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Autumn grass treated with a hydrolysable tannin extract versus lactic acid bacteria inoculant: Effects on silage fermentation characteristics and nutritional value and on performance of lactating dairy cows

Tine Van den Bossche[1] | Karen Goossens[1] | Geert Haesaert[2] | Eva Wambacq[3] | Leen Vandaele[1] | Johan L. De Boever[1]

[1]Flanders Research Center for Agriculture, Fisheries and Food (ILVO), Melle, Belgium

[2]Department of Plants and Crops, Ghent University, Gent, Belgium

[3]AgroFoodNature, HOGENT, Bottelare, Belgium

Correspondence

Karen Goossens, Flanders Research Center for Agriculture, Fisheries and Food (ILVO), Scheldeweg 68, 9090 Melle, Belgium.
Email: Karen.Goossens@ilvo.vlaanderen.be

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Abstract

Hydrolysable tannins (HT) show potential as silage additive for autumn herbage silages, high in (rumen degradable) protein, as they may reduce proteolysis. Additionally, they have abilities to form pH-reversible tannin–protein complexes, non-degradable in the rumen but degradable in the abomasum and intestines of ruminants. Therefore they can improve milk N efficiency and shift N excretions from urine to faeces, possibly mitigating the environmental impact of ruminants. In this study, two small bunker silos were filled with autumn grass. One was treated with 20 g/kg DM HT extract (TAN) (TannoSan-L), the other with 8 mg/kg DM inoculant containing lactic acid bacteria (INO) (Bonsilage Fit G). Secondly, micro-silos (2.75 L) were filled with four treatments; (1) grass without additive (CON) ($n = 5$); (2) TAN ($n = 5$); (3) INO ($n = 5$); and (4) TAN + INO ($n = 5$). The bunker silos were used in a cross-over feeding experiment with periods of 4 weeks involving 22 lactating Holstein cows (average ± SD: 183 ± 36.3 days in milk, 665 ± 71.0 kg body weight, and 33.8 ± 3.91 kg/day milk yield). The HT dose was insufficient to reduce proteolysis or alter chemical composition and nutritional value in the micro- and bunker silages. Including grass silage added with TAN (3.2 g HT/kg DM) in the diet, did not affect feed intake nor fat and protein corrected milk yield in comparison to feeding the grass silage added with INO in a similar diet. The TAN-fed cows had an increased faecal N excretion and decreased apparent total-tract N and organic matter digestibility, but no improvement in the cows' N utilization could be confirmed in milk and blood urea levels. Overall, feeding an autumn grass silage treated with 20 g/kg chestnut HT extract did not affect the performance of dairy cows in comparison to feeding an autumn grass silage treated with a lactic acid bacteria inoculant.

KEYWORDS
autumn herbage, dairy cows, homo- and heterofermentative lactic acid bacteria, hydrolysable tannins, silage additive

1 | INTRODUCTION

The chemical composition of herbage varies during the growing season. Autumn herbage, in comparison to spring herbage, has less sugar and fibre and a higher crude protein (CP) and fat content (Hurley et al., 2021; Larsen et al., 2016). In the temperate climate of Belgium, the herbage season approaches its end in October. By then, grasslands have been mowed and harvested four to five times, depending on weather conditions during the growing season. Many dairy farmers in Belgium consider autumn herbage silage to be of poorer quality as compared to spring herbage silage. This can be related to the lower sugar content, as sugars are important for a fast and intense silage fermentation. Further, the wet autumn mowing conditions make it difficult to wilt autumn herbage to the desired dry matter (DM) content. Both factors jeopardize the palatability of the silage and result in lower uptake by cattle (Hurley et al., 2021; Larsen et al., 2016). Improving the protein quality of autumn herbage may be a promising strategy on dairy farms for two reasons. First, autumn herbage generally contains large amounts of CP and rumen degradable protein (RDP) (O'Connor et al., 2019; Pontes et al., 2007). Highly productive cows with access to adequate herbage of high nutritive value can achieve milk production with lower use of concentrates (Peyraud, 2017). Second, global warming and summer droughts have adverse effects on summer plant growth, increasing the importance of qualitative autumn herbage cuts (Doležal et al., 2022; Schils et al., 2020; Tello-García et al., 2020).

Silage additives may help to improve the preservation and quality of autumn herbage silages. Tannins are natural polyphenolic secondary plant metabolites, which have the property to form pH-reversible tannin–protein complexes (pH 3.5–7) that cannot be degraded in the rumen, but can be degraded in the acidic abomasum (pH ≤ 3.5) and the intestines (pH > 7) of ruminants (Bunglavan & Dutta, 2013; Frutos et al., 2004; Min et al., 2003). Multiple experiments, the majority of which focus on condensed tannins (CT), have shown beneficial effects on health, production and protein utilization of cattle that receive tannins as feed additive to a maximum dose of 50 g CT/kg DM in feed (Jayanegara et al., 2012; Min et al., 2003; Mueller-Harvey, 2006). Hydrolysable tannins (HT) show potential to be used as silage additive. Numerous studies describe a reduction of ammonia (NH_3) concentration in silages added with HT (Herremans, Vanwindekens, et al., 2020; Jayanegara et al., 2019; Tabacco et al., 2006). This indicates that HT could be an interesting additive to assure protein quality in autumn herbage silages, which are typically rich in RDP.

Furthermore, tannins can improve the milk nitrogen efficiency (MNE) of dairy cows by reducing CP degradation in the rumen and increasing the duodenal protein flux (Frutos et al., 2004). Several studies describe a decrease in urinary nitrogen (N) output and a lower milk urea N (MUN) content when feeding tannins, but sometimes a decrease in the N apparent total-tract digestibility has also been found, suggesting a shift from urinary to faecal N excretions (Aguerre et al., 2016; Henke et al., 2017). This is an interesting property of tannins, as dairy cows have a rather poor MNE of on average 24.7 ± 4.1% (Huhtanen & Hristov, 2009). The excess N is excreted through urine and faeces, potentially causing pollution to the environment. Nitrogen losses result in NH_3 emissions (harmful to ecosystems and toxic for animals) as well as nitrous oxide (NO_2) emissions (a greenhouse gas with a global warming potential 298 times higher than CO_2) (Castillo et al., 2000; Matassa et al., 2023). Furthermore, N losses are a major cause of water pollution, eutrophication and acidification (Castillo et al., 2000; Dijkstra et al., 2013; Mottet et al., 2018). Additionally, increasing tannin levels can lead to decreased ruminal methane (CH_4) emissions as described in the meta-analysis of Jayanegara et al. (2012) on in vivo experiments. However, this effect seems only reliable and distinguishable from tannin levels exceeding 20 g/kg DM. Overall, tannin utilization can thus be a mitigation strategy to lower the environmental impact of ruminants as long as its use does not result in lower milk production (Castillo et al., 2000).

Only a few studies have examined the effects of HT on both the silage characteristics and the performance of dairy cows consuming these silages (Colombini et al., 2009; Herremans, Decruyenaere, et al., 2020). Moreover, the potential of HT to reduce proteolysis in the silo as well as in the rumen has not yet been studied for autumn herbage, an important forage source with a high but strong RDP content. Therefore, the objective of our experiment was to study the effects of an HT extract on silage fermentation characteristics, chemical composition and nutritional value of autumn herbage, and the effects on animal performance, enteric CH_4 emissions and N partitioning of dairy cows fed this HT-treated autumn herbage silage.

2 | MATERIAL AND METHODS

2.1 | Forage and treatments of micro- and bunker silos

The grass used in this experiment originated from three temporary grassland plots, sown with ryegrass (*Lolium perenne)* at ILVO (Flanders Research Institute for Agriculture, Fisheries and Food). The grass was mown as a fourth cut on the 27th and 28th of October 2019 (disc cutter with conditioner, mowing height 7–8 cm). After 2–3 days of wilting under cloudy weather, the grass was ensiled at a presumed DM content of about 25%. Two small bunker silos (65 m^3) were filled

alternately until each contained about 28 t of fresh grass. Contamination between silos during ensiling was avoided by using separate tractors, loading wagons and machinery. The first silo was filled in five times and the second silo in four times (different capacity of loading wagons), so each silo contained an equal proportion of grass from each plot. One silo was treated with an HT extract and the other with a lactic acid bacteria (LAB) inoculant. A third bunker silo was filled with the remaining autumn grass (27 t) without silage additive (UNT) and was fed to the cows in the pre-experimental period of the feeding trial.

