Effects of Feeding Condensed Tannin-containing Plants on Natural Coccidian Infection in Goats*

Sam N. Hur, Abdul L. Molan[1] and Jang O. Cha**

Department of Animal Resources and Biotechnology, College of Agriculture

Chunbuk National University, Chonju, Korea

ABSTRACT : Twelve Korean native goats, spontaneously infected with mixed species of *Eimeria* were used to study the possible direct anticoccidial effect of feeding condensed tannin-containing plants on the production of *Eimeria* oocysts. The effects of feeding pine (*Pinus densifora*) needles, oak (*Quercus acutissima*) leaves and lucerne chaff on coccidia oocyst output were studied for a period of 10 days post-feeding. The results indicate that feeding fresh pine needles (40 g condensed tannins (CT) dry matter (DM)/day/goat) and oak leaves (40 g CT DM/day/goat) in combination with lucerne chaff had rapid anticoccidial activities in goats as demonstrated by a sharp decrease in oocyst production. Two days after feeding, the numbers of oocysts per gram of faeces (OPG) from the goats fed pine needles with lucerne chaff, and from goats fed oak leaves reduced by 40% and 44% compared to pre-feeding, respectively. On the sixth day after commencing feeding pine needles and oak leaves, the reduction was 81% and 72%, respectively. Ten days after feeding pine needles and oak leaves, the OPG was reduced by 93% and 85%, respectively compared to pre-feeding. Statistical analysis showed that feeding pine needles and oak leaves to goats naturally infected with coccidia significantly ($p<0.001$) reduced the numbers of oocysts compared to the control group fed lucerne chaff only. Four clinically important species of coccidia, *Eimeria parva, Eimeria ninakohlyakimovae, Eimeria christenseni* and *Eimeria arloingi* were identified in Korean native goats. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 9 : 1262-1266)***

Key Words : *Eimeria*, Goats, Pine Needles, Oak Leaves, Anticoccidial Activity

INTRODUCTION

Coccidiosis, caused by infection with *Eimeria* species affects cattle, deer, sheep, goats, pigs, horses, rabbits, turkeys, ducks and poultry (Cox, 1998), is probably the most important parasitic disease of veterinary importance throughout the world. This disease costs the US poultry industry more than $1.5 billion in annual losses (Yun et al., 2000).

The signs of damage in lambs can range from loose diarrheic faeces to severe dysentery and death (Samizadeh et al., 1979). Scanning electron microscopic observations of the small intestine of lambs infected with *Eimeria* species showed disturbed intestinal surfaces and the villi were short and flat with sloughing of the epithelium (Samizadeh et al., 1979).

Currently, chemotherapy is used extensively to control coccidiosis, but the worldwide and rapid development of drug resistance (Greif et al., 1996; Haberkorn, 1996), coupled with the increasing costs of developing new drugs and the public's distrust of drug-treated meat demonstrate the urgent need to explore alternative methods of controlling this disease.

Condensed tannins (CT) are polyphenolic compounds commonly found in the seed coats of many plants and in the foliage of several legumes and grass cultivars (Waghorn et al., 1990; Terrill et al., 1992). Some studies (Niezen et al., 1995; Robertson et al., 1995) have shown that CT-containing forages such as sulla (*Hedysarum coronarium*) had significant effects on intestinal nematodes in sheep but they could not determine whether the effect was indirect, ie. by increasing the amount of protein reaching the small intestine which may increase animal resistance to the parasites, or to the direct effect of condensed tannins on parasites in the small intestine.

Recently, Molan et al. (1999) have shown that CT extracted from some forages have direct antiparasite activity as evidenced by their ability to reduce the viability of the eggs of sheep gastrointestinal nematodes. They also showed that CT were able to reduce the viability of the infective larvae of sheep and deer gastrointestinal nematodes and lungworms (Molan et al., 2000a, b, c, d).

To our knowledge there is no published report of the effects of CT-containing forages on *Eimeria* infections. The main objective of this study was to investigate the effect of feeding pine needles and oak leaves that contained CT on natural coccidian infection in goats.

