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NUTRIENT COMPOSITION, FERMENTATION QUALITY, *in vitro* DEGRADABILITY, AND METHANE EMISSIONS OF WHOLE-CROP CORN SILAGES TREATED WITH ARBUSCULAR MYCORRHIZAL FUNGI BIOFERTILIZERS DURING PLANTING

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ABSTRACT

Silage fermentation quality and nutrient composition are significantly influenced by the raw material used before ensiling, including agronomic practices such as fertilizer application. This study evaluated the effects of different levels of Arbuscular Mycorrhizal Fungi (AMF) biofertilizer applied during corn cultivation on nutrient composition, silage fermentation characteristics, *in vitro* rumen fermentation dynamics, and methane emissions. Corn plants were harvested 82 days post-planting, cut 10 cm above ground level, and manually chopped into pieces of 3–5 cm in length. The chopped forage was packed into 10-liter silos, compacted to expel air, and sealed to ensure anaerobic conditions. All samples were ensiled for 30 days. A completely randomized design was employed with three AMF biofertilizer levels (0, 10, and 20 g per planting hole) and six replications. Data were analyzed using ANOVA followed by Tukey's test for multiple comparisons. The results indicated that AMF application had a limited effect on nutrient composition but significantly increased ($P < 0.05$) water-soluble carbohydrate (WSC) content, which is crucial for efficient fermentation. All silages were well preserved, as evidenced by a pH below 4. Enhanced *in vitro* rumen fermentation, indicated by increased total short-chain fatty acid (TSCFA) production, higher gas output, and a slight improvement in degradability, was associated with higher WSC content. Elevated WSC levels also contributed to reduced methane emissions by promoting more efficient microbial fermentation.

Keywords: Environmental sustainability, nutrient content, silage, whole-crop corn.

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INTRODUCTION

Arbuscular Mycorrhizal Fungi (AMF) function as a natural biofertilizer, forming a symbiotic relationship with plant roots (Zhu et al., 2022; Khaliq et al., 2022). This mutual interaction benefits plants by supplying water, nutrients, and pathogen protection, while the fungi receive photosynthetic products from the host (Carrara and Heller 2022; Berruti et al., 2016). AMF relies on photosynthetic products from its host plant for growth and development (Wahab et al., 2023). Plant roots associated with AMF have a greater absorption capacity due to the extended network of AMF hyphae, which are smaller and finer than roots, allowing them to access soil pores more effectively (Berger and Gutjahr, 2021). This enhances water and nutrient uptake, particularly phosphorus (Wahab et al., 2023), and improves soil structure by producing specific organic acids and compounds (Fall et al., 2022). Additionally, AMF can boost plant resistance to root pathogens (Weng et al., 2022).

Whole-crop corn is considered a high-quality forage due to its balanced nutrient composition, including crude protein (CP) around 7 to 9% of dry matter (DM), neutral detergent fiber (NDF) ranging from 40 to 50% of DM, and starch content between 25 and 35% of DM (Dong et al., 2022a). It is also an ideal silage material because of its high water-soluble carbohydrate (WSC) content, which typically ranges from 8 to 12% of DM, supporting an efficient fermentation process, along with its optimal DM content and degradability (Pang et al., 2024).

Feeding animals whole-crop corn silage increases milk productivity and body weight performance and reduces the use of concentrate feed (Krämer-Schmid et al., 2016; Zhang et al., 2019). Whole-crop corn used as forage is usually harvested at the optimal age at the beginning of the dent phase (Zaralis et al., 2014). This will affect the nutritional quality (Horst et al., 2020) and the characteristics of the material (Garcia, 2019).

The use of AMF as a biological fertilizer supports the sustainability of agricultural land by enhancing water absorption and plant nutrient uptake (Varghese and Ray, 2023). Püschel et al. (2023) demonstrated that AMF can enhance water absorption through hyphal networks, with AMF hyphae increasing water uptake in *Medicago truncatula* by more than twofold compared to non-mycorrhizal plants. These improvements in water absorption can influence the nutrient content of host plants, including corn.

The effect of AMF addition at planting on corn yield or biomass production has been widely documented in the literature (Husein et al., 2022; Stoffel et al., 2020; Santana et al., 2023). However, the influence of AMF on the nutrient composition of forage corn, silage fermentation quality, and *in vitro* rumen fermentation dynamics remains insufficiently explored. Therefore, this study aimed to evaluate the impact of different AMF application levels during planting on the nutrient profile of corn crops, silage fermentation characteristics, and *in vitro* rumen fermentation parameters, including degradability, gas production, and methane emissions in whole-crop corn forage.

