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Variation in milk production, fat, protein, and lactose responses to exogenous feed enzymes in dairy cows

H. A. Rossow,[1] PAS, H. M. Golder,[2] and I. J. Lean[2]*

[1]Veterinary Medical Teaching and Research Center, University of California–Davis, Tulare 93274; and [2]S*cibus*,
Camden, NSW, Australia 2570

ABSTRACT

Objective: Our objectives were to evaluate milk production and constituent responses to changes in the diet for pens of cows over time and whether differences in response were attributable to fibrolytic enzymes and dairy.

Materials and Methods: A multiherd trial used 7,507 cows in 8 control and enzyme-treated (750 mL/t of DM feed) replicates (16 pens) on 3 dairies. Feed composition and milk production and constituents by pen (n = 12) were analyzed weekly. Time-series cross-correlation estimates by pen of feed component intakes (kg/d) and milk responses were pooled to produce effect size (ES) estimates.

Results and Discussion: We observed differences between treatment and control pens for soluble protein (ES = 0.249) in the same week, acid detergent–insoluble CP (ES = 0.293) and lignin (ES = 0.237) 1 wk before with milk protein percentage, and acid detergent–insoluble CP (ES = 0.276) and lignin (ES = 0.246) 1 wk before with milk protein yield. These differences are consistent with enzymes improving feed digestibility, particularly for protein and fiber fractions. Differences in production responses to intake of feed components among dairies were observed. More significant and larger differences occurred among dairies than for treatments. The dairy that increased milk production most with treatment had an estimated MP excess from the diet, whereas the least responsive had an estimated MP-deficit diet and was the highest producing.

Implications and Applications: We provide evidence for variability in enzyme response and that changes in dietary feed components influence production outcomes immediately and up to 3-wk later.

Key words: diet, fibrolytic enzyme, milk protein percentage, time series

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The authors declare no conflict of interest at the time the research was conducted, analyzed, and summarized.
*Corresponding author: ianl@scibus.com.au

INTRODUCTION

Fibrolytic enzymes have been applied to feed to increase milk production (Rode et al., 1999; Yang et al., 1999, 2000). A meta-analysis of several enzyme products indicated that the use of exogenous fibrolytic enzymes (**EFE**) increased milk by 0.83 kg/d and milk protein production, but responses to intervention were variable (Arriola et al., 2017). Similar milk production responses (0.70 kg/d) to those of Arriola et al. (2017) occurred for this large multiherd study, as described in a companion paper (Golder et al., 2019); however, herd responses were also variable. Milk production responses ranged from an increase of 0.20 to 3.6 kg/d per head (Golder et al., 2019).

Factors that influence responses to EFE are not well characterized. Inconsistent responses to EFE have been attributed to differences in enzyme activity, application rate and composition, stage of lactation of dairy cows, mode and time of enzyme delivery, ruminal microbial activity, ruminal enzyme stability, enzyme-feed specificity, and the portion of the diet to which enzymes are applied (Beauchemin et al., 2004; Adesogan, 2005). Additional factors that may explain variability include insufficient statistical power, inappropriate experimental designs or durations of application, inappropriate enzyme choices, inappropriate measures of enzyme activity, and misleading enzyme designations (Arriola et al., 2017). A meta-analysis by Tirado-González et al. (2018) indicated that responses to EFE differed with forage-to-concentrate ratio. Diets defined with >50% forage had a more positive effect on milk production (1.96 L/d) than diets lower in forage, which did not show a production response. Variations in milk responses associated with the forage content of the diet suggest that dietary components influence production responses over time to EFE; studies are needed to examine that hypothesis.

Our objectives were to evaluate milk production and constituent responses to changes in diet composition for pens of cows over time and whether differences in responses were attributable to EFE treatment and dairy. We hypothesize that there would be associations between

changes in dietary components and milk production parameters over time and that these associations may differ between treatments among dairies.

MATERIALS AND METHODS

This study was approved by the University of California, Davis, Animal Care and Use Committee and was conducted from December 2015 to January 2018.

Experimental Design

A multiherd trial was conducted as described by Golder et al. (2019) using 7,507 cows in 8 control and 8 enzyme-treated replicates (16 pens) on 3 dairies in the United States. To address the hypothesis in the current study, data were used from 6 of these control and enzyme-treated pens (12 pens in total).

Dairy Selection Criteria

The 3 dairies were purposively selected for use in the study on the basis that they had good record keeping and a history of performance that suggested that they would be capable of maintaining attention to detail consistent with successful trial conduct. Details of the criteria are provided in a companion paper (Golder et al., 2019); however, the focus of this study was on pen responses to feeding and enzyme treatment. Specifically, the dairies enrolled met the following criteria: recorded daily milk and weekly milk solids production; provided clear identification, parity, and pregnancy status of cows; conducted feeding accurately and weighed feed offered and orts; weighed cows daily or more often; maintained a monitor for each dairy to assist with protocol compliance; and had uniform pens that were as identical as possible, with a minimum of 2 per treatment per dairy. If necessary, a pen rotation was used to establish replicates and ensure similarity of environmental exposure for pens. Further selection criteria included that records were easily retrieved and were able to be validated to evaluate anomalies in data and the herds were willing to comply with a 150-d study minimum to allow an evaluation of reproduction. Last, enzyme treatments were planned to start before calving.

Dairy 1 was located in Hamlin County, South Dakota, and was positively ventilated, with freely available drinking water. Dairy 2 was in Tulare County, California, and Dairy 3 was in Kerman, California. The Californian dairies were freestall sheds with fans and soakers, and drinking water was freely available. It was considered that a geographical spread of the dairies would help to improve external validity of the study. Feeding systems were TMR, and the number of feed deliveries each day and push-ups varied with the dairies and diets fed. Standard operating procedures were developed to describe the methods of treatment application and feeding for each dairy.

Sample Size Determinations

Sample sizes for cows and number of pens were determined for the study to evaluate production responses to an EFE intervention as previously described (Golder et al., 2019). For this part of the study, sample sizes were determined based on time series considerations and used previous studies to establish a sample size based on a determination that a difference in effect size (**ES**) of 0.1 (approximately an r of 0.1) between treatments and controls would be worthy of detection. A target of a minimum of 20 weekly samples was determined based on previous studies.

Study Treatment

Pens used in the study were randomly assigned to enzyme or control treatments; parity 1 and older pens were allocated separately by the toss of a coin. All cows in the dry pens with a projected calving date entered the study and were allocated systematically based on odd and even ear tags that were randomly assigned by the toss of a coin to control or enzyme treatment. Enrollment of cows occurred on a weekly basis. The DIM, parity, and milk yield of cows existing in lactation pens at study commencement were checked. Cows were moved before commencement to balance these parameters if necessary. All treated pens were exposed to a fibrolytic enzyme (AB Vista, Marlborough, United Kingdom), that is, a liquid pretreatment. It is a fermentation product of *Trichoderma reesei* and contains declared minimum activities of 350,000 BXU/g of xylanase (EC 3.2.1.8) and 10,000 ECU/g of cellulase (EC 3.2.1.4), where 1 BXU is the amount of enzyme that will release 0.06 µmol of reducing sugars (xylose equivalents) from birch xylan per minute at pH 5.3 and 50°C. One ECU is the amount of enzyme that will release 0.06 µmol of reducing sugars as glucose from hydroxyethyl cellulose per minute at pH 4.8 and 50°C.

Pens were administered enzyme based on DMI, as per label directions, at a rate of 750 mL/t of DM of feed. The enzyme was mixed with water and administered directly onto the TMR in mixer wagons. To validate the amount of enzyme being used, there was a weekly enzyme reconciliation to ensure that the volume of enzyme used was consistent with the anticipated rate of use. Xylanase and cellulase activities were measured by wet chemistry by Enzyme Services & Consultancy, Innovation & Technology Centre (Ystrad Mynach, UK) to identify the presence of active enzyme in samples of the TMR. The xylanase activity (EC 3.2.1.8) was analyzed using xylazyme AX (60 mg) tablets (Megazyme T-XAX200; Megazyme International Ireland Ltd., Bray, Wicklow, Ireland) as a substrate. The cellulase activity (EC 3.2.1.4) was analyzed using Cellazyme-C tablets (Megazyme International Ireland Ltd.) as a substrate. Cows received enzyme-treated feed or control feed for a minimum of 150 d in lactating cow strings and for variable periods before calving.

Feeding and Feed Analysis

Details of typical lactation diets are provided in Table 1. Ration samples were collected weekly for nutrient analyses from each pen with a differently formulated TMR. Samples were obtained according to standard operating procedures described by Golder et al. (2019) to obtain representative samples from each pen. Samples were frozen at −20°C before submission for laboratory analysis.

Dairy 1 contributed 2 control (13 and 19 wk of data) and 2 treatment (12 and 21 wk of data) observations from mixed parities. Dairy 2 contributed 2 control (1 primiparous and 1 multiparous) and 2 treatment (1 primiparous and 1 multiparous) observations with 31 wk of data. Dairy 3 contributed 2 control and 2 treatment observations with 58 wk of data.

