Use of ammoniated rice straw and tree leaves containing tannin as scarcity feed to Bakri lambs

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Abstract

To cover a shortage of animal food in arid regions in Egypt, this investigation has been carried out to compare the consequences of using ammoniated rice straw (ARS) with different proportions of tree foliage leaves (*Cassava*, CA; *Acacia saligna*, AS; and *Acacia neloitca*, AN) on ruminal fermentation and sheep performance. The superior six formulations of pastoral lime mixtures from CA and Acacia with ARS were selected according to chemical compositions and IVDMD values from among 72 formulations, which were tested *in vitro*. Thirty five growing Barki male lambs four months old and weighed 20.16±0.11 kg were divided into seven groups(5 each); T0: 40% concentrate feed mixture (CFM) + 60% ARS and served as control, T1: 40% CFM + 30% ARS + 30% CA, T2: 40% CFM + 30% ARS + 30% AS, T3: 40% CFM + 30% ARS + 30% AN; T4: 40% CFM + 30% ARS + 15% CA + 15% AS, T5: 40% CFM + 30% ARS + 15% CA + 15% AN, and T6: 40% CFM + 30% ARS + 15% AS + 15% AN. Protein content was higher in CA (22.22%) compared to AS (16.67%) and AN (15.99%). ADF and NDF content in CA was the lowest. T1 and T2 showed the lowest potential ruminal gas and methane production than T4 and T5. T4, T5, and T6 improved ($P < 0.05$) the degradability of DM, OM, and NDF, while reduced condensed tannins (CT) levels and increased ammonia-N, short-chain fatty acids, microbial protein, and bacterial count. No influence of various treatments on pH, protozoa count, butyric, and propionic acid. T3 group showed an increase in lambs'performance compared with the other groups.It could be concluded that the use of feed mixtures of tannin-rich foliage and ammoniated rice foddercan used as a partial substitute for ARS andmay help in cover the scarcity of forage resources and resolve the negative effects of CT and reduce the methane released**.**

Keywords: Foliage leaves; ammoniated rice straw; tannin; neutral detergent fiber; microbial protein; Barki lambs

Introduction

The scarcity of forage resources is one of the fundamental constraints of improving animal production in Egypt's arid and semi-arid regions. The indigenous natural pastures are the main feed sources in the Egyptian deserts, broadly spread in many areas of Egypt of the Mediterranean Sea and the Red Sea and also in Chile Archipelago (Martinez et al., 2012 & 2015). Halophytes make up a large part of the natural range, especially shrubs and perennials, which Almost 70 percent of the total coverage accounts f plant species (Sleimi and Abdelly, 2003). So, feeding halophytes and crop residue is a feasible solution to minimize the expected problems of feed shortage in such areas.

Under semi-arid area conditions, small ruminants fed on trees and shrubs such as Cassava and Acacia to solve the attendant problems of low productivity (Eissa *et al.,* 2016). Cassava leaves have been found to have a high nutritional value which can effectively boost the nutrition of small ruminants when preserved as hay. The crude protein (CP) content of Cassava leaves is in the range of 22–29 % of dry matter (DM) (El Shaer, 2010). Furthermore, it has been proven that Cassava leaves are rich in amino acids, comparable to soybean meal. However, such trees and shrubs foliage are generally rich in anti-nutritional factors, particularly tannins (Makkar, 2003). Feeding a mixture of these fodder shrubs could minimize or overcome the problems of palatability and toxic effects (Anbarasu et al., 2001; Patra et al., 2002; Shaker et al., 2014).The conventional approach to fodder trees is to study and exploit "single" species. While the use of mixtures of species thus appears to be desirable, there is little scientific information on which to base practical recommendations. Recent results quantified the associative effects *in vitro* of mixtures of different species of Egyptian fodder trees, identifying significant interactions. These suggest that there is considerable potential to develop feeding systems based on strategic combinations that result in added nutritive value. They can be achieved by capitalizing on the interactive processes, such as: protecting dietary protein with natural tannins to increase the amount of nitrogen that by-passes the rumen; diluting the effects of deleterious compounds; inducing associative impacts that result in an increased voluntary intake, and inducing associative effects on digestibility between the components of the mixture. Appropriate mixtures of tree foliage can result in an overall improvement in nutritive value and contribute to making more efficient use of the natural diversity of trees and shrubs in the arid regions, and hence helping to sustain it(Mohamed *et al.*, 2020).

The lack of information about feeding the mixture of Cassava and Acacia shrubs with ammoniated rice straw and their effect on animal performance in arid areas of Egypt was the investigation's motive. Therefore, this experiment was intended to verify whether the blending of foliage leaves with roughage can enhance these plants' use and affect the fermentation kinetics of *in vitro* gas production and Barki lamb performance.

Material and methods

This study conducted at the Animal Production Research Station Borg El-Arab. Which stretches along 525 km on the Mediterranean Sea, west of Alexandria city at latitudes 21 and 31 north and longitudes 25 and 35 East.

Preparation of plants

Cassava (CA), *Acacia* ***s****align a*(AS),and *Acacia neloitca* (AN) using careful leaf harvesting within 30 minutes in the morning mowed 5 cm above ground level, dried, and then separated the leaves from the woody branches. Leaves and twigs were obtained**.** After CA, AS, and AN dried, they were cut into small pieces (5 mm) to avoid bunching.

Experimental design

This study has been carried out to compare the consequences of using treated ammoniated rice straw (ARS) with different proportions of tree foliage leaves (*Cassava*, CA; *Acacia saligna*, AS; and *Acacia neloitca*, AN or their mixture) on ruminal fermentation and productive performance of growing lambs. The superior six formulations of pastoral lime mixtures from Cassava and Acacia with ARS were selected according to chemical compositions and IVDMD values from among 72 formulations, which were tested *in vitro*. Thirty five growing Barki male lambs four months old and weighed 20.16±0.11 kg were divided into seven groups (5 each);included T0: 40% concentrate feed mixture (CFM) + 60% ARS and served as control, T1: 40% CFM + 30% ARS + 30% CA, T2: 40% CFM + 30% ARS + 30% AS, T3: 40% CFM + 30% ARS + 30% AN; T4: 40% CFM + 30% ARS + 15% CA + 15% AS, T5: 40% CFM + 30% ARS + 15% CA + 15% AN, and T6: 40% CFM + 30% ARS + 15% AS + 15% AN. Animals were weighed biweekly for 21 weeks, the first three weeks was an adaptation period, and the other 18 weeks were a feeding period. Feeds were given at 07:00 and 18:00 h in two balanced meals according to NRC (2007). The level of the ingredients in the concentrate portion was adjusted to maintain iso-protein -iso-caloric experimental rations. The chemical composition of the ingredients and tested rations are presented in Table (1).

