JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Octanol–Water Partition Coefficients for Predicting the Effects of Tannins in Ruminant Nutrition

IRENE MUELLER-HARVEY,*,† VICTOR MLAMBO,†,‡ JOE L. N. SIKOSANA,§ TIM SMITH,† EMYR OWEN,† AND RON H. BROWN†

Department of Agriculture, University of Reading, 1 Earley Gate, P.O. Box 236, Reading RG6 6AT, United Kingdom, and Department of Agricultural Research and Extension, Matopos Research Station, P. Bag K5137, Bulawayo, Zimbabwe

Tannins can cause beneficial or harmful nutritional effects, but their great diversity has until now prevented a rational distinction between tannin structures and their nutritional responses. An attempt has been made to study this problem by examining the octanol−water solubilities of tannins. A relatively simple HPLC method has been developed for screening mixtures of plant tannins for their octanol−water partition coefficients (K_{ow} coefficients). Tannins were isolated from the fruits and leaves of different *Acacia*, *Calliandra*, *Dichrostachys*, and *Piliostigma* species, which are known to produce beneficial or harmful effects. The K_{ow} coefficients of these tannins ranged from 0.061 to 13.9, average coefficients of variation were 9.2% and recoveries were 107%. *Acacia nilotica* fruits and leaves had the highest K_{ow} coefficients, that is, 2.0 and 13.9, respectively. These *A. nilotica* products also have high concentrations of tannins. The combined effects of high octanol solubilities and high tannin concentrations may explain their negative effects on animal nutrition and health. It is known that compounds with high octanol solubilities are more easily absorbed into tissues, and it is, therefore, proposed that such compounds are more likely to cause toxicity problems especially if consumed in large quantities. According to the literature, tannins in human foods tend to have low K_{ow} coefficients, and this was confirmed for the tannins in *Piliostigma thonningii* fruits. Therefore, unconventional feeds or browse products should be screened not only for their tannin concentrations but also for low octanol−water partition coefficients in order to identify nutritionally safe feeds and to avoid potentially toxic feeds.

KEYWORDS: Condensed tannins; flavanol gallates; hydrolyzable tannins; K_{ow} coefficients; HPLC; tree fruits; tree leaves; animal nutrition; toxicity

INTRODUCTION

Dietary tannins, that is, condensed and hydrolyzable tannins, can affect animal nutrition and health in several different ways. Beneficial effects range from better absorption of feed proteins (*1*−*3*) to anthelmintic effects against intestinal nematodes (*4*), prevention of bloat, and treatment against diarrhea (*5*, *6*). Harmful effects range from intake reduction and impaired protein utilization to toxic effects and even animal deaths (*7*, *8*). More recently, research has been focusing on dietary tannins to reduce nitrogen pollution from intensive animal feeding systems via lower urinary nitrogen excretions from ruminants and ammonia emissions from silages and slurries (*2*, *9*, *10*). In order to exploit these natural plant products for their full potential, insight is needed into their structure−activity relationships so that the effects of plants that have different types of tannins can be predicted. Such understanding would also facilitate the use of alternative feed resources and the breeding of new plant varieties with improved nutritional and veterinary properties.

Min et al. (*1*) proposed an upper limit of 5 g condensed tannins/100 g dry matter in ruminant feeds as a safe level. However, while this limit may be appropriate for *Lotus* species, it does not appear to hold for other tanniniferous feeds, such as sainfoin (*Onobrychis viciifolia*) or browse products (*8*). At present, there are no laboratory-based methods that can differentiate between plants containing beneficial or potentially harmful tannins.

It has been recognized for some time now that the affinity and binding strengths between different tannins and proteins can vary as much as 10 000-fold (*11*−*13*). However, this fact has received little attention until now in the context of animal nutrition and health. Two types of bonds are involved in tannin−protein interactions: hydrogen bonds and hydrophobic interactions. There has been considerable discussion as to their relative

* To whom correspondence should be addressed. Phone: +44-118 378 6619. Fax: +44-118 935 2421. E-mail: i.mueller-harvey@reading.ac.uk.
† University of Reading.
‡ Present address: Faculty of Agriculture, University of Swaziland, P.O. Luvengo, Swaziland.
§ Matopos Research Station.

10.1021/jf070308a CCC: $37.00
Published on Web 06/14/2007

importance (*14*). Siebert et al. (*15*) suggested that hydrophobic bonding was more important than hydrogen bonding, and Haslam (*16*) hypothesized that nonpolar tannins formed stronger links with proteins than polar tannins. However, Hagerman et al. (*14*) analyzed the relative importance of hydrogen bonds versus hydrophobic interactions in different tannin−protein complexes and concluded instead that tannins with greater polarity (e.g., epicatechin$_{16}$ (4 → 8) catechin and heptagalloyl glucose) bound via strong H bonds but those with weaker polarity (e.g., pentagalloyl glucose and epicatechin gallate) bound via weaker hydrophobic bonds.

