EFFECTS OF SUPPLEMENT AND ANTHELMINTIC TREATMENTS ON PARASITE ESTABLISHMENT AND THE PERFORMANCES OF LAMBS ARTIFICIALLY INFECTED WITH *HAEMONCHUS CONTORTUS*

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ABSTRAK

GINTING, S.P. 1998. Pengaruh pemberian suplemen dan obat cacing terhadap perkembangan parasit cacing dan kinerja domba yang diinfeksi buatan dengan *Haemoncus contortus*. *Jurnal Ilmu Ternak dan Veteriner* 3 (2): 117-123.

Penelitian bertujuan untuk mempelajari pengaruh pemberian suplemen dan obat cacing terhadap perkembangan parasit cacing pada domba yang diinfeksi secara buatan dengan *Haemonchus contortus*. Digunakan 24 ekor anak domba persilangan St. Croix dengan domba Sumatera lokal yang sedang bertumbuh. Ternak diberi suplemen sebanyak 0,5% atau 1,6% bobot badan (BB) dan diinfeksi dengan larva *H. contortus* (L_3) sebanyak 3.000 larva sebagai dosis awal dan 1.000 larva setiap minggu berikutnya atau diberi obat cacing (Ivermectin). Perlakuan merupakan rancangan faktorial 2 x 2 dengan enam ulangan untuk setiap perlakuan. Interaksi tingkat suplemen dan infeksi nyata (P<0,0001) terhadap total telur per gram tinja (TTGT) dan terhadap protein serum, namun tidak nyata terhadap PCV darah (P>0,10). Interaksi ini disebabkan oleh perubahan besaran perbedaan antar tingkat suplemen atau infeksi dan bukan karena perubahan peringkat antar perlakuan. Ternak yang mendapat suplemen 1,6% BB memiliki rataan TTGT yang lebih rendah (P<0,0001) dibandingkan dengan kelompok 0,5% BB suplemen (1.588 vs. 7.880). Kadar PCV darah dan protein serum lebih tinggi (P<0,0001) pada kelompok 1,6% BB suplemen dibandingkan dengan kelompok 0,5% BB suplemen (masing-masing 28,3 vs. 23,8 dan 5,2 vs. 4,6). Infeksi *H. contortus* menurunkan konsumsi pakan dan konsumsi nitrogen (N), namun tidak berpengaruh (P>0,10) terhadap N tinja. Total N urin dan N tinja tidak dipengaruhi (P>0,10) oleh infeksi *H. contortus*. Kelompok 1,6% BB suplemen tumbuh lebih cepat (P<0,0001) dibandingkan dengan kelompok 0,5% BB suplemen, baik yang diinfeksi dengan *H. contortus* maupun yang diberi obat cacing. Kelompok 0,5% BB suplemen dan diinfeksi dengan *H. contortus* mengalami penurunan bobot badan selama penelitian. Disimpulkan bahwa pemberian suplemen dapat mengurangi pengaruh parasit cacing pada kinerja domba, dan kombinasi pemberian suplemen dengan obat cacing dapat mengurangi intensitas penggunaan obat cacing dalam mengendalikan parasit cacing.

Kata kunci: Suplemen, obat cacing, *Haemonchus contortus*, anak domba

ABSTRACT

GINTING, S. P. 1998. Effects of supplement and anthelmintic treatments on parasite establishment and performance of lambs artificially infected with *Haemonchus contortus*. *Jurnal Ilmu Ternak dan Veteriner* 3 (2): 117-123.