Digestibility and nitrogen balance trial

Digestibility trial conducted using 21Barki rams, 2-3 years old, and weighted 43.00±1.85 kg, holding individually in metabolic cages. They have been divided into the previous seven groups. This trial continued for 22 days (15 days as an adaptation period followed by seven days as a collection period). Feed amounts, residuals, fecal output, and urine volume were recorded daily.Nitrogen balance (NB, g/h/d) was mathematically determined by the following equation; NB = total N intake– (fecal N + urine N).

Chemical composition of samples

Feedstuff rations and fecal samples were analyzed according to AOAC (2003). Neutral and acid detergent fiber (NDF & ADF) according to Van Soest *et al.* (1991), condensed tannins (CT) according to Makkar*et al.* (1993), total flavonoid according to Zhishen *et al.* (1999), and free radical scavenging activity on α, α-diphenyl-β-picrylhydrazyl (DPPH; $C_{18}H_{12}N_5O_6$) was determined according to Blois (1958). Siddhuraju and Becker (2003) defined the total phenolic content of leaves calculated by the FolinCiocalteu process. Rumen fluid short-chain fatty acids (SCFA's) are evaluated by gas chromatography (Isac *et al.*, 1994). Ammonia-N before incubation, buffer mixture, and after incubation was estimated by the phenol-hypochlorite method according to Broderick and Kang (1980).

Determination of gas production (GP)

A modified version determined rumen GP and methane (CH_4) production *in vitro* for foliage, according to Navarro-Villa *et al.* (2013). Kinetic GP parameters are determined by matching GP (mL/g OM) output in nonlinear regression models as defined in the model presented by France *et al.* (2000).The ratio of genuinely degraded organic matter (mg) to gas volume (ml) at twenty-four hours of incubation was used as a microbial synthesis efficiency index (Blümmel *et al.*,1997).Using the Menke *et al.* (1979) equation, the metabolizable energy (ME) are determined as follows: ME (MJ/kg DM) = 2.20 + 0.136GP + 0.057CP.

Blood samples

At the end of the experiment, blood samples were taken from each animal in all treatments. The blood samples were taken at three hours post morning feeding. A sample of 15 ml of blood per animal waswithdrawn from the jugular vein and directly collected into clean dried glass culture tubes after adding EDTA. The blood plasma was obtained by centrifuging the blood samples soon after collection at 4000 rpm for 15 minutes. Blood plasma was transferred into clean dried glass vials and then stored in a deep freezer at -20º C for subsequent specific chemical analysis.

Economic efficiency and Statistical analysis

Based on local prices, economic efficiency was determined as total output/total input (where one-ton of CFM, CA, AS, ARS & kg BW/lambs were 2850, 550, 550, 500 &45 L.E, respectively). Data were expressed as means (±S.E.), and statistical analyses were performed with the General Linear Model (GLM) of SAS (2008). Duncan's New Multiple Range Test of the same SAS program was applied to determine significant differences among all tested treatments.

Results and discussion

The compositions of foliage leaves offered to Barki lambs are presented in Table (1). The crude protein (CP) was higher in CA (22.22%) compared to AS (16.67%) and AN (15.99%), and ARS (3.09%). The present results are consistent with the contents of the AS values reported by Shumuye and Yayneshet (2011). However, their values of CA tended to be smaller than those estimated by Oni *et al.* (2011), who reported that the CP content of CA ranged from 177 to 240 g/kg DM, while AN was in the range from 14 to 26% (Vimal *et al.*, 1986).These salt-tolerant plants had higher crude protein (CP), which could safely cover the essential nutrients requirements for animals**.**

Table (1) shows that the CP and EE were higher in CA, AS, and AN than their mixing as salt-tolerant plant mixture. These results agree with Shaker *et al.* (2014) and Helal *et al*. (2018), who said that the results showed that CP, EE, and ash contents were higher in alfalfa hay and berseem hay respectively than tree legumes mixture (*Prosopis juliflora, Acacia saligna, Leucaena leucocephala*), while this mixture had higher CF, NFE, and OM%, These salt-tolerant plants had higher crude protein (CP), crude fiber (CF), nitrogen-free extract (NFE), and organic matter (OM) which could safely cover the essential nutrients requirements for animals.

In the present study, all diets have adequate supplies of CP ranging from 8.13% (T0) to 13.87% (T1). These crud protein levels above the necessary microorganism's requirement (7%) to encourage appropriate ruminal microflora growth and the host ruminant's maintenance requirement for CP (McDonald *et al.*, 2002). These findings were agreed with Helal *et al.* (2018)**,** who illustrated that salt-tolerant plants mixture was higher in CP (9.37%) than

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

Items	Chemical composition (% on DM basis)							CT (g/kg DM)
	DM	OM	CP	EE	Ash	NDF	ADF	
CFM	91.20	91.20	15.70	3.13	6.10	43.00	17.30	nd
ARS	90.73	83.90	3.09	1.40	16.10	64.02	40.01	nd
CA	88.76	90.36	22.22	4.24	9.64	49.14	33.28	20.58
AS	88.65	90.65	16.67	2.95	9.35	55.23	35.58	25.03
AN	90.02	90.82	15.99	3.66	9.82	52.55	34.77	15.61
T0	90.92	86.82	8.13	2.09	12.10	55.61	30.93	nd
T1	90.33	88.76	13.87	2.94	10.16	51.15	28.91	11.27
T2	90.29	88.85	12.21	2.56	10.08	52.98	29.60	12.31
T3	90.71	88.90	12.00	2.77	10.22	52.17	29.35	6.18
T4	90.31	88.80	13.04	2.75	10.12	51.25	29.25	11.79
T5	90.52	88.83	12.94	2.86	10.19	51.66	29.13	8.73
T6	90.50	88.87	12.11	2.66	10.15	52.57	29.48	9.25

