

Effects of different methods and additives on the quality of ensilage from rehydrated maize grain with industrial tomato waste

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ABSTRACT. The objective of this study was to evaluate different rehydration methods (water vs. industrial tomato waste, ITW) and additives in the ensiling of ground maize grain in terms of losses and fermentation profile, chemical composition, digestibility and ruminal degradability. The experiment was carried out at the State University of Montes Claros, and included five treatments: 1) maize silage rehydrated with water without additives, 2) maize silage rehydrated with ITW without additives, 3) maize silage rehydrated with ITW and bacterial-enzymatic inoculant, 4) maize silage rehydrated with ITW and 2% molasses powder, and 5) maize silage rehydrated with ITW, bacterial-enzymatic inoculant, and 2% molasses powder, on a natural matter basis. A completely randomised experimental design with five treatments and seven replicates was applied. Polyvinyl chloride silos were used to incubate the ensiled mass, and they were opened after 72 days. A higher ($P < 0.01$) pH value was observed in maize silages rehydrated with ITW (mean 4.25), while greater losses of ammoniacal nitrogen were observed in maize silages rehydrated with water compared to those with ITW. Among the additives, silages rehydrated with ITW and bacterial-enzymatic inoculant showed reduced effluent losses. Additionally, silages rehydrated with ITW and inoculant showed higher concentrations of acetic acid ($P < 0.01$), and propionic acid ($P < 0.01$). ITW can be used for rehydration of ground maize grain and ensiling because it does not alter dry matter losses and improves the levels of nitrogenous compounds in the silage.

Introduction

Tomato (*Solanum lycopersicum*) is one of the most widely produced and consumed agricultural commodities in the world, with an annual production of approximately 150 mln t (Almeida et al., 2021). In Brazil, 54 502 ha are dedicated to tomato cultivation, yielding around 3.8 mln t in 2022 for human consumption. A significant fraction of tomato is processed into various products, generating large amounts of by-products, such as waste

or discarded fruits, pulp, peels, and seeds, whose management is a problematic (Bugatti et al., 2019; Vidyarthi and Simmons, 2020). Every ton of processed tomatoes generates up to 420 kg of byproducts. These byproducts are rich in nutrients such as nitrogenous compounds, non-forage fibre, bioactive compounds (carotenoids, vitamins E and C), and minerals. Moreover, they do not contain antinutritional factors, making them a potentially useful resource for ruminant feeding. Industrial tomato waste (ITW) generated by the processing industries contains

approximately 250 g/kg dry matter (DM), 190 g/kg crude protein DM, up to 100 g/kg DM of ether extract and has DM digestibility exceeding 60%. However, its high moisture content is considered by farmers as a factor making storage and use for animals difficult, requiring the development of effective preservation strategies. This high moisture content of ITW can be utilized in rehydrating ground maize grain for ensiling, offering a preservation alternative, while improving nutrient digestibility in flint maize grown in Brazil (Jacovaci et al., 2021). Typically, pure water is used to rehydrate ground maize (Silva et al., 2018; Cruz et al., 2021; Roseira et al., 2023; Durães et al., 2024). However, Soares et al. (2024) recently demonstrated that forage cactus could also be utilized for rehydration, resulting in improved digestibility of DM and starch in maize silage. The rationale for this research is that ITW can also be used to rehydrate maize grain and contribute to improving the nutritional value of the resulting silage. Rehydrating maize grain using ITW and subsequent ensiling is intended to primarily improve starch digestibility and the crude protein content of maize grain silage. In addition, using ITW as a rehydration method can reduce DM losses, as the water present in ITW is not free but rather adheres to the cells, allowing for slow rehydration of maize meal, reducing effluent production. However, the low content of water-soluble carbohydrates in ITW and maize, as well as the composition of the epiphytic flora in this material, raises concerns regarding fermentation quality (Diogénes et al., 2023). Thus, the use of bacterial additives containing selected homofermentative (i.e., *Lactobacillus acidophilus*, *L. plantarum*, *L. lactis*) and heterofermentative (i.e., *L. buchneri*) microorganisms associated with a source of water-soluble carbohydrates, such as sugarcane molasses, can help reduce DM losses in the ensiled mass. Jesus et al. (2021) reported that bacterial-enzymatic inoculant in food ensilage reduced DM losses during fermentation and limited the growth of fungi and yeasts after opening the silo. Additionally, Jesus et al. (2022) observed an increase in water-soluble carbohydrate content when sugarcane molasses was added to millet grain silage rehydrated with whey. Furthermore, sugarcane molasses plays a crucial role in increasing lactic acid content and effective degradation of silage DM. Water-soluble carbohydrates are utilized by lactic acid bacteria to produce lactic acid, responsible for a rapid decrease in pH and inhibition of the growth of undesirable microorganisms in the ensiled mass (Cruz et al., 2021; Monção et al., 2024). In this study,

we hypothesised that a suitable method for rehydrating ground maize grain, combined with technological additives, would improve the fermentation profile and nutritional value of the silage produced.

Based on the above, the aim was to evaluate different rehydration methods (water vs. ITW) of ground maize grain for silage production, and to evaluate the effects of various additives on the fermentation profile and nutritional value of silages produced using ITW-rehydrated maize.

Material and methods

Ethics Statement

The care and handling procedures of the animals used in the experiment followed the guidelines established by the Animal Use Ethics Committee of the State University of Montes Claros (Protocol No. 011/2023).

Experiment location

The experiment was conducted at the UNIMONTES Experimental Farm, located in the municipality of Janaúba, Minas Gerais, Brazil (geographic coordinates: 15°52'38"S, 43°20'05"W). The climate of the region, classified as Aw according to the Köppen system, is characterised by summer rains and well-defined dry periods in winter. The average annual precipitation is below 800 mm, with an average annual temperature of 27 °C.

Treatments and experimental design

Five treatments involving rehydration of ground maize (particle size: 1–2 mm) with water or ITW were evaluated to produce silages with approximately 35% moisture content. The treatments were as follows: 1) maize silage rehydrated with water without additives, 2) maize silage rehydrated with ITW without additives, 3) maize silage rehydrated with ITW and bacterial-enzymatic inoculant, 4) maize silage rehydrated with ITW and 2% powdered sugarcane molasses, and 5) maize silage rehydrated with ITW, bacterial-enzymatic inoculant, and 2% powdered sugarcane molasses, on a natural matter (NM) basis. The experiment was conducted according to a completely randomised design with five treatments and seven replicates.

