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Lemongrass essential oil reduces whole-plant sorghum silage gas losses and does not affect silage *in vitro* degradation

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Abstract

Lemongrass essential oil (LEO) has been evaluated as a silage additive to improve silage fermentation and reduce fermentative losses. The present study aimed to evaluate the effects of increasing levels of LEO on whole-plant sorghum silage (WPSS) fermentation profile, fermentation losses, chemical composition, dry matter (DM) and neutral detergent fibre (NDF) *in vitro* degradation and aerobic stability. Five cultivars and fifty experimental silos were used to evaluate the following LEO levels: 0, 1, 2, 3 and 4 ml kg^{-1} DM of WPSS. The material was compacted (650 kg m^{-2}) and the silos were sealed, weighed and stored at room temperature for 167 days. The addition of LEO linearly decreased butyric acid content and the ratio between lactic and acetic acids. Intermediate levels of LEO increased NH_3-N and reduced lactic and acetic acids content. LEO linearly decreased silage gas losses. However, LEO did not affect total silage losses and DM recovery. Intermediate levels of LEO addition decreased organic matter and crude protein in the WPSS. The addition of LEO did not affect other chemical composition parameters, DM and NDF *in vitro* degradation, and pH and temperature of the silage after aerobic exposure. Thus, LEO supply in WPSS reduces gas losses, butyric acid concentration, and the ratio between lactic and acetic acids. However, LEO does not improve the chemical composition, *in vitro* degradation, and aerobic stability of WPSS.

Introduction

Maize silage is the main forage source in the Brazilian dairy cows' systems (Bernardes and Rego, 2014) because it is palatable, important as an energy and highly digestible forage source (Scheler and Cavichioli, 2021). However, weather factors such as temperature, precipitation and solar radiation can affect the quality and productivity of whole-plant maize silage (Maldaner *et al.*, 2014). Whole-plant grain sorghum silage (WPSS) can be an alternative forage source that has higher adaptability to warm and dry environments (Zegada-Lizarazu *et al.*, 2012) and shows high productivity and energy content.

Although sorghum is a more productive crop than maize, losses can be high during silage fermentation (Pinho *et al.*, 2015). Several studies have attempted to improve the silage fermentation process, but the conditions are not always adequate to guarantee sufficient silage quality (McDonald *et al.*, 1991). Microbial inoculants are the main group of additives used to improve dry matter (DM) recovery (Muck *et al.*, 2018) and the aerobic stability of sorghum silage (Thomas *et al.*, 2013). Kung *et al.* (2003) evaluated a blend of essential oils (EO) as an additive in maize silage. EO are natural secondary metabolites extracted from plants (Benchaar *et al.*, 2008) and are known to have antimicrobial properties (Calsamiglia *et al.*, 2007). Despite the antimicrobial effects of known EO, few studies using these substances as additives for silage have been carried out (Besharati *et al.*, 2020).

The ban on antibiotics used as growth promoters is negatively impacting the livestock sector (Laxminarayan *et al.*, 2016). Innovative alternatives are needed to produce animal feed and help combat rising antibiotic resistance (Czaplewski *et al.*, 2016). In a recent study of our research group, Cantoia *et al.* (2020) observed positive effects using 2 ml of lemongrass essential oil (LEO) per kg of sugarcane silage (as-fed) on DM recovery, nutritional value and aerobic stability. This study also showed decreased yeast and mould count in LEO-treated silages, which are considered one of the main factors responsible for ethanol production and fermentation losses. Kholif *et al.* (2021) observed improvements in rumen fatty acids and milk yield and better nutrient utilization efficiency in lactating ewes fed lemongrass. However, to the best

of our knowledge, there is no study evaluating LEO addition during the ensiling of a less fermentable material, such as whole-plant sorghum silage (WPSS). Although fermentability of fresh material affects additives' effects on silage fermentation profile (Oliveira *et al.*, 2017), we hypothesized that increasing doses of LEO would reduce fermentation losses, silage butyric acid concentration, and increase the aerobic stability of WPSS. The present study aimed to evaluate the effects of increasing LEO levels on silage fermentation profile, fermentation losses, chemical composition, *in vitro* degradation of DM and fibre, and pH and temperature after aerobic exposure.

Materials and methods

The present trial was conducted at the Itaqui Campus of the Federal University of Pampa (29.2° South, 56.6° West, and 57 m above sea level). Experimental procedures were previously approved by the Animal Welfare Ethics Committee from the Federal University of Pampa (approval number 042/2019).

Treatments, experimental design and ensiling

Fifty experimental silos were made in PVC tubes with 28 cm diameter and 25 cm height. A completely randomized design was used to evaluate increasing doses of LEO addition during whole-plant sorghum ensiling: 0 (control); 1, 2, 3 and 4 ml per kg of sorghum DM. Lemongrass EO was obtained from Quinarí (Ponta Grossa, Brazil) and the doses were defined to be lower than those evaluated by Cantoia *et al.* (2020).

