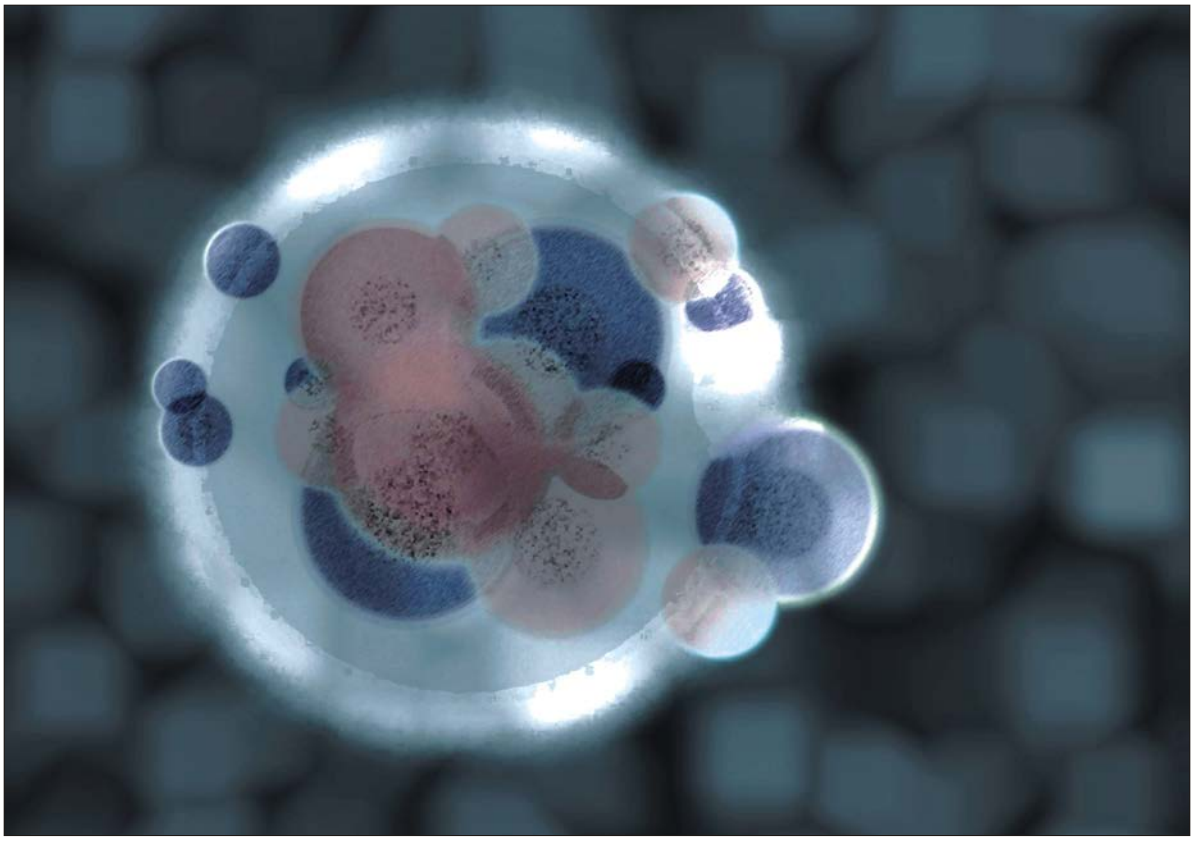

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 6 (6): 1108-1112, 2006
ISSN 1727-3048

Faecal Microbial Flora of Tswana Goats Fed *Cenchrus ciliaris* Hay as Basal Diet and *Terminalia sericea* or *Boscia albitrunca* as Supplement

A.A. Aganga, U.J. Omphile and T.M. Sebolai
Botswana College of Agriculture, P/Bag 0027, Gaborone, Botswana

