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Goats fed tannin-containing leaves do not exhibit toxic syndromes

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Abstract

The blood metabolic profile was examined in 2–3 year old non-lactating and non-pregnant Mamber goats consuming *Quercus calliprinos* (oak), *Pistacia lentiscus* (pistacia) and *Ceratonia siliqua* (carob) leaves to determine whether intake of tannin-rich fodder induces subclinical systemic toxicity. Total phenolic and condensed tannin content ranked in the order pistacia > oak > carob. Goats did not exhibit toxic effects following consumption of 10–23 g kg^{-1} day^{-1} of tannin-rich leaves. Metabolite blood concentrations did not differ from goats fed wheat straw, and were within the normal range. Certain serum metabolic indices that are known to be sensitive indicators of damage to the liver (gamma glutamyltranspeptidase, alkaline phosphatase, cholesterol) and kidneys (urea, uric acid, minerals) were within the normal range for goats. Thus, it appears that goats used in this study were well adapted to the nutritional environment and may consume large amounts of tannins (1.1–2.7 g per kg BW per day condensed tannins and 0.4–0.9 g kg^{-1} BW day^{-1} soluble phenolics) without suffering any ill effects.

Keywords: Goats; Browse; Tannins; Toxicity; Metabolic profile

1. Introduction

Trees and shrubs are an important source of fodder for livestock in tropical and dry environments (Topps, 1992). However, the use of browse sources by herbivores is restricted, in many cases, by defending or deterring mechanisms related to high tannin content woody plants (Provenza, 1995). The inverse relationship between high tannin level in forage and palatability, voluntary intake, digestibility and N retention in some mammalian herbivores is well established (Robbins et al., 1987; Silanikove et al., 1994; Silanikove et al., 1996). Reduced palatability, low rate of evacuation of digesta out of the rumen and toxic effects are factors that were considered in the explanation of the negative effects of tannins on feed intake in ruminants (Kumar and Singh, 1984; Provenza, 1995). Toxic effects have been described as the most important factor (Bryant et al., 1991) and are reflected by a damage in the liver, kidneys and the epithelium of the digestive tract (Kumar and Singh, 1984; Reed, 1995).

Goats are predominantly browsers, and on Mediterranean scrubland, browse constitutes 60–80% of goat diets (Kababya, 1995). Goats are able to consume larger amounts of tannin-rich browse than sheep under similar conditions (Gilboa et al., 1995;

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Silanikove et al., 1996), which may increase the risk of exposing goats to subclinical systemic toxicity.

In the present experiment, the blood metabolic profile was examined in 2- to 3-year-old non-lactating and non-pregnant Mamber goats consuming *Quercus calliprinos* (Common oak), *Pistacia lentiscus* (pistacia) and *Ceratonia siliqua* (carob) leaves to determine whether intake of tannin-rich fodder induces subclinical systemic toxicity. Subclinical systemic toxicity was evaluated by applying a wide-range metabolic profile test (Silanikove and Tiomkin, 1992).

2. Materials and methods

2.1. Animals

The experiments were carried out each time with four non-lactating and non-pregnant 2–3 year old female Mamber goats (a breed indigenous to the Mediterranean woodland) weighing 35 (SD ± 5) kg, chosen from a stock of eight goats. The animals were stall-fed individually in a protected yard and equipped with troughs which enabled quantitative measurement of feed intake.

2.2. Feeds

Evergreen leaves attached to small branches (2–3 mm in diameter) of Common oak, carob and pistacia were harvested by clippers once a week early in the morning and stored at −20°C. The daily allotment was removed from the freezer, let to thaw for at least 3 h, and fed once daily ad libitum at 08:00. Water was always available.

