The effect of polyphenolics on ruminant gut metabolism

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1. Objectives of the project:

(i) to evaluate whether the type and quantity of polyphenolics likely to be ingested by ruminants in the tropics and subtropics have negative effects on absorption and metabolism by the gut tissues.

(ii) to encourage plant and animal scientists to become more aware of the possible post-ruminal effects of polyphenolics.

2. Summary

The aim of the experimental programme was to quantify the extent to which polyphenolic compounds escaped rumen fermentation and to investigate the effect of these materials on the metabolism of the animal. Most of the previous information in this area had come from feeding plant material in which the phenolic compounds had not been fully identified and in which there may have been other compounds toxic to the animals. Following an extensive search of the literature and consultations with several other workers in the field, it was concluded that if significant advances were to be made it would be preferable to investigate the fate of individual compounds rather than to use plant materials with ill-defined phenolic content. Such data would not then be specific to any one plant and could be widely applied. Initial studies concentrated on determining the resistance of selected compounds to degradation in the rumen. Subsequently the influence of a phenolic compound which was found to survive passage to the lower gastrointestinal tract on the digestive tract morphology and peripheral tissue metabolism was investigated.

Initially the fate of 5 individual phenolic compounds (quercetin, rutin, gallic acid, tannic acid and quebracho tannin) in the rumen was investigated. Using a combination of *in vivo* and *in vitro* techniques it was shown that the low molecular weight compounds were rapidly degraded in the rumen and had little effect upon rumen fermentation. Degradation was rapid even when the materials were present in plant material. Tannic acid and to a greater extent quebracho tannin did however escape metabolism in the rumen and reached the duodenum. Quebracho tannin was used as a model in the subsequent studies.

Administration of a single dose (6 g) of quebracho tannin directly into the rumen of 4 rumen-fistulated sheep along with the liquid phase marker, CrEDTA, showed that the average half life of quebracho tannin in the rumen was in the order of 11.0 ± 3.15 h while that of Cr was 16.25 ± 5.16 h. Continuous administration of quebracho tannin directly into the rumen of 4 sheep for 7 days confirmed that the material was substantially resistant to microbial attack. When fed to sheep as part of a grassmeal diet, quebracho tannin reduced the digestibility of both the fibre and nitrogenous components of the diet. There was also evidence of focal surface epithelial degeneration in the jejunum and ileum. Damage to the specialised epithelium overlying Peyers patches was also

evident and the tannin appeared to be taken up by Peyers patch M cells which, coupled with other changes to the gut wall, could result in a modification of the immune competence of the animal. Other studies indicated that the volatile fatty acid patterns in the rumen were modified by quebracho tannin, possibly due to it inhibiting microbial fermentation.

The effects of dietary quebracho tannin on the tissue metabolism were investigated further by feeding the material to rats. Voluntary feed intake was reduced in the tannin-fed animals and the apparent digestibility of dietary nitrogen was also reduced. Consequently, N retention and body weight gain were lower in the tannin-fed animals. The weight of three different muscles as a proportion of body weight was not changed by quebracho tannin but there was a marked reduction in body fat. Surprisingly no effect on the fractional synthetic rate of the protein in the mucosa obtained from the first 30 cm of the duodenum was seen.

The conclusion of the study is that there is a *prima facia* case that some tannins when ingested by ruminants have marked effects both on rumen and digestive function and also on tissue metabolism.

3. Results

Following an extensive search of the literature and consultations with several other workers in the field, it was concluded that if significant advances were to be made in determining the effects of phenolics on ruminant gut metabolism it would be necessary to investigate the effect of individual compounds on rumen fermentation and digestion in the lower digestive tract, rather than using plant material with ill-defined phenolic content. Most of the previous information in this area had come from feeding plant material in which the phenolic compounds had not been fully identified and in which there may have been other compounds toxic to the animals. The approach taken in this project was to investigate first the fate of individual compounds in the rumen to determine whether they survived passage to the lower digestive tract and subsequently to study the influence of those resistant compounds on the digestion and metabolism in lower digestive tract. Initially the effect of 5 individual phenolic compounds on ruminant gut metabolism was examined. Quercetin, rutin, gallic acid, tannic acid and quebracho tannin were used. The first 4 of these are readily available commercially, the last being available from specialist sources, namely the tanning industry.

These compounds were investigated using a combination of *in vitro* and *in vivo* techniques. Initial studies involved incubating the phenolic compound with rumen fluid *in vitro* but subsequently an *in vitro* rumen simulation technique known as RUSITEC (Czerkawski & Brackenridge, 1977) was used to further investigate the effects of these compounds on rumen metabolism. The RUSITEC offers a convenient means by which a relatively normal microbial population can be maintained *in vitro* over a relatively long time period. By using the 4 separate vessels of the RUSITEC simultaneously, each containing

either buffer (artificial saliva) alone, buffer plus feed, or buffer plus feed plus rumen contents, the extent of spontaneous degradation, binding to feed particles and microbial degradation could be assessed for each individual compound. *In vivo* studies involved administration of the phenolic compound directly into the rumen of fistulated sheep, either as a single dose or as a continuous infusion, or by oral administration after incorporation of the phenolic into the diet. Sheep fitted with duodenal T-piece cannulae were used to obtain samples of duodenal digesta to determine whether the phenolic survived passage to the lower digestive tract. Samples of phenolic-containing leaf material were also incubated in Dacron bags within the rumen of fistulated sheep to compare rates of disappearance of the phenolic with that of dry matter. In all *in vivo* studies, animals were fed a basal diet of pelleted grassmeal containing about 10% protein to maintain a relatively low protein intake and thereby prevent excess dietary protein from overcoming the effects of the phenolics. It is acknowledged that animals in the tropics and subtropics may be on a considerably lower protein intake than this.

