PAPER

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 (Benchaar *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; Benchaar *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 (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 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.

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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 3×3 Latin Square design experiment with 14 d adaptation periods followed by 7 days of collections. Cows were housed in individual pens of 3×3 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 L jars. 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±20kg 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 O_2-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 inoculation 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 metabolites of *Salix babylonica* extract.

	Concentrate	Oat hay
Ingredients, g/kg		
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	
$NaCO_3$	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

°Mineral and vitamin mixture: Ca, 190 g/d; P, 115 g/d; Mg, 63 g/d; Cl,167 g/d; K, 380 g/d; Na, 70 g/d; S, 53 g/d; Co, 3.3 mg/d; Cu, 197 mg/d; Fe, 360 mg/d; Mn, 900 mg/d; Se, 2 mg/d; Zn, 810 mg/d; Vit. A, 940 mg (1000 U/d); Vit. D, 165 mg (1000 U/d); Vit. E, 374 mg (1000 U/d). #Aqueous 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:

$$=\left(\frac{((41.63\text{Fat})+24.13\text{Protein})+21.60\text{Lactose})-11.72}{1000}\right)\times 2.204$$

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) 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 3×3 in 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:

$$\gamma = \mu + Cj\ (S)i + Pk + Tl + eijkl$$

where
γ = dependent variable;
μ =overall mean of the population;
Cj (S)i = random effect of cow j nested within square I ((j = 1 to 3);
Pk = fixed effect of period k (k = 1 to 3);
Tl = fixed effect of extract doses 1 (1= 1 to 3);
eijkl = 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) (*b,* 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). Deceased 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.

Table 2. Milk production and composition in dairy cows supplemented with different doses of Salix babylonica extract.

	Doses of *S. babylonica*, mL cow/d				
	SB0	SB150	SB300	SEM	P linear
Intake, kg/d					
Total DM	17.8	18.9	17.5	1.35	0.062
Oat hay	12.9^{b}	14.4^{a}	13.0^{a}	1.12	0.041
Water	77. 6	81.4	78.3	4.70	0.058
Milk					
Production, kg/d	11.2	12.7	12.2	1.02	0.061
Energy, MJ/kg	34.6	33.6	34.0	1.57	0.064
Milk composition, %					
Fat	4.9^{a}	4.8^{b}	4.7b	0.25	0.049
Protein	4.5	4.3	4.5	0.23	0.723
Lactose	3.1	3.0	3.2	0.18	0.768
Milk component yield, kg/d					
Fat	0.55	0.58	0.57	0.033	0.063
Protein	0.50	0.53	0.55	0.030	0.408
Lactose	0.35	0.37	0.39	0.028	0.062
kg DMI/kg milk	1.59^{a}	1.55^{b}	1.35^{b}	0.210	0.042

SB, *Salix babylonica*; DM, dry matter; DMI, dry matter intake. ^{a,b}Different superscripts following means in the same row indicate differences at P<0.05.

Table 3. *In vitro* gas production parameters and after 24 and 72 h of incubation as well as dry matter degradability of the concentrate and oat hay (1:1, w/w) with different doses of *S. babylonica* extract.

	Doses of *S. babylonica*, mL cow/d					
	0	0.6	1.2	1.8	SEM	P linear
b, mL/g DM	127^{c}	209^{b}	278^{ab}	299^{a}	11.8	0.011
c, /h	0.060	0.069	0.051	0.044	0.0036	0.213
L, h	2.14	1.1	1.09	0.76	0.587	0.565
GP_{24}, mL/g DM	100^{d}	131^{c}	167^{a}	144^{b}	11.1	0.034
GP_{72}, mL/g DM	125^{c}	199^{b}	257^{a}	271^{a}	11.0	0.012
DM degradability, mg/g DM	671^{b}	685^{b}	713^{a}	725^{a}	19.4	0.025

b, asymptotic gas production (mL/g DM); *c*, rate of gas production (/h); *L*, initial delay before gas production begins (h). $^{a-d}$Different superscripts following means in the same row indicate differences at P<0.05.

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).

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