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Corn and foxtail millet silages: effects of particle size and bacterial inoculation on intake, digestion, rumen Parameters, and feeding behavior in sheep

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Abstract This study compared the effects of silage particle size (8 vs. 16 mm) and inoculation with lactic acid bacteria on the nutritional and physiological responses of sheep fed with diets containing corn or foxtail millet silage. Two separate experiments were conducted for 90-day periods arranged as 2×2 factorial experiment within a randomized complete block design. While particle size and inoculation had no significant effect on dry matter (DM), crude protein, or neutral detergent fiber concentrations in corn silage, in millet silage DM content was significantly higher with long particles (395 vs. 350 g/kg DM; $P<0.01$). Silage pH was significantly affected by particle size in both corn ($P<0.05$) and millet ($P<0.01$), with short particles yielding lower pH. Bacterial inoculation further reduced pH only in millet silage ($P<0.01$). The DM and organic matter (OM) intake were greater with short-particle silages, but digestibility improved only in the sheep fed with short, inoculated millet silage diet ($P<0.01$). Ruminal pH remained unaffected by two factors in corn silage diets, but in millet silage diets, it was significantly lower at 2 and 4 h post-feeding with short silage particles size diets ($P<0.01$). Ruminal microbial nitrogen and protein synthesis were higher with short-particle corn silage ($P<0.01$), whereas millet silage showed a significant particle size×inoculation for these parameters. Chewing behavior also differed; sheep fed on short corn silage chewed longer ($P<0.01$), while in millet silage, feeding time increased with long particles silage diet and rumination time was longer with short particles ($P<0.05$). Overall, corn silage responses were driven mainly by physical form, whereas millet silage benefited from the combined optimization of chop length and microbial inoculation.

Keywords: bacterial inoculation; microbial protein; physical characteristics of diet; silage particle size

Introduction

Ensiling is a widely used method for preserving high-moisture forages and maintaining the nutritional quality of ruminant feed. The process relies on anaerobic fermentation, primarily driven by lactic acid bacteria (LAB), which convert water-soluble carbohydrates in the forage into organic acids—chiefly lactic acid (McDonald et al., 2011). This acidification rapidly lowers the pH of the

silage, creating an environment that effectively suppresses the growth of undesirable microorganisms such as molds, yeasts, and spoilage bacteria. A swift and substantial drop in pH is therefore essential for successful preservation, as it minimizes nutrient losses and prevents feed deterioration (Guo et al., 2023). Silage is typically produced from whole-plant materials—such as corn stalks, leaves, and ears—or other green forages, and properly produced silage can be

stored for extended periods without significant loss in quality. The resulting feed is energy-dense and highly palatable, making it a valuable component of the ruminant diets (Johnson, 2005).

Corn forage is widely regarded as a vital crop for ruminant animal feed owing to its high yield potential and excellent nutritional quality. Rich in soluble carbohydrates and starch, it typically produces 80 to 100 metric tons per hectare (Khan et al., 2015) and exhibits favorable traits for silage production (MacDonald et al., 2011). Its high dry matter (DM) content and low buffering capacity promote efficient fermentation, resulting in greater lactic acid production compared to silages made from other forage grasses (Ferraretto and Shaver, 2018). According to reports from Iran's Ministry of Agriculture, the country harvested approximately 13.5 million metric tons of corn forage during the 2022-2023 agricultural year years (Agricultural Statistics for the 2022-2023 Crop Year, 2024).

While corn silage remains a cornerstone of ruminant diets, alternative forages such as foxtail millet are increasingly attracting attention due to their adaptability to challenging environmental conditions and favorable nutritional attributes. A major limitation of corn silage production, particularly during the hot growing season is its high-water demand (Nadeem et al., 2020). In Iran, where water resources are increasingly constrained, this has spurred interest in more water-efficient forage crops (Nouri et al., 2023). Foxtail millet, a C4 plant, benefits from a highly efficient photosynthetic pathway that enables it to perform well under water-limited conditions, making it a promising alternative in arid and semi-arid regions (Singh and Prasad, 2020). Additionally, foxtail millet has a short growth cycle, typically reaching maturity in just 60 to 90 days. This rapid development allows for multiple harvests within a single growing season, providing farmers with greater flexibility in forage planning and the ability to respond swiftly to shifting climatic conditions (Nadeem et al., 2020).

Forage particle size plays a pivotal role in several physiological processes critical to optimal digestion and overall rumen health in ruminants. Larger particles require more extensive chewing, which stimulates saliva secretion, a key factor in buffering Ruminant pH and maintaining a stable environment for efficient microbial fermentation. (Beauchemin et al., 2008; Sharifi Hosseini et al., 2018). The physical length of fiber particles is especially influential in regulating rumen motility, digesta retention time, and chewing behavior. Longer forage and silage particles are typically chewed more thoroughly, further enhancing saliva production and supporting rumen function (Beauchemin et al., 2008). Diets with higher concentrations of physically effective neutral detergent fiber (peNDF) promote increased chewing activity, which elevates Ruminant pH and improves fermentation efficiency (Cao et al., 2021). However, while adequate peNDF is essential for rumen health, excessive levels can lead to rumen fill that limits overall

DM intake (DMI), potentially reducing feed intake (Zebeli et al., 2010).

Inoculating the ensiled materials with LAB can significantly improve the fermentation process. These beneficial microorganisms accelerate the decline in pH, promoting a more rapid and stable ensiling environment (Ke et al., 2025). Moreover, research has demonstrated that dairy cows fed corn silage inoculated with LAB exhibit greater DMI compared to those fed non-inoculated silage (Kristensen et al., 2010; Kok et al., 2025).

