Animal Nutrition 1 (2015) 229–238

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: www.www.keaipublishing.com/en/journals/aninu

Original Research Article

Effect of exogenous fibrolytic enzymes on performance and blood profile in early and mid-lactation Holstein cows

Anja Peters *, Ulrich Meyer, Sven Dänicke

Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Braunschweig 38116, Germany

ARTICLE INFO

Article history:
Received 27 July 2015
Received in revised form
25 August 2015
Accepted 1 September 2015
Available online 11 September 2015

Keywords:
Fibrolytic enzymes
Dairy cows
Milk yield
Feed efficiency

ABSTRACT

The supplementation of exogenous fibrolytic enzymes (EFE) to dairy cows diets could be a strategy to improve fiber degradation in the rumen which is especially important for the early lactating cows characterized by a high milk energy output and an insufficient energy intake. The objective of this study was to examine the effects of a fibrolytic enzyme product (Roxazyme G2 Liquid, 3.8 and 3.9 mL/kg total mixed ration [TMR] DM) supplemented to a TMR on production performance and blood parameters of dairy cows during early (trial 1) and mid-lactation (trail 2). In addition, rumination activity was measured in trial 2. The nutrient digestibility of the experimental TMR was obtained by using wethers. In the digestibility trial, EFE was supplemented at a rate of 4.4 mL/kg Roxazyme G2 Liquid TMR-DM. The TMR contained 60% forage and 40% concentrate (DM basis). Twenty eight 50 ± 16 days in milk (DIM) and twenty six 136 ± 26 DIM Holstein cows were used in two 8-wk completely randomized trails, stratified by parity and milk yield level. One milliliter of the enzyme product contained primarily cellulase and xylanase activities (8,000 units endo-1,4-ß glucanase, 18,000 units endo-1,3(4)-ß glucanase and 26,000 units 1,4-ß xylanase). No differences in digestibility of DM, OM, CP, NDF and ADF were observed ($P > 0.05$) between the control and the EFE supplemented TMR. Addition of EFE to the TMR fed to early (trial 1) and mid-lactation cows (trial 2) did not affect daily dry matter intake (DMI), milk yield, 4% fat-corrected milk, energy-corrected milk (ECM), concentration of milk fat, protein, fat-protein-quotients, somatic cell score, energy balance, and gross feed efficiency of early and mid-lactation cows ($P > 0.05$). Mid-lactation cows (trial 2) fed with TMR enzyme showed a tendency of a slightly higher ECM yield ($P = 0.09$). The tested blood parameters were not affected by treatment in trials 1 and 2 ($P > 0.05$). Exogenous fibrolytic enzymes supplementation did not alter daily time spent ruminating in trial 2 ($P = 0.44$). In conclusion, under the conditions of this study, no positive effects of enzyme supplementation on dairy performance and health status of dairy cows during early and mid-lactation were observed.

© 2015 Chinese Association of Animal Science and Veterinary Medicine. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The genetic progress in increasing milk yields of dairy cows over the last decades, mainly peak-lactation yields, led to a remarkable increase in energy requirement for milk synthesis. However, the associated improvements in feed intake did not compensate the increased energy demands during early lactation with the consequence of more pronounced negative energy balance and the need to mobilize body reserves. As reported by Ingvartsen and Moyes (2013), this may result in a physiological imbalance, a situation where the regulatory mechanisms are insufficient and the risk for digestive, metabolic and infectious problems is enhanced.

The negative interaction between high milk yield and a prolonged severe negative energy balance has initiated investigations into feeding strategies aimed at improving the energy supply. One of the more recent attempts are directed to improve energy balance by decreasing milk energy output through supplementing conjugated linoleic acids (Pappritz et al., 2011; von Soosten et al.,

* Corresponding author.
E-mail address: boening.peters@web.de (A. Peters).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.aninu.2015.09.001

2405-6545/© 2015 Chinese Association of Animal Science and Veterinary Medicine. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

2011). However the most common practical nutritional strategies are aimed at improving the energy balance by raising energy density in the diet. Substituting the forage component with non-fiber carbohydrates in the diet increases the energy intake, but also increases the concentrate induced risk of developing subacute ruminal acidosis (Krause and Oetzel, 2006). A further way of improving the energy balance in early lactation is optimizing the gastro-intestinal degradation of fiber components in the ration (Jung and Allen, 1995). This line of thought led to examine option for improving fiber degradation.

Supplementing dairy cow diets with exogenous fibrolytic enzymes (EFE) has the potential to improve plant cell wall digestibility and therefore, the efficiency of feed utilization (Meale et al., 2014). Most EFE contain mainly xylanases and cellulases of fungal or bacterial origin applied to the ration before consumption with the expectation to improve feed efficiency and animal performance (Beauchemin and Holtshausen, 2010). Several studies with early lactation cows ($<$ 100 days in milk [DIM]) reported a significant higher milk performance due to EFE supplementation (Gado et al., 2009; Schingoethe et al., 1999; Yang et al., 1999). Other feeding trials with early lactation cows did not find significant effects of EFE supplementation on milk yield (Arriola et al., 2011; Beauchemin et al., 2000; Bernard et al., 2010; Dhiman et al., 2002; Elwakeel et al., 2007; Holtshausen et al., 2011; Miller et al., 2008c; Vicini et al., 2003). Inconsistencies of results may be due to differences in energy status of experimental cows, diet composition, type and activity of enzyme used, and method of application (Adesogan et al., 2014; Beauchemin and Holtshausen, 2010). Only a few studies using mid-lactation cows reported significant but lower effects of EFE supplementation on milk yield (Schingoethe et al., 1999) whereas others found no effect (Bernard et al., 2010; Bowman et al., 2002; Dean et al., 2013; Knowlton et al., 2002). Irrespective of these inconsistent findings it seems that adding of EFE to the diets during early lactation are likely to be more responsive due to the higher energy requirement of cows.

We hypothesized that enhancing fiber digestion with EFE supplementation would improve energy balance, milk production, milk composition and gross feed efficiency, anticipating a different animal response depending on the stage of lactation. This experiment aimed at further investigating the effect of EFE supplementation on dairy performance and selected health parameter in dairy cows during early and mid-lactation under European dairy cow production conditions based on maize and grass silage.

Table 1
Ingredients and chemical composition of dietary treatments (as DM basis).

Item	Trial 1		Trial 2	
	Control	Enzyme	Control	Enzyme
Ingredients, %				
Corn silage	40.2	40.2	40.2	40.2
Grass silage	20.3	20.3	20.1	20.1
Concentrate[1]	39.6	39.6	39.7	39.7
Chemical composition				
Dry matter, g/kg	404	405	363	361
Nutrients, g/kg of DM				
Organic matter	928	931	930	932
Crude protein	144	142	135	136
Ether extract	35	36	34	34
NDF	408	385	428	420
ADF	206	196	226	218

NDF = neutral detergent fiber; ADF = acid detergent fiber.

[1] Composition of concentrate: 25% soybean meal, 20% barley, 27% wheat, 24% sugarbeet pulp dried, 2% soybean oil and 2% mineral vitamin premix. Per kg mineral and vitamin premix: 14.0% Ca; 7.0% P; 12.0% Na; 4.0% Mg; 1,000,000 IU VA; 100,000 IU VD_3; 1,500 mg VE; 6,000 mg Zn; 5,400 mg Mn; 1,000 mg Cu; 25 mg Co; 100 mg I; 40 mg Se.

