

## Influence of sugar beet pulp and wheat bran as silage additives on the chemical composition and *in vitro* gas production of watermelon residue silage

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**ABSTRACT-** This study examined the chemical composition and gas production of watermelon residue (WMR) silage supplemented with sugar beet pulp (SBP) and wheat bran (WB) as additives. After seed removal, the WMR was manually sliced and ensiled either without additives or with 100, 200, or 300 g/kg of SBP or WB. Chemical composition and *in vitro* gas production parameters were then analyzed. The results showed that adding SBP or WB increased the dry matter content and digestibility of WMR silage ( $P < 0.0001$ ). Neutral detergent fiber concentrations rose with higher levels of SBP and WB, while acid detergent fiber concentrations and the potential degradability (a + b) of silages decreased linearly across treatments ( $P < 0.0001$ ). In contrast, WB supplementation significantly reduced metabolizable energy, short-chain fatty acids, and organic matter digestibility ( $P < 0.0001$ ). Gas production increased when WMR silage was treated with 10%, 20%, and 30% SBP and with 10% WB. Overall, these findings indicate that incorporating SBP or WB can improve the nutritional value of WMR silage.

### INTRODUCTION

The incorporation of agricultural by-products into ruminant diets is important for several reasons. First, they provide a cost-effective source of nutrients, as many by-products are readily available at low cost or even free of charge. Second, their use helps reduce agricultural waste and the associated environmental impacts by diverting material from landfills. Finally, by-products allow farmers to adopt more sustainable feeding strategies and reduce their dependence on conventional feeds such as alfalfa hay and silage. Several studies have highlighted the benefits of utilizing agricultural residues in ruminant nutrition (Bayat Kouhsar et al., 2021; Malenica et al., 2022). For example, Romero et al. (2020) reported that incorporating rice straw into dairy goat diets increased milk fat production and improved milk quality. Similarly, Bayat Kouhsar et al. (2021) investigated the potential of biologically treated palm date kernels as a feed resource. These findings demonstrate that agricultural by-products can enhance animal performance while promoting more sustainable agricultural systems. According to statistics from the Iranian Agriculture Organization (2021),

watermelon cultivation covers approximately 63,500 hectares in Iran. Watermelon residue (WMR), an industry by-product, is typically discarded after seed removal for nut production (Fig. 1). However, WMR contains nutrients that make it a promising feed resource (Gladvin et al., 2017). The rind, in particular, is rich in essential vitamins such as vitamin C and vitamin A, as well as minerals including phosphorus and calcium (Egbonu, 2015; Gladvin et al., 2017). Although several studies have explored the potential benefits of WMR in animal diets, limited research has directly evaluated its effects on ruminant performance under live feeding conditions. Agbana et al. (2022), however, reported that ensiling watermelon rinds increased crude protein (CP) and crude fiber contents while reducing anti-nutritional factors, thereby improving its overall feed value. Various methods are available to enhance the nutritional quality of agricultural by-products for animal feeding. Among these, ensiling—a process of preserving forage—can be particularly effective. The addition of silage additives has been shown to improve fermentation quality, leading to higher nutritional value and, ultimately, better animal performance (Erukainure et al., 2010; Muck et al., 2018).

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**Fig. 1.** A variety of watermelon cultivated primarily for its large, edible seeds, with the pulp being less commonly consumed, and Manual separation of watermelon seeds from the pulp.

Absorbents are a class of silage additives that reduce moisture content by absorbing water. When added to forages with low dry matter (DM) content, they can enhance silage quality and minimize nutrient loss during the ensiling process (Kordi & Naserian, 2020). Wheat bran (WB), a by-product of the milling industry, is valued for its high protein and fiber content, making it a useful feed ingredient. Sugar beet pulp (SBP), a by-product of sugar extraction, is rich in digestible fiber and energy, providing strong nutritional support for ruminants. Both WB and SBP can improve the nutritional quality of low-protein silages, although the optimal additive-to-feedstock ratio depends on specific conditions (Muck et al., 2018). Because watermelon residue (WMR) has a relatively low DM concentration and is difficult to ensile, WB and SBP were used in this study to increase absorbability and improve preservation. While silage additives such as SBP and WB have been widely studied for their ability to enhance the nutritional value of various agricultural by-products, little is known about their effects on WMR silage. To date, no studies have evaluated the nutritional value or chemical composition of WMR silage treated with WB or SBP. We hypothesized that supplementing WMR silage with WB and SBP would improve fiber digestibility and fermentation characteristics. Therefore, the objective of this study was to investigate the effects of different inclusion levels of WB and SBP on the chemical composition, digestibility, *in vitro* gas production, and fermentation characteristics of WMR silage.

