学位論文の要旨

Abstract of Thesis

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学位論文題目 Title of Thesis（学位論文題目が英語の場合は和訳を付記）

Genetic polymorphism of β-casein variants in Jersey cows and nutritional assessment of A1 and A2 caseins in mice

（ジャージー牛における β-カゼイン変異体の遺伝子多型とマウスにおける A1 および A2 カゼインの栄養学的評価）

学位論文の要旨 Abstract of Thesis

Following reports that β-casein A1 variant consumption is related to gastrointestinal disorders, type 1 diabetes, and coronary heart disease, A2 milk, which does not contain the β-casein A1 variant, has started to be produced and marketed. The benefits of A2 milk on health are controversial, but the global A2 milk market is expected to grow to 2.5 trillion yen by 2027. Several manufacturers have started selling A2 milk made from Holsteins milk in Japan.

A2 milk can be produced if cows with the *CSN2* gene A2A2 homozygous are gathered, and their milk is separately processed. Holsteins are many with A1A1 and A1A2 genotypes; hence, it is not easy to gather A2A2 genotype cows. On the other hand, Jerseys reportedly have a high percentage of A2A2 genotype. The Hiruzen area of Okayama Prefecture is known as a center of Jersey farming, but data for *CSN2* gene polymorphisms are lacking.

In the first experiment of this thesis, the frequency of *CSN2* gene polymorphisms was evaluated for the Jersey herds in the Hiruzen area, western Japan. A total of 590 cows were diagnosed and their genotype and allele frequencies were clarified. Raw milk was obtained from A1A1 and A2A2 genotype cows, and casein was fractionated by electrophoretic coagulation. In the second experiment, lyophilized A1 and A2 caseins were offered to mice and the effects on gut microbiota and fermentation were evaluated. Standard casein (a mixture of A1 and A2 casein), soy protein, and egg albumin were also fed to mice for comparison.

Experiment 1. Frequency evaluation of *CSN2* gene polymorphisms for Jersey cows

Blood samples of Jersey cows were collected from eight farms during October and November 2019 and 2020. Sampling was made at the same time that the periodic diagnostic tests for Johne's disease were performed. The plasma was used to determine metabolites concentration and buffy coat was processed to purify genomic DNA. The exon 7 regions of the *CSN2* gene were amplified and their DNA base sequences were determined. Polymorphisms at positions 67, 72, 88, 93, 106, 122, and 138 of exon 7 were analyzed to detect β-casein variants. The composition of bulk milk was monitored three times per month using a CombiFoss FT+ analyzer.

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Four β-casein variants (A1, A2, B, and I) in 12 variants at all eight farms. Variants A3, F, G, H1, and H2 were not found in the Jersey herds. The frequencies of homozygous (A1A1, BB, A2A2, and II) and heterozygous (A1A2, A1B, A1I, A2B, A2I, and BI) alleles were different between farms. One farm (F5) had all ten genotypes; three farms (F3, 4, and 8) lacked A1A1, A1B, BB, and A2I genotypes; and six farms (F1, 2, 3, 4, 6, and 7) lacked the II genotype. The A2 allele was the most frequently found across the eight farms, but the second most frequent allele was B or I, depending on the farm. The average frequencies of A1, A2, B, and I alleles were 0.059, 0.746, 0.125, and 0.070, respectively.

Considering that the B, C, F, and G variants are the A1 group and the A3, D, E, H1, H2, and I variants are the A2 group, the data obtained in this study were compared with other published data. The frequency of the A2 allele (0.816) was numerically higher than those reported for Holsteins (0.508–0.744), crossbreeds (0.568–0.606), Mexican Jerseys (0.738), and Danish Jerseys (0.700).

The A1 allele was rarely observed in Jersey herds examined in this study. Even if A1A1, A1A2, and A2A2 group-based genotypes were applied, there were no A1A1 cows at the three farms (F3, 4, and 8). The maximum number of A1A1 cows at one farm was only six (F2 and 6); hence, blood biochemical analyses were restricted to Jersey cows at the two farms. The BUN and P concentrations were higher for the cows on one farm (F6), and the Ca concentration was greater for the cows on another farm (F2). Meanwhile, no differences were observed in albumin, BUN, cholesterol, NEFA, AST, ALT, Ca, and P concentrations; hence, the β-casein genotypes did not affect the metabolism of the major nutrients.