The polarity of a compound dictates the extent to which it dissolves in organic solvents rather than water. This property has been used to measure its partition coefficient (K_{ow}) in octanol and water (*16*). K_{ow} coefficients are calculated from the ratio of a compound's octanol-to-water phase concentrations and are therefore without units. Published K_{ow} coefficients of just a few tannins analyzed so far cover a surprisingly wide range, that is, from 0.0002 to 160 (*14*, *16*, *17*). It is also of note that these K_{ow} coefficients do not distinguish between condensed or hydrolyzable tannins (*8*).

Many studies report good correlations between the K_{ow} coefficients of compounds and their in vivo effects as compounds with higher K_{ow} coefficients, that is, greater octanol solubility, tend to be absorbed more easily into tissues (*18*). The K_{ow} coefficients of tannins also correlated positively with their cellulose affinity and the extent to which they conferred hydrothermal stability to collagen (*19*). Taken together, this suggests that K_{ow} coefficients may be relevant not only for understanding the interactions between tannins and other biological polymers but possibly also for predicting the in vivo nutritional effects of tannins.

Plants tend to produce complex mixtures of closely related tannin structures (*20*, *21*). Techniques are needed which are suitable for measuring and comparing the properties of such mixtures of biologically active tannins. The objectives of the present study therefore were (i) to explore the variation in K_{ow} coefficients of naturally occurring tannin mixtures and (ii) to relate these K_{ow} coefficients to published information on the nutritional value of these tannin-containing feeds.

MATERIALS AND METHODS

Chemicals. (−)-Epicatechin (>96%) was purchased from Sigma, Poole, U.K. Octanol (>98%), methanol, and acetone (HPLC grades) were purchased from Fisher Scientific Ltd, Loughborough, U.K. Pelargonidin and cyanidin chlorides were purchased from Apin Chemicals Ltd, Abingdon, U.K., and delphinidin chloride was purchased from Extrasynthèse, Genay, France.

Plant Samples. The following samples were harvested by hand in June 2001 in Mbembeswana communal areas, Bulawayo, Zimbabwe (latitude−longitude: 20° 23′ S and 28° 28′E, at an altitude of 1340 m): leaves from *Acacia nilotica* and dry, mature fruits from *Acacia erioloba A. erubescens*, *A. nilotica*, *Dichrostachys cinerea*, and *Piliostigma thonningii* trees. *Calliandra calothyrsus* plants were grown under controlled conditions in a greenhouse at the Plant Environment Laboratory, Department of Agriculture, University of Reading (*22*). Samples were ground to <1 mm in a Retsch cutting mill (SM100; Haan, Germany) and stored at room temperature.

Extraction of Plant Samples. Air-dried and finely ground plant material (<1 mm; 500 mg) was extracted with acetone/water (7:3, v/v; 50 mL) at 6 °C for 10 min using ultrasound. The solution was whirly mixed with an MS2 Minishaker (IKA Werke GmbH & Co. KG, Staufen, Germany) and then extracted with ultrasound for another 10 min. The mixture was passed through a cotton wool plug, and the extract was kept at 6 °C until HPLC analysis.

Isolation of Tannins. Plant samples were extracted with acetone/water (7:3, v/v), and tannins were isolated by chromatography on Sephadex LH20 as described previously (*22*). Tannins (50 mg) were dissolved in acetone/water (50 mL, 7:3, v/v) and kept at 6 °C until HPLC analysis.

Octanol−Water Partitioning. Aliquots from the above plant extracts or tannin solutions in 70% acetone/water (10 mL) were transferred to a graduated test tube and placed into a heating block at ≤38 °C. Acetone was removed by directing a stream of nitrogen over the solution until only water (3 mL) remained. Then, *n*-octanol (3 mL) was added, and the mixture stirred at ≤38 °C for 40 min by placing a vigorous stream of nitrogen at the bottom of the test tube. Samples were centrifuged at 3000 rpm in a Centaur 2 centrifuge (MSE; London, U.K.) to separate the layers; the water phase was made up to 3 mL if necessary. Samples were whirly mixed for 3 min and centrifuged for 5 min to separate the upper and lower phases. Aliquots (1.5 mL) were removed from each phase, and acetone (1 mL) was added to each in order to ensure that tannins remained in solution.

