

Effect of Feeding Raw and Processed Meskit (*Prosopis Juliflora*) Pods on Serum Biochemistry and Histopathology of the Liver and Kidney of Omani Sheep

Said Al-Khalasi^{1*}, Osman Mahgoub², Halimatun Yaakub³ and Mohammed Tageldin²

¹Ministry of Agriculture and Fisheries, Oman.

²Department of Animal and Veterinary Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, P.O Box 34, Al-Khod 123, Oman.

³Department of Animal Science, Faculty of Agriculture, Universiti Putra, Malaysia.

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ABSTRACT

An experiment was conducted to study the effects of incorporating raw or processed Meskit pods on histopathology of kidney and liver of sheep. Twenty-four Omani sheep were fed one of four diets: Rhodes grass hay (RGH) plus commercial concentrate (CC); RGH plus raw Meskit pods based diet (RMP); RGH plus roasted Meskit pods based diet (ROMP); RGH plus soaked Meskit pods based diet (SMP) for 84 days. Histological and serum biochemical analyses were used to assess sheep health. Animals were slaughtered and kidney and liver tissue samples were collected. The microscopic results showed that, incorporating raw, roasted or soaked pods to level of 50% of formulated rations resulted in no histological changes in kidney and liver tissues and most serum biochemical values were similar among the treatments. In general, feeding formulated rations based on raw or processed Meskit pods did not cause histological changes in kidneys or livers of Omani sheep even at high intake.

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Introduction

Meskit (*Prosopis juliflora*), is a member of family Legumi nosae, native to Peru, Chile and Argentina and has spread to central and North America. Meskit had been introduced to many arid zone countries, with low rainfall, including Oman, to combat desertification. Although, it tolerates moderate draught and salinity to yield well under these adverse growing conditions and livestock consume its pods, the tree is invasive to natural rangeland and regarded as a weed. Kidney and liver are important organs in body which have vital role in body detoxification.

The liver is the largest gland in body which can produce serum protein and bile [1]. Disorders in liver functions cause many diseases and syndromes in body, such as, icterus, ascites and edema. Dysfunction of kidney can cause uremic syndromes [2],[3]. Because the liver and kidney can be exposed to toxins, this may damage their tissues [4]. Ruminants that graze or browse toxic plants have adapted rumen organisms that detoxify many, but not all secondary metabolites [5], [6]. However, the detoxification process may cause adverse effects in ruminants as a consequence of increased enzymatic demand in the liver, kidney, gut mucosa and other tissues [7].

Meskit pods are becoming an increasingly important source of energy and protein in animal nutrition especially in arid regions. In various regions of the world (particularly where animals are raised on natural range grazing in the tropics), it is customary to feed low-quality non-conventional feeds such as agricultural by-products. These feeds usually contain high fiber and unbalanced mineral

and vitamin content. They may also contain plant secondary compounds, such polyphenols (including condensed tannins) and saponins that may have anti-nutritional effects [8]. Anti-nutritional substances may also affect lipid metabolism by regulating excretion of bile acids and by inhibiting the activity lipase in the digesta [9].

Meskit pods contain cytotoxic alkaloids that may cause intoxications to cattle, horses, sheep and goats in diets constituents of high levels of pods (>50 %). Problems have been observed in Peru, Brazil and USA [10], [11]. Poisonings were also recorded from pods eaten after exposure to rain [12]. [13] reported that feeding low quality non-conventional feeds containing anti-nutritional factors for relatively long periods might produce subtle negative effects on the physiology and chemistry of the digestive system and blood parameters which might negatively affect sheep health and make them more susceptible to diseases.

Histopathological studies revealed degenerative changes in renal tubules, rarefaction of lymphoid tissue and necrotic lesions in the liver [14].

The current study was conducted to investigate any effect of incorporating raw or processed Meskit pods as main ingredient on the serum biochemical values and the histopathology of kidney and liver of Omani sheep.

Materials and methods

Meskit pods collection and processing

Dry Meskit pods were collected during the fruit production season and stored in a cool dry shed. The pods

were then chopped to lengths of 0.5- 1.0 cm to ensure thorough soaking and roasting. Meskit pods were roasted at 150°C for 30 minutes using a locally-made roasting device made up of a 40 kg steel container rotated by an electric motor and heated by gas flame. Ten kg of chopped pods were added to a 30 litre capacity buckets containing 20 liters of tap water. The mixture was left for 24 hours with frequent manual stirring. The pods were washed and allowed to dry for 2 days under the sun with frequent turning over. The *Prosopis* pods concentrates were formulated and pelleted in diet made of 500 g/kg ground Meskit (*Prosopis juliflora*) pods, 240 g/kg wheat barn, 200 g/kg barley, 50 g/kg fish meal and 10 g/kg of salt.