Abstract: Fifteen female and ten castrated yearling Tswana goats were weighed and randomly divided into five groups of five goats of which 3 were females and 2 were males. The objectives of the project was to determine effects of *T. serecia* and *B. albitrunca* at two levels on faecal egg worm count, bacterial count and bacterial identification. All the goats were fed buffel grass hay (*Cenchrus ciliaris*) as a basal diet, while *Medicago sativa* (0% tannin content) was fed to the control group as a supplements. The other four groups were fed low *B. albitrunca* (0.267% tannin in diet), high *B. albitrunca* (0.497% tannin in diet), Low *T. serecia* (0.342% tannin in diet) and high *T. serecia* (0.497% tannin in diet) as a supplement. The basal diet comprised of 60% of the ration, while Lucerne or the browses made up the remaining 40%. Wheat bran was provided at 250 g to provide energy for the goats. Water was provided daily. The study lasted for 60 days and faecal sampling was done fortnightly from the rectum of the goats in the morning. The faecal samples which were collected fortnightly from rectum of the goats were used for evaluation of egg worm count and bacterial identification. After a week of feeding *T. sericea* there was significant reduction on egg worm count ($p<0.05$), while on other treatments there were no significant differences in all faecal sampling dates ($p>0.05$).

Key words: Tswana goats, *Terminalia serecia*, *Boscia albitrunca*, faecal egg worm count, bacterial count

INTRODUCTION

Botswana is semi-arid with Kalahari Desert stretching from the South-west to the North-west part of the country. The scanty and erratic rainfall however, sustains well adapted browses. Aganga *et al.* (2000) stated that tree leaves and twigs form a natural part of the diet of ruminant animals and have been used conventionally as sources of forages for livestock in Botswana and the rest of Africa. The part of tree fodder commonly used as feed is referred to as browse which can be defined as the tender shorts, twigs and leaves of shrub and woody plants and also fruits and pods. Fodder trees have high crude protein content (12.5-20.7% in the dry matter) but also contain very high levels of secondary plants metabolites such as tannins. There are two major nutritional advantages of consumption of feeds high in tannins in ruminants. One of them being prevention of bloat when animals eat pasture that is rich in soluble proteins. Another one is the ability of tannins to complex with free protein in the rumen and that protect the protein from degradation in the rumen (Aganga *et al.*, 2000).

Studies by Molina *et al.* (1999) showed that tannins produce toxins in the rumen which have adverse outcomes to some micro-organisms. They mentioned that recent studies indicated the presence of bacteria able to tolerate elevated levels of condensed tannins in the rumen of animal fed high levels of tannins. There have been high rates of infectious diseases transmitted to human by micro-organisms shed in faeces in New Zealand. One of the microbes is *Campylobacter jejuni*, which accounts for more than half of all the infectious diseases and research have demonstrated that a high prevalence rate of *C. jejuni* in pastoral animals and shown that *C. jejuni* is frequently found in rivers and streams draining farmland. The contamination of water occurs through the delivery of faecal material to water coarse in overland flow, surface flow and where livestock have access to a stream, direct deposition of faecal material. Paolini *et al.* (2002) observed that there are also pathogenic microbes such as *Haermonchus contartus* which is highly pathogenic and widely distributed, particularly in tropical areas. Warriss (2000) stated that faecal microbes are one of the major source of contamination in meat industry. This show that there is need to come up with ways of minimizing and if possible eradicating contamination by faecal microbe's especially pathogenic ones. Paolini *et al.* (2002) reported that one of the ways of trying to control the faecal microbes is the use of drugs as up to now the control of gastrointestinal parasites is through the use of antihelmentics. This way of controlling parasitic diseases has largely relied on repeated use of antihelmentics.

Corresponding Author: A.A. Aganga, Botswana College of Agriculture, P/Bag 0027, Gaborone, Botswana

Repeated use of drugs result in pathogen developing resistance to it constantly and the last host species. Due to development of resistance to drugs used to control microbes and the increasing demand of consumers to limit the use of chemicals in farm animals, there have been an interest on coming up with alternative measures of controlling microbes.