MATERIALS AND METHODS

Experimental design

A total of 12 male and female naturally parasitised

* This research was supported by Agricultural R&D Promotion Center.

** Corresponding Author: S. N. Hur. Tel: +82-63-270-2610, Fax: +82-63-270-2612, E-mail: hsn@chonbuk.ac.kr

[1] Human Nutrition and Health Group, Institute of Food, Nutrition and Human Health, Massey University, New Zealand.

Received September 26, 2004; Accepted March 18, 2005

Table 1. Species of *Eimeria* identified in the faecal samples of naturally infected Korean goats and the percentage of infection

% of faecal oocysts	Species
54	*Eimeria arloingi*
23	*Eimeria christenseni*
16	*Eimeria ninakohlyakimovae*
7	*Eimeria parva*

Korean native goats from Chonbucck National University farm were selected and *Eimeria* infections were confirmed by faecal oocyst counts. Goats were weighed and faecal samples were collected (for determining oocyst counts) on two consecutive days before the goats were treated (base line data). The goats were then divided into three equal groups based on oocyst counts and sex. The goats in the first group were fed lucerne chaff only (1,750 g DM/day), to serve as a control group. The goats in the second group were fed lucerne chaff (1,278 g dry matter (DM)/goat/day) and fresh pine needles (472 g/goat/day; equivalent to 40 g CT/day/goat) whilst the goats in the third group were fed lucerne chaff (205 g DM/day) and dry oak leaves (1,545 g DM/goat/day; equivalent to 40 g CT/day/goat). The amount of feed intake was not changed during the trial and the palatability of feed was high in the order of fresh pine needles, dry oak leaves, and Lucerne chaff. The goats were fed at 0900 and 1700 h each day and had free access to water. Faecal samples for oocyst counts were collected 2, 4, 6, 8 and 10 days after commencing the nutritional treatments.

Faecal examinations

In order to determine the level of infection, faecal samples were collected from the rectum of each goat and the numbers of oocysts were determined within 3 h of collection using a modified McMaster method where a count of one oocyst was equivalent to 50 oocysts/gram faeces.

Speciation

The procedure of Harper and Penzhorn (1999) was followed with slight modifications. Briefly, faecal samples with more than 3,000 OPG were thoroughly mixed with at least 20×their volume of an aqueous 2.5% (w/v) potassium dichromate solution, placed in thin layers in Petri-dishes and the oocysts allowed to sporulate for 10-14 days at room temperature. To recover oocysts, the solution was centrifuged at 1,200 rpm for 10 minutes, the supernatant discarded and the sediment placed into a vials filled with 2 M sucrose solution. A cover slip was placed on the top of the vials for about 10 minutes to allow oocysts to float onto the underside of the cover slip which was carefully removed, placed on a microscope slide and the edges sealed with nail polish. The slide was scanned in parallel sweeps and the first 100 oocysts seen were identified. Measurements (length and width) were made using Scmascan, Scientific Measurement System at the Faculty of Veterinary Science, Chonju National University, Chonju, Korea. Species identification was based on descriptions and illustrations by Levin (1985), Craig (1986), O'callaghan (1989), Al-Yousif et al. (1992) and Rhee (1999).

Table 2. Extractable and bound condensed tannins (CT) content of pine needles, oak leaves and lucerne, measured by butanol-HCl method

Plant	CT (g/kg dry matter basis)			
	Extractable	Protein-bound	Fibre-bound	Total
Pine needles	73.1	4.3	3.1	80.5
Oak leaves	16.2	8.3	1.4	25.9
Lucerne	0.19	1.97	0.19	2.3

Determination of the level of condensed tannins

The extractable, protein-bound and fibre-bound CT concentrations in pine needles, oak and lucerne chaff were determined by the butanol-HCl method (Jackson et al., 1996).

