



FIBROLYTIC ENZYMES INCREASE FERMENTATION LOSSES AND REDUCE FIBER CONTENT OF SORGHUM SILAGE

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Abstract

Fiber digestibility is a key-point of forage usage in ruminant production systems. The present study aimed to evaluate the effect of fibrolytic enzyme blend on whole-plant sorghum silage fermentation profile, fermentative losses, chemical composition, *in vitro* degradation, and aerobic stability. It used fifty experimental silos (plastic bucket, 28 cm i.d. and 25 cm in height) in a blocked randomized design to evaluate the following fibrolytic enzyme levels: 0, 150, 300, 450, and 600 mL per ton of dry matter (DM). Enzymes had 300 U/mL of xylanase and 300 U/mL of cellulase. Enzymes linearly increased acetic acid, branched-chain organic acids, and ethanol concentration of silage. Although there was no treatment effect on silage DM recovery over the ensiling process, enzyme levels linearly increased gas, effluent, and total losses. Intermediary levels of enzymes (from 312 to 342 mL/ton DM) decreased fiber content (NDF and ADF), whereas improved NFC silage content. Besides, enzymes did not affect DM and NDF degradation and tended to linearly reduce acid detergent fiber *in vitro* degradation. The enzymes addition linearly decreased silage temperature after aerobic exposure. However, there was no treatment effect on silage pH after aerobic exposure also during the time that silage remained with a temperature lower than 2°C above environmental temperature. Thus, fibrolytic enzymes reduce fiber content, promote a heterolactic fermentation, and reduce silage temperature after aerobic exposure. However, this increases fermentative losses and has no positive effect on *in vitro* degradation and the time of aerobic stability.

Key words: acetic acid, cellulase, fiber degradation, neutral detergent fiber, xylanase

Forage production for ruminant feeding has become a challenge with the increasing human population, food and biomass requirements for feeding and fuel production (Drews et al., 2020). These activities compete for land and water usage, becoming essential to evaluate alternative forage sources that could show higher biomass per area and demand lower water. Under drought conditions, sorghum (*Sorghum bicolor*) has been proposed as a resilient alternative to corn due to its water use efficiency and photosynthetic rate (Roby et al., 2017; Yang et al., 2019).

Despite the agricultural advances relative to corn, sorghum silage has higher lignin (Miron et al., 2005; Bernard and Tao, 2015) and neutral detergent fiber (NDF; Yang et al., 2019) content, which comprises NDF and dietary dry matter (DM) digestibility. In this context, exogenous fibrolytic enzymes have shown a positive effect on NDF digestibility and dairy cows productive performance (Arriola et al., 2017). Yang et al. (2019) evaluated xylanase addition in dairy cows diets and observed no ef-

fect on animal digestion and performance. According to these authors, this absence of enzyme effect results from the enzymes ruminal instability, the low substrate to enzyme interaction, and the fast feed passage rate (Wang and McAllister, 2002).

The inclusion of exogenous fibrolytic enzymes during the forage ensiling could increase the substrate to enzyme interaction and reduce the enzymes ruminal degradation (Del Valle et al., 2019). In addition, the sorghum grain's mechanical processing is often complicated under field conditions, and sorghum grains are rich in a high hydrophobic prolamin (kafirin), which restricts starch digestibility (McCary et al., 2020). Increased fibrolytic activity could improve pericarp degradation, acids production, and karifins solubilization. Therefore, we hypothesized that exogenous enzymes during sorghum ensiling could improve the NDF and DM degradation and DM recovery through the ensiling process by positively affecting acid production. We aimed to evaluate the effects of increasing levels of exogenous fibrolytic enzymes on fermenta-

tive losses, fermentation profile, aerobic stability, chemical composition, and *in vitro* degradation.

Material and methods

The trial was carried out in the Group of Agricultural Studies and Works (GETAP) of Agrarian Sciences Center (CCA), Federal University of São Carlos (UFSCar), Araras, São Paulo State, Brazil.

Sorghum, treatments, and experimental design

Sorghum (cultivar AGX-213, Volumax®, Agrocere, Rio Claro, Brazil) was seeded after a traditional soil preparation (tillage) and using the following fertilization: 400 kg/ha of a commercial product containing 40 g/kg of N, 140 g/kg of P, and 80 g/kg of K during the seeding; and 200 kg/ha of a product containing 200 g/kg of N; 50 g/kg of P, and 200 g/kg of K, 20 days after the seeding. The agronomic plots were defined as blocks in the ensiling assay. From each plot, two experimental units (silos) were made in consecutive days. Harvest was manually performed at 5-cm height. All harvested material from plot (on each day of harvest) was processed in a stationary mill (TRF300®, Trapp, Jaguará do Sul, Brazil).

Before the treatment supply, a sample of each area ($n = 5$) was collected for chemical analysis and particle size evaluation, using a Penn State Particle Separator (Maulfair et al., 2011). Each silo's material was weighed to provide 650 kg/m³ of density, and enzyme level was individually provided using a pipette. The following levels of fibrolytic enzyme were evaluated: 0, 150, 300, 450, and 600 mL per kg of DM. Enzymes were obtained from Kera Animal Nutrition® and had 300 U/mL of xylanase and 300 U/mL of cellulase. After enzyme supply, the material was manually mixed and compacted in a plastic bucket with 28 cm i.d. and 25 cm in height.

