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Original Research Article

# Wheat silage partially replacing oaten hay exhibited greater feed efficiency and fibre digestion despite low feed intake by feedlot lambs

Zhao-Yang Cui, Wen-Juan Li, Wei-Kang Wang, Qi-Chao Wu, Yao-Wen Jiang, Ailiyasi Aisikaer, Fan Zhang, He-Wei Chen, Hong-Jian Yang*

*State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China*

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ABSTRACT

This study aimed to investigate the feeding effect of wheat silage on growth performance, nutrient digestibility, rumen fermentation, and microbiota composition in feedlot lambs. Sixty-four male crossbred Chinese Han lambs (BW = 27.8 ± 0.67 kg, 3 months of age) were randomly assigned to four ration groups with wheat silage replacing 0% (WS0), 36% (WS36), 64% (WS64), and 100% (WS100) of oaten hay on forage dry matter basis. The concentrate-to-forage ratio was 80:20 and the feeding trial lasted 52 d. Increasing wheat silage inclusion linearly decreased dry matter intake by 4% to 27% ($P < 0.01$). However, increasing the wheat silage replacement of oaten hay by no more than 64% improved the feed efficiency by 14% as noted by the feed-to-gain ratio ($P = 0.04$). Apparent digestibility of organic matter ($P < 0.01$), neutral detergent fibre ($P = 0.04$) and acid detergent fibre ($P < 0.01$) quadratically increased. Ammonia nitrogen ($P = 0.01$) decreased while microbial protein production ($P < 0.01$) increased with the increase of wheat silage inclusion. Total volatile fatty acids concentration increased quadratically with the increase of wheat silage inclusion ($P < 0.01$), and the highest occurred in WS64. The molar proportion of acetate ($P < 0.01$) and acetate-to-propionate ratio ($P = 0.04$) decreased while butyrate ($P < 0.01$) and isovalerate ($P = 0.04$) increased. Increasing wheat silage inclusion increased the Firmicutes-to-Bacteroidota ratio by 226% to 357%, resulting in Firmicutes instead of Bacteroidota being the most abundant phylum. The relative abundance of cellulolytic *Ruminococcus* numerically increased but that of amylolytic *Prevotella* ($P < 0.01$) decreased as increasing wheat silage inclusion. Taken together, increasing wheat silage replacement of oaten hay by no more than 64% exhibited greater feed efficiency and fibre digestion despite low feed intake by feedlot lambs due to the change of Firmicutes-to-Bacteroidota ratio in the rumen.


## 1. Introduction

According to forecasts in FAO Biannual Report on Global Food Markets, world wheat production in 2022/23 reached at a record 784 million tonnes, increasing 0.6% from the previous season. Most of the projected accumulation of wheat stocks was believed to occur in China and the Russian Federation (Food Outlook, FAO, November 2022). Nevertheless, even if forage resources are in short supply in China's livestock farming, wheat is often fed to monogastric animals only in the form of grains. Wheat straws and other crop residues are removed or burned, resulting in a waste of resources and environmental pollution (Wang et al., 2022a). Even though these by-products were used as low-quality forages, their digestibility and crude protein content were proved too low to improve dry matter intake (DMI) and milk production (Dong et al., 2022; Molavian et al., 2020). Therefore, feeding whole-crop wheat, especially feeding as silage, could be a feasible strategy to solve the above problems.

In the past 50 years, whole-crop wheat silage has been widely researched and applied in Western countries. Some studies noted

* Corresponding author.
*E-mail address:* yang_hongjian@cau.edu.cn (H.-J. Yang).

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that the nutritive value, silage quality, and feeding effect would vary depending on the different maturity stages of wheat silage. For instance, wheat crops harvested at the milk stage owned high fermentation quality, while those harvested at the dough stage had high forage value (Fisher and Lessard, 1977; Randby et al., 2019; Xie et al., 2012). It was reported that wheat silage at the dough stage had a comparatively higher DM content than corn silage (Turan, 2020). In an earlier study, wheat silage replacing partial corn silage was reported no adverse effects on feed intake, nutrient digestibility, milk yield as well as milk composition (Sinclair et al., 2007). Harper et al. (2017) and Gunal et al. (2019) in a subsequent study reported no significant differences in DMI and digestibility in dairy cows fed wheat silage in comparison with corn silage. Regarding the feeding application in sheep, Higgins et al. (2020) stated that feeding wheat silage in comparison with grass silage had no impacts on dry matter and metabolizable energy intake in the ewes. It is worth noting that some variations would exist in response between cattle and sheep. For instance, wethers fed wheat silage exhibited a higher organic matter intake per kilogram body weight than steers (Sudekum et al., 1995). Taken together, the above studies implicated that wheat silage could be applied as an interim forage during the time when the previous year's hay or corn silage has run out and the present year's has not been harvested. However, several studies have shown that feeding wheat silage alone is not as effective as mixing it with other forages (Sinclair et al., 2007; Turan, 2020).

In recent years, intensive sheep feedlotting is increasingly popular in some county areas of China provinces, such as Tangxian of Hebei, Yanwo of Shandong, Huairen of Shanxi, and Gulang of Gansu. At the same time, the demand for high-quality forage is increasing due to the high price of traditional forage in China. Oaten hay is an annual grass cereal used as a prime source of digestible fibre for livestock. It is considered “sweet” hay, making it highly palatable for dairy cows, especially in arid and cold areas because of its strong adaptability and cold resistance (Su et al., 2022). Due to its high price, however, farmers consider that feeding oaten hay with other forages could be more cost-effective. For instance, when it was combined with comparatively cheap corn silage, there was no significant difference in feed intake and the digestibility of organic matter and fibre compared to feeding oaten hay alone (An et al., 2020). In such studies where different forages were partly replaced, associative effects which involved the balance and complementarity of feed nutrients, were often mentioned and discussed. Associative effects mean that the intake or growth performance of livestock can be enhanced when animals are provided with a mixture of forage (Marsh et al., 2006; Niderkorn et al., 2015; Yang et al., 2017).