MATERIALS AND METHODS

Sample preparation

The corn crop was cultivated in Ngadiluwih, Kediri, East Java, Indonesia (7.83333°S, 112.16667°E). During the November-December planting period, the region experienced a mean temperature of 28°C, with daily highs of 32 °C and lows of 24 °C. Precipitation was 201.1 mm, while relative humidity averaged 82%, providing favorable conditions for crop growth. The predominant soil type in the area is Andosol, a fertile volcanic-derived soil rich in organic matter and minerals, which enhances nutrient availability and water retention, making it highly suitable for agriculture.

The experimental plots measured 1.2 × 4.2 m each, with 36 individual plants per plot. AMF inoculation and seed planting were performed in the same hole at a depth of 5-10 cm below the soil surface, with one plant per hole. The planting distance was 70 × 20 cm. No irrigation was applied, but rainfall occurred on 15 days between 35 and 56 days after planting, with intensities ranging from light to heavy.

Corn seeds (*Zea mays* Nasa 29) were used, and NPK compound fertilizer (Phonsa 15) was applied as a basal fertilizer at a rate of 400 kg ha^{-1}. The AMF inoculant, containing *Gigaspora* sp., *Acaulospora* sp., and *Glomus* sp., was obtained from the Agrostology Laboratory, Faculty of Animal Science, IPB University, Bogor (Indonesia). The AMF inoculant was applied directly to the planting hole alongside the corn seeds at sowing, following the method of Karti et al. (2021), at doses of 0, 10, and 20 g per planting hole, with six replications.

Corn plants were harvested 82 days after planting by cutting at 10 cm above the ground level. The whole crop was manually chopped into pieces approximately 3-5 cm in length and packed into 10-liter polyethylene bucket silos. Each treatment had six replicates. The chopped forage was compacted to expel air and sealed tightly to maintain anaerobic conditions. The silos

were then stored at ambient room temperature (25-27 °C) for 30 days to allow for fermentation.

The flow of AMF biofertilizer application on corn plants for silage production is shown in Fig. 1.

Chemical analysis

Dry materials of whole-crop corn and silages were prepared using the oven method. In contrast, the soluble extract was prepared by immersing 40 g of fresh silage sample in 400 mL of distilled water as described by Ridla and Uchida (1998).

The conventional chemical assessment was applied to determine the silage fermentation quality, such as pH value by a pH meter (HANNA HI98190), nutrient content (crude ash, crude protein, ether extract, crude fiber, and N-free extract) by AOAC (2015) method, WSC by spectrophotometer method using anthrone solution as described by Deriaz (1961), total volatile basic nitrogen (TVBN) by steam distillation method, and DM recovery by oven method. To determine the quality of corn silage using pH and DM, the equation calculated the Flieg point: Flieg point = 220 + (2 × % DM silage - 15) - 40 × pH (Kilic, 1986).

***In vitro* fermentation technique**

Rumen fluid was sourced from three Ongole Crossbred cattle at the IPB University (Indonesia) slaughterhouse at 3:00 AM, with the cattle maintained on a diet of 40% forage and 60% concentrate. To preserve anaerobic conditions and ensure microbial viability, the rumen fluid was immediately placed in pre-warmed (40 °C) insulated thermos bottles (Ridla and Nahrowi, 2025). All collection, handling, and slaughter procedures complied with Indonesian animal welfare and health regulations (Government Regulation No. 41/2014; Law No. 39/2021).

Forage degradability and ruminal fermentation characteristics were evaluated using an *in vitro* fermentation technique, following the two-stage method of Tilley and Terry (1963). In this procedure, 0.5 g of dried and ground forage was incubated with 50 mL of a rumen fluid-to-buffer mixture (1:4 ratio) at 39 °C for 48 h, followed by a secondary digestion phase in an acid-pepsin solution (pH ~2) for another 48 h to simulate abomasal digestion. The remaining residues were dried at 105 °C to determine *in vitro* DM degradability (IVDMD) and subsequently ashed

