Effects of Bruchid-Resistant Mungbean Meal on Growth and Blood-Biochemical Values in Mice

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Received February 2, 1996

Abstract

Breeding of grain legumes for insect resistance has recently made rapid progress. It had been found that a wild mungbean accession TC1966 has strong resistance to the bruchid beetles (*Callosobruchus chinensis* and *C. maculatus*) which is controlled by a single dominant gene producing insecticidal substance(s). We reared up mice with a diet containing 65% of mungbean seeds of the $B_{14}F_4$ line which is isogenic to a susceptible mungbean cultivar "Osaka Ryokutou" except for the bruchid resistance gene derived from TC1966 for a period of 4 weeks. The seeds of "Osaka Ryokutou" (OR) was used as control and commercial standard diet was used as reference. Aliquot portions of $B_{14}F_4$ and OR were heated to inactivate the antitrypsin activity in the seeds. There were no significant differences in the feed consumption and the body weight gain between the mice fed $B_{14}F_4$ and those fed OR or the standard diet. No difference was observed in the growth of mice between the groups fed $B_{14}F_4$ with and without heat inactivation. Changes in the weight of lungs, heart, kidneys, spleen and liver were not conspicuous between the mice fed $B_{14}F_4$ and those fed OR or the standard diet. No macroscopic and histopathologic changes were detected in almost all the organs examined. The counts of red blood cells, white blood cells, hemoglobin concentration, hematocrit value, alkaline phosphatase activity and glucose concentration were not different between the mice fed $B_{14}F_4$ and the mice fed the OR diet. However, the

glutamic-oxaloacetic transaminase activity was higher and the total cholesterol concentration was lower in female mice fed $B_{14}F_4$ compared to other female mice. Thus the $B_{14}F_4$ mungbean diet did not cause any retardation of growth or pathological changes in mice within the 4-week period of feeding. However, based on the changes of the blood biochemical values listed above, another feeding test during a long-term period should be carried out to confirm the safety of bruchid resistant mungbean seeds derived from TC1966.

Additional key words : *Vigna radiata*, *Callosobruchus chinensis*, insect-resistance gene, histopathological changes, feed safety test

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek), one of the important tropical grain legumes, has been widely cultivated in Thailand for a long period of time. Although mungbean is one of the major export commodities of the country, it is usually grown under low input cultural practices by small landholders and its productivity still remains at the low level of around 0.7 tons[10]. Among the major constraints on mungbean production in Thailand, bruchids create one of the most serious problems for mungbean farmers in the country. Visarathanonth and Promsatit (1989) reported that two species of bruchids caused serious damage to mungbean seeds during storage: adzuki bean weevil *Callosobruchus chinensis* and cowpea weevil *C. maculatus*[12].

Breeding for bruchid resistance in mungbean in Thailand has been initiated since 1987 under the collaborative research project between the Japan International Research Center for Agricultural Sciences, Japan (formerly the Tropical Agriculture Research Center) and Chai Nat Field Crops Research Center, Field Crops Research Institute, Department of Agriculture of Thailand. Since TC1966, one of the wild mungbean (*Vigna radiata* (L.) Wilczek var. *sublobata* (Roxb.) Verdc.) accessions from the Asian Vegetable Research and Development Center, displays complete resistance to *C. maculatus*[1] and *C. chinensis*[2] which is controlled by a single dominant gene[5], and is cross-compatible with cultivated mungbean[8], the resistance gene of TC1966 has been successfully utilized in mungbean breeding programs[11]. Several promising mungbean lines with favorable agronomic characters along with the resistance to the bruchids have been developed and are being tested in multilocation trials[10].

Recently Kitamura et al. (1995) have isolated an insecticidal substance that exhibit a growth inhibitory activity on the bruchids from the seeds of a mungbean isogenic line $B_{14}F_4$ harboring the corresponding gene derived from TC1966, for which the structure is being investigated[6]. Since mungbean is used as bean sprouts or other foods for human consumption, it is essential to confirm the safety of the mungbean seeds containing the insecticidal substance(s) for animals and human beings. In this study, we investigated the effect of the diets prepared from seeds on the growth of mice and also carried out several blood analyses in mice.

