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The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

Optimising substrate conditions for microbial fermentation of vinegar residue

Y. Gu, S. Xia, H. Chen, W. Liao, K. Deng and G. Bian*

Jinling Institute of Technology, College of Animal Science and Food Engineering,
Department of Animal Nutrition and Feed Science, 210038 Nanjing, China

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* Corresponding author:
e-mail: grbian@jit.edu.cn

ABSTRACT. The aim of this study was to investigate the effects of additives on the nutritional value of fermented vinegar residue. Molasses, urea, and wheat bran were added to the vinegar residue fermentation system as a carbon source, nitrogen source, and a combined source of both carbon and nitrogen, respectively. Molasses was added at the following proportions: 0, 1.5, 3 and 4.5%, urea at 0, 0.5, 1.0 and 1.5%, and wheat bran at 0, 5, 10 and 15%. Fermentation was performed using five strains of fermenting bacteria in laboratory silage bags. After four weeks, the quality of vinegar residue fermentation was evaluated using sensory index, pH value, raw ash, and crude protein contents, as well as levels of ammonia-nitrogen and short-chain fatty acids (including lactic, acetic, propionic, and butyric acids). The results revealed that the addition of 3 and 4.5% molasses significantly improved the pH value, and lactic acid concentration in the vinegar residue ($P < 0.05$). The addition of 4.5% urea significantly increased the pH value, lactic acid concentration, ash and crude protein contents, while decreasing the acetic acid concentration in the vinegar residue ($P < 0.05$). The inclusion of 15% wheat bran significantly elevated the sensory index and crude protein content, but reduced the pH value, dry matter content and acetic acid concentration in the vinegar residue ($P < 0.05$). So, the nutritional quality of vinegar residue can be improved by incorporating carbon and nitrogen sources into the fermentation system. The addition of 15% wheat bran appears to be an optimal choice for stimulating vinegar residue fermentation, surpassing the effectiveness of utilising carbon (molasses) and nitrogen (urea) sources individually.

Introduction

In recent years, animal husbandry has experienced a 70–80% increase in the costs of feed raw materials, highlighting the pressing need to explore and develop alternative feed resources not reliant on grains (Kim et al., 2018). China, as a significant producer of solid-state fermented vinegar, generates 600–700 kg of vinegar residue waste for every 1 000 kg of vinegar produced (Zhou et al., 2017). Vinegar residue represents a significant environmental hazard and poses a potential threat to its safety in China (Song et al., 2021). Proper disposal of vinegar residue is crucial to prevent any adverse environmental impacts. Vinegar residue contains specific nutritional components, such as nitrogen-free extract, crude protein, and short-chain fatty acids, making it a potential feed ingredient (Wang et al., 2022a). However, the content of these components is insufficient to be fed directly to animals. Therefore, improving its nutritional value and making it suitable for animal production is conducive to the sustainable development of China's animal husbandry.

Microbial-transformed feed components, especially roughage fermentation, has become a significant trend in animal feed development. The process of fermentation acts as a pre-treatment for roughage, either with or without the use of microbial inoculants, to effectively decompose crude fibre, reduce its content and transform it into essential nutrients such as protein and sugar (Wang et al., 2022a). Additionally, microorganisms produce organic acids during fermentation, which can acidify feed, stimulate the secretion of digestive enzymes in the animal's gastrointestinal tracts, and facilitate gastrointestinal motility and digestion. This process results in the formation of small molecular substances, such as soluble peptides, thereby improving the nutritional quality and utilisation of fermented raw materials (Missotten et al., 2015). Consequently, microbial fermentation has the potential to enhance the dietary nutritional value of coarse roughage raw materials.

The addition of nutrients is the primary factor that determines the nutritional value of microbial-processed feeds. Molasses, urea, and wheat bran are commonly incorporated into the fermentation systems as nutrients. According to a previous study (Jiang et al., 2021), the addition of molasses to fermented feed resulted in a linear reduction in pH value, propionic acid, and ammonia nitrogen while the lactic acid content linearly increased. It has been widely reported that the addition of urea leads to an increase in the crude protein content in silage (Santos et al., 2018; Hou et al., 2022; Nascimento et al., 2023). However, when the supplemented amount surpassed 1.0%, silage quality deteriorated, and the smell of ammonia intensified. A study showed that the addition of 15% wheat bran to a mixed fermentation system with tea residues and lactic acid bacteria was an excellent ingredient for producing high quality feed from tea residues (Zhu et al., 2019). This finding indicates that the inclusion of wheat bran can improve the nutritional quality of coarse roughage residues.

