Influence of oral administration of *Salix babylonica* extract on milk production and composition in dairy cows

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Abstract

A 3×3 Latin Square design was used to evaluate effects of 0, 150 and 300 mL of *Salix babylonica* (SB) extract mixed into the diet on daily milk production and composition in cows. Three Brown Swiss dairy cows (420±30.3 kg body weight), at late lactation (220±25.1 d in milk), were fed a diet with a restricted amount of concentrate and oat hay *ad libitum* twice daily in equal amounts. The SB extract was mixed daily with a small amount of concentrate and fed to the cows. *In vitro* gas production of the diet fed to the cows was recorded at 2, 4, 6, 8, 10, 12, 24, 48, and 72 h of incubation with 0, 0.6, 1.2 and 1.8 mL SB/g DM. Intake of oat hay was increased (P<0.05) by 11.5% with the SB addition at 150 mL/d. Milk production was also increased with extract addition at 150 or 300 mL/d by about 13.3 and 8.9% respectively, compared with control. Milk fat was lower (P<0.05) with SB addition, while milk protein and lactose were not affected by the extract addition. Milk efficiency was improved (P<0.05) with extract addition versus control. *In vitro* gas production of the diet increased (P<0.05) dramatically with increasing levels of extract addition with a short lag time and high rate of gas production per hour vs control. Addition of SB extract at 150 mL/d improved milk production by 13.3%, while it decreased its fat content and improved milk efficiency.

Introduction

Ruminal fermentation is accompanied by losses of the energy and protein consumed by dairy cows (Tamminga, 1992; Busquet et al., 2006) which may limit productive performance and contribute to release of pollutants to the environment (Calsamiglia et al., 2007). Ionophores have been used to reduce these losses (McGuffey et al., 2001), but the use of antibiotics in animal feeds has been banned in the European Union since January 2006 (Jiménez-Peralta et al., 2012) due to potential appearance of residues in milk (Russell and Houlihan, 2003). For this reason, there is substantial interest in evaluating the potential of using natural antimicrobials, such as plant extracts generally recognized as safe for human consumption (Busquet et al., 2006; Fandiño et al., 2008), to modify rumen microbial fermentation. Extract of *Salix babylonica* (SB) have been evaluated for their anti-microbial effects and for their potential to modulate ruminal fermentation and improve nutrient utilization in ruminants (Mejía-Hernández et al., 2013; Salem, 2012; Salem et al., 2010, 2011). The antimicrobial activity of SB extracts has been attributed to a number of plant secondary metabolites (PSM) such as alkaloids, saponins and phenolics (Jiménez-Peralta et al., 2011). Rumen microorganisms have the ability to degrade low concentrations of PSM without negative effects on rumen fermentation. Rumen microorganisms can also degrade alkaloids (Lanigan, 1970; Wachenheim et al., 1992), saponins (Lu and Jorgensen, 1987; Hu et al., 2005; Hart et al., 2008) and phenolics (Varel and Jung, 1986; Varel et al., 1991) and utilize them as an energy source. Some PSM can enhance protein metabolism and decrease methane production (Benchaa et al., 2007), and have the ability to suppress or stimulate microbial growth, increase binding of ammonia during urea ammoniation of straw and reduce odours from cattle manure in dairy barns (Makkar et al., 1998; Salem et al., 2012). In addition, some PSM reduce nutritional stress such as bloat and/or improve animal health and productivity (Patra et al., 2006; Benchaa et al., 2007, Xhomfulana et al., 2009; Salem et al., 2010). Positive effects on daily gain, voluntary feed intake and milk production (Salem et al., 2011) have been demonstrated, as well as a protective effect on dietary protein in the rumen in order to promote duodenal absorption, minimize excretion of nitrogen, modify the acetate to propionate ratio in rumen fluid and decrease the parasitic load (Salem et al., 2010; Jiménez-Peralta et al., 2011). Use of plant extracts could be limited by their secondary compound concentrations as consumption of large amounts of tannins or saponins may have a direct haemolytic effect and may even cause death (Athanasiaidou 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 determine effects of SB extract mixed in the diet on milk production and composition in Brown Swiss cows in addition to *in vitro* gas production of the diet fed to the cows with different doses of SB.
Materials and methods

