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**Volume 6 (2); 25 March 2016****Research Paper****Effect of supplementation, birth type and sex on lambs' growth rate under range condition.**

IDRIS A., Kijora C., Salih A. M., Eltaher H. and Bushara I.

*Online J. Anim. Feed Res.*, 6(2): 24-29, 2016; pii: S222877011600005-6**Abstract**

Supplementary feeding experiment was carried out with desert ewes and their lambs prior to late pregnancy days and during lactation period at Agricultural Research Station, El-Obeid, North Kordofan, Sudan. The aim of the study was to investigate the effect of supplementation on body weight of lactating ewes and their lambs' growth rate in the dry season. The ewes were allocated to one of four treatment groups, one group was the control (CTL) as in farmer practice. The second, third and fourth groups were supplemented with rations composed of local ingredients, diet 1 composed of groundnut seed cake, Roselle seeds, sorghum and 5% molasses (GRS-5%M), diet 2 composed of groundnut seed cake, Roselle seeds, and sorghum (GRS), and diet 3 composed of groundnut seed cake, Roselle seeds, sorghum and 7.5% molasses (GRS-7.5%M). Ewes and lambs were recorded within 4 h after birth. Lambs weights were recorded weekly before weaning weight (day 60) and till 120 days. The results indicated that, lambs growth rate was highest for supplemented dams before weaning, lambs suckling dams supplemented with GRS-7.5%M recorded heavier ( $P < 0.05$ ) weights, and then followed by GRS and GRS-7.5%M. Lambs suckling dams on control group (CTL) recorder lower ( $P < 0.05$ ) growth rate in 75 days. Ewe's age had effect on lambs weight change, lambs suckling older animals gained weight earlier compared with younger, also the study showed that, male lambs had higher ( $P < 0.05$ ) growth rate than female lambs. Single lambs were significantly heavier than twins before weaning.

**Keywords:** Supplementation; Late Pregnancy, Desert Ewes, Lambs Growth Rate, Dry Season.[PDF](#) [XML](#) [DOAJ](#)**Research Paper****Kitchen waste - a promising feed resource for livestock.**

Hossain M.E., Ahmed M.I., Sultana S.A. and Karim M.H.

*Online J. Anim. Feed Res.*, 6(2): 30-37, 2016; pii: S222877011600006-6**Abstract**

The study was conducted to find out the chemical composition of different vegetable wastes to use them as feed for livestock to enhance their productivity as well as to reduce feed cost. Total 10 different types of vegetable wastes like Banana tree (*Musa paradisiaca*), Bean leaf (*Lablab purpureus*), Bilimbi leaf (*Averrhoa bilimbi*), Cabbage (*Brassica oleracea* var. capitata), cauliflower (*Brassica oleracea* var. botrytis), Pumpkin (*Cucurbita maxima*), Pumpkin leaf (*Cucurbita maxima*), Radish (*Raphanus sativus*), Ridge guard (*Luffa acutangula*) and Spinach (*Spinacea oleracea*) available in different areas of Chittagong, Bangladesh were collected. Samples were chopped and tested immediately for moisture content and remaining samples were sun-dried and processed using standard procedure. Chemical analyses of the samples were carried out in triplicate for Dry matter (DM), Crude protein (CP), Crude fiber (CF), Nitrogen free extract (NFE), Ether extract (EE) and Ash. Metabolizable energy (ME) was calculated mathematically for all samples by using standard formula. Results indicated that, crude protein content in Banana tree was 15.6 g/100g, Bean leaf 28.2 g/100g, Bilimbi leaf 11.9 g/100g, Cabbage 18.9 g/100g, Cauliflower 17.3 g/100g, Pumpkin 12.9 g/100g, Pumpkin leaf 25.0 g/100g, Radish 14.9 g/100g, Ridge guard 23.4 g/100g and Spinach 11.4 g/100g. In addition to crude protein, all samples contained substantial amount of crude fibre, nitrogen free extracts, ether extracts and ash. It could therefore be inferred that, the vegetable wastes could be incorporated in appreciable quantities for substituting the conventional feed resources of animal diet.

**Keywords:** Ash, Crude Fiber, Crude Protein, Ether Extract, Kitchen Waste, Moisture, Nitrogen Free Extract.[PDF](#) [XML](#) [DOAJ](#)**Research Paper****Minerals disappearance rate of leaves of some acacia trees after digestion in goats' rumen using nylon bags technique.**

Al shafei N. K and Nour A.

*Online J. Anim. Feed Res.*, 6(2): 38-44, 2016; pii: S222877011600007-6**Abstract**

The browse plants, including acacia species, provide excellent forage with high nutritive value for ruminants especially in dry areas of Africa. In this study, some minerals (P, K, Na, Mg, Ca, Mn, Cu and Zn) were determined in leaves of browse plants (*Acacia albida*, *Acacia nubica*, *Acacia sieberiana*, *Balanites aegyptiaca* and *Ziziphus spina- christi*) collected from different areas of Sudan before and after digestion of the sample in goat's rumen by using nylon bag technique. Nylon bags containing the samples were inserted through the rumen fistula into the goat's rumen, and were incubated for 6, 12, 24, 48, and 72 hrs. After incubation periods, it was found that there were a high loss of minerals and this is attributed to the rumen digestion and solubility of minerals in rumen liquor. The results indicate that the time of incubation and the type of mineral likely had a significant effect on the loss of minerals in the rumen. It can be observed from these figures that the disappearance rates (slope of the curves) vary across mineral types and species of acacia trees. Disappearance rates suggest that the rumen microorganisms have a significant role in the digestion of minerals and their disappearance

rates are due to the solubility of minerals in the rumen liquor and the loss of minerals due to utilization of microorganisms to a certain amount for their maintenance.

**Keywords:** Acacia Trees, Browse Plants, Minerals, Rumen, Nylon Bag Technique, Disappearance Rates.

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## Review

### Effect of dietary tannin source feeds on Ruminal fermentation and production of cattle; a review.

Addisu Sh.

*Online J. Anim. Feed Res.*, 6(2): 45-56, 2016; pii: S222877011600008-6

#### Abstract

Generally, tannins are widely distributed throughout the plant kingdom, especially among trees, shrubs and herbaceous leguminous plants. Tannins are naturally occurring polyphenols with different molecular weights and complexity that are synthesized during the secondary metabolism of plants. Tannins might bind to macromolecules (proteins, structural carbohydrates and starch) and decrease their availability to digestion. Tannins based on their chemical structure and properties divided into two groups, hydrolyzable tannins (HT) and Condensed tannins (CT, proanthocyanidins). Tannins are polyphenols, which directly or indirectly affect intake and digestion. They are the primary source of astringency in plants, which results from binding to proteins, forming soluble or insoluble complexes. The nature of the interaction is greatly dependent on the structure of the polyphenols and the proteins involved. Relatively low concentration of tannins (0.5% of DM intake) is sufficient to destabilize the blood proteins while high concentration (2-4% of DM intake) is needed for improvement of protein utilization. High concentration (> 5% of dry weight reduces feed intake and feed conversion efficiency. Tannins containing forages will be important for small ruminants to control of gastrointestinal parasites. Animals fed condensed tannin had lower dressing percent than controlled one; with dressing percent being intermediate for animals fed hydrolysable tannin. Neither tannin source affected the animal's consumption of the diet or the animal's growth. Additionally, the tannin sources did not affect the meat or by-product tissues, making tannin supplementation a viable option in finishing beef cattle. Therefore, tannin source feed will have its own advantages and disadvantages on animals' performance.

**Keywords:** Dietary Tannin, Digestion, Ruminal fermentation

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## Research Paper

### Effect of medicated urea molasses blocks on sub-clinical parasitic infestations in goats.

Abid R, Khan I, Bhatti J A, Shah Z, Zahoor A, Ahmad Sh.

*Online J. Anim. Feed Res.*, 6(2): 57-61, 2016; pii: S222877011600008-6

#### Abstract

The aim of this study was to evaluate the effect of medicated urea molasses blocks (MUMB) on sub-clinical parasitic infestations and urea molasses blocks (UMB) to replenish nutrients scarcity. Twenty four goats were divided randomly into three groups of eight animals each (n=8) according to Completely Randomized Design (CRD) a group was no supplement (control) and the other were supplemented with UMB and MUMB for 90 days. Data were recorded and statistically analyzed under CRD through one way analysis of variance (ANOVA). Mean daily dry matter intake was higher ( $1.502 \pm 0.121$  kg) in MUMB supplemented group and lowest in control group (Lenovo). Mean daily weight gain of goats in control, UMB, MUMB was  $64 \pm 23$ ,  $71 \pm 22$  and  $85 \pm 21$  grams, respectively. Body condition score (BCS) was recorded in 1-5 scale of meat goats. The mean BCS in control, UMB and MUMB was  $2.741 \pm 0.193b$ ,  $2.816 \pm 0.185ab$  and  $2.903 \pm 0.248a$  respectively. Mean fecal egg count was lowest in MUMB as followed by UMB and control group. It is concluded that feeding of MUMB have significant effects for the control of sub-clinical gastrointestinal worm's infestation and replenishes nutrients deficiency by providing energy and protein.

**Keywords:** Goats, Nutrients Deficiency, Gastrointestinal Worm, Infestations

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# EFFECT OF SUPPLEMENTATION, BIRTH TYPE AND SEX ON LAMBS' GROWTH RATE UNDER RANGE CONDITION

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**ABSTRACT:** Supplementary feeding experiment was carried out with desert ewes and their lambs prior to late pregnancy days and during lactation period at Agricultural Research Station, El-Obeid, North Kordofan, Sudan. The aim of the study was to investigate the effect of supplementation on body weight of lactating ewes and their lambs' growth rate in the dry season. The ewes were allocated to one of four treatment groups, one group was the control (CTL) as in farmer practice. The second, third and fourth groups were supplemented with rations composed of local ingredients, diet 1 composed of groundnut seed cake, Roselle seeds, sorghum and 5% molasses (GRS-5%M), diet 2 composed of groundnut seed cake, Roselle seeds, and sorghum (GRS), and diet 3 composed of groundnut seed cake, Roselle seeds, sorghum and 7.5% molasses (GRS-7.5%M). Ewes and lambs were recorded within 4 h after birth. Lambs weights were recorded weekly before weaning weight (day 60) and till 120 days. The results indicated that, lambs growth rate was highest for supplemented dams before weaning, lambs suckling dams supplemented with GRS-7.5%M recorded heavier ( $P < 0.05$ ) weights, and then followed by GRS and GRS-7.5%M. Lambs suckling dams on control group (CTL) recorded lower ( $P < 0.05$ ) growth rate in 75 days. Ewe's age had effect on lambs weight change, lambs suckling older animals gained weight earlier compared with younger, also the study showed that, male lambs had higher ( $P < 0.05$ ) growth rate than female lambs. Single lambs were significantly heavier than twins before weaning.

**Keywords:** Supplementation; Late Pregnancy, Desert Ewes, Lambs Growth Rate, Dry Season.

## INTRODUCTION

Sudan desert sheep and their crosses are raised mainly under harsh dry land farming conditions for meat production in western Sudan (Khalafalla and Sulieman, 1992). Sudan desert sheep are entirely dependent on grazing natural rangeland and forest. They prefer short grasses and they have difficulty in eating coarse feedstuff. While they are not credited with browsing on bush, thorn bush and trees. The most critical period for rearing sheep in the semi-desert zone of Sudan is from February to June, when the ambient temperature becomes hot and range grazing is scanty and depleted of nutrients (Cook and Fadlalla, 1987). The nutritional limitation, low nutritive value of the range, high ambient temperature, scarcity of feed and water have great effect on the production of the sheep in semi arid area of Kordofan state (El-Hag, 2001).

This study was undertaken to evaluate the effects of ewes' supplementation, sex and birth type on lambs' daily growth rate before and after weaning in the dry season. The main ultimate objective was to introduce supplementation concept to nomadic herd owners.

## MATERIAL AND METHODS

### Study area

The study was conducted at Agricultural Research station, El-Obeid, North Kordofan state (latitudes 11°:15'-16°:30' N and longitudes 27°-32° E), Sudan. Most of North Kordofan state lies within arid and semi-arid ecological zones. The average maximum temperature varies between 30 and 35°C during most of the year with peaks above 40°C during hot summer. The rainy season extends from July to October, reaching to peaks in August. The annual rainfall ranges from 75 mm in north and about 500mm in south and with average 280 mm (Technoserve, 1987). The vegetation varies from north to south. In the north grass land and shrubs predominate while bushes and trees are common in the south (Harrison and Jackson 1958; Vogt 1995).

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### Experimental procedure and feed supplementation

This experiment was carried out in ewes that lambd in the dry season (March-April) when there is not enough feed and the pasture is poor. A total of 290 ewes 2 to 6 years old reared in natural range conditions were selected during late pregnancy days. Ewes were allocated to one of four treatment groups, the control group (n = 37) was kept as in farmer practice without any supplementation (CTL). The second group (n = 86), third (n = 87) and fourth groups (n = 80) were supplemented with rations composed from local ingredients, diet 1 composed of groundnut seed cake, Roselle seeds, sorghum and molasses (GRS-5%M), diet 2 composed of groundnut seed cake, Roselle seeds, and sorghum (GRS), and diet 3 composed of groundnut seed cake, Roselle seeds, sorghum and molasses (GRS-7.5%M). The amount of the diets is illustrated in Table 1 and all the ingredients used in this experiment were grown in the study area and Roselle seed (*Hibiscus sabdariffa*) is a species of *Hibiscus* native to the Old World tropics. All ewes were allowed for 2 weeks as adaptation period before lambing. Experimental animals were maintained on pasture and supplemented with treatments until day 120. During late pregnancy days and after birth animal were offered individually 200 g / head daily in the morning at 6:00 am. The lambs were separated from their mothers after suckling in the morning.

Live weight and of the ewes and lambs were recorded within 4 h after birth using, weights lambs were recorded weekly before weaning weight (day 60) and tell 120 days. Date, type of birth and sex of lamb were recorded. At weaning, lamb survival was calculated for each treatment group.

### Analytical procedures:

The analysis is performed to experimental diets according to A.O.A.C. (1995) and Goering and Van Soest (1991). The content of the metabolizable energy (ME, MJ / kg DM) was calculated from table values of energy content of the components.

### Statistical analyses

Data on lambs' weight at birth and weekly growth rate changes were analyzed using a least square model with supplement, type of birth and sex as fixed effects and the random error (Harvey 1990). Significant differences among means were tested using Duncan's new multiple range test (Duncan, 1995).

**Table 1 - Composition of the diets (%) and Chemical composition (g/kg dry matter) of the supplements and calculated energy content.**

Ingredlents	Treatments		
	Diet 1 (GRS-5%M )	Diet 2 (GRS)	Diet 3 (GRS-7.5%M )
Molasses	5	—	7.5
Sorghum	29	34	26.5
Roselle seeds	25	25	25
Groundnut seed cake	40	40	40
Common salt	0.75	0.75	0.75
Salt lick	0.25	0.25	0.25
<b>Chemical composition</b>			
Dry matter	923	926	934
Crude protein	381	369	330
Crude fibre	90	98	88
Crude fat	53	65	88
NDF	191	192	205
ADF	141	149	130
ADL	45	44	40
HEMI	51	44	75
CELLU	96	104	90
Ca g / kg DM	24	17	32
P g / kg DM	53.2	54	42
Energy density (Mcal DE/kg DM)	11.25	11.29	11.71
In vitro OM digestibility (%)	73.38	71.53	71.21
NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; HEMI: hemi cellulose (NDF-ADF); CELLU: cellulose (ADF-ADL). OM: organic matter. GRS-5%M; Ground nut cake, Roselle seeds, Sorghum and 5% Molasses; GRS ;Ground nut cake, Roselle seeds and Sorghum; GRS-7.5%M; Ground nut cake, Roselle seeds, Sorghum and 7.5% Molasses.			

## RESULTS AND DISCUSSION

The effect of supplementation on lamb's growth rate during dry season was shown in Table 2. Analysis of variance revealed significant differences ( $P < 0.05$ ) in daily growth rate of lambs. Before weaning (0-60 days), lambs growth rate were highest ( $P < 0.05$ ) for supplemented groups. When lactation curve advanced the significant differences among the three treatments began to appear on the growth rate after 60 days post partum. Lambs suckling dams supplemented with GRS-5%M recorded heavier weights, and then followed by GRS and GRS-7.5%M. Lambs suckling dams on control group (CTL) recorder lower growth rate in 75 days. Also the study showed that, there were no significant differences ( $P \geq 0.05$ ) in daily growth rate of lambs from 75-90 days post-partum. Lambs growth rate were highest ( $P < 0.05$ ) for CTL in 120 days post-partum. Over all daily growth rate was highest for GRS-5%M, followed by GRS and GRS-7.5%M, and the control group recorded lower growth rate.

Supplementation of dams during early lactation had significant effect on body weight change. Lambs suckling dams on framer's practice had lower body weight compared with fed groups, and this may be due to that, dams may not get their enough nutrient requirements to meet mammary growth and milk production. Supplementation of pregnant ewes during late two weeks of gestation may provide adequate energy and protein so they produce more milk yield that reflected on growth rate of their lambs, which supports embryonic and foetal growth and maintenance of animal physiological needs. Similar results were noted by Oeak et al. (2005), Sairanen et al. (2006) and El-Hag et al. (1998). Non supplemented ewes lost more body reserves compared with supplemented groups, same results were recorded by Rafiq et al. (2006), also the dams produce less milk yield.

Supplementation had effect on lamb weight changes. Lambs suckling supplemented dams recorded heavier weights at birth and during the experimental period. Findings of Rafiq et al. (2006) had revealed that lamb's birth weight is significantly and positively correlated with ewe body weight. Similar observation was supported by El-Nasr et al. (1994) and Zahari et al. (1994). These workers reached a conclusion that feeding a balanced concentrate promotes better growth.