2.3. Experimental procedures

The animals were adapted for 2 weeks to consume the experimental diet as their sole feed by gradually replacing their previous diet (wheat hay ad libitum + 100 g concentrates per day) with that consisting of one of the experimental leaves. The animals were fed oak, pistacia, or carob solely for 2 weeks before the commencement of blood sampling. Blood (10 ml) was taken from each goat from the jugular vein by a syringe at 08:00 h during the second week, and the serum was separated and stored at −20°C. Voluntary feed intake was monitored at the end of the second week; body weight was determined every 2 days.

2.4. Chemical and statistical analyses

Total phenolic compounds and condensed tannins were measured as described by Silanikove et al. (1994). Briefly, total phenolics were determined by the Folin–Denis method, and expressed as tannic acid equivalent. Condensed tannins were measured by the butanol/HCl method, and expressed as purified quebracho condensed tannin equivalent.

The choice of biochemical measurements is that conventionally used for diagnosing human and domestic animal hepatic and kidney damage and general metabolic disorders (Silanikove and Tiomkin, 1992). Some of the parameters used in the present metabolic profile were found specifically useful in detecting damage to the liver and kidneys caused by tannins (Garg et al., 1992; Murdiati et al., 1990; Zhu et al., 1992). The serum was analysed in the Biochemistry Laboratory of the Kimron Veterinary Institute (Bet Dagan, Israel) by procedures similar to those described by Silanikove and Tiomkin (1992). Components measured were total protein, albumin, cholesterol, β-hydroxybutyrate (B-HB), uric acid, calcium, phosphorus, sodium, potassium and chlorine. In addition, the serum enzymes aspartate aminotransferase (AST), alkaline phosphatase (AP) and gamma glutamyltranspeptidase (GGT) were measured.

Comparisons between treatments were made by two-way analysis of variance, with goats and feeds as independent terms in the model. Statistical significance was assessed by the Duncan's test using SAS Institute Inc. (1982) procedures.

3. Results

3.1. General (Table 1)

The DM content of oak, pistacia, carob and wheat straw was 67%, 60%, 54% and 91%, respectively.

Table 1
Chemical composition of the leaves of *Quercus calliprinos* (oak), *Pistacia lentiscus* (pistacia), *Ceratonia siliqua* (carob) and wheat straw (WS) during the feeding trials (% of dry matter), and voluntary dry matter intake (DMI) (g day^{-1})

	Oak		Pistacia		Carob		WS	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM	67 [c]	1.7	60 [b]	1.3	54 [a]	1.5	91 [d]	2.3
Ash	6.3 [a]	0.5	5.4 [a]	0.4	5.5 [a]	0.4	3.2 [b]	0.3
CP	6.4 [b]	0.4	6.5 [b]	0.4	9.0 [a]	0.6	4.8 [c]	0.2
NDF	50 [b]	6	44 [b]	3	48 [b]	5	80 [a]	9
WSM	20.0	1.3	20.9	1.2	21.9	1.1	23.9	1.5
SP	4.0 [c]	0.35	7.1 [d]	0.6	1.8 [b]	0.9	0.1 [a]	0.0
CT	9.5 [c]	0.5	20.5 [d]	1.7	5.0 [b]	3.4	0.04 [d]	0.0
DMI	664 [b]	26	465 [a]	31	799 [c]	42	801 [c]	41

Within rows, values followed by different superscript letters are different ($P < 0.05$). DM, dry matter; CP, crude protein; NDF, neutral-detergent fiber; WSM, water-soluble material; SP, soluble phenolics, expressed as tannic acid equivalent; CT, condensed tannins, expressed as purified quebracho condensed tannin equivalent.

Crude protein content was highest in carob and neutral-detergent fibre content was highest in wheat straw. Condensed tannins content ranged from 5.0% in carob to 9.5% in oak and 20.5% in pistacia, whereas in wheat straw it was nil. In parallel, the content of soluble phenolics ranged from 1.8% in carob to 4.0% in oak and 7.1% in pistacia, and only 0.1% in wheat straw. DM intake was 799, 664 and 465 g day^{-1} in goats fed carob, oak, and pistacia, respectively.

