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Effects of Feeding a Fibrolytic Enzyme on Milk Production and Reproduction in Commercial Dairy Cattle

By

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THESIS

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## ABSTRACT

Fibrolitic enzymes have been shown to enhance nutrient availability by increasing fiber digestibility of dairy cow rations. The objective of this study was to determine if a TMR supplemented with an exogenous fibrolitic enzyme product (EFE; RumaThrive; Wilbur-Ellis, Portland, OR) increased milk production or improved reproduction on a commercial dairy. A total of 2,990 Holstein cows were randomly assigned to 1 of 4 study groups: 1) primiparous control (PC: 2 pens) fed only a TMR; 2) primiparous EFE supplement (PT: 2 pens) fed a TMR with added EFE; 3) multiparous control (MC: 3 pens) fed only a TMR; 4) multiparous EFE (MT: 3 pens) fed a TMR with added EFE. The enzyme product had a declared minimum of 44,750 units/g of xylanase. Supplementation of EFE began 2 wk prior to parturition (pre-calving period) and continued 180 d into their lactation. Cows on PT and MT received EFE at a rate of 2 g/cow/d during the pre-calving period and 4 g/cow/d during the lactation period. Data were collected during the first 180 d of lactation, analyzed as a completely randomized design with repeated measures for milk production using the Mixed procedure of SAS (Statistical Analysis System, v.9.4) and Cox proportional hazard regression model with RStudio (v1.4.1106, RStudio: Integrated Development for R, Boston, MA) for time to conception. Primiparous and multiparous pens were analyzed separately. There was no difference in mean days of exposure to EFE between control and EFE primiparous or multiparous pens. The PC had higher ( $P \leq 0.05$ ) milk fat yield, percentage fat, FCM and ECM compared to PT. The MT also had lower ( $P \leq 0.05$ ) milk fat yield, FCM and higher milk protein percentage compared to MC. The PT had a 18% higher risk of conception when compared to PC pens ( $P \leq 0.02$ ). Results of this study indicate that feeding EFE to primiparous dairy cows decreased time to conception.

Keywords: Fibrolitic enzyme, risk of conception, parity

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## **LITERATURE REVIEW**

### **INTRODUCTION**

To meet the projected increase in milk demand worldwide (OECD - FAO, 2018), the already high producing and intensive milk production system of the US dairy industry will have to overcome current obstacles suppressing animal production. It is well established that forages, which make up the majority of dairy cattle feed, tend to be high in indigestible fiber, leading to a reduction in nutrient availability and limiting milk production (Adesogan et. al., 2014).

Exogenous fibrolytic enzymes (EFE), which are derived from bacterial or fungal processes, have been investigated for their potential role in improving fiber digestion and milk yield in lactating dairy cows.

The use of EFE is already well established in the paper and textile industry, as well as the swine and poultry industry where they are added to their feed to improve its digestibility (Beauchemin et al., 1995; Refat et al., 2018). In the last twenty-five years, EFE started being supplemented routinely to dairy cattle in attempts to improve forage digestibility and increase milk production, but results have been equivocal. Gado et al (2009) discovered that dairy cows that were fed a TMR supplemented with EFE produced almost 3 kg/d more milk when compared to cows not receiving EFE supplementation. Similarly, El-Bordeny et al (2015) found that EFE supplemented cows also produced nearly 3 kg/d more milk in addition to having a greater milk fat and milk protein yield compared to control cows. However, research trials conducted by Elwakeel et al (2007) and Peters et al. (2015) concluded that the addition of EFE to the TMR of lactating dairy cows had no effect on milk yield or any milk components, highlighting the heterogeneity of results in EFE research.

Inconsistencies in results have been attributed to the incorrect rate of EFE application, method of EFE application, incorrect enzyme choice, and supplementation to cows in the wrong stage of lactation (Beauchemin et al., 2004a; Adesogan et al., 2014). Inappropriate experimental design and duration have been proposed as other possible sources of result variation (Adesogan et al., 2014; Arriola et al., 2017). Addressing sources of result variation in dairy cow EFE research might lead to improvements in EFE research and allow researchers to better investigate the influences of EFE supplementation on cow health and reproduction, two topics that are seldom explored in EFE trials.

The objectives of this literature review are to review sources of variation in current published responses to EFE application on dairy cow diets and consider experimental designs that could properly measure the effects of EFE supplementation on milk production, fiber digestibility and reproduction in lactating dairy cows.

## **SOURCES OF VARIATION IN CURRENT PUBLISHED RESPONSES**

### **Incorrect Stage of Lactation**

Silva et al. (2016) set out to evaluate increasing levels of EFE application on intake, total-tract digestion, milk yield and composition in lactating Holstein cows. The researchers used 24 multiparous cows averaging 176 DIM. Treatment groups included: 1) Control (CT), no EFE supplementation 2) Low Enzyme (LE), 8 g/d EFE supplementation 3) Medium Enzyme (ME), 16 g/d EFE supplementation 4) High Enzyme (HE), 24 g/d EFE supplementation. Exogenous

fibrolytic enzyme product contained xylanase extracted from *Trichoderma longibrachiatum* (*T. longibrachiatum*) and was applied dry to the concentrate portion of the TMR before feeding. Dry matter intake was linearly increased with EFE supplementation, but total-tract digestion, milk yield, and milk components were similar between all treatment groups.

The cows used in the Silva et al. (2016) were nearing the end of mid-lactation and about to enter late-lactation, a time in which milk yield drops and peripheral tissue insulin resistance increases, leading to weight gain. Researchers reason that the cow's stage of lactation was not optimal for EFE supplementation, and that responses to EFE supplementation would be more pronounced in early-lactation dairy cows. Indeed, in a study conducted by Schingoethe et al. (1999) in which they compared milk production responses to EFE supplementation based on stage of lactation found that milk production was increased in cows with less than 100 DIM and not in cows with over 100 DIM.

In another trial that used a similar EFE product extracted from *T. longibrachiuatum*, El-Bordeny et al. (2015) fed a TMR supplemented with cellulase and xylanase to 116 multiparous Holstein cows, 24 in early-lactation, 36 in mid-lactation, and 56 in late-lactation. Treatments included a CT group fed a TMR with no EFE supplementation and an enzyme treated (ET) group fed a TMR supplemented with 15 g/cow/d of EFE. Cows that were fed a TMR with EFE supplementation had increases in milk yield, 4% FCM, and ECM of 11, 21, and 20%, respectively. Enzyme treated cows had higher production of milk fat, milk protein, and milk lactose by 28, 14, and 12%, respectfully. However, the milk production responses were greatly affected by the cow's stage of lactation. Enzyme treated cows in early- and mid-lactation had a

larger response to EFE supplementation, producing 5.37 and 12.68% more milk, respectively, compared to only 3.84% in late-lactation cows. The El-Bordeny et al. (2015) trial suggests that responses to EFE supplementation are optimized when provided to cows in early- and mid-lactation.

Yet in another study, cows were exposed to EFE treatment 4 wk pre-calving, immediately after calving, and 6 to 8 wk post-calving until week 18 post-calving (Zheng et al., 2000). Cows exposed to EFE treatment pre-calving and immediately after calving produced more ECM when compared to the 6 to 8 wk post-calving cows. Cows that received EFE treatment immediately after calving also produced more milk compared to the other cows. Cows are in negative energy balance during the 2 – 4 wk pre- and post-calving, a time in which cows have a higher energy demand. Production responses to EFE supplementation are greater in situations when energy demand is high or when it is the first-limiting nutrient in the diet, so it would be beneficial to add EFE supplementation to cows before or immediately after parturition (Beauchemin et al., 2003).

### **Method and Type of EFE Application**

The method of EFE application and type of EFE used in trials have shown to affect fiber digestibility and milk responses in lactating dairy cows (Beauchemin et al., 2003, 2004b). Research suggests that EFE are more effective when added wet to feed, as it helps with the dissemination of EFE throughout fiber polymers (Mendoza et al., 2014), and water is a fundamental requirement in the hydrolysis of soluble sugars from complex fiber polymers (Beauchemin et al., 2004a). Adding dry EFE supplement to TMR might not result in a positive response, as was seen in Silva et al. (2016) where they added the dry EFE supplement into the



concentrate portion of the TMR. In contrast, Yang et al. (2000) reported increases in milk production in cows fed a diet with EFE supplementation added to the concentrate portion. Even though most recent EFE trials added EFE supplementation to the TMR portion of the diet (Gado et al., 2009; Refat et al., 2018; Golder et al., 2019), there is evidence that adding EFE to a larger portion of the diet enhances the binding of the enzymes to feed particles, pre-ingestion (Bowman et al., 2002; Beauchemin et al., 2004b).

