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Influence of *Salix babylonica* extract addition on *in vitro* rumen gas production and degradability of ryegrass silage harvested in different cutting days

A Z M SALEM[1], A E KHOLIF[2], M M Y ELGHANDOUR[3], J HERNÁNDEZ[4], A G LIMAS[5], M CIPRIANO[6], L M CAMACHO[7], S ROJAS[8] and J OLIVARES[9]

Universidad Autónoma del Estado de México, Toluca, México

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ABSTRACT

Four cutoffs of ryegrass after 15 days (CD15), 30 days (CD30), 45 days (CD45), and 60 days (CD60) with *Salix babylonica* (SB) extract at 0, 30, and 60 ml/kg ryegrass silage were ensiled for 40 days and then evaluated for the *in vitro* dry matter (DM) digestibility and gas production (GP). No interactions occurred between cutting day and SB extract for silage's nutrient contents and *in vitro* GP. The DM and organic matter (OM) contents were decreased linearly with decreased crude protein (CP), neutral detergent fibres, acid detergent fibres, and acid detergent lignin contents with advancing of days. In contrary, addition of SB extract increased silages' OM and decreased CP contents. Addition of SB extract for CD15 and CD60 silages, quadratically decreased the lag time. However, SB extract increased the rate of GP and GP during the first 12 h of incubation at the level of 30 ml/kg with CD30 silage and asymptotic GP with the level 60 ml/kg of CD60 silage. Increased DM degradability (DMD) of CD30 and CD60 silages versus decreased DMD with CD15 with increased relative GP (ml gas/g DMD). It could be concluded that CD15 had the highest DM and OM content; however, higher GP was noted with CD45 and CD60. SB extract had weak effects on nutrient content and GP, and the level of 30 ml/kg DM was more effective than the level of 60 ml/kg DM.

Key words: Cutting day, Gas production, Ryegrass, *Salix babylonica*, Silage

Producers seek grazing alternatives that allow them to maximize utilization of their forage resources through the use of pastures with grasses especially in temperate regions. Ryegrass is a highly nutritious grass that can be utilized for grazing or production of silage, haylage and hay.

The addition of phytogenic extracts rich in plant secondary metabolites (PSM) in ruminant feed is used to improve the ruminal fermentation activity for better feed utilization (Salem *et al.* 2014a). Extract of *Salix babylonica* (SB) has antimicrobial activity and ability to modulate rumen fermentation activity and improve nutrient utilization in ruminants (Salem *et al.* 2014b). Therefore, this study was aimed to evaluate the nutritional value of 40–d silages of different cutoffs of ryegrass associated with white clover in the form of chemical composition and *in vitro* gas production (GP) and digestibility in the presence of different level of SB extract.

Present address: [1,3](asalem70@yahoo.com, Facultad de Medicina Veterinaria y Zootecnia. [4,5]Facultad de Ingeniería y Ciencias de la Universidad Autónoma de Tamaulipas, Cd. Victoria. [6-9]Unidad Académica de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Guerrero, Altamirano, Guerrero. [2]Dairy Science Department, National Research Centre, 33 Bohouth St. Dokki, Giza, Egypt.

MATERIALS AND METHODS

Study location: The current study was conducted in Los Padlocks farm in the municipality of San Bartolo Morelos, State of Mexico, in cooperation with the university of state Mexico. Ryegrass samples were obtained from meadow of temperate grasslands, with an organic fertilizing. The climate in this area is temperate, semi-humid with rains in summer. The average annual temperature is 15°C, with a maximum of 37°C and minimum 3°C. The average annual rainfall is 970 mm. The prevailing winds are from the north.

Meadow and ensiling: Ryegrass associated with white clover (70:30) was sampled from an area of 1 ha (Table 1). Ryegrass sampling was started in January (day zero) and then every 15 days until 60 days. Forage cutting was performed by cutting the forage with a sickle to a height of approximately 2 cm from the surface of soil surface. For random sampling of forage collection, 3 samples of areas with 1.0 m² of the central rows were considered as the experimental units, at 15 (CD15), 30 (CD30), 45 (CD45) and 60 (CD60) days. The weight of fresh forage (g) was recorded and a subsample was placed in an oven at 60°C for 48 h for determining DM content.

