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Pathological Features in Sheep Fed Rations Containing Phenolsand Condensed Tannins

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Abstract: A study was carried out to investigate the effects of feeding low quality non-conventional feeds (NCFs) containing phenols and condensed tannins on the pathological features of sheep. Thirty-two sheep were fed one of 4 ration combinations of 2 roughages, urea treated palm frond and Rhodesgrass hay and 2 concentrates, commercial concentrate and by-products concentrate for 120 days. Non-conventional feeds (palm frond and by-product concentrate) contained higher levels of phenols and condensed tannins than conventional ones (Rhodesgrass hay and commercial concentrate). Villus height and height: Crypt depth ratio were significantly lower in animals on the NCFs. There were signs of chronic inflammation in the intestine; the kidneys showed signs of nephritis and the liver showed congestion and hemorrhagic changes. This experiment indicated that feeding non-conventional feeds containing phenols and condensed tannins for extended periods may produce subtle negative effects on animal health and production.

Key words: Non-conventional feeds, tannins, phenols, pathology, sheep

INTRODUCTION

In many parts of the world, especially the arid and semi-arid regions with scarce animal feed resources, low quality non-conventional feeds (NCFs) are frequently fed to livestock. Although, some may be a good source of protein such as *Prosopis* sp. (Mahgoub *et al*., 2004), these feeds usually contain high fiber but low mineral and vitamin contents. They may also contain secondary plant compounds which may have anti-nutritional effects in farm animals. These include: Non-protein amino acids, memosine, indospecine, glycosides (cyanogens and saponins), phytohemagglutinins, polyphenolic compounds (tannins and lignins), alkaloids, triterpenes and oxalates (Kumar, 2000).

Anti-Nutritional Factors (ANFs) such as condensed tannins protect plants from degradation in the rumen. They are reported to form combinations with proteins in the rumen rendering them unavailable for digestion and consequently increase their output in faeces (Robins and Brooker, 2005). In non-ruminants, they interfere with nutrient digestion and promote excretion of endogenous nitrogen through formation of tannin-enzyme complexes (Margaret and McNab, 1991). Antinutritional factors may also affect lipid metabolism by promoting excretion of bile acids and by decreasing the activity of the digesta lipase (Margaret and McNab, 1991). Tannins inhibit enzymes such as dipeptidase and disaccharidase in the intestinal mucosa of birds (Ahmed *et al*., 1991). Therefore, feeding high levels of NCF may lead to malnutrition in animals especially if fed for extended periods.

Feeding farm animals some rangeland plants containing high levels of anti-nutritional compounds such as *Prosopis juliflora* produced detrimental effects on animal health (Tabosa *et al*., 2000). High levels of tannins and saponins may cause tissue and animal death (Adedapo *et al*., 2005). There are some reports that they might also cause physical damage to the digestive system (Walton *et al*., 2001). Condensed tannins resulted in marked histological effects in sheep including a marked increase in villous height and crypt dept in the abomasum and intestine as well as discrete areas of tissue fragility (Robins and Brooker, 2005). Because of the tannins affinity to proteins, they might bind proteins from feed as well endogenous proteins (Van Leeuwen *et al*., 1995) such as those of the digestive tract thus causing structural

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damage to these organs. However, effect of ANFs in NCFs on body organs other than the digestive track has not been thoroughly investigated. This study aimed to investigate the pathological features of feeding non-conventional feeds on the intestine, kidney and liver of sheep.

MATERIALS AND METHODS

Thirty two, 1-year old male Omani native sheep (BW 32.5±1.13 kg) wereused in a 120-d feeding trial with a 2 × 2 completely randomized factorial experimental design including 2 types of roughages Rhodesgrass hay (RGH) and urea-treated palm fronds (UTPF) and 2 types of concentrates commercial concentrate (CC) and by-product concentrate (BC). The BC contained 25% ground palm fronds, 15% barley, 20% Meskit pods, 15% dried sardine fish, 22% wheat bran, 1% of each ground limestone, vitamin mineral mix and salt. The four diet combination groups were: RGH plus CC; RGH plus BC; UTPF plus CC; UTPF plus BC. Animals were daily fed 450 g of the concentrate plus *ad libitum* roughage. Water and mineral blocks were offered *ad libitum*. Ration formulation and chemical composition of experimental diets has been reported (Mahgoub *et al*., 2007).

