MORPHOLOGY AND MORPHOMETRY OF Haemonchus contortus EXPOSED TO Gigantochloa Apus CRUDE AQUEOUS EXTRACT

By Budi Purwo Widiarso

WORD COUNT 2330
TIME SUBMITTED 20-MAR-2020 08:31AM
PAPER ID 56640670

MORPHOLOGY AND MORPHOMETRY OF *Haemonchus contortus* EXPOSED TO *Gigantochloa Apus* CRUDE AQUEOUS EXTRACT

Budi Purwo Widiarso,[1,2], Kurniasih[3], Joko Prastowo[4] Wisnu Nurcahyo[5]

Introduction

Haemonchus contortus is the most pathogenic nematode infesting the digestive tract of goats and sheeps throughout the world. It leads to tremendous loss in variety of routes [1]. Poor weight gain, retarded growth, loss of production and mortality usually cause economic losses because of haemonchosis in subtropic and tropic areas [2,3]. Prevalence of *Haemonchosis* in Indonesia is at 89.4% in goat and annual loss achieved 1 million dollar US [4].

For decades the use of chemicals anthelmintic for the therapy of gastrointestinal nematode infections in cattle has present of drug resistance. In addition to spend a considerable cost to find new types anthelmintic also often pose a problem for the safety of food products of animal origin [5]. Anthelmintic resistance is responded by making the discovery of natural substances with low toxicity to reduce the burden of worms in livestock [6]. 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 tanninferous plants can be considered as potential strategic alternatives for control of nematode infestation in small ruminants [7]. Plant biological resources have been widely used by breeders and researchers to help increasing the growth of their farms. One source of biological tanninferous plants that can be a new alternative is the use of bamboo leaves to help the farm business [8].

. This study evaluated *in vitro* effects of *Gigantochloa apus* crude aqueous extract as an anthelmintic on *Haemonchus contortus* morphology and morphometry

Materials and Methods

Ethical approval

All stages of the research were approved by the Ethical Committee of Gadjah Mada University (number 00118/04/LPPT/IX/2017)

Tools and Materials:

The research materials include tools and materials used during the research. The tools used are: petri dish to observe adult worms motility and mortality rate, object glass to make worm preparation, microscope to observe part of the worm, camera lucida to paint the worm, stopwatch to measure *Haemonchus* killing time submerged by *Gigantochloa apus* crude aqueous extract, oven to make apus bamboo crude aqueous extract, erlenmeyer flask to make apus bamboo crude aqueous extract in various dosage, electric scale to measure bamboo leaves weight, surgical scissor to cut off the abomasal line for worms exploring and the length and body part of *Haemonchus contortus* were measured by micro caliper. The materials used are *Gigantochloa apus* crude aqueous extract, *Haemonchus contortus* adult worm, aquadestilata, ethanol, and 0.62% NaCl.

Parasites collection

Bligon goats which is naturally infected were collected from slaughtered goat from local slaughterhouses namely Besi Sleman. Bligon Goat's abomasums part were carefully examined and transported to the Parasitology Laboratory, University of Gadjah Mada , Yogyakarta. *Haemonchus contortus* were collected from abomasum and put into a petri dish containing 0.62% water saline.

The figure of the parasites or parts of parasites were captured using camera lucida and they were measured using, both objective micrometer and objective-ocular micrometer. All the capturing processes were done by the help of Olympus Digital Camera under Olymphus CX21

microscopic. Parasites morphology was identified in morphological and morphometric characters [6].

Gigantochloa apus crude aqueous extract

In making 1% *Gigantochloa apus* crude aqueous extract, according to Daryatmo *et al*.[9], simplicia of apus bamboo leaves was made by chopping bamboo leaves into smaller pieces. Chopped apus bamboo leaves were weighed according to the desired weight or concentration, which was 1 gram and 10 grams for stock solutions. Both chopped apus bamboo leaves are put into separate beakers. The beakers filled with bamboo leaves were then filled with 100 ml of 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 concentration 0.1% and 1% *Gigantochloa apus* crude aqueous extract.