Five plots (each 180 m^2) were conventionally prepared, and sown using five different sorghum cultivars (Nusil 426®, Taguá®, Nucover 100® and Qualysilos® from Sementes Nuseed, Curitiba, Brazil and AG2501®, from Agroceres, Rio Claro, Brazil) on 01 November 2019. Within each cultivar, two silos were prepared for each of five levels of LEO. The harvest was conducted on two subsequent days from each plot. Harvest commenced when the first cultivar (plot) reached 400 g kg^{-1} of DM content, and in subsequent days, it was performed in other plots. Plants were harvested on 7 (Soft dough stage) to 8 (Hard dough stage) phenological stage (Rao *et al.*, 2007). The harvests occurred from February 11–17, 2020 (105 ± 2.44 days after the seeding). Plants were harvested at 5 cm of height and processed in a stationary mill (GP 1500 ADI, Garthen, Navegantes, Brazil). Representative samples of each cultivar were collected for chemical analysis, *in vitro* assay, and particle evaluation according to Maulfair *et al.* (2011) (Table 1). Dried sand (5 kg) was positioned inside the silo in a layer below the silage to quantify effluents. The sorghum material for each silo was individually weighed, and LEO (or placebo) was added using a pipette. Then, the sorghum was manually mixed and compacted to 650 kg m^{-3} of bunker density, sealed, and stored with shelter from light and heat. The temperature was not controlled and averaged 19°C during the storage period.

Data record and sampling

The silos were opened 168 ± 2.44 days after the ensiling. The extended period of storage occurred due to the COVID-19 pandemic condition. Silos were weighed before opening to assess the gas losses through the storage period. Once the silos were opened, the silage was completely removed from the silos; 5-cm of the top and bottom layer was discarded, and the silage was manually mixed to obtain samples for fermentation profile evaluation (500 g), chemical analysis and *in vitro* assay (500 g) and aerobic stability evaluation (3 kg).

Table 1. Chemical composition and particle size of whole-plant *Sorghum bicolor* (L) before the ensiling ($n = 5$)

Item	Mean	S.D.[a]
Chemical composition, g kg^{-1} DM unless stated		
Dry matter, g kg^{-1} fresh matter	316	79.9
Organic matter	945.9	3.38
Neutral detergent fibre	615	10.9
Acid detergent fibre	302	11.0
Non-fibre carbohydrates	243	11.5
Crude protein	71.4	5.30
Acid detergent lignin	35.7	8.46
Ether extract	17.0	2.73
Particle size, g kg^{-1} fresh matter		
>19 mm	364	198.5
8–19 mm	337	101.1
4–8 mm	137	38.2
<4 mm	162	70.0

[a]Stardard deviation.

After sampling, silage fluid was extracted using a hydraulic press, without any water addition. Silage pH was immediately evaluated using a bench pH metre (LUCA-210®, Lucadema, São José do Rio Preto, Brazil). Fluid was frozen (−20°C) without acid addition, prior to subsequent analysis. Samples for chemical and *in vitro* analysis were dried in a forced-air oven at 60°C for 72 h, and processed in a knives mill (SL-31®, Solab Científica, Piracicaba, Brazil) to pass through a 2 (*in vitro* assay) or 1-mm (chemical analysis) sieve before the storage until analysis. Samples for aerobic stability assay were packed in PVC pipes without compression and stored for 168 h in a temperature-controlled room (21.2 ± 2.27°C) (Wilkinson and Davies, 2012). The temperature at the centre of silage mass was evaluated every 12 h using a spit thermometer (K29-5030®, Kasvi – Produtos Laboratoriais, Pinhais, Brazil). Silage pH was recorded every 12 h after 15-min of water homogenization (dilution rate 15 g: 100 ml; Kung *et al.*, 1984).

Chemical analysis and in vitro assay

Silage fluid was thawed at room temperature, and centrifuged (500 × ***g*** for 15 min.) to remove solid contaminants. The supernatant was used to analyse ammonia (NH_3-N), ethanol, and organic acids. Ammonia-N was analysed using the Kjeldahl method (984.13, AOAC, 2000) without sample digestion. The concentration of lactic acid was assessed after sulphuric acid solubilization and heating (75°C for 2.5 min). Samples were cooled and heated at 90°C for 1.5 min., after the addition of a colour reagent (4-phenylphenol, Sigma Aldrich, St. Louis, USA) addition. Readings were performed in a spectrophotometer at 560 nm (Pryce, 1969). Ethanol and other organic acids were evaluated using a gas chromatographic method. The sample was acidified with ortho-phosphoric acid (1.8 ml sample: 0.2 acid) and

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injected in a gas chromatograph (GC-2010 plus chromatograph, Shimadzu, Barueri, Brazil), equipped with an auto-sampler AOC-20i, capillary column Stabilwax-DA™ (30 m, 0,25 mm ID, 0.25 µm *df*, Restek©), and a flame ionization detector. It was used 1 µl of sample and 40:1 split ratio. Helium was the carrier gas and injection velocity was 42 cm s^{-1}. The injector and detector temperatures were 250 and 300°C, respectively, whereas the initial temperature of the column was 40°C. The temperature increased from 40 to 120°C at 40°C min^{-1} rate, followed by increases from 120 to 180 and from 180 to 240°C at 10 and 120°C min^{-1}, respectively. Then, the temperature remained for 3 min at 240°C. Fatty acids were quantified based on the peaks areas, and qualifications were realized using GC solution v. 2.42.00 software.

Unfermented sorghum and silage samples (processed at 1-mm sieve) were analysed for DM (method 930.15; AOAC, 2000), ash (method 942.05; AOAC, 2000), crude protein ($N \times 6.25$; Kjeldahl method 984.13; AOAC, 2000), ether extract (method 920.39; AOAC, 2000), acid detergent fibre and lignin (method 973.18; AOAC, 2000), and neutral detergent fibre (NDF) using thermal-stable alpha-amylase without sodium sulphite (Van Soest *et al.*, 1991). Fibre contents were expressed including residual ash.