CFM = Concentrate feed mixture; ARS= Ammoniated rice straw; CA= *Cassava*; AS= *Acacia saligna*; AN=*Acacia neloitca*; nd= not detected; DM= Dry matter; OM= Organic matter; CP= Crud protein; EE= Ether extract; CF= Crud fiber; NDF= Neutral detergent fiber; ADF= acid detergent fiber; CT=condensed tannins;T0= 40% CFM + 60% ARS; T1= 40% CFM + 30% ARS + 30% CA; T2= 40% CFM + 30% ARS + 30% AS; T3= 40% CFM + 30% ARS + 30% AN; T4=40% CFM + 30% ARS + 15% CA + 15% AS; T5= 40% CFM + 30% ARS + 15% CA + 15% AN; T6= 40% CFM + 30% ARS + 15% AS + 15% AN.

Table 2. Quantitative phytochemical analysis of the tested plants.

Item	Total Phenol (GAE mg/g DM)	Total Flavonoid (mg/g DM)	Antioxidant activity (% of DPPH scavenging activity)
CA	99.73	12.22	65.68
AS	108.60	14.97	48.21
AN	121.37	16.30	72.84
CA + AS	110.37	14.38	57.32
CA + AN	120.27	15.71	68.82
CA + AS + AN	105.50	14.72	62.09

CA= *Cassava*; AS= *Acacia saligna*; AN=*Acacia neloitca*

Table 3. Prediction of total gas, methane production and degradabilities of DM, OM and NDF% and ME

Item	Treatments							±SE
	T0	T1	T2	T3	T4	T5	T6	
P-GP (ml/g OM)	99.77^{a}	75.56^{d}	72.32^{e}	84.75^{c}	92.38^{b}	94.14^{b}	96.38^{a}	4.018
GP-R (ml/h)	0.078^{a}	0.039^{c}	0.034^{c}	0.063^{b}	0.031^{c}	0.046^{b}	0.025^{d}	0.004
P-CH4 (ml/gOM)	23.90^{a}	13.37^{d}	12.04^{e}	15.37^{c}	17.87^{b}	19.24^{b}	21.93^{a}	1.275
CH4-R (ml/h)	0.055^{a}	0.039^{c}	0.037^{c}	0.044^{b}	0.037^{c}	0.042^{b}	0.018^{d}	0.004
P-CH4 / P-GP	0.240^{a}	0.176^{d}	0.169^{e}	0.182^{c}	0.193^{b}	0.205^{b}	0.228^{a}	0.016
IVDMD(%)	29.54^{a}	21.74^{d}	20.90^{e}	22.87^{d}	24.33^{c}	26.07^{b}	25.09^{b}	0.989
IVOMD(%)	28.26^{a}	24.06^{d}	23.96^{d}	24.92^{d}	25.21^{c}	27.05^{b}	26.08^{b}	0.533
IVNDFD(%)	29.76^{a}	22.56^{d}	21.35^{e}	23.62^{c}	24.77^{c}	25.36^{b}	26.62^{b}	1.271
ME (MJ/kg DM)	5.41^{c}	9.71^{b}	9.69^{b}	11.97^{a}	10.26^{b}	11.44^{a}	9.41^{b}	0.265

a, b,c,d and e Means in the same row with different superscript are significantly different (P< 0.05).P-GP= Potential gas production; GP-R= Gas production rate; P-CH4= Methane production efficiency; CH4-R= Methane production rate; P-CH4/P-GP= methane production/ gas production; IVDMD= *In vitro* dry matter degradability; IVOMD= *In vitro* organic matter degradability; IVNDFD= *In vitro* neutral detergent fiber degradibility; ME= Metabolizable energy.

Table 4. Correlation between CT intakes *vs*. in-vitro NDF degradability, SCFA's, MP, GP, CH4 production for diets containing foliage leaves.

Item	CT	IVNDFD	SCFA's	MP	CH4
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* neutral detergent fiber degradability; SCFA's= Short chino fatty acids; MP=Microbial protein; CH4= Methane production; GP= Gas production.

Table 5. Effect of tested diets on pH, NH3-N and SCFA's, MP and Bacteria count *in vitro* incubation (at 96 hrs) of lambs' rumen fluid.

Item	Treatments							±SE
	T0	T1	T2	T3	T4	T5	T6	
pH	6.**90**	6.72	6.87	6.69	6.8	6.71	6.82	0.138
NH3-N (mg/dl)	**3**8.**5**9[a]	12.69[d]	7.19[e]	23.71[c]	19.41b[c]	28.19[b]	14.56[d]	2.185
SCFA's (meq/dl)	7.**99**[b]	7.55b[b]	7.05[c]	8.53[a]	8.59[a]	8.98[a]	7.70[b]	0.333
MP (mg/dl)	40.**2**9[b]	36.59[c]	34.13[d]	38.08[c]	40.03[b]	40.81[b]	43.03[a]	0.764
Bacteria count, (×10^5 /ml)	6.29[c]	7.56[b]	7.42[b]	7.68[b]	8.68[a]	9.03[a]	8.30[a]	0.402
Protozoa count, (×10^3 /ml)	10.13	4.88	4.07	4.94	5.48	5.54	5.23	0.582
SCFA's fractionation (%) Acetic (A)	49.72[**d**]	53.91[c]	53.73[c]	54.84[c]	56.48[b]	58.74[a]	58.40[a]	0.624
Propionic (P)	20.72	23.57	23.67	23.50	24.93	23.95	24.66	0.781
A/P	2.45	2.29	2.27	2.33	2.28	2.46	2.37	0.076
Butyric	9.**3**2	11.52	11.44	11.71	12.06	12.09	12.10	0.212

a, b,c,d and e Means in the same row with different superscript are significantly different (P<0.05). NH3-N= Ammonia nitrogen; SCFA's= Short chain fatty acids; MP= Microbial protein.

