Lignocellulose degradation and subsequent metabolism of lignin fermentation products by the desert black Bedouin goat fed on wheat straw as a single-component diet*

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(*Received 8 November 1988 – Accepted 10 April 1989*)

Bedouin goats were fed on wheat straw as a single-component diet under two watering regimens, drinking once daily or once every 4 d, in order to clarify whether lignin-degradation products were absorbed, metabolized and excreted in urine. Acid-soluble lignin accounted for 220 g/kg total lignin, its digestibility was the highest (0·87) and was unaffected by water deprivation. Acid-insoluble lignin accounted for 780 g/kg total lignin and its digestibility increased during water deprivation from 0·21 to 0·41. Alkali-soluble lignin accounted for 320 g/kg total lignin and its digestibility increased during water deprivation from 0·44 to 0·53. Digestibility of structural carbohydrate was considerably higher than that observed in other domesticated ruminants fed on wheat straw. It responded positively to water deprivation, increasing from 0·63 to 0·73 with cellulose and from 0·61 to 0·68 with hemicellulose. The amount of urinary aromatic acids, mainly in the form of hippuric acid, considerably exceeded the potential contribution of any non-lignin component which might affect the excretion of aromatic acids. A considerable percentage (71–76) of the apparently digested lignin was not accounted for as soluble phenolic compounds in faeces or as aromatic acids in urine, and hence was apparently completely metabolized. Lignin is a key substrate which is extensively digested in goats fed on low-quality forage, with subsequent absorption of end-products. This enhanced the availability of structural carbohydrates for fermentation and was associated with excretion of high-energy metabolites in the form of benzoic and hippuric acids.

Lignin fermentation: Lignocellulose: Goats

Lignification of plant cell walls is the single most important factor that limits forage digestibility by ruminants. Although lignin traditionally has been considered indigestible (Van Soest, 1982), recent evidence indicates that at least part may be degraded and solubilized in the digestive tract of some ruminants (Fahey & Jung, 1983).

Desert and tropical breeds of cattle (Hungate, 1966; Hunter & Siebert, 1985) and goats (Silanikove *et al.* 1980; Silanikove, 1984, 1986*a*, *b*) fed on poor-quality diets showed a higher feed intake and better energy and nitrogen utilization in comparison with breeds from temperate climates.

The extent of dry matter and structural carbohydrate digestibility found in Bedouin goats fed on wheat straw has been observed in other domesticated ruminants only following chemical processing of the straw (Silanikove, 1986*a*). Based on the higher apparent lignin digestibility in the Bedouin goat in comparison with Saanen goats, it was suggested that the unusually high digestion of structural carbohydrates reflected cell-wall delignification (Silanikove, 1986*a*), which may possibly reduce the encrustation of structural carbohydrates and render it more susceptible to microbial degradation.

* Contribution of the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel, no. 2179-E, 1987.

In addition, if lignin is solubilized by forming smaller fragments, these may be metabolized further (Chen *et al.* 1985, 1986; Colberg & Young, 1985*a*, *b*) and absorbed in the form of phenolic acid (Martin, 1969*a*, *b*, 1970, 1973, 1982*a*, *b*). It is well-established that ruminants excrete larger amounts of aromatic acid in their urine than do non-ruminant species (Martin, 1969*a*, *b*, 1970). The main component of the aromatic acids was found to be benzoic acid, which was excreted mainly in conjugation with glycine as hippuric acid. However, the role of lignin as a source for the excretion of large quantities of aromatic acids in ruminant urine remains questionable (Martin, 1970).

The objectives of the present study were to evaluate the relationships between lignin and structural carbohydrate digestion in Bedouin goats fed on wheat straw as a single component diet, and to clarify whether lignin-degradation products were absorbed, metabolized and excreted in urine. The study examined Bedouin goats sustained on a wheat-straw diet, as wheat straw is almost free of non-lignin phenolic compounds (Silanikove & Levanon, 1987). Thus, excretion of aromatic acids (as benzoic acid and hippuric acid) under these conditions could support the conclusion, based on the balance of phenolics in the gut, that lignin-degradation products were absorbed and metabolized.

MATERIALS AND METHODS

Animals. Experiments were conducted on four adult, non-lactating, non-pregnant black Bedouin goats weighing 19·4 (SD 3·1) kg. The goats were fed for 60 d before and throughout the experiments on baled wheat straw containing (/kg dry matter) 16·7 MJ, 36 g crude protein (nitrogen × 6·25) and 390 g crude fibre (Brosh *et al.* 1986).