HPLC Analysis for Determining Octanol−Water Partition Coefficients. Aliquots (20 μL) from the original acetone/water extract, the lower phase (i.e., water/acetone, 1.5:1, v/v) and the upper phase (i.e., octanol−acetone, 1.5:1, v/v) were injected into the HPLC system (Gilson; Anachem, Luton, U.K.) attached to a UVD340S diodearray detector and using Chromeleon vs 6.10 software (Dionex, Macclesfield, U.K.) and column (Phenomenex Gemini C18, 5 μm, 110 Å, 150 mm × 4.6 mm). Water/acetic acid (99:1, v/v; solvent A) and methanol (solvent B) were used for gradient elution at 1.5 mL/min. The linear gradient profile was as follows: 5% B (0−5 min); 5 to 50% B (5−40 min); 50 to 100% B (40−45 min); 100 to 5% B (45−50 min). Absorption spectra were recorded between 200 and 595 nm. The total peak area was measured at 280 nm between 10 and 42 min using a flat baseline integration method (*17*, *23*) as illustrated in **Figure 1**.

Calculations. K_{ow} coefficients were calculated according to:

$$K_{ow} = \frac{\text{area of octanol phase}}{\text{area of water phase}}$$

Recoveries (%) were calculated according to:

$$\text{Recovery} = \frac{(\text{octanol area} + \text{water area})}{2} \times \frac{100}{\text{area of acetone−water extract}}$$

Characterization of Condensed Tannins. *Acacia erioloba*, *A. erubescens*, *Dichrostachys cinerea*, and *Piliostigma thonningii* samples were extracted with acetone/water and treated with HCl/butanol as described previously (*22*). The resulting anthocyanidins were identified in comparison with authentic delphinidin, cyanidin, or pelargonidin chlorides using the above HPLC system linked to a Waters μBondapak C_{18} (3.9 × 300 mm) column. Water/acetic acid (96:4, v/v; solvent A) and acetonitrile/ethyl acetate (7:3, v/v; solvent B) were used for gradient elution at 1.07 mL/min. The linear gradient profile was as follows: 5 to 30% B (0−10 min), 30 to 40% B (10−15 min), 40 to 100% B (15 to 20 min), 100 to 5% B (20−25 min), 5% B (25−28 min).

RESULTS AND DISCUSSION

Octanol−Water Partitioning of (−)-Epicatechin and Tannins. Preliminary tests showed that polyphenolic compounds could be kept in solution at 6 °C by adding acetone to the water or octanol phases after phase separation. This was necessary to allow sufficient time for HPLC analysis of replicates. Rothwell et al. (*18*) pointed out that measurements of K_{ow} coefficients are not straightforward as insolubility of compounds in either phase can pose a major problem. The variability of published data demonstrates that it is important to check recoveries by determining the concentrations in both the octanol and the water phase. It is, therefore, necessary to compare the results either to the expected concentration or to the original extract in order

