



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

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Received: 27 September 2015; Accepted: 24 February 2016

### 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 phytochemical 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.

### 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 m<sup>2</sup> 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

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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 two 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<sub>2</sub> 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<sub>2</sub> 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;  $\mu$ , 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<sup>1</sup> (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 |

<sup>1</sup>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 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 |  |
| CD30                   | 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 |  |
|                        | 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   |  |
| CD45                   | 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 |  |
|                        | 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    |  |
| CD60                   | 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 |  |
|                        | 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    |  |
| SEM pooled             | 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 |  |
|                        | CD × SB             | 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                 |                     |                           |        |        |                                   |        |        |        |        |        |        |        |        |        |  |
| Quadratic              |                     |                           |        |        |                                   |        |        |        |        |        |        |        |        |        |  |
| Extract (SB)           |                     |                           |        |        |                                   |        |        |        |        |        |        |        |        |        |  |
| Linear                 |                     |                           |        |        |                                   |        |        |        |        |        |        |        |        |        |  |
| Quadratic              |                     |                           |        |        |                                   |        |        |        |        |        |        |        |        |        |  |
| CD × SB                |                     |                           |        |        |                                   |        |        |        |        |        |        |        |        |        |  |

<sup>1</sup>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, standard error of the mean.

Table 4. Fermentation kinetics<sup>1</sup> 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 |

<sup>1</sup>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, standard 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.

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