The objective of this study was to investigate the effect of nutritional additives on the fermentation of vinegar residue. Molasses was introduced into the fermentation system as an additional carbon source, urea as an additional nitrogen source, and wheat bran as a combined carbon and nitrogen source. The nutritional value of the fermented vinegar residue was evaluated by measuring the fermentation quality based on the sensory index, raw ash and crude protein contents, as well as the concentration of ammonia-nitrogen and short-chain fatty acids. The findings of this study are intended to provide both theoretical insights and practical guidance for utilising vinegar residue as a raw material in fermentation processes.

Material and methods

Raw materials and equipment

The vinegar residue used in this study was obtained from a vinegar producer located in Zhenjiang city, Jiangsu province, China. Fermentative bacterial strains, including *Bacillus subtilis*, *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, and *Lactobacillus plantarum*, were sourced from our laboratory. Each bacterial strain was incubated in MRS medium at 37 °C for 24 h at > 10^8 CFU/ml. The fermentation inoculum consisted of equal proportions of the five aforementioned strains.

The experimental design, as outlined in Table 1, involved adding varying proportions of molasses (M) set at 0% (0M group), 1.5% (1.5M group), 3% (3M group), and 4.5% (4.5M group), each with four replicates. Urea addition (U) levels in the study ranged from 0% (0U) to 1.5% (0.5U, 1.0U, and 1.5U groups), while wheat bran addition (WB) percentage varied from 0% (0WB group) to 15% (5WB, 10WB, and 15WB groups).

The fermentation substrate of each group had a total weight of 100 g with a moisture content of 60%. Bacterial inoculum was introduced at a rate of 5%, as shown in Table 1. The vinegar residue and additives were mixed, and then hermetically sealed using a vacuum machine in vacuum bags. After undergoing a four-week fermentation process at room temperature, the silage bags were opened for further assessments.

Table 1. Experimental design of fermentation vinegar residue

Treatment group	Vinegar residue, g	Molasses, g	Urea, g	Wheat bran, g	Water, ml	Bacteria, %
0M	100	0	0	0	132	5
1.5M	98.5	1.5	0	0	131	5
3M	97	3	0	0	130	5
4.5M	95.5	4.5	0	0	130	5
0U	100	0	0	0	125	5
0.5U	99.5	0	0.5	0	125	5
1U	99	0	1	0	125	5
1.5U	98.5	0	1.5	0	125	5
0WB	100	0	0	0	160	5
5WB	95	0	0	5	159	5
10WB	90	0	0	10	158	5
15WB	85	0	0	15	157	5

Molasses (M) addition levels at 0% (0M group), 1.5% (1.5M group), 3% (3M group), and 4.5% (4.5M group), urea addition (U) levels ranging from 0% (0U group) to 1.5% (0.5U, 1.0U, and 1.5U groups), and wheat bran (WB) addition levels ranging from 0% (0WB group) to 15% (5WB, 10WB, and 15WB groups)

Sensory evaluation

The evaluation of silage quality was conducted following the 'German DLG Silage Sensory Scoring Standard' (Nussbaum et al., 2004). This methodology involved evaluating scent, texture, and colour attributes, with the aroma segment assigned 14 points, while texture and colour were each scored out of 4 and 2 points, respectively. Ratings falling within the range of 16–20 was categorised as excellent, while 10–15 as fair, 5–9 as moderate, and 0–4 as poor.

Quality and nutritional determination

The pH of fermented samples was determined using a standard pH meter, following the procedure outlined in Yu et al. (2009). Ammonia nitrogen content in the samples was measured using an ammonia nitrogen kit (Solarbio, Beijing, China), and lactic acid content was determined with a lactic acid kit (Jiancheng, Nanjing, China). Short-chain fatty acid levels were measured with a GC-14B gas chromatograph (Shimadzu, Kyoto, Japan) (Liu et al., 2019). Dry matter content was assessed using the drying method (Shipley and Vu, 2002), while crude protein content was determined using the Kjeldahl method (Sáez-Plaza et al., 2013). Ash content was measured using the dry ashing technique in a muffle furnace (Harris and Marshall, 2017).

