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The effect of pH and polyunsaturated C18 fatty acid source on the production of vaccenic acid and conjugated linoleic acids in ruminal cultures incubated with docosahexaenoic acid

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Abstract

Previously, combining docosahexaenoic acid (DHA) with linoleic acid in rumen cultures enhanced vaccenic acid (VA) and conjugated linoleic acid (CLA) accumulations. The objective of this experiment was to examine the effect of two pH levels and two polyunsaturated C18 fatty acid (FA) sources on VA and CLA accumulations in rumen cultures incubated with DHA. High pH culture treatments consisted of 10 mg DHA + 20 mg linoleic acid (LOH), or 10 mg DHA + 20 mg linolenic acid (LNH). Low pH culture treatments consisted of 10 mg DHA + 20 mg linoleic acid (LOL), or 10 mg DHA + 20 mg linolenic acid (LNL). Treatments were incubated in triplicate in 125 ml flasks containing 500 mg finely ground TMR, 10 ml strained ruminal fluid, 40 ml media, and 2 ml reducing solution. Ruminal fluid was collected from fermenters fed high forage (pH 6.7) or high grain diets (pH 5.4). A 5-ml sample of culture contents was taken at 0 and 24 h for fatty acid analysis by gas liquid chromatography. After 24 h of incubation, VA was the predominant *trans* C18:1 FA isomer in the high pH cultures and its concentration was greater with LOH (20.9 mg/culture) than with LNH (9.3 mg/culture). Similarly, *t*10 C18:1 was the predominant *trans* C18:1 FA isomer in the low pH cul-

Abbreviations: CLA, conjugated linoleic acid; DM, dry matter; DHA, docosahexaenoic acid; FA, fatty acid; TMR, total mixed ration; VA, vaccenic acid

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tures and its concentration was greater with LOL (12.8 mg/culture) than with LNL (4.5 mg/culture). The *c*9*t*11 CLA (0.74 mg/culture) and *tt* CLA (0.67 mg/culture) were the predominant CLA isomers in the LOH cultures, while *t*10*c*12 CLA (1.47 mg/culture) and *tt* CLA (1.27 mg/culture) were the predominant CLA isomers in the LOL cultures. The *t*11*t*13 CLA increased in linolenic acid cultures, particularly when linolenic acid incubated under low pH condition (0.95 mg/culture). Additions of linolenic acid to rumen cultures caused a dramatic increase in the concentration of *t*11*c*15 C18:2 (9.84 and 8.45 mg/culture, for treatments LNH and LNL, respectively). The concentrations of VA and *c*9*t*11 CLA in rumen cultures were greatest when DHA was incubated with linoleic acid at high pH. In contrast, *t*10 C18:1 replaced VA and *c*9*t*11 CLA disappeared when linoleic and linolenic acids were incubated under the low pH condition. This study shows that the best approach to increase ruminal VA production, and potentially *c*9*t*11 CLA levels in ruminant food products, is by combining a DHA source (such as fish oil or algae) and linoleic acid source under high ruminal pH condition.

Keywords: Docosahexaenoic acid; Conjugated linoleic acid; Vaccenic acid; pH; Linoleic acid; Linolenic acid

1. Introduction

Trans C18:1 fatty acids (FA) and conjugated linoleic acid (CLA) are produced by the incomplete biohydrogenation of long chain unsaturated FA in the rumen (Harfoot and Hazlewood, 1988), and are subsequently incorporated into milk and meat of ruminant animals. Formation of *trans* C18:1 FA and CLA in the rumen are influenced by dietary supplementation with unsaturated vegetable oils (AbuGhazaleh et al., 2003; Loor et al., 2004a) or changes in rumen pH as a result of alterations in dietary forage to concentrate ratios (Piperova et al., 2002; Sackman et al., 2003).

Previously, researchers (Kelly et al., 1998; Dhiman et al., 2000; Chouinard et al., 2001; AbuGhazaleh et al., 2003; Loor et al., 2005) used soybean oil, peanut oil, sunflower oil, linseed oil, and fish oil to increase ruminal production of VA and CLA. However, the production of *trans* C18:1, particularly vaccenic acid (VA), and *c*9*t*11 CLA in response to dietary fat supplements high in linoleic and linolenic acids has not been consistent. AbuGhazaleh et al. (2003) and Ward et al. (2002) reported greater increase in milk VA and *c*9*t*11 CLA concentrations with diets supplemented with linoleic acid compared with linolenic acid fat source. Loor et al. (2004a, 2005) and Chow et al. (2004) on the other hand, reported no difference in VA and *c*9*t*11 CLA concentrations when sunflower oil and linseed oil were compared *in vivo* and *in vitro*. Feeding lactating dairy cows a blend of fish oil and a high linoleic acid source resulted in greater increases in ruminal and milk concentrations of VA and *c*9*t*11 CLA than when fed separately (AbuGhazaleh et al., 2002; Whitlock et al., 2002). Recently, AbuGhazaleh and Jenkins (2004b) demonstrated that docosahexaenoic acid (DHA) is the component in fish oil which promotes *trans* C18:1 accumulation, including VA, when incubated with linoleic acid fat source.

Ruminal pH is also known to effect biohydrogenation of unsaturated FA and trans FA formation (Kalscheur et al., 1997; Piperova et al., 2002; Sackman et al., 2003; Loor et al., 2004a,b). When cows were switched from a control diet (60:40 forage:concentrate) to a high-concentrate diet (25:75 forage:concentrate) the proportion of *t*10 increased, concomitant