**Effects of different doses of Salix babylonica extract on growth performance and diet in vitro gas production in Pelibuey growing lambs**

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**Abstract**

Twenty Pelibuey 3-4 month old and 23.7±3.3 kg body weight male lambs were used in a randomised design to study the effects of daily oral administration of Salix babylonica (SB) extract on dry matter (DM), water intake, average daily gain (ADG), and feed efficiency for 72 days. Animals were divided into four groups with the same total mixed ration with different doses of SB: 0 (Control), 20 (SB20), 40 (SB40) and 60 (SB60) mL/lamb/d. In vitro gas production (GP) of the same diet fed to lambs as a substrate was measured with different doses of SB (0, 0.3, 0.7, 1.0 mL/g DM). Daily administration of SB to lambs had no effects (P=0.05) on growth performance and DMI (linear effect, P=0.2805; quadratic effect, P=0.3747). Both low and moderate doses of SB (SB40>SB20) tended to increase (linear effect, P=0.4010; quadratic effect, P=0.9166) ADG. The asymptotic GP quadratically increased (P<0.001) with decreased GP rate and with increasing SB extract doses. In vitro GP increased (P<0.05) with advancing of incubation time in all SB doses. During the first 24 h of incubation, 0.3 mL SB/g DM had the highest GP, whereas 1.0 mL SB/g DM quadratically increased (P<0.001) GP. The low dose of SB extract increased ME (linear effect, P=0.024) and short chain fatty acids (SCFA) (linear effect, P=0.023). However, the highest dose quadratically decreased (P=0.02) DM degradability. In conclusion, administration of SB extract at 40 mL/lamb/d tended to increase DM intake, improve daily weight gain in growing lambs with increasing asymptotic in vitro ruminal GP and SB dose.

**Introduction**

Public concern over use of antibiotics in livestock production has increased in recent years because of their possible contribution to emergence of antibiotic resistant bacteria (Busquet et al., 2006) and their transmission from livestock to humans; hence, their use has been banned in the European Union. For this reason, ruminant microbiologists and nutritionists have to explore alternative methods of favourably altering ruminal metabolism to improve feed efficiency and animal productivity, including the use of yeasts, organic acids, plant extracts, probiotics, and antibodies (Calsamiglia et al., 2007; Elghandour et al., 2014). Accordingly, plant extracts contain specific secondary metabolites that give them potential alternatives as feed additives to manipulate rumen microbial population’s activity (Jiménez-Peralta et al., 2011; Salem, 2012; Salem et al., 2014b). Plant secondary metabolites in Salix babylonica (SB) extract (Jiménez-Peralta et al., 2011; Salem et al., 2011, 2014b) or in herbs such as organic acids and essential oils (Hernández et al., 2004) seem to be alternatives to replace chemical feed additives (Patra et al., 2006; Jiménez-Peralta et al., 2011).

A combination of positive impacts of secondary metabolites on ruminal microorganism activity (Jiménez-Peralta et al., 2011; Salem et al., 2014b), nutrient digestion (Mapiye et al., 2010; Salem et al., 2011, 2014b) increased supply of amino acid to the duodenum (Mueller-Harvey, 2006), and microbial protein production. This could improve and increase muscle deposition as well as carcasses weights and meat quality (Gleghorn et al., 2004; Mapiye et al., 2010). In addition, secondary metabolites have been shown to enhance protein metabolism, decrease CH4 emission, and suppress or stimulate microbial growth (Makkar et al., 1998). Some metabolites also reduce nutritional stress such as bloat and/or improve animal health and productivity (Xhomfulana et al., 2009; Salem et al., 2010) resulting in higher daily gain, voluntary feed intake and milk production (Salem et al., 2011, 2014a). Also, it may have a protective effect on feed protein within the rumen with promoting duodenal absorption, minimising the excretion of N, modifying the acetate to propionate ratio, and it also has been shown to have anthelmintic effects (Athanasiadou and Kyriazakis, 2004; Mejía-Hernández et al., 2014). Careful aspects should be considered during plant extract feeding. Consumption of large amounts of tannins or saponins may have a direct haemolytic effect and may even cause death (Athanasiadou and Kyriazakis, 2004). Moreover, long-term feeding of plants, rich in secondary compounds, may have detrimental effects on animal health (Mahgoub et al., 2008).