Materials and Methods

Diet preparation. Seeds of the isogenic mungbean line $B_{14}F_4$ with resistance to bruchids and those of the mungbean cultivar "Osaka Ryokutou" (*V. radiata*) which does not carry the bruchid resistance gene were used. $B_{14}F_4$ had been developed at the National Agriculture Research Center, Japan, by recurrent back crossing using TC1966 as a source of resistance and "Osaka Ryokutou" as a recurrent parent. Seed samples of $B_{14}F_4$ and Osaka Ryokutou were split

into two parts: One was subjected to heat treatment at the temperature of 105°C for 25 minutes under humid conditions in order to inactivate trypsin inhibitors and other enzymes. Then the seeds were freeze-dried and milled into flour. Using the flour, a blended diet (Oriental Yeast Co., Ltd, Japan) was prepared which contains the flour at the maximum level (65 percent of total weight), approximately fulfilling the criteria for the formulation of the standard purified diet[13] recommended by AIN (American Institute of Nutrition) (Table 1).

Using seeds of Osaka Ryokutou (OR) diets with and without heat treatment were prepared in the same way and used as a control. In addition, standard commercial flour-type diet (standard diet) purchased from Funabashi Farm Co.Ltd., Japan, was used as reference. Therefore, five kinds of diets were tested in this study: diet containing bruchid-resistant $B_{14}F_4$ seeds (A), heat-treated $B_{14}F_4$ seeds (B), OR seeds (C) and heat-treated OR seeds (D) along with the standard diet (E).

Feeding test. Five-week-old ICR mice, which were reared at the National Institute of Animal Health, Japan, were divided into five groups of ten consisting of five male and female mice each. Mice were housed in stainless-steel bedded cages containing five males or five females and were reared for four weeks with one of the five kinds of diets mentioned above using flour-feeding vessels in the temperature-controlled room at 25°C. Body weight and food consumption with small loss from vessels were measured twice a week and every other day, respectively.

Table 1. Composition of diets used

Ingredients	%
Mungbean flour	65.0[a]
Cornstarch	9.0
α -Starch	10.0
Granulated sugar	5.0
Cellulose	2.5
Corn oil	4.0
AIN-76™ Mineral mixture[13)b]	3.5
AIN-76™ Vitamin mixture[13)b]	1.0

Experimental diets were prepared by mixing $B_{14}F_4$ or OR mungbean flour to other components so that the nutrient composition was as follows: 16.1% crude protein, 59.4% carbohydrates, 5% crude fat, 5% fibers and 5% minerals.

Standard flour type diet (F-2, Funabashi Farm Co.Ltd., Japan) contains 20.8% crude protein, 58.2% carbohydrates, 5% crude fat, 3.2% fibers, 5% mineral and vitamins, fulfilling the nutrient requirements recommended National Research Council (USA) for mice and rats.

[a] Protein content of mungbean was estimated at about 25%.

[b] Reference No. cited

At the end of the experimental period, blood was collected from ether-anaesthetized mice, then hematological analyses were immediately carried out for all the mice and blood plasma was separated and stored at -20°C for blood biochemical analyses. Major organs were examined macroscopically and weighed, then tissue samples were fixed in 10% buffered formalin.

Hematology and blood biochemical analyses. Red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb) and hematocrit value (Ht) were determined using a Micro Cell Counter (Toa Iyou Densi K.K., Japan). Glutamic-oxaloacetic transaminase activity (GOT), glutamic-pyruvic transaminase activity (GPT), alkaline phosphatase activity (ALP), urea nitrogen concentration (BUN), total cholesterol concentration (Tch) and glucose concentration (Glu) were determined using commercial kits (Wako Pure Chemical Industries, LTD. Japan).