## MATERIALS AND METHODS

### Chemicals and treatments

This experiment was conducted using a completely randomized design with three replications. Fresh watermelons were collected from local farms in Golestan Province, located in northeastern Iran. After separating the

large red seeds (commonly consumed as nuts) (Fig. 1), the remaining residue, including the juicy and fleshy portions, was manually chopped into pieces of 4–5 cm in length. The chopped watermelon residue (WMR) was either left untreated or supplemented with sugar beet pulp (SBP) or wheat bran (WB) at inclusion levels of 100, 200, or 300 g/kg. Each treatment was ensiled in micro-silos, which were tightly packed with approximately 3 kg of untreated or treated WMR and then sealed. The silos were stored in the dark at room temperature (22 °C) for 60 days. At the end of the ensiling period, the silos were opened, and the contents were thoroughly mixed before sampling for chemical analysis.

### Chemical analysis

The chemical composition of each sample was analyzed in five replicates. Proximate analysis was conducted to determine DM, CP, and ash content following the procedures of AOAC (2005). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were measured using the standard methods described by Van Soest et al. (1991).

The equations below were utilized to calculate the total digestible nutrients (TDN), growth net energy (NE<sub>g</sub>), and lactation net energy (NE<sub>l</sub>; NRC 2001).

$$\text{TDN} = (\text{CP} \times 0.36) - (\text{ADF} \times 0.77) + 81.38 \quad \text{Eq. (1)}$$

$$\text{NE}_g = (0.029 \times \text{TDN}) - 1.01 \quad \text{Eq. (2)}$$

$$\text{NE}_l = (0.0245 \times \text{TDN}) - 0.12 \quad \text{Eq. (3)}$$

### *In vitro* gas production test

Through the *in vitro* gas production experiment, untreated WMR silage (control) and WMR silage treated

with SBP or WB were evaluated as treatments. Gas production was measured using the Menke technique (Menke, 1988). Rumen fluid was collected from three rumen-fistulated male Dalagh sheep ( $45 \pm 2.5$  kg) prior to their morning feeding. The animals were fed a diet consisting of 30% concentrates (cottonseed meal, bran, barley, and supplement) and 70% forage (equal proportions of corn silage and alfalfa) and had free access to water. The incubation medium was prepared by mixing artificial saliva and rumen fluid at a ratio of 2:1. Aliquots of 0.2 g from each treatment (treated or control) were placed into 30 mL serum bottles. Each bottle was flushed with carbon dioxide for 10 seconds, sealed with a rubber stopper and aluminum cap, and then incubated in a shaking water bath at 39 °C for 2, 4, 6, 8, 12, 24, 36, 48, and 96 hours. Cumulative gas production and gas production parameters were determined according to the method of Orskov and McDonald (1979).

$$y = b (1 - e^{-ct}) \quad \text{Eq. (4)}$$

where:

y: Volume of gas produced during incubation

b: Fermentable insoluble fraction produces gas.

c: The b portion's gas production rates

t: Incubation time

e: Euler's number

The organic matter digestibility (OMD), short-chain fatty acids (SCFA), and metabolizable energy (ME) values were computed using the subsequent equations (Makar 2004; Menke et al. 1979).

$$\text{OMD (\%)} = 0.889 \text{ GP} + 14.88 + 0.0651 \text{ XA} + 0.45 \text{ CP} \quad \text{Eq. (5)}$$

$$\text{SCFA (mmol)} = 0.0222 \text{ GP} - 0.00425 \quad \text{Eq. (6)}$$

$$\text{ME (MJ/kg DM)} = 0.136 \text{ GP} + 2.2 + 0.0029 \text{ CF} + 0.057 \text{ CP} \quad \text{Eq. (7)}$$

where:

GP: Net gas production (per 200 mg DM after 24 hours)

XA: Ash (%)

CF: Crude fiber (%)

CP: Crude protein (%)

The analysis of the data was conducted utilizing a completely randomized design within the SAS statistical program (version 9.1; SAS, 2003).