Cows, diets and extract supplementation

Three Brown Swiss cows (420±30.3 kg body weight), at late lactation (220±25.1 d in milk), for each treatment, were used in a 3x3 Latin Square design experiment with 14 d adaptation periods followed by 7 days of collections. Cows were housed in individual pens of 3x3 m and fed a restricted amount of concentrate and oat hay ad libitum (NRC, 1985), twice daily in equal amounts at 06:30 and 18:30 h after milking at 06:00 and 18:00 h. The ingredients and chemical composition of the concentrate mixture and oat hay is in Table 1. The treatments were: Control group fed a basal diet without SB extract (SB0); SB150 group, fed basal diet (as Control) plus SB extract at 150 mL/cow/d; SB300 group, fed basal diet (as Control) plus SB extract at 300 mL/cow/d. A weekly stock volume of 10 L each of the extract was prepared for administration. Extract was administrated daily by mixing the extract dose with a small amount of the concentrate mixture to ensure that the cow received its extract dose and then the cows were offered the rest of basal diet during the day. Feed and water intake were recorded daily. Fresh water was always available.

Preparation of extract

Plant leaves of S. babylonica were collected randomly from several young and mature trees during summer. Leaves were freshly chopped into 1 to 2 cm lengths and immediately extracted at 1 g leaf/8 mL of water. Plant materials were individually soaked and incubated in water in the laboratory at 25 to 30°C for 48 to 72 h in closed 20 mL bottles. After incubation, jars were heated to 39°C for 1 h, then immediately filtered and the filtrates were collected and stored at 4°C for further use.

Milk production and composition

Cows were milked in their tie stalls at 06:00 and 18:00 h, and milk yield was recorded daily and sampled on two days during the collection period (i.e., 7 days) of each period. Milk samples were preserved with potassium dichromate, stored at 4°C, and sent to the laboratory for milk composition analyses. Milk samples were analyzed for fat, total protein and lactose with near mid-infrared procedures using a Milk-O-Scan 605 (Foss Electric, Hillerod, Denmark). Final milk composition for each week was expressed as the weighted yield of the two daily milking. Average fat and total protein yields were calculated by multiplying milk yield by fat and protein contents of milk on an individual cow basis.

In vitro experiment

Treatments

Four extract doses [i.e., 0, 0.6, 1.2, 1.8 mL/g dry matter (DM)] of the same diet fed to cows in three replicates for each treatment on the resultant in vitro fermentation kinetic profile of the substrate. The diet contained, on DM basis: organic matter, 973 g/kg; crude protein, 208 g/kg; ether extract, 12 g/kg; neutral detergent fibre, 364 g/kg; acid detergent fibre, 41 g/kg (Table 1), which was also used to feed the rumen fluid donor cows.

In vitro incubations

In vitro gas production was measured using the Control diet fed to cows as substrate with different doses of SB extract (i.e., 0, 0.6, 1.2, 1.8 mL/g DM). Rumen fluid was collected from two ruminally cannulated Brown Swiss (450±20 kg body weight) fed the Control diet. Samples (1 g) of substrate were weighed into 120 mL serum bottles and the SB extract doses (i.e., 0, 0.6, 1.2, 1.8 mL/g DM) were applied directly onto the substrate inside the bottles immediately before adding buffer medium and rumen fluid. Ruminal contents of each cow were obtained immediately before the morning feeding, mixed and strained through four layers of cheesecloth into a flask with an O2-free headspace. Ten mL of particle-free ruminal fluid was added to each bottle and 40 mL of the buffer solution of Goering and Van Soest (1970), with no trypticase added, was immediately added in a 1:4 (v/v) proportion. A total of 36 bottles (three bottles for each extract dose (i.e., 0, 0.6, 1.2, 1.8 mL) in 3 different runs with 3 bottles as blanks (i.e., rumen fluid only), were incubated for 72 h. Once all the bottles were filled, they were immediately closed with rubber stoppers, shaken and placed in the incubator at 39°C. Volume of gas produced was recorded at incubation times of 2, 4, 6, 8, 10, 12, 24, 48 and 72 h after incubation using the reading pressure technique (RPT; DELTA OHM, Italy) of Theodorou et al. (1994). At the end of the incubation (i.e., 72 h), bottles were uncapped, pH was measured (GLP 22, Crison Instruments, Barcelona, Spain). Contents of each bottle were then transferred to filtered fermentation residue for determination of apparent degraded substrate.

