INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 16, No. 6, p. 220-226, 2020

RESEARCH PAPER OPEN ACCESS

Treatment of madre de cacao (*Gliricidia sepium* Jacq.) forage and crude protein utilization in native goats (*Capra hircus* Linn.)

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Key words: Tannin forage, Crude protein utilization, Sun-drying, Alkali treatment, Digestibility.

http://dx.doi.org/10.12692/ijb/16.6.220-226 Article published on June 29, 2020

Abstract

High levels of plant secondary metabolites, including tannins, are often considered important factors limiting the use of tropical trees and shrubs for small ruminants. Thus, an experiment was conducted to determine the effectivity of potential methods of ameliorating high-tannin forage (*Gliricidia sepium* Jacq. Steud.) by sun-drying and calcium hydroxide treatment on the intake, and digestibility of dry matter (DM) and crude protein in native goats. An *in vivo* digestibility trial for twenty-two (22) days was conducted. Twelve (12) Philippine native goats were randomly distributed to the three treatment diets laid out in a randomized complete block design (RCBD) with an age-sex combination as the basis for blocking. Block 1 consisted of young male goats, block 2 young, female goats, block 3, older male goats, and block 4, older female goats. The dietary treatments were: T0 (fresh form), T1 (sun-dried), and T2 (calcium hydroxide-treated) *Gliricidia sepium* forage. Sun-drying (T1) significantly increased dry matter intake and digestibility and crude protein intake and digestibility of the forage. Calcium-hydroxide treatment (T2) did not have any significant effects in improving the nutrients intake and digestibility of the fresh form of the forage. Sun-drying inactivates the detrimental effects of tannin making the protein available for animal use and thereby improving the nutritive value of the feeds.

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Introduction

Shrubs and legumes are important sources of feeds for ruminants as most of them contain high amounts of protein and produce great quantities of biomass and are potentially promising to overcome nutrient deficiencies. However, they also have some anti-nutritional factors such as tannins and other secondary compounds (Wina *et al*., 2005) as cited by Buenoa, *et al.* (2008). Tannins are widely prevalent in plants, both leguminous and non-leguminous species, and most of the species of forage and browse legumes used as ruminant feed contain tannins (Mueller-Harvey, 2006). Makkar (1993) listed the tannin polyphenolic contents of 62 species of trees and shrubs from India and this included important fodder species in the Philippines such as "madre de cacao "(*Gliricidia sepium Jacq Steud*).

Tannins are naturally occurring plant polyphenolic compounds of high molecular weight containing sufficient phenolic hydroxyl groups that permit the formation of stable cross-links with proteins. In general, shrub and tree foliages are likely to be higher in tannins than pasture plants, and leguminous forages from the tropics are generally higher in tannin than those from the temperate countries (Makkar and Becker, 1998). Tannins are polyphenolic compounds that occur widely in plants and have the ability to bind proteins and other nutrients. Some tannins can also produce toxic and anti-nutritional effects in monogastric and ruminant animals and cause reduced intake, lower nutrient digestibility, and protein availability. Although shrubs and legumes are protein sources, their digestibility is restricted by relatively high levels of endogenous tannins.

Because high levels of condensed tannins are detrimental to livestock production, there is a need for developing ways of neutralizing the anti-nutritional effects of tannins. Conversely, finding ways to utilize the potentially beneficial effects of condensed tannins that bind proteins and protect it from rumen degradation, thus increasing the availability of amino acids for direct absorption in the intestines is also of utmost importance. Approaches towards amelioration of excessive tannins in forages by several techniques have been used in legumes and these include sun-drying and soaking in alkali such as calcium hydroxide (Bhat *et al*., 2013). A more thorough understanding of these factors may then lead to the design of specific reagents and processes that may inactivate or reduce the inhibitory effects of tannins on dietary protein utilization without affecting its capacity to protect proteins from rumen degradation to provide an adequate supply of amino acids in the intestines. Thus this study was conducted to determine the affectivity of potential methods of ameliorating high-tannin forage (*Gliricidia sepium Jacq. Steud.*) by sun-drying and calcium hydroxide treatment on the intake, and digestibility of dry matter (DM) and crude protein in native goats.

