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THE INFLUENCE OF REGULAR AND GENETICALLY MODIFIED SOYBEANS ON POSTNATAL DEVELOPMENT OF RATS

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Abstract: *Ukraine is one of the European and world leaders in soybean cultivation. According to some estimation, 30-90 % of the crops area is used currently for cultivation of genetically modified varieties. As modified soybean areas are expanding, the potential nutritional threats related to this product should be thoroughly assessed.*
The influence of nutrition ration consisting of 20 % of the thermo-treated genetically modified soybeans on postnatal development of rats has been investigated over two generations in comparison with nutrition by regular thermo-treated beans. The number of alive and dead newborns, average number of offspring and the survival rate has been calculated as well as general estimation of physical development of the newborn rats was made.
The experimental results prove that the reproductive function of rats and the offspring development are not seriously influenced by nutrition with genetically modified soybeans within the first and second generations. No statistically reliable difference was found between the characteristic parameters of the experimental and control groups, which remained within their regular physiological limits. However, some decrease in the suckling age rats' number was registered in the experimental group.

Keywords: *regular soybean, genetically modified soybean, rats, postnatal development, survival rate.*

1. Introduction

As molecular biology, biotechnology and gene engineering reach new advance, construction of new plants varieties and cattle breeds becomes easier and more available for agricultural and industrial users avoiding traditional selection methods and procedures. Moreover, it is quite feasible now to construct even the genotypes that never existed before by introducing the alien genes into DNA of a given variety. This way new functions or characteristics of the variety can be obtained. Such genetically modified organisms (GMO) are becoming more and more popular in many fields of medicine, agriculture, cattle breeding, food production and processing. The widest introduction of GMO in crop production is known for soybean, maze, cotton, rape, sugar beet, papaya, pumpkin, paprika, tomato, rice, potato, plum, haricot bean, alfalfa, wheat, groundnuts, mustard, cauliflower and chili pepper [1, 2].

Soya is extensively used in many fields of food production and agriculture because of its useful merits. Soybean consists of 36-48 % of proteins, 13-27 % of oils, 17-34 % of carbohydrates and brings other useful components like vitamins (thiamin, riboflavin, pyridoxine, folic acid, choline, vitamin E and C), macro- and micro elements (K, P, Ca, Mg, Fe, Zn, Mn and Se) and bioactive compounds (phospholipids, tocopherols, phytoestrogens and some others) [3-5]. High nutritional values and optimal composition ensure wide distribution of soybeans as a component of various

foods and food additives. Soya is used in human nutrition directly as well as soybean oil, milk, tofu cheese and as an additive for a wide variety of food such as chocolate and sausage. It is reported that about 60 % of regular food consist of some soybean products [4].
Some gene modifications (GM) are being made in order to achieve higher crop productivity and better resistance of soya against diseases, pests, herbicides, abiotic stresses and other negative influences. First commercial GM soya varieties have been introduced in 1996 while according to the estimation of International Service for the Acquisition of Agri-Biotech Applications [6], current crop area occupied by various GM plants has reached 185.1 mln ha in 2016. More than 50 % of this area is occupied by four cultures: soybean, cotton, maze and rape). More than 30 countries are involved in the GM crops cultivation while more than 60 countries – in the consumption.
Ukraine is one of world leaders and the European leader in GM soya cultivation. Current estimation shows that 30-90 % of all soya crops in Ukraine is occupied by the GM varieties [7] still remaining beyond strict government control of the seed grain quality and amount. This estimation has also been proven by independent analysis of the cattle forage and additives performed in 2013-2015 [8]. Further uncontrolled spreading of GMO can provoke more or less deeper ingression of the modified soya in human food.
There are still vivid discussions on possible threats and food safety of GMO. As seen from analysis of hundreds scientific papers dealing with this problem reviewed in [9], there is still no doubtful evidence of distinct negative effects of GMO or GMO-containing food on human health. There is still no correlation found between consumption of the GMO-containing food and digestion, kidney diseases, diabetes, cancer, autism, obesity and various allergy symptoms.
On the other hand, the idea of potential threat of genetically engineered products is also popular and has many supporters stating that these components should be considered as dangerous as long as no indisputable safety evidences are found. Moreover, some primary deviations were found in the viscera morphogenesis as a result of nutrition with GMO components. Chronic intoxications and some diseases have also been reported for next generations of the lab animals [10-18].
That is why the discussion of possible safety/unsafety of GMO is still open and requires more data and evidences.
This work deals with investigation of possible influence of nutrition with traditional and genetically modified soybeans on postnatal development in the first and second generations of rats.

