Effect of substituting *Pennisetum sinese* with bamboo shoot shell (BSS) on aerobic stability and digestibility of ensiled total mixed ration

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**ABSTRACT**

This study aimed to evaluate the effect of substituting *Pennisetum sinese* with bamboo shoot shell (BSS) on the fermentation quality, chemical composition, *in vitro* digestibility and aerobic stability of ensiled total mixed ration (TMR). Four ensiled TMRs were designed on fresh matter basis: (i) 45% *P. sinese* (BSS0); (ii) 15% BSS + 30% *P. sinese* (BSS15); (iii) 25% BSS + 20% *P. sinese* (BSS25); (iv) 35% BSS + 10% *P. sinese* (BSS35). All silages were moderately fermented according to the V-score. Substituting *P. sinese* with BSS increased acetic acid content and decreased ethanol and ammonia nitrogen contents (*p* < .05). With the increasing proportion of BSS, neutral detergent fibre decreased, and relative feed value increased (*p* < .05). Under aerobic exposure, BSS-substituted (BSS15, BSS25 and BSS35) silages were more stable than BSS0 silage with lower silage temperature and yeast population. No significant differences in BSS substitution were observed in *in vitro* gas production, digestibility, metabolisable energy and net energy for lactation (*p* > .05). The substitution of *P. sinese* with BSS had no adverse effect on the fermentation quality and *in vitro* digestibility while efficiently improving the aerobic stability of ensiled TMR. The BSS35 substitution level is recommended considering the maximum utilisation of BSS.

**HIGHLIGHTS**

- Bamboo shoot shell (BSS) was explored for ensiled TMR.
- BSS had no effect on the silage digestibility.
- BSS improved aerobic stability.

**Introduction**

With the increasing demand for animal products, the contradiction between food and feed is prominent worldwide and more specifically in China. Exploring local forages or unconventional feedstuffs as alternatives to cereal has become the focus of livestock producers and animal nutritionists (Zhao et al. 2016; Chen et al. 2017). *Pennisetum sinese*, a hybrid of *Pennisetum purpureum* and *Pennisetum americanum*, is widely cultivated as one of the most promising forage grasses in the tropical and subtropical regions due to its high yield and wide adaptability. At present, *P. sinese* has become the main forage source of local animal husbandry in the Loess Plateau and the Yangtze River Delta of China (Deng et al. 2019). Meanwhile, bamboo (*Bambusoideae*) shoots as traditional food are popular in many Asian countries with good taste and rich nutrition (Devi and Pamba 2015). China is the largest producer of bamboo shoots in the world, with more than 3 million tons of bamboo shoots per year (Chen, Chen, et al. 2019). However, up to 70% of bamboo shoots, consisting of the nonedible sheaths and the basal part of bamboo shoots, were discarded as waste residues (Lin et al. 2018), resulting in waste of resources and environmental pollution. Making full use of the bamboo shoot shell (BSS) can not only increase the added value of BSS, but also is of great significance to environmental protection. Actually, BSS is rich in protein, amino acids and carbohydrates and can be used as a new source of roughage (Liu et al. 2000). Chen et al. (1979) reported that BSS could be easily digested by cattle, with total digestible nutrients (TDN) as high as 68.5%. Inclusion of BSS to diet improved daily live weight gain of pigs (Zhou et al. 1992), the growth rate and feed conversion of ruminants (Liu et al. 2000).
Huge quantities of BSS generated seasonally call for effective storage. Ensiling, as a promising technology, is applicable for long-term preservation and year-round availability of BSS. But BSS is seldom used for silage making due to the high moisture content. The traditional method of processing high-moisture materials is wilting or drying, which causes extensive consumption of labour and time. In recent years, total mixed ration (TMR) silage has been widely developed to not only supply a complete formula diet for a long time but also provide the possibility to exploit more local materials as roughage resources (Chen et al. 2015). We assumed that the straw and hay frequently prepared in TMR or ensiled TMR could be worked as moisture absorbents to balance the high moisture content of BSS. In view of the high crude protein (CP) content of BSS, substituting \textit{P. sinese} with BSS in TMR may be the preferred method to improve the utilisation rate of BSS and reduce the dependence on concentrate. Moreover, BSS was extensively studied due to its polysaccharides having an excellent biological antioxidant activity (Luo et al. 2018; Chen, Fang, et al. 2019). Antioxidants have been broadly applied in food and feed industries to prevent deterioration. It was speculated that the inclusion of BSS might improve the aerobic stability of ensiled TMR. While to our knowledge, little information regarding the correlation between BSS and aerobic stability of silage is available yet.

