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Influence of alpine forage either employed as donor cow's feed or as incubation substrate on *in vitro* ruminal fatty acid biohydrogenation

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ABSTRACT

The effects of alpine grazing on ruminal fatty acid biohydrogenation were studied *in vitro* in comparison to different lowland controls, by (i) adapting a fistulated ruminal fluid donor cow to alpine pasture and (ii) by incubating alpine forages with the resulting ruminal fluid. The cow first received a grass–maize silage based diet in the lowlands. Subsequently, the cow grazed for 10 weeks a species-rich alpine pasture at about 2000 m elevation. This was followed by another lowland period on the mixed silage diet. Ruminal fluid was collected during the initial lowland period, after 2, 6, and 10 weeks of alpine sojourn, and 2 and 6 weeks after returning to the lowlands. Always 3.5 h after collection, 24-h *in vitro* incubations with batch cultures were started. Incubations were done in triplicate with either alpine pasture hay, lowland ryegrass hay or a grass–maize silage mixture. Non-incubated rumen fluid and all incubation liquid samples were analysed for fatty acid composition. Contents of total phenols were about twice as high in forage of alpine compared to lowland forage. Incubations with ruminal fluid of alpine origin compared to lowland origin increased the apparent net production and the proportion in total fatty acids of vaccenic acid (18:1 *trans*-11) by more than 1.5 times ($P<0.05$).This happened across all incubated forages and indicated that alpine pasture may have an inhibitory effect on the last step of ruminal fatty acid biohydrogenation. Linoleic acid (18:2*n*-6) and α-linolenic acid (18:3*n*-3) proportions of total fatty acids remained unaffected like also apparent stearic acid production. No clear influence of the type of forage incubated was observed, except for a higher 18:2*n*-6 disappearance rate during incubation with the mixed silage compared to the two hay types. Incubating the alpine hay resulted in a trend towards a lower 18:3*n*-3 disappearance rate during incubation. These results indicate that adapting the ruminal fluid donor cow for several weeks to different diets was more decisive for ruminal fatty acid biohydrogenation *in vitro* than the immediate effects of the different incubated forages. The present results provided no clear evidence which would help to explain the repeatedly described elevation of 18:3*n*-3 concentration in alpine milk fat. Different from that, indications explaining the extra conjugated linoleic acid were obtained.

1. Introduction

Alpha-linolenic acid (18:3*n*-3) and rumenic acid (18:2 *cis*-9, *trans*-11), are considered to benutraceuticals in the human diet (Barceló-Coblijn and Murphy, 2009; Benjamin and Spener, 2009). In the animal, dietary factors largely influence passage through and modification of these fatty acids (FA) in the rumen and thus the FA profile of ruminant-source food (Chilliard et al., 2007; Lourenço et al., 2008b). Proportions of 18:3*n*-3 and 18:2 *cis*-9, *trans*-11 were found to be higher in the lipids of products from ruminants grazing on pasture than from those fed on conventional maize- and concentrate-based diets (Jahreis et al., 1997; Kay et al., 2005; Leiber et al., 2005; Razminowicz et al., 2006). Particularly high concentrations of 18:3*n*-3 have been repeatedly measured in dairy

Abbreviations: NDF, neutral-detergent fibre; CP, crude protein; CT, condensed tannin; DM, dry matter; FA, fatty acid; FAME, FA methyl ester; GC, gas chromatograph; MUFA, monounsaturated FA; OM, organic matter; PUFA, polyunsaturated FA; PSC, plant secondary compound; SFA, saturated FA.

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products derived from cows grazing on alpine pastures (Collomb et al., 2002b; Hauswirth et al., 2004; Kraft et al., 2003). However, it appears that this is not related to a correspondingly higher 18:3*n*-3 intake of the cows (Leiber et al., 2004; Leiber et al., 2005). The most probable cause to explain this particular quality of alpine-originated milk fat is rather the inhibited or modified ruminal FA biohydrogenation. Biohydrogenation is the stepwise hydration and isomerisation of ingested polyunsaturated fatty acids by the ruminal bacteria, which causes losses of more than 90% of these FA (Chilliard et al., 2007). However, it is assumed, that a partial inhibition of the ruminal biohydrogenation can be caused by plant secondary compounds (PSC) occurring in typical alpine pasture herbs (Cabiddu et al., 2009; Leiber et al., 2005). Changes in ruminal FA metabolism as caused by certain PSC were reported *in vitro* (Cabiddu et al., 2010; Durmic et al., 2008; Khiaosa-ard et al., 2009; Vasta et al., 2009a) and *in vivo* (Cabiddu et al., 2009). Cabiddu et al. (2009 and 2010) demonstrated that 18:3*n*-3 proportions in rumen fluid and milk lipids are positively affected by plant phenols, likely through an inhibition of the first biohydrogenation step.