Statistical data analysis

The original data were processed in Excel 2008 (Microsoft) and statistically analysed using a one-way ANOVA implemented in SPSS v.18.0 software (SPSS Inc., Chicago, USA). Multiple comparisons between groups were conducted using the LSD method. All results were presented as mean ± standard error of the mean, and were considered significantly different at $P < 0.05$.

Results

Effect of molasses addition on vinegar residue fermentation

The inclusion of molasses lowered the pH value, and increased the concentration of lactic acid (Table 2). There were no significant differences in sensory scores between the groups. The pH values in all three treatment groups fell below 3.96, with a highly significant ($P < 0.001$) reduction observed in the 3M and 4.5M groups compared to control. Lactic acid concentration was expected to increase with the addition of molasses ($P = 0.059$), and the 3M and 4.5M groups showed a very significant raise in this value compared to the control group. The concentration of acetic acid did not significantly differ between the groups. Other short-chain fatty acids, such as propionic acid, butyric acid, and isobutyric acid were detected in some samples collected from the treatment groups. Ash and crude protein contents showed no significant differences across the groups. However, the ash content in the 3M group was lower compared to the control group, and the crude protein content in the 4.5M group was lower than that of the control group.

Table 2. Effects of molasses addition on sensory scores, nutritional content and short chain fatty acid levels in fermented vinegar residue

Index	0M	1.5M	3M	4.5M	*P*-value
Sensory scores	18.00	18.25 ± 0.25	17.75 ± 0.48	18.25 ± 0.25	0.566
pH	4.11 ± 0.01^{A}	3.77 ± 0.02^{B}	3.67 ± 0.01^{C}	3.63 ± 0.01^{C}	<0.001
Lactic acid, mmol/kg	34.90 ± 2.19^{b}	40.08 ± 0.68^{ab}	41.31 ± 1.96^{a}	41.58 ± 1.65^{a}	0.059
Acetic acid, mmol/kg	1.98 ± 0.31	1.65 ± 0.42	0.97 ± 0.31	2.05 ± 0.41	0.237
Butyric acid, mmol/kg	ND	0.01 ± 0.01	0.04 ± 0.04	0.27 ± 0.22	0.279
Propionic acid, mmol/kg	ND	0.02 ± 0.02	0.04 ± 0.02	0.08 ± 0.05	0.470
Isobutyric acid, mmol/kg	ND	ND	0.07 ± 0.07	ND	--
Dry matter (DM), %	37.99 ± 0.19	38.35 ± 0.10	38.04 ± 0.09	38.35 ± 0.22	0.309
Ash, %DM	6.60 ± 0.06	6.64 ± 0.05	6.53 ± 0.02	6.77 ± 0.24	0.597
Crude protein, %DM	10.75 ± 0.15	11.01 ± 0.20	11.07 ± 0.34	10.65 ± 0.33	0.562

Molasses (M) addition levels at 0% (0M group), 1.5% (1.5M group), 3% (3M group), and 4.5% (4.5M group). $P > 0.05$ indicated insignificant differences, $P < 0.05$ indicated significant differences. Results are expressed as mean ± standard error of the mean. ^{ab} and ^{AB} means with different superscripts are significantly different at $P < 0.05$ and $P < 0.01$, respectively; ND – not detected

Overall, the addition of molasses resulted in more favorable pH values and lactic acid content in the fermented vinegar residue. However, the presence of propionic acid, butyric acid, and isobutyric acid could potentially have adverse effects when the fermented vinegar residue is fed to ruminants.

Effect of urea addition on vinegar residue fermentation

Table 3 illustrates that the inclusion of urea had no significant impact on the sensory scores of the fermented vinegar residue ($P > 0.05$). However, the addition of urea effectively increased the ash

Table 3. Effects of urea addition on sensory scores, nutritional content and short chain fatty acid levels in fermented vinegar residue