Treatment management

The maize grain used for silage production was obtained from local stores, pre-ground and sieved through a 0.5–1.5 mm mesh to achieve the desired

particle size. Granulometry analyses were conducted using overlapping sieves, with the results expressed as the geometric mean diameter of the particles. Industrial tomato waste (ITW) was donated by Bestpulp® (<http://www.bestpulp.com.br/>), located in Janaúba, Minas Gerais, Brazil (geographic coordinates: 43°16'18"S, 15°49'49"W), Minas Gerais, Brazil.

Maize grain was rehydrated with water and/or ITW. No additives were used in the ensilage of maize grains rehydrated with water. For maize rehydrated with ITW, additives were included in each treatment to improve silage fermentation. For the enzymatic-bacterial inoculant treatments, 1 g of the lyophilized enzymatic-bacterial inoculant SILOTRATO™ (Basso Pancotte, Nova Alvorada - RS, Brazil; <https://www.bassopancotte.com.br/>) was sprayed per t of natural maize mass, according to the manufacturer's recommendations. The inoculant was a combination of *L. curvatus*, *L. acidophilus*, *L. plantarum*, *L. buchneri*, *L. lactis*, *Pediococcus acidilactici*, and *Enterococcus faecium* at concentrations of 10¹⁰ CFU/g each, along with 5% of an enzymatic cellulase-based complex. The manufacturer verified the product's compliance with its quality specifications. The same volume of dechlorinated water (2 ml/kg) was added to all treatments. The inoculant bacterial composition and enzymatic activity were verified independently of the manufacturer's information. Powdered sugarcane molasses, used as an additive rich in water-soluble carbohydrates, was provided by Mellaço de Cana (<https://mellacodecana.com.br/melaco-de-cana-emp/>), Saltinho, São Paulo, Brazil.

Silage process

Experimental polyvinyl chloride (PVC) silos of mean weight 1 068 kg, 50 cm in length and 10 cm in diameter, were used in the experiment. The bottom of each silo was filled with 10 cm of dry sand (400 g), separated from the rehydrated maize mass by foam to determine the amount of effluent produced. The material resulting from each treatment was placed into the silo and compacted with a wooden plunger. Silage density (~900 kg of natural material m³) was determined for each treatment, and approximately 4 kg of mixed material transferred to each silo. After filling, the silos were closed with PVC lids equipped with Bunsen valves, sealed with adhesive tape and weighed. The silos were incubated at room temperature for 72 days for the ensiling process.

Aerobic stability

Aerobic stability was determined by placing a silage sample (approximately 2.5 kg) into a mini-

silo, which was maintained at controlled ambient temperature (24.5–25.5 °C). The silage temperature was measured every hour using an AK172mini-USB datalogger (AKSO, São Leopoldo, RS, Brazil) placed in the centre of the mass for seven days. The ambient temperature was also monitored every hour using a datalogger placed near the mini-silos. Aerobic stability was defined as the number of hours the silage temperature remained stable before increasing more than 2 °C above ambient temperature (Moran et al., 1996). Aerobic stability was measured over a 7-day period.

Fermentation losses

Total DM losses in silages in the form of gases and effluents were quantified by weight difference following the method described by Jobim et al. (2007). For effluent loss, equation 1 was used:

$$E = (Pab - Pen) / (GMfe) \times 1000, \quad (1)$$

where: E – effluent production (kg/ton of green mass); Pab – weight of the set (silo + lid + wet sand + foam) at opening (kg); Pen – weight of the set (silo + lid + dry sand + foam) during ensiling (kg); GMfe – green mass of ensiled forage (kg). DM loss in the form of gases was calculated based on the difference between the gross weight of the initial and final ensiled DM in relation to the amount of DM ensiled, accounting for the weight of the silo and dry sand set, according to equation 2:

$$G = [(PCen - Pen) \times DMen] - [(PCab - Pen) \times DMab] \times 100 [(PCen - Pen) \times DMen], \quad (2)$$

where: G – gas losses (% DM); PCen – weight of the full silo during ensiling (kg); Pen – weight of the set (silo + lid + dry sand + foam) during ensiling (kg); MSen – DM content of the feed mixed during ensiling; PCab – weight of the full silo at opening (kg); DMab – DM content of silages at opening. DM recovery for each silo was calculated based on the initial and final weight of DM content of the feed mixture during ensiling and the silage at opening, according to Jobim et al. (2007).

Assessment of ammoniacal nitrogen, organic acids and pH

Directly after opening the silos, a 25-g sample from each treatment was mixed with 100 ml of distilled water. Subsequently, a 10-ml aliquot was used to measure ammoniacal nitrogen (N-NH₃), according to the method described by Detmann et al. (2021). A 10-ml aliquot of the solution was acidified with 2% metaphosphoric acid to quantify

organic acids. Volatile fatty acids were determined by liquid chromatography using a 20A Shimadzu® Prominence System (Kyoto, Japan) equipped with a UV-Vis detector adjusted to 210 nm, an automatic injector calibrated for 5 µl of sample volume, and a 300 × Rezex™ ROA-Organic Acid + 7.8 mm column (Phenomenex), maintained at 60 °C in an oven. The analytes were diluted with 2.5 mM H₂SO₄ at a flow rate of 0.6 ml/min. External standards were used for quantitative calibration. After allowing the solution of each sample to stand for 30 min, the pH was measured using a Hanna potentiometer (Hanna® Instruments, Barueri, SP, Brazil; <https://hannainst.com.br/>).

Chemical composition and ruminal kinetics

A portion of the silages was pre-dried in a forced ventilation oven at 55 °C. Subsequently, in case of maize silages rehydrated with ITW, all samples were ground using a knife mill with a 2-mm diameter mesh sieve. Part of these samples were ground again using a 1-mm sieve for laboratory analysis. The portion of the samples with 2-mm particles was used for *in situ* incubation (rumen degradability and indigestible fibrous fractions). In water-hydrated ground maize grain silages, a portion of all samples was ground and sieved through a 1-mm mesh for laboratory analysis. For ruminal incubation, particles of the original material size were used.

