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EFFECTS OF ADDITION OF EXOGENOUS FIBROLYTIC ENZYMES ON DIGESTIBILITY AND MILK AND MEAT PRODUCTION – A SYSTEMATIC REVIEW

Deli Nazmín Tirado-González[1§], Gustavo Tirado-Estrada[1], Luis Alberto Miranda-Romero[2], Rodolfo Ramírez-Valverde[2], Sergio Ernesto Medina-Cuéllar[3], Abdelfattah Z.M. Salem[4♦]

[1]División de Estudios de Posgrado e Investigación (DEPI), Tecnológico Nacional de México (TecNM), Instituto Tecnológico El Llano Aguascalientes (ITEL), Km. 18.5 Carr. Aguascalientes-S.L.P., El Llano, Ags., México, C.P. 20330
[2]Departamento de Zootecnia, Posgrado en Producción Animal (PPA), Universidad Autónoma Chapingo, Km. 38.5 Carr. México-Texcoco, Estado de México, México, C.P. 56230
[3]Departamento de Ciencias y Artes, Universidad de Guanajuato, Campus Salamanca, Km. 3.5+1.8 Carr. Salamanca-Valle de Santiago, Guanajuato, México, C.P. 36885
[4]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, Estado de México, C.P. 50130
[§]Current address: Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP)/Centro Nacional de Investigación Disciplinaria AF (CENID AF), Km. 8.5 Carr. Ojuelos-Lagos de Moreno, Jalisco, México, C.P. 47540
[♦]Corresponding author: salem@uaeme.mx; asalem70@yahoo.com

Abstract

Exogenous fibrolytic enzymes (EFE) added to the ruminant diet can increase fiber digestibility and production efficiency. A systematic review was conducted to understand the interactions between EFE and diet on digestibility and animal performance. The database included variables from 384 experiments with EFE and 264 controls from 85 papers published since 2000 (classification criteria: 1) type of study (*in vitro*, *in situ*, *in vivo*), 2) type of ruminants (sheep, buffaloes, goats, beef and dairy cattle), 3) primary EFE activity (cellulases (Cel) or xylanases (Xyl)), 4) forage proportion (FP), 5) type of plant (TP: legumes or grasses), 6) number of ingredients in diets, and 7) application time (AT)). In over 52.85% of cases, EFE improved the degradability of dry matter (DMD), neutral and acid detergent fiber (NDFD and ADFD), *in vitro* gas production (GP), volatile fatty acids (VFA), the acetate: propionate ratio (A:P ratio), protein and fat milk, milk yield and average daily gain (ADG) (by 7.78–21.85%). Cel improved organic matter degradability (OMD), GP, VFA, milk yield, and milk protein and fat content. EFE in FP≥40% diets enhanced the ADG, and in grass-based diets increased the dry matter intake (DMI). The AT of EFE affected the DMD, NDFD, and ADFD. Significant correlations were found between the improvements of NDFD or ADFD with DMD (r>0.59), milk yield (r=0.64), and ADG (r=0.59). In conclusion, many factors interact with EFE supplementation effects, but EFE consistently enhanced the DMD, NDFD, and ADFD of ruminant diets, which are related to improvements in productive performance.

Key words: exogenous enzymes, forage proportion, ruminal dynamics, animal performance, systematic review

The inclusion of agricultural wastes as energy sources in livestock production can help to reduce the environmental pollution derived from emissions of greenhouse gases associated with extensive grain production (Knapp et al., 2014; Kholif et al., 2017). Straw is an agricultural by-product used to feed animals, but the high cell wall content can limit ruminal degradation by enzymes and microorganisms, and nutrient availability (Kholif et al., 2014; Gado et al., 2017), because of the barely hydrolyzable β-1, 4 bonds that link the monomers that form polymers of cellulose and hemicellulose, and lignin that cross-links polysaccharides and strengthens the cell wall during plant maturation (Hatfield and Fukushima, 2005; Jung et al., 2006 a, b).

Cellulases (endo-β-glucanases, exo-β-glucanases or cellobiohydrolases and β-glucosidases) and xylanases (arabinofurosidases, acetylxylan esterases, glucuronidases, β-xylosidases, and endo-β-xylanases) are enzymes that break the links in cellulose and hemicellulose to release soluble sugars (Tirado-González et al., 2016). These enzymes hydrolyze components of the cell wall to produce substrates that favor selected populations of microorganisms (Nsereko et al., 2002; Salem et al., 2015 a).

Supplementing the ruminant diet with EFE can increase the availability of energy in fibrous feed by improving ruminal fermentation and fiber and DM degradability (Alsersy et al., 2015; Salem et al., 2013, 2015 b; Vallejo et al., 2016 b), improving characteristics of productive animal performance (Tirado-González et al., 2018) such as the composition and production of milk (Gado et al., 2014; Morsy et al., 2016), average daily gain (ADG) (López-Aguirre et al., 2016 a), and feed conversion (FC) (Salem et al., 2013), and also reduces methane production (Beauchemin et al., 2008; Elghandour et al., 2016; Hernández et al., 2017 a, b) . However, responses can vary because many factors interact with the EFE effects (Tirado-González et al., 2018), including the type of enzymes in EFE products that have different specific domains, temperature, pH, active site affinity, isoelectric points, molecular weights, spectral characteristics and sugar content (Zhou et al., 2007; Alsersy et al., 2015; Vallejo et al., 2016 b), the type of diet (Elghandour et al., 2016) , the dose (Salem et al., 2015 b; López-Aguirre et al., 2016 b; Vallejo et al., 2016 b), and the application time (Wang et al., 2012).

Using previously published data collected in *in vitro, in situ* and *in vivo* studies, the present study aimed to statistically relate differences in the type of diet to the overall effects of using EFE on digestibility, fermentation patterns and milk and meat production.

Material and methods

Sampling method

A database was generated by extracting information from a non-recurring list of peer-reviewed articles published between 2000 to 2013, obtained by a literature search of Google Scholar (http://www.scholar.google.com/) and CAB Abstracts (http://www.cabi.org/) using the following combinations of words: 1) “fibrolytic enzymes”; 2) “cellulases xylanases ruminant”; 3) “exogenous fibrolytic enzymes ruminant”; 4) “exogenous fibrolytic enzymes”; and 5) “exogenous enzymes ruminant”, according to the procedures of Arriola et al. (2017) and Tirado-González et al. (2018). Criteria for identification, screening, election, and inclusion of cites were made according to the PRISMA flow of Mohar et al. (2009).

Articles included

The search procedure identified 423 non-recurring items from Google Scholar, and 226 articles from CAB Abstracts. Additionally, 50 articles published from 2013 to 2019 were selected from a random searching in Google Scholar and were also included for comparison.

Exclusion criteria

The 226 articles from CAB Abstracts gave a representative sample of all articles published from 2000 to 2013 presented in Google Scholar. Therefore, studies were excluded from the list of 226 articles (from CAB Abstracts) if it was not possible to separate the EFE effect from other factors, or if they did not address variables related to fermentation patterns, in vivo, in situ, or in vitro digestibility, or productive animal performance. Reviews or longitudinal studies and manuscripts written in languages other than English, Spanish or Portuguese were also excluded. Statistical analysis was performed in 85 non-recurrent items from the original list of articles (see Appendix A1), discussion was made using additional 44 articles.

Coding of data and study factors

Experiment definition

Articles included treatments nested into experiments that were individually coded as a record; therefore, each experiment contained at least two treatments (control without EFE supplementation, and treatments with EFE supplementation).

