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THE EFFECT OF APUS BAMBOO (*Gigantochloa apus*) LEAVES INFUSION ON MORTALITY RATE AND MORPHOMETRY OF *Haemonchus contortus* ADULT WORM IN VITRO

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ABSTRACT

This research was carried out to determine the effect of apus bamboo (*Gigantochloa apus)* leaves infusion on mortality rate and morphometry of adult *Haemonchus contortus (H. contortus)* in vitro. The research was started with the preparation of 0.1% and 1% apus bamboo leaves infusion. Adult worms were directly collected from abomasum of naturally infected goats which were slaughtered at Animal Slaughter House. Investigation on mortality rate of adult worms at various doses and times of observation as well as differences in *H. contortus* morphometry were analyzed using analysis of variance (ANOVA). The findings disclosed that apus bamboo leaves infusion significantly affected the mortality rate of adult *H. contortus* at various doses and times of observation. This research observed that the best dose to kill the adult worms was 1% bamboo leaves infusion. Moreover, apus bamboo leaves infusion decreased the morphometry measurements of adult *H. contortus*, especially in body length, cervical papillae width, and spicules length in males, and body length, cervical papillae width, and vulva length in females.

Key words: apus bamboo, morphometry, mortality, *Haemonchus contortus*

ABSTRAK

Penelitian ini bertujuan mengetahui pengaruh infusa daun bambu apus (Gigantochloa apus) terhadap tingkat mortalitas dan morfometri cacing dewasa Haemonchus contortus (H. contortus) secara in vitro. Penelitian diawali dengan pembuatan infusa daun bambu apus 0,1% dan 1% . Sampel didapat dari koleksi H. contortus. Cacing dewasa diperoleh secara langsung dari abomasum kambing yang terinfeksi secara alami yang dipotong di Rumah Potong Hewan. Tingkat mortalitas cacing dewasa pada berbagai dosis dan waktu pengamatan serta perbedaan morfometri H. contortus dianalisis menggunakan analisis varian (ANAVA). Infusa daun bambu apus mempunyai perbedaan yang nyata terhadap tingkat kematian cacing dewasa H. contortus pada berbagai dosis dan waktu pengamatan. Dosis terbaik untuk meningkatkan mortalitas cacing adalah infusa daun bambu 1%. Infusa daun bambu apus memengaruhi morfometri cacing dewasa H. contortus terutama panjang tubuh, lebar papila cervical, dan panjang spikula pada jantan, serta panjang tubuh, lebar papila servikal, dan panjang vulva pada betina.

Kata kunci: bambu apus, morfometri, mortalitas, Haemonchus contortus

INTRODUCTION

Indonesian livestock business should have a stronger competitiveness in facing competition with similar products from abroad. The competitiveness may vary according to the types of livestock, such as ruminants (big and small) namely beef cattle, dairy cows, buffaloes, horses, goats and sheep, and non-ruminant livestock such as pigs and poultry (Simatupang and Prajogo, 2004). The main target of goat farmers in addition to improve competitiveness is to gain adequate benefits from livestock activities performed. The success of goat farming business is determined by many things such as selection of the goat, feed, cage, diseases, and management. These benefits can be gained from the daily weight gain of livestock as well as the quality of the produced meat. One of the constraints of livestock farming is parasitic diseases. The success of raising goats requires effective control of parasitic diseases. Control failure may lead to disease, growth disorders, and death. Haemonchosis caused by *Haemonchus contortus (H. contortus)* is one of the major gastrointestinal parasitic diseases in goats in Indonesia. The biggest economic losses due to this disease are mortality, reduced production, stunted growth, and low weight (Mengist *et al.*, 2014). According to Zaman *et al.* (2012), economic losses caused by Haemonchosis in goats in Indonesia are estimated to reach 4.7 million US dollars. Animal infected with *H. contortus* may develop hypo-proteinemia, hypocalcemia, and hypophosphatemia, resulting in a decrease in carcass weight (Soulsby, 1986). In addition, this worm can suck 0.05 mL of blood per sheep per day (Menzies, 2006).

Control program based on the use of commercial anthelmintics recently often fails to control gastrointestinal nematodes due to the prevalence of increased drug resistance (Mortensen *et al.*, 2003). Anthelmintic resistance is responded by making the discovery of natural substances with low toxicity to reduce the burden of worms in livestock (Alawa *et al.*, 2003). Innovations to find out a replacement for the anthelmintic is by selecting some types of plants that have tannins substance, because it is reported that the substance is able to reduce the incidence of worm infestation. The development of new anthelmintics suggests that tannin ferous plants can be considered as potential strategic alternatives for control of nematode infestation in small ruminants (Akkari *et al.*, 2008). Plant biological resources have been widely used by breeders and researchers to help increasing the growth of their farms. One source of biological plants that can

be a new alternative is the use of apus bamboo (*Gigantochloa apus*) leaves to help the farm business.