To the best of our knowledge, there are no studies on wheat silage in combination with oaten hay applied in feedlot lambs. As the nutritional value in the 2016 Feed Composition Table reported by Preston (2016) in Beef Magazine, wheat silage in comparison with oaten hay presented higher total digestible nutrients (59% vs. 54%), net energy for maintenance (5.43 MJ/kg vs. 4.97 MJ/kg) and gain (2.57 MJ/kg vs. 1.84 MJ/kg), whereas crude fibre in wheat silage was reported lower than in oaten hay (28% vs. 31%). The above data implicate the potential feasibility of combining or replacing oaten hay with wheat silage in feeding practice. Thus, the current work aimed to understand the effect of different proportions of whole-crop wheat silage and oaten hay on the growth performance, nutrient digestibility, rumen fermentation characteristics, and microbial community of fattening sheep. It was hypothesized that replacing a certain proportion of oaten hay with wheat silage in lamb diets could achieve at least a similar or higher fattening effect than feeding oaten hay alone, thus achieving a reduction in diet cost for the fattening farm.

## 2. Materials and methods

### 2.1. Animal ethics statement

The Animal Ethics Committee of China Agricultural University approved all procedures with animals. All experimental animals, design, and animal management in the present study followed the Guidelines of the Beijing Municipal Council on Animal Care (with protocol CAU20171014-1), and complied with the ARRIVE guidelines.

### 2.2. Animals and feeding

As shown in Table 1, wheat silage and oaten hay were chosen as the forage materials. Whole wheat crops were harvested at the dough stage in April 2020 in the rural area of Shijiazhuang, Hebei province, China, pre-wilted in the field to approximately 30% DM content, and chopped to 2 to 5 cm. After being baled and wrapped with plastic polyethylene sheeting, crops were fermented anaerobically without additives at 25 ± 4 °C for up to 4 weeks. Oaten hay harvested at early dough stage was pre-chopped to a length of 2 to 5 cm before feeding.

Sixty-four male, small-tailed crossbred Chinese Han lambs (27.8 ± 0.67 kg) were housed with four animals per pen (2 m × 2 m) with bamboo slotted floors equipped with individual feeding and drinking troughs. All animals were vaccinated for common infectious diseases and dewormed prior to the experiment. Pens of lambs were randomly assigned to one of four rations with four pens per ration and a fixed concentrate-to-forage ratio (80:20 on a dry matter basis) as shown in Table 2: (1) WS0, a control ration with a sole forage of oaten hay; (2) WS36, a ration replacing 36% oaten hay with wheat silage; (3) WS64, a ration replacing 64% oaten hay with wheat silage; and (4) WS100, a ration completely replacing 100% oaten hay with wheat silage. All four diets were formulated to meet the minimum nutrient requirements for energy, protein, minerals, and vitamins based on the NRC (2007) for lambs with a target growth rate of 300 g/d. All lambs had ad libitum access to diets (fed twice daily at 08:00 and 16:00) and fresh water. Before morning feeding, daily orts were removed from the troughs and weighed to calculate feed intake as fed per pen. After 7-d adaptation, the entire feeding experiment lasted for 52 d.

### 2.3. Sampling and measurements

During the feeding experiment, the amount of fresh total mixed ration (TMR) offered was adjusted daily according to the actual feed intake of each ration group. The feed offered and refused was weighed daily at per pen level to calculate the DMI. Live body weight was measured before morning feeding every 26 d, and the average daily gain (ADG) throughout the entire experimental

**Table 1**
Chemical composition of oaten hay and wheat silage (g/kg, DM basis).

| Item | Oaten hay | Wheat silage |
|---|---|---|
| DM, g/kg as fed basis | 882 | 303 |
| ME,[1] MJ/kg | 7.8 | 8.9 |
| CP[2] | 90 | 124 |
| ADF | 361 | 264 |
| NDF | 629 | 455 |
| EE | 24 | 36 |
| Ash | 116 | 125 |

DM = dry matter; ME = metabolizable energy; CP = crude protein; ADF = acid detergent fibre; NDF = neutral detergent fibre; EE = ether extract.
[1] The ME was estimated based on NRC (2007).
[2] CP = N × 6.25.

**Table 2**
Ingredients and Nutrient level of four total mixed rations offered to sheep (g/kg, DM basis).

| Item | Total mixed rations[1] | | | |
|---|---|---|---|---|
| | WS0 | WS36 | WS64 | WS100 |
| Ingredients | | | | |
| Wheat silage | 0 | 72 | 128 | 200 |
| Oaten hay | 200 | 128 | 72 | 0 |
| Corn grain | 500 | 500 | 500 | 500 |
| Soybean meal | 150 | 150 | 150 | 150 |
| Corn DDGS | 110 | 110 | 110 | 110 |
| Vitamin–mineral premix[2] | 40 | 40 | 40 | 40 |
| Nutrient levels | | | | |
| ME,[3] MJ/kg | 11.7 | 11.8 | 11.7 | 11.9 |
| CP[4] | 158 | 160 | 161 | 163 |
| ADF | 125 | 115 | 108 | 99 |
| NDF | 233 | 220 | 210 | 198 |
| EE | 33 | 34 | 35 | 35 |
| Ash | 88 | 89 | 90 | 90 |
| NFC[5] | 488 | 497 | 504 | 514 |

DM = dry matter; DDGS = distillers dried grains with solubles; ME = metabolizable energy; CP = crude protein; ADF = acid detergent fibre; NDF = neutral detergent fibre; EE = ether extract; NFC = non-fibre carbohydrate.
[1] Four rations with wheat silage replacing 0%, 36% , 64%, and 100% of oaten hay (dry matter in forage).
[2] The vitamin–mineral premix contained the following per kilogram premix: vitamin A 75,000 to 350,000 IU, vitamin $D_3$ 12,500 to 142,500 IU, vitamin E ≥ 500 mg, Ca ≥ 120 g, Cu 100 to 500 mg, Fe 750 to 17,500 mg, Zn 1,250 to 4,250 mg, Mn 750 to 5,000 mg, I 3.75 to 350 mg, Se 2.5 to 17.87 mg.
[3] The ME was estimated based on NRC (2007).
[4] CP = N × 6.25.
[5] NFC = 1,000 − (neutral detergent fibre + crude protein + ether extract + ash), g/kg dry matter (Song et al., 2018).

period was calculated according to live body weight on d 1, 26, and 52. The feed-to-gain ratio (F:G ratio) was calculated as the DMI divided by the ADG. Rectal faeces samples of the animals in each pen were sampled via anus and collected consecutively for 3 d after morning feeding at the end of the feeding period. The samples per pen were pooled equally and oven-dried at 65 °C for 48 h for subsequent chemical analysis and nutrient digestibility calculation.