The HT extract (TannoSan-L, Sanluc) is a syrup-like extract of the shredded wood of sweet chestnut (*Castanea sativa* Mill.). The chemical composition of this natural product is complex and variable. According to the company, the total HT concentration varies from 75% to 85% on DM basis (HT extract 45% DM). For this experiment an assumed HT concentration of 80% on DM basis was considered. The LAB inoculant (Bonsilage Fit G, Barenburg) is a silage additive containing both homofermentative (*Lactobacillus plantarum* and *L. rhamnosus*) and heterofermentative (*L. buchneri*) LAB. The homofermentative LAB are fast and efficient producers of lactic acid, so they can increase the rate of acidification and reduce protein degradation. The heterofermentative LAB can enhance the aerobic stability in the silage by converting lactic acid into acetic acid and CO_2, which inhibits the growth and survival of yeasts (Driehuis et al., 2001).

The LAB inoculant (1.5×10^{11} CFU per g product) was applied at the recommended dose of 2 g per t fresh grass (=8 mg/kg DM, based on a presumed DM content of 25% for the grass). Economic calculations showed that the HT extract could be administered at a dose of 4.3 g/kg DM grass at the same price as the LAB inoculant. This low dose would be biologically irrelevant. Based on previous dosage experiments with micro-silos (0–20–40–60 g/kg DM, not published) a dose of 20 g HT extract per kg grass DM (=7.2 g HT/kg DM) was chosen based on a presumed grass DM content of 25% for the grass. The HT extract (45% DM) was 1/1 (vol/vol) diluted with water to facilitate manual application. To standardize treatments, the LAB inoculant was dissolved in a similar total volume of water as the HT extract (31 g/kg DM grass). Both products were poured with a watering can on each grass layer unloaded in the silos. The grass silage with 20 g/kg DM HT extract is referred to below as TAN; the grass silage with 8 mg/kg DM LAB inoculant as INO.

During filling of the two bunker silos prior to application of the additives, samples were taken from each loading wagon and pooled, resulting in a representative sample of 80 kg fresh grass. This sample was used to perform an experiment with micro-silos, which are polyvinyl chloride (PVC) tubes (height 35 cm, diameter 10 cm, volume 2.75 L) closed with a cover equipped with a CO_2-valve. Four treatments were compared in the micro-silos: (1) grass without additive + 31 g/kg DM water = negative control (CON); (2) grass with 20 g/kg DM HT extract (45% DM) + 20 g/kg DM water (TAN); (3) grass with 8 mg/kg DM LAB inoculant + 31 g/kg DM water (INO); and (4) grass with 20 g/kg DM HT extract (45% DM) + 20 g/kg DM water + 8 mg/kg DM LAB inoculant (TAN + INO). For each treatment 15 kg of fresh grass was prepared based on a presumed grass DM content of 25%. All treatment solutions were applied homogeneously on chopped material using hand held sprayers. Separate sprayers were used per treatment. Five micro-silos were ensiled per treatment at a silo density of 215 kg DM/m^3 (2.3 kg fresh grass per micro-silo). Micro-silos were stored for 90 days at ambient temperature in a barn.

2.2 | Samplings and laboratory analysis of the micro-silages

The micro-silos were weighed immediately after filling and after 90 days of storage (on January 29, 2020) to calculate fresh weight losses during the ensiling period. At silo opening after 90 days, samples were taken from five micro-silages per treatment for evaluation of silage fermentation characteristics, chemical composition and nutritional value.

For the silage fermentation characteristics, pH, ammonia N fraction (%) (NH_3-N/total N) (ISO 5983-2, without previous destruction), lactic acid (enzymatic) (Gawehn, 1984), volatile fatty acids and alcohols (Jouany, 1981) were analysed on an aqueous extract from 100 g silage.

Another sub-sample of the grass silage was dried in a ventilated oven at 65°C and then ground through a 1 mm screen (Wiley, Rheotec). Residual moisture was determined by drying at 103°C (EC, 1971b). Crude ash was obtained by incineration at 550°C (ISO:5984, 2002). Crude protein (Nx6.25) was determined according to Kjeldahl (ISO:5983-2, 2005). Crude fat (CF) was extracted with petroleum-ether (ISO:6492, 1999). The aNDFom was determined with the Ankom Fiber Analyser using α-amylase and sodium sulphite was expressed on ash-free basis (Van Soest et al., 1991). The lignin(sa) content was determined with the Ankom Fiber Analyser and was expressed exclusive ash; the residue was then treated with sulphuric acid to obtain ADL (Van Soest et al., 1991). Sugars were extracted with 40% ethanol and analysed according to the Luff Schoorl method (EC, 1971a).

The net energy for lactation (NE_L) content according to Van Es (1978) was estimated with a regression equation (De Boever, 1999) based on the cellulase digestibility of the organic matter (OM) (De Boever et al., 1986) and chemical parameters. The content of true protein digested in the small intestine (DVE) and the degraded protein balance (OEB) according to the Dutch protein system (Van Duinkerken et al., 2011) were estimated using own developed regression equations based on the chemical composition, cellulase digestibility and the ammonia-N fraction derived from a dataset of 37 grass silages of which rumen degradation characteristics were determined in situ with three fistulated cows according to the protocol of CVB (2004).

2.3 | Experimental design and diets of the feeding trial

The feeding trial was carried out at the ILVO experimental farm in 2021. All animal handling and sampling procedures were approved by the ILVO Animal Ethics Committee (EC 2020/387).

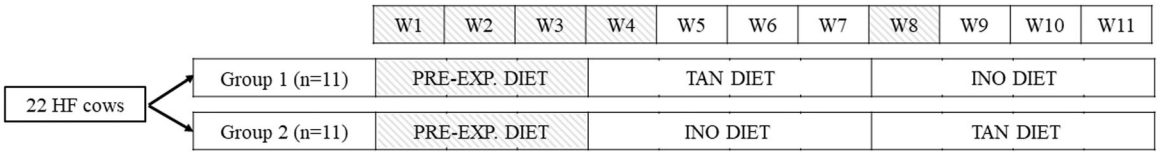

FIGURE 1 Overview of the experimental design. All cows received a pre-experimental partial mixed ration with an untreated autumn grass silage during the pre-experimental period (W1–W3). Then a cross-over experiment with two periods (W4–W7 and W8–W11) was conducted during which the cows alternately received an experimental partial mixed ration including an autumn grass silage treated with 20 g/kg DM hydrolysable tannin extract (TAN) or an experimental partial mixed ration including an autumn grass silage treated with 8 mg/kg DM lactic acid bacteria inoculant (INO). Both doses are based on a presumed DM content of 25% for the grass. Other feed ingredients were equal between the three partial mixed rations. Shaded weeks were removed for statistical data analysis. HF, Holstein Friesian; W, Week.

Before the start of the experiment, three partial mixed rations were formulated to be identical in feed ingredients (grass silage, maize silage, pressed beet pulp, rolled barley and corn meal in a ratio of 46/35/13/3/3 on DM basis) except for the grass silage. The UNT silage was used in the pre-experimental partial mixed ration and the INO or TAN silages in the two experimental partial mixed rations. Further, all pre-experimental and experimental diets were formulated to be iso-nitrogenous and the two experimental diets were also iso-DVE and iso-NE_L.