Bamboo leaves have a high potential to be an alternative because bamboo population is high and the leaves are rarely used for useful purposes. Bamboo leaf is known as *Gold of The Poor* because its value is high and its role as pharmacological agent which is spontaneous motor, antibacterial, antioxidant, and antitumor. Bamboo leaf also contains a variety of nutrients that are beneficial to the performance of livestock. The content of tannin in bamboo leaf is reported to be able to provide benefits to kill the worms in livestock so that it needs to be studied further as anthelmintic in farm animals. The potency of apus bamboo leaf as anthelmintic agent was examined in vitro for mortality rate of *H. contortus* adult worm and its morphometry.

MATERIALS AND METHODS

The tools used in this research were petri dish to observe adult worms motility and mortality rate, object glass, microscope to observe part of the worm, camera lucida to snapshot the worm, stopwatch to measure Haemonchus death time after soaking in apus bamboo infusion, oven, erlenmeyer flask to dilute apus bamboo infusion in various dosage, electric scale to measure bamboo weight, surgical scissor to dissect the abomasum for worms collection, and micro caliper to measure the length and body part of *H. contortus*. The materials used are bamboo leaves infusion, *H. contortus* adult worm, aquadest, ethanol, and 0.62% NaCl.

Bamboo Leaves Infusion

The 1% bamboo leaves infusion was prepared according to procedure used by Daryatmo and Widiarso (2010). Simplicia of apus bamboo was made by chopping bamboo into smaller pieces. Chopped apus bamboo weighed 1 g and 10 g were put into separate beakers for stock solutions. The beakers filled with bamboo were then filled with 100 mL aquadest. The beakers were then put into an oven with a temperature of 90° C for 15 minutes. The remaining liquid in the beakers were taken and filtered to obtain 1% and 10% bamboo infusion.

Haemonchus contortus Collection

Adult worms were obtained directly from abomasum of naturally infected goats that slaughtered at animal slaughter house. Abomasum adjacent to the duodenum and rumen was separated. Abomasum content was carefully removed and visible parasites were collected in containers containing phosphate buffered saline (PBS) solution (Kuchai *et al.*, 2012). Worms taken with fine wire were then carefully crushed in a mortar to remove worm eggs. Egg suspension obtained was sprayed with physiological NaCl using a pipette and put in a bottle. The bottle was then closed and stored at room temperature for 3-5 days to observe the development of worm eggs treated with bamboo infusion.

Analysis of Worm Mortality

The test was performed according to Peterson *et al.* (1997) as cited in Alemu *et al.* (2014). A total of 80 adult worms were divided into 8 treatment groups with each petri dish consisted of 10 worms. Each group was immersed in apus bamboo infusion with various doses as follows: 0.1%, 0.2%, 0.4%, 0.6%, 0.8%, 1%, albendazole (positive control) and aquadest or 0.62% NaCl (negative control). Each group was observed every hour up to 4 hours.

Data Analysis

Mortality rates of adult worms at various doses and times of observation was analysed using two way analysis of variance (ANOVA), while the differences in morphometry of *H. contortus* was analyzed using one way ANOVA (Steel and Torrie, 1990).

RESULTS AND DISCUSSION

Mortality Rate of Worms

Apus bamboo infusion was capable of killing *H. contortus* adult worms after 2 hours and 4 hours of immersion in vitro. Table 1 showed that at a dose of 0.1% was unable to kill the worms after 2 hours immersion in the apus bamboo infusion, but 23.33% of worms was killed after 4 hours immersion. At a dose of 0.2% bamboo infusion can kill 20% worms after 2 hours and 30% after 4 hours. At dose of 0.4% can kill the worms 30% after 2 hours and 53.33% after 4 hours. At dose of 0.6% bamboo infusion able to kill 53.33% worms after 2 hours and 63.33% after 4 hours. At a dose of 0.8% can kill the worms 70% after 2 hours and 80% after 4 hours. At the highest dose of 1% bamboo leaves infusion able to kill the worms 96.67% after 2 hours and 100% after 4 hours. On the positive control has the same effect with the dose of 1% which was

Table 1. Efficacy of *Gigantochloa apus* infusion on mortality rate of adult worms after 2 hours and 4 hours immersion