Data Analysis

Morphology of *Haemonchus contortus* adult worms at various concentration were determined by microscopic observation. The differences in morphometry variables of *H. contortus* were recorded and analyzed using SPSS software 16.0.

Result

Gigantochloa apus leaves crude aqueous extract on morphology of female and male *Haemonchus contortus* adult worms

Comparative characteristics have been shown in figure 1 until figure 4. The body is filiform (slender) tapering towards the anterior end in male and female. Anterior end is relatively wide and blunt. The buccal cavity is small with a conspicuous tooth extending from dorsal wall. There are no buccal capsules. In addition to transverse striation longitudinal lines are also present on the body.

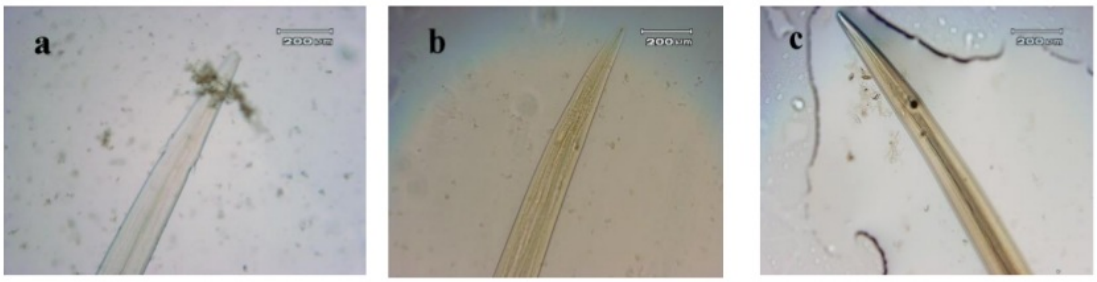

Figure 1. Morphology cervical papillae (a.cervical papillae which is not soaked in Gigantochloa apus crude aqueous extract; b. cervical papillae which is soaked in *Gigantochloa apus* crude aqueous extract 0.1 mg/ml concentration; c. cervical papillae which is soaked *Gigantochloa apus* crude aqueous extract 1 mg/ml concentration;

Figure 1 explained that cervical papillae in control (a) appears clearly and conspicuous. Part of cervical papillae at the concentration 1 mg/ml, and 0.1 mg/ml. Anterior end is more tapered and the cervical papillae bulge appears unclear shape(b/c)

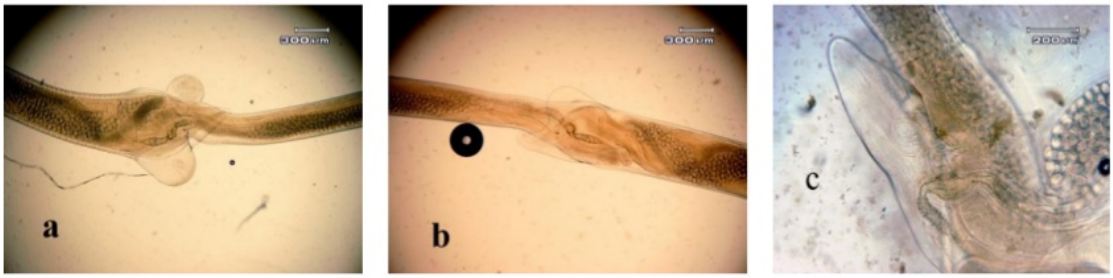

Figure 2. Morphology of vulvar flab (a.vulvar flab which is not soaked in *Gigantochloa apus* crude aqueous extract; b. Vulvar flab which is not soaked in *Gigantochloa apus* crude aqueous extract 0.1 mg/ml concentration; c. Vulvar flab which is not soaked in *Gigantochloa apus* crude aqueous extract 1 mg/ml

Figure 2 observed that vulvar flab control is not taper (a). Vulvar flab that which getting aware from *Gigantchloa apus* concentration 0.1 mg/ml and 1 mg/ml crude aqueous extract of *Gigantochloa apus* looks more pointed. Vulvar flab in concentration 1 mg/ml more sharpener and pointed than 0.1 mg/ml concentration.

