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## Ruminal biohydrogenation and abomasal flow of fatty acids in lactating cows fed diets supplemented with soybean oil, whole soybeans, or calcium salts of fatty acids

J. E. Freitas Jr.,\* C. S. Takiya,† T. A. Del Valle,† R. V. Barletta,† B. C. Venturelli,† T. H. A. Vendramini,† R. D. Mingoti,† G. D. Calomeni,† R. Gardinal,† J. R. Gandra,‡ V. P. Bettero,§ E. Ferreira de Jesus,§ M. D. S. Oliveira,§ and F. P. Rennó<sup>1</sup>

\*Department of Animal Science, Federal University of Bahia, Salvador, Brazil, 0170-110

†Department of Animal Nutrition and Animal Production, University of São Paulo, Pirassununga, Brazil, 13635-900

‡Department of Animal Science, Federal University of Grande Dourados, Dourados, Brazil, 79825-070

§Department of Animal Science, Sao Paulo State University, Jaboticabal, Brazil, 14884-900

### ABSTRACT

Ruminants have a unique metabolism and digestion of unsaturated fatty acids (UFA). Unlike monogastric animals, the fatty acid (FA) profile ingested by ruminants is not the same as that reaching the small intestine. The objective of this study was to evaluate whole raw soybeans (WS) in diets as a replacer for calcium salts of fatty acids (CSFA) in terms of UFA profile in the abomasal digesta of early- to mid-lactation cows. Eight Holstein cows ( $80 \pm 20$  d in milk,  $22.9 \pm 0.69$  kg/d of milk yield, and  $580 \pm 20$  kg of body weight; mean  $\pm$  standard deviation) with ruminal and abomasal cannulas were used in a  $4 \times 4$  Latin square experiment with 22-d periods. The experiment evaluated different fat sources rich in linoleic acid on ruminal kinetics, ruminal fermentation, FA abomasal flow, and milk FA profile of cows assigned to treatment sequences containing a control (CON), with no fat source; soybean oil, added at 2.68% of diet dry matter (DM); WS, addition of WS at 14.3% of diet DM; and CSFA, addition of CSFA at 2.68% of diet DM. Dietary fat supplementation had no effect on nutrient intake and digestibility, with the exception of ether extract. Cows fed fat sources tended to have lower milk fat concentration than those fed CON. In general, diets containing fat sources tended to decrease ruminal neutral detergent fiber digestibility in relation to CON. Cows fed WS had lower ruminal digestibility of DM and higher abomasal flow of DM in comparison to cows fed CSFA. As expected, diets containing fat supplements increased FA abomasal flow of C18:0 and total FA. Cows fed WS tended to present a higher concentration of UFA in milk when compared

with those fed CSFA. This study suggests that under some circumstances, abomasal flow of UFA in early lactation cows can be increased by supplementing their diet with fat supplements rich in linoleic acid, regardless of rumen protection, with small effects on ruminal DM digestibility.

**Key words:** fat source, linoleic acid, milk fatty acid profile, ruminal digestibility

### INTRODUCTION

Polyunsaturated fatty acids are an efficient source of energy and have been used as a tool to modulate the metabolism, reproduction, and immune system of dairy cows (Gandra et al., 2016a,b; Gardinal et al., 2018a,b). In addition, dietary supplementation of PUFA to ruminants has aimed to produce dairy products able to decrease the risk of cardiovascular diseases and obesity in humans (Bauman et al., 2006; Santos et al., 2017; Shokryzadan et al., 2017). However, ruminants are singular in terms of fatty acid (FA) digestion because, unlike monogastric animals, the FA profile ingested by ruminants is not the same as that reaching and absorbed from the small intestine. Rumen microorganisms hydrogenate double bounds within carbon chains to minimize toxic effects of PUFA on rumen bacteria (Oldick and Firkins, 2000), thus limiting the amount of PUFA absorbed by ruminants.

Providing dairy cow diets with rumen-protected fat sources such as calcium salts of fatty acids (CSFA) and oilseeds has increased the amount of PUFA reaching the small intestine. The protein complex surrounding the cotyledon in seeds protects their lipid content from enzymatic biohydrogenation (BH; Doreau et al., 2016). In addition, dietary supplementation of dairy cows with whole flaxseed or whole raw soybeans (WS) has increased milk content of PUFA (Chilliard et al.,

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<sup>1</sup> Corresponding author: francisco.renno@usp.br

2009; Venturelli et al., 2015). Furthermore, raw oilseeds might be more economically feasible than other PUFA sources, such as CSFA, because they do not undergo industrial processes, thus decreasing nutrient wastes and costs associated with electricity or fuel utilization.

