Effects of brewers’ spent grains on fermentation quality, chemical composition and in vitro digestibility of mixed silage prepared with corn stalk, dried apple pomace and sweet potato vine

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ABSTRACT
The experiment was conducted to evaluate the effects of brewers’ spent grains (BSG) on the fermentation quality, chemical composition and in vitro parameters of mixed silage prepared with corn stalk, dried apple pomace and sweet potato vine. In the experiment, a ternary mixture of corn stalk, sweet potato vine and dried apple pomace (50/30/20) was ensiled with 0%, 10%, 15% and 20% BSG on a fresh weight (FW) basis for 1, 3, 5, 7, 14 and 30 days, respectively. The application of BSG significantly (p<0.05) increased lactic, acetic and total volatile fatty acids content, and significantly (p<0.05) decreased pH, lactic acid/acetic acid, ammonia nitrogen, dry matter and water-soluble carbohydrates content during ensiling. None or tiny amounts of propionic and butyric acid were detected in all silages. Compared with the control, higher (p<0.05) Flieg points, crude protein content and lower (p<0.05) neutral detergent fibre, acid detergent fibre, cellulose and hemicellulose content were found in BSG-treated silages on day 30 of ensiling. After 72 h of incubation, the application of BSG significantly (p<0.05) increased the cumulative gas production, potential gas production, in vitro dry matter, crude protein digestibility and metabolisable energy. The 20% BSG silages had better fermentation quality and higher in vitro digestibility, as indicated by higher lactic acid content, in vitro dry matter, crude protein digestibility, metabolisable energy, lower pH, and lower ammonia nitrogen content than other silages. The application of 20% BSG was recommended to ensure good quality silages.

HIGHLIGHTS
- Ensiling in the form of mixed silages is a good way to consume largely agro-food by-products.
- Reducing the energy waste and environmental pollution caused by high-quantity agro-food by-products.
- 20% brewers’ spent grains application had positive effects on in vitro parameters.

Introduction
The shortage of forage sources and costly prices of commercial forages restrict the potential of ruminant production. Therefore, it is essential to effectively utilise unconventional roughages. Agro-food by-products are ideal alternatives to commercial forages owing to high-quantity and low-cost characteristics (Xu et al. 2020). However, most of them are discarded or incinerated as fuels, resulting in environmental pollution and resource waste (Li et al. 2019). Moreover, the hot and humid environment in southeast China usually induces decomposition, and reduces the availability of these agro-food by-products. The utilisation of agro-food by-products in the form of silages is regarded as an efficient method for reutilisation of resources and reducing disposal costs (Monllor et al. 2020).

Agro-food by-products have been proposed as replacements for commercial forages. For instance, corn stalk is a widely available agricultural by-product, and the annual yield is about 18.8 million tons in southeast China. The fresh corn stalk, after harvesting the cob, is rich in water-soluble carbohydrates (WSC), which are widely used as unconventional roughages.
for ruminants (Zhou et al. 2019). Apple pomace has a low pH ranging from 3.20 to 4.10 (Pirmohammadi et al. 2006). The dried apple pomace provides 1.86 MJ/kg metabolisable energy (ME) and 1.06–1.12 MJ/kg net energy for lactation for dairy cattle (National Research Council 2001). Abdollahzadeh et al. (2010) reported that the addition of 30% apple pomace to total mixed rations significantly increased total volatile fatty acids, the digestibility of dry matter (DM) and decreased ruminal pH. Sweet potato vine is characterised by high palatability, moderate levels of crude protein and water-soluble carbohydrates and high digestibility of DM (>62%; Galla et al. 2020). These by-products have been widely used as fodders for ruminants after ensiling. However, it is difficult to acquire good fermentation quality when corn stalk is ensiled alone, which is mainly because of its high moisture content (>75%; He et al. 2020). High-moisture silages are more prone to clostridial fermentation and greater DM loss (Zhao et al. 2021). Sweet potato vine may have the potential to reduce DM loss because of the rapid and extensive formation of lactic acid (LA) converted from water-soluble carbohydrates (WSC) and the quick decline of the pH (Pedrosa et al. 2015). Furthermore, considering the high moisture content of corn stalk and sweet potato vine, a practice in this work is to mix wet by-products (corn stalk and sweet potato vine) with dried apple pomace to prepare mixed silages.

Brewers’ spent grains (BSG) is the main by-product of the beer brewing industries and gradually used in animal production because of its antioxidant, antimicrobial activity and nutritional value (Mccarthy et al. 2013; Socaci et al. 2018; San Martin et al. 2021). Antioxidant and antimicrobial properties are mainly applied in food and feed industries, respectively, to inhibit spoilage and deterioration. Previous research has found that wet brewers’ grains could improve fermentation quality and had no adverse effects on in vitro digestibility of total mixed ration (Kim et al. 2015; Wang et al. 2020). Therefore, it is assumed that BSG had the potential to improve fermentation quality and digestibility of mixed silages ensiled with various agro-food by-products. The objective of this experiment was to evaluate the effects of BSG on the fermentation quality and in vitro parameters of mixed silage prepared with corn stalk, sweet potato vine and apple pomace.