The proximate chemical composition of the various components of feeds and faeces were determined according to the standard methods of AOAC (2000). Gross Energy (GE) was measured using a bomb calorimeter. Levels of phenols and condensed tannins were analysed following the methods of Makkar (2003). Condensed tannins were determined by extracting 200 mg samples overnight in 10 mL aqueous acetone (70: 30 acetone: water) solution at 4°C. after centrifugation (3000×g at 4°C for 10 min), the supernatants were analysed for condensed tannins using leucocyanidin standard. Total extractable phenols expressed as gramme equivalent tannic acid per kg dry matter. Extractable condensed tannins expressed as gramme equivalent leucocyanidins per kilogramme dry matter.

At the end of the experiment, the animals were slaughtered and the duodenum, liver and kidney were removed within 30 min of slaughter, cleaned and fixed in 10% buffered formalin for at least 48 h. Fixed samples were trimmed, processed, sectioned and stained with hematoxylin and eosin and special stain (Congo red stain). Morphometry measurements were taken on the duodenal sections of stained slides. Digital images of tissues were taken using Olympus DP70 attached to the microscope. Villus height, crypt depth and epithelium width measurements of small intestinal were taken using Analysis 5, Life Science Soft Image System. Five villus height and width measurements were taken per sample.

For electron microscopy, samples were collected in fresh Karnovesky's fixative (2% gluteraldehyde and 4% paraformaldehyde containing 1M cacodylate buffer) and fragmented under stereomicroscope using razor blade in fresh Karnovesky's fixative for 2 h at 4°C. Specimens were washed in three, 10 min changes of 1M cacodylate buffer and a secondary fixation was carried out in osmium Tetroxide then washed in 3 changes of 10 min in 1M cacodylate buffer twice. The samples had 3 changes of absolute ethanol to ensure complete removal of water. En bloc blocks were then infiltrated with graded ethanol/ araldite epoxy resin dilution of 1:1, 1:3 and 100% araldite resin for 1 h in each change. Further 2 changes in fresh 100% araldite resin were carried out for 1 h each. The samples were then embedded in fresh resin and polymerized at 60°C for 18 h. Ultra-thin sections, 60-90 nm, of the embedded samples were microtomed using a diamond knife and a Leica ultracult UCT ultramicrotome and stained with aqueous uranyl acetate for 30 min and lead citrate for 15 min. The stained sections were examined using Jeol Jem-1230 transmission electron microscope equipped with Gatan 792-CCD camera operated at 100 k V. Data were subjected to the analysis of variance to study effects of diet (SAS, 1991). Significant differences between treatment means were assessed using Least Significant Difference procedures.

RESULTS AND DISCUSSION

Feeds and animal performance: The details of feed formulation and chemical analyses have been described (Mahgoub *et al*., 2007). The by-product concentrate and urea-treated palm fronds contained higher (57 and 112.6 g kg^{-1}) total extractable phenols than the commercial concentrate and Rhodesgrass hay (16.6 and 32 g kg^{-1}). They also contained higher condensed tannins (2 and 12.8 g kg^{-1} DM) than commercial concentrate and Rhodesgrass hay (zero tannins). The experimental feeds based on the urea treated palm frond, especially those contained the by-product concentrate, had lower digestibility coefficients for DM, CP, ADF, NDF, gross energy and ash than those contained in the commercial concentrate and the Rhodesgrass hay.

Animals fed the NCFs consumed less feed mainly due to lower intake of roughage (urea treated palm fronds) and they consequently gained less body weight during the experimental period especially for those fed the by-product concentrate plus the urea-treated palm frond. The roughage intake (g/d) was 680, 640, 600 and 560; the concentrate intake (g/d) was 430, 420, 420 and 430; the

Table 1: Morphometric measurements on the duodenum and kidneys of Omani sheep fed diets containing phenols and condensed tannin

