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Effects of Citrus Pulp and Wheat Bran on Chemical Composition, Fermentation Profile, and Ruminal Degradability of Sunflower Silage

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Abstract

Sunflower (*Helianthus annuus* L.) is an alternative forage for silage production in tropical systems; however, its high moisture content and limited fermentable carbohydrates may impair fermentation and nutrient preservation. This study evaluated the effects of citrus pulp (CP) and wheat bran (WB) on the chemical composition, fermentation characteristics, dry matter losses, and ruminal degradation of sunflower silage during 21, 42, 63, and 84 days of ensiling. Sunflower forage was ensiled with control (SG), 5% CP (SG + CP), or 5% WB (SG + WB). Ruminal degradation was evaluated using the in situ nylon bag technique with three rumen-cannulated Jersey steers. Citrus pulp increased DM potential degradability (76.30 vs. 70.02%; $p < 0.001$) and effective degradability (62.17 vs. 57.88%; $p < 0.001$), while reducing the indigestible fraction (23.69 vs. 29.97%; $p < 0.001$). For NDF, CP increased potential degradability (89.55 vs. 84.87%; $p < 0.001$) and reduced the indigestible fraction (10.44 vs. 15.12%; $p < 0.001$). Wheat bran increased the DM degradation rate (0.21%/h; $p = 0.027$) and N-NH_3 accumulation. Ensiling duration affected DM degradation rate ($p = 0.010$) and effective degradability ($p = 0.013$), with the highest values at 42 days. Citrus pulp provided the most consistent improvements in ruminal utilization of sunflower silage.

Keywords: citrus pulp; ruminal degradability; sunflower silage; wheat bran; wilting additives

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1. Introduction

Sunflower (*Helianthus annuus* L.) has gained increasing attention as an alternative forage crop for silage production due to its high biomass yield, adaptability to different edaphoclimatic conditions, and tolerance to water deficit when compared with traditional silage crops such as maize and sorghum [1]. In tropical production systems, sunflower silage may represent an important strategy for forage conservation, particularly in regions characterized by irregular rainfall distribution and seasonal forage shortages.

Despite its agronomic advantages, sunflower silage presents important nutritional and fermentative limitations. The crop is frequently characterized by low dry matter

concentrations at harvest and elevated ether extract contents, which may impair fermentation, increase effluent losses, and reduce silage preservation efficiency [2]. Furthermore, sunflower forage generally exhibits high buffering capacity and limited concentrations of water-soluble carbohydrates, restricting the rapid decline in pH required for optimal ensiling conditions and favoring undesirable microbial activity [3,4]. As a consequence, these characteristics may promote proteolysis, nutrient losses, accumulation of ammonia nitrogen, and reductions in overall silage quality and animal performance [3,5].

The use of absorbent and energy-rich additives has been proposed as an effective strategy to improve the fermentation profile and nutritional value of high-moisture silages. Among the available additives, citrus pulp has received considerable attention due to its high dry matter content, abundance of readily fermentable carbohydrates, and elevated pectin concentration, characteristics that favor lactic acid production and improve silage preservation [6,7]. In addition, citrus pulp has been identified as one of the most effective agro-industrial by-products for reducing effluent losses, lowering pH, decreasing ammonia nitrogen concentrations, and enhancing the overall fermentation quality of tropical silages [8].

Citrus pulp can improve fermentation by increasing substrate availability for lactic acid bacteria while simultaneously reducing moisture content in the ensiled mass. Furthermore, pectin-rich feeds are rapidly degraded in the rumen without promoting excessive lactate accumulation, favoring ruminal stability and microbial efficiency. Previous studies have reported improvements in digestibility and reductions in fibrous fractions following citrus pulp inclusion in tropical silages [9].

Wheat bran also represents a potential additive for silage production because of its high dry matter concentration and moderate crude protein content. However, increases in crude protein concentration do not necessarily reflect improvements in nitrogen utilization efficiency, since proteolysis during ensiling may convert true protein into non-protein nitrogen compounds with lower nutritional value [10]. Therefore, evaluating the effects of wheat bran on both fermentative characteristics and ruminal degradability is essential for understanding its practical value as a silage additive.

Beyond the fermentative profile, ruminal degradation kinetics constitute an important indicator of silage nutritional quality because they directly influence nutrient availability and microbial synthesis in the rumen. Additives capable of reducing structural carbohydrates and increasing the proportion of degradable fractions may improve dry matter utilization and enhance the feeding value of sunflower silage. Nevertheless, information regarding the effects of citrus pulp and wheat bran on the degradation kinetics of sunflower silage during different ensiling periods remains limited, particularly under tropical conditions.

Therefore, this study aimed to evaluate the effects of citrus pulp and wheat bran inclusion on the chemical composition, fermentative profile, dry matter losses, and ruminal degradation kinetics of sunflower silage during different ensiling periods.

2. Materials and Methods

The experiment was conducted at the Animal Science Sector and Animal Nutrition Laboratory of the Faculty of Agricultural Sciences, Universidade Federal da Grande Dourados (UFGD), located in Dourados, Mato Grosso do Sul, Brazil (22°12′ S, 54°56′ W; altitude 452 m). According to the Köppen classification, the regional climate is classified as Cwa, characterized as humid mesothermal with rainy summers. The soil of the experimental area was classified as a very clayey dystrophic Red Latosol, according to the Brazilian Soil Classification System [11].

The sunflower hybrid used was EMBRAPA 122-V2000, characterized by early maturity (approximately 100 days), oil content ranging from 40 to 44%, average plant height of 155 cm, and capitulum diameter of approximately 18 cm [12].

Before sowing, the area was desiccated using glyphosate at 1.6 kg ha^{-1} of active ingredient combined with 2 kg ha^{-1} of boric acid (H_3BO_3, 17%). Sunflower sowing was mechanically performed 10 days after desiccation, in December 2009, using 0.90 m row spacing and a density of 4.5 viable seeds per meter. Basal fertilization at sowing consisted of 28 kg ha^{-1} of N, 70 kg ha^{-1} of P_2O_5, 70 kg ha^{-1} of K_2O, and 1 kg ha^{-1} of B supplied through the 8–20–20 fertilizer formulation. At 28 days after emergence, an additional 22 kg ha^{-1} of nitrogen was applied as urea, totaling 50 kg ha^{-1} of N during the crop cycle. Weed control was carried out manually.

