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Morphometry of Organs in Sheep Fed a Diet Containing Tannins and Polyphenols

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ABSTRACT: The aim of this study was to use morphometry to investigate the essential organs of sheep fed with non-conventional diets. Twelve male Omani sheep were fed with one of two types of roughages supplemented with a commercial concentrate for 63 days and then slaughtered. The volumes of their kidneys, testes, and livers were measured. Random samples from the organs were fixed in formalin and embedded in paraffin. Random five micrometer sections from each block were stained with hematoxylin and eosin. Images from four random fields were taken from each slide using a digital camera attached to the microscope and then loaded onto a stereology software installed on a personal computer. Morphometric measurements of body organs were taken. Animals fed diets containing tannins had lower final total body weight and lower weights and volumes of their livers, testes and kidneys. There was high variability in the results of intestinal parts measurements, but there was a general trend of the treated animals having higher duodenal and ilial height, villus height, crypt depth and epithelial thickness. The only significant diet effect was higher liver l and lower Vfrac cytoplasm in the control animals than in those treated. The only significant treatment effect on the kidney measurements was a higher tube length/density in treated animals. There were no treatment effects on the testes measurements. The current study indicates that sheep may safely utilize diets containing the levels of polyphenols and tannins used here with no drastic effects on the digestive system and vital organs.

Keywords: Oman; Sheep; Tannins; Morphometry.

استخدام تقنية قياس التراكيب الدقيقة لأعضاء جسم الضأن التي تمت تغذيتها على أعلاف تحتوي على التانين والبوليفونولات

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الملخص: كان الهدف من هذه الدراسة هو استخدام تقنية قياس التراكيب الدقيقة لبعض أعضاء الجسم المهمة في الضأن التي تمت تغذيتها على علف غير تقليدي. تمت تغذية اثني عشر من ذكور الضأن العماني على أحد نوعي علف خشن بالإضافة إلى علف تجاري مركز لمدة 63 يوماً ثم ذبحت بعد ذلك. تم قياس حجم كل من الكلية والكبد والخصية ومن ثم تم أخذ عينات من كل عضو وحفظها في الفورمالين ثم طمرها في البارافين. تم صبغة شرائح ميكرومترية اختيرت عشوائياً من كل مكعب بصبغة الهيماتوكسلين والإيوزين. تم أخذ صور من أربع مجالات عشوائياً من كل شريحة مجهرية باستخدام كاميرا رقمية موصلة بمجهر ومن ثم تحميلها على برنامج تجسيم حاسوبي على جهاز كمبيوتر شخصي ثم تم أخذ قياسات تراكيب الأعضاء. كان وزن جسم الحيوانات التي تغذت على العلف المحتوي على التانين عند نهاية التجربة ووزن وحجم الكبد والكلية والخصية في نهاية التجربة أقل عنه في الحيوانات التي تغذت على الغذاء العادي. كان هناك تباين كبير في قياسات مناطق الأمعاء المختلفة ولكن كانت هناك اتجاه عام بأن الحيوانات المعالجة لها عرض أكبر في الجزء الأول والثاني من الأمعاء والزغابات المعوية وعمق السراديب المعوية وسمك الخلايا الطلائية. وكانت هناك فروقات معنوية بسيطة في قياسين في الكبد وقياس في الكلية بينما لم تكن هناك تأثيرات معنوية في الخصية. بينت الدراسة بأن الضأن يمكن تغذيته بصورة مأمونة على أعلاف محتوية على التانين والبوليفينولات على المستويات التي تم استخدامها في هذه الدراسة بدون تأثيرات ضارة على الجهاز الهضمي وبعض الأعضاء المهمة في الجسم.

الكلمات المفتاحية: عمان ، الضأن ، التانين ، قياس الأشكال.

1. Introduction

Low quality non-conventional feeds (NCF) usually contain high fibre and low protein, minerals and vitamin levels. The NCF feeds usually contain polyphenols and condensed tannins that may cause antinutritional effects [1]. The tannins have been proved to form combinations with proteins in the rumen rendering them unavailable for digestion [2,3]. Tannins interfere with nutrient digestion and promote excretion of endogenous nitrogen in monogastric animals by forming tannin-enzyme complexes [4]. Tannins might also cause physical damage to the digestive system and other vital organs such as the kidney and liver [5,6]. Research has indicated different responses to tannin consumption in various animal species. For instance, oral administration of tannic acid produced coagulative and hemorrhagic necrosis in the liver of mice but produced no similar effects in sheep [7]. The current study aimed to investigate the effects of feeding feeds containing phenols and tannins on the morphometry of a number of organs in sheep.

