Soy sauce residue in total mixed ration silage: fermentation characteristics, chemical compositions, in vitro digestibility and gas production

Guofeng Xu, Zhe Han, Siran Wang, Tongtong Dai, Dong Dong, Cheng Zong, Xuejing Yin, Yushan Jia and Tao Shao

Institute of Ensiling and Processing of Grass, College of Agro-grassland Science, Nanjing Agricultural University, Nanjing, China; Key Laboratory of Forage Cultivation, Processing and High Efficient Utilization, Ministry of Agriculture, Inner Mongolia Agricultural University, Hohhot Inner Mongolia, China

ABSTRACT
The work aimed to evaluate the effect of different levels of soy sauce residue (SSR) replacement on chemical compositions, fermentation quality and in vitro digestibility of total mixed ration (TMR) silages. The experiment followed a completely randomised design, and four treatments were designed according to different mixing ration of SSR, Napier grass and concentrate (based on fresh weight): (1) 65% Napier grass + 35% concentrate (control); (2) 15% SSR + 55% Napier grass + 30% concentrate (SS15); (3) 25% SSR + 50% Napier grass + 25% concentrate (SS25); (4) 35% SSR + 45% Napier grass + 20% concentrate (SS35). TMR was ensiled in a laboratory silo (10 L) after mixing. All silos were opened after 60 days of ensiling and then were sampled for chemical compositions, fermentation quality and in vitro digestibility analysis. All TMR silages were preserved well, as indicated by the low butyric acid (BA; < 1.60 g/kg dry matter), ammonia nitrogen (NH₃-N; < 50.0 g/kg total nitrogen) contents, and the high V-Score value (> 80.0). However, SS25 and SS35 significantly (P < 0.05) decreased the BA, NH₃-N, ethanol contents and yeast counts, and significantly (P < 0.05) increased the V-Score value compared with the control. SS35 significantly (P < 0.05) increased 72 h cumulative gas production (GP₇₂), in vitro dry matter digestibility (IVDMĐ) and crude protein digestibility (IVCPD) compared with control and SS15. There was no significant (P > 0.05) difference in vitro neutral detergent fibre digestibility (IVNDFD) among all TMR silages. In conclusion, SS35 replacement was recommended for TMR silage making.

HIGHLIGHTS
- Ensiling is an effective method to utilise soy sauce residue.
- Soy sauce residue replacement had no unfavourable effect on the fermentation quality of the total mixed ration.
- 35% soy sauce residue replacement improved the in vitro digestibility of total mixed ration silages.

Introduction
Total mixed ration (TMR) consists of concentrate, roughage, vitamin-mineral and so on, which is widely used to provide adequate and balanced nutrition for ruminants (Zhao et al. 2020). However, general TMR is prone to spoilage, which limits long-period preservation and long-distance transport. Thus, TMR is made into TMR silage to prolong preservation and prevent deterioration. TMR silage also contributes to stabilising rumen function and improving protein utilisation in the rumen (Nishino et al. 2003).

In recent years, the rising cost of feed is a serious challenge for farmers. Therefore, it is of great significance to develop unconventional feed, which could replace part of commercial forages to reduce feed costs. Soy sauce is brewed from soybean and wheat, which is a traditional condiment, and was more than 7.37 million tons every year in China (Duan et al. 2019). With the rapid development of the soy sauce industry, the disposal of soy sauce residue (SSR) should be concerned. Although SSR is considered a waste traditionally, it is characterised by rich protein, oil, soy isoflavones and other useful components.
SSR contains approximately 21.8% crude protein (based on the dry matter), which is a potential low-cost protein resource (Zhao et al. 2014). Moreover, soy isoflavone is a natural phenolic compound, which could enhance immune function, antioxidant ability and bone metabolism in livestock and poultry production (He et al. 2021). In previous work, Sadarman et al. (2020) suggested that the in vitro dry matter digestibility and in vitro organic matter digestibility of ensiled soy sauce by-product was 51.0% and 49.5%, respectively, which had no remarkable difference compared with fresh soy sauce by-product. Furthermore, soy sauce by-product had high pH (6.32) after 30 days of ensiling indicating it was hard to preserve well. Uddin et al. (2010) found that the supplementation of soy sauce cake with a total mixed ration silage-based diet increased crude protein digestibility, whereas there was no obvious effect on dry matter intake and neutral detergent fibre digestibility. However, there was limited information on the application of SSR in TMR silage making in China.