Feeding trial

Twenty four 10 months-old Omani intact male sheep with average live weights 27.3 (± 0.5) kg were used in feeding trial for 84 days. Experimental animals were kept at research farm of Sultan Qaboos University and were subjected to routine animal health management practices. They were weighed, drenched with Ivermectin, vaccinated against small pox and divided into four groups with similar average body weights. Lambs were randomly assigned to receive one of the four dietary treatments (Table 1) with six animals per treatment in a completely randomized design (CRD) assignment. Animals were adapted for 2 weeks before starting the experiment. Each pen was provided with feed trough and a water container, and feed was offered once daily.

Serum biochemistry analysis

Two blood samples from each animal were collected by jugular venipuncture into plain (serum) and EDTA vacutainers tubes (Becton Dickinson, USA) on day 0 and at the end of the feeding period. The blood tubes of all animals were centrifuged for serum separation at 2700 rpm for 10 minutes. The separated serum samples were transferred into 5 ml vials and frozen at -20 °c until serum chemistry studies. The serum biochemistry parameters were determined using a CX7/CX7 serum chemistry analyzer (Synchron; Beckman, USA). These parameters were: total serum protein (TP), glucose (GLU), blood urea nitrogen (BUN), gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST) and Alanine Aminotransferase (ALT).

Preparation of samples for histopathology

After the animals were slaughtered, the kidney and liver tissue samples were collected and samples were fixed in 10% formalin and transmitted to histological laboratory of department of animal and veterinary sciences of Sultan Qaboos University. Then the samples passage for dehydration infiltration and immersion by autotechnicon (JUNG HISTOKINET 2000 Leica) equipment. Samples were embedded in solid paraffin. The paraffinized blocks were sectioned by microtome (JUNG HISTOCUT 820 Leica) in 0.7 micrometer diameters. The sections were stained by H & E staining. The images from slides were captured with the digital Olympus camera (DP12) and light fleurcent microscope (BX60).

Data analysis

Analysis of variance procedure [15], was made to evaluate the effect of commercial concentrate, raw, roasted or soaked Meskit pods based diets on serum biochemistry parameters and feed intake using [16] package. Significant differences between treatments means were assessed using the least the Tukey's Multiple Range Test.

The interaction between the treatments were excluded from the model when not significant ($P > 0.05$).

Results and Discussions

Chemical composition of the feeds

The Meskit pods based concentrate feeds mainly contained 50% of Meskit pods, 24% of barley, 20% of wheat bran and 5% of ground sardine (Table 1). Similar ingredients have been used in previous experiments but have been offered as a totally mixed ration [17]. The animal in those experiments was able to select a feed and, therefore, avoided some of the unpalatable ingredients. The chemical composition of Meskit pods in the current study was comparable with finding of Mahgoub et al., (2005) who reported that *Prosopis* pods contained 127 g/kg CP, 254 g/kg CF, 26 g/kg EE and 48 g/kg ash. This shows that Meskit (*Prosopis juliflora*) pods are ideal livestock feed compared to most available feed resources. It is relatively high in protein content and hence if incorporated into animal feeds; it will improve growth and productivity of livestock. Other studies have also shown that *Prosopis* pods are a good source of protein and energy, with 12-14% crude protein content [18].

Serum biochemistry

The level of serum iron were significantly lower ($P < 0.05$) in sheep fed SMP based diet (Table 3) compared to the other at the end of the feeding trial. Blood glucose levels were lower ($P < 0.05$) in all experimental animals at the end of the trial ($P < 0.001$). Blood urea nitrogen levels were lower in animals fed SMP based diet at the end of the feeding trial. The levels of total protein were significantly higher at the end of experiment for all animals groups at the end of the trial. Level of gamma glutamyl transpeptidase (GGT) was not significantly affected by diet. The level of Alkaline phosphatase (ALP) declined significantly ($P < 0.001$) by the end of the trial for all groups of diet. SMP diet group had significantly lower ($P < 0.05$) Aspartate amino transferase (AST) level than the other groups. The level of alanine amino transferase (ALT) were higher in animals fed the SMP based diet and CC ($P < 0.05$) by the end of the trial however, the levels of GGT were not significantly affected by diet.