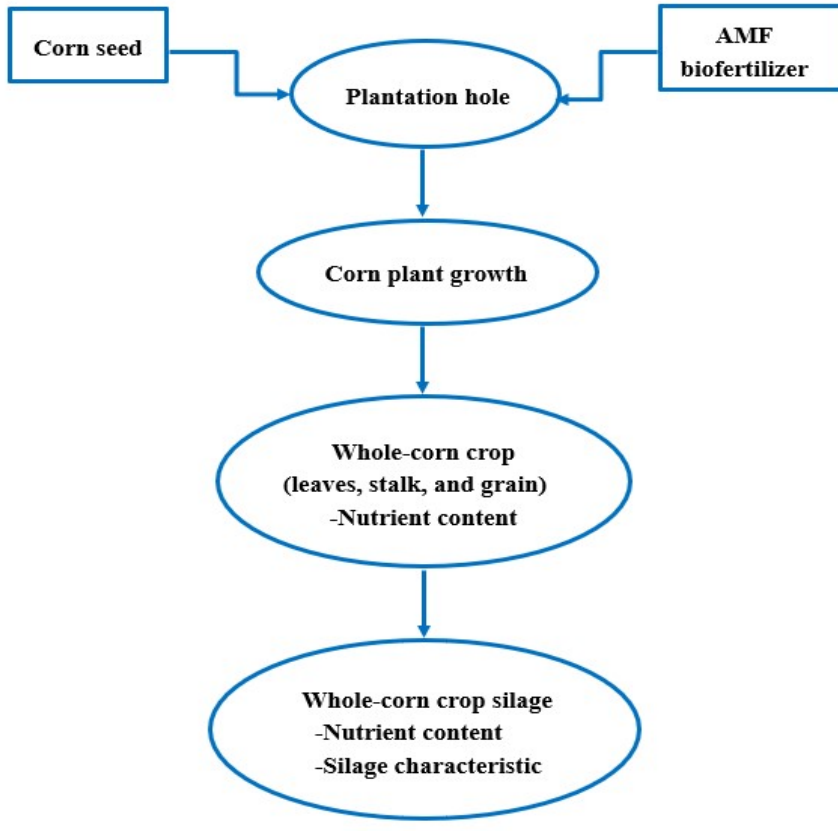

Fig. 1. Flowchart of AMF biofertilizer use in corn plants for silage production.

at 550 °C to measure *in vitro* organic matter degradability (IVOMD).

To evaluate ruminal fermentation parameters, a separate *in vitro* incubation was conducted. Briefly, 0.5 g forage samples were anaerobically fermented with 50 mL of a 1:4 rumen fluid-to-buffer solution at 39 °C for 4 h. After incubation, the supernatant was analyzed for rumen pH using a digital pH meter (Horiba-18), ammonia nitrogen ($N\text{-}NH_3$) using the micro-diffusion method (Conway and O'Malley, 1942), and total short-chain fatty acids (TSCFAs) via steam distillation (Kromann et al., 1967).

Gas and methane production were assessed following the method of Theodorou et al. (1994). Briefly, 0.75 g of feed sample was incubated in airtight fermentation vessels containing rumen fluid and buffer at 39 °C. Total gas production recorded after 24 h using a 50 mL syringe, while methane concentration was determined using a Shimadzu 14B gas chromatograph equipped with a Propak column and a flame ionization detector (FID).

All incubations were performed in triplicate, with single independent fermentation runs. Blank controls (rumen fluid and buffer without substrate) were included to account for endogenous gas and metabolite production.

Statistical analysis

A completely randomized design was employed, consisting of three levels of AMF addition (0, 10, and 20 g per planting hole) with six replications per treatment. Data were analyzed using analysis of variance (ANOVA) at a 95% significance level ($P < 0.05$). When significant differences were detected, Tukey's Honestly Significant Difference (HSD) test was performed for mean separation. Statistical analyses were conducted using SPSS software, version 22 (IBM Corp., 2015).

RESULTS

Nutrient content of whole-crop corn

The nutrient composition of harvested whole-crop corn intended for silage is presented in Table 1. The findings indicate that AMF addition had no significant effect on crude ash, crude protein, ether extract, crude fiber, or nitrogen-free extract content. Likewise, the concentrations of NDF, acid detergent fiber (ADF), and hemicellulose remained unchanged across treatments. However, WSC content significantly increased ($P \leq 0.05$) with AMF addition, rising from 9.19% in the control to 10.25 and 11.99% for the 10 and 20 g AMF treatments, respectively.

