Egyptian Journal of Sheep & Goat Sciences, Vol. 13, No. 1, P: 61-78, April 2018

Nutritive evaluation of foliage combinations from some fodder trees and shrubs

Abo-Donia, F. M.; Hanim, A. El-Sheikh; Amal M. A. Fayed; M.H. Abo El-fadel and M.M. Eissa
Animal Production Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.

ABSTRACT

Six combinations of the tree foliage leaves, *Cassava* (CL), *Acacia Saligna* (ASL) and *Prosopis juliflora* (PL) were tested, CL (100%), ASL (100%), PL (100%), CL plus ASL (50:50), CL plus PL (50:50) and CL plus ASL plus PL (50:25:25), respectively. Then these six combinations were mixed with ammoniated rice straw (ARS) (50: 50) on dry matter basis. Protein content in tested plants was higher in CL (22.22%) compared to ASL (16.67%) and PL (17.02%), while protein content in mixtures of tree foliage leaves have close protein contents. The content of NDF and ADF in CL was lower than the rest of plants tested and their mixtures. The lowest amounts of potential-GP and potential-CH_4 were shown with diets containing CL (T1) and ASL (T2) with ammoniated rice straw, while mixing the leaves of CL with ASL and/or PL raised the quantities of GP and CH_4 released. Combination of foliage leaves with ammoniated rice straw in T4, T5 and T6 diets significantly ($P<0.05$) improved DM, OM and NDF degradability compared to T1, T2 and T3 diets containing tested leaves separately with ARS. The mixture of PL with ARS in T3 and T6 significantly ($P<0.05$) increased benefit of metabolizable energy (ME) compared to the CL or ASL with ARS in T1, T2, T4 and T5 diets. There was a positive correlation ($r = 0.66$, $P<0.05$) and ($r = 0.60$, $P<0.05$) between *in vitro* neutral detergent fiber degradability (*IVNDFD*) and both microbial protein (MP) and CH_4, respectively. Also, positive correlation ($r = 0.76$, $P<0.05$) and ($r = 0.65$ $P<0.05$) where shown between MP and both GP and CH_4, respectively. The decrease of CT concentration increased NH_3-N, SCFA's, MP, and bacteria count and vice versa. There was no effect of different treatments on pH, protozoa count, propionic, A/P and Butyric. Mixture of separate foliage leaves with ARS in tested diet significantly ($P<0.05$) increased the amount of refused feed, especially for T1 and T2 diets. In contrast, the amount of feed refusal declined when combining different species of foliage leaves together.

The presence of CL and PL separate in tested diets (T1 and T3) or combined into the tested diets (T5) improved digestion of DM, OM, NDF, and ADF% compared to ASL. No significant differences found in protein digestion among tested diets. The results of the N balance showed a significant ($P<0.05$) increase in T3, T4 and T5 compared to other tested diets (T1, T2 and T6).

In conclusion, using foliage leaves rich in tannins to form of feed mixtures could help overcoming the negative effect of CT and reduce CH_4 emitted.

KEYWORDS: Foliage leaves, secondary metabolite, *in vitro* fermentation, *in vivo* digestibility

INTRODUCTION

Trees and shrubs such as *Cassava* (CL), *Acacia Saligna* (ASL) and *Prosopes* (PL) survive under harsh climatic and has been suggested as a solution to feeding animals. Although tree leaves have a high protein content, their contents of tannins and other secondary compounds may have negative effects on palatability, digestibility and may are also poisonous (Abo-Donia and Nagpal 2015 and Salem *et al*., 2011 and 2014).

Tannins are polyphenolic compounds which bind to protein thus rendering it to be unavailable to the animal thus can be used for protecting and decreasing ruminal fermentation of proteins for ruminant (Makkar, 2003). Tannins from different plants might show different response in digestibility, gas production (GP) and methane production (Makkar, 2003; Guglielmelli *et al*, 2011).

Interestingly, there are numerous reports shown the reduction of enteric methane, which is a greenhouse gas that causes significant loses of energy in ruminants, due to inclusion of tannin rich browsed plants because the tannins have anti-methanogenic activity, either by direct

inhibition of methanogens or indirectly through inhibition of protozoa (Animut *et al*., 2008; Hristov *et al.*, 2013). So, when targeting methane reduction, the nutritional effects of these compounds must fully understood to avoid any limitation for rumen function.

This experiment aimed to investigate whether mixing together of foliage leaves without or with roughage will lead to an improvement in utilization of these foliage leaves and thus effect on *in vitro* rumen GP and fermentation kinetics.

MATERIAL AND METHODS

Experimental diets

Cassava (CL), Prosopis *juliflora* (PL) and *Acacia Saligna* (ASL) were mown 5 cm above ground level using a precision chop foliage harvester within 30 min at 10:00 am, then plants sundried. After which, the leaves were separated from wooden branches which manually picked out. Finally, leaves and twigs, as non-lignified stems, were collected. After CL, PL and ASL sun-dried they chopped into small pieces (5 mm) to prevent selection. Six combinations of foliage were tried as following, CL (100%), ASL (100%), PL (100%) and CL plus ASL (50:50), CL plus PL (50:50) and CL plus PL plus ASL (50:25:25). Then these six combinations were mixed with the ammoniated rice straw (50: 50) based on dry matter.

Chemical composition

Samples of each ingredient, each combine and feces were dried in an oven with force air circulation at 65°C for 48 hrs. Another samples collected fresh of each green fodder and feces were taken directly to estimate protein. Dried samples milled through a 1-mm sieve prior to chemical analysis. Milled samples assayed for ash concentration following complete combustion in a muffle furnace at 550°C for 5 hrs. The content of N in feed, feces and acidified urine were analyzed according to the procedures of AOAC (1990, method no. 968.06). The crude protein (CP) content calculated as 6.25× the N content. Fiber fractions as neutral detergent fiber (NDF) and acid detergent fiber (ADF) determined by the method of (Van Soest *et al.*, 1991). Condensed tannins (CT) were determined according to description of Harinder *et al*., (1993). Individual short chain fatty acids (SCFA's) in rumen fluid determined by gas chromatography (Isac *et al.*, 1994). The ammonia- N concentration in pre-incubation fermentation medium, buffer mixture, and post-incubated fluid samples were determined by the phenol-hypochlorite method using spectrophotometric determination according to Broderick and Kang (1980). The total phenolic content of leaves determined by Folin Ciocalteu method as described by Siddhuraju and Becker (2003). The total flavonoid content determined by the method described previously (Zhishen *et al*. ,1999). Free radical scavenging activity on α, α-diphenyl-β-picrylhydrazyl (DPPH; $C_{18}H_{12}N_5O_6$) was measured according to the method of Blios (1958). DPPH assay is based on the measurement of the scavenging capacity of antioxidants towards it.

In vitro gas production technique and sampling procedure

The *in vitro* rumen gas and methane production of foliage was measured using a modified version of the *in vitro* gas production technique of Navarro-Villa *et al.* (2012). Briefly, 0.6 g foliage samples were transferred into a 100 mL glass bottle containing 60 ml of diluted rumen fluid. All the bottles closed with rubber stoppers, crimped with aluminium seals, shaken and placed in the incubator at 39°C. Two fistulated rams, used as rumen fluid inoculum donor, were fed on berseem hay and concentrate feed mixture (CFM 14% CP) at ratio 70:30 (DM basis) for two weeks prior experiment. Rumen fluid collected prior morning feeding from each donor sheep and immediately moved to the laboratory in a pre-warmed (39°C) vacuum flask, composited, strained through 4 layers of cheesecloth, and mixed with buffer (McDougall, 1948) at ratio 1:4 (rumen fluid to buffer, v/v), and this mixture was flushed with a continuous stream of CO_2 until its pH stabilized at 6.85 which measured by a portable pH meter (HNNA pH meter, model HI8424).

Six bottles per treatment were used, three of them to estimate the gas production (GP) and DM and OM degradability and three others to

measure the extent of NDF degradability. Three bottles contained the buffer and rumen fluid mixture was included in each run as blank. All fermentation bottles were flushed with CO_2, sealed and placed into a semi-automatic shaking incubator at 39°C for 96 h. During the anaerobic fermentation, the gas production measured intermittently throughout the incubation using the Reading Pressure Technique (RPT) (Hangzhou Runchen Electron Com., Hangzhou, China). Headspace gas pressure measured at 3, 6, 12, 24, 36, 48, 60, 72 and 96 hrs. Gas production was calculated by the following equation:

$$GV = 7.365 \times p \quad (n = 500;\ r^2 = 0.99;\ \text{data not reported})$$

where: GV - gas volume (ml); p - measured pressure (psi).

A hole made in the lid of each fermentation bottle, then inter-connected with a plastic tube with a three-way valve. After reading, at each time, 10 ml of total gas withdrawn by syringe with 2 mL NaOH (10 M) which introduced to estimate methane concentration following the method by Fievez, *et al.* (2005). Data were obtained included volume of gas and methane (CH_4) produced. Net methane and gas production were calculated by the differences of methane and gas from the corresponding blank; the methane concentration was determined as Jayanegara, *et al* (2009).

At the end of incubation, content of each bottle filtrated by Quartz Fiber Crucibles, 44mm diameter, 20ml Capacity (100/PK) in glass cone and then the bottle washed with distilled water many times. A 10 mL of *in vitro* fermentation liquor collected to determine SCFA's and ammonia-N concentration. The residue of substrates in crucibles dried at 65°C for 48 hr to calculate apparent DM disappearance which determined by subtracting sample weight from the difference between residue and blank.

