

Effect of condensed tannin on controlling faecal protein excretion in nematode-infected sheep: *in vivo* studyShahin Hassanpour¹, Mohammad Sadaghian², Naser MaheriSis¹, Behrad Eshratkhhah², Majid ChaichiSemsari¹¹. Department of Animal science, Shabestar Branch, Islamic Azad University, Shabestar, Iran.². Department of Veterinary medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran.hassanpour.shahin@gmail.com

Abstract: The main propose of this study was to investigating the short term effects of different levels of wattle tannin to protein excretion control during a naturally acquired nematode infection in Moghani sheep. Twenty Moghani ewes (aged 7-12 months and body weight 32±3 kg) were selected randomly and divided into 4 treatment groups. The animals of the experiment had not received any anthelmintic drugs for 4 month. During the study all animals fed *ad libitum* on Moghan plateau and free access to water. Group 1 received placebo. Animals in groups 2, 3 and 4, were drenched 1, 1.5 and 2 gr per kg body weight (BW) wattle tannin (WT) as water solution for one day, respectively. At 0, 24 and 48 hours after drenching WT from each animal faecal sample was taken and stored in individual containers less than 4°C carried out to laboratory as soon as possible. Faeces nitrogen (N), dry matter (DM), organic matter (OM), Ash and Wet levels determined following the standard procedures. Data were analyzed as a complete randomized design for repeated measurements using SAS (9.1) software and the least square means compared with Tukey multiple range tests. According to our result there was a significant difference between the groups. Twenty-four hours after drenching highest amount of faecal crude protein excretion observed in group 4 ($P < 0.0001$). Additionally, Forty-eight hours after drenched WT there was a significant difference in Group 4 compare to the other groups which has lower FCP excretion ($P < 0.0001$). There was no significant difference in faecal OM, DM, Ash and Wet content between groups ($P > 0.05$). In conclusion it is observed that administration of 2 gr WT per kg BW leads to decreasing faecal protein excretion and so resulted in nitrogen retention in animals.

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Keywords Condensed tannin, Nematode, Faecal content, Moghani sheep

1. Introduction

Tannins are the secondary shrubs and trees metabolites and can found in various species of plants (Waghorn, 2008). Previous studies have demonstrated tannins can defense plants against insects and Herbivores by Astringent taste (Hagerman and Butler, 1991; Rosales, 1999) which usually divided into two Major Groups: Hydrolysable tannin (HT) and condensed tannin (CT). These components have differences in structure and Molecular weights. There is D-glucose in base structure of HT and there isn't any glucose in CT whereas CT has higher molecular weight and insoluble in water compare to HT (Mueller-Harvey and McAllan, 1992). Condensed tannin (poranthocyanidins, PAs) is composed by polyhydroxyl flavan-3-ol oligomers and linked by carbon-carbon bonds together (scholfield et al, 2001). Many varieties of fruits, vegetables and leguminous families have different levels of CT (Barry et al, 1984). Previous researchers have shown that tannins of various plant species have different nutritional effects on animals especially on ruminants (Mangan, 1988).

Condensed Tannin interacts with protein and polysaccharides in the rumen by formation hydrogen and covalent bonds (Mueller-Harvey and McAllan, 1992; Schofield et al., 2001; Waghorn, 2008). It could to reduce rumen protein degradation and increase dietary crude protein bypass or non ammonia nitrogen (NAN) and dietary amino acid flow to small intestine (McNabb et al., 1993; Waghorn, 2008). This mechanism is a pH-dependent manner (Jones and Mangan, 1977). Tannin-protein complex was formed at pH <3.5 in the abomasul. This complex is stable in this situation and can be deformed in alkali pH of the small intestine. Condensed tannins shift the site of protein degradation from rumen to the lower digestive tract and large intestine which in small intestine because of pH dissociated protein-tannin complex, caused to degradation protein by proteolysis enzymes which simultaneously increase amino acid absorption, live weight gain, reproductive efficiency and wool production. Researchers suggested high levels of tannins capable to inhibit proteolysis bacteria which these effects on the rumen bacteria, protozoa and fungi populations are variable. There are many reports indicating inhibitory effect of tannins on