**Materials and methods**

**Experimental design**

Corn stalk, sweet potato vine, dried apple pomace and BSG were collected from Wushan Dairy Farm (Zhejiang, China: latitude, 29°06’~29°32’ N; longitude, 121°09’~121°49’ E; altitude, 447.4 m; annual mean temperature, 17.5 °C and average annual rainfall, 1835.2 mm). Corn stalk and sweet potato vine were chopped into about 2 cm using a mechanical chopper (9ZT-0.4, Zhengzhou Hualong Agriculture and Animal Husbandry Machinery Co., Ltd., Zhengzhou, China) equipped with four and six blades (production efficiency, 600–1500 kg/h; chopped length, 1.5–5 cm). A mixture of corn stalk, sweet potato vine and dried apple pomace was mixed at the ratio of 5:3:2 on a fresh weight (FW) basis, and then BSG (0%, 10%, 15% and 20% FW) was ensiled with the mixture. After mixing thoroughly, all ingredients (3.5 kg) of each treatment were packed into 5-L plastic silos and stored at the ambient temperature (15–20 °C) after being sealed with screw tops and plastic tape. A total of 120 silos (4 treatments × 6 ensiling days × 5 replicates) were prepared, and five silos of each treatment were opened on 1, 3, 5, 7, 14 and 30 days, respectively.

**Chemical and microbiological analyses**

Approximately 100 g subsample was dried in an oven at 65 °C for 48 h to determine DM content. The samples were ground to pass 1 mm screen with laboratory knife mills (FW100, Taisite Instrument Co., Ltd., Tianjin, China) and stored for later analysis of acid detergent fibre (ADFom), neutral detergent fibre (aNDFom), acid detergent lignin (ADL) and WSC. The WSC was determined via the modified phenol-sulfuric acid method (Thomas 1977). The content of aNDFom, ADFom and ADL were measured according to the methods of Van Soest et al. (1991) using an ANKOM 200i fibre analyser (ANKOM Technologies, Inc., Fairport, NY). Heat stable α-amylase and sodium sulphite was used in the aNDFom analysis and the results of aNDFom and ADT were expressed on a DM basis exclusive of residual ash. Hemicellulose (HC) was calculated as aNDFom minus ADT and cellulose (CEL) as ADFom minus ADL. Total nitrogen (TN) was determined by a Kjeldahl nitrogen analyser (Kjeltar 8200; FOSS, Sweden) and the crude protein (CP) content was calculated as TN × 6.25.

About 20 g subsample was mixed with 60 mL of distilled water and stored in the refrigerator at 4 °C and macerated for 24 h. The extract was filtered through two layers of medical gauze and a Whatman filter paper (pore size of 11 μm, Xinhua Co., Hangzhou, China), and a glass electrode pH metre (HANNA pH 211; Hanna Instruments Italia Srl, Villafrance Padovana, Italy) was used to measure the pH value of filtrate.
Ammonia nitrogen (NH$_3$-N) was determined by the phenol-hypochlorite reaction method of Broderick and Kang (1980). The analyses of organic acids were conducted in high performance liquid chromatography system (1260 HPLC, Agilent Technologies, Inc., Waldbronn, Germany) equipped with a refractive index detector (column: Carbomix® H-NPS, Sepax Technologies, Inc., Newark, DE; eluent, 2.5 mmol L$^{-1}$ H$_2$SO$_4$, 0.5 mL min$^{-1}$; temperature, 55°C). Total volatile fatty acids (total VFAs) were calculated as the sum of acetic acid (AA), propionic acid (PA) and butyric acid (BA), expressed on a DM basis. The analyses of organic acids were conducted in high performance liquid chromatography system (1260 HPLC, Agilent Technologies, Inc., Waldbronn, Germany) equipped with a refractive index detector (column: Carbomix® H-NPS, Sepax Technologies, Inc., Newark, DE; eluent, 2.5 mmol L$^{-1}$ H$_2$SO$_4$, 0.5 mL min$^{-1}$; temperature, 55°C). Total volatile fatty acids (total VFAs) were calculated as the sum of acetic acid (AA), propionic acid (PA) and butyric acid (BA), expressed on a DM basis.

According to the equation of Kilic (1986), Flieg point was calculated to assess the quality of mixed silage, in which points by the means of the pH values and DM content at the end of ensiling:

$$\text{Flieg points} = \frac{220 + (2 \times \% \text{DM} - 15) - 40 \times \text{pH}}{}$$

When the Flieg point was below 20, the quality of a silage was very bad; 21–40, poor; 41–60, medium; 61–80, good and 81–100, very good.

About 10 g of raw material was serially diluted 10-fold with sterilised saline solution (0.85% sodium chloride). LA bacteria (LAB), aerobic bacteria (AB) and yeasts were enumerated according to the description of Tao et al. (2021).