#### DM and OMD

This experiment followed the same procedures for rumen fluid preparation, basal diet, and treatments as described for the gas production test. The batch culture technique was used to evaluate *in vitro* DM and OMD digestibility, following the method of Theodorou et al. (1994). Briefly, 0.5 g DM samples were placed into glass vials, each of which was filled with 50 mL of an incubation medium consisting of rumen fluid and artificial saliva in a 1:2 ratio. The pH of the medium was adjusted to 6.8 with buffer. Vials were then sealed with plastic caps and aluminum foil and incubated in a water bath at 39 °C for 24 hours. After incubation, microbial activity was halted by immersing the vials in cold water. The pH of the contents was measured using a pH meter (Metrohm Company, Model 691). The contents were then filtered through polyester cloth (42-μm pore size) to separate undigested particles. For ammonia nitrogen analysis, 5

mL of the filtrate was mixed with an equal volume of 0.2 N hydrochloric acid and stored at -20 °C. Ammonia nitrogen concentration was determined using the phenol-hypochlorite method (Broderick and Kang, 1980). Dry matter disappearance was calculated by drying the undigested residues at 60 °C for 48 hours, followed by ashing at 540 °C for 6 hours to determine OM concentration. Gas pressure was recorded at 2, 4, 6, 8, 10, 12, and 24 hours of incubation using a pressure gauge, after which the accumulated gas was released. The efficiency of gas production was calculated according to the equation described by Getachew et al. (2004).

$$\text{Efficiency of gas production (G}_y\text{)} = \text{GP}_{24} / (0.5 - \text{DM weight following oven drying}) \quad \text{Eq. (8)}$$

where:

GP<sub>24</sub> = the gas produced after 24 hours

The subsequent equation was employed to compute microbial biomass production (Blummel et al. 1997).

$$\text{Microbial mass production (MCP; mg)} = (\text{PF} \times \text{GP}) - 2.2 \quad \text{Eq. (9)}$$

where:

PF = Partitioning factor [the amount of digested OM (mg) / mL of gas produced].

GP = Pure produced gas after 24 h (mL)

The microbial protein efficiency was calculated using the following equation:

$$\text{Efficiency of microbial mass production} = \text{MCP} / \text{disappeared OM} \quad \text{Eq. (10)}$$

A completely randomized design was utilized to analyze the data through the General Linear Model (GLM) procedure in SAS statistical software, version 9.1 (SAS, 2003).

#### Statistical analysis

Data were analyzed using a completely randomized design. Gas production data were analyzed via ANOVA using the General Linear Model (GLM) procedure, while all other data were analyzed using the MIXED procedure in SAS (2003). The least-square differences (LSD) test was employed to compare the means. The model used for the analysis was:

$$Y_{ij} = \mu + T_i + e_{ij} \quad \text{Eq. (11)}$$

where  $\mu$  represents the overall mean,  $T_i$  denotes the effect of the treatments, and  $e_{ij}$  signifies the random error. Statistical significance of linear and quadratic contrasts is reported.