In vitro assay was performed according to Tilley and Terry (1963) and Holden (1999) methods. Samples (processed at 1-mm sieve) were placed in nonwoven fabric (5 × 5 cm and 100 g m^{-2}; Casali *et al.*, 2008) and incubated for 48-h in ruminal inoculum using an *in vitro* incubator (NL162®, New Lab, Piracicaba, Brazil). The inoculum was prepared using ruminal fluid from two Dairy heifers maintained fed with Mombaça Guinea grass (*Megathyrsus maximus*) with no supplementation. Buffer was as described by McDougall (1948). After incubation, bags were washed in running water, and analysed for NDF content, as previously described.

Calculations and statistical analysis

Gas (GL, Eqn 1) and effluent losses (EL, Eqn 2) were calculated using the following equations (Jobim *et al.*, 2007):

$$GL\left(\frac{g}{kg\ DM}\right) = \frac{WSWen(g) - WSWop(g)}{EDM\ (kg)} \quad (1)$$

$$EL\left(\frac{g}{kg\ DM}\right) = \frac{ESW_{op}(g) - ESW_{en}(g)}{EDM\ (kg)} \quad (2)$$

where WSW and ESW are whole and empty silos weight, respectively; *en* is weight at ensiling, whereas *op* is weight at silos opening, and EDM is ensiled DM. DM recovery is the ratio between DM obtained after storage and EDM.

Data were analysed using the PROC MIXED of SAS (version 9.4) according to the following model:

$$Y_{ijk} = \mu + LEO_i + C_j + LEO \times C_{ij} + e_{ijk} \quad (3)$$

With $e_{ij} \approx N\left(0,\ \sigma_e^2\right)$, where: Y_{ijk} is the observed value of the dependent variable; LEO_i is the fixed effect of LEO level ($i = 1$ to 5); C_j is the fixed effect of cultivar ($j = 1$ to 5); $LEO \times C_{ij}$ is the LEO and cultivar fixed interaction effect; e_{ijk} is the random residual error; N stands for Gaussian distribution; σ_e^2 is the error variance. The LEO level effect was studied using polynomial regression: it evaluated the linear, quadratic, and non-quadratic (cubic) effect of LEO on evaluated variables.

Temperature and pH data obtained after aerobic exposure were analysed using the following model:

$$Y_{ijkl} = \mu + LEO_i + C_j + LEO \times C_{ij} + \omega_{ijk} + T_l + LEO \times T_{il} + C \times T_{jl} + LEO \times C \times T_{ijl} + e_{ijkl} \quad (4)$$

with $\omega_{ij} \approx N\ (0,\ \sigma_\omega^2)$; and $e_{ijkl} \approx MRN\ (0,\ R)$; where Y_{ijkl} is the observed value of the dependent variable; μ, LEO_i, C_j, $LEO \times C_{ij}$, and N were previously defined; ω_{ijk} is the error associated with parcels (silos); T_l is the fixed effect of time after aerobic exposure; $LEO \times T_{il}$, $C \times T_{jl}$, and $LEO \times C \times T_{ijl}$ are the fixed interaction effects between previously defined effects; e_{ijkl} is the experimental error; σ_ω^2 is the variance associated with parcels (silo); *MRN*: stands for multivariate analysis with approximately Gaussian distribution; R is the matrix of variance and covariance due to repeated measures. The following matrices were evaluated according to the Bayesian method: CS, CSH, AR, ARH, TOEP, TOEPH, UN, FA, ANTE. Treatment effect was decomposed when $P \leq 0.10$. Significance was declared at $P \leq 0.05$.

Results

Fermentation profile

Utilization of LEO linearly decreased ($P \leq 0.01$) butyric acid concentration and lactic to acetic acids ratio and linearly increased ($P = 0.05$) propionic acid concentration in WPSS (Table 2). Except for propionic acid silage concentration, there was no LEO and cultivar interaction effect ($P \geq 0.07$) on the silage fermentation profile. In addition, LEO quadratically affected ($P \leq 0.05$) silage NH_3-N, lactic, and acetic acids concentrations. Intermediary levels of LEO increased NH_3-N and reduced acetic and lactic acids concentrations in relation to control and upper level (4 ml kg^{-1} DM). However, treatments showed no effects ($P \geq 0.12$) on silage pH and concentrations of ethanol and branched-chain fatty acids.

Fermentative losses and DM recovery

There was no LEO and cultivar interaction effect ($P \geq 0.17$) on fermentation losses and DM recovery. The addition of LEO linearly decreased ($P \leq 0.01$) gas losses of silage. Lemongrass EO did not affect ($P \geq 0.89$) effluent and total losses. Therefore, treatments showed no effect ($P = 0.44$) on DM recovery.

Chemical composition and in vitro degradation of DM and NDF

There was no LEO and cultivar interaction effect ($P \geq 0.06$) on silage chemical composition and *in vitro* degradation (Table 3). The intermediate levels of LEO addition increased ($P \leq 0.03$) the organic matter and crude protein of the silage. However, increasing levels of LEO did not affect ($P \geq 0.41$) NDF, acid detergent fibre, acid detergent lignin, non-fibrous carbohydrates and ether extract content of the silage. Therefore, LEO had no effect ($P \geq 0.11$) on *in vitro* degradation of DM and NDF.