Sampling and aerobic stability evaluation

After ensiling, whole silos were weighed every 10 days (Mettler Toledo, Barueri, Brazil). Forty days after ensiling, silos were opened; 5 cm of top and bottom layer was discarded, and silage was homogenized and sampled. Silos without silage were weighed to quantify effluent losses. A subsample (almost 400 g) was pressed using a hydraulic press (PHE-45®, Engehidro, Piracicaba, Brazil) to obtain silage fluid. Silage pH was evaluated using a digital potentiometer (LUCA-210®, Lucadema, São José do Rio Preto, Brazil) immediately after getting the fluid. Another subsample was dried at 60°C for 72 h and ground in a knife mill (SL-31, Solab Científica, Piracicaba, Brazil) using 2-mm and 1-mm sieves. Another subsample (3 kg) was placed in a plastic bucket and maintained in a room with controlled temperature (19.1 ± 1.28 ; mean $\pm$ SD) for 144 hours.

Silage temperature was evaluated every 8 h (K29-5030®, Kasvi Produtos Laboratoriais, Pinhais, Brazil). The aerobic stability period was defined as the time

(hours) that silage temperature remained up to 2°C above room temperature. Silage pH after aerobic exposure was evaluated every 24 hours: samples (15 g) were obtained, diluted in distilled water (250 g), processed in a blender for 30 seconds, and silage pH was analyzed as previously described.

Chemical analyses

Silage fluid was analyzed for NH₃-N concentration using Kjeldahl method (method 984.13, AOAC, 2000), without sample digestion; lactic acid content by methodology described by Pryce (1969); ethanol, acetic, butyric, and propionic acids concentrations using a gas chromatograph (GC-2010 Plus chromatograph, Shimadzu, Barueri, Brazil). The chromatograph was equipped with an AOC-20i auto-sampler, Stabilwax-DA™ capillary column (30 m, 0.25 mm internal diameter, 0.25 μ m², Restek®), and a flame ionization detector. Helium was the carrier gas (42 cm/s) and temperatures of injector and detector were 250 and 300°C, respectively. The column temperature started with a gradient from 40 to 120°C at 40°C/min rate, followed by heating at 10°C/min between 120 and 180°C and 120°C/min from 180 to 240°C, totaling 11.5 min. of run. WSFA-2 standard (Ref. 47056, Supelco®) and ethanol (Ref. 459828, Sigma-Aldrich®) were used to identify the peaks. GCsolution v. 2.42.00 software (Shimadzu®) has been used to perform the calculations.

Samples (1-mm processed) were analyzed for DM (method 950.15), ash (method 942.05), crude protein (CP; N $\times$ 6.25; method 984.13), and ether extract (EE, method 920.39) according to AOAC (2000). The neutral detergent fiber was analyzed using α -amylase, without the addition of sodium sulfite, and expressed including residual ash (aNDF-NDF), as soon as acid detergent fiber (aADF-ADF), according to Van Soest et al. (1991). Non-fiber carbohydrate (NFC) was calculated as: $NFC (g/kg DM) = 1,000 - (NDF + CP + ash + EE)$. Dry matter and NDF *in vitro* degradation assay was performed according to Tilley and Terry (1963) modified by Holden (1999). 2-mm processed samples were placed in non-woven fabric tissue bags (5 $\times$ 5 cm and 100 g DM/m²; Casali et al., 2008) and incubated for 48 h in an *in vitro* incubator (NL162®, New Lab, Piracicaba, Brazil). The ruminal fluid was from two dairy heifers maintained in *Megathyrus maximus* pasture, without concentrate supplementation and inoculum was obtained using McDougall (1948) buffer. After removal, bags were washed in running tap water and NDF analysis was performed as previously described.

Calculations and statistical analysis

Fermentative losses and dry matter recovery (DMR) were calculated according to Jobim et al. (2007):

$$GL \left(\frac{g}{kg DM} \right) = \frac{[WSWE(g) - WSWO(g)]}{EDM (g)} \times \frac{1000 g}{1 kg}$$

where GL is gas losses, $WSWE$ is the whole silo weight at ensiling, $WSWO$ is the whole silo weight at the opening (or intermediary weight), and EDM is the ensiled dry matter.

$$EL \left(\frac{g}{kg DM} \right) = \frac{[ESWO(g) - ESWE(g)]}{EDM(g)} \times \frac{1000 g}{1 kg}$$

where EL is effluent losses, $ESWO$ is the empty silo weight at the opening, and $ESWE$ is the ensiling weight of the empty silo.

$$DMR \left(\frac{g}{kg DM} \right) = \frac{ODM(g)}{EDM(g)} \times \frac{1000 g}{1 kg}$$

where ODM is the DM at the opening.

