Evaluation of Gamal Leaves (*Gliricidia Sepium*) as Anthelmintic Forages Against Gastrointestinal Nematodes in Sheep

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Abstract. Several studies have demonstrated that ruminant-fed forages containing bioactive anthelmintics (tannins, alkaloids, and flavonoids) can be used to suppress nematode worms. The objective of this study was to examine any potential direct anthelmintic effects of *G.sepium* leaves containing Plant Secondary Metabolites (PSM) on the Gastrointestinal Nematode (GIN) in sheep. Twenty-five adult female and male sheep that were naturally infected with nematodes were used in this study. The experimental design utilised a randomised layout with five ~~t~~reatment groups (K1-K5). Each group was composed of five sheep. K1 and K2 received 30% and 50% of their forage feed from *G.sepium* leaves, respectively, while K3 received 60 g of *G.sepium* leave powder. Group K4 was a positive control getting the recommended dose of albendazole, and K5 was a negative control (no treatment). *Gliricidia sepium* leaves and flour was administered every day for 21 days (3 weeks). Evaluation of the anthelmintic effect was conducted using the Fecal Egg Count Reduction Test (FECRT) once a week for 56 days (8 weeks), Packed Cell Volume (PCV) values and faecal culture to detect the dominating growth of nematode larvae types. The results showed that the administration of withered fresh gamal leaves as much as 30% and 50% forages decreased eggs per gram faeces (EPG) in the first until the third week of therapy with faecal egg count reduction of 61–63% and 47–56%, respectively. The effectiveness of *G.sepium* flour at 60 g/head/day ranged from 26 to 39%. The effectiveness of all group treatments increased after the fourth week of treatment cessation. From the first week of observation until the end of the trial, the EPG of the negative control increased by 30 to $>$ 100%. Most of the sheep showed normal PCV values. The dominant nematode larvae identified were *Haemonchus contortus* (83.10%),Trichostrongylus sp(15.8%),Oesophagostomum sp (0.83%) and Cooperia sp (0.27%). *Haemonchus contortus* larvae pose the greatest threat to sheep productivity; consequently, effective control measures are required. These results are highly valuable for farmers, particularly in breeding groups with high helminthiasis prevalence that is challenging to treat with anthelmintics because the majority of ewes are pregnant. The combination of the utilised anthelmintic and *Gliricidia sepium* leaves will produce the best results in preventing nematodiasis.

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1 Introduction

Sheep and goats are the most common small ruminants raised by Indonesian farmers. Infestation with gastrointestinal nematode worms is one of the most significant diseases that reduce sheep productivity and cause the death of young livestock [1]. These animals are susceptible to the pathogens *Hemonchus contortus*, Trichostrongylus spp, Oesophagostomum sp, Cooperia sp, Tricuris sp, and Bunostomum sp [2–4], which are reported to generate significant economic losses to ruminants. The *Haemonchus contortus* worm, a bloodsucking roundworm that lives in the abomasum, is the most prevalent cause of helminthiasis in small ruminants in Indonesia [5]. Due to subclinical nematodosis infection in small ruminants, feed intake, body weight, milk production, and mortality in young animals are diminished [6].

Generally, synthetic anthelmintic compounds are utilized to control these nematodes [7]. However, the overuse of anthelmintics without rotation, time-indiscriminate administration, and insufficient dosage has favoured the selection of parasites resistant to commercial drugs [8] and has prompted research into alternative parasite control methods, such as the use of bioactive forage. It has been discovered that optimal nutrition increases an animal's resistance to the detrimental effects of worm infestation [9].

Gliricidia sepium is a widespread shrub, particularly in Indonesia and other tropical regions. Wood et al. (1998) [10] reported that *G.sepium* is a tropical legume that is widely utilized during dry seasons in cattle production units as a natural defence and nutritional resource as Fodder Forage (FF). *Gliricidia sepium* leaf phytochemical analysis reveals the presence of coumarins, saponins, steroids, tannins, and terpenoids [11, 12]. According to Santos et al. (2019) [7], the primary plant secondary metabolites associated with the anthelmintic effect are condensed tannins, saponins, and flavonoids. Utilization of feedstock condensed tannin reduces GIN levels and improves animal performance through both direct and indirect mechanisms [13]. Tannins, alkaloids, and flavonoids found in *G.sepium* leaf extract were not significantly different from albendazole in inhibiting the growth of Tricostrongylus spp according to an in vitro study [14]. Romero et al. (2020) [15] discovered that *G. sepium* contained a greater variety of compounds that may be active against the control of *H. contortus* third-stage larvae and eggs. According to [11], the decrease in the 50% FEC of sheep given a 15 g/kg BW water extract of fresh *G.sepium* leaves in comparison to the untreated group.

