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THE INFLUENCE OF FEEDING GMO-PEAS ON GROWTH OF ANIMAL MODELS

Petr Mares, Tunde Jurikova, Jiri Sochor, Ladislav Zeman, Mojmir Baron, Jiri Mlcek, Stefan Balla

ABSTRACT

Introduction of genetically modified (GM) food or feed into the commercial sale represents a very complicated process. One of the most important steps in approval process is the evaluation of all risks on the health status of people and animal models. Within our project the genetically modified peas was breeded that showed significant resistance against *Pea seed-borne mosaic virus* and *Pea enation mosaic virus*. Preclinical studies have been conducted to found out the effect of GMO peas on animals - rats of outbreeding line *Wistar*. In a total, 24 male, specific pathogen free *Wistar* rats were used in the experiment. At the beginning of the experiment, the animals were 28 days old. The three experimental groups with 8 individuals were created. The first group of rats was fed with GMO peas, the second group of rats consumed mix of pea cultivar Raman and the third group was control without pea addition (wheat and soya were used instead of pea). In the present study we focused our attention on health, growth and utility features of rats fed with GM pea. All characteristic were observed during the experiment lasting 35 days. Consumed feed was weighted daily and the weight of the animals was measured every seven days. The average values were compared within the groups. The aim of the experiment was to verify if resistant lines of pea influence the weight growth of animal models. The results of our experiment showed that even a high concentration (30% of GM pea) did not influence growth rate of rats to compare with both rats fed with pea of Raman cultivar and control group. We did not observe any health problems of animal models during the experiment.

Keywords: geneticaly modified crops; pea preclinical studies

INTRODUCTION

Crop that have been genetically modified include plants with changes in DNA structure through genetic engineering **(Morisset et al., 2008)**. Genetic modifications have been considered as modern plant breeding methods in biotechnologies and depend on spontaneous processes in nature. It does not mean creating and transfer of artificial genes. GMO crops have some specific features such as resistance to harmful conditions including pests and diseases **(Han and Jung 2013)**, low temperatures **(Sakamoto et al., 2004)**, drought **(Lawlor, 2013)** etc., or tolerance to affusion against non-selective herbicides used to kill ineligible plants **(Gryson, 2010; Ujhelyi et al., 2012)**. Genetic engineering facilitates the transfer of desired characteristics into other plants, which is not possible through conventional plant breeding **(Ahmad et al. 2012)**.

Pea (*Pisum sativum L.*) is attacked by wide range of pests and pathogens. Some of them can cause economically significant diseases and losses. The pea is sensitive to a large number of viruses transferred by pea weevil. More than 120 species of viruses that are able to infect a pea has been noticed. Only some of them occurred in such rate that they could be considered as economically significant **(Larsen et al., 2007; Pflughoft et al., 2012)**.

Recently researches detected that only two of the viruses - Pea enation mosaic virus and Pea seed-borne mosaic virus had economic effect on pea production. The Pea enation mosaic virus (PEMV) is unique within plant viruses. In fact, it is occurred in the form of two viruses in obligatory symbiosis - PEMV-1 (Enamovirus) and PEMV-2 (Umbravirus). The presence of both viruses is necessary for induction of wild type infection **(Hodge and Powell 2010)**. Pea seed-borne mosaic virus (PSMV) is a typical representant of genus Potyvirus. The disease was first described in the former Czechoslovakia by Musil **(Musil, 1966)**, and a year later in Japan **(Inouye, 1967)** and two years later it was recorded in the USA **(Stewenson and Hagedorn, 1969)**. Typical symptoms of infection with this virus are pea leaf roll leaves, shortening of internodes, degree of stunting of infected plants, further deformation of the flowers and development of small deformed pods. Transfer by seeds is a reason for its easy spreading as it has been noticed in worldwide important crops as pea, lentic or broad bean. The spreading by seeds up to 30% in sensitive pea seeds has been noticed, but there were also cases in which 90% of commercial seed was infected. Nowadays these two previously mentioned species of viruses are extended in leguminous plants worldwide **(Safarova et al., 2008)**. The viruses are naturally transferable by vectors, namely by aphids, non-persistent type such as *Mysus persicae*, *Aphis craccivora*, *A. fabae* **(Aapola and Mink, 1973; Kvicala and Musil, 1973)**. Nowadays four pathotypes PSbMV called P1, P2, P3 and P4 has been described, from which in the Europe commonly occurs the pathotype P1 and last year the occurrence of pathotype P4 was confirmed **(Alconero et al., 1986, Hjulsager et al., 2002; Johansen et al., 1991)**. Resistance to standard pathotypes PSbMV is based on recessive genes *sbm-1*, *sbm-2*, *sbm-3* and *sbm-4*, which correspond to the mentioned pathotypes PSbMV **(Nicaise et al., 2003)**.

