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The Influence of Diets Containing Phenols and Condensed Tannins on Protein Picture, Clinical Profile and Rumen Characteristics in Omani Sheep

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ABSTRACT: A study was carried out to investigate the effects of feeding low quality non-conventional feeds (NCF) containing phenols and condensed tannins on health and performance characteristics in Omani sheep. Twelve Omani sheep were fed one of two base roughages, urea-treated palm frond (UTPF) or Rhodesgrass hay, (RGH) plus a commercial concentrate for 63 days. Haematological, serum biochemical and urine analyses were used to assess sheep health. Serum protein fractions were measured using electrophoresis. Urea-treated palm frond contained higher levels of polyphenols and condensed tannins and fiber than Rhodesgrass hay or concentrate feed. Animals fed UTPF had lower feed intake ($P<0.05$) and lower body gain ($P<0.001$) than those fed RGH. Rumen liquor of UTPF-fed animals had higher pH, ammonia-nitrogen and butyric fatty acid but lower acetic fatty acid ($P<0.05$). Animals fed UTPF had higher neutrophil ($P<0.05$) but lower lymphocyte ($P<0.05$) and monocyte ($P<0.001$) counts by the end of the trial than those fed RGH. There were no effects of diet on serum albumin or globulin fraction levels or albumin:globulin ratio. There were no major effects on urine analysis but there was a trend by control animals to have higher protein and specific gravity than treated ones. This study indicated that feeding low quality non-conventional feeds containing polyphenols or tannins would reduce body gain and may produce some effects on clinical parameters. Although tannins are known to influence protein digestion and absorption in ruminants, it did not significantly affect the serum protein picture in sheep.

Keywords: Phenols; Tannins; Hematology; Protein; Sheep.

تأثير العلف المحتوي على الفينولات والتانين المكثف على صورة البروتين وصحة الحيوان وخصائص الكرش في الضأن العماني

عثمان محجوب ، عصام توفيق كاظم ، حمزة على بابكر ومحمد بن ناصر الكندي

ملخص: تم إجراء تجربة لدراسة تأثير التغذية على أعلاف غير تقليدية ذات نوعية متدنية تحتوي على مركبات الفينول والتانين المركز على صحة الضأن العماني وأدائه. تمت تغذية اثني عشر من الضأن العماني على أحد نوعين من العلف المالئ أي جريد النخيل المعالج بمحلول اليوريا أو دريس حشيشة الرودس ، بالإضافة إلى غذاء مركز تجاري لمدة 63 يوماً. تم الاستعانة بنتائج فحوص الدم وتركيبه البيو كيميائي لتقييم الحالة الصحية للحيوان. وقد احتوى جريد النخيل المعالج على مستويات أعلى من مركبات الفينول والتانين المركز و الألياف عن حشيشة الرودس أو العلف المركز. كان معدل تناول الغذاء ومعدل زيادة الوزن الحي في الحيوانات التي تغذت على جريد النخيل أقل عن تلك التي تغذت على حشيشة الرودس. كان الأس الهيدروجيني في سائل الكرش في الحيوانات التي تغذت على جريد النخيل ومحتوي أمونيا النيتروجين وحمض البيوتيرك الدهني أعلى بينما كان حمض الأسيتيك الدهني أقل عن مستواه في الحيوانات التي تغذت على حشيش الرودس. أحتوى دم الحيوانات التي تمت تغذيتها على جريد النخيل المعالج على عدد أكبر من خلايا العدلات في الدم وعدد أقل من الخلايا اللمفاوية والأحادية عند نهاية التجربة مقارنة بالحيوانات التي تغذت على حشيشة الرودس. لم يلاحظ أثر للتغذية على مستويات الزلال والغلوبيولين بأنواعه المختلفة ولا نسبة الزلال للغلوبيولين. كما لم تلاحظ آثار واضحة للتغذية على تحليل البول ولكن كان هناك إرهاص بأن الحيوانات التي غذيت على حشيش الرودس كان مستوى البروتين والكثافة النوعية أعلى في البول. بينت التجربة الحالية أن تغذية الضأن العماني على أعلاف غير تقليدية تحتوي على معدلات عالية من مركبات الفينول والتانين تؤدي إلى انخفاض في معدل وزن الحيوان وربما يكون لها تأثير سلبي على صحة الحيوان. بالرغم من أن التانين له تأثير معروف على هضم والبروتين وامتصاصه في الحيوانات المجترة ولم يلاحظ له تأثير واضح على صورة البروتين في الدم.

