pharmacy ethyl alcohol) diluted in 30% crude propolis, which was subsequently stored in a dry, aerated and protected place of light in a period of 30 days and shaken to homogenize it daily. After this period it was filtered on filter type paper.

Variables and data collections

Fecal samples of approximately 15g were collected individually, directly from the animals' rectal bulb, 1 before the start of the experiment and 2 more every 21 days. Coprological exams were performed immediately after collection of fecal samples. Tests were performed using the Willis Method for light eggs, which has flotation as its principle, using a high density sodium chloride hypersaturated solution (1: 1200) and the Hoffmann method used for the research of heavy eggs, qualitative methods direct (Hoffmann, 1987). For the counting of eggs per gram of faeces (FEC) of gastrointestinal nematodes, the

modified Mc Master technique described by Gordon & Whitlock (1939) was employed. The FEC reduction test was done by means of comparison of mean FEC before and every 21 days after the evermination.

To calculate the efficacy of propolis and after the anthelmintic in relation to the reduction of the number of eggs per gram of feces (FEC), the formula proposed by Coles and Rousch (1992):

$$\% \, efficiency = 100 \, x \, \{1 - \frac{Mean \; FEC \; in \; the \; propolis \; group}{Mean \; FEC \; in \; the \; control \; group}\}$$

$$\% \, efficiency = 100 \, x \, \{1 - \frac{Mean \; FEC \; in \; the \; group \; with \; anthelmintic}{Mean \; FEC \; in \; the \; control \; group}\}$$

Statistical analysis

For statistical analysis, the total number of eggs of gastrointestinal nematodes was transformed by means of equation Y = log (y + 1) to stabilize the error and analyzed statistically by analysis of variance and simple correlation analysis by SISVAR in the version 5.6 (Ferreira, 2014), using the Student Newman Keuls test 5% significance.

Results and discussion

The results obtained during the experimental phase demonstrated that there was a significant difference between the treatments, where the animals treated with vantihelmintic and the alcoholic extract of propolis reduced the FEC in relation to the control group (Table 1).

Table 1. Eggs per gram of faeces (FEC), light eggs and heavy eggs in sheep as a function of treatments.

Variables	Treatments			CV (%)	Pr>F
	Control	Propolis	Anthel-mintic		
FEC	0.835[b]	0.292[a]	0.342[a]	142.26	0.03
Light eggs	1.088[a]	0.688[a]	0.503[a]	103.66	0.07
Heavy eggs	0.480[a]	0.150[a]	0.212[a]	167.21	0.07

It should be clarified that in the present study a low incidence of verminose occurred, which may be justified by the low rainfall of only 3.8 mm during the experimental period, thus reducing the development of eggs and larvae in pastures. Yamamoto *et al.,* (2008), observed that in infested animals, it is possible not to detect eggs in the faeces, as long as the parasites are not ovopositive, remaining in latent stage, or simply in hyobobiosis until the environment condition improves to start their activities. The same authors also point out that the elevation of the ambient temperature causes a reduction in the number of larvae in the pastures, corroborated by Borba *et al.,* (1993), reported that, in small ruminants, there is a higher parasite concentration in the pastures than in the digestive tract of the animals.

Githigia *et al.,* (2001) in Greece and Papadopoulos *et al.,* (2003) in Kenya, where they verified the relationship between the number of eggs in the faeces and parasitic load with rainfall. Regardless of the low contamination of animals by endoparasites, propolis was effective in reducing FEC. Another factor that may have influenced the difference in infestation of the herd is that among the selected animals there were pregnant and some lactating females.

In a study conducted by Loureiro (2007), which used Ile de France lambs submitted to parasitic control, it was observed that with the addition of 30mg of propolis extract, a greater effectiveness was observed in reducing the number of Strongylida type eggs per gram of feces, than that of 15mg and that which did not contain the extract, indicating a possible reduction in the presence of endoparasites. Also corroborated by the results obtained by Principal *et al.,* (2002), where the antiparasitic effects of propolis were also observed, when they tested propolis levels in the control of West African sheep helminths using doses of 5mL, 10mL and 15mL of 3% propolis alcohol solution, comparing them with alcoholic solution without propolis (control). The authors concluded that propolis reduced the parasitic infection of sheep, with the 10mL dose being the most effective for the species.

In Table 2, it is possible to verify the percentage of effectiveness of the treatments, it is noticed that in the first collection there was no difference between the treatments adopted, but in relation to the second collection the propolis was better, obtaining a margin of 96% of efficacy.

Table 2. Percentage of efficacy of treatments.

Collections	Treatments (% de efficacy)	
	Propolis	Anthelmintic
1	94	94
2	96	94

Data confirming the efficacy of albendazole were obtained by McKellar *et al.,* (1993), where they found that sheep treated with albendazole had a 100% reduction in the number of eggs per gram of faeces. Similar results were obtained by Ghouse & Radhakrishnan (1993), also obtained a 100% reduction in helminths in sheep and goats. Marques *et al*., (1995), also demonstrated the efficacy of a cobalt-associated 10% albendazole treatment, a once-oral dose administered in the amount of 5mg/kg body weight, and which had a 100% reduction in infestation.

Ramos *et al.* (2002), when evlluating 65 flocks of sheep studied, they observed that seventy-seven percent presented parasite resistance to ivermectin, with Haemonchus larvae only (100%); sixty-five percent to albendazole, with Haemonchus (74%), Ostertagia (15%) and Trichostrongylus (11%); thirteen percent to closantel, with Haemonchus (100%); and fifteen percent to levamisole, with Thichostrongylus (44%), Ostertagia (39%) and Haemonchus (17%). According to the authors, the results detected the presence of a multi-resistance to anthelminthics in the great majority of the sheep flocks. Cunha Filho *et al.,* (2009), found 100% resistance to albendazole, 80% resistance to ivermectin and 20% to moxidectin, in a total of 850 animals from 10 herds, with predominance of the genus Haemonchus spp., And similar data were observed by Hong *et al*., (1996), in England with respect to *O. circumcincta*.

The antiparasitic effect of propolis has already been demonstrated by some authors in other studies. The effectiveness of propolis in other animal species has been verified, as can be seen in a study carried out by Holland *et al*., (1988), where rabbits demonstrated the coccidiostatic action of propolis administered orally to the concentration of 3% in drinking water, and a significant reduction of oocysts of *Eimeria spp*. present in the faeces of treated animals.

Principal *et al*., (2002), tested propolis in sheep and verified that the solution of 3% propolis is effective in the reduction of *Strongyloides* eggs, and the dose of 10mL was the most effective for this species of parasite. Morsy *et al*., (2013) when studying the effects of the administration of propolis ethanolic extract on hematological, biochemical and parasitic responses of Santa Inês sheep during and after the flushing period, the authors reported that the addition of 3 g of propolis decreased (P<0.05) to count of fecal eggs of endoparasites, concluding that propolis had a good impact on the sheep's health and could be used as a promising food additive for the sheep.

Conclusion

The efficacy of propolis in the treatment of gastrointestinal helminths has been evaluated and its use is recommended.

Recommendation

It should be emphasized that further studies are necessary in relation to ideal concentrations, its mode of action, residual effect and which active principle of the extraro of propolis acts in the control of the eggs of the gastrointestinal parasites.

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