The aim of the present experiment was to study the effect of GM pea resistant to PEMV and PSMV viruses on morphological parameters and weight gain in rat models.

MATERIAL AND METHODOLOGY

The experiment was conducted at Department of animal nutrition and forage production of Agronomic faculty of Mendel University in Brno (in according with animal cruelty law No. 246/1992 Sb).

In a total, 24 male, specific pathogen free *Wistar* rats (Biotest, Konarovice, Czech Republic) were used in the experiment. At the beginning of the experiment, the animals were 28 days old and differences in body weight were in a range ±5 g. The animals were kept in an air-conditioned room with stable temperature 23 ±1 °C, light period 12 hours and humidity 60%. Photoperiod was based on pattern 12 hour/day and 12 hour/night with maximum intensity 200 lx. The monitored air content of CO_2 was - max. 0.25% and NH_3 max. 0.0025%. Food and water was provided ad libitum.

The experiment started after 8 days of quarantine period. The animals were divided into three groups, each of 8 animals. The experiment lasted 35 days. One group served as a control and these rats had not been fed with GM peas. Instead of pea the mixture of whey and soya was used. The remaining two groups were supplemented with 30% of GM pea or *Raman* pea according to the following scheme:

Composition of feed mixture is given in Table 1. Feed mixture KS1 was used as a negative control, the second KS 2 contained GMO pea and the third KS 3 contained was created by pea cultivar Raman.

The data were processed using MICROSOFT EXCEL® (USA) and STATISTICA.CZ Version 10.0 (Czech Republic). Differences with p-value ≥0.05 (α = 5%) were considered significant and were determined by T-test, which was applied for means comparison.

Following parameters were monitored and calculated individually in groups of rats during the experiment: net intake of feed, conversion of feed, weight increment and health (were studied anatomical pathology and bacteriology parasitological virological indicators) status of animals.

The animals were treated and fed every day and once a week they were weighted.

The conversion of feed mixture was calculated according to the following formula:

Conversion = Feed consumption / (Final weight - Starting weight)

RESULTS AND DISCUSSION

During the last years the research and breeding activities have been focused on problems of pea viruses **(Safarova et al., 2008).** This concern was caused by frequent and repeating pea viruses occurrence **(Jeger et al., 2012; Soylu and Dervis 2011)**. It has been confirmed that only using of cultivars resistant to economically significant viruses represent effective measures against their negative influence on pea production and its seeds.

Changes in body weight of experimental animals

The changes in body weight were observed daily during the period of 35 days. The weights of the animals in three experimental groups are recorded in tables 2, 3 and 4.

The average weight values of the three groups were compared in. In the first group (rats feeded by mixture 1, negative control) was noticed the average weight 343 g at the end of experiment. In the second group (experiment utilized GMO pea as feeding mixture), the average weight reached up 331 g and in the third group (positive control) it was 348 g. There have not been noticed any statistically significant differences among compared groups. Standard deviations between groups were less than 3 %. Average values of weights at the end of experiment (after 35 days) are presented in Figure 1.

