EFFECT OF ENSILING OF ACACIA SALIGNA AND LEUCAENA LEUCOCEPHALA LEAVES WITH DIFFERENT LEVELS OF UREA ON THE CHEMICAL COMPOSITION, IN VITRO GAS PRODUCTION, ENERGY VALUES AND ORGANIC MATTER DIGESTIBILITY

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ABSTRACT

The effects of ensiling of acacia (Acacia saligna, AC) and leucaena (Leucaena leucocephala, LE) leaves with different levels of urea (U, 0, 1, 3 or 5%) on gas production, energy value and organic matter digestibility (OMD%) of AC and LE were assessed. The acacia and leucaena were ensiled for 35 days. Ground samples (200 mg DM) of the ensiled materials from the eight treatments were incubated in glass syringes with rumen fluid obtained from fistulated sheep fed berseem hay and commercial concentrate mixture twice a day. Cumulative gas production was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation and the kinetics of gas production was described by using the equation: Gas (Y) = a + b (1 - a)exp-cs). Ensiling of AC and LE leaves with U increased crude protein and ash, while the contents of tested samples of total phenol (TP), total tannins (TT) and condensed tannins (CT) were decreased. Also, ensiling of AC and LE leaves with Usignificantly (P < 0.05) decreased gas production. The gas production volume was significantly (P < 0.05) higher for ensiled AC and LE leaves without U than ensiled AC with 5%U or ensiled LE with 3%U. The maximum rate of gas production increased after ensiling AC and LE leaves with 5% and 3% U, respectively. The calculated values of metabolizable energy (ME) and net energy (NE) were significantly (P<0.05) increased for ensiled AC with 3 and 5%U, while ensiled LE with Uwas not significantly affected. The organic matter digestibility (OMD %) and microbial protein production were significantly (P<0.05) higher for ensiled AC and LE with U, while short chain fatty acids (SCFA) were significantly (P<0.05) decreased. The concentrations of TP, TT and CT were strongly correlated (p<0.01). The TP, TT and CT were negatively related (p<0.01) with neutral detergent fiber (NDF) and acid detergent fiber (ADF), but not with hemicelluloses (HEMI). The crude protein was strongly correlated (p<0.01) with NDF, ADF and CT and negatively related (p<0.01) with TF and TT, but not with HEMI.

In conclusion, there were negative effects on the in vitro gas production occurring more consistently when AC and LE were ensiled with different levels of U, while OMD% and microbial proteins were significantly (P<0.05) increased. The in vitro digestibility and gas production parameters were significantly correlated with chemical composition of shrubs. Finally, were treatment improved the degradation of AC and LE.

Keywords: Acacia saligna, Leucaena leucocephala, gas production, organic matter digestibility, energy value, microbial protein production.

INTRODUCTION

nimal agriculture is one of the most important components of global agriculture, and livestock is one of the main users of the natural food resources base (De Haan et al, 1996). The ability of ruminants to use fibrous material, a widely available resource, matched with the sharp increase in the human population, mainly in developing countries, and therefore increasing the demand for food. In opposition to that in many developed countries, foods that are suitable for human consumption are very often used for fish, monogastric and ruminant animal feeding. The ruminants should be fed, as far as possible, on roughages and other feeds that are not in competition with human food (Ørskov, 1998). The use of browse species as fodder for ruminants is increasingly becoming important in many parts of the tropics. Generally, tree fodder is richer in crude protein (CP), minerals and digestible nutrients than grasses (Abdulrazak et al. 2000). The use of tree legume fodder as supplement has improved digestibility and animal performance (Abdulrazak et al., 1996). In Egypt, there is limited information on the nutritive value of tree shrubs fed to livestock.

