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# Immune and production responses of dairy cows to postruminal supplementation with phytonutrients

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## ABSTRACT

This study investigated the effect of phytonutrients (PN) supplied postruminally on nutrient utilization, gut microbial ecology, immune response, and productivity of lactating dairy cows. Eight ruminally cannulated Holstein cows were used in a replicated 4 × 4 Latin square. Experimental periods lasted 23 d, including 14-d washout and 9-d treatment periods. Treatments were control (no PN) and daily doses of 2 g/cow of either curcuma oleoresin (curcumin), garlic extract (garlic), or capsicum oleoresin (capsicum). Phytonutrients were pulse-dosed into the abomasum of the cows, through the rumen cannula, 2 h after feeding during the last 9 d of each experimental period. Dry matter intake was not affected by PN, although it tended to be lower for the garlic treatment compared with the control. Milk yield was decreased (2.2 kg/d) by capsicum treatment compared with the control. Feed efficiency, milk composition, milk fat and protein yields, milk N efficiency, and 4.0% fat-corrected milk yield were not affected by treatment. Rumen fermentation variables, apparent total-tract digestibility of nutrients, N excretion with feces and urine, and diversity of fecal bacteria were also not affected by treatment. Phytonutrients had no effect on blood chemistry, but the relative proportion of lymphocytes was increased by the capsicum treatment compared with the control. All PN increased the proportion of total $CD4^+$ cells and total $CD4^+$ cells that co-expressed the activation status signal and CD25 in blood. The percentage of peripheral blood mononuclear cells (PBMC) that proliferated in response to concanavalin A and viability of PBMC were not affected by treatment. Cytokine production by PBMC was not different between control and PN. Expression of mRNA in liver for key enzymes in gluconeogenesis, fatty acid oxidation, and response to reactive oxygen species were not affected by treatment. No difference was observed due to treatment in the oxygen radical absorbance capacity of blood plasma but, compared with the control, garlic treatment increased 8-isoprostane levels. Overall, the PN used in this study had subtle or no effects on blood cells and blood chemistry, nutrient digestibility, and fecal bacterial diversity, but appeared to have an immune-stimulatory effect by activating and inducing the expansion of CD4 cells in dairy cows. Capsicum treatment decreased milk yield, but this and other effects observed in this study should be interpreted with caution because of the short duration of treatment.
**Key words:** capsicum, curcumin, garlic, dairy cow

## INTRODUCTION

Many plants produce secondary metabolites that may be useful as feed additives because of their biologically active constituents (Wallace, 2004). Plant-derived bioactive compounds, also referred to as phytonutrients (**PN**) or phytobiotics, such as phenolic compounds, essential oils, and saponins have been shown to express antimicrobial activities (Cowan, 1999; Bakkali et al., 2008) and have been investigated as alternatives to rumen modifiers, such as ionophoric antibiotics, in animal nutrition (Greathead, 2003; Rochfort et al., 2008). Phytonutrients have also been studied as inhibitors of pathogens having an effect on animal health and productivity (Panda et al., 2006; Jouany and Morgavi, 2007; Giannenas and Kyriazakis, 2009).

Natural products originating from plants have been used in traditional medicine and as feed supplements to livestock diets for centuries. Antimicrobial activities, immune enhancement, and stress reduction are among the beneficial characteristics of these preparations (Wang et al., 1998). Garlic (*Allium sativum*) and garlic oil, for example, have a wide spectrum of activities from anti-

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microbial, antioxidant, and anticarcinogenic properties to beneficial effects on the cardiovascular and immune systems (Lang et al., 2004; Mirunalini et al., 2010). The antimicrobial properties of PN, demonstrated in monogastric farm species (Youn and Noh, 2001; Ando et al., 2003; Youn et al., 2004; Michiels et al., 2005, 2007), can also be advantageous in suppressing intestinal protozoa and pathogenic bacteria in cattle. Other effects such as immune stimulation, again primarily through inhibition of intestinal parasites (Applegate, 2009), and prebiotic effects have been reported for PN in pigs and poultry (Guo et al., 2004a,b; Maass et al., 2005; Applegate, 2009).