EFE/control differences

Treatment means were expressed relative to the control by dividing each mean by their respective control (C: without EFE; EFE/C). The percentage of change due to EFE effects was also calculated (% changes = (EFE/C-1)*100).

Classification of the experiments

According to the statistical analyses reported in each paper, means were also classified and grouped as statistically significant (Dif; differences at $P<0.05$ and $P>0.05$) positive or negative effects (Ef) related to the use of EFE.

Data were classified and grouped according to: 1) type of study: *in situ*, *in vitro*, or *in vivo*; 2) animal species used in the experiment: sheep, goats, lactating dairy cows, beef cattle, or buffaloes; 3) primary forage in the diet: grasses or legumes; 4) dietary forage proportion (FP): <50 and ≥50%; 5) primary supplemented EA: no enzyme, cellulases (different types of β-glucanases), or xylanases; 6) number of ingredients included in experimental diets; and 7) application time (AT) of EFE to the feed or diet (added to feed in a liquid form for a pre-treatment or provided as a powder in the concentrate or mixed with the diet): <1 h, 1–24 h, 25h–10 d, >10 d prior to evaluation.

Evaluated variables

The means of variables were extracted and entered into a database. For *in vitro* studies (24 and 48 h incubations), the variables were: DM, organic matter (OM), NDF, acid detergent fiber (ADF) digestibility, *in vitro* degradability and *in situ* disappearance of DM, NDF and ADF (DMD, NDFD, and ADFD, respectively). *In vitro* gas production (GP), *in vivo* and *in situ* volatile fatty acids (VFA), proportions of individual VFA, the acetate: propionate ratio (A:P), N-ammonia concentration, and ruminal pH (24 to 48 h incubations) were also extracted. For *in vivo* studies, the variables DM intake (DMI), average daily gain (ADG), feed conversion (FC: DMI/ADG), and milk production and composition were also evaluated.

Statistical analysis

Distribution tests

The distribution of the data for each variable was checked using the Shapiro-Wilk, Kolmogorov Smirnov, Cramer Von Mises, and Anderson Darling tests, through the Univariate procedure of SAS statistical software (Statistical Analysis System V. 9.2, 2013). Although the present study only included normally distributed variables, the type H structure (or spherical) of variance-covariance was tested using the *Repeated* statement and *Printe* option of the GLM procedure. The variability of studies within the factors was tested and compared using the chi-squared test of heterogeneity using the option Proc Frec of SAS.

Multiple linear and logistic models

Logistic models were used to determine the relationship between the effects of adding EFE and other factors such as the type of study, ruminant, FP, TP, number of ingredients, AT and primary EA. Forest plot graphs were obtained using the SgPlot procedure.

Analysis of variance

Statistical analyses were performed using the Glimmix (general mixed linear models) procedure of SAS statistical software, and DM, OM, NDF, ADF, DMD, NDFD, ADFD, GP, VFA, A:P, N-ammonia, pH, DMI, ADG, FC, milk yield, milk fat and protein were evaluated considering the fixed effects described in models (1), (2) and (3), and the random effect of the repetition of an experiment (Exp) nested in the articles (Art). The estimated means and standard mean error (SEM) were reported (*LsMeans/PDiff* statement). Determination and variation coefficients (R^2 and VC) were obtained through variance analysis using the GLM procedure. The three models were as follows:

$$Y = \mu + [Exp(A)]_{i(j)} + Dif_k + Ef_l + (Dif * Ef)_{kl} + \varepsilon_{ijkl} \qquad (1)$$

where:

Y = response variable,

μ = general mean,

$[Exp(A)]_{i(j)}$ = random effects of the ith experiment within the jth article,

Dif_k = effect of the kth effect reported by the author,

Ef_l = effect of the lth effect reported by the author,

$(Dif * Ef)_{kl}$ = interaction of factors Dif_k and EF_l,

$\mathcal{E}_{ijk}$ = residual effects.

$$Y=\mu+ [Exp(A)]_{i(j)} +Factor_k + \mathcal{E}_{ijk} \qquad (2)$$

where:

Y = response variable,

μ = general mean,

$[Exp(A)]_{i(j)}$ = random effects of the ith experiment within the jth article,

$Factor_k$ = effect of the kth factor (type of study, type of ruminant, type of EFE, primary EA, type of plant in diets, number of ingredients evaluated, and forage proportion),

$\mathcal{E}_{ijk}$ = residual effects.

$$Y=\mu+ [Exp(A)]_{i(j)} +AT_k + TP_l+ (AT * TP)_{kl} + \mathcal{E}_{ijklm} \qquad (3)$$

where:

Y = response variable,

μ = general mean,

$[Exp(A)]_{i(j)}$ = random effects of the ith experiment within the jth article,

AT_k = effect of the kth application time of EFE,

TP_l = effect of the lth primary type of plant indiets,

$(AT * TP)_{kl}$ = interaction between AT_k and TP_l factors,

$\mathcal{E}_{ijklm}$ = residual effects.

Pearson correlation coefficients

To assess the possible two-way linear association between pairs of normally distributed variables, Pearson correlation coefficients were computed using the CORR procedure of SAS.

Results

General description of data

The 85 sampled papers contained 407 experiments and 648 treatments (243 controls, and 343 treatments for *in vitro*, *in situ*, and *in vivo* studies, and 21 controls and 41 treatments for fiber composition studies (see Appendix A2).

Appendix A1. List of authors and variables included in meta-analysis

Author	Variables
1	2
Almaraz et al., 2010	DMD, ADFD, Propionate, pH, N-ammonia, A:P ratio
Álvarez et al., 2009	DMD, ADFD, VFA
Arriola et al., 2011	DMD, OMD, ADFD, VFA, GP, pH, N-ammonia, A:P ratio, milk yield, protein milk, fat milk, DMI
Assoumaya et al., 2007	DMD, NDFD, ADFD, VFA, DMI
Avellaneda-Cevallos et al., 2007	DMD, ADFD, VFA
Avellaneda-Cevallos et al., 2009	DMD, OMD, NDFD, ADFD, VFA, GP, pH, A:P ratio
Awawdeh and Obeidat, 2011	DMD, NDFD, ADFD, VFA, milk yield
Baah et al., 2005	DMD, OMD, ADFD, VFA, GP, N-ammonia, A:P ratio
Bala et al., 2009	DMD, NDFD, ADFD, VFA, milk yield, protein milk, DMI
Balci et al., 2007	DMD, NDFD, ADFD, ADG
Bassiouni et al., 2011	DMD
Bilik et al., 2009	Milk yield, protein milk, fat milk, DMI, ADG
Boonthep et al., 2011	NDFD, Propionate
Bowman et al., 2002	DMD, OMD, ADFD, pH
Cano et al., 2003	DMD, DMI, ADG
Carreón et al., 2010	DMD, OMD, ADFD, VFA, GP, pH, N-ammonia, A:P ratio
Chaji and Mohammadabadi, 2010	DMD, ADFD
Chung et al., 2012	OMD, pH, N-ammonia, A:P ratio, milk yield
Colombatto et al., 2003 a	DMD, NDFD, VFA
Colombatto and Beauchemin, 2003	OMD, Propionate, N-ammonia, A:P ratio, DMI
Colombatto et al., 2006	NDFD, ADFD, VFA, Propionate
Cruywagen and Zyl, 2007	DMI, ADG