Analytical procedures

Samples of concentrate mixture and oat hay were collected twice weekly during each period to calculate DM intake. Samples were ground to pass a 1 mm screen on a model 4 Wiley Mill and

Table 1. Ingredients and chemical composition of the concentrate mixture and oat hay and secondary metabolities of Salix babylonica extract.

| Ingredients, g/kg | Concentrate | Oat hay |
|------------------|-------------|---------|
| Corn grain, flaked | 200 | |
| Corn grain, cracked | 260 | |
| Sorghum, grain | 154 | |
| Molasses, sugar cane | 100 | |
| Dried distillers grains with solubles | 100 | |
| Soya bean, meal | 96 | |
| Wheat, bran | 70 | |
| NaCO₃ | 10 | |
| Mineral mix | 10 | |

Chemical composition, g/kg

Organic matter 920±3.1 922±4.3
Crude protein 157±1.1 80±2.1
Ether extract 120±1.6 nd
Neutral detergent fibre 160±2.2 661±3.1
Acid detergent fibre 28±0.9 417±2.8

Secondary compounds in S. Babylonica extract, g/kg

Total phenolics 13±0.8
Saponins 4.8±0.5
Aqueous fraction 73±2.2

aMineral and vitamin mixture: Ca, 190 g/d; P, 115 g/d; Mg, 63 g/d  Cu, 1.67 g/d; Fe, 380 mg/d; Mn, 900 mg/d; Se, 2 mg/d; Zn, 810 mg/d; Vit. A, 940 mg (1000 IU); Vit. D, 165 mg (1000 IU); Vit. E, 374 mg (1000 IU).

bAqueous fraction (lectins, polypeptides, starch; Cowan, 1999). nd, not determined.
analyzed according to the AOAC (1997) for DM (method #934.01) and ether extract (method #920.39). Acid detergent fibre was determined using Ankom Technology (AOAC, 1997, Method #973.18). Neutral detergent fibre analyses included a heat stable amylase (Van Soest et al., 1991) and are expressed both inclusive and exclusive of residual ash. Ash was determined by incineration at 550°C for 3 h (AOAC, 1997, method #942.05). Total N was determined with a N gas analyzer utilizing an induction furnace and thermal conductivity (LECO FP-528, AOAC, 1997, Method #990.03). Two samples of extract were collected each week for secondary metabolite determination as described in Salem (2012). Briefly, extract, 10 mL, was fractionated by funnel separation with a double volume of ethyl acetate to determine total phenolics by drying and quantifying the total phenolics layer in the funnel. After phenolics separation, a double volume of n-butanol, was added to fractionate the saponins. The remaining solution was considered the aqueous fraction.

Dry matter degradability was determined at the end of in vitro incubation (i.e., 72 h). Contents of each serum bottle were filtered through sintered glass crucibles under vacuum. Fermentation residues were dried at 105°C overnight to estimate potential DM disappearance. Loss in weight after drying was the measure of undegradable DM. The DM degradability at 72 h of incubation was calculated as the difference between DM content of substrate and its undegradable DM.

