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Original Research Article

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

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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.

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.,

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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,