As described by Golder et al. (2019), nutrient analysis was performed by wet chemistry at Dairy One Cooperative Inc., Forage Testing Laboratory (Ithaca, NY) for dairies 1 and 3 and at Analab (Fulton, IL) for dairy 2. The DM of the feed was also estimated from the feed analyses (Dairy One and Analab). Simple sugars and TDN measurements were not available for dairy 2; therefore, the study power is lower for these estimations. Neutral detergent fiber was measured as ash-free amylase- and sodium sulfite–treated NDF (aNDFom) for dairies 1 and 3 and as amylase- and sodium sulfite–treated NDF for dairy 2. For NDF intake (kg/d) relationships, both were used in the analysis of pen responses.

Feed analysis results and details of subsequent statistical analyses are provided by Golder et al. (2019). The data in Table 2 were presented as kilograms of intake of the feed components. The diets, 5 per farm, were entered using the diets as formulated and matched to feed analyses, pen data including DIM, average BW, milk yield, fat, protein, and lactose, weather, and other inputs as required using CNCPS 6.55 (NDS Version 3.9.7.02, Emelia, Italy) and Molly (Baldwin, 1995) to provide an evaluation of limiting nutrients (data not shown; available on request). These findings are briefly discussed in context in the Results and Discussion section.

DMI Determination

The DMI was estimated from daily pen feed delivery weights from the mixer wagon and recorded using the Feed Supervisor feed management software for dairy 1 (Feed Supervisor, Supervisor Systems, K S Dairy Consulting Inc., Dresser, WI) and EZFeed (DHI-Provo, Provo, UT) for dairies 2 and 3. The dairies monitor feed residuals carefully and were evaluated for this during the study. For all 3 dairies DMI were on a per-pen basis and corrected for residual feed. Then total corrected DMI was divided by numbers of cows in the pen that day to estimate individual cow DMI. Cow numbers were obtained from Feed Supervisor and EZFeed programs and cross validated with the herd management records that identified the number of cows in pens. If there were differences between esti-

Table 1. Typical lactation diets by dairy (adapted from Golder et al., 2019[1])

Ingredient (% of DM)	Dairy 1	Dairy 2	Dairy 3
Forage:concentrate	56.0	28.1	32.2
Alfalfa hay		13.0	3.62
Alfalfa silage	18		
Almond hulls		5.80	12.1
AMINOPLUS[2]	1.60		
Blood meal	1.54		
Calcium salts of long-chain fatty acids	0.77		
Canola meal		15.0	11.0
Corn gluten		4.60	2.07
Corn ground	12.0		
Corn rolled		25.0	23.8
Corn silage	38.0	13.0	16.4
Cottonseed		6.20	
Cracked pita			6.89
Dried distillers grains			9.48
Energy booster 100[3]			0.84
EnerGII[4]		1.40	
Haylage			4.31
Mepron[5]	0.07		
Milo		3.70	
Minerals and vitamins[6]	3.13	2.49	1.50
Molasses whey		0.96	
Prilled fatty acids	1.04		
Sodium sesquicarbonate	1.18		
Soybean hulls	10.2		
Soybean meal	6.00		
Tallow	0.15		
Urea		0.20	0.11
Winter forage			3.96
Wheat hay			3.96
Wheat silage		2.10	
Wet citrus pulp		1.40	
Wet corn distillers	6.00	5.10	
Yeast	0.36[7]	0.05[8]	0.03[8]

[1]Republished with permission of Elsevier Science and Technology Journals, from "Effects of in-feed enzymes on milk production and components, reproduction, and health in dairy cows," H. Golder, H. Rossow, and I. Lean, vol. 102, pp. 8011–8026, 2019; permission conveyed through Copyright Clearance Center, Inc.

[2]AGP Ag Processing Inc., Omaha, NE.

[3]Milk Specialties Global Animal Nutrition, Eden Prairie, MN.

[4]Virtis Nutrition LLC, Corcoran, CA.

[5]Arm and Hammer Animal Nutrition, Princeton, NJ.

[6]Includes sources for Ca, P, Mg, K, Na, Cl, Mn, Zn, Cu, I, Co, Se, vitamin A, vitamin D, and vitamin E that will meet NRC (2001) requirements. Contains Rumensin 90 (Elanco, Greenfield, IN) at 200 mg/head per day at dairy 1 and at 450.4 mg/head per day at dairy 3.

[7]Cel-Con (Western Yeast Co., Chillicothe, IL), Omnigen AF (Phibro Animal Health Corp., Teaneck, NJ), Integral A+ (Alltech, Nicholasville, KY), and Procreatin 7 (Lesaffre, Marcq-en-Barœul, France).

[8]Diamond V XPC (Diamond V, Cedar Rapids, IA).

Table 2. Intake (mean ± SD) of constituents of typical lactating diets for control and enzyme-treated[1] pens by dairy

Item (kg of intake, unless otherwise specified)[2]	Dairy 1				Dairy 2				Dairy 3			
	Control		Enzyme		Control		Enzyme		Control		Enzyme	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CP	3.33	0.44	3.78	0.62	4.15	0.81	4.12	0.77	4.35	0.41	4.64	0.39
Soluble protein	1.61	0.35	1.38	0.22	1.13	0.25	1.11	0.23	1.45	0.22	1.34	0.17
Neutral detergent–insoluble CP	0.51	0.15	0.55	0.10	0.53	0.10	0.52	0.09	0.74	0.14	0.81	0.16
Acid detergent–insoluble CP	0.26	0.10	0.28	0.11	0.23	0.04	0.23	0.04	0.44	0.09	0.47	0.10
NDF					7.20	1.28	7.26	1.28				
aNDFom[3]	5.81	0.78	7.17	1.07					7.88	0.92	8.36	0.83
Lignin	0.69	0.13	0.79	0.21	0.76	0.14	0.76	0.12	1.37	0.25	1.48	0.28
Nonfiber carbohydrates[4]	7.40	1.05	8.46	1.52	5.11	1.07	5.16	1.07	10.17	1.12	10.94	1.03
Starch	4.99	0.77	5.74	1.18	5.11	1.07	5.14	1.09	6.21	0.98	6.61	0.87
Simple sugars	0.36	0.08	0.54	0.33					1.45	0.39	1.64	0.55
Crude fat	0.98	0.14	1.09	0.18	1.29	0.22	1.29	0.23	1.58	0.20	1.64	0.22
TDN	14.24	1.54	16.05	2.23					18.60	1.52	19.80	1.09
Ca (%)	0.14	0.01	0.18	0.04	0.22	0.03	0.22	0.04	0.13	0.02	0.14	0.02

[1]Enzyme-treated cows were exposed to a specific mix of fibrolytic enzymes (AB Vista, Marlborough, United Kingdom) that is a liquid pretreatment at a rate of 750 mL/t of DM of feed.

[2]Values are derived from feed analysis conducted at Dairy One (Ithaca, NY) for dairies 1 and 3 and at Analab (Fulton, IL) for dairy 2. The number of feed samples varied with pen and dairies.

[3]aNDFom = ash-free amylase- and sodium sulfite–treated NDF.

[4]Nonfiber carbohydrates = 100 − (NDF + CP + Fat + Ash).

Milk Production Data

All dairies milked 3 times per day. Milk samples were collected weekly from each pen during the a.m. milking using a 4-L milk bag with a hypodermic needle (gauge 14) inserted into a 7-port sampling cartridge installed in the milking line proximal to the cooling plate (QMI Aseptic Sampling System, Oakdale, MN). A different bag was used for each milking string, and bags were well mixed before taking a 5-mL subsample, which was analyzed for fat, protein, lactose, MUN, and SCC by the Minnesota (dairy 1), Tulare (dairy 2), and Fresno (dairy 3) DHI organizations (Table 3). The effects of enzyme, parity, dairy, and the interactions of these were described by Golder et al. (2019) and are adapted in Table 3. Milk urea nitrogen and SCC data were not examined. Daily milk production was recorded using GEA Group (Düsseldorf, Germany; dairy 1), BouMatic LLC (Madison, WI; dairy 2) and DeLaval (Tumba, Sweden; dairy 3) milking equipment. Individual cow milk fat, protein, and SCC were also recorded monthly at a.m. herd recording tests by the same DHI organizations. Pen movements were reported weekly and used to monitor whether cattle were present in the correct pens or were transferred to hospital pens. Data on BW and environment are not shown but available on request.