	Diet					P-value significance			
Parameter	CC+RGH	BC+RGH	CC+UTPF	BC+UTPF	SEM	Diet	Roughage	Concentrate	Contrast BC+ UTPF vs. Others
Intestine morphometry (µm)									
Villus height	303a	293a	280a	219b	20	*	NS	NS	*
Crypt depth	252	264	391	315	73	NS	NS	NS	NS
Villus:crypt ratio	1.21a	1.10a	0.81c	0.73c	0.08	**	**	NS	*
Total height	553	554	668	530	65	NS	NS	NS	NS
Epithelium thickness	23	23	26	21	2	NS	NS	NS	NS
Kidney morphometry (µm)									
Number of nephrons	11.14	11.97	11.14	10.96	0.74	NS	NS	NS	NS
Capsule area (µm)	11900.61	11170.49	12270.54	10950.06	594.13	NS	NS	NS	NS
Nephron area (µm)	8925.25 a	8374.99 a	8823.66 c	7322.57 c	498.79	*	NS	NS	*

CC: Commercial Concentrate; BC: By-products Concentrate; RGH: Rhodesgrass Hay; UTPF: Urea-Treated Palm Frond SEM, Standard Error of Means; *, $p<0.05$; **, $p<0.01$; ***,$p<0.001$

average daily gain (g/d) was 80, 60, 30 and 10 for animals fed RGH plus CC; RGH plus BC; UTPF plus CC; UTPF plus BC, respectively (Mahgoub *et al*., 2007).

Morphometry of the gut: Condensed tannins are reported to bind to proteins, carbohydrates and mineral and inhibit microbial and digestive processes in ruminants and consequently increasing their passage rate in the faeces (Robins and Brooker, 2005). They may also bind to indigenous proteins of the alimentary tract (Van Leeuwen *et al*., 1995) and form protein/enzyme complexes (Margaret and McNabb, 1991). Therefore, their effects on sheep gut structure were to be investigated in the current study. Villus height and height: Crypt depth ratio were significantly lower in animals on BC and UTPF (Table 1). There was a trend of increasing crypt depth and epithelium thickness in the CC + UTPF diet group. These structural effects on the gut would result in functional effects such as digestion and absorption rate in these animals. Beside the marked increase in villous height and crypt dept in the abomasum and intestine, condensed tannins resulted in marked histological effects in sheep including discrete areas of tissue fragility (Robins and Brooker, 2005). However, more studies are needed to determine whether these effects observed in the current experiment are permanent or not.

Reports on effects of tannin-containing diets on the intestine are conflicting. For instance, Walton *et al*. (2001) found no effects of 5.5% condensed tannins *Lotus pedunculatus* on gut morphometry in sheep. However, their data indicated a trend of a decreasing villus height and villus surface area in the proximal duodenum similar to observations in the current study. Van Leeuwen *et al*. (1995) also reported no significant effects of feeding tannin-containing faba beans on villus height, crypt depth or villus: Crypt ratio in weaned pigs. Their findings however, indicated a trend of increasing villus height with increasing level of tannins. Feeding *Acacia aneura* caused elongation of duodenal and ileal villi which was reversed using the anti-tannin polyethylene glycol (Robins and Brooker, 2005). These conflicting reports might be attributed to differences in species and to the site of sampling. There is a wide variation in intestinal structure along the digestive tract. Villus height and crypt depth decreases from the proximal to the distal part of the small intestine (Van Leeuwen *et al*., 1995). The rate of turnover of intestinal epithelia is also high and would correct structural damage within 48-72 h (Robins and Brooker, 2004). Effects on intestine structure may not be directly caused by anti-nutritional factors but may probably be attributed to lower levels of nutrition or physical factors such as the coarse nature of the diet.

Light microscopy pathological findings: The duodenum of animals fed BC and UTPF showed signs of chronic inflammation characterized by severe infiltration with lymphocytes, plasma cells and few macrophages (Fig. 1a) indicating chronic infection probably due to direct effect of the coarse feeds or a compromise of the immune system as reflected in the lower monocyte and lymphocyte counts (Mahgoub *et al*., 2008) and presence of local lymphoid nodules. There are reports that condensed tannins resulted in discrete areas of tissue fragility (Robins and Brooker, 2005). The latter authors attributed these changes to dietary effects due to poor protein utilization rather than toxic effects of tannins.