Research on millet silage, particularly regarding its particle size and the effects of bacterial inoculation on sheep, is limited. Furthermore, there has been no comparison to date between the bacterial inoculation of millet and corn in terms of their impacts on intake, digestibility, rumen parameters, and feeding behavior. Consequently, this study aimed to compare the effects of bacterial inoculation and particle size in corn and millet silage on intake, digestibility, rumen parameters, and feed intake behavior in sheep.

Materials and methods

The experiments were conducted at the Sheep Breeding Station of Shahid Bahonar University of Kerman, located in southeastern Iran (latitude 30°15'19.10"N, longitude 57°6'20.40"E). Approximately 1,200 kg of foxtail millet (local variety) and 1,200 kg of forage whole corn plant (Iranian variety 704) were harvested at the milk-dough stage of maturity, and then processed into two distinct particle lengths: short (8 mm) and long (16 mm), using a forage chopper.

Ensiling materials and measurements of silage characteristics

To enhance the fermentation process, a commercial bacterial inoculant, Biomin® Biostabil Mays (produced in Austria) was applied to 50% of the corn forage and to both short and long particle millet forage. This inoculant contains a synergistic blend of beneficial LAB, including *Enterococcus faecium* (a homofermentative starter culture), *Lactobacillus brevis* (a heterofermentative species), and *Lactobacillus plantarum* (a homofermentative strain). Each gram of the product provides approximately 2.5×10^{10} colony-forming units (CFU) of viable bacteria. The inoculant was applied at a rate of 4 g per metric ton of freshly chopped forage. For practical application, 1.2 g of the powder was dissolved in 0.3 liters of dechlorinated water to treat 300 kg of forage. The resulting solution was thoroughly mixed and evenly sprayed onto 50% of both the short- (8 mm) and long-particle (16 mm) millet forage batches. Treated forages were then packed into thick nylon bags (45×90 cm) and sealed and allowed to ferment for 60 days before sampling to evaluate their fermentation quality. For pH analysis, a 20-g subsample of silage was homogenized with 200 mL of distilled water for one min. The pH of the resulting slurry was then measured using

a calibrated digital pH meter (Elmetron, Model 103CP, Poland).

Experimental design, rams and diets

Both experiments were conducted for 90 days, comprising a 6-day pre-adaptation period followed by an 84-day main experimental phase. The main phase was subdivided into four consecutive 21-day periods. Within each period, the first 14 days served as an adaptation phase to allow the sheep to adjust to their assigned diets, followed by a 6-day data collection during which data on feed intake, feces, urine, and rumen fluid samples were gathered. The final day of each 21-day period was dedicated specifically to assessing the feeding behavior.

For each experiment, eight two-year-old male sheep were used in a 2×2 factorial arrangements within a completely randomized block design. The average body weight of the animals was 36.1±1.6 kg in the corn silage trial and 37.3±2.6 kg in the millet silage trial. All procedures involving animals were conducted in

accordance with the ethical guidelines for the care and use of livestock established by the Iranian Council for Animal Care (1995). The sheep were individually housed in metabolic cages equipped with separate collection systems for urine and feces to ensure precise measurement of excreta and accurate assessment of nutrient digestibility.

The experimental diets were formulated with a 50:50 forage-to-concentrate ratio to provide a nutritionally balanced profile aligned with the nutrient requirements of Kermani sheep, as recommended by the National Research Council (NRC, 2007). In both experiments, corn and the millet silage trials, the dietary treatments included inoculated and uninoculated long-particle silage and inoculated and uninoculated short-particle silage (Table 1). All diets were prepared as total mixed rations (TMR) to ensure homogeneous nutrient distribution. Sheep were fed twice daily-at 08:00 and 16:00-and had *ad libitum* access to fresh, clean water throughout the study.

Table 1. Experimental diets in two experiments of corn and millet silages

Ingredients (g/kg)	Corn silage diets					Millet silage diets			
	Part size	Long		Short		Long		Short	
	Inoc	+	-	+	-	+	-	+	-
Alfalfa		-	-	-	-	100	100	100	100
Corn silage		500	500	500	500	-	-	-	-
Millet silage		-	-	-	-	400	400	400	400
Barley grain		230	230	230	230	270	270	250	250
Corn grain		100.0	80.0	60.	75.0	50	70	70	70
Soybean meal		100.0	110.0	100.0	100.0	40	40	60	60
Wheat bran		50.0	60.0	85.0	75.0	120	100	100	100
Min and Vit supplement [†]		7.0	7.0	7.0	7.0	5.0	5.0	5.0	5.0
Limestone		5.0	5.0	5.0	5.0	7.0	7.0	7.0	7.0
DCP		3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Salt		5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Chemical composition (DM%) and ME									
DM (% as-fed)		58.5	58.9	58.4	59.6	68.5	68.9	68.4	69.6
ME (Mcal/kg DM)		2.3	2.3	2.4	2.4	2.37	2.34	2.34	2.37
CP		13.2	13.3	13.3	13.2	13.2	13.3	13.3	13.2
OM		90.8	90.6	90.5	90.0	90.8	90.6	90.5	90.0
EE		4.0	3.9	4.0	3.9	3.95	3.93	3.97	3.91
NDF		38.7	38.6	39.5	39.6	36.9	38.7	37.4	38.8
ADF		21.5	20.5	19.2	19.4	2.0	20.8	20.7	19.8
Ca (g)		6.30	6.41	6.40	6.50	7.97	7.84	7.87	7.91
P (g)		3.90	4.01	5.20	4.21	4.04	3.89	3.89	3.96

