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Anthelminthic Efficacy of Chili *(Capsicum annum longum)* and Nutritional Supplementation on Ovine Fascioliasis in Ethiopia

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ABSTRACT

A 90 days study was conducted in Gewane Agricultural Technical and Vocational Education Training College, Ethiopia, to compare the anthelminthic effect of chili or Mitmita (*Capsicum annum longum*) and a conventional treatment such as Tricalbendazole on *Fasciola* infections to nutritional levels. A total of 72 Afar and Blackhead local breeds of sheep naturally infected by fascioliasis were randomly allotted into 3 groups, two nutrition levels (supplemented with wheat middling, cotton seed cake, corn, lime and common salt and not supplemented group) and three section treatments. One treatment group received 10 mg/kg of Tricalbendazole drenching orally on the 1, 21, and 42 days during the study period. The second group received 100 gm of crushed or chopped chili solution drenching orally on the 1, 21, and 42 days during the experimental period and the control group received no treatment. The control group divided into two groups of supplemented and not supplemented or grazing only. The treatment efficacy was assessed using the percentage of the egg or fluke reduction, body weight gain, blood parameters results post treatment on 30, 60 and 90 days. At the end of the experiment, animals were slaughtered and postmortem were performed for adult *Fasciola* recovery in the liver. Tricalbendazole and chili treated groups of animals had an efficacy of 100% and 84%, respectively. Animals treated by Tricalbendazole and chill in addition supplementation had high reduction of fecal egg count (FEC) compared to the treatment groups with no supplementation ($p<0.05$). No significant difference within treatments between two breeds was observed. The result indicated that chili could be an alternative treatment for subclinical ovine fascioliasis to alleviate the production loss in sheep herds.

Key words: chili, *Capsicum annum longum,* Ethiopia, fascioliasis, tricalbendazole, sheep

INTRODUCTION

Fascioliasis, caused by *Fasciola hepatica* and *Fasciola gigantica,* is one of the most prevalent helminth infections of ruminants in all parts of the world. The parasites can cause significant mortality and morbidity (WHO, 1995). In Ethiopia, Ngategize *et al*. (1993) reported that the annual loss due to ovine fascioliasis were estimated at 48.4 million Ethiopian Birr (1.00 US$ = 2.07 Ethiopian Birr). Eradication of fascioliasis is rarely a practical option and control needs to be

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established for the reduction of the disease to allow economic livestock production. The specific aims of the control program may be to prevent the build-up of parasites in the environment and to avoid areas of heavily contaminated pasture (Brunsdon, 1980).

Reductions of pasture contamination are accomplished through the use of anthelminthic, management regimes, molluscicides, and biological methods of snail control. In addition, the use of resistant animals to reduce the impact of infection may be mostly appropriated, especially where treatment costs are relatively high (Roberts and Suhardono, 1996).

The uses of chemotherapeutics and proper grazing have been recommended as a control measurement. Constraints to the use of anthelminthics by poor farmers caused by the high cost are reported to be a problem, in Indonesia (Dorny *et al*., 1996), India (Cheah and Rajamanickam, 1997) and Kenya (Onieke, 1999).

Tobacco (*Nicotiana tabacum L.*) is used in Zaire, Tanzania and central Ethiopia for the treatment of the internal parasitism including fascioliasis. The powdered drug kamala, derived from the fruit of *Mallouts philippinesis* has historically been used as an anthelmintic in the Indian sub continent (Akhtar and Ahmed, 1992). Chili is used worldwide for the treatment of internal parasites. In Mexico, 13 chili peppers blended with water to treat ovine fascioliasis, chili (*Capsicum annum*) is used by 19% of the respondent of Rake community to cure liver fluke diseases (McCorkle *et al*., 1995).

On the other hand, the interaction between the level of the nutrition and the ability of animals to cope with internal parasites has long been recognized (Mukasa *et al*., 1991). Protein supplementation has been shown to improve the resilience and the resistance of lambs to single and mixed parasites (Knox and Steel, 1996). The objective of this study was to compare the anthelminthic effect of chili or Mitmita (*Capsicum annum longum*) and a conventional treatment such as Tricalbendazole on *Fasciola* infections to nutritional levels.