Dams' age had significant ( $P < 0.05$ ) effect on lambs growth rate (Table 3). The results showed that, lambs suckling dams their age more than 5 years began to gain more weight in the first 15 days of lactation, compared with animals less than 5 years. After weaning (60-75 days) the growth rate improved for the lambs born from dams whose age is less than 5 years. Ewe's age had effect on lambs weight change, older animals gained weight earlier compared with younger ones. These findings highlight that, nutritional status of older ewes mobilizes their body weight more than younger ewe and lactation curve may be affected with dam's age. These findings showed that, lambs from younger ewes would be lighter than lambs from the older ewes. These findings highlight that, lambs from supplemented dams were suckling more milk than lambs born from control ewes. Similar results reported by Nnadi et al. (2006) and Njoya et al. (2005).

The effects of lamb sex had no significant ( $P > 0.05$ ) effects on lambs' daily growth rate. Male lambs had higher growth rate during the experimental period than female lambs (table 4). Type of birth had a highly significant ( $P < 0.05$ ) effect on lamb growth rate before and after weaning birth (table 4). Single lambs were significantly heavier than twins from 15 days to 105 day post-partum.

The birth type had no significant effect ( $P > 0.05$ ) on growth of the lambs before the first two weeks of the lactation and 120 days post-partum. Single lambs were heavier than twins at birth and in the most experimental period. Lactation might have been inadequate in ewes with multiple births to satisfy all the lambs. Single lambs are suckling more milk than twins, for this reason single lamb had faster growth rate than twins. The results are supported by many researches obtained by Macit et al. (2002); Macit et al. (2001); Analla et al. (1998); Cloete et al. (2007); Boujenane and kansari (2002); Rastogi (2001); Njoya et al. (2005), Tuah and Baah (1985). These results disagree with El-Toum (2005) and Ngere and Aboagye (1981), the authors found that, live weights of single and twin were similar.

The study showed that, the interaction between birth type and sex had significant effect ( $P < 0.05$ ) on daily lambs, growth rate over all the period (Table 4). Female single and male single were recorded higher growth rate than female twin and male twin respectively.

The fluctuation of lamb's weight during the last weeks may be due to the result of decrease in milk yield. Male and female single lambs were slightly heavier than male and female twins. This result was in line with findings of El-Toum (2005). The current results explained that, birth type had more effect on lamb's weight than sex. It is logic that, single lambs grew faster than twins, because twins lambs consumed lower milk than singles. However many researchers have reported a significant influence of type of birth with single born lambs being heavier than their twins (Sandford et al. 1982; Sulieman et al. 1990

**Table 2 - The effect of supplementary rations on lambs growth rate (g/day)**

Day Treatment	0-15 day	15-30 day	30-45 day	45-60 day	60-75 day	75-90 day	90-105 day	105-120 day	Over all (0-120)
GRS-5%M	242.6± 6.6 <sup>a</sup>	249.4±10.8 <sup>a</sup>	212.0±9.1 <sup>a</sup>	224.8±7.5 <sup>a</sup>	226.0±7.5 <sup>a</sup>	197.3±7.5 <sup>NS</sup>	232.5±11.7 <sup>a</sup>	171.2±6.4 <sup>b</sup>	207.6±2.2 <sup>a</sup>
GRS	239.3±7.2 <sup>a</sup>	255.8±6.5 <sup>a</sup>	222.8±6.5 <sup>a</sup>	221.3±9.5 <sup>a</sup>	205.2± 5.8 <sup>b</sup>	199.5± 7.0 <sup>NS</sup>	195.0± 5.9 <sup>b</sup>	170.1± 5.6 <sup>b</sup>	195.8± 2.1 <sup>b</sup>
GRS-7.5%M	233.9±4.9 <sup>a</sup>	241.0±5.0 <sup>ab</sup>	225.0±6.6 <sup>a</sup>	238.8±8.0 <sup>a</sup>	196.8± 5.5 <sup>b</sup>	180.9± 4.7 <sup>NS</sup>	210.9± 8.6 <sup>a</sup>	155.5± 4.3 <sup>b</sup>	200.3± 2.3 <sup>ab</sup>
CTL	211.1±6.4 <sup>b</sup>	204.3±12.2 <sup>b</sup>	178.1±8.0 <sup>b</sup>	191.6± 9.9 <sup>b</sup>	187.7±11.0 <sup>b</sup>	186.0± 7.2 <sup>NS</sup>	190.3± 11.7 <sup>b</sup>	208.2± 24.2 <sup>a</sup>	182.0± 5.5 <sup>b c</sup>

<sup>abcd</sup> Means in the same column bearing different superscripts are significantly ( $P<0.05$ ) different; GRS-5%M; Ground nut cake, Roselle seeds, Sorghum and 5% Molasses; GRS ;Ground nut cake, Roselle seeds and Sorghum; GRS-7.5%M ;Ground nut cake, Roselle seeds, Sorghum and 7.5% Molasses; CTL: Control

**Table 2 - The effect of dam age's on lambs growth rate (g/day)**

Day Age	0-15 day	15-30 day	30-45 day	45-60 day	60-75 day	75-90 day	90-105 day	105-120 day	Over all (0-120)
3≥ years	224.3± 5.0 <sup>b</sup>	234.0± 7.8 <sup>NS</sup>	212.5± 6.5 <sup>NS</sup>	230.7± 9.0 <sup>NS</sup>	210.1± 6.2 <sup>ab</sup>	193.9± 6.1 <sup>NS</sup>	207.5± 6.1 <sup>NS</sup>	166.5± 5.0 <sup>b</sup>	197.1± 2.0 <sup>NS</sup>
4-5 <years	236.0± 6.6 <sup>ab</sup>	233.0± 7.9 <sup>NS</sup>	222.2± 7.5 <sup>NS</sup>	225.2± 10.0 <sup>NS</sup>	217.9±7.9 <sup>a</sup>	192.5± 5.6 <sup>NS</sup>	208.3± 8.5 <sup>NS</sup>	187.6 ± 11.1 <sup>a</sup>	199.4± 3.5 <sup>NS</sup>
5 <years	244.4± 5.9 <sup>a</sup>	244.7± 7.6 <sup>NS</sup>	212.5± 6.7 <sup>NS</sup>	217.6± 5.4 <sup>NS</sup>	195.3±4.3 <sup>b</sup>	190.9± 6.3 <sup>NS</sup>	210.8± 6.3 <sup>NS</sup>	160.2± 3.9 <sup>b</sup>	199.4± 1.5 <sup>NS</sup>

<sup>abcd</sup> Means in the same column bearing different superscripts are significantly ( $P<0.05$ ) different.

**Table 4 - The effect of sex and birth type on lambs growth rate (g/day)**

Items	0-15 day	15-30 day	30-45 day	45-60 day	60-75 day	75-90 day	90-105 day	105-120 day	Over all (0-120)
<b>Sex</b>									
Female	234.0± 6.4 <sup>NS</sup>	219.8± 8.0 <sup>NS</sup>	203.4± 6.5 <sup>NS</sup>	212.8± 8.4 <sup>NS</sup>	206.0± 6.5 <sup>NS</sup>	183.5± 6.4 <sup>NS</sup>	212.1± 8.6 <sup>NS</sup>	173.0 ±7.5 <sup>NS</sup>	195.3± 2.5 <sup>NS</sup>
Male	232.3± 6.5 <sup>NS</sup>	225.2± 6.6 <sup>NS</sup>	217.0± 6.6 <sup>NS</sup>	217.5± 6.5 <sup>NS</sup>	201.5± 6.6 <sup>NS</sup>	188.5± 6.6 <sup>NS</sup>	212.6± 8.8 <sup>NS</sup>	170.1± 7.7 <sup>NS</sup>	198.2± 2.5 <sup>NS</sup>
<b>Birth type</b>									
Single	236.5± 3.8 <sup>NS</sup>	245.6± 4.8 <sup>a</sup>	220.6± 3.9 <sup>a</sup>	227.5± 5.0 <sup>a</sup>	207.2± 3.9 <sup>a</sup>	195.1± 3.9 <sup>a</sup>	203.5± 5.2 <sup>b</sup>	170.7± 4.5 <sup>NS</sup>	199.4± 1.5 <sup>NS</sup>
Twin	229.7± 8.3 <sup>NS</sup>	199.4± 10.4 <sup>b</sup>	199.7± 8.4 <sup>b</sup>	203.2± 10.0 <sup>b</sup>	200.4± 8.4 <sup>b</sup>	177.0± 8.3 <sup>b</sup>	230.2 ± 11.1 <sup>a</sup>	172.4± 9.7 <sup>NS</sup>	194.1± 3.2 <sup>NS</sup>
<b>Interaction</b>									
<b>Sex X birth type</b>									
Female single	235.8± 5.6 <sup>NS</sup>	242.1± 7.1 <sup>a</sup>	212.7± 5.7 <sup>a</sup>	215.4± 7.3 <sup>NS</sup>	199.5± 5.7 <sup>NS</sup>	188.6± 5.6 <sup>a</sup>	193.6± 7.5 <sup>b</sup>	173..6± 6.6 <sup>NS</sup>	197.2± 202 <sup>a</sup>
Female twin	232.1± 11.5 <sup>NS</sup>	197.5± 14.5 <sup>b</sup>	194.0± 11.6 <sup>b</sup>	210.3± 15.0 <sup>NS</sup>	212.5± 11.6 <sup>NS</sup>	178.4± 11.5 <sup>b</sup>	248.6± 15.4 <sup>a</sup>	172.4± 13.5 <sup>NS</sup>	193.3± 4.4 <sup>b</sup>
Male single	237.3± 5.3 <sup>a</sup>	249.1± 6.6 <sup>a</sup>	228.5± 5.3 <sup>a</sup>	238.9± 6.9 <sup>a</sup>	214.8± 5.3 <sup>a</sup>	201.5± 5.3 <sup>a</sup>	213.4± 4.1 <sup>NS</sup>	167.8± 6.2 <sup>NS</sup>	201.6± 2.0 <sup>a</sup>
Male twin	227.3±11.9 <sup>b</sup>	201.3± 15.0 <sup>b</sup>	205.4± 12.1 <sup>b</sup>	196.0± 15.6 <sup>b</sup>	188.2± 12.1 <sup>b</sup>	175.5±12.0 <sup>b</sup>	211.8± 16.0 <sup>NS</sup>	172.3± 4.0 <sup>NS</sup>	194.9± 4.6 <sup>b</sup>

<sup>ab</sup> Means in the same column bearing different superscripts are significantly ( $P<0.05$ ) different.

## CONCLUSION AND RECOMMENDATION

The results of the present study indicated the importance of the nutritional status of the dams during lactation on the body weight changes of dams and their lambs. Accordingly, it is considered necessary to develop nutritional strategies, using locally available concentrate feeds, for supplementary feeding of grazing ewes that lambed in the dry season in order ensure adequate body weight at late gestation and after lambing, and so satisfy the different nutrient requirements. These strategies should consider the seasonal availability and nutritive quality of the natural pasture.

### Recommendations

It is recommended that, Supplementation dams during lactation should be undertaking using groundnut seed cake, Roselle seeds or other relevant local ingredients. Supplementation of lactating ewes was necessary in dry season.

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### Competing Interests

The authors declare that they have no competing interests.

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# KITCHEN WASTE - A PROMISING FEED RESOURCE FOR LIVESTOCK

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**ABSTRACT:** The study was conducted to find out the chemical composition of different vegetable wastes to use them as feed for livestock to enhance their productivity as well as to reduce feed cost. Total 10 different types of vegetable wastes like Banana tree (*Musa paradisiaca*), Bean leaf (*Lablab purpureus*), Bilimbi leaf (*Averrhoa bilimbi*), Cabbage (*Brassica oleracea* var. capitata), cauliflower (*Brassica oleracea* var. botrytis), Pumpkin (*Cucurbita maxima*), Pumpkin leaf (*Cucurbita maxima*), Radish (*Raphanus sativus*), Ridge guard (*Luffa acutangula*) and Spinach (*Spinacea oleracea*) available in different areas of Chittagong, Bangladesh were collected. Samples were chopped and tested immediately for moisture content and remaining samples were sun-dried and processed using standard procedure. Chemical analyses of the samples were carried out in triplicate for Dry matter (DM), Crude protein (CP), Crude fiber (CF), Nitrogen free extract (NFE), Ether extract (EE) and Ash. Metabolizable energy (ME) was calculated mathematically for all samples by using standard formula. Results indicated that, crude protein content in Banana tree was 15.6 g/100g, Bean leaf 28.2 g/100g, Bilimbi leaf 11.9 g/100g, Cabbage 18.9 g/100g, Cauliflower 17.3 g/100g, Pumpkin 12.9 g/100g, Pumpkin leaf 25.0 g/100g, Radish 14.9 g/100g, Ridge guard 23.4 g/100g and Spinach 11.4 g/100g. In addition to crude protein, all samples contained substantial amount of crude fibre, nitrogen free extracts, ether extracts and ash. It could therefore be inferred that, the vegetable wastes could be incorporated in appreciable quantities for substituting the conventional feed resources of animal diet.

**Keywords:** Ash, Crude Fiber, Crude Protein, Ether Extract, Kitchen Waste, Moisture, Nitrogen Free Extract.

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## INTRODUCTION

Bangladesh is an agricultural country. Livestock is one of its important components which provide protein, solve unemployment and earn foreign exchange (Taylor and Roese, 2006; Cole, 1996). Dairy sector is playing an important role in the economy of Bangladesh. It provides a large part of the increasing demands for animal protein like meat and milk. It also helps to earn cash income by exporting leather and leather products and also by creating employment opportunities. Although, dairying is the most ancient occupation established in the rural setting of Bangladesh, its development is unsatisfactory due to several problems (Shamsuddoha et al., 2000). In commercial dairying, feed cost alone accounts 60-70% of total production cost. Therefore, this is a demand of time to explore locally available cheaper alternative feed resources to reduce feed cost.

Most of the developing countries have been battling against the problem of how to adequately feed their livestock because of inadequate production of conventional feed ingredients for livestock feeding. Many of these countries are also well blessed with considerable good fertile, arable land, good sunshine and abundant and well distributed rainfall. The inadequate quantities of concentrated feedstuffs they produce yearly are competed by humans and their livestock. Usually humans have to have their needs satisfied first leaving the remainder for livestock (Babatunde, 1992).

Cattles have been fed various crop residues and unconventional feedstuffs for years. Proper utilization of unconventional feeds by ruminants will not only benefit the animal industry but will increase the economic return of many cash crops (Mustafa, 2002). Vegetable and fruit by-products have a good potential for use of ruminant and non-ruminant rations so that the gap between the demand and supply of feeds and fodders can be shortened. Efforts are focused on determining the seasonal availability and nutritive value of locally available fruit and vegetable by-products with a view to formulate adequate year round feeding system (Kumar et al., 2010). Therefore, present study was undertaken to find out the chemical composition of some neglected fruits and vegetable wastes to bridge the gap between the demand and supply of the conventional feeds for livestock.

## MATERIAL AND METHODS

### Study area

There are lots of small and large scale farm in Chittagong metropolitan area where most of the farmer usually feed their livestock with unconventional feed along with conventional based on availability. Therefore, local unconventional feeds available in these areas were selected as the study area.

### Collection of sample

Samples were collected by using simple random sampling technique. Total 10 different vegetable waste samples were collected randomly. Approximately 2000 grams of each sample was collected. Samples were wrapped up by polythene bag and preserved in the laboratory for chemical analysis.

### Preparation of sample

Samples were subjected to grinder to make it homogenous powder after sun drying. Later on, it was mixed properly and exposed to shade to cool down for sampling. Individual samples were identified by marker and subjected to chemical analyses.

### Analysis of sample

Chemical analyses of the samples were carried out in triplicate for moisture, dry matter (DM), crude protein (CP), crude fiber (CF), nitrogen free extracts (NFE), ether extracts (EE) and ash in the animal nutrition laboratory and PRTC laboratory in Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh as per AOAC (1994).

### Calculation of ME

Metabolizable energy (ME) was calculated separately for all 10 different feed samples. Calculation was performed by mathematical formula as per Lodhi et al. (1976).

### Statistical analysis

Data related to chemical composition of unconventional feeds were compiled by using Microsoft Excel 2007. Chi-square ( $\chi^2$ ) test was performed to analyze the data by using SPSS 16.0. Statistical significance was accepted at 5% level ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

Chemical composition of the vegetable wastes particularly, moisture, dry matter (DM), crude protein (CP), crude fiber (CF), nitrogen free extract (NFE), ether extract (EE) and total ash contents in different samples have been presented in Table 1. In this study proximate components were determined to make a decision as to whether they could be a suitable alternative for conventional feeds or not.

**Table 1 - Chemical composition (g/100g DM) of the vegetable wastes available in Chittagong district, Bangladesh**

English name	Scientific name	ME	DM	CP	CF	NFE	EE	Ash
Banana tree	<i>Musa paradisiaca</i>	1909.7	4.0	15.6	27.7	40.3	1.3	15.1
Bean leaf	<i>Lablab purpureus</i>	2510.4	18.8	28.2	15.7	41.0	3.5	11.6
Bilimbi leaf	<i>Averrhoa bilimbi</i>	1980.8	32.2	11.9	32.3	43.3	2.6	9.9
Cabbage	<i>Brassica oleracea</i>	2521.1	6.1	18.9	13.8	56.4	0.9	10.0
Cauliflower	<i>Brassica oleracea</i>	2316.8	9.7	17.3	21.0	50.5	1.5	9.7
Pumpkin	<i>Cucurbita maxima</i>	2889.2	12.6	12.9	9.9	70.8	2.1	4.3
Pumpkin leaf	<i>Cucurbita maxima</i>	1802.0	14.0	25.0	20.4	40.1	0.7	13.8
Radish	<i>Raphanus sativus</i>	2544.0	6.3	14.9	13.6	61.1	0.9	9.5
Ridge gourd	<i>Luffa acutangula</i>	2577.5	18.3	23.4	12.1	53.0	1.2	10.3
Spinach	<i>Spinacea oleracea</i>	2469.1	8.7	11.4	13.9	59.4	2.2	13.1
SEM		109.7	2.7	1.8	2.3	3.3	0.3	0.9
Level of sig.		***	***	*	***	*	NS	NS

ME=Metabolizable energy (kcal/kg DM); DM=Dry matter; CP=Crude protein, CF=Crude fibre, NFE=Nitrogen free extract, EE=Ether extract; NS=Non-significant ( $P > 0.05$ ); SEM=Standard error of mean; \*=Significant at 5% level ( $P < 0.01$ ); \*\*\*=Significant at 0.1% level ( $P < 0.001$ )

### Banana tree (*Musa paradisiaca*)

The local name of banana tree is Kola tree and scientific name is *Musa paradisiaca*. The banana tree is the largest herbaceous flowering plant in the world. It has been found that the starch-rich bananas have curative properties both scientifically and traditionally. Birds and animals, especially monkeys and elephants love bananas. There are many healing and medicinal properties of banana tree. The high content of iron in bananas increases the production of hemoglobin in the blood. Therefore, bananas are very good for anemia. Basically, all part of the banana tree have medicinal application. Fruits, leaves, peels, roots and stalks from banana plants have been used orally as a medicine for treating diarrhoea and dysentery as well as for healing the intestinal lesions in colitis (Stover and Simmonds, 1987). The banana plant is used in folkloric medicine for treating inflammation, pain and snake-bite by the Sumu (Ulwa) people of south-eastern Nicaragua (Coe and Anderson, 1999; Lim, 2012). In our study, banana tree contained 1909.7 kcal ME/kg DM, 4.0 g/100g dry matter, 15.6 g/100g crude protein, 27.7 g/100g crude fiber, 40.3 g/100g nitrogen free extracts, 1.3 g/100g ether extracts and 15.1 g/100g ash (Table 1). Banana tree is very common and available in all region of Bangladesh. It is one of the leading sources of iron and advisable for anemic animals. Thus, banana tree can be an alternative feed source for livestock.



