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Rumen and milk odd- and branched-chain fatty acid proportions are minimally influenced by ruminal volatile fatty acid infusions

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ABSTRACT

The objective of this study was to determine if ruminally infusing volatile fatty acid (VFA) increased concentration of their homologous odd- and branched-chain fatty acid (OBCFA) in rumen contents and milk. The influence of VFA on dry matter intake (DMI), blood metabolites, and blood insulin was also evaluated. Four mid-lactation cows were assigned to a 4 × 4 Latin square design with 48-h periods. Infusion treatments were acetate (AC), propionate (PR), isovalerate (IV), and anteisovalerate (AIV). Infusions began (time = 0) 5.5 h before feeding at 17.4 mmol of VFA/min and were terminated at 18 h. Infusions rates were well above physiological levels for IV and AIV. Surprisingly, the greatest differences in rumen OBCFA were increases in rumen liquid *iso* C15:0 and nonbranched C17:0 for AIV. In addition, infusing AIV increased *anteiso* C15:0 and *anteiso* C17:0 in rumen solid contents. Infusing IV increased *iso* C15:0 in both rumen solids and milk. Propionate increased milk C15:0 and C17:0. Both gluconeogenic compounds, PR and AIV, had similar proportions of milk C15:0, which was greater than that obtained with AC and IV. Rumen and blood VFA were as expected, with increased concentrations of the VFA present in the infusate. At 23 h, and consistently throughout infusions, DMI was similar for AC compared with PR and for AIV compared with IV. Both IV and AIV decreased DMI and energy balance; however, only IV increased plasma nonesterified fatty acids (121, 78, 172, and 102 m*M* for AC, AIV, IV, and PR), increased β-hydroxybutyrate (10.8, 5.9, 51.9, 5.4 mg/dL for AC, AIV, IV, and PR), and reduced plasma glucose (56.3, 59.1, 31.9, and 64.3 mg/dL for AC, AIV, IV, and PR). Rumen and milk OBCFA responses were minimal following infusion of large amounts of IV and AIV, suggesting limited use of IV, and AIV for de novo OBCFA synthesis, either pre- or postabsorption. Minor increases in milk odd-chain fatty acids following large doses of ruminal PR support the presence of postabsorptive synthesis of these milk odd-chain fatty acids.
Key words: odd- and branched-chain fatty acid, dairy cow, volatile fatty acid infusion, rumen

INTRODUCTION

Ruminant lipids contain nonbranched fatty acids with an odd carbon number and branched-chain fatty acids with an odd or even carbon number, collectively termed odd- and branched-chain fatty acids (**OBCFA**). Because these OBCFA are not usually found in animal feeds, the appearance of OBCFA in lipids of herbivores led Akashi and Saito (1960) to suggest that these FA arise from microorganisms populating the gut. The theory was supported by Keeney et al. (1962), who determined that OBCFA comprised the majority of total lipids within culturable rumen bacteria, further suggesting that milk OBCFA have the potential to estimate rumen microbial yield.

Bacterial branched-chain FA can be synthesized from branched-chain AA (**BCAA**) or extracellular branched-chain VFA (**BCVFA**; Kaneda, 1991). The BCAA Leu, Ile, and Val are incorporated into branched-chain FA intracellularly via deamination and decarboxylation into the acyl-CoA esters 3-methylbutyryl-CoA, 2-methylbutyryl-CoA, and isobutyryl-CoA, followed by elongation into branched-chain FA. Acyl-CoA esters can also be formed from BCVFA by fatty acid synthetase. The acyl-CoA esters are elongated into *iso* methyltetradecanoic acid, *iso* C15:0; *iso* methylhexadecanoic acid, *iso* C17:0 (from 3-methylbutyryl-CoA); *anteiso* methyltetradecanoic acid, *anteiso* C15:0; *anteiso* methylhexadecanoic acid, *anteiso* C17:0 (from 2-methybutyryl-CoA); *iso* tetradecanoic acid, *iso* C14:0; and *iso* hexadecanoic acid, *iso* C16:0 (from isobutyryl-CoA). Formation from BCVFA, 3-methylbuyrate (isovalerate, **IV**), 2-methylbutyrate (anteisovalerate, **AIV**), and isobutyrate primarily occurs by the deamination and decarboxylation of BCAA, although BCVFA may also be synthesized through acyl transferases or fatty acid kinase activity (Kaneda, 1991). Amino acid catabolism in one species and use of released BCVFA by another species can result in conversion of BCAA to OBCFA, with transfer

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of BCVFA through the ruminal fluid space. The odd and nonbranched FA pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) are formed by the elongation of propionyl-CoA or valeryl-CoA (Kaneda, 1991).

Alterations of the OBCFA content of the rumen microbial mass can be due to a shift in microbial species as the innate OBCFA content differs across microbial species. Alternatively, within-species shifts in OBCFA pattern might be caused by altering the substrate pattern offered (Allison et al., 1962; Wegner and Foster, 1963; Allison and Peel 1971; Emmanuel, 1978).