Index	0U	1.5U	3U	4.5U	*P*-value
Sensory scores	18.00 ± 0.00	17.80 ± 0.25	17.50 ± 1.19	18.0 ± 0.40	0.935
pH	4.11 ± 0.01^{C}	4.54 ± 0.04^{B}	5.80 ± 0.17^{A}	4.36 ± 0.11^{AB}	<0.001
Lactic acid, mmol/kg	39.04 ± 4.88^{B}	37.68 ± 8.31^{B}	26.45 ± 3.51^{B}	68.65 ± 4.52^{A}	<0.001
Acetic acid, mmol/kg	1.98 ± 0.31^{a}	1.44 ± 0.35^{ab}	1.24 ± 0.17^{b}	0.83 ± 0.41^{b}	0.019
Butyric acid, mmol/kg	ND	ND	ND	ND	--
Propionic acid, mmol/kg	ND	ND	ND	ND	--
Isobutyric acid, mmol/kg	ND	ND	ND	ND	--
Ammonia nitrogen, mmol/l	168.65 ± 23.04	180.50 ± 36.61	123.61 ± 25.95	146.95 ± 21.23	0.498
Dry matter (DM), %	37.99 ± 0.19	38.19 ± 0.17	38.40 ± 0.09	37.87 ± 0.20	0.211
Ash, %DM	6.97 ± 0.07^{D}	7.51 ± 0.05^{C}	7.88 ± 0.04^{B}	8.35 ± 0.06^{A}	<0.001
Crude protein, %DM	10.61 ± 0.06^{b}	10.80 ± 0.11^{ab}	10.84 ± 0.12^{ab}	11.16 ± 0.20^{a}	0.068

Urea (U) addition levels ranging from 0% (0U group) to 1.5% (0.5U, 1.0U, and 1.5U groups). $P > 0.05$ indicated insignificant differences, $P < 0.05$ indicated significant differences. Results are expressed as mean ± standard error of the mean. ^{ab} and ^{AB} means with different superscripts are significantly different at $P < 0.05$ and $P<0.01$, respectively; ND – not detected

contents of the fermented vinegar residue ($P < 0.01$). Additionally, it caused a rise in pH value and a decrease in acetic acid concentrations ($P < 0.01$), while also demonstrating a tendency to increase the crude protein content ($P = 0.068$).

The pH values in the treatment groups were significantly higher than those in the control group ($P < 0.05$). The 4.5U group showed a significant increase in lactic acid concentration compared to the control group ($P < 0.05$), whereas the 1.5U and 3U groups exhibited a lower lactic acid concentration as compared to the control group, although the difference was not significant. Moreover, acetic acid levels in all treatment groups were significantly lower compared to the control group ($P < 0.05$) and no other short-chain fatty acids were detected. In addition, no effect of urea addition on the ammonia nitrogen content was observed ($P > 0.05$).

In general, the addition of urea caused an upward trend in the crude protein content and a significant increase in the ash content in the fermented vinegar residue. The 1.5U group exhibited a significant increase in the crude protein and ash contents compared to the control group ($P < 0.05$).

Effect of wheat bran addition on vinegar residue fermentation

The addition of wheat bran led to an improvement in the sensory score ($P < 0.05$), a decrease in the pH value of the fermented vinegar residue ($P < 0.01$), a significant increase in the crude protein content ($P < 0.05$), and a decrease in the dry matter content ($P < 0.05$) (Table 4).

In all three treatment groups, a significant increase in the sensory scores of the fermented vinegar residue ($P < 0.05$) was recorded, with the 15WB group receiving the highest possible sensory score. The pH values in the three wheat bran fermentation groups were significantly lower compared to the control group ($P < 0.05$). There were no significant differences in lactic acid concentration between any of the groups. Meanwhile, acetic acid concentration

Table 4. Effects of wheat bran addition on sensory scores, nutritional content and short chain fatty acid content in fermented vinegar residue

Index	0WB	5WB	10WB	15WB	*P*-value
Sensory scores	18.25 ± 0.25^{B}	19.25 ± 0.25^{A}	19.50 ± 0.29^{A}	20.00 ± 0.00^{A}	0.010
pH	4.03 ± 0.02^{A}	3.51 ± 0.02^{B}	3.51 ± 0.02^{B}	3.51 ± 0.01^{B}	<0.001
Lactic acid, mmol/kg	39.04 ± 4.88	43.09 ± 3.52	36.52 ± 1.63	49.58 ± 2.49	0.080
Acetic acid, mmol/kg	1.98 ± 0.13^{a}	2.03 ± 0.24^{a}	1.95 ± 0.36^{a}	1.03 ± 0.19^{b}	0.039
Butyric acid, mmol/kg	ND	ND	ND	ND	--
Propionic acid, mmol/kg	ND	ND	ND	ND	--
Isobutyric acid, mmol/kg	ND	ND	ND	ND	--
Dry matter (DM), %	37.99 ± 0.19^{a}	37.36 ± 0.22^{b}	37.46 ± 0.07^{b}	37.25 ± 0.10^{b}	0.024
Ash , %DM	6.97 ± 0.07	7.07 ± 0.03	7.09 ± 0.03	7.07 ± 0.05	0.296
Crude protein, %DM	11.21 ± 0.09^{B}	11.59 ± 0.30^{B}	11.90 ± 0.15^{B}	12.48 ± 0.27^{A}	0.010