Techniques for analysing the individual phenolic compounds by high performance liquid chromatography (HPLC) were developed. However with some the higher molecular weight phenolics, analysis by this method became more difficult as they were no longer discrete peaks. The acid butanol method for condensed tannins (Porter et al, 1986) and radial diffusion protein precipitation assay (Hagerman, 1987) were subsequently used to corroborate data obtained by HPLC in some studies. Quebracho tannin was routinely analysed by the acid butanol method. Phenolics were routinely extracted from feed, digesta or faeces with 70% aqueous acetone.

(a) Initial screening of the resistance of phenolic compounds to rumen fermentation

Quercetin, a compound present in high concentrations in some browse species (eg *Quercus*) was selected as the first compound to be evaluated. Although good analytical procedures were developed for fresh solutions of quercetin, the compound appeared to be unstable, especially under the conditions required to extract it from rumen and duodenal digesta. It was found to be rapidly degraded when mixed with rumen fluid *in vitro* but due its instability, it was unclear whether this was due to metabolism by the microorganisms or to natural degradation. Instability problems were mainly associated with pH, light and certain solvents eg alcohol and acetone, the latter being used to extract phenolics from the solid phase of digesta. Consequently, no *in vivo* studies were carried out using quercetin.

Rutin a much more commonly found substance, and a monomeric unit of many condensed tannins is reported to occur at levels of 3-5% of dry weight in many browse species (Swain, 1979). It was found to be stable in solution (at neutral pH) but was rapidly degraded when incubated with rumen fluid *in vitro*. An *in vitro* rumen simulation technique known as RUSITEC (Czerkawski & Brackenridge, 1977) was used to investigate this material further. Continuous infusion of rutin (1mg/ml) into a 1 litre RUSITEC vessel containing neither feed

(grassmeal) nor rumen fluid showed the expected build up of rutin concentration to 1 mg/ml. When feed was included in the vessel, the rutin concentration detectable was only 0.75 mg/ml, suggesting that about 25% of the rutin was binding to the feed particles. When rumen contents and grassmeal were included in the RUSITEC vessel, 97.5 ± 0.5 % of the rutin disappeared. When binding to feed particles was accounted for (25.8 ± 2.3%), it was concluded that the remaining loss (71.3 ± 1.8%) was due to microbial degradation. This would agree with observations of Griffiths and Barrow (1972) who showed that rutin fed to germ-free rats was excreted in the urine virtually unchanged but when fed to conventional rats, metabolic degradation products of rutin (ie phenolic acids and benzoic acid and their conjugates) rather than rutin itself were found in the urine.

Intraruminal administration of rutin (200 mg) to sheep indicated a half life of 0.45 ± 0.05 h.

Gallic acid, the monomeric unit of many hydrolysable tannins was also shown to be degraded by rumen microbes *in vitro*. Administration of gallic acid (6 g) directly into the rumen of 4 fistulated sheep along with the liquid phase marker, CrEDTA, showed that the rate of disappearance was much faster than the outflow of the liquid phase. Gallic acid had a half life of about 0.2 ± 0.06 h in the rumen while Cr had a half life of about 10 ± 2.7 h. Extraction of the microbial plus feed fractions from rumen contents with 70% acetone indicated that gallic acid was not bound to these components suggesting that gallic acid was being rapidly degraded in the rumen, presumably by the rumen microorganisms. These data were consistent with our *in vitro* studies using the RUSITEC. Murdiati et al (1992) published similar results shortly after completion of this work, also showing that gallic acid undergoes rapid microbial degradation in the rumen of sheep.

Prolonged oral administration of gallic acid to sheep (55 g gallic acid /kg diet dry matter for 14 days) had no adverse effects on the health of the animals, nor did it reduce feed intake. At no time during the 14 day trial period was gallic acid detectable in rumen or duodenal contents, whole blood, urine or faeces and no breakdown products of gallic acid were observed. This is perhaps surprising as Murdiati et al (1992) reported that gallic acid is metabolised in sheep to methyl gallate, pyrogallol, resorcinol and phloroglucinol which they detected in blood and urine. Despite this, all evidence suggested that gallic acid was being rapidly metabolised in the rumen and was therefore unlikely to reach the lower digestive tract.

Commercially available tannic acid is not a pure compound, rather it is a heterogeneous substance which makes it somewhat more difficult to quantify. However, using the total peak area from HPLC profiles, some of the components of this mixture of compounds appeared to be resistant to rumen degradation in *in vitro* studies with RUSITEC. There were however indications that appreciable quantities were degraded in the rumen, as reported by Murdiati et al (1992) who found that the degradation product of tannic acid was gallic

acid which was then metabolised further. Some changes in rumen fermentation were also observed in the presence of tannin acid, with a reduction in dry matter (DM) losses and a small increase in the acetate:propionate ratio.

Administration of 30 g tannic acid directly into the rumen of 3 fistulated sheep indicated a half life of approximately 2 ± 0.5 h, considerably longer than that of rutin or gallic acid. This suggests that the resistance to rumen degradation may possibly be related to the molecular weight of the phenolic compounds. Gallic acid has the lowest molecular weight (168 g/mole) followed by rutin (668 g/mole) and tannic acid (approximately 1700g/mole) and the estimated rumen half lives increased with increasing molecular weight (Table 1).