### In vitro incubation

The experiment was approved by the Ethics Committee of the Nanjing Agricultural University (Jiangsu, China). The rumen fluid was obtained from three rumen-fistulated Boer male goats (100 ± 2.4 kg of live weight) 2 h before the morning feeding. The goats had free access to water and fed twice with the diet consisting of 6% alfalfa, 59% guinea grass and 35% concentrates on a DM basis. The rumen fluid was immediately filtered through four layers of gauze and mixed with the buffer at the ratios of 1:2 (v/v; Menke and Steingass 1988) . Before incubation, 60 mL of the mixture fluid was transferred into a serum bottle and stored at 39°C in a water bath. One filter bag was prepared for each silo. About 1 g ground sample was put into the filter bag (FS7; ANKOM Technology, Macedon, NY). The filter bag was previously washed with acetone, dried at 65°C to a constant weight. All filter bags were heat-sealed and placed into pre-heated serum bottles with the mixture fluid at 39°C. Another five serum bottles with empty filter bags were served as blanks. Continuous carbon-di-oxide was flushed to the serum bottles to keep anaerobic condition. Gas production (GP) was measured using a pressure transducer after 4, 8, 12, 24, 36, 48 and 72 h, respectively and corrected with the blanks.

The data of GP were fitted to the exponential equation: $y = b \left(1 - e^{-ct}\right)$, where $y$ is the cumulative volume of GP (mL) at time $t$, $b$ is the potential GP (mL), $c$ is the rate constant of GP and $t$ is the incubation time (h). The (ME) was estimated with the method of Menke and Steingass (1988).

After 72 h of incubation, all filter bags were rinsed with tap water and then dried at 65°C for 48 h. The in vitro dry matter digestibility (IVDMD), in vitro crude protein digestibility (IVCPD) and in vitro neutral detergent fibre digestibility (IVNDFD) were calculated as weight lost in DM, CP and NDF after in vitro test, respectively. The analyses of DM, CP and NDF of the digested sample and filter bags were conducted according to the methods described previously.

### Statistical analyses

The collected data were analysed using the general linear model (GLM) procedure of SAS 9.2 (SAS Inst.
Table 2. Chemical and microbial composition of mixed ensiling materials.

| Items                  | Control         | 10% BSG         | 15% BSG         | 20% BSG         | SEM | $P$ value |
|------------------------|-----------------|-----------------|-----------------|-----------------|-----|-----------|
| DM, g kg$^{-1}$ FW     | 336.85          | 328.29          | 325.82          | 321.16          | 2.145 | .074      |
| pH                     | 4.10            | 4.12            | 4.14            | 4.13            | 0.874 | .146      |
| WSC, g kg$^{-1}$ DM    | 70.52           | 65.23           | 64.17           | 60.82           | 1.214 | .085      |
| CP, g kg$^{-1}$ DM     | 69.24$^\text{a}$ | 83.37$^\text{b}$ | 89.51$^\text{c}$ | 95.15$^\text{a}$ | 3.412 | $<.001$  |
| aNDFom, g kg$^{-1}$ DM | 458.28$^\text{a}$ | 440.62$^\text{b}$ | 432.94$^\text{b}$ | 425.90$^\text{a}$ | 5.216 | $<.001$  |
| ADLom, g kg$^{-1}$ DM  | 321.37$^\text{a}$ | 304.35$^\text{b}$ | 296.43$^\text{c}$ | 289.18$^\text{a}$ | 3.232 | $<.001$  |
| CEL, g kg$^{-1}$ DM    | 272.48$^\text{a}$ | 257.10$^\text{b}$ | 250.26$^\text{b}$ | 245.70$^\text{c}$ | 2.013 | .147      |
| HC, g kg$^{-1}$ DM     | 136.91          | 136.26          | 136.50          | 136.73          | 0.011 | .514      |
| LAB, log$_{10}$ cfu g$^{-1}$ FW | 5.98 | 5.86 | 5.80 | 5.75 | 1.021 | .074 |
| AB, log$_{10}$ cfu g$^{-1}$ FW | 5.31 | 5.25 | 5.22 | 5.19 | 0.875 | .152 |
| Yeasts, log$_{10}$ cfu g$^{-1}$ FW | 5.03 | 6.37$^\text{b}$ | 7.07$^\text{bc}$ | 7.37$^\text{a}$ | 2.341 | $<.001$ |

*Means with different letters in the same row show significant differences ($p <.05$). FW: fresh weight; DM: dry matter; WSC: water-soluble carbohydrates; CP: crude protein; aNDFom: neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; ADLom: acid detergent fibre expressed exclusive of residual ash; ADL: acid detergent lignin; CEL: cellulose; HC: hemicellulose; LAB: lactic acid bacteria; AB: aerobic bacteria; cfu: colony-forming unit; BSG: spent grains; SEM: standard error of the mean. Mixture: 50% corn stalk, 30% sweet potato vine and 20% apple pomace (equal to Control); Control: no BSG; 10% BSG: with 10% BSG of FW; 15% BSG: with 15% BSG of FW; 20% BSG: with 20% BSG of FW.

