Meta-analysis: effects of exogenous fibrolytic enzymes in ruminant diets

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ABSTRACT
There are unknown interactions between supplements of exogenous fibrolytic enzymes (EFE) and the cell walls of feedstuff in ruminal conditions. The quantitative effects of using EFE in ruminant diets were evaluated using meta-analysis. Records (586) were extracted from 74 journal articles from a list of published papers (2000–2012). Statistical analyses were performed considering fixed [type of forage-based diet, forage-to-concentrate ratio (F:C) ratio] and primarily enzyme activities in the EFE, and random effects [Experiment(Article)]. In dairy cows fed high-forage (F:C ≥50%), the supplementation of primarily mixtures of cellulases and xylanases (Cel:Xyl: 1:4–1:1) increased milk production and milk composition of legume-based diets, and primarily xylanases (Xyl): EFE improved those variables of grass-based diets. In F:C <50% grass-based diets, Cel:Xyl improved the average daily gain (ADG) and feed conversion [FC/DM intake (DMI)/ADG] of beef cattle. DMI of dairy cows was not affected by EFE supplementation, but EFE improved the DMI of beef cattle. EFE effects were inconsistent in sheep productive performance variables. Cellulases (Cel) and Xyl enhanced in vivo dry matter (DM) digestibility (DMD) in low-forage (F:C <50%) grass-based diets. In F:C ≥50% legume-based diets, EFE enhanced the in situ DM disappearance (ISDMD), and mainly Cel:Xyl improved the in situ neutral detergent fibre (NDF) disappearance (ISNDFD), but there were no effects in those variables in F:C ≥50% grass-based diets. Regardless of the type of ruminal liquid (RL) or forage, in F:C ≥50% diets, in vitro DM degradability (IVDMD) was improved mainly by Cel, but fibre degradability only was improved by Cel:Xyl when sheep RL was used for in vitro evaluations. Overall, EFE could improve the productive performance of dairy cows and beef cattle, but the response depends upon the proper mixture of Cel and Xyl according to the diet composition.

Abbreviations: ADF: acid detergent fiber; ADG: average daily gain; A:P: acetate:propionate ratio; BW: initial body weight; Cel:Xyl: cellulases:xylanases; DM: dry matter; DMD: in vivo dry matter digestibility; DM: dry matter intake; EA: enzyme activities; EFE: exogenous fibrolytic enzymes; F: type of forage; FC: feed conversion; F:C: forage-to-concentrate ratio; ISDMD: in situ dry matter disappearance; ISNDFD: in situ neutral deterrent fiber disappearance; IVADFD: in vitro acid detergent degradability; IVDM: in vitro dry matter degradability; IVNDFD: in vitro neutral deterrent fiber degradability; NDF: neutral deterrent fiber; VFA: in vitro volatile fat acids

1. Introduction
Supplementation with exogenous fibrolytic enzymes (EFE) is thought to enhance ruminal fermentation and to increase the degradability of forage cell walls, potentially reducing feed costs and sustaining the productive performance of ruminants; however, the underlying interactions are unknown and the effects of using EFE are highly variable. Within the rumen, EFE hydrolyse certain components of the cell wall and produce substrates that favour selected populations of microorganisms, even with low-forage diets (Beauchemin, Colombatto, Morgavi, Yang, and Rode 2004; Bedford and Cowieson 2012). In the ruminal environment, EFE can affect bacterial attachment and colonization, and microbial populations (Colombatto and Mould et al. 2003; Beauchemin, Colombatto and Morgavi 2004; De Souza et al. 2008; Wang et al. 2012), affecting in vitro (Giraldo, Carro, Ranilla and Tejido et al. 2007; Ranilla et al. 2008; Srinivas et al. 2008), in situ (Tirado-Estrada et al. 2015) and in vivo NDF digestibility.

Some models have suggested that the increase in in vitro digestibility of forage NDF is associated with a higher production (Oba and Allen 1999; Jung et al. 2004), which could reduce feed costs (Oba and Allen 2000a, 2000b; Oba and Allen 2005; Staton et al. 2007).

Previous studies have reported positive effects of using EFE as supplements for ruminants on dry matter and neutral deterrent fibre in vivo digestibility (DMD and NDFD) (Gralzin 2005; Knowlton et al. 2007; Arriola et al. 2011; Gómez-Vázquez, Mendonza-Martinez, Aranda, Pérez, Hernández and Pinos-Rodríguez 2011), reducing the costs and sustaining the productive performance of ruminants; however, the underlying interactions are unknown and the effects of using EFE are highly variable.

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total volatile fatty acids (VFA), propionic acid proportion, acetate: propionate ratio (A:P ratio) (Miller, Granzin, Elliot and Norton 2008; Gurbuz 2009; Arriola et al. 2011; Gado et al. 2011; Tirado-Estrada et al. 2015), dry matter intake (DMI) (Chung et al. 2012), milk production (Mohamed et al. 2013; Kholif and Aziz 2014) and average daily gain (ADG) (McAllister et al. 2000; Wang et al. 2003; Tirado-Estrada et al. 2011). However, in some cases, EFE did not positively influence DMD, NDFD, VFA, fermentation patterns (Sutton et al. 2002; Tití 2003; Elwakeel et al. 2007; Holtshausen et al. 2011) or animal performance (Arriola et al. 2011).

To improve the consistency of the results, it is important to know the interaction within the ruminal ecosystem, the cell walls of plants and the type of EFE (Beauchemin, Colombatto and Morgavi 2004; Meale et al. 2014). Previously, the authors have considered factors such as Cel:Xyl ratio (Eun and Beauchemin 2007b; Eun and Beauchemin 2008b), and dose (Dean et al. 2008), stability and structure of the enzymes in the EFE preparations (Morgavi et al. 2000) along with their interactions with the different types of forages (Jililvand et al. 2008). However, despite the studies and reviews of the effects of EFE supplementation, some conclusions remain unclear (Beauchemin, Colombatto and Morgavi 2004; Beauchemin, Colombatto, Morgavi, Yang, and Rode 2004; Bedford and Cowieson 2012; Tirado-González et al. 2015).

