A meta-analysis on the effect of dietary application of exogenous fibrolytic enzymes on the performance of dairy cows

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ABSTRACT

The aim of this study was to use meta-analytical methods to estimate effects of adding exogenous fibrolytic enzymes (EFE) to dairy cow diets on their performance and to determine which factors affect the response. Fifteen studies with 17 experiments and 36 observations met the study selection criteria for inclusion in the meta-analysis. The effects were compared by using random-effect models to examine the raw mean difference (RMD) and standardized mean difference between EFE and control treatments after both were weighted with the inverse of the study variances. Heterogeneity sources evaluated by meta-regression included experimental duration, EFE type and application rate, form (liquid or solid), and method (application to the forage, concentrate, or total mixed ration). Only the cellulase-xylanase (C-X) enzymes had a substantial number of observations ($n = 13$ studies). Application of EFE, overall, did not affect dry matter intake, feed efficiency but tended to increase total-tract dry matter digestibility and neutral detergent fiber digestibility (NDFD) by relatively small amounts (1.36 and 2.30%, respectively, or <0.31 standard deviation units). Application of EFE increased yields of milk (0.83 kg/d), 3.5% fat-corrected milk (0.55 kg/d), milk protein (0.03 kg/d), and milk lactose (0.05 kg/d) by moderate to small amounts (<0.30 standard deviation units). Low heterogeneity (I^2 statistic $<25\%$) was present for yields and concentrations of milk fat and protein and lactose yield. Moderate heterogeneity ($I^2 = 25$ to 50%) was detected for dry matter intake, milk yield, 3.5% fat-corrected milk, and feed efficiency (kg of milk/kg of dry matter intake), whereas high heterogeneity ($I^2 > 50\%$) was detected for total-tract dry matter digestibility and NDFD. Milk production responses were higher for

the C-X enzymes (RMD = 1.04 kg/d; 95% confidence interval: 0.33 to 1.74), but were still only moderate, about 0.35 standardized mean difference. A 24% numerical increase in the RMD resulting from examining only C-X enzymes instead of all enzymes (RMD = 1.04 vs. 0.83 kg/d) suggests that had more studies met the inclusion criteria, the C-X enzymes would have statistically increased the milk response relative to that for all enzymes. Increasing the EFE application rate had no effect on performance measures. Application of EFE to the total mixed ration improved only milk protein concentration, and application to the forage or concentrate had no effect. Applying EFE tended to increase dry matter digestibility and NDFD and increased milk yield by relatively small amounts, reflecting the variable response among EFE types.

Key words: fibrolytic enzymes, cellulase, digestibility, xylanase, fiber

INTRODUCTION

Several studies have examined the efficacy of using exogenous fibrolytic enzymes (EFE) to improve milk production by lactating dairy cows. Milk production was increased by dietary application of EFE in some studies (Rode et al., 1999; Kung et al., 2000; Yang et al., 2000) but not others (Beauchemin et al., 2000; Kung et al., 2002; Sutton et al., 2003). Also, EFE application improved DM digestibility in some studies (Rode et al., 1999; Yang et al., 2000; Arriola et al., 2011) but not in others (Lewis et al., 1999; Weiss et al., 2011). Notable increases in feed efficiency were reported in some studies (Lewis et al., 1999; Klingerman et al., 2009; Arriola et al., 2011) but not in others (Schingoethe et al., 1999; Rode et al., 1999; Kung et al., 2002). This inconsistency in response has been attributed to differences in enzyme activity, application rate and composition, stage of lactation of dairy cows, mode and time of EFE delivery, ruminal microbial activity, ruminal EFE stability, EFE-feed specificity, and the portion of the diet

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to which EFE are applied (Beauchemin et al., 2004; Adesogan, 2005). Additional factors that may explain the variability include using experiments with insufficient statistical power, inappropriate experimental designs and or durations, inappropriate enzyme choices, as well as use of inappropriate measures of enzyme activity and misleading enzyme designations (Adesogan et al., 2014).

Although there have been several excellent reviews on effects of EFE application on forage quality and animal performance (McAllister et al., 2001; Beauchemin et al., 2003; Meale et al., 2014), which provide a synopsis of the literature, they only provided qualitative summaries. To our knowledge, no study has summarized published responses to EFE application to dairy cow diets using a statistically robust approach such as meta-analysis. Meta-analyses are a more rigorous alternative to narrative discussions of research studies (Glass, 1976). Methods for applying meta-analysis in animal and veterinary science have been described (Lean et al., 2009).

We hypothesized that across studies, EFE application would improve DM and NDF digestibility and hence improve the performance of dairy cows. The objective of this paper was to review critically and summarize published research studies on effects of dietary treatment with EFE on diet digestibility and the performance of lactating dairy cows. A second objective was to examine the existence of heterogeneity and publication bias among the studies.

MATERIALS AND METHODS

Relevant papers were identified by searching for peer-reviewed manuscripts that were published in English using online manuscript retrieval databases [Web of Science (<https://login.webofknowledge.com>), PubMed (<https://www.ncbi.nlm.nih.gov/pubmed>), Scirus (<http://www.sciencedirect.com/scirus/>), Google Scholar (<http://www.scholar.google.com/>), ScienceDirect (<http://www.sciencedirect.com/>)]. Up to 114 publications were retrieved using search terms including “fibrolytic enzyme,” “dairy cow,” and “fiber” and “fibre.” We also contacted researchers and organizations involved with this field of study for input. Of the papers that were retrieved, only those that satisfied the predetermined inclusion criteria were included in the analysis.

