CHARACTERISTICS OF SERUM ORGANIC ACID FRACTIONS FROM STEERS FED *AD LIBITIUM* AND IN RESTRICTED FEEDING

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Introduction

Blood-bone metabolites and hormones have been considered to be important in the control of food intake. Although plasma short- and long-chain fatty acids levels change with feed intake, there is no explicable evidence that these fatty acids were directly regulating food intake in ruminants (De Jong, 1986). Oomura (1984) suggested that two endogenous polyhydroxy acids, 3,4-dihydroxybutanoic acid (2-deoxytetronic acid; 2-DTA) and 2,4,5-trihydroxypentanoic acid (3-deoxypentonic acid; 3-DPA) have possible relations to satiefy in the former and hunger in the latter in rats. These substances are found in serum of other monogastric animals but not in ruminants.

The present study was undertaken to investigate the precise profile of the organic acids in serum from the steers fed *ad libitum* and in restricted feeding by the techniques of gas chromatrography-mass spectrometrical analysis.

Materials and Methods

Four Holstein steers weighing 300 kg were kept in individual pens for 14 days and were offered mixed rations of concentrate and chopped hay. During the first period of 7 days they were fed *ad libitium* twice a day. In the second period of 7 days they were given half the amount of food eaten in the first period. The blood samples (300 ml) were taken at once from their catheterized juglar vein at 8:30 AM (before meal) and 0:30 PM (after meal). The serums separated from the blood sample were freeze-dried for preservation and condensation. The freeze-dried powder equivalent to 10 ml of raw serums were used for analysis of organic acid fractions by gas chromatography-mass spectrometry techniques. The outline of procedure of analysis is described by Kuhara et al. (1980). Gas chromatography was performed with a Hitachi Model 163 gas chromatograph. GC-MS analysis was carried out by a Hitachi Modelel M-80D instrument with a wide-bore CBP-I column.

Results and Discussion

During the period of *ad libitum* feeding, the steers consumed food approximating 2.3% of their initial body weight and gained 1.8 kg a day in body weight. They lost 3.8 kg a day during the period of restricted feeding. There were no significant changes in the blood glucose concentration throughout the experimental periods. The concentrations of the plasma free fatty acids in the steers in the restricted feeding tended to be higher than in *ad libitum* feeding. Figure 1 shows the representative TIM chromatograms of organic acids in serum obtained from the steer in the restricted feeding. The compounds named in the chromatogram were tentatively identified as their trimethylsilyl derivartives by comparison with the authentic mass spectra and retention times in the gas chromatograms. The mass spectra of the peaks No.3 and 4 are shown in figure 2. Although Kuhara(1980) pointed out that the mass spectrum of 2-DTA had no peak at m/z 103, which distinguishes 2-DTA from 3-DTA, it seems to be necessary to compare the mass spectrum of authentic 2-deoxytetronate for identification of the peak No.3. The spectrum of the peak No.4 in this study was superimporsable to that of 2,3-dideoxy pentonate reported by Kuhara (1980).

Changes in the level of organic acids in serum of the steers with the level of feeding were shown in table 1. Relative amounts of lactate and 2-hydroxybutyrate were greater in *ad libitum* feeding than in the restricted feeding in contrast to the results of starved rats described by Kuhara (1980). In *ad libitum* feeding in this experiment, steers might have consumed enough concentrates to elevate the levels of lactate and acetate. While in the restricted feeding, the level of serum palmi-