The samples were analysed for the content of DM (INCT-CA G-001/1 and G-003/1), ash (INCT-CA M-001/2), crude protein (CP; INCT-CA N-001/2), ether extract (INCT-CA G-004/1), and neutral detergent fibre (NDF; INCT-CA F-001/2) using heat-stable α-amylase (Liquozyme Supra 2.2X, Novozymes, Araucária, Paraná, Brazil), as well as for acid detergent fibre (ADF; INCT-CA F-003/2), indigestible neutral detergent fibre (iNDF) (INCT-CA F-008/2), lignin (INCT-CA F-005/2), and non-fibrous carbohydrates, following the methods described in Detmann et al. (2021). The total digestible nutrient (TDN) content was estimated according to NRC (2001). The chemical composition of the natural ingredients used in silage production is presented in Table 1.

For the ruminal kinetics assay, the methodology (Method G-009/1) described by Detmann et al. (2021) was applied. Two rumen-cannulated crossbred steers, with an average body weight of 550 ± 30 kg, were used. The animals were adapted for 14 days to a diet with a forage-to-concentrate ratio of 70:30 on a DM basis, with 13.5% crude protein and 64% TDN. The roughage fraction of the diet consisted of silages (50% BRS 716 sorghum silage and 25% cactus pear) and 30% concentrate containing rehydrated maize

Table 1. Chemical composition of ingredients used in silage production, % g/kg DM

Item	Ground maize	Industrial tomato waste	Cane molasses
pH	6.8	3.00	6.9
DM, g/kg as fed	879.8	244.6	946.3
Ash	14.8	44.6	48.5
Crude protein	92.8	194.9	35.2
Ether extract	35.3	90.7	8.5
Neutral detergent fibre	130.0	562.9	12.6
Acid detergent fibre	45.0	376.2	0.5
Indigestible DM	87.2	425.1	64.8
Indigestible neutral detergent fibre	36.8	141.7	18.4
Indigestible acid detergent fibre	16.6	81.7	5.9

DM – dry matter

silages with ITW. Water and mineral salt were provided *ad libitum*. The DM intake of the animals was estimated at 2.1% of body weight, and the average pH and ruminal ammonia nitrogen during incubation were 6.5 and 12.04 mg/dl, respectively.

The *in situ* degradation technique was performed using 7.5 × 15 cm nonwoven fabric bags (weight – 100 g/m²) with approximate porosity of 60 µm, according to Casali et al. (2009). The number of samples was determined based on the ratio of 20 mg of DM/cm² of bag surface area (Nocek, 1988). The samples were deposited in the ventral sac region of the rumen for 0, 3, 6, 12, 24, 48, 72, 96, 120 and 144 h. All samples were removed and washed in cold water. Subsequently, the samples were transferred to an oven at 55 °C for 120 h. The remaining residues were analysed for DM and NDF contents (using alpha amylase) according to the previously described methodology. The percentage of degradation was calculated as the proportion of feed remaining in the bags after ruminal incubation.

The resulting data were fitted to a Gauss-Newton non-linear regression using SAS (2024), SAS software (<https://welcome.oda.sas.com/>; SAS Institute Inc., Cary, NC, USA), according to the equation proposed by Detmann et al. (2021):

$$Dt = A + B \times (1 - e^{-c \times t}), \quad (3)$$

where: Dt – cumulative degradation of the analysed nutritional component at time t; A – intercept of the degradation curve at t = 0, representing the water-soluble fraction of the analysed nutritional component; B – potential degradability of the water-insoluble fraction of the analysed nutritional component; A + B – total potential degradability of the analysed nutritional component when time is not a limiting factor; c – fractional degradation rate (h⁻¹); t – incubation time (h).

The coefficients a , b , and c were subsequently applied to the equation proposed by Detmann et al. (2021) to estimate effective degradability (ED):

$$ED = a + [b \times c / (c + k)], \quad (4)$$

where: ED – effective ruminal degradation of the analysed nutritional component; k – particle passage rate in the rumen, assumed at $5\% \text{ h}^{-1}$ (AFRC, 1993).

Multivariate analysis

Principal component analysis (PCA) was applied to better understand the relationship between the experimental and independent variables. For this analysis, 32 characteristics were considered. Using the correlation matrix of the characteristics, the data were standardised to have a mean of zero and a variance of one. A correlation matrix was chosen over a covariance matrix to ensure comparability across variables. The method proposed by Kaiser (1960) was applied to select the principal components that best simplified the variability present in the dataset and informed subsequent analyses and interpretations. In this method, only components with eigenvalues equal to or greater than one were retained, as the standardized original variables had a variance equal to one.

Statistical analysis

The data were analysed using a model incorporating the fixed effects of the silages (treatments). Means were compared using the Scott-Knott test at 5% probability level. The contrast between the control silage (maize rehydrated with water) and maize silage rehydrated with ITW were compared using the F test. The UNIVARIATE procedure was used to detect outliers or influential values and to test the normality of the residuals.

Variables related to the fermentation profile and chemical composition were analysed using the following model:

$$Y_{ij} = \mu + t_i + e_{ij}, \quad (5)$$

where: Y_{ij} – observed value for variable i in relation to the treatment in the j th repetition; μ – mean of all experimental units for the variable under study; t_i – effect of treatment i on the value of observation Y_{ij} ; e_{ij} – error associated with the independent observation Y_{ij} , assumed to follow a normal distribution with mean zero and variance δ^2 .

The DM and NDF ruminal degradability tests were conducted using a randomised block design in split plots, with five treatments (plots) and 10 incubation times (subplots). The data were analysed using a model incorporating the fixed effects of the silages (treatments). Mean comparisons were performed using contrasts at a 5% probability level.

The following statistical model was applied:

$$Y_{ijk} = \mu + T_i + B_j + e_{ij} + P_k + TP_{ik} + e_{ijk}, \quad (6)$$

where: Y_{ijk} – observation related to time (P) in subplot k of treatment (T) i in block j ; μ – constant associated with all observations; T_i – effect of treatment “ i ”, with $I = 1, 2, 3, 4$ and 5 ; B_j – effect of block j , with $j = 1$ and 2 ; e_{ij} – experimental error associated with the plots, assumed to follow a normal distribution with zero mean and variance δ^2 ; P – effect of incubation time k , with $k = 1, 2, 3, 4, 5, 6, 7, 8, 9$ and 10 ; TP_{ik} – interaction effect between treatment (i) and incubation time (k); e_{ijk} – experimental error associated with all observations, assumed to follow a normal distribution with zero mean and variance δ^2 . For exploratory data, PCA was performed using PAST[®] 4.03 software (Hammer et al., 2001).

