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Effect of 2-hydroxy-4-(methylthio)butanoate (HMTBa) on risk of biohydrogenation-induced milk fat depression

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ABSTRACT

Diet-induced milk fat depression (MFD) is a multifactorial condition resulting from the interaction of numerous risk factors, including diet fermentability and unsaturated fatty acids concentration, feed additives, and individual cow effects. 2-Hydroxy-4-(methylthio) butanoate (HMTBa) is a methionine analog that has been observed to increase milk fat in some cases, and interactions with MFD risk factors may exist. The objective was to evaluate the effect of HMTBa supplementation on milk fat synthesis in cows with different levels of milk production and fed diets with increasing risk of biohydrogenation-induced MFD. Sixteen high-producing cows (44.1 ± 4.5 kg of milk/d; mean ± SD) and 14 low-producing (31.4 ± 4.3 kg of milk/d) were used in a randomized block design. Treatments were unsupplemented control and HMTBa fed at 0.1% of diet dry matter (25 g/d at 25 kg of dry matter intake). The experiment was 70 d and included a 14-d covariate period followed by 3 phases whereby diets were fed with increasing risk of MFD to determine the interaction of treatment and diet-induced MFD. During the low-risk phase, the base diet was balanced to 33.5% neutral detergent fiber (NDF) and had no exogenous oil (28 d); during the moderate-risk phase, the diet was balanced to 31% NDF and contained 0.75% soybean oil (14 d); and, during the high-risk phase, the diet was balanced to 28.5% NDF and contained 1.5% soybean oil (14 d). An interaction of treatment, production-level, and dietary phase was observed. Low producing cows neither experienced substantial biohydrogenation-induced MFD nor a response in milk fat to HMTBa supplementation. In high-producing cows, HMTBa maintained higher milk fat concentration during the moderate- (2.94 vs. 3.49%) and high-risk (2.38 vs. 3.11%) phases. High-producing cows receiving HMTBa also had greater milk fat yield (0.94 vs. 1.16 kg/d) and lower *trans*-10 C18:1 (6.11 vs. 1.50) during the high-risk phase. In conclusion, HMTBa increased milk fat in situations with a high risk of biohydrogenation-induced MFD by decreasing absorption of alternate biohydrogenation intermediates.
Key words: 2-hydroxy-4-(methylthio)butanoate, biohydrogenation, milk fat, milk fat depression

INTRODUCTION

Fat is one of the most important components of milk, as it significantly affects the value of milk, the yield and quality of dairy products, and because of the recent associations between consumption of milk bioactive fatty acids (**FA**) and human health (Bainbridge et al., 2016). Consequently, the mechanisms regulating milk fat synthesis in the mammary gland, as well as nutritional and management strategies that improve milk fat synthesis, have been studied extensively. Low-milk fat syndrome, contemporarily called diet-induced milk fat depression (**MFD**), is a condition in which milk fat yield decreases up to 50% with generally no change in yields of milk and other milk components (Harvatine et al., 2009b). Milk fat synthesis is affected by several factors (e.g., genetics, physiological state, and environment), but is especially responsive to nutrition. The biohydrogenation (**BH**) theory (Bauman and Griinari, 2003) of MFD is a unifying concept that mechanistically explains the inhibition of milk fat synthesis when feeding highly fermentable and high-UFA diets. This model attributes the causal mechanism to changes in ruminal BH pathways, leading to increased formation and rumen outflow of specific bioactive FA that inhibit mammary lipid synthesis.

The understanding of dietary regulation of milk fat synthesis has improved, yet varying levels of MFD still commonly occur on dairy farms. This is primarily because MFD is a multifactorial condition resulting from the interaction of several factors, such as dietary FA level, FA profile and availability, diet fermentability, feeding strategies, rumen modifiers, and individual cow effects (Harvatine, 2017). Diet UFA level and

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carbohydrate fermentability have been characterized as central risk factors for BH-induced MFD (Rico and Harvatine, 2013; Rico et al., 2014a). Additionally, an association between level of milk production and risk of BH-induced MFD has been proposed. For instance, supplementation with calcium salts of UFA decreased milk fat in high-producing cows, whereas no MFD was observed in the low-producing cows (Harvatine and Allen, 2006; Rico et al., 2014b).

