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In vitro gas production and digestibility of oat and triticale forage mixtures ensiled with fibrolytic enzymes and inoculants

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ABSTRACT

Objective: To assess the effects of adding fibrolytic enzymes (FE) or lactic acid bacteria (LAB) inoculants to 40 d silages with oat and triticale (O:T) mixtures on the ratio and composition of neutral detergent fiber (NDF) and its subsequent *in vitro* gas production (GP) and *in vitro* dry matter digestibility (IVDMD) at 24 h.

Design/Methodology/Approach: Silages elaborated with two O:T ratios (60:40 and 80:20) treated with low (LD), medium (MD), and high (HD) doses of FE (0.75, 1, and 1.25 g/kg forage in wet basis (WB), respectively), and LAB (0.188, 0.25, and 0.31 g/kg WB, respectively). In both cases (FE and LAB), the control had a value of 0. Subsequently, pH, NDF, acid detergent fiber (ADF), acid detergent lignin (ADL), hemicellulose (HEM), cellulose (CEL), dry matter (DM), crude protein (CP), GP parameters, and IVDMD were assessed. GP parameters included maximum velocity (Vmax), fractional rate (S), and lag. Experiments were planned in complete randomized designs (CRD), including factorial and split-plot arrangements. Variance analysis (ANOVA) models included fixed (doses, additives, and FR) and random (place/moment of sampling) effects.

Results: LAB improved the IVDMD at 24 h of 60:40 and 80:20 O:T silages. FE did not reduce the NDF of 60:40 silages, but LD and MD increased the HEM and CP, and reduced the ADF, ADL, and CEL; these results are correlated (r) with the improvement of pH pattern, GP, and IVDMD.

Study Limitations/Implications: The differences in the NDF of FR mixtures could affect the effectiveness of FE and LAB.

Findings/Conclusions: Although FE and LAB did not reduce the NDF, they changed the ratios of ADF, ADL, HEM, CEL, and CP of silages, potentially improving the GP and IVDMD.

Keywords: Oats and triticale, ensiling, fibrolytic enzymes, lactic acid bacteria, gas production, ruminal degradability.

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INTRODUCTION

In semi-arid regions, the high frequency of droughts has a negative effect on the availability and nutritional value of forages (Acosta *et al.*, 2003), limiting the ability of

producers and ranchers from mainly extensive and family systems to maintain the production of bovine milk and sheep, goat, and bovine meat. Therefore, maximizing the ratio of fibrous forages in the diet of ruminants, without reducing the quality and quantity of the products (Tirado-González *et al.*, 2018; Tirado-Estrada *et al.*, 2020), can help to reduce production costs (Oba and Allen, 1999, 2000a, b; Tirado-Estrada *et al.*, 2015), improve animal health (Saleem *et al.*, 2012; Petri *et al.*, 2013), and diminish the environmental impact of deforestation, consequently maintaining intensive grain production (McGinn *et al.*, 2004; Knapp *et al.*, 2014; Mora de Alba *et al.*, 2018).

The ensiling process contributes to the conservation and modification of the degradability of the cell walls of the forages, improving the nutritional characteristics of the forage through the formation of lactic acid and alcohols and the increase in crude protein (CP) (Tiwari *et al.*, 2008; Ajila *et al.*, 2015; Chen *et al.*, 2016). Meanwhile, the use of lactic acid bacteria (LAB) inoculants can improve the carbohydrate fermentation period, while the volatile fatty acid (VFA) profile and the formation of lactic acid increase the aerobic stability and quality of silages (Tabacco *et al.*, 2011; Skládanka *et al.*, 2012; Guo *et al.*, 2013; Schroeder, 2013). Fibrolytic enzymes (FE) can help to break the bonds of the cellulose and hemicellulose components (Arriola *et al.*, 2017; Kholif *et al.*, 2017; Tirado-González *et al.*, 2015, 2018), favoring the fermentation process through the increase of the sugars available for the microorganisms (Gado *et al.*, 2013; Salem *et al.*, 2015; Kholif *et al.*, 2017). However, the efficient action of LAB and FE depends on the interaction of various factors, such as: type and ratios of forages (Dehghani *et al.*, 2012), LAB strain or FE mixture (Dean *et al.*, 2005; Lynch *et al.*, 2012), dose (Del Valle *et al.*, 2019), and ensiling time (Lynch *et al.*, 2012). This phenomenon is caused by the high specificity of the enzymatic components, which record small differences in their neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) composition (Tirado-González *et al.*, 2016).