MATERIALS AND METHODS

Description of the study area

This work was carried out from January to March 2006 in Gewane Agricultural Technical and Vocational Education Training College, Gewane district, Ethiopia. Gewane district is an endemic area for ovine fascioliasis with a hot and Semi-arid climate. The location of the study area is 10º9′59N latitude and 40º 8′43E longitude. The college is 344 km away from Addis Ababa. The altitude of the area is 560 meters above the sea level.

Animal management and supplementation

Seventy two local breeds of sheep (Afar and Blackhead) naturally infected with fascioliasis, were collected from different sites with age above two years and weights between 19 and 30 kg. Prior to the treatment they were housed for 15 days. During this period, animals were weighed and fecal and blood samples were examined. During the experimental period all animals were housed separately in the pens at night while at feeding time; they were kept together on the same pasture. Water, from natural sources was provided *ad libitum*. The presence of fasciola was confirmed by pre-examination and all sheep were vaccinated against the common infectious diseases of the area like Anthrax, Pasteurellosis and Sheep Pox with the vaccine obtained from the National Veterinary Institute, Debre Zeit, Ethiopia. Spraying of acaricides against ectoparasites was applied regularly every month with Diazinol 60% E.C. (Kafr El Zayat Pesticides and Chemical Co., Egypt) throughout the study period.

The concentrate supplementation was provided for daily dry matter intake (DMI), crude protein (CP) (g) and metabolizable energy (ME)

(MJ/kgDM) requirements of the lambs per head estimated according to (McDonald *et al.,* 1988) of 615g, 113g and 6.25MJ, respectively. It was assumed that about 52% of the total DM (318g), CP (25g), and ME (2.8MJ) were obtained from grazing, while the rest were provided from mixed concentrate supplementation. Composition of the concentrate was wheat middling (50%), cotton seed cake (30%), corn (15%), lime (4%) and common salt (1%). The concentrate was analyzed to contain 20.2% CP and 11.8 MJ/kgDM, metabolized energy. All sheep were allowed to graze on native communal grazing land for 8 hours (8:00 AM up to 4:00 PM) per day. The efficacies of the treatments were evaluated based on pre and post treatments evaluation of fecal, hematological and biochemical examinations, as well as post mortem findings on slaughtered animals.

Fecal examination

Five grams of fecal samples were collected from the rectum using a plastic glove. The efficacies of the Tricalbendazole treatment and chili with or without supplement were evaluated by measuring egg shedding. Fecal samples were collected monthly. For the detection of fecal egg count (FEC) per gram, the sedimentation method according to Tiber (1999) and Garcia (1997) was used. FEC was determined according to the following equations:

FEC = Amount of egg counted consistency × correlation factor × 100

Where consistency value was given for a form of stool, (1 = normal stool and hard; 2 = soft, 3 = moisture, 4 = diarrhea and 5 = watery)

Evaluation of the efficacy

The efficacy or the percentage or de-parasitism was extrapolated according to the method used by Carvier (1973). The formula was as follows.

$$\textbf{Efficacy}\ (\%) = \frac{(N - n) \times 100}{N}$$

Where N = average fecal egg count (FEC) in negative control animals

n = average FEC in treated animals

Percentage reduction in FEC

The percentage reduction in fecal helminthes egg output following treatment administration was calculated for the test group and control group. The formula was as follows.

$$\text{Reduction } \% = \frac{(Mean\ FEC\ day_0 - Mean\ FEC\ day_X) \times 100}{Mean\ FEC\ day_0}$$

Where; day $_0$ = before administrating treatments and Day $_X$ = the days post treatment administration.

Treatment and administration

One treatment group received 10 mg/kg of Tricalbendazole drenching orally on the 1, 21, and 42 days during the study period. The second group received 100 gm of crushed or chopped local chili (*Capsicum annum*) was mixed with 200 ml water so that the extracted mixture of the solution could be delivered through a 300 ml plastic container drenched orally on the 1, 21 and 42 days during the experiment and the control group received no treatment.

Blood sample collection

From the commencement of the experiment and monthly thereafter, 10 ml blood samples were collected through the jugular vein into vacutainer tubes containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Red blood cell (RBC) count was measured using a cell counter and the Packed Cell Volume (PCV %) was determined by the micro-haematocrit centrifugation technique as described by Murray *et al.* (1983). The hemoglobin concentration was measured in the form of cyanmethaemoglobin and total protein (TP) in plasma was determined by using commercial kits supplied from Life Science Dynamic Division, Arnaparn, and Nonthaburi, Thailand.