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

Item	**Treatments**							**±SE**
	T0	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	
Feed intake (FI)								
Offer feed (g/h/d)	770	750	734	799	775	784.67	773.33	38.14
Refusal feed (g/h/d)	338.90[b]	250.90[a]	132.93[b]	121.17[c]	108.73[d]	107.07[d]	96.10[e]	5.56
Refusal feed %	44.01[b]	33.45[a]	18.11[b]	15.27[c]	14.03[d]	13.65[d]	12.43[e]	0.814
Real FI (g/h/d)	431.10	499.03	601.07	677.83	666.27	677.60	677.23	36.15
Real FI (%)	55.98[d]	75.03[e]	81.89[d]	84.73[c]	85.97[b]	86.35[b]	87.57[a]	0.814
FI(g/kg weight)	13.80	10.20	13.03	14.73	14.50	14.77	14.77	0.847
FI(g/kg weight (0.075))	35.93	23.93	33.97	38.37	37.77	38.43	38.37	2.167
CT intake (g/kg)	0.00	11.27[a]	12.31[a]	6.18[d]	11.79[a]	8.73[c]	9.25[b]	0.737
Digestion coefficients (%)								
DM	49.60[e]	56.60[b]	55.38[d]	59.20[a]	56.02[c]	57.57[b]	56.91[b]	0.706
OM	43.85[e]	58.85[c]	57.31[d]	61.31[a]	58.13[c]	59.24[b]	58.50[c]	0.838
CP	48.92	56.92	55.41	58.17	56.21	57.54	57.03	1.337
EE	51.43[d]	63.43[c]	62.58[c]	65.75[b]	63.03[c]	64.56[b]	67.81[a]	0.726
NDF	47.84[d]	55.84[b]	53.76[c]	57.03[a]	53.23[c]	55.91[b]	53.59[c]	0.548
ADF	48.12[d]	51.12[b]	50.15[c]	52.58[a]	50.09[c]	51.81[a]	51.17[b]	0.477
Nitrogen utilization (g/h/d)								
N intake	12.83[a]	12.83[a]	9.53[c]	10.90[b]	12.00[a]	12.33[a]	12.27[a]	0.620
N output	12.30[a]	12.30[a]	9.22[c]	10.12[c]	11.55[b]	11.76[b]	11.64[b]	0.622
N balance	0.28[d]	0.53[c]	0.31[d]	0.78[a]	0.45[c]	0.69[b]	0.51[c]	0.015
Nutritive value								
TDN (%)	48.51[c]	53.51[b]	51.73[d]	55.39[a]	52.66[d]	53.72[b]	53.15[b]	0.734
DCP (%)	4.21[d]	7.21[a]	5.47[c]	5.85[c]	6.33[b]	6.54[b]	6.45[b]	0.155

a, b,c,d and e Means in the same row with different superscript are significantly different (P< 0.05). DM= Dry matter; OM= Organic matter; CP= Crud protein; EE= Ether extract; CF= Crud fiber; NDF= Neutral detergent fiber; ADF= acid detergent fiber; CT= condensed tannins; TDN= Total digestible nutrients; DCP= Digestible crude protein.

the maintenance requirements for ruminants as recommended by Norton(2003), who determined that feeds contain less than 8% CP could not provide the ammonia levels required by rumen microbes for optimum activity.

Furthermore, the ADF and NDF levels in CA were inferior to those of the other plants and their mixtures. These findings were agreed with Fayed *et al.* (2010), Shaker et al. (2014), and Helal *et al.* (2018) said that the mixing of plants affecting crude fiber (CF) values were decreased as increased portions of *Atriplex*. While, El-Waziry (2007) reported that mixing of fresh *Acacia* and *Atriplex* had increased the degradation of neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and hemicellulose.

The present results showed higher CT content in AS (25.03g/kg DM) than CA (20.58g/kg DM), while AN (15.61g/kg DM) had the least content. The content of CT in AS was lower than this reported by Salem (2005) that was 113 and 63 g/kg DM in the winter and summer seasons, respectively, in comparison, Cassava's CT value ranges from 1.0 to 3.8 g/kg, estimated by Oni *et al.* (2011) while mixing of fresh shrubs indicated that tannins content was lower than three tolerant plants, these agree withEl-Waziry (2007),Fayed *et al.* (2010)andShaker *et al.* (2014) who reported that mixing of fresh shrubs indicated that tannins content was lower in the mixture than that of individual form. CT values are almost in the useful category ranged from 20 to 40 g / kg DM confirmed by Nguyen *et al.* (2005).

Quantitative analysis of leaves' phytochemicals and their combinations are presented in Table (2). Total phenolic content ranged from 99.73 to 121.37 GAE mg/g DM. Average flavonoids content varied from 12.22 to 16.30 mg/g DM, besides the concentration of antioxidant potential differed from 48.21 to 72.84%. Ebana*et al.* (1995), Alali *et al.* (2007), and Aryal *et al.*(2019) reported that total flavonoid content and phytochemicals have potent antimicrobial activity and free radical scavengers that avoid harm to oxidative cells, enhancing the productivity of animals.

As shown in Figures (1 & 2), *in vitro* cumulative GP and CH_4 released for the experimental rations were roughly similar at various incubation times, except for T6, which showed more significant methane release. Potential-GP (P-GP) and Potential-CH_4 (P-CH_4) from *in vitro* fermentation using an exponential equation of the various substrates of the experimental rations are shown in Table (3). Several studies have documented CT content's effects on methane reduction(Abo-Donia and Nagpal, 2015). Conversely, some studies found that tannins in sheep did not influence methanogenesis or even improved methane production (Śliwiński *et al.*, 2002). In the current study, T1 and T2 were seen with the lowest P-GP and P-CH_4 when utilized as fermentation substrates. The volume of total gas and methane released was increased by combining CA with AS or AN or their combinations with ARS. These findings are attributed to CT's presence, where the effect of ingested tannins on gas and methane production is shown in Figure (3). These findings are compatible with Hatew *et al.* (2016), who illustrated that raising CT values reduced peak rates of gas and methane production linearly and evaluated the digestibility of OM *in vitro*. The higher amount of GP found for diet supplemented AN leaves relative to AS or CA having relatively high CT levels (Table 3). Some scholars have pointed out many theories for how methanogenesis is prevented by CT; none of them have been confirmed. The 1st assumption is that CT serves as a drain of hydrogen (Naumann *et al.*, 2013a). The 2nd theory is that CT specifically acts on rumen methanogens (Ng *et al.*, 2016). The 3rd theory is that indirect prevention reduces the supply of nutrients to the rumen's microorganisms. Thereafter, the digestibility of the substrate decreases, which implicitly inhibits microbial communities of the rumen. Because CT binds to proteins (Saminathan *et al.*, 2014), minerals (Lavin, 2012), lipids (Delehanty *et al.*, 2007), or carbohydrates (Soares *et al.*, 2012) also, CT binding to microbial enzymes can modulate their activity (Gonçalves *et al.*, 2011).Naumann *et al.* (2013b) revealed a poor association between the CT-bound protein and decline in CH_4.

