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Composition of rumen volatile fatty acids and blood components at pre- and post- partum in healthy, ketonuric and ketotic dairy cows

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健康, 케톤尿症 및 케톤症 乳牛에 있어서 出産前後時 第1胃內 揮發性脂肪酸과 血液像의 變化 比較觀察

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초록 : Holstein種 乳牛에 있어서 第1胃內의 揮發性脂肪酸(VFA)과 血脂質의 濃度가 食餌造成, 繁殖狀態 그리고 케톤尿症과 케논症에 미치는 효과를 알아보고저 試圖하여, 出産 13~15日前과 出産後 15日에 6頭의 健康한 乳牛(第I群), 9頭의 케톤尿症 乳牛(第II群) 그리고 8頭의 케톤症 乳牛(III群)들을 中心으로 檢事하여 보고 그 結果를 다음과 같이 摘要한다.

可消化營養總量(TDN)과 可消化蛋白質(DCP)은 産後에 增加趨勢를 보이고, 各群의 揮發性脂肪酸濃度는 産前에 比하여 産後에 낮았는데, 그 中에서도 第I群(健康)의 경우에는 産前과 後에 第II群(케톤尿症)에 比하여 多少 높았다. 第I群에 있어서 産後에 醋酸當量比率은 減少 傾向을 보였으나, 第II群과 第III群에서는 繼續 增加 되었다. 第I群에서의 産後 프로피온酸當比率은 增加되는 反面에 第I群과 第III群에서는 繼續 減少 傾向을 보였다. 血中 콜레스테롤, 遊離콜레스테롤 및 콜레스테롤-에스텔, HDL-cholesterol, 燐脂質 및 遊離脂肪酸 등의 濃度는全試驗群에서 産後에 減少 傾向을 보였으며, 그 中에서도 第I群은 第III群에 比하여 보다 낮았으며, 그 中間은 第II群 이었다.

中性脂肪은 産後에 減少 傾向을 보였으며, 特히 케톤症의 第III群은 顯著한 減少가 觀察되었다.

Key words : VFA & blood lipid, healthy, ketonuric & ketotic, pre- & post-partum in Holstein cows

Introduction

Ketosis is a common metabolic disease in dairy cows that frequently occurs at post-partum(POP). In cows diagnosed with ketosis, treatment may take several weeks[5,6,16]. As a result, milking volume is considerably decreased, inflicting serious losses on dairy farms. In the clinical field, the main interests are in the mechanism of onset of the disease and in prevention. The mechanism of onset in particular is not well un-

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derstood. Energy sources in dairy cows, unlike those in monogastric animals, are volatile fatty acids(VFA), ie, products of terminal fermentation of cellulose digested by bacteria and protozoa in the rumen, particularly propionic acid, acetic acid, and butyric acid. These VFA are absorbed from the rumen wall and transferred to the liver[10,11,13], where propionic acid is converted to glucose, and acetic acid and butyric acid are utilized as energy substrates. In dairy cows, however, ingested energy exceeds expended energy during the POP increase in milking volume, which frequently leads to an imbalance between energy supply and demand. As a result, since somatic fat is mobilized as an energy source, resulting in the development of ketosis. In this study, the composition of VFA in the rumen and blood lipid concentrations at day 13-15 pre-partum (PRP) and day 15 POP were determined in healthy, ketonuric, and ketotic dairy cows.

Meterials and Methods

Dairy cows : The experiment included 22 Holstein dairy cows that had been reared in dairy farms in the eastern region of Saitama Prefecture. They had a history of 3-5 parturitions. Body weight ranged from 600 to 710kg, with a mean of 660kg. Of these cows, 5 that showed a clinically healthy course at POP were assigned to Group I, 9 that had ketonuria were assigned to Group II, and 8 with ketosis were assigned to Group III(Table 1).

Composition of diet : For each dairy cow examined, the supplied diet was weighed at days 13-15 PRP and at day 15 POP, and the ratio of condensed diet, and the fulfillment rates of total digestive nutrient(TDN) and digestive coarse protein(DCP) were calculated based on the NRC diet standard.