Histopathological examination. Tissue samples were fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin (HE), and examined under a microscope.

Statistics. The data obtained were statistically analyzed using one-way analysis of variance and Student's t-test.

Results

Differences in body weight among the five groups of mice fed on five different kinds of diets are shown in Table 2. In female mice, there was no significant difference in the body weight gain during the experimental period among the five groups of diets, that is, unheated and heated $B_{14}F_4$

Table 2. Body weight gain of mice fed the mungbean diet

Mice		Diet		Time (days of feeding)									Body weight gain (g)	
Group	Sex			1	5	8	12	15	19	22	26	28	mean	sd*
				body weight (g)										
A-1	Female	$B_{14}F_4$	Unheated	21.2	22.1	22.6	23.4	24.0	24.7	24.8	25.6	25.7	4.2	0.8
A-2	Male		Unheated	29.2	28.7	30.1	30.1	30.6	31.7	32.0	32.2	32.4	3.1	0.8
B-1	Female	$B_{14}F_4$	Heated	22.6	23.8	23.4	24.2	24.8	25.5	25.8	26.7	26.9	3.6	0.5
B-2	Male		Heated	27.8	28.0	28.5	29.3	29.5	30.4	30.4	30.8	31.1	3.6	1.3
C-1	Female	Osaka	Unheated	22.3	23.5	23.4	24.5	24.8	25.3	25.7	26.8	27.0	4.2	1.6
C-2	Male		Unheated	28.7	29.1	29.3	29.8	30.2	30.8	30.9	31.1	31.5	2.8	1.0
D-1	Female	Osaka	Heated	22.0	23.0	23.1	24.0	24.4	25.5	25.9	26.6	26.5	4.5	0.7
D-2	Male		Heated	27.8	28.8	29.7	30.3	30.5	31.1	31.4	32.0	32.0	4.3	0.8
E-1	Female	Standard		22.2	23.1	23.2	24.5	24.8	25.1	24.9	26.3	26.9	4.4	1.6
E-2	Male	Standard		27.3	28.4	29.2	30.3	30.8	31.5	31.7	32.2	32.5	5.5	1.0

$B_{14}F_4$: Diet contains 65% of bruchid-resistant mungbean seeds from plant which was developed by repeated backcrossing using TC1966 and mungbean cultivar "Osaka Ryokutou" as a recurrent parent.
Osaka : Diet contains 65% of "Osaka Ryokutou" mungbean seeds from plant which does not carry bruchid-resistance gene.
Standard : See the legend in Table 1.
Heated : See text.
*Standard deviation

diet (A-1, B-1), unheated and heated Osaka Ryokutou diet (C-1, D-1) and standard diet (E-1) ($p = 0.97$). In male mice, there was no significant difference in the body weight increase between the $B_{14}F_4$ diets (A-2, B-2) and Osaka Ryokutou diets (C-2, D-2) ($p > 0.2$). A small difference was observed in the body weight increase between male mice fed the $B_{14}F_4$ diet (A-2, B-2) and those fed the standard diet (E-2) ($p < 0.05$). This difference may be related to the fluctuations in the feed consumption caused by fierce competition among male mice.

Regarding feed consumption, there was no significant difference between unheated $B_{14}F_4$, unheated OR diets and standard diet both in female amd male mice, although heat treated OR diets were consumed in slightly larger amounts compared with the other two kinds of diets (Table 3).

After being subjected to feeding experiments for four weeks, all the mice were dissected to examine almost all the organs and no significant differences among the diets were observed. Weight of major visceral organs of mice fed on each of the five kinds of diets, i.e. liver, lungs, heart, kidneys and spleen along with their proportion to body weight at the end of the experimental period is shown in Table 4. In female mice there were no significant differences among the five groups in the proportional weight of the liver, lungs, kidneys and spleen. The proportional weight of heart was significantly smaller in mice fed unheated $B_{14}F_4$ diet (A-1) than in mice fed the standard diet (E-1), though it was not different from that in mice fed unheated OR diet (C-1). In male mice there were not significant differences among the five groups in the proportional weight of the lungs, heart, kidneys and spleen. The proportional weight of the liver was slightly larger in mice fed unheated $B_{14}F_4$ (A-2) than in mice fed unheated and heated OR diets (C-2, D-2). But the value in A-2 was similar to that in mice fed the standard diet (E-2).

