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Milk odd- and branched-chain fatty acids as biomarkers of rumen function—An update☆

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ABSTRACT

The main odd- and branched-chain fatty acids (OBCFA) in milk of dairy cows are isomers of tridecanoic acid (*iso* C13:0), tetradecanoic acid (*iso* C14:0), pentadecanoic acid (C15:0, *iso* C15:0 and *anteiso* C15:0), hexadecanoic acid (*iso* C16:0) and heptadecanoic acid (C17:0, *iso* C17:0 and *anteiso* C17:0). OBCFA are suggested to reflect rumen function (*e.g.* ruminal fermentation pattern, including methane, duodenal flow of microbial protein and acidosis). This relies on their predominant origin, *i.e.* bacteria leaving the rumen. The OBCFA are synthesized *de novo* by ruminal bacteria and incorporated in their cell membrane, suggesting a direct relation with bacterial biomass. Their potential as duodenal markers to quantify bacterial protein is strengthened by their constant relation with bacterial N content over a diversity of bacterial groups. From a limited database, evidence was shown of a useful relation of milk OBCFA yield with microbial protein flow from the rumen, but more research is needed to elucidate some discrepancies under diverse dietary regimes.

Further, variation in the OBCFA profile of pure strains of ruminal bacteria were reported and are, in the current review, linked with their production of metabolites. From this, it can be assumed that the rumen fermentation pattern is related to the rumen OBCFA profile, which seems consistent for milk OBCFA. The close stoichiometric relation between ruminal VFA and methane further opens perspective for the use of OBCFA profiles in milk to quantify methane emissions. OBCFA consistently contributing to the predictive models, irrespective of the modeling approach are: *iso* C14:0 and *iso* C15:0, which positively relate to acetate and methane and negatively to propionate; and C15:0 and C17:0 which show an inverse relationship. *Anteiso* C15:0 seemed only relevant in the prediction of butyrate proportions.

Abbreviations: ANN, artificial neural network; ANTE, sum of *anteiso* C13:0, *anteiso* C15:0 and *anteiso* C17:0; CCC, concordance correlation coefficient; DAPA, diaminopimelic acid; DMI, dry matter intake; F:C, forage to concentrate ratio; FA, fatty acids; FA ≤ C16, fatty acids with chain length of 16 carbons or shorter; FAME, fatty acid methyl esters; GA, genetic algorithm; GC, gas chromatography; IR, infrared; LAB, liquid associated bacteria; MIR, mid-infrared; MLR, multiple linear regression; MP, milk production; MPr, microbial protein; OBCFA, odd- and branched-chain fatty acids; ODD, sum of odd-chain fatty acids with chain length from 5 to 23 carbons; PB, purine bases; RMSPE, root mean square prediction error; R^2, Coefficient of determination; PLS, partial least square; SAB, solid associated bacteria; SARA, subacuteruminal acidosis; VFA, volatile fatty acids; Var PE, variance of the prediction error; VIP, variable of importance of projection; VLDL, very low density lipoproteins.

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As changes in the ruminal microbial population (*e.g.* increased dominance of *Streptococcus bovis*) sometimes initiate a chain of events that eventually might lead to (sub-acute) ruminal acidosis, OBCFA in milk fat are targeted as candidates for the early detection of ruminal acidosis. Increasing C17:0 + C17:1 *cis*-9 and decreasing *iso* C14:0 concentration show potential as indicators of sub-acute acidosis or were obvious before clinical symptoms of acute acidosis occurred. Collection of more experimental data is currently on-going for the development of more robust models to classify rumen health in continuous probability classes rather than discrete acidotic *vs.* non-acidotic cases.

1. Introduction

There is an increased interest in the use of milk components to give additional information on rumen fermentation, rumen feed degradability and utilization in the dairy cow and its metabolic condition. Research at our laboratory focuses on components in milk that may act as a marker of rumen function and microbial synthesis. In this respect, odd- and branched-chain fatty acids (OBCFA) in milk fat are specifically emphasized as they are largely derived from rumen bacteria. Obviously, these specific components of rumen microbes, which are transferred to milk, could be useful non-invasive indicators of rumen fermentation. In our former review paper on this topic (Vlaeminck et al., 2006a), we extensively documented concomitant variation in milk OBCFA and dietary composition, and related this to OBCFA composition of pure strains of rumen bacteria. Further ongoing research focuses on the potential of OBCFA in milk as biomarkers of major nutrients and emissions from the rumen and its health status. Prediction of rumen volatile fatty acids (VFA), microbial protein and methane as well as early detection of ruminal acidosis were the main focus and are summarized here. Moreover, progress was made on the fast analysis of these compounds which is essential for practical purposes.