Colombatto et al. (2002) demonstrated that there was a significant positive relationship between xylanase activity and alfalfa hay digestibility, indicating that xylanase supplementation could increase DM digestibility in dairy cattle. Yet, they also demonstrated that xylanase activity and corn silage digestibility share a negative relationship. Ensiled feed, such as corn silage in a TMR, could have an inhibitory effect on xylanase enzymes. Instead, a combination of cellulase and xylanase could reduce variability in EFE trial responses, as it has been reported that products with a combination of enzymes could have higher enzymatic activity (Beauchemin et al., 2004a; b). Arriola et al. (2011) demonstrated that cows fed a TMR supplemented with a combination of cellulase and xylanase, diluted in water and sprayed onto the TMR, showed increases in DM digestibility and increased efficiency of milk production.

Since cellulose and hemicellulose make up a large portion of the structural polysaccharides in plants (Mendoza et al., 2014), it is understandable that the majority of current EFE trials have supplemented a combination of cellulase and xylanase (Arriola et al., 2017). “Cellulase” and “xylanase” are generic terms for a collection of enzymes that hydrolyze cellulose and hemicellulose. In general, the major enzymes involved in the breakdown of cellulose that fall

under the generic cellulase title are endocellulases, exocellulases, and  $\beta$ -glucosidases (Beauchemin et al., 2004a; Mendoza et al., 2014). The major enzymes in hemicellulose degradation, under the title of xylanases, are endoxylanases and  $\beta$ -xylosidases, and other accessory enzymes which are responsible for side chain degradation (Beauchemin et al., 2004). Most commercially available EFE products advertising “cellulase” or “xylanase” activity might contain enzymatic activity from more than one cellulase or xylanase, and often, only the main activity is displayed on the product label (Adesogan, 2005). In a meta-analysis, Eun and Beauchemin (2008) demonstrated that specific enzymes, and combination of enzymes, designated as cellulases and xylanases have a range of effects on fiber digestibility in different feeds. Thus, commercial products should be marketed for specific types of feed based on the enzyme additives included.

Some EFE products might also contain additional enzymes like amylases, ferulic acid esterases and proteases (Beauchemin K A and Holtshausen L, 2010; Mendoza et al., 2014). Ferulic acid esterases aid in NDF digestibility by removing side chains and the cross linkages in plant polymers (Williamson et al., 1998). Several in-vitro studies have demonstrated that enzymes products containing ferulic acid esterase improve NDF digestibility in several types of feed (Krueger et al., 2008; Yu et al., 2005). Amylase supplementation, however, is not as widespread as other EFE, because the apparent total-tract digestibility of starch in high producing cows is relatively high (> 90%; Firkins et al., 2001). DMI and milk production have been improved in studies supplementing amylase extract to dairy cows (Tricarico et al., 2008; Klingerman et al., 2009). Ferulic acid esterases and amylases are oftentimes present in commercial EFE products, but their enzymatic activity is not printed on the product label (Beauchemin K A and

Holtshausen L, 2010). Similarly, protease supplementation has been shown to increase NDF degradation in certain low-forage diets fed to lactating dairy cows (Eun and Beauchemin, 2005). Colombatto et al. (2003) suggested that protease supplementation increases fiber digestibility by removing nitrogen rich components of cell walls which act as physical barriers for other EFE. Exogenous fibrolytic enzyme supplementation containing amylases, proteases and ferulic acid esterases can have a positive impact on NDF digestibility and milk production in dairy cows, but there is a need for more research on their interactions with cellulases, xylanases and the specific types of forages they are added to.

### **Rate of EFE Application**

Current and past research has shown that it is possible to under- and over-supplement EFE, rendering them ineffective (Kung et al., 2000; Holtshausen et al., 2011). Refat et al. (2017) conducted a lactation trial set out to evaluate the effects of low, medium, and high EFE supplementation on milk production in lactating Holstein cows. Exogenous fibrolytic enzyme treatment consisted of cellulase and xylanase and was diluted in water and sprayed directly onto the TMR just before feeding. Treatments consisted of 1) CT, no EFE supplementation 2) LE, 0.5 ml EFE/ kg DM of TMR 3) ME, 0.75 ml EFE/ kg DM of TMR 4) HE, 1.0 ml EFE/ kg DM of TMR. Cows on ME treatment had significantly higher DM, OM, and NDF digestibility, and produced higher milk yields, milk fat yields, FCM, and ECM compared to the other treatments. Cows on HE treatment produced significantly less milk, milk fat, and milk protein than CT, LE, and ME treatment cows.

Holtshausen et al. (2011) fed a TMR supplemented with low and high EFE to 60 early-lactation Holstein cows. Enzyme treatment consisted of a combination of cellulase and xylanase which was also diluted in water and added to the TMR. Treatments included 1) CT, no EFE supplementation 2) LE, 0.5 ml EFE/ kg DM of TMR 3) HE, 1.0 ml EFE/ kg DM of TMR, very similar to the treatments in the Refat et al. (2017) trial. Dry matter intake decreased proportionally with the dose of EFE, with HE cows having the lowest DMI, followed by LE cows. There were no differences in milk yield between CT and LE, but HE had numerically lower milk yields when compared to the other treatments. Decrease in milk yield and milk components seen in cows fed a TMR supplemented with high doses of EFE might be attributed to shifts rumen microbial populations, which can lead to lower acetate concentrations and interrupt fiber fermentation by ruminal microorganisms (Beauchemin et al., 2004a; Refat et al., 2018). Lower doses of treatment might not provide enough EFE to increase fiber digestibility and nutrient availability. Enzyme treatment dose level might be dependent on the diet being fed.

### **Experimental Design and Duration**

In a meta-analysis, Adesogan et al. (2014) compared two similar trials that were conducted by the same researchers using identical EFE supplementation (cellulase and xylanase), EFE batch, and lactating cow diet but had different trial designs. One trial was conducted as a 10 wk continuous design (Holtshausen et al., 2011) while the other was a 3 x 3 Latin square design with 21 d experimental periods (Chung et al., 2012). The continuous trial used 60 lactating Holstein cows while the Latin square design trial only used 9 lactating Holstein cows. In the continuous trial, EFE supplementation decreased DMI and increased feed efficiency while no changes were observed in the Latin square design trial. In their conclusion, Adesogan et al. (2014) stated that

short changeover designs are not appropriate for measuring the performance responses of EFE treatment in dairy cows and that EFE treatment needs to be fed for a minimum of 3 wk for milk production to be affected.

In another meta-analysis conducted by Arriola et al. (2017), researchers critically reviewed published research studies on the effects of dietary EFE treatment on the performance of lactating dairy cows. They discovered that increasing the duration of experiments by one day tended to increase the standardized mean difference (SMD) of both milk protein yield and milk protein concentration by 0.007 and 0.006 standard deviation units, respectively. Acknowledging the small effect size, they stated that continuous long-term experiments would better represent the benefits of sustained EFE application and the lactational performance of lactating dairy cows (Arriola et al., 2017). Moreover, continuous long-term trial could lead to increases in statistical power by facilitating the involvement of more cows (Adesogan et al., 2014).

### **OPTIMIZING EXPERIMENTAL DESIGN FOR HEALTH AND REPRODUCTION**

The transition period is often defined as the 2 – 4 wk before and after parturition, a time in which dairy cows require additional energy for fetus growth and the sudden onset of lactation, but also experience a reduction in DMI. Improper nutrition during the transition period has been linked to an increase in diseases and reduced reproductive performance in dairy cows (Shah and Murphy, 2006; Santos, 2008; Tucho and Ahmed, 2017), so it is judicious to implement strategies that would optimize nutrient uptake during this time. Few trials have investigated the effects of EFE supplementation on health and reproduction of dairy cows (Łopuszańska-Rusek and Bilik, 2011; Golder et al., 2019).