Kinetic parameters of gas production (i.e., GP) estimated by fitting GP results (mL/g OM) in the nonlinear regression models (NLIN), option of SAS (2008), according to the model described by France *et al*. (2000):

$$\mathbf{GP = b \times (1 - e^{-c(t-L)})}$$

where: GP is the volume of GP at time t; b is the asymptotic GP (mL/g OM); c is the rate of GP (ml/h) from the slowly fermentable feed fraction b; and L is the lag time prior to GP.

The ratio of organic matter, truly degraded (mg) to gas volume (ml) at 24 hrs incubation, was used as an index of microbial synthesis efficiency (Blümmel *et al*., 1997).

The metabolizable energy (ME; MJ /kg DM) of samples was calculated using equation of Menke *et al.* (1979) as follows:

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136GP + 0.057CP \quad (R2 = 0.94)$$

where, GP is 24 h net gas production (ml/600 mg) and after correction for the day-to-day variation in the activity of rumen liquor using the Hohenheim standard, CP is crude protein (%).

Digestibility and nitrogen balance trial

Digestibility trial conducted using eighteen Barki males, aged 2 to 3 years, average live body weight (LBW) 43±1.85 kg, which kept individually in metabolism cages. They randomly assigned to the six dietary treatments. The digestibility trial lasted 22 days, the first 15 days out metabolism cages as a preliminary period, followed by a 7 days collection period in metabolism cages. The animals weighed at the beginning and end of the metabolism trial.

During the metabolism trials, tested diets and drinking water offered ad-libitum. Daily feed intake, feces and urine recorded every morning. Feces and urine quantitatively collected from each animal. The dry matter (DM) of feed and feces determined by drying to a constant weight in a forced air oven at 55°C then ground to pass a 1 mm screen and preserved for chemical analysis. Nitrogen balance, NB (g/h/d) values were mathematically calculated by subtracting (fecal N + urine N) values from total N intake values.

Statistical methods

Data of feed intake, digestibility, *in vitro* gas production kinetics, OMD and ME contents of samples were subjected to the random completely design analysis using General Linear Model (GLM) of SAS (2002). Statistical model applied for analysis was:

$$Y^{ijk} = \mu + S_i + eijk$$

where Y^{ijk} represents the general observation of chemical composition, *in vitro* gas production kinetics, OMD and ME contents, μ is the expected score, S_i is the subject i' s effect of tested treatments on the observed parameters and e^{ijk} the standard error term common for all observations. Significant differences among individual means were identified using the Duncan's New Multiple Range Test (1955). Mean differences were considered significant at ($P < 0.05$).

RESULTS AND DISCUSSION

The composition of foliage leaves offered to sheep are summarized in Table (1). The protein content was higher in CL (22.22%) compared to ASL (16.67%) and PL (17.02%), while protein contents of foliage leaves mixtures were close. The chemical composition of ASL is consistent with what has been reported by Sawe *et al.,* (1998) while higher than the value reported by Ahmed (2007) and Mousa (2011). Crude protein (CP) contents of CL ranged from 177 to 240 g/kg dry matter (DM) (Oni *et al.*, 2010). The concentration of protein in PL was in the range shown by various authors (14 - 26%) (Vimal *et al.*, 1986). The same trend was also seen with EE content. On the other hand, the NDF and ADF contents in CL were lower compared to the rest of plants and their mixtures. The NDF and ADF contents of A. *saligna* are in agreement with the values reported by Shumuye and Yayneshet (2011). However, NDF and ADF contents of CL appeared to be less than values estimated by Oni *et al*. (2010) which ranged from 596 to 662 and 418 to 546 g/kg DM, respectively.

The analysis showed higher CT content in ASL compared to CL, while PL had the least content. Content of CT in ASL in this study is lower than values reported by Abdel-Fattah (2005) which was 63 to 113 g/kg DM in summer and winter seasons, respectively. The estimates of CT are almost within the beneficial category of 20 to 40 g/kg DM reported by Thi *et al.* (2005). While CT value of CL is within the range from 1.0 to 3.8 g/kg determined by Oni *et al.* (2010).

Table (1): Chemical composition of the ingredients and tested combinations (% on DM basis).

Treatments	DM	OM	CP	EE	Ash	NDF	ADF	CT (mg/g)
CL	88.76	90.36	22.22	4.24	9.64	49.14	33.28	37.58
ASL	88.65	90.65	16.67	2.95	9.35	55.23	35.58	41.03
PL	89.52	90.57	17.02	3.24	9.43	52.14	34.87	20.32
CL and ASL	88.71	90.51	19.45	3.60	9.49	52.19	34.43	39.31
CL and PL	89.14	90.47	19.62	3.74	9.53	50.64	34.08	28.95
CL and ASL and PL	88.92	90.49	19.53	3.67	9.51	51.41	34.25	34.13
ARS	90.73	83.90	3.09	1.40	16.10	64.02	40.01	nd*
Chemical composition of the experimental diets (foliage+ ARS) (% on DM basis)								
T1	89.75	87.13	12.66	2.82	12.87	56.58	36.65	18.79
T2	89.69	87.28	9.88	2.18	12.72	59.63	37.80	20.52
T3	90.13	87.24	10.06	2.32	12.76	58.08	37.44	10.16
T4	89.72	87.20	11.27	2.50	12.80	58.10	37.22	19.65
T5	89.94	87.18	11.36	2.57	12.82	57.33	37.04	14.48
T6	89.83	87.19	11.31	2.53	12.81	57.72	37.13	17.06

* = not detective

CL= Cassava leaves, ASL= Acacia Saligna leaves, PL= *Prosopis juliflora* leaves, CL&ASL= Cassava: Acacia Saligna leaves, CL&PL= Cassava: *Prosopis juliflora* leaves, CL+ASL+PL= Cassava: Acacia Saligna leaves: *Prosopis juliflora* leaves, ARS, ammoniated rice straw.

T1: CL + ARS (50:50), **T2:** ASL+ ARS (50:50), **T3:** PL+ ARS (50:50), **T4:** CL+ASL + ARS (25:25:50), **T5:** CL+PL+ARS (25:25:50), **T6:** CL+ASL+PL + ARS (25:12.5:12.5:50).

The contents of OM, EE and Ash in all experimental diets had minor differences. The content of NDF was higher in T2, while ADF was lower in T1 compared to other diets but the differences were minor.

All diets had reasonable levels of crude protein which ranged from 9.88% (T2) to 12.66% (T1) of DM. All experimental diets also had CP content above the minimum microbial requirement (7%) to support acceptable ruminal microbial activity and the maintenance requirement of CP for the host ruminant (McDonald *et al*., 2002).

The quantitative phytochemicals analysis of the foliage leaves and their combinations are shown in Table (2). The total Phenol concentration ranged from 65.90 to 76.33 GAE mg/g DM. The total flavonoid concentration in the combinations ranged from 14.17 to 25.24 mg/g DM while anti-oxidant activity concentration (DPPH scavenging activity) ranged from 50.31 to 61.30 %. These phytochemicals are known to have antimicrobial activity (Ebana *et al.,* 2009) and free radical scavengers which prevent oxidative cell damage (Salah *et al*., 2002; Okeke and Elekwa, 2003) which may improve animal productivity.

Data of i*n vitro* cumulative gas production (GP) and methane (CH_4) released for the tested diets were approximately comparable at different incubation times as illustrated in Fig. (1 and 2) except T6 which showed higher methane release. Potential total gas (P-GP) and methane production (P-CH_4) from *in vitro* fermentation by exponential equation of different tested diets substrates presented in Table (3).

Table (2): Quantitative phytochemical analyses of the tested plants.

Item	Total Phenol*	Total Flavonoid**	Anti-oxidant activity***
CL	65.90	14.17	50.31
ASL	75.23	21.32	58.45
PL	76.33	25.24	61.30
CL and ASL	75.37	16.38	57.32
CL and PL	69.27	21.71	56.82
CL and ASL and PL	71.50	20.72	56.09

* Total Phenol as Gallic acid equivalent (GAE mg/g DM)
**Total Flavonoid content (mg/g DM)
***Anti-oxidant activity (% of DPPH scavenging activity)

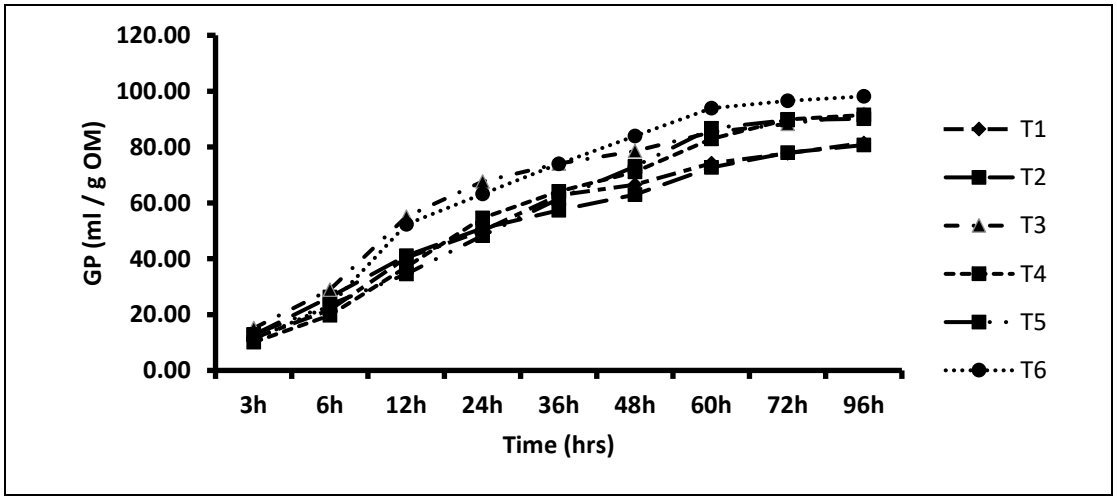

Fig. (1): Influence of using some foliage leaves on cumulative gas production at different incubation times in sheep rumen fluid.

