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Effect of Replacing Alfalfa Hay with Acacia Foliage on the Growth Performance, In Vitro Gas Production and Rumen Fermentation in Goats

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Abstract | This work examined the effect of substituting acacia for traditional alfalfa hay as an alternative goat fodder on in vitro gas production, pH, ammonia nitrogen (NH_3-N), total volatile fatty acids (VFA) concentrations and growth of goats . A total of 12 cannulated Ardi goats (initial weight: 27.66±0.28 kg) were used in gas production and rumen fermentation trials, and 60 male kid goats for growth performance trial. The goats were randomly assigned to 4 groups. Goats in group 1 were fed a control diet (C) containing 40% alfalfa hay and 60% concentrate mixture. Goats in groups 2, 3 and 4 were fed diets in which 20, 30 and 40% (A20, A30 and A40) of the control amount of alfalfa hay was replaced with acacia, respectively. There were significant differences in gas production and potential degradability (a+b) among the experimental diets. The lowest gas production rate (c) was recorded for the A30 diet, and the highest value was recorded for the A40 diet. There were no significant differences in the NH_3-N concentrations among the experimental diets after feeding, but significant differences in the VFA concentrations were observed. There were significant differences in the average daily gain between the A40 diet and other diets. The greatest daily gain was observed for the C diet, followed by the A20, A30 and A40 diets. Thus, there was no negative impact of replacing alfalfa with acacia on digestion up to 30% in feeding goats.

Keywords | Acacia foliage, Alfalfa hay,Gas production, Rumen fermentation, Growth performance

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INTRODUCTION

Some countries suffer from a lack of water resources, leading to a shortage of green fodder and more depending on concentrate feeds. These changes in turn lead to higher costs of animal feeds and a subsequent increase in the prices of meat and milk. It is therefore necessary for nutritionists and researchers to search for other feed alternatives to reduce the cost of animal feeds and hence reduce the prices of milk and meat. Many leguminous fodder trees and shrubs have high protein levels and are potential supplements that may overcome the nutrient deficiencies in animal feeds. Some of these fodders have anti-nutritional factors, such as tannins and other secondary compounds, which can be controlled (El-Waziry, 2007; Abarghuei et al., 2010; Abarghuei et al., 2015; Abdu et al., 2012; Sharifi et al., 2013; Makkar, 2003; Min et al., 2003; Reed, 1995; Brown et al., 2017). Acacia (*Acacia saligna*), a leguminous shrub species are available in numerous countries, it had high phenolic compounds such as tannins (condensed tannins and hydrolysable tannins), and it can be used in ruminant diets to protect highly degradable protein sources such as soybean meal (SBM) from degrading in the rumen (Abarghuei et al., 2015). However, this goal may have been pursued to an extreme, and it is now apparent that the rumen is occasionally deficient in nitrogen from amino acids

(AAs). Therefore, additional experiments are needed to determine the best treatment for ruminant diets. Potential solutions may be physical treatments (such as heat treatment) or physiochemical treatments. This latter treatment class includes the addition of shrubs to the protein sources and the extraction of phenolic components from shrubs before they are added to the diet. Therefore, the objective of this work was to evaluate the effect of replacing alfalfa hay with acacia foliage as a non-traditional fodder (alternative green fodder) on gas production, rumen fermentation and growth performance.