Calculation of data and statistical analysis

The efficacy of treatment was determined by using the formula:

$$\frac{A-B}{A} \times 100$$

Where A and B are the numbers of oocysts per gram of faeces at pre- and post-treatment, respectively. Data were analyzed using SAS ver. 6.11 (1991).

RESULTS

All the goats were infected with one or more species of *Eimeria*. Cultures of pooled faecal material revealed the presence of four species of *Eimeria*, *Eimeria parva* (Kotlan, Mocsy, and Vajda, 1929) (7% of total oocysts), *E. ninakohlyakimovae* (Yakimoff and Rastegaieff, 1930) (16%), *E. christenseni* (Levine, Ivens and Fritz, 1962) (23%) and *E. arloingi* (Martin, 1909) (54%; Table 1).

Table 2 shows the extractable, protein-bound and fibre-bound CT concentration in pine needles, oak leaves and in lucerne chaff. Pine needles had the highest CT concentration (81 g/kg DM) followed by oak leaves (26 g/kg DM), and lucerne chaff (2 g/kg DM).

The effects of feeding fresh pine needles, dry oak leaves and lucerne chaff on the natural coccidian infection in Korean native goats are shown in Table 3 and Figure 1. Feeding fresh pine needles had anticoccidial activity as evidenced by the reduction in the numbers of oocysts exceeded by 40% two days post-feeding, 71% four days

Table 3. Comparative anticoccidial activities of fresh pine needles, dried oak leaves in combination with lucerne chaff or lucerne chaff only fed to goats naturally infected with different species of *Eimeria* based on oocysts per gram faeces (OPG) counts

Group	Feed	Pretreatment mean OPG	Mean OPG per day					OPG reduction (%)
			2	4	6	8	10	
A	PN	6,300	3,800	1,813	1,200	538	463	75.24
B	OK	4,463	2,488	1,363	1,263	863	625	70.44
C	LC	3,813	5,250	4,300	4,625	3,725	4,513	0

LC: lucerne chaff; OK: oak leaves; PN: pine needles.

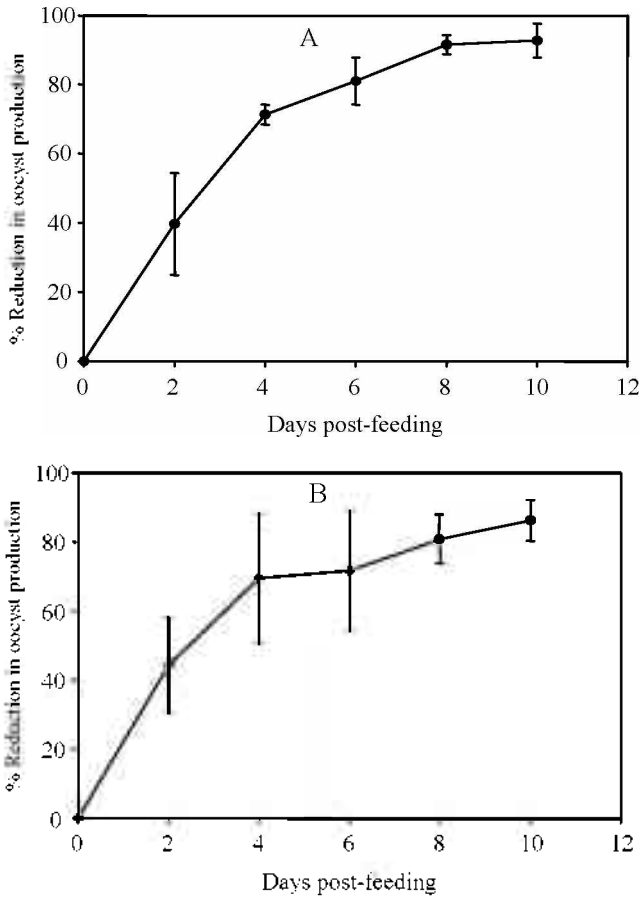

Figure 1. Effect of feeding fresh pine needles (A) and dry oak leaves (B) in combination with lucerne chaff on the natural coccidian infection in Korean native goats. The anticoccidial activity was measured by determining the reduction in the production of oocysts of different *Eimeria* species post-feeding compared to pre-feeding mean oocyst output values. Each point represents the mean of four observations with the standard error of the mean.

post-feeding, 81% six days post-feeding, 92% eight days post-feeding and by 93% ten days post-feeding relative to the numbers of oocysts shed pre-feeding (Table 3 and Figure 1A). The differences were highly significant ($p<0.001$) in comparison to control group fed Lucerne chaff only.