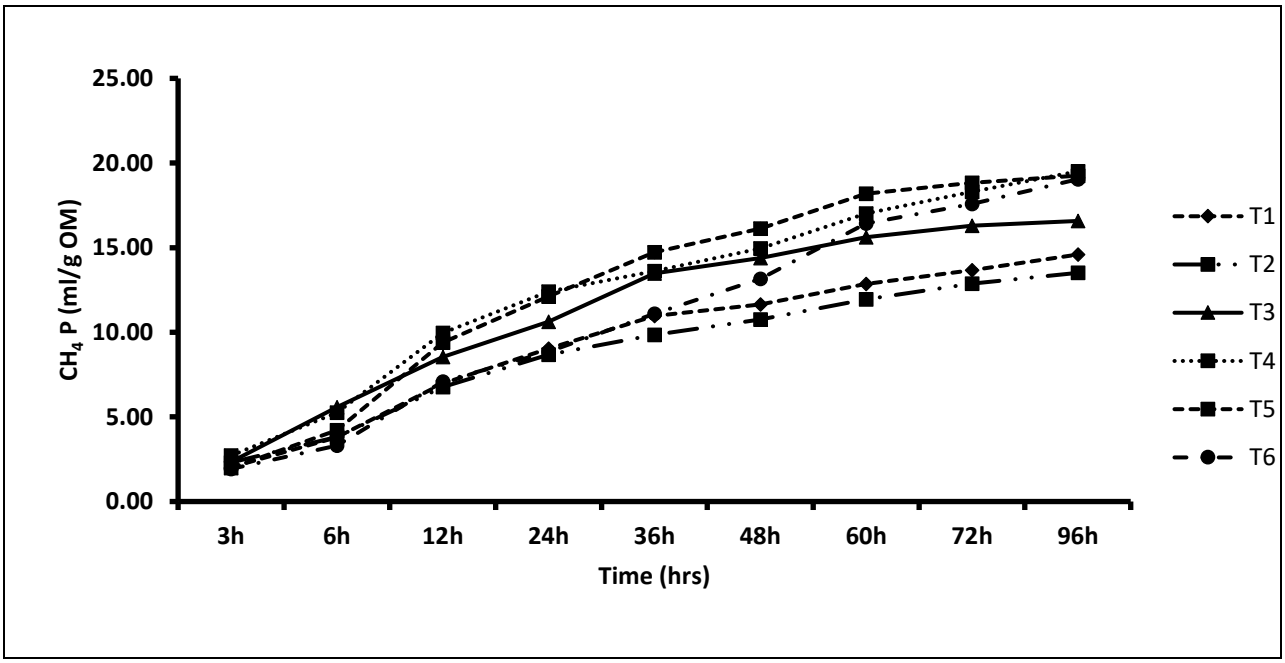

Fig. (2): Influence of using some foliage leaves on cumulative CH_4 production at different incubation times in sheep rumen fluid.

The effects of CT concentration on CH_4 reduction had reported in several studies (Abo-Donia and Nagpal 2015, Animut *et al*., 2008 and Tan *et al*., 2011). On contrary, some researchers reported that tannins did not show any effect on methanogenesis or even enhanced CH_4 production in sheep (Sliwinski *et al.*, 2002).

In this study, the lowest amounts of P-GP and P-CH_4 were shown with diet T1 and T2 when used as fermentation substrates, while mixing the leaves of CL with ASL or PL leaves or their combinations with ammoniated rice straw raised the quantities of total gas and methane emitted. These results due to the presence of the condensed tannins (CT) where Fig (3) shows the effect of tannins consumed on the production of gas and methane. This agree with Hatew *et al.,* (2015) who reported that increasing level of CT (0, 40, 80 and 120 g CT/kg of substrate DM) linearly reduced the maximum rates of GP and CH_4 production, and the estimated *in vitro* organic matter digestibility. The higher rate of gas production observed with diets containing leaves of PL compared with ASL or CL which contain a relatively high concentration of CT (Table 3).

Some authors pointed out multiple hypotheses for how CT inhibits methanogenesis, none of which have been definitely proven. One hypothesis is that CT act as a hydrogen sink (Naumann *et al*., 2013a). Another hypothesis is that CT act directly upon methanogens in the rumen (Ng *et al*., 2016). A third hypothesis is that indirect inhibition occurs by decreasing the availability of nutrients to rumen microorganisms, subsequently substrate digestibility reduces which indirectly inhibit rumen microbial populations. Because CT bind to; minerals (Lavin, 2012), organic molecules such as proteins (Saminathan *et al*., 2014), carbohydrates (Soares *et al*., 2012a), or lipids (Delehanty *et al*., 2007), it is possible that CT bind to microbial enzymes modulate their activity (Gonçalves *et al*., 2011). However, Naumann *et al.* (2013c) demonstrated a weak relationship between protein bounded to CT and the decrease in CH_4.

Mixture of the tree foliage leaves with ammoniated rice straw (ARS) (T4, T5 and T6) showed significant ($P<0.05$) improve of DM, OM and NDF degradability compared to separate trees' leaves mixed with ARS (T1, T2 and T3). The incorporation of ARS with PL significantly ($P<0.05$) increased benefit of metabolizable energy (ME) compared to incorporation with CL or ASL. These results supported by the study of Huang *et al.,* (2010) who showed that lower levels of pure CTs, of

20–40 mg g^{-1} DM, could significantly reduce CH_4 production with no significant adverse effects on DM degradability. Accordingly, there were negative relationships between tannin contents of the leaves and *IVDMD*. These results are similar to those reported by Frutos *et.al* (2002) and Seresinhe and Iben (2003). Abo-Donia and Nagpal (2015) and Tan *et al.* (2011) referred the decrease in CH_4 production to that the high CT concentrations associate with reduction in DM degradability. Therefore, in foliage leaves, tannins present in significant amounts in the NDF and ADF fractions, and the binding of the proteins to the cell wall seem to be a factor in decreasing digestibility (Reed *et al* 1990).

Table (3): Prediction of total gas and methane production and degradabilities of DM, OM and NDF% and ME (MJ/kg DM).

Treat.	P-GP (ml/g OM)	GP-R (ml/h)	P-CH_4 (ml/g OM)	CH_4-R (ml/h)	P-CH_4/ P-GP	IVDMD (%)	IVOMD (%)	IVNDFD (%)	ME (MJ/kg DM)
T1	75.56^{b}	0.039^{b}	13.37^{c}	0.039^{a}	0.176^{ab}	21.74^{c}	24.06^{c}	22.56^{ab}	9.71^{b}
T2	72.32^{b}	0.034^{bc}	12.04^{c}	0.037^{a}	0.169^{b}	20.90^{c}	23.96^{c}	21.35^{b}	9.69^{b}
T3	84.75^{ab}	0.063^{a}	15.37^{bc}	0.044^{a}	0.182^{ab}	22.87^{bc}	24.92^{bc}	23.62^{ab}	11.97^{a}
T4	92.38^{a}	0.031^{bc}	17.87^{ab}	0.037^{a}	0.193^{ab}	24.33^{ab}	25.21^{bc}	24.77^{ab}	10.26^{b}
T5	94.14^{a}	0.026^{c}	19.24^{ab}	0.042^{a}	0.205^{ab}	25.07^{a}	26.05^{ab}	25.36^{ab}	9.41^{b}
T6	96.38^{a}	0.045^{b}	21.93^{a}	0.018^{b}	0.228^{a}	26.09^{a}	27.08^{a}	26.62^{a}	11.44^{a}
±SE	4.018	0.004	1.275	0.004	0.016	0.989	0.533	1.271	0.265

P-GP= Potential Gas production., **GP-R=** Gas production rate., **P-CH_4=** Methane production efficiency., **CH_4-R=** Methane production rate., **P-CH_4/P-GP** = methane production/ Gas production, **ME (MJ/kg DM)** = Metabolizable energy.

T1: CL + ARS (50:50), **T2:** ASL+ ARS (50:50), **T3:** PL+ ARS (50:50), **T4:** CL+ASL + ARS (25:25:50), **T5:** CL+PL+ARS (25:25:50), **T6:** CL+ASL+PL + ARS (25:12.5:12.5:50).