Chopped fresh forage samples were ensiled in microsilage tubes of polyvinylchloride (PVC; 20 × 10 cm) with a capacity of 2.5 kg. *S. babylonica* extract was added

Table 1. Characteristics of the meadow or grassland of rye grass used

Variable	
Area	1 ha
Plant composition	70% rye grass + 30% white clover
Fertilization	Yes
Type of fertilizer	Organic
Dose of fertilizer	1 tonne/ha
Fertilization frequency	90 days
Irrigated	Yes
Type irrigation	Spray irrigation
Irrigation frequency	15 to 20 days during the dry season
Age of meadow	1 year

at levels of 0, 30 and 60 ml/kg DM of fresh forage, in triplicate per each forage cutting day. The mixture was compacted in microsilage tubes and sealed with polyethylene bags and tapes to prevent entry of air and kept for 40 days.

At the end of ensilaging (i.e., after 40 days), the microsilage tubes were opened and sampled for 200 g. Samples were dried in a forced air oven at 60°C for 48 h and ground in a mill (2 mm diameter) and kept for later analysis.

In vitro *incubations:* Rumen inoculum was collected from tow fistulated cows (450±20 kg body weight) fed *ad lib.* a total mixed ration made up of 70:30 commercial concentrate (16% CP, 11.7 MJ metabolizable energy (ME)/ kg DM) and oat hay with a mineral supplement, formulated to cover their nutrient requirements. Cows had a full access to freshwater at all times during the rumen inoculum collection phase. Ruminal contents were obtained before the morning feeding, mixed and strained through 4- layers of cheesecloth into a flask with O_2 free headspace and kept at 39°C.

In 125 ml serum bottles, about 0.8 g (on DM basis), in triplicate, from each cutting day at different levels of SB extract were weighted. Subsequently, 90 ml of incubation solution, gassed with CO_2 was added and kept under refrigeration at 4°C for 12 h. In the next day and for each bottle, 10 ml of ruminal fluid was added.

After filling all bottles with substrates and inoculum medium, they were filled, and immediately closed with rubber stoppers, shaken and placed in the water bath at 39°C. The volume of gas produced was recorded at times of 3, 6, 9, 12, 18, 24, 36, 48, 72 and 96 h of incubation. The GP was recorded (Theodorou *et al.* 1994) using a pressure transducer.

At the end of incubation period (i.e. 96 h), the residues of each bottle were filtered, washed with distilled water and dried in an oven at 65°C for 48 h to determine the DM degradability. The relative GP (RGP; ml gas/g DM degradability (DMD)) was calculated as ml of gas/g of DMD after 96 h incubation (González *et al.* 1998).

Kinetic parameters of GP were estimated by fitting GP results (ml/g DM) in the NLIN option of SAS (2002) according to France *et al.* (2000). Metabolizable energy (MJ/kg DM) and *in vitro* organic matter digestibility (OMD, %) were estimated according to Menke *et al.* (1979). Short chain fatty acids (SCFA) were calculated (Getachew *et al.* 2002).

Chemical analysis: Samples of silage were analysed for DM, ash, and N (AOAC 1997). The neutral detergent fibres (NDF), acid detergent fibre (ADF) content and acid detergent lignin (ADL; AOAC 1997) of both silage and fermentation residues were determined using a Fibre Analyser Unit without use of an alpha amylase but with sodium sulphite in the NDF. Both NDF and ADF are expressed without residual ash. Moisture content of the silages was determined through distillation with toluene.

Statistical analysis: Data of chemical composition and *in vitro* digestibility of forage were analysed as a 3 × 4 factorial arrangement (i.e., 3 levels of SB extracts (random effect) and 4 cutting days (fixed effect) according to a randomized block design using the PROC MIXED procedure of SAS (2002). Data of each one of the 3 runs within the same sample were averaged. Mean values of each individual sample of each ryegrass cuts (3 samples of each) were the experimental unit, and the statistical model was:

$$Y_{ijk} = \mu + A_i + L_j + AL_{ij} + e_{ijk}$$

where, $Y_{ijk,}$ response variable; μ, overall mean; $A_{i,}$ effect of cutting day (n=4); $L_{j,}$ effect of extract level (n=3); $AL_{ij,}$ effect of the interaction between A and L (n=12) and $e_{ijkl,}$ experimental error. Comparisons of means were conducted by Tukey test.