**Table 1.** Effect of adding sugar beet pulp and wheat bran on the chemical composition of watermelon rind (% , DM)

Treatment	DM	OM	Ash	CP	ADF	NDF	HEMI	pH	NH <sub>3</sub> -N	TDN	NE <sub>l</sub>	NE <sub>g</sub>
Control	12.73 <sup>d</sup>	87.00 <sup>f</sup>	13.00 <sup>a</sup>	11.99 <sup>d</sup>	36.00 <sup>a</sup>	49.00 <sup>b</sup>	13.00 <sup>d</sup>	4.40 <sup>a</sup>	4.23 <sup>ab</sup>	57.98 <sup>d</sup>	1.27 <sup>d</sup>	0.67 <sup>d</sup>
Sugar beet pulp (10%)	17.97 <sup>c</sup>	89.00 <sup>e</sup>	11.00 <sup>b</sup>	11.03 <sup>e</sup>	28.00 <sup>b</sup>	42.22 <sup>d</sup>	14.33 <sup>d</sup>	4.15 <sup>c</sup>	2.86 <sup>bc</sup>	63.79 <sup>c</sup>	1.41 <sup>c</sup>	0.84 <sup>c</sup>
Sugar beet pulp (20%)	25.53 <sup>ab</sup>	90.33 <sup>d</sup>	9.67 <sup>e</sup>	10.59 <sup>f</sup>	27.00 <sup>b</sup>	45.00 <sup>c</sup>	18.00 <sup>c</sup>	4.12 <sup>cd</sup>	2.75 <sup>bc</sup>	64.40 <sup>c</sup>	1.42 <sup>c</sup>	0.85 <sup>c</sup>
Sugar beet pulp (30%)	29.85 <sup>a</sup>	90.78 <sup>c</sup>	9.22 <sup>d</sup>	11.82 <sup>d</sup>	27.00 <sup>b</sup>	48.00 <sup>b</sup>	21.00 <sup>c</sup>	4.06 <sup>d</sup>	1.43 <sup>c</sup>	64.84 <sup>c</sup>	1.42 <sup>c</sup>	0.87 <sup>c</sup>
Wheat bran (10%)	21.40 <sup>bc</sup>	90.22 <sup>d</sup>	9.78 <sup>e</sup>	13.74 <sup>c</sup>	28.00 <sup>b</sup>	49.00 <sup>b</sup>	21.00 <sup>c</sup>	4.28 <sup>b</sup>	4.24 <sup>ab</sup>	64.76 <sup>c</sup>	1.43 <sup>c</sup>	0.87 <sup>c</sup>
Wheat bran (20%)	26.20 <sup>a</sup>	92.23 <sup>b</sup>	9.67 <sup>e</sup>	14.88 <sup>b</sup>	20.00 <sup>d</sup>	52.00 <sup>a</sup>	32.00 <sup>a</sup>	4.30 <sup>b</sup>	4.66 <sup>ab</sup>	71.34 <sup>a</sup>	1.59 <sup>a</sup>	1.05 <sup>a</sup>
Wheat bran (30%)	29.78 <sup>a</sup>	92.67 <sup>a</sup>	7.33 <sup>f</sup>	16.63 <sup>a</sup>	24.00 <sup>c</sup>	49.00 <sup>b</sup>	25.00 <sup>b</sup>	4.33 <sup>b</sup>	6.14 <sup>a</sup>	68.88 <sup>b</sup>	1.53 <sup>b</sup>	0.99 <sup>b</sup>
SE	1.49	0.058	0.058	0.142	0.815	0.826	1.02	0.020	0.895	0.606	0.014	0.017
P-value	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.0001	0.046	≤ 0.0001	≤ 0.0001	≤ 0.0001

DM: Dry matter (%); OM: Organic matter (%); Ash (%); CP: Crude protein (%); ADF: Acid detergent fiber (%); NDF: Neutral detergent fiber (%); HEMI: Hemicellulose (%); Ammonia nitrogen (mg/dL); TDN: Total digestible nutrients (%); NE<sub>L</sub>: Net energy for lactating (Mj/Kg); NE<sub>g</sub>: Net energy for growth (Mj/Kg); SE: Standard error of mean.

In each column, numbers with non-identical alphanumeric characters are statistically different ( $P < 0.05$ ).



