Phytoalexins and endophyte related mycotoxins as hazards to the animal industry

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Summary

Phytoalexins, compounds produced by plants in response to pathogens, and biologically active metabolites produced by endophyte are beneficial to plant growth. There are many trials to utilize these biologically active substances for plant disease control. On the other hand, some of these substances are toxic to mammals. We experienced ryegrass staggers of cattle caused by endophyte infected ryegrass straw. In this review, toxicities of phytoalexins and endophyte related substances to animals are overviewed. Furthermore, toxic substances found in false smut balls in rice plant are cited.

Key words : phytoalexin, endophyte, lolitrem, ergovaline, loline, peramine, false smut ball

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Introduction

Phytoalexins are compounds produced by higher plants in response to attack by pathogens and to other stresses. Phytoalexins are sometimes referred to as plant antibiotics, but rather non-specific, having a general fungicidal and bacteriocidal action. Phytoalexin production is analogous to an antibody response as triggered by elicitors.

The term endophyte has been defined as an organism growing symbiotically in the plants. In the field of animal industry, the term endophyte has been used to indicate fungi in the genus Neotyphodium that infect to forage and turf grasses. Endophyte itself produces biologically active substances or endophyte acts as elicitor to induce phytoalexin production in the grass. These endophytes related substances provide benefits to the plants.

Phytoalexin induction and endophyte infection have been actively adopted for disease control of plants. On the other hand, some phytoalexins and endophyte related substances are highly toxic to mammals. For example, ryegrass staggers, a poisoning caused by endophyte infected perennial ryegrass, has been occurring in the cattle consuming ryegrass straw imported form USA.

In this paper, toxicities of phytoalexins and endophyte related substances to livestock and future tasks for the prevention of adverse effects of such substances are briefly overviewed.
Phytoalexins

Phytoalexins were low molecular weight, antimicrobial compounds that are both synthesized and accumulated in plants after exposure to microorganisms. Known phytoalexins are group of chemicals include the coumarins, isocoumarins, linear furanocoumarins, glycoalkaloids, isoflavonoids, stilbens and terpenoids. Induction of phytoalexin production can result from a plant’s exposure to many kinds of stimuli. Therefore, numerous trials have been performed to induce beneficial phytoalexins by using elicitor chemicals instead of synthetic pesticides. Although the production of phytoalexins is a major mechanism of plant defense, their toxic activity is not confined to microorganisms. Namely, many phytoalexins are toxic to mammals. Therefore, utilization of phytoalexins to control plant diseases should be carefully introduced.

Gossypol is a phytoalexin found in pigment gland of cottonseed (Gossypium spp). Cottonseed meal, the residue derived from seed oil extraction, is often utilized as animal feed. As gossypol adversely affects the color of the oil, processors attempt to leave most of gossypol in the meal. Two kinds of gossypol, free and protein bound gossypol, are found in the cottonseed meal. Free gossypol is the main concern from a toxicological point of view. The bound gossypol is physiologically inactive. All monogastric species are sensitive to gossypol. Intoxicated pig showed hydrothorax, hydropericardium, pulmonary edema, and hepatic necrosis. While adult ruminants are more resistant, gossypol poisoning cases in calves and lambs have been reported.

Mold-damaged sweet potatoes contain 3-substituted furans (4-ipomeanol (Fig. 1), 1-ipomeanol, 1,4-ipomeadiol, ipomeanine, ipomeamarone). These phytoalexins are responsible for bovine pulmonary emphysema caused by consumption of mold-damaged sweet potatoes. Cytochrome P-450-dependent monooxygenase system activates 4-ipomeanol to toxic metabolite. Pulmonary microsomal preparations were much more active than hepatic preparations in mediating this reaction.

