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Intravenous lipid infusion affects dry matter intake, methane yield, and rumen bacteria structure in late-lactating Holstein cows

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ABSTRACT

Increasing the dietary fat content of ruminant diets decreases methane (CH_4) production. This effect is caused by the toxic properties of fatty acids on rumen microbial populations, coating of feed particles diminishing the accessibility for microbes, and a reduction in dry matter intake (DMI). The latter effect is caused by postabsorptive long-chain fatty acids eliciting anorexic signaling; however, whether circulating long-chain fatty acids affect rumen CH_4 production alike is unknown. To approach this question, 5 rumen-cannulated Holstein cows in late lactation received 2 jugular catheters and were kept in respiration chambers to measure CH_4 production and DMI for 48 h. In a crossover design, cows were intravenously infused with a 20% lipid emulsion (LIPO) or 0.9% NaCl (CON). The LIPO cows received 2.1 kg of triglycerides/d [0.152 ± 0.007 g of triglycerides/(kg of BW $\times$ h)$^{-1}$] consisting of 12.1% palmitic acid, 4.2% stearic acid, 31.1% oleic acid, and 52.7% linoleic acid. Blood and rumen fluid samples were taken hourly during the day. Results showed that LIPO compared with CON infusion increased plasma triglyceride as well as free fatty acid and serotonin concentrations but reduced the proportion of de novo synthesized milk fatty acids (sum of C6 to C16). Daily CH_4 production and DMI were lower, whereas daily CH_4 yield (CH_4/DMI) was greater in LIPO than CON cows, although CH_4 yield decreased from d 1 to d 2 by 2 to 14% in LIPO-infused cows only. This effect was associated with a higher (acetate + butyrate)/propionate ratio, tending lower propionate concentrations between 24 and 34 h of infusion, reduced relative abundances of genera belonging to *Succinivibrio*, *Ruminococcaceae*, and *Ruminiclostridium*, and greater relative *Bacteroidetes* genus abundances in the rumen.
Key words: lipid infusion, milk fat synthesis, methane, microbiome, dairy cow

INTRODUCTION

Daily methane (CH_4) production from lactating dairy cows was shown to be directly related to the concentration of de novo-synthesized milk fatty acids (individual or the sum of C8:0, C10:0, C12:0, C14:0, and C16:0) but negatively related to C18 milk fatty acids (individual or the sum of C18, the sum of C18:1 *trans*, or the sum of C18:1 *cis*; Chilliard et al., 2009). Dietary supplementation with oilseeds rich in C18 UFA such as different linseed products, cottonseeds, or canola oilseeds increased total C18 but reduced C6 to C16 fatty acid contents in milk while diminishing enteric CH_4 production (Johnson et al., 2002; Martin et al., 2008; Chilliard et al., 2009). The reduction of enteric CH_4 production in response to dietary oilseeds is due to decreased accessibility of feed particles for microbes, direct toxic effects on microbial populations primarily on protozoa, and a reduction in DMI (Machmüller and Kreuzer, 1999; Chilliard et al., 2009). Apart from the dietary effects on microbes, circulating long-chain fatty acids may disturb the intestinal barrier integrity and affect bacterial composition in the lumen (Hodin et al., 2012; Harris et al., 2014; Lavallee et al., 2016), suggesting that CH_4 production may also be influenced by systemic fatty acids of the host. Furthermore, circulating long-chain fatty acids can be sensed by enterocytes or the liver to signal satiety (Leonhardt and Langhans,

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2004), suggesting that the long-chain fatty acid-induced decline in DMI and the accompanying reduction in CH_4 production may also be influenced by fatty acid metabolism of the host, although intravenously infused lipids may not affect DMI of dairy cows (Stocks and Allen, 2014).

