U. of K. J. Vet. Med. Anim. Prod., Vol.6, Issue 1 (2015) p 7-16

ISSN: 243456

UNIVERSITY OF KHARTOUM
Journal of Veterinary Medicine
and Animal Production

Effect of Methods of Polyethylene Glycol Addition on Gas Poduction Parameters of *Acacia seyal, Acacia ehrenbergiana* and *Capparis deciduas*

Rowa, I. Ahmed; Ahamed, G. Mahala; Randa, H. A. Mohamed and Ibtisam E. M. El Zubeir

رؤى اسماعيل احمد و احمد جفون مهلة و رندة حسن على محمد

وابتسام الياس محمد الزبير

Faculty of Animal Production, University of Khartoum, 13314 Khartoum North, Sudan

Abstract

The aim of this study was to estimate the nutritive value and the effect of methods of poly ethylene glycol (PEG) addition as single dose and spilt doses on in vitro gas production kinetic of *Acacia seyal*, *A. ehrenbergiana* and *Capparis deciduas* leaves. Crude protein (CP), Crude fiber (CF), Ether Extract(EE), Ash, Nitrogen Free Extract, (NFE), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL), and Total Condensed Tannin (TCT) were determined, In vitro Organic Matter Digestibility (IOMD) and Metabolizable Energy (ME) were calculated and in vitro gas production was carried out. Data were subjected to analysis of variance using complete randomize design. Chemical content were significantly (P≤ 0.05) varied among the species. Crude protein content ranged from 7.66 to 15.88%. ADF and NDF contents varied within species and ranged from 43.96 to 51.65% and 54.30 to 59.32 %, respectively. TCT content ranged from 1.98 to 3.26%. The addition of PEG as one dose and as split doses increased gas production in all species of trees. The single application of PEG resulted in higher gas production than the spilt application. The insoluble fraction (b), potential gas production (a+b), IOMD and ME were significantly (P≤ 0.05) increased with PEG addition. The improvement of IOMD and ME with the inclusion of PEG demonstrated the negative effect of tannins on digestibility and the addition of PEG by both methods may improve the nutritive value of trees leaves.

المستخلص

الهدف من هذه الدراسة هوتحديد القيمة الغذائية و تقيم اثر طرق إضافة مادة البولي ايثيلين جلايكول بجرعة واحدة وجرعة منفصلة في اوراق اشجار السيال، السلم والتنضب، وكان ذلك بإستخدام تجربة إنتاج الغاز المعملية. تم تحديد البروتين الخام (CP) الالياف الخام (CF), مستخلص الايثر (EE), الرماد (Ash) الألياف الغير ذائبة في المنظفات المتعادلة, (NDF) الألياف الغير ذائبة في المنظفات الحمضية (ADF), المستخلص الخالي من النيتروجين (NFE), محتوى التانين المكثف (TCT), وايضا تم حساب هضمية المادة العضوية (IOMD) والطاقة الأيضية (ME). تم تحليل البيانات بإستخدام التصميم الكامل العشوائي, فوجد فروق معنوية (P≤ 0.05) بين اوراق الاشجار الشوكية. تراوح محتوىCP مابين 7.66% الى15.88% وتراوحت نسبة (NDF) ما بين 54.33% الى 59.33%, و نسبة (ADF) تراوحت ما بين 43.36% حتى 51.65%, وترواح محتوى TCT في اوراق هذه الاشجار الشوكية ما بين 1.98% الى 3.26%. ادت إضافة مادة البولي ايثلين جلايكول

كجرعة واحدة و جرعة منفصلة الى زيادة محتوى الغاز الناتج في جميع انواع الاشجار. وكان الغاز الناتج من إضافة مادة البولي ايثيلين جلايكول في جرعة واحدة اعلى في محتواه من إضافتها كجرعة منفصلة. إضافة مادة البولي ايثيلين الى إحداث زيادة معنوية ($P\leq 0.05$) في الغاز الناتج من الجزء المتكسر الذائب ببطء (b) والغاز المنتج الكامن (a+b) وهضمية المادة العضوية والطاقة الأيضية. ومن الممكن ان نلخص الى انإضافة مادة البولي ايثيلين جلايكول كان لها تأثير سلبي علي التانين وذلك بزيادة الغاز المنتج وزيادة هضمية المادة العضوية والطاقة الأيضية وهذا ادى الى التحسن في القيمة الغذائية لاوراق هذة الاشجار.