MATERIALS AND METHODS

Acacia foliage was collected from Wadi Hanifa in the Riyadh region, Kingdom of Saudi Arabia, from March to June 2014. The acacia foliage was sun-dried, ground and chemically analysed. Then it was incorporated into experimental diets at 0, 20, 30 and 40% (C, A20, A30 and A40 diets, respectively) and mixed with the experimental diets. A total of 12 cannulated Ardi male goats were used for rumen fermentation and gas production trials. Goats were randomly assigned to 4 groups; 3 animals each. The animals were fed alfalfa hay for 2 weeks prior to being assigned the experimental diets. The animals were ear tagged and vaccinated with anthelminthic treatments during these 2 weeks. The components of the experimental diets are shown in Table 1. In vitro gas production tests were conducted (Menke and Steingass 1988) using rumen fluid collected from the cannulated goats before the morning feeding. These tests were used to determine the extent of degradation associated with the experimental diets. Buffer and mineral solutions were prepared and placed in a water bath at 39°C under continuous flushing with CO_2. The rumen fluid from all the goats was combined and stored in pre-warmed insulted bottles, homogenized in a laboratory blender, filtered through four layers of cheesecloth and purged with CO_2. The well-mixed and CO_2-flushed rumen fluid was added to the buffer solution (1:2 v/v), which was maintained in a water bath at 39°C, and mixed. Air-dried samples (200 mg) of the experimental diets were accurately weighed and added into syringes fitted with plungers. Buffered rumen fluid (30 ml) was pipetted into each syringe containing a sample, and the syringes were immediately placed in a 39°C water bath (Blümmel and Ørskov 1993). Three syringes with only buffered rumen fluid were incubated and considered as blanks. The syringes were gently shaken every 2 h, and the incubation was terminated after recording the 72-h gas volume. Gas production was recorded after 3, 6, 9, 12, 24, 48 and 72 h of incubation. Total gas values were corrected with the blank incubation, gas values were expressed in ml/200 mg of DM. Cumulative gas production was fitted iteratively to the exponential model proposed by Ørskov and McDonald (1979). Metabolizable energy (ME), net energy (NE) and organic matter digestibility (OMD) were calculated using gas production after 24 h of incubation and chemical analysis according to Menke and Steingass (1988). Microbial protein (MP) was calculated as g/kg of digestible organic matter according to Czerkawski, (1986).

Table 1: The components, proximate analysis and fibre fractions of the experimental diets

	Diets[1]			
Ingredients (g/kg)	**C**	**A20**	**A30**	**A40**
Alfalfa hay	400	200	100	0
Acacia	0	200	300	400
Soya bean meal	70	70	70	70
Barley	210	210	210	210
Corn	304	202	150	100
Bran	0	100	150	200
Salt	5	5	5	5
Minerals &vit.	3	3	3	3
Limestone	8	10	12	12
Proximate analysis (g/kg)				
Dry matter	917.7	919.8	917.5	917.9
Ash	67.4	89.6	101.6	111.0
Crude protein	150.3	148.3	148.9	146.4
Ether extract	25.1	25.5	23.7	25.6
Crude fibre	121.8	113.8	112.7	108.2
Nitrogen free extract	635.4	622.8	613.1	608.8
Fibre Fractions				
Neutral detergent fibre	231.4	257.3	273.1	277.6
Acid detergent fibre	147.4	146.5	152.5	151.5

[1]C, control diet without acacia; A20, 20% acacia replaced with alfalfa hay in control diet; A30; 30% acacia replaced with alfalfa hay in control diet; A40, 40% acacia replaced with alfalfa hay in control diet.

The rumen contents were collected from cannulated goats before feeding and 1, 3 and 6 h after feeding to determine the ammonia-N (NH_3-N), total volatile fatty acids (VFA's) concentrations and pH. NH_3-N concentrations were determined according to the procedure of AOAC (2004). Total VFAs were determined using steam distillation (Warner, 1964). The pH was measured immediately using a glass electrode.

Sixty male kid goats were used for the growth performance trial. The kids were randomly allocated by weight to 4 treatment groups with 15 kids each. Each group of goats was equally divided into five replicates; each replicate three kids. All kids were housed in a floored pen in an open-sided building. The goats were fed the experimental diet. Upon initiation of the feeding trial, an adequate amo-

Table 2: Cumulative gas production (ml) produced from the experimental diets with or without acacia during 72 h incubation