The conclusion at this stage of the project was that the fate of most low molecular weight phenolics, when presented in their 'pure' form to the rumen environment, is rapid microbial degradation. However, the possibility that this may not be the case for phenolics when associated with other components of the plant cell was investigated by incubating samples of plant material in Dacron bags in the rumen of sheep. Ornamental chestnut leaves (*Castanae* spp) were found to contain rutin at levels of 30-35 g/kg DM while oak leaves were found to contain appreciable quantities of a wide range of phenolic-like compounds. Phenolic loss from plant material was found to occur at a rate approximately 3 times that of DM loss. The rate of phenolic and DM loss from oak leaves during the first 6 h of *in sacco* incubation was 9.45 ± 0.05 %/h and 3.76 ± 0.34 %/h respectively. Incubation of ornamental chestnut leaves containing 15 g rutin/kg DM indicated that after 36 h, 27.7 ± 3.1 % of DM and 94.3 ± 2.4 % of rutin had disappeared from the leaves. These data suggest that phenolics in plant material have a similar fate to that of the pure compounds.

Degradation by rumen microorganisms of the phenolic compounds in *Sesbania spp* and also some Nepalese oak species (*Quercus lamellosa* and *Quercus semicarpifolia*) was also investigated which again suggested that the pure compounds were behaving in a similar fashion to phenolics present in plant extracts.

Attention was then turned to quebracho tannin, a highly polymerised condensed tannin obtained from the quebracho tree. Recent evidence in the literature suggested that this tannin may survive passage to the lower digestive tract of ruminants as considerable quantities had been reported to be recovered in the faeces of deer and sheep (Hagerman et al, 1992). Quebracho tannin was obtained from Hodgesons Chemicals Ltd, Beverly, Hull. This was an extract of the heartwood of the quebracho tree which had been treated with metabisulphite, oxalic acid and EDTA to improve its solubility and colour for use in the leather industry. Infusion of quebracho tannin into RUSITEC at a concentration of 125 mg/h for 40 h, indicated spontaneous degradation occurred at a rate of 3-5 %/h. There was little evidence for binding of quebracho tannin to feed material or for microbial degradation in the rumen. Quebracho tannin was still readily detectable in the RUSITEC contents after 40 h suggesting that quebracho tannin may well survive rumen fermentation

and thus reach the lower gastrointestinal tract. Some evidence was obtained that quebracho tannin alters rumen fermentation as the total concentration of volatile fatty acids was reduced (from 47.1 ± 5.1 to 36.7 ± 5.8 mol/l, $P<0.01$) and changes in the molar proportions of volatile fatty acids were observed (an increase in acetate and a reduction in propionate). This could be due to inhibition of microbial activity as suggested by other workers (eg Bae et al, 1994) but it is also possible that the quebracho tannin contained other fermentable substrates such as carbohydrates which were preferentially fermented by the rumen microorganisms.

Administration of single dose (6 g) of quebracho tannin directly into the rumen of 4 fistulated sheep along with the liquid phase marker, CrEDTA indicated that the average half life of quebracho tannin in the rumen was in the order of 11.0 ± 3.15 h while that of Cr was 16.25 ± 5.16 h. Thus, although the half life of quebracho tannin was less than the liquid phase outflow from the rumen, it was 5-50-fold greater than those observed for the other phenolic compounds investigated. This indicated that quebracho tannin is not as rapidly bound or microbially degraded as rutin, gallic acid or tannin acid and raised the possibility that some of it at least may reach the lower gastrointestinal tract.

Continuous administration of quebracho tannin directly into the rumen of 4 sheep for 7 days confirmed this suggestion. The total daily input of quebracho tannin was 50 g per animal which was approximately equivalent to 4 % diet DM. Quebracho tannin in the liquid phase of duodenal digesta increased steadily over the first 48 h of infusion and then appeared to plateau or to increase more slowly (Fig 1). Maximal concentrations reached were in the order of 0.3 - 0.4 mg/ml. Using an estimate of daily duodenal liquid flow, the total quantity of quebracho tannin reaching the duodenum in free form was calculated and estimated to represent 10-20% of total daily input. The quantity of quebracho tannin associated with the solid phase of duodenal digesta was not determined but approximately 45% of the total daily input was estimated to be excreted in the faeces indicating a significant proportion was probably associated with feed particles and microbial matter. Hagerman et al (1992) reported that 60% of orally administered quebracho tannin was recovered in the faeces of sheep and 100% in deer.

(b) Feeding of Quebracho tannin to sheep

As a substantial quantity of intraruminally administered quebracho tannin appeared to survive rumen microbial degradation to reach the lower gastrointestinal tract and eventually be recovered in the faeces, the effects of prolonged oral administration of quebracho tannin on diet digestibility and gut morphology was investigated in young sheep.

Twenty six weaned ewe lambs (27.3 ± 1.6 kg) were introduced to a diet of dried grassmeal pellets and fed at a level sufficient to maintain a daily liveweight gain (DLWG) of approximately 100 g/d (MAFF Technical Bulletin 33). Animals were then paired according to weight. One animal from each pair was maintained on

the basal diet (control animals) while the other animal was fed the same diet but with quebracho tannin included at 5 % of DM (tannin animals). Assuming the quebracho tannin had no nutritional value, the tannin animals were fed 5% more DM in order to maintain the quantity of grassmeal fed and thus intakes of nitrogen (N) and energy approximately constant. The diets were fed for 8 weeks. Animals were weighed twice weekly during this time and the quantity of feed adjusted after each weighing. Daily liveweight gains ranged between 70 and 155 g/d and tended to be about 10% lower in the tannin-fed animals (mean 109.5 g/d for tannin-fed animals, 122.2 g/d for controls, n=22, P>0.10, Table 2). No food refusals were observed on either diet suggesting that quebracho tannin did not depress food intake as reported for some other tannins. However it should be noted that the animals were fed at a restricted level and were not fed to appetite.