مفتاح الكلمات: مركبات الفينول، التانين، فحص الدم، البروتين، الضأن.

1. Introduction

Animals raised on natural range grazing in the dry tropics are customarily fed low quality non-conventional feeds such as agricultural by-products. These feeds are usually high in fibre and low in protein, minerals and vitamins. They may also contain secondary plant compounds such as polyphenols and condensed tannins which may have anti-nutritional effects [1]. Tannins which protect plants from degradation in the rumen are reported to significantly affect protein digestion and utilization. They form combinations with proteins in the rumen rendering them unavailable for digestion and consequently increasing their output in faeces [2,3]. In non-ruminants they interfere with nutrient digestion and promote excretion of endogenous nitrogen through the formation of tannin-enzyme complexes [4]. There are some reports that they might also cause physical damage to the digestive system [5] and other vital organs such as the kidney and liver [3]. However, there are no reports on the effects of these compounds on the blood protein picture.

Especially in growing animals, protein and amino acid metabolism which accounts for 20-30% of whole body protein turnover and energy expenditure, is a function of the gastrointestinal tract tissues which amounts to only 3-6% of body weight [6]. This is mainly because of their relatively high fractional rates of protein synthesis and oxygen consumption which is several fold higher than that of the peripheral tissues such as muscles [6]. Maintaining an adequate feed supply to the intestine orally is essential to maintain the intestine's major functions of digesting and absorbing nutrients and serving as a biological barrier against pathogens, toxins and antigenic molecules [6]. Therefore, the oral route is extremely important in this regard especially in young animals. For instance, in non-weaned pigs the input of amino acids from the luminal route is far greater (67-90%) than the arterial circulation (11-21%), particularly for glutamate and glutamine. Consequently, it might be hypothesized that agents which interfere with protein digestion and utilization, such as polyphenols and tannins, can influence the general protein picture including that of the serum. Electrophoresis is a useful technique for studying serum protein and it can provide useful information for determining the cause of increased, decreased or disproportion serum protein, which is usually related to an increase in immunoglobulin, except in cases of dehydration [7].

Feeding NCF to animals may not only reduce body weight growth rate but may also affect their health status and ability to withstand diseases. Macro (energy and protein) and micro (vitamin and mineral) malnutrition negatively affects the immune system, rendering animals more prone to diseases [8]. Feeding animals for extended periods on certain range land plants containing high levels of anti-nutritional compounds has been reported to produce detrimental effects on animal health [9,10].

Studies on feeding feeds containing anti-nutritional factors mostly report effects on feed intake, body weight growth, digestibility of feeds, and rumen chemistry and biology. This study aimed to investigate the effects of feeding these feeds on the protein status, clinical profiles and rumen chemistry in sheep.

2. Materials and Methods

Twelve 1-year old male Omani native sheep (body weight 31.8 ± 1.2 kg) were used in a 63-d feeding trial with a completely randomized experimental design including two types of roughages; Rhodesgrass hay and urea-treated palm frond. The animals were also fed a commercial concentrate beside the roughage. Animals were daily fed 300 g of the concentrate for the first month then 400 g thereafter plus *ad libitum* roughage. The UTPF was prepared by adding a 4% commercial urea solution to the chopped palm fronds and ensiling for 60 days. Water and mineral blocks were offered *ad libitum*. The blocks (Frank Wright Ltd., UK) contained 400 mg/kg cupric sulphite, 200 mg/kg manganous oxide, 120 mg/kg zinc oxide, 124 mg/kg BMP-cobalt carbonate, 190 mg/kg calcium iodate, 10 mg/kg BMP-sodium selenite. They also contained 36% sodium, 1.3% calcium, 0.23% phosphorus, 0.30% magnesium and 40,000 iu/kg Vitamin D3. Daily feed intakes were determined and animals were weighed bi-weekly.