Thus, the BSS was introduced into a local TMR formula to evaluate its effects on the fermentation quality, chemical composition, \textit{in vitro} digestibility and aerobic stability of ensiled TMR.

Materials and methods

\textbf{Ensiled TMR preparation}

The BSS was obtained from a local bamboo shoots processing factory in Zhejiang, China on 24 August 2014. \textit{Pennisetum sinese}, rice straw and concentrate were provided by a private beef cattle farm (29.43°N, 121.48°E, elevation 4 m, Zhejiang, China). The \textit{P. sinese} was harvested at the milk ripe stage, leaving the stubble of 30 cm. The concentrate consisted of 45% corn, 20% cottonseed, 15% rapeseed meal, 15% corn distillers’ grains, 5% vitamin-mineral premixes. All the roughages were chopped into a length of 2–3 cm by a forage chopper. The chemical compositions of each TMR ingredient were analysed in six replicates, and the data are listed in Table 1.

As shown in Table 2, the partial substitution of \textit{P. sinese} to BSS was performed to maximise BSS utilisation based on a local TMR formula. Four treatments were designed according to the different substitution proportions (based on fresh matter) of BSS in TMR: (i) no bamboo shoot shell + 45% \textit{P. sinese} (BSS0); (ii) 15% bamboo shoot shell + 30% \textit{P. sinese} (BSS30); (iii) 25% bamboo shoot shell + 20% \textit{P. sinese} (BSS25); (iv) 35% bamboo shoot shell + 10% \textit{P. sinese} (BSS35). A batch (180 kg) for each treatment was distributed and thoroughly mixed, then approximately 5.8 kg TMR was tightly packed into a 10 L laboratory silo (polyethylene bottle with a diameter of 27.5 cm and height of 31.6 cm, Lantian biological experimental instrument Co., Ltd., Jiangsu, China) and sealed with screw tops and plastic tape. The packing densities of the BSS0, BSS15, BSS25 and BSS35 were 336, 335, 334 and 333 kg DM/m³, respectively. Anaerobic storage was conducted at an ambient temperature ranging from 22 to 28°C for 90 days. Six silos per treatment were sampled for the following analyses.

\textbf{Chemical and microbial analyses}

The fresh ingredients and TMRs were divided into three subsamples and the ensiled TMR was divided into four subsamples. The first subsamples were blended with distilled water at 1:10 w/v ratio and macerated at 4°C for 24 h. After filtering through four layers of gauze, the filtrate was used to determine the buffering capacity (BC) according to the hydrochloric

| Table 1. Chemical composition of ingredients used for total mixed ration. |
|----------------|----------------|----------------|----------------|
| Item                        | Bamboo shoot shell | \textit{Pennisetum sinese} | Rice straw | Concentrate |
| Dry matter, g/kg FM         | 200              | 217             | 830          | 903          |
| Crude protein, g/kg DM      | 144              | 119             | 58.6         | 193          |
| Water soluble carbohydrate, g/kg DM | 36.4       | 89.9            | 29.6         | 119          |
| Neutral detergent fibre, g/kg DM | 62.7       | 639             | 637          | 361          |
| Acid detergent fibre, g/kg DM | 342              | 370             | 373          | 68.3         |
| Ether extract, g/kg DM      | 15.9             | 84.1            | 18.1         | 78.4         |
| Crude ash, g/kg DM          | 93.2             | 87.9            | 111          | 74.4         |
| Non-fibrous carbohydrate, g/kg DM | 120          | 70.0            | 175          | 294          |
| Buffering capacity, mEq/kg DM | 544             | 270             | 120          | 129          |