Sunflower plants were harvested at 90 days of growth, corresponding to the R9 phenological stage. Plants were chopped using a forage harvester adjusted to produce particles averaging 1.5 cm in length, with a cutting height of 20 cm above ground level. After harvesting, the forage was homogenized and ensiled in 36 experimental silos made from plastic containers measuring 150 mm in diameter and 252 mm in height. The forage was compacted to achieve an approximate density of 500 kg m^{-3} of fresh matter.

Immediately after chopping, the fresh forage was mixed with additives on a fresh matter basis as follows: (1) control treatment without additive; (2) inclusion of 5% wheat bran; and (3) inclusion of 5% citrus pulp. The inclusion level of wheat bran and citrus pulp was established at 5% of the fresh forage mass based on previous studies evaluating agro-industrial by-products as additives in grass silages [13,14]. Treatments consisted of sunflower silage (SG), sunflower silage with citrus pulp (SG + CP), and sunflower silage with wheat bran (SG + WB). The chemical composition of the additives is presented in Table 1.

Table 1. Chemical composition of wheat bran and citrus pulp used as additives in sunflower silage.

Additive	DM (%)	CP (% DM)	EE (% DM)	NDF (% DM)	ADF (% DM)	Lignin (% DM)	TDN (% DM)
Wheat bran	89.30	18.50	4.50	36.70	12.10	4.20	73.30
Citrus pulp	91.00	7.00	3.70	23.00	-	-	77.00

DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; TDN: total digestible nutrients.

The silos were sealed with plastic film at both ends using galvanized wire and adhesive tape and stored in a covered facility at ambient temperature. Silos were opened after 21, 42, 63, and 84 days of fermentation.

At silo opening, approximately 60 g of silage were collected and homogenized with 50 mL of distilled water in 250 mL containers for approximately 30 min to determine pH using a digital potentiometer calibrated with buffer solutions at pH 4.0 and 7.0, according to Silva and Queiroz [15].

For ammoniacal nitrogen (NH_3-N) determination, samples were centrifuged at 3000 rpm for 15 min, and the supernatant was collected for analysis using the micro-Kjeldahl method. Distillation was performed with 2 N potassium hydroxide (KOH), with boric acid solution (2%) used as the receiving solution, followed by titration with 0.005 N hydrochloric acid, according to Detmann et al. [16].

Samples collected at each silo opening were pre-dried in a forced-air oven at 60–65 °C for 72 h and subsequently ground in a Wiley mill fitted with a 1 mm screen. Ground samples were stored in glass containers until laboratory analyses. Dry matter (DM—Method—G-003/2), crude protein (CP Method—N-001/3), and ether extract (EE Method—G-005/2) contents were determined according to the methodologies described by Detmann et al. [16].

Neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, lignin, and ash contents were determined sequentially. Neutral and acid detergent fiber analyses were performed using a fiber analyzer (Tecnal®, model TE-149, Piracicaba, SP, Brazil), employing nonwoven textile bags (5 × 5 cm; 100 g m^{-2}) as proposed by Casali et al. [17]. Hemicellulose content was calculated as the difference between NDF and ADF concentrations. Cellulose, lignin, and ash contents were determined sequentially from ADF residues through lignin extraction using permanganate (Method—F-006/3), followed by combustion in a muffle furnace at 550 °C (Method—M-001/3), according to Detmann et al. [16].

Dry matter losses as gases were quantified by the weight difference between silo sealing and opening. Gas losses were calculated according to the following equation: $GL = (WSf - WOa)/(FMf * DMf) * 100$; com: GL = gas losses (% DM); WSf = weight of the filled silo at sealing (kg); WOa = weight of the filled silo at opening (kg); FMf = fresh forage mass at ensiling (kg); DMf = dry matter concentration of forage at ensiling (%).

In vitro dry matter digestibility (IVDMD) was determined using a Tecnal® in vitro incubator (model TE-150) according to the modified Tilley and Terry [18] methodology. After 48 h of incubation, neutral detergent fiber (NDF) analysis was performed to quantify the undigested residue. Nonwoven textile bags (5 × 5 cm; 100 µm porosity) were used according to Casali et al. [17].

Ruminal degradability was evaluated at the Animal Nutrition Laboratory using three rumen-cannulated Jersey steers, approximately 24 months old, with an average body weight of 450 ± 50 kg. Animals were maintained in individual paddocks containing *Urochloa brizantha* cv. Marandu pasture, with free access to water and mineral supplementation. All animal procedures were approved by the Ethics Committee on Animal Use of the Universidade Federal da Grande Dourados (protocol 023/2015–3 CEUA/UFGD), in accordance with Brazilian guidelines for animal experimentation.

Ruminal degradability of DM and NDF was determined using the in situ nylon bag technique described by Mehrez and Ørskov [19]. Silage samples were ground through a 5 mm screen according to recommendations by Nocek [20], dried at 65 °C for 24 h, cooled in a desiccator, and weighed. Approximately 2.0 g of sample was placed in nonwoven textile bags (5 × 5 cm; 100 µm porosity), maintaining a ratio of approximately 20 mg DM cm^{-2} of bag surface area.

The bags were sealed, attached to nylon lines, and inserted into the rumen inside mesh bags containing 100 g lead weight to ensure immersion in ruminal contents. Incubation times were 0, 3, 6, 12, 24, 48, and 96 h, with bags introduced in reverse order to allow simultaneous removal, according to Ørskov and McDonald [21]. Three replicate bags per animal and incubation time were used. For the 0 h incubation time, bags were washed in water without ruminal incubation to estimate the soluble fraction.

After removal from the rumen, the bags were washed under running water until the rinse water became clear and subsequently dried in a forced-air oven at 65 °C for 48 h. The residues were weighed to determine dry matter (DM) disappearance and subsequently analyzed for neutral detergent fiber (NDF) concentration. The potentially degradable fraction of DM and NDF was estimated from the disappearance curves obtained from the incubation periods.

The ruminal disappearance data were fitted to the first-order exponential model proposed by Ørskov and McDonald [21]: $DP = a + b\ (1 - e\hat{}(-ct))$, where DP is the potential degradability; a is the soluble fraction; b is the potentially degradable insoluble fraction; c is the fractional degradation rate of fraction b; and t is the incubation time (h). The undegradable fraction (I) was estimated as $I = 100 - (a + b)$.

Effective degradability (ED) was calculated according to Ørskov and McDonald [21]: $ED = a + [(b * c)/(c + k)]$, where k represents the ruminal passage rate, assumed to be 0.05 h^{-1} for feed intake levels, respectively.

Lag time (LT, colonization time) was estimated according to Patiño et al. [22]: $LT = [-ln(a' - a - b)/c]$, where a′ is the disappearance observed at time zero. The experiment was conducted as a completely randomized design in a 3 × 4 factorial arrangement, consisting of three silage treatments and four silo opening times, with three replicates per treatment.