Feeding dried oak leaves to the goats reduced the numbers of oocysts by 44%, 70%, 72%, 81% and 85% on days 2, 4, 6, 8 and 10 post-feeding, respectively, compared to the numbers of oocysts shed before feeding oak leaves. These differences were also highly significant in comparison to controls.

DISCUSSION

All the goats used in this study were infected with coccidia and four species were identified. This high percentage of positive specimens is consistent with the findings of Alyousif et al. (1992) who found that 90% of the goats in Saudi Arabia were positive for *Eimeria* and. Balicka-Ramisz (1999) who showed that of 110 Polish goats 81% of adults and 100% of the kids were infected with up to nine different species of *Eimeria*. Moreover, Harper and Penzhorn (1999) found that 89-100% of adult goats in South Africa were infected with up to seven species of *Eimeria*.

Published data indicate that *E. christenseni*, *E. arloingi*, *E. ninakohlyakimovae*, *E. caprina* and *E. caprovina* are the most pathogenic species in goats (Alyousif et al., 1992; Balicka-Ramisz, 1999; Harper and Penzhorn, 1999). The data reported here indicate that these *Eimeria* species are also among the most common in the Korean goats (Table 1) with *Eimeria arloingi* the most prevalent species. Harper and Penzhorn (1999) similarly reported that *E. arloingi* was the most prevalent species of *Eimeria* in South African goats. The absence of symptoms such as diarrhoea in adult goats may indicate a well-developed immunity against coccidiosis (Craig, 1986; Alyousif et al., 1992).

In this study we showed that feeding goats naturally infected with *Eimeria*, with diet containing pine needles and oak leaves had a profound anticoccidial activity as evidenced by their ability to reduce the numbers of oocysts by up to 93% during the trial period of 10 days. This anticoccidial activity may be related to the CT contents in both pine needles and oak leaves.

It is well known that CT can react and form complexes with proteins in a pH-dependent manner (Jones and Mangan, 1977) and that these reactions are highly specific for different tannins as well as different proteins (Asquith and Butler, 1986; Butter et al., 2000). Condensed tannins found in pine needles and oak leaves fed to goats might therefore interact with the dietary proteins and protect them from microbial degradation in the rumen so that more protein would reach the small intestine and the ability of the host to respond to reduce parasite establishment could be achieved by better protein nutrition. It has been clearly

established that feeding diets containing low concentrations of CT in forage (usually less than 50 g CT/kg DM), benefit ruminants by reducing degradation of dietary protein and increasing the supply of amino acids to the small intestine (Waghorn et al., 1987; McNabb et al., 1993).

Although the CT present in pine needles and oak leaves may increase bypass protein supply to the goats, it is proposed that the decrease in the production and shedding of *Eimeria* oocysts in this short-term study was more likely to have been due to direct effects of the CT on different stages of the parasite's life cycle, rather than via improved protein nutrition of the goats. This is because Molan et al. (1999) found that exposure of the eggs and first-stage (L1) larvae of the sheep nematode, *Trichostrongylus colubriformis* to low concentrations of CT extracts from different forages resulted in a reduction in the rate of egg hatching and the development of eggs and L1 larvae to infective larvae under *in vitro* conditions. Athanasiadou et al. (2000) drenched sheep experimentally infected with *T. colubriformis* with quebracho extract and found that in the treated sheep the worm burdens and number of eggs per gram faeces per worm were reduced by 30% compared with the undrenched controls. In contrast, many short-term studies have shown that increased protein supply to the small intestine does not influence parasite establishment in sheep infected with *Haemonchus contortus* (Abbott et al., 1985, 1986) and *Trichostrongylus colubriformis* (van Houtert et al., 1995; Kyriazakis et al., 1996).