The aim of the present study was to determine if, and to which extent, forages from biodiverse alpine swards, rich in various PSC, affect ruminal FA biohydrogenation. This was tested by an approach including adaptation of a ruminal fluid donor animal to alpine forage for different time periods (2 to 10 weeks) and short term (24 h) incubation of such a forage *in vitro*. Various control periods and feeds were used for comparisons. The design allowed determining interactions between the incubated forage type and the adapted *vs*. non-adapted ruminal fluid.

2. Materials and methods

2.1. Treatment of the donor cow

The experimental ruminal fluid donor cow was cared for according to the Swiss guidelines for animal welfare (permission ZG50/08 of the veterinary cantonal authority). This rumen-cannulated non-lactating Brown Swiss cow was assigned to different locations and diets. The experiment lasted for 16 weeks (from end of June to mid of October 2008).The cow had been kept in the lowlands (ETH research station Chamau, central Switzerland, 400 m above sea level (a.s.l.)) for the entire winter and spring before the start of the experiment. Subsequently, the animal was transferred to a highland alpine site (ETH research station Alp Weissenstein south-eastern Switzerland, 2000 ma.s.l.), where it grazed freely for 10 weeks. Finally, it returned to the lowland site for another 6 weeks. During this experimental period, ruminal fluid was collected six times for analysis and subsequent *in vitro* incubations, namely in week 0 (Lowland), weeks 2, 6 and 10 on the alpine site (Alpine) and in weeks 2 and 6 after return to the lowland site. The lowland diet always consisted of the same kind and quality of grass hay, grass and maize silage (1:3:6) provided at *ad libitum* access, and 1 kg per day of a mixture of commercial energy and protein concentrates (UFA 248 and UFA 249, UFA AG, Herzogenbuchsee, Switzerland). The grazing area at the high altitude station comprised more than 70 plant species, >26, >16 and >28 thereof being grasses, legumes and herbs, respectively (Leiber et al., 2005).The cow could freely move between a *Crepido aureae–Festucetum rubrae* and a *Deschampsio cespitosae–Poetum alpinae* vegetation type. One day prior to each ruminal fluid collection, a representative forage sample to be later analysed for its nutrient composition was collected by following the donor cow and mimicking its grazing behaviour as described by Berry et al. (2000). The nutrient composition, phenolic fractions and fatty acid profile of these pasture samples are presented in Table 1. The assumption was made that the grazing behaviour of that respective sampling day was similar to the days before. Samples of the lowland diet were collected from the feeding trough. Amounts of 1 l of ruminal fluid were collected in the morning at 09:30. The bottle with ruminal fluid was kept in a 38 °C water bath for transport to the laboratory. Always after 3.5 h, the bottle was opened in the lab and the fluid was processed for incubation.

2.2. In vitro incubation

An *in vitro* Hohenheim gas test apparatus was used to incubate in triplicate 200 mg air-dry matter of three forages for 24 h at 39 °C. The incubated forages were (i) pure lowland ryegrass hay (*Lolium multiflorum*, cut when budding), expected to be poor in PSC, (ii) botanically diverse alpine hay, collected from a *Crepido aureae–Festucetum rubrae* meadow at 1700 ma.s.l. and presumed to be rich in PSC, and (iii) a grass–maize silage mixture from the lowlands (other batches than those fed to the donor cow). The analysed nutrient composition and fatty acid profile of the incubated forages are presented in Table 1. Preparation of the incubation medium and the incubation procedure were performed following the protocol outlined in Soliva and Hess (2007). Briefly, 30 ml of strained ruminal fluid were mixed with buffer solution (1:2) and added to the experimental forages in the Hohenheim gas test pistons. Triplicates of blank pistons (containing only incubation liquid without feed) as well as triplicates of pistons filled with standard hay and concentrate obtained from the University of Hohenheim were incubated as well. All pistons contained 0.2 ml of a 10 ml/l aqueous solution of Tween® 80 (polyoxyethylene sorbitan monooleate, Sigma-Aldrich, Saint Louis, MO, USA). After incubation, the incubation liquid containing feed residues (later on called 'incubation liquid') was removed from the syringe and samples were stored at −20 °C. Additionally, samples of the buffered but not yet incubated ruminal fluid were taken to obtain information about the initial FA status and for quantitative calculations of the disappearance rate of FA.

2.3. Laboratory analyses

After drying for 24 h at 60 °C (where necessary) and grinding through a 1-mm sieve, all experimental feeds and the donor cow's diets were analysed by an automatic furnace (TGA-500, Leco Corporation, St. Joseph, MI, USA) for their contents of dry matter (DM) and total ash as outlined by AOAC (1997, index no. 942.05). A C/N analyser (Type FP-2000, Leco) was used to determine N, with crude protein (CP) being calculated as N × 6.25 (AOAC index no. 977.02). Neutral detergent fibre (NDF) content was analysed (Fibertec 1020 system, Tecator, Höganäs, Sweden) using α-amylase