In addition, among the five diet groups, no histopathological differences were observed when the brain, liver, kidneys, heart, lungs, trachea, thymus, mesenteric lymph nodes, pancreas,

Table 3. Feed consumption of mice fed the mungbean diet

Mice		Diet		Time (days of feeding)													Total	Mean*
Group	Sex			2-3	-5	-7	-9	-11	-13	-15	-17	-19	-21	-23	-25	-27	-29		
A-1	Female	$B_{14}F_4$	Unheated	45.8[a]	52.4	70.4	69.3	82.4	77.1	74.0	70.5	68.2	77.1	65.2	80.6	65.8	74.9	973.6	7.0
A-2	Male		Unheated	51.9	54.6	64.4	61.5	57.8	58.0	67.5	69.4	90.9	76.9	69.1	75.7	70.3	61.6	929.8	6.6
B-1	Female	$B_{14}F_4$	Heated	55.3	53.2	56.3	50.5	62.6	64.4	72.3	55.2	81.5	57.2	66.3	53.1	62.7	56.3	846.9	6.0
B-2	Male		Heated	45.8	51.9	56.1	51.4	63.7	73.6	66.0	80.5	69.4	75.0	70.4	97.0	80.8	72.0	953.6	6.8
C-1	Female	Osaka	Unheated	51.4	53.2	52.1	68.2	88.3	63.1	93.1	80.1	68.5	71.1	82.7	107	63.9	60.1	1002.7	7.2
C-2	Male		Unheated	48.9	55.5	61.5	51.1	61.5	58.3	59.8	58.1	62.0	77.0	71.6	57.9	68.4	55.1	846.6	6.0
D-1	Female	Osaka	Heated	61.0	56.8	54.4	79.8	64.9	88.2	94.0	87.8	105	89.5	81.2	78.5	76.0	77.9	1090.4	7.8
D-2	Male		Heated	56.8	65.8	68.9	66.4	85.2	72.9	74.4	73.4	70.9	97.3	72.2	106	89.6	70.2	1069.7	7.6
E-1	Female	Standard		51.6	41.5	50.1	58.6	74.3	67.8	69.9	68.0	56.7	74.0	53.7	79.0	67.8	105	917.7	6.6
E-2	Male	Standard		56.9	64.1	63.6	54.8	67.2	57.5	61.8	66.9	68.3	73.8	65.0	76.9	59.5	57.1	893.3	6.4

a: Feed consumption of five mice in each group of two-day intervals (gram)
* Mean feed consumption per mice per day (gram) : Total/(28days×5mice)
For explanation of diet, see the legend in Table 2.

Table 4. Effects of the mungbean diet on weight of visceral organs

Mice		Diet		Liver	Lungs	Heart	Kidneys	Spleen
group	Sex							
A-1	Female	$B_{14}F_4$	Unheated	1057.2[a]	165.4	123.2	255.4	89.4
				4.1[b]	0.6	0.5	1.0	0.3
A-2	Male		Unheated	1647.0	203.4	165.0	202.2	97.0
				5.1	0.6	0.5	1.3	0.3
B-1	Female	$B_{14}F_4$	Heated	1080.8	169.4	125.2	265.0	94.8
				4.0	0.6	0.5	1.0	0.4
B-2	Male		Heated	1441.4	215.4	160.0	198.2	101.0
				4.6	0.7	0.5	1.3	0.3
C-1	Female	Osaka	Unheated	1109.4	182.2	138.8	276.0	102.0
				4.1	0.7	0.5	1.0	0.4
C-2	Male		Unheated	1484.8	202.0	162.3	204.5	98.8*
				4.6	0.7	0.5	1.3	0.5
D-1	Female	Osaka	Heated	1103.6	185.6	138.0	279.0	102.2
				4.1	0.7	0.5	1.1	0.4
D-2	Male		Heated	1423.2	203.2	169.4	207.0	96.2
				4.4	0.6	0.5	1.3	0.3
E-1	Female	Standard		1214.4	172.0	154.2	304.2	113.2
				4.5	0.6	0.6	1.1	0.4
E-2	Male	Standard		1651.4	201.2	152.8	209.8	93.4
				5.1	0.6	0.5	1.3	0.3