No	Treatment	After 2 hours (%)	After 4 hours (%)
1	Aquadest/negative control	$0.00\pm0.00^{a,F}$	$0.00\pm0.00^{a,F}$
2	0.1%	$0.00\pm0.07^{a,F}$	$23.33\pm5.77^{b,G}$
3	0.2%	$20.00\pm0.00^{a,F}$	$30.00\pm0.00^{b,G}$
4	0.4%	$30.00\pm0.00^{b,F}$	$53.33\pm5.77^{c,G}$
5	0.6%	$53.33\pm5.77^{c,F}$	$63.33\pm5.77^{c,G}$
6	0.8%	$70.00\pm10.00^{d,F}$	$80.00\pm0.00^{d,G}$
7	1%	$96.67\pm5.77^{e,F}$	$100.00\pm0.00^{d,F}$
8	Albendazole/positive control	$73.33\pm5.77^{d,F}$	$100.00\pm0.00^{d,G}$

$^{A, B, C, D, E, F, G}$Different superscripts within the same row indicates significant differences (P<0.05)

$^{a, b, c, d, e, f, g}$Different superscripts within the same column indicates significant differences (P<0.05)

capable of killing 100% after 4 hours.

The result of variance analysis indicated that there was significant difference in different time and various doses on mortality rates of *H. contortus* in vitro. At the dose of 1%, all *H. contortus* adult worms were killed, while at a dose of 0.1% only *H. Contortus* adult worms were killed. This is probably due to apus bamboo have a tannin content of 8.81% w/w based on total tannin content which had been tested in Gadjah Mada University integrated testing laboratory. The high amount of tannins are able to play a significant role in binding proteins and turning nematode walls into inactivity and killing them (Hoste *et al.*, 2006). Tannins commonly found in plants (leguminous) is condensed tannins (Min and Hart, 2003). Condensed tannins are effective against gastrointestinal parasites. The effect of condensed tannins against gastrointestinal parasites was carried out directly or indirectly. Directly, tannins through the interaction of condensed tannin-nematodes affect hatching, and affect the growth of infective larvae. The condensed tannin also has the ability to bind proteins, and make nematode walls to be inactive and subsequently kill them. Indirectly, tannins may bind plant proteins in the rumen to prevent microbial degradation. Furthermore, this will increase the flow of proteins into the duodenum. Min and Hart (2003) had proven that increased protein nutrition will decrease parasite infections by increasing host immunity.

Figure 1 illustrated the increasing of worms mortality immersed in bamboo leaves infusion with increasing of doses ranging from 0%, 0.1%, 0.2%, 0.4%, 0.6%, 0.8% to 1%. Long immersion for 4 hours in apus bamboo leaves infusion killed more worms than those immersed for 2 hours.

In Figure 1, the best dose to increase mortality rate of *H. contortus* adult worms was 1%, based on its capability to kill 96.67% worms after 2 hours. Such finding was related to tannin content in apus bamboo leaves. According to Kamaraj *et al.* (2011), natural ingredients that have anthelmintic properties were active ingredients of tannins, saponins, flavonoids, and alkaloids. Plants containing 5% tannin extract can reduce contamination of larvae and can be used as anthelmintic (Min and Hart, 2003). Bamboo leaves can be an alternative to herbal anthelmintic as a substitute for commercial anthelmintic, as it has been studied by Razali *et al.* (2014) on the use of katuk leaves as anthelmintic on goats, and the research of Beriajaya *et al.* (1998) on anthelmintic of betel nut for goats. In addition, according to Hamad (2012) the continuous administration of certain commercial anthelmintics may cause resistance in sheep, therefore it needs a worm treatment program using herbal ingredients.

Apus Bamboo Leaves Infusion on Morphometry of Female *Haemonchus contortus* Adult Worms

The result showed that bamboo leaves infusion affects morphometry of female and male *H. contortus* adult worms such as body length, cervical papillae width, and vulva length (Table 2 and Table 3).

Table 2 showed that there was a significant difference in the body length of female *H. contortus* adult worms in 0.1% and 1% apus bamboo leaves groups over the control ($P<0.05$). On the width of cervical papillae, there was no significant difference between the dose of 0.1% and the control which were significantly higher ($P<0.05$) than the dose of 1% apus bamboo leaves infusion. Similarly, the length of vulva in 0.1% group and the control group were similar ($P<0.05$), which were significantly higher than the dose of 1% apus bamboo leaves infusion ($P<0.05$). The decrease on the body length of the worm, cervical papillae width, and vulva length probably due to the cuticle was damaged by tannins contained in the apus bamboo leaves. Tannins on apus bamboo may be leaves play a role in binding proteins and turning nematode walls into inactivity and killing them as reported by Hoste *et al.* (2006). In addition, Molan *et al.* (2009) stated that condensed tannins may have different effects on ruminants when consumed on the growth of adult worms and larvae.

