

# Natamycin added to maize silage does not adversely affect performance and voluntary feed intake of lambs

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**ABSTRACT.** Additives and inoculants are the focus of silage research, as new additives and their combinations can improve fermentation and silage quality. We performed two trials evaluating two doses (4 and 8 g/t, wet weight) of natamycin as an additive to maize silage compared to a control without supplementation. In the first trial, we assessed fermentation losses, yeast count, chemical composition, and aerobic stability of maize silages stored in pilot-scale silos using four replicates per treatment. The second trial was designed to evaluate the voluntary intake and performance of lambs fed the treated silages prepared in bunker silos. The lambs (10 lambs/treatment) were kept in individual pens and fed twice daily a total-mixed ration containing one of the treated silages. The highest dose of natamycin decreased dry matter and gas losses. The yeast count in the silages from the bunker silos tended to increase ( $r = 0.92$ ) over the weeks. There was no significant difference in voluntary feed intake or average daily gain of lambs fed natamycin silages compared to the control silage. Since high natamycin doses caused a decrease in fermentation losses in maize silage and exerted no deleterious effects on animal performance, this bacteriocin may soon be considered a potential component of silage additives.

## Introduction

Maize silage is a common conserved forage worldwide due to its high nutritional value for ruminants, which is associated with high productivity. Reducing fermentation losses in maize silages is the primary goal behind the use of additives to avoid the growth of yeast and other spoilage microorganisms (Woolford et al., 1980; Muck et al., 2018; Pinto et al., 2020) to prevent losses in silage quality and improve aerobic stability. New additives that can reduce fermentation losses, while improving aerobic stability of silage, remain an important subject in forage conservation research (Kung et al., 2021). Several chemical substances can inhibit the growth of undesirable microorganisms, increasing dry matter (DM) recovery and aerobic stability of silages (Muck et al., 2018).

Natamycin, initially called pimaricin, is a bacteriocin derived from cultures of *Streptomyces natalensis* and related species; it acts on ergosterol present in mould and yeast membranes. It is an important adjuvant in food preservation approved by over 150 countries and applied in fermented dairy products, meat, fruit and vegetables (Aparicio et al., 2016).

Despite its antifungal properties, data on the use of natamycin as a silage additive remain scarce. Woolford et al. (1980) first evaluated pimaricin and found a decrease in the number of yeasts and moulds during aerobic exposure of grass and maize silages. In a recent study, decreased dry matter losses (DML) and gas losses were reported in sugar cane silages (Buono et al., 2020) when natamycin was added during the storage stage.

The safety of natamycin as a food additive has been investigated in several species, such as rats, rabbits, dogs or cows (Mattia et al., 2002). No gross or microscopic lesions related to natamycin intake were found in haematological examinations or organ weight. Additionally, natamycin is the only polyene antifungal feed additive authorized in the European Union due to its low intestinal absorption and very low risk of toxicity to cattle and horses (Woodward, 2013). However, to the best of our knowledge, the potential impact of natamycin as a silage additive on voluntary intake and performance of ruminants has never been tested.

Therefore, the aim of this study was to evaluate doses of natamycin as an additive to maize silages. Specifically, we set out to (i) assess fermentation losses and aerobic stability of the silages, and (ii) examine voluntary intake and performance of lambs fed the treated silages. Moreover, we hypothesized that natamycin had no negative effects on animal performance in comparison to the control treatment.

## Material and methods

This study consisted of two trials performed using silages produced simultaneously, applying the same treatments to forage from the same plot. Both trials were conducted on the same day. The first trial was performed in the experimental silos to assess fermentation losses and aerobic stability of the silages. The second trial used small-scale bunker silos to evaluate voluntary DM intake and performance of growing lambs. The research on animals was conducted according to the guidelines of the Institutional Committee on Animal Use of the Federal University of Paraná (protocol number: 2012026289).

### Crop, ensiling and silage assessment

The Pioneer® 32R22H *stay-green* hybrid was harvested 101 days after emergence, when starch deposition was half of the “milk line” in the kernels in the centre of the cob. The following cultivation area data were collected: precipitation and relative humidity for seven days before and on the day of harvest. A pull-type forage harvester was used, set to chop maize forage at a theoretical particle size of 10 mm.