Statistical Analysis

The intention of time-series methods is to evaluate the relationships between variables that are potentially influenced by changes over time. This is achieved by removing strong trends in the data and evaluating relationships between the variables. Specifically, data from each pen and feed intake measure over time were detrended separately to produce an approximately stationary series (Shumway, 1988) using the Stata UVRS procedure (Spline fitting; Stata Version 15.1, StrataCorp LLC, College Station, TX). The data points produced effectively equate to residuals from these models. Cross-correlations (ρ) on these data are performed using the XCORR procedure of Stata for pairs of metabolites (x and y) for each lag (m) using the following model:

$$\rho_{xy}^T (m) = \frac{R_{xy}^T (m)}{\sqrt{R_x^T (0) R_y^T (0)}},$$

where it is assumed that series x and y are stationary and are observed at time points $t = 0, 1, \ldots, T - 1$ and the cross-covariance function is R as follows:

$$R_{xy}^T (m) = T^{-1} \sum_{t=0}^{T-1-m} (x_{t+m} - x^T)(y_t - y.^T), \quad m \geq 0$$

Table 3. Effects of enzyme treatment[1] at monthly DHIA herd recording and the estimated marginal means ± SE from monthly DHIA herd-recording data by dairy[2]

Outcome	Dairy 1		Dairy 2		Dairy 3		P-value						
	Control	Enzyme	Control	Enzyme	Control	Enzyme	Enzyme (E)	Parity (P)	Dairy (D)	E × P	E × D	E × P × D	DPCD
Milk yield (kg/d)	37.6 ± 0.84	38.2 ± 0.84	37.9 ± 0.82	41.5 ± 0.82	43.5 ± 0.57	43.7 ± 0.57	<0.001	<0.001	<0.001	0.180	<0.001	<0.001	<0.001
ECM (kg/d)	42.4 ± 0.76	41.6 ± 0.76	36.8 ± 0.75	41.0 ± 0.76	46.1 ± 0.51	46.2 ± 0.51	<0.001	<0.001	<0.001	0.504	<0.001	<0.001	<0.001
Fat (%)	4.56 ± 0.07	4.46 ± 0.07	3.80 ± 0.07	3.86 ± 0.07	4.03 ± 0.05	4.02 ± 0.05	0.914	0.072	<0.001	0.499	<0.001	0.782	0.541
Fat yield (kg/d)	1.73 ± 0.04	1.68 ± 0.04	1.35 ± 0.04	1.54 ± 0.04	1.77 ± 0.03	1.78 ± 0.03	<0.001	<0.001	<0.001	0.429	<0.001	<0.001	<0.001
Protein (%)	2.91 ± 0.03	2.92 ± 0.03	2.94 ± 0.03	2.91 ± 0.03	2.95 ± 0.01	2.92 ± 0.01	0.008	<0.001	0.898	0.001	0.163	<0.001	<0.001
Protein yield (kg/d)	1.10 ± 0.02	1.09 ± 0.02	1.06 ± 0.02	1.16 ± 0.02	1.29 ± 0.02	1.28 ± 0.02	<0.001	<0.001	<0.001	0.266	<0.001	<0.001	<0.001
Ln SCC	4.42 ± 0.06	4.34 ± 0.06	3.68 ± 0.06	3.87 ± 0.06	4.12 ± 0.04	4.09 ± 0.04	0.888	<0.001	<0.001	0.119	<0.001	<0.001	0.012
BW (kg)	654 ± 9.25	656 ± 9.25	564 ± 9.35	565 ± 9.44	635 ± 6.46	637 ± 6.42	0.322	<0.001	<0.001	0.058	0.895	0.605	0.894

[1]Enzyme-treated cows were exposed to a specific mix of fibrolytic enzymes (AB Vista, Marlborough, United Kingdom) that is a liquid pretreatment at a rate of 750 mL/t of DM of feed.

[2]Models used evaluated the fixed effects of days on precalving diet (DPCD), enzyme treatment, parity, dairy, and their interactions and the random effects of identity within treatment and dairy. Adapted from Golder et al. (2019). Estimated marginal means ± SE for control and enzyme groups are provided in the study by Golder et al. (2019). Republished with permission of Elsevier Science and Technology Journals, from "Effects of in-feed enzymes on milk production and components, reproduction, and health in dairy cows," H. Golder, H. Rossow, and I. Lean, vol. 102, pp. 8011–8026, 2019; permission conveyed through Copyright Clearance Center, Inc.

mates of numbers of cows in pens, data were deleted for that week (no. deleted = 3). The numbers include cows that may have been in the incorrect pen on that day.

and

$$R_{xy}^T\left(-m\right) = \ R_{yx}^T\left(m\right)$$

for negative lags (Shumway, 1988). These are assessed for lags of interest, from 0 lag (i.e., the same time) to several negative 3 lags of interest, representing relationships between feed components from 3 wk before the same week. Positive lags were not assessed as any effect of production on components of feed intake were not of interest. Cross-correlation coefficients were transformed using Fisher's transformation to allow a meta-analytic evaluation using each pen as a separate study. A random effects estimate of standardized mean difference was produced using meta-analytic methods (DerSimonian and Laird, 1986) with the METAN procedure of Stata, in which the transformed cross-correlations for each pen were treated as a separate study as described by Hedges and Vevea (1998). The ES of each observation, then, is given weight (ω):

$$\omega_i = 1 / \left[\mathrm{SE}\widehat{\left(\theta_i\right)}^2 + \hat{\tau}^2\right],$$

where (θ_i) is the ES for an individual (i) comparison, SE is the standard error of that ES, and $\hat{\tau}^2$ is the variance. The pooled ES is described by Bradburn et al. (2009), where $\hat{\theta}_{\mathrm{DL}}$ is the DerSimonion and Laird ES and estimated by

$$\hat{\theta}_{\mathrm{DL}} = \left(\Sigma\omega_i\hat{\theta}_i\right) / \left(\Sigma\omega_i\right)$$

and

$$\mathrm{SE}\left\{\hat{\theta}_{\mathrm{DL}}\right\} = 1 / \sqrt{\Sigma\omega_i}.$$

Effect sizes can be transformed back to correlations; however, final evaluations are presented using ES from meta-regression models. The effects of dairy and treatment and their interactions were tested using Knapp-Hartung meta-regression methods. The only significant effect of parity by treatment was on protein percentage in the study by Golder et al. (2019), and effects of parity were not further considered for this study.

RESULTS AND DISCUSSION

Enzyme treatments of feed for lactating dairy cattle have been characterized by variable results (Arriola et al., 2017; Tirado-González et al., 2018). This study provides unique insights to responses to enzyme treatment by using feed analysis and milk production responses over long periods in large commercial dairies. The results showed responses in milk production, composition, and component yield to intake of different dietary feed fractions over time, and they showed differences between responses to

some diet fractions for treatment and control pens that may be used to assist in the formulation of diets for cattle fed enzyme-treated TMR. We also noted very different responses to intake of nutrients among dairies, which also provide potential insights for diet formulation.

The time-series and meta-analytical methods used have been widely published including by Lean et al. (2009) and Rodney et al. (2018). The statistical models used for this study are likely to produce both type 1 and type II error, due to the relatively large number of hypotheses tested and a limited number of pen observations (n = 12). However, the time-series responses at the individual pen level had substantial power and each cross-correlation estimate is an individual study. Pooling these studies using meta-analytical methods is conservative as it relies on consistency among pens to provide a significant effect.

Differences in the tested feed analyses between treatment groups were expected as a result of the treatment (Table 2). Supplemental Figure S1A to S1C and Figure S2A to S2C (https://doi.org/10.15232/aas.2019-01943) show the CP and lignin content of lactation TMR and demonstrate that management on the dairies controlled the delivery of feed nutrients quite effectively to achieve consistent dietary formulations. The mean CV for CP % of DM among the dairies for the control and enzyme-treated pens were 5.88 and 5.70, respectively. For lignin % of DM, the most variable of the measures, mean CV among dairies were 15.95 and 17.21 for control and enzyme-treated pens, respectively. Figures 1 to 7 and Supplemental Tables S1 to S6 (https://doi.org/10.15232/aas.2019-01943) provide responses that can be evaluated in terms of milk production and constituent outcomes to changes in components of the diet. Milk constituent values are from a.m. milk samples only so may differ from mean daily milk constituent values; however, samples were consistently collected allowing treatment comparisons. Effects at time zero, that is, on the same week, are associations, whereas outcomes with antecedent intake and production outcomes 1 to 3 wk later could be considered causal. The ES are very similar to an r-value, differing only at the second or third decimal. Consequently, regression values can be approximated using the square of these.

Overall Effects of Change in Dietary Intake on Production Responses

There were few overall effects for milk production (Figure 1 and Supplemental Table S1; https://doi.org/10.15232/aas.2019-01943). Figure 1 shows that only neutral detergent–insoluble CP (**NDICP**) in the week before had a positive overall effect on milk production (ES = 0.158; *P* = 0.013), whereas starch 3 wk before (*P* = 0.045) and crude fat at the same time had negative associations (ES = −0.106; *P* = 0.029) with milk production. The ES for NDF and milk 1 wk later was large but not significant. It might be expected that fat would increase milk volume and depress milk protein percentage (Wu and Huber,
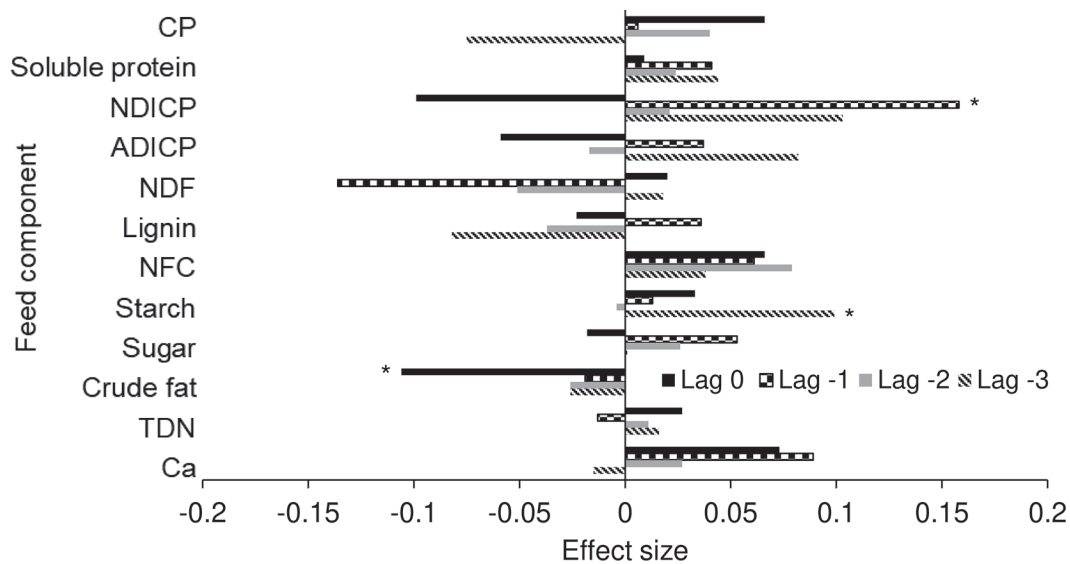
Figure 1. The effect size (ES) results of the meta-analysis of time series cross-correlations for the overall associations of intake (kg/d) of feed components of the diet with milk yield for enzyme-treated and control pens on 3 dairies. For each feed component, the ES, which approximates a correlation, is shown for the association at the same time (lag 0), for intake of the feed component 1 wk (lag −1), 2 wk (lag −2), and 3 wk (lag −3) before with milk yield. Enzyme-treated cows were exposed to a specific mix of fibrolytic enzymes (AB Vista, Marlborough, United Kingdom) that is a liquid pretreatment at a rate of 750 mL/t of DM of feed. * denotes significant ES with $P < 0.05$. NDICP = neutral detergent insoluble CP; ADICP = acid detergent insoluble CP; NFC = nonfiber carbohydrates.