Statistical Analysis

All statistical analyses were performed using R Project for Statistical Computing in the RStudio environment (RStudio 2024).

Chemical composition variables (DM, CP, MM, NDF, ADF, hemicellulose, lignin, EE, IVDMD, and cellulose) and fermentation characteristics (pH, N-NH_3, gas losses, and DM losses) were analyzed considering silage type and ensiling period as fixed effects, as well as their interaction. Because the silos were opened destructively at each sampling time, measurements obtained at 21, 42, 63, and 84 days represented independent experimental units rather than repeated measurements of the same silo.

For ruminal degradation kinetics, data were analyzed in a 3 × 4 factorial arrangement consisting of three silage types (SG, SG + CP, and SG + WB) and four ensiling periods (21, 42, 63, and 84 days). Before ensiling (day 0), samples of the fresh material were collected for initial chemical composition characterization. The silos remained sealed until the respective opening periods, and ruminal degradability and other analyses were performed on samples collected at 21, 42, 63, and 84 days of ensiling.

The evaluated parameters included soluble fraction (a), potentially degradable fraction (b), degradation rate (c), potential degradability (DP), effective degradability (ED), indigestible fraction (I), and colonization time (LT).

Data from ruminal degradability were analyzed using linear mixed models considering silage type, ensiling period, and their interaction as fixed effects, and animal as a random effect. The following model was used: $Yijk = \mu + Si + Dj + (SD)ij + Ak + \varepsilon ijk$, where (Yijk) is the observed value of the dependent variable; (μ) is the overall mean; (Si) is the fixed effect of silage type; (Dj) is the fixed effect of ensiling period; ((SD)ij) is the interaction between silage type and ensiling period; (Ak) is the random effect of animal; (εijk) is the residual error.

Mixed models were fitted using the lmer function from the lme4 package, with significance determined by Type III analysis of variance using Satterthwaite approximation from the lmerTest package. Estimated marginal means were obtained using the emmeans package.

When significant effects were detected, means were compared using Tukey's test at the 5% significance level. Results are presented as least-squares mean ± standard error of the mean (SEM).

Additionally, regression analyses were performed considering ensiling days as a quantitative variable to evaluate linear responses of effective degradability and potential degradability throughout the storage period for each silage type. Regression equations and coefficients of determination (R^2) were generated when significant effects were detected ($p < 0.05$).

3. Results

3.1. Chemical Composition of Experimental Silages

No interaction between silage type and ensiling time was observed for any of the evaluated variables ($p > 0.05$); the effects of the factors are presented independently. Therefore,

Table 2 presents the means of the five storage periods (0, 21, 42, 63, 84, and 105 days) for each silage treatment, whereas Table 3 presents the means for each storage period, averaged across silage treatments. Thus, the values presented in Table 2 should be interpreted as the overall means across the five storage periods and do not correspond to a specific storage time.

Table 2. Chemical composition and in vitro dry matter digestibility of experimental silages.

Variable (% DM)	SG	SG + CP	SG + WB	SEM	*p*-Value
DM (%)	13.18 ^{b}	18.48 ^{a}	18.42 ^{a}	0.295	<0.001
CP (%)	7.59 ^{ab}	7.08 ^{b}	9.27 ^{a}	0.321	0.0236
Ash (%)	10.06 ^{a}	9.41 ^{ab}	9.11 ^{b}	0.128	0.0151
NDF (%)	53.95 ^{a}	48.15 ^{b}	53.61 ^{a}	0.576	0.0003
ADF (%)	38.24 ^{a}	34.67 ^{b}	34.60 ^{b}	0.533	0.0126
Hemicellulose (%)	15.71 ^{b}	13.47 ^{c}	19.01 ^{a}	0.338	<0.001
Cellulose (%)	17.72 ^{a}	18.11 ^{a}	12.72 ^{b}	0.691	0.0051
Lignin (%)	10.13	8.65	8.63	0.310	0.0925
EE (%)	7.14	6.62	7.03	0.112	0.1497
IVDMD (%)	70.00 ^{a}	67.99 ^{b}	69.61 ^{a}	0.336	0.0483

SG: sunflower silage; SG + CP: sunflower silage with citrus pulp; SG + WB: sunflower silage with wheat bran. SEM = standard error of the mean. Means followed by different lowercase letters within the same row differ according to Tukey's test ($p < 0.05$). Abbreviations: DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract; IVDMD, in vitro dry matter digestibility.

Table 3. Effect of ensiling days on chemical composition and in vitro dry matter digestibility of silages.

Variable	Day 0	Day 21	Day 42	Day 63	Day 84	SEM	*p*-Value
DM (%)	15.25	16.49	16.98	17.94	16.80	0.295	0.0982
CP (%)	9.78 ^{a}	7.16 ^{ab}	9.49 ^{a}	7.28 ^{ab}	6.18 ^{b}	0.321	0.0040
Ash (%)	9.41	9.43	9.89	9.23	9.68	0.128	0.5344
NDF (%)	55.44 ^{a}	55.93 ^{a}	46.78 ^{c}	48.85 ^{bc}	52.52 ^{ab}	0.576	<0.0001
ADF (%)	38.55 ^{a}	38.77 ^{a}	29.79 ^{b}	34.80 ^{a}	37.29 ^{a}	0.533	<0.0001
Hemicellulose (%)	16.89 ^{a}	17.15 ^{a}	17.00 ^{a}	14.05 ^{b}	15.23 ^{b}	0.338	0.0243
Cellulose (%)	8.84 ^{b}	19.81 ^{a}	17.48 ^{a}	15.98 ^{a}	18.81 ^{a}	0.691	0.0002
Lignin (%)	11.02 ^{a}	9.52 ^{a}	5.36 ^{b}	10.12 ^{a}	9.67 ^{a}	0.310	<0.0001
EE (%)	4.66 ^{c}	8.21 ^{a}	7.24 ^{bc}	6.98 ^{b}	7.55 ^{bc}	0.112	<0.0001
IVDMD (%)	69.18	67.76	70.92	69.05	69.10	0.336	0.0888

SEM = standard error of the mean. Means followed by different lowercase letters within the same row differ according to Tukey's test ($p < 0.05$). Abbreviations: DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract; IVDMD, in vitro dry matter digestibility.