### Bean leaf (*Lablab purpureus*)

Scientific name of banana is *Lablab purpureus*. It is an annual or short-lived perennial dual-purpose legume. It belongs to the family Fabaceae and genus *Lablab* grown in the tropics. The seed and immature pods can be used for human food (Purseglove, 1968) while the herbage is used as a feed supplement for ruminant grazing during the dry season (Schaaffausen, 1963). Reports are however, limited on its use as a feed resource for monogastric animals. In present study, bean leaf contained 2510.4 kcal ME/kg DM, 28.2 g/100g crude protein, 15.7 g/100g crude fibre, 3.5 g/100g ether extracts, 41 g/100g nitrogen free extracts and 11.6 g/100g ash. In a study *Lablab purpureus* had 76.4 g/100g dry matter in leaf and incase of stem it was 84.1 g/100g for rongai variety (Karachi, 1997). Protein content was 25 g/100g for leaves and 11.88 g/100g for stems. Ajayi et al. (2009) found 41.8 g/100g crude fiber 26.9 g/100g dry matter, 18.1 g/100g crude protein, 28.5 g/100g crude fibre and 2.6 g/100g ether extracts in bean leaf. Aganga and Autlwetse (2000) reported 16.4 g/100g crude protein for whole plant *Lablab* hay. The DMD for the leaf and stem was 64.4 g/100g and 44.2 g/100g respectively.



### Bilimbi leaf (*Averrhoa bilimbi*)

Bilimbi leaf is a member of the Oxalidaceae family. The local name of Bilimbi leaf is Bilambu and scientific name is *Averrhoa bilimbi*. Mature bilimbi leaf is usually 3-6 cm long, alternate, imparipinnate and cluster at branch extremities. There are around 11 to 37 alternate or sub-opposite oblong leaflets. The leaves are quite similar to those of the Otaheite gooseberry. Possibly originated in Moluccas, Indonesia, the species are now cultivated and found throughout the Philippines, Indonesia, Sri Lanka, Bangladesh, Myanmar and Malaysia. It is also common in other Southeast Asian countries. In India, where it is usually found in gardens, the bilimbi has grown wild in the warmest





regions of the country. This is essentially a tropical tree, less resistant to cold. The leaf of *A. bilimbi* is used for the treatment of stomachache and parotitis. The fruit is used to treat dyspepsia, colitis and also dental caries. It is also used to treat bleeding haemorrhoids, bleeding gums, mouth ulcers, dental caries and to alleviate internal haemorrhoids. Infusion of the flowers is a remedy for mouth ulcers and oral thrush (Peter, 2007 and Orwa et al., 2009). Leaf decoction also heals inflammation of rectum and as paste of it is applied on affected surface area for mumps, rheumatism and pimples. Leaves, flower and fruit are consumed for stomachache, wounds, stomatitis, whooping cough, bleeding gums, ache and hypertension as well as antitussive and antidiabetic (Peter, 2007). It is also good for scabies (Batugal et al., 2004). In present study, bilimbi leaf contained 1980.8 kcal ME/kg DM, 32.2 g/100g dry matter, 11.9 g/100g crude protein, 32.3 g/100g crude fiber, 43.3 g/100g nitrogen free extracts, ether extracts 2.6 g/100g and 9.9 g/100g ash (Table 1). Bilimbi leaf is favorite to goat. It can also be used for cattle and sheep in our country as unconventional feed.

#### **Cabbage (*Brassica oleracea* var *capitata*)**

Cabbage (*Brassica oleracea* var *capitata*) is an herbaceous flowering plant with leaves forming a compact head characteristics. This is an abundant feedstuff both for man and animal and available throughout the whole country. This is low in calorie because of its high water content. Cabbage is a good source of fibre, provitamin A, vitamin C and B9. It is a vital source of calcium. Recently, cabbage was found to contain substances such as indole, isothiocyanates and dithiolthiones which seem to have powerful anti-cancer properties. A wide number of experiments performed over last twenty years, both on animals and people have confirmed the beneficial effect of eating cabbage on a regular basis to help prevention of colon, stomach, lung and oesophagus cancer. Akula et al. (2007) mentioned that, cabbage contained 2.4 kcal



ME/gDM, 92.0 g/100g moisture, 1.3 g/100g protein, 0.2 g/100g fat, 5.4 g/100g fibre. In present study, cabbage contained 2521.1 kcal ME/kg DM, 93.9 g/100g moisture, 18.9 g/100g crude protein, 13.8 g/100g crude fibre, 0.9 g/100g ether extract, 56.4 g/100g nitrogen free extracts and 10.0 g/100g ash. Cabbage leaf contains high levels of glucosinolates, which form compounds with antioxidant and anticancer activities during preparation (Mvere and Werff, 2004). According to Gopalan et al. (2004), brassica vegetables are highly regarded for their nutritional value as they provide higher amounts of vitamin C, soluble fibre and many other multiple nutrients with potent anti-cancer properties. It has recently been discovered that 3, 3-Diindolylmethane in Brassica vegetables is a potent modulator of the innate immune response system with potent anti-viral, anti-bacterial and anti-cancer activity. Iron in leaf cabbage is available in an easily digestible form (Mvere and Werff, 2004).

#### **Cauliflower (*Brassica oleracea* var. *botrytis*)**

Cauliflower (*Brassica oleracea* var. *botrytis*) is a vegetable. Its scientific name is *Brassica oleracea* var. *botrytis* species. It originated in the northeast Mediterranean and is presently cultivated in most of the countries of the world including Bangladesh. Cauliflower has a small compact head covered with hundreds of flower cluster attached to a short stalk. Cauliflower's nutrients make it a true champion in the fight against cancer. In addition to fibre, this is a good source of protein, thiamin, riboflavin, phosphorus, potassium, vitamin C, vitamin K, vitamin B6, folate, pantothenic acid and manganese. In present study, cauliflower contained 2316.8 kcal ME/kg DM, 91.3 g/100g moisture, 17.3 g/100g crude protein, 21 g/100g crude fibre, 1.5 g/100g ether extract, 50.5 g/100g nitrogen free extracts and 9.7 g/100g ash. Cauliflower contains 2.7 kcal ME/g DM, 97.0 g/100g moisture, 2.7 g/100g protein, 0.2 g/100g fat and 5.2 g/100g crude fibre (Akula et al., 2007). In another study, cauliflower contained 1.9 g/100g proteins, 91.95 g/100g water, 0.7 g/100g ash and 2 g/100g dietary fiber. According to Gopalan et al. (2004), cauliflower contained 66 kcl energy, 80.0



g/100g moisture, 6.0 g/100g protein, 1.0 g/100g fat, 3.0 g/100g mineral and 2 g/100g fibre. Therefore, cauliflower and its wastes could be a promising alternative feed resource for livestock.

### **Pumpkin (*Cucurbita maxima*)**

Pumpkins (*Cucurbita maxima*) are gourd squashes of the genus *Cucurbita* and family Cucurbitaceae. The pumpkins are cultivated worldwide and have high production yields. In fact, most of the species belongs to the Cucurbitaceae family is a nutritious food in Bangladesh. With a high nutritional value, pumpkins are associated with a lot of health benefits. Apart from the flesh, even the seeds of pumpkins boast of a large number of nutrition benefits. The high amount of fiber present in a pumpkin, is good for the bowel health. Pumpkin is very rich in carotenoid which is known for keeping the immune system strong and healthy. Being rich in alpha-carotene, pumpkin is believed to slow down the process of aging and also prevent cataract formation. Pumpkins have been known to reduce the risk of macular degeneration and a serious eye problem like blindness. In present study, pumpkin contained 2889.2 kcal ME/kg DM, 87.4 g/100g moisture, 12.9 g/100g crude protein, 9.9 g/100g crude fibre, 2.1 g/100g ether extract, 70.8 g/100g nitrogen free extracts and 4.3 g/100g ash. In another study, Rahman (2008) found 4.0 g/100g dry matter, 1.0 g/100g crude protein, 0.7 g/100g crude fibre, 0.1 g/100g ether extract in pumpkin which is contradictory to our finding. Jenkins, (2010) found 16.5 g/100g dry matter, 14.45 g/100g crude protein, 38.6 g/100g neutral detergent fiber, 32.5 g/100g acid detergent fiber in pumpkin. So pumpkins are a good source of energy and adequate in protein for beef cattle.



### **Pumpkin leaf (*Cucurbita maxima*)**

Pumpkin leaf is one of the important crops which belong to the family, Cucurbitaceae. The local name of pumpkin leaf is Misty kumra shak and scientific name is *Cucurbita maxima*. Pumpkin leaf is very common in Bangladesh. This is found all over the country. Most parts of the pumpkin are edible, including the fleshy shell, the seeds, the leaves, and even the flowers. It is a traditional vegetable crop, grown mainly for its leaves, fruits, and seeds and consumed either by boiling the leaves and fruits or by roasting or baking the seeds (Facciola, 1990). Pumpkins are grown all around the world for a variety of reasons ranging from agricultural purposes such as animal feed to commercial and ornamental sales. Of the seven continents, only Antarctica is unable to produce pumpkins. The biggest international producers of pumpkins include the United States, Canada, Mexico, India, and China. The traditional American pumpkin is the Connecticut field variety.



Pumpkin leaves, fruits, flowers and seeds are health promoting food. Different parts of the plant have been used as medicine in some developed world. The leaves are haematinic, analgesic, and also used externally for treating burns. Traditionally, the pulp is used to relieve intestinal inflammation or enteritis, dyspepsia and stomach disorders (Sentu and Debjani, 2007). Pumpkin fruit is an excellent source of vitamin A which the body needs for proper growth, healthy eyes and protection from diseases. It is rich in vitamin C, vitamin E, lycopene and dietary fiber (Pratt and Matthews, 2003; Ward, 2007). It has also been featured in various systems of traditional medicine for several ailments such as antihypertensive, antibacterial, intestinal antiparasitias, anti-inflammation and antalgic (Bown, 1995; Burkill, 1985; Chiej, 1984; Chopra et al., 1986; Rahman et al., 2008).

In our analysis, pumpkin leaf contained 1802.0 kcal ME/kg DM, 14.0 g/100g dry matter, 25.0 g/100g crude protein, 20.4 g/100g crude fiber, 40.1 g/100g nitrogen free extracts, 0.7 g/100g ether extracts and 13.8 g/100g ash (Table 1). The result is in agreement with Idris (2011) who found 13.0 g/100g dry matter, 8.72 g/100g crude protein, 20.17 g/100g crude fiber and 17.2 g/100g ash in pumpkin leaf. The potential of a particular feed is determined primarily by its nutrient composition. Leafy vegetables like pumpkin leaves are known to add taste and



flavour, as well as substantial amounts of protein, fiber, minerals, and vitamins to the diet (Oyenuga and Fetuga, 1975). So, it could undoubtedly be a good ration item for livestock.

### **Radish (*Raphanus sativus*)**

Radish (*Raphanus sativus*) belongs to Brassicaceae family. It is cheap and available feedstuffs in Bangladesh and found around 6 month in a year. Radishes are known for their anti-bacterial and anti-fungal properties. Radish contains vitamin C, potassium, sodium and trace amount of other minerals. Radishes are low in saturated fatty acids. They are a good source of riboflavin, vitamin B6, calcium, magnesium, copper and manganese. Radish is an excellent source of dietary fiber, folate, vitamin C and potassium. Radish is an important vegetable crop worldwide. In present study, radish contained 2544.0 kcal ME/kg DM, 93.7 g/100g moisture, 14.9 g/100g crude protein, 13.6 g/100g crude fibre, 0.9 g/100g ether extract, 61.1 g/100g nitrogen free extracts and 9.5 g/100g ash which is close to the result of Zhao-liang et al. (2008) who found 29.7 to 88.2 g/100g dry matter, 4.507 to 18.546 g/100g crude fiber, 2.233 to 15.457 g/100g total soluble sugar, 0.1416 to 0.3341 g/100g vitamin C and 0.34 to 1.15 g/100g protein on fresh weight basis.



### **Ridge gourd (*Luffa acutangula*)**

Ridge gourd (*Luffa acutangula*) locally known as Dhundol. The fruits are edible and eaten as vegetable. It is good for health. The seeds are emetic and carthartic. Young fruits are cool, demulcent, productive of loss of appetite and extive of mind bile and phlegmare (Rahman et al., 2008). This is low in saturated fat and cholesterol, high in dietary fiber, vitamin C, riboflavin, zinc, thiamin, iron, magnesium and manganese. It has blood-purifying properties. It helps to purify, restore and nourish liver from alcohol intoxication. It has high beta carotene that is good for eyes. In present study, ridge gourd leaf contained 2577.5 kcal ME/kg DM, 81.7 g/100g moisture, 23.4 g/100g crude protein, 12.1 g/100g crude fibre, 1.2 g/100g ether extract, 53.0 g/100g nitrogen free extracts and 10.3 g/100g ash. Hussain et al. (2010) found 7.31 g/100g dry matter, 13.47 g/100g crude protein, 2.09 g/100g crude fibre, 2.09 g/100g ether extract and 5.55 g/100g ash in ridge gourd leaves which is close to our findings. In another study (Abitogun, 2010) the range of the proximate components in ridge gourd leaf was crude protein (42.17-70.65 g/100g), moisture (5.69-6.42 g/100g), fat content (1.53-33.64 g/100g), ash content (3.87-3.92 g/100g), crude fibre (1.95-2.80 g/100g), carbohydrate (12.68-14.68 g/100g) and the available energy (1507.53-2177.13KJ).



### **Spinach (*Spinacea oleracea*)**

Spinach (*Spinacea oleracea*) belongs to the Amaranthaceae family. This is a wonderful green-leafy vegetable often recognized as one of the functional foods for its nutritional, antioxidants and anti-cancer constituents. Around 100g of spinach contains about 25 g/100g of the daily intake of iron. Spinach is a leafy green vegetable of winter season. Spinach is a prominent source of iron, vitamins A and C, thiamin, potassium and folic acid, carotenoids, lutein and zeaxanthin (Abbas et al., 2010). It contains 3.2 g/100g protein, 0.3 g/100g fat and 4.3 g/100g fibre (Akula et al., 2007). According to Gopalan et al. (2004) spinach contained 2.6 kcal ME/g DM, 92 g/100g moisture, 2.0 g/100g protein, 1.0 g/100g fat, 2.0 g/100g mineral and 1.0 g/100g fibre. In present study, spinach contained 2469.1 kcal ME/kg DM, 91.3 g/100g moisture,



11.4 g/100g crude protein, 13.9 g/100g crude fibre, 2.2 g/100g ether extract, 59.4 g/100g nitrogen free extracts and 13.1 g/100g ash.

## CONCLUSION

The role of unconventional feeds in livestock nutrition continues to increase. The utilization of unconventional feeds will not only benefit the livestock industry but will also increase the economic return for several crops in Bangladesh. To standardize the feeding value of unconventional feeds, a systematic evaluation system based on based direct feeding trial should be adopted in future.

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### Competing Interests

The authors declare that they have no competing interests.

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# MINERALS DISAPPEARANCE RATE OF LEAVES OF SOME ACACIA TREES AFTER DIGESTION IN GOATS' RUMEN USING NYLON BAGS TECHNIQUE

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**ABSTRACT:** The browse plants, including acacia species, provide excellent forage with high nutritive value for ruminants especially in dry areas of Africa. In this study, some minerals (P, K, Na, Mg, Ca, Mn, Cu and Zn) were determined in leaves of browse plants (*Acacia albida*, *Acacia nubica*, *Acacia sieberiana*, *Balanites aegyptiaca* and *Ziziphus spina-christi*) collected from different areas of Sudan before and after digestion of the sample in goat's rumen by using nylon bag technique. Nylon bags containing the samples were inserted through the rumen fistula into the goat's rumen, and were incubated for 6, 12, 24, 48, and 72 hrs. After incubation periods, it was found that there were a high loss of minerals and this is attributed to the rumen digestion and solubility of minerals in rumen liquor. The results indicate that the time of incubation and the type of mineral likely had a significant effect on the loss of minerals in the rumen. It can be observed from these figures that the disappearance rates (slope of the curves) vary across mineral types and species of acacia trees. Disappearance rates suggest that the rumen microorganisms have a significant role in the digestion of minerals and their disappearance rates are due to the solubility of minerals in the rumen liquor and the loss of minerals due to utilization of microorganisms to a certain amount for their maintenance.

