



Original research article

# The effect of diet supplemented with vegetable oils and/or monensin on the vaccenic acid production in continuous culture fermenters



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## ABSTRACT

Studies have shown that supplementing ruminant diets with vegetable oils modulated the rumen biohydrogenation and increased polyunsaturated fatty acid in their products. These positive values are often accompanied by a marginal loss of supplemented unsaturated fatty acids and rise in the concentrations of saturated fatty acids. This study were carried out mainly to investigate the effect of supplementing diets with sunflower oil, olive oil with or without monensin on the production and accumulation of vaccenic acid (VA) in continuous culture fermenters as a long term *in vitro* rumen simulation technique. Eight dual-flow continuous culture fermenters were used in an 8 replication experiment lasted 10 days each (first 7 days for adaptation and last 3 days for samples collection). Supplementing diets with plant oils and monensin in the present experiment increased VA and conjugated linoleic acids ( $P > 0.05$ ) in ruminal cultures. The results suggest that supplementing diets with both olive oil and sunflower oil and monensin increased VA accumulation compared to plant oils supplemented alone without affecting the rumen dry matter and organic matter digestibility.

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## 1. Introduction

Modulation of rumen processes, particularly rumen biohydrogenation, gained an increasing interest in the last decade. This intensive interest is related to some health promoting effects of fatty acids presented in ruminants' products, especially milk, which is considered as a major source of dietary lipids in human diets (Abo El-Nor and Khattab, 2012; Kromhout et al., 2002). Recent researches have shown that unsaturated trans-10, cis-12 C18:2 inhibit tumors growth in colon and gastric cancer cell lines; as well, cis-9, trans-11 C18:2 may be efficacious in reducing risk of breast

cancer in premenopausal women. The studies published about antidiabetic effect of conjugated linoleic acids (CLA) isomers also indicated that either cis-9, trans-11 C18:2 or trans-10, cis-12 C18:2 increase insulin resistances in obese men with metabolic syndrome. It has also been shown that trans-10, cis-12 C18:2 is responsible for the body mass reduction and weight change (McCrorie et al., 2011).

In ruminants, biohydrogenation of long chain unsaturated fatty acid (LCUFA) at pH 5.9 to 6.7 is related with the presence of CLA. Conjugated linoleic acids isomers naturally produced by rumen bacteria as intermediates in the biohydrogenation of dietary LCUFA mainly linoleic acid C18:2, with cis-9, trans-11 CLA being the predominant isomer found in ruminants products (Bauman and Lock, 2006). Conjugated linoleic acid is formed also from the endogenous conversion of trans-11 C18:1 (trans vaccenic acid, VA), another intermediate of rumen biohydrogenation of linoleic acid and/or linolenic acid (C18:3) by the  $\Delta$ -9 desaturase enzyme in the mammary gland (Corl et al., 2001; Buccioni et al., 2012). Several studies in the last decades were conducted mainly to illustrate the impact of oil supplementation on the rumen biohydrogenation and other parameters i.e., methane mitigation. Most of the results positively

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highlighted the impact of supplemented oils on different rumen parameters especially fatty acid modulation (Hellwing et al., 2012; Storlien et al., 2012; Beauchemin et al., 2007). According to Kellens et al., 1986; Harfoot and Hazlewood, 1997, oleic acid conversion by rumen microflora produce only stearic acid without the formation of any intermediates, while, some *in vitro* experiment studies showed that conversion of oleic acid to a variety of trans isomers (Mosley et al., 2002; Abu-Ghazaleh et al., 2005). Additionally, the impact of monensin in ruminants nutrition were noted which work in inhibiting the growth of gram-positive bacteria that produce hydrogen and also related to the biohydrogenation process (Shingfield et al., 2012; Wang et al., 2005; Chen and Wolin, 1979). Fellner et al. (1997) showed that the *in vitro* supplementation of monensin (2 g/mL) increased CLA proportion compared with the control, the same results was obtained from the *in vivo* study conducted on dairy cattle by Dhiman et al. (1999) who noticed an increase in CLA content in milk when monensin is supplemented. Thus, the use of monensin in association with lipid supplementation (sunflower oil, olive oil) may be one of the ways of increasing the CLA content in ruminant products. Subsequently, the main aim of the present study is to investigate the effect of supplementing diets with sunflower and olive oils with or without monensin on the *in vitro* vaccenic acid and CLA formation using continuous culture fermenters.