Results

Aerobic stability

There was no interaction ($P = 0.08$) between silage type and time after silo opening in relation to temperature values. After 168 h, maize silage rehydrated with ITW had a 2.55% higher temperature than the control silage (rehydrated only with water; Figure 1). For maize silage rehydrated with ITW, the use of additives had no significant effect on temperature, with an average of $25.77 \text{ }^\circ\text{C}$ recorded across treatments.

The highest temperatures were observed 144 h after silo opening (Figure 2). No signs of aerobic stability were detected during the monitored period.

Fermentation profile and dry matter losses

A significant difference ($P = 0.01$) was observed between maize silage rehydration methods in terms of pH values. Silages rehydrated with ITW showed pH values 0.16 units higher compared to the control treatment. Of the additives evaluated, maize silages rehydrated with ITW, either without additives or combined with molasses, showed the highest pH values, with an average of 4.22 (Table 2). Higher losses of ammoniacal nitrogen were recorded in the maize silages rehydrated with water compared to those rehydrated with ITW addition. However, no differences were detected in ammoniacal nitrogen content between ITW-rehydrated silages treated with additives, with an average of 4.67% of total nitrogen. No differences were observed between silages in terms of total DM losses (mean of 3.45% of DM) or gas losses (mean of 4.57%).

A difference was found between maize grain rehydration methods in relation to effluent losses

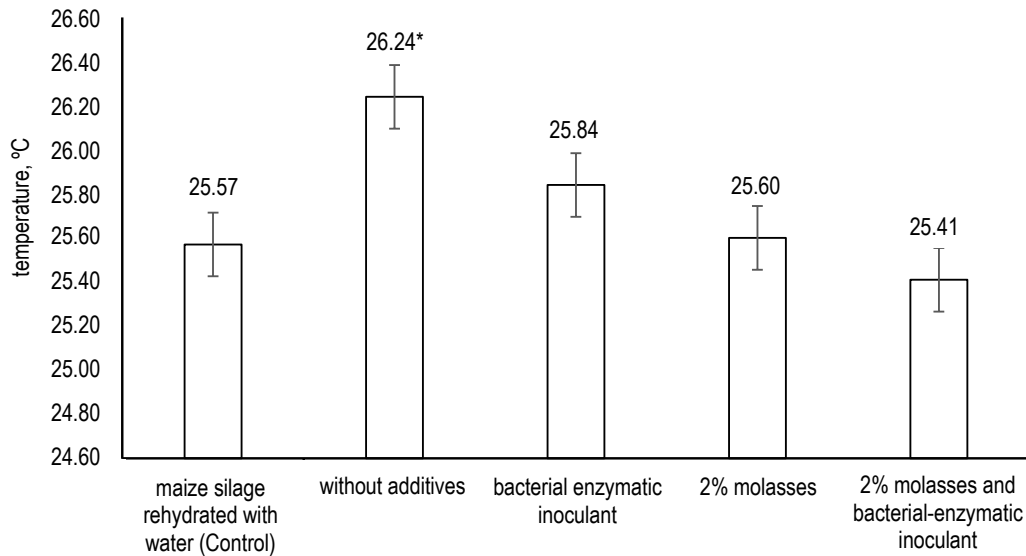


Figure 1. Average temperature values during stable aerobic conditions (168 h) of different rehydrated maize silages. Averages marked with * differ significantly from each other ($P < 0.05$)

ITW – industrial tomato waste

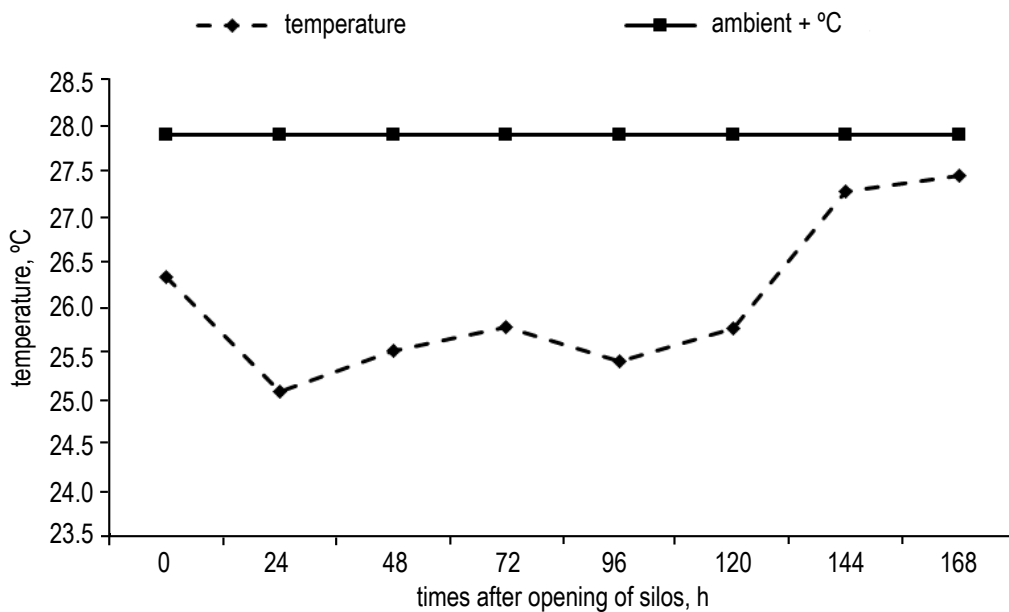


Figure 2. Average temperatures of different rehydrated maize silages during stable aerobic conditions (168 h)

during ensiling. Higher effluent losses were observed in maize silage rehydrated with water, while lower effluent losses were recorded in silages rehydrated with ITW combined with the bacterial-enzymatic inoculant. In terms of silage temperature, maize silage rehydrated with ITW exhibited significantly higher temperatures ($P = 0.01$) compared to the control silage. Among additives, lower average temperatures were observed in maize silage rehydrated with ITW when combined with molasses or molasses with inoculant. There were no differences between maize rehydration methods ($P = 0.69$) in terms of DM recovery, with an overall average of

93.12%. However, with respect to the additives, higher DM recovery was observed in maize silage rehydrated with ITW and treated with the bacterial-enzymatic inoculant (Table 2).