Experiment 2. Nutritional assessment of A1 casein and A2 casein using mice

β-casein is characterized by a large number (35 out of 209 amino acids in the A2 variant) of proline, a cyclic amino acid that complicates the formation of protein secondary structure. Because proline is substituted by histidine in the A1 variant, β-casein may be cleaved differently during digestion between the A1 and A2 variants. This difference in protein hydrolysis would generate peptides flowing into the large intestine, which may differentiate the composition and activity of gut microbiota. Meanwhile, most published studies evaluated the effects of β-casein A1 and A2 variants by administering β-casomorphin 7 or providing A1 and A2 milk. Consumers are advised to take a variety of foods; hence, comparing A1 and A2 milk may give us quite narrow-sighted outcomes. In this study, casein was prepared from A1 and A2 milk, and the effect on gut microbiota and its metabolites was compared to standard casein (A1 and A2 milk), soy protein, and egg albumin.

Raw milk was collected from Jersey cows diagnosed as A1A1 and A2A2 β-casein genotypes. Caseins were fractionated by isoelectric coagulation, lyophilized, defatted, and then formulated in a diet fed to mice. The experiment was performed using female C57BL/6 mice with five diets differing in protein source. In addition to A1 casein and A2 casein, commercial casein (a mixture of A1 and A2 variants), soy protein isolate, and egg albumin were compared. Further, mice were administered with and without a laxative agent [0.2 mL of $MgSO_4$ (100 mg/mL) solution] once a week. After four weeks of diet feeding, the mice were sacrificed and cecum contents were collected to analyze short-chain fatty acid (SCFA) levels and to extract bacterial DNA, which was subsequently used for 16S rRNA genes amplicon sequencing targeting the V4 region. Mice were sacrificed two days after the last laxative administration.

Regardless of the proteins offered, the mice were in good health and showed no difference in body weight gain. Administration of $MgSO_4$ induced excretion of soft stools, but the shape of feces quickly returned to normal after one day. No differences were seen in the cecum tissue weight between dietary treatments, but the cecum content weight increased in mice fed egg albumin. Differences between A1 casein, A2 casein, and commercial casein were marginal. There were no effects of $MgSO_4$ administration on either body weight, cecum tissue weight, or cecum content weight.

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Cecum SCFA concentrations were affected by dietary protein sources, except for *iso*-valeric acid concentration. Mice fed soy protein and egg albumin had lower SCFA concentrations, with a greater decline observed with egg albumin. Differences between A1 casein, A2 casein, and commercial casein were small, but acetic acid concentrations were lower in mice fed A2 casein than A1 casein. The $MgSO_4$ administration decreased *n*-butyric acid, *iso*-valeric acid, and *n*-valeric acid concentrations in the cecum.

Chao 1 and Shannon indices of the cecum microbiota were higher in mice fed soy protein than egg albumin. Mice fed A1 casein, A2 casein, and commercial casein were in the middle and no differences were observed between the three types of casein feeding. When cecum microbiota was compared at the family level, the effects of dietary protein sources were more apparent. However, the difference between A1 casein and A2 casein was found only in the abundance of Desulfovibrionaceae, which was higher in mice fed A1 casein than A2 casein. Changes because of soy protein and egg albumin feeding were much more obvious; the abundances of Ruminococcaceae and Eggerthellaceae were enhanced by soy protein and egg albumin feeding, respectively, and the abundance of Erysipelotrichaceae was decreased by soy protein feeding. Although not significant, the abundance of Akkermansiaceae was higher in mice fed soy protein and egg albumin than in the three casein types. Based on the principal coordinate analysis, the cecum microbiota of mice fed casein was characterized by Aerococcaceae, Bifidobacteriaceae, and Clostridiaceae, that of mice fed soy protein by Staphylococcaceae, Corynebacteriaceae, Ruminococcaceae, and Muribibacteriaceae, and that of mice fed egg albumin by Akkermansiaceae, Bacteroidaceae, Rikenellaceae, and Eggerthellaceae. It is concluded that, although the difference between A1 casein and A2 casein may exist, the effects of dietary protein sources on the activity and composition of cecum microbiota should be marginal compared with the effects of soy protein and egg albumin.

This is the first to clarify the *CSN2* gene polymorphisms of the Jersey herds in Japan and the findings obtained can be helpful additions to the debate if farmers, manufacturers, and consumers benefit from promoting A2 milk production.