## 2. Materials and methods

### 2.1. Apparatus and experimental design

Eight dual-flow continuous culture fermenters as described by Teather and Sauer (1988) in an 8 × 8 Latin square experiment lasted for 10 days each (first 7 days for adaptation and last 3 days for samples collection). Ruminant fluid was obtained from 2 ruminally fistulated bulls (450 kg BW) fed a mixture of dehydrated alfalfa hay and commercial concentrate (30:70 on DM basis). Collected rumen content were blended then strained through four layers of cheesecloth and immediately transported to the laboratory in a water bath preheated to 39°C. Approximately 600 mL of the ruminant fluid was added to each of the eight fermenters, containing 100 mL of pre-warmed buffer, anaerobic conditions in fermenters were maintained by infusing CO<sub>2</sub> at a rate 40 mL/min. Cultures were stirred continuously at 45 rpm. Fermenter temperature was maintained at 39°C using a circulating water bath. Buffer was delivered continuously at a flow rate of 1.16 mL/min, using a precision pump. Treatment diets (forage to concentrate; wt/wt) were fed at 45 g/d DM in two equal portions at 0800 and 1600 h. pH were measured daily before morning feeding and recorded. Overflow measured daily before morning feeding and recorded. The control basal after contained 16.6% CP, 27.5% NDF, and 17.4% ADF. The diet (DM basis) consisted of alfalfa hay (50%), ground barley grain (24%), ground corn grain (16%), soybean meal (9.7%), salt (NaCl, 0.3%). The diet was designed to meet or exceed nutrient recommendations for a Holstein cow (650 kg BW) producing 30 kg of milk (NRC, 2001). Treatments were T<sub>1</sub>: control diet plus monensin; T<sub>2</sub>: control diet supplemented with 5% olive oil plus monensin; T<sub>3</sub>: control diet supplemented with 5% sunflower oil plus monensin; T<sub>4</sub>: control diet supplemented with 5% olive oil and sunflower oil plus monensin, T<sub>5</sub>: control diet; T<sub>6</sub>: control diet supplemented with 5% olive oil; T<sub>7</sub>: control diet supplemented with 5% sunflower oil; T<sub>8</sub>: control diet supplemented with 5% olive oil and sunflower oil.

### 2.2. Sample collection and analysis

Starting on days 7, 8 and 9 of each period, the overflow (effluent) was collected into 2 L plastic flasks. Collected effluents were

homogenized by stirring and approximately 0.25 (vol/vol) sub-samples were pooled into one sample and stored at –20°C until further analysis. Effluent samples were carried out according to Abo El-Nor et al. (2010). Dry matter of experimental diets was determined by drying at 105°C for 48 h (AOAC, 1990; method 930.15). Samples were analyzed for ash (method 942.05) according to AOAC methods (2000). Treatment diets and effluent samples were methylated using the sodium methoxide (CH<sub>3</sub>ONa) and HCl two step procedures as outlined by Kramer et al. (1997) and analyzed in duplicate for fatty acids (FA). Fatty acids methyl esters (FAME) were separated using a Cp-Sil 88 fused-silica capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness, Chrompack, Middelburg, Netherlands) on a Perkin–Elmer chromatograph (model 8420, Beaconsfield) equipped with a flame ionization detector. The column was held at 100°C for 1 min after injection, temperature-programmed at 7°C/min to 170°C, held there for 55 min, then temperature-programmed at 10°C/min to 230°C and held there for 33 min. Helium was the carrier gas with a column inlet pressure set at 30 psig and a split ratio of 1:20. The injection volume was 0.2 μL. Total run time was of 105 min.

