Doctoral Thesis

Nutritional Studies on Plasma Formate and Amino Acids for One-carbon Metabolism in Ruminants

(Summary)

Ding Lanlan

Graduate School of Biosphere Science

Hiroshima University

March 2021

Nutritional Studies on Plasma Formate and Amino Acids for One-carbon Metabolism in Ruminants

CHAPTER 1: General summary

One-carbon metabolism is at the core of all metabolisms in vivo in living organisms, due to its provision of one-carbon units for synthesis of purines, thymidylates, and hundreds of methylated products. One-carbon metabolism is essentially the one-carbon transferring reaction that cycles from methyl donors to intermediates and eventually to products, mainly through the folate cycle and methionine cycle. Amino acids are vital throughout the cycles due to their various roles; the serine and glycine are the major one-carbon donors; methionine synthesis accepts most of the methyl groups; homocysteine is the potent intermediate between transmethylation and remethylation; and cysteine production is the salvage pathway of methionine disposal. Additionally, formate is different from other one-carbon donors for its mobile capability between cell compartments (mitochondria and cytosol) and plasma. The plasma concentration of formate and amino acids would highly reflect the status of one-carbon metabolism.

For the neonatal ruminants, the one-carbon donors are derived from colostrum in the first few days, followed by transition milk and whole milk, as well as starter diet before weaning. In addition, formate might be produced by the rumen fermentation of starter diets. Thus, by the alteration of milk and development of rumen, the one-carbon metabolism is likely to be affected , which has not been elucidated.

After weaning, the ruminants are fed with various diets, the dietary nutrients may play a role in one-carbon metabolism. Total mixed ration (TMR) is popular in some regions, especially for conserving the nutrients of high-moisture byproducts. By the way of ensilage treatment, the dietary protein is highly degradable in the rumen, which may finally reduce the absorption of amino acids from the small intestine. Besides, silage feeding is always accompanied with consumption of ethanol. TMR silage containing sake lees would contain high content of both ethanol and soluble protein. Ethanol consumption represses methionine synthesis in monogastric animals and leads to accumulation of formate precursor, methanol. However, effect of ethanol consumption on one-carbon metabolism through the methionine synthesis is not clarified for ruminants.

Ruminants consume green forages in pasture. Phytol is a moiety of chlorophyll, which is rich in green forage and could be modulated by application of nitrogen (N) fertilizer. The phytol is cleaved from chlorophyll molecule by the function of ruminal microbes, and oxidized into phytanic acid. Phytol and phytanic acid are ligands of PPARα, which regulate lipid catabolism in mouse. Particularly, PPARα activation could increase the plasma concentrations of serine and glycine, the primary one-carbon donors. Moreover, oxidation of one molecule of phytanic acid would generate a formate. Thus, the third aim is to study the effect of phytol supplementation on plasma concentration of formate, and amino acids related to one-carbon metabolism.

Therefore, the aims of this study are to clarify the effect of nutritional factors (colostrum and milk changes, protein degradability, ethanol, and phytol) on one-carbon

metabolism in ruminants. The present research paid a special attention to the plasma concentrations of formate, as well as amino acids related to one-carbon metabolism.

CHAPTER 2: The effect of colostrum and milk changes on plasma formate and amino acids related to one-carbon metabolism in neonatal calves

To clarify the effect of colostrum feeding on plasma concentrations of formate and amino acids related to one-carbon metabolism, ten neonatal calves were used for blood collection from their birthday until after weaning on the day 47. The calves were provided with the colostrum within immediately after birth and on the next day, followed by transition milk and whole milk supplemented with a starter diet and hay. The plasma concentrations of formate, serine, and methionine had a common alteration, which were doubled on the first day of birth ($P < 0.01$), compared with that immediately after birth, while their concentrations were reduced again until the day 6. In contrast, plasma concentration of glycine was simultaneously decreased on the day 1 ($P < 0.01$), and kept steady in the remaining experimental period. Plasma concentrations of homocysteine and cysteine were stable, without apparent alteration ($P > 0.05$). Notably, all calves were born with a high plasma concentration of sarcosine, which was gradually diminished until the end of experimental period. In conclusion, colostrum has significant effect on plasma concentrations of formate and amino acids related to one-carbon metabolism, while transition and whole milk feeding and rumen development caused no obvious change.

