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Full paper

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ABSTRACT Four Holstein steers (500-550 kg body weight, 22-27 months old), each prepared with a catheter for sampling from the jugular vein, were used to examine changes in concentrations of plasma insulin and metabolites after the beginning of foraging in a pasture. The results are summarized as follows. (1) Although plasma glucose levels decreased with the beginning of foraging as in previous studies with housed animals. (2) Plasma insulin levels did not show a distinct peak of secretion after the beginning of foraging, however, a suppression of insulin secretion responded to a decrease in plasma glucose levels was observed. (3) The plasma levels of free fatty acids did not increase before foraging, however, the levels tended to increase after the beginning of foraging. Rapid decrease in plasma levels of free fatty acids as seen in housed animals was not observed. (4) Plasma acetic acid levels tended to decrease after the beginning of foraging. Thus, foraging cattle show a tendency to move in the direction of catabolism with the beginning of foraging behavior, leading us to postulate a mechanism of manifestation of feeding behavior that is entirely different from that of monogastric animals.

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Keywords：foraging behavior, grazing cattle, plasma glucose, plasma insulin

Introduction

It is generally known that variations in blood constituents of monogastric animals are closely linked to feeding behavior. Both the central and peripheral nervous systems are thought to monitor these variations, regulating feeding behavior through feedback mechanisms (Aou *et al.* 1989; Nagai 1984; Oomura and Koyama 1988). Changes in levels of blood constituents with the beginning of eating have been reported in ruminants such as housed sheep (Bassett 1974a; Thye *et al.* 1970) and cattle (Bines and Morant 1983; Blom *et al.* 1976; Chase *et al.* 1977a; Hove and Blom 1973; Sato *et al.* 1984; Simkins *et al.* 1965; Vasilatos and Wangsness 1980).

There have been many observations of the foraging behavior of grazing cattle in pastures. Those reports have noted that there were two main foraging times, one was in the morning, when foraging starts in close connection with sunrise; and the other was in the evening, when foraging stops in close connection with sunset (Entsu *et al.* 1978; Hafez and Bouissou 1975). No report, however, described variations in the levels of blood constituents before and after the beginning of eating in a pasture.

In this study, to understand the signal of the beginning of eating, we attempted to clarify variations in blood constituents associated with the beginning of eating in grazing cattle.

Materials and Methods

Blood was collected on 9-10 July and 6-7 August 1991, and on 2-3 July 1992. One hectare, orchardgrass-dominant pasture, was used. The herbage mass on each of the days immediately before testing was 269 gDM/m^2 and 248 gDM/m^2 in the first year, and 242 gDM/m^2 in the second year. A group of six cattle, including the two test animals, was put to pasture from the end of April through the day of testing in each year. The test animals consisted of two 22-month-old Holstein steers each weighing 500 kg and 515 kg as of July and August 1991 and two 27-month-old Holstein steers each weighing 523 kg and 581kg as of July 1992. At 9:00 on the morning of testing, a catheter was inserted into the jugular vein using a mobile stanchion installed beside the pasture. Blood samples were collected from a three-way faucet that was jointed to the catheter by an extension tube.

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Subsequently, a rope of 5 meters long was fastened to the nose ring of each animal. Whenever it was time to collect blood, the rope was stepped on, thereby securing the animal for the procedure.

Blood samples of approximately 10 ml each were collected at half-hour intervals from 11:00 of the first day to 20:00 of the following day. The collected blood was dispensed into blood-collecting tubes containing heparin and EDTA. The tubes are temporarily stored on ice and centrifuged at 3,000 rpm for 10 min. The separated plasma was kept in a freezer at –40°C until analysis.

This study assessed the levels of the following blood constituents: plasma glucose and free fatty acids (on a Hitachi Autoanalyzer 7050, using an HA Test Wako: Wako Pure Chemical Industries, Ltd.), plasma acetic acid (by gas chromatography on a Shimadzu GC14A), and plasma insulin (by the RIA technique). The cattle were kept under constant observation. Their behavior was classified as foraging, rumination, or other, and duly recorded at every minute during the sampling period. For each of the main foraging times in the morning and evening, the value measured immediately upon the beginning of foraging was defined as the 0-min value. The values at 30, 60, and 90 min after the beginning of foraging were compared respectively with the values from 90, 60, and 30 min before foraging (hereafter, the before and after- foraging times are referred to as foraging times –90, –60, –30, 30, 60, and 90). Difference was examined by SCHEFFE's multiple range test using SAS program.

All animals received humane care as *Guide for the Care and Use of Experimental Animals* of the National Institute of Livestock and Grassland Science.

Results and Discussion

Insulin and glucose (Fig. 1)

The mean level of plasma insulin from foraging time –90 to foraging time –30 min remained at approximately 10 μU/ml, however, the level tended to decrease gradually once foraging began, with a mean 90 min value of 7.77 μU/ml.

The mean level of plasma glucose was approximately 80 mg/dl from –90 to –30 min. The level gradually decreased and reached to the lowest value of 73.8 mg/dl at 90 min after the beginning of foraging. Plasma glucose value at 60 min and at 90 min differed significantly from the mean –30 min value ($p < 0.05$).