Materials and methods

Preparation of experimental area and test animals

The study was conducted on October 2, to 23, 2018 at Cebu technological University, Barili Campus, Barili, Cebu. Twelve (12) Philippine native goats were randomly distributed to the three treatment diets laid out in a randomized complete block design (RCBD) with an age-sex combination as the basis for blocking. Block 1 consisted of young male goats, block 2 young, female goats, block 3, older male goats, and block 4, older female goats. There were three (3) goats allotted per treatment. The initial average body weights (BW) of the goats were taken before the experiment started. The goats were confined in open-top metabolism cages (Bestil and Espina, 1992). This cages were designed in a way that allows measurement of intake and separation of feces from the urine for digestibility measurements, following the standard space requirement for grower goats which is 1 meter square per animal. The animals were dewormed with Albendazole ^{R} (oral preparation) at a dosage of 1 ml/33 kg, one week before the experiment started.

Treatment of the forage

The experiment utilized *Gliricidia sepium* leaves which are high in tannin content as a supplement to an all-cogon (*Imperata cylindrica*) basal diet. For treatments requiring sun-drying of high-tannin

forage, the fresh forage was spread in a plain GI sheet under the sun until a dry matter of 86% was attained through measurement of the dry matter by oven drying (Fig. 1).

The calcium hydroxide treatment involved immersion of the fresh *Gliricidia sepium* leaves in a 4% calcium hydroxide solution before feeding to the goats. The 4 % calcium hydroxide solution was prepared by adding ten (10) grams of calcium oxide to forty (40) ml of distilled water. One thousand (1000) milliliter of distilled water was added to the solution. The solution was shaken thoroughly and allowed to stand and the supernatant was decanted. Another one thousand (1000) ml of distilled water was added to the residue and allowed to stand for an hour with occasional shaking. The supernatant was collected by draining.

Feeding the experimental animals

The fresh cogon (*Imperata cylindrica*) forage was chopped before feeding and was offered at 8:00 a.m. and 4:00 p.m. The dietary treatments were: To (fresh form), T1 (sun-dried), and T2 (calcium hydroxide-treated) *Gliricidia sepium* forage. There were three (3) experimental goats per treatment.

The supplement (treated *Gliricidia sepium*) was given at 1% body weight, dry matter (DM) basis twice a day (8:00 a.m. and 4:00 p.m) by mixing it with the basal all-cogon diet at 2%, body weight. Drinking water was made available at all times.

Digestibility trial

The digestibility trial was conducted according to the procedures of Bestil (1992). The first phase is the adjustment period which lasted for 15 days where the initial weights of experimental animals and the *ad libitum* intake of the basal diet were determined. The daily feed intake was carefully recorded during this period. The collection period started at 16 to 22 days where voluntary feed intake was measured, and samples of feed offered and refused were collected. Fecal outputs were weighed and representative samples were collected for dry matter and crude protein analysis.

Chemical/Laboratory analyses

Feed and fecal samples were analyzed for its dry matter (DM) and crude protein (CP) contents according to the methods of AOAC (1990) at the Department of Agriculture, RFO-7 Regional Feed Analysis Laboratory in Cebu City. The tannin content of the forages tested was analyzed at the Lipa Quality Control Center (LQCC) in Lipa, Batangas.

The following data were gathered as expressed by the following equations. The dry matter intake (DMI) was determined by Eq.1,

$$DMI = (FG \times DM_{FG})(FR \times DM_{FR}) \quad (1)$$

where *FG* is the amount of feed given, DM_{FG} is the dry matter (in percent) of feed given, FR is the amount of feed refused, and DM_{FR} is the dry matter (in percent) of feed refused.