Acacia (AC) and leucaena (LE) trees dominate in many parts of the arid and semi arid areas of Sub-Saharan Africa, and have multiple uses. The presence of phenolic compounds in AC species has a negative effect on their nutritional value and also on their intake by livestock (Degen et al., 1998). Tannins have been attributed to be one of the major causes of their limited use as livestock fodder (Bakshi and Wadhwa, 2007). Generally, tannins in fodder trees are known to have a negative effect on intake and digestibility (Kumar and D'Mello, 1995). Studies on some ACs have shown them to have either a positive (Ben Salem et al., 1999) or a negative effect (Degen et al., 1998) on animal performance. This variable effect could be attributed to the type of species, season and nutritive value. Many studies were conducted for decreasing the negative effect of tannins in tropical herbaceous and tree forage legumes (Conbolat et al., 2005). In vitro gas production (Khazaal and Ørskov, 1994) and in sacco rumen degradability (Apori et al., 1998) have been used to assess the nutritive values of browse species. These rapid and less expensive methods have been used to screen feed resources before making them

available to livestock (Larbi et al., 1998). The objective of this study was to assess the effect of ensiling of AC and LE leaves with different levels of urea on the chemical composition, in vitro gas production, energy values and organic matter digestibility.

MATERIAL AND METHODS

Samples and site description

Two tropical plants used in this study: Acacia saligna and Leucaena leucocephala leaves (whole leaves: rachis plus leaflets) have been sampled from the Experimental Farm, Faculty of Agriculture. Alexandria University, Alexandria, Egypt on October, 2008. Duplicate 100 g samples of AC and LE leaves were treated with water (20 ml/100 g fresh leaves) or with urea solutions at increasing levels of 1, 3 or 5%. Each sample of treated leaves was stored in a plastic bag which was closed with adhesive rubber to create anaerobic conditions. Storage time was 35 days. Bags containing treated leaves were stored in a big black plastic bag which was also closed with adhesive rubber. After storage, treated leaves were dried at 65 °C for 48 h and then ground to pass through a 1mm screen.

Chemical Analyses

Representative samples of ensiled AC and LE with different levels of urea (0, 1, 3 or 5%) were subjected to dry matter (DM), organic matter (OM), ether extract (EE), crude fiber (CF) and ash determinations following the procedure of AOAC (1990). Nitrogen (N) content was measured by the Kjeldahl method (AOAC 1990). Crude protein (CP) was calculated as N X 6.25. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicelluloses (HEMI) were determined according to Van Soest et al. (1991). Total phenols (TP), total tannins (TT) and condensed tannins (CT) were determined according to Makkar (1999). All chemical analyses were carried out in duplicate samples.

Measurement of in vitro gas production

In vitro gas production was undertaken according to Menke and Steingass (1988). Rumen fluid was collected before morning feeding from fistulated sheep fed berseem hay and commercial concentrate mixture twice a day. The rumen fluid was filtered through four layers of cheese-cloth and flushed with CO2. The CO2-flushed rumen fluid was added (1:2, v/v) to the buffered mineral solution (Onodera and Henderson, 1980), maintained in a water bath at 39 °C, and combined. All laboratory handling of rumen fluid was carried out under a continuous flow of CO2. Samples (200 mg) of the air-dried feedstuffs were accurately weighed into glass syringes fitted with plungers. The syringes were pre-warmed at 39 °C before injecting 30 ml rumen fluid-buffer mixture into each syringe and excess gas

was released. The syringes were incubated in a water bath at 39 °C. Two blank syringes containing 30 ml of the medium only were also included. All the syringes were gently shaken 30 min after the start of incubation and every one hour for the first 12 h of incubation, thereafter five times daily. The gas production was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 hours of incubation. Total gas values were corrected for blank incubation which contained only rumen fluid. Cumulative gas production (Y) at time (t) was fitted to the exponential model of Orskov and McDonald (1979) as follows: Gas $(Y) = a + b (1-exp^{-ct})$, where; a = gas production from the immediately soluble fraction, b = gas production from the insoluble fraction, c = gas production rate constant for the insoluble fraction (b), t = incubation time.

Estimation of energy values, organic matter digestibility, short chain fatty acids and microbial proteins

The energy values and the percentages of organic matter digestibility of forages can be calculated from the gas produced on incubation of 200 mg feed dry matter after 24 h of incubation with the levels of crude protein, ash and crude fat (Menke et al., 1979 and Menke and Steingass, 1988) as follows:

ME (MJ/kg DM) = 1.06 + 0.157GP + 0.084CP + 0.22CF - 0.081A

OMD (%) = 14.88 + 0.889 GP + 0.45 CP + 0.0651A Where: ME is the metabolizable energy, OMD (%) is the percentage of organic matter digestibility, GP is the 24 h net gas production (ml/200 mg DM) after 24 h of incubation. CP, crude protein (%); CF, crude fat (%) and A, ash content (%).