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Table 2. Silage fermentation profile and losses of WPSS containing increasing levels of LEOs

	LEO, ml kg^{-1} DM[a]						Probabilities[b]					
Item	0	1	2	3	4	S.E.M.	Treat.	Lin.	Qua.	Cub.	Cult.	Treat. × Cult.
pH	4.45	4.49	4.48	4.45	4.49	0.006	0.12	0.56	0.70	0.06	<0.01	0.23
NH_3-N, g kg^{-1} N	114	118	117	112	107	1.1	0.03	0.01	0.02	0.81	<0.01	0.38
Ethanol, g kg^{-1} DM	30.5	31.2	29.9	28.5	30.0	0.76	0.46	0.39	0.45	0.28	<0.01	0.48
Organic acids, g kg^{-1} DM, unless stated												
Acetic acid	5.55	4.81	5.00	5.23	5.51	0.113	0.21	0.68	0.04	0.28	<0.01	0.83
Butyric acid, mg kg^{-1}	157	102	68.8	34.3	19.9	7.26	<0.01	<0.01	0.20	0.98	<0.01	0.07
Propionic acid, mg kg^{-1}	46.9	56.8	46.5	58.2	60.7	1.96	0.09	0.05	0.66	0.44	<0.01	0.05
Lactic acid	21.5	20.1	18.4	18.6	18.8	0.29	0.02	0.01	0.05	0.53	<0.01	0.07
Lactic: acetic ratio	4.66	5.53	4.17	3.88	4.05	0.164	0.03	0.01	0.56	0.11	<0.01	0.10
BCFA, mg kg^{-3}	168	196	212	191	173	7.5	0.38	0.91	0.06	0.78	<0.01	0.06
Fermentation losses, g kg^{-1} DM												
Gas	55.4	53.9	50.5	48.7	49.2	0.72	0.03	<0.01	0.40	0.40	<0.01	0.48
Effluent losses	49.9	53.5	51.7	51.1	56.8	2.61	0.93	0.55	0.81	0.53	<0.01	0.99
Total losses	108.6	107.4	102.2	99.8	105.9	3.15	0.89	0.57	0.52	0.57	<0.01	0.99
DM recovery	925.3	926.5	919.8	927.8	928.4	2.61	0.44	0.24	0.21	0.68	<0.01	0.17

[a]Increasing levels of LEO in WPSS: 0, 1, 2, 3 and 4 ml kg^{-1} DM.
[b]Probabilities: LEO effect (Treat.), linear (Lin.), quadratic (Qua.); and non-quadratic/cubic (Cub.) of LEO.
[c]Branched-chain fatty acids.

Aerobic stability

After aerobic exposure, the addition of increasing levels of LEO did not affect ($P \geq 0.32$) the silage pH and temperature regardless of the cultivar ($P \geq 0.13$) and evaluation period ($P \geq 0.20$) (Figs 1 and 2).

Discussion

We hypothesized that LEO addition during WPSS ensiling could reduce fermentative losses and butyric acid silage content and increase silage *in vitro* degradation and aerobic stability of silage, based on previous studies in sugarcane silage (Cantoia *et al.*,

Table 3. Chemical composition and *in vitro* degradation of WPSS containing increasing levels of LEOs

	LEO, ml kg^{-1} DM[a]						Probabilities[b]					
Item	0	1	2	3	4	S.E.M.	Treat.	Lin.	Qua.	Cub.	Cult.	Treat. × Cult.
Chemical composition, g kg^{-1} DM												
Dry matter, g kg^{-1} as-fed	299	299	297	298	300	0.78	0.64	0.53	0.22	0.37	<0.01	0.37
Organic matter	939	939	932	939	939	0.63	0.01	0.93	0.03	0.99	<0.01	0.88
Neutral detergent fibre	667	660	670	669	657	2.9	0.54	0.61	0.37	0.18	<0.01	0.07
Acid detergent fibre	342	336	338	341	336	2.3	0.86	0.59	0.89	0.33	<0.01	0.21
Acid detergent lignin	43.6	42.7	43.1	42.9	40.9	0.61	0.68	0.24	0.57	0.46	<0.01	0.47
Non-fibre carbohydrates	177	187	173	179	188	2.9	0.41	0.51	0.45	0.25	<0.01	0.06
Crude protein	79.1	76.9	75.7	76.8	79.2	0.52	0.09	0.97	0.01	0.91	<0.01	0.58
Ether extract	16.1	14.8	14.4	13.8	14.9	0.57	0.76	0.38	0.34	0.84	0.08	0.75
In vitro degradation, g kg^{-1}												
Dry matter	548	549	543	526	551	4.69	0.43	0.52	0.28	0.11	<0.01	0.30
Neutral detergent fibre	449	454	475	407	469	8.97	0.11	0.86	0.55	0.14	<0.01	0.71

[a]Increasing levels of LEO in WPSS: 0, 1, 2, 3 and 4 ml kg^{-1} DM.
[b]Probabilities: LEO effect (Treat.), linear (Lin.), quadratic (Qua.); and non-quadratic/ cubic (Cub.) of LEO.