All experimental animals were in good health condition without any differences in growth and changes in behaviour.

Table 1 Composition of feed mixture in experimental groups

Feed mixture /Experimental groups	KS 1	KS 2	KS 3
Composition (%)	Negative control	GM peas	Positive control
Wheat	60.00	43.80	43.80
Peas – species *Raman*	0.00	0.00	30.00
Peas – resistant line	0.00	30.00	0.00
Maize	11.00	10.00	10.00
Pollards from soya (47.5%)	12.00	0.00	0.00
Starch	10.84	10.00	10.00
Lysine (78%)	0.46	0.00	0.00
Premix of micro and macroelements	3.00	3.00	3.00
Premix of vitamins	0.20	0.20	0.20
Sunflower oil	2.50	3.00	3.00
Total	100.00	100.00	100.00

Table 2 Increase of weight of rats fed mixture 1

Negative control	1. day	7. day	14. day	21. day	28. day	35. day
1	134	179	213	257	293	360
2	114	158	200	247	289	349
3	133	169	212	262	306	342
4	123	158	202	251	284	333
5	131	164	213	260	297	338
6	134	171	227	267	308	347
7	125	160	216	256	289	328
8	141	179	218	248	330	345
Average	129	167	213	256	300	343
Sandard deviation	8.4	8.8	8.4	7.0	14.0	9.9

Table 3 Increase of weight of rats fed mixture 2

GMO peas	1. day	7. day	14. day	21. day	28. day	35. day
1	136	168	235	277	324	358
2	124	160	214	258	306	306
3	130	172	220	270	311	338
4	120	160	211	252	292	309
5	120	164	215	262	311	352
6	121	158	202	258	289	339
7	120	154	215	271	277	321
8	120	167	215	258	297	327
Average	124	163	216	263	301	331
Standard deviation	5.8	5.9	9.3	8.3	15.1	18.9

Table 4 Increase of weight of rats fed mixture 3

Positive control	1. day	7. day	14. day	21. day	28. day	35. day
1	118	149	202	273	310	314
2	145	183	232	266	304	341
3	116	160	198	241	287	346
4	136	174	214	251	301	372
5	125	161	196	234	273	354
6	123	177	218	268	322	370
7	133	175	213	242	288	356
8	120	156	212	278	315	330
Average	127	167	211	257	300	348
standard deviation	9.9	11.9	11.9	16.6	16.2	19.6

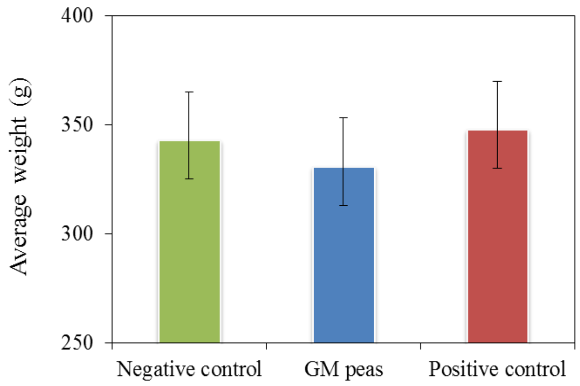
The average values of weight were compared in three groups. In the first group (rats fed mixture 1, negative control) was noticed the average weight 343 g at the end of experiment. In the second group (experiment fed GMO pea), the average weight reached up 331 g and in the third group (positive control) it was 348 g. There have not been noticed any statistically significant differences among compared groups. Standard deviations between groups were less than 3%. Average values of weights at the end of experiment (after 35 days) are presented in Figure 1.

All experimental animals were in good health condition without any differences in growth and changes in behaviour.