Introduction

Sudan is one of the largest countries in Africa with great agricultural potential in general and livestock in particular. The population of cattle sheep, goat and camel were around 132 million head. More than 90 percent of the livestock in Sudan are kept in nomadic flocks for meat, milk and skin extensive systems depending mainly on range land resources (Ministry of Animal Resources and Fisher, 2008). The feed resources available to the livestock include natural pasture, crop residues and agro-industrial by products in that order of importance.

The nutritive value of pasture and rangeland in the Sudan is greatly affected by seasonal change. In the summer (dry period), the moisture content, crude protein (CP) and total soluble sugars decreases and the plant tends to be of relatively poor nutritive value (El hag, 1985). Fodder trees and shrubs leaves constitute a vital component in livestock productivity in the arid and semi arid zoon. Trees and shrubs leaves are important sources and supply goats and camels by nutritive requirement and complement the diet for small ruminants and camels (Mahala and Fadel Elseed, 2007).

Browse species such as Acacia play major role in providing feed for ruminant in arid and semi arid zoon particularly during the dry season when quantity and quality roughages are limited (Dmello, 1992 and Karabulut *et al.*, 2007). Acacia leaves and pods are a potential source of protein especially in the dry season (Osuji and Odenyo, 1997).

Acacia a leguminous shrub species are important source of protein but most acacia species contain significant amount of phenolic compounds mainly tannins (Makker and Beckr, 1998). The primary anti-nutritional agent in acacia species and many other browse species are condensed tannins (Dmello, 1979). Tannins are plant polyphenolic substances that can bind with protein (Haslam, 1979) and has a negative effect in nutrient digestibility and voluntary intake (Barry and Balney, 1987) and decrease palatability (Provenza and Malechek, 1983).

Polyethylene glycol (PEG) may counter anti nutritional condensed Tannin through the formation of strong irreversible bond with displacing condensed tannin from the Tannin protein complex (Jones and Mangan, 1977). The objective of this study was to estimate the effect of methods of PEG addition as one and split dosages on in vitro gas production parameters of *Acacia seyal*, *Acacia ehrenbergiana and Capparis decidua* leaves.

Materials and Methods

Collection and preparation of milk samples

Sample collection and preparation

Leaves samples were collected from three species of fodder trees *A. seyal*, *A. ehrenbergiana* and *Capparis decidua* from Shandi, samples were oven dried, grinded and kept for further analysis.

Chemical analysis

Ash, DM, CP, E.E, CF and NFE were determined according to proximate analysis procedure (AOAC, 1990). Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignins (ADL) were determined according to Van Soset *et al.* (1967). Total condensed Tannins were determined by the butanol-HCL method (Makkar *et al.*, 1995).

In vitro gas production

Sample of three fodder trees namely *A. seyal, A. ehrenbergiana and Capparis deciduas* leave without PEG and with 0.1 mg PEG as one dose and 0.1 mg PEG as spilt (two) doses were incubated in vitro with rumen fluid in calibrated glass syringes using the procedure of the Menke and Steingass (1988). Rumen liquor was obtained from a fistulated steer fed Abu70 Hay and a concentrate mixture. Approximately 200mg of DM sample was weighted in triplicate into glass syringes. (T_1, T_2,T_3) for each group $\mathbf{T_1}$ for control, $\mathbf{T_2}$ treated with 0.05 mg from polyethylene glycol and completed to 0.1after 48h and $\mathbf{T_3}$ treated with 0.1mg of polyethylene glycol as single dose. The piston was lubricated with Vaseline to ease movement and to prevent escape of gas. The syringes were pre-warmed at 39°C before the addition of rumen buffer mixture, incubated in a water bath at 39±1°C and were gently shaken every 30min.after the start of incubation. Gas production was recorded before incubation (0) and 3,6,12,24,48,72 and 96h after incubation. Total gas values were corrected for cumulative gas production data were fitted to the model of (Ørskov and McDonald, 1979) using the following equation:

$P=a+b\ (1-\exp^{-ct})$

Where:

P is the gas production at time.

a+b the potential gas production (ml).

a the gas produced from soluble fraction.-

b the gas produced from in soluble but fermentable fraction.

c the rate constant of gas production during incubation (ml h^{-1}).

Metabolisable Energy (ME) was calculated as

ME= 2.20 + 0.136GV + 0.057 CP + 0.00286 EE (Menke and Steingass, 1988).