The dry matter digestibility (DMD,%) was determined using Eq. 2,

$$DMD = \left(\frac{DMI - DME}{DMI}\right) \times 100 \quad (2)$$

$$DME = FO \times DMF \quad (3)$$

where *FO* is the fecal output and *DMF* is the dry matter of feces. Likewise, crude protein intake was calculated using Eq. 4,

$$CPI = VFI \times \%CP \quad (4)$$

where *VFI* is the voluntary feed intake, and *CP* is the crude protein content (in percent) of feed. Furthermore, crude protein digestibility (CPD) was determined using Eq. 5,

$$CPD = \left(\frac{CPI - CPE}{CPI}\right) \times 100 \quad (5)$$

$$CPE = FO \times CPF \quad (6)$$

where *CPE* is the crude protein excreted, *FO* is the fecal output and *CPF* is the crude protein content (dry basis) of feces.

Analysis of data

The data on intake and digestibility were subjected to two-way analysis of variance (ANOVA) for a randomized complete block design (RCBD), and comparison of treatment means was analyzed using Honestly Significant Difference (HSD) test using the Statistical Package for Social Sciences (SPSS) version 20.

Results and discussion

Dry matter intake and digestibility

As shown in Table 2, there are significant differences between the three treatments, with sun-dried forage (T1) with the highest value. Calcium-treated forage (T2) is not significantly different from fresh forage (TO).

Table 1. Tannin content of the different forages tested.

Forage sample	Common names	Tannin content (%)
Gliricidia sepium (Jacq.) Steud	Madre de cacao	0.97
Artocarpus heterophyllus Lam.	Jackfruit	0.81
Arachis pintoi Krapov.	Pasture peanut	0.96
Leucaena leucocephala Lam.	Ipil-ipil	1.14
Centrosema pubescens Benth.	Centro	0.47

The low DMI in goats fed fresh *Gliciridia sepium* (T0) suggests that tannin may have caused a reduction in voluntary intake due to the pre-ingestive effect of astringency (Salem *et al.*, 2005). According to Wickens (2012) tannins, form a diverse group of astringent, water-soluble phenolics that can bind the protein to form soluble or insoluble complexes. This astringency adversely affects palatability with above 5% tannin dry weight levels leading to food rejection. Studies of Makkar and Singh (1991) showed that an increase in the moisture content of oak leaves followed by heat treatment decreased tannin levels, and Salem *et al.* (2005) also reported that sun-drying was effective in reducing the levels of condensed tannins (CT) in acacia foliage. The high DM intake of goats fed sun-dried forage can be due to the potential reduction in tannin content, but the higher DM content of the sun-dried forage as compared to its fresh form may also have some contribution.

Table 2. Dry matter intake and crude protein intake and digestibility of high- tannin *Gliricidia sepium* leaves fed to goats in fresh, sun-dried, and calcium hydroxide–treated forms.

Treatment	Dry matter Intake (% BW)	Dry matter digestibility (%)	Crude protein Intake (grams/day)	Crude protein digestibility (%)
(Fresh forage)	2.03^{b}	55.83^{b}	13.05^{b}	74.57^{b}
(Sun-dried forage)	2.75^{a}	72.60^{a}	16.24^{a}	83.96^{a}
($Ca(OH)_2$-treated forage)	2.18^{b}	57.01^{b}	11.07^{b}	70.82^{b}
p-value	*0.03*	*0.04*	*0.04*	*0.03*

Means within the same column having different letter superscripts are significantly different.