Data were analyzed using PROC MIXED of SAS (version 9.4, SAS Inst. Inc., Cary, NC, USA), according to the following model:

$$Y_{ijk} = \mu + E_i + s_j + e_{ijk}$$

with $s_j \approx N(0, \sigma_s^2)$ and $e_{ijk} \approx N(0, \sigma_e^2)$ where: Y_{ijk} is the observed value; μ is the overall mean; E_i is the fixed effect of enzyme level ($i = 1$ to 4); s_j is the random effect of sorghum cultivar ($j = 1$ to 4); e_{ijk} is the random residual error; N stands for Gaussian distribution; σ_s^2 and σ_e^2 are variances associated with sorghum cultivar and error, respectively.

Repeated measures data (gas losses before silos opening and temperature and silage pH after aerobic exposure) were analyzed considering the following model:

$$Y_{ijk} = \mu + E_i + s_j + \omega_{ijk} + T_l + E \times T_{il} + e_{ijk}$$

with $s_j \approx N(0, \sigma_s^2)$; $\omega_{ijk} \approx N(0, \sigma_{\omega}^2)$ and $e_{ijk} \approx MVN(0, R)$ where: Y_{ijk} is the observed value; μ , E , s_j , N and σ_s^2 were previously defined; ω_{ijk} is the error associated with experimental units (silos); T_l is the fixed effect of evaluation time; $E \times T_{il}$ is the fixed interaction effect between enzyme and time; e_{ijk} is the experimental error; σ_{ω}^2 is the variance associated with silo; MVN stands for multivariate normal distribution; R is a variance and covariance matrix, due to repeated measures. Matrix (CS, CSH, AR, ARH, TOEP, TOEPH, FA, UN, ANTE) was evaluated using the Bayesian method. For all analyses, enzyme level effects were decomposed using orthogonal contrasts for linear, quadratic, and cubic (deviation of cubic) effects. Equations of variables with the quadratic effect of the enzyme were obtained using the solution function. Significance was declared at $P \leq 0.05$, and the tendency was considered when $0.05 < P \leq 0.10$.

Results

Fermentation profile, losses, and DM recovery

The enzymes addition linearly increased ($P \leq 0.04$) acetic acid, branched-chain organic acids (BCOA), and ethanol concentration of silage (Table 2). Besides, it quadratically affected ($P = 0.04$) butyric acids and did not affect ($P \geq 0.17$) pH value and lactic acid concentrations, NH_3 -N, and propionic acid. Although there was no treatment effect ($P \geq 0.11$) on silage DM recovery over the ensiling process, enzyme levels linearly increased ($P \leq 0.01$) gas (Figure 1), effluent, and total losses. Gas and total losses increased 0.0136 and 0.0983 g/kg DM, respectively, for every mL/ton. of enzyme (Table 3).

Table 1. Chemical composition and particle size of sorghum ($n = 5$)

	Mean	SD ¹
Chemical composition (g/kg dry matter)		
dry matter (g/kg as fed)	283	43.3
organic matter	939	11.5
neutral detergent fiber	617	25.2
acid detergent fiber	339	18.9
non-fiber carbohydrate	223	32.5
crude protein	62.8	16.84
ether extract	36.3	7.89
<i>In vitro</i> degradation ² (g/kg)		
dry matter	546	40.1
neutral detergent fiber	444	42.7
acid detergent fiber	394	24.8
Particle size (g/kg as fed)		
>19 mm	359	201.7
8 to 19 mm	360	103.9
4 to 8 mm	105	38.3
< 4 mm	172	79.2

¹Standard deviation.

²48-hours of *in vitro* incubation.

Chemical composition and *in vitro* degradation

The enzymes addition quadratically affected ($P \leq 0.01$) silage NDF, ADF, and NFC concentrations (Table 4). Intermediary levels of enzymes (from 312 to 342 mL/ton DM; Table 3) decreased fiber content (NDF and ADF), whereas improved NFC silage content. However, there were no other effects ($P \geq 0.13$) of treatments on chemical composition. Besides, enzymes did not affect ($P \geq 0.28$) DM and NDF degradation and tended to linearly reduce ($P = 0.07$) the ADF *in vitro* degradation.

Aerobic stability

The enzymes addition linearly decreased ($P = 0.01$) silage temperature, regardless of evaluation time ($P = 0.42$; Table 5 and Figure 2). However, there was no treatment effect ($P \geq 0.12$) on silage pH after aerobic exposure (Figure 3) and the time that silage remained with a temperature lower than the 2°C above environmental temperature.