Due to the low cost of SSR, the high ratio of SSR possible in the TMR silage formula is most profitable. Improper disposal of SSR would lead to a waste of resources and environmental pollution, therefore, the utilisation of SSR in TMR silages contributes to alleviating environmental concerns. However, SSR has a low WSC content (Lei et al. 2014), and the high SSR replacement level is not conducive to the preservation of TMR silage. Thus, a laboratory test of different SSR replacement levels should be conducted to identify the appropriate incorporation ratio of SSR in TMR silages. The objective of the work was to evaluate the different SSR replacement levels on chemical compositions, fermentation quality and in vitro digestibility of TMR silages.

Materials and methods

Experimental design and silage preparation

The experiment was carried out at the Institute of Ensiling and Processing of Grass, College of Agro-grassland Science, Nanjing Agricultural University, Nanjing, China (32°2’N,118°50’E). Napier grass was cultivated in the experimental field of Nanjing Agricultural University (31°61’N,119°18’E) and was harvested at the vegetative stage. Soy sauce residue was provided by Nanjing Jiafu Biotechnology Company. The concentrate was provided by Xuzhou Shenniu Feedstuff Company (consisting of 27.5% distillers dried grain with soluble, 20% rape cake meal, 20% cotton seed meal, 7.5% crack corn, 20% wheat bran and 5% vitamin-mineral). The chemical compositions of raw materials are shown in Table 1. The partial replacement of Napier grass and concentrate with SSR was performed to maximise SSR utilisation based on a regular ration formula in the control TMR. The experiment followed a completely randomised design, and four treatments (Table 2) were designed according to different mixing ration of SSR, Napier grass and concentrate (based on fresh weight): (1) 65% Napier grass + 35% concentrate (control); (2) 15% SSR + 35% Napier grass + 50% concentrate (SS15); (3) 25% SSR + 50% Napier grass + 25% concentrate (SS25); (4) 35% SSR + 45% Napier grass + 20% concentrate (SS35). Napier grass was chopped into 1-2 cm lengths with a fodder chopper, and then mixed with SSR and concentrate in an ethanol-sterilised plastic tub. TMR was packed into a laboratory silo (10 L capacity) and then sealed with screw tops and plastic tape. A total of 24 silos (4 treatments × 6 replicates per treatment) were stored at ambient temperature. All silos were opened after 60 days of ensiling, and TMR silages were sampled for chemical compositions, fermentation quality and in vitro digestibility analysis.

Table 1. Chemical compositions of soy sauce residue, Napier grass and concentrate.

| Item | Soy sauce residue | Napier grass | Concentrate |
|------|------------------|--------------|-------------|
| DM (g/kg FW) | 639 ± 4.91 | 251 ± 9.75 | 840 ± 1.36 |
| CP (g/kg DM) | 204 ± 2.22 | 80.2 ± 5.92 | 276 ± 1.01 |
| NFC (g/kg DM) | 263 ± 2.12 | 172 ± 4.91 | 348 ± 4.99 |
| Ash (g/kg DM) | 84.0 ± 1.62 | 114 ± 6.18 | 60.2 ± 1.18 |
| CP (g/kg DM) | 75.0 ± 1.39 | 25.7 ± 1.28 | 42.4 ± 0.82 |
| NFC (g/kg DM) | 22.2 ± 0.85 | 72.8 ± 2.47 | 192 ± 1.89 |
| EE (g/kg DM) | 240 ± 3.69 | 379 ± 7.40 | 149 ± 1.40 |
| ADF (g/kg DM) | 65.0 ± 1.39 | 379 ± 7.40 | 149 ± 1.40 |
| WSC (g/kg DM) | 84.0 ± 1.62 | 114 ± 6.18 | 60.2 ± 1.18 |
| NFC (g/kg DM) | 22.2 ± 0.85 | 72.8 ± 2.47 | 192 ± 1.89 |
| EE (g/kg DM) | 240 ± 3.69 | 379 ± 7.40 | 149 ± 1.40 |
| ADF (g/kg DM) | 64.6 ± 1.35 | 379 ± 7.40 | 149 ± 1.40 |
| CP (g/kg DM) | 204 ± 2.22 | 80.2 ± 5.92 | 276 ± 1.01 |
| NFC (g/kg DM) | 263 ± 2.12 | 172 ± 4.91 | 348 ± 4.99 |
| EE (g/kg DM) | 75.0 ± 1.39 | 25.7 ± 1.28 | 42.4 ± 0.82 |
| ADF (g/kg DM) | 84.0 ± 1.62 | 114 ± 6.18 | 60.2 ± 1.18 |
| WSC (g/kg DM) | 22.2 ± 0.85 | 72.8 ± 2.47 | 192 ± 1.89 |