Organic matter digestibility (OMD %) was assessed as

OMD = 16.49 + 0.9042 GV + 0.0492 CP+0.0387 CA (Menke and Steingass, 1988).

Statistical analysis

The data were subjected to analysis of variance using complete randomize design (CRD) according to Steel *et al.* (1997). The Differences between means were done by least significant difference (LSD).

Results

The chemical composition of three tree leaves was shown in Table1. There were significant differences ($P<0.05$) in the chemical composition among tree leaves. The CP content in DM bases ranged from 7.66%-15.88%. *A. seyal* had significantly ($P\leq0.05$) higher CP than the other species.

Ether Extract varies significantly ($P\leq 0.05$) among tree leaves and A. *ehrenbergiana* was higher (5.93%) in EE than the other. The ash content of tree leaves ranged from 7.67%- 12.86%. The ash content of *A. ehrenbergiana* was 12.86% higher significantly ($P\leq0.05$) than the other species. NDF, ADF and ADL ranged from 54.30%-59.30% and from 43.36% to 51.65% and from 13.3% to 38.09%, respectively. NDF, ADF, ADL) were higher significantly ($P\leq0.05$) in *A. ehrenbergiana* compared to the other species. Also NFE was significantly ($P\leq0.05$) varied among species and ranged from 35.30 to 57.22%. The *Capparis deciduas* showed the highest NFE and *A. seyal revealed the* lower in NFE. The TCT was significantly ($P\leq0.05$) different among tested Acacia spp.*;* *A. seyal* had higher CT (3.26%) and *Acacia ehrenbergiana* showed low CT (1.98%).

PEG effect on total gas production volume of Acacia trees leaves in incubation time (ml/g DM) were presented in Table 2. Addition of PEG to tannin-containing feeds increased in vitro gas production in all feeds. The Soluble fraction (a) in *Capparis deciduas* was significantly ($P\leq0.05$) higher in T_2 (5.69%) followed by T_3 (3.48%) compared to T_4 (1.08%). However the Insoluble fraction (b) in *Capparis deciduas* was significantly ($P\leq0.05$) higher in T_3 (21.50%) followed by T_1

(17.19%) compared to T_2 (15.32%). The potential gas production (a+b) was increase by addition of PEG.

The soluble fraction (a) in *A. ehrenbergiana Hayne* was 0.62% for T_1, 4.61% for T_2 and 4.84% forT_3 and there was no significant (P>0.05) different between T_2 and T_3. The insoluble fraction (b) was significantly (P≤0.05) high T1 and low in T2. The Potential gas production (a+b) was high in T_3 and no significant difference between T_2 and T_1. The Soluble fraction (a) in *A. seyal* was significantly (P≤0.05) high in T3 (11.06%) followed by T_2 (2.38%) than T_1 (-0.63%). The insoluble fraction (b)was not significant (P≤0.05) between T1 (31.69%) and T2 (31.8%). The Potential gas production (a+b) from *A. seyal* was found to increase by addition of PEG. Moreover the Soluble fraction (a) in all species increased by PEG addition as one and spilt doses, while the Insoluble fraction (b) decreased by PEG addition as spilt dose in all species. This study also found that the potential gas production (a+b) in all species increased by PEG addition.

The effect of polyethylene glycol (PEG) supplementation on gas production kinetics, organic matter digestibility (OMD) and metabolisable energy (ME) was presented in Table 3. IVOMD in *Capparis decidua* was significantly (P≤0.05) high in T3 (30.9%) but there was no significant difference between T_2 (28.38%) and T_3 (29.9%). Moreover the IVOMD in *A. ehrenbergiana* showed no significant (P≤0.05) difference between T_1 and T_3 (30.49%). Similarly the IVOMD in *A. seyal* revealed no significant difference between T_1 (35.39%) and T_3 (35.84%).

The ME in *Capparis deciduas* showed no significant (P≤0.05) deference between T_1 (4.17%), T_2 (4.17%) and T_3 (3.94%). The ME in *A. ehrenbergiana Hayn*e revealed non significant (P>0.05) differences between T_1 (4.91%) and T_2 (4.67%). The ME in *A. seyal* was no significantly (P≤0.05) different between T_1 (4.97%), T2 (4.57%) and T3 (5.04%).

The present study showed that gas production after 48 hrs in all acacia species treated by T3 was significantly (P≤0.05) higher than other treatment.