The proximate chemical composition of the various components of feeds was determined according to the standard methods of AOAC [11]. Dry matter (DM) was determined by drying in an oven for 24 hours at 80 ºC (method 934.01). Crude protein (CP) was determined using a Foss Tecator Kjeltec 2300 Nitrogen/Protein Analyser (method 976.05). Fat (EE) was determined by Soxhlet ether extraction of the dry sample, using petroleum ether (method 920.39). Ash content was determined by ashing samples in a muffle furnace at 500 ºC for 24 hr (method 942.05). Acid detergent fibre (ADF) was determined using cetyl trimethyl ammonium bromide (CTAB) and 1N H_2SO_4 as described by Roberston and Van Soest [12]. Neutral detergent fibre (NDF) was determined using sodium sulphite and sodium lauryl sulphate as described by Van Soest *et al.* [13]. Alpha amylase was not used to determine NDF. ADF was expressed with ash whereas NDF was expressed without ash. Calcium and phosphorus were measured with an atomic absorption spectrophotometer (Philips Model PU 9100, single beam). Gross energy (GE) was measured using a bomb calorimeter.

Levels of phenols and condensed tannins were analysed following the methods of Makkar [14]. 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 are expressed as gram equivalent tannic acid per kilogram dry matter. Extractable condensed tannins are expressed as gram equivalent leucocyanidins per kilogram dry matter.

The use of experimental animals and methodology for the trial was approved by the College of Agricultural and Marine Science Research Committee under Project number: SR/AGR/PINT/01/01.

Rumen liquor was collected at slaughter, strained and pH was recorded. Liquor was centrifuged and the clear top liquid used for the estimation of ammonia-N by reading against optical density at 540 nm in a UV-Visible spectrophotometer (Thermo Spectronic Corporation U.K.; Type Helios Beta Model).

Ten mL of blood samples were collected from each animal in plain glass vacutainers or with EDTA by jugular venipuncture on the last day of experiment. They were analyzed for: red blood cell count (RBC), packed cell volume (PCV), haemoglobin (HGB), mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) and differential white blood cell count (WBC) of Neutrophils, Lymphocytes, Basophils, Eosinophils and Monocytes using a CELL-DYN 3700 blood analyzer (CELL-DYN 3700; Abbott Laboratories Diagnostic Division, Abbott Park, IL 60064, USA). A Giemsa stained blood smear was prepared for each sample. Serum samples were analyzed for glucose, blood urea nitrogen (BUN), albumin, creatinine, total protein, alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), magnesium (Mg), calcium (Ca), phosphorus (P) and iron (Fe) by spectrophotometric analysis using a CX7/CX7 serum chemistry analyser (Synchron, Beckman).

Total protein quantification was carried out using the Bradford protein assay method which is a reliable procedure for determining the concentration of solubilized protein. It involves the addition of an acidic dye to a protein solution, and subsequent measurement at 595 nm with a spectrophotometer or microplate reader. A NanoDrop® ND-1000 Spectrophotometer and bio-rad protein assay reagent were used to quantitate the total protein. Serum Protein Electrophoresis (SPE) was used to determine levels of two major protein groups, albumin and globulin in the blood serum. Using protein electrophoresis, these two groups can be separated into five smaller groups (fractions): Albumin, Alpha-1 globulin, Alpha-2 globulin, and Gamma globulin. The Paragon Electrophoresis System (Beckman, USA) was used to separate the two blood protein groups and the mobility pattern was visually interpreted and quantitated by densitometry at 600 nm, on a Beckman APPRAISE densitometer, in which the relative percent of each protein fraction is calculated automatically.

Urine samples were collected at the end of the experiment using a syringe to collect urine directly from the urinary bladder. They were analysed for blood, urobilinogen, bilirubin, protein, nitrite, ketones, ascorbic acid, glucose, pH, specific gravity and leucocytes using URYXXON® 200 equipment and Combi 11 urine strips.

Data were subjected to the analysis of variance [15] to study the effects of diet using the general linear models procedure [16]. Significant differences between treatment means were assessed using the least significant difference procedure at $P<0.05$ level.

3. Results

There were significant differences in chemical composition between the UTPF and Rhodesgrass hay. UTPF contained higher levels of fibre, ash, Ca and phosphorus but lower hemicellulose (Table 1). It also contained four times the total extractable phenols and much higher condensed tannins compared to the conventional feeds (Table 1).