The effect of feeding supplement and anthelmintic treatments on the establishment of parasites and the performances of lambs was studied in 24 St. Croix x Local Sumatra Crosses infected with *Haemonchus contortus* larvae (L_3). The study consisted of a 2 x 2 factorial arrangement involving two levels of supplement (1.6% and 0.5% body weight) and two levels of larval infection (0 and 3,000 L_3 initial dose followed by a 1,000 L_3-weekly dose).The supplement x infection interaction was significant (P< 0.0001) on the egg counts and total serum proteins, but not on the PCV values (P>0.10). The interaction was resulted from the shift in the magnitude of difference between supplement or between infection level and not by the shift in the rank between treatments. The 1.6% body weight (BW) group had lower (P<0.0001) mean egg count than the 0.5% BW group (1,588 vs. 7,880). Consistently, the blood PCV value and total serum proteins of lambs receiving 1.6% BW supplement were higher (P<0.0001) than the 0.5% BW supplement group (28.3 vs. 23.8 and 5.2 vs. 4.6, respectively). Infection resulted in decreased feed and nitrogen (N) intake, but had no effect on fecal-N (P>0.0001). N-excretion (fecal-N + Urine-N) was not altered (P>0.10) by *Haemonchus* infection, but N-retention decreased in infected lambs due to a reduction in N intake. The effect of supplement and *Haemonchus* infection was significant (P<0.0001) on daily gain. Lambs on the 1.6% BW supplement group grew faster than on the 0.5% BW supplement group whether they were infected or not. Infected lamb receiving 0.5% BW supplement lost weight during the experiment. It is concluded that an approach that combines the use of anthelmintics and supplement could ameliorate the influence of gastro-intestinal parasites on the performances of lambs, and reduces the intensity of using anthelmintics in controlling the parasites.

Key words : Supplement, anthelmintics, *Haemonchus contortus*, lambs

INTRODUCTION

In most parts of Indonesia the combination of confinement at night and day-time grazing is the major feeding system of sheep production. In this production setting, endoparasite infection in particular, *Haemonchus contortus* is one of the major constraints (HANDAYANI and GATENBY, 1988). It is generally accepted that there is an interaction between level of nutrition and susceptibility to infection by parasitic worms, but the reponse has varied by breed and level of infection. Although anthelmintics can provide effective control in the short term, but other management approaches are needed, which reduce the frequency of treatment. The objectives of this study is to evaluate the effects of nutrition improvement and anthelmintic treatment on the establishment of parasites and the performance of lambs infected with *H. contortus*.

MATERIALS AND METHODS

Animals and experimental treatments

Twenty four, 50% St. Croix x Sumatra crossbred lambs 3 to 4 months of age with average body weight of 13.5 kg (± 1.4 kg) were used for the study. The lambs were stratified by weight and randomly allocated to one of the four experimental treatments. The four treatments were 1) induced infection with *H. contortus* and fed 0.5% BW concentrate supplement, 2) induced infection with *H. contortus* and fed 1.6% BW concentrate supplement, 3) treatment with anthelmintic (Ivermectin) and fed 0.5% BW concentrate supplement, and 4) treatment with anthelmintic (Ivermectin) and fed 1.6% BW concentrate supplement.

Feed supplement and pen feeding procedures

Feed supplement consisted of palm kernel cake (25.5%), molasses (20.5%), rice bran (39.4%), fish meal (10.8%), limestone (2.2%), bone meal (.5%) and salt (1.1%). The supplement contained 3.9% N (DM basis). Animals were offered supplements at 1.6 or 0.5% BW on a dry matter basis. The amount of supplement offered was adjusted weekly on the same day the animals were weighed. The animals had free access to mineral blocks (20% cement, 69% salt and 11% ultra mineral). Daily allowance of feed dry matter was set at 3.6% BW. Thus, the amount of dry matter of forage (*Brachiaria humidicola* cv. Tully) offered daily was set at 2.0% BW for the 1.6% supplement group and 3.1% BW for the 0.5% BW supplement group. The forages was established in the Sungai Putih area and had not been grazed by animals for at least three months prior to the beginning of the experiment. This minimised the chances of *H. contortus* larval infection by the animals when consuming this harvested forages.

Animals were put in individual pens in a barn with raised and slotted floors. Measured supplement and forages were provided in separate troughs. The forages were manually chopped into 10 to 15 cm lengths and fed at 09.00, 13.00 and 16.30 hours in a approximately equal quantities. Forage samples of approximately 1.0 kg were collected daily from each pen for five consecutive days, composited, sub-sampled and analysed for dry matter concentration, and used to calculate the amount of forages offered for the following week.