De Souza et al., 2006	DMD, OMD, NDFD, Propionate, pH, A:P ratio, DMI
De Souza et al., 2008	DMD, ADFD, VFA
Dean et al., 2008	DMI, ADFD, VFA
Dehghani et al., 2012	ADFD, pH, N-ammonia
Elwakeel et al., 2007	DMD, OMD, protein milk, fat milk
Eun et al., 2006	DMD, NDFD, VFA, Propionate
Eun and Beauchemin, 2007 b	DMD, OMD, NDFD, ADFD, VFA, Propionate, pH, N-ammonia, A:P ratio
Eun and Beauchemin, 2008	DMD, ADFD, VFA, Propionate
Facchini et al., 2012	DMD, NDFD, ADFD
Flores et al., 2008	Milk yield, protein milk, fat milk, DMI
Franco et al., 2008	DMD, ADFD, GP, pH, milk yield
Gado et al., 2009	DMD, OMD, ADFD, VFA, GP, pH, N-ammonia, A:P ratio
Gado et al., 2011	DMD, OMD, NDFD, ADFD, VFA, Ph
Gallardo et al., 2010	DMD, ADFD, VFA
Giraldo et al., 2007 a	DMD, OMD, ADFD, VFA, Propionate, GP, pH, N-ammonia, A:P ratio
Giraldo et al., 2007 b	DMD, OMD, ADFD, VFA, N-ammonia, A:P ratio
Giraldo et al., 2007 c	DMD, OMD, ADFD, VFA, Propionate, N-ammonia, A:P ratio
Giraldo et al., 2008 a	DMD, OMD, ADFD, VFA, Propionate, GP, pH, N-ammonia, A:P ratio
Giraldo et al., 2008 b	DMD, OMD, NDFD, ADFD, VFA, GP, pH, A:P ratio
Gómez-Vázquez et al., 2011 a	DMD, OMD, ADFD, Propionate, DMI, ADG
Gómez-Vázquez et al., 2011 b	NDFD, ADFD, VFA
Granzin, 2005	OMD, NDFD, ADFD, VFA, GP, pH, A:P ratio, milk yield, protein milk, fat milk, DMI
Guerra et al., 2007	DMD, ADFD, GP, ADG
Holtshausen et al., 2011	DMD, ADFD, VFA, milk yield, protein milk, fat milk, DMI, ADG

Appendix A1. – contd.

1	2
Hong et al., 2003	DMD, OMD, ADFD, VFA, GP, pH, N-ammonia, A:P ratio
Hristov et al., 2000	DMD, OMD, ADFD, GP, pH, N-ammonia, A:P ratio, DMI
Hristov et al., 2008	DMD, OMD, NDFD, ADFD, GP, pH, N-ammonia, A:P ratio, DMI
Hwang et al., 2008	DMD, OMD, NDFD, ADFD, VFA, Propionate
Jililvand et al., 2008	DMD
Kalkan and Filya, 2011	DMD, OMD, NDFD, GP, Propionate, A:P ratio
Knowlton et al., 2007	DMD, ADFD, VFA, milk yield, protein milk, fat milk
Kozelov et al., 2008	DMD, ADFD, Propionate, GP, Ph
Krueger and Adesogan, 2008	DMD, ADFD, Propionate, N-ammonia, A:P ratio
Lopuszanska-Rusek and Bilik, 2011	Milk yield, protein milk, fat milk, DMI
Malik and Bandla, 2010	DMD, OMD, NDFD, ADFD, VFA, pH, DMI, ADG
Márquez et al., 2009	DMD
McAllister et al., 2000	DMD, NDFD, ADFD, VFA, GP, DMI, ADG
Miller et al., 2008 a	DMD, OMD, NDFD, ADFD, VFA, GP, pH, N-ammonia, A:P ratio, DMI, ADG
Miller et al., 2008 b	DMD, OMD, NDFD, ADFD, VFA, GP, pH, A:P ratio
Miller et al., 2008 c	Propionate, A:P ratio, milk yield, protein milk, fat milk, DMI
Moharrery et al., 2009	DMD, ADFD, VFA
Muwalla et al., 2007	DMD, NDFD, ADFD, DMI, ADG
Pinos-Rodríguez et al., 2002	DMD, OMD, NDFD, ADFD, VFA, GP, pH, A:P ratio, DMI
Pinos-Rodríguez et al., 2001	DMD, NDFD
Pinos-Rodríguez et al., 2008	DMD, OMD, ADFD, GP, N-ammonia, A:P ratio
Ranilla et al., 2008	DMD, OMD, ADFD, VFA, GP, pH, N-ammonia, A:P ratio
Reboucas et al., 2005	DMD, NDFD, ADFD, DMI

Salem et al., 2012	DMD, NDFD, ADFD, DMI
Shekar et al., 2010	DMD, NDFD, ADFD, VFA, milk yield, protein milk, fat milk, DMI
Srinivas et al., 2008	DMD, ADFD, Propionate
Sutton et al., 2002	DMD, NDFD, ADFD, VFA, milk yield, protein milk, fat milk, DMI, ADG
Tang et al., 2008	DMD, NDFD, Propionate
Tirado-Estrada et al., 2011	DMD, OMD, pH, N-ammonia, A:P ratio, DMI, ADG
Titi, 2003	Milk yield, protein milk, fat milk, DMI
Wang et al., 2002	Propionate, pH, N-ammonia
Wang et al., 2003	DMD, DMI, ADG
Wang et al., 2004	DMD, NADF, VFA, Propionate
Wang et al., 2012	DMD, OMD, N-ammonia, A:P ratio
Ware et al., 2005 a	DMD, NDFD, ADFD, VFA, DMI
Ware et al., 2005 b	DMI, ADG
Xu et al., 2011	DMD, ADFD
Yang et al., 2011	DMD, OMD, ADFD, VFA, Propionate, N-ammonia, A:P ratio
Yu et al., 2005	DMI

Disappearance and digestibility values reported in experiments performed *in vitro*, *in situ* and *in vivo*: OM, organic matter; DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; GP, gas production; VFA, volatile fatty acids; A:P, acetate:propionate ratio; DMI, dry matter intake; ADG, average daily gain.

Overall effects of EFE supplementation

The average effect of including EFE on the digestibility of diet components, fermentation patterns, and milk and meat production was variable among the articles, and thus the random effect of experiments nested in the article was considered in further analysis. Table 1 presents the average change caused by EFE in ruminal fermentation patterns, degradability, disappearance, digestibility, and animal performance in *in vitro*, *in situ* or *in vivo* experiments (according to Model (1)). The average increase in DMD was 11% for the 52.85% of cases reported by authors as significant, and 4.88% for the 23.58% of cases reported as showing non-significant positive responses. In over 69.79% of experiments, EFE improved the NDFD and ADFD (by 11.41±4.79 % and 16.55±6.35%, respectively).

Over 75.83% of the data included in this analysis showed that EFE supplementation increased the *in vitro* GP (average, 19.84±10.67%) and the concentration of VFA (average, 21.85±11.43%). In addition, 61.39% of the studies with EFE supplementation showed an increase in propionic acid concentration (12.61±7.30%), although in 35.64% of the studies it was decreased by an average of 8.26±6.23%.

In 53.75% of the studies in which EFE supplementation had a significant effect ($P<0.05$) the A:P ratio decreased by 8.89±0.10%. Furthermore, 55.06% of studies with EFE supplementation demonstrated an increase in N-ammonia concentration (average, 13.16±0.50%), while 40.45% of the studies showed a decrease (average, –7.78±4.70%). The pH increased in 30% of the studies (average 1.68±0.25%) but decreased in 53.33% of cases (average, –2.1±0.3%).