The chemical composition of the sunflower silages is presented in Table 2. Silage type affected DM, CP, ash, NDF, ADF, hemicellulose, IVDMD, and cellulose ($p < 0.05$). SG had a lower DM concentration than SG + CP and SG + WB ($p < 0.001$). CP concentration was highest in SG + WB, whereas SG and SG + CP showed lower values ($p = 0.0236$). Ash concentration differed among treatments, with SG presenting greater values than SG + WB, and SG + CP showing intermediate values ($p = 0.0151$).

NDF concentration was lower in SG + CP than in SG and SG + WB ($p = 0.0003$). A similar response was observed for ADF, with lower concentrations in SG + CP and SG + WB compared with SG ($p = 0.0126$). Hemicellulose concentration differed among all treatments, increasing from SG + CP (13.5% DM) to SG (15.7% DM) and SG + WB (19.0% DM) ($p < 0.001$). In contrast, lignin and EE were not affected by silage type ($p > 0.05$).

Differences were also observed for IVDMD and cellulose concentrations. SG showed the greatest IVDMD value (70.0%), SG + CP the lowest (68.0%), and SG + WB an intermediate value (69.6%) ($p = 0.0483$). Cellulose concentration was lower in SG + WB than in SG and SG + CP ($p = 0.0051$).

Ensiling time affected CP, NDF, ADF, hemicellulose, lignin, ether extract, and cellulose ($p < 0.05$), whereas DM, MM, and IVDMD were not altered throughout the fermentation period ($p > 0.05$) (Table 3). DM remained relatively stable during ensiling, varying from 15.25 to 17.94%, while ash showed only minor fluctuations among sampling days.

Crude protein concentration was affected by ensiling time ($p = 0.0040$), with higher values observed on days 0 and 42 (9.78 and 9.49%, respectively) and the lowest value observed on day 84 (6.18%). The fiber fractions also changed during fermentation. NDF decreased from 55.93% on day 21 to 46.78% on day 42 ($p < 0.001$), while ADF followed a similar pattern, declining from 38.77 to 29.79% over the same period ($p < 0.001$). Cellulose concentration was lower in the fresh forage (8.84%) than in the ensiled material, increasing to 19.81% at 21 days ($p = 0.0002$). Thereafter, cellulose concentration remained between 15.98 and 18.81% until 84 days. Likewise, lignin reached its lowest concentration on day 42 (5.36%) and was greater on all other evaluation days ($p < 0.001$). In contrast, hemicellulose showed relatively small variations, with lower values observed on days 63 and 84 compared with the remaining fermentation periods ($p = 0.0243$).

EE increased during the fermentation process, rising from 4.66% at day 0 to a maximum of 8.21% at day 21 ($p < 0.001$). Although IVDMD was not significantly affected by ensiling time ($p = 0.0888$), values tended to increase during fermentation, reaching 70.92% on day 42.

3.2. Fermentation Profile and Dry Matter Losses of Silages

Ammoniacal nitrogen (N-NH_3, % total N) was the only variable that showed a tendency for interaction between silage type and ensiling time ($p = 0.0632$; Figure 1). Therefore, the effects of silage type at each fermentation period and the effects of fermentation time on each silage were examined independently.

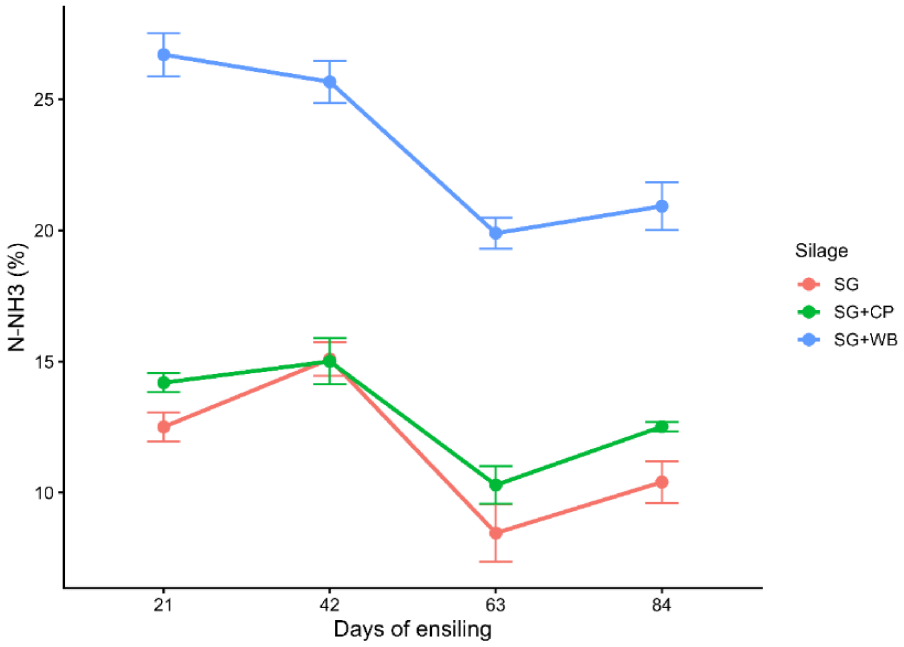


Figure 1. Changes in ammonia nitrogen (N-NH3) concentration of sunflower silage (SG), sunflower silage with citrus pulp (SG + CP), and sunflower silage with wheat bran (SG + WB) during the ensiling period ($p = 0.0632$).

The fermentation profile and DM losses of the sunflower silages are presented in Table 4. Among the evaluated variables, only N-NH_3 was affected by silage treatment ($p < 0.001$).

Table 4. Fermentation characteristics and losses of sunflower silage according to additive treatment.

Variable	SG	SG + CP	SG + WB	SEM	*p*-Value
pH	4.89	4.75	4.66	0.059	0.2948
Ammonia nitrogen (N-NH_3, % total N)	11.62 ^{b}	13.00 ^{b}	23.30 ^{a}	0.64	<0.001
Gas losses (% DM)	25.45	32.26	29.46	2.389	0.5147
Dry matter losses (% DM)	21.58	22.25	22.17	1.871	0.9875

SG: sunflower silage; SG + CP: sunflower silage with citrus pulp; SG + WB: sunflower silage with wheat bran. SEM = standard error of the mean. Means followed by different lowercase letters within a row differ according to Tukey's test ($p < 0.05$).

The pH values were similar among silages, ranging from 4.66 to 4.89 ($p = 0.2948$), indicating that the inclusion of citrus pulp or wheat bran did not alter the overall acidification pattern during fermentation. In contrast, N-NH_3 concentrations differed among treatments. Sunflower silage supplemented with wheat bran exhibited the highest N-NH_3 concentration (23.30% of total N), whereas sunflower silage and sunflower silage supplemented with citrus pulp showed lower and similar values, averaging 11.62 and 13.00% of total N, respectively.