Some interfering or confounding factors (Beauchemin et al. 2003; Bala et al. 2009) can be evaluated using a meta-analysis of different studies (Eugène et al. 2004, 2008; Kholif and Aziz 2014) in order to determine the effect of EFE supplementation and the optimal situations (Desnoyers et al. 2009). Using meta-analysis, the aim of the present study was to quantify the effects of using EFE in ruminant diets with varying proportions of legumes and grasses on in vitro, in situ and in vivo digestibility, and the productive performance of lactating dairy cows, sheep, and growing beef cattle.

2. Materials and methods

2.1. Sampling method

A list of articles published between 2000 and 2012 was generated as follows: five combinations of words were introduced as the search criteria in the CabAbstracts browser: (1) ‘fibrolytic’ and ‘enzymes’; (2) ‘cellulases’, ‘xylanases’ and ‘ruminant’; (3) ‘exogenous’, ‘fibrolytic’, ‘enzymes’ and ‘ruminant’; (4) ‘exogenous’, ‘fibrolytic’ and ‘enzymes’; and (5) ‘exogenous’, ‘enzymes’ and ‘ruminant’. The search procedure identified 226 articles after a list of non-recurring items was generated. These articles were published in the Journal of Dairy Science (48), Journal of Animal Science (44), Canadian Journal of Animal Science (19), Asian-Australasian Journal of Animal Science (14), Animal Feed Science and Technology (8) and other journals (93). The final analysis was performed with 74 articles chosen from the random list after eliminating those articles which were not performed under the set conditions, or did not analyse variables or factors included in the present study experiments (see Appendices 1 and 2).

2.2. Evaluated variables

From the articles considered, the averages for numerous variables were extracted and entered into a database (see Appendices 1 and 2). For in vitro studies (24 and 48 h incubations), the variables were: dry matter (DM), NDF, acid detergent fibre (ADF) degradability (IVDMD, IVNDFD and IVADF, respectively), total VFA, propionate proportion (Gurbuz et al. 2008) and A:P ratio. For in situ studies, the variables were: disappearance after 24, 36 and 48 h incubation times for DM and NDF (ISDMD and ISNDFD). For in vivo studies, the variables were: initial body weight (BW), DMI, feed conversion (FC (DMI/ ADG)), ADG, and milk production and composition, dry matter and neutral detergent fibre digestibility (DMD and NDFD). Prior to tabulation in the database, all data were transformed into similar units of measurements to allow direct analysis within certain parameters.

2.3. Coding of data and study factors

2.3.1. Experiment definition

According to Desnoyers et al. (2009), papers included mean treatments which were individually coded. Each article contained two or more treatments (control: without EFE supplementation; treatment means: with EFE supplementation), defined as experiments.

2.3.2. Classification of the experiments

In situ, in vivo or in vitro experiments were classified according to: (1) type of study: in situ, in vitro or in vivo; (2) animal species used in the experiment: sheep, lactating dairy cows and beef cattle; (3) primary forage in the diet: grasses or legumes; (4) dietary forage-to-concentrate ratio (F:C); <50% or ≥0%; (5) type of enzyme product (Promote, Roxazyme G2, Zado, Econase, Naturarzyme, Liquicell, Novozyme, GNC, Maxicel, Nutreco, Cattle-Ase-P, etc.); (6) primary supplemented enzyme activity (EA): no enzyme (control), Cel, Xyl or a combination of both (Cel:Xyl ratio from 1.1 to 1.4); and (7) application time of EFE to the feed or diet (added to feed in a liquid form as a pre-treatment or provided as a powder in the concentrate or mixed with the diet): <1 h, 1–24 h, 25 h–10 d, >10 d prior to evaluation.

2.4. Inclusion criteria for experiments

Experiments carried out an insufficient number of times (with minimal experimental representation), or where EFE effects could not be separated from other effects were not included in the analyses. The final analysis included 74 and 586 articles: 160 in vivo studies (94 EFE treatments/66 controls), 120 in situ studies (66 EFE treatments/54 controls) and 306 in vitro studies (206 EFE treatments/100 controls) (see Appendices 1 and 2).

2.5. Categorizing of factors

To categorize the included factors and to find a general model, multiple linear regression analysis was performed (stepwise) using the Proc REG module of SAS statistical software v. 9.4 (SAS 2013). Data were subdivided to generate subgroups of studies carried out under the same conditions but with varying levels of the following factors: (1) type of study: in
situ, in vivo and in vitro; (2) type of forage: legume or grass; (3) F: C: <50% or ≥50% and (4) primary type of supplemented EA: cellulases (Cell), cellulases xylanases proportion (Cell:Xyl: 1:4 to 1:1), xylanases (Xyl) or without enzymes (control).

2.6. Statistical analysis

Statistical analyses were performed using SAS statistical software (SAS 2013). Normal distribution of the information for all variables was verified using the Shapiro-Wilk, Kolmogorov-Smirnov, Cramer Von Mises and Anderson Darling tests, using the Univariate procedure. Experiments were carried out using completely randomized statistical designs and factorial treatment arrangements, considering fixed effects and the random effects of the number of records within the paper in which they were originally reported [Record (Paper)], according to models (1 and 2) (number of replicates are indicated in tables). Significant values for the model fixed effects were obtained using the GLIMMIX procedure, the weight factor was obtained using the GLIMMIX procedure, the weight factor was number of experiments per article minus one. Correct standard errors were obtained from the adjusted means (LSMeans/pdift) using the GLIMMIX procedure. The GLM procedure was used to obtain the coefficients of variation and of determination. Least squares means were estimated (LSMEANS) and reported.