*DM, dry matter; FW, fresh weight; CP, crude protein; aNDF, neutral detergent fibre (with a heat stable amylase and expressed inclusive of residue ash); ADF, acid detergent fibre; EE, ether extract; WSC, water soluble carbohydrate; NFC (non-fibrous carbohydrate) = 1000 – CP – NFC – EE – Ash.

bConcentrate: 7.5% crack corn, 20% rape cake meal, 20% cotton seed meal, 27.5% distillers dried grain with soluble, 20% wheat bran, 5% vitamin-mineral.

Chemical and microbial compositions analysis

The raw materials were sampled for chemical composition analysis. The TMR silages were sampled for microbial counts, chemical compositions and fermentation quality analysis. At sampling, a 300 g sample was oven-dried at 65 °C for 48 h to determine dry matter content (DM) and then ground to pass a 1-mm screen with a laboratory knife mill (FW100, Taisite Instrument Co., Ltd., Tianjin, China), and sample powders were stored for later analysis. Total nitrogen (TN) was determined by the Kjeldahl method (Krishnamoorthy et al. 1982), crude protein (CP) was
calculated by multiplying TN by 6.25. Ether extract (EE) and ash were determined in accordance with the methods 920.39 and 942.05, of the Association of Official Analytical Chemists (AOAC 1990). Neutral detergent fibre (aNDF) and acid detergent fibre (ADF) were determined in accordance with the method of Van Soest et al. (1991), the determination of aNDF with the addition of heat stable amylase and expressed on a DM basis, including residual ash. Water soluble carbohydrates (WSC) was determined in accordance with the method of colorimetry after reaction with anthrone reagent (Arthur Thomas 1977).

The 10 g of TMR silage samples were placed in a 250 mL conical flask and incubated in 90 mL 0.85% sterilised saline solution. Then, the conical flask was sealed with plastic wrap and kept in the orbital shaker at 120 rpm for 1 h, and then serially diluted with 0.85% sterilised saline solution. Lactic acid bacteria (LAB) were counted on deMan, Rogosa and Sharp (MRS) agar medium (Shanghai Bio-way Technology Co., Ltd.) after anaerobic incubation for 48 h at 37°C. Aerobic bacteria were counted on nutrient agar medium (Qingdao Hope Bio-technology Co., Ltd.) after aerobic incubation for 24 h at 37°C. Yeast was counted on Potato Dextrose agar medium (Shanghai Bio-way Technology Co., Ltd.) after aerobic incubation for 48 h at 30°C. All microbial data were transformed to log10 for presentation and statistical analysis.

Another 30 g of TMR silage samples were mixed with 90 g of deionised water and extracted at 4°C for 24 h. After 24 h of extraction, the extracted liquid was filtered through two layers of cheesecloth and a layer of filter paper. The filtrate was stored at −20°C for later analysis. The pH was determined with a glass electrode pH metre (HANNA HI 2,221; Hanna Instruments Italia Srl, Villafranca Padovana, Italy). The ammonia nitrogen (NH₃-N) was determined in accordance with the method of phenol-hypochlorite reaction (Broderick and Kang 1980). The organic acids and ethanol were determined by an Agilent 1260 HPLC system equipped with a refractive index detector (Carbomix® H-NP5 column, 2.5 mM H₂SO₄, 0.5 mL/min). The V-Score was used for assessing the fermentation quality of TMR, the V-Score was calculated using NH₃-N/TN, acetic acid (AA), propionic acid (PA) and butyric acid (BA) content in TMR (Takahashi et al. 2005).