Silage nutrient content

The nutritional composition of all whole-crop corn silages is presented in Table 2. The results indicate that DM, crude ash, crude protein, ether extract, crude fiber, and nitrogen-free extract did not differ among treatments. Similarly, the concentrations of NDF, ADF, and hemicellulose remained unchanged. However, a slight reduction in nutrient content was observed compared to the raw material (Table 1), likely due to fermentation losses during the ensiling process.

Silage fermentation quality

All silages produced (18 samples) in this experiment were well preserved, as indicated by low pH values below 4. The pH values of the silages were 3.99, 3.98, and 3.93 for the 0, 10, and 20 g AMF inoculant treatments, respectively,

Table 1. Nutrient content of harvested whole-crop corn before ensiling.

	AMF levels (g/planting hole)				
Nutrient content	**0**	**10**	**20**	**SEM**	**p-value**
Dry matter (DM), %	29.26	29.01	29.80	0.34	NS
Crude ash, % DM	5.26	6.04	5.52	0.21	NS
Crude protein, % DM	8.86	8.40	9.21	0.24	NS
Ether extract, % DM	4.15	4.05	3.95	0.59	NS
Crude fiber, % DM	29.17	30.39	29.17	1.29	NS
N-free extract, % DM	52.56	51.13	53.14	1.48	NS
NDF, % DM	70.96	71.12	71.21	0.11	NS
ADF, % DM	37.53	38.14	38.43	0.40	NS
Hemicellulose, % DM	33.43	32.98	32.78	1.45	NS
WSC, % DM	9.19c*	10.25b	11.99a	0.19	0.04

WSC = Water-soluble carbohydrates; SEM = Standard error means; NS = Not significant.
*Means within the same row with different letters differ significantly ($P \leq 0.05$, Tukey's test).

Table 2. Nutrient content of whole-crop corn silages.

Nutrient content	AMF levels (g/planting hole)			SEM	p-value
	0	10	20		
Dry matter (DM), %	27.88	27.58	27.02	0.42	NS
Crude ash, % DM	5.52	5.71	5.79	0.13	NS
Crude protein, % DM	8.52	7.99	8.45	0.36	NS
Ether extract, % DM	3.31	2.98	2.96	0.25	NS
Crude fiber, % DM	32.57	32.54	32.74	1.47	NS
N-free extract, % DM	50.07	50.47	50.06	1.85	NS
NDF, % DM	71.51	71.16	71.99	0.37	NS
ADF, % DM	38.69	38.83	39.30	0.27	NS
Hemicellulose, % DM	32.82	32.33	32.69	0.22	NS
WSC, % DM	6.72c*	7.99b	8.81a	0.07	0.03

WSC = Water-soluble carbohydrates; SEM = Standard error means; NS = Not significant, *Means within the same row with different letters differ significantly ($P \leq 0.05$, Tukey's test).

indicating that the silages were well fermented. The results show that AMF addition did not significantly affect silage quality, as no differences were found among treatments in terms of pH, total volatile basic nitrogen, DM recovery, and Flieg point (Table 3). This lack of variation suggests that the natural epiphytic microbial population present in the forage was sufficient to drive effective lactic acid fermentation, leading to similar fermentation end-products across treatments regardless of AMF addition.

In vitro rumen fermentation dynamics and degradability

Table 4 presents the rumen fermentation dynamics and *in vitro* degradability of whole-crop corn silages. No significant differences were observed among treatments for rumen fermentation parameters, including pH, ammonia concentration, IVDMD, and IVOMD, indicating that AMF addition did not alter the overall degradability or fermentation environment of the silages.

However, significant differences were found in TSCFA, total gas production, and methane emissions, which may be attributed to the increased WSC content in AMF-treated silages. The higher WSC levels likely provided more readily fermentable substrates, enhancing microbial activity and fermentation intensity, thereby influencing gas production and fermentation byproducts.

DISCUSSION

Nutrient content of whole-crop corn

Plant productivity and nutrient content are fundamental objectives in the cultivation of forage crops, as they directly influence feed ration quality. The nutrient composition of forage is essential for formulating balanced livestock diets and is influenced by factors such as harvest age (Horst et al., 2020), nutrient uptake, and plant metabolic processes (Tariq et al., 2023). Additionally, the initial nutrient profile of forage plays a key role in determining the nutritional quality of the resulting silage (Soe Htet et al., 2021).