Łopuszańska-Rusek et al (2011) conducted a study that was aimed at investigating whether feeding TMR diets with EFE, yeast, or both to periparturient dairy cows would affect milk yield and reproductive parameters. They used 36 Holstein-Friesian dairy cows from the Experimental Station of the National Research Institute of Animal Production, Kraków, Poland. For 3 weeks pre-calving and 10 weeks post-partum they fed the cows 1 of 4 TMR diets: a control TMR, a TMR with EFE, a TMR with yeast preparation, or a TMR with EFE and a yeast preparation. Although they found that the EFE treated cows experienced increased milk production by 11% in the first three weeks of lactation compared to the other treatment groups, EFE treatment had no effect on reproduction parameters. Łopuszańska-Rusek et al (2011) were able to perform a continuous long-term trial but were likely limited in the number of cows available at the Experimental Station of the National Research Institute of Animal Production, and so lacked statistical power needed to detect treatment effects.

Conducting EFE trials in commercial dairy operations would allow researchers to include significantly more cows in long-term trials, providing adequate statistical power for the detection of treatment effects. Golder et al. (2019) were able to conduct a continuous EFE trial using 7,507 Holstein dairy cows across 3 commercial dairies. They set out to characterize responses to a specific EFE treatment in a commercial dairy herd with sufficient statistical power to evaluate milk production, reproductive responses, and cow health with the enzyme treatment commenced in the pre-calving period. The EFE treatment was diluted with water and added to the TMR before feeding at a rate of 750 ml/ ton DM TMR. Treatments included a CT group and an ET group. The odds ratio for retained placenta tended to be 27% higher in the EFE treated cows, but

otherwise the disease rates seemed to be similar between both treatment groups. They did find that EFE treated cows had a 26% greater probability of being bred per day when compared to control cows, but the effect was significantly influenced by interactions between treatment, days on pre-calving diet, parity, and dairy. Enzyme treated cows also produced more milk, milk fat, milk protein, FCM and ECM when compared to cows not receiving EFE treatment. At the moment, Golder et al. (2019) is the only published continuous study conducted in a commercial setting investigating milk production, health, and reproduction responses in dairy cows to EFE supplementation.

## **CONCLUSION**

This literature review critically reviewed some sources of variation in currently published responses to EFE application on dairy cow diets. Sources of variation covered were stage of lactation, method and type of EFE application, rate of EFE application, and experimental design and duration. It also considered the effects of EFE supplementation of dairy cow diets on health and reproduction responses. It is difficult to pinpoint the exact source of variation in milk production responses to EFE supplementation, as there are very few studies that use similar experimental designs. Although, variation in responses might be reduced if future trials are conducted in a continuous long-term manner, supplement EFE treatment in a way that would maximize its effectiveness based on diet used, and supplement EFE at an adequate rate. Conducting continuous long-term trials in a commercial setting would also allow researchers to use more animals and increase statistical power and evaluate health and reproductive responses to prolonged EFE supplementation. Additionally, studies performed in a commercial setting could better reflect the performance of EFE products and provide the dairy industry and

researchers with valuable data on packaging, delivery, usage, and sensitivity of the EFE to realistic environmental conditions. In order to better understand the responses to EFE supplementation in realistic environmental conditions, more continuous studies performed in a commercial setting must be conducted.

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## INTERPRETIVE SUMMARY

Feeding a TMR supplemented with xylanase to Holstein cow pens on a commercial dairy operation, during the pre-calving period and continuing into their lactation, can lower the median time to pregnancy by 18% in primiparous cows, but lowers milk fat yield in primiparous and multiparous treatment pens.

Title: Effects of feeding a fibrolytic enzyme on milk production and reproduction to commercial dairy cattle

Running Title: Feeding a fibrolytic enzyme

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Declaration of Conflict of Interest

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## INTRODUCTION

Feeding exogenous fibrolytic enzymes (EFE) to dairy cows has shown to increase feed digestibility, fluid milk and milk component production, but, production responses have been mixed. Gado et al. (2009) fed 20 lactating cows a TMR with or without EFE supplementation from parturition to 12 wk. Cows receiving EFE produced 2.9 kg/d more fluid milk and 0.12 kg/d more milk protein compared to control cows. El-Bordeny et al. (2015) also fed 116 lactating Holstein cows with average 126 DIM a TMR with or without EFE supplementation for 5 wk. Cows receiving EFE produced 2.85 kg/d more fluid milk, 0.24 kg/d more milk fat, and 0.12 kg/d more milk protein compared to control cows. Both studies showed increased milk and milk component yields, however, not all trials agree with this response. Elwakeel et al. (2007) fed 24 lactating Holstein cows with average 106 DIM a TMR with or without EFE supplementation for a 28 d experimental period in a Latin square design. Milk production and milk components were not affected by the addition of EFE to the TMR. Similarly, Peters et al. (2015) fed 28 early-lactation averaging 50 DIM and fed 26 mid-lactation Holstein cows averaging 136 DIM, TMR that either did or did not contain EFE supplementation for a 20 d adaptation period followed by a 56 d experimental period. For both lactation groups, the addition of EFE to the TMR had no effect on milk yield or any milk parameters. These studies highlight the heterogeneity of results in EFE research conducted within the last two decades.

The inconsistency in results have been attributed to incorrect rate of EFE application, method of delivery, EFE-feed specificity, inappropriate experimental designs, and short study periods (Beauchemin et al., 2003; Adesogan, 2005; Arriola et al., 2017). Exogenous fibrolytic enzymes are commonly diluted with water and added to various portions of a TMR just before feeding

(Romero et al., 2016) or are incorporated dry into the hay or mineral mix portion of the TMR (Yang et al., 1999), with varying results. If enzymes are not well mixed in the water dilution, are exposed to high environmental temperatures, or lose activity during pelleting or mineral mix processing, then there may be reduced enzyme activity leading to mixed experimental results. The most used enzymes are cellulase and xylanase (Mendoza et al., 2014; Arriola et al., 2017), which work by catalyzing the stereoselective hydrolysis of the glycosidic bonds found in cellulose and hemicellulose (Bhat M. K. and Hazlewood G. P., 2001). If the TMR are low in feeds that contain cellulose or hemicellulose, there may be little enzyme activity and no effects on milk and milk component yields. Adesogan et al. (2014) stated that short changeover designs are not appropriate for measuring the performance responses of EFE in dairy cows and that EFE needs to be fed for a minimum of 3 wk for milk production to be affected. In a meta-analysis conducted by Arriola et al. (2017), researchers critically reviewed published research studies on the effects of dietary EFE on the performance of lactating dairy cows and stated that continuous long-term experiments would better represent the benefits of sustained EFE application and could increase in statistical power by facilitating the involvement of more cows (Adesogan et al., 2014). Longer studies would also allow researchers to investigate the effects of sustained EFE supplementation on cow health and reproduction, two topics that are often overlooked in EFE trials.

Conducting more continuous, long-term studies performed in a commercial setting could better reflect the performance of different EFE products and provide the dairy industry and researchers with valuable data on packaging, delivery, usage and sensitivity of the EFE to realistic environmental conditions. At the moment there is only one published study meeting the



previously stated criteria (Golder et al., 2019). Therefore, the objectives of this continuous field study were to determine if EFE supplementation would influence yields of milk, fat, and protein, or decrease time to conception in a commercial dairy herd when provided during the pre-calving period and continued into the lactation period.

## **MATERIALS AND METHODS**

This study was approved by the University of California, Davis, Institutional Animal Care and Use Committee.

### **Animals, Housing and Care**

This study was performed on a large commercial Holstein dairy farm located in Fresno County, California, from August 2019 to January 2020. All cows were housed in freestall pens equipped with fans and an automatic water-flushing system for manure removal. Primiparous and multiparous cows were housed in separate pens. Cows were milked 3 times daily using two 80-cow rotary milking parlors. Fresh drinking water was available ad libitum. All pens in the study received on average 3 vertically mixed loads of TMR per day, with the first load offered at 0500 hr and the last load at 1600 hr. The TMR was pushed up every 3 hr and refusals were collected and measured daily using EZ Feed (DHI-Provo, Provo, UT).