### Table 2. Formula and chemical composition of TMR.

| Itemd | Control | SS15 | SS25 | SS35 | SEMf | T | L | Q |
|-------|---------|------|------|------|------|---|---|---|
| Raw material composition (%) | | | | | | | | |
| Soy sauce residue | 0 | 15 | 25 | 35 | 35 | 35 | 35 | 35 |
| Napier grass | 65 | 55 | 50 | 45 | 50 | 50 | 50 | 50 |
| Concentrate | 35 | 30 | 25 | 20 | 25 | 25 | 25 | 25 |
| Chemical composition | | | | | | | | |
| DM (g/kg FW) | 457<sup>b</sup> | 486<sup>ab</sup> | 495<sup>a</sup> | 505<sup>a</sup> | 6.374 | 0.017 | 0.003 | 0.270 |
| CP (g/kg DM) | 149<sup>b</sup> | 157<sup>ab</sup> | 169<sup>a</sup> | 163<sup>a</sup> | 1.904 | 0.020 | 0.004 | 0.250 |
| EE (g/kg DM) | 31.5<sup>c</sup> | 38.1<sup>bc</sup> | 42.2<sup>ab</sup> | 46.3<sup>a</sup> | 1.830 | 0.003 | <0.001 | 0.534 |
| aNDF (g/kg DM) | 491<sup>c</sup> | 473<sup>ab</sup> | 466<sup>b</sup> | 459<sup>b</sup> | 4.191 | 0.012 | 0.002 | 0.287 |
| ADF (g/kg DM) | 298<sup>a</sup> | 289<sup>ab</sup> | 287<sup>b</sup> | 284<sup>b</sup> | 1.973 | 0.024 | 0.005 | 0.225 |
| Ash (g/kg DM) | 95.3<sup>c</sup> | 93.5<sup>a</sup> | 93.2<sup>a</sup> | 92.8<sup>a</sup> | 0.514 | 0.359 | 0.120 | 0.472 |
| WSC (g/kg DM) | 114<sup>a</sup> | 101<sup>ab</sup> | 89.8<sup>b</sup> | 78.8<sup>c</sup> | 4.336 | 0.002 | <0.001 | 0.766 |
| NFC (g/kg DM) | 233<sup>a</sup> | 238<sup>b</sup> | 238<sup>b</sup> | 239<sup>b</sup> | 4.457 | 0.977 | 0.722 | 0.830 |

Values in the same row (a–c) with different superscript letters are significantly different (P < 0.05).

dDM, dry matter; FW, fresh weight; CP, crude protein; aNDF, neutral detergent fibre (with a heat stable amylase and expressed inclusive of residue ash); ADF, acid detergent fibre; EE, ether extract; WSC, water soluble carbohydrate; NFC (non-fibrous carbohydrate) = 1000 – CP – ADF – EE - Ash; Concentrate: 7.5% crack corn, 20% rape cake meal, 20% cotton seed meal, 27.5% distillers dried grain with soluble, 20% wheat bran, 5% vitamin-mineral.

cControl = 65% Napier grass + 35% concentrate; SS15 = 15% soy sauce residue + 55% Napier grass + 30% concentrate; SS25 = 25% soy sauce residue + 50% Napier grass + 25% concentrate; SS35 = 35% soy sauce residue + 45% Napier grass + 20% concentrate.

fSEM, standard error of the mean.

gT, effect of soy sauce residue proportions; L, linear effect of soy sauce residue proportions; Q, quadratic effect of soy sauce residue proportions.

### In vitro gas production and digestibility measurements

The rumen fluid was obtained from Boer male goats through a rumen fistula before morning feeding (fed with a diet that contained 59% guinea grass, 35% concentrate and 6% alfalfa hay at 1.3 times the maintenance level). The rumen fluid was filtered through 4 layers of cheesecloth and mixed with buffer solution (1:2, v/v).

In vitro incubation was conducted in serum bottles following the methods of Contreras-Govea et al. (2011) with some modifications. Briefly, 1 g of ground samples was put into nylon bags (F57; ANKOM Technology, Macedon, NY, USA). Then, each nylon bag was heat-sealed and placed into a 120 mL serum bottle. About 60 mL of mixed inoculum was placed
into each serum bottle, each serum bottle was kept in the water bath at 39°C with continuous flushing with CO2. The serum bottles with the only mixed inoculum were added as blank. Gas production (GP) was measured at 4, 8, 12, 24, 36, 48 and 72 h through a pressure transducer technique. After 72 h of incubation, the samples were gently rinsed with cold tap water and then dried at 65°C to a constant weight. In vitro dry matter digestibility (IVDMD), in vitro neutral detergent fibre digestibility (IVNDFD) and in vitro crude protein digestibility (IVCPD) was calculated through the difference in their weight before and after incubation.