Study of feeding conversion

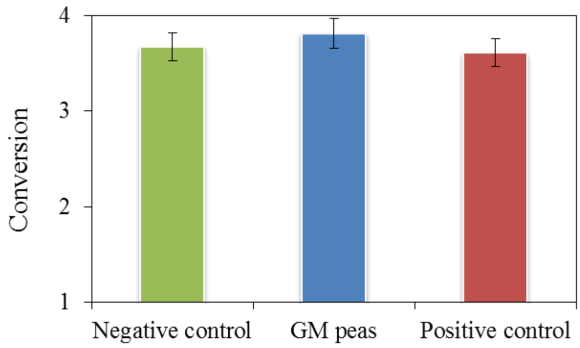
Feed conversion is defined as ratio between total increase of animal to feed consumption. It is calculated difference between weight at the begging and end of experiment. This total increase is rated by feed consumption during experimental period. All necessary values of 24 experimental animals are expressed in table 5.

Results of feeding conversion showed that there were no statistically significant differences between observed groups. Values reached up from 3.61 up to 3.81. Results of feeding conversion are given at Figure 2.



p-value ≥ 0.05, $\alpha = 5\%$

Figure 1 The average increases in weight of rats in three experimental groups



p-value ≥ 0.05, $\alpha = 5\%$

Figure 2 Feeding conversion during experimental period

Table 5 Feeding conversion during experiment period

	Starting weight	Final weight	Total growth	Feed consumption	Conversion
Negative control	1038	2746	1707	6268	3.67
GM peas	994	2651	1657	6312	3.81
Positive control	1020	2786	1766	6371	3.61

Although the genetic transformation of leguminous plants has been considered as very difficult and published protocols are reproducible with difficulties, there have been created new GM materials with declared resistance to biotic factors. From studied legumes - soybean, bean, pea, chickpea, peanut and vigna was released only soybean **(Huyghe, 1998; Wang and Brummer 2012)**. Precising and acceleration of breeding process has been possible by rapid development of information and techniques. It has been realised thank to adequate DNA markers in binding to genes of resistance, in ideal cases by direct detection of responsible genes **(Gilliland et al., 2003)**. The results of our experiment suggest that feeding GM pea to rats does not have detrimental effect on their health. There is potential possibility of GM peas utilisation and ranking GM pea into the List of approved GM crops.

CONCLUSION

The aim of presented experiment was to verify the influence of feeding of GM pea on health conditions, growth and quality parameters of rats. The results of the experiment showed that high level of GM pea in feed had no statistically significant effect on weight increase in comparison with both the group fed by pea cultivar Raman and negative control. No health complications were noticed during the experiment in model animals.

REFERENCES

Aapola, A. A., Mink, G. I. 1973. Potential aphid vectors of pea seedborne mosaic-virus in Washington. *Plant Disease Reporter*, vol. 57, no. 6, p. 552-552.

Ahmad, P., Ashraf, M., Younis, M., Hu, X. Y., Kumar, A., Akram, N. A., Al-Qurainy, F. 2012. Role of transgenic plants in agriculture and biopharming. *Biotechnology Advances*, vol. 30, no. 3, p. 524-540. http://dx.doi.org/10.1016/j.biotechadv.2011.09.006 PMid: 21959304

Alconero, R., Hoch, J. G. 1989. Incidence of pea seedborne mosaic-virus pathotypes in the United States national pisum germplasm colection. *Annals of Applied Biology*, vol. 114, no. 2, p. 311-315. http://dx.doi.org/10.1111/j.1744-7348.1989.tb02107.x

Gilliland, A., Singh, D. P., Hayward, J. M., Moore, C. A., Murphy, A. M., York, C. J., Slator, J., Carr, J. P. 2003. Genetic modification of alternative respiration has differential effects on antimycin A-induced versus salicylic acid-induced resistance to Tobacco mosaic virus. *Plant Physiology*, vol. 132, no. 3, p. 1518-1528. http://dx.doi.org/10.1104/pp.102.017640 PMid: 12857832