The experiment included 22 lactating Holstein cows (11 primiparous and 11 multiparous) and was preceded by a pre-experimental period of 3 weeks, during which the cows received the pre-experimental partial mixed ration including the UNT grass silage. The pre-experimental period was introduced to adapt the cows to the group layout, the herringbone milking system and the partial mixed ration. In the last 2 weeks of the pre-experimental period, cows (n = 22) had a mean body weight (BW) of 665 ± 71.0 kg, were 183 ± 36.3 days in milk (DIM) and produced 33.8 ± 3.91 kg of fat and protein corrected milk yield (FPCMY) per day.

Further, based on the data of the pre-experimental period, two random balanced groups were made with the package 'blockTools' in R. First, duos were created of similar cows based on predefined variables (DIM, parity, milk yield [MY], milk composition and dry matter intake [DMI]). Secondly, within each duo, each cow was randomly assigned to one of the two groups. The cows in the first group (n = 11) had a mean DMI of 23.3 ± 2.06 kg, were 180 ± 36.2 DIM and produced 34.1 ± 4.08 kg of FPCMY per day. The cows in the second group (n = 11) had a mean DMI of 22.6 ± 2.91 kg, were 185 ± 38.1 DIM and produced 33.5 ± 3.91 kg of FPCMY per day. Then, a cross-over experiment was conducted with two treatment periods of 4 weeks, during which the two groups alternately received the diets with either INO or TAN silage. Figure 1 gives an overview of the experimental design.

The partial mixed rations (pre-experimental and experimental) were supplemented with concentrates (formolated soybean meal and balanced concentrate) in automatic feeders to meet individual cow requirements of 105% NE_L and 105% DVE. The concentrate supplementation was set in the pre-experimental period and lowered in relation to the milk production curve at the start of Week 4, 6, 8 and 10 of the treatment periods with a fixed amount of 350 g for multiparous cows and 150 g for primiparous cows. The feed ingredients, chemical composition and nutritional value of the three total diets (incl. concentrates) are represented in Table 1.

2.4 | Samplings and laboratory analysis during the feeding trial

The bunker silos with INO and TAN were drill sampled on September 15, 2020 to determine the rumen degradation characteristics (rumen undegradable protein; RUP) and the intestinal digestibility of the protein (intestinal digestible RUP, dRUP) in three fistulated cows using the nylon bag method according to the protocol of CVB (2004).

During the feeding trial, the three grass silages (INO, TAN and UNT) were sampled twice weekly during the weeks of treatment and pooled per period for subsequent analysis. The maize silage was sampled every 2 weeks and pooled per period. Pressed beet pulp, formolated soybean meal, rolled barley, corn meal and the balanced concentrates were sampled once every 2 weeks and pooled over the whole experiment. Silage fermentation characteristics were analysed on an aqueous extract from 100 g silage, as described in Section 2.2. A subsample of each feed component was dried in a ventilated oven at 65°C and then ground through a 1 mm screen (Wiley, Rheotec). Residual moisture, CP, CF, crude ash, aNDFom, lignin(sa), ADL and sugars were analysed as described in Section 2.2. The DM content of the silages was corrected for volatilization losses during drying. Acid insoluble ash (AIA) was determined according to ISO 5985 (2002) on each feed component and used as a natural inert marker to estimate apparent total-tract N and OM digestibility and total faeces production (McCarthy et al., 1974). The NE_L content of the feeds according to Van Es (1978) was estimated with a regression equation (De Boever, 1999) based on the cellulase digestibility of the OM (De Boever et al., 1986) and chemical parameters. The DVE and OEB content of the grass silages according to the Dutch protein system (Van Duinkerken et al., 2011) were calculated based on RUP and dRUP obtained from the in situ incubation. The DVE and OEB content of the other feeds were estimated using chemical parameters in combination with tabular RUP and dRUP values (CVB, 2019).

The cows from the feeding trial were housed in a freestall with cubicles for the total experiment. They had access to the partial mixed ration at all times through Roughage Intake Control feed bins

TABLE 1 Feed ingredients, chemical composition and nutritional value of the total diets (including concentrates) fed during the feeding trial.[a]

	Pre-experimental period	Cross-over experiment	
	UNT diet[b]	TAN diet[b]	INO diet[b]
Feed ingredients (g/kg DM)			
Prewilted autumn grass silage			
UNT	311	-	-
TAN	-	332	-
INO	-	-	329
Maize silage	272	282	283
Pressed beet pulp	108	115	115
Maize meal	22	23	23
Rolled barley	21	23	23
Formolated soybean meal[c]	26	26	26
Balanced concentrates[c]	240	199	201
Chemical composition (g/kg DM, unless noted)			
Dry matter (g/kg)	345	343	342
Crude protein	166	166	167
Crude fat	31	30	31
Crude ash	94	86	92
Acid-insoluble ash	32	26	34
aNDFom	315	323	325
ADFom	189	194	192
Lignin(sa)	18.1	16.2	14.4
Starch	190	180	180
Sugars	27	25	24
Nutritional value (g/kg DM, unless noted)			
NE_L[d] (MJ/kg DM)	6.68	6.77	6.79
DVE[e]	96	91	91
OEB[e]	13	17	18
FOM[e]	538	545	545

[a]The proportions of the feed ingredients were calculated based on the actual average partial mixed ration and concentrate feed intake by the cows during the experiment, with exclusion of the transition weeks.

[b]UNT = untreated autumn grass silage; TAN = hydrolysable tannin extract treated autumn grass silage (20 g/kg DM = 7.2 g HT/kg DM); INO = lactic acid bacteria inoculant treated autumn grass silage (8 mg/kg DM).

[c]Concentrates fed in the individual concentrate boxes and the GreenFeed on an individual cow level.

[d]NE_L = net energy for lactation (Van Es, 1978) estimated with regression equations (J. De Boever, 1999) based on the chemical composition and the cellulase digestibility of the organic matter (J. L. De Boever et al., 1986).

[e]DVE = true protein digested in the small intestine, OEB = rumen degraded protein balance and FOM = rumen fermentable organic matter (Van Duinkerken et al., 2011). For the UNT grass silage these values were estimated using own developed regression equations based on chemical analyses and cellulase digestibility derived from a data set of 37 grass silages, of which rumen degradation characteristics were determined in situ with three fistulated cows. For the TAN and INO grass silages these values were calculated based on rumen undegradable protein and intestinal digestible rumen undegradable protein obtained from the in situ incubation with three fistulated cows of these silages. For the other feeds these values were calculated using chemical analyses in combination with tabular values (CVB, 2019).

(Hokofarm Group BV) that were filled up two times a day (at 0800 and 1900 h) to ensure continuous feed supply. The partial mixed rations were prepared in a small mixing wagon, also twice daily (at 0800 and 1900 h). Concentrates were provided via standard in-parlor and out-of-parlor feed stations (DeLaval NV). Cows were milked twice a day in a herringbone milking parlor (DelPro Farm Manager). The individual BW (scale, DeLaval) and body condition score (BCS) (automatic BCS camera, DeLaval) were measured after each milking. Methane and CO_2 emissions were recorded daily with a GreenFeed concentrate box (C-Lock Inc.) placed in the freestall. Cows were aimed to receive 1 kg/day of balanced concentrate in the GreenFeed. They collected, on average, 24 drops of concentrate per day, resulting in, on average, 3.3 successful (airflow > 26 L/min) CO_2 and CH_4 measurements per cow per day. Individual milk samples were collected and analysed at four consecutive milkings in weeks 3, 5, 7, 9 and 11 of the experiment. Each sequence of four consecutive milkings was stored in a fridge until analysis at the day of the last sampling. Milk samples were analysed separately for protein, fat, lactose and urea content with Fourier-transform infrared spectroscopy (FTIR) (Lactoscope Advanced, Delta Instruments). The FTIR results were checked and if necessary adjusted within each run by selecting four milk samples of which fat content was also determined with the Gerber method and protein content with Kjeldahl (ISO:5983-2, 2005). MUN was calculated based on the molecular mass of urea; the N content of urea is 46.22% by weight. Milk composition for the weeks without sampling was calculated from the measurements in the previous and the following week in the same period. Milk yield was corrected for fat and protein content with the formula:

$$\text{FPCMY} = \text{MY} \times [0.337 + (0.116 \times \text{milk fat\%}) + (0.06 \times \text{milk protein\%})]$$

(Subnel et al., 1994). Feed efficiency was calculated as the FPCMY (kg/day) divided by the DMI (kg/day). The MNE was determined with the formula:

$$\text{MNE} = \frac{\frac{\text{milk protein \%}}{100} \times \frac{\text{milk yield (kg / day)}}{6.38}}{\frac{\text{CP intake (kg / day)}}{6.25}}.$$

The CP intake (kg/day) through the feed was calculated based on the DMI of the cows and the analysed CP (*N*x6.25) content of the feed ingredients.