2. Materials and methods

2.1. Experimental design and animals

Two experiments were implemented at the Friedrich-Loeffler-Institute in Braunschweig (FLI). The early lactation experiment (trial 1) consisted of twenty-eighth lactating Holstein cows (5 primiparous, 23 multiparous, 50 $\pm$ 16 DIM, 33.4 $\pm$ 5.9 kg milk yield, 4.24 $\pm$ 0.86% fat, 3.07 $\pm$ 0.19% protein, 593 $\pm$ 45 kg BW), and the mid lactation experiment (trial 2) included twenty-six lactating Holstein cows (6 primiparous, 20 multiparous, 136 $\pm$ 26 DIM, 32.7 $\pm$ 3.5 kg milk, 4.13 $\pm$ 0.56% fat, 3.13 $\pm$ 0.16% protein, 625 $\pm$ 65 kg BW). Both trials used different cows and were implemented over an experimental period of 56 days. Cows were fed with a total mixed ration (TMR) and blocked by parity and milk yield, and then randomly assigned to 1 of 2 treatments in a completely randomized block design: 1) TMR control (without enzyme supplementation, water only), 2) TMR enzyme (with enzyme supplementation).

2.2. Diet ingredients and chemical composition of trial 1 and trial 2

The diet was formulated to meet the nutritional requirements of the cows as recommended by the German Society of Nutrition Physiology (GfE, 2001). The TMR consisting of 60% forage and 40% concentrate (DM basis). Components and chemical composition of the basal diet are shown in Table 1. All cows were housed together in a free-stall barn and were fed twice a day at 0730 and 1400 h. The TMR was provided in 5 self-feeding stations (TYPE RIC, Insentec, B.V., Marknesse, the Netherlands) per treatment.

2.3. Enzyme product, enzyme level and application method

A commercial enzyme mixture traded as Roxazyme G2 Liquid (RG2, Single lot Nr. 302501, DSM Nutritional Products, Ltd, Basel Switzerland) was used in these experiments. The enzyme mixture was a commercial preparation produced by a strain of *Trichoderma reesei*. One milliliter of the enzyme mixture contained 8,000 units endo-1,4-ß glucanase (EC 3.2.1.4), 18,000 units endo-1,3(4)-ß glucanase (EC 3.2.1.6) and 26,000 units 1,4-ß xylanase (EC 3.2.1.4), as specified by the manufacturer.

All cows were exposed to a ration-adaptation period of 20 days followed by a 56-day experimental period (supplementation period) on their assigned diet. The RG2 was applied at 3.9 $\pm$ 0.14 mL/kg TMR DM in the trial 1 and 3.8 $\pm$ 0.17 mL/kg TMR DM for trial 2. The RG2 liquid was diluted at a rate of 1:10 with water and added to the TMR using a sprinkler-can while being mixed in a mixer wagon. The daily TMR and the enzyme application were prepared directly before the morning feeding for the TMR.

Details of the measurement of enzyme activities in feed samples are reported by Peters et al. (2010) and results are shown in Table 2.

2.4. Measurement, sampling and analysis

Feed intake was measured daily for individual cows through a transponder assisted automatic feed weighing trough system. Cows were milked twice daily (0530 and 1530 h). Individual milk yields were recording automatically by the milking system at each milking. Milk samples from each cow were collected twice a week (a.m./p.m. composite) and treated with a preservative agent (bronopol) and stored at 8°C until analysis. Milk composition (fat,

Table 2
Enzyme activities (U/kg TMR DM) in the total mixed ration (means ± SD; $n = 8$).

Item	Trial 1		Trial 2	
	Expected minimum activity	Analyzed activity	Expected minimum activity	Analyzed activity
Endo-1,4-ß-glucanase	70,828 ± 2,574	61,938 ± 6,741	69,573 ± 3,088	57,471 ± 10,511
Endo-1,3(4)-ß-glucanase	31,479 ± 1,144	28,302 ± 3,105	30,921 ± 1,373	25,608 ± 4,034
Endo-1,4-ß-xylanase	102,307 ± 3,717	94,618 ± 13,397	100,494 ± 4,461	75,969 ± 21,664

protein, lactose, urea concentration, and somatic cell count) was determined using an infrared milk analyser (Milkoscan FT 6000 combined with a Fossomatic 5000, Foss Electric, Hillerød, Denmark). Milk composition data of the two sampling days were averaged and the average values used in the statistical analysis. Body weight was electronically recording after leaving the milking parlor twice daily using automatic walk-through scales. The two measurements were arithmetically averaged to a single value.

Ruminating activities (only in trial 2) were monitored visually for each cow on days 14, 28, 42 and 56 over a 24-h period, according to Kononoff et al. (2002) with modified observation intervals. Rumination activities were observed at 10 min intervals. If the cow was detected to ruminate within a 10 min time span it was noted as a 10-min rumination episode. To estimate the time spent ruminating per kilogram of dry matter intake (DMI) or neutral detergent fiber (NDF) unit, the average intake of the preceding 3 days was used because time spent ruminating was assumed to reflect the DMI of the previous days.

Samples of grass silage and corn silage were taken at alternate days from the mixing wagon after 5 min of mixing. All samples were pooled on a weekly basis and stored at −20°C until analysis. Feed samples of the TMR enzyme to determine enzyme activities were taken once per week and stored at −18°C until analysis. Concentrate samples were taken twice per week from the concentrate silo. All samples were pooled fortnightly and directly subjected to Weender Analysis.

Samples of TMR control/TMR enzyme for analyzing DM content were taken daily from each trough directly before feeding. All samples were pooled fortnightly and directly exposed to Weender Analysis.

All methods for analyzing silage, concentrate and TMR samples are in accordance with the book of methods Vol. III of Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (Naumann and Bassler, 2012).

The blood samples were taken on trial days 28 and 56 by puncture of the coccygeal vein/artery. The samples were then centrifuged (2,120 × *g* for 10 min and 15°C) within 2 h after collection to obtain serum, which was immediately stored in Eppendorf tubes at −20°C until analysis.

Serum samples were analyzed at the Clinical Laboratory of the Cattle Clinic Hannover University of Veterinary Medicine, Germany. Haemolysed samples were excluded. The following biochemical blood components were measured by different colorimetric techniques using an automatic multiparameter analyser for clinical chemistry (Cobas-Mira, Hoffmann-La Roche & Co. AG Diagnostika, Basel) and different commercial kits accordance with Deutsche Gesellschaft für Klinische Chemie (DGKC) and International Federation of Clinical Chemistry (IFCC) recommendations. For total bilirubin (TB), a commercial kit (Jendrassik-Grof colorimetric Diazo method; LT-SYS, Berlin) was used. ß-hydroxybutyrat (BHB) and urea were analyzed with commercial kits (Randox Laboratories Ltd., Wülfrath; LT-SYS, Berlin and Roche Diagnostics GmbH, Mannheim) using an enzymatic UV method. Serum activities of aspartate-amino transferase (AST) and gamma glutamyl transferase (GGT) were determined using an enzymatic assay (ABX Deutschland, Göppingen and Hitado Diagnostic Systems, Möhnesee Delecke). The spectrophotometric biuret method was used to quantify the concentrations of total protein (Sigma Diagnostics, Deisenhofen).

2.5. Digestibility measurements using wethers

The apparent digestibility of the applied TMR control and TMR enzyme was determined through digestibility tests using wethers according to the regulations for the determination of digestibility of crude nutrients with ruminants published by (GfE, 1991).