Weight gain

The animals were weighed once a month in the morning before they were released to graze and water was offered. Each sheep was hanged on a strap in attached to the hook of a spring scale and then weighed.

Experimental protocol

Animals were allotted to 12 treatments with 6 animals each. The treatment combination were formed from 3 treatments (Tricalbendazole), chili (*Capsicum annum longum*) and no treatment), 2 breeds (Afar and Blackhead), and 2 feeding systems (supplement and no supplement). The treatment combinations are as illustrated in Table 1.

Data analysis

The differences between treatment groups for different parameters were tested using a three way factorial analysis of variance (ANOVA). That is (3 × 2 × 2) factorial in completely randomized design (CRD). The complete model is given below. Differences between means were tested using Duncan's Multiple Range test (Steel and Torrie, 1980).

Model $Y_{ijkl} = \mu + T_i + B_k + F_j + (TxB)_{ik} (TxF)_{ij} + (BxF)_{kj} + (TxFxB)_{ijk} + e_{ijkl}$

Where, Y_{ijkl} = Response variable

μ = Overall mean

T_i = Effect of i_{th} treatment (i =1 to 3)

B_k = effect of K_{th} breed (1 to 2)

F_j = Effect of j_{th} feeding system (j=1 to 2)

$(TxB)_{ik} (TxF)_{ij} + (BxF)_{kj} + (TxFxB)_{ijk}$ = interaction effects

e_{ijkl} = Error term.

Post mortem examination

Post mortem examination on animals was carried out soon after they were slaughtered. A total of 36 sheep (three from each treatment group) were randomly selected for slaughtering and examination of all internal organs for abnormalities, with particular attention to the size, weight, color, and appearance of the livers were conducted to determine the effects of treatments. During the examination, the liver and the gall bladder were removed and the gall bladder was then separated from the liver, emptied and washed into a glass trough. The contents of the troughs were checked for adult *Fasciola spp*. The bile ducts were opened with scissors and searched for adult

Table 1 Experimental groups with 3 treatment combinations.

No.	Treatment	Feeding system	Breed	Number of animals	Total No. of animals
1	T(Tricalbendazole)	Grazing (G)	Afar (A)	6	12
			Blackhead(B)	6	
		Grazing (G) and	Afar (A)	6	12
		Supplement (S)	Blackhead(B)	6	
2	C (Chili)	Grazing (G)	Afar (A)	6	12
			Blackhead(B)	6	
		Grazing (G) and	Afar (A)	6	12
		Supplement (S)	Blackhead(B)	6	
3	N (No treatment or control)	Grazing (G)	Afar (A)	6	12
			Blackhead(B)	6	
		Grazing (G) and	Afar (A)	6	12
		Supplement (S)	Blackhead(B)	6	

Note: Treatment (T =Tricalbendazole, C= Chili, N = No treatment), Feeding system (S= Supplement, G = grazing only), breed (A = Afar B = Blackhead).

flukes. The liver was then cut into 1 cm thick slices and pressed between the fingers to expose flukes lodged in small bile ducts.

RESULTS AND DISCUSSION

The average live weight gain by anthelmintic treated groups were the highest followed by chili treated animals. The control groups (not treated) were the groups with the lowest live weight gains. The results have demonstrated that there was significant difference in weight gain of animals treated with anthelmintic, and this is supported by previous reported research (Scott *et al.,* 1974). The weights of the animals were compared to prior treatment had shown significant improvements during the experiment: the animals were well managed and the treatment did not affect the health of the experimental animals. The weight changes in infected animals were found to depend upon two variables: the fluke burden and the quality of the diet available to the host. Njau *et al*. (1990) also reported that nutrition played a key role in efficient parasite control.

Chili treatment could be used to treat infected sheep under subclinical infection of ovine fascioliasis. This was consistent with supplements and had a significant effect on treatment. Implementing the control of helminthes without adequate management of host nutrition caused weight loss or morbidity and masked the advantages of the treatment.

The overall result of the present study demonstrated that animals received treatments of T (tricalbendazole) and chili were found to have reduced fecal egg count per gram, increases in PCV, RBC, Hb, TP and live weight gain (LWT) measurement as compared with those animals that received no treatments ($P<0.001$). No differences were observed in LWT, FEC, RBC, TP and Hb ($P>0.05$) while there were significantly differences among breeds in PCV ($P<0.001$). These results are in agreement with the result of Jain (1993) who reported that several factors such as age, sex, breed, health, physiological status, nutrition and other factors may influence the normal blood values of various species.

