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Effects of Bovine Diets and Environmental Factors on Conjugated Linoleic Acid (CLA) Production by Rumen Bacteria

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Growing evidence has suggested that conjugated linoleic acid (CLA) has a wide scope of health-promoting roles and the properties in the prevention of degenerative diseases. However, the concentration of CLA in natural products is not sufficient to play such roles. Hence, our research aimed to enhance CLA contents in dairy products by providing insight into the mechanism of CLA production by rumen bacteria.

Butyrivibrio fibrisolvens A38, one of the most active rumen bacteria in CLA production was characterized *in vitro*. Washed cell suspensions that were incubated anaerobically with 350 μM LA converted most of the LA to hydrogenated products, and little CLA was detected. If the washed cell suspensions were incubated aerobically, biohydrogenation (BH) was inhibited, CLA production was at least 2-fold greater, and CLA persisted. The LA isomerase reaction was very rapid, but did not recycle like a normal enzyme to catalyze more substrate. Cells that were pre-incubated with CLA, lost their ability to produce more CLA from LA, and the CLA accumulation was directly proportional ($r^2 = 0.98$) to the initial cell density. Since growing cells were as sensitive to CLA as LA, and the LA isomerase and reductases of BH were linked, the free CLA was not released until the LA concentration was high enough to arrest BH and cell growth. The bacterium did not produce significant amounts of CLA until the linoleic acid (LA) concentration was high, BH was arrested, and the cell density declined all of which indicated that the flow of CLA from the rumen may be due to LA-dependent bacterial lysis

Furthermore, some inhibitory conditions for bacterial growth were less influential to the isomerization step than to the following reduction steps. LA was inhibitory not only to cell growth, but also to the overall BH and this effect was greater at high concentrations. The reduction steps, converting CLA to hydrogenated products (*trans*-C18:1 and C18:0) were significantly inhibited and more CLA accumulated during aerobic incubation when LA was added along with a glycolytic inhibitor, iodoacetate (IAA) to cells which were pre-adapted to LA ($P < 0.05$). Rumen fluid in the culture medium seemed to activate BH even in an aerobic condition resulting in a lower CLA level than the control group ($P<0.05$). Since the isomerization and reduction steps are coupled reactions of most hydrogenating bacteria including *B. fibrisolvens* A38 cells, the modulations of reduction steps could be key determinants for CLA accumulation in the rumen.

Extended research was performed on mixed rumen bacteria from cattle fed different diets. Mixed culture of rumen bacteria obtained from three different cows fed high-concentrate diet (HCD) or high-roughage diet (HRD) for three weeks. Mixed rumen bacteria fractionated by slow centrifugation (500 g) into particle-associated and planktonic bacteria, and incubated in a 30% rumen fluid medium with LA at various conditions. The bacteria from cattle fed HCD were more active in BH and *trans*-10, *cis*-12 CLA production than from cattle fed HRD and particle-associated bacteria produced more hydrogenated products leaving less CLA than the planktonic bacteria ($P<0.05$). CLA production did not always correlate to LA given, and CLA concentration typically dropped in prolonged incubations.

Furthermore, a bacterium isolated from a mixed rumen culture that was enriched in medium containing lactic acid was found to produce 6 times more *trans*-10, *cis*-12 CLA than *cis*-9, *trans*-11 CLA. Mixed rumen bacteria from ruminal contents of a cow fed HCD were enriched with DL-lactate and Trypticase. They produced more *trans*-10, *cis*-12 CLA than those that were not enriched (7 versus 2 μg mg protein^{-1}, $P < 0.05$). Enrichments had an abundance of large cocci that produced *trans*-10, *cis*-12 CLA from LA. Strain YJ-4 produced the most *trans*-10, *cis*-12 CLA (approximately 7 μg mg protein^{-1}), and 16S rDNA sequencing indicated that YJ-4 was a strain of *Megasphaera elsdenii*. The *trans*-10, *cis*-12 CLA production by YJ-4 was first order with respect to cell concentration (0 to 800 μg protein ml^{-1}), but kinetics were not first order with respect to substrate concentration. The results from this study indicates that rumen microbial population could affect CLA isomer balance which may lead to milk fat depression in cattle fed diets supplemented with grain and PUFAs.

Overall, the study presented herein may provide some strategies to increase CLA content in dairy products by modulating the bovine dietary regimen and environmental factors for rumen bacterial growth.