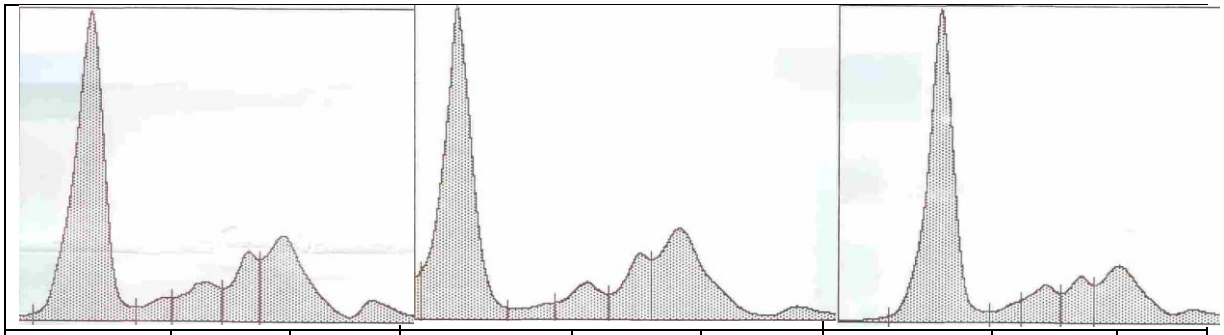
Animals fed the UTPF consumed similar daily amounts of concentrates (Table 2) but less roughage than those fed RGH. The UTPF-fed animals gained less body weight over the experimental period (3 vs. 70 g/d) than those fed the RGH. The UTPF-fed animals maintained their weight throughout the trial.

Haematological values of experimental animals were within normal values reported for sheep. However, animals fed the UTPF had higher neutrophil ($P<0.01$) but lower lymphocyte ($P<0.01$) and monocyte ($P<0.05$) counts at the end of the trial compared to the RGH-fed animals (Table 3). There were no dietary effects on the serum biochemistry parameters measured (Table 3).

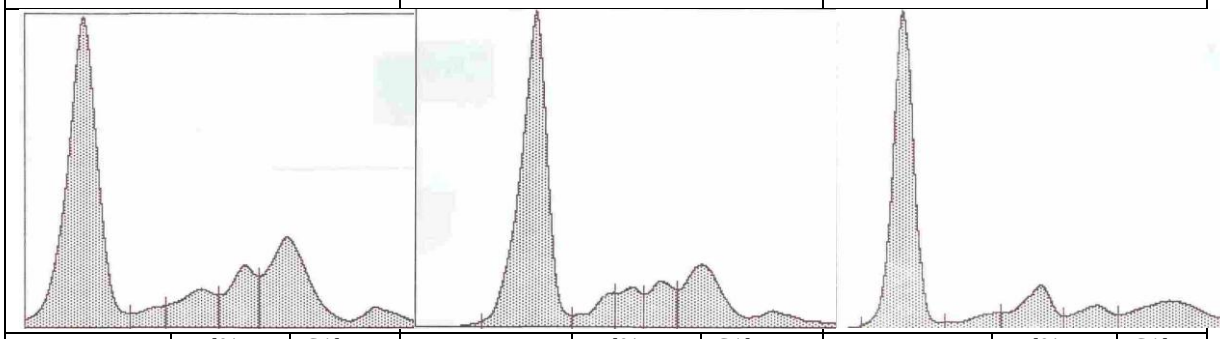
The serum protein profile is presented in Figure 1 and Table 4. Serum protein electrophoretograms exhibited a normal pattern. The serum albumin in treated and control sheep averaged 7 G/dL, while the total globulin was 12.7 G/dL (Table 4).

The albumin proportion was higher (54%), resulting in an albumin/globulin ratio of 1.3. Within the globulins, the highest proportion was made up of the Gamma globulins (3 G/dL, 24%) followed by Beta globulins (9%) and Alfa 2 globulins (7.5%), whereas the smallest proportion was made by Alpha 1 globulins (4.%). However, there were no significant treatment effects on the various protein fractions. However, there was a consistent trend of treated animals having higher albumin and Gamma globulins (Table 4).

Visual examination had shown that urine from NCF-fed animals was turbid and brownish in colour. Urine analysis indicated a trend of elevated levels of protein, leukocytes and specific gravity in animals fed UTPF compared to those fed Rhodesgrass hay (Table 5).