One of alternative method is the use of quebracho (a natural substance that contain high concentration of tannin) trees. Tannin toxicity to rumen microorganism has been described for several bacterial species such as *Streptococcus bovis*, *Butyvibrio fibrosolvens*, *Fibrobacter succinogenes*, *Prevotella ruminocola* and *Ruminobacter amylophilus*. There are three mechanisms of toxicity that have been identified such are the enzyme inhibition and substrate deprivation, action on membrane and metal ion deprivation. The tannins induce the changes in morphology of several species of ruminal bacteria. Studies by Paolini *et al.* (2003) in New Zealand where the initial results were obtained from the field studies, suggested that the consumption of tanniferous forages could affect the biology of the gastrointestinal worms, mainly by decrease in egg excretion and therefore could contribute to modulate the epidemiology of these parasitic diseases. Fodder plants used in studies by Baumann *et al.* (1997) (*Dialium guineense* and *Millettia thonningii*) can be used to prevent enterotoxaemia caused by *Clostridia perfringes* since when they were fed they resulted in reduction in *Clostridium* flora detected. This shows that plant materials containing tannins selectively kill *C. perfringes* bacteria. Effects of condensed tannins on establishment of population and on-coming larvae of *Trichostrongylus colubriformins* and *Teladorsagia circumcineta* in goats were determined by Paolini *et al.* (2003) and they found that condensed tannins affected adult worm population in goats, quebracho extracts also had impact on the early establishment of third stage larvae and lastly on worm biology depended on the timing of administration in relation to the parasitic cycle. Their main result was decrease in egg excretion observed in tannin fed goat group. The reductions started immediately after the administration of quebracho and represented an overall 50% decrease compared to the controls. Plants containing condensed tannin (pine leaves and dry oak leaves) reduced the number of coccidian oocytes count which exceeded 40% two days post-feeding (Paolini *et al*., 2003). They concluded that tannins can be used instead of chemotherapy.

Terminalia sericea is a small to medium sized well-shaped tree, usually 4 to 6 m in height but occasionally reaching 10 m. It occurs in open woodland in a range of soil frequently on sandy soils, moisture condition and drainage condition often at margin, locally very common even dominant or co-dominant. The barks are dark-grey or brownish and deeply vertically fissured. The leaves are clustered towards the tips of the slender branchlets. *B. albitrunca* is a stock tree up to 7 m, rarely a shrub, stiffly branched with a well rounded crown, widespread in dry, open woodland and bushveld and often associated with termite mounds. Its backs are smooth, conspicuously whitish-grey and leaves arise in groups of 2 to 4 on very reduced, hard, spiky side shoots and rarely alternate *B. albitrunca* has 9.04% CP in leaves and twigs (Palgrave, 1995). The objectives of the study were to determine the effects of feeding tannin containing browses (*Terminalia sericea* and *B. albitrunca*) on faecal bacteria count, faecal egg worm count and to identify feacal tannin resistant bacteria in Tswana goats.

MATERIALS AND METHODS

Location of the study: The study was conducted in Botswana College of Agriculture's Farm, which is located in between 24°33' S latitude and 25°57° E longitude at an altitude of 994 m above the sea level. The annual rain fall was about 500 mm. Monthly average minimum and maximum temperature was 12.8 and 28.6°C, respectively.

Experimental animals and management: Twenty-five yearling goats (15 females and 10) will be used in the study for seventy (70) days. The animals were obtained from BCA goat herd where they were grazed daily. On the arrival in the feeding trial, the animals were weighed and allocated in to five different treatment groups (Table 1), each with five goats, balanced for weight and sex. Each group had two males and three open, non-pregnant and non-lactating females. Goats were individually housed in the pens with concrete floor. Each of these pens had feeding trough and bucket for clean water which were replenished each day.