Diets[1]	Incubation Times (h)						
	3	6	9	12	24	48	72
C	5.94[a]	13.00[ab]	15.89[b]	16.56[b]	18.89[a]	19.44[b]	19.56[b]
A20	6.44[a]	13.78[a]	17.89[a]	18.78[a]	19.22[a]	21.33[a]	21.89[a]
A30	4.83[b]	9.44[c]	12.11[c]	13.78[c]	14.89[b]	16.00[d]	16.89[d]
A40	5.33[b]	11.56[b]	14.78[b]	14.44[c]	16.00[b]	17.89[c]	18.00[c]
SEM[2]	0.143	0.371	0.399	0.382	0.366	0.372	0.362
P-Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[1]C, control diet without acacia; A20, 20% acacia replaced with alfalfa hay in control diet; A30; 30% acacia replaced with alfalfa hay in control diet; A40, 40% acacia replaced with alfalfa hay in control diet.
[2]Standard error of mean.
[a-d] Mean values within a column with unlike superscript letters were significantly different (p<0.05).

Table 3: Parameters of gas production produced from the experimental diets with or without acacia during 72 h incubation

	Diets[1]					
Parameters[2]	C	A20	A30	A40	SEM[3]	P-Value
a+b (ml)	19.29[b]	21.13[a]	16.34[d]	17.48[c]	0.345	< 0.0001
c (ml/h)	0.224[a]	0.228[a]	0.162[b]	0.237[a]	0.009	0.068

[1]C, control diet without acacia; A20, 20% acacia replaced with alfalfa hay in control diet; A30; 30% acacia replaced with alfalfa hay in control diet; A40, 40% acacia replaced with alfalfa hay in control diet.
[2]Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979), Gas (Y) = a + b (1-exp^{-ct}), where; a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, a+b = potential degradability, c the gas production rate constant for the insoluble fraction (b), t = incubation time.
[3]Standard error of means.
[a-d] Mean values within a row with unlike superscript letters were significantly different (p<0.05).

Table 4: Predicted of metabolizable energy (ME), net energy (NE), organic matter digestibility (OMD) and microbial protein (MP) in vitro from the experimental diets with or without acacia after 24 h incubation

	Diets[1]					
Items	C	A20	A30	A40	SEM[2]	P-Value
ME (MJ/kg DM)	5.29[a]	5.16[a]	4.35[b]	4.47[b]	0.08	< 0.0001
NE (MJ/kg DM)	3.86[a]	3.88[a]	3.49[b]	3.59[c]	0.03	< 0.0001
OMD (%)	38.87[a]	39.23[a]	35.48[b]	36.41[b]	0.32	< 0.0001
MP (g/kg OMD) [3]	46.89[a]	47.32[a]	42.87[b]	43.93[b]	0.38	< 0.0001

[1]C, control diet without acacia; A20, 20% acacia replaced with alfalfa hay in control diet; A30; 30% acacia replaced with alfalfa hay in control diet; A40, 40% acacia replaced with alfalfa hay in control diet.
[2]Standard error of means.
[3]MP (g/kg OMD) according to Czerkawski[14].
[a,b,c] Mean values within a row with unlike superscript letters were significantly different (p<0.05).

unt of feed was provided. Data of feed consumption were recorded weekly, and the goats were weighed once per fortnight before feeding.

Samples of the experimental diets were chemically for moisture, ash, ether extract, crude fibre and crude protein according to the procedure of AOAC (2004). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to the method of Van Soest et al. (1991). The extractable total phenols and total , condensed tannins in acacia were determined as described by Makker and Goodchild (1996). The data were subjected to statistical analysis using SAS (1998).

RESULTS

The proximate analysis and fibre fractions of the experimental diets are presented in Table 1. The total phenols, total tannins and condensed tannins in acacia were 5.4, 3.9 and 3.5%, respectively.

Cumulative gas production for the experimental diets with

Table 5: Effect of the experimental diets with or without acacia on pH in the rumen of Ardi goats

	Diets[1]					
Feeding Times	**C**	**A20**	**A30**	**A40**	**SEM[2]**	**P-Value**
0h	6.26	6.50	6.13	6.43	0.08	0.400
1h	6.13	6.09	5.84	6.18	0.10	0.738
3h	6.06	6.18	5.96	6.19	0.07	0.717
6h	5.86	6.02	5.90	5.99	0.08	0.917

[1]C, control diet without acacia; A20, 20% acacia replaced with alfalfa hay in control diet; A30; 30% acacia replaced with alfalfa hay in control diet; A40, 40% acacia replaced with alfalfa hay in control diet.
[2]Standard error of mean.