In regard to variations in the plasma levels of insulin associated with the feeding behavior of housed animals, it was reported that the mean insulin level at 60 min before the beginning of feeding in blood collected at 10 min intervals from dairy cattle fed ad *libitum* was 18.8 μU/ml, reaching a maximum value of 33.0 μU/ml at 10 min after feeding (Vasilatos and Wangsness 1980). It was reported that the plasma level of insulin in castrated cattle before feeding was 37.2 μU/ml. The level was reaching a peak of 65.8 μU/ml at 6 min after the beginning of feeding and, after a transient decrease, increasing again to 69.4 μU/ml at 60 min after the end of feeding in the experiment (Chase LE *et al.* 1977b). In contrast, the results of the present study showed a tendency of insulin levels to decrease gradually after the beginning of foraging, although not significant.

It was reported that dairy cattle show slight however, significant decreases in plasma glucose levels after their morning feeding (Bines *et al.* 1983). In sheep, plasma glucose levels have been reported to decrease transiently by 2 mg/dl with feeding, reaching a maximum at 6 – 8 h after feeding and

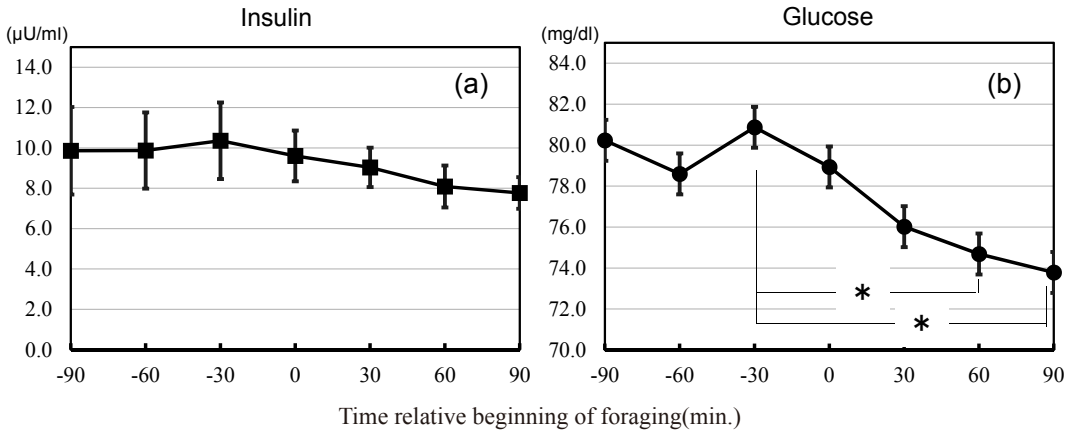

Fig.1. Changes in plasma insulin(a) and glucose(b) concentrations with beginning of foraging in grazing cattle(n = 12).
—■—: Insulin; —●—: Glucose.(＊: P<0.05)

gradually returning to pre-feeding levels (Bassett 1974b). Similar to those finding, the results of this pasture study showed a significant decrease associated with the beginning of foraging, and a marked decrease of 6 mg/dl, compared to previous reports. The concomitant decrease in plasma insulin levels indicates that the decrease in plasma glucose levels is not due to the action of insulin. On the contrary, the decrease in plasma glucose levels is interpreted as having induced a decrease in the secretion of insulin into the blood.

Free fatty acids and acetic acid (Fig. 2)

The mean plasma level of free fatty acids decreased slightly, from 0.144 mEq/l at foraging time –90 to 0.131 mEq/l at foraging time 0, thereafter increasing to 0.160 mEq/l at foraging time 30.

Although the plasma level of acetic acid remained at approximately 0.7 mmol/l from foraging time –90 to 0, the level decreased to approximately 0.5 mmol/l at foraging time 30 and thereafter.

It was reported that the plasma level of free fatty acids in dairy cattle increased up to the time of feeding, decreasing rapidly after the beginning of feeding (Bines *et al.* 1983; Hafez and Bouissou 1975; Simkins *et al.* 1965). A post-feeding decrease has also been reported in female calves (Fox *et al.* 1991). A rapid decrease in levels of free fatty acids associated with feeding was reported in sheep (Bassett 1974a). Although free fatty acids have been considered to be a possible factor as a substance that signals hunger by these previous observations, the present results for the grazing cattle showed a tendency of free fatty acids to increase slightly after the beginning of eating. This appears to rule out the role of plasma levels of free fatty acids as a signal to begin feeding. We speculate that free fatty acids play only a minor role in the transient feeding-regulating mechanism for the start of each daily foraging session.

In dairy cattle, it was reported that plasma levels of acetic acid increase to a maximum at 6 h after the beginning of eating, gradually decreasing thereafter to pre-feeding levels (Simkins 1965). Similar post-feeding increases have been reported (Hove and Blom 1973). It has also been reported that levels of acetic acid in castrated cattle are higher at 30, 60, 90, and 120 min after the beginning of eating than pre-feeding levels (Chase *et al.* 1977b). In contrast, the results of the present study for grazing cattle indicated a decrease after the beginning of eating.