Faecal sampling procedures: Faecal samples were collected twice fortnightly throughout the 60 days of the study. Faecal samples were taken at the start of the study

Table 1: Feeding ration for each treatment

Treatments	Basal diet 400 g of buffel grass	Energy concentrate 250 g wheat bran	Legume
Control	✓	✓	800 g of lucerne
Treatment 1 (Low tannin)	✓	✓	400 g of *B. albintrunca*
Treatment 2 (High tannin)	✓	✓	800 g of *B. albitrunca*
Treatment 3 (Low tannin)	✓	✓	400 g of *T. sericea*
Treatment 4 (High tannin)	✓	✓	800 g of *T. sericea*

and weekly thereafter. The numbers of eggs per gram of fresh faeces were determined. The faecal samples were taken from the rectum and placed in a clean sampling bottle. Five grams of faeces from individual goats were weighed and then crushed with a spoon. Forty-five glass beads were placed in the crushed faecal sample to further improve crushing. Twenty-eight milliliter of water were added to the sample bottle, which was tightly closed and shaken well. The mixture was sieved through a coarse sieve into a clean beaker, mixed well and transferred to centrifuge tubes. Centrifugation was done for 3 min at 1500 rpm. The supernatant fluid was then decanted and saturated aqueous sodium chloride solution was added to fill the tube up to level. Using a pipette a McMaster slide was filled and examination for presence of eggs under a light microscope using a 10x lens was carried out. The eggs were counted according to the modified McMaster method where one egg was taken to represent 50 eggs/g fresh faeces (Madibela and Jansen, 2003). Egg worm counting was done using Mcmaster procedure.

Faecal culture procedure: A sterile swap was dipped into faecal material and inoculum smeared into the Blood Agar (BA) and MacConkey Agar (MA). A culture loop was flamed with Bunsen burner flame and cooled by touching to the surface of the agar and streaks were made from the initial smear on the agar. The loop was flamed between the streaks until culturing was completed. Plates were incubated at temperature of 37°C aerobically and anaerobically for 18-24 h. The cultures were examined for microbial growth and re-incubated if there is none. The microbial colony was characterized by there appearance, shape, size and colour. Cultures were purified if mixed, by sub-culturing and incubated at the same temperature and environmental conditions of the initial cultures. The colonies were gram stained and the results were interpreted by morphology (cocci, rods, dipplococci plemorphic). Gram positive microbes were purple in colour, gram negative were pink and variable in mixed colour.

Bacterial count technique: One gram of crushed and wetted faecal samples was cultured in blood agar overnight and the colonies were used to prepare dilutions. Six dilutions were prepared in a test tube for each sample, the first or original test tube which is 10^0, was filled 10 mL of distilled water and the other five were 9 mL, using a pipette. From the blood agar plate, colonies were picked using the swab and homogenized in the 1st test tube. Then different pipettes were used to transfer 1 mL from the original dilution to the, (thus, $10^0 \rightarrow 10^{-1} \rightarrow 10^{-2} \rightarrow \ldots 10^{-6}$) in every step. The samples were thoroughly mixed before sampling and transferring.

For every dilution of a bacterial suspension the inoculum of 0.1 mL was placed on the surface of agar plate (Plate count agar). Then the inoculum was spread rapidly over the entire agar surface using a thin, bent glass rod, bent in an L-shape. The plates were incubated for 24 h at temperature of 37°C. After incubation, plates inoculated with a sample dilution yielding between 30 and 300 colonies or countable colonies were counted for greater accuracy.

Preparation of culturing media: The blood agar base was prepared from dehydrated powder, which was dissolved in cold distilled water and boiled with regular agitation. It was prepared following the instructions on the label. After boiling it was sterilized in the autoclave and cooled in water bath at 50°C. The sterile sheep blood at the rate of 5-10% vol/vol was added to the cooled agar base and mixed well before being poured in the plates. After cooling some plates were incubated at 37°C for 24 h to determine sterility of the media.

MacConkey and Plate count agar were also prepared following instructions on the label. All equipments used were sterile to avoid contamination of the media.