FM: fresh matter; DM: dry matter; mEq: milligram equivalent.
acid–sodium hydroxide method (Playne and McDonald 1966). The second subsamples were immediately oven-dried to determine dry matter (DM) content and then ground with laboratory knife mills (FW100, Taisite Instrument Co., Ltd., Tianjin, China) to pass through a 1-mm screen. The resulting powder samples were used for subsequent analyses of water-soluble carbohydrates (WSC), neutral detergent fibre (NDF), acid detergent fibre (ADF), CP, ether extract (EE) and crude ash (Ash). The contents of DM (934.01), CP (984.13), EE (920.39) and Ash (942.05) were determined following the procedure of the Association of Official Analytical Chemists (AOAC 2019). The WSC was determined via the colorimetric method after reaction with anthrone reagent (Thomas 1977). The NDF and ADF were analysed according to the procedures of Van Soest et al. (1991) and Robertson and Van Soest (1981), respectively, and expressed on a DM basis inclusive of residual ash. Non-fibrous carbohydrate (NFC) was calculated by the following formula: NFC = 100 – CP – NDF – EE – Ash (NRC 2000). The TDN were calculated by the method of Harlan et al. (1991).

The third subsample was homogenised with sterilised saline solution (0.85% NaCl) at 1:9 w/v ratio and then serially diluted for 7-fold. The LAB was counted on deMan, Rogosa and Sharp (MRS) agar medium (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) after incubation at 37 °C for 2–3 days. The LAB was counted on deMan, Rogosa and Sharp (MRS) agar medium (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and yeasts were counted on potato dextrose agar medium (Sincere Biochemical Technology Co., Ltd., Shanghai, China), agar plates were incubated at 30 °C for 2–3 days (Kozaki et al. 1992).

The remaining silage subsample was blended with distilled water at 1:3 w/v ratio and macerated at 4 °C for 24 h to obtain the extract. After filtering through two layers of gauze and a filter paper, the filtrate from the extract was used for later determination of pH, organic acids and ammonia nitrogen (NH₃–N) contents. The pH was measured with a glass electrode pH metre (Hanna HI 2221, Hanna Instruments Italia Srl, Villafranca Padovana, Italy). After centrifugation at 14,000 g for 10 min, the supernatant fluid was analysed for organic acids (Desta et al. 2016), and NH₃–N (Broderick and Kang 1980). To evaluate the fermentation quality and digestible energy intake of silage, the V-score (an evaluation method that is calculated from volatile fatty acids and NH₃–N, Takahashi et al. 2005) and relative feed value (RFV, Rohweder et al. 1978) were adopted, respectively.

### In vitro incubation

Rumen fluid was manually collected from the rumens (different positions) of three rumen-cannulated Boer male goats before morning feeding. The goats were fed the diet consisting of alfalfa (6%), guinea grass (59%) and concentrate (35%) at 1.3 times the maintenance level (Feng and Lu 2007). The whole process was...
carried out under anaerobic conditions with continuous CO₂ flushing. Rumen fluid was homogeneously mixed, immediately filtered and stored at 39 °C in a water bath. Before later incubation, rumen fluid was mixed with a buffer solution at a 1:2 v/v ratio as described by Menke (1988).

In vitro incubation was conducted in serum bottles following the method of Contreras-Govea et al. (2011) with some modifications. Approximately 1 g ground samples of ensiled TMR were transferred into filter bags (F57; ANKOM Technology, Macedon, NY, USA) that were previously washed with acetone, dried at 55 °C for 24 h and weighed. Each bag was heat-sealed and then placed into each preheated 120 mL serum bottle with 60 mL rumen fluid-buffer mixture under CO₂ at 39 °C. A total of 144 silage samples (4 treatments × 6 silos × 2 replicates × 3 runs) were prepared. Meanwhile, six serum bottles with the only inoculum added were served as blank at each run.

Gas production (GP) data were fitted to an exponential model: \( y = b (1 - e^{-ct}) \), where \( y \) is the cumulative volume of GP at incubation time \( t \) (mL), \( b \) is the potential GP (mL), and \( c \) is the rate constant of GP (Blümmel et al. 2003). The metabolisable energy (ME) and net energy for lactation (NEₗ) were calculated using the equations as described by Menke (1988).