In contrast to other therapies, T4, T5, and T6 demonstrated substantial ($P < 0.05$) change in degradability of DM, OM, and NDF, The integration of ARS with AN improved ($P < 0.05$) the benefit of ME relative to CA or AS. These findings, confirmed by Huang *et al.* (2010), revealed that lower pure CTs levels, 20-40 mg g^{-1} DM, may decrease methane production without adverse effects on DM's degradability. Thus, there were negative correlations between IVDMD and the leaves' tannin content.Abo-DoniaandNagpal(2015)reported to the lowering of methane production is correlated with a decrease in DM degradability due to high CT concentrations. Consequently, tannins present in large amounts in foliage leaves in the NDF and ADF fractions and protein binding to the cell wall appear to be agents in digestibility reduction (Reed *et al.*, 1990).

As seen in Table (4), there were positive correlations (r= 0.655 & +0.602, $P < 0.05$) between both microbial protein (MP) & CH_4 and *in vitro* neutral detergent fiber degradability (IVNDFD). Also, between MP and both GP & CH_4 (r= 0.654 & 0.756, $P < 0.05$), and between IVNDFD and GP (r = 0.479, $P< 0.05$). There have also been negative correlations noted between CT and GP (r = -0.202, $P = 0.422$). Abo-Donia and Nagpal (2015) reported a linear lowering GP with rising CT concentrations. When including CT of *L. leucocephala*, GP was decreased up to 43%. Likewise, methane production was reduced by 63%. Besides, Hariadi and Santos (2010) reported that after 24

Table 7. Effect of feeding experimental rations for Barki lambs on some blood serum parameters

Item	Treatments							±SE
	T0	T1	T2	T3	T4	T5	T6	
TP (g/dl)	5.22[c]	6.75[b]	6.35[b]	8.65[a]	7.16[b]	7.75[a]	7.31[a]	±0.40
ALB (g/dl)	2.42 [c]	3.58[b]	3.25[b]	4.82[a]	3.78[b]	4.08[a]	4.05[a]	±0.26
GLO (g/dl)	2.33	3.25	3.15	3.77	3.59	3.89	3.60	±0.19
ALB/GLO	0.92[c]	1.14[b]	1.09[b]	1.41[a]	1.21[b]	1.65[a]	1.35[b]	±0.07
Urea-N (mg/dl)	30.10[a]	24.10[b]	21.80[c]	24.95[b]	24.13[b]	24.15[b]	23.59[b]	±0.49
Creatinine (mg/dl)	1.31[b]	1.82[a]	1.82[a]	1.43[b]	1.39[b]	1.89[a]	1.98[a]	±0.12
AST (Ul)	34.29	31.48	30.89	32.85	31.04	31.99	30.95	±0.50
ALT (Ul)	19.80[a]	17.20[c]	18.10[b]	18.62[a]	17.96[b]	17.60[b]	17.51[b]	±0.38

a, b and c Means in the same row with different superscript are significantly different (*P*< 0.05).TP= Total protein; ALB= Albumin; GLO= Globulin; Urea-N= Urea nitrogen; AST= Aspartate aminotransferase; ALT= alanine aminotransferase

Table 8. Growth performance and economic efficiency of Barki lambs fed experimental rations.

Item	Treatments							±SE
	T0	T1	T2	T3	T4	T5	T6	
No. of lambs	5	5	5	5	5	5	5	
Feeding period, weeks	18	18	18	18	18	18	18	
Initial weight, (kg)	20.12	20.28	20.32	20.08	20.02	20.32	20.02	±0.20
Final weight, (kg)	36.74[c]	40.68[b]	37.73[c]	42.09[a]	39.62[b]	41.39[a]	39.90[b]	±0.50
Total gain, (kg)	16.62[c]	20.40[b]	17.41[c]	22.01[a]	19.60[b]	21.07[a]	19.88[b]	±0.38
Daily body gain, (g)	131.90[d]	161.90[b]	138.17[d]	174.68[a]	155.55[c]	167.22[b]	157.77[c]	±0.003
Economic efficiency								
Total feed coast(as feed) /h/d (L.E.)	1.30	1.33	1.28	1.34	1.31	1.32	1.23	
Price of daily gain (L.E.)	5.10	4.50	4.59	5.13	4.65	4.89	3.93	
Efficiency (%)	3.20	3.47	3.67	3.92	3.64	3.80	3.28	

a, b and c Means in the same row with different superscript are significantly different (*P*< 0.05).