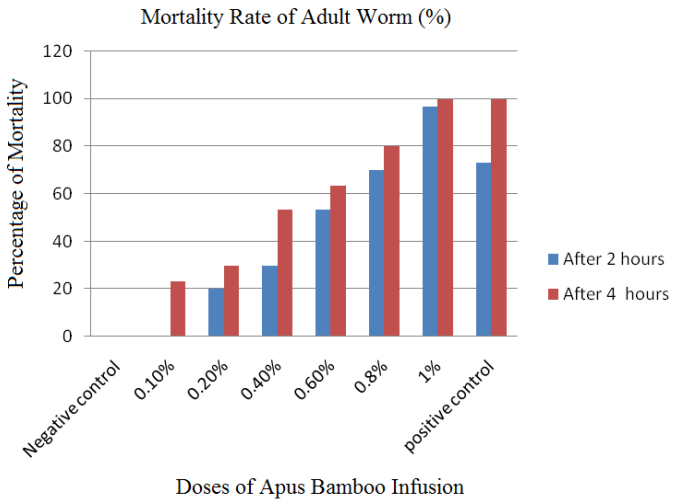

Figure 1. Efficacy of bamboo (*Gigantochloa apus*) leaves infusion on mortality rate of *Haemonchus contortus* adult worms

Table 2. Morphometry of female *Haemonchus contortus* adult worms due to administration of bamboo leaves infusion in vitro

Worm morphometry	Control (0%)	0.1% apus bamboo leaves infusion	1% apus bamboo leaves infusion
Body length	27.25±2.59[a]	26.30±1.75[b]	24.30±1.85[c]
Cervical papillae width	0.38±0.08[a]	0.36±0.06[a]	0.33±0.08[b]
Vulva length	4.67±0.31[a]	4.12±0.22[a]	3.87±0.67[b]

[a, b, c]Different superscripts within the same row indicates significant differences (P<0.05)

Table 3. Morphometry of male *Haemonchus contortus* adult worms due to the administration of bamboo leaves infusion in vitro

Worm morphometry	Control (0%)	0.1% (mm) apus bamboo leaves infusion	1% (mm) apus bamboo leaves infusion
Body length	17.25±0.79[a]	16.30±0.71[b]	14.30±1.85[c]
Cervical papillae width	0.44±0.03[a]	0.41±0.06[a]	0.38±0.08[b]
Spicula length	0.52±0.01[a]	0.42±0.22[a]	0.38±0.67[b]

[a, b, c]Different superscripts within the same row indicates significant differences (P<0.05)

Microscopic observation on *H. contortus* morphometry indicated a difference on body length, cervical papillae width, and spicula length in male worms treated with control and apus bamboo leaves infusion. Apus bamboo leaves infusion at a dose of 0.1% (16.30±0.71) and 1% (14.30±1.85) significantly (P<0.05) reduced the body length of male adult worms compared to control (17.25±0.79). The cervical papillae width and spicula length were lower (P<0.05) in 1% apus bamboo leaves infusion (0.38±0.67) compared to 0.1% (0.42%±0.22) and control (0.52±0.01). There was no significant difference between the spicula length and cervical papillae width of worms treated with the dose of 0.1% and those of the control. The presence of many morphometric differences between different doses and control may be due to the effect of tannins in bamboo leaves infusion that can damage the adult worms cuticle, interfere with the digestion process. The cuticle gives the shape of the worms and also involved in their motility and in the exchanges with the parasite environment, including the metabolic exchanges with the local environment in the digestion tract of the host (Kuchai *et al.*, 2012).

CONCLUSION

Apus bamboo leaves infusion has a significant difference on mortality rate of *H. contortus* adult worms at various doses and times of observation and reduced morphometry measurement of *H. contortus* adult worms, notably in body length, cervical papilla width, and spicules length in males and body length, cervical papilla width, and vulva length in females.The best dose to increase mortality of *H. contortus* adult worms was 1% apus bamboo leaves infusion.

REFERENCES

Akkari, H., M.A. Darghout, and H.B. Salem. 2008. Preliminary investigations of the antinematode activity of *Acacia cyanophylla* Lindl.: Excretion gastrointestinal nematode eggs in lambs browsing *A. Cyanophulla* with and without PEG or grazing native grass. **J. Small Rum. Res**. 74(b):78-83.