2.6.1. Productive animal performance

\[ Y = \mu + [\text{Exp(Art)}]_{ijkl} + \text{EFE}_k + F_i + FC_m + (\text{EFE} \times F)_i + (\text{EFE} \times FC)_km + (\text{F} \times FC)_lm + (\text{EFE} \times FC)_{klm} + \beta_1(x-x_l) + E_{ijklm}, \]

where \( Y \) is the average daily gain, dry matter intake, feed conversion (ADG, DMI, FC) milk protein, milk fat; \( \mu \) is the general mean; \([\text{Exp(Art)}]_{ijkl}\) is the random effect of the \( j \)th experiment within the \( i \)th article; \( \text{EFE}_k \) is the effect of the \( k \)th exogenous fibrolytic enzyme (EFE); \( F_i \) is the effect of the \( i \)th type of forage; \( FC_m \) is the effect of the \( m \)th F:C ratio; \( (\text{EFE} \times F)_i \) is the interaction between the \( i \)th EFE by the \( F \) type of forage; \( (\text{EFE} \times FC)_km \) is the interaction between the \( k \)th EFE by the \( m \)th F:C ratio; \( F \times FC \) is the interaction between the \( m \)th F:C ratio; \( \beta_1(x-x_l) \) is the effect of the covariate (initial weight, days in milk production, or the initial NDF in experimental diets); and \( E_{ijklm} \) is the experimental error.

2.6.2. In situ and in vivo DM and NDF digestibility

\[ Y = \mu + [\text{Exp(Art)}]_{ijkl} + \text{EFE}_k + RF_j + (\text{EFE} \times RF)_km + E_{ijkl}, \]

where \( Y \) is the in vivo dry matter digestibility (DMD), in vivo neutral detergent fibre digestibility (NDFD), in situ dry matter disappearance (ISDMD), in situ neutral detergent fibre disappearance (ISNDFD); \( \mu \) is the general mean; \([\text{Exp(Art)}]_{ijkl}\) is the random effect of the \( i \)th experiment within the \( j \)th article; \( \text{EFE}_k \) is the effect of the \( k \)th EFE; \( RF_j \) is the effect of the \( j \)th type of ruminal fluid; \( (\text{EFE} \times RF)_km \) is the interaction between the \( k \)th EFE by the \( m \)th type of ruminal fluid; and \( E_{ijkl} \) is the experimental error.

3. Results

3.1. Effect of supplementation with EFE on animal performance

3.1.1. Dairy cows

Table 1 shows that in low-forage diets (F:C <50%), EFE supplementation did not have positive effects on milk production and milk solid contents. EFE applied to high-forage diets (F:C ratio ≥50%) had positive effects on milk production, milk protein (1.96 kg/d and 94.44 g/d, increases, respectively) \( P = .06 \) and milk fat (83 g/d) \( P = .015 \). EFE negatively affected the DMI in all diets \( P = .003 \). However, correlation coefficients between DMI and the production of milk, fat and protein were \( r^2 = .58 \), \( r^2 = .61 \) and \( r^2 = .60 \), respectively \( P < .0001 \) (data not presented in Table 1).

| Variable                  | N  | Forage:concentrate ratio |               |               | VC (%) | \( R^2 \) | SE  |
|---------------------------|----|--------------------------|---------------|---------------|--------|----------|-----|
|                           |    | F:C ratio < 50%          | F:C ratio ≥50%|               |        |          |     |
|                           |    | Control | EFE | Control | EFE |             |     |
| Dairy cows                |    |            |    |         |    |            |     |
| BW (kg)                   | 52 | 543.9     | 548.8 | 621.7    | 612.9  |          |     |
| BW SD (kg)                | 52 | ±31.2     | ±26.5 | ±41.9    | ±47.5  |          |     |
| Days in milk production   | 52 | 98.7      | 98.7 | 72.2     | 83.4   |          |     |
| Days in milk production SD| 52 | ±15.4     | ±15.4 | ±40.4    | ±42.0  |          |     |
| DMI (kg/d)                | 52 | 20.3      | 20.1 | 21.8     | 21.2   | 3        | 0.99|
| ADG (g/d)                 | 52 | 0.5       | -0.5 | 1.1      | 1.5    | 38.4     | 0.8 |
| Milk production (kg/d)    | 52 | 28.4      | 28.1 | 33       | 34.9   | 42       | 0.99|
| Milk protein (g/d)        | 49 | 924.6     | 881.3 | 1007.5   | 1106.9 | 6.7      | 0.97|
| Milk fat (g/d)            | 49 | 1136.6    | 1101.2 | 1179.6   | 1262.6 | 6.6      | 0.98|

Table 1. Effect of using exogenous fibrolytic enzymes (EFE) in diets with high and low forage on dairy cows productive performance.

Note: \( N \), number of experiments; EFE, exogenous fibrolytic enzymes; \( F \), type of forage; F:C ratio, forage-to-concentrate ratio; BW, initial body weight; SD, standard deviation; DMI, dry matter intake; ADG, average daily gain; VC, variation coefficient; SE, standard error; \( R^2 \), determination coefficient.
3.1.2. Beef cattle

Table 2 presents data from experiments performed using diets at high- and low-forage concentration and based on grasses. The ADG increased by 0.30 kg/d ($P = .01$) in animals fed diets containing <50% grasses and treated with EFE. In low-forage diets (F:C <50%), EFE also had positive effects on feed conversion (FC) (control = 5.8 vs. EFE treatment = 5.6) ($P = .02$).

Although improvements were seen in DMI when EFE were included in diets containing ≥50% grasses (0.9 kg/d) ($P = .03$), and the simple linear regression (data not presented in tables) between DMI and ADG was $r^2 = 0.49$ ($P < .0001$), there were no positive effects on ADG and FC when EFE was used as a supplement of high-forage diets (F:C ≥50%).

3.1.3. Sheep

EFE supplementation did not have significantly positive effects on ADG and FC in both low- and high-forage diets (F:C <50% and F:C ≥50%) ($P > .2$) (Table 2). DMI was not affected by EFE supplementation ($P > .77$), but the simple linear regression between DMI and ADG was $r^2 = 0.38$ ($P < .0001$) (data not presented in tables).

### 3.2. Effect of type of enzyme activity

#### 3.2.1. Effect of using EFE with different enzymatic compositions on animal productive performance

Table 3 shows the effect of the addition of different EFE products to diets.

The addition of Cel:Xyl (from 1:4 to 11) in legume-based high-forage diets (≥50% F:C) increased milk production, fat milk and protein milk (2.3, 0.118 and 0.082 kg/d, respectively) ($P < .0001$) (data not presented in tables).