¹Mineral and vitamin supplement: Each kilogram of premix contained 52,000 IU of vitamin A, 50,000 IU of vitamin D₃, 1,500 IU of vitamin E, 2.25 g of manganese, 120 g of calcium, 7.7 g of zinc, 10 g of phosphorus, 20.5 g of magnesium, 186 g of sodium, 1.25 g of iron, 3 g of sulfur, 1.25 g of copper, 14 mg of cobalt, 56 mg of iodine, and 10 mg of selenium

Feed intake, digestibility, and chemical analysis, ruminal pH

The DMI was recorded daily, and feed bunk samples were collected and analyzed for DM, organic matter (OM), CP, and NDF using procedures identical to those applied to fecal samples at 0 and 24 h after feeding on days 15 to 19. Intakes of DM, OM, CP, and NDF were calculated as the difference between the amount of feed offered and the amount refused. Apparent digestibility of DM and other nutrients was determined using the total fecal collection method (Ryder, 2000).

Rumen fluid was collected on the final day of each experimental period using a suction device. Sampling occurred both before morning feeding (designated as time zero) and at 2, 4, 6, and 8 h post-feeding. Immediately after collection, the pH of each sample was measured using a portable pH meter (Elmetron, Model 103CP).

To evaluate the particle size distribution of the diets and to calculate the physically effective fiber content and the physical effectiveness factor, the sieving method described by Kononoff et al. (2003a) was used.

Feed intake behavior and microbial N and protein synthesis

On day 20 of each experimental period, feed intake, rumination, and chewing activities were visually recorded at 5-min intervals following the method described by Teimouri Yansari et al. (2004). Microbial nitrogen production (g N/day) was estimated using Equation 1 (Denek and Can, 2006). Microbial protein was estimated by multiplying microbial nitrogen by a conversion factor of 6.25.

$$\text{Equation 1) Microbial protein} = (X(\text{mmol/day}) \times 70) / (0.116 \times 0.83 \times 1000)$$

where X represents the purine absorption rate (mmol/day)

Statistical analysis

To evaluate the effects of two levels of corn and millet silage particle size and two levels of bacterial inoculation on the animals, a randomized block design with a 2×2 factorial arrangement was used. The statistical analysis was based on the model described in Equation 2. For traits measured repeatedly over time-such as ruminal pH the repeated-measures model outlined in Equation 3 was applied.

$$\text{Equation 2) } Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \delta_L + \lambda + e_{ijkl}$$

$$\text{Equation 3) } Y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \delta_L + \sigma_m + e_{ijklm}$$

In these models, Y_{ijklm} and Y_{ijk} represent individual observations, while μ denotes the overall mean. The fixed effect of silage particle size (α_i) reflects the two levels tested: long particles (16 mm) and short particles (8 mm) for both corn and millet silage. The effect of microbial inoculation (β_j) compares inoculated versus uninoculated silage. The interaction between particle size and inoculation level is represented by $(\alpha\beta)_{ij}$. Additional terms include γ_k , the random effect of the animal; δ_i , the fixed effect of the experimental period; and

σ_m , the fixed effect of time (included only in repeated-measures analyses). The residual errors are denoted by e_{ijklm} and e_{ijk} , corresponding to the repeated and non-repeated models, respectively.

Statistical analysis was conducted using SAS software (version 9.4; SAS Institute Inc., Cary, NC) with the MIXED procedure. Treatment means were compared using the Tukey's HSD test at a significance level of $P < 0.05$.

Result

Silage characteristics

Particle size and bacterial inoculation had no significant effect on the concentrations of DM, CP, or NDF in corn silage ($P > 0.05$) (Table 2). In millet silage, DM concentration was significantly influenced by silage particle size (SPS; $P < 0.01$), with long particles yielding a higher DM content (395 g/kg DM) compared to short particles (8 mm; 350 g/kg DM). Similarly, OM concentration in millet silage was significantly ($P < 0.01$) affected by both particle size and inoculation ($P < 0.05$). However, neither bacterial inoculation nor particle size had a significant ($P < 0.05$) effect on total CP content in millet silage. Additionally, short-chopped corn silage exhibited a lower pH than long-chopped silage (LCS) ($P < 0.05$).

The NDF concentration in millet silage was significantly influenced by both particle size and bacterial inoculation ($P < 0.001$). The silage prepared with long particles contained higher NDF (522 g/kg DM) than that with short particles (8 mm; 490 g/kg DM). Similarly, uninoculated silage had a higher NDF concentration (538 g/kg DM) compared to inoculated silage (490 g/kg DM). Both particle size and inoculation had highly significantly affected the silage pH ($P < 0.01$). Long SPS exhibited a higher pH (3.99) than short-particle silage (3.80), and uninoculated silage had a higher pH (4.00) compared to inoculated silage (3.79).

Table 2. Effects of particle size and bacterial inoculation on the chemical composition¹ and pH¹ of corn and millet silage

SPS ²	Long		Short		Main effects				SEM ⁶	P-value		
Inocu-le ³	+	-	+	-	Long	Short	Inocu ⁴	UnIno ⁵		SPS	Inocu-le	S×IN ⁷
Chemical composition (g/kg DM) and pH												
Effect of corn silage particle size and inoculation levels												
DM	258	252	244	258	255	251	251	255	19.3	0.65	0.67	0.92
CP	88.8	88.0	84.1	88.4	88.4	84.3	86.7	86.0	3.40	0.63	0.93	0.99
NDF	538	486	486	407	512	446	512	446	60.0	0.11	0.15	0.55
pH	4.00	3.84	3.76	3.77	3.92 ^a	3.77 ^b	3.88	3.81	0.10	0.05>	0.23	0.45
Effect of millet silage particle size and inoculation levels												
DM	395	395	365	335	395 ^a	350 ^b	380	365	15.0	<0.01	0.07	0.19
OM	359	355	332	304	357 ^a	318 ^b	345 ^a	330 ^b	12.0	<0.01	<0.05	0.12
CP	116	116	105	117	116	110	111	115	7.00	0.20	0.25	0.14
NDF	506	538	475	538	522 ^a	490 ^b	490 ^b	538 ^a	18.0	<0.01	<0.001	0.25
pH	3.89	4.09	3.69	3.91	3.99 ^a	3.80 ^b	3.79 ^b	4.00 ^a	0.06	<0.01	<0.01	0.99

a, b, c Within row, means with common superscript (s) are not different ($P > 0.05$).