Results in regard to the hematological and biochemical evaluation effect of the different levels of treatments on blood parameters are shown in Table 2. There were significant differences among treated animals in PCV ($P<0.001$) the values which were contrary to what was reported

Table 2 Effects of breed, supplement and different treatment options on body daily weight gain, fecal and blood parameters.

Factors	Daily weight gain (g)	FEC (g)	RBC ($x10^6\mu L$)	PCV (%)	TP (g %)	Hb (g/dl)
BREED						
Afar (A)	36.7[a]	219.4 [a]	8.7[a]	36.1[a]	59.6[a]	7.1[a]
Blackhead (B)	43.9[a]	222.8[a]	8.7[a]	34.2[b]	58.8[a]	7.6[a]
FEEDING						
Supplement (S)	59.3[a]	146.7[b]	9.1[a]	36.9[a]	60.7[a]	8.6[a]
No Supplement (G)	21.4[b]	295.6[a]	8.3[b]	33.4[b]	57.8[a]	6.1[b]
TREATMENT						
T (Tricalbendazole)	56.2[a]	00.00[a]	9.1[a]	38.3 [a]	66.3[a]	8.9 [a]
Chili (C)	43.0[a]	183.3[b]	8.8[a]	34.9 [b]	60.5[b]	7.2 [b]
No treatment (N)	21.7 [b]	480.0[c]	8.3[b]	32.3 [c]	50.8[c]	5.9 [c]

[abc] means in the same column within the same group with different superscripts are different ($p<0.05$).

by Davis *et al*. (1999) that in which he obtained no difference in treated goats. The RBC, PCV, Hb and TP concentration revealed significant differences among supplementation and treated levels (P<0.001). The TP result among the supplement group were higher than the non supplement group but were not significantly different (P>0.05). The PCV was highest (36.9 %) in sheep with supplement and treatment and was significantly different from those of non-supplement in non-treated animals. Oyedipe *et al*. (1984) observed that animals fed with good quality rations had higher values of RBC, PCV, TP and Hb concentration, which was in line with the current study.

The result of RBC, PCV, Hb, and TP of the two breeds are presented in Table 2. Differences in those treated with supplement in infected animals were observed between the two breeds (P<0.001). The Afar breed had slightly higher PCV value (p>0.05) than The Blackhead breed. PCV was used as an indicator of anemia; the more anemic the animal, the lower the PCV. Animals with PCV less than 24% were considered to be anemic (Kelly, 1974). The PCV of Afar and Blackhead sheep were 36.1 and 34.2 %, respectively.

At the end of the experiment, no important clinical sign was observed in treated animals. However, in the control group, 4 sheep had easily observed visible anemic mucus membranes, progressive emaciation, and two animals revealed mandibular edema from day 60 to 90. No mortality was found during the experimental period.

The result of the efficacy of treatment and the percentage reduction in fecal helminthes egg compared to negative control animals shown in Table 3 and 4, respectively. Tricalbendazole with the supplement treated group of animals recorded a fecal egg count of zero. This result indicated that this treatment was more efficacious than other treatment groups (P<0.001). The highest treatment efficiency was observed on days 60 and 90 in Tricalbendazole with the supplement treated group of animals. This was due to the treatment by Tricalbendazole in the fields using a frequent dose of treatment. It was found that all of them had 100% efficacy. Results for this study were concur with the finding of Richards *et al*., (1990) who obtained 99.0 - 100% efficacy of anthelminthic treatment of the experimental animals.

Table 3 The efficacy (%) of treatment tricalbendazole, chili and supplement in experimental animals.