Recent studies have shown that certain biological processes of *H. contortus* are extremely sensitive to low concentrations of tannins or polyphenolic compounds (75 g of extract/ml) [16]. The efficacy of *G.sepium* leaves, which has anthelmintic properties needs to be evaluated. The objective of the present study was to examine the effect of PSM-rich forages *G.sepium* against GIN, focusing on the potential direct anthelmintic effect on sheep in the field.

2 Materials and Methods

2.1 Ethical Statement

The experimental design was approved by the Ethics Committee on animal use at the Indonesian Agricultural Research and Development Agency. (Number:Balitbangtan/BBLITVET/Rm 06/2021).

2.2 Animal and Experimental Diet

Twenty-five Garut Sheep were utilized in this study (20 ewe and 5 tup lambs). The sheep are owned by the "Bina Mandiri" Farmers Group in the subdistrict of Cikeusal, the district of Serang, province of Banten. The sheep were naturally infected with GIN with the intensive animal husbandry system. Animals are fed dwarf elephant grass (Pennisetum Purpureum Cv. Mott) three times per day and tofu dregs once per day. The animals were divided into five groups (K1-K5). The animal was placed in a separate cage and given ad libitum access to clean water. Each animal consumed *P. purpureum* Cv. Mott and tofu dregs throughout the experiment. The experimental animals in Groups K1 and K2 were fed daily mixtures of *G. sepium* and *P. purpureum Cv. Mott* leaves in proportions of 30%:70% and 50%:50%, respectively. Group 3 (K3) was fed *P. purpureum* Cv. Mott leaves, tofu dregs, and 60 g of *G. sepium* leave powder per animal per day (3g/kg BW/day). This amount refers to the dose of the previous study [11] and was converted from fresh leaves to dried leaves (5:1). Group 4 (K4) constituted the control group (no treatment). Group K5 was administered 1x the recommended dose of albendazole as a positive control. Withered G. *sepium* leaves and flour was administered daily for 21 days (3 weeks).

2.3 Preparation of Withered G. Sepium Leaves

Fresh *G. sepium old* leaves with a pungent odour were collected from the Farmers Group's garden. The leaves were immediately air-dried for one day at room temperature by spreading them in a shady location (25–27 °C). The leaves then weighed between 30 and 50% of the total daily green forage.

2.4 Preparation of G. Sepium Leaves Powder

During the dry season, fresh *G. sepium* leaves were harvested from an experimental garden belonging to the Indonesian Livestock Research Center in Ciawi, West Java. The harvested leaves are old, foul-smelling leaves. The leaves were immediately air-dried for 5–7 in a room that was not exposed to direct sunlight (28–30 °C). The dried leaves were then milled, sieved, weight 60 g and incorporated into the treatment diets.

2.5 Faecal Samples Collection

Faecal samples were collected every 7 days (weekly) intervals until day 49 (Day 0–56th). Faeces samples obtained directly from the rectums of sheep were preserved in separate plastic bags and refrigerated at 4 °C for laboratory testing.

2.6 Faecal Egg Count Reduction Test (FECRT)

The anthelmintic effect was evaluated using the Fecal Egg Count Reduction Test (FECRT), modified from the Whitlock method, once per week for 56 days (8 weeks) [17, 18]. Briefly, three grams of each faeces sample was soaked in 20 millilitres of water and stirred uniformly. As the floating fluid, 40 mL of saturated NaCl (specific gravity: 1.2 kg/m^3) was added, homogeneously mixed, and pipetted into the 'Whitlock chamber' counting room (0.5 mL/room) for egg identification and counting under a microscope at 40 magnification. The result of the calculation is multiplied by 40 for egg units per gram (EPG).

2.7 Packed Cell Volume (PCV)

Packed Cell Volume is directly measured by centrifuging blood in a microhematocrit tube in a microhematocrit centrifuge.