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

### Statistical analysis

The data of chemical compositions, microbial counts, fermentation quality and in vitro digestibility were analysed by one-way analysis of variance (ANOVA). Significant differences among means were determined by Tukey’s test. Significant differences were declared at \( P < 0.05 \). All the above statistical analyses were performed using SPSS (version 26.0 for Windows, SPSS Inc.). The Pearson correlation analyses were performed using OriginPro 2021.

### Results

#### Chemical compositions of raw materials and TMR

The chemical compositions of raw materials are shown in Table 1. SSR and concentrate had high DM content (639 g/kg FW and 840 g/kg FW, respectively). The CP contents of concentrate, SSR and Napier grass were 276 g/kg DM, 204 g/kg DM and 80.2 g/kg DM, respectively. Concentrate had the highest WSC content, and SSR had the highest EE content. Napier grass had the highest aNDF, ADF and ash contents.

The formulas and chemical compositions of TMR are shown in Table 2. With the increasing levels of SSR replacement, the contents of aNDF, ADF and WSC were linearly \( (P < 0.05) \) decreased, and the DM, CP and EE contents were linearly \( (P < 0.05) \) increased. There was no significant \( (P > 0.05) \) difference in the contents of ash and NFC among all TMR formulas.

#### Fermentation quality of TMR

As shown in Table 3, after 60 days of ensiling, the contents of LA, PA, BA and NH3-N were quadratic \( (P < 0.05) \) affected by the SSR replacement levels. With the increasing levels of SSR replacement, the contents of AA and ethanol were linearly \( (P < 0.05) \) decreased. SSR replacement significantly \( (P < 0.05) \) decreased LA content and LA/AA, and significantly \( (P < 0.05) \) increased the pH compared with control. There was a negative correlation \( (r = -0.982, P < 0.01) \) between LA and pH (Figure 1). However, there was no significant \( (P > 0.05) \) difference between SS15 and SS25 in LA content and LA/AA. There was only numerical difference \( (P > 0.05) \) in the content of AA among all TMR silages. The SSR replacement significantly \( (P < 0.05) \) increased the PA content compared with control. Although the BA content of SSR replacement was significantly \( (P < 0.05) \) lower than control, trace amounts of BA was detected in all TMR silages. The NH3-N contents of SS25 and SS35 significantly \( (P < 0.05) \) decreased relative to control and SS15. SS25 and SS35 had significant \( (P < 0.05) \) lower ethanol content than the control. The value of V-Score in all TMR silages was >80.0.

With the increasing levels of SSR replacement, the LAB, yeast and aerobic bacteria counts were linearly \( (P < 0.05) \) decreased. SS25 and SS35 significantly \( (P < 0.05) \) decreased LAB and yeast counts compared with control. However, there was only a numerical \( (P < 0.05) \) difference between control and SS15 in LAB and yeast counts. SS25 significantly \( (P < 0.05) \) decreased the aerobic bacteria counts compared with control.

#### Chemical compositions of TMR silages

As shown in Table 4, the contents of DM, CP and WSC were quadratic \( (P < 0.05) \) affected by SSR replacement levels. The aNDF and ADF contents were linearly \( (P < 0.05) \) decreased with the increasing levels of SSR replacement. SSR replacement significantly \( (P < 0.05) \) increased the DM content compared with control, whereas there was no significant \( (P > 0.05) \) difference in DM contents among different SSR replacement levels. SS25 and SS35 significantly \( (P < 0.05) \) increased the CP contents compared with control and SS15. The aNDF and ADF contents of SS25 and SS35 significantly \( (P < 0.05) \) decreased compared with control. SSR replacement significantly \( (P < 0.05) \) decreased the WSC content compared with control. However, there was no significant \( (P > 0.05) \) difference in the WSC content among different SSR replacement levels.
In vitro parameters of TMR silages

The in vitro gas production profiles and in vitro digestibility parameters are shown in Figure 2 and Table 5. With the increasing levels of SSR replacement, the GP72, IVDMD, IVCPD and ME were linearly (P < 0.05) increased. SS25 and SS35 significantly (P < 0.05) increased the GP72, IVCPD and ME compared with control. SS35 had the highest (P < 0.05) potential GP among all TMR silages. SS25 and SS35 had significantly (P < 0.05) higher IVDMD than control and SS15.