1994; Rabiee et al., 2012); however, given that milk production responses to supplemental fat are heterogeneous and often negative (Rabiee et al., 2012), the negative ES is not surprising. There are often lags between the supply of additional nutrients and milk production responses, with only 50% of additional dietary energy being evident in milk in the first 21 d (Broster and Broster, 1984). Factors that influence milk production responses include the partitioning of additional nutrients to body tissue (Broster and Broster, 1984) and alterations to the rumen microflora that may not immediately stimulate additional production (Russell, 2007; Golder et al., 2014b). Cows fed enzyme did not gain significantly more weight than the controls (Golder et al., 2019), and short-term partitioning to tissue pools would be unlikely to be detected.

Milk fat percentage was significantly associated with increased CP intake (kg/d on the same week [Figure 2 and Supplemental Table S2 (https://doi.org/10.15232/aas .2019-01943): ES = 0.290; $P = 0.001$]. Similarly, fat yield increased on the same week with increased CP intake (Figure 3, Supplemental Table S3: ES = 0.217; $P = 0.001$). Both of these effects were large and explained more than 8 and 4% of the variance in milk fat percentage and yield, respectively. The intake (kg/d) of NDICP 2 wk before was associated with lower milk fat percentage (ES = −0.139; $P = 0.005$) and with milk fat yield (Figure 3; ES = −0.104; $P = 0.036$). All 3 dairies had diets containing approximately 16% adjusted CP (Golder et al., 2019). Dairy 3 was the most limited by MP of the 3 dairies as assessed by CNCPS 6.55, a finding consistent with Molly evaluation of nitrogen balances. It is very possible that the CP intake

in the diet was not sufficient to optimize milk fat percentage and production and that the quite large and positive responses to change in CP intake on the same day reflect this situation. Leonardi et al. (2003) and Colmenero and Broderick (2006) found that increased intake of CP increased milk fat content, by 0.44 and 0.3%, respectively, when CP in the diet was increased from 16 to 18.8% and linearly from 13.5 to 19.4%, respectively. Notably, neither study found increased milk yield with the increased CP content of the diet (Leonardi et al., 2003; Colmenero and Broderick, 2006).

The nonfiber carbohydrate (**NFC**) intake (kg/d) 3 wk before was associated with increased fat percentage (ES = 0.106; $P = 0.030$) and milk fat yield (kg/d; Figure 3; ES = 0.102; $P = 0.038$). Intake (kg/d) of TDN was positively associated with fat percentage on the same week (ES = 0.122; $P = 0.034$) and 2 wk before (ES = 0.118; $P = 0.041$), indicating that intake of energy either as rapidly fermentable carbohydrates or as TDN had both immediate and longer-term positive effects on milk fat content.

Figures 4 and 5 (Supplemental Tables S4 and S5, respectively; https://doi.org/10.15232/aas.2019-01943) show that, in contrast with milk production (Figure 1) and fat percentage (Figure 2) and yield (Figure 3), there were many more significant effects for milk protein percentage and yield and many of the results were consistent between change in protein percentage and yield. Milk protein percentage and yield increased with increased CP intake (kg/d) 2 wk before (Figure 4; ES = 0.147; $P = 0.003$; Figure 5; ES = 0.157; $P = 0.013$, respectively). Soluble protein intake (kg/d) was associated with increased milk
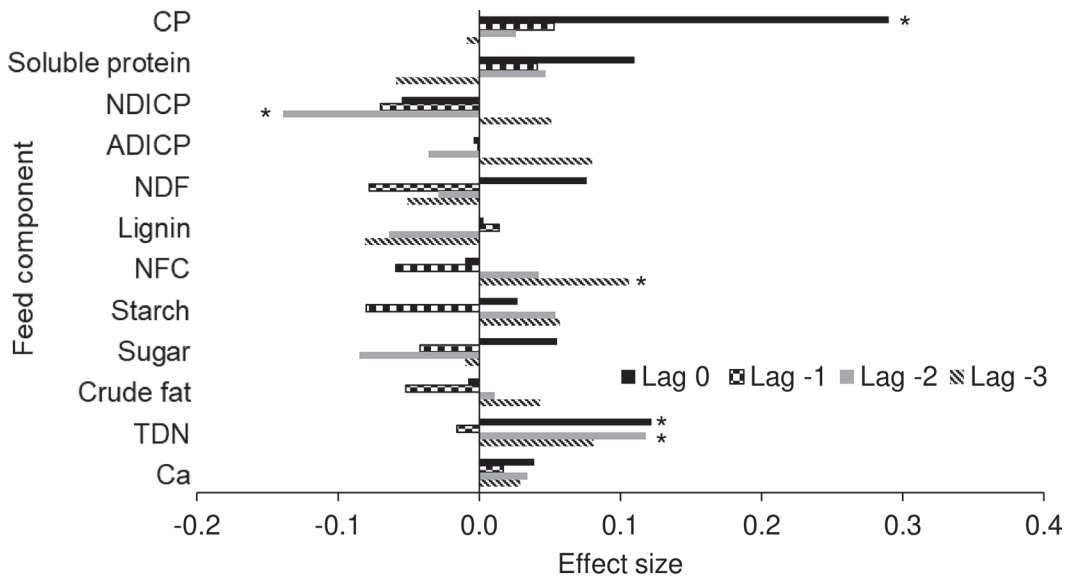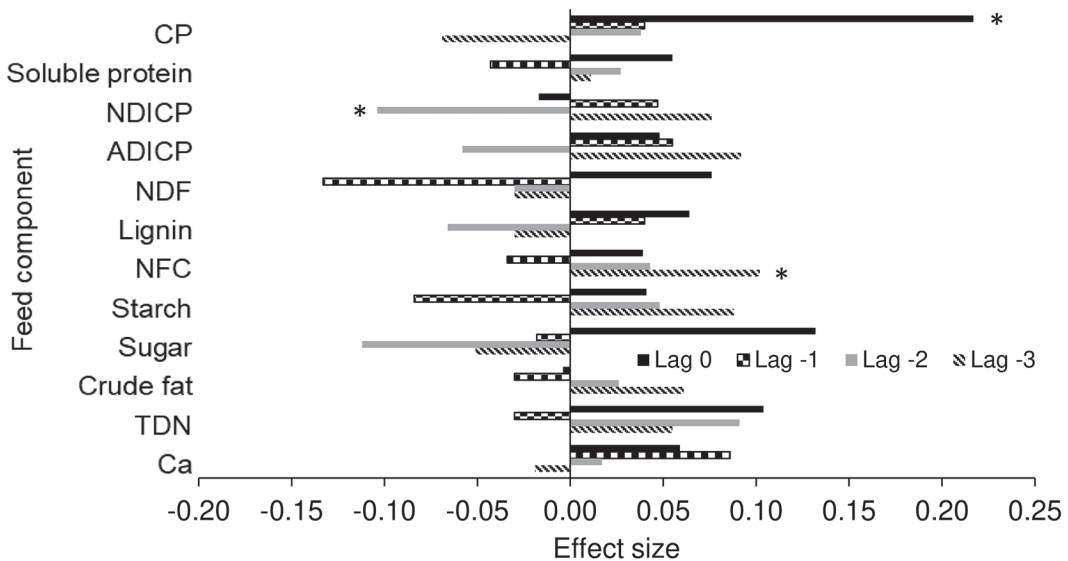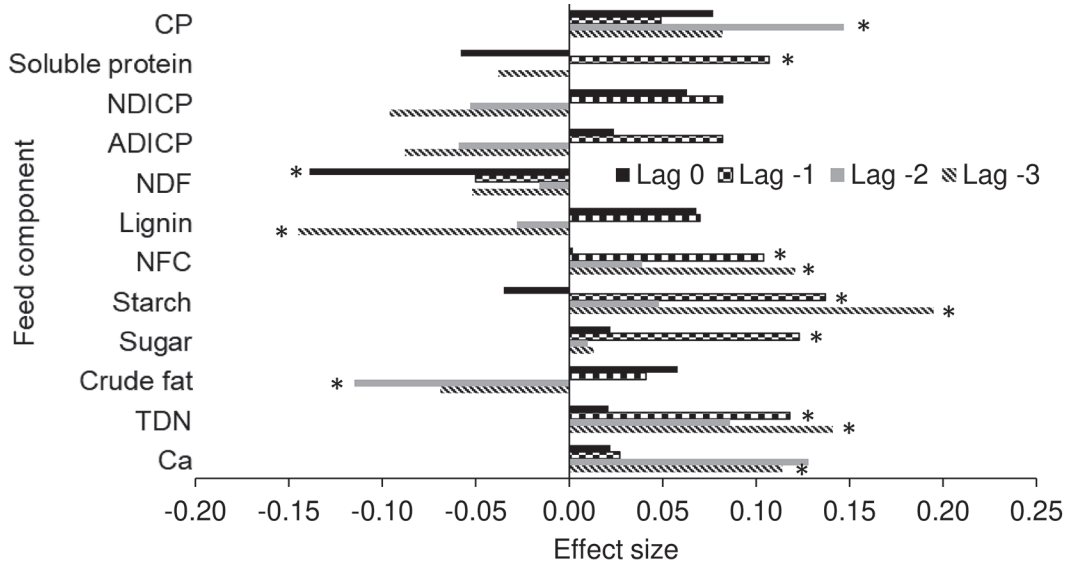
Figure 2. The effect size (ES) results of the meta-analysis of time series cross-correlations for the overall associations of intake (kg/d) of feed components of the diet with milk fat percentage for enzyme-treated and control pens on 3 dairies. For each feed component, the ES, which approximates a correlation, is shown for the association at the same time (lag 0), for intake of the feed component 1 wk (lag −1), 2 wk (lag −2), and 3 wk (lag −3) before with milk fat percentage. Enzyme-treated cows were exposed to a specific mix of fibrolytic enzymes (AB Vista, Marlborough, United Kingdom) that is a liquid pretreatment at a rate of 750 mL/t of DM of feed. * denotes significant ES with $P < 0.05$. NDICP = neutral detergent insoluble CP; ADICP = acid detergent insoluble CP; NFC = nonfiber carbohydrates.