All data were analyzed by using Duncan's Multiple Range Tests of SAS (1995), to find the effects of treatment on faecal egg count, bacterial count and identification. Bacterial counts were transferred using $\log_{10}$ (FEC) to normalize the data before analysis.

RESULTS AND DISCUSSION

There was significant difference ($p<0.0413$) on FEC of goats fed low *Terminalia sericia* on the first week of the study (Table 2 and 3) and from the second week there were no difference ($p>0.05$), compared with the control. There was no significant difference ($p>0.05$) on the bacterial count of all groups in the period of 60 days of the study. Figure 1 shows faecal egg worm count of Tswana goats used in this study. Also the difference on bacterial identification was not significant (Table 4).

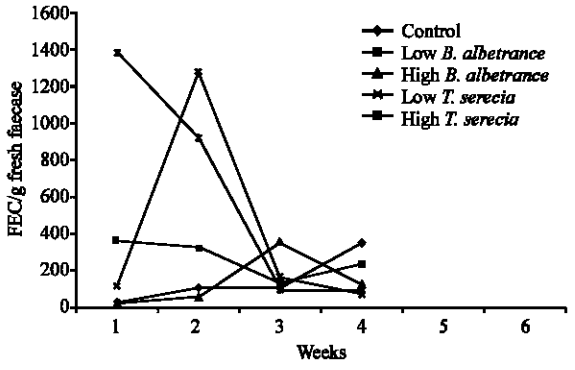

Fig. 1: Effect of fodder plants fed at different levels on faecal egg worm count

Table 2: Mean bacterial count (cfu mL^{-1} *10^5) of tswana goats faeces fed *C. ciliaris* as basal diet, *B. albitrunca* or *T. sericea* as supplements

	Bacterial count										
Treatments	Week 1	SE	p-level	Week 2	SE	p-level	Week 3	SE	p-level	Week 4	SE	Level
Control	1.46	0.18		1.92	0.18		1.97	0.15		2.2	0.3	
L.B. albit	2.04	0.33		2.18	0.36		2.04	0.16		2.3	0.1	
H.B. albit	1.37	0.20	0.701	1.71	0.16	0.5125	2.28	0.31	0.7807	2.0	0.2	0.1703
L.T. seric	1.50	0.44		2.24	0.40		2.07	0.19		1.7	0.2	
H.T. seric	1.93	0.70		2.44	0.36		1.95	0.14		1.8	0.2	

L.B. albit = Low *Boscia albitrunca*, H.B. albit = High *Boscia albintrunca*, L.T. seric = Low *Terminalia sericea*, H.T seric = High *Termenalia sericea*

Table 3: Least square means of Faecal Egg Count (FEC) (Log_{10} (FEC+1)) of Tswana goats fed diets with *B. albintrnca* or *T. sericea* as supplements

	Faecal egg count											
Treatments	Week 1	SE	p-level	Week 2	SE	p-level	Week 3	SE	p-level	Week 4	SE	p-level
Control	8.08	0.28		7.60	0.33		8.27	0.15		8.42	0.79	
L.B. albit	8.54	0.12		8.51	0.12		8.12	0.18		8.08	0.28	
H.B. albit	8.50	0.13	0.0413	8.07	0.20	0.1126	8.09	0.15	0.8147	7.10	0.75	0.4681
L.T. seric	7.66	0.21		7.81	0.20		7.98	0.27		8.32	0.13	
H.T. seric	8.30	0.25		8.36	0.36		8.32	0.20		8.09	0.43	

Table 4: Type and morphology of bacteria identified in collected faecal samples of Tswana goats fed diets with *B. albintrnca* or *T. sericea* as supplements