Experimental procedures. The animals were kept outside during mid-summer in yards fully exposed to solar radiation. Maximum daily temperature was 35°, and relative humidity 40–70%. Two goats were given water once every 4 d (treatment D) and the other two were given water once daily (treatment H). After 1 month on each drinking regimen, the treatments were reversed. Water (as appropriate) and feed were offered at 16.00 hours daily. Water was removed when the goats had finished drinking. Feed intake, feed refusals, faecal and urine output were recorded daily for 10 d. A Foley catheter was used for gravimetric urine collection. Daily subsamples (5%) of feed, urine and faeces were pooled and kept frozen for chemical analysis.

Chemical methods. Core samples from bales were taken during the 10 d collection periods. Feed and faecal samples were dried and then ground to pass through a 1 mm screen. Contents of dry matter and organic matter were determined by standard procedures. Cell walls were isolated by the neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) procedures of Goring & Van Soest (1970), with slight modifications (Silanikove & Levanon, 1987). Total amounts of phenolic compounds (total phenolics) were determined with NDF by the acetyl bromide method, or gravimetrically by the 12 M-sulphuric acid procedure (Silanikove & Levanon, 1987). The latter determination was based on the finding that under strongly acidic conditions, acid-soluble phenolic compounds condense and become insoluble (Silanikove & Levanon, 1987). Acid-insoluble lignin (AIL) was determined as the acetyl-bromide content or the 12 M-H_2SO_4 content of ADF. Acid-soluble phenolic compounds (acid-soluble lignin; ASL) have recently been shown to be composed mainly of lignin (97%), with only a small percentage (3) of phenolic acids (Silanikove & Levanon 1987). ASL was determined as the difference in lignin content of NDF and ADF (acetyl bromide and 12 M-H_2SO_4), and directly by u.v. detection in the 1 M-H_2SO_4 hydrolysate of NDF (Silanikove & Levanon, 1987). Alkali-soluble phenolic compounds (alkali-soluble lignin; AL) were determined directly with the NDF-saponification hydrolysate, as described by Silanikove & Levanon (1987). Lignin was also detected

histochemically using NDF-isolated fibre by the chlorine–sulphite and acid phloroglucinol test (Johanson, 1940). Hemicellulose was determined as the difference between NDF and ADF corrected for ASL (Silanikove & Levanon, 1987).

For the determination of soluble phenolic compounds (SP), feed or whole ground faecal samples were extracted three times successively with methanol (800 ml/l) and the extracts were combined. Phenolic concentrations were determined spectrometrically and expressed as benzoic acid equivalents, using the Folin Denis reagent and benzoic acid as a standard (Swain & Hillis, 1959).

Aromatic acids in urine samples were extracted and quantified titrimetrically as benzoic acid equivalents (Martin, 1969*a*). Benzoic acid and hippuric acid in urine samples were determined by high-performance liquid chromatography (HPLC). Filtered (SM11306 0·45 μm; Sartorius), potassium hydroxide hydrolysed (Martin, 1969*a*) and unhydrolysed samples were separated under the conditions described by Lecroix *et al.* (1986). The identities of benzoic acid and hippuric acid were confirmed by thin-layer chromatography (Scheline, 1968) of the same samples.

Statistical analysis. The results are presented as means and standard deviations, and statistical differences between treatment were evaluated by the pair-comparison *t* test analysis (Steel & Torrie, 1960).

RESULTS

Total lignin in the wheat cell wall accounted for 136 and 131 g/kg dry matter according to NDF–ADF acetyl bromide and NDF–ADF 12 M-H_2SO_4 fractionations respectively (Table 1). ASL accounted for 213 and 229 g/kg total lignin according to the above fractionations (Table 1). ASL was the main fraction (787 and 771 g/kg according to the two fractionation schemes respectively) and AL accounted for 320 g/kg total lignin.

The digestibilities of the different types of lignin (total, ASL, AIL and AL) were similar irrespective of the method of determination ($P < 0{\cdot}05$). The values obtained by the two methods of fractionation (12 M-H_2SO_4 NDF–ADF and actyl bromide NDF–ADF) were pooled (Table 2). Total lignin digestibility was much higher in treatment D (0·58) than in treatment H (0·45; $P < 0{\cdot}01$). There was good agreement (within 5%) between total lignin digestibility and the weighted average of ASL and AIL digestibilities (Table 2).