NE (Mcal/Ib) = $[2.2 + (0.0272 \times Gas) + (0.057 \times CP) + (0.149 \times CF)] / 14.64$

Where: NE is the net energy; Gas, the net gas production in ml from one-gram dry sample after 24 h of incubation; CP, crude protein (%); CF, crude fat (%) then, net energy unit converted to be MJ/kg DM. Short chain fatty acids (SCFA) were calculated according to Getachew et al. (2005) as follows:

 $SCFA = (-0.00425 + 0.0222 GP) \times 100$

Where: GP is 24 h net gas production (ml/200 mg DM).

Microbial protein was calculated as 19.3 g microbial nitrogen per kg OMD according to Czerkawski (1986).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM). Significant differences between individual means were identified using least significance difference (LSD) multiple range test (SAS, 2000). A simple correlation analysis was used to establish the relationship between chemical compositions and polyphenolic concentrations and *in vitro* gas production according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Chemical Composition

The chemical composition of ensiled AC and LE leaves with different levels of U (0, 1, 3 and 5% U) for 35 days are presented in Table 1. Results indicated great variations between the tested samples in their contents of CP, NDF, ADF, TF, TT and CT contents. The CP values ranged from 181 to 369.4 g/kg DM and were higher in LE compared with AC. These results are in agreement with those of Kumar and D'Mello (1995). The present data show that U treatments of AC and LE increased their CP contents. Also, NDF and ADF content were increased after ensiling with U for 35 days. El-Serafy et al. (1983) reported that urea addition changed the chemical composition of water hyacinth, total nitrogen increased and crude fiber (CF), NDF and ADF decreased as the period of ensiling increased. For all the samples the ADF fraction was a large proportion of the NDF, indicating high content of cellulose and lignin and low levels of hemicellulose. These results are in agreement with those of Abdulrazak et al. (2000). The values of TP, TT and CT concentration ranged between 12.3-112.2, 11.9-96.6 (eq. g tannic acid/kg DM) and 00.8-24.0 eq. g leucocyanidin/kg DM), respectively. Ensiling of AC and LE leaves without U had the highest values of all of them, while AC and LE with 5% of urea had the lowest values. The results of AC are consistent with values reported in the literature (Akkari et al., 2008). Ash content in the samples ranged from 84.7-134.7 g/kg DM and was the lowest in ensiled AC and LE without U.

In Vitro Gas Production

Gas production for the means of the ensiled AC and LE with different levels of urea (0, 1, 3, 5%) is presented in Fig.1 a, b and Table 2. The cumulative volume of gas production increased with increasing time of incubation, and the differences in gas production occurring during the early hours indicated little differences in total gas produced at 24 h. There were significant (P<0.05) differences among the tested samples in terms of total gas production and parameters (Fig. 1a, b and Table 2). The produced gas at 96 h ranged from 27.7-36.7 ml/200 mg DM, Total gas produced at 96 h of incubation was significantly (P < 0.05) higher for the ensiled AC and LE without U than in AC treated with 5% U. The highest GAS24, GAS96 and b were observed for the ensiled AC and LE leaves without U. Haddi et al. (2003) suggested that interactions between NDF, CP, ADL and ash contents influenced the kinetics of gas production. Kamalak et al. (2005) noted considerable variations among alfalfa varieties in terms of gas production at all incubation times according to the differences in the chemical composition of the varieties of alfalfa. Estimated gas production rate (c) varied from 0.044 ml/h in ensiled AC with 3% U to 0.053 ml/h in ensiled LE with the same level of urea. The values of (c)

increased after ensiling with urea. The intake of a feed is mostly explained by the rate of gas production (c) which affects the passage rate of feed through the rumen, whereas the potential gas production (a + b) is associated with degradability of feed (Khazaal et al. 1995). Although the present study showed that the ensiling AC and LE leaves with urea decreased the values of produced gas, (a) and (b), the value of (c) was significantly (P < 0.05) increased compared with ensiled samples without U.