Milk production improved by 9.28±3.03% with EFE use (in 81.82% of experiments), and fat and crude protein contents also increased by 6.03±13.68% and 4.30±2.45% (in over 54.55% of experiments), respectively. The ADG was positively affected by EFE supplementation (average, 17.57±6.98%) in 67.86% of the studies.

Effects of EFE supplementation associated with other factors

The effect of using EFE was associated with other factors using logistic models (Table 2). The type of EA (proportion of Cel and Xyl) affected the response of OMD, VFA and milk yield ($P<0.02$), and tended to affect the OMD, milk protein and fat content and ADG ($P<0.08$). The TP and types of ruminant affected the responses of GP and VFA ($P<0.01$) to the use of EFE, whereas the FP affected the GP, VFA and ADG ($P<0.04$), and the AT of diets altered the effect of EFE on GP, ruminal pH and N-ammonia, milk yield and milk protein ($P<0.02$). Although, the effects of EFE on DMD, GP, VFA, A:P ratio, and propionate were similar among *in vitro*, *in situ* and *in vivo* studies ($P>0.12$), OMD and NDFD responses varied among type of studies ($P<0.01$).

The forest plot graphs represent the changes caused by EFE addition within the confidence interval (95%); averages near to the vertical line represent no significant effect (Figure 1). Although the results were highly variable, EFE had positive effects on digestibility, GP and VFA but was primarily associated with factors such as the type of ruminant, TP, FP and EA. Depending on the FP, using EFE could improve the ADG, but some types of EA can enhance milk composition and production. The variability of the data and the adverse effects on GP, N-ammonia, and milk yield were associated with the AT of EFE.

Table 1. Effects of exogenous fibrolytic enzymes (EFE) on digestibility, fermentation patterns, and animal performance, according to authors

Response variable		Type of effects reported in articles						R^2	SE	P-Value		
		when P<0.05			when P>0.05							
		media	positive	negative	positive	negative	no-effects			Dif	Effect	Dif*Effect
1		2	3	4	5	6	7	8	9	10	11	12
Digestibility[¶]												
DM	N	246	130	20	58	29	9					
	Change (%)	6.99	11	–2.70	4.88	–0.60		0.8	1.33	0.09	<0.0001	<0.0001
OM	N	57	24	2	18	11	2					
	Change (%)	8.45	10.7	–0.31	3.51	–0.27	0	0.9	2.92	0.25	0.11	0.35
NDF	N	192	80	20	54	34	4					
	Change (%)	7.44	15.20	–3.69	5.80	–3.78		0.8	1.22	0.06	<0.0001	<0.0001
ADF	N	194	115	8	43	25	3					
	Change (%)	14.09	19.7	–6	8.14	–5.64		0.7	2.26	0.008	<0.0001	0.03
Fermentation patterns												
GP	N	106	57	11	33	2	3					
	Change (%)	17.79	27.40	–26.5	6.79	–3.35		0.8	2.22	0.14	<0.0001	<0.0001
VFA	N	120	49	7	42	21	1					
	Change (%)	14.14	33.20	–44.20	8.61	–3.20		0.6	3.9	0.11	0.003	0.05
pH	N	60	5	5	13	27	10					
	Change (%)	1	2	–1.50	1.55	–2.21		0.8	0.29	0.32	0.0005	0.61
N-*ammonia*	N	89	16	13	33	23	4					
	Change (%)	2.37	13.80	–2	12.85	–11.05		0.6	1.85	0.62	0.001	0.74

Table 1 – contd.

1		2	3	4	5	6	7	8	9	10	11	12
A:P ratio	N	80	17	23	18	20	2					
	Change (%)	1.44	23.10	–8.80	8.72	–8.99		0.7	1.69	0.0002	<0.0001	<0.0001
Propionate	N	101	36	14	26	22	3					
	Change (%)	3.19	19.80	–15.70	2.66	–3.53		0.6	1.54	0.13	<0.0001	0.008
Animal performance												
DMI	N	64	13	6	26	14	5					
	Change (%)	3.28	11.8	–6	5.14	–1.64		0.7	1.44	0.31	0.006	0.15
ADG	N	28	13	0	6	7	2					
	Change (%)	13.18	21.7	0	8.61	–9.25		0.9	4.77	0.19	0.01	0.52
milk production	N	25	7	0	14	2	2					
	Change (%)	8.15	12.9	0	6.98	–2.4		0.9	4.01	0.16	0.2	0.1
milk protein	N	25	12	2	3	3	5					
	Change (%)	3.96	5.4	–0.9	1.01	–2.86		0.8	3.72	0.3	0.46	0.58
milk fat	N	25	2	2	15	4	2					
	Change (%)	3.62	25	–7.2	2.24	–2.54		0.9	3.95	<0.0001	0.005	0.03

N, number of experimental means; EFE, exogenous fibrolytic enzymes; ¶general means for degradability, disappearance and digestibility values reported in experiments performed *in vitro*, *in situ* and *in vivo*; OM, organic matter; DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; GP, gas production; VFA, volatile fatty acids; A:P, acetate:propionate ratio; DMI, dry matter intake; ADG, average daily gain; P-Value, probability value; R^2, determination coefficient; SE, standard error; Effect, type of effect reported by authors (positive, negative, no-effects); Dif, differences reported by authors (reported $P<0.05$ and $P>0.05$).

Table 2. Logistic models to associate factors with the effects of exogenous fibrolytic enzymes (EFE) on digestibility, fermentation patterns, and animal performance

Response variable (Yi)	Intercept		EA		FP		TP		Ruminant		AT		Study	
	β0	P-Value	βi	P-Value	βi	P-Value	βi	P-Value	βi	P-Value	βi	P-Value	βi	P-Value
Digestibility¶														
DM	0.8	0.0005	0.41	0.08	–0.21	0.32	0.58	0.02	0.09	0.3	0.05	0.74	0.03	0.88
OM	11.28	0.02	1.79	0.005	–0.21	0.64	–0.4	0.55	0.3	0.09	–0.33	0.32	–2.47	<0.0001
NDF	4.77	0.05	–0.07	0.81	0.17	0.46	0.26	0.35	–0.08	0.4	–0.3	0.06	–0.56	0.01
ADF	2.57	0.33	0.43	0.2	0.32	0.23	–0.02	0.95	0.08	0.45	0.26	0.25	–0.03	0.9
Fermentation patterns														
GP	0.15	0.66	0.18	0.62	1.12	0.001	0.4	0.37	0.58	0.0002	–2.51	<0.0001	–1.13	0.2
VFA	-9.5	<0.0001	0.86	0.02	1.07	<0.0001	0.89	0.005	0.44	0.01	0.18	0.52	–0.32	0.31
pH	17.19	0.001	–0.59	0.32	–0.31	0.5	–1.32	0.01	–0.51	0.13	–1.07	0.02	–0.19	0.57
N-ammonia	6.06	0.06	–0.64	0.11	0.24	0.46	–0.53	0.23	–0.28	0.07	–0.9	0.0003	–0.87	0.006
A:P ratio	2.19	0.45	–0.02	0.98	0.36	0.23	0.42	0.28	–0.03	0.91	–0.25	0.5	0.36	0.33
propionate	5.49	0.04	–0.29	0.45	–0.14	0.59	0.46	0.22	–0.16	0.38	0.06	0.82	–0.49	0.12
Animal performance														
milk yield	3.96	0.2	4.21	0.01	0.11	0.86	1.22	0.15	–0.25	0.48	–2.5	0.0004		
protein milk	2.24	0.51	2.49	0.08	0.15	0.82	–0.01	0.99	–0.52	0.14	–2.64	0.0009		
fat milk	-0.27	0.93	2.35	0.06	–0.68	0.3	0.5	0.62	0.2	0.6	–0.19	0.73		
DMI	–1.14	0.82	–0.72	0.16	–0.32	0.34	1.06	0.06	0.16	0.42	–0.24	0.43		
ADG	–12	0.31	1.42	0.07	1.22	0.04	0.18	0.86	0.3	0.33	0.16	0.75		