In this study, the addition of AMF inoculant did not significantly affect the macronutrient content of whole-crop corn, including crude ash, crude protein, ether extract, crude fiber, and nitrogen-free extract. These findings align with Young et al. (2015), who also reported no influence of AMF inoculation on leaf nitrogen concentrations. Although AMF inoculation has been consistently associated with increased biomass and yield in various crops (Aguegue et al., 2017; Stoffel et al., 2020; Bouzeriba et al., 2021; Husein et al., 2022; Zhao et al., 2023), these improvements may not necessarily translate into changes in macronutrient composition, particularly when soil nutrients are adequate.

Contrastingly, Wu et al. (2023) observed that AMF inoculation significantly increased leaf nitrogen (N), phosphorus (P), potassium (K), and the P:K ratio while reducing the N:P and N:K ratios. This suggests that AMF can influence nutrient allocation within plant tissues. AMF primarily enhances the uptake of micronutrients, especially P and trace elements like zinc and copper. Variations across studies may result from differences in soil nutrient availability, AMF species, or environmental conditions affecting symbiotic efficiency.

Since this study focused exclusively on

Table 3. Fermentation quality of whole-crop corn silages.

Parameter	AMF levels (g/planting hole)			SEM	p-value
	0	10	20		
pH value	3.99	3.98	3.93	0.02	NS
TVBN, g kg^{-1} TN	1.02	1.05	1.16	0.08	NS
DM recovery, %	95.24	95.06	94.82	0.15	NS
Flieg point	109.16	108.87	109.84	1.27	NS

TVBN = Total volatile basic nitrogen; TN = Total nitrogen; SEM = Standard error means; NS = Not significant.

Table 4. Rumen fermentation quality and degradability of whole-crop corn silages.

Parameters	AMF levels (g/planting hole)			SEM	p-value
	0	10	20		
pH	6.92	6.87	6.97	0.02	NS
Ammonia, mM	12.03	12.08	12.13	0.06	NS
TSCFA, mM	120.32b	127.43ab	128.45a	0.49	0.04
IVDND, %	67.86	68.67	68.94	1.35	NS
IVOMD, %	66.62	66.91	67.59	1.27	NS
Total gas, mL g^{-1} DM	168.78b*	175.56a	176.31a	1.37	0.03
Methane, mL g^{-1} DM	15.62a*	13.94b	12.48c	0.13	0.04
Methane, % total gas	9.25a*	7.82b	7.31c	0.12	0.04

TSCFA = Total short-chain fatty acid; IVDMD = *In vitro* dry matter degradability; IOMD = *In vitro* organic matter degradability; SEM = Standard error means, NS = Not significant. *Means within the same row with different letters differ significantly ($P \leq 0.05$, Tukey's test).

macronutrient content, the potential effects of AMF on micronutrient dynamics remain unexplored. Further research is warranted to assess the role of AMF in enhancing micronutrient accumulation and its implications for forage and silage quality.

The increase in WSC content may be attributed to enhanced P absorption facilitated by AMF addition. P is essential for carbohydrate metabolism, and improved uptake through AMF associations may stimulate WSC synthesis, contributing to better yield outcomes, particularly under P-limited conditions (Bouzeriba et al., 2021; Kazadi et al., 2022; Mei et al., 2019; García-Caparrós et al., 2021). WSC is an essential carbohydrate, serving as a substrate for lactic acid bacteria during silage fermentation, thereby promoting lactic acid production (Li et al., 2021; Yi et al., 2023). Higher WSC levels in silage raw materials facilitate faster acidification and preserve silage quality.

In this study, WSC content ranged from 9.19 to 11.99%, surpassing the minimum threshold of 5% suggested by McDonald et al. (1991) for effective silage fermentation. However, P was omitted from the micronutrient analysis in this experiment, limiting direct evaluation of the relationship between AMF-induced P uptake and WSC accumulation. Literature indicates that P uptake efficiency via AMF can be influenced by soil nutrient status, AMF species, and plant physiological responses (Smith and Smith, 2011; Chen et al., 2018). Thus, while AMF may contribute to WSC enhancement, the direct link to P dynamics remains speculative, highlighting the need for further research into micronutrient interactions in AMF-inoculated systems.