¹Each number is based on four analytical replicates, ²Silage particle size, ³Inoculation levels, ⁴Inoculation, ⁵Uninoculation,

⁶Standard error of the mean, ⁷Interaction between silage particle size and inoculation levels.

Physical characteristics of experimental diets

Particle size and bacterial inoculation significantly influenced the particle distribution of both corn and millet silages across sieve fractions (Table 3). On the 19-mm sieve, LCS retained significantly more DM than short-chopped silage (SCS) ($P<0.01$). Uninoculated silage also retained more DM than inoculated silage ($P<0.01$), and the interaction between particle size and inoculation was significant ($P<0.05$). On the 8-mm sieve, LCS resulted in greater DM retention ($P<0.01$). In contrast, bacterial inoculation increased DM retention on this

sieve ($P<0.01$). On the 1.18-mm sieve, SCS retained more DM than LCS in both corn and millet ($P<0.01$). Additionally, in corn silage, inoculation slightly but significantly increased DM retention on this sieve ($P<0.01$). This effect was not observed in millet silage. In the pan fraction (particles <1.18 mm), no significant treatment effects were detected in corn silage. However, a significant effect was observed in millet silage, with SCS diets showing higher DM retention ($P<0.05$). Physically effective NDF (peNDF) in millet silage was significantly higher in long-particle diets than in short-particle diets ($P<0.05$).

Table 3. Effect of corn and millet silage particles size and inoculation level on physical characteristics² of diets in two experiments

SPS ³	Long		Short		Main effects				SEM ⁷	P-value		
Inocu-le ⁴	+	-	+	-	Long	Short	Inocu ⁵	UnIno ⁶		SPS	Inocu-le	S×IN ⁸
Effect of corn silage particle size and inoculation levels on DM remained on the sieves (%)												
19 mm	17.7 ^b	24.6 ^a	12.2 ^c	10.9 ^c	21.2 ^a	11.6 ^b	14.9 ^b	17.7 ^a	1.59	<0.01	<0.01	<0.01
8 mm	36.5	2.73	26.6	23.7	31.9 ^a	25.1 ^b	31.5 ^a	25.5 ^b	5.53	<0.01	<0.01	0.06
1.18 mm	35.9	39.7	49.9	56.0	37.8 ^b	52.9 ^a	42.9 ^b	47.8 ^a	2.39	<0.01	<0.01	0.23
Pan	9.86	8.36	11.3	9.41	9.12	10.4	10.6	8.90	2.68	0.22	0.11	0.82
PEF ⁹	90.1	91.6	88.7	90.6	89.8	89.6	89.4	90.0	2.67	0.22	0.11	0.82
PeNDF ¹⁰	34.8	35.4	35.0	25.9	35.1	35.4	34.9	35.6	0.40	0.39	0.09	0.81
Effect of millet silage particle size and inoculation levels on DM remained on the sieves (%)												
19 mm	16.7 ^b	25.6 ^a	13.2 ^b	14.1 ^c	21.5 ^a	13.6 ^b	14.9 ^b	19.8 ^a	1.95	<0.001	<0.01	0.05
8 mm	37.0	28.1	27.1	24.2	32.5 ^a	25.6 ^b	32.0 ^a	26.1 ^b	4.20	<0.01	<0.01	0.07
1.18 mm	38.9	38.7	46.9	45.0	38.8 ^b	45.9 ^a	42.9	41.8	2.45	<0.01	0.15	0.21
Pan	7.60	7.65	12.8	16.7	7.63 ^b	14.8 ^a	10.2	10.3	2.00	<0.05	0.89	0.06
PEF	92.4	92.3	87.2	83.3	92.3 ^a	85.25 ^b	89.8	87.8	2.80	<0.01	0.09	0.11
PeNDF _{<1.18}	34.1	35.7	32.6	32.6	34.9 ^a	32.6 ^b	33.3	34.1	2.10	0.05	0.76	0.70

a, b, c Within row, means with common superscript (s) are not different ($P>0.05$).

¹Each number is based on six analytical replicates, ²Each number is based on six analytical replicates, ³Silage particle size, ⁴Inoculation levels, ⁵Inoculation, ⁶Uninoculation, ⁷Standard error of the mean, ⁸Interaction between silage particle size and inoculation levels,

⁹Physical effective factor, ¹⁰Physical effective NDF_{>1.18 mm}.

Intake and digestibility of diets

Short-particle corn silage diets increased DM intake compared with long-particle diets ($P<0.05$). Bacterial inoculation had no significant effect on DM intake (Table 4). A similar pattern was observed for OM intake, which was higher with short-particle corn silage (1.47 kg/d) than with long-particle silage (1.34 kg/d; $P<0.05$). The NDF intake was also greater for short-particle corn silage diets (0.62 kg/d) than for long-particle diets (0.55 kg/d; $P<0.01$), with no effect of inoculation on NDF intake. In millet silage, reducing particle size increased both DM and OM intake ($P<0.01$). Furthermore, a significant interaction between particle size and inoculation was observed for both DM and OM intake ($P<0.01$), indicating that the effect of particle size on intake depended on inoculation status.