Digestibility of ASL was approximately 0·86 (range 0·74–0·97) and did not differ between treatments (Table 2). AIL digestibility was considerably lower than that of ASL, although this fraction was also digested to a considerable extent (Table 2). The digestibility of AIL was much higher in treatment D (0·41) than in treatment H (0·25; $P < 0{\cdot}01$). AL digestibility was much higher ($P < 0{\cdot}05$) than that of AIL, although considerably lower than that of ASL ($P < 0{\cdot}01$). The digestibility of AL was higher with treatment D (0·53) than in treatment H (0·4; $P < 0{\cdot}05$) but the differences between treatments were smaller than those obtained for AIL (Table 3).

Relative to the content in the original material (Table 1), AIL was enriched in faecal dry matter, while the concentration of AL was unchanged and that of ASL was reduced considerably (Table 2). Therefore, it may be concluded that not only was the lignin digested to a large degree in the present experiment, but the composition of the remaining undigested lignin was modified considerably.

Wheat-straw NDF reacted positively in the chlorine sulphite and phloroglucinol tests. After modification in the gut, NDF reacted positively only in the phloroglucinol test and gave only faint results in the chlorine–sulphite test. The results of the histochemical examination of lignin support the previous conclusion that there was extensive modification in the composition of undigested lignin.

Table 1. *Dry matter and cell-wall contents (g/kg) of wheat straw consumed by goats**

	Dry matter	Cell wall
NDF	752	1000
Cellulose	418	556
Hemicellulose†	256	340
Total lignin‡	136	181
Total lignin§	131	174
Acid-soluble lignin‡	29	39
Acid-soluble lignin‖	30	40
Alkali lignin	44	59
Acid-insoluble lignin‡	107	142
Acid-insoluble lignin§	101	134

NDF, neutral-detergent fibre.
* Average of triplicate analysis.
† Corrected for acid-soluble lignin.
‡ NDF–acid-detergent fibre (ADF) acetyl bromide fractionation.
§ NDF–ADF 12 M-sulphuric acid fractionation.
‖ Directly determined on acid hydrolysate.

Table 2. *Intake, faecal excretion and digestibility of total lignin (TL), acid-soluble lignin (ASL) and acid-insoluble lignin (AIL)* by Bedouin goats maintained on wheat straw with water once daily (treatment H) or once every 4 d (treatment D)*

(Mean values and standard deviations)

	Gross intake (g/d)		Faecal output (g/d)		Intake of apparently digested matter (g/d)		Digestibility		Concentration in faeces (g/kg dry matter)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Treatment H (hydrated goats)										
TL	40·9	12·5	22·5[a]	3·8	18·4	4·6	0·45[a]	0·21	147[a]	4
ASL	8·7	2·7	1·2	0·2	7·5	2·2	0·86	0·089	7	0·1
AIL	32·2	8·9	24·2[a]	3·7	8·0	1·4	0·25[a]	0·038	136	3
Treatment D (dehydrated goats)										
TL	34·6	8·5	16·0[b]	2·9	18·6	2·9	0·58[b]	0·055	133[b]	3
ASL	7·8	1·9	1·0	0·1	6·8	1·5	0·88	0·081	8	0·1
AIL	26·8	5·7	15·8[b]	2·7	11·0	3·1	0·41[b]	0·07	131	4

[a, b] Mean values with different superscript letters were significantly different between treatments (pair-comparison *t* test): $P < 0.05$.
* The results of the neutral-detergent fibre (NDF)–acid-detergent fibre (ADF) 12 M-sulphuric acid and NDF–ADF acetyl bromide fractionations were pooled. For details of procedures, see p. 510.

The SP content of wheat straw was very low (0·1 g/kg), and that of the faeces increased significantly (Table 3). The amount of SP excreted in the faeces during treatments H and D rose by 59 and 35 times respectively in comparison to the content in wheat straw, and accounted for about 10 and 8% of total lignin digestibility in treatments H and D respectively (Table 3).