Table 2. Fermentative losses, pH, lactic acid, and ammonia concentration of sorghum silage treated with increasing levels of fibrolytic enzymes

Item	Fibrolytic enzymes level (mL/ton DM) ¹					SEM ²	P ³			
	0	150	300	450	600		treat.	lin.	quad.	desv.
Fermentation losses (g/kg DM)										
gas	37.1	41.0	39.8	43.3	46.3	1.67	<0.01	<0.01	0.62	0.36
effluent	73.2	113	99.9	109	136	16.5	<0.01	<0.01	0.94	0.11
total	110	154	139	153	185	17.6	0.02	<0.01	0.91	0.11
Dry matter recovery	865	874	862	848	857	11.3	0.30	0.12	0.93	0.12
pH	3.92	3.94	3.91	3.93	3.90	0.006	0.40	0.17	0.45	0.96
NH ₃ -N, g/kg N	94.4	84.9	92.5	89.8	91.0	4.17	0.26	0.85	0.34	0.17
Lactic acid (g/kg DM)	73.7	71.0	69.7	75.1	70.4	1.64	0.54	0.77	0.78	0.17
Acetic acid (g/kg DM)	9.80	9.34	10.59	11.1	10.8	0.258	<0.01	<0.01	0.82	0.14
Propionic acid (g/kg DM)	0.0245	0.00632	0.0111	0.0232	0.00595	0.006033	0.42	0.49	0.79	0.18
Butyric acid (mg/kg DM)	0.00919	0.00223	0.00119	0.00138	0.0194	0.00290	0.20	0.32	0.04	0.55
BCOA ⁴ (mg/kg DM)	0.212	0.288	0.269	0.360	0.377	0.0392	0.29	0.04	0.97	0.92
Ethanol (g/kg DM)	20.7	21.0	20.8	25.8	25.4	1.67	<0.01	<0.01	0.41	0.13

¹Level of fibrolytic enzyme. Each mL of the enzyme had 300 U of xylanase and 300 U of cellulose.²Standard error of mean.³Probabilities (P): treatment/enzyme (treat.); and polynomial regression for treatment effect: linear (lin.); quadratic (quad.); and cubic (quadratic deviation; desv.).⁴Branched-chain organic acids: valeric, isovaleric and isobutyric acids.

Table 3. Regression coefficients and quadratic maximum for variables with linear and quadratic effects of enzyme level

Item (g/kg DM)	Constant		Linear coefficient		Quadratic coefficient		Estimated max./min. ²
	estimate	SE ¹	estimate	SE ¹	estimate	SE ¹	
Gas losses	37.4	3.54	1.36×10 ⁻²	3.24×10 ⁻³			
Total losses	119	37.6	9.83×10 ⁻²	3.14×10 ⁻²			
Acetic acid	9.57	0.530	2.55×10 ⁻³	8.03×10 ⁻⁴			
Ethanol	19.9	3.66	9.39×10 ⁻³	2.64×10 ⁻³			
Neutral detergent fiber	647	15.3	-0.118	3.85×10 ⁻²	1.89×10 ⁻⁴	6.22×10 ⁻⁵	313
Acid detergent fiber	374	6.38	-0.109	3.67×10 ⁻²	1.59×10 ⁻⁴	5.93×10 ⁻⁵	342
Non-fiber carbohydrate	200	11.1	0.1147	4.39×10 ⁻²	-1.79×10 ⁻⁴	7.01×10 ⁻⁵	320

¹Standard error.²Level of XYZ for maximal or minimal response = -linear coefficient/(2 × quadratic coefficient).Table 4. Chemical composition and *in vitro* degradation of sorghum silage treated with increasing levels of fibrolytic enzymes

Item	Fibrolytic enzymes level (mL/ton DM) ¹					SEM ²	P ³			
	0	150	300	450	600		treat.	lin.	quad.	desv.
Chemical composition (g/kg DM, unless stated)										
dry matter (g/kg as fed)	246	248	245	243	245	6.5	0.55	0.28	0.92	0.18
organic matter	937	938	937	937	938	2.2	0.91	0.74	0.49	0.58
neutral detergent fiber	645	639	627	629	646	6.9	0.04	0.67	<0.01	0.23
acid detergent fiber	374	368	351	358	367	2.9	0.03	0.20	0.01	0.33
non-fiber carbohydrate	202	207	223	215	203	5.0	0.09	0.57	0.01	0.43
crude protein	61.9	63.4	63.1	61.7	60.4	1.99	0.39	0.22	0.13	0.60
ether extract	28.5	28.4	23.5	30.9	28.1	1.67	0.67	0.86	0.63	0.61
<i>In vitro</i> degradation (g/kg)										
dry matter	500	497	506	500	492	6.7	0.80	0.61	0.43	0.56
neutral detergent fiber	385	386	404	381	397	8.6	0.54	0.63	0.28	0.91
acid detergent fiber	368	362	350	350	333	9.3	0.10	0.07	0.83	0.78

¹Level of fibrolytic enzyme. Each mL of the enzyme had 300 U of xylanase and 300 U of cellulose.²Standard error of mean.³Probabilities (P): treatment/enzyme (treat.); and polynomial regression for treatment effect: linear (lin.); quadratic (quad.); and cubic (quadratic deviation; desv.).