Sampling : Rumen fluid and blood samples were collected 4-5 hr after diet supply at days 13-15 PRP and at day 15 POP in all the cows. Rumen fluid samples were collected with a gastric fluid collector(Chiba Kyosai; Fujihira), and filtered with double gauze after collection. The filtrate was centrifuged at 3000rpm for 15min, and 0.6ml of the supernatant and 0.3ml of 3N sulfuric acid containing 25% metaphosphoric acid were thoroughly mixed and left to stand at room temperature overnight. Subsequently, the mixed specimen was centrifuged at 3000rpm for 15min, and 180μl of the supernatant and 20μl of 0.1N crotonic acid were mixed. An equal amount of acetone was added to the specimen, and the mixture was then used for VFA determination[9].

Blood samples were collected from the cervical vein of the dairy cow at the time of gastric fluid collection, and the sera obtained were used for the determination of blood lipid concentrations.

Observations : VFA were determined based on an internal standard material by gas chromatography using a capillary column(GC-15A, Shimazu)[8]. The conditions for determination were as follows: 60-180℃ rise in column temperature, 6℃/min rise in temperature, 60ml/min of carrier gas(He), 50ml/min hydrogen flow volume, and 500ml/min air flow volume. The internal standard material was 0.1N crotonic acid. The items determined were VFA concentration, acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valeric acid, n-valeric acid and capronic acid.

Blood lipid determinations consisted of total cholesterol(T-CHO), free-cholesterol(F-CHO), esterified cholesterol(E-CHO), HDL-cholesterol(HDL-CHO),

Table 1. Clinical symptoms at day 15 post partum in dairy cows

Group	No of cows	Symptoms	Ketone bodies in urine (mg/dl)	Glucose in blood plasma (mg/dl)
I (Healthy cows)	5	-	-	<40
II (Ketonuric cows)	9	-	<10	>40
III (Ketotic cows)	8	depression, loss of appetite, lower milk production	<10	>40

phospholipids(PL), non-esterified free fatty acids(NEFA) and triglyceride(TG). Each was determined with an autoanalyzer(Model 712, Hitachi)[1,2,4,7,15].

Results

Composition of the diet : Group I was not given any concentrated feed at PRP, or even at POP. The supplied diet was mainly roughage. In Group II, the ratio of concentrated feed was 25% at PRP, but the ratio was higher by about 2.7 times at POP. In Group III, the ratio of concentrated feed was similar to that in Group II, and the ratio at POP was about 1.5 times higher than that at PRP.

The fulfillment rates of TDN and DCP were markedly higher after parturition, at POP than those before parturition, at PRP in Group I. The fulfillment rate of TDN was lower in Group II than in Group I at both before and after parturition. Group III showed changes in the fulfillment rates of TDN and DCP that were similar to those in Group I(Fig 1). Total VFA levels were lower at POP than at PRP in the three groups. In Group I, however, the concentrations were higher than in Group II and III at both before and after parturition. The mole fraction of acetic acid was higher at POP in Group II and III, whereas the mole fraction was lower at POP in Group I. Propionic acid concentrations were lower at PRP in Groups II and III, whereas they increased at POP in Group I. The iso-butyric acid concentrations in Group I were lower than those in Groups II and III at both before and after parturiton. The iso- and n-butyric acid concentrations at PRP were higher in Group III than those in Groups I and II. The iso-valeric acid and caproic acid concentrations were lower in Group I than in Groups II and III at both before and after parturition(Fig 2).

Blood lipid : T-CHO, F-CHO, E-CHO, HDL-CHO and PL concentrations were higher at POP than at PRP in these three groups, but the levels in Group I were lower than those in Group II and III. NEFA were distinctly lower in Group I than in Group II and III. On the other hand, TG was lower at POP at PRP in the three groups, but the level at PRP was higher in Group III than Groups I and II(Fig 3).