The study selection criteria were (1) publication in English in a peer-reviewed journal, (2) use of concurrent negative control and treatment groups, (3) use of confined or housed lactating dairy cows, (4) use of continuous experimental designs rather than change-over or Latin square studies, (5) use of at least one fibrolytic

enzyme as a dietary treatment, (6) description of the main enzyme activity(ies) that were applied, and (7) presentation of least squares means and standard errors of the means for DMI, milk yield, FCM, and feed efficiency (FCM/DMI). The study exclusion criteria were: (1) did not involve feeding a TMR, (2) involved a study that was not randomized, (3) involved animals on pasture, and (4) involved low genetic merit dairy cows.

We only included peer-reviewed publications in the study because the peer review process is a proxy for assessing the quality of studies (Weisz et al., 1995). In addition, more heterogeneity existed among non-peer-reviewed studies in a similar meta-analysis on effects of yeast culture products on the performance of dairy cows (Poppy et al., 2012).

Data Extraction

Data for DMI, milk yield, milk composition, feed efficiency, total-tract DM digestibility, and total-tract NDF digestibility were used to estimate outcomes.

Sources of variability in the data set included the EFE type, form (liquid vs. solid), composition, microbial source, application rate and method, as well as the designation of the main EFE activity and the duration of the experiments. Consequently, the data were classified based on these factors. The types of EFE identified by the authors were as follows: xylanase (**X**), cellulase-xylanase (**C-X**), ferulic acid esterase (**FAE**), cellulase-FAE (**C-FAE**), endoglucanase-xylanase (**En-X**), and amylase-exogenous proteolytic enzyme (**A-EPE**). Certain EFE that included nonfibrolytic activities such as amylase and protease or bacteria were retained in the analysis because relatively few studies met our selection criteria. Rate of application was calculated for each treatment as grams of EFE/kilogram of total diet DM. Methods of EFE application examined were application to the forage, concentrate, or the TMR. Effects of such methods on the performance of cows were only analyzed for experiments in which the EFE was applied to diets in each of the 3 methods. The form of EFE application was classified as liquid or solid.

Statistical Analysis and Effect Sizes

Statistical analysis was conducted on the extracted data using Stata software version 14.1 (StataCorp LP, College Station, TX). The Z score was used to examine the null hypothesis that EFE treatment had no effect. In addition to the significance of the relationships, the magnitude of the relationships (effect size) was examined using the standardized mean difference [SMD; SMD = raw mean difference (RMD) between EFE treatment and control means divided by the pooled

standard deviation of the means of both treatments]. Consequently, the SMD data are expressed in standard deviation units (Higgins and Green, 2011). Standardized mean difference values of <0.2 , >0.2 and <0.7 , or >0.8 were considered small, moderate, or large effects, respectively (Cohen 1988). In addition, the effect size also was examined using the RMD, which allows expression of the effect size with same unit as the measurement as recommended by Takeshima et al. (2014). Both SMD and RMD data were weighted using the inverse of the study variance by the random effect method proposed by DerSimonian and Laird (1986).

Heterogeneity, Meta-Regression, and Publication Bias

Between-study variability was compared with within-study variability (i.e., heterogeneity of effect size) using both the chi-squared (Q) test of heterogeneity and the I^2 statistic, which measures the percentage of variation due to heterogeneity (Higgins et al., 2003). An I^2 value less than 25% indicated low heterogeneity, whereas values between 35 to 50% denoted moderate heterogeneity and those above 50% denoted high heterogeneity (Higgins et al., 2003).

Meta-regression analyses were used to examine heterogeneity sources using SMD or RMD as the dependent variables. The covariates for the mixed model included EFE type (A-EPE, C-X, FAE, C-FAE, En-X, or X), rate of application (g of EFE/kg of diet DM), method of EFE application (forage, concentrate, or TMR), form of EFE application (liquid or solid), and experimental duration (d). Tests of null hypothesis for the covariate coefficients were obtained from the multi-parameter Wald test (Harbord and Higgins, 2008). The adjusted R^2 for the model, which represents the proportion of between-study variance (heterogeneity) explained by the covariates (Harbord and Higgins, 2008), was calculated by comparing the estimated between-study variance when covariates were included in the model (σ^2) with corresponding values when they were excluded (σ_o^2); adjusted R^2 (%) = $(\sigma_o^2 - \sigma^2)/\sigma_o^2$. If the categorical covariates were significant ($P < 0.05$), forest plots were drawn to further illustrate their effects. Publication bias was assessed using contour-enhanced funnel plots (Light and Pillemer, 1984). Significance was declared at $P \leq 0.05$ and tendencies at $0.05 > P > 0.1$.

RESULTS AND DISCUSSION

Studies that Met the Inclusion Criteria

Fifteen studies with 17 experiments and a total of 36 comparisons met the study criteria. The citations of the

studies included in the meta-analysis as well as their EFE treatments and the rate (g of EFE per kg of total diet), method (concentrate, forage, TMR), and form of application (liquid vs. solid) are in Table 1.