### Aerobic stability test

After 90 days of ensiling, six silos per treatment on each exposure day were used for a 14-day aerobic stability test. Briefly, silage samples were taken out from each 10 L silo, fully mixed and loosely loaded into a bigger, sterile and open-top polyethylene bottle (capacity 15 L). The bottles were stored at ambient temperature (26 ± 2 °C) covering with two layers of gauze to prevent drying and contamination. The thermocouple wires of a data logger (MDL-1048A, Tianhe Automation Instrument Co., Ltd., Shanghai, China) were placed in the geometric centre of the bottle to record silage temperature every 30 min. The ambient temperature near the bottles was recorded as blank. Aerobic stability is defined as the time (h) elapsed when the silage temperature is 2 °C above the ambient temperature (Wilkinson and Davies 2013). And the dynamic changes of pH, acetic acid (AA), NH₃–N, WSC, and microbes counts after 0, 3, 6, 9, and 14 days of aerobic exposure were also analysed as indicators of aerobic deterioration using the same procedures of silage subsamples analyses.

### Statistical analyses

Analysis of variance (ANOVA) was performed using the general linear model procedure of SAS rev. 9.2. Data on chemical composition, fermentation quality and in vitro incubation were subjected to one-way ANOVA. And data on aerobic stability were subjected to two-
way ANOVA. Statistical difference between means was determined by using Tukey’s multiple comparisons and considered as significant at \(p < .05\).

Results

Chemical and microbial compositions of pre-ensiled materials

Compared with \(P. \) sinese, BSS had higher moisture, NFC and CP contents, and lower WSC, fibre and EE contents. Except for the contents of WSC, EE, BC and NFC, no significant \((p > .05)\) differences were found for DM, CP, NDF, ADF, Ash, RFV and TDN in the TMRs among the treatments (Table 2). The epiphytic LAB population in all treatments was more than 105 cfu/g FM. With the increasing proportion of BSS, the population of aerobic bacteria and yeast significantly decreased \((p < .05)\).

Fermentation quality of ensiled TMR

As presented in Table 3, substitution levels significantly affected the AA, ratio of lactic acid to acetic acid (LA/AA), ethanol and NH\(_3\)–N contents \((p < .05)\). All ensiled TMRs were moderately fermented according to the V-score (>70). The BSS substitution increased the AA content and decreased the ethanol and NH\(_3\)–N contents of resulting silages \((p < .05)\). Negligible contents of propionic acid and butyric acid (<2 g/kg DM) were recorded in all ensiled TMRs. The inclusion of BSS had no effect on the AB population but decreased the LAB and Yeast population of resulting silage.

Chemical compositions of ensiled TMR

The WSC, NDF and NFC contents, as well as RFV of ensiled TMR, were affected by BSS substitution levels (Table 4, \(p < .05\)). Among them, the WSC content was highest in BSS25 silage and lowest in BSS15 silage. In addition, the NFC content for BSS35 was higher than that for other treatments. The BSS-substituted silages had numerically or statistically lower NDF content and higher RFV than BSS0 silage.

In vitro parameters of 90-day ensiled TMR

The in vitro measured or estimated parameters of four ensiled TMRs are shown in Table 5. The potential GP \((b\) value) was ranged from 111.32 to 120.88 mL. BSS substitution had no effect on GP24, GP72, potential GP, GP rate constant \((c\) value), IVDMD, IVNDFD, ME and NEL.

Aerobic stability of ensiled TMR

After aerobic exposure for 14 days, the pH of BSS0 raised sharply from 4.58 to 5.90 \((p < .05)\), yet no significant \((p > .05)\) change was found among BSS-substituted ensiled TMRs (Table 6). The contents of AA and WSC decreased to varying degrees, with AA of BSS0, BSS15, BSS25 and BSS35 silages decreasing by 4.58 to 5.90, 31.01 and 14.67%, and WSC decreasing by 78.96, 39.74, 39.00 and 25.01%, respectively. Irrespective of the aerobic exposure days, with the increasing proportion of BSS, the population of aerobic bacteria and yeast significantly decreased \((p < .05)\). During the 14-day aerobic exposure, the population of LAB decreased while that of AB and Yeasts increased (Table 7). And the yeast population in all ensiled TMRs were always less than 106 cfu/g FM except for BSS0.