The apparent digestibility of crude nutrients and net energy for lactation (NEL)-content of the offered TMR-Control and the TMR-Enzyme in trials 1 and 2 was measured in two trials with 4 wethers (German Blackhead/SKF) per trial. The daily ration offered contained 1.1 kg TMR (40% corn silage, 20% grass silage, 40% concentrate) plus 44.4 mL water/d (TMR-control) during the first period and an enzyme addition of 4.4 mL/d diluted with 44 mL water/d during the second period, respectively.

Experimental animals were kept in metabolic crates and fed the restrictive ration daily at 0630 and 1430 h. Water was offered ad libitum. Each trial period started with a 12-day adaptation period followed by an 8-day collection period, during which the total feaces were collected after each feeding and stored at −20°C until further processing.

Before analysis the total feaces samples collected were weighed and thoroughly mixed before several samples were taken. Part of the fresh sample was used for crude protein determination using the Kjeldahl method. Feaces samples were dried at 60°C and thereafter milled to 2 mm for further chemical determinations. From each daily feed sample 200 g were separated, stored at −20°C, and at the end of each trial period dried and milled to 2 mm. All feed and feaces samples were subjected to the Weender analyses of crude nutrients and acid detergent fiber (ADF) and NDF determination.

2.6. Calculations

The apparent digestibility of nutrients was calculated as follows:

$$\text{Apparent digestibility (\%)} = \lfloor(\text{nutrient}_{\text{intake}} - \text{nutrient}_{\text{faeces}})/\text{nutrient}_{\text{intake}}\rfloor \times 100.$$

Based on the equations published by the German Society of Nutrition Physiology (GfE, 2001) the metabolizable energy (ME), gross energy (GE) and NEL were calculated as follows:

$$GE(MJ/kg) = 0.0239 \times CP + 0.0398 \times EE + 0.0201 \times CF + 0.0175 \times NfE,$$

$$ME(MJ/kg) = 0.0312 \times DEE + 0.0136 \times DCF + 0.0147 \times (DOM - DEE - DCF) + 0.00234 \times CP,$$

$$NEL(MJ/kg) = 0.6 \times [1 + 0.004 \times (q - 57)] \times ME(MJ/kg).$$

Where is: CP = crude protein (g/kg); EE = ether extract (g/kg); CF = crude fiber (g/kg); NfE = nitrogen free extract (g/kg); OM = organic matter (g/kg); D = digestible; q = ME/GE × 100.

Based on the equations published by the German Society of Nutrition Physiology (GfE, 2001) the net energy requirements for maintenance (NE_m) and NEL as well as milk energy concentration and output were calculated as follows:

$$NEm(MJNEL/d) = 0.293 \times BW^{0.75},$$

$$\text{Milk energy concentration (MJNEL/kg)} = 0.38 \times \text{milk fat(\%)} + 0.21 \times \text{milk protein (\%)} + 0.95,$$

$$\text{Requirement for milk production(MJNEL/d)} = \text{Energy content of milk(MJNEL/kg)} \times \text{milkyield(kg/d)}.$$

Fat-corrected milk (FCM) and energy corrected milk (ECM) was calculated as:

$$FCM(kg/d) = [(\text{milk fat (\%)} \times 0.15) + 0.4] \times \text{daily milk yield(kg/d)},$$

$$ECM(kg/d) = \text{milk yield(kg/d)} \times \{([(0.38 \times (\text{milk fat \%}) + 0.21 \times (\text{milk protein \%})) + 1.05])/3.28\}.$$

The net energy balance was calculated with the following equation:

$$\text{Net energy balance (MJNEL/d)} = \text{energy intake(MJNEL/d)} - [NEm(MJNEL/d) + NEL(MJNEL/d)].$$

Gross feed efficiency was determined by dividing the daily 4% FCM yield by DMI of each animal. The changes in body weight (henceforth BW change) were calculated by subtracting the BW at the end from the BW at the start of the trial.

2.7. Statistical analysis

The normal distribution of all variables included in the dataset was tested with the PROC UNIVARIATE of SAS (Version 9.3.1, SAS Inst. Inc., Cary, NC, USA) using the Shapiro–Wilk test. Variables with Shapiro–Wilk values (W) ≥ 0.98 were considered normal. The non-normal distributed variables gross feed efficiency, BHB, AST, and GGT were calculated on the log-transformed values (natural logarithm) before being subjected to further statistical evaluation. Milk somatic cell count (SCC) data were converted to somatic cell score (SCS) using a base 2 logarithmic function: SCS = log 2 (SCC/100,000) + 3 to achieve an approximate normal distribution of the test day values for the statistical analysis (Ali and Shook, 1980).

Gaussian and transformed performance (test day information) and blood data were statistically processed by PROC MIXED (Version 9.3.1, SAS Inst. Inc., Cary, NC, USA) using a mixed linear model including a REPEATED statement with subject = cow, to assess the fixed effects of treatment, parity (category 1, 2 and 3+) and trial day. Differences in milk yield between groups of cows allotted to the treatment groups were corrected using the actual deviation from the common mean obtained during the adaptation period. Cows within trial differed in DIM, thus the day in milk at the first trial day (fDIM) was introduced into the model as a covariate. Ruminating activities data were analyzed with same model, except for fDIM which tested not significant. The variance-covariance matrix structures were evaluated for each response variable using AR (1), SP (Pow), CS and VC covariance structures. Variance-covariance matrix structures were selected for each variable based on the lowest Akaike information criterion (AIC) fit statistic. Significance of the fixed effects was determined using the *F*-test. Degrees of freedom were calculated using the KENWARDROGER option. Body weight change was analyzed by using the PROC MIXED procedure (Version 9.3.1, SAS Inst. Inc., Cary, NC, USA). The model contained treatment, parity and fDIM as a covariate. Results of all normal distributed traits are shown with their LSmeans and SEM. Log-transformed traits are presented as means with their SD while *P*-values are taken from the statistical analysis of the log-transformed data. Significance was declared at $P \leq 0.05$ and a tendency to significance at $0.05 \leq P < 0.10$, differences were considered to indicate a trend.

3. Results

3.1. Digestibility trials

Results of the digestibility trials are compiled in Table 3. The total mixed ration control and TMR enzyme both showed a similar NEL-content of 7.2 MJ NEL/kg DM ($P > 0.05$). Digestibility values of DM, OM, CP, NDF and ADF did not differ between treatment groups ($P > 0.05$).

3.2. Production response in lactation trials 1 and 2

Results for performance traits and gross feed efficiency of experimental cows from trials 1 and 2 are presented in Tables 4 and 5, respectively. In general cows in trial 2 had a higher DMI and water intake than those of trial 1 ($P > 0.05$). Milk yield and milk components were also slightly higher during trail 2 ($P > 0.05$).

Addition of the fibrolytic enzyme mixture to the TMR had no significant effect on any production parameter in both trials ($P > 0.05$). The treatment effect on energy balance showed a numerical trend in the early lactation trial ($P = 0.11$) of a lower negative energy balance in the treatment group during, while no differences were observed during the mid-lactation trial ($P = 0.35$). Cows fed TMR enzyme showed a tendency of a slightly higher ($P = 0.09$) ECM yield in trial 2.

Table 3
Nutrient digestibility in sheep fed control or enzyme supplemented total mixed ration (mean ± SD).

