**Online Supporting Material**

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**Supplementary Fig. S1.** Mass spectrum of the 4,4-dimethyloxazoline derivative of *cis*-7,*cis*-12,*cis*-15-18 : 3formedfrom α-linolenic acid during incubations with strained rumen contents diluted with buffer prepared using deionized water or deuterium oxide. A molecular ion at *m*/*z* 331 confirmed the octadecatrienoic acid structure. Gaps of 12 atomic mass units between *m*/*z* 168 and 180, 236 and 248 and 276 and 288 located double bonds at Δ7, 12 and 15, respectively. Comparison of the mass spectrum of *cis*-7,*cis*-12,*cis*-15-18 : 3 formed during incubations with or without deuterium oxide provided no evidence of labelling.

**Online Supporting Material**

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**Supplementary Fig. S2.** Mass spectrum of the 4,4-dimethyloxazoline derivative of *cis*-8,*cis*-12,*cis*-15-18 : 3synthesized from α-linolenic acid during incubations with strained rumen contents diluted with buffer prepared using deionized water or deuterium oxide. A molecular ion at *m*/*z* 331 confirmed an octadecatrienoic acid structure. Gaps of 12 atomic mass units between *m*/*z* 182 and 194, 236 and 248 and an abundant ion at *m*/*z* 222 confirmed a Δ8,12 double bond arrangement. The double bond at Δ15 was located based on a gap of 12 atomic mass units at *m*/*z* 276 and 288. Comparison of the mass spectrum of *cis*-8,*cis*-12,*cis*-15-18 : 3 formed during incubations with or without deuterium oxide provided no evidence of labelling.

**Online Supporting Material**

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**Supplementary Fig. S3.** Mass spectrum of the 4,4-dimethyloxazoline derivative of *cis*-12,*cis*-15-18 : 2 synthesizedfrom α-linolenic acid during incubations with strained rumen contents diluted with buffer prepared using deionized water or deuterium oxide. Gaps of 12 atomic mass units between *m*/*z* 238 and 250 and 278 and 290 located double bonds at ∆12 and ∆15, respectively. Due to the low abundance, the position of labels on the fatty acid moiety during incubations with deuterium-containing buffer could not be established.