The liver also showed signs of congestion (Fig. 1b) with dilated capillaries filled with blood indicating localized hyperemia. This effect is substantiated by increasing levels of alanine aminotransferase (ALT) (Mahgoub *et al*., 2008) indicating hepatocellular damage. Effects on the liver indicate probable toxic effects of tannins, as the liver is the site of detoxification of harmful substances in animals.

The kidneys exhibited chronic interstitial nephritis characterized by infiltration of mononuclear cells mainly

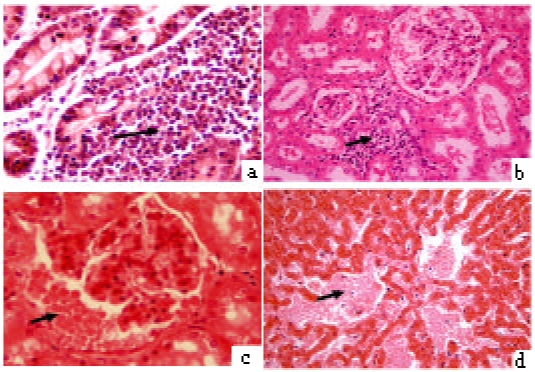

Fig. 1: Light microscope photographs showing pathological changes in organs of sheep fed rations containing phenols and condensed tannins. A: Chronic inflammation indicated by infiltration with lymphocytes and plasma cells (×40); b: Congestion and haemorrhages in the liver (×10); c: Infiltration with lymphocytes indicating chronic interstitial nephritis in the kidneys (×40); d: Presence of cast in Bowman's capsule and tubules in the kidneys (×40)

lymphocytes (Fig. 1c). There was also presence of deposits in the Bowman's space and the tubules which were of 2 types, a. homogenous one which was diagnosed as albumin. This was differentiated from amyloidosis by special stain (Congo red stain). The large pink spherical droplets of various sizes were identified as large molecular proteins most likely globulin (Fig. 1d). They were deposited in large quantities that in some cases pushed the glomerulus aside. Similarly, there are reports on experimentally-produced proteinuria-associated tubulointerstitial inflammation and fibrosis in cases of experimental membranous nephropathy in rats (Bonegio Ramon *et al*., 2005).

Electron microscopy observations: Electron microscopy micrographs indicated signs of structural damage in the kidneys including fibrosis within nephrons (Fig. 2a). There were also signs of generalized shedding and large cytoplasmic blebs of parietal endothelium in the urinary space (Fig. 2b). Blebbing is a sign of apoptosis (Coleman *et al*., 2001). Mesangial capillary junction showed loss of endothelia cytoplasm cover allowing mesangial luminal contact and mesangial matrix frail, cleavage and degeneration of cytoplasm (Fig. 2c). Endothelial cells showed sloughing of cytoplasm, degeneration vaccuolation, dense bodies cytoplasm blebbing, fraying and occasional nuclear budding (Fig. 2d).

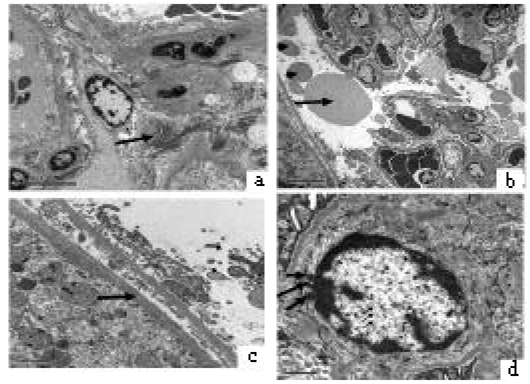

Fig. 2: Electron micrographs showing pathological changes in kidneys of sheep fed rations containing phenols and condensed tannins. A: Fibrosis in the kidney, b: Shedding of large cytoplasmic blebs of parietal endothelium in the urinary space; c: Degeneration of visceral epithelium; d: Nuclear budding

CONCLUSION

In general, the present study indicated that non-conventional feeds that contain tannins may cause structural and functional damage to animal essential organs that may negatively affect animal health, welfare and productivity. Such effects could be directly resulting from compounds such as phenols and tannins, or indirectly due to malnutrition.

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