Different maize rehydration affected organic acid levels (Table 2). The highest concentration of succinic acid ($P = 0.04$) was observed in maize silage rehydrated with ITW, which showed a 58.04% increase compared to silage rehydrated only with water. Regarding other acids, silages rehydrated with water had significantly higher concentrations of acetic acid ($P = 0.01$) and ethanol ($P = 0.03$). In contrast, the silages rehydrated with ITW con-

Table 2. Fermentation profile and dry matter losses of maize silages rehydrated with water or industrial tomato waste supplemented with different additives

Item	Silage from rehydrated maize grain					SEM	P-value	
	Methods		Additives				methods	additives
	water	ITW	bacterial-enzymatic inoculant	molasses	molasses + inoculant			
pH	4.09	4.25 ^a	4.18 ^b	4.20 ^a	4.15 ^b	0.02	0.01	<0.01
Ammoniacal nitrogen, % TN	7.52	4.44 [*]	4.8	5.17	4.27	0.56	<0.01	<0.01
Total losses, % DM	3.74	3.49	2.05	3.45	4.56	0.61	0.8	0.06
Gas losses, %	5.08	4.68	3.12	4.59	5.39	0.66	0.63	0.16
Effluent losses, kg/t	7.51 [*]	5.91 ^a	3.79 ^b	7.38 ^a	6.79 ^a	0.57	0.01	<0.01
Initial temperature, °C	25.74	27.08 ^a	26.78 ^a	26.28 ^b	25.81 ^b	0.15	<0.01	<0.01
DM recovery, %	93.58	92.67 ^b	97.75 ^a	90.49 ^b	91.71 ^b	1.71	0.69	<0.01
Succinic acid	1.33	3.17 ^{ab}	7.76 ^a	1.7 ^c	1.46 ^c	0.74	0.04	<0.01
Lactic acid	16.35	17.62 ^a	20.89 ^a	8.53 ^b	16.35 ^a	1.57	0.6	<0.01
Acetic acid	5.27	3.15 ^{d*}	7.7 ^a	6.07 ^b	4.62 ^c	0.65	<0.01	<0.01
Butyric acid	0.44	1.77 [*]	1.64	1.44	1.43	0.18	<0.01	0.49
Propionic acid	1.16	0.93 ^d	4.93 ^a	2.32 ^b	1.32 ^c	0.28	0.19	<0.01
Ethanol	1.65	0.89 [*]	0.99	0.46	1.00	0.16	0.03	0.09

water – ground maize silage (1–2 mm) rehydrated with water (Control; 35% of humidity); ITW – maize silage rehydrated with industrial tomato waste (35% moisture content) without additives; bacterial-enzymatic inoculant – maize silage rehydrated with ITW (35% moisture content) with bacterial-enzymatic additives; molasses – maize silage rehydrated with ITW (35% moisture content) with cane molasses (2% NM); molasses + inoculant – maize silage rehydrated with ITW (35% moisture content) with bacterial-enzymatic additive and 2% cane molasses; NM – natural matter, TN – total nitrogen, DM – dry matter, SEM – standard error of the mean, P – probability; means followed by the same letters within a row do not differ from each other based on the Scott-Knott test ($P > 0.05$); means followed by * differ from the control silage based on the F test ($P < 0.05$), ^{a-d} means followed by the same letter on the line do not differ from each other according the Scott-knot test ($P < 0.05$)

tained lower concentrations of butyric acid. The addition of the bacterial-enzymatic inoculant increased succinic acid concentration in maize silages rehydrated with ITW. Maize silages rehydrated with ITW and molasses had 53.33% lower lactic acid concentrations compared to other treatments. Additionally, silages rehydrated with ITW and inoculant showed higher concentrations of acetic acid ($P < 0.01$) and propionic acid ($P = 0.01$). Individual additives did not affect the concentration of butyric acid (average of 1.57% of DM) and ethanol (average of 0.83% of DM) in maize silages rehydrated with ITW.

Chemical composition

There was a significant influence ($P < 0.05$) of maize rehydration methods on chemical composition variables, except for the ash content (mean 5.69%; $P = 0.10$). Maize silages rehydrated with ITW had higher contents of DM, CP, NDF, ADF, lignin, cellulose, indigestible DM, indigestible neutral detergent fibre, and indigestible ADF compared to those rehydrated with water. The use of ITW in maize rehydration increased CP content by 34.06% compared to water-hydrated silages. Furthermore, ITW rehydration reduced ($P < 0.05$) the content of total carbohydrates, non-fibrous carbohydrates, and *in vitro* digestibility of DM of the silages (Table 3).

Regarding the inclusion of additives in maize silages rehydrated with ITW, a higher DM content was observed when bacterial-enzymatic inoculant and molasses with inoculant were applied. The different additives did not significantly modify ($P > 0.05$) the contents of ash (mean 4.04%), ether extract (mean 5.81%), NDF (mean 13.76%), ADF (mean 9.20%), lignin (mean 4.04%), cellulose (mean %), indigestible DM (mean 13.08%), indigestible NDF (mean 11.84%), and *in vitro* DM digestibility (mean 81.64%). With respect to the additives, higher CP content was observed in maize silages rehydrated with ITW without additives, and in silages with molasses. The averages in these treatments were 6.22% higher compared to silages with inoculant and molasses with inoculant. The silages rehydrated with ITW, supplemented with inoculant or molasses with inoculant had higher contents of total carbohydrates and non-fibrous carbohydrates compared to the other silages rehydrated with ITW. The highest content of indigestible ADF was found in the silage rehydrated with ITW but without additives.

