論文題目　　Studies on the Utilization of Phytol in Forages for Ruminant Production
（粗飼料中フィトールの反芻家畜生産への利用に関する研究）

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1. General Introduction

Ruminants have obvious characters to produce milk and meat through utilizing forages. Therefore, it is necessary to explore the potential value of forages and to maximize their utilization of forages. Recently, consumers have shown increased concern for livestock products enriched with bioactive compounds that impact on human health. The content of such functional compounds in ruminant products is considered to relate to their content in diets consumed by animals. Abundant chlorophyll and carotenoids in green forage are considered to be related to the function of ruminant products for human health. The ruminal degradation of chlorophyll in ingested forages liberates phytol moiety which is microbially metabolized to phytanic acid, a natural ligand of peroxisome proliferator-activated receptors. This phytanic acid particularly appears in meat and milk produced by ruminants, and presumably has positive effects on human health.

The study aimed to investigate the factors affecting phytol contents in Italian ryegrass (IR) herbages, and extent of phytanic acid production in ruminants.

2. Changes of photosynthetic pigments and phytol in herbages

Changes of photosynthetic pigments and phytol content of herbage were investigated through 4 experiments. In Experiment 1, three rates of nitrogen (N) fertilization levels (0 kg N/ha; 60 kg N/ha; and 120 kg N/ha) were applied for IR, and the contents of chemical components and photosynthetic pigments (β-carotene, lutein and chlorophylls) in fresh herbage and hay prepared by natural conditions were measured. The crude protein (CP), ether extract (EE), photosynthetic pigments and phytol in IR (fresh herbage and hay) linearly increased with increasing N fertilization levels, and decreased obviously for hay preperation (chlorophylls: 40-70%, phytol: 25-47%, β-carotene: 72-90%).

In Experiment 2, the time course changes of carotenoid, chlorophyll and phytol during ensiling were determined for IR herbage. The IR harvested at the heading stage (May 2014) were allowed to wilt under natural conditions for 1 day, and then ensiled using a small-scale pouch system (12 bags). Three bags were destructively analyzed each week to determine the contents of photosynthetic pigments and phytols (esterified and free forms) over a 5-week period (every three bags were unsealed at the 1st, 2nd, 3rd and 5th

week, respectively). β-carotene content decreased at 2 week after ensiling, while the lutein content did not change significantly. Although the chlorophyll content decreased rapidly in the first week of ensiling, the total phytol content barely changed over the five weeks. During ensiling, the chlorophyll decomposed to pheophytine, then, further degraded to pheophorbide and phytol. Re-esterification of the released phytol might have contributed the stable phytol content during the ensiling of IR.

In Experiment 3, the effects of N fertilizer application and harvesting stage on the contents of chlorophyll, phytol and carotenoids in IR herbage before and after ensiling were investigated, and the extent of phytol preservation after ensiling was clarified. Three rates of N fertilizer (same as Experiment 1) were applied for IR. The herbage harvested at the booting stage (27 weeks of age, April 25, 2014) or heading stage (29 weeks of age, May 9, 2014) were wilted for 1 day, and then ensiled for 60 days using a small-scale pouch system. The experimental results again verified that photosynthetic pigments content in pre-ensiled herbages increased with the extent of N fertilization levels. In addition, the contents of photosynthetic pigments and phytol at booting stages were higher than those at heading stage. In silage, increasing N fertilizer application also increased the contents of CP, EE, the photosynthetic pigments and their derivatives (pheophytins and pheophorbides), while harvesting stage did not affect the contents of β-carotene, chlorophylls and pheophorbides. Lutein and phytol contents were also higher in the silage at the booting stage or grown under higher N fertilizer treatment. In the pre-ensiled herbage, the molar content of phytol was higher than those of the chlorophyll content. A part of the phytol might be derived from the substances other than chlorophyll. N fertilizer application and early harvesting of herbage increased carotenoids and phytol contents in IR silage. Lutein and phytol in Italian ryegrass herbage were indicated to be well preserved during ensiling.

In Experiment 4, the effects of adding lactic acid bacteria (LAB) on the changes in photosynthetic pigments and phytol contents during ensiling of IR grown under different N fertilization levels were investigated. The IR herbages grown with three levels of N fertilizer levels (same as Experiment 1) were harvested at the heading stage, and were allowed to wilt under natural conditions for 1 day. The chopped wilted herbages were applied two different treatments: 1) without additive, 2) LAB addition (5 mg/kg fresh grass), then they were ensiled for 60 days using a small-scale pouch system. After ensiling, decreasing silage pH with addition of LAB increased β-carotene content, but, unaffected phytol content in silage

3. Phytanic aicd production in the rumen

The ruminal phytanic acid production from herbage phytol was explored in *in vitro* incubation experiments with fresh herbage (Experiment 5) and silage (Experiment 6 and 7). The herbage in both experiments were grown under three N fertilization levels and harvested at the booting and heading stages. Two adult wethers fitted with a rumen cannula were used as donors of ruminal fluid. The wethers were fed a basal diet of 50% hay and 50% concentrate diets at the maintenance energy level. The rumen fluid was collected at 2 hours after feeding in the morning and used for *in vitro* incubation. After 48 hours incubation,

the phytanic acid production was higher for both herbages at higher N fertilization levels and at booting stage. The ratio of phytanic acid production to total phytol in herbages was higher for silage (15-36%) compared with those for fresh herbages (12-17%).

4. Effects of forages sources on phytanic acid content in milk of dairy cows

Phytanic acid content in milk was investigated for the cows (n=17) fed the total mixed ration (TMR) containing IR silage or corn silage for three periods lasted for 21 days (Experiment 8). In the period 1 and period 3, the cows were fed corn silage TMR, while the cows were fed IR silage TMR in the period 2. Dietary phytol content was higher for the IR silage TMR compared with the corn silage TMR. Phytanic acid content in milk was higher for cows fed the IR silage TMR compared with those fed the corn silage TMR. The conversion ratio from dietary phytol to milk phytanic acid was found to be 2.6%. There were no differences in milk yield and milk component contents between cows fed IR silage TMR and corn silage TMR diets.

5. General summary

In summary, the results of the studies indicated that higher N fertilization levels or harvesting at early stage were an effective way to improve the phytol content in the herbages. Although the phytol content in hay prepared under natural conditions decreased largely, ensiling could effectively preserve phytol in herbage independent on the fermentation quality of silage. The forages containing higher phytol could produce higher phytanic acid in the rumen. However, the phytanic acid production rate in the rumen was relatively low, and most phytol residue may be remained in the rumen. Milk yield and milk component were not affected by phytol content in diets, however, phytanic acid in milk was higher in cows fed phytol rich diets.