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# Effect of dietary antioxidant and increasing corn oil inclusion on milk fat yield and fatty acid composition in dairy cattle

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## ABSTRACT

The objective of this study was to examine the effect of a dietary synthetic antioxidant on feed intake, yields of milk and milk components and milk fatty acids (FA), in combination with increasing concentrations of dietary corn oil to provide increasing rumen unsaturated fatty acid load (RUFAL) challenges. Twenty-six Holstein cows (177 ± 57 d in milk; mean ± standard deviation) were assigned to treatment in a randomized complete block design. Treatments were a control diet (CON; n = 13 cows) or the same diet supplemented with a synthetic antioxidant (AOX; 6.1 g/d; dry blend of ethoxyquin and propyl gallate, Novus International Inc., St. Charles, MO; n = 13 cows). In period 1 (21 d), no supplemental corn oil was fed; in periods 2, 3, and 4 (14 d each), corn oil was supplemented at 0.7, 1.4, and 2.8% of the diet [dry matter (DM) basis] to incrementally increase RUFAL. For all variables measured, no significant interactions were detected between treatment and period, indicating no differences between the CON and AOX treatments at all levels of oil inclusion. Intake of DM was lower for AOX compared with CON but AOX had no effect on milk yield or milk fat concentration and yield. Milk protein yield and feed efficiency (energy-corrected milk/DM intake) tended to be greater for AOX compared with CON. Increasing dietary corn oil concentration (RUFAL) decreased DM intake, milk yield, milk fat concentration and yield, and feed efficiency. The AOX treatment increased the concentration and yield of 16-carbon milk FA, with no effect on de novo (<16 carbon) or preformed (>16 carbon) milk FA. Milk FA concentration of *trans*-10 C18:1, *trans*-10,*cis*-12 C18:2, and *trans*-9,*cis*-11 C18:2 were unaffected by AOX but increased with increasing RUFAL. In conclusion, supplementation with AOX did not overcome the dietary-induced milk fat depression caused by increased RUFAL.

**Key words:** antioxidant, dairy cow, milk fat, unsaturated fatty acid

## INTRODUCTION

Over the last decade, significant advances have aided our understanding of the interrelationship between rumen lipid metabolism and milk fat synthesis. The available evidence indicates that milk fat depression (**MFD**) is caused by altered rumen fermentation resulting in changes in rumen biohydrogenation of unsaturated FA and the passage of specific intermediates of biohydrogenation out of the rumen that subsequently reduce milk fat synthesis in the mammary gland by altering expression of genes involved in fat synthesis (Bauman et al., 2011). Milk fat is produced by 2 mechanisms, either the incorporation of preformed FA ≥16 carbons in length or the production of short- and medium-chain FA ≤16 carbons in length (de novo synthesis) in the mammary gland (Bauman and Davis, 1974). Several intermediates produced during the biohydrogenation of unsaturated FA have been shown to directly inhibit milk fat synthesis: *trans*-10,*cis*-12 C18:2 (Baumgard et al., 2001), *cis*-10,*trans*-12 C18:2 (Sæbø et al., 2005), and *trans*-9,*cis*-11 C18:2 (Perfield et al., 2007).

Because dietary unsaturated FA are toxic to rumen bacteria (Maia et al., 2007), increasing the rumen unsaturated FA load (**RUFAL**) of dairy cows may alter rates and pathways of biohydrogenation, thereby potentially increasing the risk of MFD. Previous work has determined the key changes in biohydrogenation pathways and profiles that are indicative of the alterations that lead to MFD (Bauman et al., 2011). Much of this focus has been on *trans* C18:1 isomers, particularly *trans*-10 C18:1. Although *trans*-10 C18:1 is not a causative factor of MFD, as demonstrated by abomasal infusion of the pure FA (Lock et al., 2007), it is relatively easy to analyze compared with C18:2 biohydrogenation intermediates that are present in much lower concentrations in milk fat, and there is a robust relationship between its production in the rumen and milk fat synthesis (e.g., Loor et al., 2005; Shingfield et al., 2006). Thus, *trans*-10 C18:1 can serve as a surrogate marker for the type of alterations in rumen biohydrogenation that characterize diet-induced MFD.