protein percentage and yield 1 wk later (ES = 0.107; P = 0.028 and ES = 0.111; P = 0.022, respectively). Intake (kg/d) of NDICP tended to reduce milk protein percentage 3 wk later (ES = −0.096; P = 0.055) but increased

milk protein yield 1 wk later (ES = 0.158; P = 0.002). The findings (Supplemental Tables S1 to S6 and Figures 1 to 5) regarding overall production responses to protein fractions in the diet show that CP, soluble protein, and
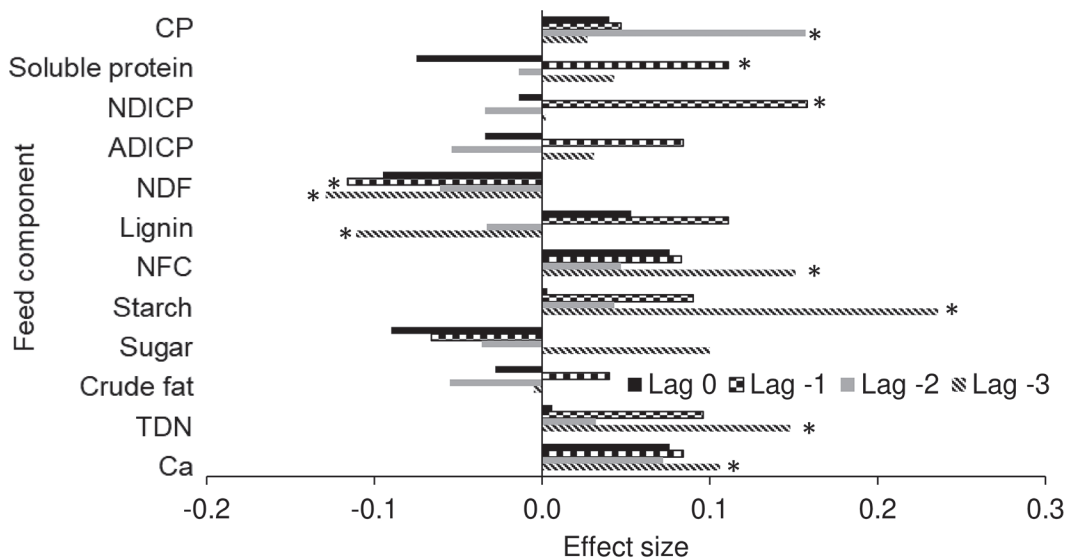

Figure 3. The effect size (ES) results of the meta-analysis of time series cross-correlations for the overall associations of intake (kg/d) of feed components of the diet with milk fat yield for enzyme-treated and control pens on 3 dairies. For each feed component, the ES, which approximates a correlation, is shown for the association at the same time (lag 0), for intake of the feed component 1 wk (lag −1), 2 wk (lag −2), and 3 wk (lag −3) before with milk fat yield. Enzyme-treated cows were exposed to a specific mix of fibrolytic enzymes (AB Vista, Marlborough, United Kingdom) that is a liquid pretreatment at a rate of 750 mL/t of DM of feed. * denotes significant ES with $P < 0.05$. NDICP = neutral detergent insoluble CP; ADICP = acid detergent insoluble CP; NFC = nonfiber carbohydrates.

Figure 4. The effect size (ES) results of the meta-analysis of time series cross-correlations for the overall associations of intake (kg/d) of feed components of the diet with milk protein percentage for enzyme-treated and control pens on 3 dairies. For each feed component, the ES, which approximates a correlation, is shown for the association at the same time (lag 0), for intake of the feed component 1 wk (lag −1), 2 wk (lag −2), and 3 wk (lag −3) before with milk protein percentage. Enzyme-treated cows were exposed to a specific mix of fibrolytic enzymes (AB Vista, Marlborough, United Kingdom) that is a liquid pretreatment at a rate of 750 mL/t of DM of feed. * denotes significant ES with $P < 0.05$. NDICP = neutral detergent insoluble CP; ADICP = acid detergent insoluble CP; NFC = nonfiber carbohydrates.

NDICP increased milk protein percentage and yield, but increased NDICP intake reduced milk fat percentage and yield 2 wk later.

A higher NDF intake (kg/d) reduced milk protein percentage in the same week (ES = −0.139; $P = 0.007$), whereas intake (kg/d) of NDF was associated with lower protein yield (kg/d) 1 wk later (ES = −0.116; $P = 0.024$) and 3 wk later (ES = −0.129; $P = 0.013$) and responses to NDF intake differed between treatments. Increased lignin intake (kg/d) was associated with lower milk protein percentage and yield 3 wk later (ES = −0.145; $P = 0.003$ and ES = −0.111; $P = 0.023$, respectively). These findings

Figure 5. The effect size (ES) results of the meta-analysis of time series cross-correlations for the overall associations of intake (kg/d) of feed components of the diet with milk protein yield for enzyme-treated and control pens on 3 dairies. For each feed component, the ES, which approximates a correlation, is shown for the association at the same time (lag 0), for intake of the feed component 1 wk (lag −1), 2 wk (lag −2), and 3 wk (lag −3) before with milk protein yield. Enzyme-treated cows were exposed to a specific mix of fibrolytic enzymes (AB Vista, Marlborough, United Kingdom) that is a liquid pretreatment at a rate of 750 mL/t of DM of feed. * denotes significant ES with $P < 0.05$. NDICP = neutral detergent insoluble CP; ADICP = acid detergent insoluble CP; NFC = nonfiber carbohydrates.

are consistent with the expected responses in milk protein production and yield to increased NDF and lignin in the diet.

Milk protein percentage increased with NFC intake (kg/d) both 1 and 3 wk before (ES = 0.104; P = 0.032 and ES = 0.121; P = 0.026, respectively), whereas increased NFC intake (kg/d) only increased milk protein yield 3 wk later (ES = 0.151; P = 0.020). Milk protein percentage and yield responses to starch were similar to NFC, with increased percentages 1 wk and 3 wk later (ES = 0.137; P = 0.005; ES = 0.195; P = 0.005) and increased milk protein yield 3 wk later (ES = 0.236; P = 0.001). Sugar intake (kg/d) the week before also increased milk protein percentage (ES = 0.123; P = 0.038). However, there were no effects of sugar on protein yield. Further, increased intake (kg/d) of TDN 1 and 3 wk before increased milk protein percentage (ES = 0.118 and 0.141; P = 0.041 and 0.014, respectively) and increased milk protein yield 3 wk later (ES = 0.148; P = 0.010). The role of NFC in stimulating microbial protein production has been long recognized (Oldham, 1984) and is integral to modern nutritional models. Our findings strongly support the importance of fermentable carbohydrates and TDN to production of milk protein and indicate that both short-term and longer responses over 3 wk may be important to consider. Longer-term responses may well reflect substrate-induced changes in the ruminal meta-taxome and rumen function over periods of weeks (Golder et al., 2014a). Such responses are implicitly recognized in the use of wash-out periods for Latin-square and crossover research study designs.

Increased fat intake (kg/d) reduced milk protein percentage 2 wk later (ES = −0.115; P = 0.018), a finding consistent with milk protein percentage responses to fat supplementation (Wu and Huber, 1994; Rabiee et al., 2012). Immediate production responses to supplemental fat are not always observed (Jenkins et al., 1998) and milk and milk fat and protein percentage and yield responses to supplemental fat intervention overall were heterogeneous (Rabiee et al., 2012), supporting observations reviewed by Palmquist and Jenkins (1980). Further, Jenkins et al. (1998) found that milk protein yield only consistently separated between treated cows supplemented with 5% tallow after 5 wk of treatment, suggesting that there may well be lags in production responses to fat treatments.