The effects of quebracho tannin on N balance and diet digestibility were determined on 6 pairs of animals after 2 weeks and again after 6 weeks. These animals were transferred to metabolism crates and allowed to acclimatize for 3 days. Total urine and faeces production was then collected in 12 h batches for the next 7 days and stored frozen at -20C before analysis.

The effect of quebracho tannin was similar at both periods with the animals fed the tannin diet excreting 10-13% more faeces (P<0.10) and 36-46% less urine per day (P<0.10)) than the controls (Table 3). Faecal dry matter excretion was also 14-22% higher in the tannin-fed animals (P<0.001) which was due to reduced DM digestibility (61.6% compared with 67.1% for the controls, P<0.001). Neutral detergent fibre (NDF) digestibility was also significantly (P<0.001) reduced by quebracho tannin (Table 4) which is probably due to inhibition of cellulose digestion by the rumen microbes, an effect shown by other workers (Bae et al, 1994; Muhammed et al, 1994) for both quebracho tannin and tannic acid. DM disappearance from the rumen has also been shown to be inhibited by some condensed tannins, including quebracho tannin (eg Bae et al, 1994). The effect of quebracho tannin on N metabolism is shown in Table 5. Again the effects of quebracho tannin were similar at both week 2 and week 6. Overall, animals fed the tannin diet excreted significantly less N in urine (P<0.01) and more N in the faeces (P<0.001) than the control animals. However overall N balance was not significantly different between the two groups of animals although apparent N digestibility was significantly (P<0.001) reduced in the tannin diet. Similar results were reported by Vallet et al (1994) with rats fed grape seed tannins at 2 g/100 g (see also results of our rat trial below). This suggests that both quebracho tannin and grape seed tannins alter the nitrogen excretion pathway, with a shift from urine to faeces, indicating reduced tissue protein metabolism. However, as N retention was similar in both groups of animals (in both studies), this suggests that the animals fed the tannin-containing diet use the protein they do receive more efficiently. This could be due to the tannin binding to specific proteins, thus making different (and possibly better quality) proteins available to the animal. Other reports have indicated that tannin-protein interactions are specific for different tannins and for different proteins (Asquith & Butler, 1986). Alternatively, if the tannin is

taken up into cells of the body (see below), it is possible that the tannin may alter the rates of protein synthesis and degradation. The faecal N excretion measured in the present study took no account of endogenous N excretion which might be expected to be higher in the tannin-fed animals (Jansman et al, 1993; Vallet et al, 1994). The results of Vallet et al (1994) indicated that true nitrogen digestibility (taking account of faecal endogenous nitrogen) was still significantly lower in the rats fed the tannin diet compared to controls. However, from their results, if faecal N excretion is corrected for endogenous N content (5.8 mg/d in control rats, 17.4 mg/d in tannin-fed rats), the amount of dietary N retained increases from 148 to 154 mg/d in the control rats while in the tannin-fed rats, N retained increases from 142 to 159 mg/d suggesting a slightly improved N retention in the tannin-fed rats compared to the controls.

Interestingly, in the present study, digestibilities of DM, N and NDF were all increased at week 6 compared to week 2 for both control and tannin diets (Tables 3, 4 and 5) which resulted in significant ($P<0.01$) differences between the two measurement periods. This would indicate improved nutrient utilisation by animals in both groups with continued feeding of the grassmeal diet but no adaptation to the presence of quebracho tannin. Approximately 60% of the measured amount of quebracho tannin in the feed was recovered in the faeces at both week 2 and week 6 (Table 6). This is similar to the recovery reported by Hagerman et al (1992) for sheep.

Two pairs of animals were slaughtered after 2, 5 or 7 weeks and samples of gastrointestinal tract (duodenum to terminal ileum) were taken at 10 cm, 50 cm, 100 cm and 1-5 m intervals from the abomasal valve, at unusual appearing areas and at Peyers patches and were examined for morphological changes, erosions, lesions etc in the Department of Histopathology in the University of Nottingham Medical School. Samples from the 6 control and 6 tannin animals were stained with haematoxylin and eosin and examined. There appeared to be some variability in the response to tannin but overall, there was evidence for focal surface epithelial ulceration in the jejunum and ileum of 2 of the 6 treated animals, but in none of the controls. Surprisingly, there was little evidence of any morphological changes in the duodenum of treated animals, although there was an increase in the number of mucosal histiocytes in this region in some of the treated animals. Mucosal histiocytes were increased in the jejunum and ileum of all the treated animals and there was also an increase in the number of histiocytes overlying the Peyers patches. It was concluded that the tannin appears to be locally toxic to surface epithelium and specialised epithelium overlying Peyers patches leading to epithelial degeneration and ulceration. It was thought that this may be an early feature of the response and may depend on local concentration. It would appear that the tannin is probably phagocytosed by lamina propria macrophages and persists there for some time. Some of the macrophages formed aggregates (either superficial or related to crypts) and some formed granulomas. Tannin also appeared to be taken up, possibly selectively, by Peyers patch M cells. This could lead to an immune response. In some animals there appeared to be local mucosal damage (mild partial villous atrophy, fusion of villi) associated with macrophage

accumulation. Other workers have also shown that tannins affect the functional state of the intestinal epithelium (eg Mitjavila et al, 1977) and in some cases the tannin has been shown to be absorbed from the gastrointestinal tract, with degradation products appearing in blood, urine and faeces (Das, 1971). Other studies have also suggested that condensed sorghum tannins and their metabolites cross the intestinal barrier (Sell & Rogler, 1983; Butler et al, 1986). The implications of damage to the intestinal epithelium include reduced nutrient absorption and/or increased endogenous protein losses either through binding to digestive enzymes or through cell sloughing. Vallet et al (1994) reported that the activity of alkaline phosphatase and sucrase was reduced at the villus tip and along the length of the villus-crypt axis of the jejunum in rats fed 2% grape seed tannins which they interpreted as evidence for a tendency for villus erosion and which presumably contributed to the increased faecal endogenous N losses observed. This effect was accompanied by an increase in ^{3}H-thymidine incorporation in the middle of the crypt zone which may suggest a stimulation in the rate of enterocyte proliferation in the tannin-fed animals. Interestingly, these authors observed no effect of the tannin on alkaline phosphatase activity in the duodenum.