The objective of this work is to assess the effect of the use of EF and LAB in silages with oat and triticale mixtures on the contents and compositions of NDF and their relationship with GP and IVDMD.

MATERIALS AND METHODS

Experiment location and biological material

Representative samples of 60:40 and 80:20 ratios of oat (*Avena sativa* L.) and triticale (*Triticosecale* Wittmack. Ex. A. Camus) were taken in two locations in the central-northern region of Mexico:

1) El Llano, Aguascalientes (21° 55' 00" N, 101° 58' 00" W; 2,021 m.a.s.l.). The climate is semi-arid (BS1kw, Köppen), with an average temperature of 17.4 °C and an average annual precipitation of 540 mm. The soil mainly consists of planosol (66%) and phaeozem (23%).
2) Lagos de Moreno, Jalisco (21° 21" 23" N, 101° 55" 45" W; 1,942 m.a.s.l.). The climate is subtropical (Csa, Köppen), with an average temperature of 18.4 °C and an average annual precipitation of 670 mm. The soil is mainly composed of lithosol, planosol, and phaeozem (31%, 29% and 25%, respectively).

The samples were taken 120 hours after the cultivation began: 10 samples (20 kg of complete plants/sample) were selected at random in each of the blocks (DM=33.1±2.7%).

Preparation of microsilages

The microsilages and chemical analyzes were carried out at TecNM/ITEL (21° 55' N, 101° 58' W; 1,840 m.a.s.l.), in El Llano, Aguascalientes (average annual temperature of 17 °C, average annual precipitation of 455 mm).

The additives applied to the microsilages were an FE preparation —with different ratios of cellulases and xylanases (Fibrozyme, Alltech Inc., Nicholasville, KY, USA)— and a LAB inoculant —with *Lactobacillus plantarum* and *Pediococcus pentosaceus* (EnziBiolac, Enzimas y Productos Químicos, S. A. de C. V., SAGARPA Reg. A-9912-001, Mexico). Samples of 1±0.1 kg (WB) of the 60:40 and 80:20 O:T mixtures collected in the two locations (DM=30±2.5%) were chopped into 2 cm particles and treated with: 1) 0 (control), 0.75 (LD), 1 (MD), and 1.25 g (LD) doses of FE/kg WB; and 2) 0 (control), 0.188 (LD), 0.25 (MD), and 0.31 g (HD) doses of LAB/kg WB.

The FE and LAB mixtures were dissolved in distilled water and sprayed uniformly on the forage. The treatments were placed in 5.08 cm wide×30 cm long polyvinyl chloride (PVC) pipes (with reinforced PVC end caps installed at both ends of the pipe). The treatments were compacted with a metal piston (to eliminate as much oxygen as possible inside the microsilages) and stored in a closed room with an average temperature of 20 °C. They were then allowed to ferment for 40 days.

Chemical analysis

At the time of ensiling and every 0, 10, 20, 30, and 40 d after the start of the ensiling process, 300 g WB/microsilage fractions were sampled from different parts of each microsilage. These samples were placed in a Felisa® AR-290 forced air oven at 60 °C, until a constant weight (initial DM; 0 d) was reached. The dried samples were ground with a 1 mm sieve.

In the chemical analysis, the pH of the samples was measured using an Orion Star™ A2110 pH-meter (Thermo Scientific). The initial and final DM, crude protein (CP), and ash (ASH) were determined using the 10.136, 990.03 and 942.05 methods, respectively (AOAC, 2005). The NDF, ADF, and ADL of the samples collected from the microsilages at 40 d were determined (Van Soest *et al.*, 1991). For this purpose, the samples were adapted for the reagents of the F57 filters (2016, Ankom Technol Technology, Macedon, NY, USA) and an ANKOM^{200} Fiber Analyzer (ANKOM Technology,). Hemicellulose (HEM) and cellulose (CEL) were calculated by difference (HEM=NDF−ADF and CEL=ADF−ADL−ASH). Each sample was analyzed in duplicate.