Fraction	Rel%	G/dL	Fraction	Rel%	G/dL	Fraction	Rel%	G/dL
Albumin	53.8	4.52	Albumin	51.7	9.77	Albumin	58.9	7.07
Alpha 1	3.3	0.28	Alpha 1	3.1	0.59	Alpha 1	3.2	0.38
Alpha 2	7.8	0.66	Alpha 2	7.2	1.36	Alpha 2	7.5	0.90
Beta	9.6	0.81	Beta	9.9	1.87	Beta	7.8	0.94
Gama	25.4	2.13	Gama	28.2	5.33	Gama	22.6	2.71
Total G/dL 8.50 A/G: 1.16			Total G/dL 18.90 A/G: 1.07			Total G/dL 12.00 A/G: 1.43		



Fraction	Rel%	G/dL	Fraction	Rel%	G/dL	Fraction	Rel%	G/dL
Albumin	53.7	7.79	Albumin	58.7	7.22	Albumin	55.4	5.93
Alpha 1	3.1	0.45	Alpha 1	5.5	0.68	Alpha 1	4.7	0.50
Alpha 2	8.0	1.16	Alpha 2	6.1	0.75	Alpha 2	11.8	1.26
Beta	9.3	1.35	Beta	7.8	0.96	Beta	7.2	0.77
Gama	25.9	3.76	Gama	21.9	2.69	Gama	20.9	2.24
Total G/dL 14.50 A/G: 1.16			Total G/dL 12.30 A/G: 1.42			Total G/dL 10.72 A/G: 1.24		

Figure 1. Serum protein electrophoretograms of Omani sheep fed Rhodesgrass hay (Control) and urea-treated palm frond (Treated) plus General Ruminant concentrate for nine weeks (Top row, treated animals; bottom row, control animals).

Table 1. Chemical composition of ingredients of experimental feeds.

Feed ingredient	Experimental diets		
	Commercial concentrate	Urea-treated palm frond	Rhodesgrass hay
DM (g/kg)	862	892	916
Crude protein (g/kg DM)	180	85	120
Ether extract (g/kg DM)	21.5	11	15
Neutral detergent fiber (g/kg DM)	182	740	614
Acid detergent fiber (g/kg DM)	56	580	370
Hemicellulose (g/kg DM)	126	160	243
Ash (g/kg DM)	73	120	95
Ca^{++} (g/kg DM)	12	7.4	5
PO_4(g/kg DM)	8	1.1	0.20
GE (kj/g)	183	192	173
Total extractable phenols [a]	16.6	112.6	32.1
Extractable condensed tannins [b]	0	12.8	0

[a] Expressed as gram equivalent tannic acid/kg DM.
[b] Expressed as gram equivalent leucocyanidins/kg DM.

Animals on UTPF had higher rumen ammonia-N ($P<0.05$) levels and their rumen pH was higher ($P<0.01$) than those fed Rhodesgrass hay (Table 6). They also had significantly lower acetic acid but higher butyric fatty acids than the controls (Table 6).

Table 2. Feed intake and body weight gain of Omani sheep fed Rhodesgrass hay (Control) and those fed urea-treated palm frond (Treated) plus General Ruminant concentrate for nine weeks.

Parameter	Treatment		PSE	Effect of diet
	Treated	Control		
Number of animals	6	6		
Days of experiment	63	63		
Daily roughage intake (g)	554	696	38	*
Daily concentrate intake (g)	365	365	0	NS
Daily feed intake (g)	919	1061	38	*
Starting body weight (kg)	31.60	31.88	0.519	NS
Final body weight (kg)	31.70	35.92	0.837	**
Average daily gain (g)	3	69	7	***

PSE, pooled standard error of means
NS, P>0.05;*, P<0.05; **, P<0.01; ***, P<0.001.

Table 3. Haematological and serum biochemistry values in Omani sheep fed diets containing phenols and tannins.