Energy contents, organic matter digestibility, short chain fatty acids and microbial protein

predicted metabolizable energy The (ME, MJ/kg DM), net energy (NE, MJ/kg DM), organic matter digestibility (OMD, %), short chain fatty acids (SCFA, mM) and microbial protein (MP, mg/kg DM) of ensiled AC and LE leaves with 0, 1, 3 and 5% U are presented in Table 3. The present data show that the ME and NE were higher (P<0.05) for ensiled AC leaves with 3% U than for ensiled AC leaves without U, while no significant differences were detected between LE samples. Khazaal et al. (1993) correlated the chemical composition (i.e. CP, NDF, ADF or ADL) with the in vitro two-stage digestibility, in sacco degradability and gas production with voluntary intake. The calculated organic matter digestibility from gas production values at 24 h was subsequently highest in ensiled AC and LE leaves with 3% U (489.2 and 536.9 g/kg DM, respectively) and lowest in ensiled AC and LE leaves without U (455 and 517 g/kg DM, respectively) (Table 3). Condensed tannin concentrations were significantly correlated with the in vitro dry matter digestibility (r=0.77, P=0.043), extent of degradation (r=0.829,P=0.021) and cumulative gas production at 24 h (r=0.798, P=0.032) (Khazaal et al., 1993).

Microbial proteins and SCFA ranged from 54.89-64.77 g/kg DOM and 40.15-57.91 mM, respectively. Microbial proteins were significantly (P<0.05) increased after ensiling with urea, while SCFA were decreased. Blümmel et al. (1997) noted an inverse relationship between *in vitro* gas production and microbial biomass yield.

The relationship between the concentration of phenolic compounds, crude protein and cell-wall component of the untreated and urea treated of AC and LE

The correlations between the phenolic compounds, cell-wall parameters and CP in ensiled AC and LE with different levels of U (0, 1, 3 or 5%) are presented in Table 4. The concentrations of TP, TT and CT were strongly correlated (p<0.01). The TP, TT and CT were negatively related (p<0.01) with NDF and ADF, but not with HEMI. The CP was strongly correlated (p<0.01) with NDF, ADF and CT and negatively related (p<0.01) with TF and TT but not with HEMI. The relationship between cell-wall parameters and phenolics contrasts with those of Abdulrazak et al. (2000), but is comparable with those of Tolera et al. (1997).

Table (1): Proximate analysis (g/kg DM) of ensiled acacia and leucaena leaves with different levels of urea (0, 1, 3, or 5% U) for 35 days

Items	СР	EE	Ash	NDF	ADF	HEMI	TF	TT	CT
AC (0%)	181.0	18.1	84.7	410.9	318.4	92.5	112.2	96.6	27.6
AC (1% U)	222.1	16.6	98.9	419.5	324.5	95.0	69.3	68.8	6.3
AC (3% U)	268.4	16.1	96.7	472.0	347.5	124.5	46.1	45.7	1.4
AC (5% U)	322.0	15.2	91.8	486.5	374.5	112	17.6	17.2	1.0
LE (0%)	251.6	53.1	93.2	428.8	361.2	67.6	88.3	59.9	4.9
LE (1% U)	350.4	53.8	134.7	473.9	412.4	61.5	23.1	22.7	1.4
LE (3% U)	369.4	45.7	131.3	489.1	418.6	70.5	13.4	13.0	1.1
LE (5% U)	368.9	34.9	108.2	559.5	457.4	102.1	12.3	11.9	0.8

AC, acacia; LE, leucaena; AC(0%, 1%U, 3%U, 5%U), acacia with 0%, 1%, 3% and 5% of urea; LE (0%, 1%U,3%U, 5%U), leuceana with 0%, 1%, 3%, 5% of urea; CP, crude protein; EE, ether extract; CF, crude fiber; NDF, nutrant detergent fiber; ADF, acid detergent fiber; HEMI, hemicellulose; TF, total phenol (eq-g tannic acid/kg DM); TT, total tannins (eq-g tannic acid/kg DM); CT, condensed tannin (eq-g leucocyanidin/kg DM).