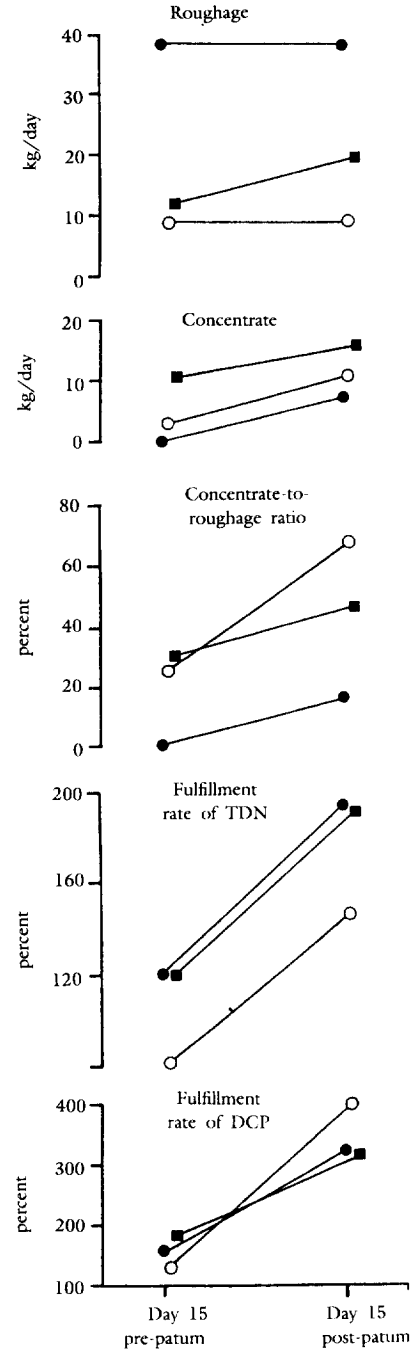

Fig 1. Composition of diet supplied day 15 pre- and post-partum in dairy cows.
●—●: Group I(healthy cows) ○—○: Group II(ketonuric cows) ■—■: Group III(ketotic cows)