There were significant effects of both the treatments and the time of blood sampling on glucose levels with commercial concentrate groups had the highest at the beginning of the experiment mainly because of highly nutritive value and SMP group had the lowest at the end of the experiment which is also an indication that Meskit pods based diets fed animals had been on a good nutritional regime. Meskit pods apparently contained sufficient levels of dietary protein as indicated by the chemical analyses. However, greater proportions of nitrogen may be lost in feces and therefore, not been available for body metabolism which is consistent with the Meskit pods based diet having lower BUN values at some stages of feeding trial. Generally BUN tends to be lower in ruminants with diets low in protein or with severe liver disease ([18] but in this case the levels of crude protein in the formulated diets were relatively high and comparable of the commercial concentrate which explains the similarity of blood urea for all the experimental animals. Most plasma enzymes come from different tissues of animal. Its activity level had a relation with metabolism and functional status of certain organs.

Table 1. Proportional and chemical compositions of ingredients used in the diets

Ingredient	Level in diet %	DM %	DM%					
			CP	Ash	ADF	NDF	EE	GE (kJ/g)
Meskit pods	50	91.85	10.45	1.98	8.12	43.21	1.53	18.53
Wheat bran	24	90.32	14.51	3.25	12.54	44.78	3.44	18.87
Barley grain	20	93.87	14.92	1.97	7.91	43.41	1.55	15.86
Dried sardines	5	91.42	65.71	26.74	6.54	4.12	4.60	15.64
Salt	1	96.0	0	0	0	0	0	0

DM= dry matter; CP: Crude protein; ADF: Acid detergent fibre; NDF: Neutral detergent fibre; EE: ether extract; GE: Gross energy

Table 2. Feed intake (kg) of Omani sheep fed diets containing raw of processed Meskit pods

	Type of diet					Effect of diet
	CC	RMP	ROMP	SMP	SEM	
Numbers of animals	6	6	6	6		
Days of experiment	84	84	84	84		
Total hay intake	43.36	43.10	39.40	40.58	1.002	NS
Total concentrate intake	40.11 ^a	26.99 ^b	36.70 ^a	28.50 ^b	1.389	***
Total feed intake	83.47 ^a	70.10 ^b	76.11 ^{a,b}	69.10 ^b	1.833	**

CC: Commercial concentrate; RMP: Raw Meskit pods based diet; ROMP: Roasted Meskit pods based diet; SMP: Soaked Meskit pods based diet; SEM: Standard error of means; *: p<0.05; **: p<0.01; ***: p<0.001; NS: Non significant.

Table 3. Serum biochemistry values in Omani sheep fed diets based on raw, roasted, or soaked Meskit pods based diets at the start and end of the experiment

Parameter	CC		RMP		ROMP		SMP		S.E.M	p-value Sig.	
	Start	End	Start	End	Start	End	Start	End		Diet	Time
Ca (mg/dL)	9.50	9.67	10.12	9.33	9.83	10.00	8.85	9.2	0.12	NS	<0.05
P (mg/dL)	7.93	8.92	8.62	9.57	8.98	8.48	8.32	9.10	0.32	NS	NS
Mg (mEq/dL)	2.45	2.48	2.72	2.50	2.55	2.58	2.23	2.38	0.04	NS	NS
Iron (µg/dL)	198.67	163.17	168.33	210.00	161.50	217.50	138.67	197.67	8.12	<0.05	NS
Glucose (mg/dL)	76.33	15.00	70.50	16.83	69.17	15.17	63.00	29.00	2.78	<0.05	<0.001
Urea (mg/dL)	25.67	34.17	29.17	35.83	33.67	37.50	27.67	39.67	1.78	NS	<0.05
Creatinine (mg/dL)	0.74	0.86	0.67	0.77	0.74	0.82	0.70	0.84	0.02	NS	NS
Albumin (g/dL)	1.50	1.57	1.52	1.48	1.52	1.67	1.27	1.57	0.03	NS	<0.001
Total protein (g/dL)	5.85	6.50	6.15	6.15	6.25	6.53	5.28	6.25	0.09	NS	<0.05
Creatine (mg/dL)	287.07	141.08	193.93	260.78	342.38	181.27	189.45	176.10	18.91	NS	NS
AST (mg/dL)	90.00 ^{a,b}	88.50	96.33 ^{a,b}	110.33	106.83 ^a	103.50	73.33 ^b	99.00	3.74	<0.05	<0.05
GGT (mg/dL)	60.67	55.50	72.00	49.00	67.00	57.00	54.67	56.17	1.86	NS	NS
ALT (µg/L)	297.33 ^{a,b}	126.00 ^c	291.17 ^{a,b}	210.00 ^d	355.33 ^a	177.00	225.33 ^b	151.67	11.18	<0.05	<0.001

