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The effect of replacing barley with glycerol in the diet of dairy cows on rumen parameters and milk fatty acid profile

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ABSTRACT

The objective of this paper was to evaluate the changes in rumen parameters (pH, NH_3-N and volatile fatty acids (VFA) proportions), caused by large amounts of dietary glycerol fed to dairy cows, and whether those changes are associated with differences in the milk fatty acid (FA) composition. For this study eight primiparous Estonian Holstein cows (134 ± 5 DIM at the beginning of the experiment) were paired and used in a 4 × 4 Latin square experimental design. Cows were fed a total mixed ration *ad libitum*, consisting of grass silage, barley meal, soybean meal and minerals (control) with crude glycerol isoenergetically replacing barley meal in experimental diets to inclusion levels of 52 g/kg, 104 g/kg and 156 g/kg of crude glycerol in the dry matter (DM) of the diets. The effect of the diets was studied with the analyses of variance followed by the tests of linear and quadratic contrasts. Pearson correlation was used to assess the suitability of models predicting rumen VFA proportions based on milk FA, when glycerol-containing diets were fed. Results showed that dietary glycerol did not affect ruminal pH but linearly increased the relative proportions of propionate and butyrate in rumen fluid, at the expense of acetate. The ratios of acetate to propionate, and lipogenic VFA to propionate, showed both linear and quadratic changes. The proportion of 2-methyl butyrate changed both linearly and quadratically; highest when the diet with 52 g/kg in DM of crude glycerol was fed. Rumen total VFA content decreased linearly according to the crude glycerol content in the diets. Replacing barley meal with glycerol in the diets affected the concentration of most of the *de novo* synthesized milk FA, as well as FA in different groups according to chain length or degree of saturation. The proportions of even-chain saturated FA C6:0–C10:0 increased linearly in milk fat of cows in the dietary glycerol treatments, whereas the proportions of C14:0 and C16:0 decreased quadratically. The proportion of saturated odd-chain FA (C5–C17) increased linearly and quadratically with increasing dietary glycerol treatments. Correlation analysis indicated that the increase in the proportion of propionate in total VFA was accordance with the changes in the milk FA profile, increasing the odd-chain (C5–C17) FA concentration.

Abbreviations: ADF, acid detergent fibre; aNDF, neutral detergent fibre assayed with heat stable amylase and expressed inclusive of residual ash; DM, dry matter; DIM, days in milk; FA, fatty acid; TMR, total mixed ration; VFA, volatile fatty acids.

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1. Introduction

Rapid growth in the alternative fuel market has increased the availability and decreased the price of glycerol, making it available as an animal feed. Glycerol is a by-product of biodiesel production, with approximately 4.5 kg of crude glycerol available from every 50 l of biodiesel produced (Donkin, 2008). The use of glycerol for preventing or treating post-partum ketosis has been a research subject for some time (Johnson, 1954; DeFrain et al., 2004). Due to the increased availability of glycerol, it has also been proposed as an additional energy-rich feed for both beef (Mach et al., 2009) and dairy cattle (Donkin et al., 2009; Kass et al., 2013). In such experiments most attention has been given to performance parameters, with occasionally some interest in the effect of glycerol on rumen environment *in vivo* (Wang et al., 2009) and *in vitro* (Abo El-Nor et al., 2010; Krueger et al., 2010). While the direct evaluation of ruminal conditions is difficult, invasive, time consuming and impractical in production conditions, biomarkers of rumen function from easily collected milk samples could be indicative. In this respect, milk fatty acids (FA) have been shown to be possible markers of rumen microbial population and fermentation pattern (Cabrita et al., 2003; Fievez et al., 2012). As previously mentioned studies mainly focused on changes in the supply of carbohydrate or protein sources, it is unknown whether they are suitable for diets supplied with high amounts of glycerol. Few papers have been published regarding the effect of glycerol on milk FA pattern, and in these studies only a limited selection of FA were reported (Khalili et al., 1997; Zymon et al., 2012). Therefore, our aim was to evaluate the changes in rumen environment and volatile fatty acid (VFA) content of rumen fluid caused by high amounts of glycerol, and how those changes affect the milk FA pattern.

2. Materials and methods

2.1. Experimental design and diets

This is the second part of a study (Kass et al., 2012) investigating the effect of dietary glycerol on production traits.

Animal use and care was in accordance with the Estonian Animal Protection Act. The experiment was conducted at the Eerika Experimental Farm of the Estonian University of Life Sciences (Märja, Estonia). Eight primiparous Estonian Holstein cows (DIM 134 ± 15 at the beginning of the experiment) were paired by milk yield (24.7 ± 1.0 kg/d) and body weight (535 ± 13.5 kg) and used in 4 × 4 Latin square experimental design. One cow from each experimental pair was ruminally fistulated. Cows were housed in individual tie stalls and milked twice daily at 05:00 and 15:00. Each experimental period lasted 21 days, of which 16 days were for adaptation. The somatic cell counts of the milk samples involved in the study were lower than 200,000 cells/ml. Single cows had elevated somatic cell counts on four occasions during the experiment. Their data were omitted for the experimental period when the problem occurred.

Cows were fed a total mixed ration (TMR) *ad libitum* twice daily at 06:00 and 16:00 (5–10% refusals). The TMR was mixed manually for each individual cow before feeding. The basal diet consisted of grass silage, barley meal, soybean meal, limestone, salt and a mineral mix prepared for lactating cows. Experimental diets consisted of the basal diet (control, G0), in which barley meal was isoenergetically replaced by crude glycerol in combination with a non-protein nitrogen source (Optigen II; Alltech, USA) to balance the nitrogen contents of the diets. The inclusion level of crude glycrerol in experimental diets was 52 g/kg (G52), 104 g/kg (G104) and 156 g/kg (G156) of diets' dry matter. Feed ingredients and chemical composition of the experimental diets are shown in Tables 1 and 2.

2.2. Sample collection

Samples of the silage, barley meal and soybean meal were taken in the last 5 days of each experimental period and pooled for chemical analysis. Feed residuals were removed and weighed twice a day before fresh feed was offered. Dry matter intake was calculated based on the dry matter weight of TMR offered and feed residuals. Rumen pH was measured during the last 2 days of each experimental period using an indwelling pH meter (PHCN-37 Microprocessor-based pH Controller, Submersible Flat Surface pH/ORP electrodes; Omega Engineering Inc., Stanford, USA) once each hour from the morning feeding 6:00 until

Table 1
Chemical composition of the feeds used in the diets (g/kg DM unless noted differently).

	Grass silage	Soybean meal	Barley meal	Crude glycerol[1]	Optigen II[2]
Dry matter (g/kg)	280	880	860	929	100
Crude protein	155	495	116	–	2,560
Ether extract	29	27	22	6.5	120
aNDF[3]	546	166	224	–	–
ADF[4]	379	123	73	–	–
Crude ash	87	61	22	100	–
Metabolizable energy[5] (MJ/kg DM)	9.4	13.0	14.3	14.0	4.8

[1] Crude glycerol (BioOil Ltd., Estonia) = glycerol – 889 g/kg in DM, methanol – 4.5 g/kg in DM.
[2] Optigen II = Alltech, USA; non-protein nitrogen source. Chemical composition as provided by producer.
[3] aNDF = Neutral detergent fibre assayed with a heat stable amylase and expressed inclusive of residual ash.
[4] ADF = acid detergent fibre.
[5] Metabolizable energy calculated according to Oll (1995).