(c) Effects of quebracho tannin on the tissue metabolism

As it had been shown that quebracho tannin escaped rumen degradation and affected diet digestibility and the gut epithelium of sheep, attention now turned to investigating the effects of this material on tissue metabolism. Tissue metabolic studies using ruminants are expensive and take considerable resource. There was no reason to believe that the response of the tissues to the tannin would differ greatly between sheep and the rat. The rat was therefore used as a model for this section of the work.

The effects of feeding quebracho tannin on protein synthesis in the intestinal mucosa of young rats was investigated. Weanling Wistar rats (initial liveweight 63.7 ± 10.0g) were housed in individual metabolism cages and fed *ad libitum* a ground rat chow. After 7 d acclimatization, the rats were randomly allocated into two groups and fed the basal diet containing either quebracho tannin at 4% diet DM (tannin group) or 4% cellulose (controls). These diets were fed for 22 d. Feed intakes and animal weights were recorded every second day. Complete collections of urine and faeces were made on twenty randomly selected animals (10 control and 10 tannin) between days 15 and 21 of feeding these diets in order to determine the effect of the tannin on N balance when animals were given free access to feed. After 22 days of feeding the diets, 10 control and 10 tannin fed animals were randomly selected and administered a flooding dose of [^{3}H]-phenylalanine (2.5 MBq/ml/100g body weight in 150 mmol/l unlabelled phenylalanine) via the tail vein in order to determine the fractional rate of protein synthesis in the gastrointestinal tract mucosa. Gut mucosa was rapidly (within 3 min of death) scraped from the upper 30 cm of the gastrointestinal tract and frozen in liquid N_2 .Fractional rates of protein synthesis (FSR) were determined according to the method of Garlick et al (1980). Average protein FSR was 235 %/d for the control animals and 228 %/d

for the tannin-fed animals indicating no significant difference in the rate of protein synthesis in gut mucosa as a result of quebracho tannin ingestion ($P>0.10$; Table 7). These values are comparable to other values reported by other workers using the flooding dose method in rats (182%/d Haji Baba 1991; 136%/d, McNurlan et al, 1979). Unfortunately this experiment was undertaken before the results of the effect of quebracho tannin on sheep gut morphology were received. In the light of these results, it is possible that any effects of quebracho tannin on the rate of protein synthesis may be more likely to be detected lower down the gastrointestinal tract as no evidence of morphological damage was observed in the duodenum of sheep, but changes were observed in the jejunum and ileum. Gut length was 126.6 cm for control rats and 119.1 cm for tannin-fed rats ($n=10$ per group). Final liveweights of the tannin-fed animals were however significantly ($P<0.05$) lower than those of the controls (201.2 g and 216.7 g respectively, Table 8) and gut length was not different when expressed as a proportion of body weight. Voluntary food intake was significantly ($P<0.01$) lower in the tannin-fed animals (20.5 g/d compared with 21.8 g/d) which must at least partly have contributed to the reduced growth rate of these animals. Feed/gain ratios were, however, not significantly different from control animals ($P>0.10$).

The weight of three hind limb muscles (gastrocnemius, soleus and plantaris) dissected from the tannin-fed animals were similar to those of controls when expressed as a proportion of final body weight (Table 9). The weights of two dissected fat depots (perirenal and epididymal) were however significantly ($P<0.001$) lower in the tannin-fed animals as a proportion of final body weight: perirenal fat comprised an average of 212 mg/100 g body weight in control animals, but only 116 mg/100 g body weight in tannin-fed animals, while the epididymal fat comprised 264 mg/100 g in control rats and 203 mg/100 g in tannin-fed rats (Table 9). This reduction in body fat content could be due to reduced feed intake, or could indicate a direct effect of the tannin on fat deposition. Poor utilisation of ingested nutrients would also result in less energy available for fat deposition. However, Barry et al (1986) reported that adipose tissue from sheep fed condensed tannins had consistently higher rates of lipolysis with a consequent reduction in the lipogenesis:lipolysis ratio which supported earlier data from Purchas and Keogh (1984) suggesting that lambs fed fresh forages containing high levels of condensed tannins have a lower carcass fat content.

Nitrogen balance data indicated that the rats fed quebracho tannin had reduced overall N retention (0.199 g/d for control animals. 0.178 g/d for tannin-fed animals; $P<0.025$; Table 10). As with the sheep, urinary N excretion was significantly ($P<0.001$) reduced in the tannin-fed animals compared to the controls while faecal N excretion was significantly ($P<0.001$) increased. The reason for the difference in the effect of quebracho tannin on N balance in the rats and in the sheep was possibly due to differences in level of feeding: the rats were fed to appetite while the sheep were fed at a restricted intake. N digestibility was again significantly ($P<0.001$) reduced on the quebracho tannin diet (63.8% compared with 71.9% on the control diet).