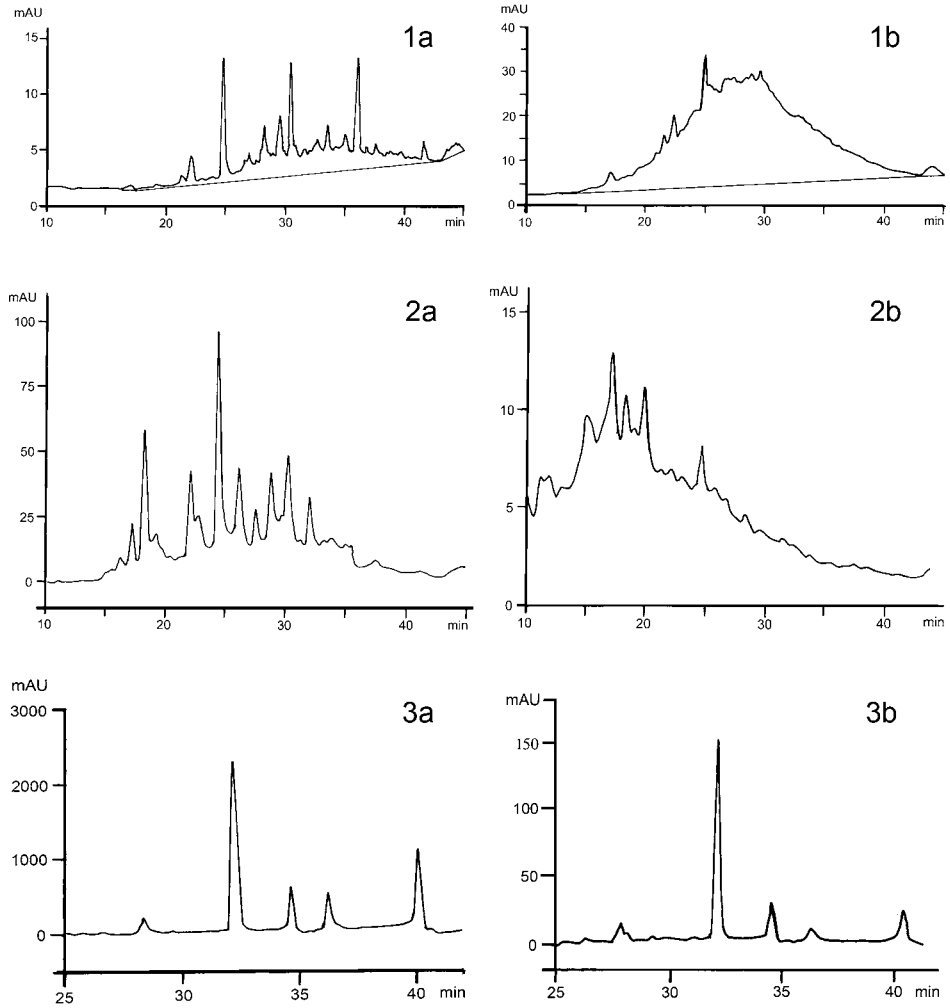

Figure 1. HPLC chromatograms of tannins isolated from (**1**) *Dichrostachys cinerea* fruits, (**2**) *Acacia nilotica* fruits, and (**3**) *Acacia nilotica* leaves after partitioning between (**a**) octanol and (**b**) water.

to detect any insolubility problems. Moreover, close control of the temperature is required to ensure reproducible results. Many workers prefer to use calculated K_{ow} coefficients because of these problems; however, as can be seen from **Table 1**, calculated K_{ow} coefficients of even simple flavan-3-ols, their galloyl esters, and tannins (*24*, *25*) can differ by orders of magnitude from experimentally determined K_{ow} coefficients.

Several different methods have been described for measuring K_{ow} coefficients, and this probably also explains some of the variation in reported values (*18*). K_{ow} coefficients are calculated from the ratio of a compound's octanol-to-water phase concentrations (*26*). The method described here uses a flat baseline for determining the total HPLC peak area (at 280 nm) of all compounds in the mixtures in order to calculate this octanol−water ratio and for determining recoveries because authentic standards are not available for most tannins. Other researchers have also used a flat baseline for integration and for determining K_{ow} coefficients of tannin mixtures (*17*, *23*) because of overlapping peaks in HPLC chromatograms.

The proposed procedure was tested with (−)-epicatechin, which yielded a K_{ow} coefficient of 1.50 and a recovery of 107.5%. This K_{ow} coefficient was comparable to data in the literature, that is, 1.34 to 2.43 (*14*, *26*, *27*; **Table 1**).

Figure 1 presents some examples of how different tannin mixtures partition between the octanol and water phases. The resulting K_{ow} coefficients of the different tannin mixtures, which had been isolated by Sephadex LH20 chromatography, are presented in **Table 2**. Tannins from the fodder trees and browse products examined in the present study showed >200-fold differences: K_{ow} coefficients ranged from 0.06 to 13.9. This procedure resulted in mean coefficients of variation of 9.2% and in recoveries that averaged 106.8%. We found that it was necessary to mix the two phases vigorously with a stream of nitrogen and to add acetone to both solutions immediately after the phases had been separated in order to obtain acceptable standard deviations and recoveries.

Octanol−Water Partitioning of Plant Extracts. Table 3 gives the K_{ow} coefficients of all compounds in the 70% acetone extracts; these same extracts had been used for isolating the corresponding tannins (**Table 2**). The K_{ow} coefficients of these compounds showed a similar range (0.134 to 25.9) as the K_{ow} coefficients of the isolated tannin mixtures (0.061 to 13.9). UV−vis spectra indicated that the acetone/water plant extracts contained some flavonoids and other polyphenols in addition to tannins, and these extracts were treated in the same way as the tannin solutions (**Figure 2**). Mean coefficients of variation were 11.4% and recoveries ranged from 87.6 to 126.4% (average = 98.8%), which demonstrated that insolubility did not pose a problem in the water or octanol phases. A highly significant relationship ($P < 0.001$; df = 7) exists between the K_{ow} coefficients of compounds in the plant extracts and those in

Table 1. Experimental and Calculated K_{ow} Coefficients for Tannins, Flavanols, Flavanol Gallates, and Flavonols