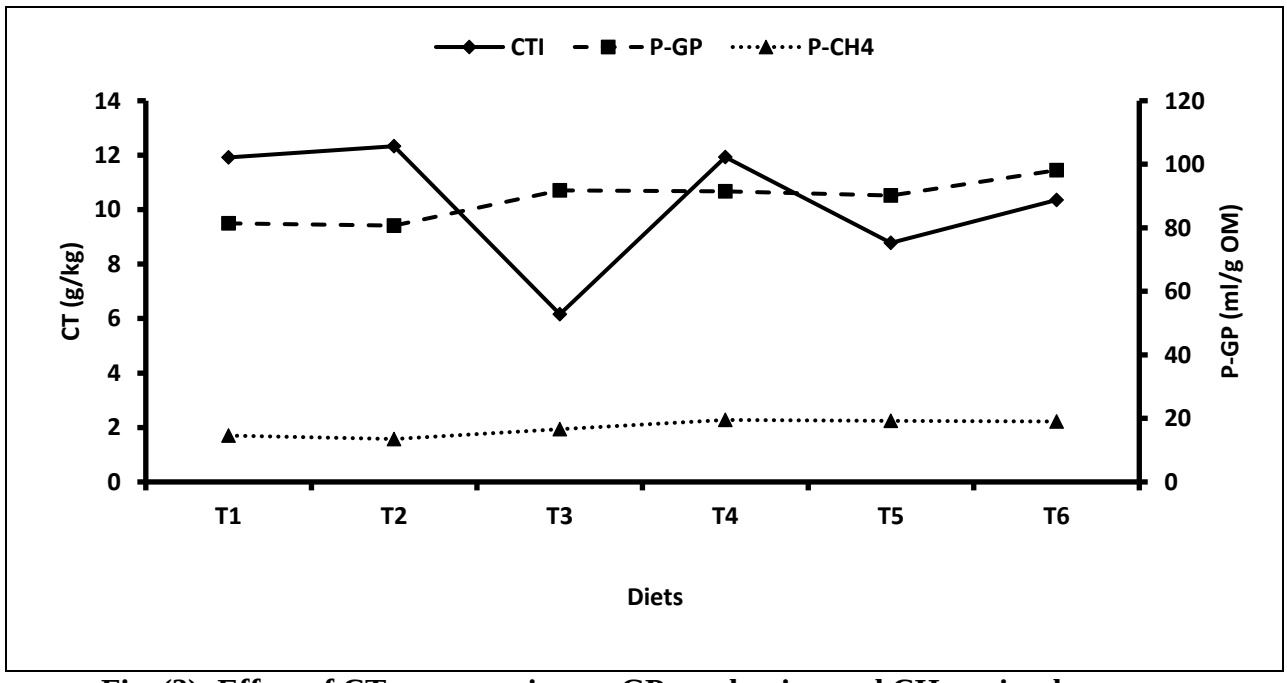

Fig. (3): Effect of CT consumption on GP production and CH_4 emitted

As seen in Table (4), there was a positive correlation (r = 0.66, P < 0.05) and (r = 0.60, P < 0.05) between *in vitro* neutral detergent fiber degradability (*IVNDFD*) and both microbial protein (MP) and CH_4, respectively. Also, positive correlation (r = 0.76, P < 0.05) and (r = 0.65 P < 0.05) where shown between MP and both GP and CH_4, respectively. Reasonable correlations were also observed (r = 0.48, P < 0.05) between *in vitro* neutral detergent fiber degradability (IVNDFD) and GP. Negative correlations were also observed between CT intake of the foliage leaves and total gas production (r = -0.20, P = 0.422). Naumann *et al*. (2013b, c) indicated that CT and CH_4 production were negatively correlated (r2 = 0.44). There was a significant (P<0.05) and negative correlation for CT with GP. Abo-Donia and Nagpal (2015) and Tan *et al.* (2011) also reported a linear reduction in gas production with increasing CT levels and when they included CT from *L. leucocephala*, gas production was reduced up to 43%. In a similar way, CH_4 production was decreased by 63%. In addition, Hariadi and Santos (2010) reported a strong negative relationship (r = −0.600) between total tannins and *in vitro* CH_4 production after 24 hrs. incubation. A reduction in total gas production, including CH_4, could due to a decrease in DM degradability which may attributed to the inclusion of foliage leaves, containing CT, in the tested feed combinations offered to animals. This result is consistent with Gemeda and Hassen (2015). Significant (P<0.05) positive correlations were observed between *IVNDFD* and both CH_4 and MP.

Table (4): Correlation analysis of relationship between CT intakes versus *in-vitro* NDF degradability, SCFA's, MP, GP, CH_4 production for diets containing foliage leaves.

Item	CT	IVNDFD	SCFA' s	MP	CH_4
IVNDFD	0.019^{ns}				
SCFA's	0.020 ^{ns}	0.245 ^{ns}			
MP	0.070 ^{ns}	0.655 *	0.346 ^{ns}		
CH4	0.169 ^{ns}	0.602*	0.591*	0.756*	
GP	-0.202 ^{ns}	0.479*	0.415^{ns}	0.654*	0.648*

CT= condensed tannins **IVNDFD=** In-vitro NDF degradability SCFA's= Short chin of fatty acids MP= Microbial protein **CH_4=** Methane production **GP=** Gas production

The pH, NH3-N, SCFA's profile, microbial protein and Bacterial and protozoal counts obtained from sampling times are given in Table 5. The decrease of CT concentration increased NH3-N, SCFA's, MP, and bacteria count and vice versa. Fermentation and VFA production by microbes in the rumen influence the production of CH_4 (Tan *et al*., 2011). In addition, Naumann *et al.* (2017) reported that CT and SCFA's were negatively correlated (R2 = 0.52). Hassanat and Benchaar (2013) also reported a decrease of *in vitro* SCFA's concentration when CT level increased from 20 to 200 g/kg of DM. For ruminal NH_3-N concentration, tannins may increase the efficiency of urea recycled to the rumen. Thus, there is a shortage in the rate of protein degradation and deamination in the rumen, therefore ruminal NH_3-N decreased (Woodward and Reed., 1989).

In this study, there was no effect of different treatments on pH, protozoa count, propionic, A/P and Butyric. In ruminants, Beauchemin *et al.* (2007) reported a linear decrease in acetic acid concentration and A/P ratio with supplement of 20 g kg−1 DM quebracho tannin extract. The decrease in the A/P ratio resulted from increased use of hydrogen for propionate formation may related to the inhibition of CH_4 production by CT (Naumann *et al.*, 2017). However, Gunun *et al.* (2014) reported that protozoal population dramatically decreased with *in vitro* supplementation of CT. Bhatta *et al.* (2014)

determined that rumen ciliated protozoa populations decreased when the feedstuff contained CT of *Ficus bengalensis* and *Azardirachta indica* at concentrations 26 and 13.8%, respectively. In contrast, Animut *et al.* (2008) reported that CT in Kobe lespedeza was not responsible of the antiprotozoal activity in goat. Concerning the inconsistent effect of CT on ruminal protozoa, Patra and Saxena (2009) suggested that effect of CT on rumen protozoa are variable and mostly depend on the type of CT, their origin and supplementation levels. Min *et al*. (2002) reported a decrease of 0.5-0.1 log in proteolytic ruminal bacteria *Clostridium proteoclasticum*, *S. bovis*, *Eubacterium* sp and *B. fibrisolvenes* when CT from *Lotus corniculatus* (32 g CT/kg DM) were fed to sheep.

Table (5): Effect of tested diets on pH, NH_3-N and SCFA's, MP and Bacteria count *in vitro* incubation (at 96 hrs) of sheep rumen fluid.

Item	T1	T2	T3	T4	T5	T6	±SE
pH	6.72	6.87	6.69	6.8	6.71	6.82	0.138
NH_3-N (mg/dl)	12.69^{cd}	7.19^{d}	23.71^{ab}	19.41^{bc}	28.19^{a}	14.56^{c}	2.185
SCFA's (meq/dl)	$7.55b^{c}$	7.05^{c}	8.53^{ab}	8.59^{ab}	8.98^{a}	7.70^{bc}	0.333
MP (mg/dl)	36.59^{d}	34.13^{e}	38.08^{cd}	40.03^{bc}	40.81^{ab}	43.03^{a}	0.764
Bacteria count, (x 10^5 /ml)	7.56^{b}	7.42^{b}	7.68^{b}	8.68^{ab}	9.03^{a}	8.30^{ab}	0.402
Protozoa count, (x 10^3 /ml)	4.88	4.07	4.94	5.48	5.54	5.23	0.582
SCFA's fractionation (%)							
Acetic (A)	53.91^{c}	53.73^{c}	54.84^{bc}	56.48^{b}	58.74^{a}	58.40^{a}	0.624
Propionic (P)	23.57	23.67	23.50	24.93	23.95	24.66	0.781
A/P	2.29	2.27	2.33	2.28	2.46	2.37	0.076
Butyric	11.52	11.44	11.71	12.06	12.09	12.10	0.212

a, b and c Means in the same column with different superscript are significantly different (P<0.05).
NH_3-N= Ammonia nitrogen, **SCFA's=** Short chain fatty acids and MP= Microbial protein.
T1: CL + ARS (50:50), **T2:** ASL+ ARS (50:50), **T3:** PL+ ARS (50:50), **T4:** CL+ASL + ARS (25:25:50), **T5:** CL+PL+ARS (25:25:50), **T6:** CL+ASL+PL + ARS (25:12.5:12.5:50).