Table 5. Aerobic stability of sorghum silage treated with increasing levels of fibrolytic enzymes

Item	Fibrolytic enzymes level (mL/ton DM) ¹					SEM ²	P ³					
	0	150	300	450	600		treat.	time	treat.×time	lin.	quad.	desv.
Temperature	2.22	2.17	2.24	1.78	1.89	0.058	0.03	<0.01	0.42	0.01	0.67	0.25
pH	4.74	4.69	4.71	4.61	4.63	0.026	0.52	<0.01	0.69	0.12	0.91	0.74
Time (2°C)	42.5	44.4	39.8	46.4	41.5	2.00	0.53	–	–	0.99	0.82	0.59

¹Level of fibrolytic enzyme. Each mL of the enzyme had 300 U of xylanase and 300 U of cellulose.

²Standard error of mean.

³Probabilities (P): treatment/enzyme (treat.); and polynomial regression for treatment effect: linear (lin.); quadratic (quad.); and cubic (quadratic deviation; desv.).

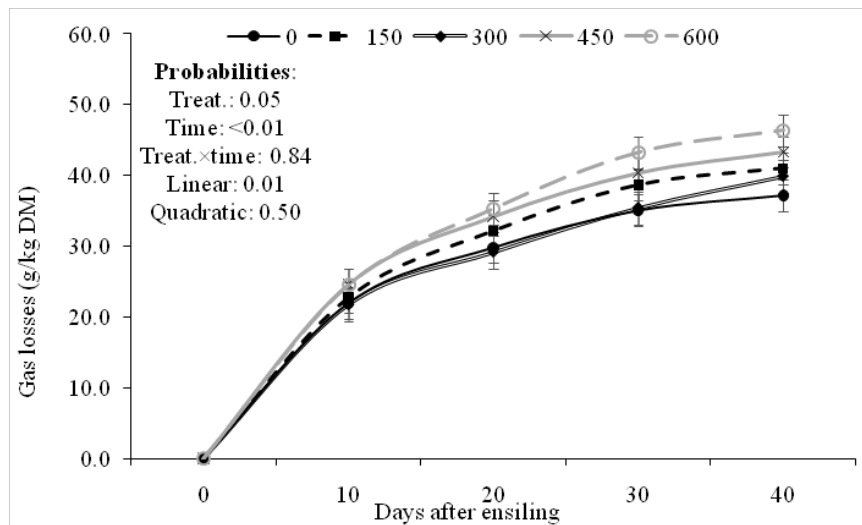


Figure 1. Effects of fibrolytic enzyme level on sorghum silage gas losses after ensiling

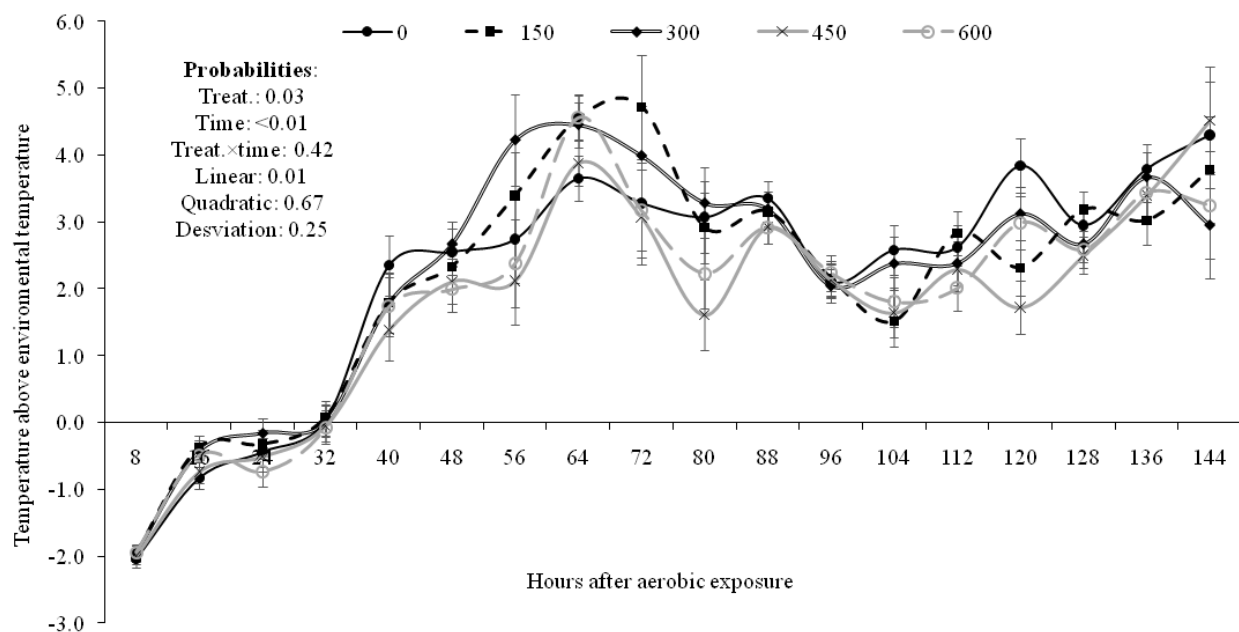


Figure 2. Effects of fibrolytic enzyme level on sorghum silage temperature after aerobic exposure