**Keywords:** Acacia Trees, Browse Plants, Minerals, Rumen, Nylon Bag Technique, Disappearance Rates.

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## INTRODUCTION

The growing patterns, leaf longevity and growing dynamics that characterize plants species (González et al., 2010) provide habitat to wildlife (González and Cantu, 2001). Further, leaf longevity has an impact on the vegetation biomass (Zhang et al., 2016). Forage plants provide small ruminants and other livestock with good quality forage which is high in protein, fiber, vitamins and essential fatty oils for (Moya et al., 2002). Although ruminants in the tropics naturally graze growing forages which are poor in quality and have low digestibility (Minson, 1980), acacia trees provide browse that is important for goats and camels nutrition. Browse and shrubs can provide supplemental feeds for ruminants grazing tropical pastures, and provide very much needed nutrients and minerals (Minson, 1980). Edible browse tree parts serve as an insurance against seasonal feed shortages. In that regards, acacia trees and shrubs are important for sustaining livestock productivity in arid and semi-arid zones (Backlund, 1991). The main feature of browse plants is their high crude protein and mineral contents, with higher calcium and potassium content than other minerals (Backlund and Belskog, 1991). Because quantity and quality of grasses decrease in the dry season, livestock growth, development, and animals' productivity suffer (Darrag, 1995). Brows plants, which are well adapted to water stress by means of their morpho-physiological traits, thrive under drought conditions (Fardous et al., 2011) and provide valuable feeds for browsing animals. In the semi-arid rangeland of Sudan where this study was conducted, free grazing of rangelands sustains traditional livestock production. The contributions of browse acacia trees to livestock nutrition needs to be investigated. The objective of this study was to determine the minerals composition of some acacia browse plants collected from different areas of Sudan before and after the digestion in the goat rumen using nylon bags technique for different periods of time.

## MATERIAL AND METHODS

### Animals

Three female Nilotic goats approximately 20 kilograms of weight and 2 years of age, were used in this experiment. All goats were apparently healthy. The animals were housed separately under hygienic conditions in clean rooms, with adequate light and good ventilation. All animals were fed with Berseem (*Medicago sativa*). The rumen fistula was made as described by Alshafie and Nour (2016). Animals were kept for two weeks before experiment for acclimatization. The experimental period was two weeks.

### Collection of feed material (Acacia leaves)

In this study, leaves were obtained from *Acacia albida*, *Acacia nubica*, *Acacia sieberiana*, *Balanites aegyptiaca* and *Ziziphus spina-christi* acacia trees growing in different parts of the Sudan. The leave samples were carefully cleaned and freed from stones, dirt, and other materials; they were then numbered and carefully stored in polythene bags. The leaves were analyzed for their chemical composition as described by Alshafie and Nour (2016)

### Nylon bags

To ensure that rumen fluid can easily enter the bag and mix with sample, the size of the bags was selected to be large enough relative to sample used and small enough so it can easily be withdrawn through the rumen fistula. The mesh size of these bags allows entry of rumen microbes and exit of accumulated gases. On the other hand, mesh size ensures that the losses of solid particles will be minimal.

### Analytical methods

#### Preparation of the samples for minerals analysis

Approximately, three grams of each test sample were weighed and placed into pre-weighed empty, clean and dry crucibles. All the crucibles were numbered and were then transferred to an oven and the temperature was adjusted to 100°C. The samples were left in the oven for 24 hrs and then the crucibles were weighed several times until a constant weight was reached. The dry

samples weights were calculated and recorded. Crucibles and their contents of the dry matter were transferred to a muffle furnace set at 250°C. The temperature was then increased by 25°C gradually until it reached 450°C. The samples were left over night to ash. The crucibles and their contents were left to cool at room temperature.

Material extraction was done by adding 10 ml of 25% HCL to the dry ash. The extract was then dried on a sand bath, and then the contents were boiled and filtered into 50-ml volumetric flask using 1% HCL. The final volume was made up to 50 ml with 1% HCL solution. 20 ml of each treated sample were then pipetted into a clean acid washed dry plastic vials, the vials were closed, and saved for mineral analysis. Calcium (Ca), Magnesium (Mg), Copper (Cu), Zinc (Zn) and Manganese (Mn) were determined by the atomic absorption spectrophotometer, while the flame photometer (FP 410 Corning) was used to estimate the Sodium (Na) and Potassium (K) levels in the samples. Phosphorus (P) concentration was determined calorimetrically as described by Parkinson and Allen (1975).

#### Preparation of the sample for incubation

Prior to the use, the nylon bags were thoroughly washed under tap water, dried to a constant weight at 105° C in a hot air oven and weighed. Four grams of each sample were placed in the bag.

#### Incubation of the bags in the rumen

Minerals disappearance rate of acacia leaves were determined using the nylon bags technique described by Alshafie and Nour (2016). Briefly, the nylon bag containing four grams of the browse leave samples were administered into the rumen directly through the fistula, and were incubated for 6, 12, 24, 48 and 72 hrs. Each sample was replicated 3 times in one of three goats used. At the end of the incubation period, the bags were washed cleaned, and dried as described by Alshafie and Nour (2016). The losses were determined by calculating the mean of the three replications.

#### Statistical analysis

Data were subjected to standard methods of statistical analysis that was performed using windows-based Statistical Package for Social Sciences (SPSS) Version 17.0. Descriptive statistic (percentages, means and standard deviation) was used to evaluate the minerals disappearance rates in leaves of acacia trees.

## RESULTS

Minerals composition of the browse plants studied is given in table 1. The content of phosphorus in the browse samples analyzed was within the range of 0.09- 0.21 %. Some browse species, including *Ziziphusspina Christi*, had high phosphorus content. These include *Z. spina-christi* and *A.siebriana*. On the other hand, *Balanities. Aegyptiaca* had low phosphorus content. The content of calcium in the browse samples analyzed ranged between 4.05- 10.92%. *Balanites aegyptiaca* had the highest calcium content, while, *A. albida* leaves has the lowest calcium content. On the other hand, the content of magnesium in browse samples analyzed ranges between 0.80- 1.92%. The browse species with the high magnesium content was *Z. spina-Christi*, while *A. seibriana* and *A. albida* contained low amount (Table 2). Sodium content in the browse samples ranged between 12.23-17.06%. Generally, all samples had high sodium content. The potassium content in the browse samples analyzed ranged from 0.29% to 2.49%. *A. albida* had the lowest value, while *Balanites aegyptiaca* had the highest potassium in their leaves. Samples content of zinc was between 0.30 to 1.22%. The browse species with the high zinc content was *A.seibriana*, while *Balanites aegyptiaca* contained low amount. The content of copper in the samples analyzed was within the range of 0.049-0.079%. The acacia trees with the highest copper content was *Balanites aegyptiaca*, and the sample that contained the lowest amount of copper was *A. albeda*. Manganese content in the acacia leaves analyzed was within the range of 0.0135 to 0.0487%. Generally, all samples were low in manganese (Table1).

Tables 2 through 7 and figures 1 through 5 present the concentration of minerals after the incubation of the samples in the rumen. There is a marked loss in the mineral concentration of the samples analyzed after incubation in the rumen of goats compared to that of un-incubated samples. The difference between mineral levels of the incubated samples and un-incubated ones is clear as shown in the tables. It can be said that from figures 1 through 5 that showed the pattern of the change in the concentration of Mg, Ca, Na, Cu, Mn and Zn of different browse plants incubated in the rumen of goat; and that all the curves showed regular loss of minerals. In all samples, the loss of element increased with increasing incubation time.

**Table 1 - Percentages of phosphorus, potassium, magnesium, calcium, manganese, copper and zinc of leaves of some Acacia browse trees in Western Sudan.**

Botanical name	P%	K%	Na%	Mg%	Ca%	Mn%	Cu%	Zn%
<i>Acacia albida</i>	0.18	0.29	14.01	0.80	4.05	0.0135	0.049	1.02
<i>Acacia nubica</i>	0.14	1.03	12.73	1.02	5.35	0.0312	0.057	0.42
<i>Acacia seiberiana</i>	0.19	1.06	13.32	0.80	5.95	0.0214	0.069	1.22
<i>Balanites aegyptiaca</i>	0.09	2.49	17.00	1.70	10.92	0.0484	0.079	0.30
<i>Ziziphusspina christi</i>	0.21	1.22	17.06	1.92	8.92	0.0217	0.057	0.37

**Table 2 - The mean values  $\pm$  SD of Mg<sup>+2</sup> % in different acacia plants incubated in the rumen of goats for different periods of time by using nylon bag technique.**

Botanical name	Incubation period in hours				
	0	12	48	72	Means $\pm$ SD
<i>Acacia albida</i>	0.80	0.60	0.40	0.20	0.5 $\pm$ 0.26
<i>Acacia nubica</i>	1.02	0.50	0.45	0.30	0.57 $\pm$ 0.31
<i>Acacia seiberiana</i>	0.80	0.80	0.60	0.20	0.6 $\pm$ 0.28
<i>Balanites aegyptiaca</i>	1.70	0.60	0.42	0.30	0.76 $\pm$ 0.64
<i>Ziziphusspina christi</i>	1.92	0.70	0.50	0.40	0.88 $\pm$ 0.70



**Table 3 - The mean values  $\pm$  SD of  $\text{Ca}^{+2}$  % in different plants incubated in the rumen of goats for different periods of time by using nylon bag technique.**

Botanical name	Incubation period In hours				Means $\pm$ SD
	0	12	48	72	
<i>Acaciaalbida</i>	4.05	1.90	1.70	1.00	2.16 $\pm$ 1.32
<i>Acacianubica</i>	5.35	1.50	1.30	1.10	2.31 $\pm$ 2.03
<i>Acacia seiberiana</i>	5.95	1.70	1.20	0.80	2.41 $\pm$ 2.39
<i>Balanites aegyptiaca</i>	10.95	1.80	1.60	0.50	3.71 $\pm$ 4.68
<i>Ziziphus spinachristi</i>	8.92	1.90	1.70	0.60	3.28 $\pm$ 3.80

Comparison between different incubation times are given as Mean  $\pm$  SD

**Table 4 - The mean values  $\pm$  SD of  $\text{Na}^{+}$  % in different plants incubated in the rumen of goats for different periods of time by using nylon bag technique.**

Botanical name	Incubation period In hours				Means $\pm$ SD
	0	12	48	72	
<i>Acacia albida</i>	14.01	13.75	11.00	10.25	12.25 $\pm$ 1.91
<i>Acacia nubica</i>	12.73	12.50	11.25	9.75	11.56 $\pm$ 1.37
<i>Acacia seiberiana</i>	13.32	12.75	10.25	8.25	11.14 $\pm$ 2.34
<i>Balanites aegyptiaca</i>	17.00	15.50	11.50	9.25	13.31 $\pm$ 3.57
<i>Ziziphus spinachristi</i>	17.06	16.25	12.50	10.50	14.08 $\pm$ 3.10

Comparison between different incubation times are given as Mean  $\pm$  SD

**Table 5 - The mean values  $\pm$  SD of  $\text{Cu}^{+2}$  % in different plants incubated in the rumen of goats for different periods of time by using nylon bag technique.**

Botanical name	Incubation period In hours				Means $\pm$ SD
	0	12	48	72	
<i>Acacia albida</i>	0.049	0.04	0.03	0.01	0.03 $\pm$ 0.012
<i>Acacia nubica</i>	0.057	0.05	0.04	.02	0.04 $\pm$ 0.02
<i>Acacia seiberiana</i>	0.069	0.06	0.05	0.015	0.05 $\pm$ 0.02
<i>Balanites aegyptiaca</i>	0.079	0.07	0.04	0.025	0.05 $\pm$ 0.03
<i>Ziziphus spinachristi</i>	0.057	0.05	0.04	0.01	0.04 $\pm$ 0.02

Comparison between different incubation times are given as Mean  $\pm$  SD

**Table 6 - The mean values  $\pm$  SD of  $\text{Zn}^{+2\%}$  in different plants incubated in the rumen of goats for different periods of time by using nylon bag technique.**

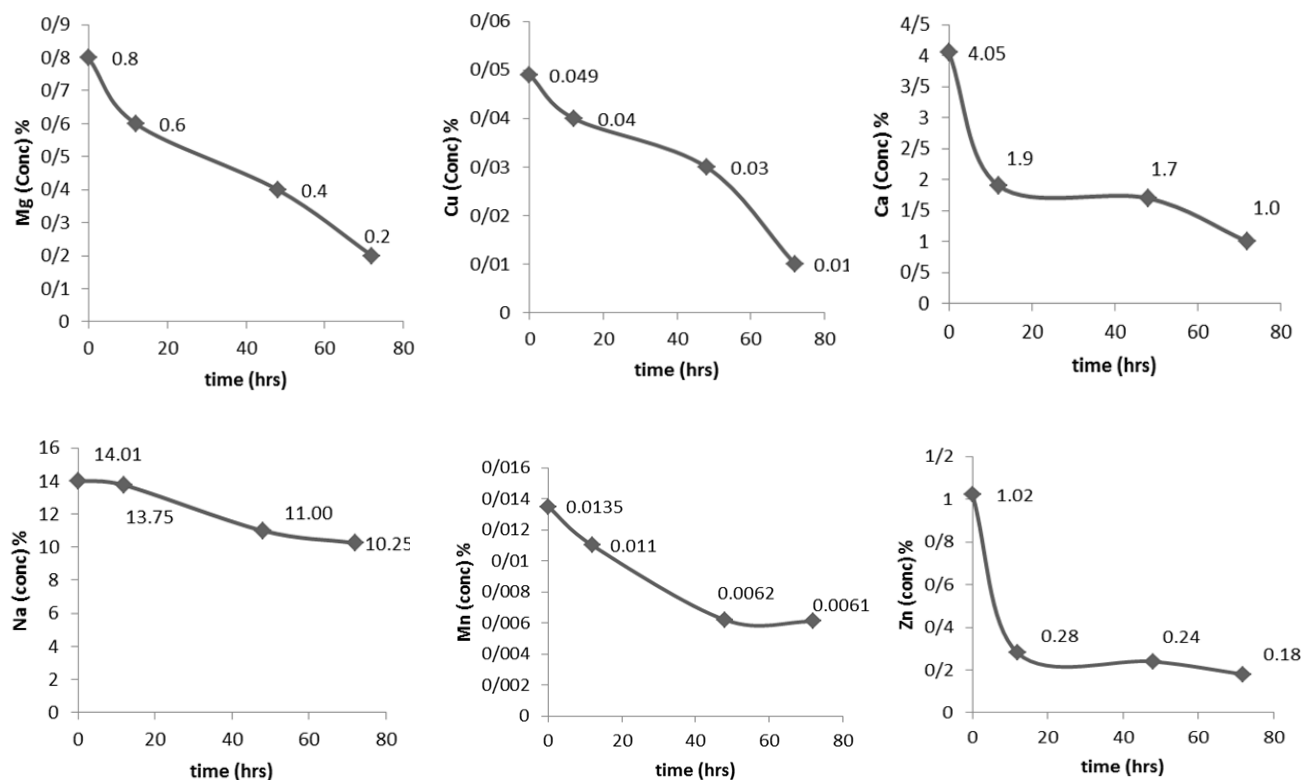
Botanical name	Incubation period In hours				Means $\pm$ SD
	0	12	48	72	
<i>Acacia albida</i>	1.02	0.280	0.240	0.180	0.430 $\pm$ 0.395
<i>Acacia nubica</i>	0.42	0.202	0.165	0.110	0.224 $\pm$ 0.136
<i>Acacia seiberiana</i>	1.22	0.250	0.182	0.110	0.441 $\pm$ 0.523
<i>Balanites aegyptiaca</i>	0.30	0.265	0.110	0.075	0.188 $\pm$ 0.1115
<i>Ziziphus spina Christi</i>	0.37	0.3	0.125	0.100	0.224 $\pm$ 0.132

Comparison between different incubation times are given as Mean  $\pm$  SD

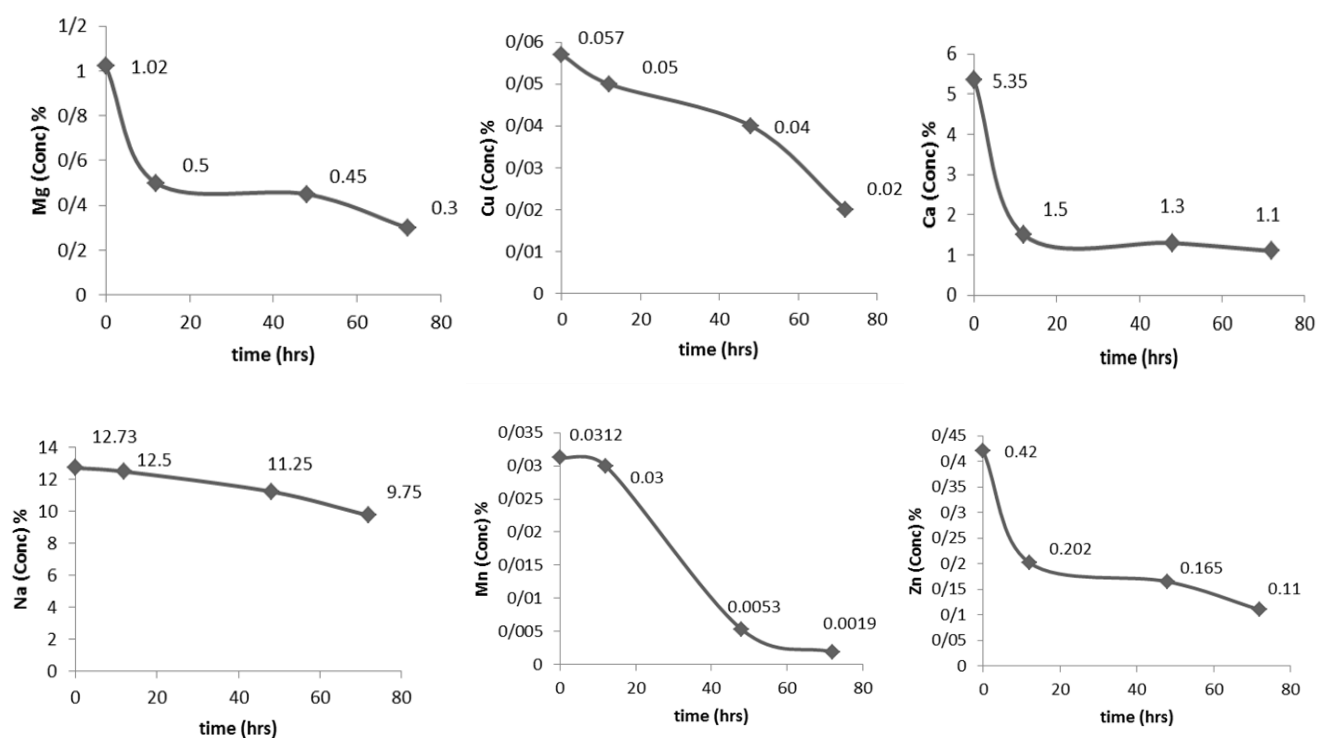
**Table 7 - The mean values  $\pm$  SD of  $\text{Mn}^{+2}$  % in different plants incubated in the rumen of goats for different periods of time by using nylon bag technique.**