Parameters	Experimental groups		PSE	Effect of diet
	Treated	Control		
White blood cells ($X10^3$/ml)	9.4	9.3	0.48	NS
Neutrophils (% of WBC)	61.1	46.9	1.82	***
Lymphocytes (% of WBC)	30.0	39.9	2.24	*
Monocytes (% of WBC)	3.3	6.5	0.43	***
Eosinophils (% of WBC)	1.7	1.8	0.44	NS
Basophils (% of WBC)	4.0	4.9	1.73	NS
Neutrophils ($X10^3$/m)	5.8	4.4	0.42	*
Lymphocytes ($X10^3$/m)	2.8	3.9	0.24	*
Monocytes ($X10^3$/m)	0.3	0.6	0.04	***
Eosinophils ($X10^3$/m)	0.2	0.2	0.04	NS
Basophils ($X10^3$/m)	0.3	0.5	0.14	NS
Red blood cells ($X10^6$/ml)	13.4	13.6	0.40	NS
Hemoglobin (g/ll)	10.3	10.1	0.19	NS
Haematocrit (%)	43.8	44.7	1.61	NS
Mean corpuscular vol. (fl)	32.72	32.9	1.26	NS
Mean cell hemoglobin (pg)	7.7	7.4	0.17	NS
Mean cell hemoglobin (pg)	23.9	22.5	0.89	NS
Ck	75.7	89.8	24.8	NS
GGT (µ/L)	62.9	90.5	5.57	NS
GOT (µ/L))	95.8	120.4	11.20	NS

PSE, pooled standard error of means
NS, P>0.05;*, P<0.05; **, P<0.01; ***, P<0.001.

4. Discussion

Feed composition analyses indicated that animals fed the urea-treated palm fronds were subjected to a lower nutritional regime compared to those fed the Rhodesgrass hay as the UTPF had much higher fibre contents than the RGH. UTPF has been reported to have lower digestibility coefficients and sheep fed on it produced larger volumes of faeces and had lower viscosity of gut contents, a characteristic of highly fibrous diets [17]. Moreover, the UTPF also contained higher levels of phenols and condensed tannins that are known to have anti-nutritional effects [18, 2]. This would have further reduced its nutritive value, resulting in animals consuming less feed and gaining less body weight. Although the nutritional insult was not severe enough to cause experimental animals fed the UTPF to lose body weight, the diet characteristics indicated that animals that consumed them had been subjected to malnutrition. Malnutrition was defined by [8] as an "inadequate or unbalanced diet, or a failure to absorb or assimilate dietary elements". If severe enough, malnutrition may produce detrimental effects on the animal body. These may include macronutrient

deficiencies that may lead to body cell mass depletion and micronutrient deficiencies that may impair body immune function [8].

Animals fed the UTPF would have ingested considerable amounts of condensed tannins as they had consumed approximately 550 g/d of UTPF. Similar levels of condensed tannins in sheep have been shown to reduce protein degradation in the rumen and increase protein flow to the intestine [19, 20]. A digestibility trial using UTPF with sheep [17], indicated classical features of tannin-containing feeds in ruminants. A higher level of nitrogen in the faeces (less nitrogen retention) is a characteristic of dietary tannins, which bind to proteins in the rumen and consequently reduce digestion and absorption in the gut [4,5,2]. Tannins also form complexes with carbohydrates and minerals and inhibit microbial and digestive processes in ruminants [2]. Therefore, the presence of condensed tannins in the UTPF in the current study could have resulted in depressed rumen digestibility [18].

The UTPF-fed animals had higher rumen pH values than those fed Rhodesgrass hay. Ammonia-N and pH are indicators of rumen fermentation efficiency. This suggests that these animals had lower fermentation levels, which produced less volatile fatty acids, especially acetic fatty acid, as confirmed by chemical analyses of rumen liquor. Contrary to the findings of Mahgoub *et al*. [17], ammonia nitrogen levels in the current study were higher in UTPF-fed animals than in Rhodesgrass-fed animals. This could mainly be attributed to the fact that palm fronds in the current experiment had been ensiled in a solution of higher urea levels (4%) which must have increased non-protein nitrogen levels in the rumen. This was reflected in diet chemical composition. Higher nitrogen levels in the faeces are usually accompanied by lower urine nitrogen level, a characteristic of animals fed condensed tannins [H.P.S. Makkar, personal communication, 2007 and 17] most probably due to the higher proportion of nitrogen excreted through the faeces.