Exchange Service for the first author is acknowledged

Plant samples	E.E	CP	CF	Ash	NFE	NDF	ADF	ADL	TCT
Capparis decidua	4.45^{ab}	11.18^{b}	24.68^{b}	7.67^{b}	57.22^{a}	58.78^{a}	43.92^{b}	30.67^{a}	2.95^{b}
A. ehrenbergiana	5.93^{a}	7.66^{b}	28.82^{ab}	12.86^{a}	41.99^{b}	59.33^{a}	51.65^{a}	38.09^{a}	1.98^{c}
A. seyal	3.13^{b}	15.88^{a}	31.67^{a}	8.70^{b}	35.30^{c}	54.30^{b}	43.36^{b}	13.3^{b}	3.26^{a}
±S.E.M	0.739	1.464	2.050	2.862	1.77	4.06	6.90	86.5	0.21

E.E= Ether extract, CP= crude protein; CF= Crude Fiber, NFE = Nitrogen Free Extract, NDF= Neutral detergent fiber; ADF= acid detergent fiber; ADL= acid detergent lignin.TCT= Total condensed tannin; S.E.M= standard error of mean.

Table 2: The effect of polyethylene glycol (PEG) supplementation on gas production kinetics in three fodder trees

(a)= Gas produced from soluble fraction (ml); (b) = gas produced from insoluble fraction but fermentable fraction (ml), the potential gas production (*a*+b) (ml); and (c) = rate of gas production during incubation (ml/ h^{-}

Plant samples	Treatment	A	b	c	a+b
Capparis decidua	T_1	1.08^{c}	17.19^{b}	0.053^{a}	18.27^{c}
	T_2	5.69^{a}	15.32^{c}	0.02^{b}	21.02^{b}
	T_3	3.48^{b}	21.56^{a}	0.033^{c}	25.04^{a}
SME±		0.53	0.76	3.96	0.88
A. ehrenbergiana	T_1	0.62^{b}	31.07^{a}	0.027^{a}	31.69^{b}
	T_2	4.61^{a}	27.18^{c}	0.027^{a}	31.8^{b}
	T_3	4.84^{a}	29.14^{b}	0.027^{a}	33.98^{a}
SME±		0.26	0.5	1.39	0.53
A. seyal	T_1	-.63^{c}	27.5^{a}	.053^{a}	26.87^{c}
	T_2	2.38^{b}	25.94^{b}	0.032^{b}	28.32^{b}
	T_3	11.06^{a}	25.14^{b}	0.028^{b}	35.99^{a}
SME±		0.55	0.78	2.6	0.28

[1]).**T_1** = without PEG; **T_2** = treated with PEG 0.1g as two dose; **T_3**= treated with PEG0.1g as one dose.

U. of K. J. Vet. Med. Anim. Prod., Vol.6, Issue 1 (2015) p 7-16

ISSN: 243456

Table 3: The effect of polyethylene glycol (PEG) supplementation on gas production after 48 hrs, organic matter digestibility (OMD) and metabolisable energy (ME) in three fodder trees

(IVOMD) In vitro organic matter digestibility, ME MJ/kg= metabolisable energy; 48h= the gas production after 48hrs

Plant samples	Treatment	IVOMD	ME MJ/kg	48h
Capparis decidua	T_1	29.9^{ab}	4.17^{a}	16.5^{b}
	T_2	28.38^{b}	3.94^{b}	14.16^{c}
	T_3	30.94^{a}	4.17^{a}	20.33^{a}
SME±		o.89	0.13	0.57
A. ehrenbergiana	T_1	30.49^{b}	4.91^{a}	20.33^{c}
	T_2	33.8^{a}	4.67^{a}	22.83^{b}
	T_3	30.49^{b}	4.26^{b}	24.67^{a}
SME±		0.39	0.08	0.24
A. seyal	T_1	35.39^{a}	4.97^{a}	24.17^{ab}
	T_2	32.68^{b}	4.57^{b}	23.17^{b}
	T_3	35.84^{a}	5.04^{a}	26.0^{a}
SME±		0.26	0.39	0.44