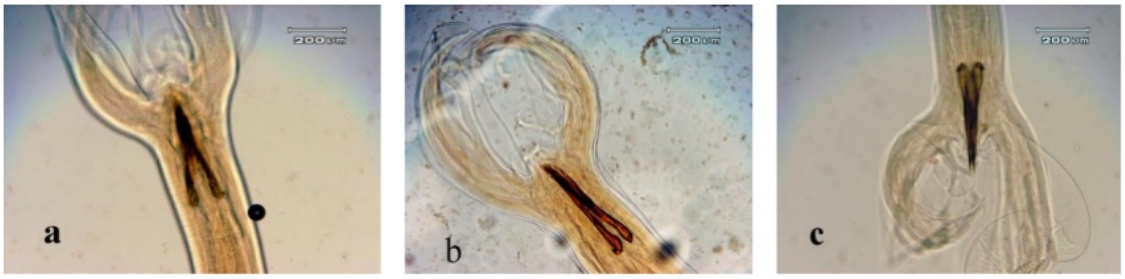

Figure 3. Morphology of gubernaculum (a.gubernaculum which is not soaked in *Gigantochloa apus* crude aqueous extract; b. gubernaculum which is soaked in *Gigantochloa apus* crude aqueous extract 0.1 mg/ml concentration; c. gubernaculum which is soaked in *Gigantochloa apus* crude aqueous extract 1 mg/ml

The gubernaculum form exposed to *Gigantochloa apus* (b/c) appears irregular compared to control gubernaculum which tends to be more compact. Spiculas exposed to *Gigantochloa apus* appear more budding than control spicula (a) (Figure 3).

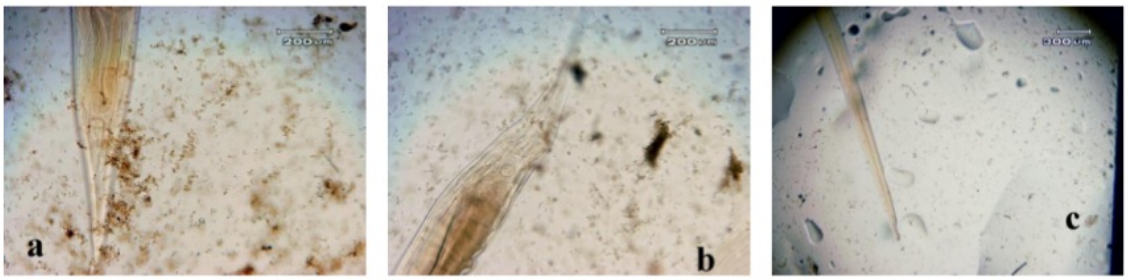

Figure 4. Morphology of posterior end (a.posterior end which is not soaked in *Gigantochloa apus* crude aqueous extract; b. Posterior end which is soaked in *Gigantochloa apus* crude aqueous extract 0.1 mg/ml concentration; c. Posterior end which is soaked in *Gigantochloa apus* crude aqueous extract 1 mg/ml

The posterior end form exposed to *Gigantochloa apus* crude aqueous extract 0.1 mg/ml and 1 mg/ml appears irregular (b/c) more than posterior end control (a) (Figure 4).

Apus bamboo leaves crude aqueous extract on morphometry of female and male *Haemonchus contortus* adult worms

The result reports that bamboo leaves crude aqueous extract affects morphometry of female and male *Haemonchus contortus* adult worms such as body length of the worm, body width of the worm, cervical papillae width, spicula length, gubernaculum length and vulva length (Table 1 and Table 2).

Table 1. Morphometry of female *Haemonchus contortus* adult worms due to administration of *Gigantochloa apus* crude aqueous extract in vitro

Worm morphometry	**Control (0%)**	**0.1mg/ml *Gigantochloa apus* crude aqueous extract (mm)**	**1 mg/ml *Gigantochloa apus* crude aqueous extract (mm)**
Body length	27.25±2.59[a]	26.30±1.75[b]	24.30±85[c]
Body width	0.64±0.08[a]	0.48±0.09[b]	0.42±0.07[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]	4.1 ±0.22[a]	3.87± 0.67[b]

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

Morphometry of female *H.contortus* produces a body length and body width 27.25 ± 2.59 mm and 0.64 ±0.08 respectively . Vulva is located in the posterior third of the body at a distance of about 4.67 ± 0.31mm from posterior end.The vulvular lips are inconspicuous s but a linguiform process is invariably present. Vulva is covered with valves.