Three treatments were applied: C – control, without additive; N4 – natamycin (4 g/t, wet basis – WB); and N8 – natamycin (8 g/t, WB). The proposed dose of 8 g natamycin per tonne of forage was determined to be cost-equivalent to commercial microbial inoculants. The additives were diluted in 2.5 l of

distilled water per tonne of forage and homogeneously sprayed over the chopped forage. The same volume of distilled water per tonne without additives was evenly sprayed over the control forage.

During ensiling, samples were collected from each treatment to determine the DM content, pH, and chemical composition of the forage. The average composition of the maize forage used in both trials was expressed on a dry matter basis.

In the first trial, the treated forage was ensiled (625 kg/m<sup>3</sup>, WB) in a total of 12 (four replicates/treatment) plastic buckets (290 mm in diameter and 340 mm in height; 20 l volume). After mixing the additives, the forage was individually weighed to fill the silos. The silos were equipped with a bunsen-type stopcock in the lid to release fermentation gases and a 2-cm-high plastic platform was placed at the bottom to recover the effluents. The silos were stored for 170 days in a barn without temperature control (22 ± 5 °C), representing normal climate variability. After the storage period, all silos were individually weighed once again to assess gravimetric losses in the form of gas, effluent, and total DM losses (Jobim et al., 2007). At the time of opening, the silage was removed and homogenized for sampling and aerobic stability assessment.

The second trial was performed using maize silage from the small-scale bunker silos (0.5 m width × 0.5 m height × 12.5 m length; n = 3) designed to allow 20 cm/day of feeding-out. The bottom and sidewall of the silos were covered with double-sided polyethylene plastic sheets (200 µm thickness). The forage was manually compacted by feet protected by disposable boots to obtain 800 kg/m<sup>3</sup> bulk density. Four tracer nylon bags (1 kg of forage) were placed inside each silo (n = 12) to estimate fermentation losses with the difference between the DM content during ensiling and after storage (Jobim et al., 2007). The silos were properly sealed and kept closed for 170 days before opening.

The aerobic stability of silages from the first trial was assessed on 5 kg samples from each replicate. The silages were placed in plastic buckets and stored at uncontrolled room temperature of 15.3 ± 0.8 °C, which corresponded to normal climate change, as in the second trial with the small-scale bunker silos. Silage temperature was measured every three hours for five days. Aerobic stability (AS) was defined as the time (h) required for the temperature to rise 2 °C above room temperature (Keady and O’Kiely, 1996). The accumulated temperature (sum of all temperature measurements) and the maximum temperature during air exposure were also recorded.

After five days, the buckets were reweighed to estimate the aerobic DM loss (Ranjit and Kung et al., 2000).

Silage temperature in the bunker silos was measured daily during 63 days of the feed-out. The temperature from the silo panel was taken before the daily 20-cm silage removal (08:00). Bulb thermometers were horizontally inserted (10 cm depth) at three heights from bottom to top (10, 25, and 40 cm) at the centre of the exposed front panel of the silo; the ambient temperature was recorded simultaneously. The average ambient temperature observed during the experimental period after air exposure was  $10.8 \pm 4.4$  °C. Weekly samples from each bunker silages ( $n = 10$ ) were collected to determine the pH, DM content, chemical composition and yeast counts.

### Chemical analyses and microbial analyses

The pH was directly determined after fresh sample collection and dilution in 225 ml of deionized water using a glass electrode meter (HANNA pH 211, Hanna Instruments Italia Srl, Padova, Italy). The DM content was determined (method No. 934.01; AOAC International, 1990) in 350-g samples collected from each silo, which were dried in a forced air circulation oven (55 °C for 72 h) and subsequently ground using a Wiley mill (1-mm sieve) for further chemical analysis. Nitrogen content for crude protein (CP) was determined using the Dumas method (FP-528, Leco, combustion N analyzer, Leco Instruments Inc., St. Joseph, MI, USA), as described by Wiles et al. (1998). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to the Van Soest procedures. The content of neutral detergent fibre after amylase treatment (aNDFom, organic matter basis) was determined using  $\alpha$ -amylase and sodium sulphite. ADF content was adapted to the ANKOM200 Fiber Analyzer (ANKOM Technology, Fairport, NY, USA), using the procedures described by the manufacturer. Hemicellulose (HEM) was calculated as the difference between aNDFom and ADF contents. The concentration of ash was measured according to the method No. 924.05 of AOAC International (2012).