Individual blood samples were taken from the tail vein at 10 a.m. on the penultimate day of each period. Blood heparin plasma was separated after centrifugation at 1890*g* for 8 min at 21°C and was analysed for urea according to the manufacturer's instruction of the Urea Nitrogen2 assay (methodology: urease) (ARCHITECT c System, Abbott). Plasma urea N (PUN) was calculated from plasma urea based on the molecular structure of urea; the N content of urea is 46.22% by weight.

One faeces spot sample of 100 g from each cow was taken daily during the last 4 days of each period, either directly from the rectum or during voluntary defecation. The spot samples were taken at a different time point for each sampling day (07.30 a.m., 10.30 a.m., 01.30 p.m., 04.30 p.m.) and were pooled per cow and per period. Total N and NH_4-N were determined on fresh faeces according to ISO 5983-2 (2009) with and without previous destruction, respectively. The content of AIA was determined following ISO 5985 (2002) on oven-dried (65°C) and ground (1 mm screen [Wiley, Rheotec]) samples to estimate total faeces production (McCarthy et al., 1974), apparent total-tract N digestibility with the formula $100 \times (1 - (N_{faeces}/N_{feed}) \times (AIA_{feed}/AIA_{faeces}))$ and apparent total-tract OM digestibility with the formula $100 \times (1 - (OM_{faeces}/OM_{feed}) \times (AIA_{feed}/AIA_{faeces}))$. Total N intake (g/day) through the feed was calculated based on the DMI of the cows and the analysed CP (*N*x6.25) content of the feed ingredients. Total N output via milk (g/day) was calculated based on the MY of the cows and the FTIR analysed protein (*N*x6.38) content of the milk. A correction for N in milk urea was made based on the molecular weight of N and urea and the FTIR analysed urea content of the milk. Total N and NH_4-N output via faeces (g/day) was calculated based on the estimated total faeces production (McCarthy et al., 1974) and the analysed N and NH_4-N concentration in the faeces samples. Based on the study of Spanghero and Kowalski (2021), the retained N (g/day) was calculated as 6.7% of N intake. No urine samples were available to determine total N output via urine, but residual N (g/day) was estimated by subtracting N output via milk and faeces and retained N from the total N intake.

2.5 | Statistical analysis

For the micro-silages, all variables (weight loss, pH, NH_3-N/N, lactic acid, acetic acid, ethanol, DM, CP, crude ash, aNDFom, sugars, NE_L, DVE and OEB) were averaged per treatment (CON, TAN, INO, TAN +INO; five repetitions per treatment). The variance in the data was analysed with treatment as fixed factor. Pairwise comparisons between treatment effects were explored using a Tukey corrected post hoc test. All statistical analysis regarding the micro-silages were performed using the statistical software programme Statistica V14.

The differences in silage fermentation characteristics (pH, NH_3-N/N, lactic acid, acetic acid, propionic acid, butyric acid and ethanol), chemical composition (DM, CP, CF, crude ash, AIA, aNDFom, ADFom, lignin(sa), sugars), and OM digestibility and nutritional value (NE_L, DVE, OEB and FOM) between the TAN and INO grass bunker silages were analysed with treatment as fixed factor (two repetitions per treatment). The third bunker silo (UNT) was only analysed once on pooled samples taken during the pre-experimental period; therefore, no statistical analysis was possible on this data.

Daily data (BW, BCS, DMI, MY, CO_2 and CH_4 emission) were averaged for each cow per week. The weekly CO_2 and CH_4 enteric emission was only calculated when a cow had 15 or more successful measurements in a week. To evaluate the effects of the treatments, all variables (BW, BCS, DMI, MY, FPCMY, milk fat, milk protein, milk lactose, MUN, MNE, feed efficiency, CO_2 and CH_4 emission, CO_2/CH_4 ratio) were averaged per period for each cow per treatment (TAN and INO diet) with exclusion of the pre-experimental period

(Weeks 1–3) and adaptation weeks (Weeks 4 and 8). The pre-experimental period was introduced only to acclimate the cows to the group layout, the herringbone milking system and the partial mixed ration (only grass silage changed in the treatment period). Additionally, this provided relevant data to make two balanced groups for the experimental period. The adaptation weeks were included to adapt the cows to the new grass silage (TAN or INO) during the experimental period. For the other variables (PUN, feed N input, milk N output, faecal N output, faecal NH_4-N, retained N, residual N, faeces NH_4-N output, apparent total-tract N and OM digestibility) only data of the last week of each period were available. The model for all variables to evaluate the effects of INO versus TAN contained period, DIM and treatment as fixed effects and cow as random effect. The results were presented as least-square means ± standard error of the lsmeans per treatment. All statistical analyses regarding the feeding trial were performed using the statistical software programme R (version 4.1.2, www.r-project.org) and its packages 'lme4', 'lsmeans' and 'nlme'. The analysed outcomes were assumed to be normally distributed based on the graphical evaluation of the residuals of the model used (histogram and quantile-quantile plot). Differences were considered significant at $p \leq 0.05$ and tendencies at $0.05 < p \leq 0.10$.

3 | RESULTS

3.1 | Composition and nutritional value of the micro-silages

The results of the silage fermentation characteristics, chemical composition and nutritional value of the micro-silages, as the mean of five technical replicates per treatment, are presented in Table 2. Butyric acid and propionic acid were not detected in the samples. In comparison to CON, the addition of TAN resulted in a tendency towards a lower ethanol concentration ($p = 0.10$), a higher DM content ($p = 0.03$), more CP ($p = 0.002$), less crude ash ($p \leq 0.001$) and

TABLE 2 Silage fermentation characteristics, chemical composition and nutritional value of autumn grass micro-silages (means of five silos per treatment) (g/kg of dry matter (DM) unless noted).

	CON[1]	TAN[1]	INO[1]	TAN + INO[1]	SEM	*p* value[2]
Silage fermentation characteristics						
Weight loss (%)	0.60^{bc} ± 0.023	0.57^{c} ± 0.006	0.74^{a} ± 0.012	0.62^{b} ± 0.004	0.016	≤0.001
pH	4.09^{b} ± 0.014	4.11^{ab} ± 0.009	4.15^{a} ± 0.024	4.15^{a} ± 0.003	0.009	0.017
NH_3-N/N (%)	9.01^{ab} ± 0.212	7.79^{bc} ± 0.171	9.41^{a} ± 0.582	7.51^{c} ± 0.324	0.247	0.004
Lactic acid	73 ± 1.3	71 ± 1.9	76 ± 3.6	70 ± 1.1	1.2	0.265
Acetic acid	10.6^{c} ± 0.31	11.1^{c} ± 0.49	21.1^{a} ± 0.81	14.6^{b} ± 0.52	0.99	≤0.001
Ethanol	3.8^{a,b} ± 0.13	3.1^{b} ± 0.25	4.1^{a} ± 0.25	3.2^{b} ± 0.08	0.13	0.006
Chemical composition						
Dry matter (g/kg)	269^{bc} ± 4.1	283^{a} ± 3.2	265^{c} ± 2.5	280^{ab} ± 3.1	2.3	0.003
Crude protein	219^{b} ± 2.4	231^{a} ± 1.4	233^{a} ± 2.3	232^{a} ± 1.8	1.6	≤0.001
Crude ash	165^{a} ± 5.0	140^{b} ± 2.9	154^{ab} ± 2.6	143^{b} ± 2.9	2.7	0.001
aNDFom	391 ± 2.8	396 ± 2.4	401 ± 1.8	399 ± 2.2	1.3	0.062
Sugar	6^{a} ± 0.4	6^{a} ± 0.2	4^{b} ± 0.1	6^{a} ± 0.3	0.2	≤0.001
Nutritional value						
NE_L[3] (MJ/kg DM)	6.21^{b} ± 0.036	6.41^{a} ± 0.028	6.21^{b} ± 0.035	6.36^{a} ± 0.033	0.026	0.001
DVE[4]	65^{b} ± 0.7	69^{a} ± 0.4	67^{ab} ± 0.7	69^{a} ± 0.7	0.5	0.002
OEB[4]	107^{b} ± 1.8	115^{a} ± 1.0	120^{a} ± 2.2	116^{a} ± 1.0	1.3	≤0.001