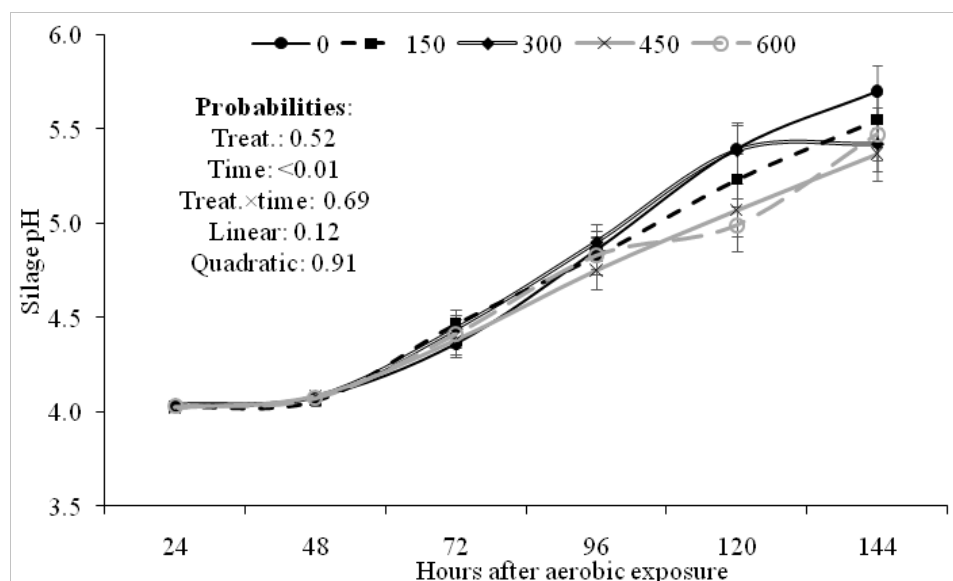


Figure 3. Effects of fibrolytic enzyme level on sorghum silage pH after aerobic exposure

Discussion

The enzymes addition increased the silage concentration of acetic acid, branched-chain organic acids, and ethanol. Another study of our research group observed a positive effect of xylanase's intermediary levels on sugarcane silage acetic acid concentration (Del Valle et al., 2019). These enzyme effects are associated with improved xylan degradation (Dehghani et al., 2012) once the blend evaluated in the present study had 300 U/mL of xylanase activity. As Muck et al. (2018) reported, cellulases and hemicellulases can increase the release of plant cell wall carbohydrates, making them available to LAB for fermentation. However, enzymes did not affect lactic acid concentration, and improved ethanol and acetic acid production are prone to heterolactic fermentation, as observed in silages with high enterobacteria count. According to Kung Jr. (2018), wet silages tended to a high acetic acid level because enterobacteria dominated fermentation. These bacteria consume glucose and produce acetic acid, ethanol, and CO_2 as end-products (McDonald et al., 1991).

Produced CO_2 from enterobacteria in enzyme-treated silos was volatilized, which resulted in increased gas losses. Besides, as we evaluated gas losses considering oven DM, a possible technique bias could be associated with increased gas losses of enzyme-treated silages. However, the most prominent effect observed in fermentative losses was the linear increase in enzymes' increasing levels. Increased solubilization of the substrate could positively affect the water metabolism in silage. This effect of xylanase has been observed in sugarcane silage (Del Valle et al., 2019). However, it did not affect dry matter content and DM recovery of silage in the present study.

We had hypothesized that enzyme use could increase acetic acid, and it was confirmed in the present study. However, an increased concentration of lactic acid was also expected, and there was no effect of enzymes on lactic acid concentration, which is prone to improving a heterofermentative partner. According to Muck (2010), homofermenters produce 2 moles of lactic acid from one glucose mole, whereas heterofermenters produce one mole of lactic acid, one carbon dioxide mole, and one mole of ethanol or one mole of acetic acid from glucose. As observed in Figure 1, 55.6% of gas losses occurred during the first 10 days of ensiling, just during the aerobic phase of ensiling (McDonald et al., 1991).

Increasing levels of enzymes did not affect $\text{NH}_3\text{-N}$ concentration. A linear increase of BCOA concentration was observed, prone to increasing branched-chain amino acid degradation (Miura et al., 1980). Although there was no effect of enzyme addition on NDF and DM *in vitro* degradation, these increased BCOA could positively affect fiber *in vivo* degradation, as reported in previous studies (Gorosito et al., 1985; Yang, 2002). However, butyric acid, which is a marker of clostridial fermentation, was reduced by enzyme treatment. Clostridial fermentation is one of the main ones responsible for protein degradation in wet silages (Muck, 2010). Butyric and propionic acid were observed in a shallow concentration (16.3 mg/kg propionic and 3.50 mg/kg butyric acid) regardless of treatment. Fernandes et al. (2020) observed propionic and butyric levels under detectable levels in sorghum silage. According to Kung et al. (2018), it is doubtful if Propionibacteria natural populations can flourish in most silages and high propionic acid concentration observed in silages are a consequence of clostridial fermentation. In the present study, the low concentration of these acids is an indicator of good silage conservation.