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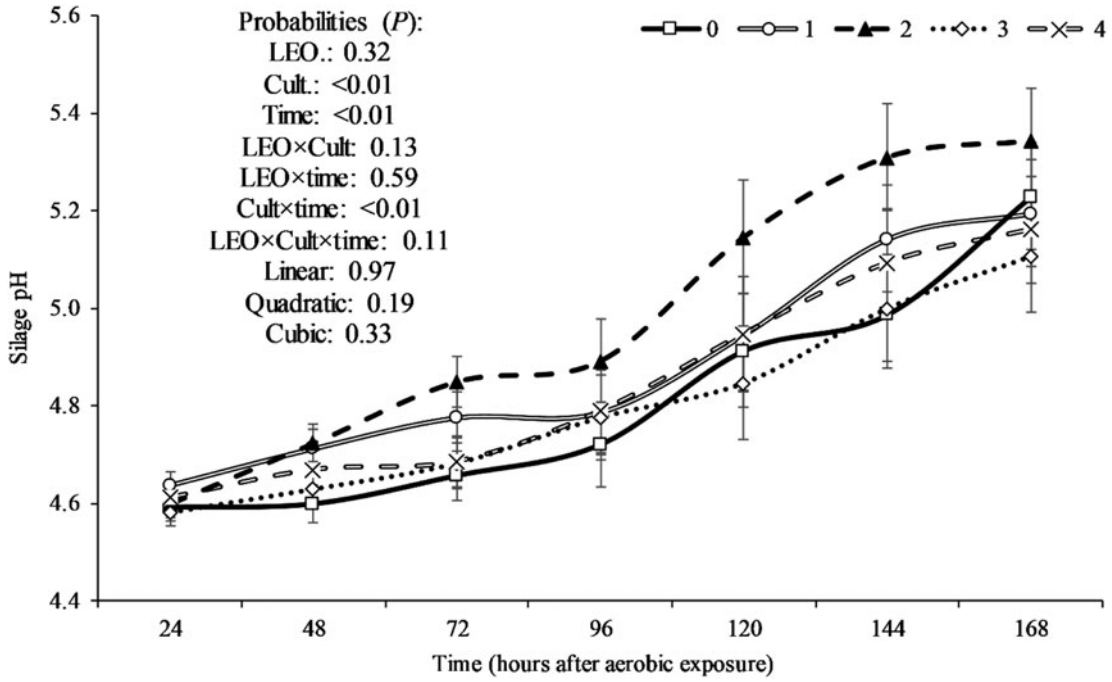

Fig. 1. pH after aerobic exposure of WPSS containing increasing levels of LEOs.

2020). Although LEO reduced silage gas losses and butyric acid concentration, it did not affect DM recovery, *in vitro* degradation, and silage temperature and pH after aerobic exposure.

In the present study, we found a quadratic effect of LEO on NH_3-N concentration, once silages containing 1 ml kg^{-1} of LEO had higher NH_3-N concentration than control-silage. In a previous study of our research group (Cantoia *et al.*, 2020), LEO increased NH_3-N in sugarcane silage. Microbial and plant enzymes are the main accountable for protein solubilization and proteolysis (Junges *et al.*, 2017). In addition, NH_3-N and butyric acids are produced in high humidity silages by clostridial fermentation (Kung *et al.*, 2018). Studies evaluating LEO in beef cattle diet observed a reduction in NH_3-N *in vivo* (Wanapat *et al.*, 2008) and *in vitro* (Nanon *et al.*, 2014) and associated this with a negative effect of LEO on rumen ammonia bacterial production. In another study by our research group, Garcia *et al.* (2022) observed a reduced NH_3-N concentration in Guinea grass silage when LEO was added during ensiling. Ammonia-N has been associated with increased silage pH observed in LEO-treated silages: reduced pH could inhibit clostridial growth, showing a negative effect on NH_3-N concentration (Kung *et al.*, 2018). In the current study, LEO had no effect on

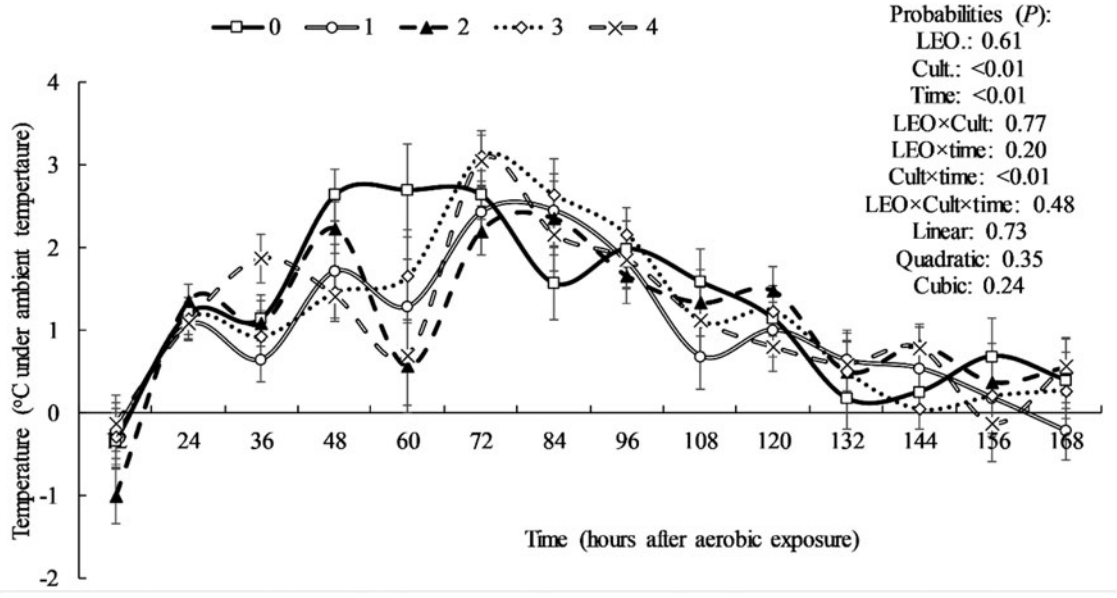

Fig. 2. Temperature after aerobic exposure of WPSS containing increasing levels of LEOs.