[a,b,c]Treatment means within a row with different superscripts significantly differ ($p \leq 0.05$) as determined by Tukey's post hoc test.

[1]CON = grass micro-silos without additive; TAN = hydrolysable tannin (HT) extract treated grass micro-silos (20 g/kg DM = 7.2 g HT/kg DM); INO = lactic acid bacteria (LAB) inoculant treated grass micro-silos (8 mg/kg DM); and TAN + INO = HT extract (20 g/kg DM = 7.2 g HT/kg DM) + LAB inoculant (8 mg/kg DM) treated grass micro-silos.

[2]*p* value for treatment effect.

[3]NE_L = net energy for lactation (Van Es, 1978) estimated with regression equations (De Boever, 1999) based on the chemical composition and the cellulase digestibility of the organic matter (De Boever et al., 1986).

[4]DVE = true protein digested in the small intestine and OEB = rumen degraded protein balance (Van Duinkerken et al., 2011) estimated using own developed regression equations based on the chemical analyses and cellulase digestibility derived from a data set of 37 grass silages, of which rumen degradation characteristics were determined in situ with three fistulated cows.

a higher NE_L ($p = 0.002$), DVE ($p = 0.004$) and OEB ($p = 0.007$). The addition of INO compared to CON resulted in higher weight loss ($p \leq 0.001$), a higher pH ($p = 0.04$), more acetic acid ($p \leq 0.001$) and CP ($p \leq 0.001$), less sugar ($p \leq 0.001$) and a tendency towards a higher DVE ($p = 0.08$) and OEB ($p \leq 0.001$). The combination of TAN+INO compared to CON resulted in a higher pH ($p = 0.04$), lower NH_3-N/N fraction ($p = 0.04$), more acetic acid ($p \leq 0.001$), a tendency towards a higher DM ($p = 0.09$) and CP content ($p \leq 0.001$), less crude ash ($p = 0.002$) and a higher NE_L ($p = 0.02$), DVE ($p \leq 0.001$) and OEB ($p = 0.004$). Compared to INO, TAN resulted in lower weight loss ($p \leq 0.001$), a lower NH_3-N fraction ($p = 0.03$), less acetic acid ($p \leq 0.001$) and ethanol ($p = 0.01$), a higher DM content ($p = 0.008$), a tendency towards a lower crude ash content ($p = 0.06$), more sugar ($p \leq 0.001$) and a higher NE_L ($p = 0.003$). The crude ash content in the TAN micro-silages was respectively 15% and 9% lower compared to CON and INO, respectively. Compared to TAN, TAN+INO had more weight loss ($p = 0.05$) and a higher acetic acid concentration ($p = 0.002$). Weight loss ($p \leq 0.001$), NH_3-N/N fraction ($p = 0.009$), acetic acid ($p \leq 0.001$) and ethanol ($p = 0.02$) were higher in INO compared to TAN+INO, whereas DM ($p = 0.02$), sugar ($p = 0.002$) and NE_L ($p = 0.02$) were lower in INO compared to TAN+INO.

3.2 | Composition and nutritional value of the bunker silages

Table 3 presents the silage fermentation characteristics, chemical composition and nutritional value of the three grass silages (bunker silos) used in the dairy cow experiment (UNT, TAN and INO). Statistical comparison was only possible between TAN and INO ($n = 2$) as only one sample was available for UNT. The TAN silage tended to have lower propionic acid ($p = 0.07$) and had lower CF ($p < 0.01$), crude ash ($p = 0.03$) and OM digestibility ($p = 0.04$) compared to the INO silage. The crude ash content was 13% lower for TAN compared to INO and 18% lower compared to UNT. The sugar content tended ($p = 0.09$) to be higher for the TAN silage compared to the INO silage. Energy and protein value of the TAN and INO silages did not significantly differ.

3.3 | Animal feeding trial

The animal performance parameters are presented in Table 4 as a mean for all cows while feeding the TAN and INO diets in the cross-over experiment. The BW ($p = 0.99$), BCS ($p = 0.30$), MY ($p = 0.11$), FPCMY ($p = 0.57$), milk protein content ($p = 0.62$) and MUN concentration ($p = 0.30$) did not differ between the two treatment diets TAN and INO. DMI ($p = 0.09$) and milk fat content ($p = 0.07$) tended to be higher for TAN, whereas milk lactose was lower ($p = 0.02$) in comparison to INO. The MNE ($p \leq 0.01$) and feed efficiency ($p = 0.02$) were lower for TAN compared to INO. The CO_2 (kg/day, kg/kg of digested OM, kg/kg of FPCMY) and CH_4 (g/day, g/kg of DMI, g/kg of digested OM, g/kg of FPCMY) emissions, as well as the ratio of CH_4/CO_2, were all higher ($p \leq 0.02$) with the TAN diet in comparison with the INO diet. The CO_2 emission expressed as kg/kg of DMI tended ($p = 0.06$) to be higher with the TAN diet compared to INO.

The PUN concentration, feed N input, milk N output, faecal N output and faecal NH_4-N, retained N, residual N and apparent total-tract N and OM digestibility are presented in Table 5. The PUN concentration ($p = 0.79$), feed N input ($p = 0.12$), milk N output ($p = 0.96$) and retained N ($p = 0.12$) did not differ between TAN and INO. The faecal N output ($p \leq 0.01$), NH_4-N output ($p = 0.05$) and residual N ($p \leq 0.01$) were higher for TAN in comparison to INO. The apparent total-tract N and OM digestibility (%) decreased ($p \leq 0.01$) on the TAN diet in comparison to the INO diet.

4 | DISCUSSION

4.1 | Silage fermentation characteristics of the micro-silages

The fourth cut of grass used in our experiment was typical autumn grass with low DM (26.6%), low sugar (6.6%) and high CP (23.0%), thus poor fermentation could be expected. Contrary to expectations, the CON micro-silages had an acceptable silage pH, high lactic acid content and relatively low NH_3-N/N fraction (Devisser et al., 1993; Larsen et al., 2016; Nadeau et al., 2016).