#### 3.2.2. Effect of using EFES with different enzymatic compositions on in vivo and in situ digestibility

Table 4 shows the improvements of in vivo DMD and NDFD following EFE supplementation of different diets composition. Among the rumen fluid sources, the addition of primarily Xyl and Cel EFE products to low-forage (F:C ≤50%) grass-based diets (F:C <50%) positively affected milk production (3.1 kg/d) ($P < .02$), but primarily Xyl added to grass-based diets (F:C ≥50%) positively affected milk production (3.1 kg/d) ($P = .01$), milk protein content (0.74 kg/d) ($P = .02$), and tended to positively affect meat protein content (0.11 kg/d) ($P = .09$). DMI was not affected by EFE supplementation on legume- and grass-based diets ($P > .14$).

In low-forage diets (F:C <50%) (Table 3) based on grasses, Xyl and Cel:Xyl EFE positively affected the beef cattle productive performance ($P < .02$). EFE primarily Xyl improved the ADG (50.9 g/d) and FC (0.5 units), but increasing amounts of Cel of EFE using a Cel:Xyl product had better results on the ADG (114 g/d) and FC (0.9 units) than control and Xyl treatments. All types of EFE products negatively affected the DMI of beef cattle (200–400 g/d) ($P = .02$).

A trend to improve the ADG in sheep (14.7 g/d) was observed when low-forage diets (F:C <50%) based on grasses were supplemented with EFE primarily Cel ($P = .096$). However, the use of EFE with primarily Xyl activities slightly decreased the ADG (−14.3 g/d) ($P = .096$), and both Cel and Xyl EFE products negatively affected the sheep FC (−0.6 to −0.8 units) ($P = .02$). DMI was not affected by adding EFE products to low-forage diets ($P = .74$).

### Table 2. Effect of using exogenous fibrolytic enzymes (EFE) in diets with high and low forage on beef cattle and sheep productive performance.

| Variable      | N  | F:C ratio < 50% | F:C ratio ≥50% | VC (%) | $R^2$ | $SE$ |
|---------------|----|----------------|----------------|--------|------|------|
| **Beef cattle** |    |                |                |        |      |      |
| BW (kg)       | 45 | 324.1          | 305.2          | 219.8  | 233.3|      |
| BW SD (kg)    |    | ±81.9          | ±63.8          | ±124.8 | ±112.9|      |
| DMI (kg/d)    | 45 | 8.3            | 8.1            | 8.3    | 9.4  | 6.6  |
| ADG (g/d)     | 45 | 1542.9         | 1573.3         | 1124   | 1128 | 5.3  |
| FC (DMI/ADG)  | 45 | 5.8            | 5.6            | 10.1   | 10.5 | 15.3 |
| **Sheep**     | 45 |                |                |        |      |      |
| BW (kg)       | 45 | 24.8           | 24.6           | 36.1   | 32.5 |      |
| BW SD (kg)    |    | ±7.1           | ±7.6           | ±16.0  | ±14.2|      |
| DMI (g/d)     | 45 | 1450.3         | 1533.9         | 920.9  | 971.3| 14.1 |
| ADG (g/d)     | 33 | 280.3          | 302.6          | 179    | 185.6| 13.2 |
| FC (DMI/ADG)  | 33 | 6.2            | 6.4            | 7.6    | 7    | 39   |

Source of variation (P-value) Covariate

| Source | E | F | F:C ratio | $E^*F$ | $E^*F:C$ | $E^*F:C$ | $E^*F:C$ | $E^*F:C$ |
|--------|---|---|-----------|--------|---------|---------|---------|---------|
|        |   |   |           |        |         |         |         |         |
| **Beef cattle** |     |   |           |        |         |         |         |         |
| DMI (kg/d) | 0.54 | 0.0009 | 0.03 | 0.0001 |         |         |         |         |
| ADG (g/d) | 0.05 | 0.0001 | 0.01 | 0.0001 |         |         |         |         |
| FC (DMI/ADG) | 0.3 | 0.0001 | 0.02 | 0.0001 |         |         |         |         |
| **Sheep** |     |   |           |        |         |         |         |         |
| DMI (g/d) | 0.77 | 0.0001 | 0.05 | 0.09 | 0.84 | 0.06 | 0.14 | 0.061 |
| ADG (g/d) | 0.185 | 0.0001 | 0.01 | 0.28 | 0.67 | 0.8 | 0.34 | 0.0006 |
| FC (DMI/ADG) | 0.73 | 0.02 | 0.01 | 0.73 | 0.63 | 0.22 | 0.83 | 0.0001 |

Note: $N$, number of experiments; EFE, exogenous fibrolytic enzymes; F, type of forage; F:C ratio, forage-to-concentrate ratio; BW, initial body weight; SD, standard deviation; DMI, dry matter intake; ADG, average daily gain; FC, feed conversion; VC, variation coefficient; $E$, standard error; $R^2$, determination coefficient.
diets improved the DMD (8 and 30 g/kg DM) (*P* = .02). NDFD was not affected by the addition of EFE treatments (*P* = .86).

In high-forage diets (F:C ≥50%), using Xyl EFE products in legume-based diets enhanced in situ DMD (25 g/kg DM) (*P* = .0005), but Cel:Xyl EFE supplementation primarily improved the in situ NDFD (154.2 g/kg DM) (*P* = .008). Despite the species of ruminant, EFE in grass-based diets did not affect either ISDMD or ISNDFD (*P* > .25).
3.2.3. Effect of using EFEs with different enzymatic compositions on in vitro digestibility

Table 5 presents in vitro evaluations of high-forage diets (F:C ≥50%) supplemented with EFE. Cel enzyme activities primarily supplemented either legume- or grass-based diets, increased the IVDMD \((P < .0001)\) regardless of the type of ruminal fluid used during in vitro evaluation \((P > .19)\). Among the rumen fluid sources and diets, the addition of EFE composed primarily of Cel improved the IVDMD by an average of 90 ± 30.1 g/kg DM.

Adding primarily Cel:Xyl also enhanced the DMD of grass-based diets according in vitro experiments performed with sheep ruminal fluid \((80.7 \text{ g/kg DM})\); however, this improvement was not consistent with other rumen fluids.