Yeasts and moulds were counted in the samples collected from the experimental silos at the opening or from the bunker silos weekly. All samples underwent 1/10 pre-dilution (25 g of silage in 225 ml of sterile 8.5 g/l NaCl solution); the solution was stirred and filtered, and 2 ml of the extract were used to prepare further dilutions ranging from  $10^{-1}$  to  $10^{-7}$ .

The plate method was carried out in triplicate on agar Sabouraud medium (Himedia®, Mumbai, India) with 100 g/l tartaric acid (1 ml of acid/100 ml of agar) with a pH of 4.5. All plates were incubated in a Bio-Oxygen Demand (BOD) incubator at 26 °C for 144 h. The colony-forming units per gram of silage (cfu/g) expressed the measured amounts of yeast and moulds and were based on the average values of three replicates for each sample.

### Animal assessment

Thirty non-castrated lamb crossbreeds (White Dorper  $\times$  Suffolk,  $55 \pm 8$  days old,  $18.3 \pm 3.3$  kg) were included in the experiment. They were housed in a naturally ventilated barn in indoor individual pens (2 m<sup>2</sup>), and had free access to feed and water. The animals were vaccinated against clostridial diseases, treated against ecto- and endoparasites, and weighed before the 10-day adaptation period. Clinical observations were carried out daily to evaluate the health status of each animal, and the Famacha® method was applied and the body condition score was calculated every 10 days.

The experiment was conducted in a completely randomised design, with three total mixed ration (TMR) treatments (10 lambs/treatment). TMRs were formulated based on the requirements of the National Research Council (NRC, 2007), predicting 200 g/day weight gain on a DM basis of the 70:30 forage to concentrate ratio. TMRs were isoproteic and isoenergetic. The concentrate mixture was composed of 705 g/kg soy bran, 195 g/kg ground corn, 95 g/kg mineral mixture, and 5 g/kg sodium bicarbonate, giving 910 g/kg DM, 357 g/kg CP, 208 g/kg NDF, and 760 g/kg total digestible nutrients (TDN). The samples of each TMR were collected weekly to determine DM content and chemical composition. The animals were fed twice a day (08:00 and 16:00). Orts were retrieved every day before the morning feeding. The offered feed was adjusted daily to allow 10% of Orts.

The animals were weighed at the beginning of the 53-day experimental period and every 10 days throughout this period. Animal performance was assessed based on the voluntary DM intake (DMI, kg/day), total weight gain (TWG, kg) during the whole experimental period, average daily gain (ADG, g/day), and feed conversion (g/kg).

Ultrasound evaluation (Landwind C40® with a linear transducer of 5 MHz, Shenzhen, GD, China) of the animals was carried out at the beginning and end of the experimental period. After shaving the left side between the 13<sup>th</sup> thoracic vertebra and



**Figure 1.** Ultrasound measurements of the thickness of subcutaneous fat (✱) and loin eye area (+ – vertical, × – horizontal) of the lambs. SF – subcutaneous fat in cm, LEA – loin eye area in cm<sup>2</sup>, L – *longissimus dorsi*

first lumbar vertebra, perpendicular to the *longissimus dorsi* muscle, the thickness of subcutaneous fat (SF, in cm) and loin eye area (LEA, in cm<sup>2</sup>) were measured (Figure 1).

### Statistical analyses

The first trial was carried out in a completely randomized design with four replicates (silos) per treatment. The second trial was performed in a completely randomized design with ten replicates (lambs) per treatment. The descriptive statistics (mean value  $\pm$  SD) of maize silages from bunker silos and the TMR from the treatments were performed. The normality and homogeneity of the data were tested using the Bartlett and Shapiro-Wilk tests. Yeast counts from both trials were log<sub>10</sub>-transformed to obtain log-normal distributed data and presented on a wet weight basis. The data were analysed for statistical significance using ANOVA at  $\alpha = 0.05$ . The means of the treatments were compared using the Tukey test ( $\alpha = 0.05$ ). All analyses were performed using JMP 13.1.0 software (SAS Institute Inc., Cary, NC, USA).

