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Effects of rumen-protected *Capsicum* oleoresin on productivity and responses to a glucose tolerance test in lactating dairy cows

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ABSTRACT

The objective of this experiment was to investigate the effects of rumen-protected *Capsicum* oleoresin (RPC) supplementation on feed intake, milk yield and composition, nutrient utilization, fecal microbial ecology, and responses to a glucose tolerance test in lactating dairy cows. Nine multiparous Holstein cows were used in a replicated 3 × 3 Latin square design balanced for residual effects with three 28-d periods. Each period consisted of 14 d for adaptation and 14 d for data collection and sampling. Treatments were 0 (control), 100, and 200 mg RPC/cow per day. They were mixed with a small portion of the total mixed ration and top-dressed. Glucose tolerance test was conducted once during each experimental period by intravenous administration of glucose at a rate of 0.3 g/kg of body weight. Dry matter intake was not affected by RPC. Milk yield tended to increase for RPC treatments compared to the control. Feed efficiency was linearly increased by RPC supplementation. Concentrations of fat, true protein, and lactose in milk were not affected by RPC. Apparent total-tract digestibility of dry matter, organic matter, and crude protein was linearly increased, and fecal nitrogen excretion was linearly decreased by RPC supplementation. Rumen-protected *Capsicum* oleoresin did not affect the composition of fecal bacteria. Glucose concentration in serum was not affected by RPC supplementation post glucose challenge. However, compared to the control, RPC decreased serum insulin concentration at 5, 10, and 40 min post glucose challenge. The area under the insulin concentration curve was also decreased 25% by RPC. Concentration of nonesterified fatty acids and β-hydroxybutyrate in serum were not affected by RPC following glucose administration. In this study, RPC tended to increase milk production and increased feed efficiency in dairy cows. In addition, RPC decreased serum insulin concentration during the

glucose tolerance test, but glucose concentration was not affected by treatment.

Key words: *Capsicum* oleoresin, insulin, milk production, dairy cow

INTRODUCTION

Capsicum oleoresin, an acetone or hexane extract from *Capsicum* fruits, has been studied as a modifier of ruminal fermentation in cattle (Calsamiglia et al., 2007). Capsaicin, the main active compound in *Capsicum* oleoresin, has a phenolic structure and has been shown to exhibit antimicrobial effects in the rumen and to modify rumen fermentation (Calsamiglia et al., 2007). In beef cattle studies, *Capsicum* oleoresin applied as a feed additive decreased acetate proportion and increased ammonia concentration (Fandiño et al., 2008; Rodríguez-Prado et al., 2012). *Capsicum* oleoresin, however, had no effect on rumen fermentation in dairy cows (Tager and Krause, 2011; Oh et al., 2015).

Recent studies with dairy cows suggested that *Capsicum* may exhibit physiological effects directly on the host animal. For example, abomasal infusion of *Capsicum* oleoresin increased a subtype of T lymphocytes related to adaptive immunity (Oh et al., 2013). In addition, dietary supplementation of *Capsicum* oleoresin increased serum BHB concentration and neutrophil counts with no effect on rumen fermentation (Oh et al., 2015). In studies with nonruminants, *Capsicum* or capsaicin has been investigated with regard to its physiological effects (Lee et al., 2013a; Liu et al., 2014; Srinivasan, 2016). The regulatory effects of capsaicin include changes in feed intake, digestive enzyme secretion, fat mobilization, and hormone regulation. Capsaicin treatment stimulated gastric emptying and decreased leptin levels, which resulted in higher food intake in humans and rats (McCann et al., 1988; Debrececi et al., 1999; Hsu and Yen, 2007). Capsaicin increased the activity of digestive enzymes such as lipase and trypsin in pancreatic homogenate of rats (Platel and Srinivasan, 2000). Dietary inclusion or topical administration of capsaicin reduced adipose tissue and increased blood free fatty

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acids (FA) in rats (Yoshioka et al., 2000; Lee et al., 2013b). In addition, capsaicin reportedly increased or decreased pancreatic hormones in rats and human subjects (Dömötör et al., 2006; Chaiyasit et al., 2009). In particular, insulin concentration in blood was decreased by capsaicin after an intravenous glucose challenge in rats (van de Wall et al., 2005, 2006). Because insulin has a pivotal role in glucose homeostasis and homeorhesis in dairy cows (Bell and Bauman, 1997), the effect of capsaicin on insulin secretion may increase glucose availability to the mammary gland and thus milk production in dairy cows.