Fecal egg counts and hematological analyses

Starting from week three following the first infection, fecal samples were taken directly from the rectum of each animal once per week to determine the number of eggs per gram of fresh feces. The feces were examined the same day by a modified McMaster technique (URQUHART *et al.*, 1989). Blood for hematological analyses was collected once a week at 0800 hours by jugular vein puncture using an evacuated glass tube. Blood samples for packed cell volumes (PCV) analysis were collected in 5-ml evacuated glass tubes containing sodium ethylene diamine tetra acetate (NaEDTA). Hematocrit levels were determined in microhematocrit capillary tubes centrifuged at 12,000 rpm in a microhematocrit centrifuge (Hawksley Microhematocrit centrifuge). Blood samples for analysis of serum protein was collected in 5-ml evacuated glass tubes without NaEDTA. Blood was allowed to clot for 30 minutes and centrifuged at 3,000 rpm for 10 minutes. Serum was separated and protein concentration in the serum was determined using a clinical refractometer (American Caduceus Industries, Toledo, OH). Blood sampling and hematological analyses were conducted on the same day.

Animal weight and condition score

Animals were weighed each week prior to the morning feeding using a hanging scale with a sensitivity of 0.1 kg. During the same day, animals were scored for body condition. The tissue depth in spinous processes and transverse processes were estimated according to the procedures described by BRINK (1990).

Balance trial

The N balance study commenced at week 14 after the first infection and continued for 15 days. All animals were placed in metabolism cages designed to enable separate collection of feces and urine from each animal. The cages were equipped with separate troughs for forage, supplements and water. Weekly dosing with *Haemonchus* larvae continued during the ten-day preliminary and five-day collection period. Forages were offered in approximately three equal feedings at 08.00, 13.00 and 16.00 hours. The forage offered and the orts for each animal were sampled daily (each of approximately 1.0 kg), placed in a freezer, then composited for the five-day collection phase at the end of the trial. Each day, a 25% representative sample of feces of each animal was placed in a freezer for later analyses. Fecal samples were composited by animal for the five-day collection phase. Total urine was collected in plastic containers containing 100 ml 10% H_2SO_4. Each day, subsamples (10%) were taken from each animal and put into tightly-covered plastic containers stored in a refrigerator. At the end of the balance trial, all the infected animals were slaughtered and worm burdens were determined.

Culture of *Haemonchus* larvae for induced infection

Two donor animals (Local Sumatra Thin-tail sheep) that had been repeatedly infected with *Haemonchus* larvae were used as the source of feces containing *Haemonchus* eggs. The recovery of larvae from the cultures was processed according to the procedures described by ROBERTS and O'SULLIVAN (1950).

Two weeks prior to artificial infection with the *Haemonchus* larvae animals were drenched with Ivermectin at 0.3 ml per kg body weight. On day 12 after drenching, fecal samples were taken from the rectum of each animal to confirm that all animals were free of gastro-intestinal parasites. The animals designated to treatment of induced infection were initially orally dosed with 3,000 larvae (L_3) per animal. These animals were then dosed repeatedly once per week with 1,000 L_3 per animal. Larval collections were kept under refrigeration and were monitored regularly for concentration of active larvae. When 5% or more of the larvae population were found dead, a new larval population was established.

Anthelmintic treatments

Anthelmintic was given to animals designated as treated groups only when infection was detected as measured by fecal egg counts. During the experiment, anthelmintics were given to these groups twice when fecal egg counts of animals in these groups ranged from 0 to 90 epg.

Chemical analyses

Samples of forage offered, orts, feed supplements and feces were dried in a air-forced oven at 100°C for 24 hours to determine the dry matter concentration. Feed and fecal samples for N analysis were dried at 60°C, and analysed for the Kjeldahl-N content (AOAC, 1990). The content of NDF and ADF was determined according to the procedures of GOERING and VAN SOEST (1970).

Statistical analyses

Data were analysed as completely randomised design with a 2 x 2 factorial arrangement with six animal replicates, according to the general linear model procedures of SAS (1985). A set of three orthogonal contrasts were used to extract information from the four treatment combinations (Table 1). Treatment means were examined using the Waller-Duncan k-ratio (k=100) t-test (STEEL and TORRIE, 1980) when a significant ($P<0.05$) treatment *F*-test was detected.