**Calculations**

Milk energy (Mcal/kg) was calculated on an individual cow basis using milk fat, protein and lactose content of milk (Tyrrell and Reid, 1965) as:

\[
\text{Milk energy (Mcal/kg)} = \frac{(0.61 \times \text{Fat} + 24.13 \times \text{Protein} + 21.60 \times \text{Lactose}) 
- 11.72}{1000} \times 2.204
\]

where
- fat = milk fat g/kg milk;
- protein = milk protein g/kg milk;
- lactose = milk lactose g/kg milk.

To estimate kinetic parameters of gas production, gas production results (mL/g DM) were fitted using the NLIN option of SAS (2002) according to the France et al. (2000) model as:

\[
A = b \times (1 - e^{-c(t-L)})
\]

where
- A = volume of gas production at time t;
- b = asymptotic gas production (mL/g DM);
- c = rate of gas production (h/DM), from the slowly fermentable feed fraction b, and L is the discrete lag time prior to gas production.

**Statistical analysis**

Differences in in vitro measurements were determined using the PROC GLM procedure of SAS (2002) with SB levels in a completely randomized design. SB effects were determined as linear contrasts within SAS as defined by Steel and Torrie (1980). Significance was accepted if P<0.05. All data were analyzed as a 3x3 Latin Square with a factorial arrangement of treatments using the MIXED model procedure in SAS (2002). The model for DM intake, water consumption, milk production and milk composition was:

\[
\gamma = \mu + C_j(S) + P_k + T_i + e_{ijk}
\]

where
- \( \gamma \) = dependent variable;
- \( \mu \) = overall mean of the population;
- \( C_j(S) \) = random effect of cow j nested within SB levels;
- \( P_k \) = fixed effect of period k (k = 1 to 3);
- \( T_i \) = fixed effect of extract doses 1 (1 = 1 to 3);
- \( e_{ijk} \) = unexplained residual element assumed to be independent and identically distributed.

The SB extract effects for DM intake, water consumption, milk production and milk composition were determined as linear contrasts within SAS as defined by Steel and Torrie (1980). Significance was accepted if P<0.05.

**Results**

Neither total dry matter intake (DMI) nor water intake (Table 2), were influenced by addition of SB extract at 150 or 300 mL/d supplementation. Only oat hay intake increased (P<0.05) by ~11.5 and 1.2%, respectively (Table 2), compared to Control.

Output of milk tended to increase (P=0.061) for cows fed SB extract at 150 or 300 mL/d by ~13.3 and 8.9%, respectively. Milk content of protein and lactose were not affected (P>0.05) by SB extract administration, while milk fat content was decreased (P<0.05), causing a decrease of milk energy output (Mcal/d). Increased milk yield for cows fed SB extract was not accompanied by changes in yield of milk components. Milk efficiency (kg DM intake/kg of milk produced) was improved (P<0.05) with the SB extract doses (i.e., 150 and 300 mL/d) vs Control (i.e., 0 mL/d; Table 2). As doses of SB extract increased, the asymptotic gas production (mL/g DM) (P<0.05) and DM degradability (P<0.05) increased, whereas rate of gas production (c) and the initial delay before gas production begins (L) was not affected (Table 3). The gas production (mL/g DM) at 24 (P<0.05) and 72 h (P<0.05) of incubation increased with increasing the doses of SB extract administrated (Table 3).

**Discussion**

**Feed intake**

Addition of low dose of SB extract (i.e., 150 mL/d) tended (P=0.062) to increase DMI by about 6.3%, and increased (P<0.05) the intake from oat hay by about 11.5% compared to Control (Table 2), while high dose of the SB extract (i.e., 300 mL/d) did not affect total DMI or oat intake. However, increased DMI with low SB extract administration may have been due to positive impacts of low dose of PSM on ruminal fermentation, whereas the high SB extract dose (i.e., high PSM administration) with antimicrobial activity decreased microbial activity and diet fermentability, which negatively affected DMI (Jiménez-Peralta et al., 2011; Salem et al., 2011). Administration of low doses of SB extract likely encouraged some rumen bacterial species to metabolize phenolic compounds (Chen et al., 1988; Salem et al., 2010), and may act as catalysts for fibre degradation by increasing access of fibrolytic bacteria to cell wall polysaccharides in the diet. This action will lead to increased rates of disappearance in the rumen, with increased rates of passage and DM intake as a result (Conrad, 1966). Our results are consistent with Salem et al. (2011) who stated that addition of SB extract at 30 mL/d had no effect on DM intake compared to Control (S. babylonica extract at 0 mL/d) of growing lambs.