tannins	measured K_{ow} coefficients	calculated K_{ow} coefficients (MDL QSAR) (*24*)	calculated K_{ow} coefficients (KowWin) (*25*)	typical examples of occurrence (*56*)	refs
Hydrolyzable Tannins (Gallotannins and Ellagitannins):					
sanguiin H-6	0.0002	0.51		raspberry leaves and fruits (*Rubus idaeus*); *Sanguisorba officinalis*	(*17*)
punicalagin	0.002	16.2	0.001096	pomegranate apple (*Punica granatum* L.); *Terminalia cattapa*	(*17*)
terflavin	0.007			*Terminalia cattapa* leaves	(*17*)
pedunculagin	0.008			raspberry fruits (*Rubus idaeus*); *Sanguisorba officinalis*	(*17*)
1-*O*-galloyl glucose	0.013				(*17*)
vescalagin/castalagin	0.017–0.1	26.9	0.158	*Castanea sativa* wood; oak galls (*Quercus robur*)	(*16*, *17*, *29*)
corilagin	0.064	128.8	0.000102	*Bischofia javanica*; myrobalan fruit (*Terminalia chebula*)	(*17*)
galloyl pedunculagin	0.086				(*17*)
bischofianin	0.22			*Bischofia javanica*	(*17*)
2,3-di-*O*-galloylglucose (hamamelitannin)	0.45	0.095	0.038	witch hazel leaves (*Hamamelis virginiana* L.*)*	(*17*)
1,6-di-*O*-galloyl glucose	0.47	0.063	0.091		(*17*)
1,2,6-tri-*O*-galloyl glucose	4.1				(*17*)
eugeniin	10			clove (*Syzygium aromaticum* (L.) MERR. & L. M. PERRY)	(*17*, *29*)
casuarinin	19	7.8	8.7		(*17*)
phillyraeoidin A	26				(*17*)
1,2,3,6-tetra-*O*-galloyl glucose	36			Turkish galls (*Quercus infectoria*)	(*17*, *29*)
penta-*O*-galloyl glucose	32–160			tannic acid	(*14*, *17*, *27*, *29*)
geraniin	0.23; >100	6.2	3.7	*Bischofia javanica;* cocoa leaves (*Erythroxylum coca var. coca*)	(*17*, *29*)
Flavanols or Proanthocyanidins:					
epicatechin$_{16}$ (4 → 8) catechin	0.00212			sorghum seeds (*Sorghum vulgare*)	(*14*)
procyanidin B4		1.66	1.88	raspberry, blackberry	
procyanidin B5, B6, B7		1.73	1.88		
procyanidin C1		0.22	2.18		
procyanidin B2	0.2	1.66	1.88	apple, quince, cherry, hawthorn	(*27*)
procyanidin B3	0.3	1.66	1.88	strawberry, hops, rose hips, willow	(*27*)
procyanidin A2		1.94	2.67		
epigallocatechin	0.281 -- 0.5	0.09	1.12	tea leaves (*Camellia sinensis* (L.) KUNTZE)	(*14*, *27*)
epicatechin	1.3–2.43	4.57	15.1	tea leaves (*Camellia sinensis* (L.) KUNTZE)	(*14*, *26*, *27*)
epigallocatechin gallate	12.1	0.66	2.56	tea leaves (*Camellia sinensis* (L.) KUNTZE)	(*14*)
epicatechin gallate	48.0	1.08	2.62	tea leaves (*Camellia sinensis* (L.) KUNTZE)	(*14*)
Flavonoids:					
daidzein	324	1349	354.8	soybean seeds (*Glycine max* (L.) MERR.)	(*18*)
kaempferol	1288	436.5	30.2	*Brassica* spp., *Allium* spp.	(*18*)
quercetin	66.1	8.5	2.7	*Allium cepa bulb;* tea leaves *(Camellia sinensis*)	(*18*)
quercetin 3-*O*-β-D-glucoside	5.0			vegetables and fruits	(*55*)

the tannin mixtures (**Tables 2** and **3**) and is described by the following equation:

$$K_{ow}\text{ coefficients of extracts} = 1.864 \times K_{ow}\text{ coefficients of tannins} - 0.008 \qquad (R^2 = 0.999)$$

This may not be too surprising as the tannins were obtained from these acetone/water extracts and account for up to 23% of the dry matter in some of these browse products (**Table 3**). More importantly, however, this indicates that co-occurring compounds in the plant extracts either have greater octanol solubility than tannins, enhance the octanol-solubility of the tannins, or both. This finding contrasts with reports that a variety of compounds and some constituents in crude herbal drugs can increase the water-solubility of tannins, which is thought to promote the medicinal effects of tannins in these preparations (*17*, *28*−*30*).

***K*$_{ow}$ Coefficients for Predicting Nutritional Value and Toxicity Problems. Table 1** lists K_{ow} coefficients of pure tannin compounds from the literature and covers gallotannins, ellagitannins, procyanidins, and several flavan-3-ols. The K_{ow} coefficients of these tannins vary by six orders of magnitude and range from 0.0002 (Sanguiin H-6) to >100 (geraniin and pentagalloyl glucose) (*14*, *17*, *29*). K_{ow} coefficients of flavanols

Table 2. Octanol–Water Partition Coefficients (K_{ow} Coefficients) of Tannin Mixtures and Total Recoveries by HPLC Chromatography (S.D. in Brackets, $n = 3$)

tannin mixtures isolated from	main tannin types[a]	K_{ow} coefficients (S.D. in brackets)	recoveries (%)	references
Piliostigma thonningii (fruits)	PC	0.061 (0.0061)	111.6 (1.29)	present study
Dichrostachys cinerea (fruits)	PC > PD, PP	0.111 (0.0121)	108.7 (5.06)	present study
Calliandra calothyrsus San Ramón (leaves)	PD > PC	0.181 (0.0344)	116.1 (13.49)	(*22*)
Acacia erubescens (fruits)	PD	0.209 (0.0267)	100.2 (10.17)	present study
Calliandra calothyrsus Patulul (leaves)	PC > PD	0.372 (0.0101)	99.9 (3.3)	(*22*)
Acacia erioloba (fruits)	PC	0.770 (0.0410)	100.8 (4.2)	present study
Acacia nilotica (fruits)	epigallocatechin gallates	2.00 (0.141)	117.5 (0.98)	(*57*)
Acacia nilotica (leaves)	catechin gallates	13.9 (0.77)	99.9 (3.1)	(*58*)
average			106.8 (7.6)	

[a] PC = procyanidins; PD = prodelphinidins; PP = propelargonidins.