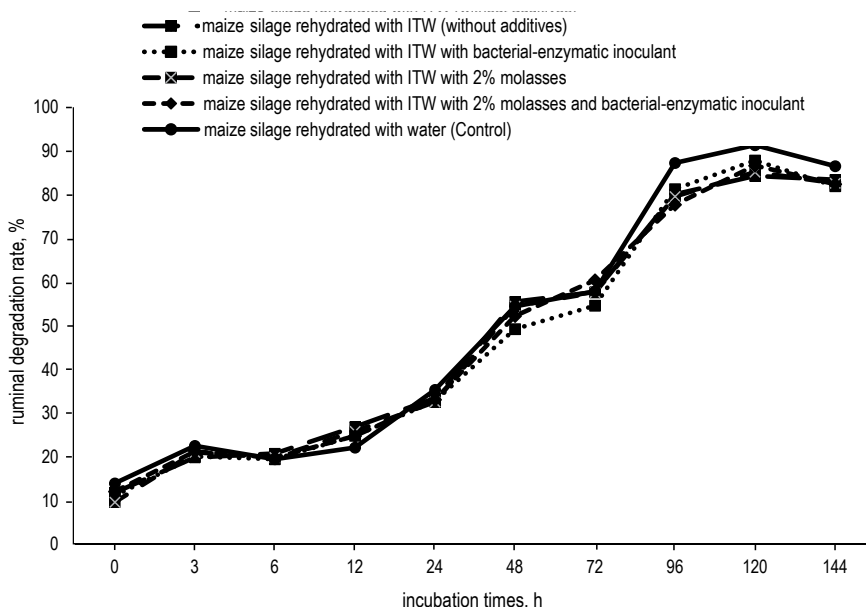
Rumen kinetics

An interaction between silages and ruminal incubation time was observed regarding the potential degradability of dry matter (Figure 3).

Table 3. Chemical composition of maize silages rehydrated with water or industrial tomato waste supplemented with different additives

Item	Silage from rehydrated maize grain					SEM	P-value	
	Methods		Additives				methods	additives
	Water	ITW	bacterial-enzymatic inoculant	molasses	molasses + inoculant			
Chemical composition, %								
dry matter	59.87	63.11 ^{ab}	65.88 ^a	60.63 ^b	65.00 ^a	1.08	<0.01	<0.01
ash	6.42	4.97	3.51	3.94	3.75	0.48	0.1	0.12
crude protein	8.36	12.68 ^{ab}	12.14 ^b	13.02 ^a	11.96 ^b	0.2	<0.01	<0.01
ether extract	4.95	6.12	5.6	6.17	5.35	0.29	<0.01	0.21
neutral detergent fibre	6.86	13.68 [*]	13.89	13.95	13.53	0.4	<0.01	0.86
acid detergent fibre	3.2	9.36 [*]	9.16	9.57	8.74	0.39	<0.01	0.58
lignin	0.64	4.13 [*]	4.01	3.96	4.07	0.2	<0.01	0.95
cellulose	2.56	5.24 [*]	5.15	5.6	4.67	0.28	<0.01	0.22
total carbohydrates	80.25	76.21 ^{ab}	78.74 ^a	76.86 ^b	78.92 ^a	0.57	<0.01	<0.01
non-fibrous carbohydrates	73.39	62.52 ^{ab}	64.84 ^a	62.90 ^b	65.39 ^a	0.76	<0.01	0.02
<i>In vitro</i> digestibility								
dry matter	90.78	82.26 [*]	81.75	82.52	80.06	1.27	<0.01	0.53
neutral detergent fibre	78.67	68.30 ^{ab}	66.92 ^b	71.28 ^a	72.55 ^a	1.51	<0.01	<0.05
crude protein	84.17	80.73 ^{ab}	82.46 ^a	83.42 ^a	80.94 ^b	0.59	<0.01	0.01
Indigestible fraction								
dry matter	6.13	15.23 [*]	12.04	13.07	11.99	1.04	<0.01	0.12
neutral detergent fibre	5.33	14.05 [*]	11.04	11.42	10.86	0.93	<0.01	0.08
acid detergent fibre	2.68	10.21 ^{ab}	8.15 ^b	7.72 ^b	7.30 ^b	0.73	<0.01	0.05

water – ground maize silage (1–2 mm) rehydrated with water (Control; 35% moisture content); ITW – maize silage rehydrated with industrial tomato waste (35% moisture content) without additives; bacterial-enzymatic inoculant – maize silage rehydrated with ITW (35% moisture content) with bacterial-enzymatic additives; molasses – maize silage rehydrated with ITW (35% moisture content) with cane molasses (2% NM); molasses + inoculant – maize silage rehydrated with ITW (35% moisture content) with bacterial-enzymatic additive and 2% cane molasses; NM – natural matter, SEM – standard error of the mean, P – probability; means followed by the same letters within a row do not differ from each other based on the Scott-Knott test ($P > 0.05$); means followed by * differ from the control silage based on the F test ($P < 0.05$), ^{ab} means followed by the same letter on the line do not differ from each other according to the Scott-Knott test ($P < 0.05$)

**Figure 3.** Ruminal dry matter kinetics of maize silages rehydrated with water or industrial tomato residue combined with different additives

In general, the potential degradability of silages showed an exponential increase as the incubation time progressed. No differences in ruminal degradability of dry matter between the silages were

observed at 0, 3, 6, 12, 24, 48, and 72 h. After 96 h of incubation, greater potential degradability was observed in silages produced from maize rehydrated with water and those rehydrated with ITW and supplemented

with the bacterial-enzymatic inoculant. At 120 h of incubation, silages made from maize rehydrated with water showed 91.35% ruminal dry matter degradation, which did not differ significantly from maize silages rehydrated with ITW and with the addition of the bacterial-enzymatic inoculant (mean 87.85%).

An interaction between silages and ruminal incubation time was observed regarding the potential degradability of neutral detergent fibre (Figure 4). Overall, the potential degradability of silage fibre ranged from 18.23 to 76.12%. No differences were found between silages in terms of the potential degradability of the fibrous fraction at 0, 3, 6, 12, and 24 h. At 48 h of incubation, higher

potential degradability was observed in silages made from maize rehydrated with water and maize rehydrated with ITW without any additives but with molasses (mean 49.67%). At 72 h of incubation, greater potential degradability was observed in silages made from maize rehydrated with water and those produced from maize rehydrated with ITW and supplemented with 2% molasses and the bacterial-enzymatic inoculant (average of 54.54%).