2. Experimental

All experiments were carried out with three generations of the Wistar line rats: parents (F0), first (F1) and second (F2). The source population consisted of males and females aged 3-3.5 months. The experiment lasted for 9 months. The animals were kept in a vivarium, in the standard plastic boxes with thermo-treated wood shavings substrate at 20-22 ^{0}C, relative humidity 50-60 % and under the standard day/night regime.
All rats were divided into three groups: one control and two experimental. All the groups were formed by random selection of the animals with the only account of the body weight. Each group consisted of 12 rats: 8 females and 4 males. The control group animals were fed according to the standard vivarium ration. The second group animals were fed with the food containing 20 % of thermo-treated soybeans of variety Chernivetska 9. Unlikely to the traditional procedure, the soybeans were

steeped in water during 12 hours then boiled during 60 minutes and finally dried at 115-125 ^{0}C. The third group animals were fed using the above procedure but with the genetically engineered soya Roundup Ready, line GTS 40-3-2: 20 % of their ration consisted of this GM material. All rations were optimized according to the standard norms and requirements and provided to the respective groups throughout the entire experimental period.

The reproduction capacity characteristics and some postnatal development data during first two months of life were investigated in all the groups. Following postnatal development characteristics were controlled: ratio between alive and dead youngs, average offspring value, visual estimation of general physical development, body weight and survival rate. Further development of the rats was assessed by the auricle opening time, body hair eruption time, teething and eyes opening time.

Male and female rats were combined for the fertilization with ratio 2:1 for a single estrous period (5 days). The baby rats were put apart from their mothers on the 30th day and provided with the same adult ration. Further experiments were carried out with the descendants obtained from different mothers in order to randomize the investigation and avoid incest.

3. Results and discussion

Investigation of reproductive capacity/fertility is one of integral indicators for the hygienic evaluation of various potentially unsafe or threatening processes or phenomena. This capacity is very sensitive function that can be disordered under influence of many unfavorable impacts and effects. On the other hand, evaluation of reproductive capacity is very labor and time demanding procedure, which puts obstacles on wide introduction of this method to the food and food additives safety analysis. This approach is recommended only for an optional usage in case its necessity is obvious. Even after more than 20 years long efforts in investigation of possible adverse effects of GMO on the human and cattle health, there is still not enough evidence to support or reject this assumption. That is why results of investigation of reproductive capacity should shed more light on this problem.

It has been found that the pregnancy time was unchanged in both experimental groups comparing to the value of the control group. All baby rats were delivered after 21-23 days of pregnancy.

An average offspring number was 9.4 ± 2.4 individuals in the control group F1 generation; 8.0 ± 2.1 individuals – in the second group and 8.5 ± 1.5 individuals in the third group. No deviations were found in physical development of young rats from all the groups [19]: the auricle opening time was 3-4 days; body hair erupted after 5-6 days; teething time was 9-10 days and eyes opening time was 15-16 days. No visible mutilations were registered.

Survival rate of the F1 rats was quite high in all the groups (see Table 1). It is easy to see that the corresponding values for different groups are rather close. Death rate for the first five days was 5.3 % (control group), 6.2 % (second group) and 7.3 % (third group). Same rates for the next 25 days period were correspondingly 4.2 %, 8.3 % and 12.7 %.

Gender ratio in the control group was balanced while insignificant prevalence of the young males was registered in both experimental groups.

It has been shown [20] that physical condition of the female rats is the key factor governing reproductive capacity of the rodent. Fat deposits and body weight are growing during the pregnancy time to store enough metabolic resource to be used for lactation.

Table 1.

Survival rate indexes for the F1 offspring

Group	Number of baby rats, individuals	First 5 days survival rate		Next 25 days survival rate	
		Individuals	%	Individuals	%
I	75	71	94.7	68	95.8
II	64	60	93.8	55	91.7
III	68	63	92.7	55	87.3

Gaining body weight during pregnancy is the key factor for the successful pregnancy ratio, fertility, offspring gender balance, baby rats' survival ratio, the rate of their development and overall reproductive capacity in the population. Insufficiently fed females bring more male babies. This gender regulation occurs due to the embryonic mortality and results in lowering of the female number in the population, which decreases its reproductive potential.