**Cow performance**

Reasonably, if the cows’ rumen fermentation kinetics and digestion were improved by administration of low dose of SB extract, which paralleled increased intake of oat hay, it would be expected that they would produce more milk, as occurred. Administration of low doses of SB extract could lead to reduction in the proportion of methane in eructated gas thereby resulting more digestible energy (Jiménez-Peralta et al., 2011), which can be utilized to support milk production. However, increased milk production may also be due to improved synchronization between energy and protein release in the rumen in the presence of some chemical constituents of the plant extracts. Some of these phenolic compounds may interact with biosynthesis of aromatic amino acids, as both biosynthesis pathways are linked...
through cinnamic acid. Phenylpropanoic acid and phenylacetic acid have been reported to enhance cellulose degradation and growth of several strains of *Ruminococcus albus* (Stack et al., 1983; Stack and Cotta, 1986). In addition, administration of SB extract eliminated >40% of the intestinal worm burdens (Mejía-Hernández et al., 2013) which means more utilization of dietary energy for milk production.

Increased milk production was paralleled with a decreased milk fat content which confirms the negative relationship between milk fat content and milk yield as stated by Alphonsus and Essien (2012). Decreased milk fat content in SB cows, at both 150 and 300 mL/d compared with Control, may be due to reduction of acetate to propionate portion in the rumen.

### Gas production

Gas production is generally a good indicator of digestibility, fermentability and rumen microbial protein production (Sommart et al., 2000). SB extract addition was expected to be beneficial to rumen function based on their stimulating effect on fermentation, and by increasing degradabilities of crude protein and plant cell wall constituents, as well as by increasing microbial protein production. Increased ruminal gas production and rumen fermentation activities were paralleled with increasing doses of SB extract. This may be due to the ability of rumen microorganisms to degrade these chemical constituents, as they have the ability to degrade low levels of secondary metabolites in plant extracts (Wachenheim et al., 1992 (alkaloids); Hu et al., 2005 (saponins); Hart et al., 2008 (saponins); Varel et al., 1991 (phenolics)) and utilize them as an energy source without negative effects on rumen fermentation. Increased gas production at 24 and 72 h suggests a higher extent of fermentation in the rumens of cows fed SB extract at all doses (*i.e.*, 0.6, 1.2, and 1.8 mL/g DM) compared to Control (*i.e.*, 0 mL/g DM). Salem et al. (2012) and Jiménez-Peralta et al. (2011) all found that administration of SB extract to high concentrate diet increased asymptotic gas production and gas production after 24 and 72 h of incubation.

### Conclusions

*Salix babylonica* extract supplementation to diets of lactating dairy cows has been a renewed subject of research by ruminant nutritionists in the search for ionophore mimicking natural products, which can be safely fed to ruminants. Our results show that SB extract was an effective way of increasing milk production and improving feed utilization by Brown Swiss dairy cows by improving rumen fermentation.

The present results suggest that the low doses of SB administration (150 mL/d) were better than the high dose (300 mL/d).

### References

Alphonsus, C., Essien, I.C., 2012. The relationship estimates amongst milk yield and milk composition characteristics of Bunaji and Friesian × Bunaji cows. Afr. J. Biotechnol. 11:8790-8793.

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

Athanasiadou, S., Kyriazakis, I., 2004. Plant secondary metabolites: antiparasitic effects and their role in ruminant production systems. P. Nutr. Soc. 63:631-639.