Voluntary feed intake and digestibility of the tested diets

Results in Table (6) showed that using foliage leaves separately with ammoniated rice straw significantly (P<0.05) increased the amount of feed refusal, especially for T1 and T2 diets. On the other hand, the amount of feed refusal declined when combining different species of foliage leaves together. Thus, the actual amount of feed consumed decreased significantly (P<0.05) when foliage leaves were separately incorporated in the tested diets (T1, T2 and T3). Waghorn *et a*l., (1994a) showed that consumption of plant species with medium or low CT (< 50 g kg-1 DM) seems not affecting voluntary feed intake, while, high CT contents (generally > 50 g kg-1 DM) significantly reduced it. Also, Pathak *et al.*, (2016) reported that intake of DM and OM (g day $^{-1}$ and % a live weight) were statistically similar between groups of sheep fed diets supplemented with 0 and 1.5 % of CT, respectively. In addition, camel and sheep are more resistance to tannin rich feed than other livestock. This idea supported by Abdel-Fattah (2005) who reported that it possible to use sheep as models for cattle to characterize tanniniferous feeds. In this study, the change in the amount of feed intake and refused feed was due to increasing content of tannins in some foliage leaves. The consumption of CT intake (g/h /d) which corresponds to the same trend of feed refusal and the actual consumed feed of the tested diets are illustrated in Fig. (4).

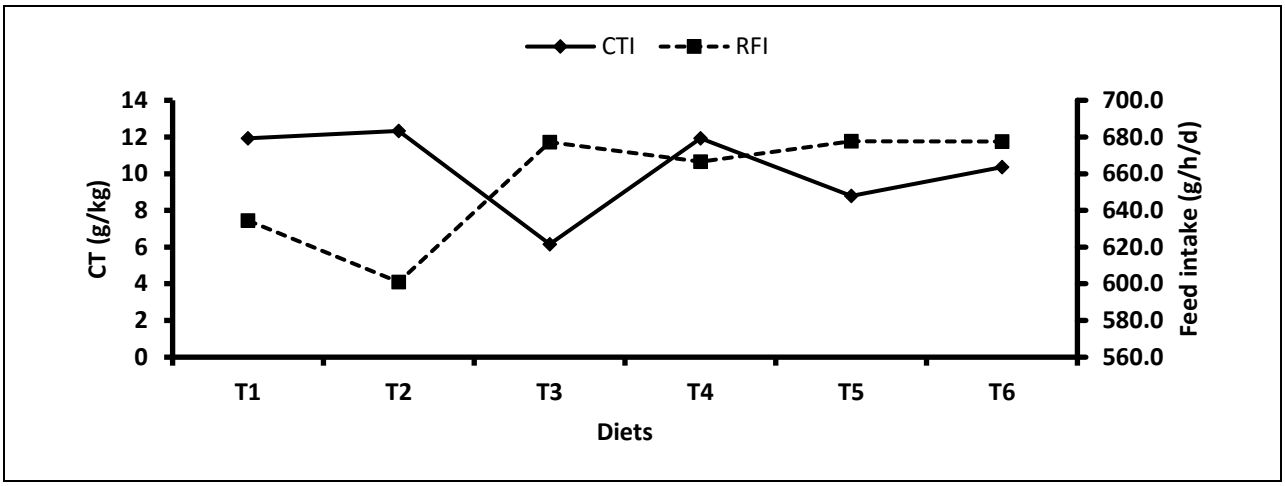

Fig. (4): Effect of CT consumption on feed intake

The results of the digestion trials (Table 6) showed that the diet containing PL was significantly superior compared to other diets. The presence of CL and PL separately in tested diets (T1 and T3) or the incorporation of foliage leaves of different species into the tested diets improved digestion of DM, OM, NDF, and ADF% compared with ASL diet. No significant differences found in the protein digestion among tested diets.

This might firstly due to that CT considerably reduced the proteolytic enzyme activity and growth of bacteria in rumen of sheep (Jones *et al.,* 1993). Secondly, this may due to that condensed tannin-protein complexes are formed at the pH level of rumen which increase the amount of available protein in the small intestine that known as ruminal by-pass protein (Min *et al*., 2003), while, dissociated at pH either lower (2.5-3.5) in the intestines (Jones and Mangan, 1977) or greater than 8 (for example in the duodenum, pH 8) (Hagerman *et al.,* 1992). These protein molecules undergo enzymatic hydrolysis in the small intestine leading to availability of enormous number of amino acids which absorbed from the intestine of sheep (McNabb *et al*., 1998). However, higher tannin levels (above 50 g/kg DM) in plant material can become an anti-nutritional factor and can result in reduced feed intake and digestibility (Barry and McNabb, 1999) or growth rate and milk quality and quantity in animals (Waghorn *et al*., 1994a).

Therefore, the beneficial effects of tannins in sheep are associated with the greater outflow and absorption of amino acids especially for sheep fed forages containing tannin percentage range from 2-4% (Min *et al*., 1999).

Concerning fiber digestion, several studies showed that fiber degradation in rumen can drastically reduce in animals consume tannin-rich feeds (McSweeney *et al.,* 2001). Whereas, CT could reduce fiber digestion by complexing with lignocelluloses, just like that suppressing microbial digestion (Auwal *et al*., 2014) or reducing the number of cellulolytic microorganisms (Kavitha *et al.,* 2013) and/or activities of fibro-lytic enzymes (Bae *et al.*,1993). Thus, it could suggest that intake of under 50 g CT kg-1 DM (10 - 40 g kg-1 DM) improves the digestive utilization of feed by ruminants (Barry and McNabb, 1999; Min et a*l*., 2003).

Feeding diets containing combination of foliage leaves or separate CL or PL with ARS were more nitrogen consuming than that in second diet (T2) containing ASL. Nitrogen release take the same trend of food consumption, where output of nitrogen was significantly ($P<0.05$) lower with T2 (containing ASL) compared to cassava diets (T1) and diet containing the mixture of leaves (T4, T5 and T6). The results of N balance showed significant ($P<0.05$) increase in T3, T4 and T5 compared to other tested diets. These findings supported by those reported by Grainger *et al.* (2009) that when 0.86 and 1.4% CT consumed (143 and 266 g/day) the level of

Table (6): Mean nutrient intake, apparent digestibility coefficients, nutritive value and nitrogen balance of experimental ration offered to sheep.

Item	T1	T2	T3	T4	T5	T6	±SE
Feed Intake							
Offer feed (kg/h/d)	773	734	799	775	784.67	773.33	38.14
Refusal feed (kg/h/d)	138.90^{a}	132.93^{a}	121.97^{ab}	108.73^{bc}	107.07^{bc}	96.10^{c}	5.56
Refusal feed %	17.97^{a}	18.11^{a}	15.27^{b}	14.03^{bc}	13.65^{bc}	12.43^{c}	0.814
Real Feed Intake (g/h/d)	634.10	601.07	677.03	666.27	677.60	677.23	36.15
Real feed intake (%)	82.03^{c}	81.89^{c}	84.73^{b}	85.97^{ab}	86.35^{ab}	87.57^{a}	0.814
FI g/kg weight	13.8	13.03	14.73	14.50	14.77	14.77	0.847
FI g/kg weight (0.075)	35.93	33.97	38.37	37.77	38.43	38.37	2.167
CT intake (g/kg)	11.92^{a}	12.33^{a}	6.16^{c}	11.93^{a}	8.78^{b}	10.35^{ab}	0.737
Digestion coefficients (%)							
DM	56.60^{b}	55.38^{b}	59.20^{a}	56.02^{b}	57.57^{ab}	56.91^{b}	0.706
OM	58.85^{ab}	57.31^{b}	61.31^{a}	58.13^{b}	59.24^{ab}	58.50^{b}	0.838
CP	56.92	55.41	58.17	56.21	57.54	57.03	1.337
EE	63.43^{bc}	62.58^{c}	65.75^{ab}	63.03^{c}	64.56^{bc}	67.81^{a}	0.726
NDF	55.84^{a}	53.76^{b}	57.03^{a}	53.23^{b}	55.91^{a}	53.59^{b}	0.548
ADF	51.12^{ab}	50.15^{b}	52.58^{a}	50.09^{ab}	51.81^{a}	51.17^{ab}	0.477
N utilization (g/h/d)							
N intake	12.83^{a}	9.53^{b}	10.90^{ab}	12.00^{a}	12.33^{a}	12.27^{a}	0.620
N output	12.30^{a}	9.22^{c}	10.12^{bc}	11.55^{ab}	11.64^{ab}	11.76^{ab}	0.622
N balance	0.53^{c}	0.31^{e}	0.78^{a}	0.45^{d}	0.69^{b}	0.51^{c}	0.015
Nutritive value							
TDN (%)	53.51^{ab}	51.73^{b}	55.39^{a}	52.66^{b}	53.72^{ab}	53.15^{ab}	0.734
DCP (%)	7.21^{a}	5.47^{c}	5.85^{c}	6.33^{b}	6.54^{b}	6.45^{b}	0.155

a,b,c Values on the same row with different superscripts differ ($p<0.05$).
T1: CL + ARS (50:50), **T2:** ASL+ ARS (50:50), **T3:** PL+ ARS (50:50), **T4:** CL+ASL + ARS (25:25:50), **T5:** CL+PL+ARS (25:25:50), **T6:** CL+ASL+PL + ARS (25:12.5:12.5:50).

inclusion of CT in ration was increased, the amount of N excreted in the feces increased, while N retained and that excreted in the urine was reduced. On the contrary, Pi~neiro-V_azquez *et al.,* (2017) found that nitrogen balance in the rumen of sheep not affected ($P \geq 0.05$) by the increase in the levels of condensed tannins (0, 1, 2, 3 and 4% CT/kg DM) in the ration.

These results might due to the CT content of foliage leaves as illustrated in Fig. (5). Disharmony in the effects of CT on nitrogen balance may be interfered by the chemical nature of CT and the source and molecular weight of the CT fed (Naumann *et al.,* 2013c).

