

## Existence of antibodies to various feed antigens in beef cattle and goats

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### Background;

Grass components including plants body and pollen are possible to be allergens to human being. The plant antigens were possible to cross over the intestinal barrier, and were also detected in breast milk as allergy sources for neonates. There is a possibility that the antigens contribute serious allergy to human through the livestock products due to the allergens originated from domestic livestock feed. Once the antigens in the plants invade the immune systems of domestic livestock then the specific antibodies of IgG against the antigens are detectable in the livestock body. The detections of IgG antibody specific to plant antigen in serum and/or other tissues of domestic livestock are available to prove the invasion of plant antigen to the livestock body. However, there are few previous reports that demonstrating the existence of plant antigens derived from the feed and antibody to feed materials in beef cattle and goats.

### Objectives;

It is desired to confirm the safety of livestock products for human health because the products probably contain the plant antigens that affect human immune systems disorder. The objective of this study is to clarify the existence of the specific IgG antibodies to feed derived plant antigens in serum and/or muscles of beef cattle and goats, fed with Italian ryegrass, Bahia grass and some concentrates including Wheat and Corn grain.

### Methods;

The beef cattle and goats were applied in this study, and were fed with Italian ryegrass hay, fresh Bahia grass and concentrates such as Wheat and Corn. The serums and muscles of the cattle and goats were sampled, and were stored at  $-20^{\circ}\text{C}$  to keep the conditions. Muscle samples (*m. longissimus dorsi*, *m. biceps femoris*, *m. psoas major* and *m. gastrocnemius*) were divided into small pieces, and the pieces were homogenized (1:20 wt/vol) in 50mM Tris-HCl (pH 7.5) by using teflon homogenizer at  $4^{\circ}\text{C}$ . The extracts were centrifuged at 3000rpm for 20min, and the supernatant was collected (frozen at  $-20^{\circ}\text{C}$  until analysis). The antigens of Italian ryegrass, Bahia grass, Timothy, Orchard grass, Alfalfa pollen, Corn pollen, Wheat and Cottonseed (GREER lab. USA) were used in this study. The plant antigens treated by SDS and  $\beta$ -ME were transferred on PVDF membrane for the detection of IgG antibody in each sample by the dot blot analysis. The membrane was incubated with collected serum or muscle extracts at ambient temperature. After second antibody conjugated HRP applied to the membrane to detect the immuno-complex of first antibody and plant antigens, the staining was performed by chemiluminescent methods using Amersham ECL reagents. To determine the distributions of molecular weight of antigens, each antigen was applied to 10% gel for SDS-PAGE (Laemmli, U.K. 1970). Subsequently, the western blot analysis using the serum and muscle extracts as a source of first antibody and second antibody conjugated HRP were performed.

### Results and discussions;

The IgG antibodies to Italian ryegrass, Bahia grass, Corn Pollen and Wheat were detected in the beef cattle serum, and the IgG antibodies specific to Italian ryegrass and Bahia grass were also found in the serums of goats, which were fed with Italian ryegrass hay, fresh Bahia grass, Wheat grain and Corn grain (Table1). It is thought that the plant antigens in the feed were able to induce rising antibody in domestic livestock through invasion of plant antigen to live stock immune systems. In human, some food antigens were digested partially, but the remaining antigenicity was adsorbed or/and excreted in breast milk (Fujiwara, Y. 1996). From the literature view, it was suggested that the plant antigen with relative high molecular weight could cross the intestinal epithelial cell of human without complete digestion. The relatively high molecular weight of antigens, greater than 10kDa, have permeability of human intestinal monolayers in some condition (Jensen-Jarolim, E. 1997), and it supports our results. Timothy have never fed and exposed

to the livestock, although some feasible IgG antibodies to Timothy were detected in the serum of beef cattle and goats unexpectedly. Detail mechanisms of rising IgG to Timothy antigen were not clear, but it seemed it was due to the cross-reactivity to Timothy antigens and some others. Cross-reactive immune reaction responses in patients with pollinosis had often reported (Ipsen, H. 1997). The muscle extracts from goats showed negative reactions to the feed antigens, although the beef muscle extracts reacted only with Wheat antigen. The partially adsorbed Wheat antigen seemed to induce the IgG antibody in serum since the beef cattle had been fed with a lot of concentrates including Wheat grains. It is not clear whether the positive response have been derived from contamination of serum components to muscle or the other cross-reaction by unknown substance. In this study, only IgG antibody (not IgE antibody) to feed derived plant antigens was detected to prove the invasion of feed antigen, because IgG antibodies specific to the allergens had been synthesized before rising IgE antibody to the exposed allergens (Eysink, P.E.D. et al. 1999). It may be appeared that the domestic livestock that have the plant antigens through the feed and the detectable IgG antibody to the feed are suffering from the immune diseases like allergy. From this investigation, it is possible that the meat products contained the plant derived antigens from livestock feed might induce the allergic reaction in human who have not been exposed to the allergen. Further investigation is required to clarify the effects of plant allergen in the livestock feed on human immune system from the viewpoint of supplying safety meat products to the market.

### Conclusions;

The detection of specific IgG antibody to some feed staffs of domestic livestock was observed in only serum not muscle extracts of beef cattle and goats without antibody to Wheat in two kinds of muscles in beef cattle. The feed antigen with relatively high molecular weight could be adsorbed across small intestine barrier with remaining its antigenicity. These observation showed the possibility that the allergic reaction to grass antigen in human was originated partially from the livestock products which included feed grass antigens. Further investigations are required to conform the possibilities that some allergy to plant allergen in human is derived from livestock products.

### Pertinent literature;

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- Ipsen, H. and Lowenstein, H. (1997).Basic features of crossreactivity in tree and grass pollen allergy. Clin. Rev. Allergy Immunol., 15,389-396.
- Jensen-Jarolim, E., Gajdzik, L., Haberl, I., Kraft, D., Scheiner,O., and Graf, J.(1997). Hot-spices influence permeability of human intestinal epithelial monolayers. J. Nutri.,128,577-581.
- Fukushima, Y.(1996). Consumption of cow'milk in lactating women and transfer of food antigen in breast milk. Jpn.J.Dairy and Food Sci.,45,A123-A129.
- Laemmli, U.K.(1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature,277,680-685.

Table1. Detection of IgG antibodies to feed staffs in serums and muscle extracts of beef cattle and goats

	Italian ryegrass	Bahia grass	Corn Pollen	Timothy	Cotton Seed	Orchard grass	Wheat	Alfalfa Pollen
<b>Beef cattle*</b>								
Serum	++	++	+	±	-	-	++	±
<i>m.longissimus dorsi</i>	-	±	-	±	-	-	+	-
<i>m.biceps femoris</i>	-	-	-	-	-	-	-	-
<i>m.pasas major</i>	-	±	-	±	-	-	+	-
<i>m.gastrocnemius</i>	-	-	-	-	-	-	-	-
<b>Goat*</b>								
Serum	+++	++	-	+	-	-	-	-
<i>m.longissimus dorsi</i>	-	-	-	-	-	-	-	-
<i>m.biceps femoris</i>	-	-	-	-	-	-	-	-
<i>m.pasas major</i>	±	±	-	-	-	-	-	-
<i>m.gastrocnemius</i>	-	-	-	-	-	-	-	-

\*Beef cattle and goat fed Italiana ryegrass hay, fresh Bahia grass and some concentrates including Wheat and Corn grain but not Cotton Seed, Orchard grass, Timothy grass.