Effect Sizes

The SMD (and corresponding RMD) analysis shows that EFE treatment did not affect DMI ($P = 0.276$) but increased milk yield ($P = 0.001$; RMD = 0.83 kg/d, 95% CI: 0.36 to 1.30), 3.5% FCM yield ($P = 0.001$; RMD = 0.55 kg/d, CI: -0.005 to 1.11), milk protein yield ($P = 0.014$; RMD = 0.03 kg/d, CI: 0.007 to 0.04), and milk lactose yield ($P = 0.001$; RMD = 0.05 kg/d, CI: 0.02 to 0.08), but did not affect feed efficiency (3.5% FCM/DMI; $P = 0.219$; Table 2). However, the magnitude of the increases ($P \leq 0.05$) in these performance parameters was relatively small, with SMD amounting to less than 0.3 standard deviation units. Application of EFE reduced milk fat content ($P = 0.006$; RMD = -0.05%, CI: -0.09 to -0.01), but tended to increase milk fat yield ($P = 0.099$; RMD = 0.02 kg/d, CI: 0.001 to 0.03), both by small amounts (SMD <0.2 SD units). These results indicate that EFE treatment resulted in positive but small to moderate increases in yields of milk and milk components without increasing DMI or feed efficiency. The small to moderate increases reflect the variability in the types of EFE used as well as that in application rates, methods, and forms of the EFE.

Dietary treatment with EFE tended to increase total-tract DM digestibility ($P = 0.08$) and NDF digestibility ($P = 0.09$) by moderately small amounts as shown by the respective RMD values of 1.36% (CI: -0.14 to 2.86) and 2.30% (CI: -0.42 to 0.503), and SMD values of 0.31 (CI: -0.04 to 0.66) and 0.31 (CI: -0.05 to 0.67) standard deviation units. These results confirm that EFE application increased the digestibility of dairy cow diets, which likely caused or contributed to the increased yields of milk and milk components by the EFE.

Some authors (Schingoethe et al., 1999; Knowlton et al., 2002; Beauchemin et al., 2003) have emphasized the importance of using cows in early lactation to examine responses to EFE treatment of diets because they are more responsive to such treatments than those in mid to late lactation. This is because the energy supplied by the EFE-induced increases in DM or NDF digestibility is critically required for milk production in early lactation. In this meta-analysis, 70% of the studies started in early lactation (<60 DIM) and 30% started in mid-lactation (>60 DIM).

The most commonly used EFE was C-X (13 studies). Each of the other types of enzymes were used in no more than 2 studies (Table 1, Figure 1). As a result of

Table 1. Publications summarized in this meta-analysis on the effects of exogenous fibrolytic enzyme (EFE) application to dairy cow diets

Citation	Study	Exp.	n ¹	Treatment ²	Rate of application ³	Method ⁴	Form ⁵
Chen et al., 1995	1	1	9	CT	0	C	S
	1	1	9	A-EPE	0.084	C	S
	1	1	9	CT	0	C	S
	1	1	9	A-EPE	0.084	C	S
Lewis et al., 1999 (trial 2) ⁶	2	2	10	CT	0	F	L
	2	2	10	C-X	0.5	F	L
	2	2	10	CT	1.0	F	L
	2	2	10	C-X	2.0	F	L
Rode et al., 1999	3	3	10	C-T	0	C	L
	3	3	10	C-X	1.3	C	L
Yang et al., 2000	4	4	21	CT	0	TMR	S
	4	4	21	C-X	0.05	TMR	S
	4	4	21	C-X	0.05	C	S
Zheng et al., 2000	5	5	12	CT	0	F	L
	5	5	12	C-X	0.81	F	L
	5	5	12	C-X	0.63	F	L
	5	5	12	C-X	0.63	F	L
Kung et al., 2000 (trial 1) ⁶	6	6	10	CT	0	F	L
	6	6	10	C-X	1.0	F	L
	6	6	10	C-X	2.5	F	L
Kung et al., 2000 (trial 2) ⁶	6	7	10	CT	0	F	L
	6	7	10	C-X	1.0	F	L
	6	7	10	C-X	0.6	F	L
	6	7	10	C-X	0	F	L
Dhiman et al., 2002	7	8	10	CT	0	F	L
	7	8	10	C-X	0.66	F	L
	7	8	10	C-FAE	0.66	F	L
	7	8	10	FAE	0.001	F	L
	7	8	10	FAE	0.015	F	L
Kung et al., 2002	8	9	10	CT	0	F	L
	8	9	10	C-X	10.8	F	L
	8	9	10	C-X	10.8	F	L
Vicini et al., 2003 (trial 1) ⁶	9	10	56	CT	0	F	L
	9	10	58	C-X	0.59	F	L
	9	10	58	C-X	2.00	TMR	L
Vicini et al., 2003 (trial 2) ⁶	9	11	26	CT	0	F	L
	9	11	31	C-X	0.59	F	L
	9	11	29	C-X	2.00	TMR	L
Bernard et al., 2010	10	12	11	CT	0	TMR	S
	10	12	11	C-X	0.16	TMR	S
	10	12	11	C-X	0	TMR	S
	10	12	11	C-X	0.16	TMR	S
Holtshausen et al., 2011	11	13	20	CT	0	TMR	L
	11	13	20	En-X	0.5	TMR	L
	11	13	20	En-X	1	TMR	L
Arriola et al., 2011	12	14	15	CT	0	TMR	L
	12	14	15	C-X	3.4	TMR	L
	12	14	15	CT	0	TMR	L
	12	14	15	C-X	3.4	TMR	L
Mohamed et al., 2013	13	15	60	CT	0	TMR	S
	13	15	60	X	0.607	TMR	S
Peters et al., 2015	14	16	14	CT	0	TMR	L
	14	16	14	En-X	3.9	TMR	L
	14	16	13	CT	0	TMR	L
	14	16	13	En-X	3.8	TMR	L
Romero et al., 2016	15	17	22	CT	0	TMR	L
	15	17	22	X	1	TMR	L
	15	17	22	C-X	3.4	TMR	L