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silage pH and, therefore, it was possible to confirm that LEO inhibits WPSS proteolysis, once LEO reduces NH_3-N and butyric acid content on silage.

As observed in NH_3-N concentration, LEO showed a quadratic effect on silage CP and OM content. Increased protein solubilization increases N losses through the effluents, resulting in a lower CP content in the silage. As observed by Chaves *et al.* (2008), EO' effects on silage fermentation and aerobic stability are greatly affected by supplying level. On the other hand, LEO effect on OM content has limited implications on silage nutritional value.

Besides reported effects on NH_3-N and butyric acid concentrations, intermediary levels of LEO reduced the acetic and lactic concentrations on WPSS. Evaluating increasing levels of cumin EO in alfalfa silage, Turan and Önenç (2018) also observed a quadratic effect on NH_3-N and organic acids production. According to those authors, the inhibition of lactic acid bacteria metabolism reduces organic acids production and proteolysis. However, increasing LEO levels linearly decreased the lactic to acetic acid ratio. This effect agrees with heterolactic fermentation, as observed using second-generation (heterolactic) microbial inoculants (Arriola *et al.*, 2011). Besides linear negative of LEO on both acids production, Cantoia *et al.* (2020) also observed a lactic to acetic ratio of 1.62 in control and 1.36 using 3 ml of LEO per kg of sugarcane. Heterolactic fermentation normally has a more positive effect on high fermentable substrates, as sugarcane evaluated by Cantoia *et al.* (2020) than WPSS evaluated in the present study (Oliveira *et al.*, 2017).

Although heterolactic fermentation results in higher water and CO_2 production than homolactic fermentation (Muck, 2010), the addition of LEO linearly reduced silage gas losses. Furthermore, possible increased gas production due to heterolactic fermentation largely reduced butyric acid content. According to Kung *et al.* (2018), silages with higher clostridial growth have high concentrations of fibre and low DM digestibility because much of the readily available soluble nutrients have been degraded (Mills and Kung, 2002). This degradation seems to increase gas losses of low-LEO treated silages. Besides the reduced lactic to acetic acid ratio, LEO increased propionic acid concentration in the present study. Inoculation with propionic acid bacteria has been used to increase propionic acid and increase aerobic stability by inhibiting yeast and mould growth (Filya *et al.*, 2004). Although LEO did not affect WPSS aerobic stability, increased propionic concentration contributed to decreased fermentation gas losses in the present study.

Besharati *et al.* (2021) evaluated increasing levels of lemongrass seed essential oil on alfalfa silage fermentation profile and *in vitro* de gradation kinetics. Intermediary levels evaluated in that study (60 ml /kg^{-1} DM) increased the potential DM degradation. However, in the present study there were no treatment effects on DM recovery, silage fibre concentration, and *in vitro* degradation of DM and NDF. It is possible to infer that LEO positive effects on silage fermentation and gas losses were slight to affect these variables. Other questions, such as effluent losses and fluidification of DM, could affect DM recovery and result in a lack of LEO impact on this variable. Although increased acetic acid content of silage can reduce feed intake (Steen *et al.*, 1998), and butyric acid has been associated with decreased animals' performance (Scherer *et al.*, 2015), the absence of LEO effect on DM recovery, fibre content, and *in vitro* degradation limits its application in practical conditions, once these variables are the most associated with the financial viability of the additive.

The addition of LEO showed no effect on WPSS temperature and pH after aerobic exposure. A linearly reduced lactic to acetic ratio could lead us to expect increased aerobic stability of WPSS: acetic acid inhibits yeast and mould growth after aerobic exposure, whereas lactic acids serve as the substrate for these microorganisms' growth (Danner *et al.*, 2003). However, butyric has been associated with extended aerobic stability (Kung *et al.*, 2018). Consequently, LEO did not improve the aerobic stability of WPSS in the present study. Kung *et al.* (2003) evaluated a blend of essential oil addition during whole maize plant ensiling. Besides positive effects observed on *in vitro* ruminal fermentation and animal performance, EO had no effects on silage fermentation and aerobic stability. Similarly, LEO altered WPSS silage fermentation and has limited effects on silage nutritional value and aerobic stability.

As previously mentioned, fermentability is one of the main factors influencing the effects of EO on silage parameters (Chaves *et al.*, 2008). EO could be considered inhibitors of silage fermentation by their negative effects on microbial growth. Considering the lower fermentability of WPSS compared to sugarcane silage, we prefer to evaluate lower LEO doses than the optimal doses recommended by Cantoia *et al.* (2020). Low-fermentability material response is better when treated with fermentation promoters rather than inhibitors. It also explains the lower effects of LEO on Guinea Grass (Garcia *et al.*, 2022) and WPSS (current study) when compared to sugarcane silage (Cantoia *et al.*, 2020).