Inc., Cary, NC) according to the following 4 × 6 factorial model:

$$Y_{ij} = \mu + R_i + D_j + (R \times D)_{ij} + \varepsilon_{ij}$$

where $Y_{ij}$ is the response variable; $\mu$ is the overall mean; $R_i$ is the effect of treatments $i$ ($i = 1, 2, 3, 4$); $D_j$ is the effect of ensiling days $j$ ($j = 1, 3, 5, 7, 14$ and 30 days); $(R \times D)_{ij}$ is the effect of interaction between treatments $i$ and ensiling days $j$ and $\varepsilon_{ij}$ is a residual error term. Fermentation quality, and chemical composition during ensiling were analysed by two-way analysis of variance (ANOVA) to evaluate the effects of treatments, ensiling days and their interactions. Structural carbohydrates compositions and in vitro digestibility of mixed silages on day 30 of ensiling were subjected to one-way ANOVA. The polynomial orthogonal contrasts (linear and quadratic) were used to determine the response to an increasing proportion of BSG. Statistical differences among means were determined using Turkey’s multiple comparison, and significance was declared at $p <.05$.

Results

The chemical and microbial compositions of raw materials

As shown in Table 1, apple pomace (900.04 g kg$^{-1}$ FW) contained relatively high DM content, almost quadruple than that of corn stalk (229.44 g kg$^{-1}$ FW). Corn stalk contained higher content of WSC, aNDFom, ADFom and HC than other raw materials. The pH (3.61) of BSG was lower than apple pomace (3.81), corn stalk (5.58) and sweet potato vine (5.63). BSG had the highest CP content among all raw materials. The epiphytic LAB, AB and yeast counts in raw materials were less than $1.0 \times 10^6$ cfu g$^{-1}$ FW, except the LAB counts in corn stalk and sweet potato vine.

The chemical and microbial compositions of mixed ensiling materials are presented in Table 2. With the increasing proportion of BSG, the aNDFom, ADFom and CEL contents decreased, and the CP content and yeast counts increased ($p <.05$). Compared with the silage containing 10% BSG, the silage including 20% BSG had significantly ($p <.05$) higher CP content and yeast counts, and significantly ($p <.05$) lower aNDFom, ADFom and CEL content. However, no significant ($p >.05$) differences were observed on CP, aNDFom, ADFom and CEL content and yeast counts between 10% BSG and 15% BSG silages. The counts of epiphytic LAB and AB in all treatments were below $1.0 \times 10^5$ cfu g$^{-1}$ FW ($p >.05$). No significant ($p >.05$) difference was observed for pH, DM, WSC, ADL and HC content in all treatments.

Chemical compositions and fermentation quality of mixed silages during ensiling

As presented in Table 3, treatments, ensiling days and their interaction had significant effects on pH, DM, WSC, NH$_3$-N and total VFAs content ($p <.001$). The pH of all silages was lower than 4.20 during the first 14 days of ensiling, whereas there was a significant ($p <.05$) increase during 14–30 days of ensiling. BSG-treated silages had lower ($p <.05$) pH than the control during 14–30 days of ensiling. Though no significant ($p >.05$) difference was observed between 10% BSG and 15% BSG silages, the pH values were numerically or statistically lower than the control during ensiling. Compared with 10% BSG and 15% BSG silages, 20% BSG silage had significantly ($p <.05$) lower pH value on days 3, 14 and 30 of ensiling. DM content in all silages significantly ($p <.05$) decreased during ensiling.
Table 3. Effects of brewers’ spent grains on pH, dry matter, water-soluble carbohydrates, ammonia nitrogen and total volatile fatty acids content of mixed silages during ensiling.