Digestibility of structural carbohydrates was much higher than expected (National Research Council, 1981). Cellulose digestibility was 0·63 and 0·73 ($P < 0.01$) with

Table 3. *Intake, faecal excretion and digestibility of soluble phenolic compounds (SP) and alkali lignin (AL)* by Bedouin goats maintained on wheat straw with water once daily (treatment H) or once every 4 d (treatment D)*

(Mean values and standard deviations)

	Gross intake (g/d)		Faecal output (g/d)		Intake of apparently digested matter (g/d)		Digestibility		Concentration in faeces (g/kg dry matter)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Treatment H (hydrated goats)										
SP	0·03	0·004	1·84	0·06	−1·81	0·05	—	—	11	1
AL	13·1	3·1	7·4[a]	0·5	5·7	0·7	0·435[a]	0·035	45	6
Treatment D (dehydrated goats)										
SP	0·03	0·006	1·47	0·04	−1·45	0·4	—	—	12	1
AL	11·2	2·9	5·3[b]	0·5	5·9	0·8	0·527[b]	0·029	44	4

[a, b] Mean values with different superscript letters were significantly different between treatments (pair-comparison *t* test): $P < 0·05$.

* For details of procedures, see p. 510.

Table 4. *Intake, faecal excretion and digestibility of structural carbohydrates* by Bedouin goats maintained on wheat straw with water once daily (treatment H) or once every 4 d (treatment D)*

(Mean values and standard deviations)

	Gross intake (g/d)		Faecal output (g/d)		Intake of apparently digested matter (g/d)		Digestibility		Concentration in faeces (g/kg dry matter)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Treatment H (hydrated goats)										
Cellulose	125·4	44·8	47·1[a]	19·5	78·3	24·1	0·62[a]	0·02	282	12
Hemicellulose	76·7	26·0	30·1[a]	2·1	46·6	19·2	0·61[a]	0·04	182	18
Treatment D (dehydrated goats)										
Cellulose	111·5	34·3	31·2[b]	17·2	80·3	22·3	0·72[b]	0·04	258	15
Hemicellulose	66·0	19·9	21·2[b]	3·5	44·8	11·4	0·68[b]	0·06	178	21

[a, b] Mean values with different superscript letters were significantly different between treatments (pair-comparison *t* test): $P < 0·05$.

* For details of procedures, see p. 510.

treatments H and D respectively, and the respective hemicellulose digestibilities were 0·61 and 0·68 ($P < 0·05$) (Table 4).

When the goats were given water once every 4 d their intake was 3609 ml or 902 ml/d compared with 940 ml/d in goats that consumed water daily (not significant).

Drinking once every 4 d (treatment D) resulted in a small (15%) but consistent and statistically significant ($P < 0·05$) reduction in feed intake (from 314 to 267 g dry matter/d). However, due to the increased efficiency in the utilization of cell-wall components, intakes of apparently digested cellulose and hemicellulose were similar in both treatments (Table 4).

Table 5. *Urine production and aromatic, benzoic and hippuric acid excretion as benzoic acid equivalent (BAE) by Bedouin goats maintained on wheat straw* with water once daily (treatment H) or once every 4 d (treatment D)*

(Mean values and standard deviations)

	Urine production (ml/d)		Aromatic acid excretion (g BAE/d)		Benzoic acid excretion (g BAE/d)		Hippuric acid excretion (g BAE/d)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Treatment H (hydrated goats)	257	57	3·7[a]	0·5	2·9[a]	0·3	2·5[a]	0·3
Treatment D (dehydrated goats)	226	48	2·9[b]	0·5	2·5[b]	0·4	2·0[b]	0·3

[a,b] Mean values with different superscript letters were significantly different between treatments (pair-comparison *t* test): $P < 0{\cdot}05$.

* For details of procedures, see p. 510.

Urine production was 257 and 226 ml/d (Table 5) in treatments H and D respectively, and the corresponding aromatic acid concentrations in urine were about 13 and 14 g/l. The amount of aromatic acid excreted was approximately 4 and 3 g benzoic acid equivalent/d for the two treatments respectively (Table 5).

Benzoic acid was the major component of the aromatic acids in alkali (KOH)-hydrolysed urine, accounting for 84 and 86% of the total aromatic compounds excreted with treatments H and D respectively (Table 5). Most of the excreted benzoic acid was conjugated with glycine as hippuric acid, accounting for 68 and 76% of the total aromatic compounds excreted, or 79 and 86% of the total benzoic acid excreted with treatments H and D respectively.

