J. Dairy Sci. 97:1067–1071
http://dx.doi.org/10.3168/jds.2013-6860

Short communication: Effect of blackberry and pomegranate oils on vaccenic acid formation in a single-flow continuous culture fermentation system

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ABSTRACT

A single-flow continuous culture fermenter system was used to study the effect of blackberry and pomegranate oils on vaccenic acid (*trans*-11 C18:1; VA) formation. Four continuous culture fermenters were used in a 4 × 4 Latin square design with 4 periods of 10 d each. Diets were (1) control (CON), (2) control plus soybean oil (SBO), (3) control plus blackberry oil (BBO), and (4) control plus pomegranate oil (PMO). Oil supplements were added at 30 g/kg of diet dry matter. Effluents were collected from each fermenter during the last 3 d of each period and analyzed for nutrient and fatty acid composition. The concentration of VA in effluents increased with oil supplements and was greatest with the BBO diet. The concentration of stearic acid (C18:0) increased with the addition of soybean oil but decreased with the addition of pomegranate oil compared with the CON diet. The concentration of *cis*-9,*trans*-11 conjugated linoleic acid increased with oil supplements and was greatest with the PMO diet. In conclusion, all 3 oil sources were effective in increasing the production of VA. The effect of PMO and BBO on VA may have resulted in part from inhibition of the final step in the biohydrogenation of VA to stearic acid.
Key words: soybean oil, blackberry oil, pomegranate oil, *trans* fatty acids

Short Communication

Conjugated linoleic acids (**CLA**) are naturally occurring fatty acids in foods derived from ruminants. Conjugated FA have recently attracted significant attention because of their health benefits in a variety of models of metabolic and chronic inflammatory diseases. Among the many CLA isomers, *cis*-9,*trans*-11 (***c9t11***) and *trans*-10,*cis*-12 CLA have received the most attention because of their recognized potential health benefits (Crumb, 2011;Tanaka et al., 2011). The *c*9*t*11 CLA is synthesized either in the rumen as an intermediate during the biohydrogenation of linoleic acid (Harfoot and Hazlewood, 1997) and linolenic acid (Lee and Jenkins, 2011) or in tissues by Δ^9-desaturase from vaccenic acid (*trans*-11 C18:1; **VA)**, another important intermediate in ruminal biohydrogenation of C18 unsaturated FA (Griinari and Bauman, 1999). Bichi et al. (2012) estimated that more than 74% of *c*9*t*11 CLA in milk fat is synthesized through the activity of the Δ^9-desaturase enzyme; therefore, increasing VA flow from the rumen to the lower digestive tract would be necessary to increase milk *c*9*t*11 CLA content.

Previous studies have demonstrated an increase in VA flow from the rumen with the feeding of plant oils high in C18 unsaturated FA (AbuGhazaleh and Buckles, 2007; Varadyovaet al., 2007) or fish oil (Lee et al., 2008; Potu et al., 2011). Plant oils high in unsaturated C18 FA increase the availability of VA precursors in the rumen, whereas the effect of fish oil is believed to occur because of the inhibitory effect of docosahexaenoic acid (C22:6n-3) in fish oil on the reduction of VA to stearic acid (AbuGhazaleh and Jenkins, 2004). Pomegranate and blackberry oils are characterized by their high C18 unsaturated FA content. Fang et al. (2012) compared the FA composition in the seed oil of 4 blackberry varieties and reported that linoleic acid was the main FA (from 51.7 to 69.3% of total FA) followed by linolenic acid (from 10.6 to 31.6% of total FA). Kyralan et al. (2009) also compared the FA composition of 15 different pomegranate seeds and reported that punicic acid (C18:3 *cis*-9,*trans*-11,*cis*-13) was the predominant FA in pomegranate oil, accounting for 74.4 to 76.2% of total FA. Recently, Modaresi et al. (2011) reported an increase in milk *c*9*t*11 CLA content when goat diet was supplemented with pomegranate seed pulp. Pomegranate and blackberry seeds are also rich in polyphenolic compounds (proanthocyanidins, hydrolyzable tannins, and flavonols) that have potent antioxidant and antimicrobial properties (Wang et al., 2004; Dahham et al., 2010). During cold processing, these compounds are extracted into the cold pressed oil in significant quantities (Parry and Yu, 2004).