¹Number of animals in each experiment.²Treatments: control (CT), amylase-exogenous proteolytic enzymes (A-EPE), cellulase-xylanase (C-X), ferulic acid esterase (FAE), cellulase-FAE (C-FAE), endoglucanase-xylanase (En-X), xylanase (X).³Rate of application: g of EFE/kg of total diet.⁴Method of EFE application to the forage (F), concentrate (C), or TMR.⁵Form of EFE applied: liquid (L), solid (S).⁶Lewis et al., 1999: 2 trials were conducted by these authors but only the second one met the inclusion criteria. Kung et al., 2000: 2 trials were conducted by these authors and both met the inclusion criteria. Vicini et al., 2003: 2 trials were conducted by these authors and both met the inclusion criteria.

Table 2. Effect of applying exogenous fibrolytic enzymes (EFE) on the performance of lactating dairy cows across all trials and EFE types

Item	n ¹	RMD ² (95% CI)			Heterogeneity			SMD ³ (95% CI)		
		Control mean	Random effect	P-value	Chi-squared (Q)	P-value	I ² (%)	Random effect	P-value	P-value
DMI, kg/d	36	23.2 (2.18)	0.22 (-0.07, 0.49)	0.132	58.4	0.008	40.1	0.09 (-0.07, 0.24)	0.276	
Milk yield, kg/d	36	36.0 (3.26)	0.83 (0.36, 1.30)	0.001	51.4	0.037	31.8	0.25 (0.10, 0.39)	0.001	
FCM, kg/d	26	35.7 (3.43)	0.55 (-0.005, 1.11)	0.052	38.3	0.044	34.6	0.16 (-0.003, 0.33)	0.001	
FCM/kg of DMI	21	1.59 (0.42)	0.02 (-0.02, 0.05)	0.353	36.9	0.012	45.7	0.14 (-0.08, 0.36)	0.219	
Milk fat, %	36	3.59 (0.41)	-0.05 (-0.09, -0.01)	0.008	30.2	0.697	0.0	-0.16 (-0.27, -0.04)	0.006	
Milk fat yield, kg/d	25	1.28 (0.18)	0.02 (0.001, 0.03)	0.038	20.1	0.693	0.0	0.12 (-0.02, 0.26)	0.099	
Milk protein, %	36	3.07 (0.18)	0.002 (-0.02, 0.02)	0.815	46.8	0.087	25.2	0.005 (-0.13, 0.89)	0.938	
Milk protein yield, kg/d	27	1.13 (0.14)	0.03 (0.007, 0.04)	0.007	32.8	0.169	20.6	0.20 (0.04, 0.37)	0.014	
Milk lactose, %	24	4.80 (0.14)	-0.009 (-0.03, 0.02)	0.479	34.2	0.063	32.7	-0.09 (-0.27, 0.10)	0.357	
Milk lactose yield, kg/d	13	1.77 (0.19)	0.05 (0.02, 0.08)	0.001	7.43	0.828	0.0	0.31 (0.13, 0.48)	0.001	
DM digestibility, %	9	68.4 (4.44)	1.36 (-0.14, 2.86)	0.077	16.7	0.033	52.2	0.31 (-0.04, 0.66)	0.080	
NDF digestibility, %	9	50.1 (7.45)	2.30 (-0.42, 0.503)	0.098	17.5	0.025	54.4	0.31 (-0.05, 0.67)	0.093	

¹Number of comparisons between enzyme treatment and control treatment.²RMD = raw mean difference between enzyme and control treatments (and the associated 95% CI).³SMD = standardized mean difference between enzyme and control treatments (and the associated 95% CI). This SMD was estimated by dividing the RMD by the pooled standard deviation of both treatment groups.

the greater number of studies that used C-X and the apparently greater effect of C-X enzymes than most other types in the forest plot in Figure 1, we analyzed separately the effects of application of C-X enzymes. The SMD (and corresponding RMD; Appendix Figure A2) analysis showed that application of C-X enzymes did not affect DMI, FCM, feed efficiency, milk protein content, yields of milk fat and protein, or DM digestibility ($P > 0.10$; Table 3), but increased milk yield ($P = 0.006$; RMD = 1.04 kg/d; 95% CI: 0.33 to 1.74), milk lactose yield ($P = 0.046$; RMD = 0.041 kg/d, CI: -0.003 to 0.086), and NDF digestibility ($P = 0.031$; RMD = 2.844%, CI: 0.52 to 5.169), and decreased milk fat content ($P = 0.005$; RMD = -0.07%, CI: -0.121 to -0.019) and milk lactose content ($P = 0.024$; RMD = -0.043%, CI: -0.084 to -0.001). However, the magnitude of the increases or decreases in these performance parameters was small to moderate, with SMD values of less than 0.35 standard deviation units (Table 3). Nevertheless, the 24% increase in the RMD resulting from examining only C-X enzymes instead of all enzymes (RMD = 1.04 vs. 0.83 kg/d) suggests that we did not have enough statistical power to detect differences between enzyme treatments due to the paucity of studies that met the inclusion criteria. The 24% increase nonetheless indicates that it was important to analyze separately C-X enzymes and that such enzymes were more effective on average than all enzymes at increasing milk production.