Increased soluble protein intake (kg/d) decreased lactose yield in the same week (ES = −0.126; P = 0.030; Supplemental Table S6; https://doi.org/10.15232/aas .2019-01943). Increased NDICP intake (kg/d) was associated with increased lactose yield 1 wk later (ES = 0.135; P = 0.010) but decreased lactose yield 3 wk later (ES = −0.108; P = 0.030). Similarly, the intake of acid detergent–insoluble CP (**ADICP**) was associated with increased lactose yield 1 wk later (ES = 0.131; P = 0.033). Sugar intake (kg/d) 2 wk before (ES = −0.161; P = 0.007) was significantly associated with lower lactose yield. These findings suggest that increased soluble protein availability influenced lactose yield by either providing additional sub-

strate for microbial protein synthesis and reducing availability of glucose precursors or by reducing energy availability for lactose production with energetic costs of urea formation (Huntington and Reynolds, 1987; Eisemann and Nienaber, 1990). The changes in lactose yield response to NDICP and ADICP may reflect increased lactose yield responses to intestinal supply of AA from feed (Oldham, 1984).

Increased Ca intake (kg/d) increased milk protein percentages (ES = 0.114; P = 0.024) 3 wk later, and Ca intake (kg/d) 1 wk before was associated with increased lactose yield (ES = 0.141; P = 0.005).

Treatment Differences in Response

There were relatively few instances of differences between the enzyme-treated and control pens to changes in nutrient intakes over time, as there were no differences in treatment effects on milk production, fat percentage, fat yield, or lactose yield (Supplemental Tables S1 to S3 and S6, respectively; https://doi.org/10.15232/aas.2019 -01943). Differences were evident for milk protein percentage and yield (Figures 6 and 7; Supplemental Tables S4 and S5, respectively). Quite frequently, as detailed later, either the enzyme-treated or control pens had significant associations with changes in nutrient intakes over time but did not significantly differ in effect from the other group.

Milk protein percentage responses to soluble protein differed for the treatments (ES = 0.249; P = 0.028; Figure 6 and Supplemental Table S4) with control pens having a negative response (ES = −0.196; P = 0.005; Supplemental Table S4) to extra intake of CP on the same week. Milk protein percentage responses to intake (kg/d) of ADICP also differed between treated and control pens (ES = 0.293; P = 0.017; Figure 6), with the treated pens having a positive response (ES = 0.240; P = 0.025). Although changes in the intake (kg/d) of ADICP did not increase milk protein yield (kg/d), there was a difference between treated and control pens (ES = 0.276; P = 0.023) as treated pens increased protein yield 1 wk after an increase in ADICP (ES = 0.213; P = 0.003). Rode et al. (1999) found an increase of 8.1% in digestibility of protein for a diet with a basal protein digestibility of 61.7 when applying a similar cellulase–xylanase enzyme to feed, indicating that changes in protein availability are possible following enzyme application. Rode et al. (1999) also found increased DM digestibility and increased digestibility of protein and ADF and NDF fractions of the diet with the enzyme treatment. Increased intake of soluble protein could be expected to reduce milk protein percentage, if the availability of ammonia was in excess of needs and resulted in energy loss in urea formation (Huntington and Reynolds, 1987; Eisemann and Nienaber, 1990). However, increased digestibility of protein fractions could lead to increased availability of peptides and AA and increase microbial nitrogen synthesis (Russell and Strobel, 1993; Van Kessel and Russell, 1996), and the addition of urea to AA in vitro increased
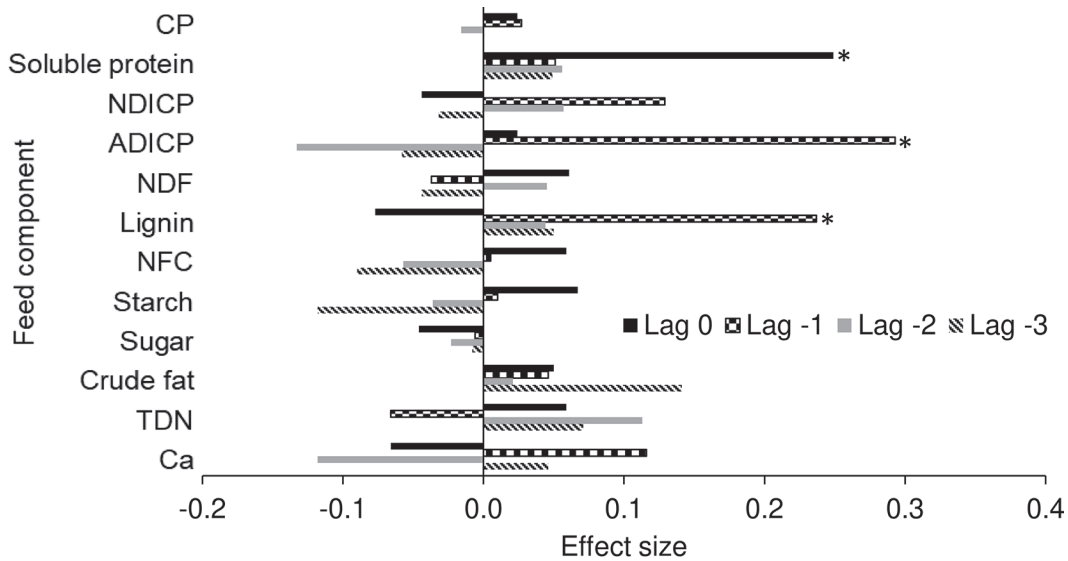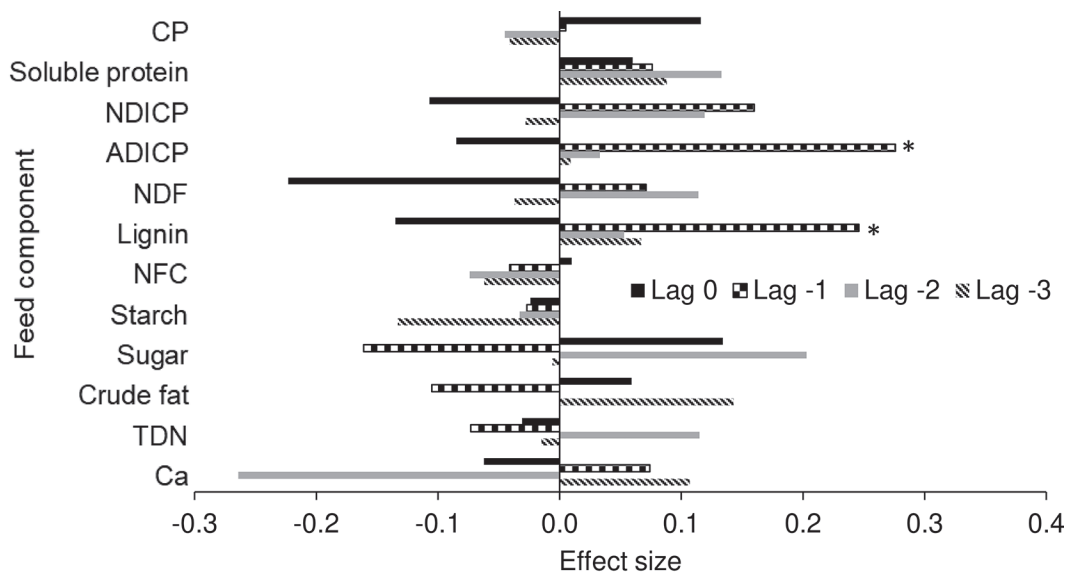
Figure 6. The effect size (ES) results of the meta-analysis of time series cross-correlations for differences between the enzyme-treated and control pens for intake (kg/d) of feed components of the diet with milk protein percentage for treated and control pens on 3 dairies. For each feed component, the ES, which approximates a correlation, is shown for the association at the same time (lag 0), for intake of the feed component 1 wk (lag −1), 2 wk (lag −2), and 3 wk (lag −3) before with milk protein percentage. Enzyme-treated cows were exposed to a specific mix of fibrolytic enzymes (AB Vista, Marlborough, United Kingdom) that is a liquid pretreatment at a rate of 750 mL/t of DM of feed. * denotes significant ES with $P < 0.05$. NDICP = neutral detergent insoluble CP; ADICP = acid detergent insoluble CP; NFC = nonfiber carbohydrates.

microbial protein yield (Maeng and Baldwin, 1976). Yang et al. (1999) found increased microbial protein production with a similar enzyme treatment and a point direction to higher milk protein percentage in treated cattle. Hence, a differential effect for treatment for soluble protein responses and also for the protein-associated fiber fractions is consistent with expected changes in rumen function and the observed responses.

