論文の内容の要旨

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氏　　名　　董　　梅
指導教員　　熊谷　進

論文題目　The Study of Zearalenone Metabolism in Goats

（ヤギにおけるゼアラレノン代謝に関する研究）

Zearalenone (ZEA), 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcyclic acid lactone, is an estrogenic mycotoxin produced by several *Fusarium* species and has been found to be a common and widespread contaminant in cereal grains and animal feedstuffs. Various health problems associated with this mycotoxin have been documented in farm animals. The most important toxic effect of ZEA is its estrogenic effect, which has recently led to identification of ZEA and zearalenols (ZOLs) as endocrine disrupters. ZEA can bind to estrogen receptors and cause diseases in the reproductive system, impaired fertility and abnormal fetal development in farm animals. In addition to these estrogen-related effects, hepatic and renal lesions including hepatocarcinogenesis, and hematotoxicity in rodents, immunotoxicity in mice and humans, the reduction of milk production in cows have been observed. The impact of ZEA on human and animal health is now increasingly recognized. Ruminants are less known for their sensitivity to the negative effects of ZEA than non-ruminants. In order to characterize the metabolism of ZEA in ruminants, a series of studies focusing were

performed using adult goats.

In chapter 1, in order to clarify the features of ZEA metabolism in various organs, the *in vitro* zearalenone-reducing activity was investigated by postmitochondrial, microsome and cytosolic fractions of various organs of adult male and female goats. The results indicated that in the liver, α-ZOL was a major metabolite in cytosol, whereas β-ZOL was a predominant metabolite in microsomes. Post-mitochondrial fractions of the liver converted ZEA predominantly to more potent estrogenic compound, α-ZOL, indicating that the goat liver may function as an activation organ rather than as an inactivation organ. Such a feature of ZEA metabolism was confirmed by the K_m and V_{max} values from an enzyme kinetics experiment. In most other tissues including rumen tissue, the activity converting ZEA to α-ZOL was also higher than that to β-ZOL.

In chapter 2, in order to clarify whether 3α-hydroxysteroid dehydrogenase (HSD) and 3β-HSD are involved in ZEA metabolism, the effects of endogenous substrates for 3α-HSD and 3β-HSD were studied *in vitro* using liver. The results showed that the reduction of ZEA to α-ZOL and β-ZOL was competitively inhibited by the endogenous substrates, suggesting that 3α-HSD and 3β-HSD are involved in the ZEA to α-ZOL and to β-ZOL conversion. The expression of 3α-HSD mRNA in liver was increased, but that in the jejunum decreased, by intravenous injection of ZEA to goats, showing possible effects of ZEA on estrogen metabolism by these organs.

In chapter 3, in order to delineate the fate of ZEA in goats, the pharmacokinetics of ZEA were investigated by determining changes in blood plasma concentrations of ZEA and its metabolites in intravenously injected goats. Also the excretion of ZEA and its metabolites in urine and feces, and tissue residues of these metabolites in the liver were investigated. Kinetic evaluation of the plasma data revealed that the distribution

half-life ($t_{1/2\alpha}$) and elimination half-life ($t_{1/2\beta}$) of ZEA were 3.15 h and 28.58 h, respectively. The urine and feces samples revealed that ZEA is excreted as ZEA, α-ZOL and β-ZOL, β-ZOL being predominant metabolite. Urinary excretion showed that ZEA and its metabolites were mostly in glucuronide and /or sulfate conjugated forms. Faecal excretion showed that free forms of ZEA and ZOLs were major constituent. A large proportion of ZEA and its metabolites were excreted into urine during 24 h, and into feces during 48 h after administration of ZEA. In liver tissue samples, small amounts of α-ZOL and β-ZOL were identified, while ZEA was below detection limits.

In chapter 4, the effect of ZEA on expression of ERα and ERβ mRNA, and histopathological changes were investigated after intravenous administration. The expression of ERα mRNA was significantly increased in the liver, but no clear effect was found in the jejunum and rumen. The expression of ERβ mRNA was significantly increased in the jejunum, but not in the liver and rumen. These results suggest that ZEA can change the estrogenic response of the liver and jejunum. Histopathological changes such as lymphocytic infiltration were observed in the liver, kidney, uterus and epididymis, but not in gastrointestinal tract, ovary and testis. Although whether these ZEA-induced histopathological changes are caused via ERα and ERβ remains uncertain, the result shows that ZEA induces toxicity other than estrogenic effect in goats.

Allover the results of these studies delineated firstly a characteristic feature of ZEA metabolism in goats, i.e., ZEA is metabolized to more potent α-ZOL and less potent β-ZOL not only in the liver but also in the other organs, β-ZOL being more rapidly excreted in feces and urine. Toxic effects other than estrogenic effects of ZEA were also observed in the liver and other organs. Thus this study may provide a better understanding on ZEA metabolism in relation to its toxic effects in goats, and background information for other ruminant species.