Heterogeneity

Most outcomes had low ($I^2 < 25\%$) to moderate ($I^2 = 25\%$ to 50%) heterogeneity levels, indicating low to moderate inconsistency in SMD estimates. No heterogeneity (Table 2) was detected for milk fat concentration ($P = 0.70$, 0%) or yield ($P = 0.69$, 0%) or milk lactose yield ($P = 0.83$, 0%), whereas low heterogeneity was evident for milk protein yield ($P = 0.17$, 20.6%). Moderate heterogeneity was evident for milk yield ($P = 0.037$, 31.8%; Figure 1), DMI ($P = 0.008$, 40.1%; Figure 2), milk protein concentration ($P = 0.087$, 25.2%; Figure 3), 3.5% FCM yield (0.044, 34.6%), feed efficiency ($P = 0.012$, 45.7%), and milk lactose concentration ($P = 0.06$, 32.7%). Only total-tract DM digestibility ($P = 0.033$, 52.2%) and NDF digestibility ($P = 0.025$, 54.4%) had high heterogeneity, possibly because few of the included studies ($n = 9$) examined this measure.

Heterogeneity analysis is perhaps one of the most critical measurements in meta-analysis because it compares the amount of variance within the group of studies to the within-study variance (Lean et al., 2009). Heterogeneity analysis helps to determine whether meta-regression or subgroup analysis (or both) is nec-

Figure 1. Forest plot of random effects of standardized mean difference (SMD) between control and treatment means for milk yield classified by enzyme type. A-EPE, C-X, C-FAE, FAE, En-X, and X represent studies involving the following enzyme types: amylase-proteolytic enzymes, cellulase-xylanase, cellulase-ferulic acid esterase, ferulic acid esterase, endoglucanase-xylanase, and xylanase, respectively. The solid diamonds represent individual studies and their weighting by the inverse of their respective variances. The horizontal lines represent the 95% confidence interval for the study. The open diamond represents the overall SMD and its width represents the associated 95% confidence interval. The solid line is the line depicting no treatment effect. The dotted line indicates the average response to exogenous fibrolytic enzyme treatment across studies as well as the heterogeneity of the studies by not passing through all the solid diamonds.

Table 3. Effect of applying cellulase-xyylanase enzyme (C-X) on the performance of lactating dairy cows

Item	n ¹	RMD ² (95% CI)			Heterogeneity			SMD ³ (95% CI)		
		Control mean	Random effect	P-value	Chi-squared (Q)	P-value	I ² (%)	Random effect	P-value	
DMI, kg/d	24	22.7 (2.12)	0.28 (−0.014, 0.575)	0.062	36.8	0.046	34.7	0.125 (−0.04, 0.29)	0.135	
Milk yield, kg/d	24	35.6 (3.43)	1.04 (0.33, 1.74)	0.004	59.2	0.0001	59.5	0.27 (0.08, 0.46)	0.006	
FCM, kg/d	19	35.7 (3.69)	0.67 (−0.121, 1.468)	0.097	42.5	0.002	55.3	0.148 (−0.057, 0.353)	0.167	
FCM/kg of DMI	12	1.59 (0.18)	−0.009 (−0.059, 0.041)	0.728	22.1	0.036	45.7	−0.013 (−0.308, 0.282)	0.93	
Milk fat, %	24	3.64 (0.40)	−0.070 (−0.121, −0.019)	0.007	27.4	0.287	12.3	−0.200 (−0.339, −0.062)	0.005	
Milk fat yield, kg/d	15	1.29 (0.20)	0.012 (−0.022, 0.047)	0.486	16.9	0.326	11.2	0.059 (−0.137, 0.255)	0.553	
Milk protein, %	24	3.07 (0.18)	−0.009 (−0.035, 0.017)	0.505	35.9	0.055	33.3	−0.064 (−0.232, 0.103)	0.452	
Milk protein yield, kg/d	17	1.11 (0.12)	0.014 (−0.011, 0.039)	0.273	20.4	0.253	16.8	0.126 (−0.065, 0.318)	0.195	
Milk lactose, %	12	4.80 (0.08)	−0.043 (−0.084, −0.001)	0.044	26.9	0.008	55.4	−0.309 (−0.577, −0.040)	0.024	
Milk lactose yield, kg/d	8	1.75 (0.21)	0.041 (−0.003, 0.086)	0.070	6.28	0.616	0.0	0.248 (0.005, 0.492)	0.046	
DM digestibility, %	5	70.6 (4.93)	1.464 (−0.485, 3.413)	0.141	11.3	0.046	55.7	0.299 (−0.103, 0.701)	0.145	
NDF digestibility, %	5	52.1 (7.72)	2.844 (0.52, 5.169)	0.016	6.41	0.268	22.0	0.344 (0.031, 0.657)	0.031	

¹Number of comparisons between enzyme treatment and control treatment.²RMD = raw mean difference between enzyme and control treatments (and the associated 95% CI).³SMD = standardized mean difference between enzyme and control treatments (and the associated 95% CI). This SMD was estimated by dividing the RMD by the pooled standard deviation of both treatment groups.

essary even though meta-regression can be of value in homogeneous data sets. In this study, the high heterogeneity of total-tract NDF and DM digestibility responses and moderate heterogeneity of milk yield, DMI and feed efficiency responses indicate that the effect sizes differed considerably among studies. In this study, EFE type, rate, method, and form of EFE application, and experimental period duration were among the important potential heterogeneity sources. Consequently, meta-regression and sub-grouping analysis were used to further examine their influences.