Weeks		1		2		3		4	
Treat	ID	BA	MC	BA	MC	BA	MC	BA	MC
Cont	3025	+ve rods	L.Fs	-ve cocci	L.Fs	+ve rods	L.Fs	-ve cocci	L.Fs
	4519	+ve rods	L.Fs	+verods	L.Fs	+vecocci	L.Fs	+ve rods	L.Fs
	C1	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
	3015	+ve rods	L.Fs	-ve cocci	L.Fs	-ve cocci	L.Fs	-ve cocci	L.Fs
	3518	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs	-ve cocci	L.Fs
L.B. alb	T1a	-ve rods	L.Fs	-ve cocci	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
	T1b	+ve rods	L.Fs	-ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
	T1c	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
	3022	-ve rods	L.Fs	-ve rods	L.Fs	+ve cocci	L.Fs	+ve rods	L.Fs
	4516	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
H.B. alb	T2a	-ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
	T2B	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
	3021	-ve rods	L.Fs	+ve rods	L.Fs	+ve cocci	L.Fs	+ve rods	L.Fs
	3515	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
	4513	-ve cocci	L.Fs	+ve cocci	L.Fs	+ve cocci	L.Fs	+ve cocci	L.Fs
L. T. ser	T3A	+ve cocci	L.Fs	-ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
	T3B	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
	T3C	+ve cocci	L.Fs	+ve cocci	L.Fs	+ve cocci	L.Fs	+ve rods	L.Fs
	3001	+ve cocci	L.Fs	+ rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
	4501	+ve rods	L.Fs	-ve cocci	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
H.T. ser	T4A	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
	T4B	-ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
	T4C	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
	3010	-ve cocci	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs
	4505	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs	+ve rods	L.Fs

Lfs, Lactose fermenters, Treat Treatment, Cont, Control, L.B. alb, Low *Boscia albintrunca*, H.B. alb, High *Boscia albintrunca*, L.T. ser, L ow *Terminalia sericea*, H.T. ser High *Terminalia sericea*

The data obtained showed that browses fed to Tswana goats in this study had no effect on faecal microbial flora of the goats. This may be due to the fact that Tswana goats used in this study were used to browsing on plants relatively high in tannin content before the study. Therefore the microbes in the gut were used to the tannins in the browses evaluated. Previous researches on this issue used tannins extracts which showed negative impact on FEC. Also tannin extracts were fed in high concentration as compared to feeding browse twigs and leaves which is what the Tswana goats browse daily. Paolini *et al.* (2003) observed that FEC was significantly lowered in goats receiving quebracho extracts, which represented 5% of the diet DM while in this study the browse plants contained lower tannin contents (Table 5). Low concentration of tannins over time resulted in microorganisms getting adapted to the tannins. Results obtained in this study is in line with the findings of Max *et al.* (2004) that stated that it was possible that both the small ruminants and parasites from Tanzania were adapted to diets high in tannins. There could also be some chemicals contained in the plants that inhibit the action of tannins. Paolini *et al.* (2002) one of these chemicals that have anti-tannin action is polyethylene glycol (PEG) which is a specific inhibitor of tannins. PEG is a polymer that can bind tannins irreversibly and its presence reduces the formation of tannin-protein complex. The role of tannin could be more

Table 5: Percentage chemical composition of feedstuffs fed to goats during the trial

Name of feedstuff	DM	CP	Tannin
Medicago sativa	86.9	10.4	-
Cenchrus ciliaris	87.0	6.84	-
Terminalia serecia	41.1	5.72	4.13
Boscia albetrunca	55.5	6.10	0.32
Wheat bran	87.0	9.00	-

specific based on data acquired in a survey in Uganda, where two groups of Anglo-Nubian goats bred under rangeland environment, received or not an inhibitor of tannin (PEG) for six months. This induced a significant rise in parasitic egg excretion suggesting a possible repressive role of tannin on worm fertility. While tannin extracts are pure and are able to inhibit or reduce microbial action and growth, browse twig may contain other chemicals which affects tannin activities.

After gram staining procedure most of the identified bacteria were gram positive especially from faeces of goats supplemented with browses and they were lactose fermenters (Table 4). Representative samples were selected from all of the treatments including the control to perform the oxidase and catalase test to identify the genera group in all treatments and *Staphylococcus* and *Streptococcus* were identified, showing that they are tannin resistant. Also *Klebsiella* sp. and *Corynebacterium* were isolated.