## Results

### Maize silages

The average composition of the fresh maize forage used in both trials was as follows (dry mass basis): 225.4 g/kg DM, 79.9 g/kg CP, 494.9 g/kg aNDFom, 230.6 g/kg HEM, 264.3 g/kg ADF, and 30.0 g/kg ash. The pH of the forage was 5.43.

**Table 1.** Descriptive statistics of the chemical composition of maize silages from bunker silos and TMR

Variables	Treatments (n = 10)		
	C	N4	N8
<b>Silages</b>			
DM	219.4 $\pm$ 1.19	221.6 $\pm$ 1.04	223.1 $\pm$ 0.76
CP	78.2 $\pm$ 0.32	79.2 $\pm$ 0.33	79.1 $\pm$ 0.30
aNDFom	481.6 $\pm$ 2.41	482.3 $\pm$ 1.45	489.7 $\pm$ 1.59
HEM	186.4 $\pm$ 2.18	193.8 $\pm$ 1.81	198.9 $\pm$ 1.41
ADF	295.2 $\pm$ 2.30	288.5 $\pm$ 1.64	290.1 $\pm$ 1.85
ash	32.3 $\pm$ 0.31	32.3 $\pm$ 0.21	32.4 $\pm$ 0.15
pH	3.50 $\pm$ 0.07	3.51 $\pm$ 0.10	3.52 $\pm$ 0.09
<b>Total mixed ration</b>			
DM	462.0 $\pm$ 0.99	462.7 $\pm$ 1.14	467.5 $\pm$ 1.01
CP	174.5 $\pm$ 1.30	169.7 $\pm$ 0.49	171.2 $\pm$ 0.44
aNDFom	391.5 $\pm$ 4.47	379.5 $\pm$ 0.86	383.3 $\pm$ 0.82
HEM	161.0 $\pm$ 1.93	161.1 $\pm$ 0.48	168.9 $\pm$ 0.85
ADF	208.3 $\pm$ 4.11	218.3 $\pm$ 0.50	219.0 $\pm$ 0.47
ash	72.6 $\pm$ 0.53	70.1 $\pm$ 0.20	72.0 $\pm$ 0.15

C – control, N4 – natamycin (4 g/t), N8 – natamycin (8 g/t); TMR – total mixed ration, DM – dry matter, CP – crude protein, aNDFom – neutral detergent fibre after amylase treatment on organic matter basis, HEM – hemicellulose, ADF – acid detergent fibre; data are presented as mean value  $\pm$  SD, g/kg DM

The chemical composition of the maize silages from bunker silos and the TMR used during the animal assessment are shown in Table 1.

The chemical composition of maize silages from the experimental silos is presented in Table 2. The N8 treatment showed a higher DM content ( $P < 0.01$ ) compared to the control and N4 treatment,

**Table 2.** Chemical composition (g/kg DM), fermentation losses, and aerobic stability of maize silages from the experimental silos

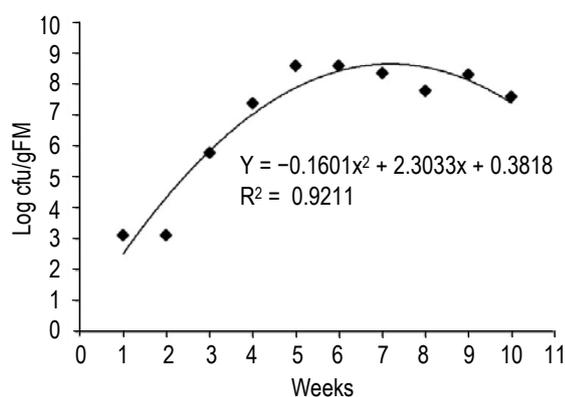
Variables	Treatments (n = 4)				
	C	N4	N8	SEM	P-value
DM, g/kg	210.1 <sup>a</sup>	206.5 <sup>a</sup>	227.2 <sup>b</sup>	0.157	0.001
CP	87.2	89.9	84.5	0.184	0.177
aNDFom	492.1	500.0	495.8	1.537	0.929
HEM	215.2	224.3	220.6	0.947	0.781
ADF	276.9	275.7	275.2	0.684	0.983
Ash	41.3 <sup>b</sup>	44.6 <sup>c</sup>	38.7 <sup>a</sup>	0.065	0.001
pH	3.58	3.67	3.68	0.028	0.068
DML, %	10.8 <sup>b</sup>	11.4 <sup>b</sup>	2.9 <sup>a</sup>	0.065	0.001
Gases, g/kg DM	83.0 <sup>b</sup>	88.5 <sup>b</sup>	13.5 <sup>a</sup>	0.664	0.001
Effluent, kg/t WB	26.7	27.6	29.1	0.618	0.074
Yeast, log <sub>10</sub> cfu/g WB	6.1 <sup>b</sup>	5.0 <sup>a</sup>	5.9 <sup>b</sup>	0.073	0.001
Aerobic stability, h	>120	>120	>120	–	–
Temperature, °C	15.7	15.6	15.4	0.152	0.668
DMLae, %	0.6 <sup>a</sup>	0.2 <sup>a</sup>	3.2 <sup>b</sup>	0.701	0.031