2. Materials and Methods

Twelve 1-year old male Omani sheep (body weight 31.8 ± 1.2 kg) were fed one of two types of roughage: Rhodesgrass hay (RGH) or urea-treated palm frond (UTPF) for 63 days. Both groups also received 400 g of a commercial concentrate daily. At the end of the feeding period, the animals were slaughtered. The use of experimental animals and the methodology for the trial was approved by the College of Agricultural and Marine Science Research Committee under Project number: SR/AGR/PLNT/01/01.

Levels of phenols and condensed tannins were analysed following standard methods [8]. The reference volumes of the kidneys, testes, and liver were estimated using water immersion volumetry [9,10]. Organs including the abomasum, duodenum, jejunum, and ileum were fixed in 10% buffered formalin. Systematic random samples of tissue were embedded in paraffin. Random five micrometer sections were obtained from each block and stained with H&E and then observed with a Leica microscope (microscope specs). Images from four random fields were taken from each slide using an Olympus digital camera (DP12) attached to the microscope. The images were then loaded onto *Histometrix*, a stereology software (Histometrix MIL6, Kinetic Imaging Ltd UK) installed on an IBM compatible personal computer. The volume density (V_f) of the nucleus in hepatocytes was estimated by point counting by generating an array of random points grid on the image and counting the total number of points, $\sum P$, falling within the nucleus and dividing it by the number of points falling within the whole cell [11].

$$V_{f(nuc)} = \frac{\sum P_{nuc}}{\sum P_{cell}} \qquad (1)$$

The absolute volume of the hepatocyte nucleus ($V_{(nuc)}$) was determined by the point-sampled intercept method [12]. The four random sections showing whole cells from each animal, an unbiased counting frame with superimposed random test points was applied on each selected section. If a test point fell on a nucleus profile, a line, l_0, in an isotropic uniform direction (IUR) was drawn through the point to the nuclear boundary. The volume of the nucleus, $\overline{V}_{nuc}$, was then estimated from the following equation:

$$\overline{V}_{nuc} = \frac{\pi}{3} \times \sum l_0^3 \qquad (2)$$

Estimates of absolute hepatocyte cell volume (V) were obtained by using the volume density of the nucleus, and volume-weighted mean nuclear volume:

$$V_{cell} = \frac{V_{nuc}}{V_{V(nuc)}} \qquad (3)$$

The surface density measurements (S_V) of intestinal villi and the filtration barrier of the kidney were estimated by applying line probes on to an image of the villi/glomerulus and counting the intersections between the line probe and villus/glomerular surface ΣI. The classical relationship which describes S_V, the surface density, in terms of the intersection count Σ I and the total length of line probe, L, is

$$S_V = \frac{2\Sigma I}{L} \qquad (4)$$

where *Sv* is the surface density
Σ I is the number of intersections
L is the total length of line probe falling within the object.

For the length density measurements (L_V), the probability that a given structure is hit by a randomly positioned and randomly oriented section is proportional to its linear dimension or length. The number of times a linear structure is cut by or intersects a section will depend on the total length of the structure and the area of the section surface. The length density of renal tubules and seminiferous tubules was estimated by counting the number of tubule profiles, ΣQ, in an area of the section. The relationship which describes L_V, the length density, in terms of the intersection count ΣQ and the area of the section is

$$L_V = \frac{2\Sigma Q}{A} \qquad (5)$$

where *Lv* is the length density
Σ Q is the number of intersections
A is the total area of section.

The volume of renal glomeruli was determined by the point-sampled intercept method [12]. On each of four random sections showing whole glomeruli at a magnification of ×40, an unbiased counting frame with superimposed random test points was applied. If a test point fell on a glomerulus profile, a line, l_0, in an isotropic uniform direction (IUR) was drawn through the point to the glomerular boundary. The volume of the glomerulus, $\overline{V}_{Glom}$, was then estimated from the following equation:

$$\overline{V}_{Glom} = \frac{\pi}{3} \times \sum l_0^3 \qquad (6)$$

To study the effects of diet, data were subjected to the analysis of variance using the general linear models procedure in the SAS Windows version [13,14]. Significant differences between treatment means were assessed using the least significant difference procedure at the *P<0.05* level.

3. Results

Feed analyses indicated that animals fed the urea-treated palm fronds (UTPF) were subjected to a lower nutritional regime compared to those fed the Rhodesgrass hay (RGH) as their diet had a higher fibre content and higher levels of phenols and condensed tannins (Table 1).