RESULTS AND DISCUSSION

Linear effects for cutting days were observed (P<0.05) for OM, CP, NDF, ADF, and ADL contents without any effects for SB extract addition during ensiling. Regardless of SB extract presence (i.e., 0 ml SB/kg DM), the DM and OM contents were decreased linearly (P<0.05) with advancing days. However, there were gradual linear increases (P<0.05) for CP, NDF, ADF, and ADL contents. The level of 60 ml SB/kg DM of silage had linearly increased (P<0.05) NDF, and ADL contents with increasing (P<0.05) CP content for the level of 30 ml SB/kg DM, during the first period (i.e., CD15). For CD30 and CD45 silages, the high level of SB increased (P<0.05) CP content compared to the other levels. In contrary, increased OM content and decreased CP content were noted with SB extract addition (P<0.05) before ensiling (Table 2). These results may be because the active compound of the extract can affect only in the ruminal fermentation (Salem *et al.* 2014a; Valdes *et al.* 2015) not during ensiling.

The increased DM content with CD15 indicates a suitable forage age for silage quality and less DM loss as a result of fermentation. Accordingly, Castro (2006) observed an increased DM content from day 0 to 28 for ryegrass forage grown in the temperate region of Mexico.

Organic matter content decreased linearly with about 2 to 3% with advancing of cutting days. However, CP contents increased about 17.8, 27.4, 53.4% for CD30, CD45, and

Table 2. Chemical composition[1] (g/kg) of ryegrass (*Lolium perenne*) with white clover silage, with addition of *Salix babylonica* (SB) extract at different cutting days during ensiling

Cutting day (CD, day)	Extract (SB, ml/kg)	DM	OM	CP	NDF	ADF	ADL
CD15	0	462	894	146	365	360	33
	30	415	887	265	370	366	42
	60	402	897	199	421	416	51
	SEM	8.8	13.4	5.6	4.4	4.2	2.3
	Linear	0.0327	0.8421	0.5014	0.0325	0.4423	0.0437
	Quadratic	0.4206	0.4224	0.0046	0.0542	0.0672	0.6270
CD30	0	264	877	172	423	417	45
	30	256	862	138	401	396	57
	60	277	887	198	412	407	40
	SEM	9.0	16.2	4.4	5.7	7.6	4.0
	Linear	0.1235	0.4335	0.0784	0.0871	0.3840	0.0889
	Quadratic	0.4420	0.6817	0.0360	0.0689	0.6697	0.0499
CD45	0	238	866	186	366	362	50
	30	231	866	218	404	399	60
	60	220	848	265	379	374	59
	SEM	5.2	18.8	3.7	7.3	8.2	5.2
	Linear	0.0400	0.7142	0.0021	0.4449	0.4444	0.2345
	Quadratic	0.8032	0.5147	0.0451	0.5398	0.0681	0.4120
CD60	0	240	865	224	439	434	46
	30	235	876	212	368	363	50
	60	230	898	199	429	423	48
	SEM	9.4	5.7	4.9	10.1	9.9	6.2
	Linear	0.0724	0.0327	0.0455	0.1101	0.1025	0.4478
	Quadratic	0.1134	0.0614	0.0662	0.0632	0.2697	0.6281
SEM pooled		9.4	3.9	7.9	7.9	7.7	8.2
Cutting day (CD)							
Linear		0.0442	0.0211	0.0160	0.0033	0.0031	0.0220
Quadratic		0.5540	0.0773	0.0742	0.0914	0.0754	0.1417
Extract (SB)							
Linear		0.5710	0. 3714	0.1888	0.5421	0.2478	0.0971
Quadratic		0.624	0. 3661	0.1421	0.3618	0.2978	0.1004
CD × SB		0.0751	0.0997	0.1124	0.3011	0.3541	0.1119

[1]DM, dry matter; OM, organic matter; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre; ADL, acid detergent lignin; SEM, standard error of means.

CD60 with days advancing. Ariciaga (2005) evaluated the chemical composition of ryegrass hay at 21 and 35 days old, and reported a decreased OM content from 84.6 to 80.4%. These results were consistent with the values described by Castro (2006) where lower values of 86 to 72.5%, from day 0 to day 28 were obtained for ryegrass forage. Highest NDF and ADF contents were noted with the late cutoff compared to the early ones.