After 2 h of morning feeding, two lambs per pen from each ration group were randomly selected for collecting rumen fluid samples. A flexible polyvinyl chloride oral stomach tube (2 mm of wall thickness, 6 mm of internal diameter) with an electric vacuum pump (Ramos-Morales et al., 2014) was used. Then 30 mL of rumen fluid first drawn was discarded to exclude the contamination of saliva. Samples were strained through four layers of cheesecloth and immediately frozen at −20 °C for subsequent analyses of volatile fatty acids (VFA), ammonia nitrogen (N), microbial protein (MCP), and microbiota composition.

### 2.4. Chemical analyses

Representative samples of the distributed TMR and faeces were dried in a forced-air oven at 65 °C for 48 h and then grounded to pass through a 1-mm screen. Chemical compositions of the samples were analysed following the AOAC (1999) methods, for DM, crude protein (CP, calculated as N × 6.5), ether extract, and crude ash. Indigestible acid-insoluble ash (AIA) was used as an intrinsic marker for subsequent calculation of apparent nutrient digestibility (Van Keulen and Young, 1977). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to the methods of Van Soest et al. (1991).

The pH value of rumen fluid samples was measured using a digital pH meter (PHS-3E; Shanghai INESA & Scientific Instrument Co., Ltd., Shanghai, China). Samples for the determination of VFA, ammonia N, and microbial protein were centrifuged at 3,000 × *g* for 15 min at 4 °C. The VFA concentrations were determined using gas chromatography (GC-1120; Shanghai Sunny Hengping Scientific Instrument Co., Ltd., Shanghai, China) according to the method of Erwin et al. (1961). Ammonia N and microbial protein content were determined using a microplate spectrophotometer (RT-6500; Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China) using the methods of Verdouw et al. (1978) and Makkar et al. (1982), respectively.

The rumen fluid samples for the microbiota were melted on ice, mixed well, and centrifuged, and a small amount of specimen was taken for concentration, purity, and integrity testing. Genomic DNA was extracted from rumen fluid using a FastDNA SPIN kit for soil (MP Biomedicals, USA). The amplified primers were 338F (5′-ACTCCTACGGGAG-GCAG-3′) and 806R (5′-GGACTACHVGGGT-WTCTAAT-3′) for the V3–V4 regions of the bacterial 16S rDNA gene. Polymerase chain reaction amplification products were used for high-throughput sequencing using an Illumina MISeq system, and the sequencing company was Shanghai Majorbio Technology Co. Ltd.

Sequence analysis was performed using Quantitative Insights into Microbial Ecology (QIIME) V1.9 (Caporaso et al., 2010) (http://qiime.org/install/index.html). Low-quality sequences were screened and removed based on sequence length, quality, primers, and tags. Short sequences were removed using Mothur V.1.30.2 (Schloss et al., 2009) (https://www.mothur.org/wiki/Download_mothur). The clustering command in Mothur was used to tag high-purity, high-quality sequences as bacteria. The operational taxonomic unit (OTU) was defined by Edgar (2010) at a 97% similarity cut-off. The OTU clustering was performed using the Usearch software platform (http://www.drive5.com/usearch/), and sample OTU representative sequences were compared using the silva138/16s_bacteria species taxonomy database (https://www.arb-silva.de/). After classifying the corresponding species information of OTUs, the Ribosomal Database Project (http://rdp.cme.msu.edu) V11.5 classification algorithm was used. Representative OTU sequences were analysed comparatively, annotated with species at the phylum and genus levels, and calculated using QIIME V1.9.1 software. The Spearman correlation coefficients between the rumen fermentation characteristics and genus abundance were calculated using R language, and a heat map was drawn based on the obtained numerical matrices.

### 2.5. Calculation and statistical analysis

The apparent digestibility of nutrients was calculated using indigestible AIA as an internal marker in the diets and faeces samples as Eq. 1 (Brestensky et al., 2017):

$$\text{Nutrient digestibility (\%)} = [1 - (N_f \times D_{AIA})/(N_d \times F_{AIA})] \times 100\%, \quad (1)$$

where $N_d$ and $N_f$ are the nutrient contents, and $D_{AIA}$ and $F_{AIA}$ are the AIA contents in the diets and faeces samples, respectively.

The non-glucogenic-to-glucogenic acid ratio (NGR) and fermentation efficiency (FE) were calculated as Eq. 2 and Eq. 3, respectively (Abdl-Rahman et al., 2010):

$$\text{NGR} = (\text{acetate} + 2 \times \text{butyrate} + \text{valerate})/(\text{propionate} + \text{valerate}), \quad (2)$$

$$\text{FE} = (0.622 \times \text{acetate} + 1.092 \times \text{propionate} + 1.56 \times \text{butyrate})/(\text{acetate} + \text{propionate} + 2 \times \text{butyrate}), \quad (3)$$

where VFA are expressed in molar proportion.

Data were subjected to a one-way analysis of variance using the MIXED procedure of the software SAS package for Windows (version 9.4, SAS Institute Inc., Cary, NC, USA). The model applied was Eq. 4:

$$Y_{ij} = \mu + T_i + A_j + e_{ij}, \quad (4)$$

where $Y_{ij}$ is dependent variable under examination; $\mu$ is the overall mean; $T_i$ is the fixed effect of wheat silage replacing oaten hay level in forage source of TMR ($i = 4$, WS0, WS36, WS64, WS100); $A_j$ is the random effect of pen ($j = 4$ for dry matter intake and F:G ratio) or animals ($j = 16$ per TMR for ADG and nutrient digestibility or $j = 8$ for all rest variables), and $e_{ij}$ is the residual error. The least square means and standard errors of the means were calculated using the LSMEANS procedure of SAS software. Orthogonal polynomial contrasts were performed to determine the linear and quadratic effects of wheat silage replacing oaten hay in four sources of the total mixed rations. Significance was set to $P < 0.05$.