Received March 27, 2013.
Accepted October 26, 2013.
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Table 1. Ingredient, chemical and fatty acid composition of treatment diets

	Treatment[1]			
Item	CON	SBO	BBO	PMO
Ingredient (g/kg of DM)				
Alfalfa hay	500.00	500.00	500.00	500.00
Soybean meal, 48% CP	70.00	70.00	70.00	70.00
Corn	320.00	320.00	320.00	320.00
Soy hulls	100.00	100.00	100.00	100.00
Minerals mix[2]	10.00	10.00	10.00	10.00
Oil supplement (% of diet DM)				
Soybean oil	—	3.00	—	—
Blackberry oil	—	—	3.00	—
Pomegranate oil	—	—	—	3.00
Chemical composition (% of DM)				
CP	14.32	14.14	14.04	14.12
ADF	24.91	25.23	25.68	25.78
NDF	39.82	37.54	37.67	37.33
Ether extract	3.85	7.29	7.17	7.22
NFC	36.35	35.32	35.44	35.61
Ash	5.66	5.71	5.68	5.72
Fatty acid (mg/g of DM)				
C18:0	0.89	3.23	2.14	1.82
C18:1 *cis*-9	4.81	16.35	12.07	6.87
C18:2 *cis*-9,*cis*-12	11.79	39.32	39.77	14.13
C18:3 *cis*-9,*cis*-12,*cis*-15	2.03	5.07	9.59	2.17
C18:3 *cis*-9,*trans*-11,*cis*-13[3]	ND[4]	ND	ND	39.60

[1]CON = control diet; SBO = control diet + soybean oil at 30 g/kg of DM; BBO = control diet + blackberry oil at 30 g/kg of DM; and PMO = control diet + pomegranate oil at 30 g/kg of DM.

[2]Contained (g/kg): NaCl (975.3), Zn (10.0), Mn (7.5), Fe (6.0), Mg (0.5), Cu (0.32), I (0.28), and Co (0.11).

[3]Punicic acid.

[4]Not detected or detected at <0.01.

To our knowledge, the effects of pomegranate and blackberry oils on VA formation have not been investigated. Therefore, the main objective of this study was to compare the effects of 3 oil sources high in C18 unsaturated FA (soybean, blackberry, and pomegranate oils) on VA formation in a continuous culture system.

Four single continuous culture fermenters, as described by Teather and Sauer (1988), were used in 4 × 4 Latin square designs with 4 periods of 10 d each. The first 7 d were used for adaptation and the last 3 d for sample collection. Treatment diets (50:50 forage: concentrate ratio; DM basis) were fed at 45 g/d (DM basis) in 3 equal portions during the day at 0800, 1500, and 2200 h. Diets were (1) control (**CON**), (2) control plus soybean oil (**SBO**), (3) control plus blackberry oil (**BBO**), and (4) control plus pomegranate oil (**PMO**). The forage consisted of alfalfa pellets, and the concentrate mix contained corn, soybean meal, soy hulls, and minerals (Table 1). Oil supplements were added at 30 g/kg of diet DM. Cold-pressed blackberry oil (57.4% linoleic acid, 18.0% linolenic acid) and pomegranate oil (75.9% punicic acid, 5.4% linoleic acid) were purchased from Botanic Innovations LLC (Spooner, WI), whereas the soybean oil (52.8% linoleic acid, 6.3% linolenic acid) was purchased from a local store.

Two ruminally fistulated Holstein cows fed a TMR (55:45 forage: concentrate ratio; DM basis) were used for collection of ruminal contents. Whole ruminal contents were collected approximately 4 h after the morning feeding and then immediately transferred to the laboratory in sealed bags. Samples were then strained using a double-layered cheese cloth, and approximately 600 mL of the ruminal fluid was added to each of the 4 fermenters, containing 100 mL of prewarmed buffer. Cultures were stirred (BCD Caframo Stirrer, Fisher, St. Louis, MO) continuously at 45 rpm, and fermenter pH was measured daily before addition of feed using a portable pH meter at 0800, 1500, and 2400 h. Anaerobic conditions in fermenters were maintained by infusing CO_2 at 40 mL/min and fermenter temperature was maintained at 39°C using a circulating water bath. The buffer solution (Weller and Pilgrim, 1974) was prepared in one container and delivered continuously to each fermenter at a flow rate of 1.16 mL/min (0.10 h^{-1} liquid dilution rate), using a precision pump. The flow rate of each fermenter was recorded every day at 0800 h.

On d 8, 9, and 10 of each period, the overflow (effluent) was collected into 2-L plastic flasks immersed in ice. The collected effluents from each fermenter during the last 3 d were then homogenized by stirring and 25%