C – control, N4 – natamycin (4 g/t), N8 – natamycin (8 g/t); DM – dry matter, WB – wet basis, DML – dry matter losses, CP – crude protein, aNDFom – neutral detergent fibre after amylase treatment on organic matter basis, HEM – hemicellulose, ADF – acid detergent fibre, DMLae – DML after aerobic exposure, SEM – standard error of the mean, <sup>abc</sup> – means within a row with different superscripts are significantly different at  $P < 0.05$

while ash concentration in the N8 treatment was lower than in the control and N4 treatment. The remaining variables were not significantly affected by the additives ( $P > 0.05$ ). The N8 treatment presented the lowest DML and gas losses in the laboratory-scale silos ( $P < 0.01$ ), which resulted in higher DM and ash content in these silages. The N4-treated silages showed the lowest yeast count ( $P < 0.01$ ) upon opening the experimental silos. Moulds were not detected ( $< 2.0$  cfu/g) in our experiment.

None of the silages exceeded the room temperature during the five-day period of aerobic exposure. The average temperature of the silages was  $15.6 \pm 0.37$  °C (mean  $\pm$  SD). Moreover, despite its small magnitude, DML after aerobic exposure (DMLae) was greater in the N8 treatment ( $P < 0.01$ ) in comparison to the control and N4 treatment (Table 2).

Despite the daily removal of 20 cm of silage, the yeast count tended to increase over the weeks in the silages from the bunker silos (Figure 2). A quadratic pattern of growth was observed. The yeast count increased until the fifth week. No significant differences were detected between the treatments ( $P > 0.05$ ).



**Figure 2.** Average yeast counts ( $\log_{10}$  cfu/g) in the maize silage from bunker silos during feed-out

Fermentation losses in the silages from the bunker silos showed no differences between the treatments ( $P > 0.05$ ), averaging  $8.8 \pm 1.43\%$  DM,  $7.8 \pm 1.74\%$ , and  $9.2 \pm 2.97\%$  for the C, N4, and N8 treatments, respectively. Similarly, no differences were observed in aerobic stability between these silages ( $P > 0.05$ ). The average temperatures observed in the silo panel were  $16.9 \pm 2.40$  °C for C,  $16.2 \pm 2.51$  °C for N4, and  $16.4 \pm 2.51$  °C for N8.

### Animal performance

The inclusion of natamycin as a silage additive did not affect voluntary intake or performance of the lambs (Table 3). No adverse effects were observed during the clinical assessment of the lambs throughout the experimental period.

**Table 3.** Average performance of lambs fed individual silage treatments

Variables	Treatment (n = 10)			P-value
	C	N4	N8	
ADG, kg/day	0.272	0.255	0.247	0.425
TWG, kg	14.400	13.515	13.090	0.425
FC, intake:weight gain	3.237	3.270	3.342	0.778
DMI, g/kg/d body weight	25.728	25.943	25.845	0.976
DML, kg/day	0.879	0.833	0.826	0.722
Initial LEA, cm <sup>2</sup>	7.181	7.615	7.118	0.420
Final LEA, cm <sup>2</sup>	11.015	10.966	10.529	0.726
Initial SF, cm	0.267	0.280	0.275	0.788
Final SF, cm	0.367	0.344	0.353	0.741

C – control, N4 – natamycin (4 g/t), N8 – natamycin (8 g/t); ADG – average daily gain, TWG – total weight gain, FC – feed conversion, DMI – dry matter intake, LEA – loin eye area, SF – subcutaneous fat, SEM – standard error of the mean;  $P > 0.05$

## Discussion

### Silage composition

The maize hybrid used in this experiment presented strong *stay-green* characteristics. The vegetative parts of the plants exhibited high moisture content (225.4 g/kg) at harvest, even when senescence of the spike bracts and half-filled milk line in the grain was observed. Similar to our study, Yuan et al. (2022) reported lower DM concentrations in the whole-crop corn (219 g/kg), than the recommended DM concentration range (300–350 g/kg) (Ferraretto et al., 2018).