Table 6: Effect of the experimental diets with or without acacia on ammonia nitrogen concentrations (mg/ 100 ml rumen liquid) in the rumen of Ardi goats

	Diets[1]					
Feeding Times	**C**	**A20**	**A30**	**A40**	**SEM[2]**	**P-Value**
0h	9.82^{a}	10.15^{a}	7.34^{b}	7.00^{b}	0.46	0.095
1h	12.17	9.96	10.56	9.90	1.19	0.936
3h	7.70	8.25	7.46	7.36	0.78	0.902
6h	8.26	9.12	8.13	8.30	0.43	0.981

[1]C, control diet without acacia; A20, 20% acacia replaced with alfalfa hay in control diet; A30; 30% acacia replaced with alfalfa hay in control diet; A40, 40% acacia replaced with alfalfa hay in control diet.
[2]Standard error mean.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($p<0.05$).

Table 7: Effect of the experimental diets with or without acacia on volatile fatty acids concentrations (mmol/ 100 ml rumen liquid) in the rumen of Ardi goats

	Diets[1]					
Feeding Times	**C**	**A20**	**A30**	**A40**	**SEM[2]**	**P-Value**
0h	2.58^{b}	3.13^{a}	2.64^{b}	2.76^{ab}	0.12	0.117
1h	4.18^{a}	3.50^{ab}	2.39^{c}	3.19^{bc}	0.19	0.002
3h	3.05^{b}	3.77^{a}	2.11^{c}	3.64^{ab}	0.17	0.0002
6h	3.10^{b}	3.80^{a}	3.05^{b}	3.51^{ab}	0.12	0.068

[1]C, control diet without acacia; A20, 20% acacia replaced with alfalfa hay in control diet; A30; 30% acacia replaced with alfalfa hay in control diet; A40, 40% acacia replaced with alfalfa hay in control diet.
[2]Standard error mean.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($p<0.05$).

Table 8: Performance growth of Ardi male goats fed the experimental diets[1]

	Diets[2]					
Item	**C**	**A20**	**A30**	**A40**	**SEM[3]**	**P-Value**
Feeding period, day	84	84	84	84		
Initial weight, kg	20.66	20.32	20.38	20.60	0.265	0.9684
Final weight, kg	34.70^{a}	31.05^{b}	30.68^{b}	27.31^{c}	0.752	0.0008
DM intake, kg d^{-1}	1.08^{a}	1.03^{a}	1.04^{a}	0.902^{b}	0.038	0.0113
Gain, kg d^{-1}	0.167^{a}	0.128^{b}	0.123^{b}	0.080^{c}	0.018	0.0003

[1]Values represent means of 5 pens, 3 kids each per treatment.
[2]C, control diet without acacia; A20, 20% acacia replaced with alfalfa hay in control diet; A30; 30% acacia replaced with alfalfa hay in control diet; A40, 40% acacia replaced with alfalfa hay in control diet.
[3]Standard error of means.
[a, b, c] Means within rows not sharing the same letter (s) differ ($p<0.05$).

or without acacia during 72 h of incubation is shown in Table 2. There were significant differences in gas production

among the four diets; moreover, dietary acacia supplementation had an effect on gas production during incubation. The potential degradability (a+b) of the experimental diets is presented in Table 3. The values were 16.34, 17.48, 19.29 and 21.13 ml for the A30, A40, C and A20 diets, respectively, with significant ($p<0.05$) differences. The lowest gas production rate (c) was recorded for A30 diet, followed by C, A20 and A40 diets (Table 3). The predicted values of ME, NE, OMD and MP are shown in Table 4. The statistical analysis revealed significant differences in ME, NE, OMD and MP among the experimental diets.