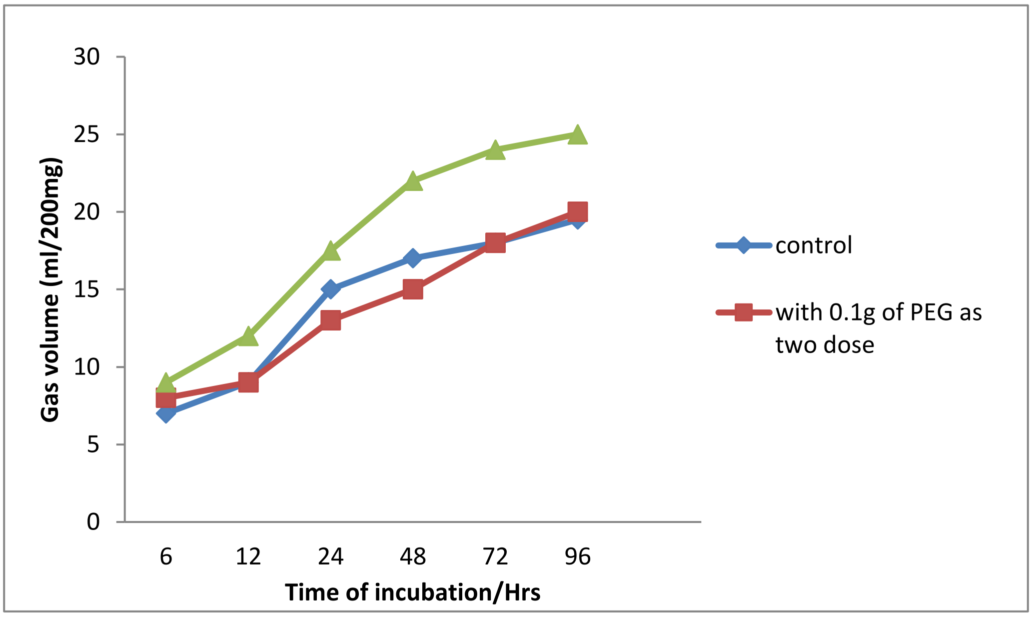

Figure 1: Gas production volume from *A. Capparis deciduas* with (one dose and spilt doses) and without PEG

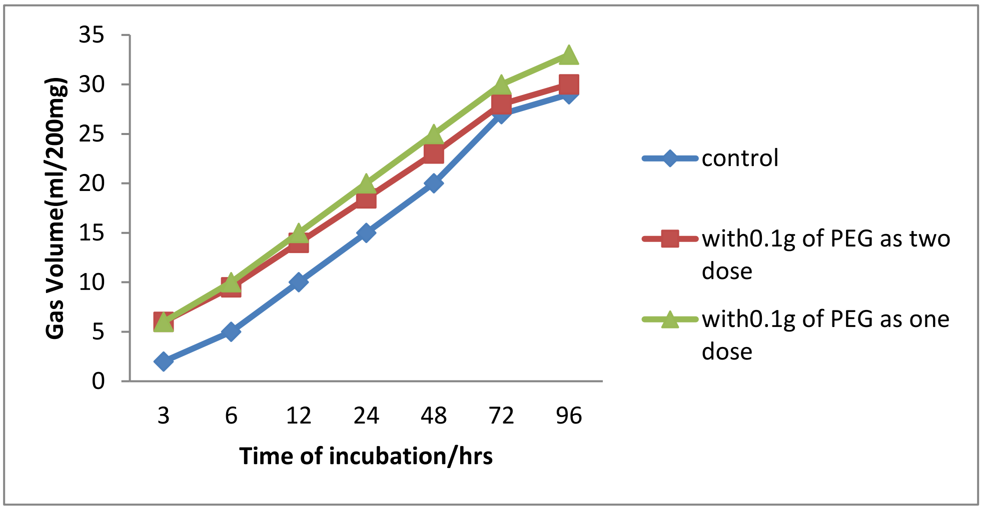

Figure 2: Gas production volume from *A. ehrenbergiana Hayne* with PEG (one dose and spilt doses) and without PEG.