Table (2): Cumulative gas production (ml/200 mg DM) after 12, 24, 48, 72, 96 h of incubation and gas production parameters in ensiled acacia and leuceana leaves with different levels of urea (0, 1, 3, or 5% U) for 35 days

Items	12	24	48	72	96	a	b	c
AC (0%)	16 ^b	25 ⁵	34 ^{ab}	35 ^b	35*b	3.584 ⁶	32.737ª	0.045 ^{bc}
AC (1% U)	16 ^b	24 ⁵	32 ^{bc}	32°	33 ^{bc}	3.631 ^b	30.133 ^{ab}	0.046 ^{bc}
AC (3% U)	15.1 ^{bc}	24 ^b	31.1 ^{cd}	32°	33.1 ^{bc}	3.328 ^b	30.441 ^{ab}	0.044 ^{bc}
AC (5% U)	12 ^d	20°	26°	26 ^f	27.7°	1.504°	26.336 ^{cd}	0.047ª
LE (0%)	18ª	28ª	34.7ª	36ª	36.7ª	6.156ª	31.522ª	0.044 ^c
LE (1% U)	15 ^{bc}	24 ^b	30 ^d	30 ^d	30 ^d	3.459 ^b	27.517°	0.052ª
LE (3% U)	14 ^c	24 ^b	27.3°	28 ^e	29.3 ^{de}	3.590 ^b	25.735 ^d	0.053ª
LE (5% U)	15 ^{bc}	24 ^b	31 ^{cd}	31 ^{cd}	31 ^{cd}	2.852 ^b	29.352b	0.049ab

a,b,c,d,e means within the same column with different superscripts are significantly different (P<0.05).

Table (3): Metabolizable energy (ME), net energy (NE), organic matter digestibility (OMD), short chain fatty acids (SCFA) and microbial protein (MP) synthesis prediction in ensiled acacia and leuceana leaves with different levels of urea (0, 1, 3, or 5% U) for 35 days

Items	ME	NE	OMD	SCFA	MP
AC	6.17°	4.41°	45.50°	50.51 ⁶	54.89 ^e
AC (1%U)	6.26°	4.48°	46.85 ^d	49.03 ^b	56.52 ^d
AC (3%U)	6.65 ^b	4.65 ^b	48.92°	49.03 ^b	59.02°
AC (5%U)	6.49 ⁶	4.49°	47.75 ^{cd}	40.15°	57.60 ^{cd}
Le	7.98ª	5.29a	51.70 ^b	57.91ª	62.36 ^b
Le (1%U)	7.86ª	5.31ª	52.86 ^{ab}	49.03 ^b	63.76 ^{sb}
Le (3%U)	7.87ª	5.30°	53.69ª	49.03 ⁶	64.77 ^a
Le (5%U)	7.82ª	5.20ª	53.52*	49.03 ^b	64.56ª

ME, metabolizable energy (MJ/kg DM); NE, net energy (MJ/kg DM); OMD, organic matter digestibility (%); SCFA, short chain fatty acids (mM), MP, microbial protein (g/kg DOM).

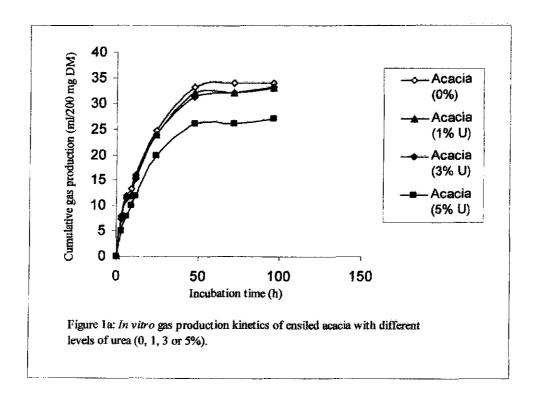
Table (4): Correlation coefficients (r) of the relationship between the concentration of phenolic compounds, crude protein (CP) and cell-wall component of the untreated and treated acacia and leucaena with different levels of urea