IVNDFD and IVADFD were not affected by EFE supplementation \((P > .17)\) in legume-based diets \((F:C ≥ 50%)\), and in grass-based diets, although EFE with primarily Cel:Xyl tended to improve the IVNDFD and IVADFD using ruminal liquid from sheep \((83.1 \text{ and } 86.9 \text{ g/kg DM})\), EFE had negative effects on IVNDFD and IVADFD evaluated with dairy cows \((-76.8 ± 40 \text{ and } -60.1 ± 109.6 \text{ g/kg DM, respectively})\) and in beef cattle ruminal fluids \((-137.9 ± 53.2 \text{ and } -49.2 ± 47.7 \text{ g/kg DM, respectively})\) \((P < .07)\).

The A:P ratio was not affected when EFE supplemented diets composed primarily of grasses \((P > .25)\), there were also negative or null effects related to EFE use on in vitro VFA. EFE treatments comprised primarily of Xyl and Cel had less VFA than control \((P = 0.0001)\) in ruminal fluid from dairy cows \((-9.6 ± 8.6 \text{ mM/100 mM})\), beef cattle \((-11 ± 28.6 \text{ mM/100 mM})\) and sheep \((-2.5 ± 5.5 \text{ mM/100 mM})\).

4. Discussion

4.1. Effect of supplementation with EFE on animal performance

We analysed the productive performance separately in dairy cows, beef cattle and sheep fed with four different diets: <50% and ≥50% of forage content in grass- or legume-based

### Table 5. Effect of fibrolytic enzyme type on in vitro digestibility in diets containing high-forage contents.

| Variable | Enzymatic activity (EFE) |
|----------|--------------------------|
|          | Cel | Cel:Xyl | Xyl | Control |
| F:C ratio ≥50%. Legume-based diets | | | | |
| Dairy cows, beef cattle, sheep | | | | |
| IVDMD (g/kg DM) | 596.8 | 466.1 | 493.4 | 477.9 |
| Dairy cows | | | | |
| IVNDFD (g/kg DM) | 305.6 | 280.8 | 334.4 | 346.6 |
| IVADFD (g/kg DM) | 189 | 271.5 | 290.1 | 287.3 |
| Beef cattle | | | | |
| IVNDFD (g/kg DM) | 239.7 | 319.5 | 407 | 255.1 |
| Sheep | | | | |
| IVNDFD (g/kg DM) | 455.8 | 480.1 | 385.7 | 414.3 |
| IVADFD (g/kg DM) | 378.5 | 408.5 | 278.5 | 313 |
| F:C ratio ≥50%. Grass-based diets | | | | |
| Dairy cows | | | | |
| IVDMD (g/kg DM) | 484.7 | 306.3 | 470.2 | 435.9 |
| IVNDFD (g/kg DM) | 249.6 | 267.7 | 342.1 | 363.3 |
| IVADFD (g/kg DM) | 151.9 | | 306.9 | 290.1 |
| In vitro VFA (mM/100 mM) | 99.8 | | 112 | 115.5 |
| A:P ratio (in vitro) | 1.9 | | 2 | 1.9 |
| Beef cattle | | | | |
| IVDMD (g/kg DM) | 592.3 | 359.1 | 508.1 | 503.5 |
| IVNDFD (g/kg DM) | 336.7 | 372.6 | 267.9 | 463.6 |
| IVADFD (g/kg DM) | 107.5 | | 120.4 | 135.9 |
| In vitro VFA (mM/100 mM) | 119 | | 78.6 | 109.8 |
| Sheep | | | | |
| IVDMD (g/kg DM) | 594.8 | 572 | 522.3 | 491.3 |
| IVNDFD (g/kg DM) | 425.4 | 486.3 | 419.2 | 403.2 |
| IVADFD (g/kg DM) | 346.4 | 415.1 | 280.6 | 328.2 |
| In vitro VFA (mM/100 mM) | 36.3 | | 44.1 | 42.7 |
| A:P ratio (in vitro) | 2.6 | | 2.4 | 2.5 |

### Note:

N, number of experiments; EFE, exogenous fibrolytic enzymes; Cel, primarily cellulases; Xyl, primarily xylanases; Cel:Xyl, mixture of cellulases:xylanases (1:4 to 1:1); F:C, forage-to-concentrate ratio; IVDMD, in vitro dry matter digestibility; IVNDFD, in vitro neutral detergent fiber digestibility; IVADFD, in vitro acid detergent fiber digestibility; VFA, volatile fat acids; A:P ratio, acetate:propionate ratio; TR, type of ruminant; VC, variation coefficient; SE, standard error; \(R^2\), determination coefficient.
diets. The proportions and populations of ruminal bacteria vary according to the type of diet (Petri et al. 2013; Zhao et al. 2014), stage of production (Li et al. 2012) and type of ruminant (Lee et al. 2012).

4.1. Dairy cows

EFE supplementation increased the production of milk and milk solids with high-forage-based diets. These results agree with studies published subsequently to those included in our meta-analysis (Kholf and Aziz 2014). Mohamed et al. (2013) found that the use of a preparation with primarily Xyl activity increased milk production by 1.5 kg/d (3.8%). It might be possible to increase the amount of forage in the diets of dairy cows if it is treated with fibrolytic enzymes. The use of EFEs as a supplement for ruminants could increase the digestible energy of high-fibre and forage-based diets and reduce the amount of feed required per unit of milk or live weight (Meale et al. 2014).

Maximizing the amount of forage included in the diet not only might reduce feed costs (Oba and Allen 2005; Mendoza et al. 2014), but might also have beneficial effects on the health and welfare of dairy cows. For example, including more forage in diets increases the diversity of rumen microorganisms up to 3.45 times, and reduces the potential for ruminal acidosis caused by *Acetitomaculum*, *Lactobacillus*, *Prevotella* and *Streptococcus* (Petri et al. 2013). Higher forage diets also reduce the occurrence of abnormal metabolites in the rumen (Saleem et al. 2012; 2013).