Dry matter digestibility is the proportion of the dry matter in the feed that is digested by the animal, and results presented in Table 2 showed that the sun-dried form (T1) of the forage had significantly higher values than the T0 and T2 groups. Condensed tannins will bind with feed proteins, the microflora themselves or microbial enzymes, and CT–protein complexes are poorly degraded under anaerobic conditions. However, when the high-tannin, frozen while still fresh forage was fed in hay (dried) form, intake and digestibility improved which indicated that drying has benefits than feeding in fresh form,

particularly with animals that do not like to graze such forage. Although Ca $(OH)_2$–treated (T2) and fresh (T0) forms showed no significant differences in digestibility, T0 group had lower values than T2, indicating some effects of the alkali treatment. Condensed tannins reduce microbial numbers and cause a substantial inhibition of proteolytic activity for many bacteria (Waghorn, 2008). Although not shown in the results of this study, calcium hydroxide treatment (T2) of the forage can be effective in ameliorating the effects of high-tannin forage on the dry matter intake and dry matter digestibility. This is manifested by the study of Alam *et al.* (2005), as cited by Bhat *et al.* (2013), that calcium hydroxide treatment reduced the concentrations of extractable tannin by as much as 92 % in *Clidemia hirta* leaves. Tannins are water-soluble polymers which form complexes essentially with proteins that can be broken under conditions of high acidity or high alkalinity.

Fig. 1. Fresh (T0), sundried *Gliricidia sepium* (T1), CaOH Treated (T3).

Crude protein intake and digestibility

Dietary crude protein values give a good indication of whether or not a particular feed will satisfy the protein needs of the animal, and the crude protein intake and digestibility of high-tannin *Gliricidia sepium* forage subjected to sun-drying and Ca $(OH)_2$ treatment is shown in Table 2. Results showed a similar trend as that of the dry matter (DM) intake and digestibility, with sun-drying (T1) having the highest value, significantly different from fresh (T0) and Ca $(OH)_2$ treated (T2).

The low crude protein intake of calcium hydroxide – treated forage (T2) as compared to sun-dried forage agrees with Bhat *et al.* (2013) which stated that treatment with various chemicals under alkaline conditions led to a decrease in tannin content and activity up to 90 % in agro-industrial by-products and tree leaves, but a major disadvantage is the loss of soluble nutrients which affects voluntary intake, for which methods have to be devised to minimize such loss during chemical treatment.

The low digestibility of crude protein in goats fed fresh *Gliricidia sepium* (T0) is in agreement with Yousuf (2005), low digestibility of crude protein in goats on *Gliricidia* leaf meal treatment could be related to the presence of a relatively higher concentration of tannins in *Gliricidia* leaves. The consequences are reduced availability of crude protein to animals, increased fecal nitrogen excretion and lowered fermentation rate.

This might be connected to a theory that tannins form complexes with natural polymers such as proteins and carbohydrates (McSweeney *et al.*, 2001; Makkar, 2003; Mueller-Harvey, 2006; Jayanegara *et al.*, 2009) as cited by Bhat (2013) and, therefore, may reduce their digestibility in the digestive tract of ruminants. The binding property of tannins resulted from a large number of free phenolic groups that form strong hydrogen bonds at multiple sites with proteins

(Silanikove *et al.*, 2001). Tannins may also form complexes with proteins through hydrophobic binding between the aromatic ring structure of tannins and hydrophobic regions of the proteins (Smith *et al.*, 2005). Sun drying of the forages (To) inactivates the detrimental effects of tannin making the protein available for animal use and thereby improving the nutritive value of the feeds.

Calcium hydroxide treatment did not improve the crude protein digestibility is in consonance with a study by Dias,*et al.* (2011) where they did not found the effect of the treatment with sugar cane with 1% calcium oxide on the digestibility of nutrients. This contradicts the principle of alkaline hydrolysis, the phenomenon known as swelling of the alkali cellulose, which causes expansion and rupture of the cellulose molecules, which would improve the utilization of the feed by the rumen microorganisms.

Acknowledgment

This study was funded by the DOST (Department of Science and Technology) ASTHRDP-NSC (Accelerated Science and Technology Human Resource Development Program-National Science Consortium) Scholarship Funds.

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