On day 10 of each period, samples were collected from each fermenter at 3 h post morning feeding for total volatile fatty acids (TVFA) and ammonia–nitrogen (NH<sub>3</sub>–N). Before collection of samples, the fermenter speed was increased to 190 to 200 rpm to ensure thorough mixing. Ammonia–N samples were centrifuged at 2,000 × g at 4°C for 10 min and the supernatant was acidified with 0.5 mL of 0.1 N HCl then analyzed for NH<sub>3</sub>–N by a Teco Diagnostics Kit (Anaheim, CA, USA) using a spectrophotometer (Thermo Spectronic Genesys 5 Spectrophotometer, Artisan Scientific, Champaign, IL, USA) (Abo El-Nor and Khattab, 2012).

### 2.3. Statistical analysis

Data was analyzed as an 8 × 8 Latin square design with a 2 × 4 factorial arrangement of treatments using the PROC MIXED of SAS (SAS Institute, Inc., Cary, NC). The statistical model included: fermenters, diet, and period. Fixed effects were diet and period, Random effect was fermenter. Fermentation parameters collected over different times analyzed by ANOVA for repeated measures. In case of NH<sub>3</sub>–N, TVFA zero time means used as a covariate to adjust 3 & 6 h. Results are expressed as least square means with standard error of the means. The significance threshold was set at  $P < 0.05$ .

## 3. Results

The effect of the experimental diets on fermentation is presented in Table 1. No differences were found on fermenters pH ( $P > 0.05$ ). Ammonia–nitrogen concentration was decreased ( $P < 0.05$ ) in T<sub>2</sub>, T<sub>4</sub>, T<sub>6</sub> and T<sub>8</sub> while increased in T<sub>3</sub> and T<sub>7</sub> ( $P < 0.05$ ) compared with T<sub>1</sub>. Total volatile fatty acids concentrations results showed that T<sub>7</sub> and T<sub>3</sub> recorded the highest values while T<sub>4</sub> and T<sub>8</sub> recorded the lowest values ( $P < 0.05$ ).

The effects of the experimental diets on effluent FA are presented on Table 2. Results showed that T<sub>7</sub> recorded the highest value of C18:2 and T<sub>4</sub> recorded the lowest value and there were significant differences among treatments ( $P < 0.05$ ). Vaccenic acid values were increased ( $P < 0.05$ ) in T<sub>3</sub> and T<sub>4</sub>, while decreased in T<sub>2</sub>, T<sub>5</sub>, T<sub>6</sub> and T<sub>7</sub> compared with T<sub>1</sub> ( $P < 0.05$ ). Conjugated linoleic acids values were higher in T<sub>5</sub> and T<sub>6</sub> compared with other treatments ( $P < 0.05$ ). The effects of the experimental diets on DM and OM digestibility are presented in Table 3. Supplementing diets with olive oil and sunflower oil plus monensin (T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>7</sub> and

**Table 1**  
Effect of experimental diets on *in vitro* ruminal parameters using continuous fermentor.

Item	Treatment <sup>1</sup>								SE	P-value
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>	T <sub>8</sub>		
<b>pH</b>										
Zero	6.5	6.53	6.48	6.53	6.45	6.45	6.50	6.53	0.032	0.21
3 h	6.28	6.2	6.15	6.15	6.30	6.25	6.15	6.15	0.06	0.16
6 h	6.45	6.45	6.4	6.5	6.43	6.48	6.40	6.40	0.035	0.15
Mean	6.41	6.39	6.34	6.39	6.39	6.39	6.35	6.36	0.045	0.11
<b>Ammonia-N concentration, mg/dL</b>										
Zero	8.88	5.18	9.77	6.03	8.70	5.08	9.25	5.90	1.84	0.044
3 h	16.47	11.4	19.1	11.15	15.95	10.82	19.15	11.05	3.44	0.02
6 h	13.15	6.63	16.8	6.8	13.98	5.85	16.60	6.75	4.46	0.039
Mean	12.83	7.74	15.22	7.99	12.88	7.25	15.00	7.90	3.23	0.023
<b>TVFA concentration, mmol/L</b>										
Zero	7.83	8.31	9.74	7.97	7.22	7.73	9.64	7.89	0.85	0.038
3 h	9.57	10.0	10.42	8.84	9.68	10.06	13.16	8.86	1.27	0.042
6 h	9.49	10.52	11.38	8.10	9.61	9.69	10.98	8.55	1.06	0.029
Mean	8.96	9.61	10.51	8.30	8.84	9.16	11.26	8.43	0.96	0.034