4.1.2. Beef cattle

The ADG increased in beef cattle fed low-forage (<50%) grass-based diets treated with EFE. In similar diets, Gómez-Vázquez, Mendonza-Martínez, Aranda, Pérez, Hernández and Pinos-Rodríguez (2011) reported improvements of 0.31 kg/d in ADG (70.4%), 0.12 units in DMD (16.6%) and 4.1 units in NDFD (6.2%) in beef cattle in feedlots with use of an enzyme preparation; similarly, Balci et al. (2007) also found an increase in ADG of 0.38 kg/d.

In beef cattle experiments, DMI improvements could be related to how enzymes act to increase the availability of fibre (Bradford and Allen 2004). Using EFE as supplement in high-grain diets can contribute to breaking seed hulls which contain an average of 96% cellulose; EFE acts not only through the direct hydrolysis of cellulose and xyllose links, but also by changing the structure of cell walls and the successive populations of microorganisms (Yu et al. 2005; Giraldo, Ranilla, Tejidio and Carro 2007; Giraldo, Tejidio, Ranilla and Carro 2007; Wang et al. 2012; Vyver and Cruywagen 2013).

4.1.3. Sheep

Although in the present analysis we found some negative and inconsistent effects because of using EFE in diets for sheep, some studies have demonstrated improvements in ADG and FC following EFE supplementation related to an increase in in vivo DMD and increases in the proportions of propionic acid and VFA (McAllister et al. 2000; Miller, Granzin, Eliot and Norton 2008; Gado et al. 2011). Tirado-Estrada et al. (2011) found similar results when using EFE in diets with more than 56% corn stover.

4.2. Effect of supplementation with EFE on DMI

The results of present meta-analysis suggest that, although EFE had negative of null effects on DMI of dairy cows and sheep, DMI was positively correlated with the production of milk, fat and protein, and sheep ADG ($r^2 = 0.58$, $r^2 = 0.61$ and $r^2 = 0.60$, and $r^2 = 0.39$, respectively). In high-grain diets (F:C ≥50%), EFE supplementation improved the beef cattle DMI, which was also positively correlated with ADG ($r^2 = 0.48$). The correct selection of the type and application of EFE could contribute to improve DMI by enhancing DM and NDF digestibility. Improving in vitro NDF digestibility of feedstuff should be reflected in the increment of passage rate, dry matter intake and yield of fat corrected milk (Oba and Allen 1999, 2005; Jung et al. 2004).

EFE effects on DMD and NDFD depend upon the enzyme-substrate specificity. Before, authors have considered the DMD improvement (Faramarzi-Garmroodi et al. 2013) or positive changes in patterns of fermentation (Yang et al. 2011) to make the selection of EFE products; nevertheless, some authors have hypothesized that NDFD and ADFD improvements should be considered in a proper EFE selection (Phakchoed et al. 2013). Independently from VFA, DMD or NDFD improvements, certain types of enzyme preparations change the structure of cell walls of some forages (Vyver and Cruywagen 2013), enhance colonization of the substrate by bacteria (De Souza et al. 2008; Mao et al. 2013) and promote the activity of certain endogenous enzymes (Colombatto and Mould et al. 2003; De Souza, Figueiredo and Berchielli et al. 2006), which could change the passage rate (De Souza, Figueiredo and Tere-sinha et al. 2006) and therefore the DMI (Balci et al. 2007; Arriola et al. 2011).

4.3. Effect of type of enzyme activity

4.3.1. Effect of cellulases:xylanases proportion on dry matter and neutral detergent fibre digestibility

Multi-enzyme cocktails may work better than extracts of almost pure enzymes (Yu et al. 2005); the correct mixture of xylanases and cellulases improves glucose release, first by removing the xylose (Eun and Beauchemin 2007b), which increases the accessibility to cellulose (Grabber et al. 2002).

Although the excess of Cel in EFE could limit the access of the enzyme to the hydrolysable portion of carbohydrates and reduce microbial adherence (Morgavi et al. 2001; Wang et al. 2004), previous studies suggest that the deficiency of Cel activities is the main limitation for the release of reducing sugars, and the excess of Xyl activities could also adversely affect the ruminal microorganism population (Eun et al. 2007a, 2007b; Eun and Beauchemin 2008a).

It seems that there is a relationship between the activity of certain types of cellulases (endoglucanases, exoglucanases and β-glucosidases) or the combination of Cel:Xyl, and the improvement of alfalfa or corn silage IVNDFD, according to assays with mixtures of cellulases:xylanases (ranging from 1:10 to 1:5:1) (Eun et al. 2007b; Eun and Beauchemin 2007b; Eun and Beauchemin 2008b).

In many cases, the products evaluated for ruminant feed applications do not contain the appropriate mixture of
enzymes, which compromises the consistency of the results. However, supplementing the correct dose and EFE product depends on the type of diet (Tirado-González et al. 2015).

For example, Eun and Beauchemin (2007b) analysed the effect on in vitro DM and NDF degradability of enzyme activities of extracts from T. longibrachiatum, regression models showed that the units of enzyme activities of Cel were linearly associated with alfalfa IVNDFD (r=0.26), and the improvement of corn silage IVNDFD (r=0.72). Colombatto and Morgavi et al. (2003) also performed an in vitro study with 22 EFE products; using multiple linear regression modelling, they found that Xyl activities were positively correlated with an improvement in alfalfa hay IVNDFD and negatively correlated with an improvement in corn silage IVNDFD.

Cel:Xyl balance in EFE should be different for supplementing grass or legume-based diets; thus, optimal doses would vary according to EFE composition (Fortes et al. 2010). EFE for legume-based diets could contain more Xyl than EFE for grass-based diets. Yang et al. (2011) evaluated 26 enzyme products in alfalfa forage; after the first selection, they choose EFE including 1:7.4 and 1:2.3 endoglucanase:xylanase ratios; EFE with a 1:7.4 endoglucanase:xylanase ratio increased the IVNDFD and IVADF of alfalfa hay, while a ratio of 1:2.3 did not affect the digestibility. Moreover, Yu et al. (2005) reported a multiple linear regression model ($r^2 = 0.74$) that related the IVDMD of oat hulls with the activities of Xyl, two types of endoglucanases, ferulic acid esterases, β-glucosidases and Cel, but 55% of the DMD was explained by the activity of Cel and β-glucosidases of an enzymatic product with 1024 units of Cel and 4096 units of Xyl (Cel:Xyl = 0.25:1).