## 3. Results

### 3.1. Growth performance

As shown in Table 3 and Fig. 1A, increasing dietary wheat silage replacement of oaten hay linearly decreased the DMI during the period of not only 0 to 26 d ($P < 0.01$) but also 27 to 52 d ($P < 0.01$), and finally DMI in WS36, WS64 and WS100 rations compared with the WS0 ration decreased by 4%, 12% and 27%, respectively. After 52 d of feeding, body weight gain per animal and ADG did not differ among WS0, WS36 and WS64, but the lowest occurred in WS100 ($P = 0.02$) as shown in Fig. 1B. Consequently, increasing the wheat silage replacement of oaten hay decreased F:G ratio, and the lowest F:G ratio occurred in WS64 ($P = 0.04$).

### 3.2. Nutrient digestibility

As shown in Table 4, increasing dietary wheat silage replacement of oaten hay did not alter the apparent digestibility of DM, CP, and ether extract, but quadratically increased the digestibility of organic matter ($P < 0.01$), non-fibre carbohydrate (NFC) ($P < 0.01$), NDF ($P = 0.04$), and ADF ($P < 0.01$), and their highest digestibility occurred in WS64.

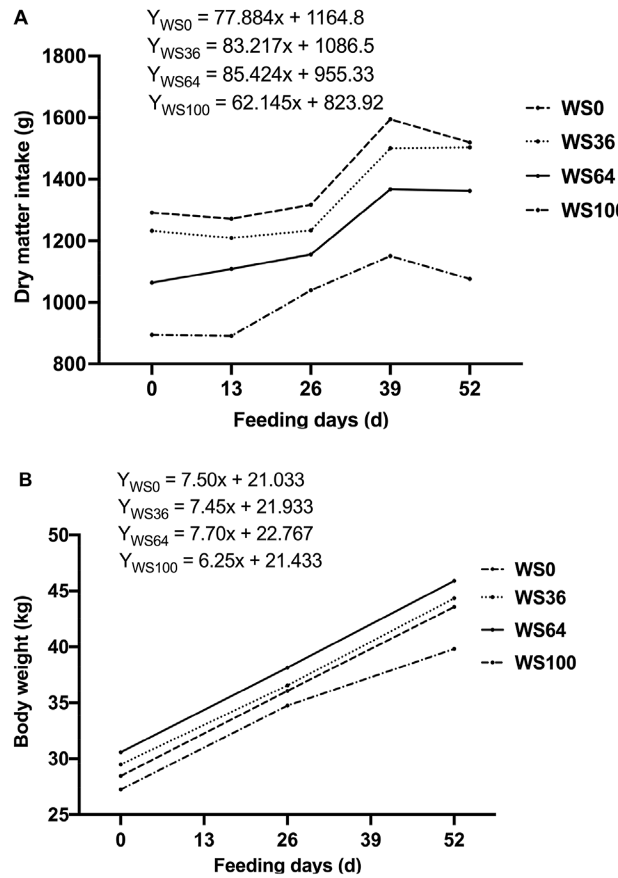


**Fig. 1.** Dry matter intake (A) and body weight (B) of fattening sheep in response to four rations with wheat silage replacing 0% (WS0), 36% (WS36), 64% (WS64), and 100% (WS100) of oaten hay (dry matter in forage).

**Table 3**
Growth performance of fattening sheep in response to different ratios of wheat silage to oaten hay in four total mixed rations.

| Item | Total mixed rations[1] | | | | SEM | P-value | |
|---|---|---|---|---|---|---|---|
| | WS0 | WS36 | WS64 | WS100 | | Linear | Quadratic |
| Dry matter intake, g/d | | | | | | | |
| 0 to 26 d | 1,294[a] | 1,222[a] | 1,132[ab] | 965[b] | 51.6 | <0.01 | 0.41 |
| 27 to 52 d | 1,557[a] | 1,502[ab] | 1,365[b] | 1,113[c] | 56.9 | <0.01 | 0.12 |
| 0 to 52 d | 1,425[a] | 1,362[a] | 1,248[a] | 1,039[b] | 53.5 | <0.01 | 0.22 |
| Body weight, kg | | | | | | | |
| Initial | 28.5 | 29.5 | 30.5 | 27.3 | 2.04 | 0.60 | 0.05 |
| 26 d | 36.1 | 36.6 | 38.1 | 34.7 | 2.40 | 0.65 | 0.11 |
| 52 d | 43.5[a] | 44.4[a] | 45.9[a] | 39.8[b] | 2.76 | 0.10 | 0.01 |
| Body weight gain, kg | | | | | | | |
| 0 to 26 d | 7.6 | 7.1 | 7.6 | 7.5 | 0.45 | 0.93 | 0.60 |
| 27 to 52 d | 7.5[a] | 7.8[a] | 7.8[a] | 5.1[b] | 0.48 | <0.01 | <0.01 |
| 0 to 52 d | 15.1[a] | 14.9[a] | 15.3[a] | 12.6[b] | 0.83 | 0.02 | 0.07 |
| Average daily gain, g/d | | | | | | | |
| 0 to 26 d | 292.2 | 272.1 | 290.9 | 288.2 | 22.96 | 0.93 | 0.60 |
| 27 to 52 d | 288.5[a] | 299.5[a] | 298.6[a] | 195.0[b] | 25.43 | <0.01 | <0.01 |
| 0 to 52 d | 290.3[a] | 285.8[a] | 294.7[a] | 241.6[b] | 21.09 | 0.02 | 0.07 |
| Feed-to-gain ratio | | | | | | | |
| 0 to 26 d | 4.4[a] | 4.5[a] | 3.9[ab] | 3.3[b] | 0.29 | 0.01 | 0.47 |
| 27 to 52 d | 5.4 | 5.0 | 4.6 | 5.7 | 0.43 | 0.76 | 0.05 |
| 0 to 52 d | 4.9[a] | 4.8[ab] | 4.2[c] | 4.4[bc] | 0.27 | 0.04 | 0.42 |

[a, b, c] Within a row, values with no common superscripts differ significantly ($P < 0.05$).
[1] Four rations with wheat silage replacing 0% (WS0), 36% (WS36), 64% (WS64), and 100% (WS100) of oaten hay (dry matter in forage).

### 3.3. Rumen fermentation characteristics

As shown in Table 5, dietary substitution of wheat silage in lambs numerically decreased the rumen pH ($P = 0.12$). The inclusion of wheat silage as forage decreased ammonia N ($P = 0.01$) but increased microbial protein ($P < 0.01$) in the rumen. Feeding wheat silage as a replacement for oaten hay quadratically increased the total VFA concentration in the rumen, with the highest total VFA occurring in WS64 ($P < 0.01$).