***In vitro* fermentation**

The gas production of the silage samples taken at 40 d was analyzed using the gas production technique published by Menke and Steingass (1998). Ruminal fluid was obtained from two cannulated Dorper sheep (live weight: 60±5 kg) fed with a 76:24 forage:concentrate ratio (forage: barley (*Hordeum vulgare*) straw; concentrate: mixture of

corn and soybean grain). The chemical composition of the forage was: 58% NDF, 10% CP, and 44.1% crude fiber (CF). Meanwhile, the chemical composition of the concentrate was: 22.4% NDF, 16% CP, and 5.4% CF.

The ruminal fluid was filtered with an 8-layer gauze and mixed with the mineral solutions reported by Cobos and Yokoyama (1995). Once the ruminal inoculum was prepared, it was placed in amber bottles with 0.5 g of DM and 90 mL of inoculum from the samples taken from the microsilages at 0 and 40 d (each sample was analyzed in duplicate). Incubation was carried out in a water bath (39 °C) under conditions of continuous flow of carbon dioxide (CO_2). The excess CO_2 from each bottle was extracted with the 63100 analog manometer (Metron®). The pressure of the fermentation gas (0 to 1 kg/cm^2) was measured with the manometer at 2, 4, 6, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, and 72 h of incubation and it was converted to mL.

At the end of the incubation period, the residue from each bottle was filtered through a previously weighed filter paper (Whatman® qualitative filter paper, Grade 4: 1004-110, pore size: 20-25 μm); the filter papers with residue were dried at 65 °C for 48 h and subsequently weighed. After 24 h, the fermentation of half of the flasks was stopped and the IVDMD was determined as follows:

$$IVDMD24 = 100 - \left[\left(initialDM - finalDM \right) / \left(initialDM * 100 \right) \right]$$

The maximum volume, fractional rate, and lag phase (Vmax, S, and Lag) parameters of gas production were optimized in Equation (1) of Schofield *et al.* (1994).

$$Vo = \frac{V \max}{1 + e^{2-4S(t-Lag)}} \quad (1)$$

Where: *Vo*=accumulated GP volume; *Vmax*=maximum volume of GP; *S*=fractional rate; *Lag*=lag phase.

Statistical analysis

Data analysis was performed with the SAS [9.2] statistical software (Statistical Analysis System). An analysis of variance (ANOVA) took into consideration the DCA, with factorial and split-plot arrangements with 4 repetitions per treatment, as well as 2 sub-repetitions for the fixed and random effects of Models 1 and 2. The significances, coefficients of determination (R^2), and coefficients of variation (CV) were obtained using the General Linear Procedure (Proc GLM); the LsMeans instruction was used for the adjusted means; and the standard errors (SE) were determined with the Mixed Models Procedure (Proc Mixed). The DMS were calculated using the SE values (P=0.05).

Model 1

$$Y = \mu + Rep(Loc)_{ij} + Tra_k + T_l + (Tra * T)_{kl} + \varepsilon_{ijkl}$$

Where: Y=pH; μ is the overall mean; $Rep\ (Loc)_{ij}$ is the random effect of the i-th repetition within the j-th sowing location; Tra_k is the effect of the k-th treatment; T_l is the effect of the l-th time; $(Tra*T)_{kl}$ is the interaction between fixed factors; ε_{ijkl} is the random error.

Model 2

$$Y = \mu + Subrep\left(Rep\right)_{ij} + D_k + A_l + PAT_m + (D*A)_{kl} + (D*PAT)_{km} + (A*PAT)_{lm} + (D*A*PAT)_{ijklm} + \varepsilon_{ijklm}$$

Where: Y=*initial DM*, *final DM*, *Vmax*, *S*, *Lag*, *pH*, *NDF*, *ADF*, *ADL*, *CP*, *ASH*, and *IVDMD24*; *Subrep* $(Rep)_{ij}$ is the random effect of the i-th subrepetition within the j-th locality or analysis time; D_k is the effect of the k-th dose; A_l is the effect of the l-th additive; PAT_m is the effect of the m-th ratio of *O*:*T*; $(D*A)_{kl}$, $(D*PAT)_{km}$, $(A*PAT)_{lm}$, and $(D*A*PAT)_{ijklm}$ are the interactions of the fixed factors; ε_{ijklm} is the random error.