<sup>1</sup> TVFA = total volatile fatty acids; T<sub>1</sub>: control diet plus monensin; T<sub>2</sub>: control diet supplemented with 5% olive oil plus monensin; T<sub>3</sub>: control diet supplemented with 5% sunflower oil plus monensin; T<sub>4</sub>: control diet supplemented with 5% olive oil and sunflower oil plus monensin, T<sub>5</sub>: control diet; T<sub>6</sub>: control diet supplemented with 5% olive oil; T<sub>7</sub>: control diet supplemented with 5% sunflower oil; T<sub>8</sub>: control diet supplemented with 5% olive oil and sunflower oil.

**Table 2**  
Effect of experimental diets on *in vitro* fatty acids concentrations (mg/100 mL) using continuous fermentor.

Item	Treatment <sup>1</sup>								SE	P-value
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>	T <sub>8</sub>		
C18:2	0.70	0.55	0.43	0.38	0.68	0.50	0.75	0.70	0.06	0.041
Trans vaccenic acid	2.70	2.68	3.10	3.33	1.98	1.93	2.53	2.78	0.07	0.034
C18: 2 cis-9, trans-11	0.28	0.18	0.15	0.23	0.35	0.45	0.15	0.18	0.05	0.039

<sup>1</sup> T<sub>1</sub>: control diet plus monensin; T<sub>2</sub>: control diet supplemented with 5% olive oil plus monensin; T<sub>3</sub>: control diet supplemented with 5% sunflower oil plus monensin; T<sub>4</sub>: control diet supplemented with 5% olive oil and sunflower oil plus monensin, T<sub>5</sub>: control diet; T<sub>6</sub>: control diet supplemented with 5% olive oil; T<sub>7</sub>: control diet supplemented with 5% sunflower oil; T<sub>8</sub>: control diet supplemented with 5% olive oil and sunflower oil.

**Table 3**  
Effect of experimental diets on *in vitro* dry matter and organic matter digestibility (g/100 g) using continuous fermentor.

Item	Treatment <sup>1</sup>								SE	P-value
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>	T <sub>8</sub>		
DM	56.2	61.02	63.83	65.61	54.77	56.86	60.19	62.15	1.34	0.04
OM	56.83	61.70	64.54	68.87	54.10	55.30	57.95	59.87	1.02	0.03

<sup>1</sup> T<sub>1</sub>: control diet plus monensin; T<sub>2</sub>: control diet supplemented with 5% olive oil plus monensin; T<sub>3</sub>: control diet supplemented with 5% sunflower oil plus monensin; T<sub>4</sub>: control diet supplemented with 5% olive oil and sunflower oil plus monensin, T<sub>5</sub>: control diet; T<sub>6</sub>: control diet supplemented with 5% olive oil; T<sub>7</sub>: control diet supplemented with 5% sunflower oil; T<sub>8</sub>: control diet supplemented with 5% olive oil and sunflower oil.

T<sub>8</sub>) increased ( $P < 0.05$ ) DM and OM digestibility compared with other treatments.

#### 4. Discussion

The present results of ruminal fermentation especially TVFA disagreed with many studies (Wallace et al., 1980; Fuller and Johnson, 1981; Richardson et al., 1976) who suggested that adding monensin to diet decrease TVFA which may be due to reduction of cellulolytic activity through inhibiting cellulolytic bacteria (Chalupa et al., 1980). Many studies showed that supplementing diets with oils rich on linoleic acid (sunflower oil) or rich on oleic acid (olive oil) could increase CLA contents (Whitlock et al., 2002, 2003; Ward et al., 2003; Dhiman et al., 2000; Loo et al., 2002). Fellner et al. (1997) reported that adding monensin to diets could inhibit the growth and activity of *Butyrivibrio fibrisolvens*, which is active in the ruminal hydrogenation.

It's also proven that monensin is associated with milk fat depression due to its effect on inhibiting the biohydrogenation of polyunsaturated fatty acids, and consequently alters the rumen and milk fatty acid composition (Sauer et al. 1998). He et al. (2012) also reported an increase in milk VA and CLA when diet was rich in C18 polyunsaturated fatty acids suggesting that monensin did directly alter ruminal biohydrogenation.