| Items                        | Ensiling days | Control | 10% BSG | 15% BSG | 20% BSG | Mean   | SEM  | T   | D   | T x D | B-L  | B-Q  |
|------------------------------|---------------|---------|---------|---------|---------|--------|------|-----|-----|------|------|------|
| pH                          | 1             | 4.02    | 4.02    | 4.00    | 4.00    | 4.01   | 1.305 | <.001 | <.001 | <.001 | .052 | .402 |
|                             | 3             | 4.02    | 4.02    | 4.01    | 3.84    | 3.97   |      |      |      |      |      |      |
|                             | 5             | 3.89    | 3.88    | 3.84    | 3.83    | 3.86   |      |      |      |      |      |      |
|                             | 7             | 4.00    | 3.96    | 3.84    | 3.83    | 3.91   |      |      |      |      |      |      |
|                             | 14            | 4.11    | 4.03    | 3.98    | 3.84    | 3.99   |      |      |      |      |      |      |
|                             | 30            | 4.79    | 4.24    | 4.23    | 4.09    | 4.34   |      |      |      |      |      |      |
| DM, g kg⁻¹ FW               | 1             | 333.27  | 328.75  | 323.09  | 317.23  | 325.58 | 2.835 | <.001 | <.001 | <.001 | .117 |
|                             | 3             | 322.46  | 312.20  | 309.98  | 306.14  | 312.70 |      |      |      |      |      |      |
|                             | 5             | 325.33  | 321.65  | 305.09  | 303.12  | 308.80 |      |      |      |      |      |      |
|                             | 7             | 314.52  | 313.89  | 308.63  | 303.71  | 310.19 |      |      |      |      |      |      |
|                             | 14            | 305.35  | 293.31  | 285.38  | 281.43  | 291.37 |      |      |      |      |      |      |
|                             | 30            | 273.83  | 271.68  | 256.59  | 251.75  | 268.46 |      |      |      |      |      |      |
| WSC, g kg⁻¹ DM              | 1             | 65.70   | 63.85   | 62.24   | 57.70   | 62.37  | 2.106 | <.001 | <.001 | <.001 | .150 |
|                             | 3             | 62.90   | 51.17   | 48.81   | 46.50   | 50.35  |      |      |      |      |      |      |
|                             | 5             | 61.48   | 43.23   | 34.83   | 33.47   | 43.25  |      |      |      |      |      |      |
|                             | 7             | 52.82   | 29.54   | 28.69   | 27.70   | 34.68  |      |      |      |      |      |      |
|                             | 14            | 47.46   | 37.53   | 20.34   | 17.65   | 30.75  |      |      |      |      |      |      |
|                             | 30            | 33.74   | 18.43   | 17.38   | 17.20   | 21.69  |      |      |      |      |      |      |
| NH₃-N, g kg⁻¹ TN            | 1             | 34.98   | 31.25   | 26.76   | 21.36   | 28.59  | 3.508 | <.001 | <.001 | <.001 | .681 |
|                             | 3             | 42.82   | 32.79   | 31.73   | 28.59   | 33.98  |      |      |      |      |      |      |
|                             | 5             | 51.80   | 46.74   | 40.59   | 38.05   | 44.30  |      |      |      |      |      |      |
|                             | 7             | 59.96   | 53.12   | 50.36   | 41.04   | 57.12  |      |      |      |      |      |      |
|                             | 14            | 84.63   | 81.62   | 80.65   | 66.87   | 79.42  |      |      |      |      |      |      |
|                             | 30            | 96.25   | 93.97   | 91.37   | 84.09   | 91.42  |      |      |      |      |      |      |
| Total VFAs, g kg⁻¹ DM       | 1             | 1.66    | 1.77    | 2.66    | 3.00    | 2.13   | 0.485 | <.001 | <.001 | <.001 | .084 |
|                             | 3             | 2.44    | 2.73    | 4.12    | 5.03    | 3.83   |      |      |      |      |      |      |
|                             | 5             | 0.75    | 4.37    | 5.56    | 6.24    | 4.23   |      |      |      |      |      |      |
|                             | 7             | 1.44    | 5.99    | 6.09    | 6.88    | 5.10   |      |      |      |      |      |      |
|                             | 14            | 5.02    | 6.27    | 10.68   | 10.28   | 8.13   |      |      |      |      |      |      |
|                             | 30            | 7.53    | 9.00    | 11.17   | 14.32   | 10.51  |      |      |      |      |      |      |

*Means with different letters in the same row show significant differences (p<.05).
A- Means with different letters in the same column show significant differences (p<.05).
FW: fresh matter; DM: dry matter; WSC: water-soluble carbohydrates; NH₃-N: ammonia nitrogen; TN: total nitrogen; Total VFAs: total volatile fatty acids.
Control: no brewers’ spent grains; 10% BSG: with 10% brewers’ spent grains of FW; 15% BSG: with 15% brewers’ spent grains of FW; 20% BSG: with 20% brewers’ spent grains of FW; T: treatments; D: ensiling days; T x D: interaction between treatments and ensiling days; B-L: linear effect of BSG proportions; B-Q: quadratic effect of BSG proportions; SEM: standard error of the mean.

15% BSG and 20% BSG silages had lower (p<.05) DM content than the control during ensiling, whereas the DM content in 10% BSG silages had no significant (p>.05) difference with the control. On comparing the silage containing 10% BSG, the silage containing 20% BSG had significantly (p<.05) lower DM content during ensiling, whereas no significant (p>.05) difference was observed in 15% BSG silage except on day 5 of ensiling. The WSC content in all silages significantly (p<.05) decreased during ensiling. BSG-treated silages had lower (p<.05) WSC content versus control, whereas no significant (p>.05) difference was observed among BSG-treated silages on days 5, 7 and 30 of ensiling. The NH₃-N content in all silages significantly (p<.05) during ensiling. BSG-treated silages had lower (p<.05) NH₃-N content than the control during ensiling. Compared with 10% BSG silage, 20% BSG silage had significantly (p<.05) lower NH₃-N content during ensiling, whereas no significant (p>.05) difference was found in 15% BSG silage except on day 5. Total VFAs content in all silages significantly (p<.05) increased during ensiling, and BSG-treated silages had higher (p<.05) total VFAs content than the control during ensiling.

