学位論文内容の要旨

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学位論文題名

Evaluation of ginkgo fruit as a new feed additive candidate for ruminant animals

（反芻家畜における新規飼料添加物候補 ギンナン果肉の評価）

Ginkgo (*Ginkgo biloba*) is a so-called living fossil classified as a deciduous tree. In Asian countries such as China, Japan, and Korea, ginkgo nuts are consumed as food and medicine. Ginkgo fruit is a byproduct in ginkgo nut industry but is not utilized due to its peculiar smell and the presence of nutritionally negative compounds. In Japan, ginkgo fruit is obtained from 6 cultivars, which may differ in chemical and functional properties. Recently, interest has been growing in functional phenolic compounds in ginkgo. Of these, alkylphenols represented by anacardic acid found in cashew plant are suggested to favorably change fermentation pattern (less methane and more propionate production) when supplemented to rumen fluid of cattle and sheep. The aim of this study was to evaluate the potential of gingko fruit as a new feed additive candidate to manipulate rumen fermentation, and to propose application strategy of ginkgo fruit in ruminant feeding.

1. Batch culture evaluation

In batch culture, a mixture of hay and concentrate was incubated in diluted rumen fluid with or without 1.6% (fruit equivalent) ginkgo fruit extract from two major cultivar Kyuju (K) and Tokuro (T). Extract was obtained by soaking fruit in ethanol and by concentrating supernatant with centrifugal evaporator. Another series of batch culture studies was conducted to determine the dose-response of fermentation. Although both K and T extracts led to decreased methane and increased propionate production, changes were more apparent in K and were dose dependent. Total gas production was depressed at doses ≥3.2%, suggesting that 1.6% was the optimal supplementation level.

2. Continuous culture evaluation

In continuous culture supplemented with 1.6% ginkgo K, methane decreased by 53% without affecting total gas or total VFA production but with decreased acetate and increased propionate. Disappearance of dry matter, NDF and ADF was not affected by

ginkgo, while the ammonia level was decreased. Quantitative PCR indicated that the abundance of protozoa, fungi, methanogens and bacteria related to hydrogen and formate production decreased, while abundance of bacteria related to propionate production increased. MiSeq analysis confirmed these bacterial changes and also identified archaeal community changes including a decrease of *Methanobrevibacter* and *Methanomassiliicoccaceae* and an increase of *Methanoplanus*.

3. Pure culture evaluation

Pure culture study using 17 representative rumen bacterial species was conducted to know sensitivity to ginkgo fruit extract and component phenolics. Bacterial responses were clearly divided into sensitive and insensitive groups, essentially based on the absence and presence of outer membrane. Anacardic acid was found to be a main compound responsible for surfactant action to inhibit specific rumen bacteria. The results support bacterial community changes observed in continuous culture study, demonstrating that ginkgo fruit can modulate rumen fermentation via microbial selection.

4. Evaluation under different dietary conditions

The above potential of ginkgo fruit extract was evaluated by another batch culture with feeds in different forage to concentrate ratios (1:9, 3:7, 5:5, 7:3 and 9:1). Methane production was decreased by ginkgo extract, with the greatest reduction found in 5:5. Total VFA and ammonia levels were not affected by ginkgo fruit extract in any of 5 different diets. However, ginkgo fruit extract increased propionate proportion in all dietary conditions, which was supported by increased abundance of propionate-producing bacteria such as *Selenomonas ruminantium* and *Megasphaera elsdenii*. The results suggest that rumen modulation by ginkgo fruit extract can be achieved at a wide range of diet with no adverse impact on feed digestion.

5. Application as ensiled material

As original ginkgo fruit rather than its ethanol extract is preferable for easy use as feed additive, application of ginkgo fruit as ensiled material was attempted. Grass silage was prepared with supplementation of ginkgo fruit at 0 - 6.4 % and evaluated its quality by chemical and microbial analysis. Ginkgo fruit supplementation increased lactate concentration, while decreased pH value of silage, clearly indicating that a higher quality of silage was obtained by ginkgo fruit supplementation. These were caused by higher abundance of lactic acid bacteria in ginkgo fruit-supplemented silage. Anacardic acid were stably present in silage after 33d ensiling period. This feature is surely advantageous for keeping functionality of ginkgo fruit in ruminant feed.