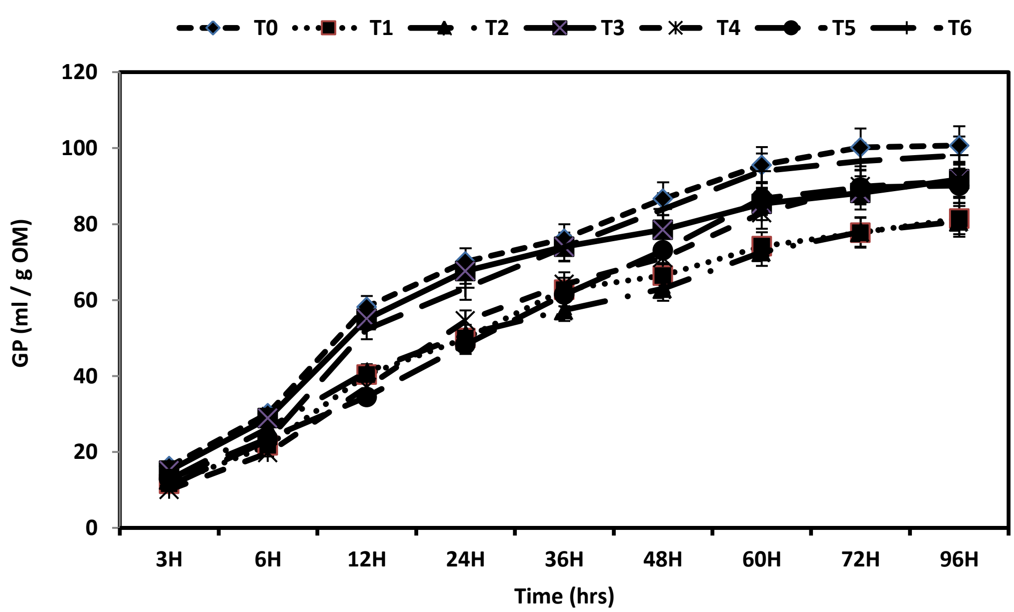

Fig. 1. Influence of using some foliage leaves on cumulative gas production (GP) at different incubation times in lambs'rumen fluid.

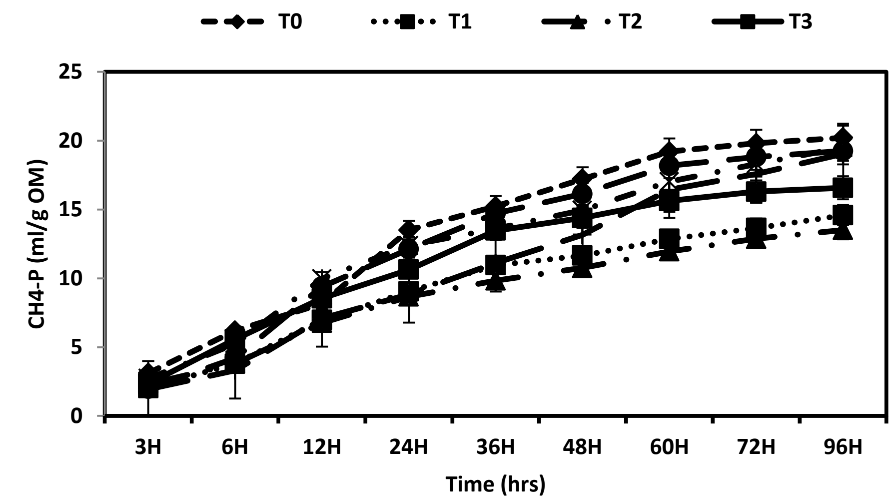

Fig. 2. Influence of using some foliage leaves on cumulative CH_4 production at different incubation times in lambs' rumen fluid**.**

hours of incubation, a strong negative correlation (r = -0.60) between total tannins and in vitro CH_4 production was recorded. The decrease in the overall production of methane may be attributed to the reduction in DM's degradability that might be related to the inclusion of CT-containing leaves in the tested diet mixtures provided to animals, as confirmed that by Gemeda and Hassen (2015).

Table (5) presents pH, NH3-N, SCFA's profile, microbial protein, and bacterial &protozoa counts. The lower CT content has increased NH3-N, SCFA, MP, and bacteria levels, and vice versa. These findings were agreed with Naumann et al. (2017), who illustrated an inverse relationship between CT and SCFA's (r = 0.52). Tannins can improve the effectiveness of urea recycled into the rumen for ruminal NH3-N concentration. Hence, protein degradation rate and delamination in the rumen are reduced. In the current investigation, various treatments did no influence on pH, protozoa count, propionic, and butyric count. Beauchemin*et al.*(2007) found that 20 g kg^{-1} DM extract of quebracho tannin supplementation reduced acetic acid level and acetate/propionate ratio in the ruminant. The reduction in the acetate/propionate ratio due to the higher use of hydrogen to form propionate may be correlated with the inhibition of methane production by CT (Naumann *et al.,* 2017). Gunun*et al.*(2014) confirmed that the protozoan population was significantly reduced with CT supplementation *in vitro.* Bhatta *et al.*(2015) illustrated that the numbers of rumen ciliary protozoa reduced when the forage included CT of *Azardirachta indica* and *Ficus bengalensis* at levels of 13.8 and 26%, respectively.

Data in Table (6) revealed that the use of foliage leaves separately with ARS raised significantly (P< 0.05) the level of feed refusal, particularly for T1 and T2 groups. Also, feed refusal decreased when various foliage was combined. Thus, feed intake reduced significantly (P<0.05) when the foliage was separately introduced into the experimental rations.Waghorn *et al.* (1994) illustrated that ingestion of medium or low CT (< 50 g kg-1 DM) plant species does not appear to affect feed intake. In contrast, it was substantially reduced by elevated CT content (> 50 g kg^{-1} DM). Also, sheep and camels are more resistant than other livestock to tannin-rich feed. Salem (2005) reported that utilizing sheep as models for livestock to characterize tanniniferous diets. In the current study, feed consumption and feed refused were due to the rising tannin content in some foliage leaves. The utilization of CT intake correlates to a similar feed refusal pattern, and the actual feed intake for the experimental diets is illustrated in Fig.(4).

The digestion experiments (Table 6) reported that the AN-containing diet was substantially superior to other diets. T1 and T3 increased the digestion coefficient of DM, OM, ADF, and NDF % relative to T2. There were no significant variations among the experimental diets were found in protein digestion. This result could happen first because the CT significantly decreased the proteolytic enzyme's function and bacterial growth in sheep's rumen (Jones *et al.,* 1993). Second, this could be attributed to condensed tannin-protein complexes produced at the rumen's pH level, which raises the obtainable protein known as ruminal bypass protein in the gastrointestinal tract (Min *et al.*,2003).