Ruminal passage rate is one of the most important factors determining the extent of ruminal BH of UFA (Bettero et al., 2017), whereas the lipid FA profile determines the duodenal FA profile (Jenkins and Bridges, 2007). Our research group has published data regarding the ruminal outflow of FA in dry and mid- to late-lactation cows fed different sources rich in linoleic acid. Dietary supplementation of WS to dry cows reduced ruminal passage rate of DM in comparison with cows fed CSFA (2.38 vs. 2.91%/h, respectively) but did not alter the abomasal flow of C18:2 (Bettero et al., 2017). On the other hand, mid- to late-lactation cows fed WS and CSFA had similar ruminal passage rate of DM (3.1 vs. 3.0%/h), whereas cows fed WS tended to have a greater abomasal flow of C18:2 than those fed CSFA (Barletta et al., 2016). Early- to mid-lactation cows have a relatively high ruminal passage rate of DM, which would influence the response to dietary supplementation of WS and CSFA on abomasal flow of FA.

Gandra et al. (2016b) reported that transition cows fed WS had a higher percentage of neutrophils positive for phagocytosis of *Staphylococcus aureus* in comparison to those fed CSFA. The positive effects of WS supplementation on the immune system and milk FA profile of early-lactating cows (Gandra et al., 2016a,b; Gardinal et al., 2018a,b) could be supported by an increase in specific FA reaching the small intestine, such as linoleic acid and CLA. Although the ruminal outflow of FA was studied in lactating cows fed WS (Tice et al., 1993, 1994), the authors failed to describe DIM of cows. Thus, literature still lack data regarding the ruminal outflow of FA in early- to mid-lactation cows fed WS.

This study was designed to determine the influence of dietary supplementation of soybean oil (SO), WS, and CSFA on ruminal BH and FA profile reaching the small intestine in early- to mid-lactating cows. We hypothesized that cows fed SO (unprotected oil) would exhibit greater BH extent and lower UFA abomasal flow in comparison with cows fed either WS or CSFA (protected oils), whereas we expect similar BH extent and profile of FA in abomasum of cows fed protected oils.

## MATERIALS AND METHODS

The experimental procedures were approved by the Ethics Committee of the School of Veterinary Medicine

and Animal Science of the University of São Paulo, São Paulo, Brazil (approval #1603/2009).

### Animals, Experimental Design, and Diets

Eight multiparous Holstein cows ( $80 \pm 20$  DIM,  $22.9 \pm 0.69$  kg/d of milk yield,  $580 \pm 20$  kg of BW; mean  $\pm$  SD), with ruminal and abomasal cannulas, were allocated to a replicated  $4 \times 4$  Latin square experiment with 22-d periods, of which 10 d allowed for diet adaptation and 12 d for sampling. Dietary treatments were (1) control (CON), no dietary fat source; (2) SO, added at 2.68% (DM basis); (3) WS, added at 14.3%; and (4) CSFA (Megalac-E, Church & Dwight Co. Inc., Trenton, NJ), added at 2.68%. Diets were formulated based on NRC (2001) recommendations (Table 1), supplied twice daily (0600 and 1400 h) as TMR, and aiming refusals between 5 and 10% on an as-fed basis. Formulation of diets with fat sources (SO, WS, and CSFA) targeted a dietary FA content of 45 g/kg. Corn silage DM was assessed weekly for dietary adjustments when necessary. Treatment sequences were randomly distributed to cows.

Corn silage and concentrate ingredients were collected during each sampling period and pooled into a composite sample per period. Samples were dried in a forced-air oven ( $55^\circ\text{C}$  during 72 h) and ground in a Wiley mill (1-mm screen). Samples were analyzed for NDF using heat-stable  $\alpha$ -amylase (Van Soest et al., 1991), DM (method #930.15), ash (method #942.05), ether extract (EE; method #920.39), and CP (method #984.13) according to AOAC International (2000). Nonfiber carbohydrate content was estimated according to Hall (2000), as follows:  $\text{NFC} = 1,000 - [(\text{CP} - \text{CP from urea} + \text{urea}) + \text{EE} + \text{ash} + \text{NDF}]$ , all values expressed in grams per kilogram. To determine indigestible NDF (iNDF), samples were ground in a Wiley mill (2-mm screen), placed in nonwoven textile bags tissue ( $5 \times 5$  cm, pore size  $50 \mu\text{m}$ ,  $100 \text{ g/m}^2$ ). Bags with ground samples were incubated for 288 h (Huhtanen et al., 1994) in the rumen of 2 dry cows, previously adapted to the CON diet as described by Casali et al. (2008). Afterward, samples were removed from the rumen, washed in running tap water, and analyzed for NDF concentration as previously described.

Feed samples for FA profile analyses were lyophilized and ground (1-mm screen) with liquid nitrogen to avoid changes in FA profile. Lipid extraction was performed according to Folch et al. (1957) and methylated according to Kramer et al. (1997). Briefly, the lipids were extracted after homogenizing samples with a chloroform and methanol solution (2:1, vol/vol). Lipids were

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