Table 1. Chemical composition of ingredients of experimental feeds.

Feed ingredient	Experimental diets		
	Commercial concentrate	UTPF	RGH
DM (g/kg)	862	892	916
Crude protein (g/kgDM)	180	85	10
Ether extract (g/kgDM)	21.5	11	15
Neutral detergent fiber (g/kgDM)	182	740	614
Acid detergent fiber (g/kgDM)	56	580	370
Hemicellulose (g/kgDM)	126	160	243
Ash (g/kgDM)	73	120	95
Ca^{++} (g/kgDM)	12	7.4	5
PO_4(g/kgDM)	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

Total extractable phenols expressed as gram equivalent tannic acid/kgDM.
[b] Extractable condensed tannins expressed as gram equivalent leucocyanidins/kgDM.

Animals fed UTPF had lower final weight, lower weight of liver, testes and kidney as well as lower volumes for the same organs either in absolute terms or as per kg/BW (Table 2).

Table 2. Body organ weight and volume in Omani sheep fed either urea-treated palm frond (Treated) or Rhodesgrass hay (Control) plus a commercial concentrate for nine weeks.

Parameter	Experimental group		PSE	Effect of diet
	Treated	Control		
Final body weight (kg)	31.70	35.92	0.837	**
Liver weight (g)	311.7	388.6	3.33	***
Liver weight/BW	9.7	10.4	0.27	NS
Testes weight (g)	110.6	128.3	6.52	*
Testes weight/BW	3.4	3.4	0.21	NS
Kidney weight (g)	39.0	40.1	1.02	NS
Kidney weight/BW	1.21	1.10	0.019	**
Liver volume (ml)	272.7	352.8	10.03	***
Testes volume (ml)	90.0	106.8	8.01	*
Kidney volume (ml)	23.3	26.1	2.55	NS

PSE : pooled standard error
NS : not significant
p<0.05; ** p<0.01; *** p<0.001

There was high variability in the various measurements of organ morphometry including those of intestinal parts. However, there was a general trend of the UTPF fed animals having higher values for duodenal and ilial height, villus height, crypt depth and epithelial thickness (Table 3). Measurements on the jejunum, however, showed an opposite trend with values from UTPF fed animals being significantly lower than those of the control group, and only significant in the villus height.

Table 3. Morphometry of the small intestine of Omani sheep fed urea-treated palm frond (Treated) or Rhodesgrass hay (Control) plus a commercial concentrate for nine weeks.

Parameter	Type of diet		PSE	Effect of diet
	Treated	Control		
Duodenum:				
Duodenum height (um)	979.0	790.2	63.78	0.09
Duodenum villus height	603.5	505.7	54.96	NS
Duodenum crypt depth	368.0	285.5	24.15	0.06
Duodenum epithelial thickness	45.0	37.3	4.28	NS
Jejunum				
Jejunum total height	844.5	1066.5	106.52	NS
Jejunum villus height	427.8	652.5	59.49	*
Jejunum crypt depth	419.5	416.0	50.0	NS
Jejunum epithelial thickness	22.3	26.7	1.84	NS
Ilium				
Ilium total height	996.3	969.2	79.83	NS
Ilium villus height	507.5	543.2	47.46	NS
Ilium crypt depth	489.5	428.7	56.17	NS
Ilium epithelial thickness	23.3	26.5	1.47	NS

PSE : pooled standard error
NS : not significant
p<0.05; ** p<0.01; *** p<0.001

There were no significant effects of diet on the morphometry of the liver except for the liver length density which was higher and the volume of fraction cytoplasm which was lower in control animals (Table 4). The only significant treatment effect on the kidney measurements was a higher value of tube length/density in treated vs. control animals. There were no treatment effects on the testes measurements, either for tube volume, length, length/density or number of nephrons per area (Table 4).

Table 4. Morphometry of the liver, kidney and testes of Omani sheep fed urea-treated palm frond (Treated) or Rhodesgrass hay (Control) plus a commercial concentrate for nine weeks.