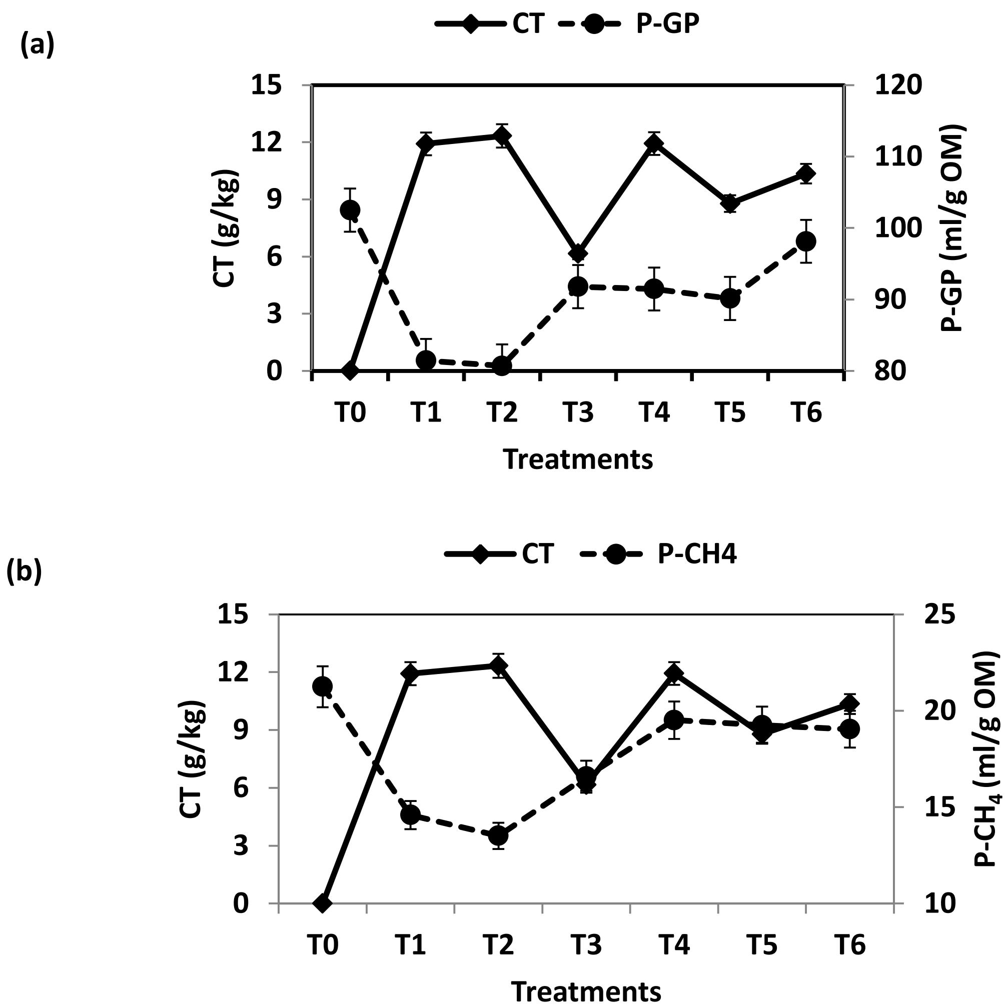

Fig. 3 Effect of condensed tannins (CT) consumption on (a) GP production and (b) CH_4 emitted

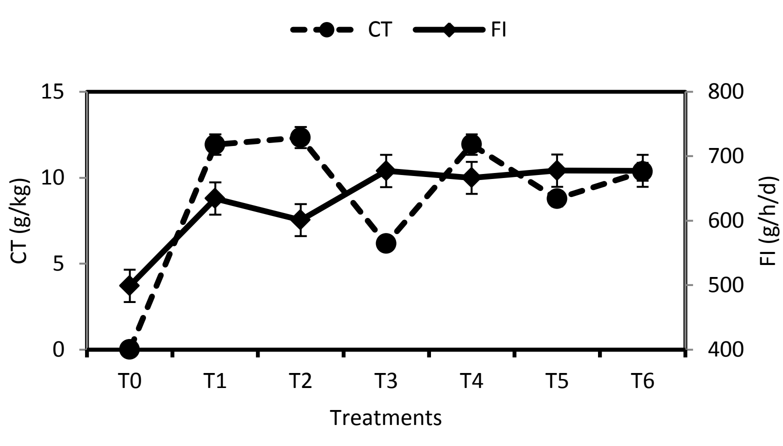

Fig. 4 Effect of condensed tannins (CT) consumption on feed intake (FI)

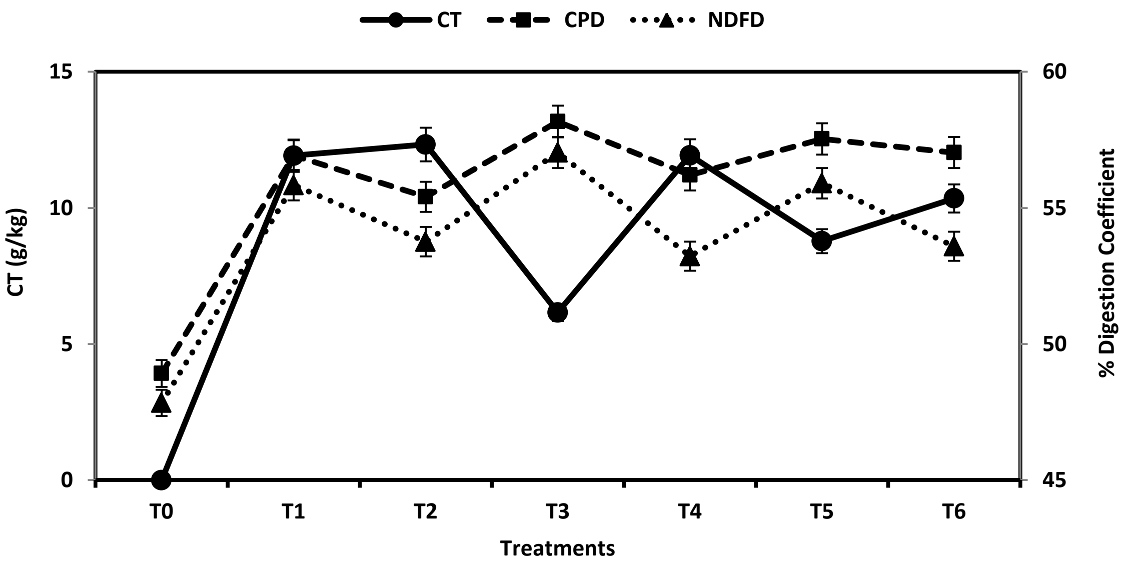

Fig. 5 Effect of condensedtannins(CT) consumption on crud protein (CP) and NDF digestibility