Table 3. Octanol–water Partition Coefficients (K_{ow}-coefficients) of Compounds Extracted with Acetone/water from Browse Products and Total Recoveries by HPLC Chromatography (S.D. in Brackets, $n = 3$)

browse species and part	K_{ow} coefficient	recovery (%)
Piliostigma thonningii (fruits)	0.134 (0.0238)	87.6
Dichrostachys cinerea (fruits)	0.165 (0.0284)	88.5
Calliandra calothyrsus San Ramón (leaves)	0.353 (0.0412)	97.5
C. calothyrsus Patulul (leaves)	0.423 (0.0139)	98.0
Acacia erubescens (fruits)	1.10 (0.206)	97.3
Acacia erioloba (fruits)	1.30 (0.184)	88.3
Acacia nilotica (fruits)	3.31 (0.041)	126.4
A. nilotica (leaves)	25.9 (3.82)	106.6
average		98.8

and flavanol gallates from green tea range from 0.28 to 48 (*14*), and K_{ow} coefficients of several dietary flavonols, flavones, isoflavones, and their glycosides range from 0.23 to 1660 (*18*). Most plant-based foods, which are consumed by humans, tend to contain low concentrations of tannins and flavonoid aglycones (*31*−*33*). Recent research (*34*) on U.S. diets revealed that proanthocyanidins account for most of the dietary flavonoids, which are valued for their antioxidant and health effects (*31*−*33*). However, animal feeds can contain much higher tannin concentrations (**Table 4**; *8*). **Table 4** summarizes the information on what is known about the nutritional value of several tree fruits and leaves in terms of intake, nitrogen retention, digestibility, or liveweight gain by ruminants (*35*−*41*).

A perusal of **Tables 1**−**4** leads us to hypothesize that animal feeds containing tannins with low K_{ow} coefficients are nutritionally safer than those with high K_{ow} coefficients. It would appear that low K_{ow} tannins, even if present in relatively large quantities, can produce positive nutritional effects in ruminants: better N-retention due to rumen escape protein and high intakes. Tannins from *Dichrostachys cinerea* and *Piliostigma thonningii* fruits and from *Calliandra calothyrsus* (San Ramón) leaves have relatively low K_{ow} coefficients (0.11, 0.06, and 0.18, respectively) and produce beneficial nutritional effects (*3*, *39*, *42*). Commercial chestnut wood extract represents another example of low K_{ow} tannins; it contains the ellagitannins, vescalagin, and castalagin (K_{ow} coefficient = 0.1) as the main tannin compounds (**Table 1**). Chestnut extract is an approved feed additive in Switzerland and improves the N supply to the duodenum by generating rumen escape protein (*42*, *43*). It is also of note that tannins present in human foods tend to have low K_{ow} coefficients; examples are raspberries, pomegranate, sorghum, apple, and *Piliostigma thonningii* fruits (**Table 1**; *40*).

However, high K_{ow} tannins, such as pentagalloyl glucose (K_{ow} > 32; **Table 1**), can be toxic to people and ruminants, especially if administered or consumed in large quantities. Barium tannic acid enemas have resulted in several deaths among people (*7*). Oak poisoning of ruminants is a recurring problem in the United States (*44*). Typical signs of tannin poisoning are gastrointestinal ulcerations, bleeding, and liver and kidney damage (*45*). Therefore, we suggest that *A. nilotica* fruits and leaves exert negative effects on ruminant nutrition and health because they contain large quantities of tannins with relatively high K_{ow} coefficients (K_{ow} = 2.0 and 13.9; **Table 2**). Both types of *A. nilotica* feeds resulted in low intakes by sheep and goats. Supplementing basal diets with *A. nilotica* fruits did not improve goat kid birth weights in contrast to the other browse fruits listed in **Table 3** (*35*−*37*, *46*). Terblance et al. (*47*) also reported that goats showed signs of toxicity and some died after consuming large quantities of *A. nilotica* fruits. Similarly, farmers in Zimbabwe reported that goats consuming excessive amounts of *A. nilotica* fruits produced tainted milk and showed signs of poisoning and abortions (information reported in informal meeting with farmers; J. Sikosana, personal communication). Furthermore, recent studies revealed that goat intakes and N-retention improved and kid mortalities decreased when the tannins in *A. nilotica* fruits were deactivated by soaking the fruits overnight in a wood-ash solution (*37*).