Botanical name	Incubation period In hours				Means $\pm$ SD
	0	12	48	72	
<i>Acacia albida</i>	0.0135	0.011	0.0062	0.0061	0.0092 $\pm$ 0.0037
<i>Acacia nubica</i>	0.0312	0.030	0.0053	0.0019	0.0171 $\pm$ 0.0157
<i>Acacia seiberiana</i>	0.0214	0.019	0.0079	0.0028	0.01280 $\pm$ 0.0089
<i>Balanites aegyptiaca</i>	0.0484	0.010	0.0062	0.0061	0.0177 $\pm$ 0.0206
<i>Ziziphus spina christi</i>	0.0217	0.019	0.0086	0.0030	0.0131 $\pm$ 0.0088

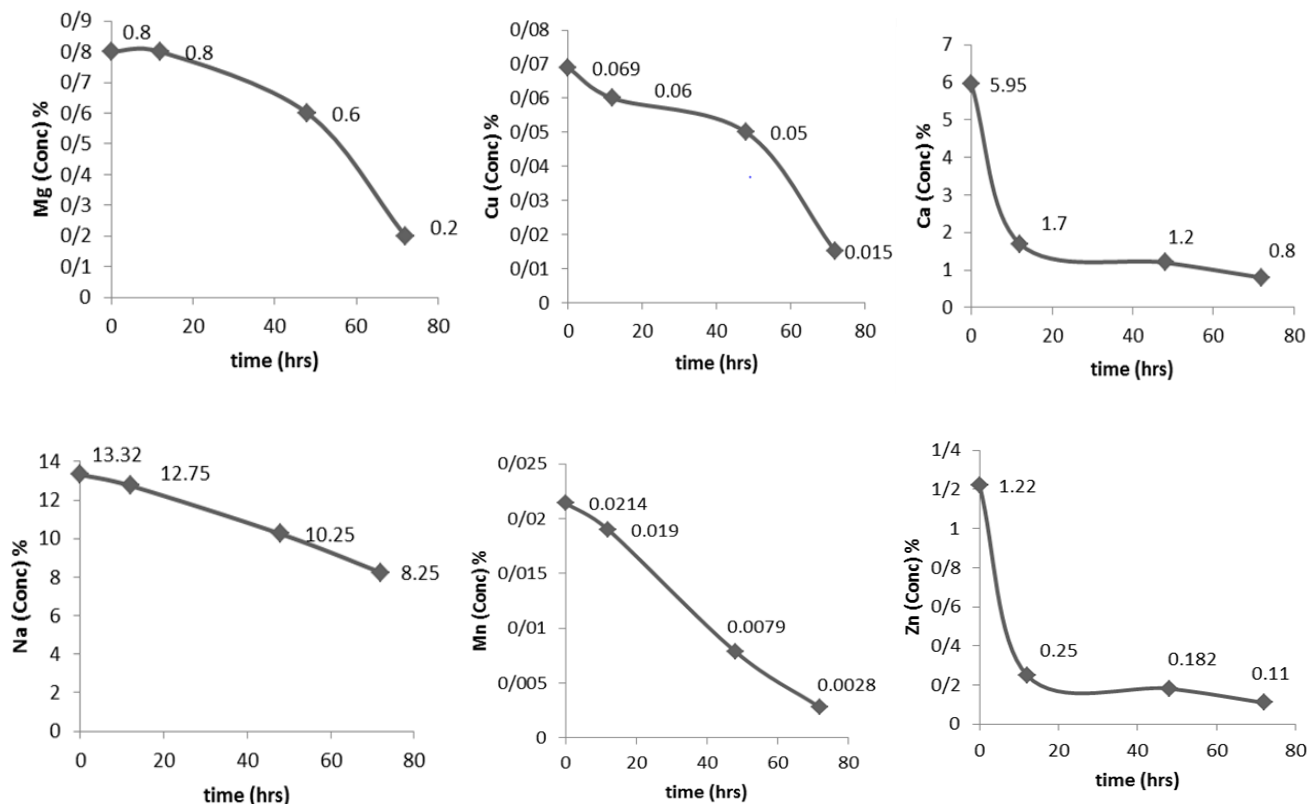
Comparison between different incubation times are given as Mean  $\pm$  SD



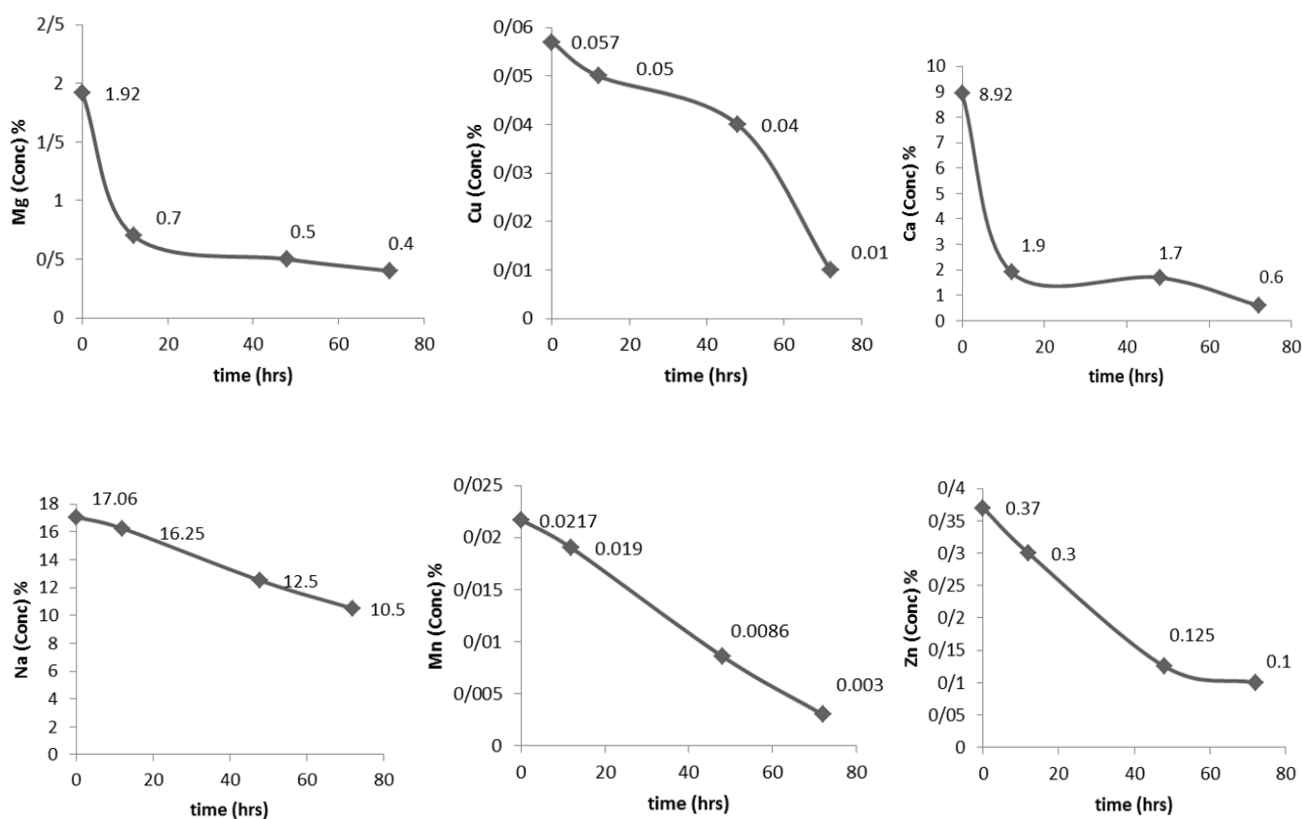
**Figure 1. Minerals disappearance rates of *Acacia albida* after incubation in the rumen for 0, 20, 40, 60, and 80 hrs.**



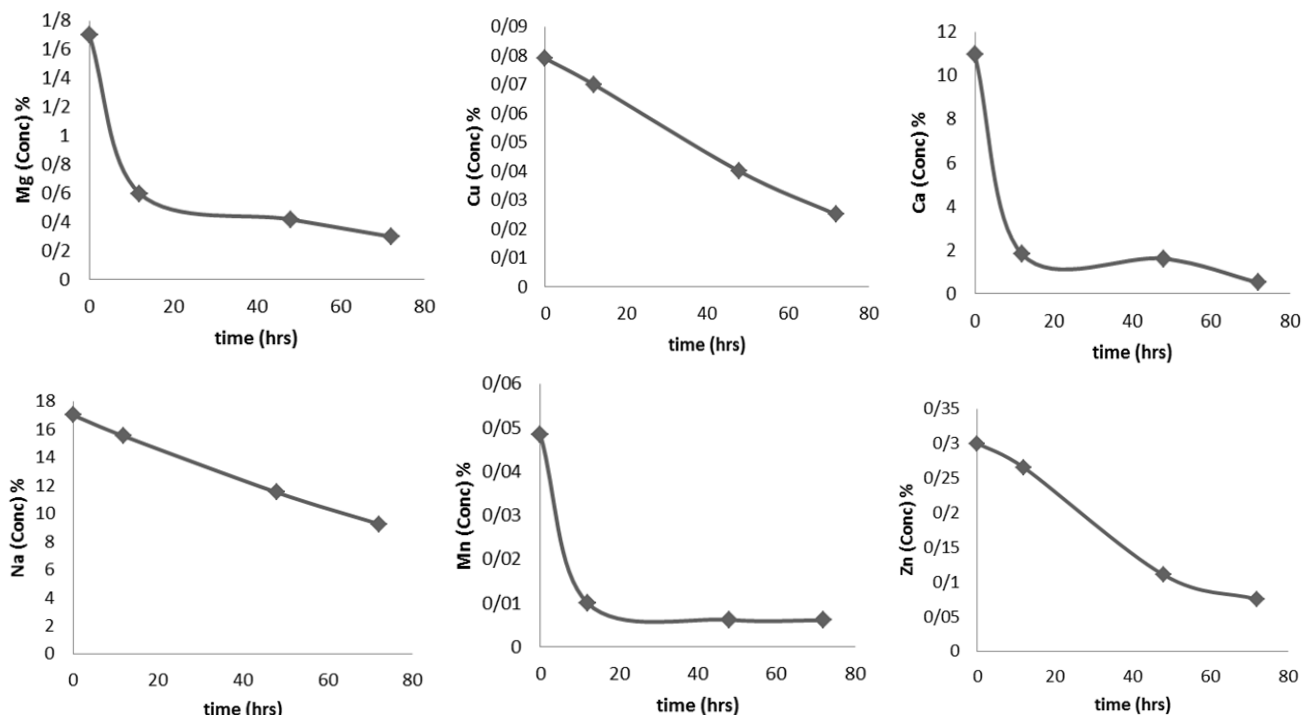
**Figure 2. Minerals disappearance rates of *Acacia nubica* after incubation in the rumen for 0, 20, 40, 60 and 80 hrs**



**Figure 3.** Minerals disappearance rates of *Acacia sieberiana* after incubation in the rumen for 0, 20, 40, 60, and 80 hrs.



**Figure 4.** Minerals disappearance rates of *Z. spina-christi* after incubation in the rumen for 0, 20, 40, 60, and 80 hrs.



**Figure 5.** Minerals disappearance rates of *Balanites aegyptiaca* after incubation in the rumen of goat for 0, 20, 40, 60, and 80 hrs.

## DISCUSSION

### Fate of minerals in the rumen of goat using the nylon bags technique

For optimal rumen function, the process of rumination is essential for maintenance of adequate salivation and optimal pH for cellulose fermenting microorganisms. The fermentation process typically yields higher volatile fatty acids comprising mainly of acetate. Acetate and other volatile fatty acids (VFAs). VFAs provide energy for maintenance, growth, reproduction, and production (Lu et al., 2005). Mineral nutrition is essential for the functions of rumen microbes, including production of VFAs the animal needs. Plants provide variable amounts of minerals for the rumen microbes and their host. For proper mineral nutrition of ruminants which rely solely on plants for their nutritional needs, mineral composition of feeds such as browse leaves needs to be quantified and their disappearance rates in the rumen needs to be assessed.

High concentrations of minerals in browse acacias leaves investigated in this study provide mineral supplements in the diets of ruminants. These feeds contained higher levels of Ca, P, Mg and S than the recommended requirements for various physiological and production functions of ruminants. In this study, phosphorus concentration was about 0.09- 0.21% (Table 1), which is similar to the values reported by Elginaid (1997) and within the range of those obtained by Dougall et al. (1964). Gomide (1978) maintained that the level of phosphorus in plants decreases with the age, and moves from the oldest tissues to the newest parts of the plant, such as the leaves. On the other hand, Rodgers (1975) observed that plants in the savannah areas of eastern Africa are deficient in phosphorus, and only browse shrubs provide animals with high phosphorus during the dry season (Wilson and Bredon, 1963). Elginaid (1997) reported similar findings in the central areas of Sudan. Mc Dowell and Conard (1977) found that in more than a thousand Latin American forages, 72.8% of their phosphorus values were 0.3% or less.

The sodium concentration of browse species in this study was found to be high 12.23 - 17.06% (Table1) compared to the values of 0.03-0.07% reported by (Dougall et al., 1964), and also is opposite to that found by (Fadel Elseed, et al., 2002) who argued that that sodium content was very low for all tree species studied and attributed this to the soil conditions. On the other hand, magnesium concentration of the browse species studied lies within the range of 0.80- 1.92% (Table 1) which is near the range of (0.20- 0.76%) for leaves reported by Le Houerou (1980). Since magnesium was found to be immobile in the plant, hence it is found to be higher in old parts of the plant (Gomide, 1978). Calcium concentration of the browse species were in the range of 4.05-10.92% (Table1) which is higher than the range of 0.48- 4.68% recorded by Dougall et al. (1964), and the range obtained by Elginaid (1997), 0.40- 2.80%, and these differences may be due to genetic factors. The concentration of potassium of the browse leaves studied was in the range of 0.29- 2.49% (Table1) which is similar to that obtained by Dougall et al. (1964). Decreased potassium concentration in the leaves was attributed to the movement of potassium to the older parts. to the roots system, and then to the soil (Gomide, 1978).

The trace minerals of the acacia browse plants investigated in this study were low in terms of Cu and Zn and this is in agreement with Le Houerou (1980) who reported that the deficiency in Cu and Zn may occur concurrently in browse species. Under wood (1977) suggested that different plant species had different abilities to absorb and return copper; this is determined by the availability of minerals from the soil, and genetic makeup of the plants. Zn concentration in the investigated samples was low and this is in agreement with (Sauchelli, 1969). The present study reports large variation in Mn concentration of plant samples analyzed (table1). These findings are in agreement with Miller (1972) who has stated that such variation is found even in the plants of the same species. Generally, variation in Mn may be due to genetic factors, nature of the soil nature and its pH. It seems also that the stage of the plant growth may have an effect on this (Under wood, 1977).

### Minerals digestion and disappearance rates in the rumen

One of the purposes of the current work is the determination of the rumen mineral digestion and mineral disappearance rates by using nylon bag technique. Two possible explanations are suggested for the disappearances rates of minerals in the rumen. The first explanation is that minerals losses after incubation of samples in the rumen of goats might be due to the

rumen digestion in which the rumen microorganisms attack various organic complexes. This microbial activity and thus releases minerals making them available for absorption by the animal or for the usage of the microorganisms. The second explanation of the losses might be due to solubility of minerals in rumen liquor. The results obtained in the current study strongly support these two suggestions. It is quite clear from all the figures (1-5). Which illustrate the effect of incubation of various browse plants in the rumen of goats. that the curves show regular and gradual disappearance of all minerals with time of incubation. Some of the minerals, such as calcium, demonstrate a sharp disappearance during the first few hours of incubation opposite to that of sodium whose disappearance rate is gradual. Further these figures indicate that the time of incubation and the type of mineral likely had a significant effect on the loss of minerals in the rumen. It can be observed from these figures that the disappearance rates (slope of the curves) vary across mineral types and species of acacia trees. Disappearance rates suggest that the rumen microorganisms have a significant role in the digestion of minerals and the minerals disappearance rates are due to the solubility of minerals in the rumen liquor and the loss of minerals due to utilization of microorganisms to a certain amount for their maintenance.

## CONCLUSION

Knowledge of browse plants in terms of availability, utilization and other related information under Sudan condition is still lacking. For proper mineral nutrition of ruminants that rely solely on plants for their nutritional needs, this study quantified mineral composition of browse leaves of acacia trees, and determined their disappearance rates in the rumen. Acacia trees provide important nutrients for livestock in arid areas of the tropics, such as that of Western Sudan. High concentrations of minerals in browse acacia leaves investigated in this study provide important nutrients and minerals for ruminants such as goats and camels. The results indicate that the time of incubation and the type of mineral likely had a significant effect on the loss of minerals in the rumen. It can be observed from these figures that the disappearance rates (slope of the curves) vary across mineral types and species of acacia trees. Disappearance rates suggest that the rumen microorganisms have a significant role in the digestion of minerals. Further, minerals disappearance rates are due to the solubility of minerals in the rumen liquor and the loss of minerals can be due to their utilization.

