J. Dairy Sci. 100:1–4
https://doi.org/10.3168/jds.2016-12233

Short communication: Effect of a citrus extract in lactating dairy cows

Y. Ying,[1] M. Niu,[2] A. R. Clarke,[3] and K. J. Harvatine[4]
Department of Animal Science, Pennsylvania State University, University Park 16802

ABSTRACT

Dry matter intake is a main driver of energy balance in lactating dairy cows, and some plant extracts have been commercially fed to dairy cows to stimulate feed intake. Citrus extracts contain several bioactive components and have been shown to modify metabolism in other animal models. Our hypothesis was that a citrus extract would increase dry matter intake. Two experiments were conducted to determine the effect of a citrus extract on intake and milk production in lactating dairy cows. In experiment one, 11 early-lactation dairy cows (experiment 1; 77 ± 15 d in milk, mean ± standard deviation) were used in a switchback design, and in experiment two, 15 mid-lactation Holstein cows (experiment 2; 157 ± 44 d in milk, mean ± standard deviation) were used in a crossover design. In both experiments, treatments were control (no supplement) or a citrus extract (4 g/d in experiment 1 and 4.5 g/d in experiment 2). Treatment periods were 21 and 14 d in experiment 1 and experiment 2, respectively, with the final 7 d used for sample and data collection. No effect was observed for treatment on dry matter intake, feeding behavior, milk yield, milk fat yield, milk protein yield, or milk composition in either experiment. Treatment also had no effect on milk *trans* fatty acid profile, but the extract increased total 16 carbon fatty acids 0.9 and 0.6 percentage points in experiment 1 and experiment 2, respectively. Plasma nonesterified fatty acids were decreased 6 h after feeding in both experiments (11.1 and 16.0 μEq/L in experiment 1 and experiment 2, respectively). Plasma insulin was increased 1 h before feeding compared with the control in experiment 1 (3.36 vs. 2.13 μIU/mL) and tended to increase 1.79 units 1 h before feeding in experiment 2. The citrus extract had no effect on feed intake or milk production at the dose investigated, but changed plasma insulin and nonesterified fatty acids, indicating some metabolic effects requiring further investigation.

Key words: citrus extract, feeding behavior, intake, insulin

Short Communication

Natural plant extracts may provide the opportunity to modify animal physiology to improve milk production and animal health. Citrus extracts contain several bioactive compounds, including alkaloids, flavonoids, tannins, phenols, and saponins, that have been investigated for their beneficial effect on several disease conditions including metabolic diseases in other model organisms (reviewed by Li et al., 2006; Favela-Hernandez et al., 2016). Citrus extracts contain polymethoxyflavones and hydroxylated polymethoxyflavones not found in other plants. The extracts have been proposed to have antimicrobial, antifungal, antiobesity, antiinflammatory, antistress, and antioxidant properties relevant to dairy cows. Additionally, citrus extracts have documented neurological effects in the central nervous system including antidepressant and sedative effects and effects on memory and learning (Roohbakhsh et al., 2014). Because intake is centrally regulated, citrus extracts may increase feed palatability and DMI of dairy cows, although little research has been conducted. Our objective was to test the effect of a citrus-based extract on milk production, feed intake, feeding behavior, and plasma metabolites in lactating dairy cows. Our hypothesis was that the extract would increase intake through either an increase in meal size or number of meals per day.

Two experiments were conducted using multiparous Holstein cows housed in a tie stall at the Pennsylvania State University Dairy Production Research and Teaching Center. All experimental procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Eleven early lactation dairy cows were arranged in a switchback design with three 21-d periods in experiment 1 (experiment 1; 77 ± 15 DIM; mean ± SD) and 15 mid-lactation

Received October 30, 2016.
Accepted April 2, 2017.
[1]Current address: Department of Medicine, Penn Center for Pulmonary Biology, Penn Cardiovascular Institute, University of Pennsylvania, Philadelphia 19104.
[2]Current address: Department of Animal Science, University of California, Davis 95616.
[3]Current address: 2118 St., Rt. 168, Georgetown, PA 15043.
[4]Corresponding author: kjh182@psu.edu