Iason and Murray (*48*) hypothesized that animals, when necessary, can reduce their intakes in order to allow time to metabolize potentially toxic compounds. Their experiments showed that urinary energy excretions increased and digestible energy intake decreased when 3,5-dihydroxytoluene and *p*-hydroxybenzene, which are two phenolic compounds that occur naturally in heather, were infused into the rumen of sheep at likely dietary concentrations. **Table 4** reveals that total dry matter intakes (fruits plus hay) were highest with *D. cinerea* (844 g/day) and lowest with *A. nilotica* supplementation (491 g/day) (*36*). Using the tannin yields from Sephadex LH20 chromatography indicates that goats ingested similar amounts of total tannins: 11.8 g tannins per day from *D. cinerea* and 10.0 g from *A. nilotica* fruits. While Foley et al. (*49*) suggested that techniques needed to be developed which were capable of

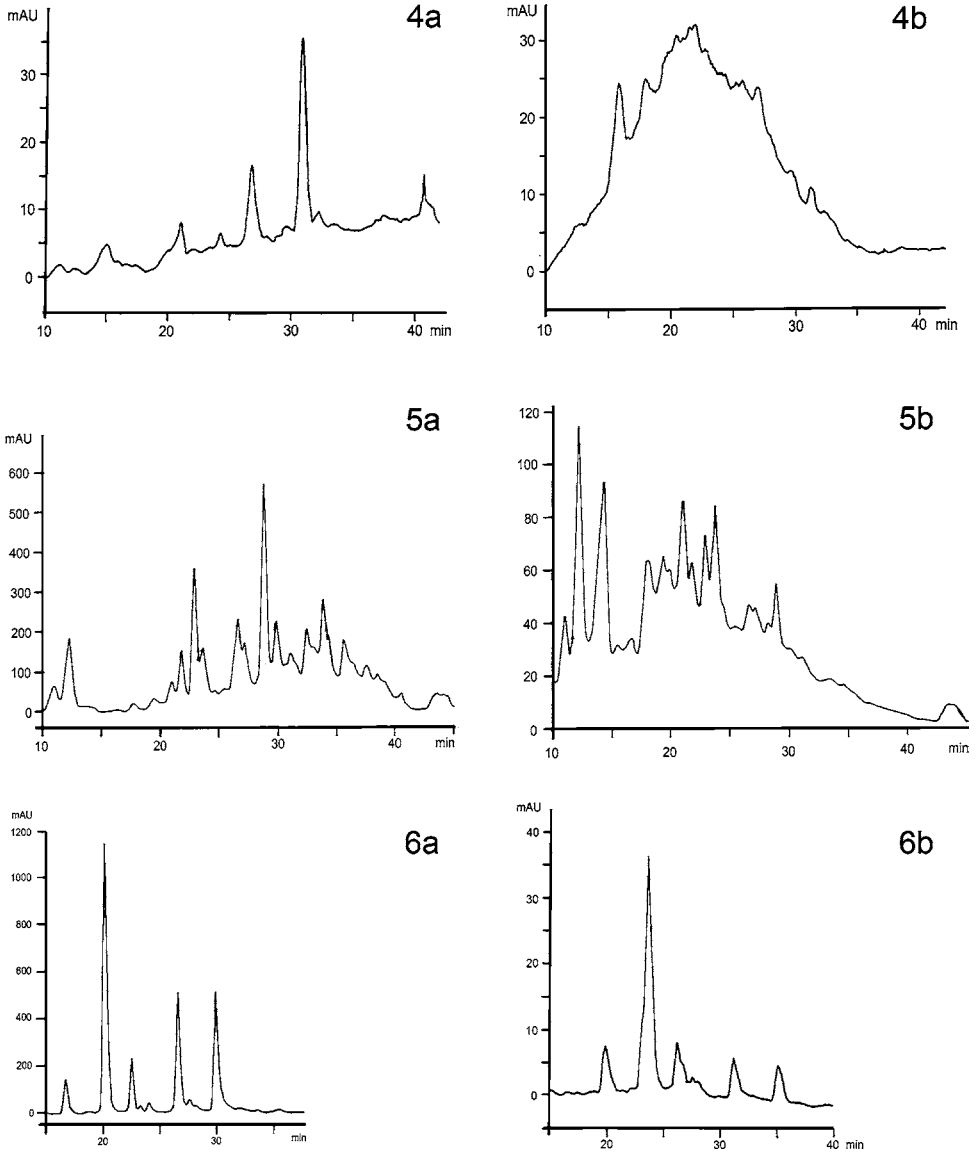

Figure 2. HPLC chromatograms of acetone–water extracts from (**4**) *Dichrostachys cinerea* fruits, (**5**) *Acacia nilotica* fruits, and (**6**) *Acacia nilotica* leaves after partitioning between (**a**) octanol and (**b**) water.