Wheat bran (WB) addition levels ranging from 0% (0WB group) to 15% (5WB, 10WB, and 15WB groups). $P > 0.05$ indicated insignificant differences, $P < 0.05$ indicated significant differences. Results are expressed as mean ± standard error of the mean. ^{ab} and ^{AB} means with different superscripts are significantly different at $P < 0.05$ and $P < 0.01$, respectively; ND – not detected was significantly lower in the 15WB group compared to the control group ($P < 0.05$), while there was no significant differences between the 5WB and 10WB groups and the control group ($P > 0.05$). No other short-chain fatty acids were detected in any of the samples. The 15WB group had a significantly higher crude protein content compared to the control group ($P < 0.05$).

Overall, the inclusion of wheat bran substantially enhanced the sensory scores, lowered the pH values, and increased the crude protein content. No traces of short-chain fatty acids, which can impair the quality of fermented vinegar residues, other than acetic acid, were found.

Discussion

Vinegar residue is a common organic waste generated by the food industry. Utilising it as animal feed, can address the issue of environmental pollution and provide an additional source of a non-grain feed ingredient. The present findings revealed that the addition of wheat bran could substantially enhance the nutritional value and sensory properties of fermented vinegar residue, suggesting its potential as a valuable resource in animal husbandry. This research also contributes to the advancement of eco-friendly and sustainable feed resources, offering practical solutions for effective utilisation of vinegar residue.

Vinegar residue is not suitable for direct animal consumption due to its low nutrient content. Studies have shown that feeding laying hens with 60 g/kg of vinegar dregs resulted in reduced nitrogen digestibility in their digestive tracts (Song et al., 2012). Similarly, it was observed that direct feeding of vinegar residue to pigs could lead to multiple problems such as constipation, discomfort, and indigestion (Jiang, 2008). However, fermenting vinegar residue with the aid of specific microbial strains was shown to effectively increase its nutrient content (Wang et al., 2022a). The present study indicated that the addition of 4.5% urea and 15% wheat bran to the fermenting mixture significantly increased the crude protein content compared to the control group. Moreover, the pH value of the fermented feed decreased significantly in all treatment groups, except for the urea group. These results collectively demonstrate an improvement in the quality and nutrient composition of the fermented vinegar residue, thereby enhancing their overall feeding value.

Moderate amounts of molasses serve as an essential carbon source for the fermentation system, enhancing feed fermentation, reducing pH levels, and creating an optimal environment for the growth of lactic acid bacteria. The results of this study showed that when the proportion of molasses reached 3%, it resulted in a higher crude protein content of the fermented vinegar residue. Additionally, a decrease in pH values was observed, which was consistent with prior research. It was determined that the addition of 5% molasses caused a significant decrease in pH values, total short-chain fatty acid levels, and the abundance of the bacterial community, while increasing titratable acidity and lactic acid concentration (Singh et al., 1985). Singh et al. (1985) tested three types of roughages (wheat straw, rice straw, and bagasse) in combination with fresh molasses to produce silage, and showed that all three mixtures reached a pH value below 4.5. A study concerning the effects of wilting and molasses addition on Guinea grass silage fermentation (Nishino et al., 2012) demonstrated increased acetic acid levels during silage fermentation (Nishino et al., 2012). The present study indicated that the addition of molasses could be an efficient approach to enhancing the nutritional quality of vinegar residue.