The addition of 7.2 g HT/kg DM did not significantly improve silage characteristics compared to CON in the present experiment (except for a tendency towards a lower ethanol concentration). In the meta-analysis of Jayanegara et al. (2019), increasing doses of tannins (from 0 to 58 g/kg DM, including both HT and CT administered through either additive or plant) were not associated with changes in silage pH or concentrations of lactic acid, acetic acid, propionic acid and, in contrast to our experiment, ethanol, but did result in lower contents of soluble N, non-protein N (NPN) and NH_3-N. This indicates a reduction in the extent and rate of proteolysis during silage fermentation. Most of the findings from Jayanegara et al. (2012) are also described in the experiment of Tabacco et al. (2006), who examined wilted alfalfa mixed with 0, 20, 40 and 60 g HT/kg DM (chestnut) in lab-scale silos. The addition of HT decreased the NPN and NH_3-N content without affecting silage pH or lactic and acetic acid content, except for the silage with 20 g HT/kg DM, which had a reduced silage pH and increased lactic acid concentration (Tabacco et al., 2006). In contrast to our experiment, weight loss decreased from 3.35% to 2.72% to 2.43% with HT applied in doses from 0 to 20 to 40 g/kg DM, whereas 60 g HT/kg DM did not further decrease weight loss (Tabacco et al., 2006). Cavallarin et al. (2002) also reported a positive influence of chestnut HT (25 g/kg DM) on the silage fermentation of alfalfa, resulting in lower NH_3-N and NPN concentrations. In contrast to results obtained by Cavallarin et al. (2002) and Tabacco et al. (2006), with much higher HT doses (20–60 g/kg DM), we observed no effect of the addition of HT (7.2 g/kg DM) on proteolysis in the present experiment. In the study of Herremans et al. (2019), an oak HT extract (10 g/kg DM) reduced

TABLE 3 Silage fermentation characteristics, chemical composition and nutritional value of the three grass bunker silages (in g/kg DM, unless noted) used in the feeding trial; for TAN and INO this is the mean of the analyses on two pooled samples (pooled from two samples per week taken during each treatment period).

	UNT[a]	TAN[a]	INO[a]	SEM	*p* value TAN vs. INO[b]
Silage fermentation characteristics					
pH	4.55	4.25	4.37	0.074	0.41
NH_3-N/N (%)	17.7	12.2	12.2	0.71	0.99
Lactic acid	55.2	71.9	62.8	6.39	0.42
Acetic acid	36.1	22.6	30.6	2.68	0.17
Propionic acid	3.63	1.76	3.40	0.328	0.07
Butyric acid	6.35	3.26	2.46	2.050	0.81
Ethanol	8.75	4.24	4.95	0.508	0.43
Chemical composition					
Dry matter (g/kg)	221	238	235	10.6	0.88
Crude protein	231	233	236	6.4	0.79
Crude fat	52	47	51	0.1	<0.01
Crude ash	165	136	157	2.5	0.03
Acid insoluble ash	68	43	69	1.73	<0.01
aNDFom	396	372	381	12.5	0.66
ADFom	259	245	241	7.1	0.72
Lignin(sa)	32	24	18	2.7	0.27
Sugars	1.4	6.1	3.6	0.57	0.09
Digestibility OM[c] (%)	74	76	78	0.3	0.04
Nutritional value					
NE_L[d] (MJ/kg DM)	5.6	6.1	6.1	0.05	0.72
RUP[e] (%)	-	19.6	18.5	0.32	<0.01
dRUP[e] (%)	-	67.5	69.9	0.68	<0.01
DVE[f]	58	53	53	0.5	0.70
OEB[f]	121	120	125	5.3	0.57
FOM[f]	453	515	515	0.7	0.74

[a]UNT = untreated autumn grass silage fed during the pre-experimental period; TAN = hydrolysable tannin extract treated autumn grass silage (20 g/kg DM = 7.2 g HT/kg DM); INO = lactic acid bacteria inoculant treated autumn grass silage (8 mg/kg DM).

[b]*p* values for the pairwise comparison between the TAN and INO silage. Means significantly differ when $p \leq 0.05$ and tend to differ when $0.05 < p \leq 0.10$.

[c]Cellulase digestibility of the organic matter (OM) (De Boever et al., 1986).

[d]NE_L = net energy for lactation (Van Es, 1978) estimated with regression equations (De Boever, 1999) based on the chemical composition and the cellulase digestibility of the OM (De Boever et al., 1986).

[e]RUP = rumen undegradable protein and dRUP = intestinal digestibility of rumen undegradable protein determined in situ with fistulated cows for TAN and INO according to the protocol of CVB (2004).

[f]DVE = true protein digested in the small intestine, OEB = rumen degraded protein balance; and FOM = rumen fermentable organic matter (Van Duinkerken et al., 2011) estimated using own developed regression equations based on chemical analyses and cellulase digestibility derived from a dataset of 37 grass silages, of which rumen degradation characteristics were determined in situ with three fistulated cows for UNT and calculated using RUP and dRUP determined in situ with three fistulated cows for TAN and INO.

silage pH by 4% and NH_3-N by 10% and lowered lactic acid and acetic acid concentrations in lab-scale grass silages, whereas a chestnut HT extract (8 g/kg DM) reduced only lactic acid and acetic acid concentrations. Tabacco et al. (2006) proved that chestnut HT can be efficient starting at 20 g/kg DM onwards, suggesting that the dose in our experiment (7.2 g/kg DM) and in the experiment of Herremans et al. (2019) (8 g/kg DM) might be too low to significantly reduce proteolysis during the ensiling process. The lowered ethanol

TABLE 4 Least-square estimates of dry matter intake (DMI), body weight (BW), body condition score (BCS), milk yield (MY) and fat- and protein-corrected milk yield (FPCMY), milk composition, N efficiency, feed efficiency and CO_2 and CH_4 emissions for the hydrolysable tannin (HT) extract silage (TAN) and lactic acid bacteria (LAB) inoculant (INO) diets fed in a crossover design.

Parameter	TAN diet[a]	INO diet[a]	SEM	*p* value[b]
BW (kg)	683	683	15.9	0.99
BCS	3.22	3.25	0.059	0.30
DMI (kg/day)	23.7	23.5	0.50	0.09
Milk yield (kg/day)	29.3	29.6	0.73	0.11
FPCMY[c] (kg/day)	32.4	32.5	0.94	0.57
Milk composition				
Milk fat (%)	4.66	4.60	0.097	0.07
Milk protein (%)	3.79	3.79	0.048	0.62
Milk lactose (%)	4.58	4.60	0.052	0.02
MUN (mg/dL)	12.8	13.0	0.29	0.30
Milk N efficiency[d] (%)	27.6	28.0	0.44	≤0.01
Feed efficiency[e]	1.36	1.39	0.027	0.02
CO_2 emission				
kg/day	13.1	12.8	0.24	≤0.01
kg/kg of DMI	0.561	0.555	0.0100	0.06
kg/kg of digested OM[f]	17.4	16.7	0.32	≤0.01
kg/kg of FPCMY	0.417	0.407	0.0171	0.02
CH_4 emission				
g/day	428	409	9.5	≤0.01
g/kg of DMI	18.3	17.7	0.36	≤0.01
g/kg of digested OM	567	532	11.9	≤0.01
g/kg of FPCMY	13.6	13.0	0.48	≤0.01
CH_4/CO_2 (g/kg)	32.7	31.9	0.43	0.01

Abbreviation: MUN, milk urea nitrogen.

[a]TAN = HT extract treated autumn grass silage (20 g/kg DM = 7.2 g HT/kg DM); INO = LAB inoculant treated autumn grass silage (8 mg/kg DM).

[b]*p* values for the pairwise comparison between the TAN and INO diet in the treatment period. Means significantly differ when $p \leq 0.05$ and tend to differ when $0.05 < p \leq 0.10$.

[c]Fat and protein corrected milk yield, calculated as FPCMY = milk production × [0.337 + (0.116 × milk fat %) + (0.06 × milk protein %)].

[d]Calculated as milk N efficiency = [(milk protein/100) × milk production/6.38]/(CP intake/6.25).

[e]Calculated as feed efficiency = kg of FPCM/kg of DMI.

[f]Digested organic matter (OM) intake calculated as the OM intake (kg/day) multiplied with the apparent total-tract OM digestibility (%) = $100 \times (1 - (OM_{faeces}/OM_{feed}) \times (AIA_{feed}/AIA_{faeces}))$.

concentration found in TAN-treated silages was not found (Jayanegara et al., 2019) or described (Cavallarin et al., 2002; Herremans et al., 2019; Tabacco et al., 2006) in previous studies. Overall, silages with high concentrations of ethanol (>3%–4%) commonly exhibit an abundance of yeast populations. Consequently, silages with lower ethanol content are expected to exhibit improved stability and a reduced likelihood of spoilage compared to those with higher ethanol concentrations (Kung et al., 2018).