There were no significant differences in pH among the experimental diets before feeding nor 1, 3 and 6 h after feeding (Table 5). Table (6) showed that the NH_3-N concentrations (mg/100 ml of rumen fluid) in goats fed the experimental diets. There were no significant differences among the experimental diets after feeding, but significant differences did occurred among the diets before feeding (Table 6). The statistical analysis revealed significant differences in total VFA concentration among all diets (Table 7).

Table 8 shows the growth performance of kids fed the experimental diets. There were significant differences in dry matter intake between A40 diet and C, A20 and A30 diets. The lowest value of dry matter intake was recorded for the A40 diet. There was a significant difference in the final weight of kids between the control Cdiet and other diets, while there was no significant difference between A20 and A30 diets. The highest average daily gain of kids was recorded for C diet, followed by A20, A30 and A40 diets, but there was no significant ($p>0.05$) difference between A20 and A30 diets.

DISCUSSION

Protein metabolism in the rumen is the result of the metabolic activity of ruminal microorganisms. Consequently, controlling the degradation of dietary protein to balance the protein supply from microbial synthesis is of great interest to ruminant nutritionists and microbiologists (El-Waziry, 2007; El-Waziry et al., 2005). The improvement of ruminant protein is a matter of great practical concern; the amounts of protein and AA's delivered to the intestine commonly limit the productivity of ruminant animals, as shown by their responses to post-ruminal supplementation (Ipharraguerre et al., 2005). It seems that rumen-protected soy products must be combined with other ruminal undegradable protein (RUP) sources and/or rumen-protected AA's that complement their essential AA profiles. The crude protein source modulates the intestinal supply of AA's by affecting the passage of RUP and microbial nitrogen (metabolizable protein) to the lower gastrointestinal tract (Ipharraguerre et al., 2005). In addition, the contribution of RUP to the ruminal outflow of total protein and its AA composition impacts the pattern of AA's available for absorption in the small intestine. This limitation has motivated the development of several methods for decreasing the ruminal degradability of proteins in SBM and soybean seeds. Some of the most effective methods for this purpose that are approved for commercial use involve the control administration of heat to soybeans and the application of heat when adding reducing sugars or tannic acid treatments. Tannins are polyphenolic compounds of plant origin that bind with proteins. There are two distinct types of tannins: hydrolysable tannins and condensed tannins. Treating SBM with tannic acid (hydrolysable tannins) using an in vitro gas production technique reduces the protein degradation of SBM and the kinetics of gas production (El-Waziry et al., 2005). However, there is still a need to perfect the conditions for protein protection with both hydrolysable and condensed tannins. This study used both hydrolysable and condensed tannins by adding acacia to experimental diets. There was high gas production after the first six hours of incubation for all the experimental diets in the present study (Table 2). The high gas production at the earlier incubation time was attributed to the fermentation of soluble and rapidly fermentable components of the diet, mainly soluble protein and soluble carbohydrates. There were significant differences ($p<0.05$) in gas production among the experimental diets; however, acacia-containing diets (except the A20 diet) reduced gas production during incubation period compared to the C diet, and the lowest gas production during the 72 h incubation period was recorded for the A30. The decrease in gas production resulting from the acacia-containing diet was due to the ability of the tannins to strongly bind to protein (Sharifi et al., 2013; Mcsweeny et al., 2001; Hassanat & Benchaar, 2013). The same pattern was observed for the A30 diet in terms of a+b and the c (Table 3); therefore, the A30 diet had the lowest values of a+b and c. A similar reduction in the c by tannin supplementation has been reported in studies using quebracho tannins (El-Waziry, 2007; Sharifi et al., 2013; Min et al., 2003). Gas production, a+b and the c were low due to the presence of tannins in the acacia-containing diets, especially the A30 diet. Tannins may affect the utilization of protein in forages by reducing the digestive enzymatic activity in the rumen and binding to feed protein (Mcsweeny et al., 2001; Tabacco et al., 2006; Khattab, 2007). There were significant differences in the predicted ME, NE, OMD and MP between the C/A20 and A30/A40 diets (Table 4). The values of ME, NE, OMD and MP attributed to the amount of gas produced at 24 h for the A30 and A40 diets whereas it lower than those for the C and A20 diets because the energy values and OMD were calculated from the amount of gas produced after 24 h of incubation period and MP was calculated using OMD (Menke and Steingass, 1988; Tabacco et al., 2006). The low values of MP in the acacia-containing