2.8 Culture and Differentiation of Nematode Larva

A total of 3 g of stool samples from day 0 (pre-treatment), days 7, 14, and 21 post-treatment were placed in a small dish and combined with vermiculite in a ratio of 1:1. The mixture was then stirred until smooth while sufficient water was provided to fertilize the eggs become damp then poured into a jar. Fertilizers containing larvae were kept at room temperature 22–27 °C with 80% humidity and incubated in the shade for seven days and were not exposed to direct sunlight. On the seventh day, the larvae that emerge from the culture in the bottle are collected in the petri dish and then placed in a 100-ml plastic bottle and identification microscopically with the MAFF manual [18].

2.9 Statistical Analysis

Experimental data collected were analysed by SPSS 23 (IBM Corp 2015). The effects of feeding on *G.sepium* on FEC of sheep on GIN were analysed with Kruskal Wallis non parametric test.

3 Results

3.1 Clinical Manifestation

All of the sheep utilized in this study appeared generally healthy with a good appetite as indicated by the PCV value in the majority of the sheep being within normal intervals value (27–43%).

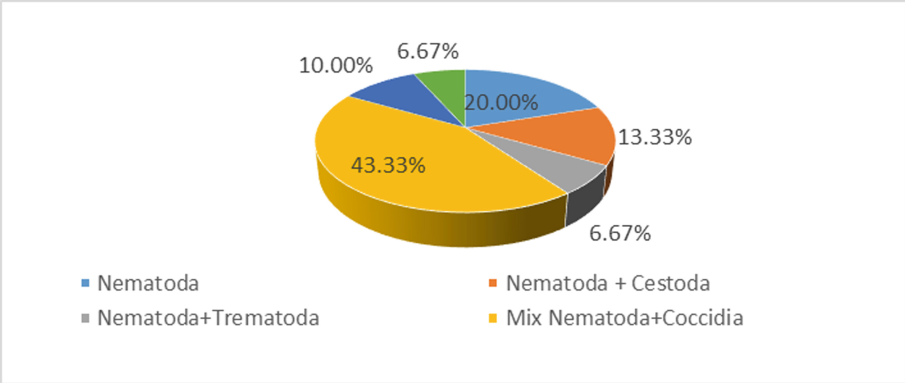

Fig. 1. Percentage of Worm and Protozoa Gastrointestinal infestation in sheep

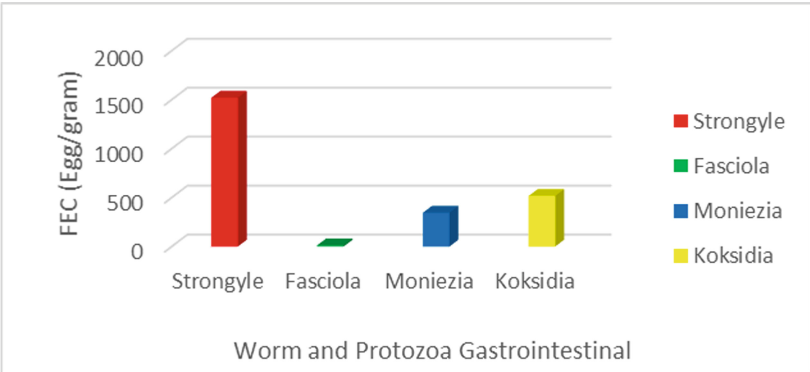

Fig. 2. Mean of FEC of Worm and Protozoa Gastrointestinal Infestation in sheep

3.2 Sheep Gastrointestinal Nematode (GIN) Infection

Numerous genera of nematodes are frequently found as a natural infection in small ruminants. Based on the FEC conducted before the experiment, the GIN infection rate was extremely high. One hundred per cent of adult sheep were infected with at least one type of GIN or a mix of Trematode (Fasciola), Cestode (Moniezea), and Protozoa (Coccidia) (Fig. 1). However, GIN is the most prevalent, with a mean infection intensity of 1520 - 5040 eggs/gram.

The GIN infection could be generally divided into mild infection (FEC $<$ 500 eggs/gram), moderate infection (FEC $=$ 500–1000 eggs/gram), and severe infection (FEC $>$ 1500 eggs/gram) [19]. In this sample set, the GIN infection was determined to be mild to severe (Fig. 2).