Bauman et al. (2000) recorded that the differences in CLA production in the rumen may be related to ruminal bacteria adaptation in which monensin-resistant species be dominant and replace monensin-sensitive bacteria responsible for bio-hydrogenation in the rumen.

#### 5. Conclusion

Supplementing diets with plant oils and monensin have no effects on fermenter pH. The supplementing diets with sunflower oil plus monensin increased the concentration of trans-vaccenic acid and CLA. Also, the supplementing diets with different plant oils plus monensin increased DM and OM digestibility.

#### References

- Abo El-Nor SAH, Abughazaleh AA, Potu RB, Hastings D, Khattab MSA. Effects of differing levels of glycerol on rumen fermentation and bacteria. *Anim Feed Sci Technol* 2010;162:99–105.
- Abo El-Nor SAH, Khattab MSA. Enrichment of milk with conjugated linoleic acid by supplementing diets with fish and sunflower oil. *Pak J Biol Sci* 2012;15(14): 690–3.
- Abu-Ghazaleh AA, Riley MB, Thies EE, Jenkins TC. Dilution rate and pH effects on the conversion of oleic acid to *trans* C18:1 positional isomers in continuous culture. *J Dairy Sci* 2005;88:4334–41.
- Association of Official Analytical Chemists (AOAC). Official methods of analysis. 15th ed. Arlington, VA, USA: AOAC; 1990.