Results from previous studies have suggested that high levels of vitamin E (α-tocopheryl acetate, 6,000

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to 12,000 IU/d) may have the potential to prevent the "*trans*-10 shift" during biohydrogenation, thus reducing the risk of MFD, although in some cases the objectives of individual studies did not focus on rumen biohydrogenation and milk fat yield (Charmley and Nicholson, 1994; Focant et al., 1998; Pottier et al., 2006). However, more recent results from studies designed to directly examine the effect of vitamin E supplementation on overcoming MFD offer no support for a role of vitamin E in mitigating MFD (O'Donnell-Megaro et al., 2012; Zened et al., 2012). Although results are inconsistent, the potential for antioxidants to prevent the *trans*-10 shift during biohydrogenation warrant further examination. An improved understanding of potential situations where antioxidants affect biohydrogenation may allow for more flexibility in ration formulation by allowing inclusion of ingredients with high or variable unsaturated FA content while reducing the risk of MFD.

Several studies have reported benefits of commercially available synthetic antioxidants on the performance and health of lactating dairy cattle. For example, Vázquez-Añón et al. (2008) reported improved milk fat yield when a synthetic antioxidant was supplemented with either 2% oxidized or fresh soybean oil, and Wang et al. (2010) observed a tendency for increased fat-corrected milk yield during early lactation. However, He and Armentano (2011) reported no effect of a synthetic antioxidant on milk fat yield for diets containing 5% additional unsaturated oils. In a recent study, we observed a trend for increased milk fat concentration when a synthetic antioxidant was supplemented in a diet containing dried distillers grains that contained a considerable amount of unsaturated FA (Boerman et al., 2014). In our previous study, the synthetic antioxidant was supplemented at the same time as the added unsaturated FA. Based on these results, we hypothesized that the timing of the antioxidant inclusion, relative to an unsaturated FA challenge, may affect the magnitude of the response. Therefore, our objective in the current study was to evaluate production responses to a synthetic antioxidant combined with increasing concentrations of dietary corn oil to provide increasing RUFAL challenges.

## MATERIALS AND METHODS

### Design and Treatments

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (East Lansing). Twenty-six Holstein cows averaging 177 ± 57 DIM (mean ± SD) from the Michigan State University Dairy Field Laboratory were used in a randomized complete block design with a preliminary period (14 d), a treatment period (21 d), and 3 RUFAL challenge periods (14 d each). During the preliminary period, all cows were fed a common diet to obtain baseline production data. For the treatment period, cows were blocked by 3.5% FCM and then randomly assigned to 1 of 2 diets: a control diet (**CON**; n = 13 cows) or the control diet supplemented with a novel blend of synthetic antioxidants (**AOX**; Agrado Ultra, a dry blend of ethoxyquin and propyl gallate; Novus International Inc., St. Charles, MO; n = 13 cows). The synthetic antioxidant blend in the AOX treatment was top-dressed daily at a rate of 6.1 g/d. Cows remained in their respective treatment group (CON or AOX) for the remainder of the experiment, including challenge periods. During RUFAL challenge periods, corn oil was added (0.7, 1.4, and 2.8% of diet DM, respectively) to incrementally increase RUFAL concentration. Corn oil replaced soyhulls in the diet.

The ingredients and nutrient composition of experimental diets are shown in Table 1. Diets were formulated to contain 28% NDF, 28% starch, and 17% CP, and mineral and vitamins were formulated according to NRC (2001) recommendations. Dry matter concentration was determined twice weekly for forages, and diets were adjusted when necessary.

### Data and Sample Collection

Throughout the experiment, cows were housed in individual tiestalls and milked twice daily (0400 and 1500 h). Access to feed was blocked from 0800 to 1000 h to allow for collection of orts and provision of feed. Cows were fed 115% of expected intake at 1000 h daily. Water was available ad libitum in each stall, and stalls were bedded with sawdust and cleaned twice daily. Feed intake and milk yield were determined daily throughout the study. Samples and data were collected on d 12 to 14 of the preliminary period, d 17 to 21 of the treatment period, and d 12 to 14 of each RUFAL challenge period. Samples of all diet ingredients (0.5 kg) and orts from each cow (12.5%) were collected daily, stored at −20°C, and composited by period for analysis. Milk yield was recorded and 2 milk samples were collected at each milking for determination of milk composition. One milk sample was collected in a sealed tube and stored with preservative (bronopol tablet; D&F Control Systems, San Ramon, CA) at 4°C for milk component analysis. The other milk sample was stored without preservative at −20°C until analyzed for FA composition. Cows were weighed on the last 2 d of each period. Three trained investigators determined BCS on a 5-point scale (in 0.25-point increments; Wildman et al., 1982) on the last day of each period.

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