Table 4. Intakes and Nitrogen Retention in Goat Feeding Trials with Tannin-rich Tree Fruits as Protein Supplements

	D. cinerea	*P. thonningii*	*A. erubescens*	*A. erioloba*	*A. nilotica*	source
K_{ow} coefficients	0.111	0.061	0.209	0.770	2.00	Table 2
K_{ow} coefficients	0.165	0.134	1.10	1.30	3.31	Table 3
total DM intake (g DM/day)	844[a]		669[ab]	731[a]	491[b]	(*36*)
fruit intake (g DM/day)	182		138	183	44	(*36*)
tannins (g /100 g fruit)	6.47	3.28	0.42	3.16	22.8	present study
tannin intake (g/day)	11.8		0.580	5.78	10.0	
N-retention (g N/day)	3.0[a]		1.3[b]	2.1[ab]	–0.5[c]	(*35*)
comments	large number of fruits eaten by cattle and game	can be eaten by people; avidly eaten by game, cattle, monkeys		fruits eaten by cattle and game; promote milk production in cows; but cyanogenic glycosides can cause poisoning	low intakes; negative N-balance; deaths	(*40*, *41*, *59*, *60*)

[a-c] Means within rows with different superscripts differ significantly ($P < 0.05$).

measuring detoxification indices in ecological studies of herbivores, it would be worth investigating if the K_{ow} coefficients of tannins could be used instead as a laboratory-based screening tool to rank unconventional feed resources for potential intake and toxicity problems. This might involve developing a model that describes dose−response relationships (*50*), which would

need to link the total amounts of tannins in feeds with their K_{ow} coefficients in order to predict nutritional or toxic responses. Such a laboratory-based approach would have highlighted that *A. nilotica* products could be harmful, if supplied in large quantities, because of their high concentrations of tannins and high K_{ow} coefficients.

K_{ow} Values, Protein Precipitation Capacity, Rumen Escape Protein, and Bioavailability. Another technique that merits examination in relation to K_{ow} coefficients is the protein precipitation capacity (PPC) or "astringency" of tannins (*51*, *52*). Although it is not known if the PPC impacts on protein digestibility, there is some evidence which indicates that tannins with lower K_{ow} coefficients precipitate more protein than those with higher K_{ow} coefficients. Hagerman et al. (*14*) found that the epicatechin$_{16}$ (4 → 8) catechin tannin (K_{ow} = 0.002; MW 4930 Daltons) was more efficient at precipitating bovine serum albumin (BSA) than pentagalloyl glucose (K_{ow} = 129; MW 940 Daltons) on a molar or mass basis. Similarly, the *C. calothyrsus* (San Ramón) tannins (K_{ow} coefficient = 0.181) precipitated more protein on a mass basis than the *C. calothyrsus* (Patulul) tannins (K_{ow} coefficient = 0.372; **Table 2**; *22*, *39*). This corresponded with results from feeding trials, which found that the San Ramón provenance generated significantly more rumen escape protein than the Patulul provenance (*39*). PPC and K_{ow} coefficients are, therefore, likely to affect both the digestibility of proteins and the bioavailability or toxicity of tannins. Although the currently available evidence suggests this to be generally the case, it is also probable that not all tannins will comply with this rule, given the large number of different tannin structures and that further fine-tuning will be needed. In fact, it will be interesting to discover what exceptions exist to this proposed rule, in analogy to the bioavailability of flavonoids. For instance, nonpolar flavonoid aglycones (higher K_{ow} coefficients) are generally absorbed more readily than the polar flavonoid glycosides (*18*; lower K_{ow} coefficients). However, the nature and position of the sugar moiety is another important parameter that influences bioavailability (*53*, *54*, *55*).

To summarize, tannins that occur in plant foods or feeds can vary greatly in their octanol or water solubilities. It is suggested that these solubilities can be used to rank tannins and tannin-rich feeds in terms of their likely toxicity: tannins with high octanol solubilities may cause toxicities, especially if present in high concentrations. Therefore, unconventional feeds or browse products should be screened not only for total tannin contents but also for low octanol−water partition coefficients in order to avoid negative nutritional responses. Further research will be needed to validate this technique in conjunction with animal feeding trials that test a wide range of different tannin-containing feeds in terms of tannin types and tannin concentrations. It is anticipated that this will lead to a feed evaluation system which includes tannin concentrations, K_{ow} coefficients, and dose−response relationships for tannin-containing feeds.

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Received for review February 2, 2007. Revised manuscript received April 25, 2007. Accepted May 4, 2007. This publication is an output from a research project (R7351, Livestock Production Program) funded by the United Kingdom Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID.

JF070308A