Some studies have reported that exogenous fibrolytic enzymes increase the cell wall polysaccharides degradation but do not always improve DM *in vitro* degradation of silage (Dehghani et al., 2012, Del Valle et al., 2019). In the present study, intermediary levels of enzymes (313 to 342 mg/kg) reduced fiber and increased non-fiber content of silages without a positive effect on *in vitro* degradation. The main explanation for those effects is that enzyme addition might degrade the most digestible fraction of the structural polysaccharides and leave less digestible residue (Liu et al., 2016). Higher acetic acid and ethanol concentration could increase the kafirin solubilization and improve starch digestibility, even at earlier stages of ensiling (Saylor et al., 2020). However, enzymes showed no positive effect on silage *in vitro* degradation in the present study.

Enzymes did not affect DM, CP, and EE silage concentration. It is possible to speculate that improved degradation of fiber constituents increased silage water activity. According to Savoie and Jofriet (2003), different from gas losses that depend on a biochemical process, effluent losses depend on the pressure within silos, overcoming a critical point of silage moisture. Although enzyme addition did not affect silage DM and lactic acid in the present study, it linearly increased effluent losses. These improved effluent losses observed in enzyme-treated silages are undesirable because of the loss of acids and highly digestible silage components and also because effluent has a high biochemical oxygen demand and a low pH, becoming a farm pollutant (Savoie and Jofriet, 2003). We agree that increased water activity resulted in higher effluent losses in enzyme-treated silos, and this effect became null treatment effects on silage DM content.

Evaluating enzyme addition during the ensiling, Kung Jr. and Muck (2015) observed that excessive release of water soluble carbohydrates could reduce aerobic stability. Once released, yeasts and molds spoilage use sugars. However, the high concentrations of acetic acid (an excellent antifungal agent; Danner et al., 2003) may explain silages' reduced temperature after aerobic exposure. However, this decreased temperature observed on enzyme-treated silos was not sufficient to affect the time of aerobic stability and silage pH after aerobic exposure.

Conclusion

Intermediary levels of fibrolytic enzymes (313 to 342 mL/ton DM) reduce fiber and increase the non-fiber carbohydrate content. Increasing levels of enzymes promote heterolactic fermentation and reduce silage temperature after aerobic exposure. However, this increases fermentative losses and has no positive effect on *in vitro* degradation and time of aerobic stability.

Conflict of interest

The authors declare that are no conflicts of interest to the current manuscript.

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References

- AOAC International (2000). Official Methods of Analysis, 17th ed. Association of official analytical chemists, Arlington, VA, USA, 2200 pp.
- Arriola K.G., Oliveira A.S., Ma Z.X., Lean I.J., Giurcanu M.C., Adesogan A.T. (2017). A meta-analysis on the effect of dietary application of exogenous fibrolytic enzymes on the performance of dairy cows. *J. Dairy Sci.*, 100: 4513–4527.
- Bernard J.K., Tao S. (2015). Short communication: Production response of lactating dairy cows to brachytic forage sorghum silage compared with corn silage from first or second harvest. *J. Dairy Sci.*, 98: 8994–9000.
- Casali A.O., Detmann E., Valadares Filho S.C., Pereira J.C., Henriques L.T., Freitas S.G., Paulino M.F. (2008). Influence of incubation time and particles size on indigestible compounds contents in cattle feeds and feces obtained by *in situ* procedures. *Rev. Bras. Zootec.*, 37: 335–342.
- Danner H., Holzer M., Mayrhuber E., Braun R. (2003). Acetic acid increases stability of silage under aerobic conditions. *Appl. Environ. Microbiol.*, 69: 562–567.
- Dehghani M.R., Weisbjerg M.R., Hvelplund T., Kristensen N.B. (2012). Effect of enzyme addition to forage at ensiling on silage chemical composition and NDF degradation characteristics. *Livest. Sci.*, 150: 51–58.
- Del Valle T.A., Antonio G., Zenatti T.F., Campana M., Zilio E.M.C., Ghizzi L.G., Gandra J.R., Osório J.A.C., de Moraes J.P.G. (2019). Effects of xylanase on the fermentation profile and chemical composition of sugarcane silage. *J. Agric. Sci.*, 156: 1123–1129.
- Drews M., Larsen M.A.D., Balderrama J.G.P. (2020). Projected water usage and land-use-change emissions from biomass production (2015–2050). *Energy Strategy Rev.*, 29: 100487.
- Fernandes T., Paula E.M., Sultana H., Ferraretto L.F. (2020). Short communication: Influence of sorghum cultivar, ensiling storage length, and microbial inoculation on fermentation profile, N fractions, ruminal *in situ* starch disappearance and aerobic stability of whole-plant sorghum silage. *Anim. Feed Sci. Tech.*, 266: 114535.
- Gorosito A.R., Russell J.B., Van Soest P.J. (1985). Effect of carbon-4 and carbon-5 volatile fatty acids on digestion of plant cell wall *in vitro*. *J. Dairy Sci.*, 68: 840–847.
- Holden L.A. (1999). Comparison of methods of *in vitro* dry matter digestibility for ten feeds. *J. Dairy Sci.*, 82: 1791–1794.
- Jobim C.C., Nussio L.G., Reis R.A., Schmidt P. (2007). Methodological advances in evaluation of preserved forage quality. *Ver. Bras. Zootec.*, 36: 101–119.
- Kung Jr. L. (2018). Silage fermentation and additives. *Arch. Latinoam. Prod. Anim.*, 26: 3–4.
- Kung Jr. L., Muck R.E. (2015). Silage additives: Where are we going? *Proc. XVII International Silage Conference*, Piracicaba, Sao Paulo, Brazil, pp. 72–81.
- Kung Jr. L., Shaver R.D., Grant R.J., Schmidt R.J. (2018). Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. *J. Dairy Sci.*, 101: 4020–4033.
- Liu Q.H., Li X.Y., Desta S.T., Zhang J.G., Shao T. (2016). Effects of *Lactobacillus plantarum* and fibrolytic enzyme on the fermentation quality and *in vitro* digestibility of total mixed rations silage including rape straw. *J. Integr. Agric.*, 15: 2087–2096.
- Maulfair D.D., Fustini M., Heinrichs A.J. (2011). Effect of varying total mixed ration particle size on rumen digesta and fecal particle size and digestibility in lactating dairy cows. *J. Dairy Sci.*, 94: 3527–3536.
- McCary C.L., Vyas D., Faciola A.P., Ferraretto L.F. (2020). Graduate Student Literature Review: Current perspectives on whole-plant sorghum silage production and utilization by lactating dairy cows. *J. Dairy Sci.*, 103: 5783–5790.