The treatment and control pens differed in milk protein percentage response to increased lignin intake (kg/d) 1 wk

Figure 7. The effect size (ES) results of the meta-analysis of time series cross-correlations for differences between the enzyme-treated and control pens for intake (kg/d) of feed components of the diet with milk protein yield for treated and control pens on 3 dairies. For each feed component, the ES, which approximates a correlation, is shown for the association at the same time (lag 0), for intake of the feed component 1 wk (lag −1), 2 wk (lag −2), and 3 wk (lag −3) before with milk protein yield. Enzyme-treated cows were exposed to a specific mix of fibrolytic enzymes (AB Vista, Marlborough, United Kingdom) that is a liquid pretreatment at a rate of 750 mL/t of DM of feed. * denotes significant ES with $P < 0.05$. NDICP = neutral detergent insoluble CP; ADICP = acid detergent insoluble CP; NFC = nonfiber carbohydrates.

before (ES = 0.237; P = 0.035; Figure 6 and Supplemental Table S4; https://doi.org/10.15232/aas.2019-01943), with treated pens having a positive response (ES = 0.186; P = 0.007). Similarly, treated pens differed from control pens (ES = 0.246; P = 0.039; Figure 7 and Supplemental Table S5) for milk protein yield response to lignin intake (kg/d) 1 wk before and had an increase in ES (0.227; P = 0.001). Although studies found increased ADF digestibility with a similar cellulase–xylanase treatment (Beauchemin et al., 1995; Krause et al., 1998; Rode et al., 1999), the effects of treatment on lignin do not appear to be reported. The herds had percentages of NDF or ash-free amylase- and sodium sulfite–treated NDF (aNDFom) of 30 to 34% of the diet (Golder et al., 2019) and, at times, lignin concentrations as low as 2 to 4% (Supplemental Figures S2A–S2C). It is possible that the additional lignin was more important for the enzyme-treated pens in providing rumen stability and preventing acidosis. However, for treated pens increased NDF intake (kg/d) was negatively associated with milk production on the same week (ES = −0.266; P = 0.001). The latter finding indicates that an increased intake of the more digestible fiber, that is, as NDF, did not have an immediately favorable change in milk production and is consistent with the decline in milk protein percentage observed for increased NDF and increase in protein percentage with increased NFC on the same week.

Treatment and Pen Responses

For the following results, changes in intake of dietary constituents for the control pens or treated pens had a significant association with the production outcomes but did not significantly differ from the other group of pens (P > 0.1) that did not have a significant association. The results are in the footnotes to each Supplemental Table (Supplemental Tables S1 to S6; https://doi.org/10.15232/aas.2019-01943). The results are similar to those for the treatment responses but highlight observations made on the overall responses and those to treatment.

Crude protein intake (kg/d) 3 wk before was associated with increased milk production in the treatment group only (ES = 0.145; P = 0.029; Supplemental Table S1), and NDICP intake tended to be associated with increased milk yield 1 wk later for treated pens (ES = 0.242; P = 0.057). Intake of NDF in the same week reduced milk yield in the treated pens (ES = −0.266; P = 0.001). Also starch intake 3 wk before for the control group was significantly associated with milk production (ES = 0.172; P = 0.019).

Milk fat percentage and intake (kg/d) of NDICP 2 wk before was negatively associated for treated pens (ES = −0.159; P = 0.027; Supplemental Table S2). Sugar intake 2 wk before was negatively associated with fat percentage for treated pens (ES = −0.218; P = 0.017; Supplemental Table S2). The NDF intake (kg/d) 1 wk before was negatively associated with milk fat percentage for the treated pens (ES = −0.181; P = 0.048). Treated pens had a more positive response than control pens for associations with

CP intake (kg/d) 3 wk before and fat yield (kg/d; ES = 0.156; P = 0.019; Supplemental Table S3); similarly, control pens had a positive association with starch intake 3 wk before (ES = 0.166; P = 0.023). The latter results are consistent with the responses to CP intake 3 wk before and milk production, suggesting that longer-term responses to nutrients are an important part of the responses to diet and form part of the adaptive response to the enzyme treatment.

Control pens had a negative association between ADICP 2 wk before and milk fat yield (ES = −0.150; P = 0.03), and milk fat yield response to sugar intake (kg/d) 2 wk before was lower in the treated pens (ES = −0.238; P = 0.026). Control pens also had a significant decrease in milk fat yield associated with change in sugar intake 3 wk before (ES = −0.208; P = 0.011). The large ES for the sugar associations represent 4 to 6% of the variance in fat yield and suggest an important role for sugars in influencing milk fat yield. The negative associations for sugars with fat percentage and yield within the treatment and control pens appear to differ from observations in a study where starch was incrementally replaced with sucrose (Broderick et al., 2008) or when molasses was increased to 2.4 to 7.4% sugars in the diet by replacing high-moisture corn (Broderick and Radloff, 2004). In both studies fat percentage remained stable and fat yield increased; however, in both studies additional sugar content increased DMI (Broderick and Radloff, 2004; Broderick et al., 2008). In evaluating the time-series relationships between sugars and additional grams of sugar intake, we are not evaluating the overall effect of dietary change as considered by Broderick and Radloff (2004) and Broderick et al. (2008).

Control pens had a decrease in milk protein percentage with increased CP intake on the same week (ES = −0.196; P = 0.005; Supplemental Table S4; https://doi .org/10.15232/aas.2019-01943) and an increase in milk protein percentage with increased CP intake (kg/d) 2 wk before (ES = 0.155; P = 0.024). The immediate responses contrast with the overall dietary effect to increase in milk fat percentage with CP intake on the same week. Intake of ADICP was associated with increased milk protein percentage in the treated pens 1 wk later (ES = 0.240; P = 0.025) but tended to decrease milk protein percentage for those pens 2 and 3 wk later (ES = −0.18; P = 0.075 and ES = −0.121; P = 0.079, respectively). A higher NDF intake (kg/d) reduced milk protein percentage in the same week for treated pens (ES = −0.163; P = 0.020); however, lignin intake was associated with a greater milk protein percentage 1 wk later in treated pens (ES = 0.186; P = 0.007). The control pens had an increase in milk protein percentage with increased NFC intake (kg/d) 3 wk before (ES = 0.121; P = 0.040). Starch intake (kg/d) increased milk protein percentage for treated pens 1 wk later (ES = 0.142; P = 0.039), and control pens had an increase in milk protein percentage associated with increased starch intake (kg/d) 3 wk previously (ES = 0.259; P = 0.011). Intake of Ca 2 wk before was associated with increased

milk protein percentage in control pens (ES = 0.188; P = 0.014).

Although milk protein percentage responses did not differ between treated and control pens to increased lignin intake (kg/d) 3 wk before, both treated and control pens were negative (ES = −0.144; P = 0.003 and ES = −0.147; P = 0.054, respectively). This finding is potentially important as the overall response and that for both control and treated pens was significant, suggesting a longer-term effect of lignin on milk protein percentage. However, the finding of an increase in milk protein percentage of the control pens to starch 3 wk previous highlights the limitation of univariable analysis, as the potential for confounding or interaction of intake of other nutrients is possible. Future studies could be conducted using multivariable time-series methods.

Milk protein yield responses for the pens were similar to those for milk protein percentage; however, the treated pens had an increase in milk protein yield (kg/d) 2 wk after an increase in CP intake (ES = 0.157; P = 0.023; Supplemental Table S5; https://doi.org/10.15232/aas.2019 -01943). Treated pens had an increase in protein yield 1 wk later (ES = 0.149; P = 0.023) with increased intake of soluble protein. Intake (kg/d) of NDICP increased protein yield for treated pens 1 wk later (ES = 0.232; P = 0.002), whereas ADICP lowered protein yield for treated pens 1 wk later (ES = 0.213; P = 0.003). These findings suggest that the current analytical methods that evaluate CP and, therefore, AA supply to the small intestine are correctly identifying the fractions of the CP that are available and unavailable in the intestine. On the same week treated pens had lower milk protein yield with increased NDF intake (kg/d; ES = −0.250; P = 0.001) and also had lower milk protein yield associated with increased NDF intake (kg/d) 3 wk before (ES = −0.149; P = 0.044). Control pens had lower milk protein yield associated with increased intake (kg/d) of NDF 1 wk before (ES = −0.145; P = 0.040) but increased milk protein yield associated with lignin intake 1 wk before (ES = 0.227; P = 0.001). Treated pens had higher milk protein yield with increased Ca intake (kg/d) 3 wk earlier (ES = 0.153; P = 0.030).

The control pens increased milk protein yield 3 wk after increased NFC intake (kg/d; ES = 0.188; P = 0.010; Supplemental Table S5; https://doi.org/10.15232/aas .2019-01943). Milk protein yield responses to starch were similar to NFC as both the treated (ES = 0.167; P = 0.033) and control pens (ES = 0.308; P = 0.001) had increased milk protein yield 3 wk after increased starch intake (kg/d). Treated pens increased protein yields 3 wk after increased TDN (ES = 0.181; P = 0.028). The responses to NFC, starch, and TDN support the important role of energy, particularly readily fermentable carbohydrates in milk protein production, but also highlight the delays in response even in pens of cattle fed for prolonged periods on similar diets.

The intake of soluble CP (kg/d) lowered lactose yield of the control pens (ES = −0.197; P = 0.015; Supplemen-

tal Table S6; https://doi.org/10.15232/aas.2019-01943) on the same week. For treated pens also, an increase in NDICP intake (kg/d) increased lactose yield 1 wk later (ES = 0.194; P = 0.038). Increased lignin intake (kg/d) was associated with decreased lactose yield (ES = −0.146; P = 0.034) on the same day for control pens. Treated pens responded to increased sugar intake 2 wk before with lower milk lactose yield (ES = −0.283; P = 0.002) but had increased lactose yield with increased sugar intake 3 wk before (ES = 0.148; P = 0.085).