diets are in agreement with Khattab (2007) and may be due to the direct adverse effect of acacia tannins on rumen fermentation and the limited nutrient supply for MP synthesis (Sharifi et al., 2013). None of the acacia-containing diets had an effect on pH before feeding nor after feeding (Table 5). The rumen pH values obtained in the present study were in the favourable range for microbial activity. In the current study, there were no significant differences in NH_3-N concentration among the experimental diets after feeding, although the concentration did decrease in response to acacia-containing diets (Table 6). The lowest value was recorded for the A30 diet, which can be attributed to the presence of more amount tannins in acacia that decreased the degradation of protein in the diet (Sharifi et al., 2013; Hassanat and Benchaar, 2013; Khattab, 2007; Min et al., 2000; Molan et al., 2001). These results are consistent with the a+b and c observed for the same diet (A30). Rumen NH_3-N concentrations for all the experimental diets were higher than the threshold (5 mg/100 ml) for maximal microbial growth (Satter and Slyter, 1974). There were significant differences in total VFAs among the experimental diets in the present study (Table 7). The lowest VFA values were recorded for the A30 diet, which was consistent with the results of gas production, a+b, the c, ME, NE, OMD, MP, and NH_3-N due to the presence of tannins in the acacia-containing diets. The formation of undegradable complexes between tannins and proteins and/or carbohydrates may have reduced the amount of substrate available for fermentation (Fall-Toure et al., 1998). The escape of undegradable complexes from the rumen to the abomasum could also explain the low concentrations of protein in the rumen fluid (Reed, 1995; Wiegand et al., 1996). The study of Woodward and Reed (1997) indicated that the low and steady production of VFAs and ruminal NH_3-N may resulted from the high fibre content in acacia and from the inhibition of rumen microbes by tannins, which lead to a low rate of deamination (Hassanat and Benchaar, 2013).

Concerning feed intake, there was a significant ($p<0.05$) difference between the A40 diet and the C, A20 and A30 diets; while no significant ($p>0.05$) differences among the C, A20 and A30 diets. However feeding animals with plant species containing a high tannin content appears to significantly reduce feed intake. The lowest values of gain and final weight were recorded in goats fed the A40 diet, which confirmed the adverse relationship between gain and tannin content. The study of Gebru et al. (2016) reported that adding *Acacia saligna* to the diets of rams improved their body weight. In the same manner, Ahmed et al. (2015a,b) established that a mixture of *Atriplex nummularia* and *Acacia saligna* (1:1) could replace berseem (*Medicago sativa*) hay by up to 50% without affecting the performance of Bakri lambs.

CONCLUSION

According to the results of the present study, the presence of tannins in acacia-containing diets, especially the A30 diet, resulted in lower gas production, a+b and c values. Tannins may affect the utilization of nitrogen in diets containing a protein source and forage by binding to the protein and reducing enzymatic activity in the rumen, causing a decrease in protein degradation and NH_3-N concentration. Consequently, acacia-containing diets can increase the amount of protein and AAs post-ruminal leading to improved animal performance, as indicated by the greatest daily gain for the C diet, followed by the A20 and A30 diets. Moreover, there was no adverse effect on the digestion of diets by goats when acacia replaced alfalfa hay by up to 30%.

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CONFILCT OF INTEREST

The authors declare no competing financial interest.

AUTHORS CONTRIBUTION

The manuscript was written by Ahmed M. El-Waziry, Abdallah N. Al-Owaimer and edited by Saeid M. Basmaeil. Planning and execution of this work were under the supervision of Ahmed M. El-Waziry; Hassan M. Metwally, Muttaher H. Ali, Muqhim S. Al-Harbi collected the samples and conducted an experiment under the supervision of Ahmed M. El-Waziry, Saeid M. Basmaeil, Abdallah N. Al-Owaimer. All authors read and approved the final manuscript.

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