Regarding the VFA pattern, the addition of wheat silage in diets WS36, WS64, and WS100 significantly reduced acetate molar proportion ($P < 0.01$). Although not significant, higher propionate occurred in these groups. The acetate-to-propionate ratio (A:P ratio) was quadratically decreased and the lowest occurred in WS64 ($P = 0.04$). Compared to the WS0 group, butyrate ($P < 0.01$) and isovalerate proportions were significantly higher in WS100 ($P = 0.04$). The NGR and FE did not indicate any difference.

### 3.4. Microbial community composition

Ten dominant genera and phyla consistently present in all rumen fluids were identified (Table 6). The wheat silage replacement of oaten hay in the total mixed rations remarkably increased the dominant Firmicutes by 46% to 67% ($P = 0.04$) and decreased

**Table 4**
Apparent nutrient digestibility of fattening sheep in response to different ratios of wheat silage to oaten hay in four total mixed rations (%).

| Item | Total mixed rations[1] | | | | SEM | P-value | |
|---|---|---|---|---|---|---|---|
| | WS0 | WS36 | WS64 | WS100 | | Linear | Quadratic |
| Dry matter | 76.5 | 78.6 | 80.9 | 77.3 | 1.34 | 0.45 | 0.06 |
| Organic matter | 78.1[c] | 81.6[b] | 83.1[a] | 81.5[b] | 1.09 | 0.08 | <0.01 |
| Crude protein | 79.4 | 79.7 | 79.4 | 74.7 | 1.24 | 0.07 | 0.13 |
| Neutral detergent fibre | 64.1[b] | 69.4[b] | 72.2[a] | 61.6[b] | 2.15 | 0.12 | 0.04 |
| Acid detergent fibre | 49.2[b] | 59.9[a] | 60.6[a] | 51.5[ab] | 2.95 | 0.60 | <0.01 |
| Ether extract | 85.8 | 86.0 | 88.7 | 89.3 | 0.94 | 0.07 | 0.87 |
| Non-fibre carbohydrate | 90.8[b] | 92.6[ab] | 93.1[a] | 75.0[c] | 1.78 | <0.01 | <0.01 |

a, b, c Within a row, values with no common superscripts differ significantly ($P < 0.05$).
1 Four rations with wheat silage replacing 0% (WS0), 36% (WS36), 64% (WS64), and 100% (WS100) of oaten hay (dry matter in forage).

**Table 5**
Rumen fermentation characteristics of fattening sheep in response to different ratios of wheat silage to oaten hay in four total mixed rations.

| Item | Total mixed rations[1] | | | | SEM | P-value | |
|---|---|---|---|---|---|---|---|
| | WS0 | WS36 | WS64 | WS100 | | Linear | Quadratic |
| pH | 6.9 | 6.8 | 6.6 | 6.8 | 0.10 | 0.12 | 0.48 |
| Ammonia N, mg/dL | 14.3[a] | 8.5[b] | 9.9[b] | 6.9[b] | 1.54 | 0.01 | 0.41 |
| Microbial protein, mg/mL | 0.4[b] | 0.7[a] | 0.6[a] | 0.7[a] | 0.04 | <0.01 | 0.05 |
| Total VFA, mmol/L | 42.9[b] | 60.3[a] | 60.9[a] | 49.9[b] | 2.62 | 0.08 | <0.01 |
| VFA pattern, % molar | | | | | | | |
| Acetate | 61.5[a] | 55.3[b] | 54.6[b] | 53.2[b] | 1.57 | <0.01 | 0.53 |
| Propionate | 26.7 | 31.7 | 32.8 | 27.8 | 2.34 | 0.67 | 0.04 |
| Butyrate | 9.0[b] | 9.7[b] | 9.2[b] | 15.1[a] | 1.27 | <0.01 | 0.06 |
| Isobutyrate | 0.4 | 0.5 | 0.4 | 0.5 | 0.04 | 0.90 | 0.61 |
| Valerate | 1.2 | 1.3 | 1.4 | 1.3 | 0.13 | 0.47 | 0.60 |
| Isovalerate | 1.1[b] | 1.6[ab] | 1.4[ab] | 2.1[a] | 0.25 | 0.04 | 0.76 |
| A:P ratio | 2.30[a] | 1.74[ab] | 1.66[b] | 1.91[ab] | 0.180 | 0.18 | 0.04 |
| NGR[2] | 2.8 | 2.3 | 2.3 | 2.9 | 0.28 | 0.82 | 0.07 |
| Fermentation efficiency[3] | 0.77 | 0.79 | 0.79 | 0.78 | 0.009 | 0.36 | 0.19 |

VFA = volatile fatty acids; A:P ratio = acetate-to-propionate ratio; NGR = non-glucogenic-to-glucogenic acid ratio.
a, b Within a row, values with no common superscripts differ significantly ($P < 0.05$).
1 Four rations with wheat silage replacing 0% (WS0), 36% (WS36), 64% (WS64), and 100% (WS100) of oaten hay (dry matter in forage).
2 NGR = (acetate + 2 × butyrate + valerate)/(propionate + valerate) (Abdl-Rahman et al., 2010).
3 Fermentation efficiency = (0.622 × acetate + 1.092 × propionate + 1.56 × butyrate)/(acetate + propionate + 2 × butyrate) (Abdl-Rahman et al., 2010).