rumen protozoa (Patra and Saxena, 2010). Recent studies have shown inclusion grazing sheep and goats with shrub species containing CT can reduce rumen fermentative protein, increase NAN flow to distal part of GIT, and improve N retention, methane production and finally improve animal performance (Waghorn, 2008). Likewise CTs has anti-parasitic effects on small ruminants (Herva's et al, 2000). Recently has been proven that consuming forages containing CT could be considerable reduce in worm burden and nematode egg hatchability in sheep and goats (Athanasidou et al, 2001). Several studies have shown that consumption tannin-rich plants due to significantly reduction in egg excretion and worm burdens in nematode infected goats and sheep. Max et al (2009) and Sadaghian et al (2011) indicated a significant reduction in Nematode faecal egg counts after drenches different levels of Wattle tannin. They suggested WT drench had significant anthelmintic activity against important nematodes in sheep. Researchers believe CT could increase the flow of essential amino acids (E. A. A.) to small intestine thus, indirectly could enhance immune system of GIT against nematodes, thus decrease endogenous protein excretion and improvement sheep performance (Coop and Kyriazakis, 2001). Also, tannins interact with proteins, structural enzymes and ion channels on glycoprotein cuticle membrane of the nematodes which inhibit substrate receiving to the parasite (Waghorn, 2008). The main propose of this study was to investigate the short term effects of different levels of WT to control of protein excretion during a naturally acquired worm infection Moghani sheep.

2. Material and Methods

The experiment was carried out in summer 2010 Moghan plateau, Ardabil province Iran (30°24'35.47" N and 48°18'12.36" E) and 98M above sea level. Twenty Moghani ewes, (aged 7-12 months and body weight 30-35 kg (none of them was pregnant or lactating) were identified to naturally acquire gastrointestinal nematode infection using McMaster flotation technique (Urquhart et al., 1987). Animals were identified by number, divided to 4 groups and raised ad libitum during the study on the Moghan plateau natural pastures and free access to water. The animals of the experiment had not received any anthelmintic drugs for 4 month. Wattle tannin powder was provided from the Tanzania leather company LTD. According to the manufacture WT powder contain 62.5gr/kg CT, 21.35gr/kg of simple phenolics. Group 1 was control and received tap water for placebo. To animals in groups 2, 3 and 4 were drenched orally 1, 1.5 and 2 gr WT per kg of BW (Max, 2010) as water suspension in tap water for one day, respectively. To all experimental animals

were given 7 ours fasting period then at 0, 24 and 48 hours after drenching CT faecal samples was collected directly from the rectum of each animal and kept in individual sampling containers under 4 C until used. Faeces protein (CP) dry matter (DM), organic matter (OM), Ash and Wet were determined in Feed Analyses laboratory using following the standard procedures of (AOAC, 1990) in Azad University Shabestar Branch. The majority size of the obvious gastrointestinal eggs in the examined faecal samples using scaled microscope were between 70 - 90 μ m. These eggs size were consistent with ones of *Trichostrongylus*, *Ostertagia*, *Haemonchus* and *Nematodirus* genus.

Amount of nitrogen as follows:

$N (\% \text{ of sample}) = (\text{Volume of acid used in sample titration} - \text{Volume of acid used in blank titration}) \times (\text{acid molarity} \times 0.014 \times 100) / (\text{weight of sample in gram} \times 1000)$. Crude protein (%) = N % x 6.25

The dry matter, Om, Ash and Wet content was then calculated using the formulas:

$DM (\%) = \{(W2-W0)\} / \{(W1-W0)\} \times 100$

$OM (\%) = \{(W2-W0)\} / \{(W1-W0)\} \times 100$

$Ash (\%) = \{(W2-W0)\} / \{(W1-W0)\} \times 100$

$Wet (\%) = \{(W2-W0)\} / \{(W1-W0)\} \times 100$

Data were processed in excel and were analyzed as a complete randomized design for repeated measurements using SAS (9.1) software and the least square means compared with Tukey multiple range tests.