Correlation analysis. Simple Pearson correlation analyzes were performed to analyze the relationship between the Vmax, S, Lag, NDF, ADF, ADL, CP, ASH, and IVDMD24 variables.

RESULTS AND DISCUSSION

Changes in chemical composition and pH

Chemical composition. Table 1 shows the chemical composition of the silages. Overall, the use of LD and MD of FE and LAB in the 60:40 O:T silages did not affect the ratio of NDF. However, the HD of both additives increased the ratio of NDF in the 60:40 O:T silages. The LD of FE reduced the ratio of NDF ($P<0.0009$) in 80:20 O:T silages. Furthermore, the ratio of ADF did not diminish in any of the FE and LAB treatments in both types of silage; even treatments with HD of LAB showed the highest ADF values compared to the control ($P<0.02$). Although the additives did not consistently reduce NDF and ADF, their inclusion did affect the ratios of HEM and CEL: the 60:40 O:T silages had more HEM and less CEL than the 80:20 silages ($P<0.008$), while silages with FE had a lower CEL content than those treated with LAB ($P<0.01$). The ratios of ADL and ASH were lower in 60:40 O:T silages than in 80:20 silages ($P<0.0002$); likewise, ASH were higher in silages treated with FE ($P<0.03$).

The 60:40 O:T silages had better CP content than 80:20 silages ($P<0.0001$). In the 60:40 O:T silages, the use of FE did not improve the CP in relation to the Control. In the 80:20 O:T silages, the use of LD, MD, and HD of FE increased the CP. Similarly, LD, MD, and HD of LAB improved the CP ratios of the 60:40 and 80:20 O:T silages ($P<0.05$).

Higher initial and final DM were observed in the 60:40 O:T silages than in the 80:20 silages ($P<0.0001$), as well as in those treated with FE with regard to those treated with LAB ($P<0.01$).

Table 1. Nutritional quality of silages of oat and triticale (O:T) forage mixtures (60:40 and 80:20 ratios), supplemented with various doses of fibrolytic enzymes (FE) and lactic acid bacteria (LAB).

Silages		NDF (%)	HEM (%)	ADF (%)	CEL (%)	ADL (%)	CP (%)	Ashes (%)	PreE-DM (%)	PostE-DM (%)
Oats: triticale 60:40%										
EFE	Control	61.38c	28.28b	33.51de	19.25cd	4.15c	7.25c	9.69cd	36.88cd	41.88cd
	LD	61.48c	28.15ab	33.34e	19.32cd	4.17c	7.80bc	9.86bc	39.65a	44.81a
	MD	62.28c	28.78ab	33.5de	20.18cd	4.27c	7.76bc	9.04d	38.15b	43.16ab
	HD	64.61a	29.23a	35.38c	21.70bc	4.53bc	7.37c	9.15d	38.59ab	43.71b
ALB	Control	62.69bc	28.37ab	34.20d	20.36cd	4.23c	6.92d	9.17d	37.13c	42.13c
	LD	62.29c	29.24a	34.05d	20.85bc	4.26c	9.06a	8.94d	37.11c	42.08cd
	MD	62.54bc	27.95b	34.59cd	21.54bc	4.31c	8.06b	8.74d	37.73bc	42.89bc
	HD	63.68ab	28.39ab	35.29c	21.55bc	4.29c	7.35c	9.46cd	35.83d	40.98d
Oats: triticale 80:20%										
EFE	Control	63.01ab	28.86ab	35.16cd	19.38cd	4.49bc	5.09h	9.28cd	28.43f	33.53f
	LD	61.89c	27.29bc	34.6cd	19.33d	4.57b	6.38e	10.69ab	28.28f	33.44f
	MD	63.27b	27.45bc	35.81ab	20.49c	4.57b	5.92f	10.75a	29.48ef	34.87e
	HD	62.66bc	27.30c	34.36d	20.53c	4.65b	5.80f	10.18b	28.26f	33.36f
ALB	Control	63.74a	28.13b	35.61c	20.56c	4.62b	4.83h	9.27cd	29.14ef	34.24ef
	LD	63.71ab	27.25c	36.46b	22.05ab	4.75ab	5.47g	9.66c	27.68f	32.58fg
	MD	64.41a	26.88c	37.53a	23.23a	5.02a	5.44gh	9.28cd	28.22f	33.16g
	HD	64.25a	27.07c	37.17a	21.85b	4.89ab	5.41gh	9.44cd	29.05ef	34.01ef
P-values										
	Doses	0.006	0.84	<0.0001	0.006	0.12	0.0004	0.36	0.56	0.89
	Additive	0.004	0.01	<0.0001	0.004	0.24	0.04	0.003	0.03	0.05
	OT	0.37	<0.0001	<0.0001	0.008	0.0002	<.0001	<0.0001	<0.0001	<0.0001
	D*A	0.009	0.03	0.17	0.06	0.83	0.003	0.23	<0.0001	<0.0001
	D*OT	0.11	0.26	0.05	0.02	0.67	0.21	0.22	<0.0001	<0.0001
	A*OT	0.10	0.17	0.02	0.99	0.11	0.002	0.10	0.13	0.01
	D*A*OT	0.47	0.38	0.66	0.93	0.26	0.05	0.40	0.004	0.05
	R^2	0.65	0.59	0.8	0.57	0.44	0.87	0.48	0.98	0.97
	V.C. (%)	1.77	3.20	2.19	4.91	11.77	7.92	6.00	2.99	4.01
	LSD (0.05) =	1.05	1.04	0.72	1.20	0.34	0.46	0.50	0.95	1.01
	S.E.	0.62	0.63	0.42	0.73	0.20	0.27	0.29	0.583	0.571