Gryson, N. 2010. Effect of food processing on plant DNA degradation and PCR-based GMO analysis: a review. *Analytical and Bioanalytical Chemistry*, vol. 396, no. 6, p. 2003-2022. http://dx.doi.org/10.1007/s00216-009-3343-2 PMid: 20012944

Han, S. W., Jung, H. W. 2013. Molecular sensors for plant immunity; pattern recognition receptors and race-specific resistance proteins. *Journal of Plant Biology*, vol. 56, no. 6, p. 357-366. http://dx.doi.org/10.1007/s12374-013-0323-z

Hjulsager, C. K., Lund, O. S., Johansen, I. E. 2002. A new pathotype of Pea seedborne mosaic virus explained by properties of the p3-6k1-and viral genome-linked protein (VPg)-coding regions. *Molecular Plant-Microbe Interactions*, vol. 15, no. 2, p. 169-171. http://dx.doi.org/10.1094/MPMI.2002.15.2.169 PMid: 11876428

Hodge, S., Powell, G. 2010. Conditional facilitation of an aphid vector, *Acyrthosiphon pisum*, by the plant pathogen, pea enation mosaic virus. *Journal of Insect Science*, vol. 10, p. 1-14 http://dx.doi.org/10.1673/031.010.14115 PMid: 21067425

Huyghe, C. 1998. Genetics and genetic modifications of plant architecture in grain legumes: a review. *Agronomie*, vol. 18, no. 5-6, p. 383-411. http://dx.doi.org/10.1051/agro:19980505

Inouye, T. 1967. A seed-borne mosaic virus of pea. *Japanese Journal of Phytopathology*. vol. 33, p. 38-42. http://dx.doi.org/10.3186/jjphytopath.33.38

Jeger, M., Chen, Z. Y., Cunningham, E., Martin, G., Powell, G. 2012. Population biology and epidemiology of plant virus epidemics: from tripartite to tritrophic interactions. *European Journal of Plant Pathology*, vol. 133, no. 1, p. 3-23. http://dx.doi.org/10.1007/s10658-011-9913-0

Johansen, E., Rasmussen, O. F., Heide, M., Borkhardt, B. 1991. The complete nucleotide-sequence of pea seed-borne mosaic-virus RNA. *Journal of General Virology*, vol. 72, p. 2625-2632. http://dx.doi.org/10.1099/0022-1317-72-11-2625 PMid: 1940858

Kvicala, B. A., Musil, M. 1967. Transmission of pea leaf rolling virus by aphids. *Biologia*, vol. 22, no. 1, p. 10-16. PMid: 6043114

Larsen, R., Timmerman-Vaughan, G., Murray, S. 2007. Molecular evidence that Pea enation mosaic virus is seed borne but not seed transmitted in *Pisum sativum*. *Phytopathology*, vol. 97, no. 7, p. S62-S62.

Lawlor, D. W. 2013. Genetic engineering to improve plant performance under drought: physiological evaluation of achievements, limitations, and possibilities. *Journal of Experimental Botany*, vol. 64, no. 1, p. 83-108. http://dx.doi.org/10.1093/jxb/ers326 PMid: 23162116

Morisset, D., Stebih, D., Cankar, K., Zel, J., Gruden, K. 2008. Alternative DNA amplification methods to PCR and their application in GMO detection: a review. European *Food Research and Technology*, vol. 227, no. 5, p. 1287-1297. http://dx.doi.org/10.1007/s00217-008-0850-x

Musil, M., 1966. Über das Vorkommen des Virus des Blattrollens der Erbse in der Slowakei (Vorläufige Mitteilung). *Biologia* (Bratislava) vol. 21, p. 133-138.

Nicaise, V., German-Retana, S., Sanjuan, R., Dubrana, M. P., Mazier, M., Maisonneuve, B., Candresse, T., Caranta, C., Le Gall, O. 2003. The eukaryotic translation initiation factor 4E controls lettuce susceptibility to the potyvirus Lettuce mosaic virus. *Plant Physiology*, vol. 132, no. 3, p. 1272-1282. http://dx.doi.org/10.1104/pp.102.017855 PMid: 12857809

Pflughoft, O., Merker, C., Von Tiedemann, A., Schafer, B. C. 2012. Incidence and Importance of Fungal Infection in Field Peas *(Pisum sativum L.)* in Germany. *Gesunde Pflanzen*, vol. 64, no. 1, p. 39-48.