No interaction effects (P>0.05) were noted between cutting days and SB extract addition on GP and ruminal fermentation kinetics with exception of DMD (P=0.003). However, cutting days linearly (P<0.05) affected *b*, *c*, GP, DMD, OMD, ME, SCFA, and RGP values. In contrary, SB addition during ensiling affected (P<0.05) only the *L* value and RGP. Addition of SB extract to the silage CD15 and CD60 decreased the lag time (P<0.05). However, for CD30, addition of SB extract increased the rate of GP (P<0.05) and GP during the first 12 h of incubation at the level of 30 ml/kg DM. A higher asymptotic GP was obtained (P=0.046) with the level 60 ml/kg DM for CD60 silage (Table 3). Higher GP indicates a better nutrient availability for rumen microorganisms.The obtained results showed weak effects for the SB extract addition during ensiling in ruminal GP. However, the level of 30 ml/kg DM shorted the initial delay before GP begins for the CD15 silage and increased the rate of GP for the CD30 cutoff silage. As we mentioned above, this may depends on the ability of the extract to modify the ruminal fermentation if added directly to the feeds just prior to feeding or directly drenched. Salem *et al.* (2014a) showed that ruminal GP and rumen fermentation activities were increased with SB extract at 0.6 and 1.2 ml/g DM, but not with the dose 1.8 ml/g DM. Salem *et al.* (2014b) explained this phenomenon based on the ability of rumen microorganisms to degrade low and moderate levels of PSM in plant extracts and utilize them as an energy source without negative effects on rumen fermentation.

The maximum production of gas and highest rates of GP were for the cutoffs after 45 and 60 d. The different

Table 3. *In vitro* gas production (ml/g DM) kinetics[1]of ryegrass (*Lolium perenne*) with white clover silage, with addition of *Salix babylonica* (SB) extract during ensiling at different cutting day

Cutting day (CD, day)	Extract (SB, ml/kg)	Gas production parameters			In vitro gas production (ml/g DM)									
		b	*c*	*L*	Gas3	Gas6	Gas9	Gas12	Gas18	Gas24	Gas36	Gas48	Gas72	Gas96
CD15	0	418	0.045	3.96	35	95	134	167	222	264	322	357	393	407
	30	411	0.041	2.96	31	85	121	152	204	246	305	348	381	398
	60	412	0.045	3.41	34	92	129	162	215	256	313	342	384	399
	SEM	15.0	0.0028	0.209	2.0	5.2	6.9	8.2	10.0	11.1	12.4	13.3	14.3	14.7
	Linear	0.7858	0.8978	0.1099	0.6973	0.6841	0.674	0.6654	0.6508	0.6411	0.6358	0.6444	0.6761	0.7081
	Quadratic	0.8564	0.2690	0.0298	0.2234	0.2336	0.2439	0.2565	0.2888	0.3307	0.4355	0.5464	0.7139	0.7987
CD30	0	399	0.044	3.59	35	94	131	164	216	256	310	342	375	389
	30	409	0.054	3.5	41	110	152	188	244	285	337	366	393	403
	60	404	0.050	3.57	34	93	130	163	216	258	314	348	381	395
	SEM	14.4	0.0015	0.173	2.0	5.3	7.3	8.9	11.3	12.9	14.4	14.8	14.7	14.5
	Linear	0.8083	0.0422	0.9303	0.7604	0.8401	0.8933	0.9405	0.9812	0.9216	0.8425	0.8005	0.7759	0.7822
	Quadratic	0.7050	0.0107	0.7141	0.0335	0.0449	0.0450	0.0465	0.0931	0.1241	0.1966	0.278	0.438	0.5578
CD45	0	430	0.052	3.25	43	115	160	199	260	304	360	391	417	425
	30	428	0.050	3.37	40	107	149	184	241	283	339	372	404	417
	60	472	0.047	3.08	41	112	157	196	258	306	370	408	446	461
	SEM	25.4	0.0028	0.147	3.7	9.4	12.5	14.9	18.1	20.1	22.3	23.4	24.5	25.0
	Linear	0.2817	0.2353	0.4374	0.7819	0.8184	0.8505	0.8865	0.9673	0.9448	0.7702	0.6245	0.4434	0.3590
	Quadratic	0.4836	0.9244	0.3002	0.6290	0.5724	0.5355	0.5033	0.4521	0.4166	0.3808	0.3747	0.3977	0.4277
CD60	0	430	0.051	3.81	42	114	159	197	256	300	356	386	414	423
	30	408	0.050	3.42	36	97	136	169	223	265	321	354	387	400
	60	472	0.046	3.66	41	111	155	193	254	300	265	401	439	456
	SEM	17.2	0.0063	0.116	3.9	9.6	12.6	14.8	17.5	18.7	19.4	19.3	18.8	18.5
	Linear	0.1390	0.5958	0.3847	0.7645	0.7921	0.8179	0.8480	0.9211	0.9917	0.7962	0.6134	0.3732	0.2606
	Quadratic	0.0463	0.9243	0.0424	0.2617	0.2366	0.2212	0.2082	0.1880	0.1738	0.1571	0.1488	0.1383	0.1262
SEM pooled		18.5	0.0038	0.165	3.04	7.68	10.20	12.11	14.68	16.17	17.58	18.12	18.53	18.67
Interactions (*P* value)														
Cutting day (CD)														
Linear		0.0440	0.0405	0.1344	0.0032	0.0030	0.0030	0.0030	0.0033	0.0038	0.0060	0.0101	0.0240	0.0397
Quadratic		0.076	0.2814	0.0795	0.7545	0.6675	0.6061	0.5481	0.4460	0.3626	0.2474	0.1819	0.1233	0.1009
Extract (SB)														
Linear		0.1262	0.1425	0.0445	0.5422	0.5967	0.6435	0.6955	0.8122	0.9399	0.8035	0.5899	0.3369	0.2282
Quadratic		0.1791	0.7380	0.0332	0.5103	0.4491	0.4104	0.3772	0.3251	0.2882	0.2448	0.2245	0.2095	0.2019
CD × SB		0.4925	0.7367	0.0608	0.4030	0.4010	0.4043	0.4108	0.4318	0.4602	0.5217	0.5686	0.5955	0.5757