Meta-Regression

The results of the meta-regression analysis of the effect of EFE type, application rate, method, form, DIM, and experimental duration on the SMD and RMD values are shown in Tables 4 and 5, respectively. These covariates explained up to 24.4, 35.9, 35.5, and 65.8% of the SMD heterogeneity in milk yield, 3.5% FCM, feed efficiency, and milk lactose yield, respectively. Notably, they explained most of the SMD heterogeneity in milk protein yield (adjusted R² = 100%). However, they did not explain the heterogeneity (adjusted R² = 0) in DMI, milk fat concentration, milk fat yield, milk protein concentration, milk lactose yield, and total-tract DM and NDF digestibility. The variation in the latter measures may have been due to other dietary factors like the forage to concentrate ratio, NDF concentration and effectiveness, and animal factors like the stage of lactation of the cows. In particular, that only 24.4 and 35.9% of the heterogeneity in milk yield and 3.5% FCM, respectively, was explained by EFE type suggests that EFE designations on product labels did not sufficiently reflect their actual activities. This supports the previous recommendation that more specific characterization and labeling of EFE products is required (Adesogan et al., 2014).

Although the forest plot for milk yield (SMD data, Figure 1; RMD data, Appendix Figure A1) and the RMD data indicated a 24% greater milk yield response to C-X enzyme addition versus addition of all enzymes, the meta-regression analysis showed that EFE type did not affect ($P > 0.10$) any of the parameters measured. The lack of an EFE type effect on milk yield in the meta-regression analysis may be due to the low number of studies examined for each enzyme type except C-X and the moderate heterogeneity (42.1%) in the C-X response (Figure 1). The former indicates a need for more studies on the subject to allow more comprehensive meta-analysis to be undertaken. The moderate heterogeneity of the milk response reflects considerable variation in effect sizes among studies, which may be due to variability in the actual enzyme activities

designated as cellulase or xylanase by enzyme manufacturers (Adesogan et al., 2014). Hence, more detailed characterization of enzymes designated as cellulases and xylanases is needed.

Method of EFE application tended to increase ($P = 0.06$) the SMD of milk protein concentration by 0.17 standard deviation units (RMD = 0.04%; Table 5), but application to the concentrate ($P = 0.25$) or forage ($P = 0.23$) had no effect (data not shown). Therefore, this study provides no support for the notion that the method of application of EFE affects the performance of dairy cows.

Increasing the duration of experiments by one day tended to increase the SMD of milk protein concentration ($P = 0.091$) and yield ($P = 0.075$) by 0.006 and 0.007 standard deviation units (Table 4), respectively, which corresponded to RMD values of 0.001% and

0.001 kg/d (Table 5), respectively. These small but significant effect size increases indicate that researchers should opt for longer-term continuous experiments rather than short-term change-over studies to examine responses to dietary treatment with EFE. Adesogan et al. (2014) suggested that short-period changeover designs are not appropriate to measure performance responses to EFE treatment. This was based on the fact that treatment with the same EFE decreased DMI and increased feed efficiency in a study with a 10-wk continuous design (Holtshausen et al., 2011) but had no effect on performance in a study with a 3 × 3 Latin square design with 21-d periods (Chung et al., 2012), yet both studies were conducted by the same researchers with the same diet, EFE, and EFE batch. Adesogan et al. (2014) also cited studies (Schingoethe et al., 1999; Romero et al., 2016) that suggest that milk production

Figure 2. Forest plot random effects standardized mean difference (SMD) between control and treatment means for DMI. The solid diamonds represent individual studies and their weighting by the inverse of their respective variances. The horizontal lines represent the 95% confidence interval for the study. The open diamond represents the overall SMD and its width represents the associated 95% confidence interval. The solid line is the line depicting no treatment effect. The dotted line indicates the average response to exogenous fibrolytic enzyme treatment across studies as well as the heterogeneity of the studies by not passing through all the solid diamonds.

Figure 3. Forest plot of random effects standardized mean difference (SMD) between control and treatment means for milk protein content as affected by enzyme application method (i.e., 1 = to the concentrate, 2 = to the forage, 3 = to the TMR). The solid diamonds represent individual studies and their weighting by the inverse of their respective variances. The horizontal lines represent the 95% confidence interval for the study. The open diamond represents the overall SMD and its width represents the associated 95% confidence interval. The solid line is the line depicting no treatment effect. The dotted line indicates the average response to exogenous fibrolytic enzyme treatment across studies as well as the heterogeneity of the studies by not passing through all the solid diamonds.

by cows may be unaffected by EFE application until EFE-treated diets are fed for at least 3 wk. Continuous, longer-term experiments may be better at indicating the effectiveness of EFE because they avoid carry-over effects, involve more animals, and are better at representing the lactation performance of cows as well as the benefits of sustained EFE application. Studies that aim to examine effects of experimental duration and dietary adaptation period on the efficacy of EFE are needed.

The lactation stage or DIM only affected the SMD of milk lactose concentration ($P = 0.028$; Table 4), resulting in an increase in RMD value of 0.001% (Table 5).

Previous studies have shown that cows in early lactation are more responsive to EFE treatment in early lactation (Schingoethe et al., 1999; Knowlton et al., 2002; Beauchemin et al., 2003). This may have not been evident in this study because the lactation stage assessment focused on DIM rather than on early versus mid to late lactation.