Higher tannin levels (> 50 g/kg DM) can become an anti-nutritional factor in plant materials. They may decrease feed consumption and digestibility (Barry and McNabb, 1999) or milk production and growth rate (Waghorn *et al.*, 1994). Therefore, tannins' useful influences in sheep are related to the flow and absorption of amino acids, especially for sheep fed on 2-4% tannin in the diets (Min *et al.*, 1999). Regarding fiber digestion, many researchers suggested that fiber degradation in the rumen can be significantly decreased in animals eating tannin-rich diets (McSweeney *et al.*, 2001). CT may reduce fiber digestibility by complicating with lignocelluloses, suppressing microbial digestibility (Auwal *et al.*, 2014), or decreasing cellulolytic micro-organisms amount(Kavitha *et al.*, 2013).

A feeding diet that includes CA or AN foliage leaves with ARS had a higher nitrogen intake than AS. The release of nitrogen follows the same food intake pattern, where nitrogen production was slightly lower (P< 0.05) with T2 (containing AS) compared to other treatments. N balance results found a significant (P< 0.05) rise in T3, T4, and T5 relative to control. These results are in agreement with Grainger *et al.* (2009). These findings might be attributed to foliage leaves' CT content, as presented in Fig.(5). The dissonance may interfere with the CT influences on nitrogen balance due to CT's source and molecular weight (Naumann *et al.*,2013b). TDN and DCP varied significantly (P<0.05) among experimental groups. Feeding AN leaves mixed with ARS or supplemented with CA and AS may be greater than other experimental foliage.

The results provided in Table (7) showed that the blood parameters of treated lambs had minor variations. The lowest total protein (TP), albumin (ALB), globulin (GLO), and ALB/GLO ratio levels were observed with T2. These results are in agreement with Eissa*et al.* (2016), Shaker *et al.* (2014),and Sadek *et al.* (2020). This decline of TP in treated animals may be attributed to the plants' protein and tannins' content. Reed *et al.* (1990) found that high tannins in Acacia would likely reduce crude protein's digestibility. Mahmoud (2001) revealed that reducing GLO level in sheep could be attributed to a high tannins level that forms complexes with ration. So, T3 was greater than T2 in the blood protein profile. The same pattern was observed in serum urea-N. ALT and AST levels were greater with salt-tolerant shrubs mixture groups. The findings suggest that the measured blood parameters show minor variations because of shrubs source, where all values were within the standard ranges.

Feed efficiency and growth performance of Barki lambs fed experimental diets are showed in Table (8). The findings showed that a wide range of CT-dietary levels varied from 6.18 to 25.03 g/kg DM (Table 1) that enhanced lambs' growth rate. There were significant differences (P< 0.05) among experimental groups in lambs' final weight, total body gain, and daily gain. T3 has the highest values (42.09 kg, 22.01 kg, & 174.68 g, respectively), while T1 has the lowest values (36.74 kg, 16.62 kg, and 131.90 g, respectively). These improvements could be attributed to the raised benefit of ME of treated diets compared to the ARS diet, which also enhanced the digestibility of DM, OM, ADF, and NDF%. There is a negative association between the elevation of CT forages level (> 50 g CT / kg

DM) and voluntary intake, palatability, digestion, and nitrogen retention in sheep. Concerning the feeding of ruminants, tannins are considered beneficial effects, such as increased protein intake, growth rate, wool and milk production, improved fertility, and animal welfare (Mueller-Harvey, 2006). This effect was attributed to the rise in proteins available post-rumen (Barry and McNabb, 1999; Eissa *et al.*, 2015,Sadek *et al.*, 2020).Shaker *et al.* (2014) said that such a salt-tolerant plant mixture's potentiality fulfills the animal requirements to maintain their body weight.

Economic efficiency (EE) represented in Table (8) indicated that feed cost/kg body gain and economic efficiency improved with T3 (3.92%) relative to other treatments. Eissa *et al.* (2015& 2016) and Sadek *et al.* (2020) found that EE was significantly enhanced by substituting legume trees with ammoniated wheat straw in sheep diets.

Conclusion

It could be concluded that a mixture of treated ammoniated rice straw with different proportions of tree foliage leaves (*Cassava*, *Acacia saligna,* and *Acacia neloitca* or their mixture) could reduce the shortage of animal food in Egyptian arid region.They are a good indicator as feeding for sheep, help overcome CT's negative effect, decrease rumen-N absorption, reduce CH4 emission for livestock and increase the utilization of the available unpalatable salt-tolerant plants without any adverse effect on performance. So, we could use a mixture of treated ammoniated rice straw with tannin-rich foliage leaves as part of the animal diets better than ammoniated rice straw only. However, researchers still need to develop a series of searches from which recommendations can be developed.

Animal Welfare Statement

All research procedures were carried out in compliance with the standards set forth guidelines for the care and use of experimental animals by the Animal Ethics Committee of APRI, ARC, Egypt. Cannulated Barki sheep were well treated under good veterinary care. The cannulas have been installed by APRI ethical approval to the animals after sterilization and anaesthesia well with the provision of all appropriate conditions and remained under intensive veterinary care during the experimental period in Animal Production Research Station Borg El-Arab.All animals were kept under the same management and hygienic conditions. Lambs were kept in a ventilated barn and housed separately in shaded pens with concrete flooring and natural lighting through windows along either side of the building. The pens were cleaned in the morning and afternoon after feeding. Diets were provided at 8 am and 3.00 pm. and refusals were collected 24 h later. Fresh water and mineral licks are provided to animals as free choices.

Declaration of interest

All authors declare that there is no conflict of interest in this study.

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