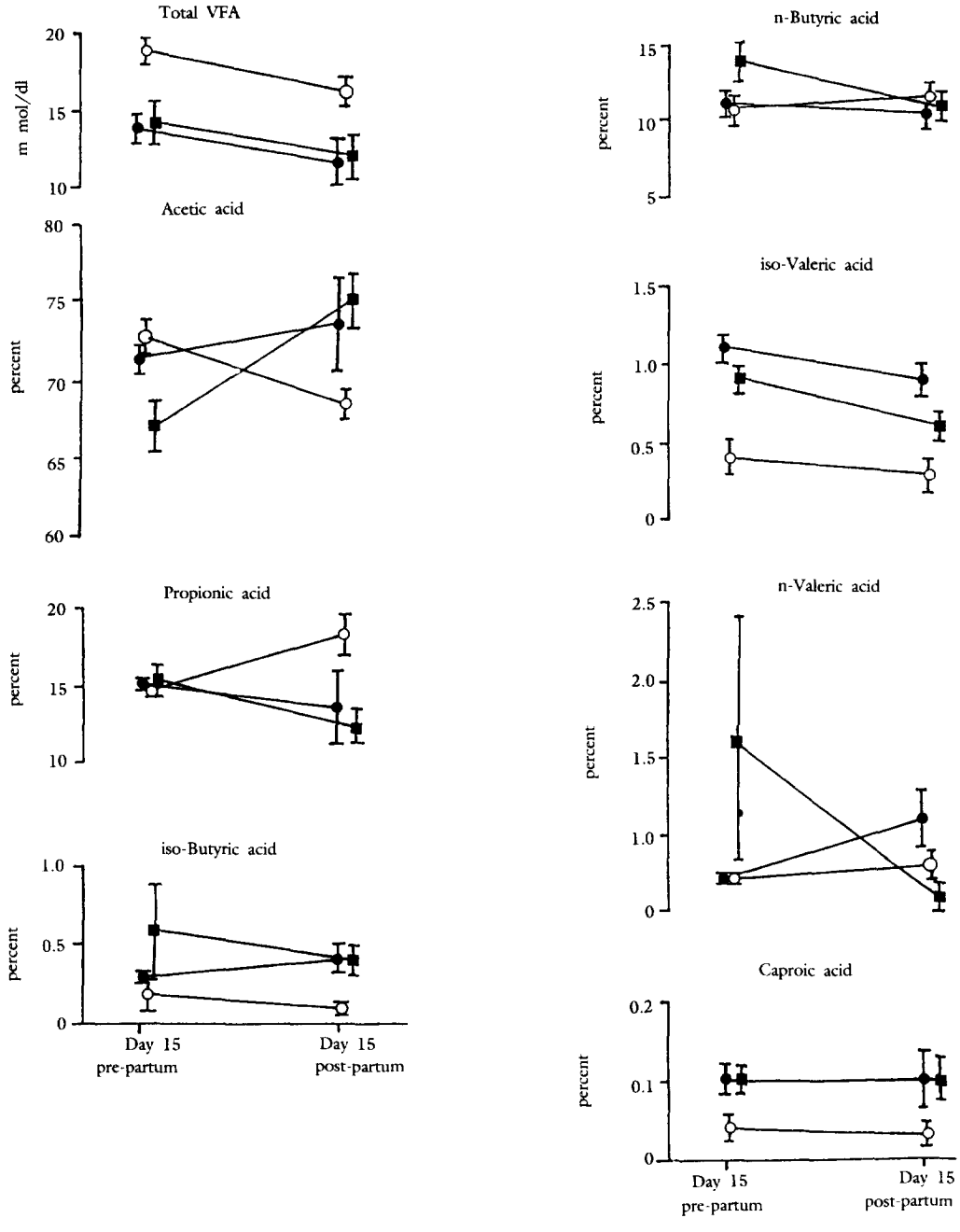

Fig 2. Composition of VFA at day 15 pre- and post-partum in dairy cows.

●—●: Group I(healthy cows) ○—○: Group II(ketonuric cows) ■—■: Group III(ketotic cows)