The chemical composition of the silages was similar in both trials. Rodrigues et al. (2002) analysed the chemical composition and losses of maize silage in various types of silos and observed no differences in the ensiled material between the experimental laboratory silos and conventional trench-type silos. In the present study, an efficient fermentation process resulted in low pH of the silages from the bunker (3.51) and experimental silos (3.64). Yuan et al. (2022), evaluating natamycin treatment, reported similar pH values in maize silages with low DM content. The high amount of soluble carbohydrates in low-DM forages contributed to the high fermentation rates in the early stage after silo closure and the rapid pH decline in comparison to the high-DM forage (Filya et al., 2006; Yuan et al., 2022).

### Silage losses and aerobic stability

Despite the low DM content of the forage, most of the losses in our study were caused by gas production during fermentation. In a study on maize silages with 307.0 g/kg DM using experimental silos to evaluate additives, the authors reported an average DML of 7.6% after 120 days of fermentation

(Junges et al., 2013). In addition, low-DM forages i.e., between 200 and 250 g/kg, produced up to 200 l of effluent per tonne of fresh matter during ensiling (Ashbell et al., 2002). This was not observed in our results ( $27.8 \pm 0.61$  kg/t WB). Dry matter losses during fermentation can be prevented by additives (Muck et al., 2018). Our results demonstrated the efficiency of natamycin addition in controlling DML and gas losses at a dose of 8 g/t WB (N8). When the same dose of natamycin was used in combination with a microbial additive (*Lactobacillus buchneri*), it decreased DML and gas production in maize silages (Pinto et al., 2020). The authors assumed a possible synergistic effect reducing the population of spoilers, such as yeasts. Shah et al. (2020) reported that natamycin in combination with *Lactobacillus* spp. reduced the abundance of unwanted microbial organisms and improved fermentation characteristics during ensiling. As opposed to conventional silos, a positive effect of natamycin was observed in our study in preventing silage losses in the laboratory-scale silos, probably due to their complete sealing. This effect was also observed in a previous study on maize silage stored in conventional silos, which showed high DML (Bernardes et al., 2012). However, comparing the losses between both silo models was not practical in our study because there were no replicates of the conventional silos. In contrast, tracer nylon bags used in the present experiment was a proper method to obtain reliable data on losses and fermentation quality in the conventional silos.

Yeast counts in the silages was high (Table 2) and could have increased due to the low DM content of the silages. However, Yuan et al. (2022) reported an average of 2.18 log cfu/g yeast in a maize silage with 219 g/kg DM stored for 60 days. Filya and Sucu (2010) observed yeast counts of 5.12 and 5.73 cfu/g at the end of the ensiling period (90 days) in the control and homofermentative maize silages, respectively. This suggests that obtaining higher yeast counts is not uncommon in the laboratory-scale maize silages. In the pilot scale silos, the N4 treatment contained a lower yeast count than the N8 silage and control. Yeasts can tolerate pH fluctuations between 3 and 8, which is a problem in silage spoilage control (Bravo-Martins et al., 2006). Conversely, after silage fermentation, low natamycin doses may be present in the recovery analysis (Woolford et al., 1980). This effect may be related to the short half-life of that compound when applied to low pH media such as silage. Thus, natamycin may inhibit the growth of yeast in the first aerobic phase of the ensiling, but then its effect is reduced,