Benchaar, C., Petit, H.V., Berthiaume, R., Ouellet, D.R., Chiquette, J., Chouinard, P.Y., 2007. Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage. J. Dairy Sci. 90:886-897.

Busquet, M., Calsamiglia, S., Ferret A., Kamel, C., 2006. Plant extracts affect in vitro rumen microbial fermentation. J. Dairy Sci. 89:761-771.

Calsamiglia, S., Busquet, M., Cardozo, P.W.,...
Castillejos, L., Ferret, A., 2007. Invited Review: Essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci. 90:2580-2595.

Chen, W., Ohmiya, K., Shimizu, S., Kawakami, H., 1988. Isolation and characterization of an anaerobic dehydrovanillin-degradin bacteria. Appl. Environ. Microbiol. 54:1254-1257.

Conrad, H.R., 1966. Symposium on factors influencing the voluntary intake of herbage by ruminants: physiological and physical factors limiting feed intake. J. Anim. Sci. 25:227-235.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12:564-582.

Fandíño, I., Calsamiglia, S., Ferret, A., Blanch, M., 2008. Anise and capsicum as alternatives to monensin to modify rumen fermentation in beef feeders fed a high concentrate diet. Anim. Feed Sci. Tech. 145:409-417.

France, J., Dijkstra, J., Dhanou, M.S., Lopez, S., Bannink, A., 2000. Estimating the extent of degradation of ruminant feedstuffs from a description of their gas production profiles observed in vitro: derivation of models and other mathematical considerations. Brit. J. Nutr. 83:143-150.

Goering, M.K., Van Soest, P.J., 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures and Some Applications). Agriculture Handbook N. 379. Agricultural Research Service, USDA Publ., Washington, DC, USA.

Hart, K.J., Yanez-Ruiz, D.R., Duval, S.M., McEwan, N.R., Newbold, C.J., 2008. Plant extracts to manipulate rumen fermentation. Anim. Feed Sci. Tech. 145:409-417.

Hu, W.L., Liu, J.X., Ye, J.A., Wu, Y.M., Guo, Y.Q., 2005. Effect of tea saponin on rumen fermentation in vitro. Anim. Feed Sci. Tech. 120:333-339.

Jiménez-Peralta, F.S., Salem, A.Z.M., Mejía, H.P., González, R.M., Albarrán, P.B., Rojo, R.R., Tinoco, J.L., 2011. Influence of individual and mixed extracts of two tree species on in vitro gas production kinetics of a high concentrate diet fed to growing lambs. Livest. Sci. 136:192-200.

Lanigan, G.W., 1970. Metabolism of pyrroloizidine alkaloids in the ovine rumen. II. Some factors affecting rate of alkaloid breakdown by rumen fluid in vitro. Aust. J. Agr. Res. 21:633-640.

Lu, C.D., Jorgensen, N.A., 1987. Alfalfa saponins affect site and extent of nutrient digestion in ruminants. J. Nutr. 117:919-927.

Mahgoub, O., Kadim, I.T., Tageldin, M.H., El Marzoogi, W.S., Khalaf, S.Q., Ambu Ali, A., 2008. Clinical Profile of sheep fed non-conventional feeds containing phenols and condensed tannins. Small Ruminant Res. 78:115-122.

Makkar, H.P.S., Sen, S., Blummel, M., Becker, K., 1998. Effects of fractions containing saponins from Yucca schidigera, Quillaja saponaria and Acacia auriculiformis on rumen fermentation. J. Agric. Food Chem. 46:4324-4328.

McGuffey, R.K., L.F. Richardson, J.D. Wilkinson. 2001. Ionophore for dairy cattle: Current status and future outlook. J. Dairy Sci. 84:E194-E203.

Mejía-Hernández, P., Salem, A.Z.M., Elghandour, M.M.Y., Cipriano-Salazariz, M., Cruz-Lagunas, B., Camacho, L.M., 2013. Anthelmintic effects of Salix babylonica L. and Leucaena leucocephala Lam. extracts in growing lambs. Trop. Anim. Health Pro. (in press).