Item	Treatment	
	Control ($n = 4$)	Enzyme ($n = 4$)
DMI, g/d	1,117	1,110
Apparent digestibility,%		
DM	76.7 ± 0.2	77.8 ± 2.6
OM	79.8 ± 0.3	80.3 ± 2.4
CP	77.2 ± 0.9	75.6 ± 2.6
NDF	67.5 ± 0.8	69.7 ± 4.4
ADF	64.2 ± 1.7	66.4 ± 4.7
Energy content		
MJ ME/kg DM	11.7	11.7
MJ NEL/kg DM	7.2	7.2

DMI = dry matter intake; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ME = metabolizable energy; NEL = net energy lactation.

Parity effects were observed for water intake, milk yield, FCM, ECM, Urea and milk energy output in both trials ($P < 0.05$). The effect of fDIM on performance parameter was different in the two trials. In the first trial a significant effect of DIM was observed on FCM, ECM, milk fat, fat-protein-quotient (FPQ), maintenance requirement and milk energy output ($P < 0.05$) but not on milk yield, DMI and water intake ($P > 0.05$). During the second trial milk yield, FCM, ECM, milk energy output, energy balance and gross feed efficiency were significantly influenced by fDIM ($P < 0.05$) but not DMI, water intake and milk components ($P > 0.05$).

Trial day affected all traits significantly ($P < 0.05$). This is also illustrated in Fig. 1. Significant interactions between treatment and trial day were observed for DMI, water intake, NEL intake and energy balance during trial 1 ($P < 0.05$), while in trial 2 this interaction also affected milk yield, FCM and ECM and milk energy output ($P < 0.05$). Gross feed efficiency showed this interaction during both trials ($P < 0.05$).

3.3. Blood parameters

The effects of EFE supplementation on selected blood parameter are shown in Table 6. No treatment effects on blood parameters were observed during both trials ($P > 0.05$). Trial day significantly influenced all blood parameter ($P < 0.05$) except total bilirubin in trial 2 ($P = 0.13$). During the first trial BHB, total bilirubin and AST were significantly affected by parity, while only AST was affected in trial 2 ($P < 0.05$). The highly significant effect of parity on BHB in trial 1 ($P < 0.05$) was caused by a markedly higher value in second parity cows (0.48, 0.69 and 0.51 mmol/L for first, second and further parity). The day in milk at the first trial day had an significant influence on BHB and AST in the first trial ($P < 0.05$) and only on total protein in the second trial ($P = 0.05$).

3.4. Ruminating activities in trial 2

The effects of EFE supplementation on ruminating are shown in Table 7. Ruminating activity parameters were not affected by treatment and parity ($P > 0.05$). The trial day was significant ($P < 0.05$) but no treatment by trial day interaction was observed ($P > 0.05$).

4. Discussion

4.1. Digestibility

The calculation of nutrient values in the total mixed ration used in the experiments was based on the results of digestibility trials using wethers. The similarity in digestibility values for TMR control and TMR enzyme treatment groups is contrary to our hypothesis that enzyme application improves digestibility of a TMR. Miller et al. (2008a) who used a same enzyme product (Roxazyme G2 Liquid) at a similar dosage fed to lambs also found no significant effects on nutrient digestibility ($P > 0.05$).

Table 4
Performance, milk composition and gross feed efficiency of cows fed the control or the enzyme supplemented total mixed ration in trial 1.

Item	Treatment		*P*-value				
	TMR control ($n = 14$)	TMR enzyme ($n = 14$)	TRT	Parity	fDIM	Trial day	TRT × Trial day
DMI, kg/d[1]	16.6 ± 0.56	17.4 ± 0.62	0.36	0.29	0.17	< 0.01	< 0.01
Water intake, L/d[1]	63 ± 3.0	63 ± 3.3	0.87	0.05	0.91	< 0.01	< 0.01
Yield, kg/d[1]							
Milk yield	30.2 ± 0.82	30.4 ± 0.90	0.84	< 0.01	0.18	< 0.01	0.13
4% FCM	30.8 ± 0.70	30.3 ± 0.77	0.64	< 0.01	< 0.01	< 0.01	0.44
ECM	30.1 ± 0.62	29.9 ± 0.68	0.77	< 0.01	< 0.01	< 0.01	0.29
Milk components, %							
Milk fat[1]	4.16 ± 0.16	4.04 ± 0.18	0.60	0.21	0.01	< 0.01	0.95
Milk protein[1]	3.14 ± 0.05	3.16 ± 0.06	0.78	0.28	0.09	< 0.01	0.95
Milk lactose[1]	4.73 ± 0.04	4.83 ± 0.04	0.08	0.02	0.19	< 0.01	0.01
FPQ[1]	1.34 ± 0.04	1.28 ± 0.05	0.43	0.07	< 0.01	< 0.01	0.86
Urea, mg/L[1]	189 ± 6.0	189 ± 6.7	0.98	0.02	0.37	< 0.01	0.03
SCS[2]	2.39 ± 0.33	2.37 ± 0.37	0.96	0.27	0.49	< 0.01	0.99
Energy measure, MJ/d[1]							
NEL intake	119.1 ± 4.0	125.6 ± 4.5	0.28	0.29	0.19	< 0.01	< 0.01
Maintenance requirement	35.4 ± 0.4	34.6 ± 0.5	0.19	0.06	0.01	< 0.01	0.08
Milk energy output	95.7 ± 2.0	94.9 ± 2.2	0.77	< 0.01	< 0.01	< 0.01	0.34
Energy balance	−12.4 ± 3.7	−3.2 ± 4.1	0.11	0.59	0.97	< 0.01	< 0.01
GFE, kg FCM/kg DMI[2]	1.99 ± 1.09	1.82 ± 0.75	0.16	0.21	0.25	< 0.01	< 0.01
BW change, kg/56 d[1]	14.0 ± 3.4	15.6 ± 3.8	0.75	0.16	0.40	nm	nm

DMI = dry matter intake; FCM = fat corrected milk; ECM = energy corrected milk; FPQ = fat-protein-quotient; SCS = somatic cell score; NEL = net energy lactation; GFE = gross feed efficiency; BW = body weight; TMR = total mixed ration; TRT = treatment; fDIM = the day in milk at the first trial day; nm = not included in the model.

[1] Least squares means ± SEM (normal distributed data).
[2] Means ± SD (log-transformed data).

Table 5
Performance, milk composition and feed efficiency of cows fed the control or the enzyme supplemented total mixed ration in trial 2.