In view of the results of the present study and the fact that goats like eating fresh pine needles and dry oak leaves, these feedstuff could be used as an alternative method for controlling coccidian infection in goats in order to reduce a dependence on chemotherapeutics as the sole method for controlling coccidian infection in goats.

Further studies are clearly needed both to determine the minimal concentration of CT required to stop the production of oocysts and to investigate the effects of feeding pine needles and oak leaves on the productive performance of goats.

ACKNOWLEDGEMENTS

This work was supported by Agricultural R&D Promotion Center, Korea, for which we are grateful. The authors would like to thank Professor H. H. Yang, Faculty of Veterinary Sciences for allowing us to use the microscope and the digital camera for measuring the dimensions of the oocysts and Professor J. K. Rhee for his kind help in the identification of *Eimeria* species.

REFERENCES

About, E. M., J. J. Parkins and P. H. Holmes. 1985. Influence of dietary protein on parasite establishment and pathogenicity in Finn Dorset and Scottish Blackface lambs given a single moderate infection of *Haemonchus contortus*. Res. Vet. Sci. 38:6-13.

About, E. M., J. J. Parkins and P. H. Holmes. 1986. The effect of dietary protein on the pathogenicity of acute ovine haemonchosis. Vet. Parasitol. 20:275-289.

Alyousif, M. S., A. A. K.asim and Y. R. AL-Shawa. 1992. Coccidia of the domestic goat (*Capra hircus*) in Saudi Arabia. Int. J. Parasitol. 22:807-811.

Asquith, T. N. and L. G. Butler. 1986. Interactions of condensed tannins with selected proteins. *Phytochemistry* 25:1591-1593.

Athanasiadou, S., I. Kyriazakis, F. Jackson and R. L. Coop. 2000. Effects of short-term exposure to condensed tannins on adult *Trichostrongylus colubriformis*. Vet. Rec. 46:728-732.

Balicka-Ramisz, A. 1999. Studies on coccidiosis in goats in Poland. Vet. Parasitol. 81:347-349.

Butter, N. L., J. M. Dawson, D. Wakelin and P. J. Buttery. 2000. Effect of dietary tannin and protein concentration on nematode infection (*Trichostrongylus colubriformis*) in lambs. J. Agric. Sci. 134:89-99.

Cox, F. E. G. 1998. Control of coccidiosis: lessons from other sporozoa. Int. J. Parasitol. 28:165-179.

Craig, T. M. 1986. Epidemiology and control of coccidia in goats. Vet. Clin. North Am. Food Anim. Pract. 2:389-395.

Greif, G., B. Stephan and A. Haberkorn. 1996. Intraspecific polymorphisms of *Eimeria* species due to resistance against coccidial drugs. Parasitol. Res. 82:706-714.

Haberkorn, A. 1996: Chemotherapy of human and animal coccidiosis: state and perspectives. Parasitol. Res. 82:193-199.

Hammond, D. M. 1982. Life cycle and development of coccidia. In: (Ed. D. M. Hammond, P. L. Long). The Coccidia. Maryland University Park Press, Baltimore, pp. 45-79.

Harper, C. K. and B. L. Penzhorn. 1999. Occurrence and diversity of coccidia in indigenous, Saanen and crossbred goats in South Africa. Vet. Parasitol. 82:1-9.

Jackson, F. S., W. C. McNabb, T. N. Barry, Y. L. Foo and J. S. Peters. 1996. The condensed tannins of a range of subtropical and temperate forages and the reactivity of condensed tannin with ribulose-1,5-bisphosphate carboxylase (Rubisco) protein. J. Sci. Food Agric. 72:483-492.

Jones, W. T. and J. L. Mangan. 1977. Complexes of the condensed tannin of sainfoin (*Onobrychis viciifolia* Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. J. Sci. Food Agric. 28:126-136.