**Table 2.** Effect of adding sugar beet pulp and wheat bran on watermelon rind gas production parameters

Treatment	a + b	C	ME	OMD	SCFA
Control	262.9±7.15	0.0391±0.0027	6.78 <sup>a*</sup>	46.94 <sup>a</sup>	0.743 <sup>a</sup>
Sugar beet pulp (10%)	243.1±5.72	0.0536±0.0036	7.12 <sup>a</sup>	49.20 <sup>a</sup>	0.798 <sup>a</sup>
Sugar beet pulp (20%)	251.1±3.40	0.0514±0.0019	7.26 <sup>a</sup>	50.13 <sup>a</sup>	0.821 <sup>a</sup>
Sugar beet pulp (30%)	242.4±3.47	0.0445±0.0017	6.83 <sup>a</sup>	47.30 <sup>a</sup>	0.752 <sup>a</sup>
Wheat bran (10%)	256.0±5.50	0.0504±0.0030	7.02 <sup>a</sup>	48.53 <sup>a</sup>	0.782 <sup>a</sup>
Wheat bran (20%)	212.5±5.68	0.0382±0.0026	5.78 <sup>b</sup>	40.25 <sup>b</sup>	0.578 <sup>b</sup>
Wheat bran (30%)	220.9±8.57	0.0342±0.0032	5.74 <sup>b</sup>	39.98 <sup>b</sup>	0.572 <sup>b</sup>
SE	-	-	0.181	1.20	0.0296
P-value	-	-	≤ 0.0001	≤ 0.0001	≤ 0.0001

(a + b): Gas production potential (mL/gDM); C: Gas production rate (mL/h); ME: Metabolizable energy (MJ/kg); OMD: Organic matter digestibility (% DM); SCFA: Short chain fatty acids (mmol); SE: Standard error of the mean.

\* Within each column, means with common superscripts do not differ ( $P > 0.05$ ).

## RESULTS AND DISCUSSION

### Chemical composition

The addition of SBP and WB significantly influenced the chemical composition of WMR silage, affecting parameters such as DM, OM, ash, CP, ADF, NDF, hemicellulose, pH,  $\text{NH}_3\text{-N}$ , TDN, NEL, and NEg, with a significance level of  $P < 0.0001$  (Table 1). The inclusion of WB and SBP resulted in significant increases in DM, OM, CP, hemicellulose, TDN, NEL, and NEg compared with the control. Notably, CP content increased with WB supplementation and rose further as the proportion of WB increased ( $P < 0.0001$ ). Among the treatments, WB at 30% produced the highest CP concentration (16.63%), whereas SBP at 20% yielded the lowest (10.59%). Across all treatments, WMR silage DM and OM contents increased (Table 1), and, as expected, higher proportions of SBP and WB further enhanced these values. Since silage quality is largely determined by DM content, which directly affects its nutritional value and palatability, these increases are particularly relevant. Previous studies have shown that supplementing silage with SBP or WB during ensiling can raise DM content by 10–15%, thereby improving overall quality (Gul, 2023; Kordi & Naserian, 2021; Kung et al., 2018). Similarly, Wang et al. (2022) reported increased DM content in corn stover silage following SBP supplementation. This improvement is likely attributable to the fibrous structure of these additives, which enhances moisture absorption and reduces overall silage moisture content when WB was added to alfalfa silage. In contrast, Kordi and Naserian (2021) found that adding three levels of barley grain (6, 12, and 18 g/kg) to citrus pulp silage did not affect ash content. Since high ash content reduces digestibility and limits nutrient availability to animals, the observed reduction in ash content following SBP or WB supplementation is considered beneficial. The CP content of WMR silage increased with WB supplementation, and this increase was directly proportional to the level of WB added. For instance, incorporating 30% WB raised CP by 38%, from 11.99% to 16.63%. Similar effects have been reported in other feedstocks: Gul (2023) found that adding 10% WB to

Caramba mix silage increased CP from 14.58% to 16.23%, while Kordi and Naserian (2021) observed higher CP levels in citrus pulp silage after barley grain supplementation. This rise in CP content can be attributed to the naturally high protein concentration of WB, which provides readily available nitrogen for ensiling microorganisms, thereby enhancing microbial protein synthesis during fermentation (Gul, 2023).

Additionally, SBP and WB contain soluble fibers such as pectin and hemicellulose, which can reduce the moisture content of silage by improving the water-holding capacity of the ensiled material (Kung et al., 2018). The resulting increase in DM content enhances fermentation and preservation, as lower moisture levels inhibit the growth of undesirable microorganisms while favoring lactic acid bacteria responsible for the fermentation process (Joanna et al., 2018). Supplementation with SBP and WB also significantly reduced the ash content of WMR silage. Similarly, Wang et al. (2022) observed decreased ash content in corn stover silage supplemented with SBP, while Kung et al. (2018) documented a reduction in ash.

