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Changes in fermentation and biohydrogenation intermediates in continuous cultures fed low and high levels of fat with increasing rates of starch degradability

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ABSTRACT

Excessive levels of starch in diets for lactating dairy cattle is a known risk factor for milk fat depression, but little is known about how this risk is affected by differences in rates of starch degradability (K_d) in the rumen. The objective of this study was to compare accumulation of biohydrogenation intermediates causing milk fat depression, including conjugated linoleic acid (CLA), when corn with low or high K_d were fed to continuous cultures. Diets contained (dry matter basis) 50% forage (alfalfa pellets and grass hay) and 50% concentrate, with either no added fat (LF) or 3.3% added soybean oil (HF). Within both the LF and HF diets, 3 starch degradability treatments were obtained by varying the ratio of processed (heat and pressure treatments) and unprocessed corn sources, giving a total of 6 dietary treatments. Each diet was fed to dual-flow continuous fermenters 3 times a day at 0800, 1600, and 2400 h. Diets were fed for four 10-d periods, with 7 d for adaptation and 3 d for sample collection. Orthogonal contrasts were used in the GLIMMIX procedure of SAS to test the effects of fat, starch degradability, and their interaction. Acetate and acetate:propionate were lower for HF than for LF but daily production of *trans*-10 18:1 and *trans*-10,*cis*-12 CLA were higher for HF than for LF. Increasing starch K_d from low to high increased culture pH, acetate, and valerate but decreased butyrate and isobutyrate. Changes in biohydrogenation intermediates (expressed as % of total isomers) from low to high starch K_d included reductions in *trans*-11 18:1 and *cis*-9,*trans*-11 CLA but increases in *trans*-10 18:1 and *trans*-10,*cis*-12 CLA. The results show that increasing the starch K_d in continuous cultures while holding starch level constant causes elevation of biohydrogenation intermediates linked to milk fat depression.
Key words: lipid, biohydrogenation, starch degradability, continuous cultures

INTRODUCTION

Diet-induced milk fat depression (**MFD**) continues to have a major economic effect on the dairy industry giving high priority to developing dietary strategies that restore milk fat yield. Recent research results link MFD with the formation of bioactive *trans* fatty acid intermediates produced from biohydrogenation (**BH**) of unsaturated fatty acids by the rumen microbial population (Jenkins and Harvatine, 2014). Among the most potent intermediates causing MFD are several CLA isomers, such as *trans*-10,*cis*-12. Baumgard et al. (2000) reported that *trans*-10,*cis*-12 infused postruminally in lactating dairy cows decreased milk fat content 42% and milk fat yield 48%. *Trans*-9,*cis*-11 CLA and *cis*-10,*trans*-12 CLA were also reported to inhibit milk fat synthesis in dairy cows (Saebø et al., 2005; Perfield et al., 2007) with the former causing a 15% reduction in milk fat yield.

Changes in the ruminal environment initiated through the diet can lead to a microbial population shift that is accompanied by a change in the type of CLA produced. For example, increasing UFA concentration in the rumen can cause rapid and dramatic microbial shifts (Rico et al. 2015) and elevate ruminal concentration of the *trans*-10,*cis*-12 CLA isomer. Addition of 3.6% soybean oil to a diet fed to continuous cultures of mixed ruminal microorganisms caused several fold increases in the daily production of *trans*-10 C18:1 and *trans*-10,*cis*-12 CLA (Jenkins et al., 2014). Low rumen pH is another key factor contributing to a microbial shift and the type of CLA produced. Lowering pH in continuous cultures caused an increase in the concentration of *trans*-10,*cis*-12 CLA but no change in *cis*-9,*trans*-11 CLA (Fuentes et al., 2009). Qiu et al. (2004) reported that reduced ruminal pH can affect microbial populations, especially cellulolytic bacteria. Total cellulolytic bacterial numbers were reduced, accompanied by reduced acetate:propionate and altered BH when pH was low.

Also, high starch diets can affect the types of BH intermediates that accumulate in ruminal contents. High

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starch diets shift BH pathways in continuous cultures, causing an increase in concentrations of *trans*-10 18:1 and *trans*-10,*cis*-12 CLA intermediates (Gudla et al., 2012). Combinations of high starch and high fat often reduce milk fat synthesis more than feeding the same amount of starch or fat alone (Zened et al., 2013). This might be attributed to starch and fat targeting different points in BH where one has a greater effect on shifting the pathway toward *trans*-10,*cis*-12 CLA production and the other acts more to inhibit the final step in the pathway, allowing *trans*-10,*cis*-12 CLA to accumulate in ruminal contents.