EA, primary enzyme activity (Cel or Xyl); FP, forage proportion; TP, type of plant; Ruminant, type of ruminant; Study, type of study; AT, application time; ¶general means for degradability, disappearance and digestibility values reported in experiments performed *in vitro*, *in situ* and *in vivo*; OM, organic matter; DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; GP, gas production; VFA, volatile fatty acids; A:P, acetate:propionate ratio; DMI, dry matter intake; ADG, average daily gain; βi, parameter value; P-Value, probability value.

Table 3. The change effects of exogenous fibrolytic enzymes (EFE) addition in ruminant diets under certain experimental conditions on ruminal digestibility and fermentation patterns

Variable	Changes (%)									
	DMD[¶]	OMD	NDFD	ADFD	GP	VFA	pH	N-*ammonia*	A:P	propionate
1	2	3	4	5	6	7	8	9	10	11
Type of study										
In vivo	4.09	3.42	6.73	8.18	0.36	16.84	–0.9	9.03	–1.95	3.06
In situ	6.55	0.25	7.94	9.67	1.17	0.97	–0.3	2.83	–2.15	2.24
In vitro	8.56	17.1	8.98	14.8	9.73	13.76	–0.8	4.84	0.42	6.34
R^2	0.79	0.88	0.68	0.58	0.66	0.59	0.74	0.59	0.41	0.21
SE	2.51	3.36	3.08	4.36	2.16	11.77	1.11	6.61	5.97	7.48
VC (%)	7.41	7.07	9.43	14.41	10.51	20.24	1.42	11.56	13.27	13.28
P-Value	0.01	<0.04	0.55	0.16	0.46	0.37	0.95	0.04	0.82	0.75
Type of ruminant										
beef steers	7.7	8.75	9.87	13.1	8.16	9.2	–1.7	12.23	0.39	–0.4
dairy cows	7.08	4.22	7.92	10.9	6.85	9.31	0.74	8.42	–1.98	3.64
sheep	3.97	1.28	6.22	8.76	6.62	15.91	–1.1	1.31	–0.27	5.9
buffaloes	11.77	13.8	3.36	2.51	17.97	1.64	–1.5	16.23	–0.2	1.88
goats	7.43	2.31	13.7	19.3	11.2	17.39		11.82		
R^2	0.78	0.88	0.69	0.59	0.66	0.59	0.74	0.58	0.41	0.21
SE	3.2	5.22	6.79	7.56	17.99	21.39	2.17	8.68	11.99	17.76
VC (%)	7.5	7.07	9.21	14.17	10.57	20.14	1.42	11.52	13.24	13.28
P-Value	0.0001	0.09	0.14	0.26	0.12	0.08	0.05	0.006	0.94	0.88
Primary EA										
Primarily Xyl	4.94	3.96	6.74	9.43	7.56	9.39	–0.2	5.97	–3.33	4.9

Primarily Cel	8.83	7.41	9.69	13.6	9.24	16.48	–1.8	5.65	3.74	1.1
R^2	0.78	0.88	0.66	0.57	0.67	0.59	0.74	0.58		
SE	1.56	1.94	2.05	3.35	6.19	6.32	8.71	4.19	3.83	5.04
VC (%)	7.5	7.2	9.69	14.62	10.48	20.14	1.41	11.5		
P-Value	0.6	0.05	0.35	0.34	0.05	0.04	0.89	0.64	0.15	0.57
Diets										
1 ingredient	9.71	11	6.22	15	9.47	21.88	1.19	-0.93	4.42	1.93
>1 ingredient	5.04	3.52	8.47	9.29	6.01	8.39	–1	7.55	–3.11	4.57
R^2	0.78	0.89	0.67	0.56	0.66	0.71	0.74	0.58	0.41	0.21
SE	2.21	3.45	2.94	4.25	8.51	8.88	1.22	5.58	4.33	5.73
VC (%)	7.51	7.07	9.58	14.69	10.51	16.87	1.42	11.44	13.18	13.19
P-Value	0.001	0.04	0.89	0.07	0.2	<0.0001	0.02	0.001	<0.0001	0.57
Forage proportion (FP)										
<20%	7.61	7.8	6.35	14.3	2.44	3.49	1.21	0.29	–0.18	2.29
≥20% and <40%	4.79	2.15	5.35	6.08	2.63	14.56	–1.3	11.09	–6.68	6.62
≥40% and <60%	4.51	5.61	9.78	9.97	16.91	38.37	–1.7	4.33	–2.84	2.14
≥60% and <80%	4.59	2.25	9.85	11.6	0.39	45.99	0.46	8.4	0.26	4.27
≥80%	26.31	25.3	7.94	19.7	6.59	1.16	–	–3.49	0.35	1.68
R^2	0.8	0.89	0.69	0.59	0.66	0.72	0.74	0.59	0.43	0.25
SE	2.92	3.23	3.6	10.41	11.28	13.12	1.24	8.68	6.11	8.43
VC (%)	7.28	7.28	9.27	14.36	10.69	16.78	1.45	11.64	13.39	13.14
P-Value	0.0001	0.21	0.61	0.13	0.0005	<0.0001	0.07	0.02	<0.0001	0.91
Type of plant (TP)										
legume-based	5.03	6.43	9.1	10.9	15.77	12.07	0.09	6.91	–2.15	1.71

Table 3 – contd.

1	2	3	4	5	6	7	8	9	10	11
grass-based	6.9	4.87	7.5	10.8	1.4	8.83	–1.3	5.33	–3.1	4.99
R^2	0.78	0.9	0.66	0.56	0.66	0.71	0.74	0.58	0.41	0.24
SE	4.84	4.16	5.86	7.6	7.61	10.48	8.77	5.05	6.23	3.49
VC (%)	7.5	6.87	9.72	14.82	10.57	16.96	1.41	11.52	13.29	13.01
P-Value	0.07	0.73	0.87	0.68	<0.0001	<0.0001	0.02	0.79	<0.0001	0.88

EA, enzyme activity; R^2, determination coefficient; VC, variation coefficient; ¶general means for degradability, disappearance and digestibility values reported in experiments performed *in vitro*, *in situ* and *in vivo*; OM, organic matter; DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; GP, gas production; VFA, volatile fatty acids; A:P, acetate:propionate ratio; DMI, dry matter intake; ADG, average daily gain; R^2, determination coefficient; VC, variation coefficient; SE, standard error; P-value, probability value.