National Research Council, 1985. Nutrient Requirements of Domestic Animals. National Academy Press, Washington, DC, USA.

Patra, A.K., Kamra, D.N., Agarwal, N., 2006. Effects of plant extracts on in vitro methanogenesis, enzyme activities and fermentation in feed in rumin liquid of buffalo. Anim. Feed Sci. Tech. 128:276-291.

Russell, J.B., Houlihan, A.J., 2003. Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS Microbiol. Rev. 27:65-74.

Salem A.Z.M., 2012. Oral administration of leaf extracts to rumen liquid donor lambs modifies in vitro gas production of other tree leaves. Anim. Feed Sci. Tech. 176:94-101.

Salem, A.Z.M., Olivares, M., López, S., González-Ronquillo, M., Rojo, R., Camacho, L.M., Cerrillo, S.M.A., Mejia, H.P., 2011. Effect of natural extracts of Salix babylonica and Leucaena leucocephala on nutrient digestibility and growth performance of lambs. Anim. Feed Sci. Tech. 170:27-34.

Salem, A.Z.M., Robinson, P.H., Lopez, S., Gohar, Y.M., Rojo, R., Tinoco, J.L., 2010. Sensitivity of sheep intestinal lactic acid bacteria to secondary compounds extracted from Acacia saligna leaves. Anim. Feed Sci. Tech. 161:85-93.

SAS, 2002. SAS User’s Guide: Statistics, ver 9.0. SAS Inst. Inc., Cary, NC, USA.

Sommart, K., Parker, D.S., Wanapat, M., Rowlinson, P., 2000. Fermentation characteristics and microbial protein synthesis in an in vitro system using cassava, rice straw and dried ruzi grass as substrates. Asian Austral. J. Anim. 13:1084-1093.

Stack, R.J., Cotta, M.A., 1986. Effect of 3-phenylpropanoic acid on growth of, and cellulose utilization by, cellulolytic ruminal bacteria. Appl. Environ. Microbiol. 52:209-210.

Stack, R.J., Hungate, R.E., Opsahl, W.P., 1983. Phenylacetic acid stimulation of cellulose digestion by Ruminococcus albus. Appl. Environ. Microbiol. 46:539-544.

Steel, R.G.D., Torrie, J.H., 1980 Principles and Procedures of Statistics, 2nd ed. McGraw-Hill Publ., New York, NY, USA.

Tammingsa, S., 1992. Nutrition management of dairy cows as a contribution to pollution control. J. Dairy Sci. 75:345-357.

Theodorou, M.K., Williams, B.A., Dhanou, M.S., McAllan, A.B., France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Tech. 48:185-197.

Tyrrell, H.F., Reid, J.T., 1965. Prediction of the energy value of cow’s milk. J. Dairy Sci. 48:1215-1223.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre, and non-starch carbohydrates in relation to animal nutrition. J. Dairy Sci.74:5383-5397.

Varel, V.H., Jungh, H.G., 1986. Influence of forage phenolics on ruminal fibibytic bacteria and in vitro fiber degradation. Appl. Environ. Microbiol. 52:275-280.

Vareli, V.H., Jung, H.G., Krumholz, L.R., 1991. Degradation of cellulose and forage fiber fractions by ruminal cellulolytic bacteria alone and in coculture with phenolic monomer-degrading bacteria. J. Anim. Sci. 69:4993-5000.

Wachenheim, D.E., Blythe, L.L., Craig, A.M., 1992. Characterization of rumin bacterial pyrrolizidine alkaloid biotransformation in ruminants of various species. Vet. Hum. Toxicol. 34:513-517.

Xhomfulana, V., Mapije, C., Chimonyo, M., Marufu, M.C., 2009. Supplements containing Acacia karroo foliage reduce nematode burdens in Nguni and crossbred cattle. Anim. Prod. Sci. 49:646-653.