## Competing Interest

The authors declare that there are no significant personnel, professional or financial competing interest that might have influenced the presentation of the results of the study described in this manuscript

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# EFFECT OF DIETARY TANNIN SOURCE FEEDS ON RUMINAL FERMENTATION AND PRODUCTION OF CATTLE; A REVIEW

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**ABSTRACT:** Generally, tannins are widely distributed throughout the plant kingdom, especially among trees, shrubs and herbaceous leguminous plants. Tannins are naturally occurring polyphenols with different molecular weights and complexity that are synthesized during the secondary metabolism of plants. Tannins might bind to macromolecules (proteins, structural carbohydrates and starch) and decrease their availability to digestion. Tannins based on their chemical structure and properties divided into two groups, hydrolyzable tannins (HT) and Condensed tannins (CT, proanthocyanidins). Tannins are polyphenols, which directly or indirectly affect intake and digestion. They are the primary source of astringency in plants, which results from binding to proteins, forming soluble or insoluble complexes. The nature of the interaction is greatly dependent on the structure of the polyphenols and the proteins involved. Relatively low concentration of tannins (0.5% of DM intake) is sufficient to destabilize the bloat proteins while high concentration (2-4% of DM intake) is needed for improvement of protein utilization. High concentration (> 5% of dry weight reduces feed intake and feed conversion efficiency. Tannins containing forages will be important for small ruminants to control of gastrointestinal parasites. Animals fed condensed tannin had lower dressing percent than controlled one; with dressing percent being intermediate for animals fed hydrolysable tannin. Neither tannin source affected the animal's consumption of the diet or the animal's growth. Additionally, the tannin sources did not affect the meat or by-product tissues, making tannin supplementation a viable option in finishing beef cattle. Therefore, tannin source feed will have its own advantages and disadvantages on animals' performance.

**Keywords:** Dietary Tannin, Digestion, Ruminal fermentation

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## INTRODUCTION

In animal production, nutrition is one of the most important factors, being determinant to productive performance. Consequently, the understanding of ingestive behavior, and particularly dietary choices and adaptation to pastures, is of extremely importance in livestock management. The major constraints to increase ruminant productivity in developing countries are the scarcity, fluctuating quantity and quality of the year-round supply of conventional feeds and the inadequate nitrogen supply from low quality forages such as straw and stovers (Leng, 1990), which often contain as low as 20–50 g/kg of crude protein which do not meet the minimum crude protein requirement (80 g/kg DM) for optimal rumen microbial function (Annison and Bryden, 1998). Differences among free-grazing ruminant species, concerning food selection, allow an efficient pasture use at the habitat scale. Additionally, an effective and sustainable animal management, as well as ecological and environmental aspects, would benefit from a well founded knowledge on animal-plant interactions. Intake is influenced primarily by hunger, which is distressing, and by satiety, which is generally pleasant (Forbes, 1996). However, food intake is not so simple. In fact, it is a complex behavior, which involves simultaneously homeostatic and hedonic aspects.

On one hand, factors related to the biological requirements for energy and nutrients modulate the beginning and the end of an ingestion episode, also influencing the type of feed chosen. On the other hand, affective factors, linked to past experiences, will affect the likeness and the consumption of a particular food item. This complexity is further increased by the interaction of these homeostatic and hedonic aspects (Berthoud, 2006). Among the substances present in herbivores' diets, plant secondary metabolites (PSMs) have an ecological function of plant protection from the attack of pathogens and consumption by herbivores. PSMs comprise a wide range of chemicals, such as terpenes, alkaloids, oxalates, saponins and tannins. In ruminant nutrition, the levels of tannins present in food items represent a major component of food choice. Tannins are obtained upon the decomposition of vegetation. They will generally be found in surface water supplies or shallow wells. Although these compounds are not a health risk, they are aesthetically displeasing. Tannins are difficult to remove from water. Tannins can cause a yellow to brown cast in water and may also affect a taste and odour (Ashok and Upadhyaya, 2012). The inverse relation between high tannin levels in forage and palatability, voluntary intake, digestibility and nitrogen retention has long been established in several herbivores (Robbins, 1987). Reduced palatability, low evacuation



rate of the digested material out of the rumen and toxicity are factors that were considered as an explanation for the negative effects of tannins on ruminants feed intake. However, according to the report of Bhatta et al. (2009) recently, tannins have been increasingly investigated as a means to reduce the methane emission of ruminants, because they are considered to represent promising substances to reduce rumen methanogenesis. According to the report of Bunglavan and Dutta (2013), protection of proteins is essential for productive ruminants, where the protein requirement of these animals cannot be met from microbial protein synthesis. Mueller-Harvey (2006) was also summarized the beneficial and detrimental effects of tannins in ruminant nutrition. One of the main benefits is their effect on protein digestion. There has been considerable interest in reducing ruminal degradation of proteins. This was also in line with that of Gxasheka et al. (2015) Tannins rich plants have a potential to improve absorption of essential amino acid and also controlling gastro-internal parasites. Moreover, these plants can also be used to mitigate feed shortage during winter since rural farmer tend to have less potential to buy supplementary feeds. However, several ruminant species seem to tolerate (or even prefer) considerable amounts of tannins in their diets. The discrepancy in the tolerance to tannins among herbivores, in general, and ruminants, in particular, can be related to different defense mechanisms that each species present to PSMs. The oral cavity plays an important role in the process of tannin ingestion, both by being the place of detection of these plant compounds, and through the presence of salivary proteins which act as defense mechanisms. Therefore the objective of this paper is to review the effect of dietary tannin source feeds on ruminal fermentation and production of cattle.

### **Tannins**

Tannins are polyphenolic secondary plant compounds that have been shown to affect microbial activity to impact fermentation, protein degradation, methane production, and potential to mitigate food borne pathogens. Tannins, a group of chemical compounds produced by a number of broadleaf forage plants, can bind proteins. Typically, grasses don't contain tannins, although sorghum (*Sorghum bicolor*) has a significant tannin content. Tannins are often found in higher concentrations in broadleaf plants adapted to warm climates (MacAdam et al., 2013) Plants contain various secondary compounds which protect them from attack by fungi, bacteria, herbivorous insects and vertebrates. Classes of compounds known to act in this way include saponins and tannins (Makkar et al., 1995; Pell et al., 2001), which are prevalent in many tropical fodder plants. Tannins are oligomeric compounds with multiple structure units that have free phenolic groups. Tannins are usually soluble in water (Haslam, 1989) except for some with high molecular weight structures. They are also capable of binding proteins and forming soluble and insoluble tannin-protein complexes. Tannins are usually divided into two groups, hydrolyzable tannins (HT) and CT (proanthocyanidins), based on their chemical structure and properties (Athanasiadou et al., 2001).

### **Hydrolyzable Tannins**

Hydrolyzable tannins are molecules with a carbohydrate, generally D-glucose as a central core. The hydroxyl groups of these carbohydrates are partially or totally esterified with phenolic groups like gallic acid (gallotannins) or ellagic acid (ellagitannins). Hydrolyzable tannins are usually present in low amounts in plants (Mueller-Harvey, 2001). These tannins are found in oak (*Quercus* spp.) Acacia, Eucalypts and a variety of browse and tree leaves (Waghorn and McNabb, 2003). The browse that contain these leaves and apices can contain anywhere from 200g per kg of dry matter (DM) and in some species they can contain phenolic compounds that can exceed 500g per kg of dry matter (Reed, 1995; Lowry et al., 1996). Hydrolyzable tannins are potentially toxic to animals, but most ruminants can adjust to a diet of these tannins (Waghorn and McNabb, 2003). Ruminants are able to adjust to these toxic tannins by reducing their urinary excretion of degradation products, thus allowing them to consume these diets (Lowry et al., 1996). Although ruminants have this ability, an excessive amount of this tannin diet can lead to liver and kidney lesions, as well as death (Waghorn and McNabb, 2003). Death usually occurs five to ten days after the first excessive consumption; the toxic compound responsible is not known. Information concerning the digestion, absorption, and impact on metabolism and productivity of hydrolyzable tannins is rare.

### **Condensed Tannins**

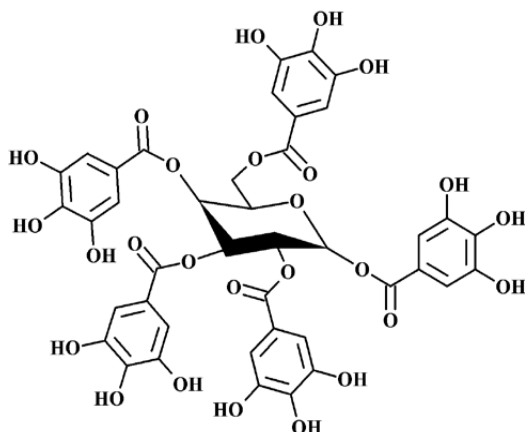
The presence of CT in forage species may provide a practical means of protecting dietary forage protein from ruminal degradation, thus increasing plant protein uptake in the small intestine with implications for animal performances (Piluzza et al., 2013). Of the tannins, condensed tannins are the most widely distributed. Condensed tannins are oligomers or polymers of flavonoid units linked by carbon-carbon bonds (Waghorn and McNabb, 2003) not susceptible to cleavage by hydrolysis (Reed, 1995). They are called condensed tannins because of their condensed chemical structure. CT, are also termed proanthocyanidins (PA), which is derived from the acid catalyzed oxidation reaction that produces red anthocyanidins through heating of PA in acidic alcohol solutions (Haslam, 1982). Cyanidin (procyanidin) and delphinidin (prodelphinidin) are the most common anthocyanidins produced (Reed, 1995). Condensed tannins can contain as little as two or greater than fifty flavonoid units. Due to the

variability of flavonoid units to some substituents and because of the variable sites for interflavon bonds, condensed tannin polymers have complex structures. Condensed tannins may or may not be soluble in aqueous organic solvents, depending on their chemical structure and degree of polymerization.

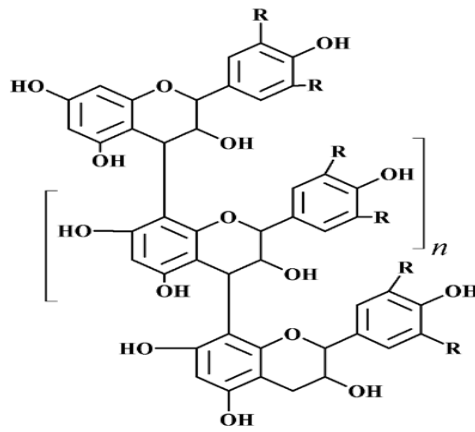
It is speculated that plants containing condensed tannins evolved over time to implore them as a defense mechanism, which protected them against pathogenic microorganisms and against being consumed by insects or grazing animals (Swain, 1979). Now they are being extracted from various plants to be used in improving animal health. Extraction of these condensed tannins was once performed using acetone-water, but full extraction of the CT was not obtained with this method (Barry et al., 1999). Condensed tannins found in tropical forages are thought to promote plant growth by reducing the release of leaf litter into the soil (Palm et al., 1991) and reducing the release of animal feces (Waghorn and McNabb, 2003). Condensed tannin containing forages have different benefits for ruminants, depending on the species of plant. For example, lotus has been proven beneficial in the prevention of bloat (Beddows, 1956). Other condensed tannins have been efficient at improving live-weight gain (Waghorn et al., 1999). In sheep, they have been shown to increase milk protein concentration (Wang et al., 1996), improve lambing percentages (Min et al., 1999), and reduce, gastrointestinal nematode infection (Niezen et al., 1995), incidence of fly strike (Leathwick et al., 1995), and methanogenesis in sheep (Waghorn et al., 2002).

### Tannin Chemistry

Tannins are oligomeric, polyphenolic compounds, often with high molecular weight, and accumulate in many plants as natural products of secondary plant metabolism (Caygill and Mueller-Harvey, 1999). They show great structural diversity among different plant species but one feature that most tannins have in common is that they precipitate protein. Tannins can be divided chemically into two important groups: the hydrolysable tannins and the condensed tannins (CT). Hydrolysable tannins are polyesters of sugars (mostly glucose) and gallic or ellagic acids (Figure 1) and are generally considered detrimental to animal nutrition (Serrano et al., 2009). Condensed tannins are polymers of flavan-3-ols (Figure 2). They form colorful anthocyanidins upon oxidative cleavage (heating in presence of acid) and are therefore also called proanthocyanidins. Each CT polymer can consist of a variety of flavan-3-ol subunits of which the most common are catechin and epicatechin or gallocatechin and epigallocatechin which form procyanidins or prodelphinidins.



**Figure 1.** Structure of the hydrolysable tannin with  $n+2$  flavan 3-ol subunits.



**Figure 2.** Possible structure of condensed Pentagalloylglucose.

### Biological Features of Tannins

Tannins are functionally known by their competence to bind with proteins which forms the bases of many biological effects of tannins (Hagerman and Butler, 1991). They can have detrimental effect against many microorganisms and fungi (Bernays et al., 1989) which may be one of the major reasons of their evolution (Swain, 1979; Bernays et al., 1989; Ayres et al., 1997; Aerts et al., 1999). Intense deposits of tannin contents occur in the epidermis of leaves and stems of many leguminous forage plants, herbs and grasses with different concentrations including *Onobtychis vicifolia* (sainfoin), *Lotus (L.) corniculatus* (birdsfoot trefoil), *L. pedunculatus* (big trefoil), *Hedysarum coronarium* (sulla), and *Lespedeza cuneata* (sericea lespedeza) (Jones et al., 1976; Terrill et al., 1989, 1992). Interestingly, it has been reported that plants higher in tannins produced fewer leaves as compared to those having low tannins (Coley, 1986).

### Effect of Tannin in Ruminal Fermentation

Tannins can be beneficial or detrimental to ruminants, depending on which (and how much) is consumed, the compound's structure and molecular weight, and on the physiology of the consuming species (Hagerman and Butler, 1991).

### Voluntary Feed Intake

Until fairly recently, most researchers believed that the consumption of tannins reduced voluntary feed intake. It would appear that the consumption of plant species with high CT contents (generally > 50 g kg<sup>-1</sup> of dry matter, DM) significantly reduces voluntary feed intake, while medium or low consumption (< 50 g kg<sup>-1</sup> DM) seems not to affect it (Barry and Duncan, 1984; Barry and Manley, 1984; Waghorn et al., 1994a). The effect of HT has also been reported variable, mainly dependent on the quantity consumed. McSweeney et al. (1988) observed no significant reduction in voluntary feed intake in sheep whose diet included *Terminalia oblongata*, a species low in HT (34 g kg<sup>-1</sup> DM). However, a reduction did occur when the same animals were fed *Clidemia hirta*, a shrub with a high HT content (> 50 g kg<sup>-1</sup> DM). Frutos et al. (2004) found no reduction in voluntary feed intake among sheep provided a feed containing soya bean meal treated with HT (20.8 g HT kg<sup>-1</sup> DM of feed).

There are three main mechanisms have been suggested to explain the negative effects of high tannin concentrations on voluntary feed intake: a reduction in feed palatability, the slowing of digestion, and the development of conditioned aversions. A reduction in palatability could be caused through a reaction between the tannins and the salivary mucoproteins, or through a direct reaction with the taste receptors, provoking an astringent sensation (McLeod, 1974). The tannin-proline-rich protein complexes formed, unlike other protein-tannin complexes, are stable across the whole pH range of the digestive tract. This might cancel their negative effect on palatability, and therefore on feed intake, and improve the digestion of tannin-rich feeds (Robbins et al., 1987; Austin et al., 1989; McArthur et al., 1995; Narjisse et al., 1995). It would seem very likely that, throughout evolution, herbivores would have developed different adaptive mechanisms for the consumption of tannin-rich plants (Robbins et al., 1987; Leinmüller et al., 1991; Hagerman et al., 1992; Narjisse et al., 1995). Browsing animals secrete proline-rich proteins constantly, while sheep, for example, only produce them when consuming plants rich in tannins (Robbins et al., 1987; Austin et al., 1989).

In cattle, however, no increase in the production of such proteins has been observed in response to tannin ingestion, although other proteins with high affinity for these polyphenols have been found in their saliva (Makkar and Becker, 1998). With respect to the second possible mechanism, Narjisse et al. (1995) infused tannins directly into the rumen to determine whether factors independent of palatability were responsible for the reduction in voluntary feed intake. Slowing the digestion of dry matter in the rumen impairs the emptying of the digestive tract, generating signals that the animal is full and providing feedback to the nerve centres involved in intake control. In agreement with some authors, this could influence voluntary feed intake more than a reduction of palatability (Waghorn et al., 1994a).

### Digestibility of the Diet

Tannins mainly exert this effect on proteins, but they also affect other feed components to different degrees (Kumar and Singh, 1984). Their main effect on proteins is based on their ability to form hydrogen bonds that are stable between pH 3.5 and 8 (approximately). These complexes-stable at rumen pH- dissociate when the pH falls below 3.5 (such as in the abomasum, pH 2.5-3) or is greater than 8 (for example in the duodenum, pH 8), which explains much about the activity of tannins in the digestive tract (McLeod, 1974; Mangan, 1988; Hagerman et al., 1992). Evidently, the modifications of the digestibility caused by tannin ingestion are mainly associated with changes in the ruminal fermentation pattern, along with changes in intestinal digestibility. The two subsections below discuss these effects, but it is worth mentioning here the repeatedly published conclusion that «one of the clearest pieces of evidence showing that tannins reduce the digestibility of feed is the increase in faecal excretion of nitrogen with increased dietary tannin content». Numerous examples of this argument exist, such as that in which sheep fed only carob (*Ceratonia siliqua*) leaves (tannin concentration = 50 g kg<sup>-1</sup> DM) lose liveweight and excrete more protein in their faeces than they consume (Silanikove et al., 1994). It is important to realise, however, that the consequences of tannin ingestion include increased secretion of endogenous proteins such as salivary glycoproteins, mucus and digestive enzymes, and increased desquamation of intestinal cells (Mehansho et al., 1987; Waghorn, 1996). This increase in faecal nitrogen could therefore be an increase in metabolic faecal nitrogen, i.e., nitrogen of endogenous origin that does not represent a fall in the amount of protein absorbed from feed.

### Ruminal Fermentation

The reduction of ruminal protein degradation may be the most significant and well-known effect of tannins (e.g., McLeod, 1974; Mangan, 1988; Hagerman et al., 1992; Mueller-Harvey and McAllan, 1992). The affinity of tannins for these molecules is very great, and the pH of the ruminal medium favours the formation of tannin-protein complexes. In general, this reduction in protein degradation is associated with a lower production of ammonia nitrogen and a greater non-ammonia nitrogen flow to the duodenum (Barry and Manley, 1984; Waghorn et al., 1994b; Waghorn, 1996). The effect of tannins on protein degradation is basically a reduction in the immediately degradable fraction, and a reduction of the fractional rate of degradation (Aharoni et al., 1998; Frutos et al., 2000;

Hervás et al., 2000). Though tannins mainly exert their effects on proteins, they also have effects on carbohydrates, particularly hemicellulose, cellulose, starch and pectins (Barry and Manley, 1984; Chiquette et al., 1988; Leinmüller et al., 1991; Schofield et al., 2001). For a long time, the effect of tannins on the degradation of fibre was seen as a secondary anti-nutritional effect. However, several studies have shown that fibre degradation in the rumen can be drastically reduced in animals that consume tannin-rich feeds (e.g., Barry and McNabb, 1999; McSweeney et al., 2001; Hervás et al., 2003a).