Neither gas losses (% DM) nor dry matter losses (% DM) were affected by silage additives ($p > 0.05$), indicating similar fermentation efficiency and dry matter recovery among treatments.

The fermentation period significantly affected pH ($p < 0.001$), whereas DM losses and gas losses were not influenced by ensiling time ($p > 0.05$) (Table 5). The pH decreased sharply from 6.13 at day 0 to 4.17 and 4.19 at 21 and 42 days of ensiling, respectively. Thereafter, pH increased slightly to 4.59 and 4.76 at 63 and 84 days, respectively, but remained substantially lower than the initial value.

Table 5. Effect of ensiling days on fermentation characteristics and losses of silages.

Variable	Day 0	Day 21	Day 42	Day 63	Day 84	SEM	*p*-Value
pH	6.13 ^{a}	4.17 ^{c}	4.19 ^{c}	4.59 ^{ab}	4.76 ^{b}	0.059	<0.0001
Dry matter losses (%)	—	19.56	23.89	21.22	23.33	1.871	0.8365
Gas losses (%)	—	24.70	31.10	29.37	31.07	2.389	0.7553

Values represent the overall mean of all silage treatments (SG, SG + CP, and SG + WB) at each ensiling time. SEM = standard error of the mean. Means followed by different lowercase letters within the same row differ according to Tukey's test ($p < 0.05$).

No significant differences were observed for DM losses ($p = 0.8365$) or gas losses ($p = 0.7553$). Dry matter losses ranged from 19.56 to 23.89%, whereas gas losses varied from 24.70 to 31.10% throughout the fermentation period.

3.3. Effects of Silage Type and Ensiling Period on Ruminal Degradability

Significant silage × storage period interactions were observed for several degradability parameters, indicating that the effects of additive inclusion changed throughout the ensiling process ($p < 0.05$). To facilitate visualization of these responses, the main interaction patterns are presented in Figures 1–5.

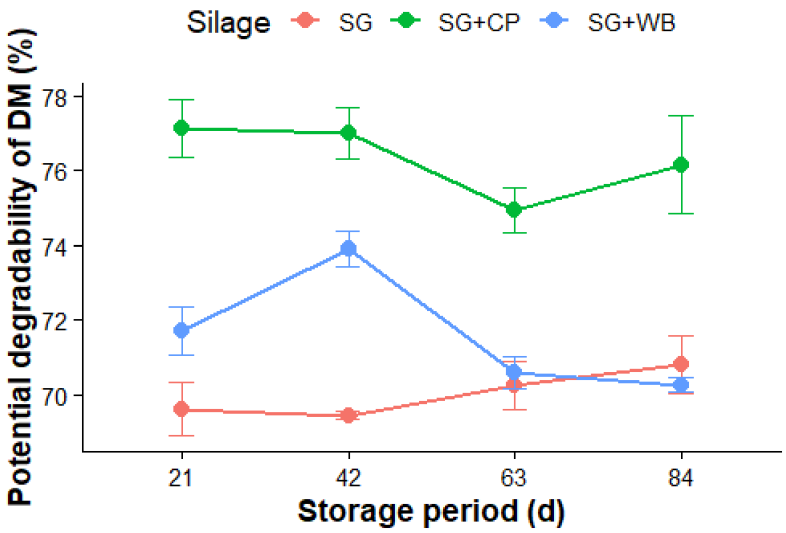


Figure 2. Interaction effect between silage type and storage period on DM potential degradability.

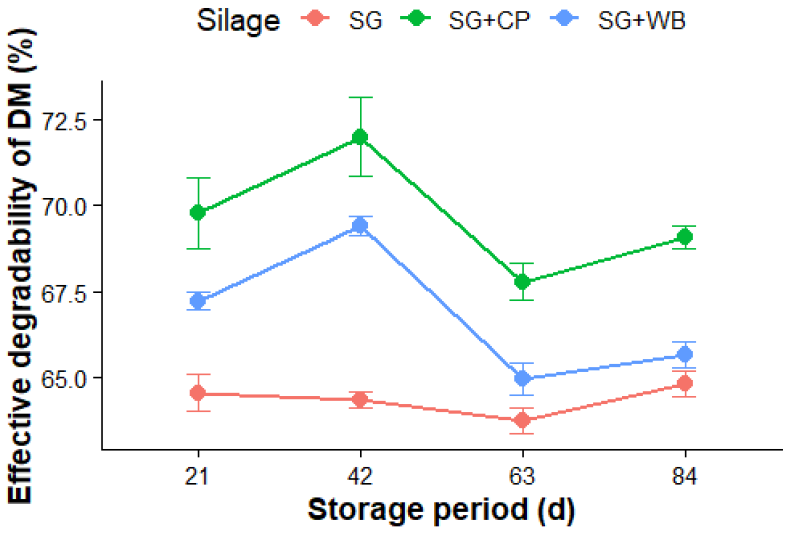


Figure 3. Interaction effect between silage type and storage period on DM effective degradability.

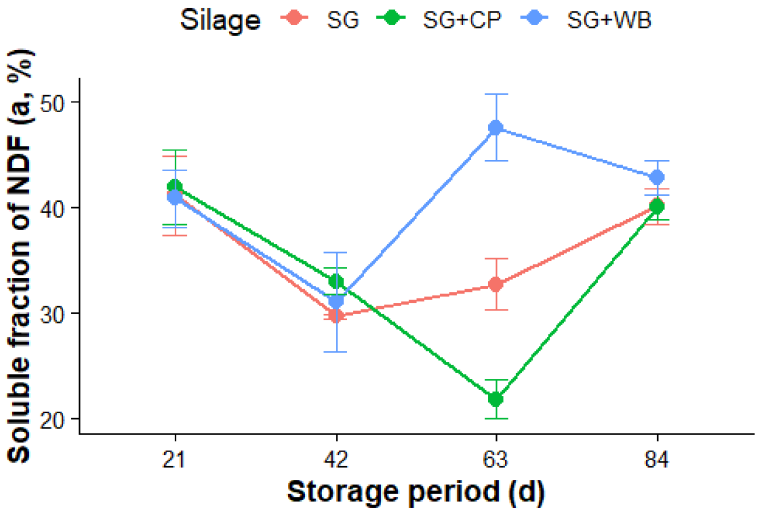


Figure 4. Interaction effect between silage type and storage period on the soluble fraction of NDF.