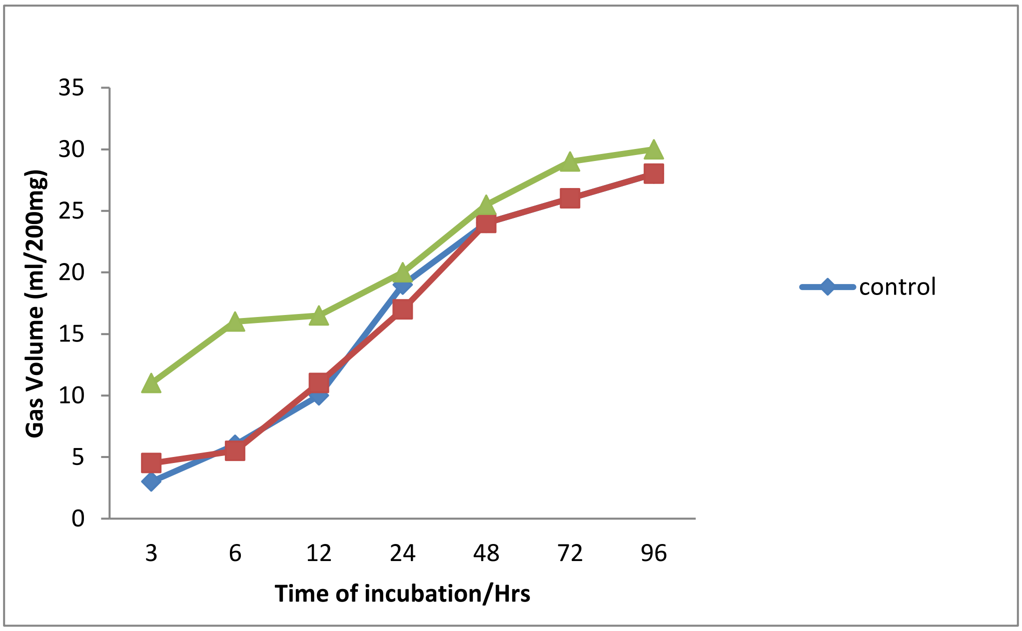

Figure 3: Gas production volume from *A. Seyal* with PEG (one dose and spilt doses) and without PEG

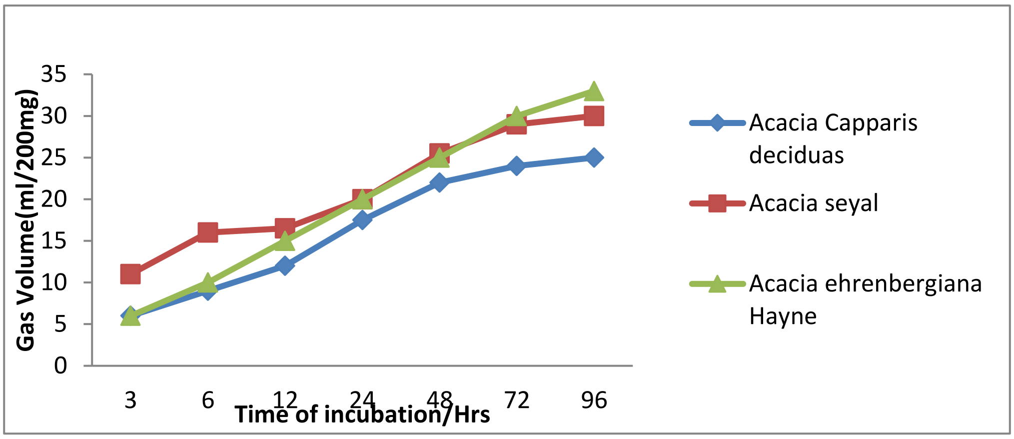

Figure 4: Gas production volumes from addition PEG as one dose to *A. Capparis deciduas*, *A. ehrenbergiana Hayne* and *A. seyal*

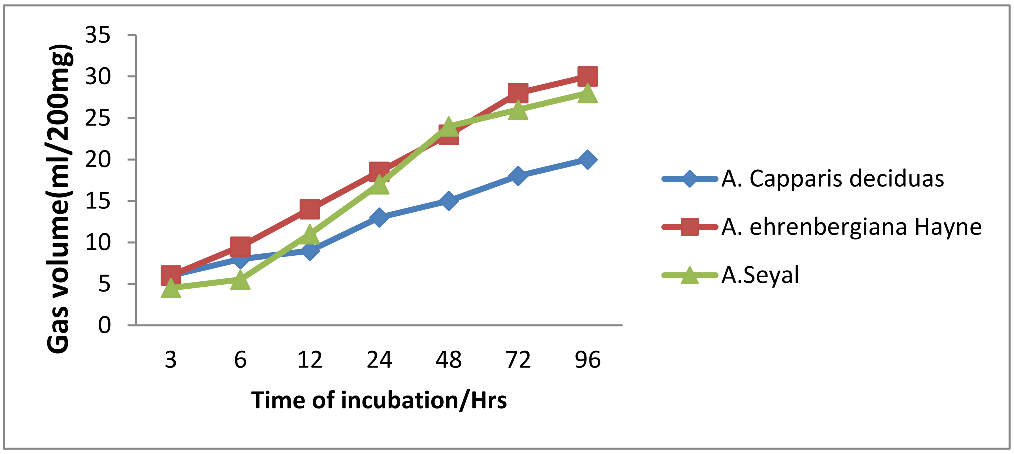

Figure 5: Gas production volume of *A. Capparis deciduas*, *A. ehrenbergiana Hayne* and *A. seyal* from addition PEG as spilt dose.