Tannins are also chelating agents, and this could reduce the availability of certain metallic ions necessary for the metabolism of rumen microorganisms (Scalbert, 1991). With respect to enzyme inhibition, tannins can react with microbial (both bacterial and fungal) enzymes, inhibiting their activity (Makkar et al., 1988; Mueller-Harvey and McAllan, 1992; McAllister et al., 1994b; McSweeney et al., 2001). Several authors (Leinmüller et al., 1991; O'Donovan and Brooker, 2001) indicate that tannins alter the activity of bacterial proteolytic, cellulolytic and other enzymes, but it is important to point out that the binding of tannins to enzymes – whether bacterial or endogenous – does not necessarily simply their inhibition (Makkar et al., 1988). With respect to fibrolytic enzymes, CT more easily inhibits the activity of hemicellulases than cellulases (Waghorn, 1996). This is possibly due to the fact that the latter are associated with bacterial cell walls while the hemicellulases are extracellular and therefore more sensitive (Van Soest, 1994). This would explain why the majority of researchers report a greater reduction in the degradability of hemicellulose in the presence of tannins (Barry and Manley, 1984; Waghorn et al., 1994a; Hervás et al., 2003a). However, this can vary depending on the tannin in question (McAllister et al., 1994a). Finally, tannins might have a direct effect on ruminal microorganisms, e.g., by altering the permeability of their membranes (Leinmüller et al., 1991; Scalbert, 1991). Nonetheless, some rumen microorganisms can tolerate tannins (Nelson et al., 1998; O'Donovan and Brooker, 2001). The degree of tolerance is specific to the microorganism in question, explaining the different susceptibility of bacterial strains. It also depends on the tannin, and the differences between HT and CT in this respect are notorious.

#### **Effects of Tannins on Nutritive Value of Forages**

Tannins in forage have both negative and positive effects on nutritive value (Reed et al., 1990; Mueller-Harvey and McAllan, 1992). Tannins in high concentrations reduce intake, digestibility of protein and carbohydrates, and animal performance (Reed et al., 1990). Tannins in low to moderate concentrations prevent bloat and increase the flow of non-ammonia nitrogen and essential amino acids from the rumen (McNabb et al., 1993). The positive effects of tannins on protein utilization have practical importance because problems associated with extensive proteolysis and (or) deamination in the rumen limit production in modern feeding systems (Beever et al., 1989).

#### **Negative Effects of Tannins**

Normally condensed tannins and hydrolysable tannins supplementation at low levels does not have detrimental effects on animal performance or other economically important traits (Krueger et al., 2010).

#### **Intake**

Tannins may reduce intake of forage legumes by decreasing palatability or by negatively affecting digestion. Astringency is the sensation caused by the formation of complexes between tannins and salivary glycoproteins. Astringency may increase salivation and decrease palatability. Waghorn et al. (1994a) suggested that decreased ruminal turnover and rate of digestion was more important than palatability in reducing intake of sheep fed pure diets of *Lotus pedunculatus* in comparison to sheep fed *L. pedunculatus* along with polyethylene glycol (PEG) the latter binds the tannins making them relatively inactive in the rumen.

#### **Growth**

Rate of gain of young animals reflects total intake and availability of nutrients in the diet. Low growth rates because of low total feed intake were observed in animals eating fruits of *A. sieberiana* and *A. nilotica*, which contained high levels of tannins (Tanner et al., 1990). Low total intake and low growth rates were also observed in animals eating *A. sieberiana* pods and leaves of *A. cyanophylla* (Reed et al., 1990). The negative effect of tannins on growth rate was caused by a combination of reduced intake and low true digestibility of protein.

#### **Digestion of Fiber Fractions**

Tannins may reduce cell wall digestibility by binding bacterial enzymes and (or) forming indigestible complexes with cell wall carbohydrates (Reed et al., 1990). Digestibility of organic matter and fiber fractions was lowest for sheep fed *A. cyanophylla*, the supplement with the highest content of CT and soluble phenolics. At high levels (5-9 %) tannins become highly detrimental (Barry, 1983) as they reduce digestibility of the fiber in the rumen

(Reed et al., 1985) by inhibiting the activity of bacteria and anaerobic fungi (Chesson et al., 1982). High levels also leading to reduced feed intake (Akin and Rigsby, 1985), and above 9 % tannins may become lethal to an animal that has no other feed (Kumar, 1983).

### Positive Effects

**Rumen Escape:** Tannins may complex protein at the pH of the rumen and protect protein from microbial enzymes. These complexes are unstable at the acid pH of the abomasum and the proteins become available for digestion (Barry and Manley, 1984). The evidence for the stability of tannin-protein complexes in the ruminal environment comes from highly simplified *in vitro* systems with purified proteins and tannins in the absence of ruminal microorganisms (Jones and Mangan, 1977). However, a tannin-protein complex that survives the ruminal environment may or may not be digested in the lower tract (Waghorn et al., 1994b).

**Urea Recycling:** Tannins may increase the efficiency of urea recycled to the rumen. Tannins lower the rate of protein degradation and deamination in the rumen and therefore lower ruminal  $\text{NH}_3\text{-N}$  (Woodward, 1989). Plasma urea nitrogen, ruminal  $\text{NH}_3\text{-N}$ , and urinary N loss were lower when sheep and goats were fed legumes that contained tannins (Woodward, 1988). Tannins may increase the glycoprotein content and excretion of saliva, which could lead to more N recycled to the rumen (Robbins et al., 1987).

**Microbial Efficiency:** Tannins increase microbial yield per unit of organic matter digested. Several researchers have observed increases in non-ammonia nitrogen (NAN) flows to the duodenum that were greater than N intake for forage legumes that contain tannins. Because N is not created in the rumen, part of the increased flow of NAN must be from endogenous sources that have been incorporated into the microbial fraction. Nitrogen flows at the duodenum that are greater than N intake are common for diets low in N (< 1%), but for forage legumes with greater than 2% N, the N flows at the duodenum are normally lower than N intake (Barry and Manley, 1984).

Moreover, no effect of CT was found on voluntary feed intake, live weight change and digestibility when beef steers were supplemented with mangosteen peel (*Garcinia mangostana*) that contained tannins. However, the efficiency of rumen microbial protein synthesis and P/E ratio were slightly higher in steers fed on mangosteen peel than the control group.

### Possible Explanations of Tannin Effects

**Nutritional Aspect:** The beneficial effects of tannins in sheep are associated with the greater outflow and absorption of amino acids especially in sheep fed with the forages containing tannin percentage ranging from 2-4% (Waghorn et al., 1987; Wang et al., 1994, 1996b; Min et al., 1999). Enhanced growth rate and increase in production performance and nutritive value of milk in sheep may be due to increased availability of essential amino acids (Waghorn et al., 1987; Wang et al., 1994). Increase outflow of sulphur containing amino acids which are key precursors of wool production may contribute to increased wool production (Wang et al., 1994; McNabb et al., 1993). Tannins make complexes with the proteins in which prevent the degradation of proteins in rumen thus increase flow of proteins to the intestines (Waghorn et al., 1987, 1994; McNabb et al., 1996). Moreover, the pH value in rumen (6.0-7.0) is very favorable for the formation of stable complexes between tannin and proteins. When the complexes come in intestines, the lower pH (2.5-3.5) separates the bond between tannin contents and proteins resulting in enhanced digestion of essential amino acids in the intestines of sheep (Waghorn et al., 1987, 1994). This greater quantity of proteins is available to be absorbed in the intestine of sheep. In contrast, high tannin concentration reduces percentage digestion of proteins thus leads to reduced growth rate, wool production and milk quality and quantity (Waghorn et al., 1994).

### Control of Parasites

Although, most of the parasitic control programs are based on chemotherapeutic control (Waller, 1999; FAO, 2002) but, various problems have been evolved with this practice such as increasing problem of development of resistance by the parasites to several families of drenches (McKenna et al., 1995; Vermunt et al., 1995; Chandrathani et al., 1999; Chartier et al., 2001; Leathwick et al., 2001), hazards of chemical residues and toxicity (Kaemmerer & Bottenkotter, 1973), un-economical, non-adaptability and non-availability of drugs in remote areas. Recent reports on the small ruminants suggest that tannin containing fodder decrease the detrimental effect of gastrointestinal parasites by killing larval and adult worms (Athanasiadou et al., 2000). Similarly, various scientists observed lower fecal count and worm burden with no parasitic species difference in the sheep drenched with several concentrations of condensed tannins (Niezen et al., 1998; Paolini et al., 2003a, 2005; Molan et al., 1999, 2000, 2002; Waghorn and Molan, 2001). Tannins bind with free available proteins in the gastrointestinal tract and reduced nutrient availability of nutrients would have resulted in larval and worm starvation and death (Athanasiadou et al., 2001). Additionally, tannins would also bind with cuticle of larvae which is high in glycoproteins ensuing in death of larvae (Thompson and Geary, 1995).

### **Bloat Safety**

Bloat is frequent muddle in small ruminants caused by the formation of stable protein broth in the rumen of animals fed with high nutritive value legumes including white clover or Lucerne. These protein foams avert the fermented gases to liberate from the rumen resulting in expansion of rumen. The course of bloat formation is very acute and leads to serious damage of vital organs such as lungs and heart (Mangan, 1959) and ultimately leading to death of animals. While, moderate concentration of tannins in the food of animals destabilizes the protein foams which refers them bloat safe (Tanner et al., 1995).

### **Reduced Proteolytic Enzyme Activity and Growth of Rumen Bacteria**

Condensed Tannins considerably reduce the proteolytic enzyme activity and growth of bacteria in the rumen of sheep (Jones et al., 1993). CTs form complexes with the cell coat polymers of bacteria and proteolytic enzymes secreted by them which enable the protein to sidestep in the rumen. These complexes subsequently release the protein when come in the acidic condition of abomasum. These protein molecules undergo enzymatic hydrolysis in the small intestine leading to availability of enormous number of amino acids to be absorbed from the intestine (Jones and Mangan, 1977; Martin and Martin, 1983; McNabb et al., 1998).

### **Effect of Tannins on Animal Production**

Since tannin consumption can affect voluntary feed intake and its digestive utilisation, there are likely to be consequences on the productivity of the animals that consume them. In general, high tannin intakes have a clear negative effect on productivity; nutrient availability is reduced because of the complexes formed between tannins and several types of macromolecules, voluntary feed intake and digestibility are reduced, the digestive physiology of the animal may be impaired, and there may be mucosal perturbations, etc. Barry (1985) observed a significant reduction in the gain of live weight in lambs fed *L. pedunculatus* (which has a high CT content; 76-90 g kg<sup>-1</sup> DM). In any event, the importance of the quantity consumed is receiving more and more recognition since tannins in several types of forage can have beneficial effects in moderate amounts (Aerts et al., 1999; Barry and McNabb, 1999; Min et al., 2003; Waghorn and McNabb, 2003). The intake of under 50 g CT kg<sup>-1</sup> DM (10 - 40 g kg<sup>-1</sup> DM) improves the digestive utilization of feed by ruminants, mainly because of a reduction in ruminal protein degradation and, as a consequence, a greater availability of (mainly essential) amino acids for absorption in the small intestine (Schwab, 1995; Barry and McNabb, 1999; Min et al., 2003). Barry and Manley (1984), by comparing with predicted values for non-tannin-containing diets, report positive effects on the retention of nitrogen in lambs fed *L. corniculatus* (< 50 g CT kg<sup>-1</sup> DM). Wang et al. (1994 and 1996a) observed that the grazing of *L. corniculatus* (34 g CT kg<sup>-1</sup> DM) reduced feed intake but increased the gain in live weight, carcass weight, and dressing proportion, compared with a group supplemented with polyethylene glycol (PEG), which binds to tannins and inactivates them. Montossi et al. (1996) observed a 23% improvement in liveweight gain when lambs grazed *Holcus lanatus* (4.2 g CT kg<sup>-1</sup> DM).

With respect to milk production, Wang et al. (1996b) reported an increase of 21% during mid and late lactation in sheep fed *L. corniculatus* (44.5 g CT kg<sup>-1</sup> DM) vs. sheep dosed with PEG. They also report significant increases in the efficiency of milk production, increased protein and lactose production, and a decrease in the fat content of the milk. This increased concentration of protein might be explained by the greater availability of intestinal amino acids, especially of methionine and lysine, which are thought to limit milk production. The greater concentration of lactose can be explained by greater glucose supply; most lactose synthesis in the mammary gland relies directly on blood glucose, and in ruminants gluconeogenesis mainly involves propionic acid and amino acids. Thus, a greater availability of amino acids would contribute to greater synthesis of glucose. The reduction in the concentration of fat was attributed to a simple dilution effect as the concentrations of lactose and protein increased. Montossi et al. (1996) also observed that grazing on *H. lanatus*, with its much lower CT concentration (4.2 g CT kg<sup>-1</sup> DM), increased wool production by 10%.

### **Treatments to Avoid the Negative Effects of Tannins**

Numerous papers offer information on how to reduce or even avoid the negative effects of tannins in certain feeds. This information is especially useful in impoverished areas with few plant resources and where the majority of available species are rich in tannins. For example, wetting the feed with water or alkaline solutions can separate these phenolic compounds from the most nutritive parts, thus reducing their activity. Treatments with wood ash, as a good and cheap source of alkali, or urea have also been commonly used. «Chopping the leaves and then storage» has been found as an easy practical application by farmers. In this process, tannin inactivation seems to be due to oxidation of tannins and polymerisation to higher inert polymers (Makkar, 2001). More recent (and more studied) alternatives include treatment with polyethylene glycol (PEG), polyvinyl- polypyrrolidone, calcium hydroxide, etc. (Murdiati et al., 1990; Makkar et al., 1995; Ben Salem et al., 1999 and 2000; Makkar, 2001).



### Treatments to Protect Dietary Protein from Ruminal Degradation

One of the basic goals of protein nutrition in ruminants is to optimise dietary protein use in order to maximize animal growth and milk production per unit of protein consumed (Schwab, 1995). Tannins could protect dietary proteins from ruminal degradation. With respect to HT, in 1972 Driedger and Halfield managed to reduce the *in vitro* ruminal protein degradability of soya bean meal through treatment with tannic acid. Its effect on intestinal digestibility however, was not very consistent. Pace et al. (1993) observed that the CT of quebracho provoked a greater reduction in the degradability of soya bean meal than commercial tannic acid, but in general the results obtained were very variable and depended on many factors.

Hervás et al. (2000) and Frutos et al. (2000) treated soya bean meal with different doses (0, 1, 4.7, 9, 13 and 20%) of tannic acid or commercial quebracho CT extract, and significantly reduced the extent of crude protein degradation in the rumen. The effect was significant even at the lowest dose. With respect to the intestinal digestibility of the non-degraded protein, no negative effects were seen until the 13% dose was reached with tannic acid and until the 20% dose was reached in the quebracho CT treatment. One of the drawbacks of using tannins as additives to protect protein rich feeds is the possibility of their degradation by rumen microorganisms. If this were to happen, the treated feeds would be just as vulnerable to ruminal degradation as untreated feeds. In the experiment of Frutos et al. (2000), the intraruminal administration of quebracho CT extract to sheep for 60 days did not increase the capacity of the microorganisms to degrade tannins. Although somewhat obvious, it is worth pointing out that proper management of natural tannin-containing resources (e.g., selective grazing or supplementing the diet with the right kind of shrubs) could provide the same beneficial effects with respect to protein degradation.

### Bloat Prevention

It is well documented that bloat occurs when grazing ruminants consume large quantities of leguminous plants (e.g., alfalfa or clover). The gases produced in the rumen during fermentation cannot be released in the normal way since they are trapped in persistent foam caused by the rapid release of soluble proteins during chewing and ruminal degradation. However, when these animals graze on leguminous plants containing CT (for example *Onobrychis viciifolia*) this does not occur (Mangan, 1988; Aerts et al., 1999; Barry and McNabb, 1999; McMahon et al., 2000). The substitution of a small amount (approximately 10%) of ingested alfalfa DM by *Onobrychis viciifolia* provides unquestionable benefits in the prevention of bloat (McMahon et al., 1999 and 2000). The problem of this strategy is, however, the low persistence of this plant species in mixed cropping with alfalfa. However, the difficulty of the molecular techniques required has made progress slow. Very recently, the preliminary results of a study on the ruminal fermentation of transgenic alfalfa were published. The modification of the alfalfa decreased its initial rate of degradation in the rumen, but not the extent of degradation. This offers an interesting way to help to prevent bloat.

### Control of Internal Parasite

The tannins of numerous plant species help to control certain internal parasites of animals, for example the nematode *Trichostrongylus colubriformis* (Butter et al., 2000). It is speculated that the positive effect on the host animal might be associated with a direct negative effect on the parasites themselves plus an indirect effect in the form of increased availability and digestive utilization of protein (Niezen et al., 1995; Min and Hart, 2003). The literature has several examples of this in sheep and goats grazing *L. corniculatus* or *Hedysarum coronarium* (Robertson et al., 1995) and after having ingested quebracho CT (Butter et al., 2000) etc.

### Competing Interests

The authors declare that they have no competing interests.