a: Average of wet weight in mg (n=5) * : n=4
b: Proportion to body weight on average (%)
For explanation of diet, see the legend in Table 2.

adrenal glands, salivary glands, stomach, small intestine, large intestine, testes, seminal ducts, ovaries and uterus were examined in all the mice.

Results of hematological and blood biochemical analyses obtained in this study are shown in Table 5. Based on the analysis of variance applied to male or female mice separately, there were no significant differences among the five groups in terms of RBC, WBC, Hb, Ht, ALP and Glu, although GOT of A-1 and BUN of A-2 were significantly high, while Tch of A-1 and GPT of B-1 were low ($p < 0.05$).

Discussion

Breeding of grain legumes for insect resistance has recently made rapid progress[3, 4, 5, 6, 9]. A gene encoding the alpha-amylase inhibitor has been transferred into an adzuki bean variety using biotechnological procedures and a bruchid-resistant azuki line has been successfully developed[4]. On the other hand, the existence of bruchid resistance in wild mungbean TC1966 was evident and enabled to breed bruchid-resistant mungbean by conventional breeding methods[10, 11]. The bruchid resistance in TC1966 is considered to be caused by some water-soluble, low molecular weight, and heat-stable insecticidal substance(s)[6] for which the structure is being investigated. Thus, a biological test to determine the safety of the insecticidal substance(s) for mammals is a prerequisite for utilizing the bruchid-resistant

Table 5. Effects of mungbean diet on hematological and blood biochemical values in mice

Mice Group	Sex	Diet			WBC	RBC	Hb (g/dl)	Ht (%)	GOT (K.U.)	GPT (K.U.)	BUN (mg/dl)	ALP (K-A U.)	Tch (mg/dl)	Glu (mg/dl)
A-1	Female	$B_{14}F_4$	Unheated	mean	46.0	942.2	14.2	55.6	101.6[a]	10.3[a]	15.1[a]	6.4[a]	91.3[a]	227.8[a]
				sd	12.0	235.1	1.0	15.3	39.6	5.3	3.0	2.8	11.6	24.5
A-2	Male		Unheated	mean	84.6	815.8	14.2	46.7	45.0	17.2	27.3	6.5	141.5	220.3
				sd	23.9	32.1	0.6	2.4	6.7	12.0	4.0	1.8	19.5	22.5
B-1	Female	$B_{14}F_4$	Heated	mean	37.0	1082.6	14.9	62.5	72.0	4.7	15.3	7.1	123.8	185.3
				sd	12.3	168.5	0.4	9.8	13.5	1.1	1.7	1.6	18.3	16.3
B-2	Male		Heated	mean	49.2	844.4	14.5	47.8	43.6	12.3	25.0	6.3	101.5	196.1
				sd	11.8	45.6	0.8	3.5	9.1	1.6	3.3	0.9	3.9	32.2
C-1	Female	Osaka	Unheated	mean	34.2	1067.6	14.6	67.0	53.8	5.2	17.0	8.5	99.4	183.0
				sd	20.2	180.2	0.5	14.0	15.8	1.2	1.9	0.3	19.5	21.7
C-2	Male		Unheated	mean	109.7[b]	83.40[b]	14.3[b]	50.8[b]	61.9	16.8	22.3	6.7	137.0	202.5
				sd	106.1	43.3	0.2	4.4	16.0	6.4	3.5	1.1	18.4	46.8
D-1	Female	Osaka	Heated	mean	31.8	985.6	14.1	60.7	75.2	9.5	16.7	7.5	132.7	216.3
				sd	8.9	173.4	0.3	11.7	18.4	2.1	3.2	1.8	12.9	58.7
D-2	Male		Heated	mean	55.4	835.6	14.2	50.4	38.1	13.4	18.3	6.92	99.6	172.5
				sd	20.9	28.4	0.5	2.7	8.9	2.5	2.1	0.3	8.6	22.1
E-1	Female	Standard		mean	36.6	939.2	14.4	60.3	55.3	11.0	17.5	8.4	115.4	195.3
				sd	15.5	89.0	0.4	5.3	10.2	1.5	3.2	0.8	13.8	28.0
E-2	Male	Standard		mean	45.8	822.4	13.8	45.9	50.0	22.4	21.1	6.1	130.2	221.1
				sd	16.2	33.0	0.4	1.9	7.8	7.5	3.5	0.8	20.1	36.4