Table 2. Morphometry of male *Haemonchus contortus* adult worms due to the administration of *Gigantochloa apus* crude aqueous extract

Worm morphometry	**Control (0%)**	**0.1 mg/ml *Gigantochloa apus* crude aqueous extract (mm)**	**1 mg/ml Gigantochloa apus crude aqueous extract(mm)**
Body length	17.25±0.79[a]	16.30±0.71[b]	14.30±1.85[c]
Body width	0.29±0.06	0.22±0.05	0.20±0.04
Cervical papillae width	0.44±0.03[a]	0.4 ±0.06[a]	0.38±0.08[b]

Spicula length	0.52±0.01[a]	0.42±0.22[a]	0.38±0.07[b]
Gubernaculum length	0.22±0.02[a]	0.20±0,01[a]	0.18±0.03[a]

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

Morphometry of male *H.contortus* produces a body length and body width 17,25 ± 0,79 mm and 0.29 ± 0.06 respectively. The tail end bears a bursa. The bursa consist of three lobes, two large lateral lobes and poorly developed dorsal lobe. Dorsal ray is asymetrical and bifurcated. Externo dorsal ray is thin and long. Lateral rays arise from a common trunk, ventral rays are fused proximally and separated dorsally.

The result of microscopic observation on *Haemonchus contortus* adult worms indicates that it has a significant difference for body length, body width, cervical papillae and spicula length in male. There is no significant difference for gubernaculum length. *Gigantochloa apus* crude aqueous extract at concentration 0.1mg/ml and 1mg/ml is able to shorten the body length and spicula length. *Gigantochloa apus* crude aqueous extract were decreased the body width in male adult worms.

Discussions

From the last study about *Haemonchus contortus* morphology [1], the shape is observed to be similar to the morphology of *Haemonchus contortus* with Reyaz *et al*.,[10] characters including : colour, total length, maximum width, spicula length, vulva length, gubernaculum length, and cervical papillae width .

Based on the table 2 above, it can be determined that there is a significant difference in the body width and body length of female *Haemonchus contortus* adult worms among the concentration 0.1mg/ml ,1mg/ml and 0 mg/ml (control). On the width of cervical papillae, there is no significant difference between the concentration 0.1mg/ml and 0 mg/ml (control), but at the concentration 1mg/ml *Gigantochloa apus* crude aqueous extract has a significant difference to the control. The length of vulva

has no significant difference at the concentration 0.1mg/ml to the control, but the concentration 1mg/ml has a significant difference to the control and the concentration 0.1mg/ml in *Gigantochloa apus* crude aqueous extract. The decrease on the length of the worm's body, the body width,the width of cervical papillae, and the length of vulva were caused the cuticle is damaged by tannins contained in the apus bamboo leaves. The tannin disturbs the physiology process of nematodes by binding nematodes protein directly and subsequently [11]

Preliminary phytochemical screening of *Gigantochloa apus* revealed saponin, alkaloid, flaovonoid, and tannin. Tannin in *Gigantochloa apus* can influence both directly and indirectly with adult worms. Direct reaction happens by attaching their cuticle and causing distress, while indirectly reaction happens by improving protein nutrition [12].

Tannins on apus bamboo leaves play a role in binding proteins and turning nematode walls into inactivity and killing them as reported by Hoste *et al*.,[13]. In addition, [14] argue that condensed tannins may have different effects on ruminants when consumed on the growth of adult worms and larvae. Calvin *et al*.,[15] have shown that condensed tannins have anthelmintic activity with varied possible mechanism of actions most especially astringent property.