This experiment was conducted to assess the effect of feeding diet with different doses of plant extract of Salix babylonica on feed intake, average daily gain and in vitro gas production (GP) of growing lambs.

**Materials and methods**

The experiments of growth performance and in vitro evaluations were performed at the
briefly, leaves collected randomly during summer season from several young and mature trees of SB were fresh chopped into 2 to 3 cm lengths and immediately extracted at 1 g leaf/8 mL of solvent which contained 10 mL methanol (99.8/100, analytical grade; Ferment, Monterrey, Mexico), 10 mL ethanol (99/100, analytical grade; Ferment) and 80 mL of distilled water. Plant materials were individually soaked and incubated in water in the laboratory temperature of 25 to 30°C for 72 h in closed jars of 5 L. After incubation, jars were heated at 39°C for 1 h, and then immediately filtered and the filtrates were collected and stored at 4°C for further use.

Secondary metabolites of SB extract were determined in triplicate according to the method described in Salem (2012). Briefly, 10 mL of extract were fractionated by funnel separation with a double volume of ethyl acetate to determine total phenolic compounds by drying and quantifying the phenolics layer in the funnel. After phenolic compounds separation, 20 mL of n-butanol were added to fractionate the saponins. The remaining solution in the funnel was considered to be the aqueous fraction that has the other secondary compounds such as lectins, polypeptides and starch (Cowan, 1999).

In vitro evaluation of the total mixed ration

Salix babylonica extract was examined at four doses (0, 0.3, 0.7, 1.0 mL/g DM of high grain diet) in four replicates for each treatment on the resultant in vitro fermentation kinetic profile of lambs diet (i.e., TMR). The substrate was the same TMR fed to lambs during the growth performance experiment.

Gas production assay was carried out as described by Theodorou et al. (1994) with arrangements of Mauricio et al. (1999). Samples (1±0.002 g) of substrate (i.e., TMR) were weighed in quadruplicate into 160-mL serum bottles. Extract doses of SB were applied directly onto the substrate inside the bottles immediately before adding buffer medium and rumen fluid. Rumen fluid was collected by stomach tube from 4 of the growing lambs fed the same TMR during the growth performance experiment for 72 days (Control group). Ruminal contents of each animal were obtained immediately before the morning feeding, mixed and strained through four layers of muslin and then kept at 39°C under a continuous CO2 stream. Ten mL of particle-free ruminal fluid and 90 mL buffer medium (containing micro- and macro-elements, a reducing agent and a reduction indicator of resazurin) were added to each bottle. Negative controls containing buffered rumen fluid with or without SB extract but no substrate, were also included in triplicate for correction of gas produced from small particles present in the ruminal fluid. Cumulative GP (mL/g DM) was recorded at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 and 96 h post incubation at 39°C. Volume of gas (mL/g DM) produced after 24 h of incubation (GP24) was used as an index of energy feed value. At the end of incubation (i.e., 96 h), the contents of each serum bottle were filtered using sintered glass crucibles (porosity 1, 100- to 160-μm pore size; Pyrex, Washington, UK) under vacuum. Fermentation residues were dried at 105°C overnight to estimate the DM disappearance.

Pressure generated by the gas accumulated in the upper part of the incubation serum bottles was measured through a pressure transducer connected to a digital reader. The equation was previously obtained using PROC REG of the SAS (2002) programme:

\[ Y=a+bX+0.083X^2 \]

where \( Y \) is volume (mL), \( X \) is pressure (psi), \( R^2=0.99 \).

Then, GP data (mL/g DM) were fitted using the NLIN option of SAS (2002) to the model of France et al. (2000) as:

\[ A=bx\left[1-e^{(-c(t-L))}\right] \]

where \( A \) is the volume of GP at time \( t; b \) is the asymptotic GP (mL/g DM); \( c \) is the rate of GP (h) and \( L \) (h) is the lag time.

Metabolisable energy (ME; MJ/kg DM) was estimated according to Menke and Steingass (1988) as:

\[ ME=2.20+0.136IVGP_{24}(mL/0, 2g DM)+0.057CP \]

where IVGP_{24} was 24 h gas volume and CP (%) DM was that of tree leaves.