As presented in Table 4, treatments, ensiling days and their interaction had significant (p<.001) effects on LA and AA content. The lactic acid content of all silages significantly (p<.05) increased during ensiling. BSG-treated silages had higher (p<.05) lactic acid content than the control during ensiling. Compared with the silage containing 10% BSG, the silages including 15% and 20% BSG had significantly (p<.05) higher lactic acid content during 7–14 days of ensiling. Acetic acid content in all silages significantly (p<.05) increased during ensiling, however, low acetic acid content was obtained in all silages. BSG-treated silages had higher (p<.05) acetic acid content than the control during ensiling. Compared with 10% BSG silage, the 15% and 20%BSG silages had significantly (p<.05) higher acetic acid content during 14–30 days of ensiling. The lactic/acetic acid ratios in all silages significantly (p<.05) increased during 1–7 days of ensiling, and then gradually decreased. As the BSG proportion increased, LA/acetic acid ratios significantly (p<.05)
decreased. Though BSG-treated silages had numerical higher propionic and butyric acid content than the control, none or tiny amounts of propionic and butyric acid content were detected in all silages during ensiling. As presented in Figure 1, BSG-treated silages had higher ($p < .05$) Flieg points than the control, and the Flieg points in all silages exceeded 41. The 15% BSG silage had the highest numerical point among all silages, which was higher ($p < .05$) than the control and 10% BSG silages. There was no significant ($p > .05$) difference between 15% and 20% BSG silages.

Crude protein and structural carbohydrates compositions of mixed silages on day 30 of ensiling

As shown in Table 5, the application of BSG significantly affected CP, aNDFom, ADFom, CEL and HC content ($p < .05$). With the increasing proportion of BSG, the CP content linearly ($p < .05$) and quadratically ($p < .05$) increased, whereas aNDFom, ADFom, CEL and HC content linearly ($p < .05$) decreased on day 30 of ensiling. However, CP, aNDFom, ADFom, CEL and HC content had no significant ($p > .05$) differences between 10% and 15% BSG silages. Compared with the silage containing 10% and 15% BSG, the silage including 20% BSG had significantly ($p < .05$) higher CP.
Table 5. Effects of brewers’ spent grains on crude protein and structural carbohydrates compositions of mixed silages on day 30 of ensiling.

| Items               | Control  | 10% BSG  | 15% BSG  | 20% BSG  | SEM       | T    | B-L | B-Q |
|---------------------|----------|----------|----------|----------|-----------|------|-----|-----|
| CP, g kg⁻¹ DM       | 33.46a   | 54.50b   | 56.46b   | 65.37a   | 4.115     | <.001| <.001| <.001|
| aNDFom, g kg⁻¹ DM   | 442.04a  | 431.46ab | 427.22a  | 383.15c  | 6.423     | <.001| <.001| <.001|
| ADFom, g kg⁻¹ DM    | 306.13a  | 296.51b  | 293.76a  | 266.73a  | 4.232     | <.001| <.001| <.001|
| ADL, g kg⁻¹ DM      | 47.25    | 48.78    | 44.38    | 40.45    | 1.324     | 0.101| 0.137| 0.852|
| CEL, g kg⁻¹ DM      | 258.88a  | 247.73b  | 249.38a  | 228.28c  | 3.443     | <.001| <.001| <.001|
| HC, g kg⁻¹ DM       | 135.91a  | 124.96ab | 118.46c  | 114.47b  | 2.715     | <.001| <.001| <.001|

a,bMeans with different letters in the same row show significant differences (p<.05).

DM: dry matter; CP: crude protein; aNDFom: neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; ADFom: acid detergent fibre expressed exclusive of residual ash; ADL: acid detergent lignin; CEL: cellulose; HC: hemicellulose; Control: no brewers’ spent grains; 10% BSG: with 10% brewers’ spent grains of FW; 15% BSG: with 15% brewers’ spent grains of FW; 20% BSG: with 20% brewers’ spent grains of FW. T: treatments; B-L: linear effect of brewers spent grains proportions; B-Q: quadratic effect of brewers’ spent grains proportions; SEM: standard error of the mean.

Table 6. Gas production kinetics, in vitro digestibility and metabolizable energy of mixed silages on day 30 of ensiling.

| Items               | Control  | 10% BSG  | 15% BSG  | 20% BSG  | SEM       | T    | B-L | B-Q |
|---------------------|----------|----------|----------|----------|-----------|------|-----|-----|
| In vitro gas production kinetics |          |          |          |          |           |      |     |     |
| GP, mL g⁻¹ DM       | 27.43a   | 29.17b   | 33.85ab  | 38.51a   | 1.314     | <.001| .004| <.001|
| Potential GP, mL g⁻¹ DM | 33.04a   | 34.03b   | 39.71a   | 44.26a   | 1.389     | <.001| .016| <.001|
| Rate constant of GP, mL h⁻¹ | 0.025    | 0.026    | 0.027    | 0.028    | 0.077     | .074 | .145| .089|
| In vitro digestibility |          |          |          |          |           |      |     |     |
| IVNDFD, %            | 28.97c   | 32.58bc  | 34.75b   | 40.13a   | 1.270     | <.001| .001| .013|
| IVCPD, %             | 28.13c   | 30.42bc  | 33.77ab  | 36.29a   | 0.967     | <.001| .001| .087|
| IVDMD, %             | 31.14    | 32.76    | 33.85    | 34.40    | 0.189     | 0.077| 0.146| 0.069|
| ME, MJ kg⁻¹          | 7.12     | 7.43     | 7.58     | 7.81     | 0.196     | 0.055| 0.546| .442|

a,bValues in the same row with different letters are significantly different (p<.05).