The differences in temperature and aerobic stability among four ensiled TMRs during aerobic exposure are shown in Figure 1. The silage temperature of BSS0 was 2°C above the ambient temperature after 127 h of

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**Table 4.** Effect of BSS substitution levels on chemical compositions of ensiled total mixed ration after 90 days of ensiling.

| Item                        | Treatments1 | SEM | \(p\)-Value |
|-----------------------------|-------------|-----|-------------|
|                             | BSS0        | BSS15 | BSS25   | BSS35   |     |
| Dry matter, g/kg FM         | 466         | 476  | 439       | 401     | 1.30 | .350 |
| Crude protein, g/kg DM      | 159         | 166  | 170       | 174     | 2.55 | .199 |
| Water soluble carbohydrate, g/kg DM | 32.1A    | 22.0B | 35.0A    | 32.3A   | 1.92 | .002 |
| Neutral detergent fibre, g/kg DM | 432A     | 427A  | 410A     | 361B    | 9.67 | .008 |
| Acid detergent fibre, g/kg DM | 209        | 207  | 201       | 191     | 6.58 | .812 |
| Ether extract, g/kg DM      | 75.00       | 84.50 | 70.30    | 71.00   | 2.68 | .225 |
| Crude ash, g/kg DM          | 97.50       | 95.80 | 96.00    | 96.90   | 0.62 | .796 |
| Non-fibrous carbohydrate, g/kg DM | 237B    | 227B  | 253B     | 297B    | 8.62 | .003 |
| Relative feed value, %      | 157B        | 159B  | 166AB    | 191B    | 4.57 | .004 |
| Total digestible nutrients, % DM | 68.00     | 68.20 | 68.60    | 69.30   | 0.46 | .812 |

FM: fresh matter; DM: dry matter; SEM: standard error of means; BSS: bamboo shoot shell.

Means with different capital letters show significant differences among treatments in the same ensiling days \((p < .05)\).

1BSS0, no bamboo shoot shell + 45% Pennisetum sinese; BSS15, 15% bamboo shoot shells + 30% Pennisetum sinese; BSS25, 25% bamboo shoot shells + 20% Pennisetum sinese; BSS35, 35% bamboo shoot shells + 10% Pennisetum sinese.
### Table 5. Gas production kinetics, in vitro digestibility, metabolizable energy and net energy for lactation of ensiled TMR after 90 days of ensiling.

| Item                      | Treatment 1 | Aerobic exposure days | Means | SEM  | p-Value |
|---------------------------|-------------|-----------------------|-------|------|---------|
|                          | BSS0        | BSS15                 | BSS25 | BSS35|         |
| pH                        |             |                       |       |      |         |
| BSS0                      | 4.58c       | 4.61c                 | 4.70Ab | 5.02Ba | 5.90ab  |
| BSS15                     | 4.54        | 4.59                  | 4.62   | 4.59b | 4.71b   |
| BSS25                     | 4.53        | 4.51                  | 4.53   | 4.53a | 4.57a   |
| BSS35                     | 4.36        | 4.47                  | 4.55   | 4.48b | 4.57a   |
| Acetic acid, g/kg DM      |             |                       |       |      |         |
| BSS0                      | 10.70 Ab    | 10.90 Ab              | 9.59Bc | 6.81Aa | 4.56a   |
| BSS15                     | 17.00a      | 17.40a                | 16.60ab | 14.10Ab | 12.60b  |
| BSS25                     | 19.50a      | 19.00a                | 18.30gb | 17.60b | 13.40b  |
| BSS35                     | 19.50a      | 19.30a                | 19.60a | 16.60a | 16.70a  |
| Ammonia nitrogen, g/kg TN |             |                       |       |      |         |
| BSS0                      | 92.70Ca     | 98.90Ab               | 99.30Ab | 102Ab | 94.00b  |
| BSS15                     | 74.00bc     | 77.10Abb              | 89.60Ab | 93.10Ab | 87.70b  |
| BSS25                     | 72.80c      | 79.30bc               | 85.20b | 73.90b | 74.50b  |
| BSS35                     | 32.10Ab     | 27.80Bb               | 20.80Ob | 16.60bc | 6.76c   |
| Water soluble carbohydrate, g/kg DM | 22.00Ab | 22.70Ac | 21.90Ab | 20.90Bc | 13.30Ab | 20.10b  |
| BSS0                      | 35.00Ab     | 31.20Ab               | 30.80Ab | 28.80Ab | 21.30Ab |
| BSS15                     | 32.30Ab     | 33.30Ab               | 27.50Ab | 23.10Ab | 24.20Ab |
| BSS25                     |              |                      |       |      |         |
| BSS35                     |              |                      |       |      |         |

