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Technical note: An in vivo method to determine kinetics of unsaturated fatty acid biohydrogenation in the rumen

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ABSTRACT

Rumen microbial biohydrogenation (BH) of unsaturated fatty acids (UFA) has been extensively studied in vitro; however, in vitro BH pathways, rates, and extents may not parallel those in vivo. The objective was to develop an assay to assess in vivo rates, pathways, and extent of BH of oleic (OA), linoleic (LA), and α -linolenic acid (ALA). Each UFA was characterized in a separate experiment, each using 4 ruminally cannulated lactating Holstein cows. A single bolus consisting of 200 g of a UFA-oil [experiment 1 (EXP1): 87% OA sunflower, experiment 2 (EXP2): 70% LA safflower, and experiment 3 (EXP3): 54% ALA flaxseed] and 12 g of heptadecanoic acid (C17:0) was mixed into the rumen through the fistula. Rumen digesta was collected at -1, -0.25, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, and 6 h relative to the bolus. Overall, the triglyceride boluses increased total fatty acids (FA) in the rumen from 3.9 (standard deviation = ± 1.4) to 7.3% (± 1.4) of rumen dry matter and enriched C17:0 from 0.4 (± 0.1) to 2.5% (± 0.5) of FA. The bolus enriched OA from 8.9 (± 1.0) to 30.1% (± 4.6) of FA in EXP1, LA from 11.1 (± 1.8) to 35.9% (± 5.0) of FA in EXP2, and ALA from 2.1 (± 0.1) to 19.8% (± 4.3) of FA in EXP3. The disappearances of C17:0, OA, LA, and ALA were fit to a single exponential decay model. The first-order rate of C17:0 rumen disappearance (turnover) was 9.1, 6.9, and 5.2%/h in EXP1, EXP2, and EXP3, respectively, and was used as a marker of FA passage. The rate of total rumen turnover of OA was 54.1%/h, LA was 60.5%/h, and ALA was 93.0%/h in EXP1, EXP2, and EXP3, respectively. Rumen concentration of all 3 UFA reached prebolus concentrations within 4 h. The calculated extent of lipolysis and initial isomerization was 85.6% for OA,

89.8% for LA, and 94.7% for ALA in EXP1, EXP2, and EXP3, respectively. Assuming that BH equals total disappearance minus passage, the rates of lipolysis and initial isomerization were 45.0, 53.6, and 87.8%/h for OA, LA, and ALA in EXP1, EXP2, and EXP3, respectively. Analysis of the data using compartmental modeling showed that the normal BH pathways proposed in the literature explained 46.0, 37.3, and 49.8% of the BH of OA, LA, and ALA in EXP1, EXP2, and EXP3, respectively. Based on the model, BH of *trans* C18:1 FA was the rate-limiting step to complete BH. Importantly, oils were provided as triglycerides and the reported rates represent the rate of lipolysis and BH. In conclusion, the rate of ruminal BH of OA, LA, and ALA was higher than that commonly observed in vitro, but the extent of BH was near expected values. The method developed provides a potential in vivo assay of ruminal BH for use in future experiments and modeling efforts.

Key words: biohydrogenation, unsaturated fatty acids, modeling, *trans* fatty acids

Technical Note

Unsaturated fatty acids (FA) are predominantly found in triglycerides in feeds and undergo biohydrogenation (BH) in the rumen, which enzymatically is lipolysis and a series of isomerization and hydrogenation steps. Metabolism of UFA by rumen microbes affects the profile of FA absorbed in the intestine and, subsequently, milk FA profile and physiology of the cow, especially milk fat yield (Bauman and Griinari, 2003). Understanding the rates and pathways of BH will allow development of improved nutritional models to maximize milk fat synthesis and modify milk FA profile. Microbial metabolism of UFA has been extensively studied in vitro using batch and continuous culture systems (Jenkins et al., 2008). These in vitro studies have provided essential insight into fundamental mechanisms and principles of BH; however, specific aspects of in vitro systems may not reflect the microbial population

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and BH kinetics occurring within the rumen (Shingfield and Wallace, 2014). For example, most in vitro systems incubate fine-ground feed substrate with strained and buffer-diluted rumen fluid and are not expected to maintain viability of all microbial populations. Importantly, the microbial populations involved in BH have not been fully characterized, and it is not clear if all key populations are culturable, even in mixed cultures.