**Table 6**
Relative abundance of dominant phyla and genera of bacteria in response to different ratios of wheat silage to oaten hay in four total mixed rations.

| Item | Total mixed rations[1] | | | | SEM | P-value | |
|---|---|---|---|---|---|---|---|
| | WS0 | WS36 | WS64 | WS100 | | Linear | Quadratic |
| Phyla, % of total observations | | | | | | | |
| Firmicutes | 44.3[b] | 74.1[a] | 64.7[a] | 69.0[a] | 6.00 | 0.04 | 0.07 |
| Bacteroidota | 48.7[a] | 24.9[b] | 15.5[b] | 19.2[b] | 5.74 | <0.01 | 0.03 |
| Actinobacteriota | 0.6 | 1.3 | 4.8 | 3.6 | 1.76 | 0.17 | 0.63 |
| Patescibacteria | 1.0 | 1.9 | 1.0 | 0.8 | 0.40 | 0.44 | 0.23 |
| Spirochaetota | 0.7 | 1.2 | 0.4 | 0.4 | 0.30 | 0.22 | 0.45 |
| Proteobacteria | 0.5[a] | 0.3[ab] | 0.4[ab] | 0.2[b] | 0.08 | 0.01 | 0.94 |
| unclassified_k_norank_d_Bacteria | 0.14 | 0.08 | 0.04 | 0.04 | 0.035 | 0.11 | 0.50 |
| Cyanobacteria | 0.11 | 0.10 | 0.06 | 0.09 | 0.022 | 0.43 | 0.36 |
| Desulfobacterota | 0.07 | 0.08 | 0.18 | 0.17 | 0.052 | 0.17 | 0.91 |
| Synergistota | 0.04 | 0.07 | 0.05 | 0.11 | 0.035 | 0.36 | 0.77 |
| Genera, % of total observations | | | | | | | |
| *Ruminococcus* | 9.7 | 29.8 | 15.6 | 19.3 | 8.17 | 0.71 | 0.35 |
| *Prevotella* | 24.5[a] | 11.6[b] | 8.0[b] | 6.3[b] | 3.73 | <0.01 | 0.19 |
| *NK4A214_group* | 3.5 | 3.9 | 2.4 | 3.7 | 0.95 | 0.87 | 0.66 |
| *Christensenellaceae_R-7_group* | 3.8 | 4.6 | 3.0 | 3.7 | 0.74 | 0.56 | 0.97 |
| *norank_f_Eubacterium_coprostanoligenes_group* | 1.9[b] | 1.6[b] | 5.6[a] | 4.8[ab] | 0.77 | 0.02 | 0.84 |
| *Rikenellaceae_RC9_gut_group* | 3.5 | 3.1 | 2.2 | 1.9 | 0.93 | 0.19 | 0.97 |
| *Olsenella* | 0.5 | 0.5 | 1.4 | 1.1 | 0.46 | 0.24 | 0.69 |
| *norank_f_Muribaculaceae* | 2.2 | 1.0 | 2.0 | 1.2 | 0.64 | 0.54 | 0.80 |
| *Erysipelotrichaceae_UCG-002* | 0.1 | 0.1 | 0.6 | 1.6 | 0.39 | 0.05 | 0.26 |
| *unclassified_f_Ruminococcaceae* | 1.2 | 2.0 | 0.1 | 0.2 | 0.53 | 0.11 | 0.61 |

a, b Within a row, values with no common superscripts differ significantly ($P < 0.05$).
1 Four rations with wheat silage replacing 0% (WS0), 36% (WS36), 64% (WS64), and 100% (WS100) of oaten hay (dry matter in forage).

Bacteroidota by 49% to 68% ($P < 0.01$) at the phylum level. Interestingly, the relative abundance of Firmicutes was slightly lower than that of Bacteroidota in the WS0 group, but as wheat silage was added to the diets, the relative abundance of these two phyla differed dramatically. Besides, the relative abundance of Proteobacteria decreased linearly with the increase of wheat silage ($P = 0.01$).

At the genera level, oaten hay substitution with wheat silage in the total mixed rations numerically increased *Ruminococcus* abundance ($P = 0.35$). The abundance of *Prevotella* decreased linearly with increasing wheat silage ($P < 0.01$). The increase in wheat silage inclusion quadratically affected the abundance of the *Eubacterium_coprostanoligenes_group* community, which was the highest in WS64 ($P = 0.02$).

The result of the Spearman correlation analysis of the microbiota and rumen fermentation characteristics is shown in Fig. 2. *Olsenella* showed a negative correlation with acetate ($P < 0.01$) and A:P ratio ($P = 0.02$). *Erysipelotrichaceae_UCG-002* exhibited a strong negative correlation with the ammonia N concentration ($P < 0.01$) and a positive correlation with butyrate ($P < 0.01$). *Unclassified_f_Ruminococcaceae* exhibited a positive correlation with acetate proportion ($P < 0.01$).

## 4. Discussion

### 4.1. DMI, ADG, and feed efficiency

In ruminant feeding practice, oaten hay is known to be much more expensive than corn silage or wheat silage. As shown in Table 1, although the CP content was greater in wheat silage than in oaten hay (124 g/kg DM vs. 90 g/kg DM), the fact that the substitution of oaten hay with wheat silage linearly decreased DM intake indicated that wheat silage was somewhat less acceptable than oaten hay for lambs. Compared to wheat hay, wheat silage has an aromatic sour smell and is more palatable. However, oaten hay has high water soluble carbohydrates and may be more acceptable to lambs (Abdelraheem et al., 2019). Rustas et al. (2011) stated that the NDF content negatively correlated to the DMI of dairy heifers fed wheat silage. However, in the present study, as NDF decreased with WS addition, an increase in feed intake did not occur as expected. It may be because the harvest stage and fermentation quality of wheat silage can affect palatability, which should be considered in future experiments and fermentation characteristics should be measured (Xie et al., 2012).

With wheat silage replacing 100% of oaten hay, the significantly lower DMI in WS100 led to a significant decrease in ADG. However, compared to feeding wheat silage as a sole forage source, positive associative effects of wheat silage and other forages seem to improve animal performance in the present and previous studies. Charmley et al. (1996) reported that the 50% wheat silage treatment exhibited higher weight gain than the 100% treatment when substituting grass silage. This was also reflected in the current study as despite slightly decreased DMI, body weight data did not differ among WS36, WS64 and the control. It is noteworthy that ADG was even numerically higher in WS36 and WS64. The substitution of OH with wheat silage in diets changed the CP levels. According to the Feed Composition Table reported by Beef Magazine, wheat silage with 120 g/kg of CP contains 59% total digestible nutrients, and oaten hay with 120 g/kg of CP contains 54% total digestible nutrients as energy sources (Preston, 2016). Besides, an increase in available energy for live body weight gain occurred with the decreased dietary NDF and ADF contents, and the improved NDFD and ADFD could support this. Presumably, the reason for the enhanced ADG being statistically insignificant is that high concentrate content masked the ADG response of lambs to different forage diets. Despite that, wheat silage replacing 64% oaten hay did remarkably decrease the F:G ratio (reciprocal of feed efficiency) by 14% as we hypothesized. This result implies an effective reduction of the farm's dietary costs.