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# EFFECT OF MEDICATED UREA MOLASSES BLOCKS ON SUB-CLINICAL PARASITIC INFESTATIONS IN GOATS

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**ABSTRACT:** The aim of this study was to evaluate the effect of medicated urea molasses blocks (MUMB) on sub-clinical parasitic infestations and urea molasses blocks (UMB) to replenish nutrients scarcity. Twenty four goats were divided randomly into three groups of eight animals each (n=8) according to Completely Randomized Design (CRD) a group was no supplement (control) and the other were supplemented with UMB and MUMB for 90 days. Data were recorded and statistically analyzed under CRD through one way analysis of variance (ANOVA). Mean daily dry matter intake was higher ( $1.502 \pm 0.121$  kg) in MUMB supplemented group and lowest in control group (Lenovo). Mean daily weight gain of goats in control, UMB, MUMB was  $64 \pm 23$ ,  $71 \pm 22$  and  $85 \pm 21$  grams, respectively. Body condition score (BCS) was recorded in 1-5 scale of meat goats. The mean BCS in control, UMB and MUMB was  $2.741 \pm 0.193^b$ ,  $2.816 \pm 0.185^{ab}$  and  $2.903 \pm 0.248^a$  respectively. Mean fecal egg count was lowest in MUMB as followed by UMB and control group. It is concluded that feeding of MUMB have significant effects for the control of sub-clinical gastrointestinal worm's infestation and replenishes nutrients deficiency by providing energy and protein.

**Keywords:** Goats, Nutrients Deficiency, Gastrointestinal Worm, Infestations

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## INTRODUCTION

Livestock sector contributes 55.4% in agricultural GDP while 11.9% in national GDP of Pakistan. Current population of sheep and goat in the country is 28.8 and 64.9 million respectively, producing about 643 thousand tons mutton annually. Agriculture, including livestock, have vital role in Pakistan's economy (Anonymous, 2013). Sheep and goat production is central to the livelihood of the rural population in the country and can play vital role in poverty alleviation and keep in elating the socio-economic condition of our rural masses. Small livestock holders derive 10-25% of their income from sheep and goat farming in Pakistan. Therefore, in developing countries like Pakistan, small ruminant rearing is very significant for the livelihood of farmers (Kioumars et al. 2008). Small ruminants are more prone to gastrointestinal worms and their effects also are more severe in sheep and goats. These animals are susceptible to a lot of parasites; the most important is *Haemonchus contortus*. Who sucks blood of animal and causes loss in production, fertility and high mortality in young animals. The most prevalent regions for this parasite are temperate and tropical regions (Ijaz et al. 2008). There was a need to investigate different substitutes to overcome feed shortage and parasitic infestations.

Keeping in view the importance of these aspects, we have designed to conduct the effect of medicated urea molasses blocks on fecal egg count, daily dry matter intake, average daily weight gain, body condition score (BCS), body measurements (body length, heart girth, height at withers) and blood profile (blood glucose, blood urea nitrogen, total protein) were investigated.

## MATERIALS AND METHODS

### Experimental site

Study was conducted at Small Ruminants Training and Research Centre, University of Veterinary and Animal Sciences, Ravi Campus Pattoki, Panjab, Pakistan.

### Meteorological data

The study site is located at Latitude 31.057254 (North), Longitude 73.878469 (East) and Altitude (Above sea level) of 186 meters (613 feet). Average Annual Rainfall 550-600mm and average mean temperature of location is  $23 \pm 2^\circ\text{C}$ .



### Selection of animals

For this study, 24 castrated Beetal bucks with age  $10 \pm 2$  month and body weight  $20 \pm 3$  were used. The goats were allowed to adapt to their surroundings for at least three weeks before the start of the experiment. Special attention was taken to maintain all goats under the same management conditions. During that time they were gradually shifted to their respective feed. The goats were randomly divided into three groups (A, B and C) of eight animals in each group ( $n=8$  each) according to the Completely Randomized Design. Fresh green maize fodder *ad libitum* and 0.5Kg concentrate/animal were fed daily to all groups and refusal was weighed and recorded. The animals in group-A were treated as control group, whereas, the animals in groups B & C were treated as treatment groups, supplemented with urea molasses blocks and medicated urea molasses blocks, respectively (100g/goat/day for A and B groups). Blocks were prepared at the experimental site using different feed stuffs and ingredients (Table 1).

### Variables of interest

**Feed intake/dry matter intake:** Feed intake was calculated on dry matter basis. Dry matter intake was calculated by using the following formula:-

Feed intake (kg) = Feed offered – Feed refused

**Average daily weight gain:** Initial body weight of the individual goats was taken at the start of the experiment and then subsequent weights were taken at the end of every week. The weight gain of every week was calculated by using following formula:-

Weight gain (kg) = Weight of last week (kg) – Weight of current week (kg)

**Body condition score:** Body condition Score was taken at the start of trial of individual goats and then monthly to the end of trial. BCS was recorded in 1-5 scale.

**Fecal egg count:** Fecal egg count (FEC) for egg per gram (EPG) was performed through Modified McMaster Technique (Sloss et al. 1994) at 0 day and then the whole study period, to check parasitic infestation and efficacy of drug used in medicated urea molasses blocks.

### Statistical analysis

Statistical analysis was performed with a commercially available software program SPSS version 18, SPSS Inc, Chicago, IL, USA. The data were analyzed using one way analysis of a variance (ANOVA) between treatments. The Bonferroni test was applied when significant differences were found. The value of  $P < 0.05$  was considered to be significant.

## RESULTS

### Daily dry matter intake

Dry Matter Intake (DMI) was recorded on daily basis in goats allocated to different treatments group. The daily mean DMI (kg) of goats in control, UMB and MUMB was  $1.395 \pm 0.129$ ,  $1.499 \pm 0.128$  and  $1.502 \pm 0.121$  kg, respectively. Mean daily DMI was higher ( $P > 0.05$ ) in MUMB and UMB supplemented groups as compared to control group (Table 2). However the monthly mean DMI trend is given in Figure 1.

### Average daily weight gain

Weight gain of goats was recorded on weekly intervals throughout the trial period. Mean daily weight gained (MDWG) in control, UMB, MUMB were  $64 \pm 23$ ,  $71 \pm 22$  and  $85 \pm 21$ g, respectively. Average weight gained during the experiment were higher ( $P < 0.05$ ) in MUMB treatment group followed by UMB group ( $P > 0.05$ ) when compared to control group as shown in Figure 2.

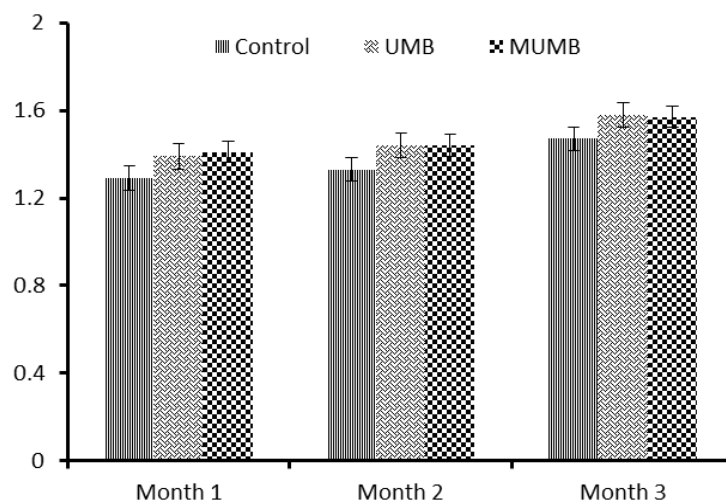
### Body condition score

Body condition score (BCS) was taken at the start of the experiment of every individual goats and then monthly basis till to the end of the study. BCS was recorded in 1-5 scale of meat goats. The mean body condition score in control, UMB and MUMB were  $2.741 \pm 0.193$ ,  $2.816 \pm 0.185$  and  $2.903 \pm 0.248$  respectively. The increase in the BCS was higher ( $P < 0.05$ ) in MUMB supplemented group compared to UMB and that of control group. However no difference ( $P > 0.05$ ) were observed among the UMB and control group into the experiment (Table 2).

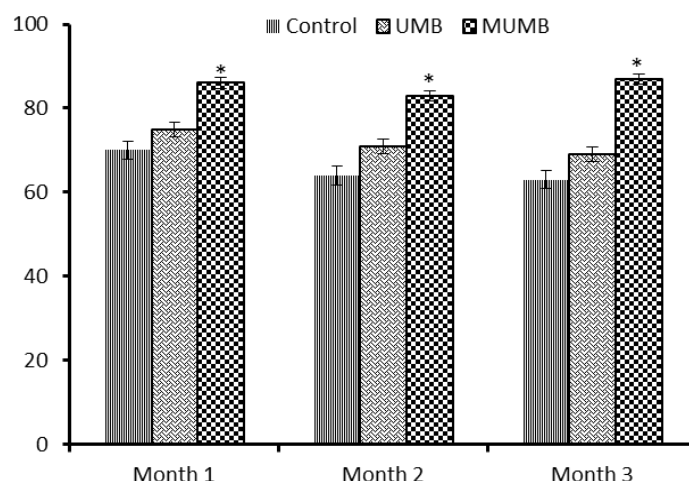
### Fecal egg count

Fecal egg count for EPG was performed at start of each trial and then at the end of study. Data for FEC were transformed by taking log 10 (count+50) before analysis to stabilize variance within groups. Our result indicated that the Mean FEC at the end of experiment in MUMB group was significantly decreased ( $P > 0.05$ ) when compared

to that of UMB and control group. There were no difference were observed among UMB and control group as shown in Table 2.



**Figure 1** - Average daily dry matter intake (Kg) trend on monthly basis (mean±SE). The values between groups do not differ ( $P>0.05$ ) from the control group, UMB and MUMB. UMB and MUMB refer to urea molasses blocks and medicated urea molasses blocks respectively.



**Figure 2** - Effects of treatment on average daily weight gain (g) trend on monthly basis (mean±SE). The values with one asterisk differ ( $P<0.05$ ) from the control group, UMB. UMB and MUMB refer to urea molasses blocks and medicated urea molasses blocks respectively.

**Table 1** - Nutritional profile of urea molasses blocks and concentrate ration

Nutrient	Urea Molasses Block	Concentrate Ration
Dry Matter	79	87.6
Protein	42.68	16.72
Fat (Ether Extract)	5.1	6.5
Crude Fiber	8.5	12.5
Ash	25.8	9.4

Weight gain (kg) = Weight of last week–Weight of current week

**Table 2** - Mean daily Dry Matter Intake (kg), Mean daily weight gain (grams), Mean Body Condition Score and Mean fecal egg count at end of experiment in Beetal male goats supplemented with different blocks.

Treatments	Mean DMI (kg)	MDWG	Mean BCS	Mean FEC at
Control	1.395± 0.129 <sup>b</sup>	64± 23 <sup>c</sup>	2.741± 0.193 <sup>b</sup>	2.764± 0.130 <sup>a</sup>
UMB	1.499± 0.128 <sup>a</sup>	71± 22 <sup>b</sup>	2.816± 0.185 <sup>ab</sup>	2.518± 0.108 <sup>a</sup>
MUMB	1.502± 0.121 <sup>a</sup>	85± 21 <sup>a</sup>	2.903± 0.248 <sup>a</sup>	1.850± 0.301 <sup>b</sup>

The values with the different lowercase superscript letters in the same column differ ( $P<0.05$ ). DMI, daily dry matter; ADWG, Average daily weight gain; BCS, body condition score; FEC, Fecal egg count; UMB, Urea molasses blocks; MUMB, medicated urea molasses blocks.

## DISCUSSION

The quantity of feed blocks offered to MUMB and UMB groups was same and concentrate offered and consumption by all groups was same. Blocks increased the green fodder intake in both groups. In agreement to our finding, study have reported that feeding UMB supplement has increased mean pasture DMI  $708.7 \pm 49.9$  gram in UMB group than  $461.9 \pm 70.9$  gram in control (Vatta et al. 2008). This is completely in agreement with the findings of this study.

These findings are also in accordance with Unal et al. (2005) as they reported that UMMB can cover weight loss due to poor quality roughages in feed scarcity seasons as in winter. Statistical analysis of data depicted that DMI increased significantly ( $P < 0.05$ ) in UMMB fed groups. However Anindo et al. (1998) studied the effect of MUMB in 120 Menz lambs of 5-7 months of age divided into 6 groups. The results showed that MUMB increased DMI  $568 \pm 11$ g (MUMB) versus  $532 \pm 11$ g per head per day in control.

Weight gain of goats supplemented with different blocks was recorded on weekly intervals throughout the trial period. Statistically mean daily weight gain was significant ( $P < 0.01$ ) between treatment groups. Mean daily weight gain of goats in control, UMB, MUMB was  $64 \pm 23$ ,  $71 \pm 22$  and  $85 \pm 21$  grams, respectively. Highest average daily weight gain ( $85 \pm 21$ g) was observed in treatment MUMB followed by UMB and Control, respectively. These results are consistence with Suresh et al (2013) who reported significantly ( $P < 0.01$ ) higher weight gain in goat on UMMB ( $63.19$  g/day) than in control ( $52.70$ g/day).

Hossain et al (1995) reported the same findings as average daily weight gain in treated urea molasses lick blocks (UMLB) group was  $70$ g as compared to  $41$ g in control group. These findings are in accordance with Aganga et al (2005) who carried a study on 16 Tswana sheep divided in two groups of 8 sheep. The results revealed that supplementation with MUB almost doubled the average daily gain of sheep ( $189.0$ g/day) as in control ( $97.30$ g/day). Kioumars et al. (2012) showed the results that molasses/mineral feed blocks and medicated blocks have significant effects ( $P < 0.05$ ) on average daily gain as  $216 \pm 9.50$ ,  $179 \pm 7.60$ ,  $193 \pm 10.50$  and  $164 \pm 9.82$  in UMMB+MUMMB, MUMMB, and control group respectively. MUMB combats with the gastro-intestinal parasites and aids in maximum growth rate. Geleta et al. (2013) reported significant ( $P < 0.05$ ) increase in average daily weight gain in grazing sheep supplemented with urea molasses blocks (UMB). Results showed that sheep supplemented with UMB had higher growth rate  $74.8 \pm 11.13$  g/head/day than control  $33.6 \pm 3.03$  g/head/day.

The mean BCS was significant higher ( $P < 0.01$ ) in MUMB goats. It indicated that increase in the mean body condition score was highest in MUMB as followed by UMB and control group. Anindo et al. (1998) studied the effect of MUMB on the BCS and found same results as discussed above. Treated animals deposited more body reserves as judged by BCS  $3.2 \pm 0.1$  versus  $2.4 \pm 0.1$  in control group after 6 months. These findings are also in line with Rafiq et al. (2003) who studied the effect of supplementation of multi nutrient UMBs on the BCS in Lohi ewes (treated,  $n = 514$ ). Ewes in treated flock had high BCS  $2.31$  as compared to  $2.08$  in control group. On the contrary, Vatta et al. (2008) stated that there was no significant difference ( $P > 0.05$ ) in BCS of goats supplemented with MUMMB and control treatment.

Fecal Egg Count for EPG of goats was performed at start of trial and then at the completion of study. The initial oral administration of dewormer before the start of trial was effective in reducing the existing nematode infections, as evidenced by the reduction of FEC to zero. At the end, MUMB group had almost zero FEC. Data for FEC were transformed before analysis by taking  $\log_{10}(\text{count} + 50)$  to stabilize variance within groups. Mean FEC at end of experiment was highest in MUMB as followed by UMB and control group. Statistically mean FEC at end of trial was significant ( $P < 0.05$ ) among goats on MUMB. These findings are in agreement with Waruiru et al. (2004) that treated MUMB group had mean worm count  $482 \pm 299$  while control group had mean worm count  $1302 \pm 410$ . The result of the study finding indicated that feeding MUMB is recommended in order to control sub-clinical gastrointestinal worm's infestation in goats and also provides nutrients requirement (energy and protein) in order to perform normal metabolism process of the body.

### Conflict of interest

None of the authors has conflict of interests with this submission. None of the authors has conflict of interests with the corporations and the software mentioned in this paper.

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Lucy MC (2000). Regulation of ovarian follicular growth by somatotropin and insulin- like growth factors in cattle. *Journal of Dairy Science*, 83: 1635-1647.

Kareem SK (2001). Response of albino rats to dietary level of mango cake. *J. Agric. Res.Dev.* pp 31-38.

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##### b) For symposia reports and abstracts:

Cruz EM, Almatar S, Aludul EK and Al-Yaqout A (2000). Preliminary Studies on the Performance and Feeding Behaviour of Silver Pomfret (*Pampus argentens euphrasens*) Fingerlings fed with Commercial Feed and Reared in Fibreglass Tanks. *Asian Fisheries Society Manila, Philippine* 13: 191-199.

##### c) For edited symposia, special issues, etc., published in a journal:

Korevaar, H., 1992. The nitrogen balance on intensive Dutch dairy farms: a review. In: A. A. Jongebreur et al. (Editors), *Effects of Cattle and Pig Production Systems on the Environment: Livestock Production Science*. 31: 17-27.

##### d) For books:

AOAC (1990). *Association of Official Analytical Chemists. Official Methods of Analysis*, 15th Edition. Washington D.C. pp. 69-88.

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##### e) Books, containing sections written by different authors:

Kunev, M., 1979. Pig Fattening. In: A. Alexiev (Editor), *Farm Animal Feeding*. Vol. III. Feeding of Different Animal Species, Zemizdat, Sofia, p. 233-243 (Bg).

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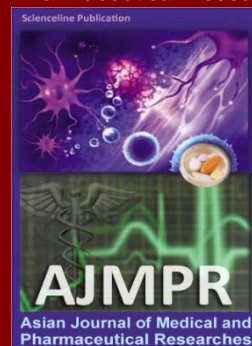
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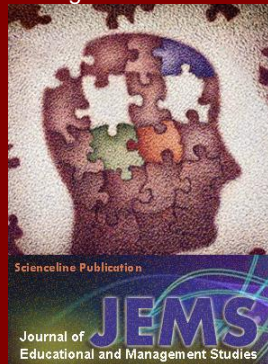
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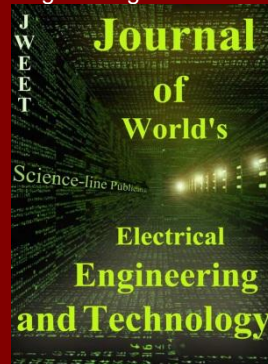
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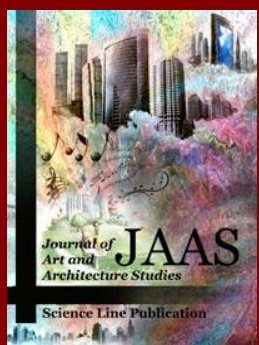
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