Item	Treatment		*P*-value				
	TMR control ($n = 13$)	TMR enzyme ($n = 13$)	TRT	Parity	fDIM	Trial day	TRT × Trial day
DMI, kg/d[1]	18.9 ± 0.44	19.5 ± 0.45	0.32	0.42	0.89	< 0.01	< 0.01
Water intake, L/d[1]	75 ± 1.9	74 ± 1.9	0.70	0.03	0.11	< 0.01	< 0.01
Yield, kg/d[1]							
Milk yield	30.7 ± 0.32	31.2 ± 0.33	0.33	< 0.01	< 0.01	< 0.01	< 0.01
4% FCM	31.9 ± 0.38	32.5 ± 0.39	0.26	< 0.01	< 0.01	< 0.01	< 0.01
ECM	31.3 ± 0.32	32.1 ± 0.33	0.09	< 0.01	< 0.01	< 0.01	< 0.01
Milk components, %							
Milk fat[1]	4.36 ± 0.16	4.23 ± 0.16	0.53	0.39	0.67	< 0.01	1.00
Milk protein[1]	3.25 ± 0.04	3.28 ± 0.04	0.61	0.58	0.38	< 0.01	0.38
Milk lactose[1]	4.77 ± 0.03	4.81 ± 0.03	0.44	0.26	0.32	< 0.01	0.02
FPQ[1]	1.34 ± 0.04	1.29 ± 0.04	0.36	0.36	0.90	< 0.01	0.96
Urea, mg/L[1]	234 ± 5.2	228 ± 5.3	0.35	< 0.01	0.95	< 0.01	0.01
SCS[2]	2.90 ± 0.32	2.47 ± 0.33	0.35	0.48	0.22	0.05	0.81
Energy measure, MJ/d[1]							
NEL intake	135.3 ± 3.2	140.9 ± 3.2	0.21	0.42	0.89	< 0.01	< 0.01
Maintenance requirement	37.4 ± 0.78	36.7 ± 0.79	0.53	0.19	0.85	< 0.01	0.99
Milk energy output	99.7 ± 1.0	102.1 ± 1.1	0.10	< 0.01	< 0.01	< 0.01	< 0.01
Energy balance	−1.84 ± 2.9	−1.94 ± 3.0	0.35	0.21	0.03	< 0.01	< 0.01
GFE, kg FCM/kg DMI[2]	1.73 ± 0.45	1.72 ± 0.82	0.86	0.20	0.01	< 0.01	< 0.01
BW change, kg/56 d[1]	15.3 ± 3.3	13.3 ± 3.4	0.68	0.95	0.28	nm	nm

DMI = dry matter intake; FCM = fat corrected milk; ECM = energy corrected milk; FPQ = fat-protein-quotient; SCS = somatic cell score; NEL = net energy lactation; GFE = gross feed efficiency; BW = body weight TMR = total mixed ration; TRT = treatment; fDIM = the day in milk at the first trial day; nm = not included in the model.

[1] least squares means ± SEM (normal distributed data).
[2] means ± SD (log-transformed data).

4.2. Dry matter intake and energy balance

This study investigated the supplementation of EFE in corn-grass silage based TMR diets for dairy cows typical for European dairy cow production systems. Exogenous fibrolytic enzymes supplementation for dairy cow diets has been applied with variable success, mainly under North American dairy feeding conditions. Especially *in vivo* experiments did often fail to show a positive production response by EFE addition (Ortiz-Rodea et al., 2013). In the present study, EFE supplementation did not significantly alter DMI, although EFE supplemented cows had a numerically higher DMI intake in both trials. The result of our study agrees with other studies which observed no effects of EFE supplementation on DMI of dairy cows during different stages of lactation (Arriola et al., 2011; Bernard et al., 2010; Chung et al., 2012; Dean et al., 2013; Dhiman et al., 2002; Elwakeel et al., 2007; Reddish and Kung, 2007). Holtshausen et al. (2011) reported a decreased DMI in dairy cows treated with EFE during early lactation (46 ± 10 DIM). Only few studies (Beauchemin et al., 2000; Gado et al., 2009) found increased DMI due to the addition of EFE. A Roxazyme G2 treatment of diets fed to beef cattle led to a positive response in DMI (Miller et al., 2008b) for forage silage but not for a barley-based diet, suggesting an EFE effect in diets with a lower OM digestibility. The mode of action of EFE is not completely understood and described because of the complexity of ruminal microbial ecosystem and the process of fiber digestion. One discussed mode of action of EFE is the increased number of cellulolytic bacteria and the population changes in the rumen (Beauchemin and Holtshausen, 2010). Zeitz et al. (2013) investigated a high dose of Roxazyme G2 Liquid (13.8 and 27.7 mL enzyme product/kg DM) on the specific ruminal bacterial population in non-lactating cows, but did not detect any effect on important rumen bacterial species.

The EFE supplementation did not significantly improve the energy balance (EB) of early and mid-lactation dairy cows. This can be possibly explained by the lack of significant differences in DMI and milk energy output between both diets. The numeric difference of the EB between the TMR control (−12.4 MJ/d) versus TMR enzyme (−3.2 MJ/d) in trial 1, may have been caused by the slightly higher DMI of EFE supplemented cows during early lactation. In the trial 2, we observed a negative energy balance (NEB) in mid-lactation dairy cows (−1.84 and −1.94 MJ/d for TMR control and TMR enzyme, respectively). This is not in agreement with Coffey et al. (2003) who reported a postpartum interval to a positive EB of below 100 DIM. Body weight change of cows was not affected by enzyme treatment. During both trials cows were able to increase BW. These positive BW changes seem not to correspond with our results in EB. This indicates that results of body weight change in our study may not be a valid indicator of energy status in dairy cows.

4.3. Milk yield, FCM, ECM and milk components

Early and mid-lactation cows of this study showed comparable milk yields. There were no significant effects of EFE on milk yield and FCM in both trials, but ECM tended to increase in mid-lactation cows. These results did not reassure our hypothesis that early lactation cows should be more responsive to an EFE supplementation than mid-lactation cows. Beauchemin and Holtshausen (2010) argued that the stage of lactation appears to be critical for dairy cows in terms of ensuring a response to enzyme additives. While this conclusion is in line with the results of Schingoethe et al. (1999) reporting that cows starting to receive enzyme treated forage during the first 100 days postpartum produced more FCM than cows treated during mid-lactation (> 100 DIM), it is not supported by the results obtained in the current study, where no significant differences were observed for cows in early and mid-lactation.

Increased milk yields or FCM and ECM were published mainly in earlier studies investigating the potential use of EFE (Schingoethe et al., 1999; Yang et al., 1999). In the experiment implemented by Gado et al. (2009) considerable positive effects of EFE are reported (13% greater DMI, 23% greater milk production) in early lactation cows. However, the experimental conditions [milk