The fasciola and cestoda infestation rates are low, at 16% and 10%, respectively, and the average infection intensity is below 13.4 ± 12.75 eggs per 3 g for fasciola and 346 ± 266.3 eggs per gram for cestode. The rate of coccidia infection was 53%, with an average infection intensity of 517.78 ± 517.17 oocysts/gram: No infection (Oocysts Per Gram

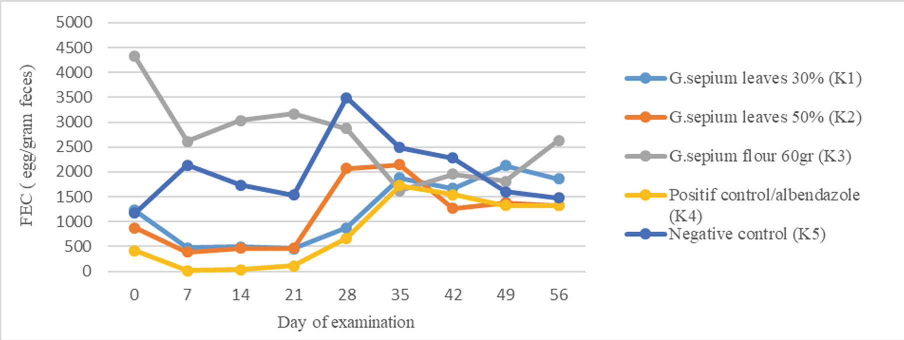

Fig. 3. Reduction of FEC in feed treatment with supplementation of various *G.sepium* leaves preparations

(OPG) = 0 oocysts/gram), mild infection (OPG 1800 oocysts/gram), moderate infection (OPG = 1800–6000 oocysts/gram), and severe infection (OPG > 6000 oocysts/gram) are the grades of coccidia infection [20, 22]. However, the fasciola, cestode, and coccidia infections in our sample set were mild, so we only considered gastrointestinal nematodes in this investigation.

3.3 The Dominant Nematode Genus in Sheep

The evaluation of nematode worm larvae from larval fertilization shows that the larva comprises Haemonchus sp (83.10%), Trichostrongylus (15.8%), Oesophagostomum (0.83%), and Cooperia (0.20%).

3.4 Application of Gliricidia Sepium Leaves, Oil Extract and Flour

From the faecal examination results, it can be seen that the K1 group of sheep treated with 30% fresh *G.sepium* leaves forage (FF) for 21 days had a significant decrease in the amount of EPG ($p < 0.05$) in the first week compared to the negative control, from 1232 ± 945.3 egg/gram (high infestation) to 472 ± 295.4 egg/gram (low infestation) and maintain until the third week (21 days), with a 62–63% reduction in the average FEC based on weekly observations (Fig. 3). When the administration of *G.sepium* leaves was discontinued, however, worm infestation at week 4 tended to increase and remained high infestation during observation. (Fig. 3). In the first week of treatment, sheep given fresh *G.sepium* leaves with 50% FF exhibited a significantly different decrease in EPG compared to negative controls ($P < 0.05$), from 872 ± 373.5 EPG (moderate infection) until the third week, with an average FECR of 47–56%. In addition, results indicated that there was no significant difference between the K1 and K2 groups during the first three weeks ($p > 0.05$). The K3 group, which received a mixture of feed containing 60 g of *G.sepium* powder for 21 days, exhibited a 27–39% decrease in EPG. The FEC of K4-positive control decreased by 96% in 1st week. The K5 group, which served as negative controls, exhibited fluctuating increases in FEC between 30- 100%.

4 Discussion

In this study, all sheep (young and adults) were infected naturally with various species of worms, with gastrointestinal nematode worms, specifically *H. contortus*, being the most prevalent species, despite the farmer's efforts to keep the cages and surrounding area clean. *H. contortus* is a highly pathogenic parasite that is capable of sucking 0.05 ml of blood per day [21]. At this location, the sheep were infected with chronic haemonchosis, but no clinical symptoms were observed. This is because the sheep received an adequate diet, but their growth and weight were not optimal. Infections can have both direct and indirect effects on sheep producers, as a major constraint [22]. In the seasonally endemic zone, chronic hemochosis typically occurs in an environment less suitable for the development of infective larvae [23]. Infections can manifest as reduced productivity, feed conversion, growth rate, or milk production [6].