Compared to the study of Scerra et al. (2001), which 30% oaten hay was compared with 6% wheat straw silage and 14% citrus pulp in lambs' diets, greater ADG occurred in our study with similar initial body weight and duration. We speculate that high concentrate content and wheat silage proportion resulted in great CP and

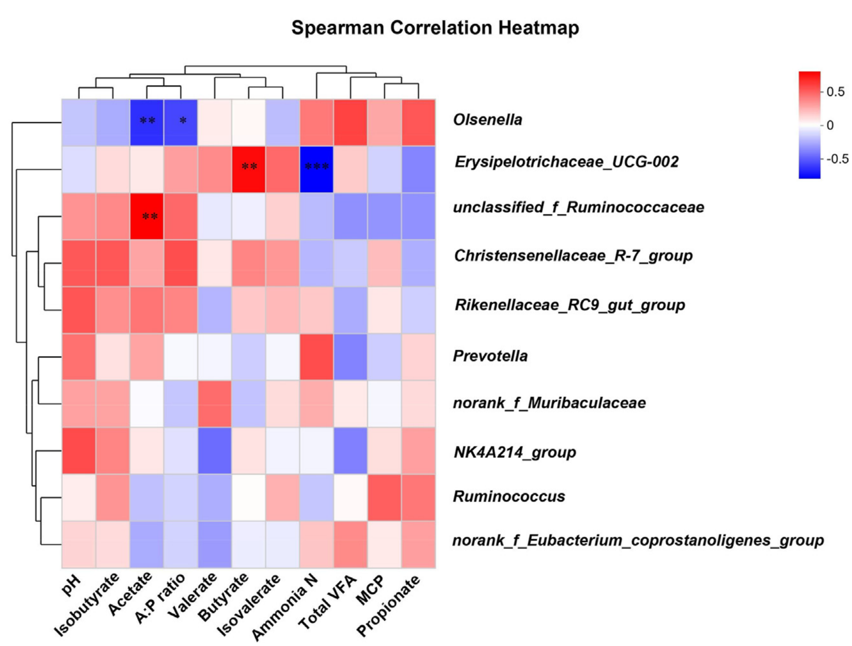


**Fig. 2.** Spearman correlation analysis of the ruminal microbiota and rumen fermentation parameters. A:P ratio = acetate-to-propionate ratio; VFA = volatile fatty acids; MCP = microbial protein. Colour represents the correlation coefficient, with red representing a positive correlation and blue presenting a negative correlation. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

low ADF content, and further led to high ADG. However, in the study of Fisher and Lessard (1977), cows fed wheat silage instead of corn silage lost live weight compared to the initial, whilst milk yield and composition were not altered. Unlike lambs, cows partitioned dietary energy toward lactation, thus sacrificing the energy for maintenance and growth.

In general, our findings indicated that feeding wheat silage as the sole forage source is not an appropriate strategy for lambs. In contrast, forage combinations of 64% and 36% wheat silage can lower the F:G ratio. This is a meaningful reference for cost-efficient use of wheat silage in feedlots.

### 4.2. Nutrient digestibility

Compared to WS0, higher organic matter, NDF, and ADF digestibilities (OMD, NDFD, ADFD) occurred in lambs fed WS36, WS64, and WS100. Digestibility is closely related to feed intake as previous studies suggested that when DMI decreases, the animals fully digest fibre to meet their energy requirements (de Souza et al., 2018). Another reason is the decreased fibre content or increased NFC (represents the content of starch) in three treatments. Song et al. (2018) found a positive relation between NFC and nutrient digestibilities. Neutral detergent fibre (i.e. hemicellulose and cellulose) occupies a lot of space in the gastrointestinal tract and is less digestible than the other digestible fractions in feeds. With less fibre, nutrients in the diet are retained in the digestive tract for a longer period of time and thus are fully digested (Pino et al., 2018).

However, higher OMD, NDFD, and ADFD occurred in WS36 and WS64 which consist of different forages rather than sole forage source. Consistent with the present study, Turan (2020) reported that the ADFD and NDFD of Hungarian Vetch Silage mixed with wheat silage in a 4:1 ratio were significantly higher than those of the whole WS diet. The reasons may be twofold. First, the appropriate combination of wheat silage and oaten hay ratios enhanced the rumen microbial growth, especially cellulolytic bacteria. Second, excessive content of NFC has been observed to depress forage fibre digestion (Arroquy et al., 2005). Too much rapidly fermenting starch in wheat also decreased DM and OM digestibility (Gonzalez-Rivas et al., 2021). Lactating dairy cows fed a large amount of crushed wheat in diets had a linear decrease in NDF and ADF digestibility (Leddin et al., 2009), which we presume is the reason for the slightly lower digestibility compared to WS36 and WS64 in WS100.

Compared with the studies of Walsh et al. (2008) and Leibovich et al. (2013), they observed a lower NDFD than ours at higher NDF content. This can also partially explain the effect of NDF content on NDFD. However, at similar forage content, lambs fed whole wheat silage in the study of Benghedalia et al. (1995) (conducted in dairy cows) consumed lower NDF than all lambs in this study (27.1% DM), but NDFD only reached similar to that of WS100 (49.6% vs. 51.9%). We assume that this is related to the difference in the nutrient utilization capacity of cattle and lambs. It has been indicated that sheep digest nutrients in wheat silage at a higher rate than cattle fed the same wheat silage diet (Mannerkorpi and Brandt, 1993), although the opinion is not yet unified (Charmley et al., 1996; Sudekum et al., 1995).

The NDF digestibility plays an important role in determining OM digestibility in diet (Gehman and Kononoff, 2010). This can be regarded as the reason why the trend of OMD was basically the same as NDFD. However, OMD was inevitably affected by the digestibility of other nutrients and had slight differences.

It is worth noting that perhaps due to the higher digestibility, lambs fed WS36 and WS64 showed similar body weight gain with WS0 on the basis of a slightly lower feed intake. Thus, in general, these results suggested that wheat silage should be combined with other forages, rather than fed alone, as a viable feeding strategy.

### 4.3. Rumen fermentation characteristics

The pH of all groups in this study was higher than the defined threshold pH values of subacute ruminal acidosis (pH > 5.6) (Ramos et al., 2021) and also higher than the daily average pH of some welfare farms (6.2 ± 0.2) (Baek et al., 2022). Thus, the wheat silage proportions in this study were still within the range of rumen fermentation regulation and buffering capacity of lambs, and did not cause abnormal rumen fermentation.