dairy cows were arranged in a crossover design with two 14-d periods in experiment 2 (experiment 2; 157 ± 44 DIM; mean ± SD). Cows were fed the basal diet for 14 d before initiation of the experiment and there were no wash-out periods. Treatments were (1) control (no supplement) and (2) a citrus-based plant extract. The extract was fed at a target rate of 4 g/d in experiment 1 and 4.5 g/d in experiment 2 in a ground corn premix. Because peer-reviewed data are not available in cows, the dose was selected based on the feeding rate of similar supplements in the field. The dose was slightly increased in the second experiment, as a response was not observed in the first experiment and because of the high level of intake observed. Cows were fed once daily at 0900 h at 110% of expected intake, milked 2×/d in a milking parlor, and received bST on d 1 and 10 of each period in experiment 1 and on the first day of each period in experiment 2 (500 mg of sometribove zinc, Posilac, Elanco Animal Health, Greenfield, IN). Data were analyzed using the Fit Model procedure and the REML method of JMP (version 9.0.2, SAS Institute Inc., Cary, NC). Daily DMI was averaged over the last 3 d of each period, and milk yield and composition were averaged over the days observed. The model included the random effect of sequence, period, and cow nested in sequence and the fixed effect of treatment. The interaction of treatment and blood sampling time was tested and was significant for key variables, so data were separated by time point for analysis. Significant differences were declared at $P < 0.05$ and tendencies declared at $0.05 < P < 0.10$.

Diet ingredients (~250 g of DM) and individual orts (12.5% of orts) were sampled daily from on the last 3 d of each period and composited by period. All feed and orts were dried in a forced air oven at 65°C. Feed samples were subsequently ground in a Wiley mill (A. H. Thomas Co., Philadelphia, PA) using a 1-mm screen and analyzed by wet chemistry procedures [CP (AOAC International, 2000) by amylase-treated NDF (Van Soest et al., 1991) by Cumberland Valley Analytical Services (Maugansville, MD; analytical methods are available at http://www.foragelab.com/Resources/Lab-Procedures]. The corn-silage- and alfalfa-haylage-based diets contained 32.0 and 33.4% NDF and 29.4 and 28.1% starch in experiment 1 and experiment 2, respectively (Supplemental Table S1; https://doi.org/10.3168/jds.2016-12233).

Dry matter intake was not modified by the extract in the first experiment, which used earlier lactation cows (77 ± 15 DIM; mean ± SD) that had higher milk yield (53 kg/d) and DMI (33.0 kg/d; Table 1). It is possible that physical fill limited intake in this situation. Intake is regulated through integration of multiple signals in the brain and a stimulatory signal would not be expected to overcome physical fill limitations (Allen, 1996). Therefore, the second experiment was conducted in a separate group of mid-lactation cows with more moderate milk production and DMI (37 kg/d of milk and 27.5 kg of DMI). However, the extract also had no effect on DMI in the second experiment (Table 1). Feeding behavior was observed in a feed intake observation system with hanging feed tubes suspended from an electronic load cell wired into a data acquisition system (Niu et al., 2017). Extract had no effect on meal number, length, size, or interval or time spent eating (Table 1). The extract had a tendency to increase eating rate in experiment 2 (98 vs. 89 g/min; $P = 0.09$). Also, no apparent differences were observed in the pattern of feed intake over the day (Supplemental Figure S1; https://doi.org/10.3168/jds.2016-12233). The extract has the potential to act through an olfactory mechanism (Nagai et al., 2014). Treatment sequence was randomly assigned to stall in both experiments and cows were housed next to each other in the barn. Control cows were therefore exposed to the olfactory characteristics of feed in their vicinity. Additionally, cows were housed in tie stalls, and motivation for feeding behavior may be different in tie stalls compared with free stalls and the effect of the extract on feeding behavior in free stall barns should be investigated. Based on the current experiments, consumption of the extract had no effect on intake and feeding behavior of healthy lactating cows.

Milk yield determined by an integrated milk meter and milk composition at the 4 consecutive milkings on the last 2 d of each period was determined by infrared spectroscopy (AOAC International, 2000; method 972.160, Dairy One Lab, State College, PA). No effect was observed of the extract on milk yield or milk composition in either experiment. An additional milk sample was collected at each milking on the last day of each period, and was analyzed for fatty acid (**FA**) profile as described by (Rico and Harvatine, 2013). No effect was observed on milk *trans* FA profile, indicating no change in ruminal biohydrogenation pathways or capacity (Table 2; Supplemental Table 2; https://doi.org/10.3168/jds.2016-12233). The extract increased total 16 carbon FA 0.9 and 0.6 percentage units in experiment 1 and experiment 2, respectively, due to an increase in C16:0, but had no effect on de novo or preformed FA concentration (Table 2). The FA profile of the extract was not analyzed, but this increase is not expected to be due to palmitic in the product because of the low dose fed (4 and 4.5 g/d). Hydroxylated polymethoxyflavones from *Citrus sinensis* reduced fat synthesis in 3T3-L1 adipocytes and decreased adiposity and fatty liver in C57BL/6 mice fed a high-fat diet (Lai et al., 2013). Increased C16:0 in the current experiment may indicate modification of activity of FA synthase or