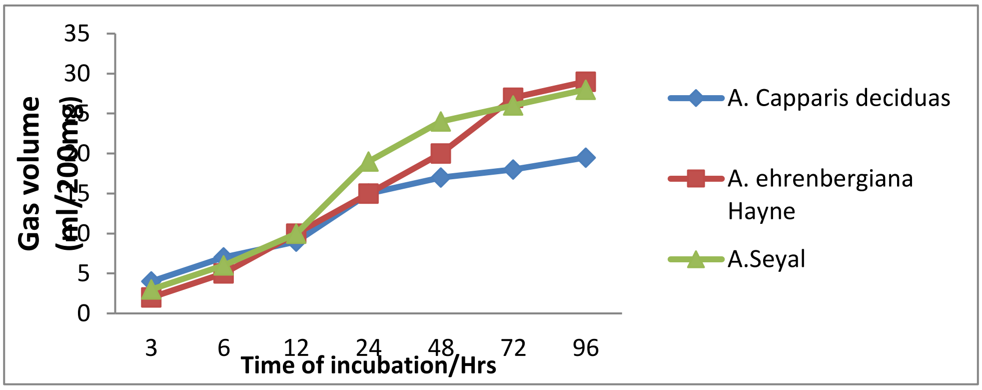

Figure 6: Gas production volume of A. Capparis deciduas, A. ehrenbergiana Hayne and A. seyal without PE

Discussion

The fodder trees and shrubs are important feed for livestock in arid areas. The nutritive value of their leaves can be superior to that of herbaceous plants, particularly in the case of leguminous trees (Osuji and Odenyo, 1997).

CP content of leaves ranged from7.6 to 15.8% this level of protein may satisfy animal requirement, and result is similar to that obtained by Alsequeer (2008) who found from 8 to 16.7% but these values were lower than that reported by Mahala and FadelElseed (2007) who's found the CP content from 17.5 to 38.6% and also inconsistent with Abdulrazak *et al.* (1999) who found the CP content ranged from 15.23 to 23.8%. The NDF and ADF content of leaves varies from 54.3 to 58.7% and from 43.3 to 51.6% respectively, this level of NDF may negatively affect the feed while the level of ADF affect negatively the digestibility, this result is comparable with finding of Mahala and FadelElseed (2007) who reported 35.7 to 51.6% and 18.4 to 43.7% and Alsoqeer (2008) who found 33.6 to 56% and 20.9 to 45% respectively.

The TCT content of leaves ranged from 1.9 to 3.2% this level of TCT might positively affect of protein utilization in ruminants (Aerts *et al.*, 1999) who stated that the tannin level between 20-40g/kg DM has been proven beneficial to protein digestion result is similar to Abdulrazak *et al.* (1999) who found the TCT 1.6 to 2.83%. The higher level of TCT in *A. seyal* was similar to that reported by Mahala and FadelElseed (2007) who reported the TCT in *A. seyal* 3.4%. This variation in the chemical composition may be due to several factors such as species, stage of maturity and harvesting parts. Gas produced from fermentable fraction (b) and the potential gas production (a+b), was higher in *A. ehrenbergiana Hayne* and *A. seyal* than the *Capparis deciduas.* Fraction (b) and potential gas production (a+b) in *A. seyal (*27.5% and 26.87% respectively this result was higher to that reported by Mahala and FadelElseed (2007who found 23.82% and24.55%respectively.this may be due to different part selected and different location. Addition of PEG as spilt dose produced lower gas volume than in one dose, this result inconsistent with findings of Ben Salem *et al.* (2004) and Getachew *et al*. (2001) who found the gas production from addition PEG as spilt dose to the incubation at different times during fermentation resulted higher gas volume compared to single dose. This may be attributed to differences in the Species of fodder trees selected and different in the type of PEG used. The addition of PEG as one dose increased the gas produced from soluble and insoluble fraction (a) and (b), potential gas production (a+b), in vitro organic matter digestibility (IVOMD) and metabolic energy (ME). This may be attributed to the high affinity of PEG to tannin and make tannin inert by forming tanning PEG complex (Makkar *et al.*, 1995).