Source of starch may also be a factor affecting the intensity of starch-fat interactions on BH. In some reports, starch sources with faster rates of degradation yield higher *trans* intermediates than sources with slower rates of degradation at any given level of added fat. For example, substitution of barley for corn caused an increase in *trans*-10 C18:1 concentrations in ruminal contents from continuous cultures (Jenkins et al., 2003). The objective of this investigation was to determine if varying the rates of starch degradability while maintaining a constant starch level in high or low fat diets fed to continuous cultures had an effect on the type of BH intermediates produced.

MATERIALS AND METHODS

Treatments

The study was a randomized complete block design consisting of 7 experimental diets fed to 7 dual-flow continuous fermenters. Fermenters were fed either 54 g of DM/d of a 0% soybean oil diet (**LF**) or 55.7 g of DM/d of a 3.3% soybean oil diet (**HF**), divided equally among 3 feedings at 0800, 1600, and 2400 h. The higher feeding rate for HF maintained the same nutrient input into the fermenters as LF except for fatty acids (Table 1). Within both the LF and HF diets, 3 starch degradability treatments were obtained by varying the ratio of processed and unprocessed corn sources obtained from Matrix Nutrition LLC, Phoenix, Arizona (Table 2). One corn source was received from the supplier as whole corn with no prior processing (unprocessed) and was then ground within 3 d of receipt to pass a 2-mm sieve in a centrifugal mill. The second corn source was received in finely ground particle size and was reported by the supplier to have been prepared from the unprocessed whole corn. The supplier stated that processing involved proprietary heat and pressure treatments that sheared the prolamine protein structure, which eliminated the crystalline and hydrophobic properties of the vitreous unprocessed corn. For this study, the term unprocessed refers to the whole corn sample and processed refers to the same whole corn subjected to the heat and pressure treatment as received and before they were ground at Clemson University.

Table 1. Diet composition and nutrient inputs into fermenters for the low fat (LF) and high fat (HF) diets

Item	LF	HF
Ingredient, % of DM		
Alfalfa pellets	32.9	31.8
Ground hay	16.6	16.1
Ground corn	33.3	32.2
Soybean meal	8.5	8.2
Soy hulls	8.7	8.4
Soybean oil	0.0	3.3
Nutrient input per fermenter, g/d		
DM[1]	54.0	55.7
CP	10.5	10.1
NDF	21.3	21.4
ADF	18.0	19.2
Starch	13.0	12.8
Fatty acids	1.01	2.73

[1]Nutrients supplied by LF and HF diets were equalized by adjusting diet composition and amounts fed to provide the same nutrient input (except fatty acids) into each fermenter per day.

After grinding, both the unprocessed and processed corn sources were analyzed for rates of starch degradability in a 7-h in vitro test performed by Cumberland Valley Analytical Services (Hagerstown, MD). The high starch degradability treatment (**HI**) was obtained by addition of only the ground processed corn grain in the diet, which analyzed at 80% starch degradability in the 7-h in vitro test. The low starch degradability treatment (**LO**) contained only the ground unprocessed corn grain in the diets, which analyzed at 48% starch degradability in the 7-h in vitro test. An intermediate level of starch degradability (**MED**) was a 50:50 mix of the same processed and unprocessed corn sources.

The expectation was that the HI treatment would lower culture pH and that might account for any observed differences in the pattern of BH intermediates. To determine the influence of a pH drop, a seventh treatment was tested that fed the HF-HI diet to a culture flask, but pH of this flask was adjusted daily

Table 2. Composition of unprocessed and processed corn sources used in dietary treatments to vary rates of starch degradability

Item	Unprocessed corn[1]	Processed corn
DM, %	87.7	87.9
CP, % of DM	8.6	7.9
Soluble protein, % of DM	0.5	0.9
ADF, % of DM	1.3	2.4
NDF, % of DM	2.7	3.3
Starch, % of DM	82.5	82.3
7-h starch degradability, % of starch	48.4	84.0

[1]Unprocessed and processed corn sources were supplied by Mark Holt of Matrix Nutrition LLC (Phoenix, AZ).