Some PN (particularly phenols) are known to be resistant to microbial degradation in the rumen and could reach the small intestine in a biologically active form. Franz et al. (2010), for example, reported resistance (up to 60% recovery) of phenolic compounds, such as carvacrol, to microbial degradation in a continuous culture fermentor study. It is possible that these escape rates of phenolic compounds occur in vivo because the rumen is a dynamic system and the passage rate of the liquid phase of ruminal contents can be as high as 20%/h (Hristov et al., 2003). Thus, ruminal fluid and any compound associated with it may have a short residence time in the rumen (around 5 h) and a high probability of reaching the intestine of the host animal. Once PN reach the absorptive sites in the small intestine, they can be rapidly absorbed (as shown recently for essential oil terpenes from caraway, *Carum carvi*, and oregano, *Origanum vulgare*; Lejonklev et al., 2013) and have various physiological effects. Thus, if PN bypass the rumen, they could exhibit the same activities in the lower part of the ruminant digestive tract as previously described in monogastric animals. Improvements in digestibility, of the mucosal and antioxidant status, and immune response could lead to improved animal health, milk production, and feed efficiency in dairy cattle.

Based on evidence in monogastric species, we hypothesized that PN could have immune-stimulating and other beneficial effects in dairy cows. The specific objectives of the study were to investigate postruminal physiological effects of PN in relation to nutrient utilization, gut microbial ecology, immune response, and productivity of lactating dairy cows.

## MATERIALS AND METHODS

### Animals and Treatments

This experiment and all procedures were reviewed and approved by The Pennsylvania State University Animal Care and Use Committee (IACUC protocol no. 35632). Animals were cared for according to the guidelines of the committee.

The design of the experiment was a replicated 4 × 4 Latin square with 1 multiparous and 7 primiparous Holstein cows averaging 533 ± 75.3 kg of BW, 175 ± 19.8 DIM, and 30.3 ± 8.01 kg/d of milk yield at the beginning of the trial. All cows were fitted with soft plastic rumen cannula (10-cm internal diameter; Bar Diamond Inc., Parma, ID). Experimental periods were 23 d, including 14-d washout and 9-d treatment periods. Treatments were control (99.9% ethanol dissolved in distilled water, see below, without PN) and 3 PN (Pancosma S.A., Geneva, Switzerland): a daily pulse dose of 2 g/cow of curcuma oleoresin (curcumin; from *Curcuma longs* L., containing 95% curcumin), a daily pulse dose of 2 g/cow of garlic extract (garlic; containing by weight, 60% polysorbate 80 and 40% organosulfur compounds), or a daily pulse dose of 2 g/cow of capsicum oleoresin (capsicum; from *Capsicum frutescens* L. and *Capsicum anum* L. var. *concoides*, containing 6% capsaicin). Phytonutrients were dissolved in 99.9% ethanol (2 g of PN in 40 mL of ethanol) and further diluted with 250 mL of distilled water. Solutions were pulse-dosed into the abomasum of the cows through the rumen cannula, 2 h after feeding during the last 9 d of each experimental period (i.e., the treatment period) using a 500-mL plastic bottle attached to a 155-cm-long polyvinyl tube (10-mm internal diameter). The infusion tube was flushed with an additional 500 mL of distilled water following the delivery of the treatment solutions. The relatively short treatment period was necessary to reduce, as much as possible, stress to the cows caused by the invasive abomasal infusion procedure. Cows were housed in a tiestall barn, were fed once daily at approximately 0800 h, and had free access to fresh water. The basal diet (Table 1) was fed ad libitum as a TMR to achieve approximately 5 to 10% refusals. Feed was pushed up 3 to 5 times daily. Cows were milked twice daily at 0500 and 1700 h and treated with recombinant bST (Posilac; Elanco Co., Greenfield, IN; 500 mg, i.m.) at 14-d intervals. Although not analyzed, we assumed that, because of the experimental design of the trial (i.e., Latin square), the bST treatment did not affect the experimental results in this study.

### Sampling and Analyses

Feed intake was measured daily, TMR samples were collected twice weekly, and individual feed ingredients were sampled once weekly. Composite samples of the TMR, forages, and concentrates were stored frozen, oven-dried for 48 h at 65°C (forced-air oven), and ground in a Wiley mill (A. H. Thomas Co., Philadelphia, PA) through a 1-mm screen before being analyzed for CP (N