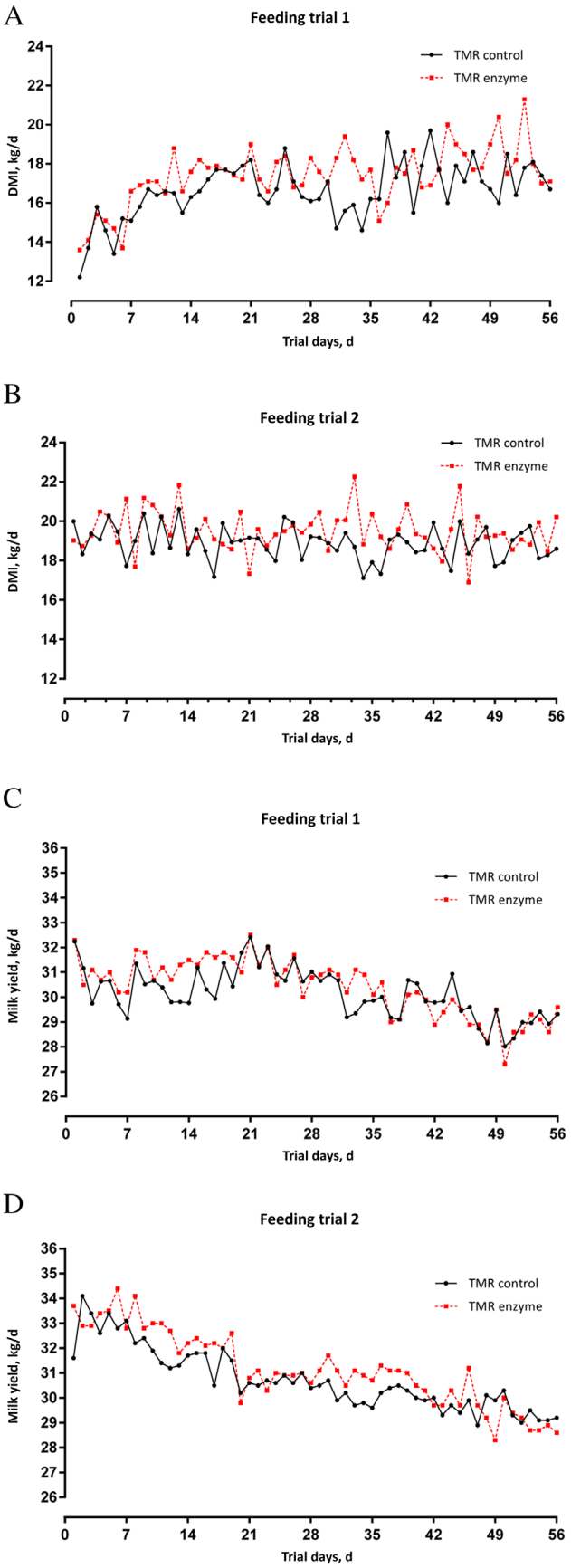

Fig. 1. (A,B) Dry matter intake (DMI) and (B,C) milk yield over time by dairy cows fed the control or the enzyme supplemented total mixed ration (TMR) in trials 1 and 2. The values shown are treatment by trial day LSmeans; the SEM for DMI in trials 1 and 2 averaged 0.81 and 0.89 kg, respectively; the SEM for milk yield in trials 1 and 2 averaged 2.26 and 1.04 kg, respectively.

yield 12.8 vs. 15.7 kg/d, protein yield 0.45 to 0.57 kg/d, respectively ($P < 0.05$)] for cows fed the EFE supplemented diet suggest a low yield environment which is not comparable with our study. Dean et al. (2013) observed a decreased milk yield for cows fed with a low EFE supplementation (1.3 mL EFE/kg DM) applied to concentrate as compared to cows fed the control diet ($P = 0.10$). These findings indicate a high variability of EFE production responses. However, our results agree with the findings of a recent meta-analysis by Ortiz-Rodea et al. (2013), who evaluated the effect of the addition of EFE in ruminant feeding on milk production and chemical composition (29 experiments including 52 treatments, 9 enzymes, and 1,187 animals) and found no increment in milk yield ($P = 0.16$) due to enzyme addition. Under conditions of a low DMI, as observed in this study, we suggest that the amount of rumen fibrolytic enzymes of the rumen microorganisms and the rumen retention time for NDF should not be limiting factors for fiber digestion which could be an explanation for the lack of production response in both trials. This corresponds with an earlier experiment (Peters et al., 2010), which reported that the EFE (Roxazyme G2 Liquid) supplementation did not affected apparent ruminal and total tract digestibility of DM, OM, NDF and ADF in diets for dairy cows under restricted feeding conditions.

We hypothesized that EFE supplementation improves fiber degradation and energy status, which could influence milk composition. In the present study, no significant effects on milk fat and protein were found. Lack of difference in milk fat content between the treatment groups in both trials is probably related to the absence of improved fiber digestion due to the EFE supplementation. Dietary differences in milk composition are generally reflective of differences in ruminal fermentation patterns. In an earlier study (Peters et al., 2010), we observed no effect of EFE addition on ruminal fermentation using the same EFE product, comparable dosage and the same basal diet. Due to the high energy requirement for protein synthesis, the milk protein yield can be affected by the energy content in the diet (Reynolds et al., 1994). Therefore, the lack of treatment effect on energy intake in both trials of our study is consistent with the absence of differences in milk protein content between cows fed control and enzyme TMR. The results of the our study agree with results of a meta-analysis by Ortiz-Rodea et al. (2013), which showed no effects of EFE addition on milk components (fat content, $P = 0.88$; lactose, $P = 0.39$; protein, $P = 0.95$). In the present study, early lactation cows fed TRM enzyme showed a tendency of a higher milk lactose percentage compared with the TMR control. A significant increase in lactose content was reported by Yang et al. (1999), who explained this effect due to the higher nutrient availability as a result of digestibility improvements caused by EFE application. In our digestibility trial with wethers we did not find any improved digestibility due to EFE treatment. Elwakeel et al. (2007) observed higher lactose content in EFE treated cows, though with a small magnitude of response, while Bowman et al. (2002) found a decrease milk lactose percentage in dependency to a specific EFE application mode. The biological reasons for varying lactose concentrations are not obvious and may relate to specific conditions of the experiment and the statistical analysis since lactose content is rather very constant.

In our study, the fat-protein-quotient (FPQ) of 1.3, with minor differences between trails and treatment, is within the range of reported reference values (H rle and Sundrum, 2013). During the

Table 6
Blood parameters of cows fed the control or the enzyme supplemented total mixed ration.

Item	Trial 1						
	Treatment		*P*-value				
	TMR control ($n = 14$)	TMR enzyme ($n = 14$)	TRT	Parity	fDIM	Trial day	TRT × Trial day
BHB, mmol/L[1]	0.67 ± 0.417	0.56 ± 0.228	0.91	< 0.01	< 0.01	< 0.01	0.16
Total protein, g/L[1]	70 ± 0.73	72 ± 0.81	0.10	0.07	0.20	< 0.01	0.68
TB, μmol/L[1]	2.9 ± 0.14	2.7 ± 0.16	0.37	0.03	0.09	< 0.01	0.88
AST, U/L[2]	61.5 ± 12.1	69.8 ± 15.8	0.16	< 0.01	0.01	0.05	0.13
GGT, U/L[2]	24.9 ± 7.6	29.5 ± 10.6	0.24	0.96	0.74	< 0.01	0.05
Item	Trial 2						
	Treatment		*P*-value				
	TMR Control ($n = 13$)	TMR Enzyme ($n = 13$)	TRT	Parity	fDIM	Trial day	TRT × Trial day
BHB, mmol/L[1]	0.64 ± 0.263	0.62 ± 0.327	0.41	0.65	0.65	< 0.01	0.37
Total protein, g/L[1]	73 ± 1.04	72 ± 1.06	0.56	0.16	0.05	< 0.01	0.61
TB, μmol/L[1]	2.7 ± 0.11	2.5 ± 0.11	0.14	0.11	0.15	0.13	0.47
AST, U/L[2]	64.3 ± 18.0	65.8 ± 19.3	0.56	< 0.01	0.31	< 0.01	0.64
GGT, U/L[2]	34.3 ± 15.1	30.3 ± 10.3	0.69	0.10	0.96	< 0.01	0.12

BHB = β-hydroxybutyrate; TB = total bilirubin; AST = aspartate-amino transferase; GGT = gamma glutamyl transferase TMR = total mixed ration; TRT = treatment; fDIM = the day in milk at the first trial day.

[1] Least squares means ± SEM (normal distributed data).

[2] Means ± SD (log-transformed data).

Table 7
Ruminating activity of cows fed the control or the enzyme supplemented total mixed ration trial 2 (LSmeans ± SEM).