DISCUSSION

Overall lignin digestibility

Lignin digestibility, as measured by the gravimetric methods for total lignin, ASL, AL and AIL, clearly indicated that under the conditions of this experiment lignin was degraded. Since accumulation of SP accounts for only 10% of the apparently digested lignin (total), the rest could be regarded as truly digested lignin.

In u.v. spectrophotometry, the typical absorbance maximum of lignin at about 280 mm should be attributed to the π–π transition in the aromatic ring of the polymer (Janshekar & Fiechter, 1983). The decrease in the absorbability coefficient at this wavelength can be due to the cleavage of the aromatic ring structures within the polymer, or to absorption of polymer degradation products.

The term 'lignin' really defines a structurally variable water-insoluble polymer. However, based on its universal phenolic subunit structure, different types of lignin may be potentially converted into similar phenolic intermediates (Crawford & Crawford, 1984). As will be discussed later, this work has shown that different fractions of chemically isolated lignin, which are probably associated with different botanical fractions, were degraded to different extents. However, it may also be concluded that the metabolic fate of the phenolic intermediates produced during lignin fermentation is quite similar.

ASL

The ASL fraction, which was the most highly digestible component in the wheat-straw cell wall, can be liberated by a mild acidic treatment as a lignin–carbohydrate complex (LCC) (Vered *et al.* 1981). Parallel to the high digestibility of ASL, the lignin fraction stained in the chlorine–sulphite test disappeared almost completely. Reaction with chlorine–sulphite indicates that syringyl units are involved (Vance *et al.* 1980), possibly located in parenchyma cells (Akin *et al.* 1977). Scalbert *et al.* (1986) have shown recently that AL contains equivalent amounts of guaiacyl and syringyl units, and Silanikove & Levanon (1987) have presented evidence that the ASL is part of AL.

Furthermore, the lignin stained in the chlorine–sulphite test occurs with advanced maturity in parenchyma cells of grasses and results in the inhibition of cell-wall digestibility (Akin *et al.* 1977). It was proposed recently that the fraction that increased during maturation of cereal straws and inhibited cell-wall digestibility is the ASL component (Silanikove & Levanon, 1987). Based on the previously mentioned evidence it is suggested that the ASL and chlorine sulphite test measured mostly the same component of lignin in the cell wall of Graminea.

AL

Wheat-straw lignin (total lignin) was solubilized to a large extent by alkali treatment at room temperature (Table 1), confirming previous results (Beckman *et al.* 1923; Scalbert & Monties, 1986; Silanikove & Levanon, 1987). This property may be responsible for most of the improved straw digestibility caused by alkali treatment (Chesson *et al.* 1983; Silanikove & Levanon, 1987). Enzyme-isolated lignin resembled AL in yield and in chemical characteristics such as percentage of complexation with polysaccharides, molecular weight distribution and phenolic composition (Scalbert & Monties, 1986; Scalbert *et al.* 1986). Due to the extraordinary efficiency of the microbial system, the Bedouin goat appears to be capable of delignifying LCC, which otherwise must be modified chemically. In vitro and in vivo studies of the fermentation processes in the rumen of the Bedouin goat support the conclusion that this process is more efficient than that noted in other domestic ruminants (Silanikove, 1986*b*).

The digestibility of AL was lower than that of ASL and, unlike ASL, it responded positively to an increase in mean retention time in the gut brought about by water deprivation.

AL of wheat straw has a wide molecular-weight-distribution range of 300–20000 (Scalbert & Monties, 1986). The lower digestibility of AL compared with ASL is related to the larger fraction of high-molecular-weight lignin, as the latter is expected to be more resistant to digestion (Chen *et al.* 1985, 1986; Ohmiya *et al.* 1986).

Formation of SP

It has been shown that the formation of soluble LCC by the action of rumen micro-organisms on grass may account for the dissolution, and hence the apparent digestion, of about half the total lignin intake in cattle (Gaillard & Richards, 1975). The magnitude of the LCC formed in the rumen is within the range of AL content in Graminea (Table 1). It is also consistent with the observation of Scalbert & Monties (1986) that enzyme-isolated lignin and AL are similar in yield and chemical composition in wheat straw. The appearance of SP in faeces in the present experiment, while it was absent in the feed consumed, could be regarded as indirect evidence for the formation of soluble LCC.