Publication Bias

Some publication bias toward studies with positive outcomes was evident by the asymmetry in the con-

Table 4. Parameters of the meta-regression of the effect of enzyme application rate, method, form, experimental duration, and DIM on standardized mean differences (SMD) between enzyme and control treatment means

Dependent variable (Y, SMD)	Meta-regression parameter (<i>P</i> -value) ¹						Adj. R ² (%) ²	No. of obs. ³
	Intercept	Enzyme	Rate	Method	Form	Duration	DIM	
DMI, kg/d	0.86 (0.246)	0.02 (0.806)	0.02 (0.588)	-0.21 (0.181)	-0.09 (0.718)	-0.002 (0.611)	-0.001 (0.656)	36
Milk yield, kg/d	-0.24 (0.694)	0.08 (0.192)	0.03 (0.474)	-0.14 (0.305)	0.145 (0.515)	0.005 (0.194)	-0.001 (0.641)	36
FCM, kg/d	-0.48 (0.546)	0.09 (0.247)	0.04 (0.327)	-0.17 (0.410)	0.24 (0.461)	0.006 (0.189)	-0.002 (0.519)	26
FCM/DMI	-0.37 (0.773)	-0.02 (0.863)	-0.02 (0.677)	0.29 (0.320)	0.50 (0.165)	-0.01 (0.352)	0.002 (0.618)	21
Milk fat, %	-0.79 (0.109)	0.03 (0.455)	0.02 (0.631)	0.02 (0.866)	0.05 (0.761)	0.004 (0.192)	0.001 (0.513)	36
Milk fat yield, kg/d	-0.40 (0.504)	0.05 (0.426)	-0.01 (0.908)	-0.06 (0.969)	0.09 (0.644)	0.006 (0.118)	-0.002 (0.405)	0.0
Milk protein, %	-1.45 (0.014)	0.05 (0.334)	0.009 (0.782)	0.24 (0.060)	0.14 (0.474)	0.006 (0.091)	0.001 (0.765)	36
Milk protein yield, kg/d	-0.83 (0.143)	0.08 (0.145)	0.008 (0.813)	-0.04 (0.770)	0.37 (0.045)	0.007 (0.075)	-0.003 (0.179)	100
Milk lactose, %	-0.64 (0.357)	0.05 (0.469)	0.106 (0.294)	0.05 (0.767)	-0.26 (0.282)	0.002 (0.585)	0.007 (0.028)	24
Milk lactose yield, kg/d	-0.15 (0.857)	0.09 (0.188)	0.14 (0.345)	-0.17 (0.389)	0.17 (0.486)	0.003 (0.486)	-0.002 (0.557)	0.0
DM digestibility, %	13.7 (0.485)	-0.56 (0.487)	-1.07 (0.496)	0.03 (0.968)	-2.55 (0.373)	-0.09 (0.608)	-0.01 (0.682)	9
NDF digestibility, %	12.7 (0.632)	-0.54 (0.619)	-1.00 (0.637)	-0.01 (0.996)	-2.10 (0.566)	-0.08 (0.731)	-0.02 (0.680)	9

¹Enzyme = type of enzyme; rate = application rate of enzyme (g/kg of diet DM); method = application to the concentrate, forage, or TMR; form = liquid, solid; duration = number of days of the study.

²Adjusted R² = proportion of between-study variance (heterogeneity) explained by the covariates.

³Number of comparisons between enzyme treatment and control treatment. obs = observations.

Table 5. Meta-regression of the effect of enzyme type, application rate, method and form, experimental duration, and lactation stage (DIM) on raw mean differences (RMD) between enzyme and control treatment means

Dependent variable (Y, RMD)	Meta-regression parameter (<i>P</i> -value) ¹						Adj. R ² (%) ²	n ³
	Intercept	Enzyme type	Rate	Method	Form	Duration	DIM	
DMI, kg/d	2.02 (0.179)	0.007 (0.955)	0.04 (0.612)	-0.50 (0.160)	-0.02 (0.970)	-0.007 (0.491)	-0.002 (0.652)	36
Milk yield, kg/d	-0.44 (0.850)	0.14 (0.482)	0.06 (0.645)	-0.54 (0.283)	0.22 (0.782)	0.02 (0.121)	-0.004 (0.547)	36
FCM, kg/d	-1.10 (0.736)	0.24 (0.413)	0.13 (0.417)	-0.76 (0.363)	0.58 (0.632)	0.03 (0.151)	-0.009 (0.438)	26
FCM/DMI	0.15 (0.539)	-0.01 (0.994)	-0.01 (0.835)	0.05 (0.356)	0.08 (0.742)	-0.004 (0.059)	0.001 (0.352)	21
Milk fat, %	-0.35 (0.154)	0.02 (0.249)	0.008 (0.438)	0.01 (0.773)	0.07 (0.505)	0.002 (0.283)	0.001 (0.730)	34
Milk fat yield, kg/d	0.01 (0.904)	0.004 (0.672)	-0.01 (0.734)	-0.02 (0.500)	0.001 (0.955)	0.001 (0.168)	-0.001 (0.234)	0.0
Milk protein, %	-0.26 (0.014)	0.007 (0.387)	0.002 (0.780)	0.04 (0.052)	0.02 (0.541)	0.001 (0.08)	0.001 (0.622)	36
Milk protein yield, kg/d	-0.05 (0.484)	0.004 (0.543)	-0.01 (0.760)	-0.01 (0.801)	0.03 (0.169)	0.001 (0.190)	-0.001 (0.170)	100
Milk lactose, %	0.07 (0.693)	0.002 (0.911)	0.01 (0.483)	-0.01 (0.805)	-0.11 (0.110)	-0.001 (0.948)	0.001 (0.026)	24
Milk lactose yield, kg/d	0.02 (0.988)	0.01 (0.304)	0.02 (0.451)	-0.04 (0.361)	0.03 (0.480)	0.001 (0.416)	-0.001 (0.673)	13
DM digestibility, %	68.0 (0.375)	-3.15 (0.348)	-5.66 (0.372)	0.15 (0.954)	-14.6 (0.253)	-0.38 (0.550)	-0.067 (0.603)	9
NDF digestibility, %	82.7 (0.663)	-3.56 (0.653)	-6.56 (0.669)	-0.05 (0.994)	-13.3 (0.617)	-0.53 (0.754)	-0.14 (0.696)	9