### **Experimental Design**

Holstein cows were randomly assigned to control or EFE supplemented pens using odd or even ear-tags during the dry-off period. Primiparous cows were housed in separate pens from multiparous cows and remained on their respective control or EFE TMR from 14 d before freshening to 180 DIM. Study groups were: 1) primiparous control (PC; 2 pens; 486 cows) fed a

TMR or 2) primiparous EFE (PT; 2 pens; 483 cows) fed a TMR with EFE, and 3) multiparous control (MC; 3 pens; 1023 cows) fed a TMR or 4) multiparous EFE (MT; 3 pens; 998 cows) fed a TMR with EFE. During the closeup period, PT and MT pens received EFE at a rate of 2 g/cow/d via pre-calving mineral pellet in the TMR. Upon freshening, PT and MT cows received EFE at 4 g/cow/d via lactating mineral mix in the TMR until the earlier of these two events: confirmation of pregnancy or 180 DIM. Post-fresh treatment and control pens housed cows enrolled in the study and cows which were not participating in the study. This was done to continue regular milk production in the commercial dairy farm. The PC and MC pens received a separate mineral mix that did not contain EFE. The enzyme mixture was a fermentation product of the fungal organism *Trichoderma reesei* (*T. reesei*) (Rumathrive, Wilbur-Ellis Nutrition, Vancouver, WA), with declared minimum 44,750 units/g of xylanase (EC 3.2.1.8), where 1 unit of xylanase is the amount of enzyme that releases 0.48  $\mu$ mol of reducing sugars (xylose equivalents) from birch xylan per minute at pH 4.2 and 50°C.

### **TMR Sample Collections**

Representative TMR samples from PC, PT, MC, and MT pens were collected weekly using the tub sampling and quartering method (Rossow and Aly, 2013) and pooled monthly by study pens for nutrient analysis. Samples of alfalfa hay, corn silage and mineral mix were also collected weekly using the hand grab method (Robinson and Putnam, 1998) and composited monthly for nutrient analysis.

### ***TMR Sample Analysis***

All nutrient analyses were sent to Cumberland Valley Analytical Services Inc. (Cumberland Valley Analytical Services Inc., Maugansville, MD). Mineral mix, TMR, alfalfa and silage samples were analyzed for DM, CP, soluble protein, lignin, fat (EE), NFC, ADF, ash, mineral analysis (Ca, P, K, Mg, Na, Fe, Mn, Zn, Cu, Cl, S, Zn and Mn) and pH. Silage samples were also analyzed for ammonia, total volatile fatty acids (TVFA), acetate and lactate. Assay of the mineral mix for xylanase activity was also conducted. Analysis of the mineral mix, TMR, Alfalfa and silage samples were performed according to wet chemistry AOAC International (1999) methods as follows: DM TMR (method 930.15); DM forage - method from Goering and Van Soest, 1970 modified per National Forage Testing Association recommendations, 2002; CP (method 990.3); lignin - methods from Goering and Van Soest, 1970 with modification; Fat (method 2003.5); ADF (method 973.18); Ash (method 942.05); metals and mineral analysis (method 985.01); chloride was extracted with 0.5% nitric acid and analyzed by potentiometric titration with silver nitrate using Brinkman Metrohm 848 Titrino Plus (Brinkman Instruments Inc., Westbury, NY). A wet silage sample (25g) was diluted with 200ml deionized water, left to sit overnight then blended and filtered through a filter paper (25 um particle retention). Extract produced was used for analysis of: ammonia - 25ml sample was mixed with 75ml of deionized water and placed onto a Labconco Rapidstill II model 65200 analyzer, titrated with 0.1 N HCL; lactic acid - silage sample was placed into a 1:1 ratio with deionized water and placed into a YSI 2700 Select Biochemistry Analyzer (YSI Inc, Yellow Springs, OH); TVFA - 1.0ul sub-sample was injected into a Perkin Elmer AutoSystem (Perkin Elmer, Shelton, CT). Sub-sample injection used a Restek column packed with Stabilwax-DA. The NFC was calculated as  $NFC = 100 - (NDF + CP + \text{crude fat} + \text{ash})$ . Xylanase (EC 3.2.1.8) activity was analyzed using wet chemistry

and Xylazyme AX tablets (Megazyme T-XAX200; Megazyme International Ireland Ltd., Bray, Wicklow, Ireland) as a substrate.

### **DMI and Milk Production**

Dry matter intake was estimated and recorded using the feed management software of EZFeed (DHI-Provo, Provo, UT) on a pen basis, and both treatment and control pens included additional cows that were not enrolled in the project. Total feed delivery weights from the vertical mixer wagon were corrected for residual feed for each pen, then divided by the number of cows in the pen that day to estimate individual cow DMI by pen.

Individual cow's milk yield and milk components were recorded monthly and analyzed by the Kings Dairy Herd Improvement Association (DHIA). Milk was analyzed using Bentley Instruments (Bentley Instruments Inc., Chaska, MN). Milk production data was collected from DHI-Plus (Amelcor Software Company, Provo, UT). Energy corrected milk was calculated as  $ECM = [0.327 \times \text{milk yield (kg)}] + [12.95 \times \text{fat (kg)}] + [7.65 \times \text{protein (kg)}]$  (Tyrrell and Reid, 1965) and 3.5% fat corrected milk as  $FCM = [0.432 \times \text{milk yield (kg)}] + [16.23 \times \text{fat (kg)}]$  (Tyrrell and Reid, 1965). Feed conversion ratio (FCR) was calculated as  $FCR = \text{DMI (kg)} / \text{Milk yield (kg)}$ . Herdtest 1 data was collected between 0-30 DIM and subsequent herdtests (2-5) were collected at 30 d interval following first herd test. Cow pen movements were reported weekly and recorded using DHI-Plus (Amelcor Software Company, Provo, UT).

### ***Health and Reproduction***

All health events, including breeding, pregnancies, calving events, disease diagnosis, disease treatments, and culling events were extracted from DHI-Plus cow management software (Amelior Software Company, Provo, UT). Estrus was detected by tail chalking, and breeding was conducted via artificial insemination (AI). Herd veterinarian conducted pregnancy examinations weekly by rectal palpation and ultrasound.

### ***Sample Size Determination***

A sample size of 245 cows per pen was determined assuming a 5% increase in FCM in multiparous cows in response to supplementation with a fibrolytic enzyme, and a standard deviation of 7.5 kg/d FCM which was typical for pens at this dairy, an alpha of 0.05 and power of 0.80 using the Power procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC).

### **Statistical Analysis**

Differences between least square means were considered significant at  $P \leq 0.05$ . The experimental unit was pen, except for health and reproduction data analysis which was evaluated at the cow level. Differences between control and EFE pens were analyzed separately for primiparous and multiparous cows.

Results from the TMR analysis were analyzed using the GLIMMIX Procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC) with month as a repeated measure. The independent variables were study group and pen nested within study group. Dependent variables were DMI, DM, CP, soluble protein (SP), ADF, NDF, Starch, NFC, lignin, EE, Ash, and minerals. Critical value for determining significance was  $P \leq 0.05$ .

Milk production data was analyzed using the Mixed Procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC) with DHIA test month relative to calving day as a repeated measure. Dependent variables were milk yield, fat yield, protein yield, fat and protein percent, ECM, 3.5% and FCM. Study group, pen nested within study group, days on EFE in the pre-calving period and in lactation, and the interaction of study group with cow test were independent variables. Independent variables that were not different were removed by backwards elimination.

Health event data were analyzed using the Logistic Procedure of SAS that accounted for study group, parity (0 = primiparous, 1 = multiparous) and season (season: 1 = fall, 2 = winter). Health events analyzed were metritis, ketosis, laminitis, mastitis, milk fever, and retained placenta, with incidence greater than 0. Final model was assessed using the Hosmer-Lemeshow test for goodness of fit. Critical value for determining significance in health event data was  $P \leq 0.05$ .

RStudio (v1.4.1106, RStudio: Integrated Development for R, Boston, MA) was used to explore and analyze cow reproduction data with a significance of  $P \leq 0.05$ . A multivariate Cox regression analysis, with milk fat yield and milk protein yield as covariates, was used for C or EFE effect on time to conception. Independent variables that were not different were removed by backwards elimination.

## **RESULTS AND DISCUSSION**

### **Diet and Treatment**

Cows were fed a typical California dairy TMR with the same ingredients within stage of lactation, differing only in the supplementation of EFE in the mineral mix between treatments (Table 1). In this study, supplementation of EFE was begun during the close-up period and continued after calving to maximize exposure and adjustment time to EFE and to determine if feeding EFE in the close-up period influenced milk production in early lactation. A previous study conducted by Zheng et al. (2000) demonstrated that cows receiving EFE treatment 4 wk pre-calving or immediately after calving had increases in milk yield and milk components.