Table 1. Orthogonal contrast coeficients for treatment combination of two levels of supplement and two levels of *Haemonchus* infection

Contrast	Treatment[a]			
	LT	LI	HT	HI
Between Supplement Levels (S)	1	1	-1	-1
Between Infection Levels (I)	1	-1	1	-1
S x I Interaction	1	-1	-1	1

aLT = Lambs on 0.5%BW supplement and treated with anthelmintic
LI = Lambs on 0.5% BW supplement and infected with *Haemonchus* larvae
HT = Lambs on 1.6% BW supplement and treated with anthelmintic
HI = Lambs on 1.6% BW supplement and infected with *Haemonchus* larvae
BW = the body weight

RESULTS AND DISCUSSION

Clinical observations

Two of the infected animals on the 0.5% BW supplement diet showed clinical signs of infection at week 11 or week 14. These animals were very anemic (PCV = 8.0 - 9.8%), had severe diarrhea, lost weight and died several days after showing these clinical signs. The last measurement on fecal egg numbers (22,000 - 23,000) indicated that the worm burden was very high in both animals. Data collected from these two animals were excluded in the statistical analyses. The infected lambs on the 1.6% BW supplement remained alert throughout the experiment.

Fecal egg counts

Table 2 shows that infected animals receiving the 1.6% BW supplement (HI) had lower (P<0.001) fecal egg counts, higher PCV and higher total serum protein than those fed the 0.5% BW supplement (LI). As expected, egg counts of the infected lamb was higher (P<0.001) than those of the treated lambs. The supplement x infection interaction (P<0.001) was caused by the shift in the magnitude of difference and not by the shift in the rank between treatments. The mean egg counts of lambs on LI (7,880 ± 582 epg) can be regarded as high (GATENBY and BATUBARA, 1994) indicating that lambs on that treatment were still susceptible. On the other hand, worm burdens in lambs on HI dropped to a moderate level (1,588 ± 531 epg). The PCV level in the HI group (28.3%) was maintained above the minimum normality (22%), while in the LI lambs (23.8%) the mean value was close to the minimum normality. The supplement x infection interaction, however, was not significant (P>0.10). The PCV levels in lambs treated with anthelmintics fed 0.5% BW (LT) or 1.6% BW (HT) remained higher that the infected group. Serum protein in the HI lambs was increased, but the value was lower than that in the HT lambs. The supplement x infection interaction was significant (P<0.001) which resulted from the shift in the magnitude of difference between supplement or between infection levels. Mean serum protein in all groups was below the minimum normality of 6 g/dl (BLOOD and RADOSTITS, 1989).

The pattern of fecal egg count, PCV and serum protein is shown in (Table 3). The fecal egg counts and serum protein levels were consistently higher, while the PCV level was lower in the LI lambs than the HI lambs over the experiment. The egg counts of the HI lambs reached peak value at week 8, and at the same time the serum protein level started to increase. The PCV levels in the LI group dropped below the minimum normality of 22% at week 11 and persisted until the experiment ended at week 16. These pattern suggest that the lambs on HI, when they were approximately five to six months old, may have the ability to acquire immune responses to *H. contortus*, while the LI group remained susceptible even when they reached on age seven to eight months.

Worm burdens

A higher (P<0.001) mean worm burden at time of slaughter occurred in the LI group (Table 4), which match their high mean egg count previously reported. This agrees with ABBOTT *et al.* (1985; 1988). BLACKBURN *et al.* (1991), however, did not find difference in worm burdens given different nutrition levels. The inconsistency of these finding may be related to difference in the level of parasite infestation and level of nutrition and the breed of the animals. The present study showed that the St. Croix crosses were responsive to nutritional level treatment in dealing with the *H. contortus*.

Table 2. Mean fecal egg counts (epg), packed cell volume (PCV) and total serum protein from lambs given two levels of supplement and artificially infected with *Haemonchus contortus* or treated with anthelmintics

Supplement[a]	*Haemonchus*[b]	Egg counts EPG	PCV %	Serum protein g/dl
Low	Infected	7,880 ± 582	23.8 ± 0.43	4.58 ± 0.06
Low	Treated	35 ± 19	28.3 ± 0.33	5.17 ± 0.08
High	Infected	1,588 ± 531	28.3 ± 0.39	5.17 ± 0.06
High	Treated	46 ± 13	31.6 ± 0.33	5.33 ± 0.06
MSD[c]		1,352	1.0	0.15
Contrast:				
Supplement levels (S)		0.0001	0.0001	0.0001
Infection levels (I)		0.0001	0.0001	0.0001
S x I interaction		0.0001	0.1366	0.0001