Ruminating activity	Treatment		*P*-value			
	TMR control ($n = 13$)	TMR enzyme ($n = 13$)	TRT	Parity	Trial day	TRT × Trial day
Min/d	540 ± 9.7	550 ± 10.0	0.44	0.42	< 0.01	0.67
Min/kg of DMI	29 ± 0.89	28 ± 0.90	0.58	0.89	0.01	0.75
Min/kg of NDFI	65 ± 2.0	64 ± 2.0	0.58	0.89	< 0.01	0.55

Min = minutes; DMI = dry matter intake; NDFI = neutral detergent fiber intake; TMR = total mixed ration; TRT = treatment.

early lactation trial FPQ showed a typical degression while in the mid-lactation trial FPQ values were comparable.

The observed milk urea content found in this study is within the range of 150 and 300 mg/L milk expected in a diet with a balanced energy and protein supply (Kirchgessner et al., 1986). The EFE supplementation did not alter content of milk urea in both trials. In the early lactation cows the milk urea level was around 190 mL/L and in mid-lactation cows around 230 mL/L. Both levels indicate a sufficient ruminal protein and energy supply.

4.4. Gross feed efficiency (GFE)

Dairy efficiency defined as yield of milk per unit of dietary DM consumed provides a readily calculated measure of dairy herd productivity. Feed additives of the group digestibility enhancer are expected to increase fiber degradation, leading to increased DMI and milk production. Increased DMI without yield response may result in decreased gross feed efficiency (GFE), but may still have positive effects on functional efficiency and decreased veterinary cost (Sudekum and Gresner, 2013). In this study, EFE supplementation did not have a significant effect on feed efficiency (FCM/kg DMI, respectively). In contrast to our results Holtshausen et al. (2011) found a significant improved GFE ($P = 0.02$; 1.50 vs. 1.67 kg FCM/kg DMI) in EFE supplemented early lactating cows as a result of a significant lower DMI, while Arriola et al. (2011) reported a numeric decrease in DMI and increase in milk yield in early lactation cows causing a significant improved GFE ($P = 0.04$; 1.44 vs. 1.60 kg FCM/kg DMI). These positive results are based on short term experiments (60 and 70 days) and it would be beneficial to evaluate EFE effects throughout an entire lactation period including health and fertility traits. A negative effect of EFE supplementation on GFE ($P = 0.08$; 1.64 vs 1.46 kg milk/kg DMI) was found by Dean et al. (2013), whereas other studies report no differences between diets (Bernard et al., 2010; Elwakeel et al., 2007). Results of available studies do not give a clear indication of EFE effects on GFE, mainly due to high variability in DMI and milk production response. The range in GFE can be quite large. The rather high GFE found in the early lactation trial of our study (TMR-control 1.93 ± 1.08 and TMR-enzyme: 1.89 ± 0.81 kg milk/kg DMI) is presumably mainly due to a low level of DMI. We observed in early lactation cows a NEB without a loss of BW which suggests that any mobilization of body tissue may have rather been associated with the change in the fat: protein ratio of body mass.

4.5. Ruminating activities

Rumination activities are determined by particle length of forage and are associated with saliva production; coarse particles stimulate chewing activity and increase saliva output which adds to bicarbonate and phosphate buffers required for an optimal rumen function (Beauchemin et al., 2003; Krause et al., 2002). Neutral detergent fiber intake influences ruminating time and EFE supplementation is supposed to reduce ruminating time. Ruminating activities measured in our study did not show treatment

differences and fall within the higher end of the range reported by Weigand et al. (1993) and Beauchemin (1991), indicating adequate amounts of dietary physically effective fiber of the TMR. In agreement with our results, EFE supplementation had no effect on rumination activity even when adjusted for DM, NDF or ADF intake in studies by Beauchemin et al. (2000) and Bowman et al. (2003).

4.6. Blood parameters

Blood indicators are used as a tool to diagnose energy balance and hepatic functions. The analysis of EFE effects on blood parameter was restricted due to the rather small number of experimental animals involved. All blood parameter tested were within a normal physiological reference range (Kraft and Dürr, 2005). In this study supplementation of EFE had no significant effect on DMI and energy balance in both trials and; thus, no treatment effects on serum BHB, total protein, total bilirubin and hepatic enzymes (AST, GGT) were observed. These observations are not in agreement with results reported by Holtshausen et al. (2011) and Dean et al. (2013), who observed that the EFE treatment decreased plasma BHB concentration, indicating a positive effect of EFE on body fat mobilization in early and mid-lactation cows. In conclusion one can state, that several blood indicators need to be investigated to detect hepatic disorders as done in this study, but EFE treatments must show marked effects on energy balance to observe differences in blood parameter.

5. Conclusion

The Roxazyme G2 Liquid enzyme applied to a TMR at a rate of 3.9 and 3.8 mL/kg TMR DM prior to feeding of dairy cows in early and mid-lactation did not significantly enhance intake or performance in the current trials. In mid-lactation cows enzyme treatment led to a tendency for a higher ECM yield, but this finding is not sufficient to indicate a beneficial production response to EFE.

Acknowledgments

We thank the barn and laboratory staff at the Institute of Animal Nutrition of the FLI for their assistance during the implementation of the experiment and the analysis of experimental samples.

The authors also thank DSM Nutritional products for the supply of Roxazyme G2 Liquid and the analysis of enzyme activities.