¹Enzyme = type of enzyme; rate = application rate of enzyme (g/kg of diet DM); method = application to the concentrate, forage, or TMR; form = liquid, solid; duration = number of days of the study.

²Adjusted R² = proportion of between-study variance (heterogeneity) explained by the covariates.

³Number of comparisons between enzyme treatment and control treatment.

tour-assisted funnel plots for milk production and DMI, particularly among larger studies (Figures 4 and 5 for SMD data, respectively; RMD data are in Appendix Figures A3 and A4). This may have been because fewer studies with negative outcomes were published. Borenstein et al. (2009) suggested that to avoid publication bias in favor of studies with positive outcomes, studies reported in the gray literature (thesis, dissertations, abstracts, technical reports, and proceedings) would need to be included in the meta-analysis. However, the quality of many of such studies may be poor or unverifiable. Focusing the meta-analysis on published studies avoids this problem because the peer-review process validates the quality of the study (Weisz et al., 1995) at least to some degree. Poppy et al. (2012) included published and unpublished studies in their meta-analysis of the effects of yeast culture on the performance of dairy cows but excluded the unpublished results from the reported SMD values due to their heterogeneity.

The results of this meta-analysis reflect the responses to EFE application in studies that met the inclusion criteria without meeting the exclusion criteria. Therefore, caution is needed when extrapolating the results to conditions that were not included in the study cri-

Figure 4. Contour-line enhanced funnel plot of the standardized mean difference between treatment and control groups in all studies in the meta-analysis of effect of exogenous fibrolytic enzyme treatment on milk yield. This funnel plot represents a slight bias toward publishing favorable studies.

Figure 5. Contour-line enhanced funnel plot of the standardized mean difference between treatment and control groups in all studies in the meta-analysis of effect of exogenous fibrolytic enzyme treatment on DMI. This funnel plot represents a slight bias toward publishing favorable studies.

teria such as responses of grazing dairy cows to dietary EFE application.

CONCLUSIONS

Across all studies included in the meta-analysis, EFE application did not affect DMI, tended to improve total-tract NDF and DM digestibility, and improved milk yield by relatively small amounts. Across studies involving only C-X enzymes, enzyme application increased the milk response by 24% relative to that for studies involving all enzymes. The C-X enzymes produced a numerically but not statistically greater milk yield response relative to that for all enzymes as we did not have enough statistical power to detect differences between enzyme treatments due to the paucity of studies that met the inclusion criteria. Therefore, more studies are needed to confirm the superiority of the C-X enzymes. Most of the performance outcomes examined had low to moderate heterogeneity levels, indicating low to moderate inconsistency in effect size (SMD and RMD) estimates. Increasing the rate of application of EFE did not affect performance measures. Application of EFE to the TMR instead of the forage or concentrate only improved milk protein concentration. Increasing the experimental duration increased the yield and content of milk protein, suggesting that longer-term experiments should be used for enzyme studies. The

type of EFE applied accounted for relatively little of the variation in milk yield and 3.5% FCM yield. This factor and the relatively small effect sizes of the EFE responses highlight the fact that differences among EFE types are not appropriately reflected by their label names or designations. Proper characterization and labeling of commercial EFE is therefore warranted.

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Appendix

Figure A1. Forest plot of the effect of enzyme application on milk yield. The solid diamonds represent individual studies and their weighting by the inverse of their respective variances. The horizontal lines represent the 95% confidence interval for the study. The open diamond represents the 95% confidence interval for the overall estimate. The solid line represents a mean difference of zero or no effect. The dotted line indicates the mean response as well as the heterogeneity of the studies by not passing through all the solid diamonds. RMD = raw mean difference; WMD = weighted mean difference.

Figure A2. Forest plot of the effect of enzyme application on DMI. The solid diamonds represent individual studies and their weighting by the inverse of their respective variances. The horizontal lines represent the 95% confidence interval for the study. The open diamond represents 95% confidence interval for the overall estimate. The solid line represents a mean difference of zero or no effect. The dotted line indicates the mean response as well as the heterogeneity of the studies by not passing through all the solid diamonds. RMD = raw mean difference.

Figure A3. Contour-line enhanced funnel plot of the raw mean difference between treatment and control groups in all studies in the meta-analysis of effect of exogenous fibrolytic enzyme treatment on milk yield. This funnel plot depicts a slight bias toward publishing favorable studies.

Figure A4. Contour-line enhanced funnel plot of the raw mean difference between treatment and control groups in all studies in the meta-analysis of effect of exogenous fibrolytic enzyme treatment on DMI. This funnel plot depicts a slight bias toward publishing favorable studies.