[a]Low = supplemented at 0.5% BW
High = supplemented at 1.6% BW
[b]Infected = infected with *Haemonchus* larvae
Treated = treated with anthelmintics
[c]Waller-Duncan minimum significant difference

Table 3. Fluctuation of egg counts, PCV and total serum protein in lambs infected with *Haemonchus contortus* and fed two levels of supplement

Week	Egg counts (EPG)		PCV (%)		Serum protein (g/dl)	
	LI[a]	HI[b]	LI	HI	LI	HI
2	422	378	29.3	28.5	5.1	5.0
3	789	603	26.5	27.5	4.8	5.1
4	2,480	153	26.9	28.8	4.9	5.2
5	2,510	166	26.8	29.9	5.1	5.4
6	2,310	194	26.9	29.6	5.0	5.1
7	2,580	326	24.3	29.7	4.7	5.0
8	5,320	584	24.1	28.3	4.6	4.7
9	5,414	373	23.8	26.1	4.7	5.0
10	9,896	562	24.0	27.1	4.6	5.1
11	8,104	412	20.1	27.5	4.5	5.1
12	10,214	446	21.3	27.7	4.6	5.1
13	13,266	433	20.8	27.5	4.4	5.2
14	14,104	533	20.1	30.1	4.6	5.6
15	12,123	476	20.9	29.8	4.5	5.4
16	13,163	423	20.6	29.9	4.3	5.6

[a]LI = Lambs infected with *Haemonchus* larvae and fed low (0.5% BW) supplement
[b]HI = Lambs infected with *Haemonchus* larvae and fed high(1.6% BW) supplement

Table 4. Mean worm numbers in lambs infected with *Haemonchus contortus* given two levels of supplement

Supplement	*Haemonchus* larvae	Worm counts				P>F
		n	Mean	Range	SE	
Low	Infected	4	2,130	1,400-2,790	251	
High	Infected	6	38	0-76	43	0.0007

Feed intake, live weight gain and body conditions

Dry matter intake, ADG and body condition scores are presented in Table 5. Supplements given at both levels were completely consumed. The forages consisted of a large proportion of stem which constituted most of the refusals. Dry matter intake, expressed as proportion of body weight (g/kg BW) was affected by supplements and infection (P<0.05), being higher on the LI than on the HI group, and being higher on the HT than HI treatment. A reduction of 5% was recorded in dry matter intake for infected lambs receiving 1.6% BW supplements. Lambs fed the 1.6% BW supplement gained faster (P<0.001) than those fed the 0.5% BW supplement whether they were infected or treated. The ADG of infected lambs on both the 0.5% BW and the 1.6% BW supplement treatments were abnormally low. The worm burdens carried by these animals contributed in part to this low ADG. The ADG of anthelmintic treated lambs fed the high supplement was also less than expected. ADG of 81 to 150 g per day have been recorded in St. Croix crosses given different kind of feed supplements (SANCHEZ and POND 1990; MERKEL, 1994). The initial condition scores ranged from 2.9 to 3.3 (1- to 5- scale) and were not different (P>0.10) among the treatment groups. The final condition scores measured at the end of the experiment differed (P<0.001) between supplementation and infection levels. Lambs on the LI, HI or LT treatment lost condition score by the end of the experiment, while those on the HT gained 0.2 unit.

Nitrogen balance

Nitrogen (N) intakes, expressed as proportion of body weight, were not different (P>0.10) between supplement levels (Table 6). The infected lambs had a lower (P<0.001) N intake than the treated lambs, which is consistent with their lower dry matter intake. The effects of supplementation on the fecal N was significant (P<0.001) being higher on the 0.5% BW supplemented group than on the 1.6% BW supplemented lambs. Fecal N of the infected and treated lambs was not different

