CHANGES IN THE PROTEIN PROFILE OF PIGS' BLOOD DURING FEEDING WITH GM SOYBEANS

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Abstract. *The study was conducted with 24 subjects (8 pigs and 4 boars in each group). The diet consisted of 10% full-fat extruded soybean; "Vorskla" varieties (without genetic modifications) in the control group and GM-soybean in the test group (GMOs, RR, GTS 40.3.2). There was a significant difference in the concentration of protein fractions in the blood of 8-month pigs fed GM soy: α2 globulin content was 21.54% lower (p = 0.001) and γ globulin content was 24.33% higher (p = 0.0005) than in the control, which indirectly indicates a decrease in estrogen levels in the blood of pigs. Changes in the individual indicators characterizing the resistance may further adversely affect the functional activity of the reproductive system of the body of a pig. Thus, by using genetically modified soybeans in diets of pigs, especially those intended for reproduction, it is necessary to monitor the biochemical composition of the blood, in particular the protein profile.*

Key words: *pigs, soybean, GMO, blood, metabolism, total protein, protein fractions*

Currently, genetic engineering is widely used in creating new varieties of crops with features that have been missing in the existing original varieties. New biotechnological methods allow for manipulation of the plants genetic code at the level of individual genes or their units [1, 2]. From the whole variety of already established and registered genetically modified (GM) plants in the world, 4-5 varieties have been successfully implemented into industrial growing. The most common in agricultural production is soybean that is steady to herbicides. Its share of the total grown soy is more than 60%, compared to certain varieties of cotton and corn that are resistant to pests, which represent 28 and 14%, respectively [3].

Despite scientific information on their adverse effects on the body of animals, GM feed has long been used in animal husbandry. From 2002 – 2005, M. Malatesta et al. [4, 5] found pathological changes in the liver of experimental mice fed with GM soybeans steady to the herbicide Roundup. In other studies on experimental mice fed with GM soy conducted by the same authors [6, 7], as well as scientists J. A. Magasa-Gumez et al. [8] pathological changes in the pancreas were revealed.

Research also found that first and second generation mice born to females who were fed with GM soy a long time had significantly increased body weight relative to control animals, an imbalanced mass of internal organs, and changed enzyme spectrum of blood: significantly decreased amylase, alkaline phosphatase and peroxidase. [9]

In 2008, an analysis of 40 experiments conducted by scientists around the world performed a risk assessment of changes in the homeostasis of experimental animals that had different lines of GM plants in their diet. 20 experiments showed statistically significant adverse shifts of indicators involved in the metabolism process in body [10].
However, there are scientific data which don't confirm the negative impact of genetically modified organisms (GMOs) on the health and physiological state of laboratory animals. In rats that received GM-soybeans within three generations, there were no significant negative or positive effects of GM-soy on their physiological state compared to animals fed with natural soy.

[11] Other studies have found insignificant influence on physiological condition and health of rats fed with genetically modified soy during three months (3Ø5423 × 40-3-2) [12]

The above-mentioned review of the literature suggests that the final answer on safety (danger) of GM feed for animals has not yet been received by the worldwide scientific community. This is emphasized by numerous modern scientific studies [13, 14, 15]. Therefore, research on the effects of their use in animal husbandry, particularly pig production, on the health and productivity of animals are highly relevant not only in terms of feed efficiency but also in terms of environmental safety and social development.

***The purpose of research** - is the study of changes in protein profile of pigs' blood with genetically modified soybeans in their feed rations.*

***Material and research methods**. Research conducted in accordance with international principles of the European Convention for the Protection of vertebrate animals used for experiments on them and other scientific purposes [16].*

The presence of genetically modified structures in the feed samples was determined in the genetics laboratory of the Institute of pig breeding and agricultural production NAAS in accordance with applicable regulatory documents on research methods: ISO 21569.2008, ISO 21570: 2008, ISO 21571.2008. For the analysis of the GM-event presence, DNA isolation from plant origin objects using a commercial kit "Sorbo-GM-B" ("Synthol", Russia) was used.

To study the effect of GM soy (RR, GTS 40.3.2) in the profile of blood proteins, scientific and economic research using "Poltava" pig meat breed was carried out. Pigs were contained in the state enterprise "Experimental basis "Nadija" of the Institute of pig breeding and agricultural production NAAS" according to method [17]. 24 clinically healthy pigs similar by weight were picked from a group of young pigs, and two experimental groups were formed. Each group included 8 pigs and 4 hogs.

Full-fat extruded soybean of "Vorskla" varieties (without genetic modifications) was included in the diet of control group of animals. In equal amounts by weight (10%), full-fat extruded GM-soybean (RR, GTS 40.3.2) was included in the diet of the research group (Table 1).