The present meta-analysis shows some positive results on animal productive performance, and in vivo, in situ and in vitro DM digestibility, mainly due to adding primarily Cel EFE. Although generally there were negative or null effects of EFE on in vivo, in situ and in vitro NDF and ADF digestibility. Eun et al. (2007b) observed that the optimum Cel:Xyl ratio for improving alfalfa hay and corn silage IVDMD ranged from 1:4 to 1:2, while Cel:Xyl mixtures ranging from 1:10 to 1:1.6 increased NDF degradability in tests including ruminal fluid (Eun et al. 2007a), suggesting that mixtures of Cel:Xyl affect in a different manner the fibre and total DM degradability.

4. Conclusions

The use of fibrolytic enzymes could allow increments in the amount of forage in the diets of dairy cows without compromising their productive performance, EFE treatments applied in high-forage-based diets increased milk production, and its protein and fat contents by an average of 1.96, 0.99 and 0.83 kg/d, respectively. For beef cattle experiments, EFE supplementation to low-forage diets increased the ADG by 0.30 kg/d. However, overall, the effects of adding EFE to diets offered to sheep were inconsistent. EFE supplementation did not affect DMI in dairy cows, but EFE improved the DMI in beef cattle. However, DMI was positively correlated with the production of milk, fat and protein, and sheep and cattle ADG ($r^2 = 0.58$, $r^2 = 0.61$ and $r^2 = 0.60$, and $r^2 = 0.39$ and $r^2 = 0.48$, respectively). In high-forage legume-based diets (F:C ≥50%), primarily Cel:Xyl (from 1:4 to 1:1) increased milk production and its fat and protein content (2.3, 0.118 and 0.083 kg/d, respectively), and Xyl EFE had similar results in cows fed with F:C ≥50% grass-based diets (3.10, 0.11 and 0.13 kg/d, respectively). In low-forage diets (F:C <50%) based on grasses, Cel:Xyl EFE supplementation had the best results on the ADG (114 g/d) and FC (0.9 units) of beef cattle, and Cel EFE supplementation tended to improve the ADG of sheep (14.7 g/d). EFE could improve sheep DMI but Xyl negatively affected FC of sheep fed high-grain (F:C <50%) diets. Primarily Xyl and Cel activities in low-forage (F:C <50%) grass-based diets improved the DMD (8 and 30 g/kg DM), but NDFD was not affected by the addition of EFE treatments. In high-forage diets (F:C ≥50%), Xyl enzyme activities on legume-based diets enhanced ISDMD (25 g/kg DM), but Cel:Xyl supplementation improved ISNDFD (154.2 g/kg DM); however, in grass-based diets, ISDMD and ISNDFD were not affected by the addition of EFE. Among different types of ruminal fluid, IVDMD was improved by the addition of primarily Cel EFE in either legume- or grass-based diets (average, 90 ± 30.1 g/kg DM) (F:C ≥50%). Regardless of the diet base, the proportions of Cel, Xyl and Cel:Xyl of EFE composition considered in this meta-analysis had negative or null effects on IVNDFD, IVADF, A:P ratio and total VFA in in vitro studies carried out with dairy cows or beef cattle ruminal liquid (RL). However, fibre in vitro degradability was improved by using Cel and Cel:Xyl in evaluations with sheep RL. However, fibre digestibility could be enhanced by supplementing the correct Cel:Xyl balance. The present meta-analysis suggests that EFE supplementation could improve dairy cows, beef cattle productive performance, DMD, ISDMD and IVDMD; however, the consistency of the results depends on the proper selection of Cel:Xyl balance in enzymatic products, according to the diet composition.

Acknowledgments

The present study was designed and directed by Luis Alberto Miranda Romero, Ph.D., and Gustavo Tirado Estrada, Ph. D., and supervised by Rodolfo Ramirez Valverde, Ph.D. All co-authors were closely involved in the collection, analysis and interpretation of data, as well as in the writing and decision to submit the present article for publication.

Disclosure statement

No potential conflict of interest was reported by the authors.

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## Appendix 1. References used for meta-analysis.