Principal component analysis

The first four PCAs had eigenvalues greater than 1, collectively accounting for 99.99% of the

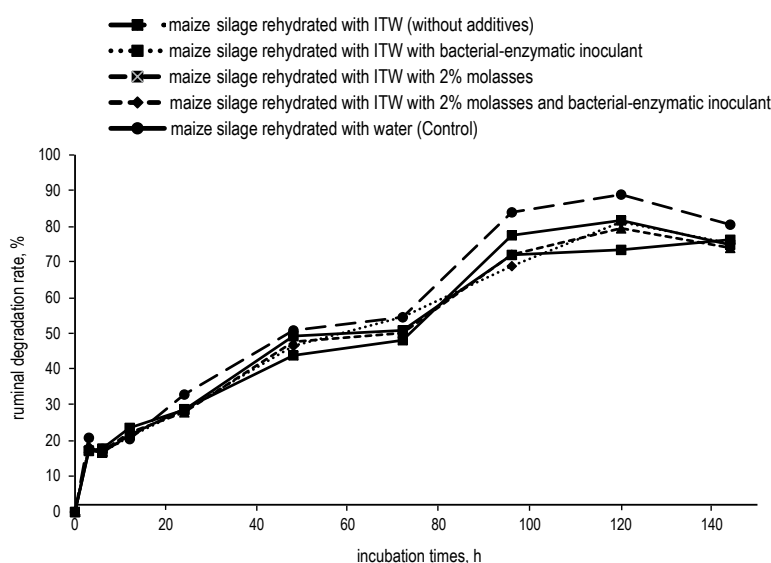


Figure 4. Ruminal kinetics of neutral detergent fibre in maize silages rehydrated with water or industrial tomato waste supplemented with different additives

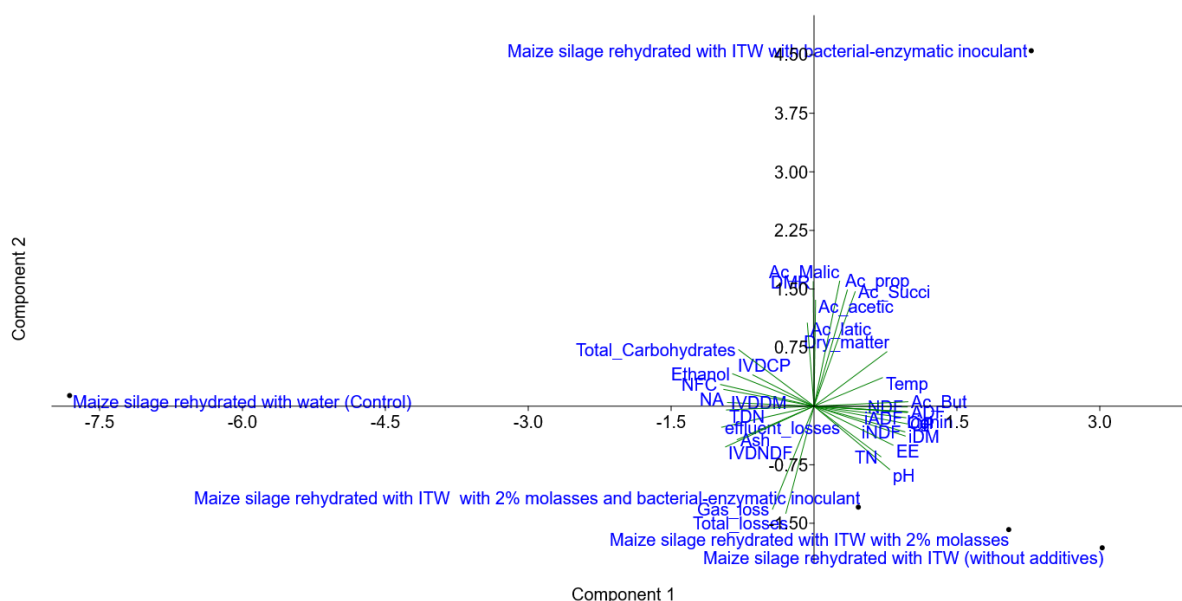


Figure 5. Schematic representation of the first (PC1) and second (PC2) principal components of the dependent variables analysed in the silages from maize grains rehydrated with water or industrial tomato waste supplemented with different additives

total variance in the results (Figure 5). PCA 1 (62.36%) and PCA 2 (22.02%) explained 84.38% of the data variance in the silages rehydrated with water or ITW with different additives. Within PCA 1, the variables with the highest weighting coefficients were: acid detergent fibre content (0.2216), butyric acid level (0.2210), and effluent losses (−0.2177). In PCA 2, the concentrations of lactic (0.2386), acetic (0.3042), and propionic (0.3336) acids had the highest weighting coefficients. As shown in Figure 4, silages with higher DM contents were negatively correlated with reduced effluent and gas losses. Regarding the silages, it was observed that certain variables, including pH, EE, indigestible DM and indigestible NDF contents showed similar behaviour in the treatments involving maize silage rehydrated with ITW combined with 2% molasses, ITW without additives, and ITW with 2% molasses and bacterial-enzymatic inoculant.

Discussion

One of the hypotheses tested in this study was that ITW can be used as a rehydration method for ground maize grain because it has a high moisture (~75%) content and favourable nutritional profile for ruminant nutrition. This hypothesis was supported by the aerobic stability of the silages, which remained consistent after silo opening. Specifically, no disturbance in aerobic stability was observed after 168 h of exposure. Aerobic stability is considered compromised when the temperature of the ensiled mass rises by 2 °C above ambient temperature. Previous studies (Arcari et al., 2016; Ferraretto et al., 2018) demonstrated that maize grain silages rehydrated with water exhibited fermentation problems, such as low aerobic stability after silo opening. However, this behaviour was not confirmed in the latter study in different silages after opening the silos. Aerobic silage stability can be defined as the resistance of the ensiled mass to deterioration after the silo is opened, i.e., the rate at which the mass deteriorates when exposed to air (Jobim et al., 2007). Loss of aerobic silage stability is generally manifested by an increase in temperature and changes in pH. The increase in temperature after opening the silo reflects the intensity of reactions induced by filamentous fungi, yeasts and aerobic bacteria (Muck et al., 2018). The respiration of aerobic microorganisms is recognised as one of the primary factors affecting silage quality (Kung Jr et al., 2018).

A higher temperature was observed during aerobic stability in maize silage rehydrated with ITW (without additives) compared to the other treat-

ments; however, there was no disruption in aerobic stability. Although the temperature variation did not exceed 1 °C in relation to maize silage rehydrated with water, potential contributing factors include the growth of filamentous fungi and yeasts. This can be attributed to the lower concentration of acetic acid in maize silages rehydrated with ITW (without additives), which is typically responsible for inhibiting the growth of these microorganisms once the silo is opened.