- McDonald P., Henderson A.R., Heron S.J.E. (1991). The biochemistry of silage, 2nd ed. Marlow, UK, Chalcomb Publications, 207 pp.
- McDougall E.I. (1948). Studies on ruminant saliva. 1. The composition and output of sheep's saliva. *Biochem. J.*, 43: 99–109.
- Miron J., Zuckerman E., Sadeh D., Adin G., Nikbachat M., Yosef E., Ben-Ghedalia D., Carmi A., Kipnis T., Solomon R. (2005). Yield, composition and *in vitro* digestibility of new forage sorghum varieties and their ensilage characteristics. *Anim. Feed Sci. Tech.*, 120: 17–32.
- Miura H., Horiguchi M., Matsumoto T. (1980). Nutritional interdependence among rumen bacteria, *Bacteroides amylophilus*, *Megasphaera elsdenii*, and *Ruminococcus albus*. *Appl. Environ. Microbiol.*, 40: 294–300.
- Muck R.E. (2010). Silage microbiology and its control through additives. *Rev. Bras. Zootec.*, 39: 183–191.
- Muck R.E., Nadeau E.M.G., McAllister T.A., Contreras-Govea F.E., Santos M.C., Kung Jr. L. (2018). Silage review: Recent advances and future uses of silage additives. *J. Dairy Sci.*, 101: 3980–4000.
- Pryce J.D.A. (1969). A modification of the Barker-Summerson method for the determination of lactic acid. *Analyst*, 94: 1151–1152.
- Roby M.C., Salas Fernandez M.G., Heaton E.A., Miguez F.E., VanLoocke A. (2017). Biomass sorghum and maize have similar water-use-efficiency under non-drought conditions in the rain-fed Midwest U.S. *Agricult. Forest Meteorol.*, 247: 434–444.
- Savoie P., Jofriet J.C. (2003). Silage storage. In: *Silage science and technology* (agronomy series No. 42), Buxton D.R., Muck R.E., Harrison H.J. (eds). American Society of Agronomy, Madison, WI., pp. 405–467.
- Saylor B.A., Fernandes T., Sultana H., Gallo A., Ferraretto L.F. (2020). Influence of microbial inoculation and length of storage on fermentation profile, N fractions, and ruminal *in situ* starch disappearance of whole-plant corn silage. *Anim. Feed Sci. Tech.*, 267: 114557.
- Tilley J.M.A., Terry R.A. (1963). A two-stage technique for the *in vitro* digestion of forage crops. *Grass Forage Sci.*, 18: 104–111.
- Van Soest P.J., Robertson J.B., Lewis B.A. (1991). Methods for dietary fiber, neutral detergent fiber, non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, 74: 3583–3597.
- Wang Y., McAllister T.A. (2002). Rumen microbes, enzymes and feed digestion – a review. *Asian-Australas. J. Anim. Sci.*, 15: 1659–1676.
- Yang C.M.J. (2002). Response of forage fiber degradation by ruminal microorganisms to branched-chain volatile fatty acids, amino acids, and dipeptides. *J. Dairy Sci.*, 85: 1183–1190.
- Yang Y., Ferreira G., Corl B.A., Campbell B.T. (2019). Production performance, nutrient digestibility, and milk fatty acid profile of lactating dairy cows fed corn silage- or sorghum silage-based diets with and without xylanase supplementation. *J. Dairy Sci.*, 102: 2266–2274.

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