Different letters represent statistical media differences; FE, fibrolytic enzymes; LAB, lactic acid bacteria; LD, low dose; MD, medium dose; HD, high dose; Initial DM, initial dry matter (0 d, prior to the ensiling process); Final DM, final dry matter (DM of the silage at 40 d); NDF, neutral detergent fiber; HEM, hemicellulose; ADF, acid detergent fiber; CEL, cellulose; ADL, acid detergent lignin; CP, crude protein; P-values, probability values; ASH, ashes; D, doses; A, additive (FE or LAB); ROT, oat:triticale ratio; R^2, determination coefficient; V.C., variation coefficient; S.E., standard error; LSD, least significant difference ($P<0.05$).

pH modifications during the ensiling process. Figure 1 shows the pH changes of the treatments over the course of the ensiling period. The pH of the 60:40 O:T silages decreased from 6.1 to 4.5 (average) in the first 10 d. The use of FE had no significant effect on the reduction of pH, but there were significant effects resulting from the use of LD and MD of LAB ($P<0.05$). On the one hand, the use of HD of LAB increased pH (on day 20 after the fermentation began) with regard to the LD and MD of LAB ($P<0.0001$). On the other hand, in the 80:20 O:T silages, the pH was reduced from 6.1 to 4.2 in the first 30 d. However, in average, the pH increased after 30 d of fermentation ($P<0.01$) in all treatments.

In vitro fermentation of DM

Table 2 shows the Vmax, S, and Lag phase of GP of the treatments assessed in vitro, as well as the IVDMD24. Overall, differences in Vmax and S were observed in the two types of silage ($P<0.0001$). In 80:20 O:T silages, the use of FE and LAB did not increase Vmax and S with regard to the control, but, in the 60:40 O:T silages, the use of LD and MD of FE and LAB resulted in higher Vmax and S than in the control treatments ($P<0.003$). Similarly, the MD of FE, and the LD, MD, and HD of LAB reduced the Lag phase time of the 60:40 O:T silages (5.73 h *vs.* 6.55 h, MD of FE *vs.* Control; 5.92 h *vs.* 6.97 h, average LD, MD, and HD of LAB *vs.* Control), but did not affect the Lag time of the 80:20 O:T silages ($P<0.03$). The use of LD of FE and MD of LAB increased the IVDMD24 of the