4. Conclusions

A series of *in vitro* and *in vivo* studies has demonstrated clearly that not all phenolic compounds ingested by ruminants survive passage through the rumen. The extent of this degradation is probably at least in part related to the molecular weight of the compound. With the low molecular weight compounds there was little evidence that they had any deleterious effects upon rumen fermentation and they were rapidly catabolized by the rumen micro-flora. This catabolism was also seen when low molecular weight phenolics were present in plant material.

Quebracho tannin was used as a model for higher molecular weight tannins. When fed to sheep it was shown to reduce digestibility of a grassmeal diet . The tannin also modified the concentrations of volatile fatty acids in the rumen, probably as a result of modifying the digestibility of the diet. Substantial amounts of the ingested material escaped rumen degradation. Examination of the intestinal tract (duodenum to the terminal ileum) yielded evidence of focal surface epithelial degeneration in the jejunum and ileum. Damage to the specialised epithelium overlying Peyers patches was also evident. The tannin also appeared to be taken up by Peyers patch M cells, which coupled with other changes to the gut wall could be indicative of a modification of the immune competence of the animal. This could influence the susceptibility of the animal to infection such as to parasites. Recent work by Waghorn et al (1994) suggested that sheep fed diets containing condensed tannins had lower numbers of gastointestinal nematodes. However this effect may not be due to the tannins *per se*.

To investigate the effects of dietary quebracho tannin on the tissue metabolism the material was fed to rats. Again, digestibility of nitrogen in the diet was reduced. Body weight gain was reduced although the weight of the three different muscles examined as a proportion of body weight was not reduced. There was however a marked reduction in body fat. Surprisingly, no effect on the fractional synthetic rate of the protein in the mucosa obtained from the first 30cm of the duodenum was detected.

There is thus a *prima facia* case that some tannins when ingested by ruminants have marked effects both on rumen and digestive function and on tissue metabolism.

5. Publication and dissemination of results

The findings have been communicated to 2 conferences, one of which specifically dealt with tropical and sub-tropical agriculture (Parrinder et al, 1992, 1993) and at least 2 seminars at NRI. Formal publications describing the results and conclusions are being prepared.

Frequent review meetings have been held with NRI staff who have then travelled overseas and communicated the results to extension workers etc.

6. Future Work

1. Prepare the results in a form for publication in Journals which are read by those interested in feeding livestock in the tropics and subtropics. It may also be necessary to produce a modified version of this report for use by NRI overseas.

2 Now that a series of effects of quebracho tannin has been established, it is necessary to see if similar effects are seen when plant materials containing similar tannin compounds are fed. These studies should ideally be undertaken in the tropics and subtropics. The profile of tannins in the plant materials used needs to be analyzed to coordinate them with those described in this report. The results of these studies will enable extension workers to give appropriate advice to farmers.

3. The effect of tannins on the immune competence of ruminants needs to be studied. Alteration in the immune competence could make the animals more susceptible to parasitic and other infections. In contrast to this there is some evidence from other published work that feeding of tannin containing plant materials can reduce the susceptibility to parasitic infection (Waghorn et al, 1994). It is not known if the tannins *per se* were responsible for this or whether the effect was due to other plant constituents.

4. The possibility of reducing the various effects of tannins by supplementing the diet with extra protein needs to be investigated. It is accepted however that supplementation of diets with extra protein may not always be feasible in practice.

5. Fundamental studies on the effects of specific tannins on the metabolism of ruminants should continue. The results of such work will greatly enhance the effectiveness (both in terms of design and interpretation) of more practical studies in the field with tannin-containing plant materials.

7. Authors of report

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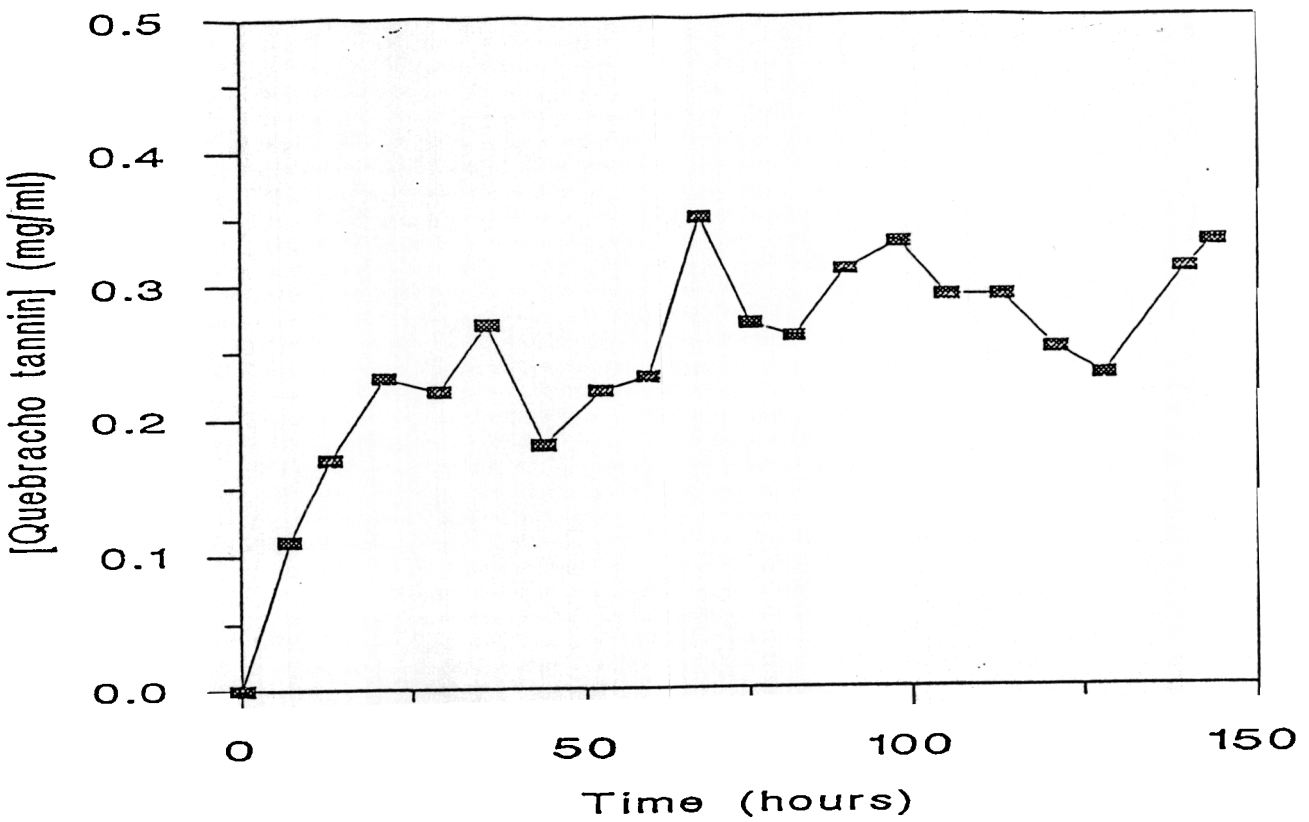
Figure 1