Table 3. Fermentation quality and microbial counts of TMR silages after 60 days of ensiling.

| Item | Control | SS15 | SS25 | SS35 | SEM | T | L | Q |
|------|---------|------|------|------|-----|---|---|---|
| pH   | 4.90a   | 5.30b | 5.47c | 5.70d | 0.089 | <0.001 | <0.001 | 0.009 |
| LA (g/kg DM) | 41.0a | 27.5b | 23.4c | 16.2d | 2.772 | <0.001 | <0.001 | 0.035 |
| AA (g/kg DM) | 10.7a | 10.4b | 9.34c | 8.03d | 0.403 | 0.052 | 0.009 | 0.440 |
| LA/AA | 3.86a | 2.65b | 2.53c | 2.01d | 0.211 | <0.001 | <0.001 | 0.020 |
| PA (g/kg DM) | 1.37a | 3.94b | 4.85c | 5.30d | 0.467 | <0.001 | <0.001 | 0.001 |
| BA (g/kg DM) | 1.51a | 1.07b | 0.98c | 1.08d | 0.072 | 0.010 | 0.008 | 0.013 |
| NH3-N (g/kg TN) | 49.4a | 48.1b | 40.6bc | 44.5d | 1.070 | <0.001 | <0.001 | 0.004 |
| Ethanol (g/kg DM) | 14.3a | 12.9ab | 11.2bc | 10.3d | 0.549 | 0.012 | 0.002 | 0.737 |
| V-Score | 80.2a | 82.1ab | 83.0a | 82.7a | 0.389 | 0.020 | 0.007 | 0.066 |
| LAB (log10 cfu/g FW) | 7.47a | 6.97ab | 6.84b | 6.68c | 0.106 | 0.016 | 0.003 | 0.242 |
| Yeast (log10 cfu/g FW) | 5.45a | 5.02ab | 4.86bc | 4.72d | 0.095 | 0.009 | 0.002 | 0.229 |
| Aerobic bacteria (log10 cfu/g FW) | 4.87a | 4.73ab | 4.39bc | 4.57d | 0.067 | 0.026 | 0.014 | 0.114 |

Values in the same row (a-c) with different superscript letters are significantly difference (P < 0.05).

- DM, dry matter; LA, lactic acid; AA, acetic acid; LA/AA, lactic acid/acetic acid; PA, propionic acid; BA, butyric acid; FW, fresh weight; NH3-N, ammonia nitrogen; TN, total nitrogen; LAB, lactic acid bacteria.
- Control = 65% Napier grass + 35% concentrate; SS15 = 15% soy sauce residue + 55% Napier grass + 30% concentrate; SS25 = 25% soy sauce residue + 50% Napier grass + 25% concentrate; SS35 = 35% soy sauce residue + 45% Napier grass + 20% concentrate.
- SEM, standard error of the mean.
- T, effect of soy sauce residue proportions; L, linear effect of soy sauce residue proportions; Q, quadratic effect of soy sauce residue proportions.

In vitro parameters of TMR silages

The in vitro gas production profiles and in vitro digestibility parameters are shown in Figure 2 and Table 5. With the increasing levels of SSR replacement, the GP72, IVDMD, IVCPD and ME were linearly (P < 0.05) increased. SS25 and SS35 significantly (P < 0.05) increased the GP72, IVCPD and ME compared with control. SS35 had the highest (P < 0.05) potential GP among all TMR silages. SS25 and SS35 had significantly (P < 0.05) higher IVDMD than control and SS15.
Table 4. Chemical composition of TMR silages after 60 days of ensiling.

| Itemd | Control | SS15 | SS25 | SS35 | SEMf | T | L | Q |
|-------|---------|------|------|------|------|---|---|---|
| DM (g/kg FW) | 428b | 474a | 492a | 499a | 8.867 | 0.001 | <0.001 | 0.033 |
| CP (g/kg DM) | 148c | 158b | 168b | 167b | 2.508 | <0.001 | <0.001 | 0.016 |
| aNDF (g/kg DM) | 431a | 401ab | 397b | 381b | 6.842 | 0.010 | 0.002 | 0.168 |
| ADF (g/kg DM) | 261a | 253ab | 237bc | 233c | 4.059 | 0.008 | 0.001 | 0.725 |
| WSC (g/kg DM) | 28.9a | 22.4b | 22.3b | 21.6c | 0.956 | 0.001 | <0.001 | 0.009 |

Values in the same row (a–c) with different superscript letters are significantly different (P < 0.05).