Short chain fatty acids (SCFA) were calculated according to Getachew et al. (2002):

\[ \text{mmol SCFA}=0.00245+0.0222(\text{mL gas at 24 h}) \]

Proximate analysis of total mixed ration

Samples of TMR were analysed for DM, ash, CP according to the AOAC (1990). Neutral detergent fibre and ADF content were analysed using the ANKOM F-57 filter bags in an AnkomTMFibre Analyzer unit (Ankom Technology, Macedon, NY, USA) according to Van Soest et al. (1991). For NDF analysis, samples were treated with α-amylase (Sigma A-
Ratios effects were calculated at P<0.05.

The dose of 0.7 mL/g DM caused a linear increased (P=0.003) in the lag time (h) while the dose 0.3 mL/g DM was linearly decreased (P=0.003) compared to control (0 mL/g DM). In vitro GP was quadratically increased (P<0.001) with increasing SB doses of SB extract, whereas the rate of GP (c/h) was quadratically decreased (P<0.001) with increasing SB doses. The dose of 0.7 mL/g DM caused a linear increased (P=0.003) in the lag time (h) while the dose 0.3 mL/g DM was linearly decreased (P=0.003) compared to control (0 mL/g DM). In vitro GP was increased (P<0.05) with the advancing of incubation time with all SB doses. During the first 24 h of incubation, the 0.3 mL SB extract/g DM had the highest GP. However, after 24 h of incubation the highest dose of SB extract (i.e., 1 mL SB extract/g DM) had quadratically increased (P<0.001) GP vs the other doses. Supplementation of 0.3 mL SB extract/g DM increased DMD (quadratic effect, P=0.020), ME (linear effect, P=0.024), SCFA (linear effect, P=0.023) vs the other doses (Table 1).

The asymptotic GP (b, mL/g DM) was quadratically increased (P<0.001) with increasing doses of SB extract, whereas the rate of GP (c/h) was quadratically decreased (P<0.001) with increasing SB doses.

The experimental design of the growth performance experiment was completely randomised, where lambs were the experimental units. The statistical model used for the analysis was:

\[ y_{ijk} = \mu + d_i + a(d)_{ij} + e_{ijk} \]

where \( y_{ijk} \) is the value measured at period \( k \) on the \( j \)th lamb assigned to the \( i \)th diet (extract dose), \( \mu \) is the overall mean effect, \( d_i \) is the \( i \)th fixed diet (extract) effect, \( a(d)_{ij} \) is the random effect of the \( j \)th lamb within the \( i \)th diet, \( e_{ijk} \) is the random error associated with the \( j \)th lamb assigned to the \( i \)th diet.

Tukey’s test was used for the multiple comparisons among mean values for each run and linear and quadratic effects were calculated at P<0.05.

Data of in vitro ruminal GP, fermentation parameters were analysed as a completely randomised design (four extract doses) using the GLM option of SAS (2002) with methods of Steel and Torrie (1980), to determine differences due to extract levels. Tukey’s test was used for the multiple comparisons among mean values for each run and linear and quadratic effects were calculated at P<0.05.

Results

No significant differences (P>0.05) were observed for the daily oral administration of SB to lambs. However, DM intake (DMI; kg/d) tended to be increased (linear effect, P=0.2805; quadratic effect, P=0.3747) with low (i.e., SB20) and moderate (i.e., SB40) doses of SB extract (SB20>SB40) compared to Control and the high SB extract dose (i.e., SB60). All SB lambs (i.e., SB20, SB40 and SB60) with different doses tended to increased (linear effect, P=0.1961; quadratic effect, P=0.8287) water intake (L/d) by about 8.1, 13.5, and 5.1% vs no SB lambs (i.e., SB0). During the 72 days of the experiment, both low and moderate doses of SB extract (i.e., SB20, SB40) tended to increase (linear effect, P=0.4010; quadratic effect, P=0.9166) the ADG (g/d) (SB40>SB20) by about 5 and 8.2%, respectively. Feed efficiency (g DM/kg ADG) was the same among all groups with the exception of SB20 group which tended to increase (linear effect, P=0.9736; quadratic effect, P=0.6510) the efficiency by about 4.3% compared to other diets (Table 1).

Tukey’s test was used for the multiple comparisons among mean values for each run and linear and quadratic effects were calculated at P<0.05.