Dewhurst et al. (2007) compared milk yields versus duodenal FA flows and measured greater secretion of C15:0, C17:0, and *iso* C17:0 in milk fat than could be accounted for solely by absorption. Milk yields greater than duodenal flow for a particular OBCFA could occur via several routes. The mammary gland could directly synthesize OBCFA, as observed previously when propionate (**PR**) was perfused into goat and cow udders and milk odd-chain FA increased (James et al., 1956; Massart-Leën et al., 1983). Other tissues have also demonstrated the ability to synthesize OBCFA from PR (Berthelot et al., 2001) and milk OBCFA could increase by incorporating OBCFA mobilized from other tissues.

Limited in vivo studies have observed the effects of VFA as FA precursors. The objective of the current study was to observe the effects of intraruminally infusing VFA in vivo on rumen fatty acid proportions and their appearance in milk. Intraruminal infusions of PR previously increased proportions of odd-chain FA in milk (Emmanuel and Kennelly, 1985; Rigout et al., 2003). Treatments in our present study included acetate (**AC**), PR, and the BCVFA AIV and IV. The effect of infusing VFA on DMI and the physiological state of the animals was also observed as mobilized FA from adipose tissue can influence milk OBCFA (Craninx et al., 2008). Infusing AIV and IV was expected to increase appearance of the respective rumen odd *anteiso* and *iso* FA via microbial synthesis that would be reflected in milk fat. As suggested previously (Vlaeminck et al., 2006a; Bessa et al., 2009), a lack of response in rumen OBCFA proportions from VFA infusions would indicate that changes in OBCFA result from alterations in the prevalence of microbial species present in the rumen, not by availability of extracellular VFA precursors.

MATERIALS AND METHODS

Experimental Design, Cows, and Treatments

Four multiparous, rumen-cannulated Holstein cows were randomly assigned to a single 4 × 4 Latin square with 48-h periods. Each cow was fitted with a 10-cm rumen cannula (Bar Diamond, Parma, ID) at least 1 mo before the start of period 1 of the Latin square. The Animal Care and Use Committee for the College of Agricultural and Life Sciences at the University of Wisconsin-Madison approved all animal procedures.

Animals were housed in tie-stalls with free access to water and fed a basal diet to meet the requirements set by the National Research Council (NRC, 2001). The diet was formulated for cows at 120 DIM, weighing 685 kg, and producing 40 kg of milk with 3.6% milk fat and 3.0% true milk protein. The TMR contained, on a DM basis (g/kg of DM): 322 alfalfa silage, 298 corn silage, 305 concentrate mixture, 78 cottonseed, with supplemented vitamins and minerals. Cows were fed the basal diet for 18 d before the start of the first infusion period. Diets were fed once daily to allow for 10% refusals. Cows were 119 ± 8 DIM (mean ± SD) and weighed 685 ± 98 kg when assigned to the basal diet and were milked twice daily at 0330 and 1530 h. At the start of the Latin square, cows averaged 137 DIM. Milk weights were recorded at each milking starting at the beginning of the 18-d adaptation period until the end of the Latin square experiment. Experimental treatments prepared for intraruminal infusion included AC (acetic acid, no. A-38P-20; Fisher Scientific, Fair Lawn, NJ), PR (propionic acid, no. W-292400; Sigma-Aldrich Co., Milwaukee, Wisconsin), AIV (2-methylbutyric acid, no. W-269506; Sigma-Aldrich Co.), and IV (isovaleric acid, no. W-310204; Sigma-Aldrich Co.). Although all solutions were approximately isomolar, only the AIV and IV treatments were isoenergetic. The experimental solutions were adjusted to a pH of 6.5 using a 3:1 (molar basis) ratio of an NaOH:KOH solution.

Treatments were infused through 3/8" o.d. × 3/16" i.d. tubing (Fisher Scientific, Fair Lawn, NJ) and extended 30 cm into the rumen with holes spaced 2.5 cm apart on alternating sides of the tubing to distribute the solution more evenly within the rumen. Treatments were infused using peristaltic pumps (Mec-o-matic VSP-20; W. W. Grainger Inc., Lincolnshire, IL). Feed was removed and infusion of the experimental solutions commenced at 0400 h (time = 0 h), and ended at 2200 h (time = 18 h). Relative to the start of infusions (0 h), feed was offered at 5.5 h. Cows were infused with 15 L of a 1.25 *M* solution at a rate of 17.4 mmol of VFA/min. The measured amount of each VFA delivered varied slightly from the expected amount of 18.8 mol over the course of the entire infusion, and averaged 19.0, 19.3, 19.0, and 18.3 mol/18 h for AC, PR, AIV, and IV. The total energy infused into each animal was 4.05, 7.03, 12.88, and 12.37 Mcal/18 h for AC, PR, AIV, and IV.

Feed Sampling and Analysis

Individual samples of the TMR offered, alfalfa silage, corn silage, concentrate mixture, and cottonseed were