[1]*b* is the asymptotic gas production (ml/g DM); *c* is the rate of gas production (/h); *L* is the initial delay before gas production begins (h); SEM, stander error of the mean.

Table 4. Fermentation kinetics[1]of ryegrass (*Lolium perenne*) with white clover silage, with addition of *Salix babylonica* (SB) extract during ensiling at different cutting day

Cutting day (CD, day)	Extract (SB, ml/kg)	DMD	ME	OMD	SCFA	RGP
CD15	0	695	10.2	691	584	301
	30	557	10.4	713	544	310
	60	587	11.2	701	567	304
	SEM	33.9	0.30	19.7	24.7	7.0
	Linear	0.0443	0.8366	0.7355	0.6419	0.8112
	Quadratic	0.0907	0.7295	0.5224	0.3305	0.3952
CD30	0	664	10.1	689	565	292
	30	694	12.0	727	630	282
	60	745	13.4	703	570	300
	SEM	18.8	0.35	23.0	28.7	4.1
	Linear	0.0228	0.7031	0.6742	0.9215	0.2112
	Quadratic	0.6786	0.2872	0.3194	0.1242	0.0326
CD45	0	676	11.5	782	672	282
	30	703	9.7	760	627	296
	60	709	14.6	822	677	303
	SEM	28.0	0.55	35.8	44.7	6.6
	Linear	0.4283	0.54	0.4581	0.9448	0.0432
	Quadratic	0.7630	0.3862	0.3736	0.4167	0.7283
CD60	0	718	11.3	792	664	287
	30	798	11.1	723	585	291
	60	754	11.7	779	664	308
	SEM	15.7	0.51	33.3	41.6	11.7
	Linear	0.1553	0.8576	0.794	0.9918	0.2415
	Quadratic	0.0175	0.1748	0.1771	0.1737	0.6412
SEM pooled		25.1	0.44	28.8	35.9	7.8
Cutting day (CD)						
Linear		0.0005	0.0018	0.0012	0.0038	0.0459
Quadratic		0.0153	0.0969	0.0408	0.3628	0.1471
Extract (SB)						
Linear		0.5604	0.6013	0.5326	0.9402	0.0244
Quadratic		0.7442	0.3864	0.4218	0.288	0.5978
CD × SB		0.0031	0.5304	0.4762	0.4603	0.5172

[1]DMD, the DM degraded substrate (mg/g DM); ME, the metabolizable energy (MJ/kg DM); OMD, the *in vitro* organic matter digestibility (g/kg DM); SCFA, the short chain fatty acids (mmol/g DM); RGP, the relative gas production (ml gas/ g DMD), SEM, slander error of the mean.

chemical compositions between silages of different cutoffs were reflected in different GP. Ariciaga (2005) evaluated ryegrass (*Lolium perenne*) silage harvested at 21 and 35 days of age, and report a value of total GP of 167 ml gas/g DM for silage of 21 days, and 166 ml/g DM for silage of 35 days of age. Castro (2006) showed that the total GP decreased with advancing of maturity stages.