Conclusion

LEO reduces WPSS gas losses, butyric acid concentration, and the ratio between lactic and acetic acids. However, LEO does not improve silage chemical composition, *in vitro* degradation, or aerobic stability.

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References

AOAC (Association of Official Analytical Chemists) (2000) *Official Methods of Analysis*, 17th Ed. Arlington, VA, USA: Association of Official Analytical Chemists.

Arriola KG, Kim SC and Adesogan AT (2011) Effect of applying inoculants with heterolactic or homolactic and heterolactic bacteria on the fermentation and quality of corn silage. *Journal of Dairy Science* **94**, 1511–1516.

Benchaar C, Calsamiglia S, Chaves AV, Fraser GR, Colombatto D, McAllister TA and Beauchemin KA (2008) A review of plant-derived essential oils in ruminant nutrition and production. *Animal Feed Science and Technology* **145**, 209–228.

Bernardes TF and Rego AC (2014) Study on the practices of silage production and utilization on Brazilian dairy farms. *Journal of Dairy Science* **97**, 1852–1861.

Besharati M, Niazifar M, Nemati Z and Palangi V (2020) Comparison study of flaxseed, cinnamon and lemon seed essential oils additives on quality and

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fermentation characteristics of lucerne silage. *Acta Agriculturae Slovenica* **115**, 455.

Besharati M, Palangi V, Niazifar M and Nemati Z (2021) Optimization of dietary lemon seed essential oil to enhance alfalfa silage chemical composition and *in vitro* degradability. *Semina Ciências Agrárias* **42**, 891–906.

Calsamiglia S, Busquet M, Cardozo PW, Castillejos L and Ferret A (2007) Invited review: essential oils as modifiers of rumen microbial fermentation. *Journal of Dairy Science* **90**, 2580–2595.

Cantoia Jr R, Capucho E, Garcia TM, Del Valle TA, Campana M, Zilio EMC, Azevedo EB and Morais JPG (2020) Lemongrass essential oil in sugarcane silage: fermentative profile, losses, chemical composition, and aerobic stability. *Animal Feed Science and Technology* **260**, 114371.

Casali AO, Detmann E, Valadares Filho SC, Pereira JC, Henriques LT, Freitas SG and Paulino MF (2008) Influence of incubation time and particles size on indigestible compounds contents in cattle feeds and feces obtained by *in situ* procedures. *Revista Brasileira de Zootecnia* **37**, 335–342.

Chaves AV, Baah J, Yang WZ, McAllister TA and Benchaar C (2008) Effect of essential oils used as inoculants on the nutritive value of barley silage. *XXI International Grassland Congress and VII International Rangeland Congress: Multifunctional Grassland in Changing World*. Inner Mogolia, China, p. 654.

Czaplewski L, Bax R, Clokie M, Dawson M, Fairhead H, Fischetti VA, Foster S, Gilmore BF, Hancock REW, Harper D, Henderson IR, Hilpert K, Jones BV, Kaioglu A, Knowles D, Ólafsdóttir S, Payne D, Projan S, Shaunak S, Silverman J, Thomas CM, Trust TJ, Warn P and Rex JH (2016) Alternativas aos antibióticos – uma revisão do portfólio do pipeline. *Lancet Infectious Diseases* **16**, 239–251.

Danner H, Holzer M, Mayrhuber E and Braun R (2003) Acetic acid increases stability of silage under aerobic conditions. *Applied and Environmental Microbiology* **69**, 562–567.

Filya I, Sucu E and Karabulut A (2004) The effect of *Propionibacterium acidipropionici*, with or without *Lactobacillus plantarum*, on the fermentation and aerobic stability of wheat, sorghum and maize silages. *Journal of Applied Microbiology* **97**, 818–826.

Garcia TM, Capucho E, Cantoia Júnior R, Burró MFB, Noernberg RR, Zilio EMC, Campana M, Del Valle TA and Morais JPG (2022) Lemongrass essential oil reduces Mombasa grass silage gas losses, fibre content and pH after aerobic exposure. *Animal Production Science* **62**, 1480–1487.

Holden LA (1999) Comparison of methods of *in vitro* dry matter digestibility for ten feeds. *Journal of Dairy Science* **82**, 1791–1794.

Jobim CC, Nussio LG, Reis RA and Schmidt P (2007) Methodological advances in the evaluation of preserved forage quality. *Revista Brasileira de Zootecnia* **36**, 101–119.

Junges DG, Morais MHF, Spoto PS, Santos AT, Adesogan LG, Nussio and Daniel JLP (2017) Short communication: influence of various proteolytic sources during fermentation of reconstituted corn grain silages. *Journal of Dairy Science* **100**, 9048–9051.

Kholif AE, Matloup OH, Hadhoud FI, Kassab AY, Adegbeye MJ and Hamdon HA (2021) Lemongrass supplementation to Farafra ewes improved feed utilization, lactational performance and milk nutritive value in the subtropics. *Animal Biotechnology* **33**, 1118–1127.

Kung Jr L, Grieve DB, Thomas JW and Huber JT (1984) Added ammonia or microbial inoculant for fermentation and nitrogenous compounds of alfalfa ensiled at various percents of dry matter. *Journal of Dairy Science* **67**, 299–306.