In corn silage-based diets, the apparent digestibility of DM, OM, CP, NDF did not differ significantly among treatments. In millet silage-based diets, DM and CP digestibility were also unaffected by treatment. However, OM digestibility (OMD) was influenced by both particle size ($P<0.001$) and bacterial inoculation ($P<0.05$).

Ruminal pH

In corn silage-based diets, neither particle size nor bacterial inoculation had a significant effect on ruminal pH at any time point measured (Table 5). In millet silage-based diets, ruminal pH at time zero (before feeding) was significantly higher in animals fed inoculated silage compared to those fed uninoculated silage (6.92 vs. 6.77; $P<0.001$). Particle size had no effect on pH at this time point. However, at 2 and 4 h post-feeding, ruminal pH was significantly lower in animals fed short-particle silage than in those fed long-particle silage ($P<0.01$ at both time points). In millet silage-based diets, the mean ruminal pH was significantly higher with long-particle silage than with short-particle silage ($P<0.01$).

Microbial protein synthesis

Sheep fed with short-particle corn silage diets exhibited significantly higher microbial nitrogen and protein production compared to those fed long-particle silage diets ($P<0.01$) (Table 6). Inoculation had no significant effect on microbial nitrogen production, nor was there a significant interaction between silage particle size and inoculation.

For millet silage, a highly significant ($P<0.01$) interaction between particle size and inoculation was observed for both microbial nitrogen and protein

production. No significant main effects of either particle size or inoculation were detected for either parameter.

Feeding behavior

In the corn silage experiment, feeding and rumination time were not significantly affected by either silage particle size or inoculation level (Table 7). However, total

chewing time (feeding+rumination) was significantly influenced by silage particle size ($P<0.01$). Sheep fed on short-particle silage spent more time chewing (718 min/day) compared to those fed long-particle silage (660 min/day).

Table 4. Effect of millet silage particle size and bacterial inoculation level on feed intake¹ and digestibility²

SPS ³	Long		Short		main effects				SEM ⁷	P-value		
Inocu-le ⁴	+	-	+	-	long	Short	Inocu ⁵	UnIno ⁶		SPS	Inocu-le	S×IN ⁸
Experiment of corn silage particle size and inoculation levels												
Intake (kg/d)												
DM ⁹	1.44	1.53	1.64	1.62	1.48 ^b	1.63 ^a	1.53	1.57	0.15	<0.05	0.35	0.21
OM ¹⁰	1.29	1.39	1.48	1.46	1.34 ^b	1.47 ^a	1.39	1.42	0.4	<0.05	0.38	0.18
NDF ¹¹	0.53	0.57	0.63	0.63	0.55 ^b	0.62 ^a	0.58	0.60	0.06	<0.01	0.27	0.13
Digestibility (%)												
DM	68.6	70.2	67.4	68.2	69.4	67.8	70.0	69.2	0.01	0.22	0.22	0.89
OM	71.3	72.7	69.4	70.0	72.0	69.8	70.4	71.4	0.01	0.13	0.50	0.63
CP	64.6	65.8	65.6	64.1	65.2	64.9	65.9	64.9	0.02	0.83	0.94	0.54
NDF	59.2	60.2	55.7	58.1	59.7	56.9	57.5	59.1	0.03	0.26	0.45	0.72
Experiment of millet silage particle size and inoculation levels												
Intake (kg/d)												
DM	1.59 ^a	1.08 ^b	1.28 ^b	1.67 ^a	1.33 ^b	1.47 ^a	1.43	1.38	0.07	<0.01	0.20	<0.01
OM	1.48 ^a	1.01 ^b	1.20 ^b	1.56 ^a	1.25 ^b	1.38 ^a	1.34	1.28	0.06	<0.01	0.20	<0.01
Digestibility (%)												
DM	55.6	53.5	52.9	59.8	54.6	56.4	54.3	56.7	4.29	0.69	0.59	0.33
OM	62.0	54.2	68.9	65.2	53.6 ^b	67.0 ^a	65.5 ^a	55.2 ^b	3.56	<0.001	<0.05	0.11
CP	68.3	56.4	69.8	68.7	62.4	69.3	69.1	62.6	2.98	0.07	0.06	0.12

a, b, c Within row, means with common superscript (s) are not different ($P>0.05$).

¹ Each value is based on eight analytical replicates, ² Each value is based on eight analytical replicates, ³ Silage particle size, ⁴ Inoculation levels, ⁵ Inoculation, ⁶ Uninoculation, ⁷ Standard error of the mean, ⁸ Interaction between silage particle size and, inoculation levels, ⁹ Dry matter, ¹⁰ Organic matter, ¹¹ Neutral Detergent fiber.