3.2. Blood metabolic profile (Table 2)

There were no consistent differences between goats fed wheat straw and any type of leaves, in any serum metabolite except urea. Therefore, results are

Table 2
Blood metabolic profile of goats fed tannin-rich leaves (*Quercus calliprinos, Pistacia lentiscus, Ceratonia siliqua*) and wheat straw (pooled values)

Metabolite	Unit	Mean	SEM	Range in goats [a]
Protein	g per 100 ml	7.7	0.4	5.9–7.8
Albumin	g per 100 ml	3.2	0.1	2.8–3.8
Uric acid	mg per 100 ml	0.8	0.4	0.2–1.1
B-HB	mg per 100 ml	8.0	0.6	6.5–10
Cholesterol	mg per 100 ml	47.0	6.0	30–108
Urea	mg per 100 ml	15.1 [b]	0.4	10–60
GGT	units l^{-1}	31	2	25–34
ALP	units l^{-1}	32	5	23–44
AST	units l^{-1}	40.0	4	30–122
K	mM	4.4	0.2	3.6–4.8
Na	mM	149	3	143–157
Cl	mM	114	4	104–120
P	mg per 100 ml	5.3	0.4	4.2–7.6
Ca	mg per 100 ml	8.1	0.4	8.2–9.8

[a] According to Mitruka and Rawnsley (1977).
[b] In goats fed wheat straw, the urea concentration (30.1 mg per 100 ml) was higher ($P < 0.01$) than in goats fed leaves (9.1 mg per 100 ml).
B-HB, β-hydroxybutyrate.

given as pooled means. All the metabolites tested fell within the normal range for goats (Mitruka and Rawnsley, 1977). Serum urea concentration was considerably lower in goats fed tannin-rich leaves than in those fed wheat straw, which is most likely related to the severe limitation in protein availability in goats fed tannin-containing leaves (Gilboa et al., 1995; Silanikove et al., 1996).

4. Discussion

Systemic tannin-related toxicity is induced by causing necrotic damage to the liver and kidney in monogastric and ruminant animals (McLeod, 1974; Kumar and Singh, 1984). The occurrence of systemic toxic effects of tannins implies that tannins or their degradation products are absorbed from the digestive tract. It is unlikely that molecules of condensed tannins are absorbed unmodified because of their usually large molecular size (McLeod, 1974). However, high tannin content can cause gastritis and damage to the intestinal mucous membranes, enabling absorption of condensed and hydrolysable tannins (McLeod, 1974). Hydrolysable tannins can be degraded in the digestive tract to phenols and sugar. The released phenols can be absorbed and cause toxic effects (McLeod, 1974). Depolymerization of condensed tannins can occur under acidic conditions in the abomasum, and the products may be toxic (McLeod, 1974; Kumar and Singh, 1984; Lindroth and Batzli, 1984; Mehansho et al., 1987).

Various oak species are generally classified as toxic to livestock because of their high tannin content (Kingsbury, 1964). In cattle, oak poisoning due to consumption of oak leaves or acorns was reported in India and Nepal (Negi et al., 1979; Lohan et al., 1983; Garg et al., 1984, Garg et al., 1992), South Africa (Nesser et al., 1982), Slovakia (Begovic et al., 1978), and the US (Kingsbury, 1964). Incorporation of *Quercus incana* leaves at an excess of 14% (on DM basis) into a mixed ration for cattle produced deleterious effects (Lohan et al., 1983), and when fed to yearling calves as a sole feed it produced toxic effect within the first 2 days and lethality within 4 days (Negi et al., 1979). Oak poisoning occurred in crossbred cattle after eating immature tender oak (*Q. incana*) leaves (Garg et al., 1992). Mortality was 70%, the surviving animals showed anorexia, severe constipation and brisket oedema. The faeces were hard, pelleted and coated with blood and mucus. Blood haemoglobin and mean corpuscular haemoglobin were reduced, and serum bilirubin was increased. Serum urea nitrogen and creatinine were greatly increased. There was bilirubinuria, proteinuria, hypoproteinaemia and hypocalcaemia, and greatly increased activities of serum AST, lactate dehydrogenase and ALP. The levels of tannins and condensed tannins were 97.7 mg tannic acid equivalent and 5.8 mg catechin equivalent per g of dry leaves. Begovic et al. (1978) found that cattle are much more sensitive than goats to tannic acid toxicosis, and related these differences to more efficient neutralization of tannic acid in goats as a result of higher activity of tannase of microbial origin in their rumen mucosa.