Alawa, C.B.I., A.M. Adamu, J.O. Gefu, O.J. Ajanusi, P.A. Abdu, N.P. Chiezy, J.N. Alawa, and D.D. Bowman. 2003. In vitro screening of two Nigerian medicinal plants (*Vernona amygdalina* and *Annona senegalensis*) for anthelmintic activity. **J. Vet. Parasitol**. 113:73-81.

Alemu, Z., Y. Kechero, A. Kabede, and A. Muhammed. 2014. Comparison of the in vitro inhibitory effect of doses of tanin rich plant extract and ivermectin on egg hatch ability, larvae development, and adult mortality of *Haemonchus contortus*. **Acta Parasitol.** 59:513-519.

Beriajaya, T.B. Murdiati, Suhardono, and C.F. Pantouw. 1998. Pengaruh ekstak biji pinang (*Arecha catechu*) terhadap cacing *Haemonchus contortus* secara in vitro. **Prosiding Seminar Hasil-Hasil Penelitian Veteriner.** Bogor:154-160.

Daryatmo, J. and B.P. Widiarso. 2014. Daun bambu sebagai agen fertilitas. **Jurnal Pengembangan Penyuluhan Pertanian.** 10(20):53-57.

Hamad, A.A. 2012. Efficacy of Some Indigenous Medicine Plant to Control Antinematicidal-Resistant *Haemonchus contortus* in Sheep. **Thesis.** University of Agriculture. Faisalabad Pakistan.

Hoste, H., F. Jackson, S. Athanasiadou, S.M. Thamsborg, and S.O. Hoskin. 2006. The effect of tannin-rich plants on parasitic nematodes in ruminants. **Trend Parasitol.** 22(6):253-261.

Kamaraj, C.A., G. Rahman, Elango, A. Bagavan, and A.A. Zahir. 2011. Anthelmintic activity of botanical extract against sheep gastrointestinal nematodes, *Haemonchus contortus*. **Parasitol. Res**. 108:37-45.

Kuchai, J.A., F. Ahmad, M.Z., Chisty, Tak, J. Achmad, S. Ahmad, and M. Rassol. 2012. A study on morpology and morphometry of *Haemonchus contortus*. **Pak. J. Zool**. 44(6):1737-1741.

Mengist, Z., N. Abebe, G. Gugsa, and N. Kumar. 2014. Assessment of small ruminant haemonchosis and its associated risk factors in and around Finoteselam, Ethiophia. **IQSR-JAVS**. 7(12):36-41.

Menzies, P. 2006. Heaven on Earth for Sheep Parasites. Ontario Sheep Flock Health and Management. **http://www. Ontario sheep.org/ LinkClick.aspx? fileticket= ofhsMvSgsmo%3D& tabid =95**.

Min, B.R. and S.P. Hart. 2003. Tanins for supression of internal parasites. **J. Anim. Sci**. 81:E102-E109.

Molan, A.L., G.C. Waghorn, and W.C. Mc.Nabb. 2009. Condensed tannin and gastrointestinal parasities in sheep. **Proc. Soc. Anim. Prod.** 69:89-92.

Mortensen, L.L., L.H.Wiliamson, T.H. Terril, R. Kircher, M. Larsen, and R.M. Kaplan. 2003. Evaluation of prevalence and clinical implications of anthelmintic resistance in gastrointestinal nematodes of goats. **J. Am. Vet. Med. Assoc.** 23:495-500.

Razali, Azhari, A. Novita, T.R. Ferasyi, Ridwan, dan A. Munandar. 2014. Potensi suspensi dan ekstrak daun katuk sebagai antelmintik terhadap nematoda gastrointestinal pada ternak kambing. **J. Ked. Hewan**. 8(2):120-123.

Simatupang, P. and U.H. Prajogo. 2004. Daya saing usaha peternakan menuju 2020. **Wartazoa.** 14(2):45-57.

Soulsby, E.J.L. 1986. **Helminths Arthropods and Protozoa of Domesticated Animals**. 7th ed. Bailiere Tindall, London.

Steel, R.G.D. and J.H. Torrie. 1990. **Principle and Procedures of Statistics.** 2nd ed. MacGraw-Hill, Singapore.

Zaman, M.A., Z. Iqbal, M.N. Khan, and G. Muhammad. 2012. Anthelmintic activity of herbal formulation against gastrointestinal nematode of sheep. **Pak. Vet. J.** 32(1):117-121.