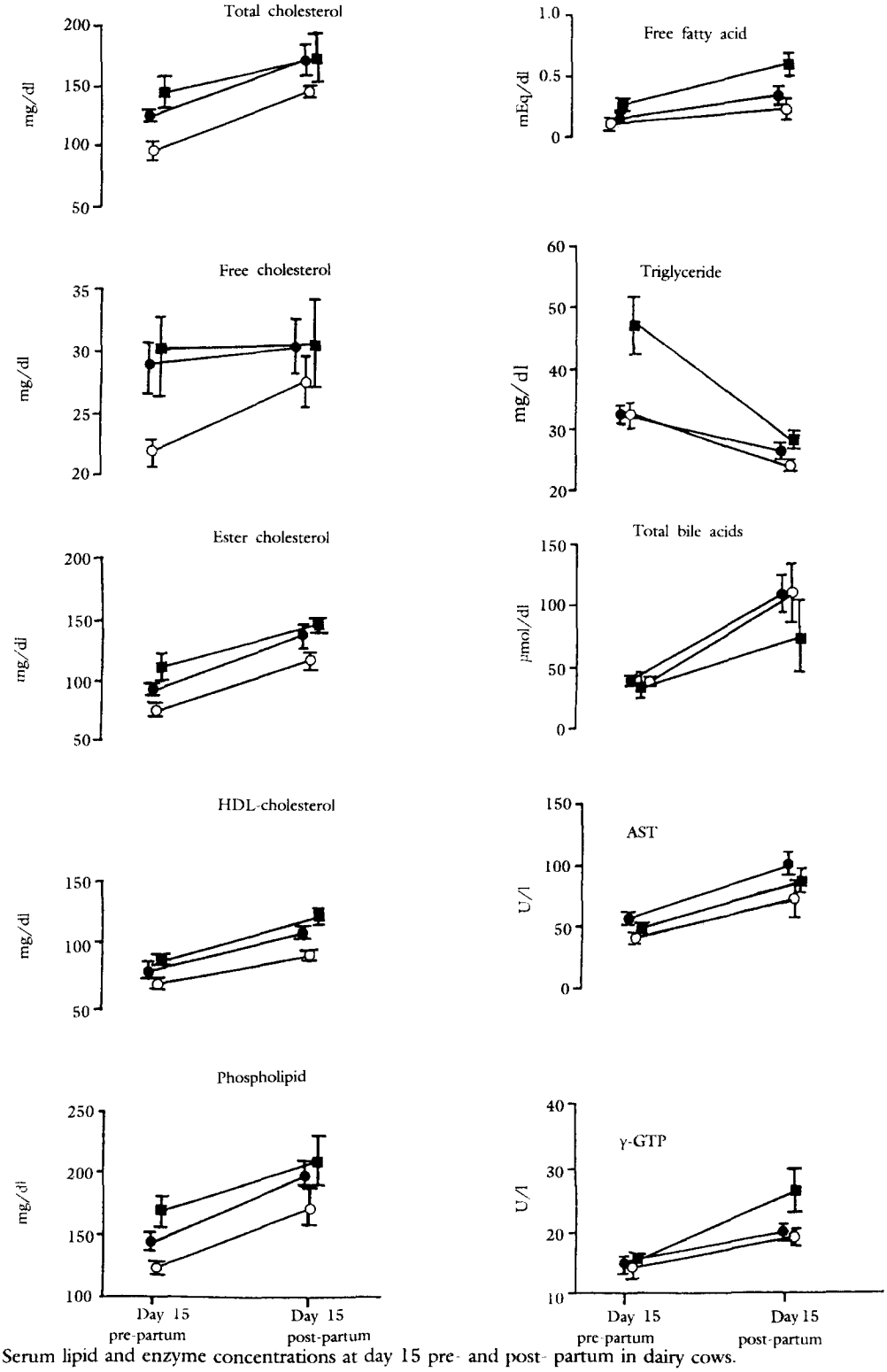

Fig 3. Serum lipid and enzyme concentrations at day 15 pre- and post- partum in dairy cows.

●—●: Group I(healthy cows) ○—○: Group II(ketonuric cows) ■—■: Group III(ketotic cows)

Discussion

In dairy cows, when energy expenditures exceed the energy supply along with the early stage of rapid lactation, gluconeogenesis occurs through the mobilization of sommatic fat. As a result, ketone bodies are produced at abnormally high levels, leading to the onset of ketosis. Clinical experience indicates that ketosis arising from an imbalance between energy intake and energy expenditures develops at days 15-20 POP, when lactation volume increases rapidly. On the other hand, energy is derived in dairy cows by utilizing cellulose fermented by bacteria and protozoa in the rumen and VFA produced from non-cellulose carbohydrates in the concentrated feed, a process unlike that in monogastric animals. In this way, disruption of the VFA production process is also connected with the onset of ketosis[12,14].

Total VFA concentrations were high at PRP and POP in Group I, in comparison with Group II and III. Acetic acid concentration decreased and propionic acid concentration increased at POP in Group I. Propionic acid is converted in the liver to an intermediate of the TCA cycle, succinyl-CoA, which is utilized as an energy source or for glucose synthesis. Succinyl-CoA also metabolizes aceto-acetic acid[3]. Therefore, in Group I, the propionic acid concentration seems to have increased, and the cows in this group were considered to have been in a state in which ketosis was unable to develop. In contrast, total VFA concentrations at PRP and POP were lower and the propionic acid concentration at POP was lower in Group III than in Group I. These results suggest that the amount of succinyl-CoA produced would have decreased, leading to an accumulation of aceto-acetic acid, and thus to an onset of ketosis in Group III.

Even Group I, consisting of clinically healthy dairy cows, showed a VFA composition in the rumen and changes in blood lipid concentration that were similar to those observed in Group III. This suggestes that all dairy cows are at risk of ketosis after partum. In Group I, no condensed diet was given at PRP, and the amount of diet supplied was small, even at POP. This provides further evidence that the supply of large amount of condensed diet provides a trigger for the onset of ketosis. To prevent ketosis, it is thus important to supply a diet that is properly matched to the cow's ability to lactate.

Summary

The effects of diet composition, reproductive condition and ketonuria and ketosis on the composition of rumen volatil fatty acids(VFA) and the blood lipid concentration were investigated in Holstein dairy cows. Six healthy cows(Group I), 9 ketonuric cows (Group II) and 8 ketotic cows(Group III) were examined at days 13-15 pre-partum(PRP) and at day 15 post-partum(POP). The ratios of concentrated feed supplied at PRP and POP were 0% and 15%, respectively, in Group I, 25% and 67% in Group II, and were 30% and 46% in Group III. The fulfillment rates of total digestive nutrient(TDN) and digestive coarse protein(DCP) increased at POP in each group. Although total VFA concentrations were lower at POP than at PRP in each group, the concentrations at PRP and POP were higher in Group I than in Groups II and III. The molar ratio of acetic acid decreased at POP in Group I, while the ratio increased at POP in Groups II and III. The molar ratio of propionic acid increased at POP in Group I, while it decreased in Groups II and III. Blood total cholesterol, free cholesterol, cholesterol esters, HDL-cholesterol, phospholipid and free fatty acid concentrations decreased at POP in all three groups, but these levels were low in Group I, high in Group III and intermediate in Group II at PRP and POP. Triglyceride concentration decreased after parturition in these three groups, but the decrease was most striking conspicuous in Group III.

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