The NDF content of WMR silage ranged from 42.22% to 52%. The treatment with 20% WB exhibited the highest NDF value, while 10% SBP supplementation resulted in the lowest ( $P < 0.0001$ ). Overall, the addition of WB and SBP decreased ADF and increased NDF levels when applied at 20% and 30%. These findings align with previous research reporting similar effects of WB and SBP on silage fiber composition (Gul, 2023). Likewise, Kordi and Naserian (2021) observed that adding barley grain to citrus pulp silage increased both NDF and ADF concentrations. The high fiber content of WB and SBP supports the proliferation of lactic acid bacteria during ensiling, which lowers pH and improves silage digestibility. As observed in this study, the increased activity of lactic acid bacteria was associated with a reduction in ADF and an increase in NDF content. Supplementation with WB and SBP also significantly reduced the pH of WMR silage (Table 1). The pH values ranged from 4.06 in the 30% SBP treatment to 4.40 in the control. Silage pH is a critical determinant of its quality and stability. A lower pH reflects higher acidity, which inhibits spoilage microorganisms and preserves

nutritional quality, whereas higher pH values encourage the growth of undesirable bacteria and toxins, ultimately reducing both nutritional value and palatability. Although no published studies have directly investigated the effect of SBP or WB on WMR silage, previous reports have shown that supplementation with WB, SBP, and other additives can improve fermentation quality by lowering pH. For instance, Kordi and Naserian (2021) found that adding barley grain to citrus pulp silage reduced pH and increased lactic acid concentration.

Similarly, Cao et al. (2011) reported that supplementing silage with SBP reduced pH and increased lactic acid production. This supplementation also prevented the accumulation of ammonia nitrogen and acetic acid during fermentation, thereby improving the preservation of vegetable residues in four types of vegetable silage. The reduction in pH observed in the present study can be attributed to the high fermentable carbohydrate content of WB and SBP, which provides a substrate for lactic acid bacteria. As these bacteria ferment the carbohydrates, they produce lactic acid, lowering silage pH and creating an acidic environment that suppresses the growth of undesirable

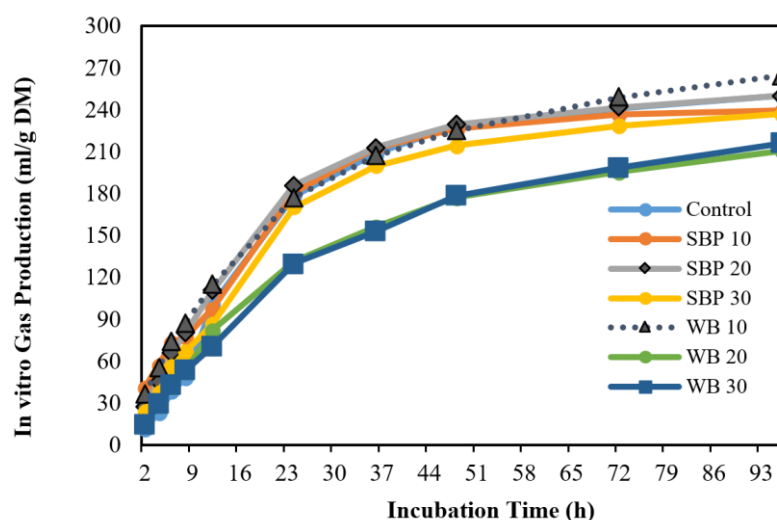
microorganisms (Cao et al., 2011; Paya et al., 2015). Interestingly, WB and SBP exerted opposite effects on the ammonia nitrogen content of WMR silage when added at 30%. WB supplementation significantly increased ammonia nitrogen levels (by more than 40%), whereas SBP supplementation significantly decreased them ( $P < 0.0001$ ). Cao et al. (2011) also reported that adding SBP to vegetable silage raised ammonia nitrogen concentrations, consistent with our findings. In contrast, Kordi et al. (2014) found that adding SBP to citrus pulp silage led to a linear increase in both ammonia nitrogen concentration and pH compared with the control. Similarly, Kordi and Naserian (2021) observed that barley grain supplementation in citrus pulp silage had no effect on ammonia nitrogen content. Ammonia nitrogen is a by-product of protein degradation during ensiling. Elevated concentrations typically indicate poor fermentation quality, as they reflect inadequate preservation of protein. Conversely, low ammonia nitrogen levels are desirable, as they suggest effective protein preservation and a higher nutritional quality of the silage.