Differences in Response Among Dairies

Differences in response to dietary intake of nutrients should be expected among dairies given the heterogeneity of responses observed in meta-analyses (Arriola et al., 2017; Tirado-González et al., 2018). The study power for dairy effects was lower than for treatment; however, there were more dairy differences, particularly between the 2 California dairies (dairies 2 and 3) and the dairy in South Dakota (dairy 1). Method of enzyme application has been identified as a possible source of variation in response to treatment (Arriola et al., 2017). All dairies used similar protocols and applied enzyme to feed in mixer wagons (Golder et al., 2019). Compliance on dairy 1, as assessed by recording of enzyme use, was similar to the other dairies (Golder et al., 2019). It appears plausible, given that dairy 1 had an intermediate production response to dairies 2 and 3 (Golder et al., 2019), that the differences in dairy responses may reflect differences in formulation and feeds. Dairy 1 had the highest NDF or ash-free amylase- and sodium sulfite–treated NDF (aNDFom) in their diet (Table 2), whereas dairy 2, the highest responding, had the highest CP but lowest soluble CP supply (Table 2).

Dairy 1 differed from dairy 2, which had a positive association between soluble protein 3 wk before and milk production (ES = 0.386; P = 0.040; Supplemental Table S1; https://doi.org/10.15232/aas.2019-01943). Dairies 2 and 3 had large positive associations with lignin intake (kg/d) 3 wk before and milk production (ES = 0.478 and 0.438, respectively) and differed from dairy 1 (P = 0.015 and 0.019, respectively). The milk production response to NFC intake or starch intake (kg/d) on dairy 3 differed from dairy 1 (ES = 0.442; P = 0.034 and ES = 0.431; P = 0.018, respectively) on the same week. The effects of fat intake 3 wk before on milk production differed for dairies 2 and 3 compared with dairy 1, which was very positive (ES = 0.510; P = 0.016 and ES = 0.429; P = 0.032, respectively). Dairies 2 and 3 produced more milk than dairy 1 (Golder et al., 2019) and had numerically higher starch and fat percentages in the diet. Notwithstanding those findings, these dairies had a more substantial response to starch and fat intake 3 wk before.

The effect of ADICP on milk fat percentage differed between dairy 1 and both dairies 2 and 3, which had marked negative associations on the same week (ES = −0.578; P = 0.014 and −0.571; P = 0.013, respectively; Supplemen-

tal Table S2; https://doi.org/10.15232/aas.2019-01943). These findings were also reflected in dairies 2 and 3 and differed from dairy 1 in large negative associations with milk fat yield and ADICP intake (kg/d) on the same week (ES = −0.545 and −0.477; P = 0.019 and 0.030, respectively; Supplemental Table S3). As noted earlier, increased CP has increased milk fat percentage (Leonardi et al., 2003; Colmenero and Broderick, 2006), and therefore, increasing the least available protein fraction to the rumen may reduce milk fat percentage on high-producing dairies, such as dairies 2 and 3, where the adjusted CP is 16% or less of diet (Golder et al., 2019).

Dairy 2, which had the lowest lignin content in the diet (Supplemental Figure S2B), had a more positive association between lignin intake (kg/d) the week before and fat percentage than dairy 1 (ES = 0.415; P = 0.027; Supplemental Table S2), and this response was reflected also in a more positive association between lignin intake (kg/d) with milk fat yield 1 wk later (ES = 0.430; P = 0.023; Supplemental Table S3) than dairy 1. Dairy 3 had a more negative association between lignin intake (kg/d) 2 wk before and fat percentage (ES = −0.349; P = 0.047; Supplemental Table S2) than dairy 1. Dairy 3 had the highest intake of lignin (Supplemental Figure S2C) and was the highest producing.

Dairy 2 differed from dairy 1 in that fat intake (kg/d) was more negatively associated with milk fat percentage on the same week (ES = −0.487; P = 0.040; Supplemental Table S2; https://doi.org/10.15232/aas.2019-01943), a result reflected in a more negative effect on milk fat yield for dairy 2 (ES = −0.442; P = 0.025; Supplemental Table S3) than for dairy 1. There were differences in supplemental fats used on all 3 dairies and also in diet structure that might explain this response. A prilled fat source was used in dairy 1 and, although the evidence base was limited, Rabiee et al. (2012) found that prilled fats produced a nonsignificant reduction in milk protein percentage, whereas other fats studied reduced milk fat percentage.

Milk protein percentage responses to NDICP intake (kg/d) 1 wk earlier differed between dairy 3 and dairy 1, with dairy 3 having a more negative response (ES = −0.474; P = 0.029; Supplemental Table S4). Dairy 2 differed from dairy 1 (ES = 0.392; P = 0.037) in milk protein percentage responses to lignin intake (kg/d) 3 wk earlier.

Dairy 3 increased milk protein percentage in the same week more than dairy 1 (ES = 0.363; P = 0.041) when NFC intake (kg/d) increased, and consistently, dairy 3 also had a more positive response in milk protein percentage and protein yield to increased starch intake (kg/d) in the same week (ES = 0.353; P = 0.044; ES = 0.382; P = 0.053, respectively) than dairy 1. Dairies 2 and 3 had increased milk protein percentages with increased fat feeding 3 wk previously compared with dairy 1 (ES = 0.385 and 0.410; P = 0.040 and 0.025, respectively), and similarly, increased fat intake (kg/d) resulted in dairy 2 having a greater increase in protein yield 3 wk later than dairy 1 (ES = 0.383; P = 0.040; Supplemental Table S5).

Dairy 3 differed from dairy 1 in having reduced milk protein percentage and protein yield responses to Ca intake (kg/d) 1 wk before (ES = −0.437; P = 0.041, ES = −0.411; P = 0.050, respectively). The effects of Ca intake were evaluated in this study based on the observation that a relatively low Ca (0.51% of DM) was present for dairy 3, which was the highest milk-producing herd of the study herds. It is notable that there were overall positive responses in milk protein yield and percentage 3 wk later to increased Ca intake, but the lesser response on dairy 3 than dairy 1 was not anticipated given the lower Ca percentage in the diet. Total DMI was numerically higher at dairy 3 than dairy 1 (Table 2), and this may have influenced responses.

Both dairy 2 (ES = 0.518; P = 0.033) and dairy 3 (ES = 0.460; P = 0.048) had more positive associations between increased NDICP intake (kg/d) and lactose yield on the same week than dairy 1 (Supplemental Table S6; https://doi.org/10.15232/aas.2019-01943). Dairy 3 had a decreased milk lactose yield response to increased lignin intake 1 wk before (kg/d) compared with dairy 1 (ES = −0.359; P = 0.041) and dairies 2 and 3 had increased lactose yield associated with increased lignin intake (kg/d) 3 wk earlier compared with dairy 1 (ES = 0.487 and 0.651; P = 0.017 and 0.003, respectively). The effect of NFC intake (kg/d) was to increase lactose yield in the same week for pens on dairy 2 compared with dairy 1 (ES = 0.449; P = 0.048). Similarly, dairies 2 and 3 had greater milk lactose yield responses to increased starch intake (kg/d) on the same week than dairy 1 (ES = 0.511; P = 0.021 and ES = 0.385; P = 0.065, respectively). Dairy 3 differed from dairy 1 in having a greater increase in lactose yield associated with increased fat intake (kg/d) 3 wk previously (ES = 0.383; P = 0.034).

The ES of differences between dairies were larger, on an absolute basis, than those between treatments and explained more than 25% of the variance (ES >0.5 or <−0.5) in production outcomes, in some cases. The dairy that responded most to treatment in terms of milk production had an estimated MP excess, whereas the least-responsive dairy had an estimated MP deficit and was the highest producing of the dairies, further suggesting that diet formulation to provide additional nutrients that facilitate responses to enzyme treatment may be important.

The responses above appear to reflect a pattern of an increased lactose yield response to positive dietary stimuli, that is, an increased NDICP, NFC, starch, and fat for dairies 2 and 3 compared with dairy 1, and a greater reduction in response to increased lignin content of the diet. It is also possible that the findings of dairy differences in response suggest that production responses to diet may not be fully characterized by current dietary analysis. Golder et al. (2018) identified marked differences in rumen VFA and lactic acid concentrations for heifers in an acidosis challenge model and related these differences to the rumen meta-taxome and to the genome of the heifers. The herd in South Dakota was more genetically diverse,

as some crossbred cattle were present, than the herds in California, and the effects of genetic differences among herds may need to be considered on responses to enzyme treatment.

APPLICATIONS

This study provides unique insights to responses to enzyme treatment by evaluating feed composition and milk production responses over long periods in 3 commercial dairies. Results show differences between responses to dietary nutrient intakes for treatment and control pens that may be used in formulation of diets for cattle fed enzyme-treated TMR. Differences in response between treatment and control pens were observed for soluble protein, ADICP, and lignin with milk protein percentage and for ADICP and lignin with milk protein yield. The differences are consistent with treatment improving the digestibility of feed, particularly the fiber fractions. Several results also highlight the importance of CP supply and NDICP to increase milk fat production and milk protein percentage and yield, which also were increased by NFC or starch or sugars. The findings indicate a need to formulate diets that may improve milk production responses to enzyme interventions.

ACKNOWLEDGMENTS

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ORCIDS

H. M. Golder ● https://orcid.org/0000-0003-1298-3107
I. J. Lean ● https://orcid.org/0000-0002-1045-7907