Kung Jr L, Stokes MR and Lin CJ (2003) Silage additives. *Silage Science and Technology* **42**, 305–360. doi: 10.2134/agronmonogr42.c7

Kung Jr L, Shaver RD, Grant RJ and Schmidt RJ (2018) Silage review: interpretation of chemical, microbial, and organoleptic components of silages. *Journal of Dairy Science* **101**, 4020–4033.

Laxminarayan R, Matsoso P, Pant S, Brower C, Røttingen JA, Klugman K and Davies S (2016) Access to effective antimicrobials: a worldwide challenge. *The Lancet* **387**, 168–175.

Maldaner LJ, Horing K, Schneider JF, Frigo JP, Azevedo KD and Grzesiuck AE (2014) Agroclimatic requirement of maize (*Zea mays*). *Revista Brasileira de Energia Renováveis* **3**, 13–23.

Maulfair DD, Fustini M and Heinrichs AJ (2011) Effect of varying total mixed ration particle size on rumen digesta and fecal particle size and digestibility in lactating dairy cows1. *Journal of Dairy Science* **94**, 3527–3536.

McDonald P, Henderson AR and Heron SJE (1991) *The Biochemistry of Silage*, 2ª Edn. Alberyst-wyth, UK: Cambrian Printers.

McDougall EI (1948) Studies on ruminant saliva, I. The composition and output of sheep's saliva. *Biochemical Journal* **43**, 99–102.

Mills JA and Kung L (2002) The effect of delayed ensiling and application of a propionic acid-based additive on the fermentation of barley silage. *Journal of Dairy Science* **85**, 1969–1975.

Muck RE (2010) Silage microbiology and its control through additives. *Revista Brasileira de Zootecnia* **39**, 183–191.

Muck RE, Nadeau EMG, McAllister TA, Contreras-Govea FE, Santos MC and Kung L (2018) Silage review: recent advances and future uses of silage additives. *Journal of Dairy Science* **101**, 3980–4000.

Nanon A, Suksombat W and Yang WZ (2014) Effects of essential oils supplementation on *in vitro* and *in situ* feed digestion in beef cattle. *Animal Feed Science Technology* **196**, 50–59.

Oliveira AS, Weinberg ZG, Ogunade IM, Cervantes AAP, Arriola KG, Jiang Y, Kim D, Li X, Gonçalves MCM, Vyas D and Adesogan AT (2017) Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. *Journal of Dairy Science* **100**, 4587–4603.

Pinho RMA, Santos EM, Oliveira JS, Bezerra HFC, Freitas PMD, Perazzo AF, Ramos RCS and Silva APG (2015) Sorghum cultivars of different purposes silage. *Ciência Rural* **45**, 298–303.

Pryce JD (1969) A modification of Barker-Summerson method for the determination of lactic acid. *The Analyst* **94**, 1151–1152.

Rao SS, Elangovan M, Umakanth AV and Seetharama N (2007) Characterizing phenology of sorghum hybrids in relation to production management for high yields. In Reddy Belum VS, Ramesh S, Ashok Kumar A and Gowda CLL (eds). *Sorghum Improvement in the New Millennium*. Patencheru, Andhra Pradesh, India: National Remote Sensing Centre (NRSC) and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), pp. 1–5. doi: 10.13140/2.1.4841.8246.

Scheler ED and Cavichioli FA (2021) Viabilidade de silagem de milho para o gado leiteiro. *Revista Interface Tecnológica* **18**, 265–275.

Scherer R, Gerlach K and Südekum KH (2015) Biogenic amines and gamma-amino butyric acid in silages: formation, occurrence and influence on dry matter intake and ruminant production. *Animal Feed Science and Technology* **210**, 1–16.

Steen RWJ, Gordon FJ, Dawson LER, Park RS, Mayne CS, Agnew RE, Kilpatrick DJ and Porter MG (1998) Factors affecting the intake of grass silage by cattle and prediction of silage intake. *Animal Science* **66**, 115–127.

Thomas ME, Foster JL, McCuistion KC, Redmon LA and Jessup RW (2013) Nutritive value, fermentation characteristics, and *in situ* disappearance kinetics of sorghum silage treated with inoculants. *Journal of Dairy Science* **96**, 7120–7131.

Tilley JMA and Terry RA (1963) A two-stage technique for the *in vitro* digestion of forage crops. *Grass and Forage Science* **18**, 104–111.

Turan A and Önenç SS (2018) Effect of cumin essential oil usage on fermentation quality, aerobic stability and *in vitro* digetibility of alfalfa silage. *Asian-Australasian Journal of Animal Sciences* **31**, 1252–1258.

Van Soest PJ, Robertson JB and Lewis BA (1991) Methods for dietary fibre, neutral detergent fibre, non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597.

Wanapat M, Cherdthong A, Pakdee P and Wanapat S (2008) Manipulation of rumen ecology by dietary lemongrass (*Cymbopogon citratus* Stapf.) powder supplementation. *Journal Animal Science* **86**, 3497–3503.

Wilkinson JM and Davies DR (2012) The aerobic stability of silage: key findings and recent developments. *Grass and Forage Science* **68**, 1–19.

Zegada-Lizarazu W, Zatta A and Monti A (2012) Water uptake efficiency and above- and belowground biomass development of sweet sorghum and maize under different water regimes. *Plant and Soil* **351**, 47–60.

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