Table 5. Effect of millet silage particle size and bacterial inoculation level on ruminal pH value¹ at different time

SPS ²	Long		Short		main effects				SEM ⁶	P-value		
Inocu-le ³	+	-	+	-	Long	Short	Inocu ⁴	UnIno ⁵		SPS	Inocu-le	S×IN ⁷
Experiment of corn silage particle size and inoculation levels												
Hours												
0	7.10	6.95	7.33	7.30	7.02	7.31	7.21	7.13	0.22	0.11	0.61	0.72
2	6.55	6.64	6.67	6.52	6.60	6.59	6.61	6.58	0.09	0.97	0.65	0.11
4	6.79	6.69	6.76	6.73	6.74	6.76	6.79	6.71	0.12	0.85	0.37	0.82
6	6.58	6.63	6.64	6.57	6.60	6.61	6.61	6.60	0.10	0.96	0.84	0.47
8	6.53	6.74	6.76	6.82	6.63	6.79	6.64	6.78	0.17	0.24	0.31	0.58
Mean	6.59	6.75	6.82	6.80	6.72	6.80	6.75	6.77	0.14	0.45	0.50	0.80
Experiment of millet silage particle size and inoculation levels												
Hours												
0	6.87	6.77	6.97	6.77	6.82	6.87	6.92 ^a	6.77 ^b	0.07	0.14	<0.01	0.71
2	6.57	6.29	6.59	6.34	6.58 ^a	6.31 ^b	6.42	6.46	0.10	<0.01	0.62	0.84
4	6.65	6.30	6.51	6.16	6.58 ^a	6.23 ^b	6.40	6.47	0.07	<0.01	0.07	0.96
6	6.67 ^a	6.58 ^a	6.50 ^b	6.54 ^b	6.62	6.58	6.52	6.55	0.09	0.41	0.21	<0.01
8	6.71	6.61	6.69	6.68	6.66	6.68	6.70	6.64	0.09	0.48	0.75	0.50
Mean	6.68	6.44	6.40	6.50	6.56 ^a	6.45 ^b	6.54	6.45	0.09	<0.01	0.91	0.20

a, b, c Within row, means with common superscript (s) are not different ($P>0.05$).

¹ Each value is based on eight analytical replicates, ² Silage particle size, ³ Inoculation levels, ⁴ Inoculation, ⁵ Uninoculation, ⁶ Standard error of the mean, ⁷ Interaction between silage particle size and inoculation levels.

In the millet silage experiment, feeding time was affected by silage particle size ($P<0.05$); sheep fed with long-particle silage spent more time feeding (247 min/day) than those fed with short-particle silage (224 min/day). Rumination time was also significantly influenced by particle size ($P<0.01$), albeit in the opposite direction: sheep consuming short-particle silage ruminated longer (426 min/day) compared to those on long-particle silage (414 min/day). Neither

feeding nor rumination time was significantly affected by inoculation or by the interaction between particle size and inoculation. Consequently, total chewing time (feeding + rumination) was not different across particle sizes, inoculation levels, or their interaction.

Table 6. Effect of millet silage particle size and bacterial inoculation level on N¹ and microbial protein synthesis²

2SPS ³	Long		Short		Main effects				P-value			
Inocu-le ⁴	+	-	+	-	long	Short	Inocu ⁵	UnIno ⁶	SEM ⁷	SPS	Inocu-le	S×IN ⁸
Experiment of corn silage particle size and inoculation levels												
Ruminal microbial Nitrogen and protein synthesis (g/d)												
Microbial nitrogen	2.81	4.57	7.98	8.01	3.68 ^b	7.98 ^a	5.39	6.29	1.06	<0.01	0.28	0.29
Microbial protein	17.5	28.5	49.8	50.0	23.0 ^b	49.9 ^a	33.7	39.3	6.66	<0.01	0.28	0.29
Experiment of millet silage particle size and inoculation levels												
Ruminal microbial Nitrogen and protein synthesis (g/d)												
Microbial nitrogen	2.22 ^b	6.49 ^a	5.21 ^{ab}	3.05 ^b	4.35	4.13	3.71	4.77	0.69	0.75	0.17	<0.001
Microbial protein	13.9 ^b	40.6 ^a	32.6 ^{ab}	19.1 ^b	25.82	27.22	29.83	23.21	6.07	0.75	0.17	<0.001

^{a, b, c} Within row, means with common superscript (s) are not different (P>0.05).

¹Each value is based on eight analytical replicates, ²Each value is based on eight analytical replicates, ³Silage particle size, ⁴Inoculation levels, ⁵Inoculation, ⁶Uninoculation, ⁷Standard error of the mean, ⁸Interaction between silage particle size and inoculation levels.

Table 7. Effect of millet silage particle size and bacterial inoculation level on feed intake behavior¹ in sheep

SPS ²	Lng		Short		Main effects				P-value			
Inocu-le ³	+	-	+	-	Long	Short	Inocu ⁴	UnInocu ⁵	SEM ⁶	SPS	Inocu-le	S×IN ⁷
Experiment of corn silage particle size and inoculation levels												
Feeding behaviors (min/d)												
Feeding time	272	271	271	302	271	286	271	287	19.7	0.20	0.20	0.45
Ruminating time	410	376	425	427	393	431	422	401	24.4	0.07	0.27	0.47
Chewing time	682	647	701	730	660 ^b	718 ^a	689	688	23.8	<0.01	0.97	0.19
Experiment of millet silage particle size and inoculation levels												
Feeding behaviors (min/d)												
Feeding time	242	253	231	236	247 ^a	224 ^b	236	244	10.4	<0.05	0.21	0.86
Ruminating time	431	397	422	430	414 ^b	426 ^a	426	414	16.5	<0.01	0.30	0.50
Chewing time	672	649	653	665	660	659	667	657	20.9	0.95	0.91	0.55

^{a, b, c} Within row, means with common superscript (s) are not different (P>0.05).

¹Each value is based on eight analytical replicates, ²Silage particle size, ³Inoculation levels, ⁴Inoculation, ⁵Uninoculation, ⁶Standard error of the mean, ⁷Interaction between silage particle size and inoculation levels.