Varying levels of evidence support the effectiveness of some rumen modifiers in attenuating BH-induced MFD. Early work observed that the methionine analog, 2-hydroxy-4-(methylthio)butanoate (**HMTBa**), increased milk fat when feeding highly fermentable diets (Rosser et al., 1971; Huber et al., 1984). A recent meta-analysis observed increased milk fat yield with HMTBa supplementation (Zanton et al., 2014), but it was inconclusive on the role of HMTBa in circumstances of MFD. As BH-induced MFD is the result of the interaction of numerous factors, studying one single factor at a time will be less informative and more inconsistent than studying multiple factors simultaneously. The current study was conducted to evaluate the interaction between dietary risk factors (UFA and carbohydrate fermentability), cow milk production level, and supplementation with a rumen modifier (HMTBa). The objective was to evaluate the effect of HMTBa supplementation on milk fat synthesis in 2 groups of cows with different levels of milk production and fed diets with increasing risk of BH-induced MFD. Our hypothesis was that high-producing cows are at higher risk of BH-induced MFD and that HMTBa would reduce the extent of BH-induced MFD.

MATERIALS AND METHODS

Experimental Design and Treatments

All experimental procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. The experiment was conducted from October to December 2013 at the Pennsylvania State University Dairy Production Research and Teaching Center (University Park). Thirty multiparous Holstein cows were used in a randomized block design that tested the effect of treatment during 3 dietary phases that progressively increased risk for diet-induced MFD. Animals were housed individually in tiestalls with rubber mattresses and sawdust bedding and had continuous access to water. Cows were blocked by milk production (high or low) at the end of a 14-d pretrial period (Table 1). The high-producing group (n = 16; 166 ± 69 d postpartum; mean ± SD) averaged 44.1 ± 4.5 kg/d of milk and the low-producing group (n = 14; 267 ± 82 d postpartum) averaged 31.4 ± 4.3 kg/d of milk. Cows were paired within block (high and low) and randomly assigned to 1 of the 2 treatments: control (**CON**) or HMTBa (0.1% of diet DM, targeting 25 g of HMTBa per cow/d at 25 kg of DMI; Table 2). The HMTBa (Alimet, Novus International Inc., St. Charles, MO) was provided in a corn carrier and mixed in the TMR. An equivalent amount of the same ground corn carrier was added to the control treatment. The experiment was split into 3 phases that fed diets formulated to have low, moderate, and high risk of BH-induced MFD, respectively (Table 3). Risk for altered BH was increased by reducing diet NDF and increasing UFA and starch content (Table 3). Diet UFA were increased using a combination of rapidly available FA from soybean oil and more slowly available FA from roasted soybeans. The low-risk phase was 28 d to allow complete adaptation of the rumen to HMTBa, and the moderate- and high-risk phases were 14 d, as previous time course work has demonstrated that changes in BH occur within 10 to 14 d (Rico and Harvatine, 2013). Importantly, phase is the repeated variable and the effect of treatment is only compared within phase, as phase and treatment are confounded. Diets were fed as a TMR once daily at 0700 h at 110% of expected daily intake. Cows were treated with rbST (Posilac, Elanco Animal Health, Greenfield, IN) every 14 d.

Sampling and Measurements

Feed intake was measured daily. Cows were milked twice daily at 0500 and 1700 h and milk yield determined by an integrated milk meter (AfiMilk, SAE Afikim, Afikim, Israel). The parlor was calibrated using a stall deviation calculated using data from the entire herd (>200 cows) over 7 d. Stall deviations were determined by modeling the effect of day, milking (a.m./p.m.), cow, and stall, excluding observations of experimental cows during treatment periods. Milk was sampled at both milkings once per week and was composited based on yield at each milking. Body weight was recorded as cows exited the parlor throughout the trial (AfiFarm 3.04E scale system, SAE Afikim). Feed ingredients were sampled once per week and stored at −20°C, thawed at room temperature, dried at 55°C in a forced-air oven for 72 h, and ground in a Wiley mill through a 1-mm screen (A. H. Thomas, Philadelphia, PA). Feed samples were composited within dietary phase (equal dry weight basis). Blood samples were collected from the tail vein using potassium EDTA vacuum tubes (Greiner Bio-One North America Inc., Monroe, NC) at 0600, 1400, and 2000 h. Blood was immediately placed on ice, centrifuged within 30 min at 1,300 × *g* for 15 min at 4°C, and plasma was harvested