Secretion of proline-rich protein, which are proteins that have very high affinity for tannins, constitutes the first line of defence against ingested tannins in rats and mice (Mehansho et al., 1987). A marked reduction of tannin content in extrusa samples collected from the oesophagus of goats and cattle consuming tannin-rich browse suggests the involvement of a salivary defense mechanism in ruminants as well (Provenza and Malachek, 1984; Burrit et al., 1987). The saliva of mule deer (*Odocoileus heminous heminous*), a browser ruminant, contains more protein, including a proline-rich protein, and has a greater tannin-binding capacity than sheep and cow saliva (Robbins et al., 1987; Austin et al., 1989). Goats produce more protein-rich saliva during eating than sheep (Dominigue et al., 1991), but Distel and Provenza (1991) did not detect proline-rich protein in the saliva of goats fed a tannin-rich diet (blackbrush twigs). However, Gilboa (1995) found that the parotid saliva of goats was relatively rich in proline (6.5%), glutamine (16.5%) and glycine (6.1%), which are known to enhance the affinity of proteins to tannins (Mehansho et al., 1987). The concentration of protein in parotid saliva was significantly higher in goats fed carob (550 $\mu g\ ml^{-1}$) than in goats fed wheat straw (212 $\mu g\ ml^{-1}$), or in goats fed carob plus polyethylene glycol (190 $\mu g\ ml^{-1}$), suggesting that exposing the goats to tannins enhanced the secretion of proteins in parotid saliva (Gilboa, 1995). This response differs from the situation in rats and mice in which exposure to tannins induced the secretion of a novel proline-rich proteins (Gilboa, 1995 vs. Mehansho et al., 1987).

Provision of 50 g day^{-1} of tannic acid caused damage to the kidneys in cattle (Cedervall et al., 1973). Murdiati (1991) (as cited in Murdiati et al., 1990) showed hepatic and renal pathology in sheep when given tannic acid at dose rate of 1 g per kg BW per day for 14 days. Rumen metabolism appeared to prevent toxicity from gallic and tannic acid at dose rate of < 0.4 g per kg BW per day. Acute tannic acid intoxication, reflected by necrotic damage to the liver, was induced in sheep following oral administration of 8 g per kg BW, intragastric administration of 1 g per kg BW, and intraperitoneal administration of 0.1 g per kg BW (Zhu et al., 1992; Zhu and Filippish, 1995). Plasma sodium and glucose levels significantly decreased ($P < 0.05$) while packed cell volume and plasma AST, ALP, creatinine and bilirubin rose ($P < 0.01$). It appears that the gut ameliorates the systemic toxic effects of tannic acid, and that the major organ which is responsible for that is the rumen. The ameliorating function of the rumen can be related to binding of tannins to protein and cell walls of plant origin, or endogenous salivary proteins, or to detoxification of the tannins by microbial enzymes. The goats in the present experiment were exposed daily to a considerable load of condensed tannins (1.1, 1.8 and 2.7 g kg^{-1} BW in goats fed carob, oak and pistacia, respectively) and to soluble phenolics (0.4, 0.8 and 0.9 g kg^{-1} in goats fed carob, oak and pistacia, respectively). Blackbrush condensed tannin intake by experienced and inexperienced goats was 2.6 and 1.7 g per kg BW per day, respectively, approximately 3 g per kg BW per day may be the maximal amount of condensed tannins goats could detoxify (this study and that of Distel and Provenza, 1991). Most of the soluble phenolics were presumably hydrolysable tannins and could be precipitated by a tannin-binding chemical (polyethylene glycol, with a molecular weight of 4000) (Silanikove, unpublished data, 1995). Makkar and Becker (1994) found also that the tannin extract from *Q. incana* is a mixture of condensed and hydrolysable tannins.