2. OBCFA profiles in rumen bacterial species and relation to their metabolites

Microbial formation of OBCFA has been outlined in detail by Vlaeminck et al. (2006a). In summary, odd-chain fatty acids (C15:0 and C17:0) are formed through elongation of propionate or valerate, whereas precursors of branched-chain fatty acids (*iso* C13:0, *iso* C14:0, *iso* C15:0, *iso* C16:0, *iso* C17:0, *iso* C18:0, *anteiso* C13:0, *anteiso* C15:0, *anteiso* C17:0) are branched-chain amino acids (valine, leucine and isoleucine) and their corresponding branched- short-chain carboxylic acids (isobutyric, isovaleric and 2-methyl butyric acids). The OBCFA profile of the rumen bacteria seems to be largely determined by the fatty acid synthase activity of the microorganism rather than by the precursor availability (Vlaeminck et al., 2006a). Hence, variation in the OBCFA profile leaving the rumen is expected to reflect changes in the relative abundance of specific bacterial populations in the rumen rather than an altered bacterial fatty acid synthesis. Accordingly, higher proportions of *iso*-fatty acids in solid associated bacteria were suggested to reflect their enrichment in cellulolytic bacteria, whereas higher proportions of *anteiso* C15:0 in liquid associated bacteria might indicate their enrichment in pectin and sugar fermenting bacteria, which seem particularly enriched in this fatty acid (Table 1) (Vlaeminck et al., 2006a; Bessa et al., 2009).

As both the fermented substrate and the main end products are of nutritional importance, detailed information on the OBCFA profile of different rumen bacteria and their main fermentation substrates and end products are shown in Table 1. Rumen cellulolytic bacteria, such as *Ruminococcus albus*, *Butyrivibrio fibrisolvens* and *Ruminococcus flavefaciens,* contain relatively high amounts of even and/or odd- *iso*-fatty acids (Table 1). These fibre digesting bacteria mainly ferment cellulose, hemicellulose and pectin to acetate, butyrate, hydrogen (H_2) and carbon dioxide (CO_2). On the other hand, amylolytic bacteria, such as *Ruminobacter amylophilus*, *Selenomonas ruminantium*, *Streptococcus bovis* and *Succinomonas amylolytica*, show low levels of branched-chain fatty acids, particularly *iso*-branched chains and are enriched in linear odd-chain fatty acids and/or *anteiso*-branched-chain fatty acids (Table 1). The latter fatty acids (FA) seem of particular importance in sugar or pectin fermenting bacteria such as *Prevotella* spp., *Lachnospira multiparus* and *Succinovibrio dextrinosolvens* (Table 1). Amylolytic or starch and sugar digesting bacteria ferment sugar, starch and peptides to propionate, butyrate, acetate, lactate, H_2 and CO_2. *Eubacterium ruminantium* and *S. bovis* are lactate producers and do not synthesize acetate. These bacterial species play an important role in the onset of acidosis. Their most important OBCFA are C15:0 and *anteiso* C15:0. During sub-acute rumen acidosis (SARA), *Megasphaera elsdenii* plays an important role (Nagaraja and Lechtenberg, 2007). *M. elsdenii* is a lactate-utilizing bacterium and the main FA in its cell wall is C15:0. Methanogens are involved in the production of methane and are members of the domain Archaea belonging to the order of the *Methanobacteriales*. The most common species of methanogens isolated from the rumen are strains of *Methanobrevibacter*, *Methanomicrobium*, *Methanobacterium*, and *Methanosarcina*. No details on the OBCFA profile of these bacteria are available yet (Whitford et al., 2001; Boadi et al., 2004).

3. Transfer of OBCFA from rumen or duodenum to the mammary gland and alternative origin of milk OBCFA

The bacterial origin of branched-chain FA in milk fat was already recognized half a century ago (Keeney et al., 1962). However, this does not guarantee that the milk OBCFA profile is a direct reflection of the OBCFA profile of the rumen bacteria