The large extent of ASL and AL digestibility in the present study (Tables 2 and 3), where ASL and AL probably first dissolved as LCC, did not adversely affect carbohydrate

digestibility (Table 4). A chemical association with other cell-wall components, mainly hemicellulose, was suggested to be the main form which protected polysaccharides from microbial degradation in Graminea (Chesson *et al.* 1983; Silanikove & Levanon, 1987). Phenolic acids esterified to hemicellulose serve as connecting agents between hemicellulose and lignin (Chesson *et al.* 1983). Esterified phenolic compounds limited the ability of the rumen microbial population to degrade cellulose and this limitation is probably related to the cellulase enzyme system (Jung & Salu, 1986).

It is suggested that this mechanism relates to the effects of LCC on cell wall porosity. Release of LCC to the water-soluble form would expose them to the influence of extracellular hemicellulases (Brice & Morrison, 1982). However, even more important, as can be extrapolated from the chemistry of wood delignification (Grethlein, 1985), is the fact that removal of hemicellulose and lignin causes larger pores to be produced in the fibre walls, thereby rendering the remaining structural carbohydrates more accessible to the rather large molecule of cellulase.

AIL

Most reports in the literature on lignin digestibility are concerned with the digestibility of AIL and small amounts of AL. This is because the present methods for lignin determination in forages include dilute acid pretreatment for hydrolyzing hemicellulose (Giger, 1985), which also hydrolyse the large lignin fraction in Graminea (Silanikove & Levanon, 1987, Table 1).

The AIL fraction seems to be the lignin fraction most resistant to digestion (Table 2). There are many reports in the literature that this fraction is indigestible, or that its apparent digestibility is so small that it could be regarded as an artifact of the method of its determination (Van Soest, 1982; Giger, 1985). Nevertheless, from the present results (Table 2), and from many other studies reviewed by Fahey & Jung (1983), it seems that the ability of ruminants in general to degrade even the more recalcitrant fractions of forage lignin is not an isolated phenomenon. The content of lignin in cell walls is particularly high (400–750 g/kg) in the compound middle lamella (Vance *et al.* 1980). This type of lignin has a higher molecular-weight distribution and is acid insoluble. It reacts positively with the acid phloroglucinol test, which indicates the presence of cinnamaldehyde groups. Phloroglucinol-stained lignin is located in forages in the parts most recalcitrant to digestion (Akin *et al.* 1977). Thus, AIL may be expected to inhibit degradation of polysaccharides by shielding them from extracellular enzymes. In accordance with this view, the degradation of AIL is slow. This can be deduced from the observation that its digestibility responded more than any other lignin component to an increase in the retention time in the gut (for the retention time measurements see Brosh, 1986; treatment D *v.* treatment H, Table 2). The larger increase in cellulose digestion in response to increases in the mean retention time of particulate matter (Table 5) seems also to be related to AIL degradation. Cellulose is distributed relatively uniformly among cell-wall layers, unlike hemicellulose, the content of which decreases from the lumen outward to the primary wall (Vance *et al.* 1980). Therefore, AIL delignification exposed relatively more cellulose than hemicellulose to enzymic degradation.

Metabolism and excretion of lignin-degradation products

Herbivores are known to excrete large quantities of aromatic acids in their urine. The principal aromatic acids in ruminant urine are hippuric acid and phenylaceturic acid, which are the glycine conjugates of benzoic acid and phenylacetic acids respectively. Phenylacetic acid was found to originate from rumen catabolism of phenylalanine (Martin, 1969*b*, 1973) and was the main source of the urinary origin of phenylaceturic acid.

Table 6. *Balance of apparently digested phenolic compounds in Bedouin goats maintained on wheat straw* with water once daily (treatment H) or once every 4 d (treatment D)*

(Mean values and standard deviations)

	Treatment H (hydrated goats)		Treatment D (dehydrated goats)	
	Mean	SD	Mean	SD
Intake of apparently digested phenolic compounds	18·4	4·6	18·6	2·9
Recovered as soluble phenolic compounds in faeces	1·8	0·05	1·5	0·04
Recovered as aromatic acids in urine	3·7[a]	0·5	2·9[b]	0·5
Apparently metabolized	12·9[a]	0·5	14·2[b]	0·6
Apparently metabolized (% of apparently digested)	70·1[a]	4·9	76·3[b]	4·5

[a, b] Mean values with different superscript letters were significantly different between treatments (pair-comparison t test): $P < 0{\cdot}05$.