Parameter	Treatment		PSE	Effect of diet
	Treated	Control		
Liver:				
Volume density (V_f)	2.73	3.53	1.021	**
Volume fraction cytoplasm	0.75	0.65	0.035	*
ANON	8.16	10.37	1.14	NS
Volume fraction sinus	0.174	0.105	0.064	NS
Nuclear size	4.57	4.28	0.789	NS
Absolute volume of hepatocyte nucleus	0.14	0.16	0.020	NS
Kidney:				
Tube volume	2.23	2.62	2.537	NS
Tube total length (µ)	2.80×10^{11}	2.15×10^{11}	3.36×10^{10}	NS
Length density	0.013	0.008	0.001	*
Glomeruli number per area	0.00005	0.00004	0.00002	NS
Glomeruli size	19140	25133	3873	NS
Surface Density	0.089	0.090	0.012	NS
Surface area	2.17×10^{-12}	2.3×10^{-12}	4.17×10^{-12}	NS
Testes:				

Tube volume	9.68	1.07	6.92	NS
Tube length	5.41×10^9	6.19×10^9	0.901×10^9	NS
Tube length/density	0.0006	0.0006	0.00009023	NS
Number/Area	0.0003	0.0003	0.00004	NS

PSE : pooled standard error
NS : not significant
P<0.05; ** p<0.01; *** p<0.001

Table 4. Contd.

4. Discussion

The UTPF with its higher fibre content and lower digestibility coefficients resulted in larger volumes of faeces and lower viscosity of gut contents in the sheep, a characteristic of highly fibrous diets [3]. The UTPF also contained higher levels of phenols and condensed tannins, which have been known to have antinutritional effects [2,15].

Animals fed the UTPF consumed approximately 550 g/d of UTPF and therefore, must have ingested considerable amounts of condensed tannins. It has been reported that similar levels of condensed tannins in sheep have reduced protein degradation in the rumen and increased protein flow to the intestine [16,17]. There was an indication of these classical effects of tannin-containing feeds in ruminants from a digestibility trial using UTPF with sheep [3]. Higher levels of nitrogen in the faeces indicate lower nitrogen retention, which is a characteristic of dietary tannins, which bind to proteins in the rumen and consequently reduce digestion and absorption in the gut [2,4,5]. There are also reports that tannins may form complexes with carbohydrates and minerals and consequently inhibit microbial and digestive processes in ruminant animals, resulting in depressed rumen digestive function [2,15].

The low body and organ weights and volumes of the animals fed diets containing tannins was expected as the highly fibrous feeds with anti-nutritional factors would restrict feed intake and consequently negatively influence the body weight of experimental animals. The size of testis is indicative of reproductive efficiency in male farm animals. The weight of the testis of the sheep in the current study was equivalent to 0.35% of body weight. This is in line with observations indicating that testis mass as a proportion of body weight decreases with increasing size of mammals [18]. It has been reported that the gonadosomatic index (testes mass/body mass) in cats was as low as 0.08% [19]. Therefore, it appears that tannin consumption did not directly affect testis size and consequently would not affect reproductive efficiency in male sheep at the levels used in the present study.

On the other hand, tannins in monogastric animals produce a significant effect on the weight of some body organs. Some reports have indicated that tannins significantly increase pancreas weight but not liver weight in chickens [20]. In the latter study, the weight of the pancreas from birds which had received the highest amount of tannins was more than double that of the control birds. Significant enlargement was detectable only at tannin concentrations above 13.5 g/kg. As the tannin content increased from 13.5 to 25 g/kg and from 25 to 50 g/kg the pancreas increased in weight by 33 and 10 % respectively.

The high variability in the measurements of the organ morphometry in the present study was in line with the conclusion of a prior study that biological variation between individuals is likely to be the major factor influencing the overall precision and efficiency of nested sampling schemes for morphometric analysis of thin sections [21]. Therefore, it was recommended that it is important to examine more animals to reduce the variance of the group mean [21].

Similar to the findings of the current study, there have been reports that duodenal villus height and height to crypt depth ratio were significantly lower in animals fed similar non-conventional feeds [6]. Also there have been findings indicating a trend of decreasing villus height and villus surface area in the proximal duodenum [5]. Conflicting reports on the effects of tannins on the morphometry of the intestine have been attributed to differences in species and to the site of sampling due to the wide variations in intestinal structure along the digestive tract [6]. Villus height and crypt depth decreases from the proximal to the distal regions of the small intestine [22].

The effects on liver morphometry were minimal and may have no biological explanation. Therefore, they were in line with those indicating that oral administration of tannic acid to sheep produced no effects on their livers, unlike inter-peritoneal administration of tannins [7]. The significant treatment effect of the higher values for kidney tube length/density in treated animals may have no serious impact on sheep health with normal glomeruli number and size, as seen in this experiment. The lack of treatment effects on the testes measurements substantiates the observations on testis size and volume discussed earlier on.

5. Conclusion

In general, the findings of the current experiment indicate that feeding non-conventional diets containing high levels of polyphenols and tannins does not produce significant effects on the morphometry of sheep intestines and vital organs.

6. Acknowledgements

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