*DM, dry matter; FW, fresh weight; CP, crude protein; aNDF, neutral detergent fibre (with a heat stable amylase and expressed inclusive of residue ash); ADF, acid detergent fibre; WSC, water soluble carbohydrate.

**Control = 65% Napier grass + 35% concentrate; SS15 = 15% soy sauce residue + 55% Napier grass + 30% concentrate; SS25 = 25% soy sauce residue + 50% Napier grass + 25% concentrate; SS35 = 35% soy sauce residue + 45% Napier grass + 20% concentrate.

*SEM, standard error of the mean.

*T, effect of soy sauce residue proportions; L, linear effect of soy sauce residue proportions; Q, quadratic effect of soy sauce residue proportions.

There was only a numerical (P > 0.05) difference in IVNDFD among all TMR silages.

Discussion

Fermentation quality of TMR

After 60 days of ensiling, all TMR silages had low BA (<1.60 g/kg DM) and NH3-N (<0.50 g/kg TN) contents and both of them were lower than the acceptable level (BA < 2.00 g/kg DM, NH3-N < 100 g/kg TN) of quality silage (Dong et al. 2017). Moreover, the V-Score was >80.0, indicating all TMR silages were preserved well (Takahashi et al. 2005). However, Chen et al. (2021) reported that the pH of quality silage should be below 4.20. In the experiment, all TMR silages had high pH ranging from 4.90 to 5.70 after 60 days of ensiling. This might be attributed to high DM content (>450 g/kg FW), which suppressed the LAB activity. The metabolic moisture for LAB growth was reduced, inhibiting the production of LA and delaying the decrease of pH (Kung et al. 2003). Herein, SSR replacement increased DM content, correspondingly, a negative correlation (r = −0.886, P < 0.01) existed between LA and DM content while pH had a positive correlation (r = 0.874, P < 0.01) with DM content. There was no significant difference in AA content among different SSR replacement levels. SSR replacement decreased the LA/AA, which indicated the growth of heterofermentative LAB might be promoted. McDonald et al. (1991) reported that 98% of the LAB in silage with high DM content were heterofermentative species. Similar to the results of Keles and Demirci (2011), who suggested high DM content could decrease the efficiency of homofermentative LAB. PA could cross the plasma membrane in undissociated form and cause the acidification in the cytoplasm of the cell, resulting in energy depletion and slow growth. Therefore, PA and its salts are often used as a food preservative and feed mildew inhibitor (Dong et al. 2017; Dijksterhuis et al. 2019). In China, suitable propionate (<2.50 g/kg) is allowed to add to soy sauce brewing as a preservative (Li et al. 2013), which might explain the PA contents increased with the increasing level of SSR replacement in TMR silages. Clostridia could ferment sugar and organic acid to form BA as fermentation end products, and the BA might be produced by other microorganisms except clostridia in silage but of minor importance (König et al. 2019). Trace amounts of BA were detected in all TMR silages, indicating that the activity of clostridia might be inhibited. König et al. (2019) reported that there was no BA detected in silage with a high DM (>300 g/kg FW). The content of NH3-N indicated the extent of proteolysis in TMR silages. Proteolysis has been mainly considered the result of plant proteases and microbial activity (Hao et al. 2020). Tremblay et al. (2001) reported that plant proteases play a key role in
the process of protein degradation, and microorganisms dominate the conversion of free amino acids to NH₃-N. In the experiment, all TMR silages had a low NH₃-N, which was probably because high DM suppressed the activity of proteolytic microorganisms, resulting in inhibition of the deamination of an amino acid (McDonald et al. 1991). Furthermore, SS25 and SS35 decreased the NH₃-N content compared with control and SS15, which could be explained by higher PA content. Fu and Diao (2007) found that PA could inhibit the activity of undesirable bacteria and reduce NH₃-N formation. Meanwhile, in the experiment, a negative correlation \( r = -0.712, \ P < 0.01 \) was observed between PA and NH₃-N. In addition, plant proteases might be mainly provided by Napier grass in TMR silage, the increase of SSR replacement levels might decrease the content of plant protease in TMR silage. To sum up, although SSR replacement could not improve LA fermentation, it remarkably suppressed the production of BA and NH₃-N.