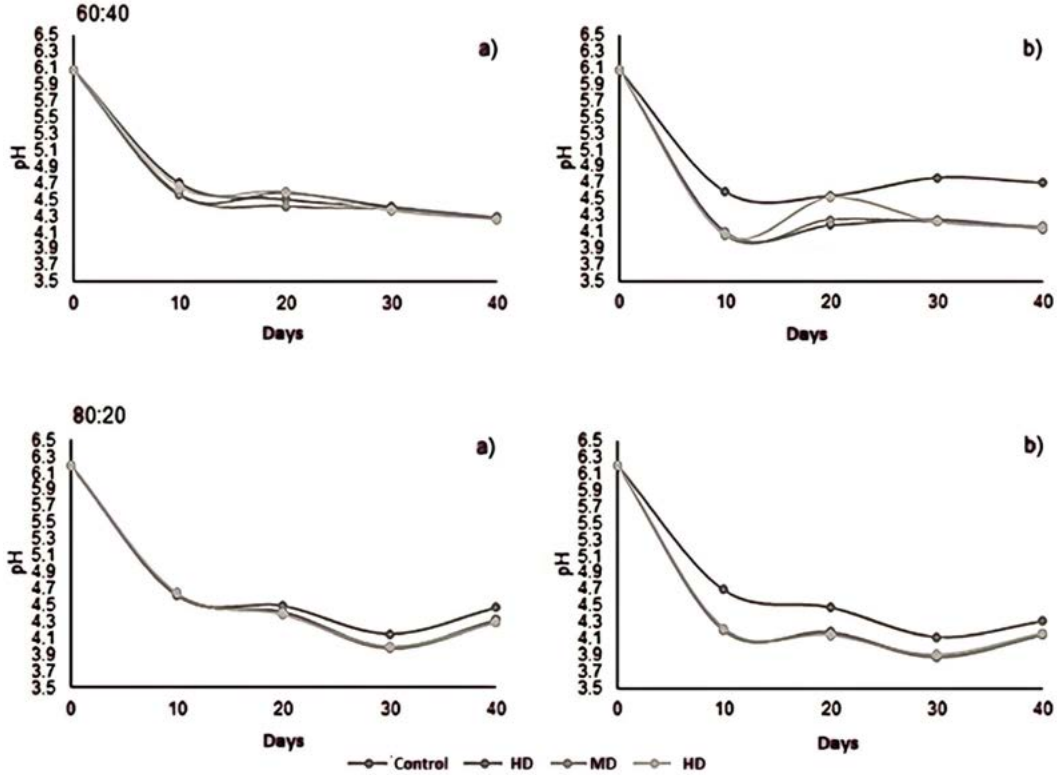

Figure 1. Effects on pH during the ensiling process of 60:40 and 80:20 oat:triticale silages supplemented with additives: a) fibrolytic enzymes (FE) and b) lactic acid bacteria (LAB).

Table 2. *In vitro* gas production (GP) and *in vitro* dry matter digestibility (IVDMD) at 24 h of silages with 60:40 and 80:20 oat:triticale (O:T) ratios supplemented with different doses of fibrolytic enzymes (FE) and lactic acid bacteria (LAB).

Silages		Vmax mL/g	S mL gas/h	Lag h	IVDMD %
Oats: triticale 60:40%					
EFE	Control	302.53cd	0.032b	6.55b	66.41b
	LD	314.24bc	0.033a	6.52b	67.70a
	MD	333.28ab	0.032b	5.73c	65.21c
	HD	322.75b	0.031c	6.60b	65.81bc
ALB	Control	308.33c	0.032b	6.97ab	65. 68bc
	LD	330.55ab	0.033a	6.02c	65.21c
	MD	331.58b	0.032b	6.01c	66.27ab
	HD	346.18a	0.033a	5.73c	62.66d
Oats: triticale 80:20%					
EFE	Control	283.83de	0.032b	6.50ab	60.95e
	LD	295.58cd	0.031c	6.61b	62.14de
	MD	294.28d	0.031c	6.79ab	59.96ef
	HD	302.01cd	0.031c	6.52b	59.95ef
ALB	Control	288.96de	0.032b	7.18a	59.76ef
	LD	300.79cd	0.031c	6.97ab	60.62ef
	MD	292.93de	0.031c	6.73ab	59.71f
	HD	277.10e	0.030d	7.01ab	59.95ef
P-values					
	Doses	0.25	0.05	0.42	0.0002
	Additives	0.05	0.84	0.83	<0.0001
	OT	<0.0001	<0.0001	<0.0001	<0.0001
	D*A	0.11	0.002	0.78	<0.0001
	D*OT	0.24	0.28	0.64	0.25
	A*OT	0.003	0.003	0.0005	0.0002
	D*A*OT	0.10	0.11	0.03	<0.0001
	R^2	0.80	0.79	0.94	0.91
	C.V. (%)	6.67	4.27	9.67	3.14
	LSD (0.05)=	17.00	0.001	0.52	1.20
	S.E.	10.33	0.0007	0.31	0.72