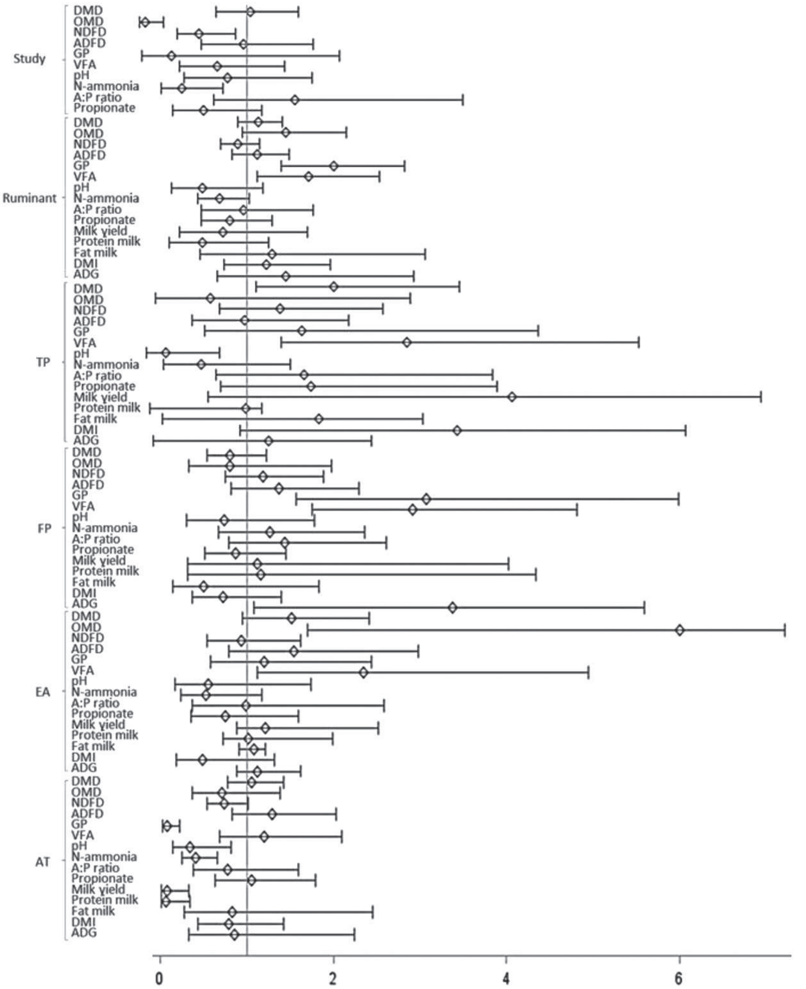

Figure 1. Forest plot graph, average effects of EFE applied under different conditions: Types of ruminant, studies (*in vitro*, *in situ* and *in vivo*), plants (TP: legume and grass-based diets), primary enzyme activity (EA: cellulases and xylanases), and application times (AT) on digestibility, fermentation patterns, and productive animal performance

The average changes for other factors on diet digestibility and productive animal performance are presented in Tables 3 and 4 (according to Model (2)). *In vitro* studies overestimated the effects of EFE on DM and OM degradability (P<0.04), primarily in experiments that evaluated one ingredient (P<0.04). The highest effects of EFE addition on DMD were observed in diets containing FP≥80% (P<0.001), but EFE had better effect on VFA and the A:P ratio in diets with FP≥40% but <80% (P<0.0005). Although EFE in FP<40% had the best effect on increasing the DMI, EFE had better effect on ADG as the FP increased over 40% (P<0.03).

Table 4. The change effects of exogenous fibrolytic enzymes (EFE) addition in ruminant diets under certain experimental conditions on productive animal performance

Item	Changes (%)				
	milk yield	protein milk	fat milk	ADG	DMI
Type of ruminant					
beef steers				19.8	1.08
dairy cows	8.54	4.68	1.86		
sheep				14.3	5.53
goats	12.99	0	1.3	15.6	7.83
R^2	0.96	0.72	0.35	0.63	0.89
SE	16.94	13.19	20.79	7.4	16.13
VC (%)	3.87	8.69	18.7	8.31	10.05
P-Value	0.9	0.66	0.99	0.42	0.15
Primary EA					
primarily xyl	4.5	1.14	-0.3	12.7	2.41
primarily cel	25.9	12.35	12.14	14.3	0.92
R^2	0.97	0.72	0.35	0.63	0.89
SE	7.6	4.61	6.95	1.77	6.47
VC (%)	3.88	8.67	18.7	8.45	10.4
P-Value	0.006	0.002	<0.001	0.07	0.73
Forage proportion (FP)					
<40%	2.78	0.91	2.08	1.4	7.57
≥40% and <60%	8.44	2.08	0.12	17.77	1.79
≥60%	9.61	7.63	4.18	25.84	2.76
R^2	0.97	0.73	0.37	0.63	0.92
SE	10.19	6.79	8.04	22.38	10.16
VC (%)	3.96	9.26	19.8	8.57	9.36
P-Value	0.37	0.19	0.74	0.03	0.008
Type of plant (TP)					
Legume-based	1.05	1.39	0.34	19.7	–4
Grass-based	10.88	4.96	2.71	12.3	2.84
R^2	0.96	0.72	0.35	0.66	0.89
SE	15.75	10.42	13.1	4.08	13.03
VC (%)	3.88	8.69	18.7	8.24	10.33
P-Value	0.29	0.54	0.71	0.37	0.02

EA, enzyme activity; ADG, average daily gain, DMI, dry matter intake; R^2, determination coefficient; VC, variation coefficient; SE, standard error; P-value, probability value.

Adding EFE to legume-based diets led to significant increases in GP and VFA and in grass-based diets had the best effect on the A:P ratio and DMI (P<0.02). Supplementing primarily Cel promoted the best improvements in OMD, GP and VFA (P<0.05), and in milk yield and milk protein and fat content (P<0.006).

Table 5. Effect of changes using fibrolytic enzymes and time of application (pre-treatment) on degradability, disappearance and digestibility (*in vitro, in situ*, and *in vivo*) of DM, NDF, and ADF

Variable	Primary enzyme activity				VC (%)	R^2	SE	Source of variation (P-values)		
	Xyl application time		Cel application time							
	0–1 h	>1 h	0–1 h	>1 h				EA	AT	EA*AT
Legume-based diets (FP≥40%)										
Change (%)										
DMD	2.4	–1.5	1.1	4.7	2.8	0.5	0.1	0.11	0.83	0.06
NDFD	9.6	4.1	2.2	18.3	5.4	0.6	0.2	0.4	0.43	0.03
ADFD	8.6	14	0.9	28.3	5.3	0.9	0.1	0.33	0.02	0.07
Grass-based diets (FP≥50%)										
Change (%)										
DMD	3.4	8.9	4.1	10.3	5.7	0.9	0.1	0.54	<0.0001	0.43
NDFD	4.2	6.3	6.5	13.9	10.8	0.7	0.2	0.08	0.07	0.12
ADFD	3.6	6.1	9.8	23.9	22	0.6	0.4	0.08	0.07	0.77

FP, forage proportion; EFE, exogenous fibrolytic enzymes; Cel, cellulases; Xyl, xylanases; DMD, dry matter digestibility; NDFD, neutral detergent fiber digestibility; ADFD, acid detergent fiber digestibility; EA, enzyme activity; AT, time of application time; VC, variation coefficient; SEM, standard error; R^2, determination coefficient.

The effects of the time of application of EFE products to the diets before feeding ruminants are presented in Table 5 (according to Model (3)). There was an interaction between the time of application of EFE and the primary type of EA supplemented to legume-based diets on NDFD (P<0.05). At least 1 h of pre-treatment with both Cel and Xyl improved the ADFD of legume-based diets (FP≥40%; P<0.02), and the DMD of grass-based diets (FP≥50%; P<0.0001) but applying EFE at least 1 h prior to feeding enhanced the NDFD and ADFD of grass-based diets (FP≥50%; P<0.07).