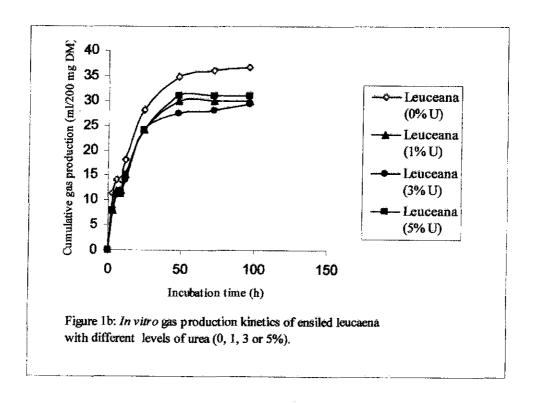
Y variable	Correlation coefficient with							
	CP	NDF	ADF	HEMI	TF	TT	CT	
CP NDF ADF		0.849**	0.897** 0.859**	-0.164 ^{ns} 0.185 ^{ns} -0.345 ^{ns}	-0.833** -0.762**	-0.966** -0.835** -0.812**	0.758** -0.615** -0.582**	
HEMI TF TT] 				-0.064 ^{ns}	0.028 ^{ns} 0.974**	-0.009 ^{as} 0.802** 0.822**	

^{**} Level of significance: p<0.01.

a,b,c,d,e means within the same column with different superscripts are significantly different (P<0.05).

ns not significant





CONCLUSIONS

Significant variations in chemical composition and in vitro rumen fermentation were observed among the ensiled AC and LE leaves with or without different levels of urea. There were negative effects on the in vitro gas production occurring more consistently when AC and LE were ensiled with different levels of urea, while organic matter digestibility and microbial proteins were significantly increased. The in vitro digestibility and gas production parameters were significantly correlated with chemical composition of shrubs. Accordingly, urea could be used to improve the degradation of AC and LE.

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الملخص للعربي

تأثير تخمير أوراق الأكاميا والليوكينا مع مستويات مختلفة من البوريا على التركيب الكيميائي وإنتاج الغاز وأيم الطاقة ومعامل هضم المادة العضوية

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إستخدم في هذه الدراسة طريقة إنتاج الغاز لتقييم تأثير المعاملة باليوريا على إنتاج الغاز وقيم الطاقة ومعامل هضم المسادة العسضوية للأكاسيا واللوكينا . تم معاملة الأكاسيا واللوكينا . تم معاملة الأكاسيا واللوكينا . تم معاملة الأكاسيا واللوكينا بثلاث مستويات مختلفة من العينسات تحت الدراسة (غير المعاملة والمعاملة باليوريا) في سرنجة زجلجية بإستخدام سائل الكرش المتحصل عليه من ثلاث رووس من الأغنام مغذاة على دريس البرسيم والعلف المركز التجاري مرتين يوميا .

تم قياس إنتاج الغاز بعد ٣ و ٦ و ١٧ و ٢٤ و ٢٧ و ٩٦ ساعة من القعضين وحركية إنتاج الغاز تم معرفتها باستخدام المعادلة . Gas (Y) = a + b (1-exp^{-ct}) .

أوضحت النتائج أن تخمير أوراق الأكاسيا واللوكينا في وجود مستويات مختلفة من اليوريا أنت إلى حدوث إرتفاع محتــوى العينـــات المختبرة من البروتين الخام والرماد بينما محتواها من الفينول الكلى والتنينات الكلية والنتينات المكثفة إنخفض .

توجد علاقة قوية بين تركيز الفينول الكلي (TP) والنتينات الكلية (TT) والتنينات المكتفة (CT). توجد علاقة معنويسة سلبة بين المركبات الفينولية (الفينول الكلي والتنينات الكلية والتنينات المكتفة) والسلام ADF و ADF لكن العلاقة مع الهيمي سليلوز غير معنويسة . توجيد علاقة معنوية معنوية معنوية معنوية سالبة علاقة معنوية سالبة الكلي والنتينات الكلية علاقة معنوية سالبة لكن العلاقة مع الهيمي سليلوز غير معنوية.

يستنتج من الدراسة أنه بوجد تأثير سالب من المعاملة باليوريا على انتاج الغاز معملياً بينما معامل هضم المسادة العـضوية البـروتين الميكروبي ازداد معنوياً . معامل الهضم ومقاييس إنتاج الغاز المقدرة معملياً ترتبط معنوياً بالتركيب الكيمياتي لنباتات العلف . معاملة الأكاســيا واللوكينا تحسن من هضمهما .