TABLE 5 Least-square estimates of the PUN concentration, feed N input, milk N output, faecal N output, faecal NH_4-N and apparent total-tract N and OM digestibility for the hydrolysable (HT) extract silage (TAN) and lactic acid bacteria (LAB) inoculant (INO) diets fed in a cross-over design.

	TAN diet[a]	INO diet[a]	SEM	*p* value[b]
PUN (mg/dL)	12.3	12.2	0.45	0.79
Feed N input (g/day)	629	621	14.3	0.12
Milk N output (g/day)	170	170	5.2	0.96
Faecal N output (g/day)	181	155	5.9	≤0.01
Faecal NH_4-N (mg/day)	4017	3579	190	0.05
Retained N[c] (g/day)	42.1	41.6	0.96	0.12
Residual N[d] (g/day)	236	255	8.5	≤0.01
Apparent total-tract N digestibility[e] (%)	71.3	74.8	0.73	≤0.01
Apparent total-tract OM digestibility[f] (%)	82.7	84.9	0.45	≤0.01

Abbreviation: PUN, plasma urea nitrogen.

[a]TAN = HT extract treated autumn grass silage (20 g/kg DM = 7.2 g HT/kg DM); INO = LAB inoculant treated autumn grass silage (8 mg/kg DM).

[b]*p* values for the pairwise comparison between the TAN and INO diet in the treatment period. Means significantly differ when $p \leq 0.05$ and tend to differ when $0.05 < p \leq 0.10$.

[c]Retained N assuming that 6.7% of the N intake is retained (body accumulation and less predominant losses) (Spanghero & Kowalski, 2021).

[d]Residual N = feed N input − milk N output − faecal N output − retained N.

[e]Apparent total-tract N digestibility (%) = $100 \times (1 - (N_{faeces}/N_{feed}) \times (AIA_{feed}/AIA_{faeces}))$.

[f]Apparent total-tract OM digestibility (%) = $100 \times (1 - (OM_{faeces}/OM_{feed}) \times (AIA_{feed}/AIA_{faeces}))$.

The addition of 8 mg/kg DM LAB inoculant did not result in improved fermentation compared to CON. The meta-analysis of Oliveira et al. (2017) showed that combinations of homofermentative and facultative heterofermentative LAB inoculants enhanced fermentation of grass silages by increasing lactic acid levels which led to reductions in DM loss, silage pH, acetic acid and NH_3-N concentrations. Borreani et al. (2018) stated that homofermentative LAB inoculants can successfully minimize DM losses, resulting in reduced silage pH and a shift in fermentation towards lactic acid in the majority of studies. The meta-analysis of Oliveira et al. (2017) appears to point mainly to the effect of the homofermentative LAB. Jatkauskas et al. (2015) and Jatkauskas et al. (2015) evaluated the fermentation characteristics of perennial ryegrass micro-silages inoculated with Bonsilage (*L. buchneri* and *Pediococcus pentosaceus*), but their results were inconsistent. Compared to the control, Jatkauskas et al. (2015) described a lower silage pH and higher lactic acid concentration, which mainly points to an effect of the

homofermentative LAB. In contrast, Jatkauskas et al. (2015) reported a higher acetic acid concentration, which mainly points to an effect of the heterofermentative LAB. This is in agreement with our experiment. Besides a higher acetic acid content, INO also had increased weight loss compared to CON, which can be explained by volatilization of CO_2 formed in addition to acetic acid during the conversion of lactic acid by *L. buchneri*. Overall, in our results mainly the effect of heterofermentative LAB is expressed and the effect of the homofermentative LAB was less pronounced.

The combination of the two silage additives resulted in silage with minor changes as compared with CON. This reflected the activity of both TAN (lower NH_3/N fraction) and INO (higher pH and acetic acid), but with similar weight loss.

4.2 | Chemical composition and nutritional value of the (micro-)silages

The NH_3-N/N fraction of the UNT silage was high (17.7%) which indicates poor conservation and loss of protein in the silage. For the TAN and INO silages, overall, the chemical composition and nutritional value were relatively similar. The addition of 20 g/kg DM HT extract resulted in silage with increased DM, sugar and energy (NE_L) content and lower ash content compared to the INO micro-silages, but only the higher sugar and lower ash content were confirmed in the bunker silages.

Herremans et al. (2019) also observed a higher sugar content for micro-silages with either chestnut (8 g/kg DM) or oak (10 g/kg DM) HT extract, compared to the control silage without additive. Tannin extracts can potentially inhibit fermentation by micro-organisms resulting in a higher residual sugar content (Herremans et al., 2019), but with the administered dose this effect was expected to be limited. Sugars could also originate from the HT extract itself, which contains some sugars (13.5% glucose on DM basis). Based on the administered dose (20 g/kg DM grass) and DM content (45%) of HT extract, 1.2 g of sugar was administered per kg DM of grass.

Another remarkable finding is the lower crude ash content in the TAN micro- and bunker silages (between −9% and −18% compared to INO, CON or UNT). In the study of Herremans et al. (2019), the ash concentration of the micro-silage with the chestnut HT additive (8 g/kg DM) was 11% lower (non-significant) compared to a negative control silage. To our knowledge no studies mention that tannins result in a lower ash content in the silage. It is known that tannins can bind numerous types of natural polymers, primarily proteins, but also carbohydrates, polysaccharides, microbes, enzymes and minerals (Bunglavan & Dutta, 2013; Frutos et al., 2004; Min et al., 2003); however, the formation of mineral complexes does not mean that they disappear. On the other hand, the overall high ash content observed in the silages indicates significant soil contamination. While this is not uncommon for autumn silages, it may also contribute to increased variability between silos.

4.3 | Performance of dairy cows

Compared to INO, the addition of 3.2 g HT/kg DM in the TAN diet tended to positively affect feed intake and did not affect the BW or BCS of the cows. Generally literature states that feed intake can be reduced with high CT concentrations (>50–55 g/kg DM), but is mostly not or minimally affected when consuming low or moderate amounts (≤50 g/kg DM) (Min et al., 2003; Mueller-Harvey, 2006; Jayanegara et al., 2012; Oliveira et al., 2023). Nevertheless, this statement is mainly based on studies with a negative control in comparison to CT as a feed additive, whereas only little information on the effects of HT as silage additive on feed intake is available. In Colombini et al. (2009), feeding a 4th cut alfalfa-silage treated with chestnut HT extract (13.4 g HT/kg DM) did not affect DMI compared to feeding a control alfalfa silage in a cross-over design with 50 lactating Holstein cows. This was confirmed in the study of Herremans et al. (2019) who also reported no effects on DMI and BW from feeding an oak tannin-treated grass (3rd cut) silage (6.1 g HT/kg DM) compared to a control treatment in a crossover experiment with six lactating Holstein cows. Taha et al. (2022) also report no effect on lambs' feed intake when fed grass silage treated with 22.5 g/kg DM chestnut HT as compared to a grass silage treated with an inoculant.

The TAN diet did not affect MY, FPCMY and milk protein content; however, milk fat content tended to be higher than with INO, whereas milk lactose content was lower in comparison to the INO diet. Literature shows that MY and milk composition are mostly not affected by tannins (Min et al., 2003; Oliveira et al., 2023); only a few studies mention a tendency towards higher milk protein content (Aguerre et al., 2016; Ramírez-Restrepo & Barry, 2005; Woodward et al., 2000). The meta-analysis of Herremans, Vanwindekens, et al. (2020) revealed that the FPCMY was unaffected when adding tannins. Again, most of the above studies mention the use of CT feed additives. In the studies of Herremans, Decruyenaere, et al. (2020) and Colombini et al. (2009) on tannin-treated grass (6.1 g HT/kg DM) and alfalfa (13.4 g HT/kg DM) silage, respectively, no effects on MY or milk composition were reported. Grainger et al. (2009) stated that when a decrease of MY in cows fed with tannins is found, it is most likely due to the combined effect of a reduced DMI and a reduced nutrient digestibility. However, the effects on milk fat and lactose content were not observed in literature on tannins specifically. The higher feed efficiency when feeding the INO diet compared to the TAN diet is most likely the result of the tendency towards lower DMI in combination with the almost equal FPCMY.