The sheep population in the study site is susceptible to worm infestation due to the *Pennisetum Purpureum Cv. Mott* grass that grows on land fertilized with fresh manure. Therefore, a high concentration of parasitic larvae in grasses infests sheep. According to Besier [23], clinical symptoms may not be apparent unless the number of ingested larvae or worms increases when the nutritional status of the host animal declines, thereby decreasing the host's tolerance to the pathogenic effects of haemonchus infestation. Many adults are infected with hemonchosis because immunity to gastrointestinal nematodes in sheep is slow to develop and incomplete [24].

The results of the study on the provision of forage mixed with various preparations of *G.sepium* leaves demonstrated the anthelmintic potential of the forage. The addition of various *G. sepium* leaf preparations to feed may result in a reduction in FEC. However, as the total egg count decreased from day 7 to day 21, the majority of the decrease in FEC during the period can be attributed to Plant Secondary Metabolites (PSM)effects. This reduction was observed just three weeks after treatment, it appears to be due to the direct action of *G. sepium* on the parasite. In addition, the *G.sepium* leaves primarily affected the fertility or egg-laying ability of female parasites but did not affect the adult parasite population. This result is consistent with previous research conducted by [25], as worm loads increased on day 28 following the cessation of *G. sepium* leaf feeding. According to Athanasiadou [26], two weeks of grazing on PSM-rich forages did not affect the immature and adult parasite populations.

According to Min and Hart (2003) [13], the administration of forages containing tannins may have two beneficial effects: direct effects on internal parasites and indirect control of the parasites by increasing animal resistance to GIN infections via improved protein nutrition. Possible direct effects may be mediated by Condensed Tanin (CT)-nematode interactions that reduce nematode viability by decreasing lamb nematode burdens and FEC. Using CT-extracted forages resulted in a 91% reduction in larval development (from eggs to L3 larvae), a 34% reduction in hatched eggs, and a 30% reduction in the mobility of L3 larvae [27]. Adding protein to diets will make sheep more resistant to *H.contortus* [28]. Therefore, by enhancing protein nutrition, dietary CT may aid parasitized ruminants, thereby enhancing the animals' immune response to parasite infection [29, 30].

Giving withered fresh *G.sepium* leaves at 30% and 50% FF significantly decreased FEC compared to untreated controls by 47–56% and 61–63%, respectively. In contrast, administration of *G.sepium* powder resulted in a decrease in FEC that was less consistent and more variable. Therefore, additional testing with larger doses is required. Even though treatment with albendazole reduced the number of FEC by 96%, the low EPG levels only lasted 3 weeks after treatment, and then began to rise to high levels at week 4 and remained elevated until the end of the study. This demonstrated that deworming alone is insufficient for long-term control of helminthiasis; therefore*, G. sepium* leaves must be added to the diet.

After ceasing the administration of *G. sepium* leaves, the EPG levels increased in all treatments. Following the study conducted by [13] on sheep fed with plants containing a high tannin content, FEC was reduced by 50% with (CT) -containing forages (45 to 55 g of CT/kg of Dry Matter) compared to non-CT-containing forages. The most common type of tannin found in forage legumes, trees, and shrubs is condensed tannins [31].

Based on these findings, it can be concluded that *G.sepium* leaves may be utilized as an FF containing anthelmintic bioactive. Giving *G.sepium* leaves can reduce moderate/high to low levels of worm infestation, but cannot eradicate worms as effectively as chemical drugs (albendazole). *G. sepium* leaves can be used to control worm infestations in the sense that if sheep are heavily infested with worms, they must be dewormed at the appropriate time, and then 30 to 50% *G.sepium* supplementation is given FF to maintain low infestation levels so that helminth infestations do not interfere with productivity. These results are highly applicable so that the livestock breeding groups can greatly benefit from these findings. Due to the condition of the sheep, anthelmintics cannot be administered to the majority of those with a high nematode worm infestation. Therefore, presenting *G.sepium* leaves as FF can be an effective means of reducing helminth infestations without the use of anthelmintics. Therefore, it is hoped that the prevalence of helminthiasis in pregnant sheep can be maintained at a low level so as not to impede fetal development. Tannins in plants function as anthelmintics by destroying microvilli [32], damaging the tegument [33], and possessing ovicidal activity [34]. Sahal [35] stated that the anthelminthic power of alkaloid compounds is achieved through the mechanism of acute toxicity, which paralyzes the worms' muscles by inhibiting their central nervous system. Flavonoid compounds have a pharmacological effect, causing a decrease in permeability and vasoconstriction of blood vessels, which disrupts the oxygen and nutrient circulation necessary for worm survival [36].

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