Kyriazakis, I., D. H. Anderson, R. L. Coop and F. Jackson. 1996: The pathogenicity and development of immunity during long-term subclinical infection with *Trichostrongylus colubriformis* of sheep receiving different nutritional treatments. Vet. Parasitol. 65:41-54.

Levine, N. D. 1985: Veterinary Protozoology. Iowa State University Press, Ames, IA.

McNabb, W. C., G. C. Waghorn, T. N. Barry and I. D. Shelton. 1993. The effect of condensed tannins in *Lotus pedunculatus* on the digestion and metabolism of methionine, cystine and inorganic sulphur in sheep. Br. J. Nut. 70:647-661.

Molan, A. L., R. Alexander, I. M. Brookes and W. C. McNabb. 2000a. Effects of sulla condensed tannins on the viability of three sheep gasrtointestinal nematodes *in vitro*. Proc. NZ. Soc. An. Prod. 60:21-25.

Molan, A. L., A. Duncan, T. N. Barry and W. C. McNabb. 2000b. Effects of condensed tannins and sesquiterpene lactones extracted from chicory on the viability of deer lungworm larvae. Proc. N.Z. Soc. An. Prod. 60:25-29.

Molan, A. L., S. O. Hoskin, T. N. Barry and W. C. McNabb. 2000c. The effect of condensed tannins extracted from four forages on deer lungworm and gastrointestinal nematode larval viability. Vet. Rec. 147:44-48.

Molan, A. L., G. C. Waghorn and W. C. McNabb. 1999. Condensed tannins and gastro-intestinal parasites. Proc. NZ. Grass. Assoc. 61:57-61.

Molan, A. L., G. C. Waghorn, B. R. Min and W. C. McNabb. 2000d. The effect of condensed tannins from seven herbages on *Trichostrongylus colubriformis* larval migration *in vitro*. Folia Parasitol. 47:39-44.

Niezen, J. H., T. S. Waghorn, W. A. G. Charleston and G. C. Waghorn. 1995. Growth and gastrointestinal nematodes parasitism in lambs grazing lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. J. Agric. Sci. Camb. 125:281-289.

O'callagham, M. G. 1989. Coccidia of the domestic and feral goats in South Australia. Vet. Parasitol. 30:267-272.

Rhee, J. K. 1999. Latest Veterinary Parasitology. Daehan Textbook Co., Korea, pp. 432-435.

Robertson, H. A., J. H. Niezen, G. C. Waghorn, W. A. G. Charleston and M. Jinlong. 1995. The effect of six herbages on liveweight gain, wool growth and faecal egg count of parasitised ewe lambs. Proc. NZ. Soc. Anim. Prod. 55:199-201.

Samizadeh, Y., C. N. Rhodes, A. L. Pope and A. C. Todd. 1979. Ovine coccidiosis: comparison of the effects of monensin and aureomycine on lambs infected with coccidia. Am. J. Vet. Res. 40:1107-1109.

SAS. 1991. User's Guide Statistics. Cary. N.C. Statistical Analysis System Institute Inc.

Terrill, T. H., A. M. Rowan, G. B. Douglas and T. N. Barry. 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. J. Sci. Food Agric. 58:321-329.

van Hotert, M. F. J., I. A. Barger, J. W. Steel, R. G. Windon and D. L. Emery. 1995. Effects of dietary protein intake on responses of young sheep to infection with *Trichostrongylus colubriformis*. Vet. Parasitol. 56:163-180.

Waghorn, G. C., W. T. Jones, I. D. Shelton and W. C. McNabb. 1990. Condensed tannins and the nutritive value of herbage. Proc. NZ. Grass. Assoc. 51:171-175.

Waghorn, G. C., M. J. Ulyatt, A. John and M. T. Fisher. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. Br. J. Nutr. 57:115-126.

Yun, C. H., H. S. Lillehoj and E. P. Lillehoj. 2000. Intestinal immune responses to coccidiosis. Dev. Comp. Immunol. 24:303-324.