as concluded by Bueno et al. (2020). Natamycin had no effect on the yeast counts in the bunker silos. The increase in the yeast population in these silages over the weeks indicated a low residual effect of natamycin on aerobic stability of the silages. The effect of silage additive was shown to reduce the initial yeast population during the first stage of forage fermentation, but in time, the combination of substrates such as soluble carbohydrates and lactic acid supported the yeast growth under air exposure (Pinto et al., 2020). Dolci et al. (2011) reported an exponential growth of yeasts in silages from bunker silos, thereby increasing the pH and benefiting bacteria and fungi responsible for high degradation rates of the material. Horizontal silos often show increased losses caused by spoiling microorganisms in the final portion due to the permeability for oxygen entering the mass over days. In the present study, the low pH of the silages associated with moderate ambient temperatures (15.3 °C, experimental silos; 10.8 °C, bunker silos) after opening the silos, provided longer aerobic stability, however, with no treatment effect in any of the trials. Pinto et al. (2020) verified 51 and 54 h of stability at a room temperature of  $23 \pm 1$  °C, for the control and N8 silages, respectively. Further studies should consider extending the evaluation time of aerobic stability. Moreover, the low DM content of our forage maximised the bulk density and fermentation process, thereby inhibiting the increase of silage temperatures. Boudra and Morgavi (2008) evaluated maize silages with different DM content and observed longer aerobic stability for silages with low DM content. Natamycin may indirectly support improved aerobic stability in silages by controlling yeast abundance in the early stages of the ensiling process. In this regard, Bueno et al. (2020) and Pinto et al. (2020) detected a synergistic effect of the association of natamycin and *Lactobacillus buchneri*.

### Lamb performance

The design and evaluation of new silage additives are a recurring theme in the literature regarding new bacterial strains, species, and combination of substances that can improve the performance of additives (Muck et al., 2018). In the present study, in addition to the chemical composition and loss assessment, we evaluated the performance of the lambs fed natamycin-treated maize silages. To our knowledge, this is the first study that assessed voluntary intake and performance of animals administered natamycin-treated silages. No detrimental effects of natamycin were observed

with respect to animal intake and performance. Clinical observations indicated no toxic effects on the lambs, which were observed daily throughout the experimental period. Potential toxicity of natamycin for several animal species has been investigated over the last 50 years (Mattia et al., 2002). Several studies in rats, rabbits, and dogs evaluated haematological parameters and organ weights, and no gross or microscopic lesions considered to be caused by natamycin were found (Levinskas et al., 1966; Mattia et al., 2002). Thus, natamycin is believed to be a safe additive due to its low absorption in the animal gut (EFSA, 2009). Additionally, natamycin is considered as GRAS (generally recognized as safe) by the US FDA (2015), and its cost-determined dose used in this trial is within the acceptable daily intake for humans and animals (0.3 mg/kg body weight/day) (EFSA, 2009).

There was no significant difference in voluntary feed intake by the lambs depending on the silage type. The findings reported here support the hypothesis that natamycin has no negative effects on feed consumption by lambs. Feed intake measured in the present study (25.8 g/kg body weight) was lower than the values reported by Ribeiro et al. (2002) (31.5 g/kg body weight). This effect could be related to the low pH and DM content of the silages, which may reduce animal feed intake (Ribeiro et al., 2003). However, the average daily gain of animals in our study (0.250 kg/day) was higher than those observed by Ribeiro et al. (2003) (0.095 kg/day) and Bueno et al. (2004) (0.193 kg/day). Moreover, Lombardi et al. (2010) reported an average daily gain of 0.150 kg/day in lambs fed maize silages with 1 g/kg DM urea supplementation. These values demonstrated the high quality of the silage used in our trial.

Carcass parameters were not affected by the treatments applied in the current study. The thickness of the subcutaneous fat indicated a satisfactory body score of the feedlot lambs fed maize silages. Sousa et al. (2008) considered subcutaneous fat of lamb carcasses greater than 0.2 mm to be an indicator of good animal condition.

Considering the silage data and animal performance, this study demonstrated the potential of natamycin in reducing fermentation losses in maize silages, without adversely affecting voluntary intake, performance or health of feedlot lambs. Our results support the potential application of natamycin for the formulation of silage additives. Further research is needed to evaluate the efficacy of natamycin as a silage additive for different forages and environments.

## Conclusions

Natamycin at a dose of 8 g/t WB decreased fermentative losses of low-DM maize silages in the experimental silos. Lambs fed maize silages treated with natamycin exhibited good health and no differences were found in voluntary DM intake or body weight gain compared to control.

## Conflict of interest

The Authors declare that there is no conflict of interest.

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