In addition to the WSC content, the DM content of the raw material before ensiling plays a crucial role in determining the success of the fermentation process. A low DM content supports the growth of clostridia bacteria (Kung et al., 2018), while a high DM content inhibits the activity of lactic acid bacteria (Comino et al., 2014). The ideal DM content to support optimal silage fermentation is around 25-35% (McDonald et al., 1991; Kung et al., 2018). Therefore, managing the DM content of silage through wilting is a key method to ensure successful silage dominated by lactic acid fermentation (Ridla et al., 2024). In this study, the

DM content ranged from 28.80 to 29.03%, which falls within the ideal range for silage production.

Nutrient content and silage quality

The nutrient content of silages was not affected by AMF inoculant addition, indicating that the initial materials used before ensiling contained almost the same nutrient content, resulting in no differences in the nutrient content among the silages. Slightly lower nutrient content in the silages (Table 2) could be attributed to first-phase fermentation activity during the ensiling process. One key process during ensiling is respiration, which occurs predominantly in the first week. In classical theory, this initial period is known as the aerobic phase (McDonald et al., 1991). Typically lasting only a few hours, this phase involves the reduction of atmospheric oxygen present between plant particles, driven by plant respiration and the activity of aerobic and facultative aerobic microorganisms such as yeasts and enterobacteria. Additionally, plant enzymes like proteases and carbohydrates remain active during this phase, provided the pH remains within the normal range (Woolford, 1984).

The pH value is an indicator of the acidic condition formed in the silage. In concurrence with other research, Kung et al. (2018) reported that a good silage pH value for corn silage is around 3.7-4.0. Acidic conditions result from the activity of lactic acid bacteria such as *Lactobacillu*s sp., *Lactococcus* sp., and *Enterococcus* sp., which produce lactic acid (He et al., 2020). Based on the research conducted by Chalisty et al. (2017), lactic acid bacteria are capable of producing bacteriocins, which can inhibit the growth of spoilage bacteria.

Total volatile basic nitrogen (TVBN) concentration in silage is an indicator of protein degradation during the fermentation process (McDonald et al., 1991). Protein degradation occurs due to the activity of spoilage bacteria such as *Clostridia* sp. and *Enterobacteria* sp. (Wang et al., 2020). The higher the TVBN level in silage, the greater the damage to feed protein. According to Tharangani et al. (2021), the standard acceptable range for TVBN in silage is 0.5 - 1.24 g/kg of DM. In the present study, TVBN levels ranged from 1.02 -1.16 g kg^{-1} of DM and were categorized as good quality silage.

DM recovery in silages could reflect their optimal fermentation profile by maintaining DM content from loss during the silage process (Borreani et al., 2018). The ensiling process can convert DM components, especially carbohydrate fractions, into CO_2, H_2O, and heat, resulting in a decrease in the DM content of silage (McDonald et al., 2010). According to McDonald et al. (1991), high-quality silage should have a minimum DM recovery value of 92%. In this study, DM recovery ranged from 93.82 to 95.24%, indicating effective fermentation and preservation. The low pH value in all treatments in this experiment supports this finding.

The Flieg point is a method used to assess silage quality based on DM content and pH. As described by Kilic (1986), the method provides a simple and practical approach for evaluating silage quality. The Flieg scores are categorized as follows: <20 (poor), 21-50 (sufficient), 51-60 (moderate), 61-85 (good), and >86 (very good). The average Flieg score produced in this study ranged from 108.87 to 109.84, corresponding to the very good category.

The results of this experiment show that an adequate WSC content (9.19 - 11.99% DM) and optimum moisture content (28.80 - 29.26%) in whole-crop corn prior to ensiling have been proven to guarantee good fermentation. This was evidenced by low pH values (3.93 - 3.99), low ammonia content (1.02 - 1.16 g kg^{-1} DM), high DM recovery (93.82 - 95.24%), and high Flieg scores (108.87 - 109.84), indicating the production of high-quality silage.

Rumen fermentation dynamics, degradability, and methane emission

Rumen pH, which measures the acidity or alkalinity of the rumen fluid, ranged from 6.87 to 6.97, indicating a slightly acidic to neutral environment typical for rumen fermentation. There were no significant differences between treatments, suggesting that variations in WSC content did not impact rumen pH. According to Holtzapple et al. (2022), optimal rumen microbial activity occurs within a pH range between 6.2 and 7.0 (neutral to slightly acidic). When the pH drops below 6.2, fiber-digesting bacteria slow down.

