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SUMMARY OF DOCTORAL THESIS

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Title: Feeding value evaluation of growing and fattening diets by *in vivo* and *in vitro* methods
(*In vivo* および *in vitro* 法によるメンヨウの育成・肥育期における給与飼料評価)

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The feeding values of two types of diets offered to growing and fattening sheep were evaluated by both of *in vivo* and *in vitro* methods in three experiments. The feeding values for ruminant animal are evaluated based on the concentration of nutrients and anti-nutritional components, digestibility, feed intake and production performance when fed the tested feed to animals. However, physiological mechanisms by which rate and extent of digested and absorbed nutrients utilization for the body tissue assimilation are not well understood; hence establishment of more detailed approaches from the stand point of ruminant physiology and molecular biology are required. In this study, the conventional feeding trial, quantitative evaluation of nutrients supply to adipose tissue and muscle tissue by physiological and pharmacokinetics approaches and a novel feeding value evaluation by utilizing adipocytes culture system applied blood serum which obtained from animals being fed with test feeds as substrates of cell culture were carried out.

In the first *in vivo* experiment, feeding value of two growing and fattening purpose diets based on timothy hay with concentrate (THD) and Italian ryegrass straw with concentrate (IRD) were evaluated by long term period of feeding trial and carcass characteristics measurement. Six growing wether lambs were used for feeding trial and four animals of them were used for carcass characteristics measurement at the conclusion of the feeding experiment. The basal forage of each diet was offered at 2% body weight, and concentrate was fed at 40% of forage intake. Feed efficiency was significantly greater for THD than that for IRD. The crude protein digestibility was slightly higher for THD than for IRD. Although the nitrogen (N) intake was significantly greater for THD than for IRD, relatively greater ratio of retained N to absorbed N for IRD was prominent as compared to THD. The EBW and carcass weight were greater for THD than IRD. The lean meat weight was numerically greater for THD as compared to IRD, and the weight of adipose tissue, its weight ratio to empty body weight was also markedly greater for THD than for IRD. The results of this experiment indicated that THD feeding had superior growth performance of growing and fattening wethers and digestible N supply as compared to IRD, but long term period of THD feeding tended to deposit adipose tissue more as compared to IRD. It is also suggested that the relatively higher N assimilation ratio of wether lambs fed with IRD than that fed with THD might have associated with the observed comparable dressing ratio and lean meat ratio to empty body weight.

In the second *in vivo* experiment, quantitative estimation of energy yielding nutrients delivered to both of muscle tissue and adipose tissue *via* blood plasma flow, in relevant to N retention in wethers

lambs being early stage of growing period, were carried out for elucidating the reason for the observed animal response in the first experiment, indicating lower daily gain for IRD group than for THD at early stage of growth period even though metabolizable energy requirement was almost sufficed for both diets and the marked difference in ratio of adipose tissue to carcass weight between the diets. Three growing wethers, formed carotid artery-skin loops and located indwelling catheters in the mesenteric vein and the hepatic portal vein, were used. Wethers were offered THD and IRD as used for 11 days of period for each diet. The daily amount of glucose supplied to both muscle tissue and adipose tissue were numerically higher for THD than those for IRD. The N retention did not differ between the diets, and which was thought to be associated with the observed lacking differences between the diets in the amount of daily energy yielding substances supplied to the muscle tissues. The results in the second experiment suggested that the difference in the amount of glucose delivered to muscle tissue might have partly reflected the N retention responses to the forage based diets in early stage of growing wether lambs.

Comparison of the feeding value between THD and IRD were carried out as whole animal level or tissue level in the first and second experiment, respectively. In the *in vitro* experiment describe in the third experiment, the effect of forage species of basal diet and difference in forage to concentrate ratio on differentiation characteristics of adipocytes were investigated. Six wether were divided into THD (n = 3) and IRD (n = 3). The sheep were fed high-roughage (HR), medium roughage (MR) and low-roughage (LR) diets in a one-way layout design for 6-day of period. Sheep serum samples were collected on the last day of each dietary treatment, and were added to an adipogenic induction medium for differentiation of preadipocytes derived from sheep subcutaneous adipose tissue. The cytoplasmic lipid accumulations in the THD serum-treated preadipocytes were higher than IRD serum treated preadipocytes. mRNA expression of CCAAT/enhancer-binding protein (C/EBP)- α , C/EBP- β , C/EBP- δ , fatty-acid-binding protein (aP2) and stearoyl-coenzyme A desaturase (SCD) were regulated by each serum treatment. This study shows that different forage source of basal diet and forage to concentrate ratio of diets can regulate adipocytes differentiation in relevant to the variation of blood components composition.

From the results obtained in these studies, the differences in characteristics of animal production responses when fed the tested diet to growing wethers in terms of growth performance, N balance and carcass component yield were shown clearly. Feed efficiency value of Italian ryegrass straw was lower as compared to timothy hay; while lean meat production property of low quality Italian ryegrass straw appeared to be superior to that of timothy hay when fed them for both growing and fattening stage of production period. The effect of metabolizable energy intake on lean meat production was masked by some factors, although the effect of glucose supply to muscle tissue on N retention and lean meat proportion of carcass was suggested. Furthermore, it was also suggested that the new feeding value evaluation method utilizing cell culture system can be used to elucidate the superiority of timothy hay for adipocytes differentiation and fat accumulation in the adipocytes in terms of adipogenic gene expression.