Endophyte Toxins

Neotyphodium endophytes provide their hosts benefits including increases in drought tolerance, shoot growth, tillering, seed production, seed germination, phosphorus uptake, and resistance to insects and parasitic nematodes. On the other hand, toxicoses in livestock that have grazed on Neotyphodium-infected grasses are well known as ryegrass staggers and fescue toxicosis. Cultivars
of perennial ryegrass (*Lolium perenne*) are infected with *N. lolii*, which produces lolitremes, primarily lolitrem B (Fig. 2), those are thought to be responsible for ryegrass staggers. Lolitremes are neurotoxic and induce nervous disorder of cattle, sheep, and horses. As for fescue toxicosis, ergopeptine alkaloids, primarily ergovaline (Fig. 3), detected in *N. coenophialum*-infected tall fescue (*Festuca arundinacea*) have been shown to have pharmacological effects. The toxic effects include poor weight gain, low milk production, poor reproduction, gangrenes of hooves, ear tips and tail tip, necrosis of the peritoneal fat tissue, depressed immune response and direct endocrine effects.

From 1997 to date, we have experienced numerous cases of ryegrass staggers in cattle and horses fed perennial ryegrass straw imported from Oregon, USA. Straws of perennial ryegrass or tall fescue are by-products of turf-type grass seed production in the US and grass seed producers have been selling them for cheap animal feed. *Neotyphodium* endophytes have been introduced into turf-type perennial ryegrass and tall fescue to enhance plant hardiness, pest resistance, and drought tolerance. Therefore, most imported straw contains endophyte toxins to some degree.

Ryegrass and fescue straws are imported as cheap alternatives for rice straw. The threshold levels of endophyte toxins in the straw were proposed by the Oregon State University for the
prevention of endophyte toxicosis\textsuperscript{18}. As an interim measure for the prevention of the disorders in Japan, import companies have been requesting information on the concentration of the toxins. These companies are importing ryegrass straw only when the concentrations of lolitrem B and ergovaline are lower than the proposed thresholds. Fertilizer and Feed Inspection Station has been monitoring the concentration of endophyte toxins in imported straws. Their survey shows that most of the imported straw contains toxins lower than the proposed threshold.

Despite the measures mentioned above, we are still facing the ryegrass staggers of Japanese black cattle caused by imported straw. This indicates the possibility that Japanese black cattle is susceptible to ryegrass staggers. We observed the perennial ryegrass straw containing 1200 µg/kg of lolitrem B, lower than proposed threshold level for cattle (1800 µg/kg), induced ryegrass staggers in Japanese black cows\textsuperscript{19}. This observation suggested that the Japanese black cattle is susceptible to lolitrem B. We are now performing feeding experiments of the imported perennial ryegrass straw to Japanese black cattle to reevaluate the toxin threshold value proposed by the Oregon State University.

Another endophyte problem of interest is the public health significance of the endophyte toxins. Ergopeprine alkaloids such as ergovaline can be metabolized by the ruminal microflora and then absorbed rapidly as the lysergic acid amide or bio-transformed ergopeptine alkaloids\textsuperscript{20}. Hill et al.\textsuperscript{21} showed that ruminal tissue has a active transport system of ergot alkaloids. In sheep, ergovaline in the blood stream diminished rapidly\textsuperscript{22}. Urine is the primary excretory route of ergot alkaloids in cattle\textsuperscript{20}. Furthermore, ergovaline can not be detected in the milk of goat received this mycotoxin intravenously\textsuperscript{21}. These findings suggest the low possibility of the contamination of milk with ergopeptine alkaloids.

There is no information on the pharmacokinetics of lolitrems in cattle. We determined lolitrem B concentrations in several organs and tissue of the Japanese black cows fed perennial ryegrass straw. Lolitrem B was not detected in muscle, liver, kidney, lung and cerebrum of the cattle showing the symptoms of ryegrass staggers. In contrast, perirenal fat tissue contained about 210 ng/g wet tissue of lolitrem B\textsuperscript{19}. Small amounts of lolitrem B less than 150 ng/g were detected even in the fat tissue of the cattle without clinical signs. This observation indicates that lolitrem B will not accumulate in major edible parts of beef cattle. However, further research is needed to make clear the fate of lolitrems in the cattle.