Factor combination	Pre-treatment FEC (g)	Days post treatment		
		30	60	90
TSA	740	40 (93.3)	0 (100)	0 (100)
TSB	750	20 (97.1)	0 (100)	0 (100)
TGA	700	100 (83.3)	50 (92.3)	0 (100)
TGB	700	100 (85.3)	40 (94.1)	0 (100)
CSA	700	400 (33.3)	200 (69.2)	100 (83.8)
CSB	720	410 (39.7)	200 (70.6)	100 (83.9)
CGA	750	500 (16.7)	433.3 (33.3)	333.3 (46.2)
CGB	750	500 (26.5)	463.3 (31.9)	333.3 (46.2)
NSA	700	500 (16.7)	350 (46.2)	330 (46.2)
NSB	750	500 (26.5)	380 (44.1)	350 (43.6)

Note: Treatment (T = Tricalbendazole, C = Chili, N = No treatment); Feeding system (S = Supplement, G = grazing only); Breed (A = Afar, B = Blackhead)

* For calculation use the control groups of treatment (NGA and NGB) results in table 4.

The chili with supplement treated group result of efficacy of treatment and percentage reduction in fecal helminthes egg was 33-40% at 30 days post treatment. However, during 60 and 90 days post treatment results observed 69.2 and 83.8%, respectively: the reduction of *Fasciola spp*. eggs were 42-43%, 71-72 and 85-86% at 30, 60 and 90 days post treatment, respectively. Chili treated animals without supplement were 16-26.5% efficacious after 30 days, and then rose to 31-33 and 46.2%, respectively after 60 and 90 days. The reductions of fecal eggs count were 33.3% after 30 days, but increased to 38-42 and 56%, respectively, after 60 and 90 days post treatment. The results of control animals are also shown in Table 4.

During post mortem examination, different parasite species were observed. The most common and easily detectable parasite species like *D. filaria*, *Paramphistomum spp. Heamonchus contrortus*, *Trichuris spp*. and *Moniezia spp*. were involved. The relative abundance of the whole parasite species involved was not determined but the adult fluke of *Fasciola spp*. had been determined and recorded. In the Tricalbendazole treated group, no liver flukes were recovered but the highest number of flukes was recovered in the control groups. Liver fluke data recovered from the slaughtered animals are presented in Table 5.

CONCLUSION

Overall, our findings have clearly demonstrated that chili with supplement might be the appropriate drug alternative in the treatment of fascioliasis for animal owners due to its affordable cost and supply. This may be of particular importance to endemic areas of fascioliasis in general in Ethiopian low lands, where there is an available growing chili and where other treatment costs are relatively high.

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Table 4 Result of fecal egg count reduction (%) in each treatment group.

Factor combination	Pre treatment FEC (g)	Days post treatment		
		30	60	90
TSA	740	40 (94.6)	0 (100)	0 (100)
TSB	750	20 (97.3)	0 (100)	0 (100)
TGA	700	100 (85.7)	50 (92.9)	0 (100)
TGB	700	100 (85.7)	40 (94.3)	0 (100)
CSA	700	400 (42.9)	200 (71.4)	100 (85.7)
CSB	720	410 (43.1)	200 (72.2)	100 (86.1)
CGA	750	500 (33.3)	433.3 (42.2)	333.3 (55.6)
CGB	750	500 (33.3)	463.3 (38.2)	333.3 (55.6)
NSA	700	500 (28.6)	350 (50.0)	330 (52.9)
NSB	750	500 (33.3)	380 (49.3)	350 (53.3)
NGA	750	600 (20.0)	650 (13.3)	620 (17.3)
NGB	740	680 (8.2)	680 (8.2)	620 (16.2)

Note: Treatment (T = Tricalbendazole, C = Chili, N = No treatment); Feeding system (S = Supplemented, G = grazing only); Breed (A = Afar, B = Blackhead)

Table 5 Numbers of recovered *Fasciola* spp. in each experimental group by post mortem examination.

Group of treatments	Fluke recovered			
	Small	Large	Total	LS Mean + SE
TSA	0	0	0	0.00 ± 0.36[a]
TSB	0	0	0	0.00 ± 0.36[a]
TGA	0	0	0	0.00 ± 0.36[a]
TGB	0	0	0	0.00 ± 0.36[a]
CSA	2	1	3	1.00 ± 0.36[c]
CSB	1	1	2	0.67 ± 0.36[b]
CGA	4	3	7	2.33 ± 0.36[e]
CGB	4	2	6	2.00 ± 0.36[d]
NSA	6	5	11	3.67 ± 0.36[g]
NSB	5	4	9	3.00 ± 0.36[f]
NGA	16	9	25	8.33 ± 0.36[h]
NGB	20	9	29	9.67 ± 0.36[i]

[abcdefghi] means in the same column with different superscripts are different ($p<0.05$).

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