Duodenal and omasal digesta sampling and use of digesta flow markers has been considered the reference technique to assess the extent of BH (Fievez et al., 2007). However, this technique is costly and subject to considerable error associated with subsampling and marker bias (Owens and Hanson, 1992). Furthermore, this technique only reports the extent of BH, unless coupled with rumen evacuation allowing calculation of FA BH and passage rate by the pool and flux method (Harvatine and Allen, 2006). The pool and flux approach limits mechanistic modeling of BH pathways and quantification of fast rates. Additionally, these intensive approaches make in vivo screening impractical. Modeling ruminal FA metabolism is also limited because ^{13}C -labeled UFA are prohibitively expensive outside of in vitro culture. Our goal was to develop an alternative economical assay to assess in vivo rates, pathways, and extent of BH. We hypothesized that rumen BH could be characterized using a perturbation trace model based on concentration pool of FA in the rumen.

Three 18-carbon UFA were characterized in separate experiments [**EXP1**: oleic acid (**OA**), **EXP2**: linoleic acid (**LA**), and **EXP3**: α -linoleic (**ALA**)], each using 4 ruminally cannulated lactating Holstein cows. Cows were housed in a tiestall barn and all experimental procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Cows received a contemporary lactating cow TMR composed of approximately (% of DM) 40% corn silage, 15% alfalfa haylage, 3% grass hay, and 42% of a concentrate mix [ground corn, canola meal, cottonseed hulls, vitamin/mineral supplement, encapsulated urea (Optigen, Alltech Inc., Nicholasville, KY), bakery by-product meal, corn gluten meal, soybean hulls, and cane molasses]. The diet contained approximately (% of DM) 17% CP, 32% NDF, 27% starch, and 3.1% total FA. Across the 3 experiments, DMI and milk yield averaged 25.3 (± 1.5) and 38.5 (± 6.8 , mean $\pm$ SD) kg/d, respectively.

The perturbation trace model included enrichment of rumen concentration pool of a targeted UFA and subsequent observation of disappearance of the UFA and formation of intermediate FA and stearic acid (Figure 1). Concentration pool enrichment was achieved by a single 200-g dose (i.e., bolus) of a UFA oil [predominantly

triglyceride; **EXP1**: 87% OA sunflower (Stratas Foods, Memphis, TN); **EXP2**: 70% LA safflower (Jedwards International Inc., Braintree, MA); and **EXP3**: 54% ALA flaxseed (Jedwards International Inc.)]. The UFA oil dose was based on the estimated rumen pool size and was evaluated in a pilot experiment in which 200 g was found adequate for enrichment of rumen UFA concentration pool without increasing total rumen FA to a level that may impair microbial activity. Under steady state conditions, either bolus or continuous infusion can be used to estimate kinetic rates of pools within a system (France and Dijkstra, 2005). A frequent feeding schedule was implemented with the goal of maintaining quasi-steady state ruminal conditions. Three days before the experiment, cows were fed in equal meals every 6 h; the day before the experiment, cows were fed equal-sized meals every 2 h; and on the day of the experiment, cows were fed hourly at 4.2%/h (1/24th) of expected daily DMI starting at 0730 h. Refusals were removed only once per day.

An odd-chain SFA (C17:0 as free FA; Tokyo Chemical Industry Co., Tokyo, Japan) was included in the oil bolus in all experiments to serve as a marker of FA passage from the rumen. This requires the assumption that C17:0 passes at the same rate as the UFA under study. Stable isotope-labeled UFA would provide an ideal approach, but they are prohibitively expensive. Further validation of FA flow rates is warranted, but difficult to truly validate. Additionally, assuming that the ratio of C17:0 to the bolused unsaturated oil remains constant in the rumen, the odd-chain SFA also corrects for heterogeneity in sampling. In a pilot experiment using a similar single bolus approach, we observed that different odd-chain SFA (C11:0, C13:0, C15:0, and C17:0) disappeared from the rumen at different rates (Supplemental Figure S1; <https://doi.org/jds.2017-13452>). Briefly, C11:0 and C13:0 disappeared at unrealistically high rates (23.9 and 12.8%/h, respectively), considering that FA in the rumen are mainly bound to the surface of feed particles and, hence, expected to have passage rates similar to the solid phase (Jenkins et al., 2008). The fast disappearance of C11:0 and C13:0 could be related to a greater solubility of these FA in the liquid phase or their absorption across the rumen wall. Ruminal metabolism of C11:0 and C13:0 has not been investigated, to our knowledge, but early investigations have shown that the rumen epithelium was able to use even medium-chain SFA (ranging from C8:0 to C14:0) to produce ketone bodies (Noble, 1978). In contrast to the shorter-chain FA, C15:0 and C17:0 total disappearance rate (turnover) was 6.1 and 7.2%/h, respectively, which is similar to rates of FA passage described using the pool flux method (Harvatine and Allen, 2006). We selected C17:0 over C15:0, as it is found at a lower

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