The overall mean effect, d

Discussion

Feed and water intakes

Daily oral administration of SB extract to lambs at doses of 20 and 40 mL/lamb/d tended to increase DMI by 10.8 and 6.4%, respectively, compared to Control and SB60 (i.e., high dose of SB extract). This tendency could be associated with the improved rumen fermentation kinetics of the same diet used as a substrate in vitro. This improvement may be due to positive impacts of plant secondary metabolites on ruminal fermentation and nutrient digestibility (Salem et al., 2011, 2014b). This action possibly leads to increase rates of dry matter disappearance in the rumen and rates of passage and consequently increased feed intake (Conrad, 1966). It is well known that some tree extracts reduced microbial protein degradability (Mueller-Harvey, 2006). The tendency to increase water consumption with administration of SB extract with SB20 and SB40 vs SB60 may be related to SB extract content of secondary metabolites. Dearing et al. (2000) and Salem et al. (2013) stated that ingestion of secondary metabolites increases water intake and could act as diuretics, resulting in more water consumption.

Growth performance of lambs

Oral administration of SB extract at appropriate doses of 20 and 40 mL/lamb/d tended to increase lambs ADG vs SB60 (i.e., high dose) or Control (SB0). Appropriate doses of plant extract, rich in secondary metabolites, ensure improved ruminal fermentation kinetics may be through reduction of CH4 emission of GP during fermentation. This action could insure more energy available for growth and increase the SCFA and ME density of the diet. Improved ADG may be due to improved GP and ruminal fermentation as well as increased microbial protein synthesis of the same diets used as substrate in vitro studies performed in our laboratory. Moreover, secondary metabolites of SB extract such as phenolic compounds or saponins could improve synchronisation between energy and N release and improve microbial protein synthesis (Salem et al.,

Table 1. Dry matter intake, average daily gain and feed efficiency in growing lambs fed concentrate diet with different doses of S. babylonica extract.

| Intake   | SB doses, mL/lamb/d | SEM  | Linear | Quadratic |
|----------|---------------------|------|--------|-----------|
| DMI, kg/d | SB0                 | 1.48 | 0.2805 | 0.3747    |
|          | SB20                | 1.64 | 0.1961 | 0.1387    |
|          | SB40                | 1.62 | 0.9087 | 0.1079    |
|          | SB60                | 1.47 | 0.6052 | 0.1569    |
| Water, L/d | SB0                 | 4.7  | 0.2805 | 0.3747    |
|           | SB20                | 4.8  | 0.1961 | 0.1387    |
|           | SB40                | 4.2  | 0.9087 | 0.1079    |
|           | SB60                | 3.9  | 0.6052 | 0.1569    |
| Initial BW, kg | SB0               | 22.7 | 0.2805 | 0.3747    |
|            | SB20                | 25.6 | 0.1961 | 0.1387    |
|            | SB40                | 22.4 | 0.9087 | 0.1079    |
|            | SB60                | 24.2 | 0.6052 | 0.1569    |
| Final BW, kg | SB0                 | 41.5 | 0.2805 | 0.3747    |
|           | SB20                | 45.4 | 0.1961 | 0.1387    |
|           | SB40                | 42.8 | 0.9087 | 0.1079    |
|           | SB60                | 43.0 | 0.6052 | 0.1569    |
| ADG, g/d | SB0                 | 216.4| 0.2805 | 0.3747    |
|          | SB20                | 227.2| 0.1961 | 0.1387    |
|          | SB40                | 234.2| 0.9087 | 0.1079    |
|          | SB60                | 216.0| 0.6052 | 0.1569    |
| g DM/kg ADG | SB0                | 6.9  | 0.2805 | 0.3747    |
|           | SB20                | 6.6  | 0.1961 | 0.1387    |
|           | SB40                | 6.9  | 0.9087 | 0.1079    |
|           | SB60                | 6.9  | 0.6052 | 0.1569    |

SB, S. babylonica; DMI, dry matter intake; SB0, 0 mL/lamb/d S. babylonica (Control); SB20, 20 mL/lamb/d S. babylonica; SB40, 40 mL/lamb/d S. babylonica; SB60, 60 mL/lamb/d S. babylonica; BW, body weight; ADG, average daily gain. P values of all parameters were more than 0.05.
decreased rate of production with increasing These effects are related to secondary metabo-
lites content of SB extract. Hig her ble sugars in the extracts (Patra et al., 2011). Saponins have antimicrobial properties on ciliate protozoa growth, peptidase-producing bacteria, and celllyticolytic bacteria (Francis et al., 2002) which normally effect SCFA production (i.e., acetate, not propionate; Wallace et al., 1994) which could improve ADG. Secondary metabolites of SB extract may improve the lamb’s health during the 72 days of experiment by their anti-helminthic effects (Xhomfulana et al., 2009; Mejía-Hernández et al., 2014) that could improve nutrient digestibilities, ruminal fermentation, and animal health. Mejía-Hernández et al. (2014) at the same experimental farm recently reported an elimination of about 40% of intestinal worm burdens when lambs administrated orally with 30 mL of SB extract daily for 60 days. Xhomfulana et al. (2009) showed that steers fed A. karroo leaves had low mean total faecal egg counts, Haemonchus contortus and Oesophagostomum columbianum worm burdens.