Correlations

Pearson correlations (Table 6) were different from zero (positively correlated) between paired DMD, NDFD, and ADFD (r>0.59, P<0.0001), DMD significantly correlated with GP and VFA (r>0.36; P<0.01), NDFD, ADFD, and DMD were positively correlated with milk yield (r>0.64; P<0.05). In addition, DMD tended to be correlated with CP milk (r=0.59; P<0.1). DMD and ADFD were positively correlated with ADG (r>0.59; P<0.05).

Table 6. Pearson correlations between digestibility, fermentation patterns, and animal performance variables

	DMD	NDFD	ADFD	GP	VFA	A:P	Propionate	CP milk	Fat milk	Milk yield	DMI
NDFD	0.65***										
ADFD	0.59***	0.71***									
GP	0.36**	0.06	–0.12								
VFA	0.36**	0.08	0.13	0.51							
A:P ratio	–0.02	–0.05	0.07	0.40**	0.51**						
Propionate	–0.01	0.19	0.16	0.34*	0.06	–0.09					
CP milk	0.59§	–0.3	-0.26	–0.51	–0.1	0.23	–0.05				
Fat milk	0.21	0.31	0.35	0.02	–0.98§	0.98§	0.03	0.72***			
Milk yield	0.76*	0.79***	0.64***	–0.75§	0.91§	0.98§	–0.21	0.77***	0.46*		
DMI	–0.16	–0.28	–0.22	0.25	0.19	0.38	0.02	0.19	0.08	0.2	
ADG	0.72***	0.43	0.59*	.	.	.	0.98*	0.31	–0.69*	–0.1	0.60§

Disappearance and digestibility values reported in experiments performed *in vitro*, *in situ* and *in vivo*: OM, organic matter; DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; GP, gas production; VFA, volatile fatty acids; A:P, acetate:propionate ratio; DMI, dry matter intake; ADG, average daily gain. Significant §P<0.10; *P<0.05; **P<0.01; ***P<0.0001.

GP tended to be negatively correlated with milk yield and the A:P ratio with milk fat (r=-0.75 and -0.98, respectively; P<0.1), but the milk yield tended to be positively correlated with VFA and the A:P ratio. (r>0.91; P<0.1). ADG was positively correlated with propionate (r=0.98; P<0.05). CP milk, milk fat and milk yield positively correlated (r>0.46; P<0.05), and ADG tended to be correlated with DMI (r=0.6; P<0.1).

Discussion

Most of the data in this review came from *in vitro* studies, mainly using sheep, and dairy and beef cattle, and with similar amounts of information on the primary activity of xylanase and cellulose enzymes. Most of the included studies used diets based on grass (73.30%) and a forage proportion ≥ 60% (63.89%). The time of enzyme application was mainly between 0 and 24 h prior to the *in vitro, in situ* or *in vivo* evaluations (see Appendix A2).

Chung et al. (2012) and Wang et al. (2012) associated the direct hydrolysis of xylose and cellulose by EFE with changes in the structure of the cell wall and the successive populations of microorganisms, ruminal microorganisms working synergistically with endogenous enzymes toward promoting the fiber digestibility of diets, and positively affecting the ruminal fermentation patterns (Yang et al., 2011; Salem et al., 2015 b). Increasing the breaking of linkages and the release of sugars in the rumen could reduce CH_4 emissions (Beauchemin et al., 2008; Knapp et al., 2014; Elghandour et al., 2016) and improve characteristics of animal production such as DMI, ADG and FC (Valdés et al., 2015).

Consistent with recent studies where Xyl and Cel improved the degradability of OM, DM, CP, NDF, and ADF, and the proportion of VFA (López-Aguirre et al., 2016 a; Vallejo et al., 2016 a; Vallejo-Hernández et al., 2018), the present analysis shows how in the majority of experiments, the use of EFE had positive effects on the digestibility of the diet components (DM, NDF, and ADF), the GP, VFA and A:P ratio, and the productive characteristics of the animals. However, the forest plot showed the high variability of EFE effects among treatments and studies, with effects primarily depending on the type of ruminant, plant, EA and FP.

Primarily cellulase enzyme products led to better responses in DM, NDF, ADF, fermentation patterns, milk protein and fat and milk yield than primarily xylanase products. This difference is significant ($P<0.05$) for VFA, production and quality of milk. The mechanism of hemicellulose removal (primarily composed of xylose, 48 to 66%, and arabinose, 10.4 to 35%) involves the breaking of some hemicellulose–cellulose bonds to increase the accessibility to cellulose (Eun and Beauchemin, 2007 a, b). Thus, any deficiency of cellulase enzyme activity and/or excess xylanase activity could adversely affect the ruminal population of microorganisms (Eun and Beauchemin, 2008; Eun et al., 2007 a). For example, Eun et al. (2007 b) observed a linear trend between increasing endoglucanase EA and the improvement of alfalfa hay and corn silage DM degradability. Also, Yu et al. (2005) reported multiple linear models for the effect of 23 enzyme products *in vitro* in ruminal fluid on the DM digestibility of oat hulls; one model indicated that 55% of the DM digestibility was attributed to cellulases and β-glucosidases, while the same model showed $r^2=0.74$ when including xylanase, endoglucanase I, endoglucanase II, ferulic acid esterase, β-glucosidase and cellulase enzyme activities.

López-Aguirre et al. (2016 b) and Vallejo et al. (2016 b) found that although in most experiments with both Cel and Xyl the ruminal degradability of fiber, DM and OM, GP and fermentation patterns improved, primarily Cel products gave the best results. Morsy et al. (2016) tested Cel and Xyl products in 21 buffaloes fed with rice straw as forage (FP=60%) and reported that the effects of supplementing primarily Cel on milk, fat, and conjugated linoleic acid yields were higher than when supplementing with Xyl. In beef steers, Salem et al. (2013) tested a primarily Cel enzymatic product, and although the EFE did not affect the DMI, Cel improved the digestibility of DM, OM, CP, NDF and ADF, the fermentation patterns, and the FC.

Differences in ruminal microbiota had been reported across the different species of ruminants according to the species, breed, and the physiological stages (Li et al., 2019, 2009; Jami and Mizrahi, 2012; Lee et al., 2012) and feeding strategies (Petri et al., 2012; Morgavi et al., 2013), affecting the potential DMD, NDFD (Petri et al., 2012; Shabar et al., 2016), related to the kinetics of GP and fermentation patterns (Miranda-Romero et al., 2020), additional studies had related changes in rumen microbial phylotypes and feed efficiency (Zhou et al., 2009; Jewell et al., 2015; Li et al., 2019).

In addition to the type of ruminant, the effects of the type of EA might depend on the type and proportion of forage included in the diet. Colombatto et al. (2003 a) conducted a multiple linear regression to determine the effect on NDF degradability of 22 enzyme products tested in ruminal fluid; at 18 h the xylanase EA was positively correlated with the improvement of alfalfa hay NDF degradability, but negatively correlated with the improvement of corn silage NDF degradability. In addition, Eun and Beauchemin (2007 a, b) assayed *in vitro* (in ruminal fluid) extracts of enzymes from *Trichoderma longibrachiatum,* reporting that the proportion of cellulases showed a coefficient of regression of 0.26 with the improvement of NDF digestibility of alfalfa, but of 0.72 with the improvement of corn silage NDF digestibility.