Conclusion

Acacia trees might provide a part of the solution to shortage of energy and protein feedstuffs during the dry season to ruminant livestock in spite of varying amounts of tannins which may constrain protein availability to ruminants.

Additions of PEG produce a response to evaluate tannin effect and led to increase the gas production and also increased the organic matter digestibility (OMD) and metabolizable energy (ME). The improvement in gas production, OMD and ME with PEG emphasizes the negative effect of tannins on digestibility. However the effect of spilt dose might be better than the one dose but the vice versa occur, this may be due to long period between dosing so we recommended to give the spilt dose after 24hrs instated of 48hrs.

References

Abdulrazak, S.A.; Fujihara, T.; Ondiek, J.K. and Ørskov, E.R. (2000b). Nutritive evaluation of some *Acacia* tree leaves from Kenya, Animal Feed Science and Technology, 85: 89-98.

Al-Soqeer, A.A. (2008). Nutritive value assessment of *Acacia* species using their chemical analyses and in vitro gas production technique, Journal of Agriculture and Biological Sciences, 4(6): 688-694.

A.O.A.C. (1990). Official Methods of Analysis of the Association of Official Analytical Chemists, 15th ed. Arlington, Virginia: AOAC.

Ben Salem, H.; Makkar, H.P.S. and Nefzaoui, A. (2004). Towards better utilization of non-conventional feed sourcesby sheep and goats in some African and Asian countries. In: Ben Salem, H.; Nefzaoui, A. and Morand-Fe, . (Eds.). Nutrition and Feeding Strategies of Sheep and Goats under Harsh Climates, 59 (Series A). INO Reproducciones, S.A., Zaragoza, pp. 177–190.

D'Mello, J. P. F. (1992). Chemical constraints to the use of tropical legumes in animal nutrition. Anim. Feed Sci. Technol., 38:237-261.

El Hage, M.G. (1985). Animal feed resource in the Sudan, potential supply and problems. Paper presented to Workshop on Live stock Policy Range and Feed Utilization Guideline for Drought-prone, African country. Khartoum,10-3 November, FAO.

Getachew, G.; Makkar, H.P.S. and Becker, K. (2001). Method of polyethylene glycol application to tannin-containing browses to improve microbial fermentation and efficiency of microbial protein synthesis from tannin-containing browses. Anim. Feed Sci. Technol., 92:51-57.

Haslam, E. (1979). Vegetable tannins. Recent Adv. Phytochem 12, 475 – 523.

Jones, D. E., 1965. Banana tannin and its reaction with polyethylene glycol. Nature. 205: 299 –300.

Jones, W. T. and Mangan, J. L. (1977). Complexes of the condensed tannins of sainfoin (Onobrychis viciifolia scop) with fractions 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. J. Sci. Food Agric., 28: 126–136.

Karabulut, A.; Canbolat, O.; Ozkan, C. O. and Kamalak, A. (2007). Determination of nutritive value of citrus tree leaves for sheep using in vitro gas production technique. Asian-Aust. J. Anim. Sci., 20:529-535.

Mahala, A.G. and FadelElseed, A.M.A. (2007). Chemical composition and in vitro gas production characteristics of six fodder trees leaves and seeds, Journal of Agriculture and Biological Sciences, (3): 938- 986.

Makkar, H.P.S.; Blümmel, M. and Becker, K. (1995). Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implicationin gas production and true digestibility in vitro techniques. Br. J. Nut., 73: 897–913.

Makkar, H. P. S. and Becker, K. (1998). Do tannins in leaves of trees and shrubs from Africa and Himalayan regions differ in level and activity? Agroforestry Syst., 40: 59– 68.

Menke, H. H. and Steingass, H. (1988)Estimation of the Energetic Feed ValueObtained from Chemical Analysis and inVitro Gas Production Using Rumen Fluid.Anim. Res. Dev., 28: 7-55.

Ministry of Animal Resources and Fisher (2008). Department of Statistic and Information, Khartoum- Sudan.

Osuji, P.O. and Odenyo A.A. (1997). The role of legume forages as supplements to low quality roughages-ILRI experience. Anim. Feed Sci. Tech., ,69:27-38.

Steel, R.G. and Torrie, J.H. (1980). Principles and procedures of Statistic. A Biometrical Approach, 2nd Edition. McGraw-Hill Book.

Van Soest, P.J. (1967). Development of a comprehensive system of feed analyses and its application to forages. Journal of Animal Science, 26: 119-128.