**Table 3.** Effect of adding sugar beet pulp and wheat bran on the digestibility and fermentation parameters of watermelon rind

Treatment	DMD	OMD	PF	MCP	EMCP	Gas yield 24
Control	67.50 <sup>c*</sup>	64.75 <sup>d</sup>	4.80 <sup>ab</sup>	152.43 <sup>b</sup>	0.532 <sup>b</sup>	176.37 <sup>c</sup>
Sugar beet pulp (10%)	78.00 <sup>b</sup>	77.25 <sup>ab</sup>	4.66 <sup>ab</sup>	180.64 <sup>ab</sup>	0.527 <sup>ab</sup>	188.91 <sup>bc</sup>
Sugar beet pulp (20%)	81.00 <sup>ab</sup>	80.50 <sup>a</sup>	4.26 <sup>b</sup>	176.07 <sup>ab</sup>	0.482 <sup>b</sup>	211.16 <sup>a</sup>
Sugar beet pulp (30%)	85.00 <sup>a</sup>	84.25 <sup>a</sup>	4.34 <sup>b</sup>	186.77 <sup>ab</sup>	0.492 <sup>b</sup>	207.40 <sup>ab</sup>
Wheat bran (10%)	70.00 <sup>c</sup>	69.25 <sup>cd</sup>	4.54 <sup>ab</sup>	160.44 <sup>ab</sup>	0.512 <sup>ab</sup>	196.50 <sup>ab</sup>
Wheat bran (20%)	70.00 <sup>c</sup>	73.00 <sup>bc</sup>	5.13 <sup>a</sup>	191.75 <sup>a</sup>	0.565 <sup>a</sup>	188.84 <sup>bc</sup>
Wheat bran (30%)	67.00 <sup>c</sup>	65.25 <sup>d</sup>	4.69 <sup>ab</sup>	161.16 <sup>ab</sup>	0.530 <sup>ab</sup>	193.04 <sup>abc</sup>
SE	0.018	0.024	0.224	12.125	0.020	6.67
P-value	$\leq 0.0001$	$\leq 0.0001$	0.0173	0.0224	0.0137	0.0230

DMD: Dry matter digestibility (%); OMD: Organic matter digestibility (%); PF: Partitioning factor (mg OM truly degraded/mL gas produced in 24 h); MCP: Microbial crude protein (mg); EMCP: Efficiency of Microbial crude protein; Gas yield 24: The amount of gas production after 24 hours of incubation (mL); N-NH<sub>3</sub>: SEM: Standard error of the mean.

\* Within each column, means with common superscripts do not differ ( $P > 0.05$ ).



**Fig. 2.** Effect of adding sugar beet pulp (SBP) and wheat bran (WB) on the *in vitro* gas production of watermelon residue silage.

During ensiling, carbohydrate fermentation lowers silage pH and helps preserve nutrients. However, the availability of fermentable carbohydrates also influences the synchronization between protein degradation and fermentation, thereby affecting the concentration of ammonia nitrogen in the final silage. Thus, adding fermentable carbohydrates can increase ammonia nitrogen levels due to the enhanced proteolysis and amino acid deamination. In contrast, incorporating fibrous materials such as WB increases the buffering capacity of the silage, which slows the decline in pH and reduces proteolysis, ultimately resulting in higher ammonia nitrogen concentrations. Thus, the type of carbohydrate source plays a critical role in shaping both the fermentation process and the nutrient profile of silage. To maximize silage quality, careful selection and management of forage additives are essential (Kung & Shaver, 2001). SBP, with its high water-soluble carbohydrate (WSC) content, has been shown to improve fermentation quality, reduce fermentation losses, and influence ruminal digestion properties. In the present study, supplementation of WMR silage with SBP and WB significantly increased TDN and net energy ( $P < 0.0001$ ). These results are consistent with earlier findings that reported positive effects of SBP and WB supplementation on silage nutritional quality (Kung et al., 2018; Zhao et al., 2019). The improvement can be attributed to the greater availability of fermentable carbohydrates in the supplemented silage, which enhances TDN and energy content. Rumen bacteria utilize these carbohydrates to produce volatile fatty acids, an important energy source for ruminants (Kung et al., 2018).