E. coli was not isolated as it is a gram negative rod bacteria which indicated that it is a tannin sensitive bacteria. Smith and Mackie (2004) reported that there were other indications that the growth of *E. coli* may be inhibited by tannins through complexation with metal ions. This occur in the environment in which tannin produce hydrogen peroxide as product of auto-oxidation leading to inhibition of micro-organisms. In the gastrointestinal system, hydrogen peroxide may be produced during mastication of plant material and in the rumen as shown by Smith and Mackie (2004) in which levels of up to 1.63 nmol of O_2 L^{-1} was measured. Hydrogen peroxide is an important molecule that contributes to oxidation to damage cells. Tannin might be one of the external sources of hydrogen peroxide.

The study shows that Tswana goats' continuously feed on browses and thus may be adapted to tannins in the fodder plants. Therefore, there was no significantly difference between the control goats fed on Lucerne and treatment groups fed browses as supplements.

ACKNOWLEDGMENTS

The authors are very grateful to Botswana College of Agriculture for funding the research, Mr. M. Mathaio and Ms. E. Samakabadi for technical assistance in laboratory analysis.

REFERENCES

Aganga, A.A., K. Tshireletso and M. Mathaio, 2000. Condensed tannin content in seeds of twenty browse species in Botswana, Optimal feeding of grazing animals in Botswana, Proceeding of Livestock Feeding Systems Workshop Held at Centre for in-Service and Continuing Education, Botswana College of Agriculture, Gaborone, Botswana, Aganga, A.A., M. Kgosimore, U.J. Omphile and R.G. Chabo (Eds.), pp: 226-229.

Baumann, M., W. Muller and J. Greiling, 1997. Effect of fodder plants containing tannin on *Clostridial* flora in the intestine of sheep. J. Anim. Res. Develop., 45: 73-81.

Madibela, O.R and K. Jansen, 2003. The use of indigenous parasitic plants (*Viscum verrocosum*) in reducing faecal egg counts in female tswana goats. J. Livestock Research for Rural Develop., http://www.utafoundation.org/Irr159/madi159.htm Downloaded on the 6th October, 2005.

Max, R.A., D. Wakelin, A.E. Kimambo, A.A. Kassuku, L.A. Mtenga and P.J. Buttery, 2004. Effect of tannin extract drenches on nematode infections in temperate and tropical small ruminants. Paper presented at the 8th International Goat Association conference held in University of Pretoria, Pretoria, South Africa.

Molina, D.O, A.N. Pell and D.E. Hoque, 1999. Effects of ruminal inoculations with tannin tolerant bacteria on fibre and nitrogen digestibility of lambs fed high condensed tannin diet. J. Feed Sci. Technol., 81: 69-80.

Palgrave, K.C., 1995. Trees of Southern Africa, 2nd Edn. Struilk Publishers, Capetown.

Paolini, V., A. Frayssines, F. Dela Farge, P. Dorchies and H. Hoste, 2002. Effects of condensed tannins on established populations and on incoming larvae of *Trichostrongilus colubriformis* and *Telaclorsagia circumcincta* in goats. Vet., Res., 34: 331-339.

Paolini, V., J.P. Bergeand, C. Grisez, F. Prevot, P.H. Dorchies and H. Hoste, 2003. Effects of condensed tannin on goats experimentally infected with *Haemonchus contortus*. J. Vet. Parasitol., 113: 253-261.

SAS, 1995. User's Guide: Statistical Analysis System Procedures. SAS Institute, Inc., Cary,NC.

Smith, A.H. and R.I. Mackie, 2004. Effects of condensed tannins on bacterial diversity and metabolic activity in the rat gastro-intestinal tract. J. Applied Environ. Microbiol., 70: 1104 -1115.

Warriss, P.D., 2000. Meat Science An introductory Text, CABI Publishing, Briston, UK. D/D and checked