* For details of procedures, see p. 510.

Based on the large proportion of aromatic acids excreted in the present experiment in the form of hippuric acid and benzoic acid, it seems that phenylaceturic acid was not a significant component of the urinary-excreted aromatic acids. This would be supported by the fact that wheat straw is a poor source of protein (only 36 g/kg dry matter), and consequently of phenylalanine. Phenylalanine entering the small intestine was not enriched by rumen microbial synthesis (Van Bruchem *et al.* 1985). Recycled urea-N was found to be the major source of gut N in Bedouin goats fed on wheat straw (Silanikove *et al.* 1980). This type of N is probably not readily converted into aromatic amino acids such as phenylalanine.

The amount of aromatic acids excreted in urine in the present experiment considerably exceeded the potential contribution of any non-lignin compounds, or other endogenous sources such as phenylalanine and alkali-labile phenolic acids. Since there is no evidence for *de novo* synthesis of aromatic compounds in mammals, the only identifiable potential source for the excretion of aromatic acids in the present experiment was lignin. This conclusion fits well with the digestion studies, which have shown considerable degradation and absorption of lignin from the gut, and with the fact that the urinary aromatic acids were excreted mainly in the form of hippuric acid and benzoic acid.

The amount of aromatic acid excreted by the Bedouin goats expressed as a proportion of intake (8 g benzoic acid equivalent/kg dry matter) exceeded the amount of aromatic acid excreted by sheep (Martin, 1970) fed on mature grass (5·7 g benzoic acid equivalent/kg dry matter).

Martin (1982*a*) suggested that β-oxidation in the liver of phenylpropionic acid absorbed from the gut, and subsequent conjugation of the formed benzoic acid, yielded hippuric acid as the principal urinary aromatic metabolite. However, Balba & Evans (1979) have shown a microbial anaerobic reductive pathway of cinnamic acids through β-phenylpropionate to benzoic acid and then reduction of the aromatic ring to cyclohexanecarboxylate.

Cyclohexanecarboxylate can be fermented relatively easily, producing volatile fatty acids and methane. From a bioenergetic consideration it could be expected that de-aromatization

of the benzene ring would be the rate-limiting step in rumen fermentation of phenylpropionic acid. In support of this possibility, it was found in the present work that the proportions of benzoic acid relative to total aromatic acid excreted increased with an increase in the mean retention time of fluid in the rumen (treatment D *v.* treatment H, Table 6). Longer mean retention time allows a longer exposure to microbial activity, which would result in accumulation of metabolites at rate-limiting steps. Therefore, it is possible that larger proportions than those assigned by Martin (1982*a*) are absorbed from the gut in the form of benzoic acid, and then conjugated with glycine in the liver.

The balance of total phenolic compounds (Table 6) further supports this possibility. Despite the large amount of urinary aromatic acids excreted, 70–76% of the phenolic compounds absorbed were not accounted for, and hence were apparently completely metabolized. In the light of the large amount of recent evidence (Balba & Evans, 1979; Chen *et al.* 1985, 1986; Colberg & Young, 1985*a*, *b*; Ohmiya *et al.* 1986) on the ability of the rumen microbial population to metabolize lignin-related compounds completely, this seems to be realistic. The increase in the proportion of phenolic compounds which were apparently completely metabolized, together with an increase in exposure time to microbial activity (treatment D *v.* treatment H, Table 6), is also in line with this conclusion.

Conclusions

Lignin undergoes extensive modification, degradation and absorption during its passage through the gastrointestinal tract in goats fed on low-quality forage. This enhances the release and microbial fermentation of structural carbohydrates. The degradation of most lignin fractions is rate limiting, and responds positively to an increase in the retention time of digesta in the gut. The extensive digestion of lignin was associated with absorption of phenolic compounds from the gut, further metabolism (probably in the liver), and finally urinary excretion as benzoic and hippuric acids.

This research was supported by grant no. 84-0035 from the US-Israel Financial Foundation (BSF), Jerusalem, Israel, and by a grant from the International Atomic Energy Agency, Vienna. The authors would like to thank Professor A. Shkolnik for his assistance in providing facilities and preparing the manuscript and to Professor C. E. Stevens for his help in preparing the manuscript.

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Printed in Great Britain