DM: dry matter; TN: total nitrogen; SEM: standard error of means; D: aerobic exposure days; T: substitution levels; D × T: the interaction between aerobic exposure days and substitution levels; BSS: bamboo shoot shell.

Means differ significantly among aerobic exposure days in the same treatment (p < .05); Values with different small letters show significant differences among treatments in the same aerobic exposure days (p < .05).

### Table 6. Effect of BSS substitution levels and aerobic exposure days on chemical compositions of ensiled total mixed ration.

| Item                                      | Treatment 1 | Aerobic exposure days | Means | SEM  | p-Value |
|-------------------------------------------|-------------|-----------------------|-------|------|---------|
|                |             | 0 3 6 9 14            |       |      |         |
| pH            |             |                       |       |      |         |
| BSS0          | 4.58c       | 4.61c                 | 4.70Ab | 5.02Ba | 5.90ab  |
| BSS15         | 4.54        | 4.59                  | 4.62   | 4.59b | 4.71b   |
| BSS25         | 4.53        | 4.51                  | 4.53   | 4.53a | 4.57a   |
| BSS35         | 4.36        | 4.47                  | 4.55   | 4.48b | 4.57a   |
| Acetic acid, g/kg DM           |             |                       |       |      |         |
| BSS0          | 10.70 Ab    | 10.90 Ab              | 9.59Bc | 6.81Aa | 4.56a   |
| BSS15         | 17.00a      | 17.40a                | 16.60ab | 14.10Ab | 12.60b  |
| BSS25         | 19.50a      | 19.00a                | 18.30gb | 17.60b | 13.40b  |
| BSS35         | 19.50a      | 19.30a                | 19.60a | 16.60a | 16.70a  |
| Ammonia nitrogen, g/kg TN         |             |                       |       |      |         |
| BSS0          | 92.70Ca     | 98.90Ab               | 99.30Ab | 102Ab | 94.00b  |
| BSS15         | 74.00bc     | 77.10Abb              | 89.60Ab | 93.10Ab | 87.70b  |
| BSS25         | 72.80c      | 79.30bc               | 85.20b | 73.90b | 74.50b  |
| BSS35         | 32.10Ab     | 27.80Bb               | 20.80Ob | 16.60bc | 6.76c   |
| Water soluble carbohydrate, g/kg DM | 22.00Ab | 22.70Ac | 21.90Ab | 20.90Bc | 13.30Ab | 20.10b  |

DM: dry matter; TN: total nitrogen; SEM: standard error of means; D: aerobic exposure days; T: substitution levels; D × T: the interaction between aerobic exposure days and substitution levels; BSS: bamboo shoot shell.

Means differ significantly among aerobic exposure days in the same treatment (p < .05); Values with different small letters show significant differences among treatments in the same aerobic exposure days (p < .05).