| N  | Refs.                  | N  | Refs.                  | N  | Refs.                  |
|----|------------------------|----|------------------------|----|------------------------|
| 1  | Hristov et al. (2000)  | 26 | Giraldo, Carro, Ranilla, Tejido and Mohamed (2007) | 51 | Avellaneda-Cevallos et al. (2009) |
| 2  | McAllister et al. (2000)| 27 | Giraldo, Ranilla, Tejido and Carro (2007)     | 52 | Bilik et al. (2009)       |
| 3  | Pinos-Rodríguez et al. (2001)| 28 | Giraldo, Tejido, Ranilla and Carro (2007)        | 53 | Eun et al. (2009)         |
| 4  | Bowman et al. (2002)   | 29 | Giraldo, Carro, Ranilla and Tejido (2007)        | 54 | Márquez et al. (2009)    |
| 5  | Pinos-Rodríguez et al. (2002)| 30 | Guerra et al. (2007)     | 55 | Moharrery et al. (2009)  |
| 6  | Sutton et al. (2002)   | 31 | Knowledge et al. (2007)  | 56 | Almaraz et al. (2010)    |
| 7  | Wang et al. (2002)     | 32 | Muwalla et al. (2007)    | 57 | Carreón et al. (2010)    |
| 8  | Colombatto and Morgavi et al. (2003)| 33 | Pinos-Rodríguez et al. (2007) | 58 | Chaji and Mohammadabadi, (2010) |
| 9  | Colombatto and Mould et al. (2003)| 34 | De Souza et al. (2008)   | 59 | Gallardo et al. (2010)   |
| 10 | Titi (2003)            | 35 | Dean et al. (2008)       | 60 | Malik and Bandla (2010)  |
| 11 | Wang et al. (2003)     | 36 | Eun and Beauchemin (2008a) | 61 | Arriola et al. (2011)    |
| 12 | Wang et al. (2004)     | 37 | Franco et al. (2008)     | 62 | Awawdeh and Obeidat, (2011) |
| 13 | Baah et al. (2005)     | 38 | Giraldo, Tejido, Ranilla, Ramos and Carro (2008) | 63 | Bassiouni et al. (2011)  |
| 14 | Granzin (2005)         | 39 | Giraldo, Tejido, Ranilla, and Carro (2008)     | 64 | Gado et al. (2011)       |
| 15 | Ware, Torreterra and Zinn (2005)| 40 | Hristov et al. (2008)   | 65 | Gómez-Vázquez, Mendonza-Martinez, Aranda, Pérez, Hernández and Pinos-Rodríguez et al. (2011) |
| 16 | Ware, Calderón, Corona and Zinn (2005)| 41 | Hwang et al. (2008)     | 66 | Gómez-Vázquez, Mendonza-Martinez and Pinos-Rodríguez (2011) |
| 17 | Yu et al. (2005)       | 42 | Jilivand et al. (2008)   | 67 | Holtshausen et al. (2011) |
| 18 | Colombatto et al. (2006) | 43 | Kozlov et al. (2008)     | 68 | Lopuszanska and Bilik (2011) |
| 19 | Assoumaya et al. (2007) | 44 | Krueger and Adesogan (2008) | 69 | Tirado-Strada et al. (2011) |
| 20 | Avellaneda-Cevallos et al. (2007) | 45 | Miller et al. (2008a)    | 70 | Yang et al. (2011)       |
| 21 | Balci et al. (2007)    | 46 | Miller et al. (2008b)    | 71 | Chung et al. (2012)      |
| 22 | Cruywagen and Zyl, (2007) | 47 | Miller, Granzin, Elliot and Norton (2008)     | 72 | Facchini et al. (2012)   |
| 23 | Elwakeel et al. (2007) | 48 | Ranilla et al. (2008)    | 73 | Salem et al. (2012)      |
| 24 | Eun et al. (2007b)     | 49 | Srinivas et al. (2008)   | 74 | Wang et al. (2012)       |
| 25 | Eun and Beauchemin (2007a) | 50 | Alvarez et al. (2009)    |      |                        |

Note: N, reference registration number; Refs., references.
Appendix 2. References and variables in the database of effects of EFE on in vivo, in situ and in vitro digestibility, and the productive performance of lactating dairy cows, sheep and growing beef cattle.

| Variables | Reference registration number (N) |
|-----------|----------------------------------|
| **Table 1** |  |
| Dairy cows |  |
| Days in milk production, milk production, fat milk, protein milk, DMI, ADG | 6, 10, 14, 23, 31, 47, 52, 61, 67, 68, 71 |
| Beef cattle |  |
| DMI, ADG, FC | 11, 15, 16, 21, 65 |
| Sheep |  |
| BW, DMI, ADG, FC | 2, 5, 19, 22, 32, 46, 56, 62, 64, 69, 73 |
| ADG, FC | 2, 22, 46, 56, 62, 64, 69 |
| **Tables 2 and 3** |  |
| F:C ratio ≥50%. Legumes forage-based diets |  |
| Dairy cows |  |
| DMI, milk production, fat milk, protein milk, ADG | 23, 61, 67, 71 |
| F:C ratio <50%. Grasses forage-based diets |  |
| Beef cattle and sheep |  |
| DMI, ADG, FC | 11, 16, 46, 56, 73, 32 |
| F:C ratio ≥50%. Grasses forage-based diets |  |
| Beef cattle and sheep |  |
| DMI | 5, 15, 19, 21, 22, 62, 64, 65, 69 |
| ADG, FC | 15, 21, 22, 64, 65, 69 |
| Dairy cows |  |
| DMI, ADG, milk production, fat milk, protein milk | 10, 47, 52, 68 |
| **Table 4** |  |
| F:C ratio <50%. Grasses forage-based diets |  |
| DMD, NDF | 1, 5, 31, 40, 45, 46, 64, 73 |
| F:C ratio ≥50%. Legumes forage-based diets |  |
| ISDMD | 2, 5, 13, 30, 33, 42, 59, 63, 67 |
| ISNDFD | 2, 5, 30, 33, 59, 67 |
| F:C ratio ≥50%. Grasses forage-based diets |  |
| ISDMD | 5, 12, 13, 19, 34, 37, 38, 50, 51, 59, 63, 67 |
| ISNDFD | 5, 13, 19, 34, 37, 38, 50, 51, 59, 67 |
| **Table 5** |  |
| F:C ratio ≥50%. Legumes forage-based diets |  |
| IVDMD | 3, 9, 17, 18, 23, 25, 38, 43, 49, 54, 55, 57, 67, 70, 74 |
| IVDNDFD | 3, 9, 23, 25, 24, 38, 43, 54, 55, 66, 67, 70, 74 |
| IVADFA | 9, 24, 25, 26, 27, 28, 29, 35, 36, 38, 48, 51, 53, 67 |
| F:C ratio ≥50%. Grasses forage-based diets |  |
| IVDMD | 3, 4, 9, 12, 17, 18, 20, 27, 28, 29, 36, 39, 41, 48, 49, 58, 59, 60, 66, 67, 70, 72, 74 |
| IVDNDFD | 4, 9, 20, 27, 28, 29, 35, 36, 39, 44, 48, 59, 60, 67, 70, 72 |
| VFA in vitro | 7, 12, 20, 26, 27, 28, 29, 36, 39, 41, 48, 60, 70, 72, 74 |
| A:P ratio | 7, 27, 29, 36, 39, 41, 48, 70, 72 |

Note: F:C ratio, forage-to-concentrate ratio; DMI, dry matter intake; ADG, average daily gain; FC, feed conversion (DMI/ADG); DMD, in vivo dry matter digestibility; NDFD, in vivo neutral detergent fiber digestibility; ISDMD, in situ dry matter disappearance; ISNDFD, in situ neutral detergent fiber disappearance; IVDMD, in vitro dry matter digestibility; IVNDFD, in vitro neutral detergent fiber digestibility; IVADFA, in vitro acid detergent fiber digestibility; VFA, volatile fat acids, A:P ratio, acetate:propionate ratio.