Serum levels of AST, GGT, ALP and cholesterol are those conventionally used for diagnosing human and domestic animal hepatic damage (Silanikove and Tiomkin, 1992). GGT has proved to be an especially sensitive indicator of minor bovine hepatic damage; ALP and cholesterol are used to detect bile obstruction, i.e. a mild and progressive damage to the liver (Silanikove and Tiomkin, 1992). Garg et al. (1984) used a similar approach to demonstrate that feeding growing cattle with sal (*Shorea robusta*) did not induce systemic toxicity. However, changes in these parameters were found when tannin-related hepatotoxicity occurred (Murdiati et al., 1990; Garg et al., 1992; Zhu et al., 1992; Zhu and Filippish, 1995). None of the parameters of the blood metabolic profile in the present study differed from those measured on tannin-free roughage (wheat straw), and all of them fell within the normal range for goats, suggesting that no damage to the liver occurred.

Damage to the kidneys would most likely lead to renal failure and to changes in serum urea, creatinine, uric acid and mineral concentrations (Garg et al., 1992; Zhu et al., 1992). Lack of increase in these metabolites above the normal values, therefore, suggests that damage to the kidney did not occur. Lack of decrease in serum mineral concentration below the normal values, particularly those of Ca, P and Mg which reflect changes in absorption from the gut, implies that: (i) absorption of these minerals from the gastrointestinal tract was not hampered, and (ii) at least in the short run, feeding tannin-rich fodder did not cause any sign of mineral depletion.

In agreement, there are no reports on tannin-related toxicity in goats raised on Mediterranean scrubland in Israel, or in other countries of the Mediterranean Basin. The large reduction in body weight during consumption of oak and particularly pistacia could be explained by negative energy and nitrogen balance due to detrimental effects of the tannins on their intake and digestion (Gilboa et al., 1995; Silanikove et al., 1996). The negative nitrogen balance in the digestive tract was a result of complexation between tannins and endogenous proteins (Silanikove et al., 1996). The negative effect of tannins on feed intake was a result of negative postingestive effects of tannins in the digestive tract (Silanikove et al., 1996) and interaction between tannins and the rumen epithelium was identified as an important factor (Gilboa, 1995).

Based on the work of Begovic et al. (1978), Garg et al. (1992) and Murdiati et al. (1990) it seems that the amount of tannins which can be consumed by goats without any signs of toxic effect, may be toxic for cattle and sheep. The lack of systemic toxicity in the goats supports the hypothesis of Freeland and Janzen (1974) that mammalian herbivores, such as prairie voles, ingest toxins in amounts that they can detoxify. The study ofMurdiati et al. (1990) suggest that this general conclusion is particularly relevant for goats. Although plants containing hydrolysable tannins can be hepatotoxic, such poisoning has not been reported in Indonesia despite the presence of these phenolics in plants. However, the hepatotoxic potential of *Climedia hirta* (harendong), which contains 19% hydrolysable tannin was demonstrated in goats when the animals were forced to eat this plant by its pelleting with other dietary ingredients.

5. Conclusions

Local goats, such as the Mamber breed used in the present experiment, have inhabited Mediterranean scrubland areas for 7000 years. Their ability to consume large amounts of tannin-rich plant material without exhibiting toxic effects can be related to their ability to avoid consuming browse in amounts exceeding their capacity to detoxify tannins and to their enhanced capacity, in comparison with other ruminants species, to detoxify tannins.

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