FE, fibrolytic enzyme; LAB, lactic acid bacteria; LD, low dose; MD, medium dose; HD, high dose; Vmax, maximum volume of GP; S, fractional gas production rate; Lag, lag phase; IVDMD, in vitro dry matter digestibility at 24 h; P-values, probability values; D, doses; A, additive (FE or LAB); ROT, oat: triticale ratio; R^2, determination coefficient; V.C., variation coefficient; S.E., standard error; LSD, last significant difference (P<0.05).

60:40 O:T silages (67.70% *vs*. 66.41%, LD of FE *vs*. Control; 66.27% *vs*. 65.68%, MD of LAB *vs*. Control) (P<0.0001); however, using FE and LAB did not have a positive effect on the IVDMD of 80:20 O:T silages.

Pearson correlations

The IVDMD24 did not correlate with NDF (Table 3); however, it showed a positive correlation with the HEM and CP ratio, but negative correlation with ADF, CEL, and ADL ($P<0.0001$). Consequently, the IVDMD24 had a positive correlation with Vmax and S and a negative correlation with the Lag phase ($P<0.01$).

FE has been used to improve the digestibility of forages intended for ruminant feed. These products can combine different ratios of forms and isoforms of cellulases and xylanases, depending on the extraction time of the FE and the substrate from which it was obtained (Tirado-González *et al.*, 2015, 2016, 2018; Carrillo-Díaz *et al.*, 2022). Using FE can have positive effects on the production and kinetics of GP and IVDMD (Phakachoed *et al.*, 2013; Salem *et al.*, 2015; Li *et al.*, 2018).

The variation in the oat and triticale ratios in this work affected the action of the FE and LAB added to the silages, confirming the high specificity of the enzymes extracted from fungi and the potential synergisms and negative interactions between enzymes and bacteria reported in previous research (Tirado-González *et al.*, 2016). Furthermore, the inconsistent fermentation and in vitro degradability results arising from the use of enzymes between silages with different O:T ratios are explained by the interaction between the types of cell walls with the exogenous enzyme preparations and the activity of bacteria and endogenous FE (Beauchemin *et al.*, 2003; Tirado-González *et al.*, 2015). The components and structures of the cell walls of forages depend on their type, maturity, and stress (Jung and Casler, 2006a, b).

Therefore, this research shows how the optimal activity of commercial FE depends more on changes in the structure and composition of NDF and its interaction with endogenous and exogenous bacteria than on the total ratio of NDF (Tirado-González *et al.*, 2018; 2021). Likewise, changes in the ratios of ADF, HEM, CEL, and ADL can improve the activity of FE. The relationships between cellulases and xylanases in commercial FEs depend on the fungus from which they were extracted, the type of substrate, and the extraction time. In addition, their action, combined with or independent from LAB, will be reflected in the pH —indicating greater production of acids during the fermentation of the silage (Skládanka *et al.*, 2012; Mora de Alba *et al.*, 2018; Li *et al.*, 2018)—, in the chemical composition of the silages at the end of fermentation (Gado *et al.*, 2013; Kholif *et al.*, 2017), in the subsequent fermentation (Tirado-Estrada *et al.*, 2015), and in the rumen degradability of NDF (Carrillo-Díaz *et al.*, 2022; Khan *et al.*, 2015).

This study shows how LD and MD of FE improved IVDMD24 in 60:40 O:T silages. However, their addition did not have positive effects in 80:20 O:T silages, partially showing the effects of interactions between the ratio and composition of NDF and the exogenous and endogenous enzymes and bacteria. This phenomenon is relevant because NDF degradability has previously been related to DM intake, as well as to the potential production and quality of ruminant milk and meat (Oba and Allen, 1999, 2000a, b; Arriola *et al.*, 2017; Tirado-González *et al.*, 2018, 2020). Likewise, changes in the productive behavior of ruminants are caused by changes in rumen kinetics —which depend on the type of successive populations of highly specific microorganisms that

Table 3. Pearson correlations among the following variables: composition, gas production (GP), and in vitro dry matter digestibility (IVDMD) at 24 h.