Discussion

In the corn silage experiment, neither SPS nor LAB inoculation level had a significant effect on the concentrations of DM, CP, or NDF. This finding aligns with numerous trials and reviews indicating that the chemical composition of whole-plant corn silage particularly DM, CP, and NDF is relatively resistant to variations in chop length or LAB inoculant strain. Instead, these compositional traits are more strongly influenced by factors such as degree of maturity at harvest, hybrid type (e.g., Vitreousness), and ensiling conditions. For instance, Gomide et al. (2023) reported that particle size in ensiled corn did not alter key compositional fractions like DM, CP, or NDF. Similarly, Kok et al. (2024) in a farm-scale inoculation trial, observed improved fermentation characteristics with inoculation but no consistent changes in basic nutrient fractions at silo opening. Despite the lack of compositional differences, lower pH value was observed in short-particle corn silage, suggesting that finer chopping enhanced fermentation efficiency and acid accumulation during ensiling. This is consistent with Salvati et al. (2021), who found that finely ground corn silage exhibited a more rapid pH decline and higher lactic acid concentrations compared to coarsely chopped material.

In contrast, millet silage responded more sensitively to both particle size and inoculation. Long-particle millet silage had significantly higher NDF values, which is in agreement with Zhao et al. (2024), who reported that reducing particle size improved fiber digestibility and lowered NDF concentration likely due to greater

microbial access to plant cell walls and more efficient fermentation. Furthermore, inoculated millet silages showed reduced NDF and acid detergent fiber (ADF) concentrations, indicating that LAB inoculation promoted partial solubilization of structural carbohydrates through accelerated carbohydrate fermentation (Bao et al., 2023). Both particle size and inoculation markedly influenced silage pH in millet, with the lowest pH values observed in silages that were both finely chopped and inoculated. This reflects synergistic effects: finer particles increase substrate surface area for microbial colonization, while inoculation enhances lactic acid production and anaerobic conditions, collectively accelerating acidification (Gomide et al., 2023).

Both SPS and bacterial inoculation significantly influenced several physical characteristics of corn and millet silage-based diets. These included the proportion of DM retained on sieves of varying mesh sizes (e.g., 19 mm and 8 mm) and key particle distribution metrics such as physical effective factor and peNDF (Sharifi Hosseini et al., 2024). Diets containing long-particle silages retained more material on the coarse sieves, reflecting a higher content of structurally effective fiber. This physical trait promotes greater chewing activity and stimulates salivary secretion both of which enhance ruminal buffering capacity and help stabilize Ruminal pH (Zebeli et al., 2010).

This physiological response has been consistently demonstrated in recent research. Yang and Beauchemin (2009) reported that diets richer in peNDF increased total chewing time and improved ruminal pH stability, thereby reducing the risk of subacute ruminal acidosis

(SARA). More recently, Randby et al. (2024) observed that increasing chop length in grass silage elevated the proportion of coarse particles, which in turn enhanced chewing behavior and supported healthier Ruminant pH profiles. Together, these findings underscore the importance of optimizing silage particle size not only for nutritional formulation but also for maintaining ruminal health and overall animal performance.

In corn silage, diets containing short-particle silage significantly increased both DM and OM intake compared to long-particle diets, while bacterial inoculation had no significant effect. This pattern is consistent with previous research: Gomide et al. (2023) reported that reducing particle size in corn silage enhanced voluntary feed intake, likely due to a softer texture, improved palatability, and more rapid ruminal fermentation. The greater surface area of finely chopped particles facilitates faster microbial colonization and digesta passage, reducing rumen fill and enabling higher feed intake key factors in the high intake potential of well-processed corn silage (Huhtanen et al., 2013).

In contrast, millet silage exhibited a highly significant interaction between particle size and inoculation on DM and OM intake. Intake was highest with long-particle, inoculated silage (1.67 kg DM/day) and lowest with short-particle, uninoculated silage (1.08 kg DM/day). This non-additive interaction indicates that inoculation mitigated or even reversed the typical intake-limiting effect often associated with coarse forages. Rather than particle size alone, it was the combination of longer particles and LAB inoculation that maximized intake suggesting that inoculation improved the fermentative quality or palatability of long-particle millet silage to a degree that overcame potential physical limitations to consumption. These findings align with Zhao et al. (2024), who observed that LAB inoculation enhanced intake and fermentation characteristics of millet silage across multiple harvest stages, and with Bao et al. (2023), who demonstrated that inoculants in tropical forages increased feed intake by promoting efficient lactic acid production and suppressing detrimental fermentation products like butyric acid.

Together, these results underscore a key contrast: corn silage intake is primarily driven by physical form (with finer particles favoring higher intake), whereas millet silage intake depends on a synergistic interaction between physical structure and microbial quality. This highlights the need for forage-specific silage management strategies optimizing chop length alone may suffice for corn, but for millet, combining appropriate particle size with effective inoculation is essential to maximize feed intake and animal performance.

In corn silage, neither DM digestibility nor OMD was significantly affected by particle size or bacterial inoculation. This indicates that the observed increases in feed intake with finer chopping were not accompanied by improvements in nutrient digestibility a dissociation between intake and digestive efficiency. These results are consistent with those of Rêgo et al. (2025), who

reported no significant differences in DM or OM digestibility between inoculated and non-inoculated corn silages despite notable improvements in fermentation quality (e.g., lower pH, higher lactic acid), underscoring the robustness of corn silage digestibility to physical and microbial modifications.

In contrast, millet silage responded strongly to inoculation: diets containing LAB-inoculated millet silage exhibited significantly higher OMD compared to uninoculated silage diets. This enhancement is likely due to the more extensive and efficient fermentation driven by LAB, which partially degrades structural components such as cellulose and hemicellulose during ensiling. As noted by Grant (2010), such microbial action weakens plant cell wall integrity and increases permeability, thereby improving the exposure of intracellular nutrients to ruminal microbes. This structural modification facilitates more complete microbial breakdown in the rumen, ultimately boosting overall feed digestibility.