Microscopic observation on *H. contortus* morphometry indicates that it has a significant difference about body length, body width, cervical papillae width, gubernaculum and spicula length in male worms (Table 2). *Gigantochloa apus* crude aqueous extract concentration 1 mg/ml and 0.1 mg/ml were able to shorten the body length of male adult worms. Both concentration of bamboo leaves crude aqueous extract 0.1 mg/ml and 1mg/ml have significant difference to the control, which are in the body length, cervical papillae width, and spicules length. The length of spicules has a significant difference between *Gigantochloa apus* crude aqueous extract at the concentration 1mg/ml (0.38 ±0.67) versus 0.1mg/ml (0.42% ±0.22) and at the concentration 1mg/ml versus control (0.52 ±0.01), but there was no significant difference between the length of spicules of worm exposed to 0.1mg/ml concentration and those of the control. The width of cervical papillae in male *H. contortus* adult worms differed

significantly between the dose of 1mg/ml *Gigantochloa apus* crude aqueous extract (0.38 ± 0.08) and the dose of 0.1mg/ml (0.41% ± 0.06), as well as betwen the dose of 1mg/ml and the control (0.44 ±0.03), but there was no significant difference between 0.1mg/ml dose and the control on width of cervical papillae. The presence of many morphometric differences between different doses and control may be due to the effect of tannins in *Gigantochloa apus* crude aqueous extract that can damage the adult worm's cuticle, interfere with the digestion process. The cuticle change with the longitudinal and transverse wrinkles after *in vitro* exposure to *Biophytum persianum* rich in condensed tannin on Haemonchus contortus were evaluated by Sambodo *et al*.[16] crude extract.The wrinkles in the cuticle and anterior end of *Haemonchus contortus* were also observed by Martinez-Ortiz-DeMontellano *et al*.[17]. The cuticle reveals the form of adult worms and also involved in its motility and in the exchanges with the parasite environment, including the metabolic exchanges with the local environment in the digestion tract of the host[1].

Conclusion

Gigantochloa apus crude aqueous extract activity revealed morphology change and reduced morphometry measurement of *H. contortus* adult worms, notably in body length, body width, cervical papillae width, gubernaculum and spicules length in males and body length, body width, cervical papillae width, and vulva length in females.

Authors ' Contributions

The research was determined, managed, and supervised by KK. BPW took samples, recorded samples and samples analysis. WN, BPW, and KK arranged, analyzed and wrote the report. JP worked overall observation of the experiment and the manuscript writing . All authors have observed and ratified the final manuscript.

Acknowledgements

The authors would like to thank to Mr. Abu Mas'ud for his goat's abomasum support for this research.Special to thanks due to State of Agriculture Human Resources Development and

Agriculture Extension, Ministry of Agriculture, Indonesia for financial support through Domestic Graduate Scholarship Grant number 547/Kpts/Kp.320/8/2016 Address: Jalan Harsono RM No 3 Gedung D Pasar Minggu Jakarta Selatan Indonesia

Competing Interest

The authors declare that they have no competing interests.

MORPHOLOGY AND MORPHOMETRY OF Haemonchus contortus EXPOSED TO Gigantochloa Apus CRUDE AQUEOUS EXTRACT

ORIGINALITY REPORT

20%

SIMILARITY INDEX

PRIMARY SOURCES

1	www.veterinaryworld.org Internet	270 words — 11%
2	zsp.com.pk Internet	131 words — 5%
3	repositorio.uam.es Internet	27 words — 1%
4	www.sciencedirect.com Internet	24 words — 1%
5	cancerimmunolres.aacrjournals.org Internet	18 words — 1%
6	ssmj.ru Internet	17 words — 1%
7	sunshine-marinabay.info Internet	9 words — < 1%
8	Chuangye Yang, Ruijuan Hao, Xiaodong Du, Qingheng Wang, Yuewen Deng, Ruijiao Sun, Zhe Zheng. "GC–TOF/MS-based metabolomics studies on the effect of protein sources in formulated diet for pearl oyster Pinctada fucata martensii", Aquaculture, 2018 Crossref	8 words — < 1%

EXCLUDE QUOTES	OFF	EXCLUDE MATCHES	OFF
EXCLUDE BIBLIOGRAPHY	OFF		