3. Results

Composition of WT is showed in table1. Effect of Wattle tannin drenches on faecal protein and other excretion components are shown in table2 and 3. In this study, what is clear from our results there is a significant difference between administrations of different levels of WT on faecal protein excretion in gastrointestinal nematodes infected ewes. According to the faecal egg count (FEC) examination using McMaster flotation technique after 4 month no drenches to experimental animals all of the ewes were infected to gastrointestinal nematodes and average faecal egg was 486 ± 21 . According to the result, Drenching WT to all animals at first 24 hours increased faecal protein excretion content in all treatment groups compare to control group ($P < 0.0001$). Forty-eight hours after drenching, group 2 that drenched 2gr WT per kg BW has significantly reduce faecal protein excretion content compare the

other groups ($P < 0.0001$) (fig.1). Additionally, there were no significant differences in faecal DM, OM, Ash and Wet content between all treatment groups during the study ($P > 0.05$).

Table1. Composition of Wattle tannin

Substance	Percent
Condensed Tannin	62.5
Simple polyphenols	21.35
Insoluble	0.2
pH	4.9
Ash	1.2
Moisture	16
Acid	30mg eq/lit

Table2. Faecal nutrient excretion 24 hours after Wattle Tannin drenching to ewes (mean \pm SE)

(%)	Control	Group1	Group2	Group3	P value
CP	13.57 \pm 0.15 ^c	14.35 \pm 0.12 ^b	14.26 \pm 0.15 ^b	15.5 \pm 0.14 ^a	$0 < .0001$
DM	33.87 \pm 0.11	33.93 \pm 0.10	33.94 \pm 0.11	34.17 \pm 0.11	0.99
OM	77.24 \pm 0.006	77.24 \pm 0.005	77.24 \pm 0.006	77.24 \pm 0.006	0.50
Ash	22.75 \pm 0.006	22.75 \pm 0.005	22.75 \pm 0.006	22.75 \pm 0.006	0.49
Wet	66.03	66.03	66.03	66.23	1.00
N	5	5	5	5	Total =20

Table3. Faecal nutrient excretion 48 hours after Wattle Tannin drenching to ewes (mean \pm SE)

(%)	Control	Group1	Group2	Group3	P value
CP	13.33 \pm 0.15 ^c	13.12 \pm 0.14 ^c	13.56 \pm 0.14 ^c	11.06 \pm 0.14 ^d	$0 < .0001$
DM	33.86 \pm 0.12	33.88 \pm 0.10	33.86 \pm 0.12	34.13 \pm 0.10	0.99
OM	77.24 \pm 0.006	77.22 \pm 0.005	77.24 \pm 0.006	77.24 \pm 0.005	0.50
Ash	22.75 \pm 0.006	22.74 \pm 0.005	22.75 \pm 0.006	22.75 \pm 0.005	0.49
Wet	66.03	66.02	66.02	66.23	1.00
N	5	5	5	5	Total =20

Control: received tap water as a placebo.

Group1: received 1gr per kg body weight WT.

Group2 received 1.5 gr per kg body weight WT.

Group 3: received 2 gr per kg body weight WT as water suspension for one day.

CP = crude protein, DM = dry matter, OM = organic matter.*Different letters indicate significantly differences between groups ($P < 0.05$). SE: Standard Error.

4. Discussions

Tannins with their specific structure can produce tannin-protein complexes in the rumen (Waghorn, 2008). This complex caused to reduce microorganism's digestibility ability in the rumen, increase bypass protein and cause to improving

microbial protein synthesis. Amount of this excretion is dependent to level of consumed tannin. The reduction of protein degradation in the rumen may occur due to the formation of tannin-protein complexes in the rumen pH and inhibition of the growth and activities of proteolytic bacterial populations. Our findings in beside of WT drenches positive effect on reduce faecal protein excretion in nematode infected sheep. Additionally, considering the short time of study (up to 48 hours), it can be concluded that the mechanisms of protein supply related to inhibitory effects of tannins against the microbial degradation of diet protein in forestomachs and also too short time for the animals to have elevated immunity response against the worms, were not at work in present study and the only probable mechanism is the direct toxicity of WT for naturally acquired gastrointestinal nematodes (GIN). Similar findings have been reported previously after feeding tanniferous plants experimentally infected animals (Patra and Saxena, 2010). Maximum protein-tannin connections performed within weight tannins 500 to 2000 Dalton. If level of consumed tannin was high this complexes weren't dissociated in small intestine alkali pH and caused to increase protein excretion. The greater faecal N excretion has shown in many studies. Also, decreasing in protein degradation can be observation in inhibiting proteolysis enzymes (Coop and Kyriazakis, 2001; Patra and Saxena, 2010). Whereas level of consumed tannin was low it could increase bypass protein which this complex dissociated in small intestine and proteins were depredated by proteolysis enzymes and absorbed.