### Table 7. Effect of BSS substitution levels and aerobic exposure days on microbial compositions of ensiled total mixed ration.

| Item                                      | Treatment 1 | Aerobic exposure days | Means | SEM  | p-Value |
|-------------------------------------------|-------------|-----------------------|-------|------|---------|
|                |             | 0 3 6 9 14            |       |      |         |
| Lactic acid bacteria, log10 cfu/g FM      |             |                       |       |      |         |
| BSS0          | 6.85a       | 5.71b                 | 5.19Ab | 4.81c | 4.15c   |
| BSS15         | 6.62a       | 6.05b                 | 5.65Bbc | 5.21hc | 4.42bc  |
| BSS25         | 6.50Ab      | 6.19b                 | 5.93Ab | 5.70b  | 5.16b   |
| BSS35         | 6.37Ab      | 6.42b                 | 5.93b  | 5.68a  | 5.31a   |
| BSS35         | 6.50      | 6.75Ab               | 6.83Ab | 6.97b  | 7.49b   |
| BSS15         | 6.30b      | 6.44Bbc              | 6.59Bbc | 6.77ab | 7.07ab  |
| BSS35         | 6.35c      | 6.32Bbc              | 6.56Bbc | 6.79ab | 6.93b   |
| BSS25         | 6.17c      | 6.18Bbc              | 6.33Bbc | 6.61b  | 6.62b   |
| BSS35         | 3.18Ad     | 3.66Ac               | 4.32Ac | 5.29ab | 6.33a   |
| BSS15         | 2.87Ad     | 3.36Ac               | 3.61Ac | 4.41Ac | 5.31b   |
| BSS25         | 2.54Ad     | 2.95Ad               | 3.52Ac | 3.80Ab | 4.43b   |
| Yeast, log10 cfu/g FM                     |             |                       |       |      |         |
| BSS0          | 2.39c      | 2.90Cc               | 2.87Cc | 3.46cb | 3.97a   |

CFU: colony-forming units; FM: fresh matter; SEM: standard error of means; D: aerobic exposure days; T: substitution levels; D × T: the interaction between aerobic exposure days and substitution levels; BSS: bamboo shoot shell.

Means differ significantly among aerobic exposure days in the same treatment (p < .05); Values with different small letters show significant differences among treatments in the same aerobic exposure days (p < .05).

1BSS0: no bamboo shoot shell + 45% Pennisetum sinesis; BSS15: 15% bamboo shoot shells + 30% Pennisetum sinesis; BSS25: 25% bamboo shoot shells + 20% Pennisetum sinesis; BSS35: 35% bamboo shoot shells + 10% Pennisetum sinesis.
aerobic exposure. But all BSS-substituted silages were no more than 2°C above the ambient temperature under the 14-day aerobic exposure (Figure 1(A)). Namely, the aerobic stability of all BSS-substituted silages exceeded 336 h (Figure 1(B)).

Discussion

**BSS substitution on fermentation quality**

The four TMRs had high WSC content (>50 g/kg DM) and sufficient LAB population (>10^5 cfu/g FM), which are conducive for a successful fermentation theoretically (Weinberg 2008). Indeed, all silages were well preserved after 90 days of ensiling according to the V-score, indicated by low BA and NH₃–N contents (Catchpoole and Henzell 1971). In the study, the fermentation products of TMR, especially LA, was lower than that of the forages with similar WSC content, and this could ascribe to its high DM content. High DM content of silage delays the multiplication of microbes including LAB (Chen et al. 2015). The lower WSC content of BSS-substituted TMRs explains the lighter LA production in BSS-substituted ensiled TMR since less substrate is available for LAB. However, the AA content in BSS-substituted ensiled TMR increased with an increasing proportion of BSS, which was difficult to explain but could ascribe to the material specificity of BSS (Yuan et al. 2019). Moreover, when WSC is limited, hetero-fermentative LAB becomes active and several homo-fermentative strains such as *Lactobacillus plantarum* could turn to the lactate/acetate conversion pathway (McDonald et al. 1991). Conversely, the NH₃–N content decreased with an increasing proportion of BSS, which corresponded well to the respective pH. The BSS substitution decreased the yeast population, thereby reducing the ethanol content of the resulting silages.