In corn silage, neither particle size nor LAB inoculation significantly affected final silage pH. In most cases, feed intake increases as forage particle size decreases (Schroeder et al., 2003). While higher intake stimulates salivation to buffer Ruminant pH, it also accelerates fermentation and volatile fatty acid (VFA) production, which lowers pH (Castillo-Lopez et al., 2021). Therefore, the buffering capacity of saliva is often offset by the increased acid load from VFAs. These mechanisms operate simultaneously and are not contradictory. The lack of effect of bacterial inoculation indicates that fermentation proceeded efficiently across all treatments, with sufficient lactic acid produced to achieve the target pH range (typically 3.8-4.2) required for stable aerobic preservation. These findings align with Arriola et al. (2021) and Ferraretto and Shaver (2018), who reported minimal effects of LAB inoculants on corn silage pH under well-managed ensiling conditions. Corn's high concentration of readily fermentable water-soluble carbohydrates (WSC) support rapid proliferation of indigenous LAB, enabling a swift and robust pH decline early in fermentation. Under such favorable conditions, exogenous inoculants provide little additional benefit in terms of acidification. Similarly, variations in chop length did not alter final pH, likely because particle size primarily influences physical characteristics such as packing density and digestibility rather than the extent of acid production once ample fermentable substrates are available (Sultana et al., 2023; Zhang et al., 2022).

In contrast, millet silage exhibited strong sensitivity to both particle size and inoculation in determining final pH. Shorter particle size and LAB inoculation each contributed to significantly lower pH values, with the lowest pH observed in silages that were both finely chopped and inoculated. This suggests that millet, which generally contains lower WSC and higher buffering capacity than corn, relies more heavily on optimized physical and microbial conditions to achieve adequate acidification. Finer chopping increases surface area, enhancing microbial access to limited fermentable substrates, while LAB inoculation compensates for a

potentially less competitive native microbiota, together accelerating lactic acid production and improving fermentation stability (Zhao et al., 2024; Bao et al., 2023).

In corn silage-based diets, both microbial nitrogen production and microbial protein synthesis nearly doubled when animals were fed short-particle silage compared to long-particle silage, indicating markedly more efficient microbial growth in the rumen. This aligns with findings by Ferraretto and Shaver (2018) and Khafipour et al. (2016), who demonstrated that reducing particle size enhances ruminal fiber degradability and increases the availability of fermentable energy key drivers of microbial protein synthesis.

In contrast, millet silage diets exhibited a highly significant interaction between particle size and bacterial inoculation. The greatest microbial nitrogen and protein synthesis occurred with short-particle, inoculated silage, substantially outperforming both uninoculated and long-particle treatments. This synergy suggests that combining LAB inoculation with adequate particle size reduction optimizes fermentation quality and enhances nitrogen recycling in the rumen. The resulting boost in microbial growth likely stems from improved synchronization between energy (from rapidly fermentable carbohydrates) and nitrogen supply a mechanism previously highlighted by Zhao et al. (2024) and Nadeau et al. (2023) as critical for maximizing microbial efficiency. These results confirm that reducing particle size is a strategy for enhancing rumen microbial protein synthesis in both corn and millet silage diets. However, the added benefit of inoculation is crop-dependent: while corn silage microbial output responds primarily to physical form, millet silage requires the combined action of fine chopping and LAB inoculation to unlock its full potential for microbial growth and nitrogen utilization.

In a trial involving corn silage-based diets, particle size significantly influenced total chewing time, although it had no effect on feeding or rumination time when considered individually. Sheep fed short-particle silage exhibited longer total chewing durations than those fed long-particle silage, suggesting that reduced particle size increased DM intake (Table 4), and that higher DM intake, in turn, stimulated chewing activity (Kononoff et al., 2003b). This response may be attributed to the greater bulk density and faster ruminal passage rate of smaller particles, which necessitate increased chewing effort to maintain an adequate rumen mat structure. These results align with previous findings by Ferraretto and Shaver (2018) and Yang et al. (2020), who reported that decreasing particle size in corn silage modifies chewing behavior and salivation patterns without adversely affecting total eating time.

In contrast, with millet silage, particle size significantly affected both feeding and ruminating times, while total chewing time remained unaffected. Sheep fed long-particle millet silage spent more time feeding and ruminating than those offered short-particle silage. This pattern suggests that longer particles require more

extensive mastication and additional regurgitation cycles to achieve sufficient particle size reduction for swallowing and efficient digestion. Similar behavioral adaptations to fibrous structure have been documented by Nadeau et al. (2023) and Zhao et al. (2024), who demonstrated that longer silage particles extend rumination duration to support effective fiber digestion and help prevent ruminal acidosis.

Conclusion

Corn and millet silages responded differently to particle size and bacterial inoculation. Corn silage showed no changes in DM, CP, or NDF content across treatments, but finer chopping increased DM intake and microbial protein synthesis without affecting digestibility. In contrast, millet silage was highly sensitive to both factors: long particles increased fiber content, while inoculation reduced NDF and improved OMD. Millet intake and microbial protein synthesis depended on a synergistic interaction between particle size and inoculation. Ruminal pH remained stable with corn silage but decreased with short-particle millet. Chewing behavior also differed—short corn silage increased total chewing time, whereas long millet silage prolonged feeding and rumination. These findings highlight that optimal silage management must be forage-specific: chop length alone is sufficient for corn, but millet requires integrated optimization of both particle size and bacterial inoculation to maximize its nutritional value especially in water-limited regions where millet serves as a sustainable alternative.

Conflicts of Interest

The authors declare no conflicts of interest.

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