Safarova, D., Navratil, M., Petrusova, J., Pokorny, R., Plakova, Z. 2008. Genetic and biological diversity of the pea seed-borne mosaic virus isolates occurring in Czech Republic. *Acta Virologica*, vol. 52, no. 1, p. 53-57.

Sakamoto, A., Sulpice, R., Hou, C. X., Kinoshita, M., Higashi, S. I., Kanaseki, T., Nonaka, H., Moon, B. Y., Murata, N. 2004. Genetic modification of the fatty acid unsaturation of phosphatidylglycerol in chloroplasts alters the sensitivity of tobacco plants to cold stress. *Plant Cell and Environment*, vol. 27, no. 1, p. 99-105. http://dx.doi.org/10.1046/j.0016-8025.2003.01131.x

Soylu, S., Dervis, S. 2011. Determination of prevalence and incidence of fungal disease agents of pea (*Pisum sativum L.*) plants growing in Amik plain of Turkey. *Research on Crops*, vol. 12, no. 2, p. 588-592.

Stevenson, W., Hagedorn, D. J. 1969. A new seed-borne virus of peas. *Phytopathology*, vol. 59, no. 8, p. 1051.

Ujhelyi, G., Van Dijk, J. P., Prins, T. W., Voorhuijzen, M. M., Van Hoef, A. A., Beenen, H. G., Morisset, D., Gruden, K., Kok, E. J., 2012. Comparison and transfer testing of multiplex ligation detection methods for GM plants. *Bmc Biotechnology*, vol. 12, p. 4. http://dx.doi.org/10.1186/1472-6750-12-4 PMid: 22257760

Wang, Z. Y., Brummer, E. C., 2012. Is genetic engineering ever going to take off in forage, turf and bioenergy crop breeding? *Annals of Botany*, vol. 110, no. 6, p. 1317-1325. http://dx.doi.org/10.1093/aob/mcs027 PMid: 22378838

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Contact address:

Petr Mares, Mendel University in Brno, Faculty of Agronomy, Department of Animal Nutrition and Forage Production, Zemedelska 1, 613 00 Brno, Czech Republic, E-mail: maresp@mendelu.cz

Tunde Jurikova, Constantine the Philosopher University in Nitra, Faculty of Central European Studies, Institut for education of pedagogics, Drazovska 4, 949 74 Nitra, Slovakia, E-mail: tjurikova@ukf.sk

Jiri Sochor, Mendel University in Brno, Faculty of Horticulturae, Department of viticulture and enology, Valtická 337, 691 44 Lednice, Czech Republic, E-mail: sochor.jirik@seznam.cz

Ladislav Zeman, Mendel University in Brno, Faculty of Agronomy, Department of Animal Nutrition and Forage Production, Zemedelska 1, 613 00 Brno, Czech Republic, E-mail: ladislav.zeman@mendelu.cz

Mojmir Baron, Mendel University in Brno, Faculty of Horticulturae, Department of viticulture and enology, Valtická 337, 691 44 Lednice, Czech Republic, E-mail: sochor.jirik@seznam.cz

Jiri Mlcek, Tomas Bata University in Zlin, Faculty of Technology, Department of Food Analysis and Chemistry, T. G. Masaryk Sq. 275, 762 72 Zlin, Czech Republic, E-mail: mlcek@ft.utb.cz

Stefan Balla, Constantine the Philosopher University in Nitra, Faculty of Central European Studies, Institut for education of pedagogics, Drazovska 4, 949 74 Nitra, Slovakia, E-mail: sballa@ukf.sk