The ammonia N concentrations in WS36, WS64, and WS100 were consistent with the best ammonia N concentration (7 to 9 mg/dL) for synthesizing MCP, as recommended by previous studies (Hume et al., 1970). As a result, more N sources were used to synthesize MCP instead of being absorbed by the rumen wall (Fanchone et al., 2013). Also, increased dietary CP content should also be another contributor to enhancing microbial protein production in rumen. Such a result suggested an improved energy and N balance, which means a high N utilization and reduced waste (Zhang et al., 2020). However, this result is not in accordance with that of Abazinge et al. (1994), who found that ammonia N was higher for wethers fed wheat straw silage than for those that received the basal diet based on 75% orchardgrass hay. Whole-crop wheat silage incorporates more fermentable carbohydrates than wheat straw silage for including grains, thus potentially promoting energy and N synchronization and N utilization. In addition, vigorous microflora in whole-crop wheat silage, which can pass from the silage to the rumen liquor and survive (Weinberg et al., 2004), may enhance the utilization of protein and the synthesis of microbial protein. According to our study, the substitution of oaten hay by WS promoted the synthesis of MCP by 50% to 75%.

The substitution of oaten hay with wheat silage facilitated the total VFA concentration and decreased A:P ratio in WS64. Propionate molar proportion also increased numerically although not notable. Previous studies also indicated that compared to hay, silage forages which contain more starch and fermentable carbohydrate could decrease A:P ratio (Calabro et al., 2004) and enhance the abundance of Fibrobacteres which fermented cellulose (Niu et al., 2020). Easy-to-ferment carbohydrates, such as starch and cellulose, were broken down into monosaccharides, then converted into pyruvate and eventually propionate. This may result in an improved fattening effect, since as a precursor of body fat and glucose, propionates offered more energy than acetates (Parra, 2013).

### 4.4. Ruminal microbial community composition and relevance analysis

To gain insight into the mechanism behind the changes in rumen characteristics, microbiota composition and correlation were examined. As previously proved, Firmicutes and Bacteroidota are the two most predominant phyla in rumen. Their relative abundance varied depending on the age, physiological condition, and diet of the animal. In accordance with previous studies, we found that the relative abundance of Firmicutes increased with NFC (Liu et al., 2019; Wang et al., 2020; Zhao et al., 2022). Conversely, a higher relative abundance of Bacteroidota tended to occur in diets with a high proportion of forage or more crude fibre. The inclusion of wheat silage improved the Firmicutes-to-Bacteroidota ratio, completely changing the most abundant phylum.

There are many drivers for the change in the predominant microbiota in our results. Species from Firmicutes and *Ruminococcus* (main member of Firmicutes) are related to the degradation of fibre, and the species from Bacteroidota and *Prevotella* (main member of Bacteroidota) are in charge of the degradation and assimilation of protein and polysaccharide (Jami et al., 2013). The relative abundance changes provided a better understanding of the differences in nutrient utilization. Given the high abundance of Firmicutes, NDFD and ADFD were changed by the inclusion of wheat silage, while CP digestibility showed no remarkable variation because of the decreased abundance of Bacteroidota. As mentioned earlier the pH did not change drastically with the addition of wheat silage, which also created favourable conditions for the survival and reproduction of cellulolytic bacteria.

To predict the microbiota involved in changing rumen fermentation characteristics, a correlation analysis was performed. A negative correlation was observed between *Olsenella* and acetate and A:P ratio. This genus has been proved to ferment carbohydrates into lactate (Kraatz et al., 2011) which could increase the molar proportion of propionate via the acrylate pathway (Mamuad et al., 2017).

*Erysipelotrichaceae_UCG-002* has been previously proved to be involved in fibre digestion and VFA synthesis (Kong et al., 2022), but the exact mechanism is still elusive. The butyrate is mainly produced by the *Butyrivibrio fibrisolvens*, and the vaccenic acid produced by the *Butyrivibrio fibrisolven* is negatively correlated with *Erysipelotrichaceae_UCG-002* (Daghio et al., 2021). It seems to be some interactions between *Erysipelotrichaceae_UCG-002* and the *B. fibrisolven* indirectly affect the butyrate proportion. Wang et al. (2022b) found that *Erysipelotrichaceae_UCG-002* in early weaned lambs was positively correlated with ammonia N concentration, inconsistent with the findings of this study. The age difference (21 d vs. 100 d) of lambs and the feeding strategies may be the primary drivers, but the detailed function of these genera remains unclear and requires further investigation.

Compared to WS0 which included oaten hay as the sole forage source, the diet incorporating 100% wheat silage showed a lower relative abundance of Proteobacteria, which is Gram-negative bacteria that includes pathogens such as *Escherichia coli* and *Salmonella*. The members of this phylum tend to increase the risk of diseases in host animals. Lambs fed a wheat silage diet have the potential to improve adaptation to environmental oscillation and mitigate the risk of disease.

## 5. Conclusion

Collectively, increasing the wheat silage inclusion significantly decreased the dry matter intake of lambs by 4% to 27%, but no more than 64% substitution increased fibre digestibility (13% in NDF and 23% in ADF), and decreased the F:G ratio by 14% without compromising on weight gain. Additionally, 36% or 64% wheat silage in forage increased total VFA and microbial protein production as well as the relative abundance of cellulolytic bacteria. In conclusion, wheat silage can be used as a relatively cheaper temporary feed when oaten hay is scarce or too expensive. Nevertheless, feeding wheat silage as sole forage is not recommended in feedlot lambs.

## Author contributions

**Hong-Jian Yang:** Conceptualization, Methodology, Software. **Zhao-Yang Cui:** Validation, Investigation, Formal analysis, Writing-Original draft preparation. **Wen-Juan Li:** Methodology, Investigation, Supervision. **Wei-Kang Wang:** Writing – Review & Editing. **Qi-Chao Wu:** Software, Project administration**: Yao-Wen Jiang:** Investigation, Writing – Review & Editing. **Ailiyasi Aisikaer:** Investigation, Resources. **Fan Zhang:** Investigation. **He-Wei Chen:** Resources.

## Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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