#### *Gas production parameters and digestibility*

Table 2 summarizes the effects of WB and SBP supplementation on the gas production parameters of WMR silage. Overall, all treatments significantly reduced gas production potential, with the greatest reductions observed at 20% and 30% WB. At these levels, WB supplementation also significantly decreased ME, OMD, and SCFA. Compared with the control (WMR without additives), WB and SBP treatments showed a marked reduction in gas production potential (Table 2). In contrast, supplementation with 10%, 20%, and 30% SBP, as well as 10% WB, led to higher gas production relative to the control (Fig. 2), suggesting that silage additives can enhance *in vitro* ruminal gas production. The gas production rate (c) was also higher in these treatments compared with the control. These findings align with Kordi and Naserian (2021), who reported that barley grain supplementation decreased the potential degradability of citrus pulp silage but increased its degradability rate. Similarly, Xia et al. (2018) observed reduced gas production in molasses-treated wheat silage. Other studies also support these results: WB addition increased gas production in citrus pulp silage (Kordi & Naserian, 2014), SBP supplementation had a similar effect (Kordi et al., 2014), and Zhao et al. (2019) reported increased gas production with lactic acid bacteria and molasses addition to rice straw silage. In addition to gas production, WB and SBP

supplementation significantly affected DMD, OMD, partitioning factor (PF), microbial CP (MCP), efficiency of MCP (EMCP), and gas yield after 24 h of incubation ( $P < 0.05$ ; Table 3). SBP supplementation improved both DMD and OMD compared with the control at all levels, while WB increased OMD only at 20% ( $P < 0.0001$ ). The highest DMD value (85.00%) was recorded in the 30% SBP treatment. These findings are consistent with Gul (2023), who observed improved DMD when WB was added to Caramba mix silage, and with Kordi et al. (2014), who reported that SBP addition to citrus pulp silage enhanced DM and OM digestibility as well as ME content. The observed increase in OMD in WMR silage with WB or SBP supplementation can likely be attributed to their high fiber content and the associated increase in silage DM content.

## CONCLUSION

This study demonstrated that supplementing watermelon residue silage with sugar beet pulp and wheat bran can significantly enhance its nutritional quality across multiple parameters. Additive inclusion increased total digestible nutrients, energy, protein, NDF, organic matter, and DM digestibility. These results indicate that sugar beet pulp and wheat bran are effective silage additives and may serve as practical strategies for improving the nutritional value of silages produced from other by-products with low DM content. Such improvements can contribute to more sustainable and efficient livestock production systems by reducing feed costs while enhancing animal health and performance. Further research is warranted to determine the optimal combinations and proportions of sugar beet pulp and wheat bran under varying forage types and storage conditions. Overall, watermelon residue exhibits high nutritive value and *in vitro* DM digestibility, and its fermentation quality can be further improved through moisture adjustment with sugar beet pulp or wheat bran.

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## CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Reza Mirshahi and Javad Bayatkouhsar; Methodology: Javad Bayatkouhsar; Software: Farzad Ghanbari; Validation: Javad Bayatkouhsar, Farkhondeh Rezaii, and Farid Moslemipoor; Formal analysis: Javad Bayatkouhsar; Investigation: Farkhondeh Rezaii; Resources: Farkhondeh Rezaii; Data curation: Javad Bayatkouhsar; Writing—original draft preparation: Farkhondeh Rezaii; Writing—review and editing: Farkhondeh Rezaii; Visualization: Farkhondeh Rezaii; Supervision: Javad Bayatkouhsar; Project administration: Javad Bayatkouhsar and Farkhondeh Rezaii; Funding acquisition: Javad Bayatkouhsar.

## DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

## DATA AVAILABILITY

All data that support the findings of this study are included within the article.

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