It has been found that the body weight increase rate stayed within normal values for all three groups over early postnatal time and mixed feeding period. No difference has been found between the first generation of rats raised under traditional and genetically modified soybean feeding. All indexes of their development didn't exceed corresponding normal values.

Survival rate parameters remained high for the F2 generation animals as well (see Table 2). For instance, the initial five days death percentage was 6.7 % (control group); 6.1 % (second group) and 8.9 % (third group) while the next 25 days death percentages were correspondingly 7.1 %, 8.1 % and 16. 4 %.

Table 2.

Survival rate indexes for the F2 offspring

Group	Number of baby rats, individuals	First 5 days survival rate		Next 25 days survival rate	
		Individuals	%	Individuals	%
I	60	56	93.3	52	92.9
II	66	62	93.9	57	91.9
III	67	61	91.1	51	83.6

It is seen that postnatal death rate for the third group F2 animals was somewhat higher than that in the second and control group. This effect can be caused by a long term influence of phytoestrogens and other bioactive compounds coming to the organism from GM soybeans. Normal embryonal development can be disturbed by these compounds resulting in the birth of debilitated or unviable individuals. This effect is especially dangerous for the reproductive hormones balance both for males and females [21] and, according, to some recent data affects primarily normal functioning of hypothalamus and hypophysis through the regime of growth hormone generation [22]. All adverse effects of phytoestrogens disappear after stopping of feeding with GM materials.

An average F2 offspring number in the control group was 7.5 ± 1.7 individuals; in the second group – 8.3 ± 1.8 and in the third group – 8.4 ± 1.9. As seen from these data, the F2 offspring number in the control group was somewhat lower than that in the other groups but these values remained comparable and within the physiological norms.

No relevant difference has been found between the groups in the F1 baby rats' weight. This parameters also stayed within physiological norms for the corresponding age (m (group I) = 5.7 ± 0.54 g; m (II) = 5.2 ± 0.49 g; m (III) = 5.3 ± 0.52 g). Same

pattern was registered also for the F2 animals' weight after 28 days (see Fig. 1).
No statistically relevant disturbances in the offspring gender balance have been registered. Besides, overall physical development of all F2 baby rats was found satisfactory: the auricle opening time was 3-4 days; body hair eruption time was 4-6 days; teething time was 9-10 days and eyes opening time was 15-17 days. All these values also lay within corresponding physiological norms [19]. No difference was found between animals' development for the control and experimental groups.

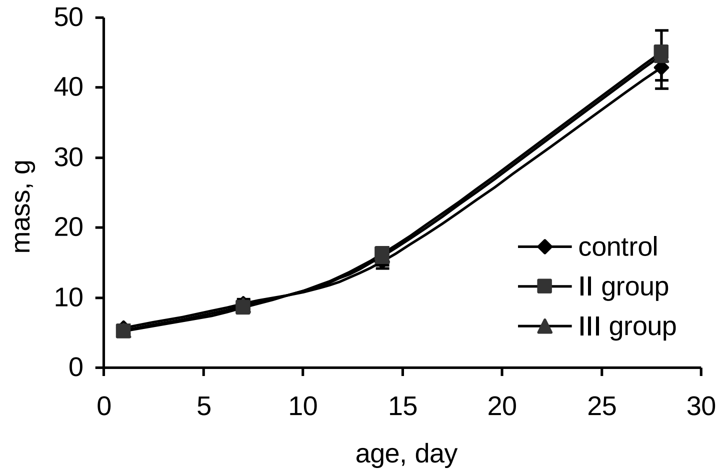

Fig. 1. Body weight dynamics for the F2 baby rats

4. Conclusions

No relevant evidence of negative or positive effect of feeding the rats with a food containing 20 % of thermo-treated genetically modified soya on reproductive capacity and physical conditions of the rats were found. All the animals showed similar rates and indexes of physical shape and postnatal development parameters despite different feeding regimes over the experimental and control groups. The parameters remained within physiological norms with the exception of some decrease in the suckling age rats number in the population fed with the genetically engineered soybean ration.

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