However, the higher proportion (4.5%) of supplemented molasses did not increase CP levels in the fermented feeds. This suggested that the addition of 3% molasses has already provided sufficient carbohydrate levels necessary for lactic acid bacterial fermentation, and additional amounts could not enhance their crude protein production capacity. In fact, it could result in the production of different, potentially undesirable, fermentation products. A previous study suggested that a high concentration of molasses did not lead to a significant increase in organic acid content (Denek et al., 2017). Although short-chain fatty acids are important nutrients in the rumen, they are undesirable in silage fermentation, as they can interfere with lactic acid fermentation (Kung Jr et al., 2018). The addition of molasses resulted in the detection of butyric acid in the fermented vinegar residue in the current study. Although butyric acid is beneficial to animal health, it can exert negative effects on the quality of fermented feeds and the eating habits of animals.

In this study, urea, a common non-protein nitrogenous compound, was observed to increase the crude protein content of the fermented vinegar residue at different levels of addition. When the results were compared with the control group, the group with 4.5% urea addition had a significantly higher crude protein content. Similar results have been previously reported, showing that the nutritional value and *in vitro* rumen fermentation of green forage increased with higher urea concentrations (Phesatcha and Wanapat, 2016). Additionally, higher levels of urea supplementation increased the crude protein content of silage. Previous studies supporting this finding, attributed the increase in crude protein content to the addition of urea during the fermentation process (Yunus et al., 2000; Maeda et al., 2011; Santos et al., 2018). The addition of urea in the preparation of fermented diets in the current study resulted in a significant increase in the crude protein content, and an insignificant decrease in ammonia levels. It is known that the microorganisms present in the rumen require ammonia nitrogen for protein synthesis. A previous study (Firkins et al., 2007) showed that the presence of ammonia at an optimal concentration of 5 mg/dl in rumen fluid promoted the growth of rumen microorganisms. Additionally, research conducted by Boucher et al. (Boucher et al., 2007) indicated that the addition of urea to conventional maize silage diets resulted in an optimal microbial protein synthesis at an average rumen ammonia concentration of 12.8 mg/dl. This optimisation reduced feed protein consumption in the rumen, thereby promoting its more efficient utilisation. Meanwhile, elevated pH levels can facilitate the balancing of organic acid contents in the rumen, thus promoting rumen homeostasis (Elmhadi et al., 2022).

Wheat bran serves as a substrate for fermentation and balances carbon and nitrogen sources during fermentation. The present study demonstrated that the pH value of the fermentation mixture with wheat bran addition was lower compared to the control and molasses groups. This pH reduction can effectively prevent the growth and activity of harmful microorganisms such as spoilage bacteria. Additionally, our findings indicated that the addition of wheat bran caused an increasing trend in lactic acid levels and a significant decrease in acetic acid concentration. The addition of wheat bran was previously found to accelerate the microbial response to anaerobic stress, leading to increased dominance of lactic acid bacteria in the microbial community of woody plant silage (Du et al., 2022). This, in turn, facilitated enhanced lactic acid fermentation of the silage, ultimately improving its flavor and overall quality by influencing specific microbial metabolic pathways. A previous study discovered that silage prepared with varying levels of wheat bran resulted in a significant rise in the degradation rate of hemicellulose and neutral detergent fibre (Wang et al., 2022b). Moreover, the activities of four enzymes, including filter paper enzyme, endoglucanase, acid protease, and neutral protease, also exhibited an increase. Furthermore, it was found that increasing the proportion of wheat bran in silage led to a higher crude protein content in the fermented feed (Kordi and Naserian, 2012; Gül et al., 2019; Gül, 2023). These previous findings suggest that wheat bran is an efficient substrate for enhancing the fermentation quality and nutrition value of silage, which is consistent with the outcomes of the present study.

Conclusions

Based on the results of the experiment, the addition of 3% molasses improved the pH value, lactic acid concentration, and crude protein content of vinegar residue after fermentation. Furthermore, the introduction of 4.5% urea significantly elevated the pH value, lactic acid concentration, as well as ash and crude protein content of the vinegar residue. On the other hand, the addition of 15% wheat bran to the fermentation system improved the sensory scores, pH value, and crude protein content of the vinegar residue. Wheat bran appears to be a better option for enhancing vinegar residue fermentation, surpassing the effectiveness of adding carbon (molasses) and nitrogen (urea) sources separately. This study provides insight into the integration of different additive sources during vinegar residue fermentation, which could be useful in utilising non-cereal feed ingredients for ruminants.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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