In the present study, total digestible nutrients (%) and digestible crude protein (%) differed significantly ($P < 0.05$) among treatments. Regarding the nutritive value of the tested feeds, feeding *Prosopis juliflora* (PL) foliage either mixed with urea-treated rice straw or combined with CL and ASL could be better than the other experimental foliage leaves.

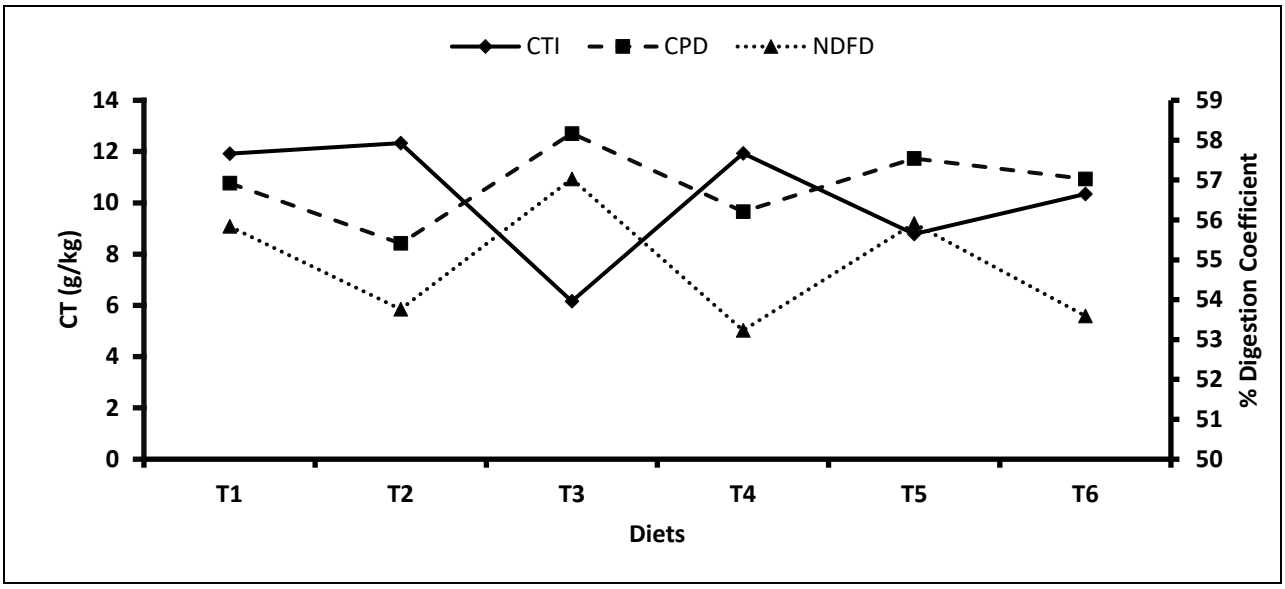

Fig. (5): Effect of CT consumption on CP and NDF digestibility

CONCLUSION

It became clear from this study that tannins reduce N absorption in the rumen and shifted it towards the higher protein flow to the small intestine which known as ruminal by-pass protein. In addition, concerning reduction of the emissions of greenhouse gases, it is important to increase production of animals. Thus, we could conclude that, using foliage leaves rich in tannins in the form of feed mixtures might help to overcome the negative effect of CT and reduce CH_4 emission

REFERENCES

A.O.A.C. (1990) Official Methods of Analysis. 15th Edition, Association of Official Analytical Chemist, Washington DC.

Abdel-Fattah, Z.M.S. (2005). Impact of season of harvest on *in vitro* gas production and dry matter degradability of Acacia saligna leaves with Inoculum. Animal Feed Sci. Technol., 123: 67-79.

Abo-Donia, F.M. and A.K. Nagpal (2015). *In Vitro* Evaluation of Moth (*Phaseolus aconitifolius*) Fodder, Khejri (*Prosopis cineraria*) Leaves and Their Combinations in Camel. Indian J. Anim. Nutr. 32: 192-197.

Ahmed M. El-Waziry. (2007). Nutritive Value Assessment of Ensiling or Mixing Acacia and Atriplex Using *In Vitro* Gas Production Technique. Res. J. of Agri and Biol Sci, 3(6): 605-614.

Animut, G., R. Puchala., A. L. Goetsch., A. K. Patra., T. Sahlu., V. H. Varel, and J. Wells. (2008). Methane emission by goats consuming different sources of condensed tannins. Anim. Feed. Sci. Technol. 144:228-241.

Animut, G., R. Puchala., A.L. Goetsch., A.K. Patra., T. Sahlu and V.H. Varel. (2008). Methane emission by goats consuming diets with different levels of condensed tannins from lespedeza. *Anim Feed Sci Technol* 144:212–227.

Auwal, M. S, S. Saka., I.A. Mairiga., K.A. Sanda., A. Shuaibu and A. Ibrahim (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (thorn mimosa). Vet Res Forum (5):95-100.

Bae, H.D., T. McAllister, J. Yanke., K.J. Cheng and A.D. Muir. (1993). Effects of condensed tannins on endoglucanase activity and filter paper digestion by *Fibrobacter succinogenes* S85. Appl Environ Microbiol: 59:2132–2138.

Barry, T. N and W.C. Mcnabb (1999). The implications of condensed tannins on the nutritive value of temperate foliages fed to ruminants. Brit J Nutr 81(4):263-72.

Beauchemin, K.A., S.M. McGinn., T.F. Martinez and T.A. McAllister. (2007). Use of condensed tannin extract from quebracho

trees to reduce methane emissions from cattle. *J Anim Sci* **85**:1990–1996.

Bhatta, R.., M. Saravanan., L. Baruah and C.S. Prasad. (2014). Effects of graded levels of tannin-containing tropical tree leaves on *in vitro* rumen fermentation, total protozoa and methane production. Journal of Applied Microbiology 118 (3):557-564.

Blios, M.S. (1958). Antioxidant determinations by the use of a stable free radical. Nature. 26:1199–1200.

Blümmel M., H. Steingss., K. Becker. (1997). The relationship between *in vitro* gas production, *in vitro* microbial biomass yield and ^{15}N incorporation and its implications for the prediction of voluntary feed intake of roughages. *British J. of Nutr.* **77**: 911–921.

Broderick, Glen and J.H. Kang.(1980). Automated Simultaneous Determination of Ammonia and Total Amino Acids in Ruminal Fluid and In Vitro Media. Journal of Dairy Science 63(1):64-75 ·

Delehanty, J. B., B.J. Johnson., T.E. Hickey., T. Pons, and F.S. Ligler. (2007). Binding and neutralization of lipopolysaccharides by plant pro-anthocyanidins. J. of Natural Products 70:1718-1724.

Duncan, D.B. (1955). Multiple ranges and multiple F test biometrics, 11:1- 42.

Ebana, R.U., A.I. Essien and O.D. Ekpa (2009). Nutritional and potential medicinal value of the leaves of *Lasianthera Africana* (Beauv). Glob. J. Pure Appl. Sci., 1: 1-7.

Fievez, V., O. Babayemi, and D. Demeyer. (2005). Estimation of direct and indirect gas production in syringes: A tool to estimate short chain fatty acid production that requires minimal laboratory facilities. *Anim Feed Sci and Tech*, 123:197-210.

France, J., J. Dijkstra., M.S. Dhanoa., S. Lopez and A. Bannink. (2000). Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*: derivation of models and other mathematical considerations. *Brit. J.l of Nutr*. 83, 143–150.

Frutos P., G. Hervas., G. Ramos., F.G. Giraldez and A.R. Mantecon (2002). Condensed tannin content of several shrub species from a mountain area in northern Spain, and its relationship to various indicators of nutritive value. Anim. Feed Sci and Tech. 95:215-226.

Gemeda, B. S and A. Hassen. (2015). Effect of Tannin and Species Variation on *in vitro* Digestibility, Gas, and Methane Production of Tropical Browse Plants. Asian Australas. J. Anim. Sci. Vol. 28, No. 2: 188-199.

Gill, L.S (1999). Medicinal uses of trees and plants in Africa. University of Benin Press: Benin. Nigeria, p. 276.

Gonçalves, R., N. Mateus. and V. De Freitas (2011). Inhibition of alpha-amylase activity by condensed tannins. Food Chemistry, 125:665-672.

Grainger, C., T. Clarke. M.J. Auldist., K.A. Beauchemin., S.M. McGinn., G.C. Waghorn and R.J. Eckard (2009). Potential use of Acacia mearnsii condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. Canadian J. of Anim Sci, 89:241-251.

Guglielmelli, A., S. Calabro., R. Primi., F. Carone., M. I. Cutrignelli., R. Tudisco., G. Piccolo., B. Ronchi, and P. P. Danieli. (2011). *In vitro* fermentation patterns and methane production of sainfoin (*Onobrychis viciifolia* Scop.) hay with different condensed tannin contents. Grass Forage Sci. 66:488-500.

Gunun, P., N. Anantasook, M. Wanapat, S. Sirilaophaisan, A. Cherdthong, C. Wachirapakorn, and C. Yuangklang. (2014). Effect of Mao (*Antidesma thwaitesianum* Mull. Arg.) seed supplementation on *in vitro* rumen protozoal population and digestibility using a gas production technique. Khon Kaen Agr. J. 42(Suppl. 4):47-53.