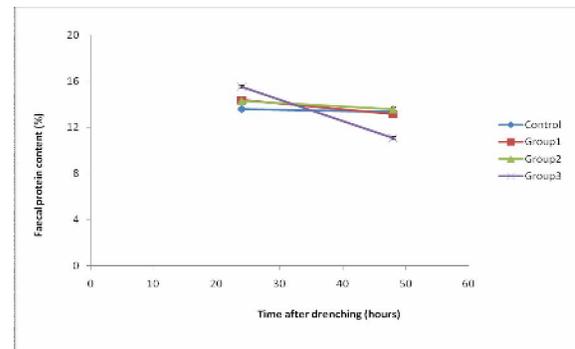


Figure 1. Effect of Wattle tannin drenches to all treatments animals during the study.

Also, Urea cycle performance may give rise in the rumen (Patra and Saxena, 2010). Gastrointestinal blood sucker parasites lead increase in protein excretion by lesions in small intestine, decreasing serum albumin and overall total serum protein content (Athanasidou et al, 2001). In these animals

consumed tannins have 2 benefits. Condensed tannins can directly inhibit phosphorilation oxidative; link to glycoprotein and ions in membrane structures of nematodes. Previous researches showed Wattle and Quebracho tannins capable to decrease nematode infection in sheep (Sadaghian et al 2011). Low levels of WT are efficiency for grazer sheep (Max, 2010). Poncet and Rémond (2002) were demonstrated feeding animal with tanniniferous plants could increase non-Ammonia nitrogen flow to duodenum. Simultaneously, previous researchers were observed fed dairy cows with chestnut tannin for 8 week could reduce 50% in faecal excretion protein (Sliwiński et al, 2004). In this study drenching WT can decrease protein excretion in during a naturally acquired nematode infection after 48 hours. It seems CT reduced infection in these ewes and subsequently improved protein absorption. However, the inclusion of high levels of the Quebracho tannin in diets of cattle and feeding legume shrubs to sheep reduced feed intake and apparent protein digestibility (Patra and Saxena, 2010). Previous researchers have been shown feeding chestnut decrease protein excretion in dairy sheep and cows (Kriaa and Thewis, 1999; Sliwiński et al, 2004). Excreted protein has two sources: Endogenous and diet base protein. The ability of tannins to protect protein degradation in the rumen are well documented, but it's too difficult demonstrate kind of this protein (Patra and Saxena, 2010). Finally, our result was illustrated that drenches WT had reduced faecal protein excretion and improved nitrogen retention in Nematode infected sheep.

5. Conclusion

Tannins interact with proteins predominately via hydrogen and covalent bonds, forming tannin-protein complexes and thus preventing degradation of protein in the rumen. These tannin-protein complexes are dissociated in the abomasum, releasing protein and increase non-ammonia N flow in the intestine for absorption. Moderate concentrations (depending upon the type of tannins) of tannins in diets improve body weight and wool growth, milk yields and reproductive performance. While tannins may shift N metabolism from rumen to intestine, the effects of tannins on large intestinal protein metabolism and microbial populations are not known. Tannins can exert beneficial effects environmentally by shifting N excretion from urine to faeces and decreasing methane output. Not all types of tannins produce beneficial nutritional and environmental responses. There is evidence that the structure of tannins may influence physiological effects such as nitrogen metabolism, rumen microbial populations, intake and performance of animals, which has not been studied

well in ruminant nutrition (Patra and Saxena, 2010). Many studies are requiring to identifying clear advantage of tannins on the ecology of rumen.

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