Repeated sampling and analyses of the TMR throughout the trial showed that nutrient composition within parity groups for both treatments were consistent (Table 2). Therefore, nutrients delivered in the TMR to the study pens were uniform.

### **Exclusion Criteria**

The proportion of cows removed from the study, separated by treatment, are shown in Table 3. Cows that were removed from their study pen due to mastitis, laminitis, metritis, milk fever, or retained placenta had subsequent milk data removed from the study at the time of disqualifying health event. Cows that were sold or died before the first herdtest, and cows that were in the incorrect treatment pen pre-calving were removed from the trial and did not contribute any data. Data from cows that died or were sold post-calving were also removed at time of death or sale. Cows in the incorrect treatment pen on herd test day had milk production data censored from the statistical analysis for the corresponding herdtest. To be included in the health and reproduction data analysis, cows had to be in the correct pre-calving treatment pen for at least 14 d and bred at least once, so cows that were marked as “Do not breed” were excluded from the reproduction

data. The total percent of cows removed from the study (12%) was consistent with another similar study where ~ 9% of cows were excluded from the trial (Golder et al., 2019).

### **Health events**

Supplementation of EFE did not affect the incidence of health events (Table 4). The probabilities of cows experiencing ketosis, mastitis, and retained placenta were lower in the colder season of November – January. As expected, multiparous cows experienced higher incidences of laminitis, mastitis, and retained placenta. No primiparous cows experienced ketosis or milk fever. It is known that dairy cows experience higher rates of health disorders with an increase in parity (Grohn and Rajala-Schultz, 2000; Seifi et al., 2011; Ruprechter et al., 2018), and during summer months when ambient temperatures increase the probability of heat stress (Steele, 2016; Becker et al., 2020). The effects of EFE supplementation on health in this trial are similar to that of Golder et al. (2019) in which they found no significant differences in health with supplementation of EFE.

### **DMI**

There were no significant differences in DMI overall between MC and MT pens or PC and PT pens (Table 5). But DMI was significantly higher for primiparous and multiparous control pens compared to the EFE treated pens during the first 2 wk of the study (Figure 1). Increased DMI by the control pens could have been due to irregular feed intake cows experience during the first 3 wk of lactation (Shah and Murphy, 2006), or there may have been palatability issues, or an adjustment period may have been needed for the cows that were not enrolled in the project and already in the treatment pens when EFE administration was begun. These cows did not have the



benefit of EFE supplementation during the dry period and would have immediately been switched to a TMR containing a higher dose of EFE. During weeks 16 – 21 (Figure 1), mold was identified in the corn silage via lab analyses. All pens were fed this corn silage, and diarrhea was observed in all pens during this period. Dry matter intakes decreased at 17-19 wk for MT, MC, and PT pens. Romero et al. (2016) in a long-term trial did observe that cows receiving a moderate amount of EFE supplementation had an increase of 0.9 kg/d in DMI. But that study did not indicate if there were any feed ingredient quality challenges.

### **Milk Production**

Including EFE in the TMR significantly decreased milk fat yield in PT (Table 5). There were no significant differences in milk yield or milk protein yield between PC and PT pens. Because milk fat yield was increased, percent milk fat, ECM and FCM were also increased in PC pens (Table 5; Figure 2C, Figure 2G; Figure 2H). The decreased milk fat yield in PT pens was not expected. Only a few studies investigating the effect of EFE supplementation on dairy cow milk production have reported a lower milk fat yield with EFE treatment (Kung et al., 2000; Sutton et al., 2003; Peters et al., 2010). Peters et al (2010) fed a TMR supplemented with cellulase and xylanase to 6 lactating multiparous Holstein cows. The EFE supplement was diluted in water and added to the TMR at a rate of 6.2 ml EFE/kg DM TMR. They discovered that cows receiving EFE treatment had numerically lower milk yields and milk fat yields compared to control cows, which led to non-significant decreases of both ECM and FCM. Rode et al (1999) also found that EFE supplementation decreased milk fat yield in lactating Holstein cows when given at a rate of 1.3 kg/ ton DM TMR to 20 lactating Holstein cows. They postulated that EFE supplementation could have increased post-ruminal digestion, leading to increases in ruminal propionic acid,

glucose, and the release of insulin, of which the net effect led to adipose tissue lipogenesis and reduced milk fat synthesis, or the effective NDF content of the diet was reduced by the increased fiber digestion brought on by EFE treatment leading to a mild rumen acidosis. In this study, we did not observe more signs of rumen upset in PT vs. PC, and there was no significant difference in odds of laminitis between EFE supplemented and non-supplemented pens (Table 4). However, we did not measure body weights, and PT cows could have increased body weight due to increased fat deposition.

Multiparous control pens produced significantly more milk fat yield and FCM, but MT pens produced significantly more % milk protein (Table 5). Both MC and MT pens had similar protein yields, but MT pens had numerically lower milk yields with increased milk protein percent. In MC pens, milk fat yield and FCM were significantly increased at 3, 4 and 6 mo compared to MT pens (Figure 3C, 3H), and milk protein percentage was significantly higher in MT pens at 4 and 5 mo (Figure 3F).

Similar milk production results were found in previous trials also using EFE derived from *T. reesei* (Peters et al., 2015; Romero et al., 2016). Romero et al. (2016) supplemented dairy cow TMR with xylanase and determined that, even though there were periodic increases in milk yield throughout the trial, cows that received EFE treatment did not have any overall increases in milk yield, milk fat yield, milk protein yield, milk fat percent, milk protein percent, ECM, or 3.5% FCM. Periodic significant increases in milk yield observed in wk 3, 6, and 7 in the Romero et al. (2016) study were not seen in our current trial, where EFE treated pens and control pens had similar milk yield productions throughout the 6-month period (Figure 2A; Figure 3A). Peters et

al. (2015) supplemented both xylanase and cellulase in two trials, one trial consisting of early-lactation cows and the other consisting of mid-lactation cows. The researchers hypothesized that early-lactation cows would be more responsive to EFE treatment. They discovered that both early and mid-lactation cows produced comparable milk yields (30.4 kg/d and 31.2 kg/d, respectively), and concluded that EFE treatment did not have any effect on milk yield or any milk components. Although, like the Romero et al. (2016) trial, milk yield had periodic increases throughout the 56 d experimental period.

Golder et al. (2019) incorporated cellulase and xylanase into dairy cow TMR and fed it to cows during the pre-calving period until approximately 5 months into their lactation on three commercial dairy farms. They observed that EFE increased milk yield by 0.7 kg/d, milk fat yield by 0.04 kg/d, milk protein yield by 0.01 kg/d and ECM by 0.8 kg/d, but responses varied by dairy. The differences in milk production responses to EFE supplementation between the Golder et al. (2019) study and this study could be attributed to the type of EFE product used and how it was mixed into the TMR. Both EFE products used enzymes derived from *Trichoderma reesei*, but the Golder et al. (2019) trial used cellulase and xylanase, which was diluted with water and sprayed onto the TMR before feeding. In the current study, the EFE product only contained xylanase and was incorporated into the mineral mix and added to the TMR. A recent meta-analysis found that studies involving the combination of cellulase and xylanase increased milk production responses by up to 24% when compared to other enzymes (Arriola et al., 2017). It is possible that EFE supplementation containing only xylanase does not illicit a large enough response to increase milk production in high producing dairy cows and instead, the additional

energy released by EFE application to TMR could have been diverted into body weight or reproduction.

## **SCC**

Multiparous treatment pens had significantly higher SCC compared to MC pens (Table 5). When viewed by monthly herdtest, all treatment groups had variable SCC throughout the trial period (Figure 2B, 3B). Aside from intramammary infections, SCC can be altered by seasonal changes, a cow's stage of lactation and parity, and variations in SCC can be higher than expected (International Dairy Federation, 2013; Norstebo et al., 2019). Both treatment groups had an average SCC below 200,000 cells/ml (133,000 and 187,000 cells/ml for PT and MT, respectively), indicating no active infections (Bradley and Green, 2005), and all herdtest SCC were below federal (750,000 cells/ml) and state (600,00 cells/ml) standards (CDFA, 2022).