- Association of Official Analytical Chemists (AOAC). Official methods of analysis. 17th ed. Arlington, VA, USA: AOAC; 2000.
- Bauman DE, Lock AL. Conjugated linoleic acid: biosynthesis and nutritional significance. In: Fox PF, McSweeney PLH, editors. Advanced dairy chemistry, lipids. 3rd ed., vol. 2. New York, NY: Springer; 2006. p. 93–136.
- Bauman DE, Baumgard LH, Corl BA, Grinari JM. Biosynthesis of conjugated linoleic acid in ruminants. *Proc Am Soc Anim Sci* 2000;1–15.
- Beauchemin KA, McGinn SM, Petit HV. Methane abatement strategies for cattle: lipid supplementation of diets. *Can J Anim Sci* 2007;87:431–40.
- Buccioni A, Decandia M, Minieri S, Molle G, Cabiddu A. Review: lipid metabolism in the rumen: new insights on lipolysis and biohydrogenation with an emphasis on the role of endogenous plant factors. *Anim Feed Sci Technol* 2012;174:1–25.
- Chalupa W, Corbett W, Brethour J. Effects of monensin and ampicloral on rumen fermentation. *J Anim Sci* 1980;51:170–9.
- Chen M, Wolin MJ. Effect of monensin and lasalocid-sodium on the growth of methanogenic and rumen saccharolytic bacteria. *Appl Environ Microbiol* 1979;38:72–7.
- Corl BA, Baumgard LH, Dwyer DA, Grinari JM, Phillips BS, Bauman DE. The role of delta (9)-desaturase in the production of cis-9, trans-11 CLA. *J Nutr Biochem* 2001;12:622–30.
- Dhiman TR, Anand GR, Satter LD, Pariza MW. Conjugated linoleic acid content of milk from cows fed different diets. *J Dairy Sci* 1999;82:2146–56.
- Dhiman TR, Satter LD, Pariza MW, Galli MP, Albright K, Tolosa MX. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. *J Dairy Sci* 2000;83:1016–27.
- Fellner V, Sauer FD, Kramer JKG. Effect of nigericin, monensin, and tetronasin on biohydrogenation in continuous flow-through ruminal fermenters. *J Dairy Sci* 1997;80:921–8.
- Fuller JR, Johnson DE. Monensin and lasalocid effects on fermentation *in vitro*. *J Anim Sci* 1981;53:1574–80.
- Harfoot CG, Hazlewood GP. Lipid metabolism in the rumen. In: Hobson PM, editor. The rumen microbial ecosystem. New York, NY: Elsevier; 1997. p. 382–426.
- He M, Perfield KL, Green HB, Armentano LE. Effect of dietary fat blend enriched in oleic or linoleic acid and monensin supplementation on dairy cattle performance, milk fatty acid profiles, and milk fat depression. *J Dairy Sci* 2012;95:1447–61.
- Hellwing ALF, Sørensen MT, Weisbjerg MR, Vestergaard M, Lund P. Can rapeseed lower methane emission from heifers? *Acta Agric Scand Sect A – Anim Sci* 2012;62(4):259–62.
- Kellens MJ, Goderis HL, Tobback PP. Biohydrogenation of unsaturated fatty acids by a mixed culture of rumen microorganisms. *Biotechnol Bioeng* 1986;28:1268–76.
- Kramer JKG, Fellner V, Dugan MER, Sauer FD, Mosoba MM, Yurawecz MP. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. *Lipids* 1997;32:1219–28.
- Kromhout D, Menhotti A, Kesteloot H, Sans S. Prevention of coronary heart disease by diet and lifestyle: evidence from prospective cross-cultural, cohort, and intervention studies. *Circulation* 2002;105:893–8.
- Loor JJ, Herbein JH, Jenkins TC. Nutrient digestion, biohydrogenation, and fatty acid profiles in blood plasma and milk fat from lactating Holstein cows fed canola oil or canolamide. *Anim Feed Sci Technol* 2002;97:65–82.
- McCrorie TA, Keaveney EM, Wallace JMW, Binns N, Livingstone MBE. Human health effects of conjugated linoleic acid from milk and supplements. *Nutr Res Rev* 2011;24:206–27.
- Mosley EE, Powell GLP, Riley MB, Jenkins TC. Microbial biohydrogenation of oleic acid to *trans* isomers *in vitro*. *J Lipid Res* 2002;43:290–6.
- National Research Council. Nutrient requirements of dairy cattle. 7th rev. ed. Washington, D.C: National Academy of Sciences; 2001.
- Richardson LF, Raun AP, Potter EL, Cooley CO. Effect of monensin on rumen fermentation *in vitro* and *in vivo*. *J Anim Sci* 1976;43:657–64.
- Sauer FD, Kramer JKG, Cantwell WJ. Antiketogenic effects of monensin in early lactation. *J Dairy Sci* 1989;72:436–42.
- Shingfield KJ, Kairenius P, Arola A, Paillard D, Muetzel S, Ahvenjarvi S, et al. Dietary fish oil supplements modify ruminal biohydrogenation, alter the flow of fatty acids at the omasum, and induce changes in the ruminal butyrvibrio population in lactating cows. *J Nutr Nutr Physiol Metab Nutrient-Nutrient Interact* 2012;142(8):1437–48.
- Storlien TM, Harstad OM, Narvaez N, Wang Y, McAllister TA. Effects of different oils and plant extracts on *in vitro* ruminal methane production. *Acta Agric Scand Sect A – Anim Sci* 2012;62(4):300–4.
- Teather RM, Sauer FD. A naturally compartmented rumen simulation system for the continuous culture of rumen bacteria and protozoa. *J Dairy Sci* 1988;71:66–73.
- Wallace RJ, Cheng KJ, Czerkawski JW. Effect of monensin on fermentation characteristics of the artificial rumen. *Appl Environ Microbiol* 1980;40:672–4.
- Wang JH, Zhu BW, Song MK, Choi YJ. Effect of monensin, fish oil or their combination on *in vitro* fermentation and conjugated linoleic acid (CLA) production by ruminal bacteria. *Anim Feed Sci Tech* 2005;120:341–9.
- Ward AT, Wittenberg KM, Froebe HM, Przybylski R, Malcolmson. Fresh forage and solin supplementation on conjugated linoleic acid levels in plasma and milk. *J Dairy Sci* 2003;86:1742–50.
- Whitlock LA, Schingoethe DJ, Hippen AR, Kalscheur KF, Abughazaleh AA. Milk production and composition from cows fed high oil or conventional corn at two forage concentrations. *J Dairy Sci* 2003;86:2428–37.
- Whitlock LA, Schingoethe DJ, Hippen AR, Kalscheur KF, Baer RJ, Ramaswamy N, et al. Fish oil and extruded soybeans fed in combination increase conjugated linoleic acids in milk of dairy cows more than when fed separately. *J Dairy Sci* 2002;85:234–42.