Generally the serum protein fractioning was of a normal picture, as indicated in the electrophoretograms and individual animal values. However, there was a pattern of lower albumin and higher globulin levels. Albumin, produced by the liver, is major protein within the blood, and its levels are reduced by malnutrition and chronic liver disease such as cirrhosis. It appears that the UTPF feeding did not produce enough insult to produce severe malnutrition or affect the liver of the experimental animals. Globulins play an important role in immunity (e.g., IgA, IgG, IgE). The trend of slightly higher globulins in treated animals may indicate some effects of tannins on the immune system, most probably indirectly through the leptin axis.

This lack of effect on the protein picture suggests that, although tannins are known to interfere with protein digestion and utilization [2,17], apparently they did not affect the serum protein pattern in the current study. This could be due to the lower dosages and levels of tannins and polyphenols used in the present study.

Table 4. Serum protein values in Omani sheep fed diets of urea-treated palm frond or Rhodesgrass hay plus a concentrate.

ID	TRT[1]	Albumin (%)	Albumin (G/dL)	Alpha 1 globulins (%)	Alpha 1 globulins (G/dL)	Alpha 2 globulins (%)	Alpha 2 globulins (G/dL)	Beta globulins (%)	Beta globulins (G/dL)	Gamma globulins (%)	Gamma globulins (G/dL)	Total globulins (G/dL)L	Albumin Globulin ratio
2380	T	55.00	4.95	5.40	0.49	9.10	0.82	5.30	0.48	25.10	2.26	9.00	1.22
2400	T	51.70	9.77	3.10	0.59	7.20	1.36	9.90	1.87	28.20	5.33	18.90	1.07
2442	T	57.50	8.40	2.70	0.39	7.70	1.12	9.10	1.33	23.00	3.36	14.60	1.35
2467	T	53.80	4.52	3.30	0.28	7.80	0.66	9.60	0.81	25.40	2.13	8.40	1.16
2470	T	58.90	7.07	3.20	0.38	7.50	0.90	7.80	0.94	22.60	2.71	12.00	1.43
2494	T	58.00	7.83	4.50	0.61	3.80	0.51	7.70	1.04	26.10	3.52	13.50	1.38
Mean		55.82	7.09	3.70	0.46	7.18	0.90	8.23	1.08	25.07	3.22	12.73	1.27
2401	C	55.30	6.91	6.20	0.78	5.50	0.69	8.00	1.00	24.90	3.11	12.50	1.24
2420	C	55.40	5.93	4.70	0.50	11.80	1.26	7.20	0.77	20.90	2.24	10.70	1.24
2466	C	53.70	7.79	3.10	0.45	8.00	1.16	9.30	1.35	25.90	3.76	14.50	1.16
2471	C	49.90	8.03	7.00	1.13	10.00	1.61	12.20	1.96	20.90	3.36	16.10	1.00
2486	C	46.00	4.69	3.10	0.32	8.50	0.87	18.30	1.87	24.20	2.47	10.20	0.85
2495	C	58.70	7.22	5.50	0.68	6.10	0.75	7.80	0.96	21.90	2.69	12.30	1.42
Mean		53.17	6.76	4.93	0.64	8.32	1.06	10.47	1.32	23.12	2.94	12.72	1.15
PSE		1.53	0.69	0.55	0.09	0.85	0.14	1.32	0.20	0.86	0.38	1.29	0.07
Significance		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] TRT; T = treated (fed UTPF); C = control (fed Rhodesgrass hay)
PSE, pooled standard error of means
NS, P>0.05;*, P<0.05; **, P<0.01; ***, P<0.001.

Although they did not gain much weight, UTPF-fed animals did not show explicit signs of disease such as diarrhoea, constipation or anorexia. This could be mainly attributed to genetic disease resistance in such animals of the arid tropics, which have been naturally selected over generations for survival and ability to utilize low quality feeds. However, negative health effects may be produced and go unnoticed in animals fed under similar systems. The lower

lymphocyte and monocyte and lower neutrophil counts in animals fed UTPF could be an important indication of the health status of experimental animals. Monocytes are essential for the immune system as they are precursors of macrophages and lymphocytes essential for humoral and cell-mediated immunity responses. Similar findings had been reported by Mahgoub *et al*. [17].