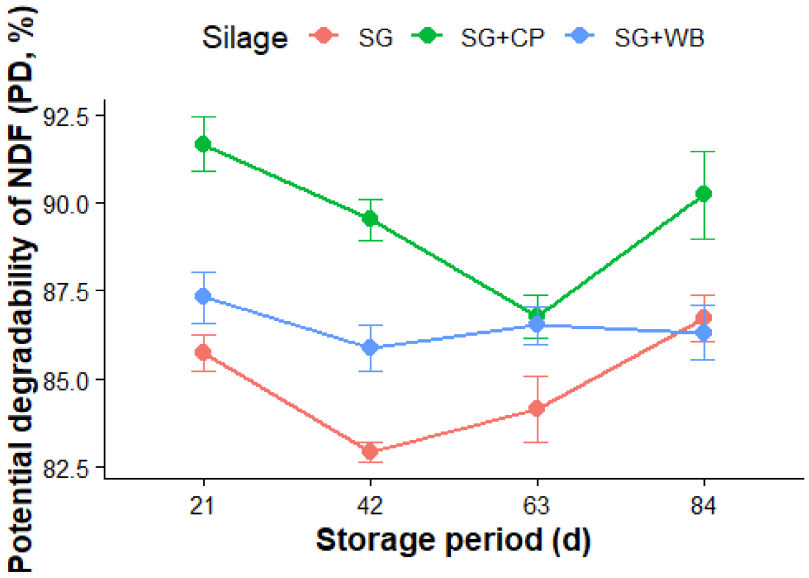


Figure 5. Interaction effect between silage type and storage period on NDF potential degradability.

The interaction for DM degradation kinetics was characterized by different temporal responses among silage formulations. Both DM potential degradability (p = 0.021; Figure 2) and DM effective degradability (p = 0.014; Figure 3) remained higher in SG + CP throughout the storage period, whereas SG and SG + WB showed different temporal patterns, particularly across the intermediate storage periods. Notably, the differences among silages were greatest during the early stages of storage and became less evident after prolonged ensiling.

For NDF degradation characteristics, the interaction was particularly evident for the soluble fraction ($p < 0.001$; Figure 4), which exhibited contrasting trajectories among silages during storage. The greatest divergence among treatments occurred at 63 d of ensiling, reflecting distinct patterns of fiber modification during fermentation. Similar interaction patterns were observed for NDF potential degradability (p = 0.029; Figure 5) and effective degradability at a passage rate of 0.08 h^{-1} (p = 0.003; Figure 6). In general, SG + CP maintained greater NDF degradability during the initial stages of storage, whereas SG + WB showed greater stability in degradability parameters over time.

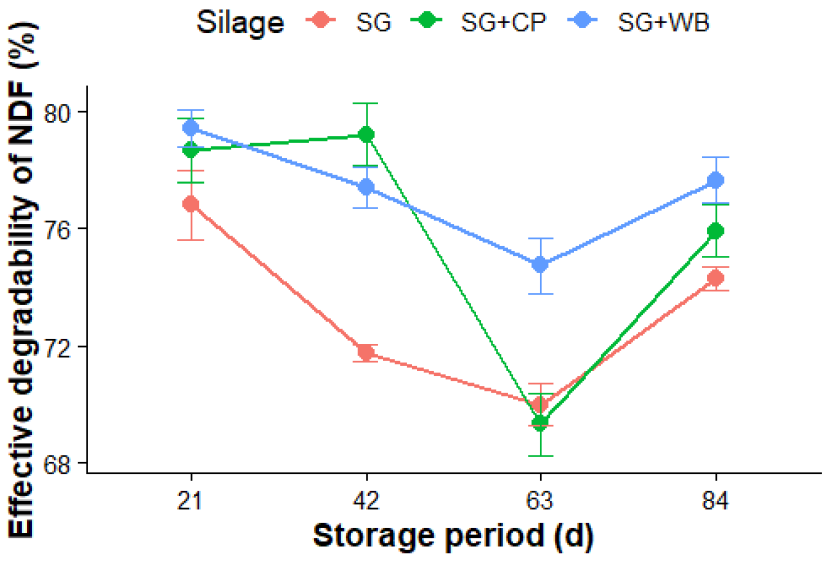


Figure 6. Interaction effect between silage type and storage period on NDF effective degradability.

The inclusion of citrus pulp (CP) and wheat bran (WB) affected several ruminal degradation characteristics of DM and NDF (Table 6). For DM, the soluble fraction (a) was not affected by the treatments ($p = 0.931$), whereas the potentially degradable fraction (b) differed among treatments ($p = 0.004$). The SG + CP treatment resulted in the highest b fraction (59.38%), compared with SG (52.41%) and SG + WB (54.31%). The degradation rate (c) was also affected by treatment ($p = 0.027$), with the highest value observed for SG + WB (0.21%/h), while SG + CP showed the lowest value (0.16%/h); SG presented an intermediate value (0.17%/h).

Table 6. Main effects of silage type on dry matter and neutral detergent fiber degradability parameters.

Variable	SG	SG + CP	SG + WB	SEM	*p*-Value
DM					
a	17.61	16.93	17.31	0.724	0.931
b	52.41 ^{b}	59.38 ^{a}	54.31 ^{b}	0.936	0.004
c	0.17 ^{ab}	0.16 ^{b}	0.21 ^{a}	0.008	0.027
DP	70.02 ^{c}	76.30 ^{a}	71.61 ^{b}	0.509	0.000
ED	57.88 ^{b}	62.17 ^{a}	61.09 ^{a}	0.782	0.000
I	29.97 ^{a}	23.69 ^{c}	28.38	0.509	0.000
TC	5.74 ^{ab}	5.89 ^{a}	5.56 ^{b}	0.044	0.005
NDF					
a	35.88	34.15	40.51	1.330	0.128
b	49.00 ^{ab}	55.40 ^{a}	46.00 ^{b}	1.260	0.005
c	0.16	0.15	0.21	0.011	0.054
DP	84.87 ^{b}	89.55 ^{a}	86.50 ^{b}	0.437	0.000
ED	73.20 ^{b}	75.77 ^{ab}	77.28 ^{a}	0.600	0.015
I	15.12 ^{a}	10.44 ^{b}	13.49 ^{a}	0.438	0.000
TC	5.70 ^{ab}	5.88 ^{a}	5.42 ^{b}	0.060	0.005

Means within a row followed by different lowercase letters differ significantly according to Tukey's test ($p < 0.05$). SEM = standard error of the mean; a = soluble fraction, immediately degradable; b = potentially degradable fraction; c = degradation rate of fraction b (%/h); DP = potential degradability; ED = effective degradability; I = indigestible fraction; TC = colonization time (h).