CC: Commercial concentrate; RMP: Raw Meskit pods based diet; ROMP: Roasted Meskit pods based diet; SMP: Soaked Meskit pods based diet; SEM: Standard error of means; *: p<0.05; **: p<0.01; ***: p<0.001; NS: Non significant, on the same row with same superscripted letter do not differ significantly (p>0.05).

Body's ability to adjust and adapt depends on the function of tissues and organs largely [20]. Concentrations of its enzymes alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) are those conventionally used for diagnosing hepatic damage. Liver enzymes such as ALT, which is a liver specific hepatocellular enzyme released by hepatocellular was both effected by diet and time of bleeding with ROMP diet group had the highest at the start of the feeding but dropped at the end. However, GGT, were similar in all experimental animals. This indicates that livers of these animals have not been affected by the anti-nutritional factors in the diets which is in contrast with reports that such compounds cause animal tissue damage [21], [22] which might be because of low feed intake in both the RMP diet and SMP diet groups, and the roasting treatment could help

reducing the affects of the anti-nutritional factors such as phenol and alkaloids compounds.

Minerals play an important role in the regulation of body fluids, acid base balance and metabolic process [23].

Regarding to calcium concentration in the plasma, the results showed that MPBDs fed groups had non-significant effect in lowering the mean value compare with CC fed group. With Meskit pods accounting for 30% of concentrate diet of cattle there was no effect blood glucose, calcium, phosphorus, copper, zinc and iron levels in the blood [24]. These results were confirmed for hemoglobin, blood calcium and phosphorus levels for bullocks fed *Prosopis juliflora* pods up to 45% DM of their diet [25].

Histopathology of liver

The liver is a large gland in digestive system. It is storing nutrient material to absorb from small intestine as glycogen. Additionally, the liver produces plasma protein and

detoxifying exotic toxin. Therefore, hepatocytes are very active cells with euchromatin nucleus and eosinophilic cytoplasm. The liver has capillary plexus as the sinusoid. The hepatic sinusoids are facilitating rapid transportation of material between blood and hepatic tissue. [26]. Due to hepatocytes are exposing directly to blood materials exits in blood; it can be effect on hepatocytes. Some of the plant secondary products in these feeds may have anti-nutritional effect. Besides reducing protein and carbohydrate digestibility, they could be have direct effect on the structure and function of body essential organs such as the digestive tract, kidneys and liver resulting in tissue damages and system malfunction. Hepatocytes of the liver of sheep which fed with the high amount of Meskit pods did not show pyknotic nucleus, degeneration or necrosis hepatocytes. Also, there was no serious damage in the hepatic tissues of animals which received processed Meskit pods (Figure 2), and it is supported by [27] who reported that sheep fed raw or processed Meskit pods based diets did not show signs of disease such as diarrhea, constipation or anorexia. This is could be mainly due to roasting treatment for the ROMP based diet and the low concentrate intake for the other formulated diets and also it might be due to genetic diseases resistance in of the Omani sheep which have been naturally selected over generations for survival and ability to utilize low quality feeds.

Histopathology of kidney

The kidney is important organ for detoxification and filtration of blood from the metabolites which are produced in the metabolic activity of cells. Also kidney is able to detoxify the toxins which enter directly to body. This resulted kidney expose to the toxins directly, therefore, maybe it can cause serious damages on the kidney tissue [26]. Findings of this research showed that incorporating raw or processed Meskit in the ration did not cause any serious damage to kidney tissue such as degeneration of proximal convoluted tubule (PCT) cells, distal convoluted tubule (DCT) cells, congestion or hemorrhage. There were also no necropsy changes in kidney structure (Figure1).