	S	**Lag**	**IVDMD**	**PreE-DM**	**Ash**	**NDF**	**HEM**	**ADF**	**CEL**	**ADL**	**pH**	**CP**	**PostE-DM**
Vmax	0.32**	−0.74***	0.66***	0.68***	−0.43***	−0.03	0.46***	−0.41***	−0.03	−0.44**	0.03	0.56***	0.68***
S		−0.50***	0.64***	0.43**	−0.09	−0.23§	0.24§	−0.45**	−0.30*	−0.31*	−0.17	0.61***	0.43**
Lag			−0.76***	−0.60***	0.21§	0.16	−0.38**	0.50***	0.22§	0.46***	−0.08	−0.62***	−0.60***
IVDMD				0.74***	−0.18	−0.21	0.48***	−0.62***	−0.37**	−0.47***	0.03	0.76***	0.74***
PreE−DM					−0.40**	−0.13	0.57***	−0.61***	−0.25*	−0.46***	0.09	0.77***	0.87***
Ash						0.06	−0.12	0.17	−0.40**	0.15	0.11	−0.33§	−0.40§
NDF							0.50***	0.70***	0.64***	0.22	0.01	−0.22	−0.13
HEM								−0.27*	−0.05	−0.38**	0.15	0.52***	0.57***
ADF									0.75***	0.56***	−0.12	−0.68***	−0.61***
CEL										0.07	−0.11	−0.36**	−0.25*
ADL											−0.16	−0.45***	−0.46***
pH												−0.14	0.11
CP													0.77***

*, **, ***, statistical significances at P<0.001, P<0.01, and P<0.05, respectively; Vmax, maximum volume of GP; S, fractional rate; Lag, lag phase; Initial DM, initial dry matter (0 d, prior to the ensiling prwocess); ASH, ashes; NDF, neutral detergent fiber; HEM, hemicellulose; ADF, acid detergent fiber; CEL, cellulose; ADL, acid detergent lignin; CP, crude protein; IVDMD24, *in vitro* dry matter digestibility at 24 h; Final DM, final dry matter (DM of the silage at 40 d).

degrade the components of the NDF and non-fibrous carbohydrates of the diets, and on their efficient use to provide net production energy (Knapp *et al.*, 2014).

The use of LAB can contribute to the stability and quality of silages by promoting an increase in the lactic acid:acetic acid ratio (Guo *et al.*, 2013; Chen *et al.*, 2016; Mora de Alba *et al.*, 2018) and by limiting the growth of yeast in the silage (Tabacco *et al.*, 2011).

In this study, LD and MD of LAB improved the quality of the silages, based on the CP contents of the 60:40 O:T silages; in addition, they improved the IVDMD24, Vmax, S, and Lag of the 80:20 O:T silages. Lynch *et al.* (2012) and other authors have found that some strains and/or doses of Lactobacillus may not improve or that they may even worsen the quality of silage, as a consequence of the reduction in the concentration of lactic acid that can be used as a substrate by some lactic acid bacteria.

Finally, this study proves the relationship between IVDMD24 and GP during *in vitro* fermentation with ruminal fluid. This relation is deeply connected with the changes in the ratios of hemicellulose and cellulose caused by the use of additives during the ensiling process. However, they are not always related to the total NDF content, which may not even be affected by the use of FE or LAB.

CONCLUSIONS

This work shows the degree of specificity with which enzyme preparations and lactic acid bacteria act on the different types of cell walls of different oat and triticale ratios in a silage. The contents of cell walls (NDF) and CP, *in vitro* degradability and fermentation were better in mixtures containing a lower ratio of oat. Although the additives did not consistently reduce NDF and ADF, their inclusion did affect the hemicellulose and cellulose ratios: the 60:40 O:T silages had more hemicellulose and less cellulose, ADL, and ASH than 80:20 silages, while silages with FE had a lower cellulose content than those treated with LAB. Although the use of additives does not directly improve the ratio of NDF during the ensiling process, it can increase the ratios of hemicellulose and reduce the ratios of cellulose. Hemicellulose and cellulose could be highly correlated with the fermentation and degradability of forages in the rumen.

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