Potential degradability (PD) of DM differed among treatments ($p < 0.001$), with the highest value observed for SG + CP (76.30%), followed by SG + WB (71.61%) and SG (70.02%). Effective degradability (ED) was also affected by treatment ($p < 0.001$). Both SG + CP (62.17%) and SG + WB (61.09%) showed higher ED than SG (57.88%). Conversely, the indigestible fraction (I) was lower for SG + CP (23.69%) than for SG (29.97%), whereas SG + WB showed an intermediate value (28.38%). Colonization time (CT) differed among treatments ($p = 0.005$), with the longest time observed for SG + CP (5.89 h), the shortest for SG + WB (5.56 h), and an intermediate value for SG (5.74 h).

For NDF, the soluble fraction (a) was not affected by treatment ($p = 0.128$), whereas the potentially degradable fraction (b) differed among treatments ($p = 0.005$). The SG + CP treatment resulted in the highest b fraction (55.40%), while SG + WB showed the lowest value (46.00%); SG presented an intermediate value (49.00%). The degradation rate (c) did not differ among treatments ($p = 0.054$), although numerically it was higher for SG + WB (0.21%/h) than for SG (0.16%/h) and SG + CP (0.15%/h).

Potential degradability of NDF was affected by treatment ($p < 0.001$), with SG + CP showing the highest value (89.55%), whereas SG (84.87%) and SG + WB (86.50%) had lower values. Effective degradability also differed among treatments ($p = 0.015$), with the highest value observed for SG + WB (77.28%), followed by SG + CP (75.77%) and SG (73.20%). The indigestible fraction of NDF was lower in SG + CP (10.44%) than in SG (15.12%) and SG + WB (13.49%) ($p < 0.001$). Colonization time differed among treatments ($p = 0.005$),

with SG + CP presenting the longest time (5.88 h), SG + WB the shortest (5.42 h), and SG an intermediate value (5.70 h).

The ruminal degradation characteristics of DM and NDF were affected differently by ensiling duration (Table 7). For DM, ensiling duration did not affect the soluble fraction (a), the potentially degradable fraction (b), potential degradability (PD), indigestible fraction (I), or colonization time (CT) ($p > 0.05$). The soluble fraction ranged from 14.80 to 19.78%, whereas the potentially degradable fraction ranged from 52.15 to 58.65%. Similarly, PD remained relatively constant among ensiling periods, ranging from 71.92 to 73.45%, and the indigestible fraction ranged from 26.55 to 28.07%.

Table 7. Effects of storage period on ruminal degradability parameters of dry matter (DM) and neutral detergent fiber (NDF).

Variable	21 d	42 d	63 d	84 d	SEM	*p*-Value
DM						
a	16.82	14.80	19.78	17.73	1.380	0.101
b	55.99	58.65	52.15	54.67	1.770	0.093
c	0.19 ^{ab}	0.22 ^{a}	0.14 ^{b}	0.17 ^{ab}	0.014	0.010
DP	72.82	73.45	71.92	72.41	1.046	0.768
ED	60.63 ^{ab}	62.64 ^{a}	58.44 ^{b}	59.81 ^{ab}	0.854	0.013
I	27.18	26.55	28.07	27.59	1.046	0.768
TC	5.70	5.57	5.88	5.76	0.084	0.089
NDF						
a	41.26 ^{a}	31.19 ^{b}	34.01 ^{ab}	40.93 ^{a}	2.307	0.007
b	46.98	54.92	51.81	46.83	2.355	0.056
c	0.20 ^{ab}	0.22 ^{a}	0.13 ^{b}	0.16 ^{ab}	0.018	0.006
DP	88.24	86.10	85.81	87.75	0.838	0.127
ED	78.29 ^{a}	76.11 ^{a}	71.34 ^{b}	75.94 ^{a}	0.879	0.000
I	11.76	13.89	14.18	12.24	0.840	0.127
TC	5.49 ^{b}	5.49 ^{b}	5.98 ^{a}	5.71 ^{ab}	0.103	0.005

Means within a row followed by different lowercase letters differ significantly according to Tukey's test ($p < 0.05$). SEM = standard error of the mean; a = soluble fraction, immediately degradable; b = potentially degradable fraction; c = degradation rate of fraction b (%/h); DP = potential degradability; ED = effective degradability; I = indigestible fraction; TC = colonization time (h).

The degradation rate (c) of DM was affected by ensiling duration ($p = 0.010$). The highest value was observed at 42 days (0.22%/h), while the lowest value occurred at 63 days (0.14%/h). The values at 21 (0.19%/h) and 84 days (0.17%/h) were intermediate. Effective degradability (ED) was also affected by ensiling duration ($p = 0.013$), with the highest value observed at 42 days (62.64%), followed by 21 (60.63%) and 84 days (59.81%), whereas the lowest value was observed at 63 days (58.44%).

For NDF, ensiling duration affected the soluble fraction (a) and degradation rate (c) ($p < 0.01$). The soluble fraction was higher at 21 and 84 days (41.26 and 40.93%, respectively) than at 42 days (31.19%), while the value at 63 days (34.01%) was intermediate. The degradation rate was highest at 42 days (0.22%/h) and lowest at 63 days (0.13%/h), with intermediate values at 21 (0.20%/h) and 84 days (0.16%/h).

The potentially degradable fraction (b), PD, and indigestible fraction (I) of NDF were not affected by ensiling duration ($p > 0.05$). Potential degradability ranged from 85.81 to 88.24%, while the indigestible fraction ranged from 11.76 to 14.18%. In contrast, ED was significantly affected by ensiling duration ($p < 0.001$), with the highest value at 21 days (78.29%) and lower values at 42 (76.11%) and 84 days (75.94%), while the lowest ED was observed at 63 days (71.34%).

Colonization time of NDF was also affected by ensiling duration (p = 0.005). The longest colonization time occurred at 63 days (5.98 h), whereas the shortest times were observed at 21 and 42 days (5.49 h). The value at 84 days (5.71 h) was intermediate.

4. Discussion

Dry matter (DM) content is an important parameter in silage production because the incorporation of dry, moisture-absorbing additives can increase the DM concentration of the ensiled mass and contribute to more suitable fermentation conditions [23]. The observed increase in dry matter (DM) content in silages supplemented with citrus pulp or wheat bran underscores the capacity of these moisture-absorbing additives to optimize the ensiling process [8,24]. In sunflower silage, moisture control is particularly critical because excessive water activity promotes clostridial development, proteolysis, and nutrient losses through effluent runoff [25,26]. High-DM agro-industrial by-products generally improve fermentation kinetics by lowering water activity and increasing the availability of fermentable substrates for lactic acid bacteria. In this regard, Freitas et al. [8] highlighted citrus pulp as an effective additive for tropical silages due to its hygroscopic properties and substantial concentration of readily fermentable carbohydrates.