GP: 72 h cumulative gas production; Potential GP: potential gas production; Rate constant of GP: rate constant of gas production; IVNDFD: in vitro neutral detergent fibre digestibility; IVCPD: in vitro cumulative gas production; IVDMD: in vitro dry matter digestibility; ME: metabolizable energy; Control: no brewers’ spent grains; 10% BSG: with 10% brewers’ spent grains of FW; 15% BSG: with 15% brewers’ spent grains of FW; 20% BSG: with 20% brewers’ spent grains of FW. T: treatments; B-L: linear effect of brewers’ spent grains proportions; B-Q: quadratic effect of brewers’ spent grains proportions; SEM: standard error of the mean.

Figure 2. Gas production profiles (mL g⁻¹ DM) of mixed silages during 72 h of incubation (average, five replicates per treatment, bars stand for standard error of the mean). Control: no brewers’ spent grains; 10% BSG: with 10% brewers’ spent grains of FW; 15% BSG: with 15% brewers’ spent grains of FW; 20% BSG: with 20% brewers’ spent grains of FW.

Gas production parameters and in vitro digestibility of mixed silages are summarised in Table 6 and Figure 2. The application of BSG had significant effects on cumulative GP, potential GP, IVNDFD and IVCPD (p<.05). Compared with the control, BSG-treated silages had higher (p<.05) cumulative GP, potential GP, IVNDFD and IVCPD. On comparing the silage containing 10% BSG, the silage including 20% BSG had significantly (p<.05) higher cumulative GP, potential GP, IVNDFD and IVCPD, whereas no significant (p>0.05) differences were found in the silage including 15% BSG after 72 h of incubation. Compared with the silage including 15% BSG, the silage containing 20% BSG had no significant (p>0.05) difference on cumulative GP, potential GP, rate constant of GP, IVNDFD, IVCPD, ME and rate constant of GP.

In vitro parameters of mixed silages on day 30 of ensiling

The gas production parameters and in vitro digestibility of mixed silages are summarised in Table 6 and Figure 2. The application of BSG had significant effects on cumulative GP, potential GP, IVNDFD and IVCPD (p<.05). Compared with the control, BSG-treated silages had higher (p<.05) cumulative GP, potential GP, IVNDFD and IVCPD. On comparing the silage containing 10% BSG, the silage including 20% BSG had significantly (p<.05) higher cumulative GP, potential GP, IVNDFD and IVCPD, whereas no significant (p>0.05) differences were found in the silage including 15% BSG after 72 h of incubation. Compared with the silage including 15% BSG, the silage containing 20% BSG had no significant (p>0.05) difference on cumulative GP, potential GP, rate constant of GP, IVNDFD, IVCPD, ME and rate constant of GP.

neutral detergent fibre expressed exclusive of residual ash; ADL: acid detergent lignin; CEL: cellulose; HC: hemicellulose; Control: no brewers’ spent grains; 10% BSG: with 10% brewers’ spent grains of FW; 15% BSG: with 15% brewers’ spent grains of FW; 20% BSG: with 20% brewers’ spent grains of FW. T: treatments; B-L: linear effect of brewers’ spent grains proportions; B-Q: quadratic effect of brewers’ spent grains proportions; SEM: standard error of the mean.

In vitro parameters of mixed silages on day 30 of ensiling

The gas production parameters and in vitro digestibility of mixed silages are summarised in Table 6 and Figure 2. The application of BSG had significant effects on cumulative GP, potential GP, IVNDFD and IVCPD (p<.05). Compared with the control, BSG-treated silages had higher (p<.05) cumulative GP, potential GP, IVNDFD and IVCPD. On comparing the silage containing 10% BSG, the silage including 20% BSG had significantly (p<.05) higher cumulative GP, potential GP, IVNDFD and IVCPD, whereas no significant (p>0.05) differences were found in the silage including 15% BSG after 72 h of incubation. Compared with the silage including 15% BSG, the silage containing 20% BSG had no significant (p>0.05) difference on cumulative GP, potential GP, rate constant of GP, IVNDFD, IVCPD, ME and rate constant of GP.
Discussion

BSG typically has high CP and is widely used as an feed resources for ruminants (Silva et al. 2020). San Martin et al. (2021) reported that ethanol or antimicrobial peptides existing in BSG had the potential to inhibit the growth of undesirable microorganisms, and the application of BSG is favourable in enhancing fermentation quality during ensiling.