## **Reproduction**

Median time to pregnancy for PC, PT, MC, and MT cows were 111 d, 101 d, 113 d, and 112 d, respectively (Figure 4). Primiparous treatment cows had a 18% higher risk of conception when compared to PC cows (Figure 4A), i.e., PT cows were getting pregnant 18% faster than PC cows. Energy is one of the major nutritional factors that directly influences reproductive performance in dairy cows (Santos, 2008; Fahar et al., 2018). The effects of energy availability for reproduction are more pronounced in primiparous cows as they have additional energy requirements for their own growth (Bell, 1995). Several studies investigating the relationship between energy intake and reproduction have concluded that dairy cows with higher energy intakes tend to get pregnant faster (Ferguson et al., 1990; Kendrick et al., 1999), and it has been

established that EFE supplementation provides more energy and nutrients for dairy cows by increasing fiber digestibility and nutrient availability (Holtshausen et al., 2011; Golder et al., 2019). Dietary EFE application has been shown to decrease days open, first heat incidence and number of services per conception in dairy buffalos (Abou-seri et al., 2020), but its effect on dairy cow reproduction has not been thoroughly investigated. Golder et al (2019) discovered that EFE treated cows had a higher chance (26%) of being bred per day than the control treated cows. In the current study, it is possible that EFE supplementation improved energy balance in PT cows, or increased NDF digestibility and unlocked more nutrients and energy from the feed, increasing the conception rates in PT cows.

### **CONCLUSION**

Supplementation of an EFE (xylanase) to Holstein cows grouped by pens on a commercial dairy operation, beginning pre-calving and continuing into their lactation, significantly decreased milk fat yield in primiparous and multiparous cows. But there were no significant differences in health events, and median time to pregnancy for primiparous cows was decreased.

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## TABLES AND FIGURES

**Table 1.** Pre-calving and Lactating TMR ingredients as percent of TMR on a DM basis and the forage to concentration ratio.

	Pre- Calving	Lactating
Forage:Concentrate	63.7	30.3
Ingredients		
Almond Hulls	7.26	11.8
Alfalfa Hay	21.5	10.7
Alfalfa Haylage		2.96
Alfalfa Silage		1.15
Canola Meal	12.6	12.9
Corn, Rolled	15.2	29.6
Corn Silage	34.2	14.8
Cottonseed, Pima		7.54
DDG		6.22
Wheat Hay	5.83	
Mineral <sup>1,2,3</sup>	3.41	2.33

<sup>1</sup>Mineral ingredients: Calcium Carbonate, Ammonium Chloride, Ammonium Sulfate, Calcium Chloride, Magnesium Oxide, Saccharomyces Cerevisiae yeast, Monocalcium Phosphate, Dicalcium Phosphate, Cane Molasses, Zinc Sulfate, di-Alpha Tocopherol Acetate, Chromium Propionate, Copper Sulfate, Manganese Sulfate, Natural and Artificial Flavor Ingredients, Sodium Selenite, Calcium Iodate, Vitamin D3 Supplement, Vitamin A Acetate, Cobalt Sulfate, Monensin 500 g/ton and Diflubenzuron 64.09 mg/lb.

<sup>2</sup>Pre-calving and lactating mineral shared the same ingredients but provided at different proportions

<sup>3</sup>Pre-calving exogenously fed enzyme (EFE) mineral and lactating EFE mineral also contained dried *Trichoderma reesei* fermentation product, ground limestone, extracted citric acid press-cake and vegetable oil. Pre-calving EFE supplemented pens received EFE at 2g/d/cow and lactating EFE supplemented pens received EFE at 4g/d/cow.

**Table 2.** Nutrient composition of lactating Holstein cow TMR separated by parity and treatment

Item, % of DM <sup>1</sup>	Primiparous				Multiparous			
	C <sup>2</sup>	EFE <sup>3</sup>	SEM	<i>P</i> -value	C	EFE	SEM	<i>P</i> -value
DM	57.1	57.3	0.61	0.9	57.7	57.1	1.0	0.6
CP	16.4	16.6	0.33	0.8	16.9	16.7	0.36	0.7
SP, % CP	29.5	28.9	1.4	0.8	28.6	29.2	0.74	0.6
ADF	19.7	19.0	0.76	0.6	18.9	19.4	0.23	0.2
NDF	27.5	26.3	0.77	0.3	26.5	26.8	0.21	0.3
Starch	25.8	27.9	1.1	0.2	26.7	26.6	1.1	0.9
NFC	45.3	46.9	0.92	0.3	46.2	46.1	0.69	0.9
Lignin	5.56	5.40	0.27	0.7	5.28	5.47	0.13	0.4
EE	5.69	5.53	0.29	0.7	5.53	5.35	0.19	0.5
Ash,	6.98	6.76	0.29	0.6	7.04	7.13	0.15	0.7
Minerals, % <sup>4</sup>								
Calcium,	0.702	0.780	0.073	0.5	0.802	0.745	0.057	0.5
Phosphorus	0.470	0.475	0.013	0.7	0.477	0.482	0.017	0.8
Magnesium	0.297	0.297	0.0044	1	0.300	0.300	0.0077	1
Potassium	1.63	1.57	0.043	0.4	1.59	1.58	0.049	0.9
Sulfur	0.267	0.275	0.0091	0.6	0.280	0.282	0.0087	0.9
Sodium	0.322	0.325	0.021	0.8	0.325	0.332	0.015	0.7
Chloride	0.590	0.620	0.027	0.5	0.555	0.607	0.028	0.2
Iron, ppm	293	325	26	0.4	315	303	24	0.7
Manganese, ppm	57.3	62.0	2.8	0.3	59.8	61.8	2.2	0.6
Zinc, ppm	79.5	90.0	3.5	0.08	82.3	90.0	3.6	0.2
Copper, ppm	20.0	21.0	1.4	0.6	21.3	21.8	1.8	0.9

<sup>1</sup>TMR samples (n=24) were collected throughout the trial period

<sup>2</sup>C = Control pens not fed the exogenous fibrolytic enzymes mineral (2 primiparous pens and 3 multiparous pens)

<sup>3</sup>EFE = Pens fed mineral containing exogenous fibrolytic enzymes in a mineral pellet at a rate of 4 g/d to 2 primiparous pens and 3 multiparous pens.

<sup>4</sup>Minerals are in % unless otherwise stated

**Table 3.** Proportion and actual number of Holstein cows excluded from the trial

Exclusion reason	Supplement <sup>1</sup>	
	C	EFE
Health events <sup>2</sup>		
Mastitis	0.0134 (40)	0.0161 (48)
Metritis	0.0154 (46)	0.0138 (41)
Laminitis		0.00101 (3)
Milk fever	0.000334 (1)	0.000334 (1)
Retained Placenta	0.000334 (1)	
Cows died	0.00268 (8)	0.00368 (11)
Cows sold	0.00569 (17)	0.00603 (18)
Incorrect pen pre-calving <sup>3</sup>	0.00469 (14)	0.00302 (9)
Incorrect pen post calving <sup>4</sup>	0.0150 (45)	0.0231 (69)
Total	0.0575 (172)	0.0671 (200)

<sup>1</sup>C = Control pens not fed exogenous fibrolytic enzymes, EFE = pens fed exogenous fibrolytic enzymes in a mineral pellet at a rate of 4 g/d.

<sup>2</sup>Cow data were removed from the trial following the disqualifying health events of the following: treatment of mastitis, treatment of laminitis, treatment of metritis, treatment of milk fever, or treatment of retained placenta.

<sup>3</sup>Cows that spent less than 14 d in the correct pre-calving supplemented (C or EFE) pens

<sup>4</sup>Cows that switched supplements during lactation due to being in the incorrect pen

**Table 4.** Effects of supplement, season and parity on control and exogenous fibrolytic enzyme supplemented pens on health events during the study.