Pretreatment at least one hour before feeding enables the partial hydrolysis of fiber, increasing the availability of initial non-structural carbohydrates, which can change the patterns of colonization of ruminal microorganisms (Yu et al., 2005; Wang et al., 2012; Díaz et al., 2015). During the ensiling process, the addition of EFE can reduce the proportions of ADF, NDF, cellulose and hemicellulose (Alsersy et al., 2015; Gado et al., 2017; Kholif et al., 2017).

Previous studies associated improvement in NDF digestibility with better animal performance. Oba and Allen (1999, 2000 a, b, 2005) published regression models, associating each unit improvement in NDF degradability with the increase in DMI (0.168 g/d) and fat-corrected milk production (0.249 kg/d). The analysis in the present study found that EFE positively affected the NDFD, which is correlated with greater milk yield (r=0.79) and not the ADG (r =0.01) Salem et al. (2013) also reported positive EFE effects on the degradability of fiber and dietary components, fermentation patterns, and the live-weight gain and FC of beef steers.

Limitations

Some effects related to the use of enzymes in ruminants are inconsistent due mainly to enzymatic products handling, the dose, diet ingredients, time and application methods (Beauchemin et al., 2003). In many cases, the products evaluated for ruminants feed application do not contain the appropriate mixture of types of enzymes decreasing their effect on fiber digestibility (Yu et al., 2005; Eun and Beauchemin, 2007 a, 2008).

Appendix A2. Overall description of database: factors, levels, and number of records registered

Factor	Type of study				Total
	In vitro	*In vivo*	*In situ*	composition studies*	
1	2	3	4	5	6
Types of ruminant			N (records)		
beef cattle	76	45	30	–	151
cows	78	62	43	–	183
sheep	104	45	44	–	193
goats	12	6	3	–	21
buffaloes	12	2	0	–	14
without ruminal fluid[§]	24	0	0	62	86
total	306	160	120	62	648
Types of ruminant			N (animals)		
beef cattle	116	1157	78	–	1351
cows	136	935	86	–	1157
sheep	404	606	130	–	1140
goats	16	132	12	–	160
buffaloes	90	54	0	–	144
total	762	2884	306	–	3952
Primary EA			N (records)		
xylanase	95	46	50	23	214
cellulase	109	25	18	18	170
control (without EFE)	102	89	52	21	264
total	306	160	120	62	648

Appendix A2 – contd.

1	2	3	4	5	6
Type of plant (TP)			N (records)		
legume-based diets	91	38	32	12	173
grass-based diets	215	122	88	50	475
total	306	160	120	62	648
Forage proportion (FP)			N (records)		
<40%	14	74	25	24	137
≥40 and <60% and	31	34	28	4	97
≥60%	261	52	67	34	414
total	306	160	120	62	648
Enzymes application time (AT)			N (records)		
0 h	99	79	68	9	255
1–24 h	192	58	52	40	342
1 d–10 d	15	23	0	13	51
total	306	160	120	62	648

§Data included in this level of classification was not included in present study.

Supplementary Information

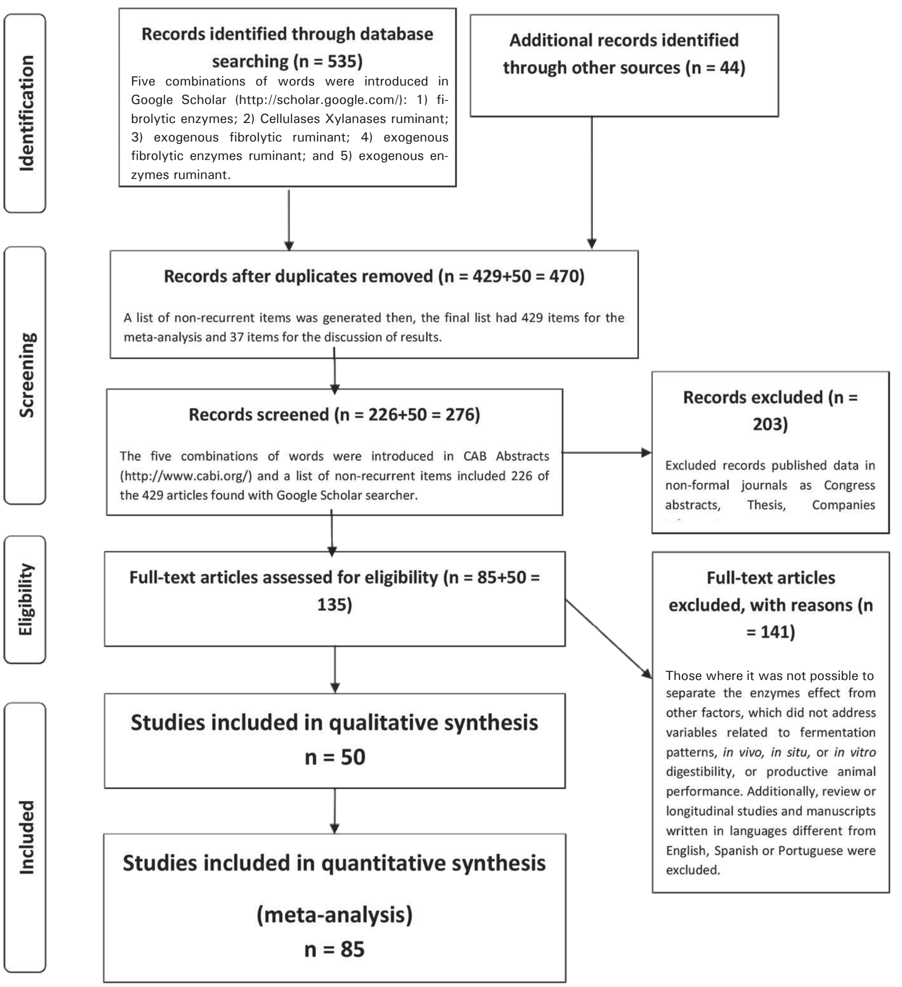

Figure Suppl. 1. Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) flow diagram (Moher et al., 2009)

In order to find products which contain specific forms of enzymes for improving the fiber degradation and combine appropriate proportions of different enzymatic activities to increase the results consistency an adequate biochemical characterization of the products is also required, considering that the optimal conditions for the enzyme activities could be different than those under enzymes are expected to act, for ruminant feed applications, the pH and temperature used in the assays should be at 39°C and pH between 5.8 and 6.8, similar to the ruminal conditions (Colombatto and Beauchemin, 2003; Tirado-González et al., 2016). Present study included random selected articles, some of them did not include information about the doses of Cel and Xyl supplemented according to the EA of products. Further systematic reviews should provide an analysis of the association between doses of EA and the degradability of DM and NDF.

Conclusions

The present systematic review showed high variability among treatments and studies. However, EFE supplementation consistently improved the NDFD, ADFD and DMD, although the type of EFE (Cel or Xyl) and the FP interacted: Xyl and Cel improved milk yield, milk protein and fat content, while adding EFE to diets with FP≥40% increased the ADG, but on grass-based diets EFE had the best effects on OMD and DMI. Since DMD, NDFD and ADFD were correlated with milk yield (r>0.64), and DMD and ADFD with ADG (r=0.59 for ADFD), the effects of EFE on digestibility were consistently associated with an improvement in milk and ADG yields.

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