Regarding the fermentation parameters, a higher pH value was observed in maize silage rehydrated with ITW (without additives) compared to the control silage rehydrated only with water, which could be attributed to the higher content of nitrogen compounds in silages with ITW, which may act as a buffering agent for the ensiled mass. However, the pH values in all silages were within the range recommended by Kung Jr et al. (2018), i.e. between 3.8 and 4.2. One of the key findings of this research that supports the use of ITW for rehydration of maize grains is the lack of total DM losses during fermentation. The use of additives in maize silages rehydrated with ITW did not cause any differences between treatments in terms of total DM losses (3.45% DM on average), and gas losses (4.57% DM on average). Although no significant differences were observed between treatments, it should be noted that there were DM losses in the silages. These losses were associated with the presence of yeasts that utilised non-fibrous carbohydrates in the silage as an energy source and released CO₂, resulting in DM loss. In addition, the release of heat from this process contributes to an increase in the products of the 'Maillard' reaction (Kung Jr et al., 2018).

When analysing effluent losses, lower average values were found in silages with ITW combined with the use of the bacterial-enzymatic inoculant. In the same treatment, higher DM recovery was also observed. Effluent contains a high concentration of organic compounds, such as sugars, organic acids, proteins and other components from the ensiled material (McDonald et al., 1991). The volume of effluent produced in a silo is mainly influenced by the DM content of the ensiled mass and the degree of compaction, along with other factors related to fermentation dynamics. In this study, the use of a bacterial-enzymatic inoculant may have contributed to the rapid reduction of the pH of the ensiled mass, thereby altering the fermentation dynamics (Bernardes et al., 2018). However, such effects are still rarely documented in the literature.

One of the important functions of using microbial inoculants in ensilage is to reduce DM losses. The purpose of evaluating the use of additives in silages made from rehydrated maize with ITW is to assess the variation in the composition of the epiphytic bacteria population and to determine whether the low content of soluble carbohydrates in maize and ITW is insufficient for the synthesis of lactic acid by homolactic bacteria. During fermentation, lactic acid bacteria utilise water-soluble carbohydrates to produce lactic acid. Based on the results, it was not necessary to add molasses as a source of soluble carbohydrates, as there was no difference in lactic acid content compared to the silage produced from rehydrated maize with ITW without additives. However, silage made from rehydrated maize with a bacterial-enzymatic inoculant had a higher concentration of acetic acid. The concentration of lactic acid and acetic acid in silages is crucial for the preservation of the ensiled mass. Lactic acid is particularly important as it is produced rapidly after the aerobic phase, largely reducing the pH of the ensiled mass to 3.8–4.2, which is initially close to neutral. This acidification is important for inhibiting the growth of undesirable microorganisms such as *Clostridium* (Kung Jr et al., 2018). Acetic acid also plays an important role after opening the silo, influencing the growth of filamentous fungi and yeasts. The fermentation of silage nutrients by fungi and yeasts after opening the silos leads to an increase in the temperature of the silages. In this study, the lowest temperature was observed in maize silage with ITW supplemented with molasses and molasses with inoculant. The exploratory data analysis revealed positive correlation coefficients for lactic, acetic, and succinic acids in both PC1 and PC2, underscoring the significance of these variables in the decision-making process when rehydrating maize with ITW. The PCA results also demonstrated that PC1 and PC2 explained higher concentrations of acids and DM recovery in the rehydrated maize silage with ITW supplemented with the bacterial-enzymatic inoculant. Based on the multivariate analysis, silages with higher acid concentrations, except for butyric acid, showed a negative correlation with DM losses, effluent losses and gas emissions. Regarding effluent losses, the silages rehydrated with water and those rehydrated with ITW and supplemented with molasses or molasses with inoculant demonstrated similar behaviour.

The chemical composition of the silages rehydrated with different methods demonstrated a higher DM content in maize silage rehydrated with ITW, which can be attributed to the slower

displacement of water present in ITW into maize. This process also contributed to the lower losses of ammoniacal nitrogen in the silages with ITW compared to water-rehydrated maize. The ammoniacal nitrogen content is an indicator of the quality of the fermentation processes, particularly in relation to proteolysis. Poorly preserved silages tend to have a high ammoniacal nitrogen content, typically exceeding 10%. However, none of the silages evaluated in this study had ammoniacal nitrogen content above 10%.

The ITW is an agroindustrial byproduct that contains high levels of CP, crude fat, fibrous fraction, and lignin, along with lower levels of total carbohydrates. As an industrial residue, the composition of ITW may vary, depending on the harvesting method and the processing procedures used in the industry. The nutritional components present in ITW can explain the differences observed between silages made from maize rehydrated with water versus those rehydrated with ITW. For example, ADF and lignin contents significantly contributed to the lower DM digestibility, increased fibrous fraction of silages containing ITW and ruminal degradability of DM and NDF. Lignin is a phenolic compound toxic to ruminal microorganisms, causing reduced DM digestibility and a higher proportion of indigestible fractions in these silages. Furthermore, during the processing of tomatoes for pulp, the mass can be heated to temperatures up to 120 °C. In this study, ITW temperature at the time of ensilage was 62 °C, which may have contributed to protein denaturation. This could explain the lower digestibility of crude protein and its impact on ruminal degradability. ITW has a high fibre content and can be classified as a non-forage fibre source, justifying the increase in the fibrous fraction in maize silages rehydrated with ITW compared to those rehydrated solely with water.

Regarding the use of additives in ITW-rehydrated maize silage, specific variations were observed primarily in the chemical composition. The application of the bacterial-enzymatic inoculant and molasses in combination with the inoculant in ITW-rehydrated maize silage, increased the total carbohydrate and non-fibrous carbohydrate contents. This can be attributed to the lower mineral salt content in these silages, which resulted from the increased fibrous carbohydrates such as ITW and non-fibrous carbohydrates with the addition of molasses. Based on the multivariate analysis, the silages of maize rehydrated with ITW and with the addition of a bacterial-enzymatic inoculant exhibited a distinct pattern of dependent variables compared to the

other silages. In these silages, variables with higher positive correlation coefficients were observed in PCA 1 and PCA 2. The higher concentration of acetic and propionic acid in the silages produced from maize rehydrated with ITW and supplemented with bacterial-enzymatic inoculant suggests that this additive exerted positive effects during ensiling.

Conclusions

Industrial tomato residue can be used to rehydrate ground maize grain for ensiling. The application of a bacterial-enzymatic inoculant in the ensilage of ground maize grain rehydrated with industrial tomato residue positively affects ensilage parameters.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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