Overall, the results of our experiment regarding feed intake and milk performance are mostly in agreement with literature, especially with the two comparable feeding trials of Herremans, Decruyenaere, et al. (2020), and Colombini et al. (2009). However, in our trial another treatment (INO) was used instead of a control without additive, and literature shows that LAB inoculants can have a limited positive effect on the performance of dairy cows. Oliveira et al. (2017) performed a meta-analysis ($n = 31$) on the effects of silage inoculants (homofermentative and heterofermentative LAB) on the performance of dairy cattle. They reported an increase in MY

(+0.37 kg/day, $p < 0.01$) and tendencies towards an increase in DMI (+0.26 kg/day, $p = 0.08$), milk fat (+0.04%, $p = 0.08$) and milk protein concentrations (+0.02%, $p = 0.06$), independent of forage type, LAB species, LAB application rate, diet type and level of MY of the control cows. The positive MY response to inoculants in silages was attributed by Oliveira et al. (2017) to an increased DMI due to a lower lignin concentration and reduced content of butyric acid, NH_3-N and biogenic amines, which are hypophagic compounds. The latter can be achieved by an effect of homofermentative LAB, whereas our results suggested mostly an effect of the heterofermentative LAB. Overall, silage fermentation characteristics, chemical composition and nutritional value did not suggest any positive effects of the LAB inoculant. Nevertheless, potential effects of a LAB inoculant-treated grass silage diet could not be verified in our experiment due to the lack of negative control. From our study we can only conclude that feeding TAN or INO did not give different results in terms of feed intake and milk performance. However, at the administered doses the HT extract appears to be almost five times more expensive than the LAB inoculant. The use of the HT extract as silage additive would only be economically feasible when its application gives rise to other economic benefits (such as increased milk or lower feed costs) for the farmer.

Another effect of feeding condensed or HT might be a partial shift in N excretion from urine to faeces, which may be a mitigation strategy to reduce the environmental impact of dairy cattle (Castillo et al., 2000; Herremans, Vanwindekens, et al., 2020; Oliveira et al., 2023). Our results showed that MUN and PUN concentrations did not differ between the TAN and INO diet, which indicates no improved protein utilization. In fact, MNE was even lower when feeding the TAN diet compared to the INO diet. Nevertheless, the faecal N and NH_4-N excretions (g/day) were higher on the TAN diet, whereas N input through feed and N output in milk were not different in the current experiment. The assumed retained N (body accumulation and less predominant losses) was calculated as 6.7% of N intake following Spanghero and Kowalski (2021), and did not differ between treatments. The residual N was lower for the TAN diet and can give an indication on the N lost in urine (although urine samples were not collected to confirm this), which might suggest a N shift from urinary to faecal N losses on the TAN diet in the present experiment. This is confirmed by the lower apparent total-tract N digestibility (−5%) and apparent total-tract OM digestibility (−3%), reported as % reduction in this study. However, it is important to emphasize that (1) no actual urine samples were taken in the current experiment and (2) digestibility results might have been influenced by variations of AIA levels within the silo. The effect of tannins on the N metabolism of cattle was also discussed in the meta-analysis ($n = 58$) of Herremans, Vanwindekens, et al. (2020) including studies on both HT and CT in a dose varying from 1 to 40 g/kg DM. The results indicated that feeding tannins lowers (−16%) the ruminal NH_3 production, so less N needs to be converted by liver and kidneys, resulting in lower PUN (−9%), MUN (−8%) and urinary N excretions (−11%). However, this was compensated by a decrease in apparent total-tract N digestibility (−7%). The faecal N excretion increased (+10%), representing a shift from urinary to faecal N losses. Therefore, tannins seem able to form undegradable tannin–protein complexes, but despite an enhanced protein flow to the intestines they did not affect the MNE by increasing the milk protein flow. Generally, the increased faecal N excretion and decreased apparent total-tract N and OM digestibility for the TAN-treated cows in our trial would indicate that a N shift has occurred, but this effect is not supported by the PUN and MUN levels. In the study of Herremans, Decruyenaere, et al. (2020), where a N shift from urine to faeces was observed, ruminal NH_3, MUN and PUN concentrations were also not significantly affected.

The enteric CO_2 and CH_4 emissions were higher when cows were fed the TAN diet compared to the INO diet. Several in vitro and in vivo studies have demonstrated a reduction of enteric CH_4 emissions by tannins, depending on their type, source and concentration (Grainger et al., 2009; Hassanat & Benchaar, 2013; Ramírez-Restrepo & Barry, 2005: Woodward et al., 2000). Jayanegara et al. (2012) conducted a meta-analysis from both in vitro as in vivo experiments ($n = 30$) and reported that dietary tannin levels clearly lead to a decrease in ruminal CH_4 emissions, but the underlying anti-methanogenic effect of tannins is associated with a decreased digestibility of OM and especially fibre in the rumen. Nevertheless, in the range of acceptable tannin levels (<50 g/kg DM) (Min et al., 2003; Mueller-Harvey, 2006; Jayanegara et al., 2012), a decrease of maximum −15% can be expected and due to contrasting results at low tannin levels, this effect seems only reliable and distinguishable at tannin levels exceeding 20 g/kg DM (Jayanegara et al., 2012), suggesting that our dose (7.2 g/kg DM) was, in either way, too low to have a clear effect on the ruminal CH_4 emissions. Although more research is required, the review of Doyle et al. (2019) suggested that some LAB strains might be capable of altering ruminal fermentation, leading to reduced CH_4 production. From the limited number of studies, in vitro experiments have shown that LAB can reduce CH_4 production effectively, but due to the lack of robust in vivo animal trials it is impossible at this time to make a clear conclusion. In contrast, the study of Ellis et al. (2016) indicated minimal responses in CH_4 emission of dairy cows fed grass silage inoculated with LAB. The inconsistent results in literature on potential effects of both low HT doses and LAB inoculants on the in vivo CH_4 emission of dairy cows make it impossible to draw well-founded conclusions from the results in our experiment.

In addition to the low HT dose used, an effect of storage time, namely a steady decrease in tannin levels over time (Kardel et al., 2013; Makkar & Becker, 1996; Price et al., 1979), cannot be ruled out as a possible (partial) explanation for the lack of effect in the current study.

5 | CONCLUSION

The HT dose (7.2 g/kg DM) in the HT extract (20 g/kg DM) treated autumn grass silages did not appear to reduce the extent or rate of proteolysis in the silage process compared to the CON and INO

silages. In addition, the fermentation characteristics, chemical composition and nutritional values show no remarkable biological advantage for HT, except for a lower ethanol and crude ash content in the silage. The addition of the LAB inoculant resulted in a worse rather than improved fermentation, most likely due to a predominant effect of the heterofermentative LAB. The results of the feeding trial with lactating dairy cows showed that the addition of 3.2 g/kg DM chestnut HT did not affect DMI nor FPCMY, compared to feeding the silage treated with the LAB inoculant. The increased faecal N excretion and decreased apparent total-tract N and OM digestibility indicated an N shift from urinary to faecal N losses. However, urine samples were not collected, nor was this reflected in the MUN and PUN levels. Furthermore, potential variations of AIA might have affected digestibility results. The enteric CO_2 and CH_4 emissions were affected in the opposite way than expected, with higher emissions on the TAN diet. Overall, the low HT dose did not appear to exert a clear effect. As higher doses are not economically profitable, practical applications of an HT extract as a silage additive for (autumn) grass are not deemed worthy of further study.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Tine Van den Bossche http://orcid.org/0000-0002-7004-6130
Leen Vandaele http://orcid.org/0000-0002-4563-6796

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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