SSR had a high salt content (Xiang et al. 2019), therefore, the salt content of TMR silage increased gradually with SSR replacement levels. Taormina (2010) considered that salt had preservative properties against microorganisms, and it not only results in the dehydration of microorganisms but also removes the oxygen from the medium and interferes with the rapid action of proteolytic enzymes to inhibit the growth of microorganisms. It could explain the fact that SSR replacement reduced the counts of LAB, yeast and aerobic bacteria in TMR silages. The production of ethanol was mainly attributed to the activity of yeast during ensiling (Nishino and Shinde 2007), the pyruvate was first formed by yeast and then decarboxylated to acetaldehyde, and the acetaldehyde was then reduced to ethanol. Thus, the ethanol content decreased with the increase of SSR replacement levels in TMR silages, which could be explained by the decrease in populations and activity of yeast.

### Chemical compositions of TMR silages

After 60 days of ensiling, the DM contents of control, SS15, SS25 and SS35 decreased by 6.3%, 2.5%, 0.7% and 1.1%, respectively. This indicated the replacement of SSR reduced DM loss, and SS25 had the minimum DM loss. There was no remarkable change in the CP content before and after ensiling, indicating the proteolysis was effectively inhibited during ensiling. With the increasing levels of SSR replacement, the aNDF and ADF contents of TMR silages decreased gradually, which was probably because of the low aNDF and ADF content in SSR. After 60 days of ensiling, the WSC content of SS15, SS25 and SS35 decreased by 77.74%, 75.17% and 72.67%, respectively. The reduction in the loss of WSC might be due to the residual salt in SSR, which could inhibit the WSC consumption by microorganisms.

### In vitro digestibility of TMR silages

Previous work had used in vitro gas production as an indicator to reflect rumen digestibility and the metabolisable energy of animal feed (Contreras-Govea et al. 2011). The GP depended on the chemical composition of the substrate. In the experiment, SS25 and SS35 remarkably increased GP72 compared with control, which could be explained by higher CP and lower aNDF content. Similar to the report of Nsahlai et al. (1994), the GP is positively correlated with CP content and negatively correlated with aNDF content. SS25 and SS35 had higher IVDMD than control and SS15, which might be attributed to lower aNDF content.
Lema et al. (2001) suggested that a negative correlation existed between IVMD and NDF, which was consistent with our results. SSR replacement remarkably increased the IVCPD, this is probably because the fermentation increased the soluble protein proportion (Da Silva et al. 2015). Protein subunits of SSR might be degraded by fermentation to form protein with smaller molecular weight and expose more hydrophilic groups, and then increased the functional groups associated with soluble protein, soluble proteins were easily hydrolysed. Overall, SSR replacement improved the in vitro digestibility and gas production of TMR silages.

**Conclusions**

The results of this work showed that SSR replacement had no unfavourable effect on the fermentation quality of TMR, while all TMR silages were preserved well as indicated by the V-Score. Furthermore, SS25 and SS35 remarkably decreased NH$_3$-N contents and the count of yeast compared with control. SSR replacement improved the in vitro digestibility of TMR silages, SS35 remarkably increased the GP$_{72}$, IVDMD and IVCPD compared with control and SS15. Thus, it is feasible to incorporate SSR into TMR silage preparation, and a 35% SSR replacement level was the most suitable for TMR silage making.

**Animal welfare statement**

The authors confirm that all experimental protocols involving animals were approved by the Animal Care and Use Committee of the Nanjing Agriculture University.

**Disclosure statement**

No potential conflict of interest was declared by the authors.

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**ORCID**

Siran Wang http://orcid.org/0000-0002-1990-550X  
Xuejing Yin http://orcid.org/0000-0003-2340-561X  
Yushan Jia http://orcid.org/0000-0001-7655-9933  
Tao Shao http://orcid.org/0000-0002-6129-145X

**Data availability statement**

All relevant data are within this manuscript.

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