The present observations on variations in the levels of blood constituents in grazing cattle were summarized below.

(1) Although plasma glucose levels decreased with the beginning of eating as in previous studies with housed animals, the decreases in plasma glucose level found in this study tended to be greater.

(2) Plasma insulin levels did not show a distinct peak of secretion after the beginning of eating, however, a suppression of insulin secretion responded to a decrease in plasma glucose levels was observed.

(3) The plasma levels of free fatty acids did not increase before foraging, however, the levels tended to increase after the beginning of eating. Rapid decrease in plasma levels of free fatty acids as seen in housed animals was not observed.

(4) Plasma acetic acid levels tended to decrease after the beginning of eating.

Of these observations, only the variation in plasma glucose levels was similar to the results observed in previous studies with housed animals (Bassett 1974b; Bines *et al.* 1983). The variations in other blood constituents differed from those in

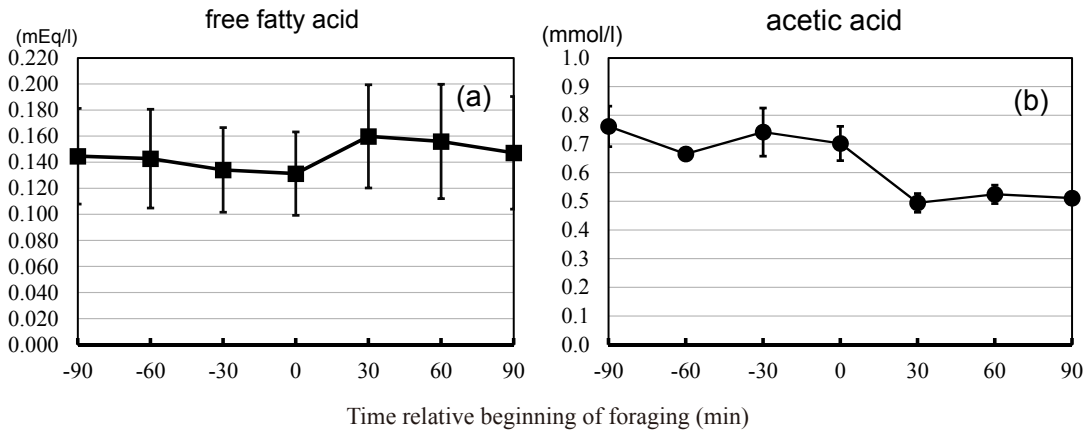

Fig. 2. Changes in plasma free fatty acid (a) and acetic acid (b) concentrations with beginning of foraging in grazing cattle (n = 12).
—■—: Free fatty acid; —●—: Acetic acid

housed animals. It is reasonable to postulate that the amount of energy expended by grazing cattle during foraging greatly exceeds the amount of energy that is concurrently produced by both the gluconeogenesis and the digestion and absorption of the food ingested. This assertion is supported by three observations. First, free fatty acids are increased by the mobilization of fat from body fat in the event of low energy; second, the extent of the decrease in the plasma glucose level after the beginning of eating was greater than in reported studies with housed animals; and third, acetic acid as well as glucose in domestic ruminants is used directly as an energy source.

In monogastric animals, including humans, blood levels of glucose and other nutrients that have been digested and absorbed increase concurrently with the beginning of eating, and the secretion of hormones such as insulin is regulated in response, with the individual's physiology entering an anabolic phase through homeostatic mechanisms. In contrast, foraging cattle show a tendency to move in the direction of catabolism with the beginning of foraging behavior, leading us to postulate a mechanism of manifestation of foraging behavior that is entirely different from that of monogastric animals.

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要　約

放牧牛の採食に伴う血漿インスリン及び代謝関連物質濃度変化

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血液サンプリングのために頸静脈カテーテルを装着した 4 頭のホルスタイン去勢雄牛（体重 500 – 550 kg，22 – 27 ヵ月齢）を用いて，放牧採食行動の開始前後における血漿インシュリンと代謝関連物質濃度の変化を調査した．得られた結果を以下に示す．(1) 血漿グルコース濃度は従来の舎飼いでの結果と同様に採食開始に伴い減少した．(2) 採食開始後のインシュリン分泌ピークは観察されず，血漿グルコース濃度に伴う低い血漿インスリン濃度が観察された．(3) 血漿遊離脂肪酸は採食開始前の上昇は見られなかった．また採食開始に伴って舎飼い時には見られる血漿遊離脂肪酸濃度の急速な減少は，観察されなかった．(4) 血漿酢酸濃度は，採食開始後に減少する傾向が見られた．以上より，単胃動物とは異なり，放牧牛では採食開始とともに代謝が異化の方向に動く傾向を示し，そのことは単胃動物とまったく異なる採食調節機序を示唆する．

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キーワード：血漿インスリン，血漿グルコース，採食行動，放牧牛