sd : Standard deviation
WBC : White blood cells ($\times 100/\mu l$)
RBC : Red blood cells ($\times 100/\mu l$)
Hb : Hemoglobin
Ht : Hematocrit values
Tch : Total cholesterol
GOT : Glutamic-oxaloacetic transaminase activity
GPT : Glutamic-pyruvate transaminase activity
BUN : Urea nitrogen
ALP : Alkaline phosphatase activity
Glu : Glucose
K.U. : Karmen unit
K-A U. : K-A unit
a : n=4 ; b : n=3 (abnormal values were statistically omitted)
For explanation of diet, see the legend in Table 2.

mungbean seeds for human consumption.

In the present study, young mice were reared for four weeks on the seeds of the isogenic line $B_{14}F_4$ which carries a bruchid resistance gene derived from TC1966 and produces insecticidal substance(s). The body weight gain, development of major visceral organs and hematological and histopathological changes were investigated and compared with those in mice fed the seeds of the recurrent parent mungbean OR line that does not contain the insecticidal substance(s) or the standard diet.

The mice fed the $B_{14}F_4$ mungbean diet showed an almost equivalent feed consumption to that of the group fed the standard diet and did not display any growth retardation. The weight of the five visceral organs (liver, lungs, heart, kidneys and spleen) in the mice fed the $B_{14}F_4$ diet did not differ from that in mice fed OR or the standard diet. The weight of the liver in male mice showed slight fluctuations among the five diet groups, though no histopathological changes were detected in these tissues as in all the organs examined.

Since various kinds of growth inhibitors are known to occur in seeds of grain legumes, diets made from mungbean seeds subjected to heat treatment in order to inactivate growth inhibitors in the seeds were also included in this study. There was no difference between heated and unheated mungbean seeds as mentioned above. These results suggest that a mammalian toxicity test of the bruchid-resistant mungbean meal can be carried out using unheated materials.

As for the results of blood analyses, there was no difference among the five diets in the RBC, Hb and Ht values which are indexes of anemia, WBC value which indicates the presence of inflammation, along with the values of GPT and ALP, which are considered to be enzymes released from injured cells. Regarding GOT which is also an enzyme associated with tissue injury, female mice fed on the unheated $B_{14}F_4$ diet (A-1) displayed a higher GOT value than the other 4 groups of female mice. Besides, the Tch value of this group tended to be low and the heart was significantly smaller as compared with that of the group fed the standard diet although the weight was almost equal to that in mice fed the OR diet. On the other hand, the BUN value which is an indicator of kidney function and tissue injury, did not show any difference among the five female groups, while the BUN values of male mice fed on the unheated $B_{14}F_4$ diet (A-2) tended to be high. Although the significance of such changes in mice fed on the $B_{14}F_4$ diet is not clear, more attention should be paid to the changes observed in these traits when long term toxicity tests of the insecticidal substance(s) are carried out.