Based on existing literature with nonruminants and our previous experiments, we hypothesized that *Capsicum* acts in the digestive tract postruminally and may positively affect feed intake, nutrient utilization, gut microbial ecology, fat mobilization, and hormone regulation in dairy cows. Thus, the objective of the experiment was to investigate the effects of rumen-protected *Capsicum* oleoresin (RPC) supplementation on feed intake, milk production and composition, total-tract digestibility, nitrogen excretion, fecal bacterial population, and responses during a glucose tolerance test (GTT) in lactating dairy cows.

MATERIALS AND METHODS

Animals and Treatments

The Pennsylvania State University Animal Care and Use Committee approved all procedures used in this experiment. The experiment was a replicated 3×3 Latin square design, balanced for residual effects, and it was conducted at the tie-stall barn of The Pennsylvania State University's Dairy Teaching and Research Center. The experiment involved 9 Holstein cows (milk yield, 47 ± 5.7 kg/d; DIM, 100 ± 9.1 d; and BW, 665 ± 83.3 kg, at the beginning of the experiment). Cows were grouped in squares based on parity and milk yield. Each period consisted of 28 d: 14 d for adaptation and 14 d for data collection including sampling. An immune challenge was conducted on d 24 in each experimental period, and data generated after the challenge, including intake, milk production, and acute phase immune responses are presented in our companion paper (Oh et al., 2017). Cows were fed once daily ad libitum targeting 5 to 10% refusals and had free access to fresh water. During feeding, RPC was mixed with a small amount of the TMR and top-dressed on the feed. Treatments in this experiment were 3 levels of RPC: 0 mg/d (control), 100 mg/d (C100), and 200 mg/d (C200). The RPC product used in the experiment was Nexulin (X50-7035; 15.5% *Capsicum* oleoresin; 0.93%

capsaicinoids; Pancosma, S. A., Geneva, Switzerland). The dose amounts were determined based on previous work in our laboratory (Oh et al., 2015). In a separate in situ test, ruminal DM disappearance rate of RPC ranged from 3.30 to 27.8% in 24-h incubation (data not shown). All cows were fed the same basal TMR (Table 1). Intake, refusal weights, and milk production were recorded daily. Recombinant bST (500 mg, i.m., Posilac; Elanco Co., Greenfield, IN) was administered at the beginning and middle of each experimental period.

Sampling and Analyses

Weekly composite samples of the TMR and refusals were prepared from subsamples collected twice weekly. Forages and concentrate feeds were sampled weekly, and composite samples were made for each experimental period. Composite samples of the TMR, forages, and concentrates were stored frozen, oven-dried to constant weight (65°C), and ground through a 1-mm sieve before being analyzed for CP (AOAC International, 2000), NDF (Van Soest et al., 1991), ADF (AOAC International, 2000), ether extract (AOAC International, 2006), Ca (AOAC International, 2000), P (AOAC International, 2000), and estimated NFC (NRC, 2001) and NE_L (NRC, 2001) by Cumberland Valley Analytical Services (Maugansville, MD). Fecal and TMR samples were incinerated for 4 h at 600°C for analysis of ash and OM. Fecal, urine, blood, and milk samples were collected during the last week of each experimental period (Figure 1). Fecal and urine samples were collected by stimulating defecation from the rectum and by massaging the vulva, respectively, at 1000, 1600, and 2200 h on d 20; 0400, 1300, and 1900 h on d 21; and 0100 and 0700 h on d 22. Fecal samples (approximately 300 g) were oven-dried at 65°C in a forced-air oven for 48 h. After drying, samples were ground through a 1-mm sieve (Wiley mill), composited on an equal weight basis per cow and period, and analyzed for OM (as indicated previously for feed samples) and NDF and ADF (Ankom A200 fiber analyzer; Ankom Technology, Macedon, NY; Van Soest et al., 1991). Heat-stable amylase (Ankom Technology) and sodium sulfite (Fisher Scientific, Waltham, MA) were used in the NDF procedure. Dried fecal samples were pulverized at 30 Hz/s for 2 min in a Mixer Mill MM 200 (Retsch GmbH, Haan, Germany) and analyzed for CP ($N \times 6.25$) on a Costech ECS 4010 C/N/S elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA). Fecal and TMR samples were analyzed for indigestible NDF as an intrinsic digestibility marker to estimate apparent total-tract digestibility of nutrients according to Huhtanen et al. (1994), with the exception

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