Toxic alkaloids produced by endophyte result in low performance in livestock. However, beneficial functions of endophyte to the plants should be utilized effectively. Many trials have been performed to develop endophyte infected cultivars of the grass with reduced or nil production of toxic substances. Perennial ryegrass and tall fescue cultivars infected with non-toxin-producing endophyte are already in the market. These cultivars contain negligible amount of toxic alkaloids with enough amount of beneficial alkaloids such as loline alkaloids (Fig. 4) and peramine (Fig. 5). However, there is little information on the toxicity of these alkaloids to mammals. Loline alkaloids are classified into a group of pyrrolizidine alkaloids. Pyrrolizidine alkaloids are widely found in the plants of Asteraceae. Typical pyrrolizidine alkaloids with CH\textsubscript{2}OR function at C(1) position (Fig. 6) are potent hepatotoxins and carcinogens to mammals\textsuperscript{24}. On the other hand, loline alkaloids have an additional nitrogen function rather than CH\textsubscript{2}OR. Although restricted reports showed low toxicity of loline alkaloids to mammals\textsuperscript{25}, toxicological information is not sufficient for risk assessment of loline alkaloids. As for
peramine, there are essentially no reports of its toxicity to higher animals. Furthermore, pharmacokinetics of these substances in ruminants is unknown. Therefore, further toxicological examinations are crucial for the effective utilization of non-toxin-producing endophyte to introduce beneficial nature to the grass.
Toxicity of False Smut Balls on Rice Panicles

The false smut balls (Fig. 7) growing parasitically on panicles of rice plant (Ina-kouji, in Japanese) are caused by a pathogen, *Ustilaginoidea virens*.

Although, Suwa first reported the toxicity of false smut balls of *Ustilaginoidea* to rabbits in 1915\(^{26}\), toxic principle has not been elucidated as yet. Koiso et al.\(^ {27, 28}\) isolated tetrapeptide substances, named ustiloxin A E from a water extract of false smut balls. Ustiloxins are a potent inhibitor of microtuble assembly of both mammals and plants cells\(^ {29}\). This means ustiloxins are mycotoxin as well as phytotoxin. The cyclic structure of ustiloxins is closely related to that of an antimitotic mycotoxin, phomopsin A, produced by *Phomopsis leptostromiformis* growing on lupins. Ustiloxin A induced acute necrosis of hepatocyte and renal tubular cell of mice\(^ {29}\). The lesions induced by ustiloxin A were similar to those observed in mice lupinosis, poisoning induced by *Phomopsis* growing lupins\(^ {29}\).

Bis (naphto-quinone) derivatives named ustilaginoidins are separated from false smut balls formed by *U. virens*\(^ {30}\). Chemical structure of ustilaginoidins is similar to that of mycotoxin chaetochromin produced by *Chaetomium* spp. Ustilaginoidins showed cytotoxicity and antitumor activity\(^ {32, 33}\).

Recently, rice plants have been used as roughage for cattle to elevate feed self-sufficiency ratio and to utilize fallow rice fields. Rice plants for feeds sometimes suffered from false smut balls formation. Although no field outbreak of disorders in the cattle fed Ina-kouji affected rice plants has been identified, effects of toxic substances such as ustiloxins found in false smut balls to the cattle should be examined carefully to secure safety of rice plants used as feed.

Toxicities of phytoalexins, endophyte related substances and false smut balls in rice plant are overviewed. The utilization of phytoalexins or endophytes is one of effective alternatives to synthetic

Fig. 7  False smut ball on rice panicles.
pesticides in controlling plant diseases. However, some phytoalexins and endophyte related substances are highly toxic to mammals as mentioned above. Furthermore, there is only little toxicological information on the beneficial phytoalexins and endophyte related substances. Toxicological evaluations of these ‘natural pesticides’ are essential for the effective utilization of them.

References


ファイトアレキシンなどの植物の代謝産物やエンドファイト代謝産物の暴露による家畜の中毒

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植物が病原菌の感染を受けたときに産生する低分子の抗生物質であるファイトアレキシンや、エンドファイトが産生する生理活性物質は、植物の生育に有益であり、植物の病害防止への積極的な利用が図られている。しかし、一部のファイトアレキシンやエンドファイトが産生する生理活性物質は、哺乳動物に対して強い毒性がある。我が国では、エンドファイトに感染した輸入ライブラストローによる牛のライブラスタッガーが散発している。本総説では、ファイトアレキシンや、エンドファイトが産生する生理活性物質の家畜に対する毒性について概説するとともに、飼料中に発生する稲こうじ菌類の毒性についても言及する。

キーワード：ファイトアレキシン、エンドファイト、ロリトレム、エルゴバリン、ロリン、ペラミン、稲こうじ