Disorder	Supplement <sup>1,2</sup>		Season <sup>3</sup>		Parity <sup>4</sup>	
	Odds ratio	<i>P</i> -value	Odds ratio	<i>P</i> -value	Odds ratio	<i>P</i> -value
Metritis	0.92	0.5	0.980	0.9	0.25	0.9
Ketosis	1.3	0.3	0.450	< 0.05		
Laminitis	0.99	0.9	0.931	0.7	2.6	< 0.05
Mastitis	0.84	0.1	0.522	< 0.05	2.9	< 0.05
Milk Fever	0.83	0.6	0.671	0.4		
Retained Placenta	0.71	0.07	0.452	< 0.05	2.3	< 0.05

<sup>1</sup>C = Control pens not fed exogenous fibrolytic enzymes, EFE = pens fed exogenous fibrolytic enzymes in a mineral pellet at a rate of 4 g/d.

<sup>2</sup>Odds ratio compares Holstein cows fed EFE mineral compared to Holstein cows not fed EFE mineral. Odds ratio compares the odds that an outcome will occur given a particular exposure to the odds of the outcome occurring in the absence of the exposure.

<sup>3</sup>Odds ratio compares Season 1 to Season 0. Season 0 = August – October; hot season, Season 1 = November – January; cold season.

<sup>4</sup>Odds ratio compares multiparous to primiparous.

**Table 5.** Least square means of milk production, DMI, and SCC, along with the mean days on supplement, of primiparous and multiparous pens fed a control or exogenous fibrolytic enzyme supplement.

Item	Primiparous (n = 4 pens)				Multiparous (n = 6 pens)			
	C <sup>1</sup>	EFE <sup>2</sup>	SEM	<i>P</i> -value	C	EFE	SEM	<i>P</i> -value
Days on supplement	157	158			158	156		
DMI, kg/d	25.4	25.5	0.27	0.2	29.2	29.1	0.15	0.3
Milk yield, kg/d	37.7	37.3	0.45	0.4	50.1	49.6	0.41	0.2
ECM, kg/d <sup>3</sup>	41.5	40.3	0.47	< 0.05	53.4	52.6	0.44	0.08
3.5% FCM, kg/d <sup>4</sup>	41.4	40.0	0.49	< 0.05	53.6	52.6	0.47	< 0.05
Fat yield, kg/d	1.54	1.47	0.020	< 0.05	1.97	1.92	0.047	< 0.05
Fat, %	4.12	3.97	0.035	< 0.05	3.94	3.89	0.049	0.1
Protein yield, kg/d	1.20	1.18	0.012	0.1	1.50	1.50	0.011	0.9
Protein, %	3.17	3.15	0.014	0.1	3.01	3.04	0.011	< 0.05
SCC, 10 <sup>3</sup> cells/ml	113	134	17	0.2	146	188	17	< 0.05

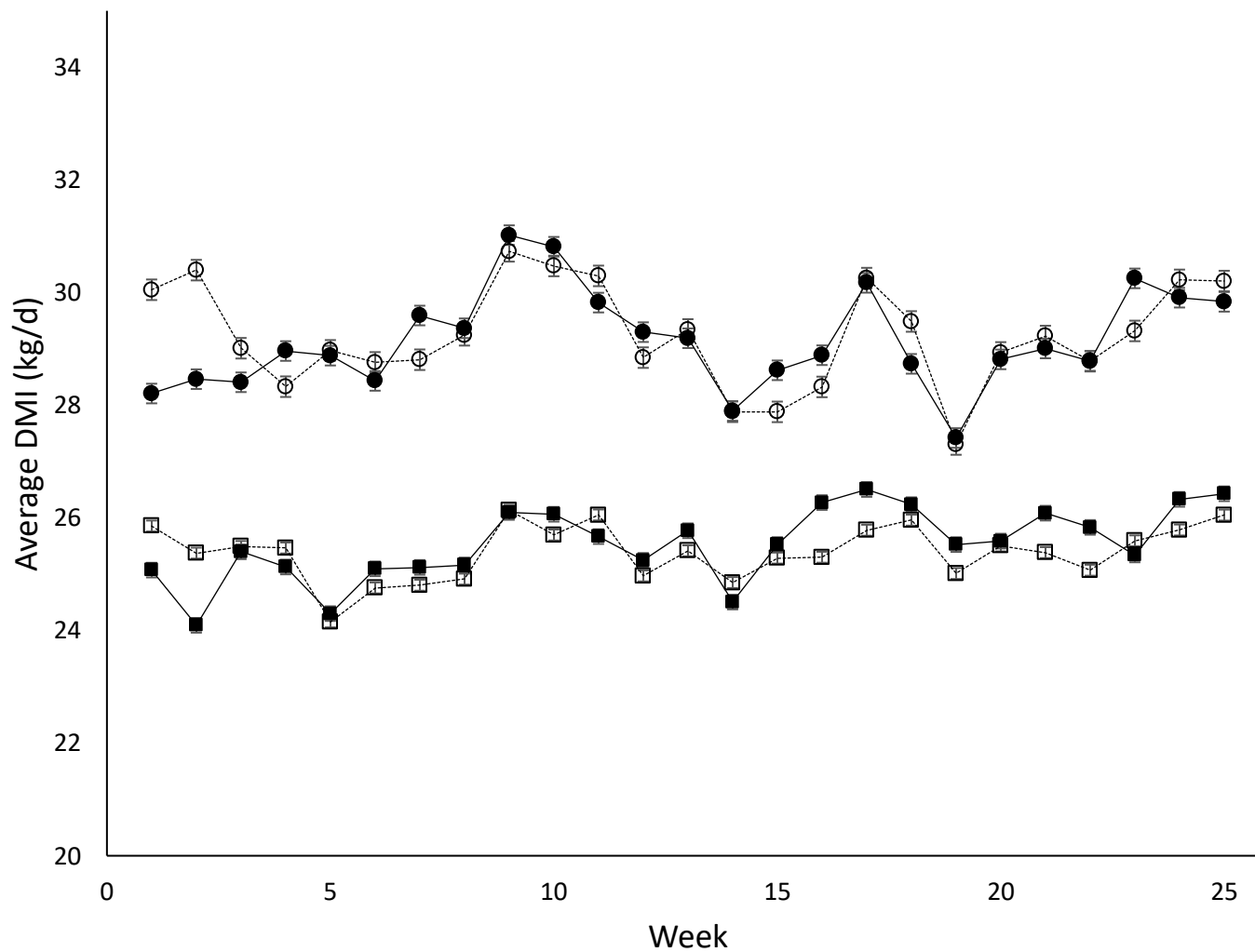
<sup>1</sup>C = Control pens not fed exogenous fibrolytic enzymes (2 primiparous and 3 multiparous pens)

<sup>2</sup>EFE = Pens fed exogenous fibrolytic enzymes in a mineral pellet at a rate of 4 g/d (2 primiparous and 3 multiparous pens)

<sup>3</sup>ECM = [0.327 × milk yield (kg)] + [12.95 × fat (kg)] + [7.65 × protein (kg)]; Tyrrell and Reid, 1965.