CHAPTER 3: The effect of dietary protein and ethanol on one-carbon metabolism in sheep

The second study aimed to explore the effect of ensiling treatment of TMR containing different types of sake lees on plasma concentrations of amino acids, methanol and formate as indicators of the status of one-carbon metabolism in sheep. Four mature Suffolk wethers (body weight, 60.9 ± 5.9 kg) were assigned to a 4 × 4 Latin square design with a 2 × 2 factorial arrangement of ensiling treatment and types of sake lees for consecutive 14-day periods. The TMR diets were formulated to contain traditional or liquefied brewing sake lees of 100 g/kg DM, mixing with oat hay, alfalfa hay, steam-flaked corn, soybean meal and wheat bran, at 1.4 times maintenance metabolizable energy. The silage was ensiled for 60 days, while the TMR without ensiling treatment were prepared freshly before feeding, using the same ingredients with their respective silages. The results indicated that N utilization was not different among treatments, but plasma concentrations of most amino acids were slightly reduced by ensilage ($P < 0.05$). Plasma concentrations of serine, glycine, methionine and homocysteine were not altered by treatments. Although ethanol intake varied between treatments, ruminal and plasma concentrations of ethanol increased after feeding, with the simultaneous elevation of ruminal and plasma concentrations of methanol. Ruminal concentration of formate was not altered after feeding, while plasma concentration of formate increased. In conclusion, the TMR containing sake lees caused a tentative increase in the ruminal and plasma concentrations of ethanol, methanol, and formate, regardless of ensilage or not. Plasma amino acids related to one-carbon metabolism

were protected.

The effect of the TMR silage feeding on the remethylation of homocysteine was investigated in experiment 2-2. On the 14th day of 1st and 2nd period of experiment 2-1, the rates of appearance of plasma methionine and its remethylation were measured by a primed and constant infusion of [1-^{13}C] and [methyl-$^{2}H_3$] methionine. The infused tracers, [1-^{13}C] and [methyl-$^{2}H_3$] methionine, reached plateau plasma enrichments of around 2.0% and 1.7 mol% excess, respectively, during 4 - 6 h after infusion. The remethylation flux calculated from the difference between methionine isotope fluxes, appeared not extensively inhibited by the higher intake of ethanol from silage, compared with that in non-silage treatment. Plasma concentrations of homocysteine and cysteine were not altered by ethanol ingestion as well. Therefore, ethanol intake from sake lees and silages had no apparent impact on methionine synthesis in ruminants.

CHAPTER 4: Phytol supplementation alters plasma concentrations of formate and amino acids related to one-carbon metabolism in sheep

The phytol moiety in chlorophyll molecules acts as an agonist of peroxisome proliferator-activated receptor-α, and absorbed as phytanic acid which releases formate by α-oxidation. The current study aimed to clarify the effect of dietary supplementation with phytol on the plasma concentrations of formate and amino acids related to one-carbon donors and its effect on lipid metabolism in sheep. Four mature sheep were fed with a mixed ration [metabolizable energy 10.7 MJ/kg DM; crude protein, 150 g/kg DM] comprising barley, rice bran, soybean meal, and oat hay at 1.5 times maintenance

metabolizable energy for three consecutive 14-day experimental periods, of which the first and third periods served as controls without phytol supplementation, while in the second period, phytol was added to the mixed ration at 12 g/kg of dietary DM per day. Dry matter intake in relation to metabolic body weight was slightly lower ($P < 0.01$) in the first period but did not differ after the second period. Dry matter digestibility was slightly lowered ($P = 0.05$) by the phytol treatment period. Nitrogen intake and retention showed similar trends with DM intake, but urinary N was not altered among the periods. Plasma cholesterol and phospholipid concentrations were decreased by the phytol treatment, while triglyceride concentration was increased ($P < 0.05$). In phytol treatment period, plasma concentrations of serine and glycine increased but the glutamate level decreased ($P < 0.01$). Plasma concentrations of formate and methionine increased ($P < 0.01$) from the first control period to the phytol period, but homocysteine and cysteine were not altered by the treatment period. In this study, dietary phytol affected lipid metabolism as well as plasma concentrations of amino acids related to one-carbon donors in sheep.

In conclusion, one-carbon metabolism, indicated by plasma concentrations of formate and relevant amino acids, was affected by colostrum and phytol supplementation, whereas irrelevant with the milk changes, rumen fermentation, and increased content of soluble crude protein and ethanol intake from ensiled TMR diet. Sheep seemed to have a high tolerance for ethanol, which may support the steady supply of one-carbon groups.