**BSS substitution on chemical compositions**

The BSS substitution effectively improved the NFC content and RFV without unfavourable effects on the CP, EE and TDN of silages. The numerically higher CP content in BSS-substituted silages than that in BSS₀ is probably due to the high CP content in BSS material. While the obvious lower NDF content in BSS₃₅ silage could be related to its relatively low pH. Hemicellulose, as a part of NDF, is known to be acid-unstable and prone to acidolysis. Moreover, the reduction in NDF content after ensiling was much larger than that in ADF content, indicating that hemicellulose is more susceptible to degradation than cellulose (McDonald et al. 1991). The highest NFC content observed in BSS₃₅ silage was mainly attributed to its lowest NDF content. The lower NDF and ADF contents observed in BSS-substituted silages than BSS₀ could ascribe to less DM loss, which in turn explained that the inclusion of BSS could enhance the RFV of ensiled TMR. Given above, BSS₃₅ silage had the best feeding value followed by BSS₂₅, BSS₁₅ and BSS₀ silages.

**BSS substitution on in vitro parameters**

*In vitro* GP and digestibility are gained wide acceptance to evaluate the nutritional value of ruminant feeds (Blümmel et al. 2003; Rymer et al. 2005). Meanwhile, the relationship between these two parameters is commonly used to predict the actual DM intake and digestion of ruminants (Getachew et al. 2017).
In the present study, BSS substitution only numerically affected all GP and digestibility parameters as well as estimated ME and NE\(_{\text{L}}\), indicating that BSS substitution had no unfavourable effects on rumen utilisation of ensiled TMR.

**BSS substitution on aerobic stability**

When the air infiltrates, the aerobic microbes begin to proliferate and silages are prone to deterioration: Yeasts, mainly lactate-assimilating yeasts, can metabolise substrates such as lactic acid to increase the pH and temperature of silage; Where after, aerobic bacteria and moulds begin to grow and further aggravate the spoilage (Kleinschmit et al. 2005). Therefore, monitoring the changes in chemical and microbial compositions during aerobic exposure is essential to prevent aerobic deterioration and toxin production resulted from seal damage or silos that are open.

It is well known that silages with lower LA content or those with less residual sugar contents were more stable when exposed to air (Weinberg and Muck 1996), which may partially explain the better aerobic stability (>336 h) of BSS-substituted silages. Furthermore, the high AA content in BSS-substituted silages is also responsible for enhanced aerobic stability as short-chain fatty acids can effectively inhibit spoilage microbes (Kung and Ranjit 2001). Another explanation for the excellent aerobic stability of the BSS-substituted silages might ascribe to the BSS material used in the study. Polysaccharides in BSS have potential antioxidant activities (Chen, Li, et al. 2019) and could inhibit the oxidation of substrates induced by aerobic microbes, which explained the low population of AB and yeasts in BSS-substituted silages under aerobic exposure. Yeast population less than \(1 \times 10^5 \text{ cfu/g FM}\) is required to inhibit the aerobic deterioration of silage as well as keep aerobic stability.

Interestingly, BSS\(_{15}\) silage still maintained aerobic stability even yeast population was above the level of \(1 \times 10^5 \text{ cfu/g FM}\) at 14 days of aerobic exposure. One possible reason is that BSS substitution favoured the establishment of the WSC-assimilating yeasts rather than lactate-assimilating yeasts. Liu et al. (2018) found that the species of yeasts rather than yeast population was more responsible for aerobic stability. Courtin and Spoolstra (1990) indicated that AB are also related to aerobic deterioration. Unlike yeasts, AB often functioned in the final phase of deterioration with relatively high pH (McDonald et al. 1991). Furthermore, AB were the critical microbes which cause the increase of NH\(_3\)–N under aerobic condition (Nussio 2005), which is consistent with this study that high AB population is accompanied by high NH\(_3\)–N content. Given the above, although the aerobic stability of all BSS-substituted silages exceeded 336 h, it was expected that BSS\(_{35}\) silage would perform better if the aerobic exposure was further prolonged.

**Conclusions**

Substituting *P. sinese* with BSS up to 35% had no adverse effect on the fermentation quality and *in vitro* digestibility while improving the aerobic stability of ensiled TMR. BSS can be a potential source of roughage for ensiled TMR production, and the BSS\(_{35}\) substitution level (proportion of BSS with roughages: 35/65) is recommended with the principle of maximum BSS utilisation. Whether BSS polysaccharides are associated with aerobic stability or not is needed for further study.

**Ethical approval**

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

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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