In vitro gas production

The asymptotic GP was increased with decreased rate of production with increasing SB extract. This phenomenon was observed with all doses of SB extract supplementation. These effects are related to secondary metabolites content of SB extract. Higher in vitro GP and ruminal fermentation patterns of TMR fed to lambs with SB could be, at least partly, due to its lower secondary metabolites concentrations (Salem et al., 2006), and/or higher soluble sugars in the extracts (Patra et al., 2006) which could positively affect ruminal microorganism activity. At low secondary metabolites concentrations, as it was in the current study, rumen microorganisms could degrade and use them as energy source. Rumen microorganisms may degrade alkaloids (Wachsenheim et al., 1992), saponins (Hart et al., 2008) and phenolics (Varel et al., 1991). The low and moderate doses of secondary metabolites can increase cell-wall constituent’s degradability, and improve synchronisation between energy and N release in the rumen resulting in more energy available for increasing SCFA production and ME density (Salem et al., 2014b).

Table 2. In vitro gas production parameters, gas volume accumulated after different hour of incubation and rumen fermentation profile of diet with different doses of S. babylonica extract.

| SB doses, mL/g DM | SEM | Linear P | Quadratic P |
|------------------|-----|----------|-------------|
| 0                | 0.872 | 0.926 | 0.889 | 0.902 | 0.0616 | 0.0232 | 0.8530 |
| 0.3              | 321.7 | 0.902 | 0.047 | 0.0046 | 0.2253 | <0.0001 |
| 0.7              | 225.5 | 0.902 | 0.1575 | 0.4766 | 0.0003 | 0.8056 |
| 1.0              | 121.2 | 0.902 | 0.1575 | 0.4766 | 0.0003 | 0.8056 |

In vitro GP, mL/g DM

| GP parameters | 0 | 0.3 | 0.7 | 1.0 | SEM | Linear P | Quadratic P |
|---------------|---|-----|-----|-----|-----|----------|-------------|
| b, mL/g DM    | 254.4 | 262.7 | 278.6 | 313.4 | 18.64 | 0.2107 | <0.0001 |
| c, h          | 9.086 | 0.07 | 0.05925 | 0.047 | 0.0046 | 0.2253 | <0.0001 |
| L, h          | 2.008 | 1.213 | 2.255 | 1.575 | 0.4766 | 0.0003 | 0.8056 |

Rumen fermentation profile

| DMD, mg/g DM  | 868.5 | 870.3 | 864.0 | 844.3 | 32.10 | 0.9417 | 0.0014 |
|---------------|-------|-------|-------|-------|-------|---------|--------|
| ME, MJ/kg DM  | 8.6 | 8.9 | 8.7 | 8.7 | 0.38 | 0.0244 | 0.8428 |
| SCFA, mmol/g DM | 0.872 | 0.926 | 0.889 | 0.902 | 0.0616 | 0.0232 | 0.8530 |

SB, S. babylonica; GP, gas production; DM, dry matter; h, asymptotic gas production; c, rate of gas production; L, initial delay before gas production begins; DMD, apparent degraded substrate; ME, metabolisable energy; SCFA, short chain fatty acids. *Calculated according to Menke and Steingass (1988) based on gas production; #calculated according to Getachew et al. (2002) based on gas production. #Means in the same row with different superscripts indicate significant differences at P<0.05.

Conclusions

Daily oral administration of SB extract to lambs tended to increase DMI with a concomitant tendency to improve lamb daily weight gain compared to control. In vitro GP and ruminal fermentation results demonstrated that SB extract with doses up to 1 mL/kg DM of diet improved in vitro GP. Results of our study indicate that the use of SB extract with a rate of 40 mL/lamb/d can improve performance of lambs.

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