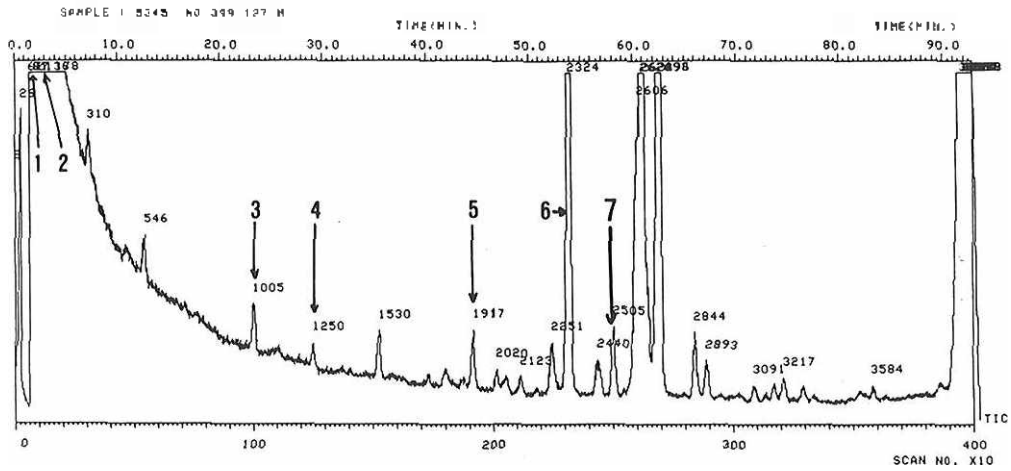

Figure 1. Total ion monitoring Chromatogram of trimethylsilyl derivertives of organic acids in serum from the steer in restricted feeding

1: Lactate
2: 2-hydroxybutyrate
3: 2(or 3), 4 dihydroxybutyrate
4: 2,3-dideoxypentonate
5: Myristate
6: Palmitate
7: n-heptadecanoate(internal standard)

TABLE 1. CHANGES IN THE LEVELS OF SEVERAL ORGANIC ACIDS IN SERUM FROM THE STEERS FED AD LIB. AND IN RESTRICTED FEEDING

Metabolites	Level of feeding		
	Ad lib.	Restricted feeding	Differences
Lactate	13.94±6.51	8.58±3.76	$p < 0.01$
2-hydroxybutyrate	9.27±2.85	7.00±2.19	$p < 0.01$
2(or 3), 4-dihydroxybutyrate*	1.22±0.58	0.96±0.63	N.S.
4,5-dihydroxypentonate	0.49±0.35	0.31±0.13	N.S.
Myristate	0.48±0.32	0.66±0.36	N.S.
Palmitate	5.53±2.06	8.98±3.47	$p < 0.01$

Values are expressed as the peak ratios relative to the internal standard on the chart of gas chromatography ($M \pm SD$; n=24)
*Tentatively identified

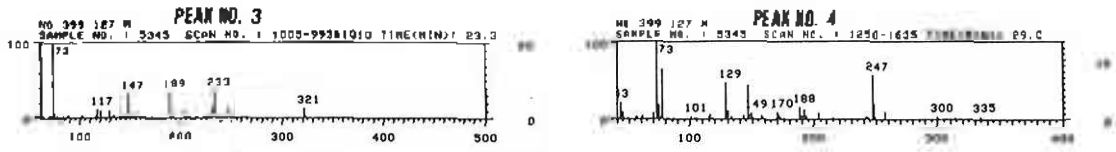

Figure 2. Mass spectrum of peak no.3 and 4 in the TIM chromatogram shown in figure 1.

tate increased in the steers. This seems to be due to the release of fatty acids from their body fat under the condition of energy deficiency. As shown in table 1, we could not get adequate evidence that 2(or 3)-DTA and 2,3-dideoxy pentonate are related to the depression or stimulation of food intake in steers.

On the relation of the control of food intake

and the chemical characteristics of 2-DTA, 3-DPA and their analogues, Oomura (1984) assumed that OH missing from the second carbon suppresses feeding, and OH missing from the third carbon enhances feeding. The present study showed that several polyhydroxy acids known as endogenous sugar acids in rats have the posibility of existing in the blood of ruminants. The role of these organic acids in the regulation of food intake in ruminants remains to be studied.

(Key Words: Serum, Polyhydroxy Acids, Food Intake)

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