There were no deleterious effects on the growth, weight and histological characteristics of various organs, and blood biochemical values in mice fed $B_{14}F_4$ mungbean containing insecticidal substance(s) derived from TC1966. However, several levels of toxicity tests had been recommended so far before the utilization of chemical substance(s) ingested as food by human[7]. Therefore, it is still necessary to carry out long term feeding experiments on this mungbean material in order to confirm the safety of the bruchid-resistant mungbean line for human consumption. In addition, it is essential to carry out biological tests such as mutagenicity tests using the insecticidal substance(s) in TC1966 which is currently being purified.

Acknowledgement

The authors thank Dr.H.Hayakawa, JIRCAS, Tsukuba, Japan, Dr.N.Terakado, National Institute of Animal Health, Tsukuba, Japan and Dr. T.Itabisashi, Chikusan Gijyutsu Kyoukai, Tokyo, Japan for their useful suggestions, and Mr.J.Obana, Mr.H.Yoshiba, Mr.Y.Hariu and Miss H.Okano, National Institute of Animal Health, Tsukuba, Japan for their technical assistance.

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アズキゾウムシ抵抗性リョクトウ給餌がマウスの生育と血液生化学値におよぼす影響

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摘　要

豆類の耐虫性育種は近年急速に進展し，リョクトウにおいては野生系統TC1966に由来するアズキゾウムシに対する抵抗性の利用が進められている。この抵抗性は単一の優性遺伝子によって支配され，本遺伝子を持つリョクトウは，アズキゾウムシその他の害虫に有害な殺虫性物質を含んでいる。本報では，アズキゾウムシ感受性品種大阪リョクトウ（OR）を反復親とした戻し交配によってこの遺伝子を導入した同質遺伝子系統$B_{14}F_4$の種子をマウスに与え，生育阻害の有無，臓器の病理組織学的変化および血液・生化学変化について検討した。$B_{14}F_4$および対照としてORの種子をそれぞれ65％含む飼料で４週間マウスを飼育した。両種子は，豆類に多い消化酵素阻害因子の作用を除くため，105℃25分間加熱したものと無加熱のものを供試した。参照のため，市販標準飼料を同様に給与した。その結果，$B_{14}F_4$で飼育したマウスの飼料消費量および４週間の体重増加は，雌雄ともORおよび市販飼料で飼育したマウスのそれとの間に差異は認められなかった。また，加熱処理の有無による体重の増加のちがいは，$B_{14}F_4$でもORでもみられなかった。

各臓器の体重に対する比率は，肺，心臓，腎臓，および脾臓において雌雄とも全ての飼料間で有意差は認められなかった。肝臓についても無加熱の$B_{14}F_4$で飼育した雄が無加熱のORに較べて大きい値を示したが，加熱飼料では$B_{14}F_4$またはORを与えた雄間で差はみられず，雌においては全ての飼料間で差はみられなかった。これらの臓器を含む16種類の臓器において病理組織学的に異常は発見されなかった。血液・生化学的検査においては，赤血球数，白血球数，ヘモグロビン濃度，ヘマトクリット値，血中アルカリフォスファターゼおよびグルコース濃度に関して雌雄とも全ての飼料間で差異は認められなかった。ただし，$B_{14}F_4$を与えた雌においてのみ血中GOT濃度が他の飼料群のいずれに較べても高く，総コレステロールはやや低いなどの変化が検出された。

以上，野生リョクトウTC1966由来の殺虫性因子をもつリョクトウの給餌によるマウス亜急性試験の結果，生育，各種臓器および血液学的検査値にほとんど影響はみられなかったものの，生化学的検査値において全く影響がみられないとは言えず，耐虫性遺伝子をもつ本リョクトウ系統の有用性を考慮すると，これを食用に供するためには，さらに長期の飼育試験等によりその安全性を確認する必要がある。

キーワード：*Vigna radiata*，*Callosobruchus chinensis*，耐虫性遺伝子，病理組織学的変化，飼料安全性試験