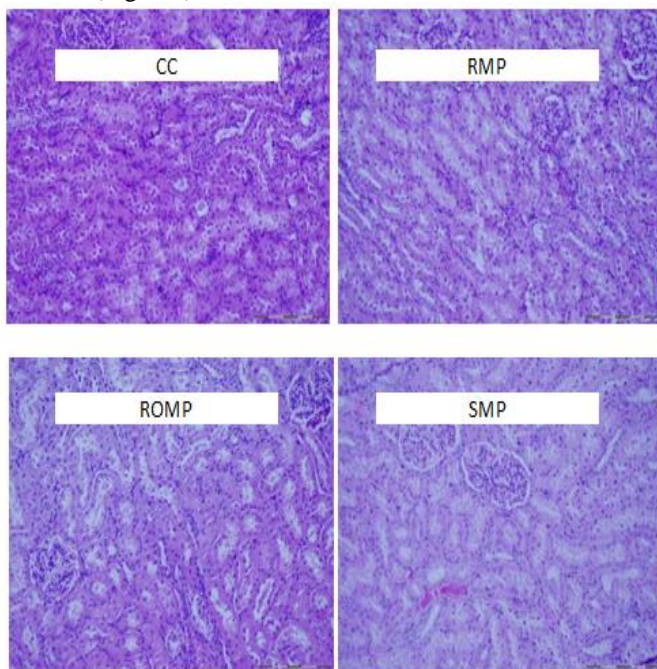


Figure 1. Micrograph from sections of sheep's kidneys fed (CC) commercial concentrate,(RMP) raw, roasted (ROMP) or soaked (SMP) Meskit pods based diets (magnification 10000-25000X)

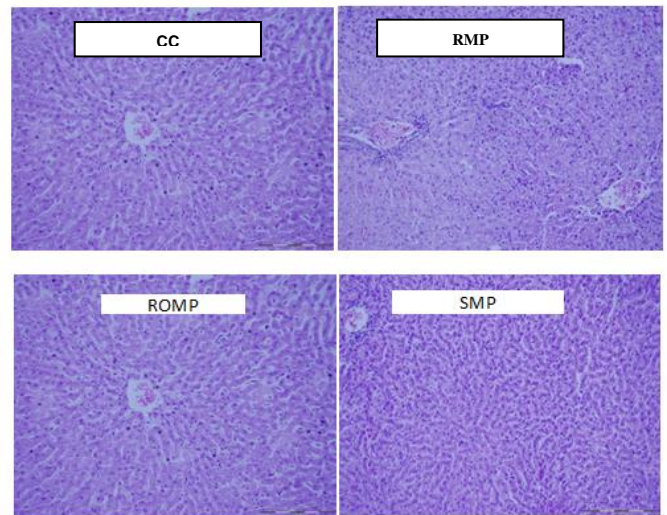


Figure 2. Micrograph from sections of sheep's livers fed (CC) commercial concentrate, (RMP) raw, roasted (ROMP) or soaked (SMP) Meskit pods based diets (magnification 10000-25000X)

The levels of urea and creatinine were similar within the groups which indicated that there was no significant effect of Meski pods based diets on the kidney.

Urea and creatinine are the two main nitrogenous compounds eventually excreted by kidney. Accordingly, any change of their concentration would reflect impaired glomerular filtration and/ or insufficiency of renal tubules [28]. In current study the level of urea and creatinine were similar within the groups which indicated that there was no significant effect of Meski pods based diets on the kidney. Furthermore, urea is the major nitrogen- containing metabolic product of protein catabolism accounting for more than 75% of the non- protein nitrogen excreted. Also, production is also too dependent on several non- real variables such as a diet and hepatic synthesis [29].

Ruminants that graze or browse on toxic plants have adapted rumen organisms that detoxify many, but not all secondary metabolites [5], [30]. However, the detoxification process may cause adverse effects in ruminants as a consequence of increased enzymatic demand in the liver, kidney, gut mucosa and other tissues [31]. However, in the current study , there was no a consequence of increased enzymatic level in the lever, or kidney (Table 3) which indicated that incorporating raw or processed Meskit pods up to 50% in diets did not cause harmful effect on histopathology of the liver and kidney of Omani sheep.

Conclusion

The results of this research showed that incorporating of raw, roasted or soaked Meskit pods up to 50% in the rations did not cause any serious damage in kidney or liver tissues of Omani sheep. This is could be mainly due roasting treatment for the roasted Meskit pods based diet and the low concentrate intake for the other types of Meskit pods based diets and also it might be due to genetic diseases resistance in of the Omani sheep which have been naturally selected over generations for survival and ability to utilize rangeland low quality feeds.

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