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授与した学位	博　士
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学位授与の要件	環境生命科学研究科　　農生命科学専攻 （学位規則第４条第１項該当）
学位論文の題目	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）
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学位論文内容の要旨

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 triple in five years. This thesis evaluated the frequency of *CSN2* gene polymorphisms for the Jersey herds in the Hiruzen area, western Japan. In addition, a nutritional assessment of A1 and A2 caseins was performed using mice with a comparison of commercial casein, soy protein, and egg albumin.

In experiment 1, blood samples of Jersey cows were collected from eight farms during October and November 2019 and 2020. The exon 7 regions of the *CSN2* gene were determined and polymorphisms at positions 67, 72, 88, 93, 106, 122, and 138 were analyzed. The composition of bulk milk was monitored three times per month. 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 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). Blood biochemical analyses were made for two farms. The BUN and P concentrations were higher for the cows on one farm, and the Ca concentration was greater for the cows on another farm. 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.

In experiment 2, 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. In addition, commercial casein (a mixture of A1 and A2 variants), soy protein, and egg albumin were fed to mice for comparison. Regardless of the proteins offered, the mice were in good health and showed no difference in body weight gain. No differences were seen in the cecum tissue weight between dietary treatments, but the cecum content weight increased in mice fed egg albumin. Mice fed soy protein and egg albumin had lower short-chain fatty acid concentrations, with a greater decline observed with egg albumin. Acetic acid concentrations were lower in mice fed A2 casein than A1 casein. 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. 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. 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.

論文審査結果の要旨

本研究は，岡山県蒜山地域のジャージー牛について，*CSN2*遺伝子多型の頻度を評価するとともに，A1およびA2カゼインの栄養評価をマウスで行ったものである。A1タイプのβ-カゼイン摂取と胃腸障害，1型糖尿病，冠状動脈性疾患等が関連するという報告を受けて，これを含まないA2牛乳が生産・販売され始めている。

実験1では，8軒の牧場から得たジャージー牛計590頭の血液サンプルを用いて，*CSN2*遺伝子の多型解析を行った。4種（A1，A2，B，I）の変異体が確認され，対立遺伝子の平均頻度はそれぞれA1（0.059），A2（0.746），B（0.125），I（0.070）であった。B変異体をA1グループ，I変異体をA2グループと考えて他データと比較したところ，A2アレルの頻度（0.816）は，ホルスタイン（0.508-0.744），交雑種（0.568-0.606），他国のジャージー（0.700-0.738）より数値的に高かった。また，血液生化学検査の結果から，β-カゼインのA1A2遺伝子変異は主要栄養素の代謝に影響しないことが確認された。　実験 2 では，A1およびA2カゼインをマウスに給与して，腸内細菌叢を指標とした栄養評価を行った。β-カゼインの遺伝子型がA1A1およびA2A2と診断されたジャージー牛から生乳を採取してカゼインを分画・調製し，これらの栄養機能を市販のカゼイン（A1およびA2変異体の混合物），大豆タンパク質，卵アルブミンと比較した。盲腸内容物の酢酸濃度はA1カゼインよりもA2カゼインを与えたマウスの方が低かったが，盲腸細菌叢の違いはDesulfovibrionaceaeの割合にのみ見られた。大豆タンパク質と卵アルブミンによる変化はより顕著で，Chao 1およびShannon指数は卵アルブミン給与で低下し，RuminococcaceaeとEggerthellaceaeの割合はそれぞれ大豆タンパク質と卵アルブミンによって増加した。A1カゼインとA2カゼインの違いは，大豆タンパクや卵アルブミンに比べれば小さかった。

本研究は，アジアにおけるジャージー牛群の*CSN2*遺伝子多型を初めて調べたものであり，A2型カゼインの機能性に関する知見は，生産者，関連産業，消費者に有益な情報となる。よって，Nuomin氏は環境生命科学研究科の博士（農学）の学位を受ける資格があるものと判断した。