Hagerman, A.E., C.T. Robbins., Y. Weerasuriya., T. Wilson and C. Mcarthur (1992). Tannin chemistry in relation to digestion. J Range Manage 45: 57-62.

Hariadi, B.T and B. Santose (2010). Evaluation of tropical plants containing tannin on *in vitro* methanogenesis and fermentation parameters using rumen fluid. *J Sci Food Agric* 90:456–461.

Harinder, M., B. Singh., S. K. Vats and R.P. Sood (1993). Total phenols, tannins and condensed tannins in different parts of Rumex hastatus. Bioresource Technology 45(1):69-71.

Hassanat, F and C. Benchaar. (2013). Assessment of the effect of condensed (acacia and quebracho) and hydrolysable (chestnut and valonea) tannins on rumen fermentation and methane production *in vitro*. *J Sci Food Agr*, 93, 332-339.

Hatew, B., E. Stringano., I. Mueller-Harvey., W.H. Hendriks., C. Hayot Carbonero., L.M.J. Smith and W.F. Pellikaan. (2015). Impact of variation in structure of condensed tannins from sainfoin (*Onobrychis viciifolia*) on *in vitro* ruminal methane production and fermentation characteristics Journal of Animal Physiology and Anim. Nutr. Vol 100, Issue 2.

Hristov, A. N., C. Oh., J. Lee., R. Meinen., F. Montes., T. Ott., J. Firkins., A. Rotz., C. Dell., A. Adesogan., W. Yang., J. Tricarico., E. Kebreab., G. Waghorn., J. Dijkstra and S. Oosting. (2013). Mitigation of greenhouse gas emissions in livestock production – A review of technical options for non-CO_2 emissions. FAO Animal Production and Health Paper No. 177 (Ed. P. J. Gerber, B. Henderson, and H. P. S. Makkar). FAO, Rome, Italy.

Huang, X.D., J.B. Liang., H.Y. Tan., R. Yahya., B. Khamseekhiew and Y.W. Ho (2010). Molecular weight and protein binding affinity of *Leucaena* condensed tannins and their effects on *in vitro* fermentation parameters. *Anim Feed Sci Technol* 159:81–87.

Isac, M. D., M.A. Garćia., J.F. Aguilera and Molina Alcaide E. (1994). A comparative study of nutrient digestibility, kinetics of digestion and passage and rumen fermentation pattern in goats and sheep offered medium quality foliages at the maintenance level of feeding. Archives of Anim Nutr, 46, 37-50.

Jayanegara, A., N. Togtokhbayar., H.P.S. Makkar and K. Becker (2009). Tannins determined by various methods as predictors of methane production reduction potential of plants by *in vitro* rumen fermentation system. *Animal Feed Science and Technology*, 150(3), 230-237.

Jones, G.A., K.D. Jakober., H.D. Bae., T.A. McAllister and K.J. Cheng (1993). Some interactions between condensed tannins of foliage legumes, bovine serum albumin, and five strains of proteolytic rumen bacteria. In: Proceedings of the World Conference on Animal Production, vol. 36, Edmonton, Canada, pp. 68–69.

Jones, W. T and J. L. Mangan (1977). Complexes of the condensed tannins of sainfoin (Onobrychis viciaefolia Scop) with fraction 1 leaf protein and with submaxillary mucoprotein and their reversal by PEG and pH. J. of the Sci. of Food and Agri, 28(2):126 - 136 ·

Kavitha, P. A., K. Pawan., T.P. Narasimah Murthy., S.M. Gopinath. (2013). Methanolic extract of *Acacia nilotica* and antibacterial activity against hospital isolates of Bengaluru district. Int J Latest Res Sci Technol; 2:522-4.

Lavin, S. R. (2012). Plant phenolics and their potential role in mitigating iron overload disorder in wild animals. Journal of Zoo Wildlife Medicine 43: S74-S82.

Makkar, H. P. S. (2003). Quantification of Tannins in Tree and Shrub Foliage. A Laboratory Manual. Kluwer Academic Publishers, Dordrecht, the Netherlands.

McDonald, P., R.A. Edwards and J.F.D. Greenhalgh (2002). Animal Nutrition. 6th Edition. Longman, London and New York. 543 p.

McDougall, E. F. (1948). Studies on ruminant saliva. The composition and output of sheep's saliva. Biochem. J. 43:99-109.

McNabb, W.C., J.S. Peters., L.Y., L.Y. Foo., G.C. Waghorn and F.S. Jackson (1998). Effect of condensed tannin prepared from several foliages on the *in vitro* precipitation of ribulose-1,5 bisphosphate carboxylase (Rubisco) protein and its digestion by trypsin (EC 2.4.21.4) and chymotrypsin (E.C. 2.4.21.1). *J. Sci. Food Agric.*, 77: 201–212.

McSweeney, C.S., B. Palmer., D.M. McNeill and D.O. Krause (2001). Microbial

interactions with tannins: nutritional consequences for ruminants. *Anim Feed Sci Technol* 91:83–93.

Menke, K. H., L. Raab, A. Salewski, H. Steingass, D. Fritz and W. Schneider. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor in vitro. J. Agric. Sci. (Camb.) 93(01):217 – 222.

Min, B. R., T.N. Barry., G.T. Attwood and W.C. McNabb (2003). The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate foliages: a review. Animal Feed Science and Technology 106:3-19.

Min, B.R., G.T. Attwood., K. Reilly., W. Sun., J.S. Peters., T.N. Barry and W.C. McNabb (2002). *Lotus corniculatus* condensed tannins decrease *in vivo* populations of proteolytic bacteria and affect nitrogen metabolism in the rumen of sheep. *Canadian J. of Microbiology* 48, 911–921.

Min, B.R., W.C. McNabb., T.N. Barry., P.D. Kemp, Waghorn and G.C. McDonald (1999). The effect of condensed tannins in *Lotus corniculatus* upon reproductive efficiency and wool production in sheep during late summer and autumn. J. of Agri. Sci, Cambridge 132: 323-334.

Mousa, M. R. M. (2011). Effect of Feeding Acacia as Supplements on the Nutrient Digestion, Growth Performance, Carcass Traits and Some Blood Constituents of Awassi Lambs under the Conditions of North Sinai Asian J. of Anim Sci, 5(2):102-117.

Naumann, H. D., J.P. Muir., B.D. Lambert., L.O. Tedeschi and M.M. Kothmann (2013a). Condensed tannins in the ruminant environment: A perspective on biological activity. J. of Agri. Sci 1:8-20.

Naumann, H. D., L.O. Tedeschi., A, E. Hagerman., B.D., B.D. Lambert and J.P. Muir. (2013c). Methane emission and protein precipitating ability of condensed tannins from warm-season perennial legumes. In: Energy and protein metabolism and nutrition in sustainable animal production, EAAP publication No. 134. p.491-492. Wageningen Academic Publishers. Wageningen, The Netherlands.

Naumann, H. D., L.O. Tedeschi., J.P. Muir., B.D. Lambert and M.M. Kothmann, (2013b). Effect of molecular weight of condensed tannins from warm-season perennial legumes on ruminal methane production in vitro. Biochemical Systematics and Ecology 50:154-162.

Naumann, H.D., O. T. Luis., E.Z. Wayne and F.H. Nichole (2017). The role of condensed tannins in ruminant animal production: advances, limitations and future directions Revista Brasileira de Zootecnia *Brazilian Journal of Animal Science*., 46(12):929-949.

Navarro-Villa, A., M. O'Brine., S. López., T.M. Boland., P. O'Kiely. (2012). *In vitro* rumen methane output of grasses and grass silages differing in fermentation characteristics using the gas-production technique (GPT). Grass Foliage Science.

Ng, F., S. Kittelmann., M.L. Patchett., G.T. Attwood., P.H. Janssen., J. Rakonjac, and D. Gagic. (2016). An adhesin from hydrogenutilizing rumen methanogen Methanobrevibacter ruminantium M1 binds a broad range of hydrogen-producing microorganisms. Environmental Microbiology 18:3010-3021.

Okeke, C.U and I.Z. Elekwa (2003). Phytochemical study of the extracts of Gongronema latifoliuin (Asclepiaclaceae). J. Health Visual Stud., 5: 47-55.

Oni, A.O., C.F. Onwuka., O.M. Arigbede., U.Y. Anele., O.O. Oduguwa., O.S. Onifade and Z.L. Tan (2010). Chemical composition and nutritive value of four varieties of cassava leaves grown in South Western Nigeria. J Anim Physiol Anim Nutr (Berl). 2011 Oct;95(5):583-90.

Pathak, A.K., N. Dutta., P.S. Banerjee., T.K. Goswami and K. Sharma (2016). Effect of condensed tannins supplementation through leaf meal mixture on voluntary feed intake, immune response and worm burden in Haemonchus contortus infected sheep. J Parasit Dis.;40 (1):100-5.

Patra, A. K and J. Saxena. (2009). Dietary phytochemicals as rumen modifiers: a review of the effects on microbial populations. *Antonie van Leeuwenhoek* 96, 363–375.

Pi~neiro-V_azquez., A. T., J. R. Canul-Solis., J. A. Alay_on-Gamboa., A. J. Chay-Canul., A. J. Ayala-Burgos., F. J. Solorio-S_anchez., C.F. Aguilar-P_erez1 and J. C. Ku-Vera. (2017). Energy utilization, nitrogen balance and microbial protein supply in cattle fed Pennisetum purpureum and condensed tannins. Journal of Animal Physiology and Animal Nutrition 101 :159–169.