A typical build-up of quebracho tannin concentration in the liquid phase of duodenal digesta of one sheep during a continuous intraruminal infusion of the tannin.



Quebracho tannin was continually infused at a rate of approximately 2 g/h into the rumen of 4 fistulated sheep for 7 days. Samples of duodenal digesta were obtained from a duodenal T-piece cannula 4 times daily. The liquid phase was separated by centifugation at 25 000 x g for 20 min and assayed for quebracho tannin content by the acid butanol method. The above figure shows results of a single, representative animal.

TABLE 1 Half lives of gallic acid, rutin and tannin acid in the rumen of sheep compared to that of chromium.

	Half life (h)	
	Chromium	Phenolic
Gallic acid	9.95 ± 2.69	0.21 ± 0.06
Rutin	10.11 ± 2.39	0.45 ± 0.05
Tannic acid	14.4 ± 0.35	2.0 ± 0.50

Each phenolic was administered directly into the rumen of 3 or 4 fistulated sheep along with the liquid phase marker CrEDTA. Samples of rumen fluid were removed at intervals for up to 24 h after administration and the concentration of chromium and phenolic was measured. Half lives were determined from the natural log plot of concentration against time. The half life of chromium gives an index of liquid phase outflow from the rumen.

TABLE 2 Initial weights and growth rates of sheep fed a dried grass diet with or without quebracho tannin.

	CONTROL	TANNIN	Pooled SED	Significance (P)
Average initial liveweight (n=26) (kg)	27.42	27.19		
Average initial liveweight (n=12) (kg)	26.99	27.03	1.049	0.969
DLWG (g/d) (n=22)	122.2	109.5	8.89	0.168
DLWG (g/d) (n=12)	119.8	112.1	13.6	0.588
Liveweight at start of digestibility period 1 (n=12) (kg)	29.14	29.30	1.109	0.889
Liveweight at start of digestibility period 2 (n=12) (kg)	31.08	31.09	1.174	0.989

Sheep were fed either pelleted grassmeal alone (controls) or containing 50 g quebracho tannin/kg diet dry matter.
Liveweights were measured twice weekly for 56 days and daily liveweight gain (DLWG) estimated from regression analysis of these data. Initial liveweights were those measured on the first day of feeding the tannin-containing diet (day 0). Initial weights and daily liveweight gains are given for all animals but also for those 12 animals (6 control and 6 tannin-fed) which were used for digestibility studies in order that these data may be related directly. Four animals were slaughtered after 2 weeks of feeding the diets for gut histology studies. These animals have not been included in DLWG estimates.

TABLE 3 The effect of feeding quebracho tannin on faeces and urine production and on DM digestibility in young sheep

	CONTROL	TANNIN	Pooled SED (20 df)	Significance (P) Diet	Period	Diet x Period
Dry matter intake (g/d)						
Week 2	818.8	839.6				
Week 6	898.5	893.7	18.81	0.553	<.001	0.349
Urine volume (litres/d)						
Week 2	2.905	1.861				
Week 6	4.275	2.326	1.068	0.061	0.239	0.556
Faeces wet weight (g/d)						
Week 2	913	1005				
Week 6	923	1048	83.2	0.079	0.662	0.781
Faecal dry matter (g/d)						
Week 2	288.2	329.8				
Week 6	275.2	334.7	12.28	<.001	0.644	0.315
DM digestibility (%)						
Week 2	64.84	60.70				
Week 6	69.30	62.55	1.246	<.001	0.002	0.154

Sheep were fed either pelleted grassmeal diet alone (control) or containing quebracho tannin at 50 g/kg diet dry matter (tannin) at a level calculated to achieve 100 g liveweight gain per day. Complete 7-day collections of urine and faeces were made on 12 sheep (6 controls and 6 tannin-fed) after 2 and 6 weeks of feeding the diets. Results are expressed as the average daily excretion rates for each of these periods. Dry matter intakes are presented for the same 12 animals over the same periods. DM digestibility was calculated as (ingested-faecal)/ingested.

TABLE 4 The effect of quebracho tannin on neutral detergent fibre (NDF) digestion in young sheep.