The reduction in CP concentration occurred without a significant change in DM concentration (p = 0.0982), indicating that it was not primarily associated with DM variation. Therefore, the decline in CP may be related to the progressive hydrolysis of true protein into peptides, amino acids, and non-protein nitrogen compounds during ensiling. The increase in NH_3-N from 63 to 84 days, particularly in the SG and SG + CP silages, corroborates the continuation of proteolytic activity during prolonged storage.

Wheat bran increased CP concentration, as expected due to its higher nitrogen content compared with sunflower forage; however, this increase was not accompanied by better protein preservation. The higher NH_3-N concentration indicates greater conversion of protein nitrogen into non-protein nitrogen during fermentation [23]. Thus, wheat bran increased the silage CP concentration, but a portion of this nitrogen was redistributed into the soluble and ammonia-N fractions during storage.

The reduction in neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations observed in citrus pulp silages is primarily associated with the chemical composition of this by-product. Citrus pulp contains low levels of structural carbohydrates and a high concentration of soluble carbohydrates, particularly pectin, which exerts a dilution effect on the fibrous fraction of the forage.

The lower NDF and ADF concentrations observed with citrus pulp supplementation are primarily explained by the compositional effect of replacing part of the sunflower forage with a low-fiber, pectin-rich ingredient. Therefore, these reductions should be interpreted mainly as a dilution effect rather than as evidence of direct fiber solubilization during ensiling. Similar reductions in structural carbohydrate concentrations have been reported in elephant grass silages supplemented with citrus pulp, which were also associated with greater digestibility [24]. Citrus pulp contains high concentrations of soluble carbohydrates and pectin and relatively low concentrations of structural carbohydrates, thereby reducing the proportional contribution of the fibrous fraction to the final silage [6].

The variation in NDF and ADF concentrations across ensiling periods should be interpreted with caution. Changes in the relative concentration of nutrients during fermentation and dry matter losses can alter the measured concentration of individual fractions without necessarily indicating their direct degradation. In the present study, the marked variation in cellulose concentration, particularly the increase from 8.84% in the fresh forage to 19.81% at 21 days, should therefore be interpreted as a change in the concentration of this fraction rather than as evidence of cellulose formation during ensiling. Because individual struc-

tural carbohydrate disappearance was not directly measured, no specific mechanism for these temporal changes can be established.

This pattern was evident in wheat bran silages, where elevated N-NH_3 concentrations were detected despite apparently adequate pH values. Increased ammonia nitrogen concentrations reflect intense proteolytic activity during fermentation, resulting in the conversion of true protein into NPN compounds. Higher N-NH_3 levels generally indicate lower preservation of the original protein fraction and reduced nitrogen retention by the animal [10,25].

The absence of significant differences in gas and DM losses among treatments indicates that neither additive altered the overall magnitude of fermentative losses. Both additives were able to maintain adequate anaerobic conditions without compromising DM retention. In tropical silages, reductions in fermentative losses are commonly associated with a faster decline in pH and improved compaction promoted by dry additives [26]. Nevertheless, the similarity among treatments suggests that the control silage also achieved satisfactory fermentative stability under the conditions evaluated.

The inclusion of citrus pulp (CP) and wheat bran (WB) resulted in distinct changes in the ruminal degradation kinetics of sunflower silage. Citrus pulp increased the potentially degradable fraction and potential degradability of DM and NDF, while reducing the indigestible fraction. In contrast, wheat bran mainly increased the degradation rate and resulted in the highest effective degradability of NDF. Thus, CP appeared to increase the extent of degradation, whereas WB mainly affected the rate of substrate utilization.

The greater degradability observed with CP is consistent with its high concentration of soluble carbohydrates and pectin, which are readily fermented by ruminal microorganisms [6]. Recent evidence confirms the high nutritional value and fermentability of citrus pulp, although its effects on animal responses depend on inclusion level and dietary composition [27,28]. In the present study, the increase in DM potential degradability from 70.02% in SG to 76.30% in SG + CP, together with the reduction in the indigestible fraction from 29.97 to 23.69%, indicates a greater proportion of substrate available for ruminal degradation. A similar response was reported when pelleted citrus pulp was included during the ensiling of elephant grass, resulting in a linear increase in in vitro DM digestibility [9].

Wheat bran showed a different response. Although SG + WB had a lower potentially degradable DM fraction than SG + CP, it presented the highest degradation rate of DM (0.21%/h) and the highest effective degradability of NDF (77.28%). The numerical increase in NDF degradation rate should be interpreted cautiously because it did not reach statistical significance ($p = 0.054$). Therefore, the main contribution of WB was associated with faster utilization of the degradable fraction rather than a consistent increase in its degradation potential.

Ensiling duration affected degradation kinetics less consistently than silage type. Potential degradability and the indigestible fraction of DM and NDF remained relatively stable throughout storage, whereas degradation rate and effective degradability varied among periods. The highest degradation rates occurred at 42 days, while the lowest values were observed at 63 days. The reduction in NDF effective degradability and the increase in colonization time at 63 days suggest reduced accessibility of the fibrous substrate during this period. However, the stability of potential degradability indicates that these changes did not result in a substantial loss of the degradable fraction.

Overall, citrus pulp produced the most consistent improvement in the ruminal degradation characteristics of sunflower silage, increasing the degradable fraction and reducing the indigestible fraction. Wheat bran, in turn, primarily increased the degradation rate. These responses indicate that the two additives modified ruminal utilization through different mechanisms, with CP increasing the overall availability of fermentable substrate and

WB favoring its faster utilization. Therefore, citrus pulp appears to be a promising additive for improving the nutritional utilization of sunflower silage.

5. Conclusions

The inclusion of citrus pulp and wheat bran differently affected the fermentation and ruminal degradation characteristics of sunflower silage. Citrus pulp showed the most consistent improvements, increasing DM content, potential and effective degradability, and reducing the indigestible fractions of DM and NDF without increasing proteolysis. Wheat bran increased CP concentration and favored degradation rate, but was also associated with greater N-NH_3 accumulation and did not consistently improve the extent of ruminal degradation. Ensiling duration mainly affected degradation kinetics, while the potential degradability and indigestible fractions remained relatively stable. Overall, citrus pulp was the most promising additive for improving the nutritional utilization of sunflower silage and represents a suitable strategy for its use in ruminant feeding.

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Data Availability Statement: All data supporting the findings of this study are included in the published article.

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