References

Adesogan AT, Ma ZX, Romero JJ, Arriola KG. Ruminant nutrition Symposium: Improving cell wall digestion and animal performance with fibrolytic enzymes. J Anim Sci 2014;92:1317–30.
Ali AKA, Shook GE. An optimum transformation for somatic-cell concentration in milk. J Dairy Sci 1980;63:487–90.
Arriola KG, Kim SC, Staples CR, Adesogan AT. Effect of fibrolytic enzyme application to low- and high-concentrate diets on the performance of lactating dairy cattle. J Dairy Sci 2011;94:832–41.
Beauchemin KA. Effects of dietary neutral detergent fiber concentration and alfalfa hay quality on chewing, rumen function, and milk production of dairy cows. J Dairy Sci 1991;74:3140–51.
Beauchemin KA, Holtshausen L. Developments in enzyme usage in ruminants. In: Bedford MR, Partridge GG, editors. Enzymes in farm animal nutrition. Wallingford: Cabi; 2010. p. 206–30.
Beauchemin KA, Rode LM, Maekawa M, Morgavi DP, Kampen R. Evaluation of a nonstarch polysaccharidase feed enzyme in dairy cow diets. J Dairy Sci 2000;83:543–53.
Beauchemin KA, Yang WZ, Rode LM. Effects of particle size of alfalfa-based dairy cow diets on chewing activity, ruminal fermentation, and milk production. J Dairy Sci 2003;86:630–43.
Bernard JK, Castro JJ, Mullis NA, Adesogan AT, West JW, Morantes G. Effect of feeding alfalfa hay or Tifton 85 bermudagrass haylage with or without a cellulase enzyme on performance of Holstein cows. J Dairy Sci 2010;93:5280–5.
Bowman GR, Beauchemin KA, Shelford JA. The proportion of the diet to which fibrolytic enzymes are added affects nutrient digestion by lactating dairy cows. J Dairy Sci 2002;85:3420–9.
Bowman GR, Beauchemin KA, Shelford JA. Fibrolytic enzymes and parity effects on feeding behavior, salivation, and ruminal pH of lactating dairy cows. J Dairy Sci 2003;86:565–75.
Chung YH, Zhou M, Holtshausen L, Alexander TW, McAllister TA, Guan LL, et al. A fibrolytic enzyme additive for lactating Holstein cow diets: ruminal fermentation, rumen microbial populations, and enteric methane emissions. J Dairy Sci 2012;95:1419–27.
Coffey MP, Simm G, Hill WG, Brotherstone S. Genetic evaluations of dairy bulls for daughter energy balance profiles using linear type scores and body condition score analyzed using random regression. J Dairy Sci 2003;86:2205–12.
Dean DB, Staples CR, Littell RC, Kim S, Adesogan AT. Effect of method of adding a fibrolytic enzyme to dairy cow diets on feed intake digestibility, milk production, ruminal fermentation, and blood metabolites. Anim Nutr Feed Technol 2013;13:337–53.
Dhiman TR, Zaman MS, Gimenez RR, Walters JL, Treacher R. Performance of dairy cows fed forage treated with fibrolytic enzymes prior to feeding. Anim Feed Sci Technol 2002;101:115–25.
Elwakeel EA, Titgemeyer EC, Johnson BJ, Armendariz CK, Shirley JE. Fibrolytic enzymes to increase the nutritive value of dairy feedstuffs. J Dairy Sci 2007;90:5226–36.
Gado HM, Salem AZM, Robinson PH, Hassan M. Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. Anim Feed Sci Technol 2009;154:36–46.
GfE. Leitlinien fur die Bestimmung der Verdaulichkeit von Rohn hrstoffen an Wiederk uern. J Anim Physiol Anim Nutr 1991;65:229–34.
GfE. Recommendations for energy and nutrient requirements of dairy cattle and heifers (in German). No. 8. In: Energy and nutrient requirements of domestic animals, DLG-Verlags-GmbH, Frankfurt; 2001. p. 136.
H rle C, Sundrum A. Animal health on the farm level 2. Communication: nutrient supply on Bavarian Dairy Farms. Zuchtungskunde 2013;85:396–412.
Holtshausen L, Chung YH, Gerardo-Cuervo H, Oba M, Beauchemin KA. Improved milk production efficiency in early lactation dairy cattle with dietary addition of a developmental fibrolytic enzyme additive. J Dairy Sci 2011;94:899–907.
Ingvartsen KL, Moyes K. Nutrition, immune function and health of dairy cattle. Animal 2013;7(Suppl. 1):112–22.
Jung HG, Allen MS. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J Anim Sci 1995;73:2774–90.
Kirchgessner M, Kreuzer M, Roth-Maier DA. Milk urea and protein content to diagnose energy and protein malnutrition of dairy cows. Arch Tierern hrung 1986;36:192–7.
Knowlton KF, McKinney JM, Cobb C. Effect of a direct-fed fibrolytic enzyme formulation on nutrient intake, partitioning, and excretion in early and late lactation Holstein cows. J Dairy Sci 2002;85:3328–35.
Kononoff PJ, Lehman HA, Heinrichs AJ. Technical note – a comparison of methods used to measure eating and ruminating activity in confined dairy cattle. J Dairy Sci 2002;85:1801–3.
Kraft W, Durr UM. Klinische Labordiagnostik in der Tiermedizin. 6. Auflage Stuttgart/New York: Schattauer; 2005.
Krause KM, Combs DK, Beauchemin KA. Effects of forage particle size and grain fermentability in midlactation cows. II. Ruminal pH and chewing activity. J Dairy Sci 2002;85:1947–57.
Krause KM, Oetzel GR. Understanding and preventing subacute ruminal acidosis in dairy herds: a review. Anim Feed Sci Technol 2006;126:215–36.
Meale SJ, Beauchemin KA, Hristov AN, Chaves AV, McAllister TA. Board-invited review: opportunities and challenges in using exogenous enzymes to improve ruminant production. J Anim Sci 2014;92:427–42.
Miller DR, Elliott R, Norton BW. Effects of an exogenous enzyme, Roxazyme (R) G2 Liquid, on digestion and utilisation of barley and sorghum grain-based diets by ewe lambs. Anim Feed Sci Technol 2008a;140:90–109.
Miller DR, Elliott R, Norton BW. Effects of an exogenous enzyme, Roxazyme (R) G2, on intake, digestion and utilisation of sorghum and barley grain-based diets by beef steers. Anim Feed Sci Technol 2008b;145:159–81.
Miller DR, Granzin BC, Elliott R, Norton BW. Effects of an exogenous enzyme, Roxazyme (R) G2 Liquid, on milk production in pasture fed dairy cows. Anim Feed Sci Technol 2008c;145:194–208.
Naumann C, Bassler R. Die chemische Untersuchung von Futtermitteln. 3rd ed. Darmstadt: VDLUFA-Verl; 2012.
Ortiz-Rodea A, Noriega-Carrillo A, Salem AZM, Castelan Ortega O, Gonzalez-Ronquillo M. The use of exogenous enzymes in dairy cattle on milk production and their chemical composition: a meta-analysis. Anim Nutr Feed Technol 2013;13:399–409.
Pappritz J, Meyer U, Kramer R, Weber EM, Jahreis G, Rehage J, et al. Effects of long-term supplementation of dairy cow diets with rumen-protected conjugated linoleic acids (CLA) on performance, metabolic parameters and fatty acid profile in milk fat. Arch Anim Nutr 2011;65:89–107.
Peters A, Lebzien P, Meyer U, Borchert U, Bulang M, Flachowsky G. Effect of exogenous fibrolytic enzymes on ruminal fermentation and nutrient digestion in dairy cows. Arch Anim Nutr 2010;64:221–37.

Reddish MA, Kung Jr. L. The effect of feeding a dry enzyme mixture with fibrolytic activity on the performance of lactating cows and digestibility of a diet for sheep. J Dairy Sci 2007;90:4724–9.
Reynolds CK, Harmon DL, Cecava MJ. Absorption and delivery of nutrients for milk protein synthesis by portal-drained viscera. J Dairy Sci 1994;77:2787–808.
Schingoethe DJ, Stegeman GA, Treacher RJ. Response of lactating dairy cows to a cellulase and xylanase enzyme mixture applied to forages at the time of feeding. J Dairy Sci 1999;82:996–1003.
Sudekum KH, Gresner N. Feed additives in relatin to feed efficiency: "Feed efficience in dairy cattle". 2013 (Online). Available at:http://www.wageningenur.nl/en/show/International-Dairy-Nutrition-Symposium-2013-Feed-efficiency-in-dairy-cattle.htm.
Vicini JL, Bateman HG, Bhat MK, Clark JH, Erdman RA, Phipps RH, et al. Effect of feeding supplemental fibrolytic enzymes or soluble sugars with malic acid on milk production. J Dairy Sci 2003;86:576–85.
von Soosten D, Meyer U, Weber EM, Rehage J, Flachowsky G, D nicke S. Effect of trans-10, cis-12 conjugated linoleic acid on performance, adipose depot weights, and liver weight in early-lactation dairy cows. J Dairy Sci 2011;94:2859–70.
Weigand E, Meyer U, Guth N. Intake, chewing activity and carbohydrate digestibility by lactating dairy-cows fed maize silage with a different physical structure. J Anim Physiol Anim Nutr 1993;69:120–32.
Yang WZ, Beauchemin KA, Rode LM. Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. J Dairy Sci 1999;82:391–403.
Zeitz JO, Guertler P, Pfaffl MW, Eisenreich R, Wiedemann S, Schwarz FJ. Effect of non-starch-polysaccharide-degrading enzymes as feed additive on the rumen bacterial population in non-lactating cows quantified by real-time PCR. J Anim Physiol Anim Nutr 2013;97:1104–13.