	CONTROL	TANNIN	Pooled SED (20 df)	Significance (P) Diet	Period	Diet x Period
NDF intake (g/d)						
Week 2	397.9	404.4				
Week 6	436.6	430.5	9.09	0.982	<.001	0.337
Faecal NDF (g/d)						
Week 2	146.1	166.3				
Week 6	137.8	167.6	6.05	<.001	0.421	0.274
NDF digestibility (%)						
Week 2	63.30	58.84				
Week 6	68.40	61.08	1.33	<.001	<.001	0.146

Sheep were fed either pelleted grassmeal alone (controls) or containing 50 g quebracho tannin/kg diet dry matter (tannin). Complete (7 day) faecal collections were made on 12 sheep (6 controls and 6 tannin-fed) after 2 and 6 weeks of feeding the diets. Results are expressed as average daily intake and excretion of NDF. NDF digestibility was calculated as (ingested-faecal)/ingested.

TABLE 5 The effect of feeding quebracho tannin on N balance and N digestibility in young sheep

	CONTROL	TANNIN	Pooled SED (20df)	Significance (P) Diet	Period	Diet x Period
N intake (g/d)						
Week 2	24.97	25.19				
Week 6	27.40	26.81	0.568	0.645	<.001	0.329
Urine N (g/d)						
Week 2	9.24	7.75				
Week6	11.70	8.78	0.979	0.005	0.020	0.311
Faecal N (g/d)						
Week 2	8.28	9.69				
Week 6	7.86	9.60	0.491	<.001	0.473	0.641
N retained (g/d)						
Week 2	7.45	7.74				
Week 6	7.84	8.43	1.065	0.562	0.485	0.842
Apparent N digestibility (%)						
Week 2	66.88	61.50				
Week 6	71.28	64.18	1.775	<.001	0.011	0.501

Sheep were fed either pelleted grassmeal alone (controls) or containing 50 g quebracho tannin/kg diet dry matter (tannin).
N intake and N excretion in urine and faeces were made on 12 sheep (6 controls and 6 tannin-fed) after 2 and 6 weeks of feeding the diets. Complete collections of urine and faeces were made for 7 days at each period. Results are expressed as average intakes and excretion over the 7 days and N retention was calculated from the difference between intake and excretion each day and averaged over the 7 day period. Apparent N digestibilities were calculated as (ingested-faecal)/ingested.

TABLE 6 Proportion of ingested quebracho tannin recovered in faeces of sheep.

	*Ingested (g/7d)	Faecal (g/7d)	% excreted
Week 2	133.5	79.4	59.4
Week 6	142.1	82.0	57.6

Total feed intake and total faecal collections were made on 6 sheep fed the tannin-containing diet after 2 weeks and after 6 weeks of feeding. Quebracho tannin was incorporated into the diet at a level of 5 g/100 g diet dry matter. However only 2.27 g quebracho tannin/100 g diet dry matter was measurable in the diet after extraction with 70% acetone although 100 ± 2% was recoverable when quebracho tannin was added to the control diet and extracted in the same way. This suggests that either after prolonged storage, some of the quebracho tannin becomes bound to feed material in such a way that is not extractable by the usual method, or that some of the tannin was destroyed by heat (and/or moisture) in the pelleting process.

*Ingested quebracho tannin was calculated assuming 2.27 g/100 g diet dry matter.

No quebracho tannin was detected in urine.

TABLE 7 Effect of quebracho tannin on the fractional rate of protein synthesis (%/day) in the gastrointestinal tract of young rats.

CONTROL	TANNIN	Pooled SED (18 df)	Significance (P)
235	228	33.2	0.836

Rats were fed a ground rat chow containing either 4% cellulose (controls) or 4% quebracho tannin for 22 days. Protein synthesis was then measured in the gastrointestinal mucosa of 10 control and 10 tannin-fed rats, following a flooding dose of [^{3}H]-phenylalanine as described by Garlick et al (1980).

TABLE 8 Effect of dietary quebracho tannin on liveweight, feed intake and feed conversion efficiency in young rats.

	CONTROL	TANNIN	Pooled SED (34 df)	Significance (P)
Initial liveweight (g)	102.5	98.0	3.84	0.254
Final liveweight (g)	216.7	201.2	6.00	0.014
DLWG (g)	5.34	4.86	0.14	0.001
Average feed intake (g/d)	21.76	20.51	0.44	0.008
Feed/gain	4.322	4.464	0.096	0.148

Rats were fed a ground rat chow containing either 4% cellulose (controls) or 4% quebracho tannin for 22 days.
Animals were weighed every 2-3 days and daily liveweight gain (DLWG) was estimated from regression analyses of these data. Feed intakes were measured daily. Average feed intake was calculated from (total consumed over the trial period/22).
Feed/gain ratio was estimated for each animal from total feed consumed and actual weight gained over the 22 day trial period.

TABLE 10 Effect of quebracho tannin on nitrogen balance in young rats

	CONTROL	TANNIN	Pooled SED	Significance (P)
N intake (g/d)	0.583	0.558	0.017	0.159
Urinary N excretion (g/d)	0.221	0.177	0.006	<0.001
Faecal N excretion (g/d)	0.163	0.203	0.004	<0.001
N retained (g/d)	0.199	0.178	0.009	0.023
Apparent N digestibility (%)	71.87	63.77	0.811	<0.001

Rats were fed a ground rat chow containing either 4% cellulose (controls) or 4% quebracho tannin for 22 days.
Complete collections of urine and faeces were made over a 7 day period between days 15 and 21 of feeding the the control or tannin-containing diets (n=10 per group). These samples were analysed individually for N content and nitrogen retained was determined from the difference between N intake and N excretion on a daily basis and then averaged. Residual degrees of freedom for these data= 132.
Apparent N digestibility was calculated for each animal from mean (7 day) (ingested N-faecal N)/ingested N . Residual degrees of freedom=18.