(P>0.10). Supplement x infection interaction was not significant (P>0.05). Lambs fed the high level of supplementation had higher (P<0.002) urine N than lambs fed the low level of supplementation. Lambs infected with *Haemonchus* had lower (P<0.002) urine N than treated lambs. Nitrogen excretion was not different (P>0.10) between supplement or infection levels. The literature on the partitioning of nitrogen losses into feces and urine is not consistent. Increased urinary N loss in sheep infected with *H. contortus* has been shown (ABBOTT *et al.*, 1988; BOWN *et al.*, 1991). In contrast to this findings, the present study showed hat urinary N decreased by approximately 32% in ınfected lambs on the 1.6% BW supplement compared to the treated lambs. The differences, however, was detected on the 0.5% BW supplemented group. Similarly, ABBOTT *et al.* (1986) did not find differences in the urinary loss of N between infected and control animals. In addition, increased fecal N in infected sheep (ABBOTT *et al.*, 1985) or no differences between the infected and control animals (BOWN *et al.*, 1991) have been reported. In the present study, no differences in fecal N were found between the treated and the infected lambs. Overall, N retention was greater (P<0.05) in lambs receiving the 1.6% BW supplement than receiving the 0.5% BW supplement, and the infected lambs had a lower (P<0.001) N retention than the anthelmintic treated lambs. The decreased N retention in infected lambs was likely due to decreased N intake by the lambs and not from the impairment of digestion, since fecal N was not affected by infection.

Table 5. Mean dry matter (DM) intake, daily gain (ADG) and body condition scores of lambs given two levels of supplement and infected with *Haemonchus contortus* or treated with anthelmintics

Supplement[a]	*Haemonchus*[b]	DM intake (g/kg BW/d)	ADG (g)	IBCS[c]	FBCS[d]
Low	Infected	39.7 ± 0.67	-5.2 ± 4.4	2.90 ± 0.08	1.90 ± 0.06
Low	Treated	40.4 ± 0.64	35.5 ± 4.1	2.95 ± 0.08	2.67 ± 0.05
High	Infected	37.3 ± 073	19.2 ± 4.1	2.85 ± 0.09	2.67 ± 0.05
High	Treated	39.4 ± 0.67	51.1 ± 4.6	3.08 ± 0.08	3.28 ± 0.05
MSD[e]		2.0	11.3	NS	0.15
Contrast:					
Supplement levels (S)		0.0386	0.0001	0.1090	0.0001
Infection levels (I)		0.0127	0.0001	0.6042	0.0001
S x I interaction		0.2899	0.3069	0.2605	0.1928

[a]Low = Supplemented at 0.5% body weight
High = Supplemented at 1.6% body weight
[b]Infected = Infected with *Haemonchus* larvae
Treated = Treated with anthelmintics
[c]Initial body condition scores
[d]Final body condition scores
[e]Waller-Ducan minimum significant difference

Table 6. Nitrogen intake, output and retention in lambs given two levels of supplement and infected with *Haemonchus contortus* or treated with anthelmintics

Supplement[a]	*Haemonchus*[b]	N intake	Fecal N	Urine N	N excreted	N retained
		....	....	g/kg BW/d	....	....
Low	Infected	0.64	0.16	0.12	0.27	0.37
Low	Treated	0.86	0.18	0.13	0.33	0.53
High	Infected	0.70	0.12	0.13	0.26	0.45
High	Treated	0.84	0.11	0.19	0.29	0.54
MSD[c]		0.06	0.03	0.03	0.07	0.06
Contrast:						
Supplement level (S)		0.3813	0.0001	0.0018	0.1594	0.0414
Infection level (I)		0.0001	0.5963	0.0018	0.1168	0.0001
S x I interaction		0.0540	0.0540	0.1206	0.1594	0.1681

[a]Low = Supplemented at 0.5% body weight
High = Supplemented at 1.6% body weight
[b]Infected = Infected with *Haemonchus* larvae
Treated = Treated with anthelmintics
[c]Waller-Duncan minimum significant difference

CONCLUSION

Improving the nutritional status of growing lambs by supplementing concentrate feeds reduced the worm burdens as indicated by decreased egg counts and increased PCV and serum protein of lambs infected with *Haemonchus contortus*. Although supplementation at 1.6% BW substantially reduced the worm burden of the infected lambs, anthelmintic treatment alone seems to be more effective in controlling the parasite. An approach that combines the use of anthelmintics and supplement could ameliorate the influence of parasites on the performances of lambs, and reduce the intensity of using anthelmintics in controlling the parasite.

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