Animals fed the UTPF had lower body weights compared to those fed RGH [21]. Although both control and treated animal groups started at similar weights (32.5 ± 1.13 kg), the group of sheep fed the Rhodesgrass hay plus the commercial concentrate gained about 4 kg over the experimental period, whereas the UTPF-fed group maintained their body weight. This indicates that the latter group had a lower body condition score and consequently lower body fat reserves. Body composition may influence the immune system, with its effect most probably mediated by leptin [8]. Leptin is a 16 Da protein produced by adipocytes and released into systemic circulation which acts as a master hormone controlling energy acquisition and utilization processes as well as the immune/inflammatory response [22]. Reduced level of leptin, a signal of energy reserve depletion (adipose tissue), appears to trigger the body to shut down non-essential body functions including those involved with immune and inflammatory responses [22]. Leptin influences lymphocyte proliferation and cytokine secretion [23]. This is in line with the low lymphocyte and monocyte counts in the current study. Pathological examination also indicated that animals fed UTPF had shown signs of chronic inflammation in the small intestine and nephritis, another sign of immune system compromise [3]. Low leptin levels caused by less adipose tissue in undernourished individuals reduced Th1 immune response resulting in increased susceptibility to infections [24].

The trend of higher levels of protein in the urine of control than in that of treated animals is in line with findings of Mahgoub *et al*. [17] and Makkar (personal communication , 2007), that animals fed higher levels of tannins tend to produce higher levels of nitrogen in the faeces and lower nitrogen levels in the urine. This is probably the cause of the trend of higher specific gravity of urine in these animals.

Table 5. Urine analyses of sheep fed urea-treated palm fronds containing higher levels of phenols and condensed tannins and those fed Rhodesgrass hay.

Diet[1]	ID No	Blood Ery/µL	Urobilinogen mg/dL	Bilirubin	Protein mg/dL	N	Ketones	ASC	Glucose mg/dL	pH	Specific gravity	Leucocytes Leu/µL
A	2380	250	norm	neg	neg	neg	neg	neg	neg	5	1.000	neg
A	2400	50	norm	neg	neg	neg	neg	neg	neg	5	1.000	neg
A	2494	250	norm	neg	100	neg	neg	neg	neg	6	1.010	neg
A	2470	250	norm	neg	neg	neg	neg	neg	neg	5	1.005	neg
A	2442	50	norm	neg	neg	neg	neg	neg	neg	5	1.005	neg
B	2486	250	norm	neg	100	neg	neg	1+	neg	8	1.005	25
B	2466	50	norm	neg	30	pos	neg	neg	nor	6	1.010	neg
B	2495	250	norm	neg	100	pos	neg	neg	neg	7	1.015	75
B	2401	250	norm	neg	30	neg	neg	neg	neg	5	1.015	neg
B	2420	250	norm	1+	100	neg	neg	neg	neg	5	1.025	25
B	2471	50	norm	neg	100	neg	neg	neg	neg	8	1.010	neg

[1] A: UTPF; B: Rhodesgrass hay
Norm: normal; neg: negative

Table 6. Rumen chemistry values in Omani sheep fed Rhodesgrass hay (Control) and those fed urea-treated palm frond (Treated) plus General Ruminant concentrate for nine weeks.

Parameters	Experimental groups		PSE	Effect of diet
	Treated	Control		
Rumen characteristics:				
pH	6.35	5.89	0.088	**
Ammonia Nitrogen (mg/L)	138.0	92.7	10.51	*
Volatile fatty acids				
Acetic	1043	1399	116.9	*
Propionic	279	222	44.6	NS
Isobutyric	92	75	7.1	NS
Butyric	231	145	42.7	*
Isovaleric	51	52	8.0	NS

PSE, pooled standard error of means
NS, P>0.05;*, P<0.05; **, P<0.01; ***, P<0.

5. Conclusions

In general the present study indicated that feeds containing anti-nutritional factors such as polyphenols and tannins, commonly fed to livestock around the world, may produce negative effects on livestock health, welfare and productivity. They appear to affect animal health in a combination of ways. Animals fed these feeds might have lower nutrient availability due to low digestibility resulting in macro and micro malnutrition. Malnutrition as a result of a combination of the above mentioned factors may result in lower body weight and consequently lower adipose tissue. This could result in a compromise of the body's immunity system, making the animal prone to infections and other ailments. The findings of this study did not show that there are significant effects of feeding sheep on diets containing tannins on the serum protein picture.

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