Increased DMD during the stage CD30 (linear effect, P=0.023) and stage CD60 (quadratic effect, P=0.018) *versus* decreased DMD at the stage CD15 (linear effect, P=0.044) were occurred with SB addition during ensiling. Moreover, addition of SB increased RGP at the level 60 ml/kg DM for CD30 (quadratic effect, P=0.033), and with the levels of 60 and 30 ml/kg DM (linear effect, P=0.043) for CD45 silage (Table 4). Results of DM degradability are parallel with the DM contents of silages after 40 days of ensiling. Results of DM content and degradability showed better fermentability for early cutoffs than the other. Higher GP with the cutoffs of low DMD was reflected in the results of RGP; where higher RGP (ml gas/ g DMD) was higher for early cutoffs than later ones. Increased OMD were noted with advancing in cutting age.

It could be concluded that the chemical composition of ryegrass silage associated with white clover and *in vitro* GP was not affected by the addition of SB extract before ensiling for 40 days. However, the effect of cutoffs was more notable. The maximum GP was obtained for silage made from cutoffs after 45 and 60 days. The level of 30 ml SB/kg DM of ryegrass silage was more effective than the level of 30 ml SB/kg DM. More studies are required with different levels of SB extract to explain the weak effect of the extract in the chemical composition and ruminal fermentation kinetics.

REFERENCES

AOAC 1997. *Official Methods of Analysis*. 16th edn. Association of Official Analytical Chemists. Arlington, VA, USA.

Ariciaga G C. 2005. Comparación de las técnicas *in sacco* e *in vitro* mediante producción de gas para estimar NA tasa de degradación y fermentación ruminal de heno y ensilado de pasto Ryegrass. Tesis de Licenciatura. Facultad de Medicina Veterinaria y Zootecnia. Universidad Autónoma del Estado de México. Toluca, México. 69 p.

Castro H H. 2006. Composición química, contenido mineral y digestibilidad *in vitro* del forraje Ryegrass (*Lolium perenne*) en relación con la madurez y época del año. Tesis de licenciatura. Universidad Autónoma del Estado de México. Toluca, México. pp 30–32.

France J, Dijkstra J, Dhanoa M S, Lopez S and Bannink A. 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. *British Journal of Nutrition* **83**: 143–50.

Getachew G, Makkar H P S and Becker K. 2002. Tropical browses: contents of phenolic compounds, *in vitro* gas production and stoichiometric relationship between short chain fatty acid and *in vitro* gas production. *Journal of Animal Science* **139**: 341–52.

González R M, Fondevila M, Barrios U A and Newman Y. 1998. *In vitro* gas production from buffel grass *(Cenchrus ciliaris L.)* fermentation in relation to the cutting interval, the level of nitrogen fertilisation and the season of growth. *Animal Feed Science and Technology* **72**: 19–35.

Menke K H, Raab L, Salewski A, Steingass H, Fritz D and Schneider W. 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*. *Journal of Animal Science* **92**: 217–22.

Salem A Z M, Kholif A E, Elghandour M M Y, Buendía G, Mariezcurrena M D, Hernandez S R and Camacho L M. 2014b. Influence of oral administration of *Salix babylonica* extract on milk production and composition in dairy cows. *Italian Journal of Animal Science* **13**: 10–14.

Salem A Z M, Kholif A E, Olivares M, Elghandour M M Y, Mellado M and Arece J. 2014a. Influence of *S. babylonica* extract on feed intake, growth performance and diet on *in vitro* gas production profile in young lambs. *Tropical Animal Health and Production* **46**: 213–19.

SAS 2002. SAS User's Guide: Statistics. Ver 9.0. SAS Institute, Cary, NC, USA

Theodorou M K, Williams B A, Dhanoa M S, Mcallan A B and France J. 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal Feed Science and Technology* **48**: 185–97.

Valdes K I, Salem A Z M, Lopez S, Alonso M U, Rivero N, Elghandour M M Y, Domínguez I A, Ronquillo M G and Kholif A E. 2015. Influence of exogenous enzymes in presence of *Salix babylonica* extract on digestibility, microbial protein synthesis and performance of lambs fed maize silage. *Journal of Agricultural Science* **153**: 732–42.