During the course of lactation, DMI increases from early to peak lactation and slowly declines afterward, and CH_4 production of cows in second or higher lactation usually follows the course of DMI (Garnsworthy et al., 2012). As first lactating cows still grow, daily CH_4 production increases from early (e.g., wk 5) to peak (e.g., wk 13) to late (e.g., wk 42) lactation, at least when cows are fed the same diet throughout the entire lactation period (Bielak et al., 2016). However, all high-yielding dairy cows mobilize endogenous fatty acids from adipose tissue in the early lactation period, thereby increasing the amount of C18:0 secreted with milk (Lerch et al., 2015). In late lactation, endogenous long-chain fatty acids are less mobilized and therefore used only to a marginal extent for milk fat synthesis (Lerch et al., 2015), underlining that the inverse relationship between the sum of C18 milk fatty acids and CH_4 production is valid for different stages of lactation and independent of dietary fat supplementation (Chilliard et al., 2009). Also, CH_4 yield (CH_4/DMI) in early but not late lactation was found to be lower in cows that mobilized high fat compared with less fat (Bielak et al., 2016). The plasma concentration of long-chain fatty acids including C18:0 reflects the extent of fat mobilization and was found to be directly related to C18:0 milk fatty acids (**MFA**; Lerch et al., 2015). It has been proposed that circulating long-chain fatty acids affect rumen-intestinal motility, which in turn could influence the retention time of the digesta in the rumen and, consequently, CH_4 yield; however, this assumption was deduced only from an association study (Bielak et al., 2016). Various rumen-intestinal hormones and neurotransmitters such as ghrelin, cholecystokinin, and serotonin are involved in the regulation of rumen motility, and their release from entero- and neuroendocrine cells is closely related to long-chain fatty acid metabolism (Plaza et al., 1996; Börner et al., 2013b; Bielak et al., 2016). Therefore, the aim of the present study was to test the hypothesis of whether increased systemic lipid levels rich in C18 fatty acids reduce CH_4 production and CH_4 yield from dairy cows and whether this effect is accompanied by changes in the rumen microbial community composition. This hypothesis was tested by an intravenous infusion of C18-rich triglycerides (**TG**), which are converted in vivo by lipases forming free C18 fatty acids.

MATERIALS AND METHODS

Animals, Experimental Design, and Infusions

The experimental protocol was approved by the local animal ethics committee (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern; approval no. 7221.3-1-056/13). Six rumen-fistulated German Holstein cows were randomly assigned to a crossover design with 2 infusions per cow in 3 blocks. One cow suffered from fever during the second infusion and was removed from the trial and data evaluation. The remaining 5 cows (4 in second lactation and 1 in third lactation) were between 270 and 310 DIM and had a BW between 515 and 667 kg. Infusions consisted of 48-h intravenous infusions of either 0.9% NaCl (B. Braun, Melsungen, Germany; **CON**) or a long-chain TG emulsion (Intralipid, Fresenius Kabi, Bad Homburg, Germany, or Lipofundin N, B. Braun; **LIPO**), both containing 20.0% soybean oil, 1.2% egg lecithin, 2.5% glycerol, and 76.3% water) to elevate plasma nonesterified fatty acids (**NEFA**). The fatty acid composition of LIPO was 12.1% palmitic acid (C16:0), 4.2% stearic acid (C18:0), 31.1% oleic acid (C18:1 *cis*-9), and 52.7% linoleic acid (C18:2 *cis*-9,*cis*-12) as determined by GC-MS (see below). Infusions were administered to the right jugular vein via a peristaltic pump at a targeted rate of 7.4 mL/min for NaCl and LIPO. Each cow received 2.1 kg of TG/d intravenously [0.152 ± 0.007 g of TG/(kg of BW $\times$ h)$^{-1}$]. A washout of at least 4 d (4–10 d) between 2 treatments prevented carryover effects because plasma NEFA concentrations declined to basal level within 24 h after the end of infusion and DMI returned to the preinfusion period level within 48 h. Four days before the first treatment and during the washout phase, cows were kept in tiestalls at 15°C with ad libitum feeding.

Measurements and Sample Collections

Twenty hours before initiation of infusion, animals were equipped with indwelling catheters (Certofix mono; B. Braun) inserted in the right and left jugular veins. Patency of the catheters was maintained by flushing with 5 mL of heparinized saline (10 IU/mL) before and between blood samplings. The BW was measured and animals in pairs of 2 (1 CON and 1 LIPO) were transferred to 2 adjacent open-circuit respiration chambers (each with a CO_2 recovery of 99.9%) separated by a window through which the animals could see each other (Derno et al., 2013). A light cycled on from 0600 to 1900 h. The airflow through the chamber was ap-