Rumen ammonia concentration serves as an indicator of protein breakdown in the rumen. In this study, values ranged from 12.03 to 12.13 mM across treatments, with no significant differences observed, indicating that variations in WSC content did not affect ammonia levels. The minimum ammonia concentration required for optimal growth of rumen microbes is 5.0 mg per 100 mL of rumen fluid, with the ideal range between 8.5 and 30.0 mg per 100 mL (Dewhurst and Newbold, 2022). In this study, ammonia concentrations ranging from 12.03 to 12.13 mM (equivalent to 20.46 to 20.62 mg 100 mL^{-1}), indicated a normal concentration.

Rumen TSCFAs (mM) are important products of fermentation in the rumen and provide energy for the animal. Normal TSCFA concentrations in

healthy ruminants typically range between 70 and 150 mM (Beckett et al., 2021). In this study, TSCFA values ranged from 120.32 to 128.45 mM, revealing significant differences (P = 0.04) across treatments. The increase in TSCFA production suggests that higher WSC content promoted more active rumen microbial fermentation. The higher short-chain fatty acid (SCFA) output is a key marker of this intensified microbial activity, indicating that microbes were thriving in the rumen, leading to more efficient fermentation processes (Liu et al., 2022).

Rumen total gas production (mL g^{-1} DM) serves as an indirect indicator of fermentation efficiency and degradability. In this study, total gas production ranged from 168.78 mL g^{-1} in the control to 176.31 mL g^{-1} in the 20 g AMF treatment, with a significant (P = 0.03) increase observed in the AMF treatments, indicating that higher WSC content significantly affected gas production during fermentation. Higher gas production reflects enhanced suitability for diets that require active rumen fermentation (Ridla and Nahrowi, 2025). This increased fermentation activity can lead to greater total TSCFA production and improved energy conversion, which benefits livestock nutrition (McDonald et al., 2010).

The elevated gas production in the AMF treatments suggests that higher WSC content supported microbial growth, likely due to the presence of more digestible material. The increased WSC content indirectly promoted rumen microbial growth and fermentation activity, as indicated by the rise in TSCFA content from 120.32 mM in the control to 128.45 mM in the 20 g AMF treatment (P = 0.04), along with a slight, though statistically nonsignificant, increase in IVDMD and IVOMD. As noted by Wang et al. (2022) and Parnian-Khajehdizaj et al. (2023), rumen gas levels typically rise in parallel with increased fermentation activity and degradability.

Methane emissions were highest in the control group (15.62 mL g^{-1} DM; 9.25% of total gas) and significantly decreased (P = 0.04) with higher WSC content to 13.94 mL g^{-1} DM (7.82%) and 12.48 mL g^{-1} DM (7.31%) in the 10 and 20 g AMF treatments, respectively. This reduction aligns with the enhanced ruminal microbial fermentation associated with increased WSC content, resulting in higher TSCFA production, gas output, and degradability, ultimately lowering methane emissions by 1.68 to 3.14 mL g^{-1} DM.

Jayanegara et al. (2016) noted that non-structural carbohydrates, such as starch and sugars, including WSC, are rapidly digested in the rumen. This quick breakdown leads to higher SCFA production compared to structural carbohydrates. Microbial fermentation of starch in the rumen, under anaerobic conditions, produces gases such as carbon dioxide (CO_2) and methane (CH_4). However, because WSC is easily fermented by rumen microbes, it can increase gas production while potentially reducing methane due to less hydrogen availability, unlike fibrous carbohydrates in forages. Fibrous carbohydrates tend to increase methane production by altering the SCFA profile, favoring acetate and butyrate, which in turn generate more hydrogen gas (H_2), a key substrate for methanogenesis (Dong et al., 2022b; Ridla and Nahrowi, 2025).

CONCLUSIONS

The application of AMF inoculant during corn planting effectively enhanced the water-soluble carbohydrate (WSC) content in whole-crop corn. This increase in WSC contributed to improved rumen fermentation, as evidenced by higher total short-chain fatty acid (TSCFA) production, increased gas output, and marginal improvements in degradability. Additionally, elevated WSC levels contributed to reduced methane emissions, indicating a positive impact on fermentation efficiency and environmental sustainability. However, AMF inoculation did not significantly affect the overall nutrient content or fermentation characteristics of the resulting silage.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

All authors were involved in the conception and design of the study, data analysis, and manuscript writing.

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