Reed, J. D., H. Soller, and A. Wood. (1990). Fodder tree and straw diets for sheep: intake, growth, digestibility and the effect of phenolics on nitrogen utilization. Anim Feed Sci and Technology 30: 39-50.

Salah, W., N.J. Miller., G. Pagauga., A.P. Tijburg., F. BoIwel and C. Evans (2002). Polyphenolic flavones as scavenger of aqueous phase radicals as chain breaking oxidant. Res. J. Biochem., 2: 339-346.

Salem, A.Z.M., A.E. Kholif. M. Olivares., M.M.Y. Elghandour., M. Mellado and J. Arece, J. (2014). Influence of *S. babylonica* extract on feed intake, growth performance and diet *in vitro* gas production profile in young lambs. *Tropical Animal Health and Production* 46, 213-219.

Salem, A.Z.M., Olivares, M., Lopez, S., Gonzalez-Ronquillo, M.,Camacho, L.M., Cerrillo, S.M.A., Mejia, H.P., Rojo, R. (2011). Effect of natural extracts of *Salix babylonica* and *Leucaena leucocephala* on nutrient digestibility and growth performance of lambs. *Animal Feed Science and Technology* 170, 27– 34.

Saminathan, M., H.Y. Tan., C.C. Sieo., N. Abdullah., C.M.V.L. Wong., E. Abdulmalek and Y.W. Ho. (2014). Polymerization degrees, molecular weights and protein-binding affinities of condensed tannin fractions from a leucaena leucocephala hybrid. Molecules 19:7990-8010.

SAS Institute Inc. (2002). JMP 5 Statistical Software. NC, USA: Cary.

SAS Institute Inc**.** (2008). SAS/STAT Software, version 9.2. SAS

Sawe, J.J., J.K. Tuitoek and J.M. Ottaro (1998). Evaluation of common tree leaves or pods as supplements for goats on rangelands area of Kenya. Small Rum. Res. 28, 31-37.

Seresinhe, T and C. Iben C (2003). *In vitro* quality assessment of two tropical shrub legumes in relation to their extractable tannin contents. J Anim Physiol Anim Nutr (Berl) ;87(3-4):109-15.

Shumuye, B and T. Yayneshet, (2011). Effect of feeding treated *Acacia Saligna* (Labill.) H.L. Wendl. leaves on growth performance and digestibility in goats. J. Dryland Agric. 4(2):341-347.

Siddhuraju P and K. Becker. (2003). Studies on antioxidant activities of mucuna seed (Mucuna pruriens var. utilis) extracts and certain non-protein amino/imino acids through *in vitro* models. J. Sci. Food. Agric. 83: 1517-1524.

Sliwinki, B.J., M. Kreuzer., H.R. Wettslein and A. Machmuller (2002). Rumen fermentation and nitrogen balance of lambs fed diets containing plant extracts rich in tannins and saponins and associated emissions of nitrogen and methane. Archives of Animal Nutrition 56, 379-392

Soares, S., N. Mateus and V. De Freitas. (2012a). Carbohydrates inhibit salivary proteins precipitation by condensed tannins. J. of Agri and Food Chem, 60:3966-3972.

Tan, H.Y., C.C. Sieo., N. Abdullah., J.B. Liang., X.D. Huang and Y.W. Ho (2011). Effects of condensed tannins from *Leucaena* on methane production, rumen fermentation and populations of methanogens and protozoa *in vitro*. *Anim Feed Sci Technol* 169:185–193.

Thi, M.U., V.B. Dinh and E.R. Ørskov (2005). Effect of foliages containing condensed tannins and on gastrointestinal parasites. Anim. Feed Sci. Technol. 121:77-87.

Van Soest, P.J., J.B. Robertson., B.A. Lewis. (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch carbohydrates in relation to animal nutrition. *J. of Dairy Sci*, 74, 3583–3597.

Vimal, O. P. and P. D. Tyagi. (1986). *Prosopis juliflora*: Chemistry and Utilization," In: V. J.

Patel, Ed., The Role of Prosopis in Wasteland Development, Agroforestry Center, Gujarat, pp. OVP1-OVP8.

Waghorn, G. C., I.D. Shelton. and W.C. McNabb. (1994a). Effect of condensed tannins in Lotus pedunculatas on its nutritive value for sheep. 1. Non-nitrogenous aspects. J. of Agri. Sci. 123:99-107.

Woodward, A and J.D. Reed (1989). The influence of polyphenolics on the nutritive value of browse: a summary of research conducted at ILCA. ILCA Bulletin, 35: 2-11.

Zhishen J, Mengcheng T and Jianming W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64, 555-559.

Nutritive evaluation of foliage combinations from some fodder trees and shrubs

التقييم الغذائى لخلطات أوراق بعض من الأشجار والشجيرات العلفية

فوزى محمد أبودنيا، هانم عبد الرحمن الشيخ، أمل فايد، مجدى أبو الفضل و محمد عيسى
معهد بحوث الانتاج الحيوانى، مركز البحوث الزراعية، الدقى ، الجيزة ، مصر.

تم اختبار ستة خلطات غذائية تحتوى على ثلاث أنواع من الاوراق الاشجار او الشجيرات العلفية أو خليط بينهما كالاتى 100% كاسافا، 100% أكاسيا، 100% بروسوبس، كاسافا : أكاسيا (50: 50)، كاسافا : بروسوبس(50: 50) و كاسافا : أكاسيا : برسوسوبس (50 : 25 : 25). وقد تم خلط هذه المخاليط الورقية بقش الارز المعامل بالامونيا (50: 50) على أساس المادة الجافة. أظهرت نتائج التحليل الكيماوى إرتفاع محتوى البروتين فى أوراق الكاسافا (22.22%) مقارنة بالاكاسيا (16.67%) أو البروسوبس (17.02%)، بينما كان محتوى البروتين متقارب فى مخاليط هذه الاوراق فى الاغذية المختبرة. كما أوضحت النتائج أن محتوى الكاسافا من NDF و ADF أقل عند مقارنتة بباقى المخاليط الاخرى. كما أظهرت النتائج أن أقل كميات ناتجة من الغاز الكلى وغاز الميثان المنطلق كان عن تحضين الاغذية المحتوية على الكاسافا أو الاكاسيا مع قش الارز المعامل بالامونيا خارج الكرش، بينما إرتفع معدل إنطلاق الغاز الكلى والميثان عند تحضين مخاليط أوراق الكاسافا والأكاسيا مع / أو البروسوبس خارج الكرش. كما تحسن معدل تكسر DM و OM NDF مع المعاملات التى بها مخاليط الاوراق (T4 و T5 و T6) مقارنة بالمعاملات التى تحتوى على الأوراق بصورة منفردة المضافة اليها قش الأرز المعامل بالأمونيا (T1 وT2 و T3) ، كما لوحظ أن المعاملات التى تحتوى أوراق البرسوبس(T3) و (T6) كانت مرتفعة بشكل معنوى فى الطاقة الممثلة مقارنة بتلك التى تحتوى أوراق الكاسافا أو أوراق الأكاسيا (T1وT2 وT4 و T5).
أظهرت نتائج معامل الارتباط أن هناك إرتباط موجب بين *IVNDFD* وكلا من البروتين الميكروبى ($r = 0.66, P < 0.05$) والميثان ($r = 0.60, P < 0.05$) عند تحضين تلك المواد المختبرة خارج الكرش. أيضا وجد أن هناك ارتباط موجب معنوى بين البروتين الميكروبى وكلا من إنتاج الغاز الكلى ($r = 0.76, P < 0.05$) والميثان ($r = 0.65 P < 0.05$) عند تحضين العلائق المختبرة خارج الكرش. كما ظهر أنة كلما إنخفض تركيز CT بالعليقة كلما زاد قيم كلا من الامونيا والاحماض الدهنية الطيارة والبروتين الميكروبى وعدد البكتريا والعكس صحيح. ولم يظهر أى تأثير معنوى لمختلف المعاملات على قيم الاس الهيدروجينى وعدد البروتوزوا وحمض البروبيونك والبيوتريك ونسبة حمض الاستيك الى حمض البروبيونك. زاد معدل الغذاء المرفوض معنويا فى العلائق المحتوية على أوراق الشجيرات المختبرة منفردة مع قش الأرز المعامل بالامونيا خصوصا مع العليقة T1 وT2.وفى المقابل , فإن كمية الغذاء المرفوض إنخفضت عند خلط أورق الشجيرات العلفية معا.
أظهرت نتائج تجارب الهضم أن وجود كلا من الكاسافا والبروسوبس منفردة (T1وT3) أو إدخالهم فى مخاليط فى العلائق المختبرة أدى الى تحسن فى معاملات هضم كلا من DM وOM و NDF وADF مقارنة بالعليقة المحتوية على الأكاسيا. كما أشارت النتائج الى عدم وجود فروق معنوية فى معاملات هضم البروتين بين العلائق المختبرة. كان هناك زيادة معنوية فى قيم ميزان النيتروجين فى الحيوانات المغذاة على العلائق T3 وT4وT5 مقارنة بباقى العلائق المختبرة (T1و T2وT6).
ونستخلص من النتائج السابقة أن أستخدام أوراق الأشجار والشجيرات العلفية الغنية بالتنينات فى صورة مخاليط علفية يمكن أن يساعد فى التغلب على التأثير الضار للتنينات وتقليل انبعاث غاز الميثان.