The effects of BSG on fermentation quality and chemical compositions during ensiling

The pH was an important indicator of fermentation quality, and the goal of ensiling is to reduce pH value to below 4.20 as rapidly as possible. The pH in all silages did not exceed 4.03 within 14 days of ensiling, and BSG-treated silages had lower pH than the control during ensiling. A low pH was mainly attributed to apple pomace, which contained malic and citric acids. The lower pH in BSG-treated silages might be caused by higher lactic acid content in these silages. A significant increase of pH in all silages was observed between 14 and 30 days, which might result from the significant increase of NH$_3$-N content at the end of ensiling. Proteolysis was influenced by the activity of plant and microbial enzymes, and NH$_3$-N content in silages revealed the extent of proteolysis (Wang et al. 2020). In the experiment, the NH$_3$-N content in all silages was less than 100 g kg$^{-1}$ TN, which indicated less proteolysis occurred during ensiling. BSG-treated silages had lower NH$_3$-N content than the control, which could be explained that lower pH in BSG-treated silages inhibited the activities of plant and microbial enzymes. Though propionic and butyric acid content in all silages maintained below 2.0 g kg$^{-1}$ DM during ensiling, they were detected in BSG-treated silages at almost each time point of ensiling, which indicated potential risk for clostridial fermentation when using BSG. The application of BSG increased lactic acid content compared with the control during ensiling, which was mostly because of the efficient conversion of WSC into LA by LAB fermentation. Meanwhile, the significant increase of LA content during ensiling confirmed that BSG is a favourable substrate for LAB (Nishino et al. 2003). Higher acetic acid content in BSG-treated silages might be related to more acetic acid-producing microorganisms in BSG. Furthermore, higher acetic acid content and lower LA/acetic acid ratios were observed in BSG-treated silages showing that, the majority of LAB were heterofermentative during ensiling (Zhao et al. 2018). This result is inconsistent with the work of Wang et al. (2020), who found that BSG increased the ratio of LA/acetic acid. The Flieg points in BSG-treated silages were above 81, which were considered as an indicator of very good quality in BSG-treated silages. Flieg points in the control exceeded 41, which was deemed to medium quality. Though numerically highest Flieg points were found in 15% BSG silages, Flieg points had no obvious difference between 15% and 20% BSG silages. The results indicated that desirable fermentation quality was obtained from the mixed silages prepared with 15% or 20% BSG.

The effects of BSG on crude protein content and structural carbohydrates compositions on day 30 of ensiling

The higher CP content was found in BSG-treated silages, which might be because of high CP content of the BSG. Structural carbohydrates could not be utilised directly as fermentation substrates to most microorganisms, whereas they may be available by the means of enzymolysis or acid hydrolysis (McDonald et al. 1991). In this experiment, lower aNDFom, ADFom, CEL and HC content were observed in BSG-treated silages on the day 30 of ensiling, which might result from acid hydrolysis of structural carbohydrates (Tao et al. 2021). In general, acid hydrolysis of structural carbohydrates was accompanied with the release of WSC (Desta et al. 2016). However, the WSC content in BSG-treated silages was lower than the control, which might be related to the rapid conversion of WSC into lactic acids.

The effects of BSG on in vitro parameters on day 30 of ensiling

In vitro DM digestibility is widely used to evaluate the nutrient value of feedstuffs. Li et al. (2021) reported that high CP and low fibre content contributed to promoting IVDM and increasing the bioavailability of nutrients. In this experiment, IVDM in BSG-treated silages was higher than that in the control, which was mainly related to higher CP and lower fibre contents in BSG-treated silages. Higher IVCPD was found in BSG-treated silages, suggesting that more soluble CP in BSG-treated silages resulted in an increase of total degradable CP fractions. The IVNDFD had no obvious difference among all silages. Probable reason is that the acidic nature of apple pomace contributed to the generation of a low pH in rumen fluid, leading to a suppression of the activity of cellulolytic bacteria (Pirmohammadi et al. 2006). In vitro gas production is
used to observe the efficiency of rumen digestibility and feed quality (Chen et al. 2016). The application of BSG linearly increased cumulative GP. It seemed probable that BSG provided more sufficient energy and substrates for microbial degradation.

Conclusion

The application of BSG significantly improved fermentation quality and in vitro digestibility of the mixed silages prepared with corn stalk, apple pomace sweet potato peel. However, the production of propionic and butyric acid in BSG-treated silages at almost each time point of ensiling indicated the potential clostridial fermentation when using BSG. The 20% BSG silage was optimum, which was indicated by higher LA content, IVDMD, IVCPD and ME, and lower pH and NH$_3$-N content. The application of BSG with 20% was recommended to ensure the quality and in vitro digestibility of mixed silages.

Ethical approval

All animal experimental protocols were approved by the Animal Care and Use Committee of Nanjing Agricultural University.

Disclosure statement

There are no conflicts of interest in this work.

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Data availability statement

The data that support the findings of this study are available from the corresponding author, ST, upon reasonable request.

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