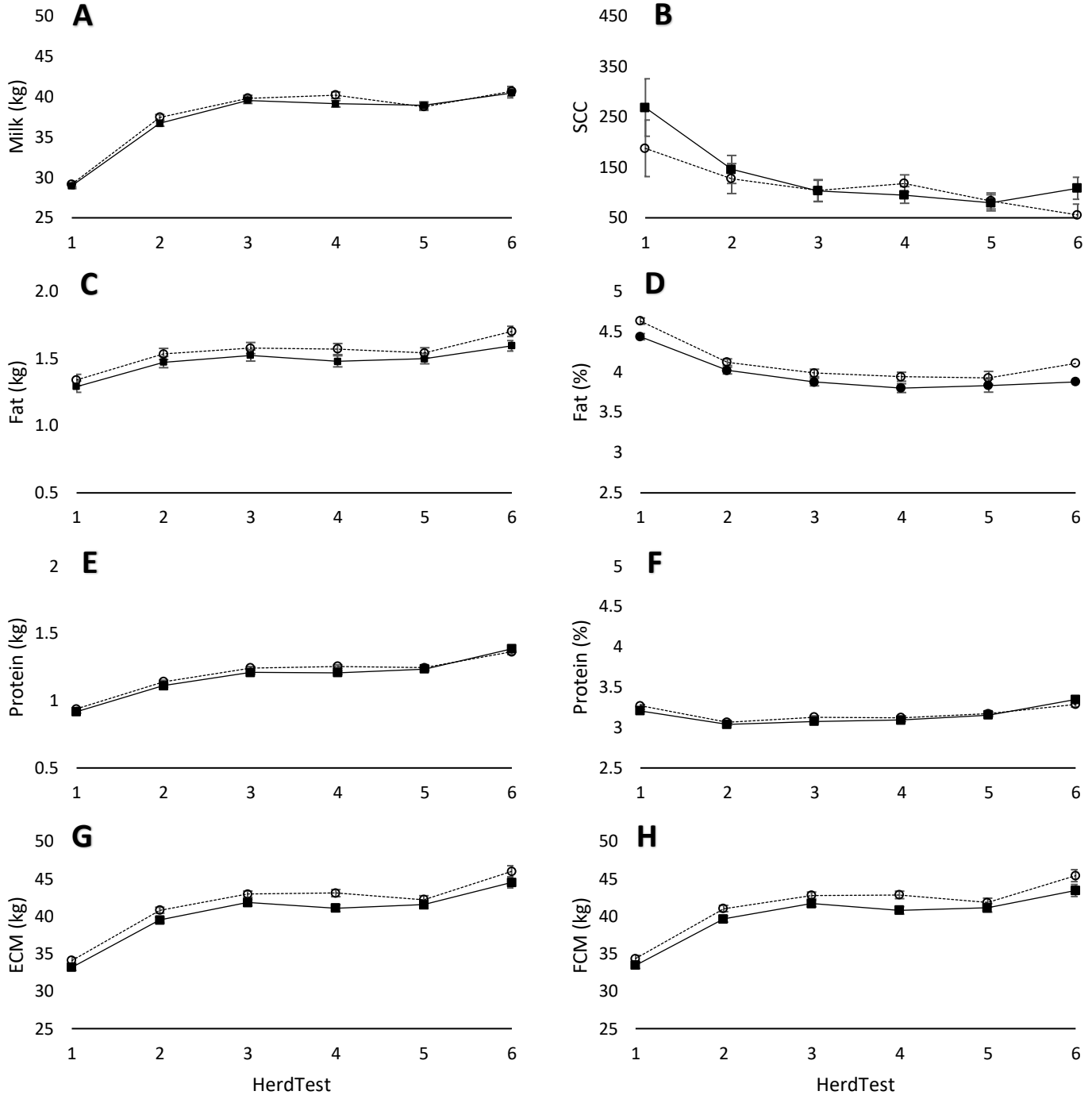
<sup>4</sup>3.5 % FCM = [0.432 × milk yield (kg)] + [16.23 × fat (kg)]; Tyrrell and Reid, 1965.

**Figure 1.** Average weekly DMI with error bars (SEM) of primiparous control ( $\square$ ) and primiparous exogenous fibrolytic enzyme supplemented ( $\blacksquare$ ) Holstein cows, multiparous control ( $\circ$ ) and multiparous exogenous fibrolytic enzyme supplemented ( $\bullet$ ) Holstein cows over the 25 wk trial period, starting at calving.

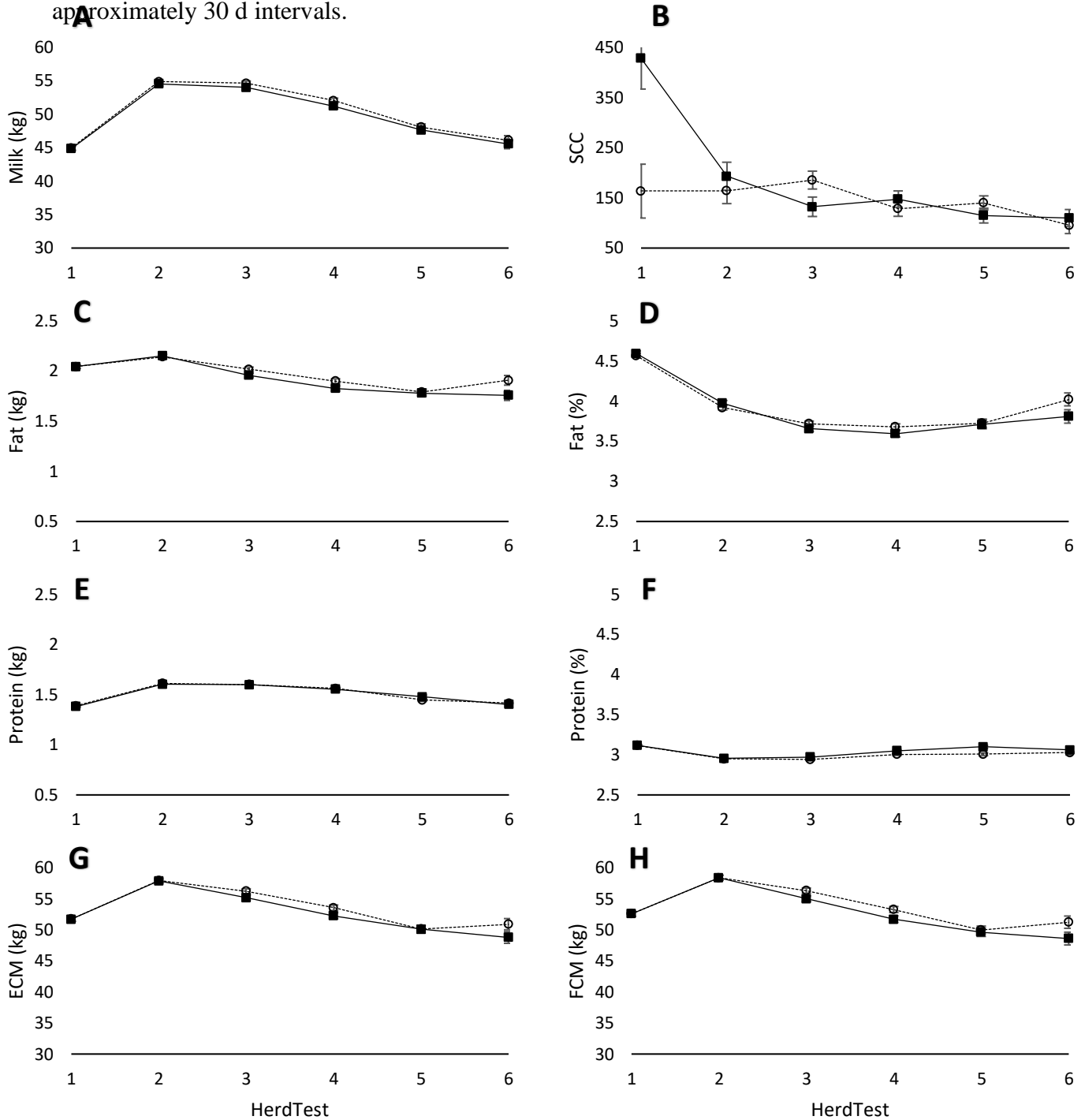




**Figure 2.** Least square mean monthly performance with error bars (SEM) for primiparous control (o) and primiparous exogenous fibrolytic enzyme supplemented pens (▪) over 6 cow tests. Cow test (x-axis) was performed once monthly. (A) Milk yield, (B) SCC  $10^3$  cells/ml, (C) fat yield, (D) fat percent, (E) protein yield, (F) protein percent, (G) ECM, (H) FCM. The first cow test was conducted 21 d after the first enrolled cows freshened. Subsequent cow tests were conducted approximately every 30 d.



**Figure 3.** Least square mean monthly performance with error bars (SEM) for multiparous control (o) and multiparous exogenous fibrolytic enzyme supplemented pens (▪) over 6 herd tests. Herd test (x-axis) was performed once monthly. (A) Milk yield, (B) SCC  $10^3$  cells/ml, (C) fat yield, (D) fat percent, (E) protein yield, (F) protein percent, (G) ECM, (H) FCM. The first herd test was conducted 21 d after the start of the trial. Subsequent herd tests were conducted at approximately 30 d intervals.



**Figure 4.** Pregnancy survival curve for primiparous (A; n = 4 pens, 915 cows) and multiparous (B; n = 6 pens, 1529 cows) cows. Hazard ratio (1.18;  $P = 0.018$ ) for EFE supplemented primiparous cows was higher than for control (Cont) primiparous cows and with 10 d less median time to conception (101 d compared to 111 d). Hazard ratio (1.04;  $P = 0.77$ ) for EFE supplemented multiparous cows getting pregnant sooner than Cont multiparous cows was not significantly different using multivariate Cox regression analysis. Dotted lines show the difference in median time to pregnancy.