1. Composition of the pigs' feeding ration

Components	Amount, %	
	by weight	by nutrition value
barley	10,0	9,7
oat	10,0	9,3
soy extruded	10,0	10,5
corn	30,0	35,3
wheat	20,0	22,3
wheat bran	10,0	6,6
sunflower expeller	5,0	4,8
premix	3,5	1,5
salt, kg	0,5	
chalk kg	1,0	
Total	100,0	100,0

Blood for analysis was taken from the ear edge vein of animal, before feeding, at the age of 4 and 8 months. Biochemical blood parameters were determined using commercial kits made by the Ukrainian company "Filisit Diagnostika": total protein - with biuret reaction (g / l), protein fractions - with turbodimetric method [18].

Statistical analysis of the data was performed using the Microsoft Exel 2010 and Statistica 8.0 programs, after checking the normality of their distribution. Determined parameters of descriptive statistics: arithmetic mean and its error (M ± m), confidence interval (95% CI),

standard deviation (SD), and coefficient of variation (Cv) in the sample. Probability of difference was calculated using t-test for dependent and independent samples [19, 20, 21].

Results and discussion. According to the obtained data, the blood protein profile of pigs that received feed with genetically modified soybeans was slightly changed compared to control animals (Table. 2). The levels of total protein in the blood of 4 month-old pigs in both groups were almost identical, but at the age of 8 months, its concentration increased to 99,11 ± 11,235 g / l in the control group and 89,29 ± 3,858 g / l in the research group. The confidence interval of the obtained data for total protein concentration of 8-month-old pigs was 63,35-134,86 g / l in the control group and 77,01- 101.56 g / l in the research group. The coefficient of variation was higher in the control group compared to the research group: 22.672% against 8.641%.

2. The blood protein profile of the pigs under feeding conditions of GM soy, g / L (n = 5)

Indicator	Control (soy without GMO)		Research group (soy with GMO)	
	4 months	8 months	4 months	8 months
total protein	**72,32 ± 1,710**	**99,11 ± 11,235**	**71,43 ± 3,260**	**89,29 ± 3,858**$^{\#\#}$
95 % CI	66,88; 77,76	63,35; 134,86	61,05; 81,80	77,01; 101,56
SD	3,419	22,470	6,521	7,715
Cv	4,728	22,672	9,129	8,641
albumin	**29,70 ± 0,404**	**48,95 ± 0,548**$^{\#\#\#}$	**24,15 ± 0,375*****	**47,40 ± 1,848**$^{\#\#\#}$
95 % CI	28,41; 30,99	47,20; 50,70	22,96; 25,34	41,52; 53,28
SD	0,808	1,097	0,751	3,695
Cv	2,722	2,241	3,108	7,795
α_1 globulin	**7,45 ± 0,606**	**5,60 ± 0,462**	**6,55 ± 0,087**	**4,95 ± 0,548**
95 % CI	5,52; 9,38	4,13; 7,07	6,27; 6,83	3,20; 6,70
SD	1,212	0,924	0,173	1,097
Cv	16,274	16,496	2,644	22,161
α_2 globulin	**23,45 ± 3,320**	**15,80 ± 0,404**	**21,20 ± 0,231**	**13,00 ± 0,289** *** $^{\#\#\#}$
95 % CI	12,89; 34,01	14,51; 17,09	20,47; 21,93	12,08; 13,92
SD	6,640	0,808	0,462	0,577
Cv	28,314	5,116	2,179	4,441
β globulin	**20,10 ± 0,462**	**14,65 ± 0,029**$^{\#\#}$	**21,10 ± 0,173**	**15,95 ± 0,779**$^{\#\#}$
95 % CI	18,63; 21,57	14,56; 14,74	20,55; 21,65	13,47; 18,43
SD	0,924	0,058	0,346	1,559
Cv	4,596	0,394	1,642	9,773
γ globulin	**19,30 ± 1,848**	**15,00 ± 0,462**	**27,00 ± 0,115****	**18,65 ± 0,260** *** $^{\#\#\#}$
95 % CI	13,42; 25,18	13,53; 16,47	26,63; 27,37	17,82; 19,48
SD	3,695	0,924	0,231	0,520
Cv	19,145	6,158	0,855	2,786
A/G	**0,42 ± 0,008**	**0,96 ± 0,021**$^{\#\#\#}$	**0,32 ± 0,007*****	**0,91 ± 0,068**$^{\#\#}$
95 % CI	0,40; 0,45	0,89; 1,03	0,30; 0,34	0,69; 1,12
SD	0,016	0,042	0,013	0,135
Cv	3,870	4,388	4,096	14,875

Note: Compared to the control group * - $p \leq 0,05$, ** - $p \leq 0,01$, *** - $p \leq 0,001$
Compared to the initial period # - $p \leq 0,05$, ## - $p \leq 0,01$, ### - $p \leq 0,001$

Despite the fact that at the initial period of research (4 months) the amount of albumin in the blood of pigs from the experimental group was significantly lower by 22.98% (p = 0.000056) compared to the control, the amount of albumin in the blood of pigs from both groups significantly (p = 0.000001, p = 0.0019) increased at the age of 8 months and did not differ each from other. At 8 months, the amount of albumin in the experimental and control groups were equal to 47.40 and 48.95 g / l respectively. The concentration of α1 globulin in the blood of pigs with GMO in their diets did not differ significantly from the control group at the ages of 4- and 8 months, but the concentration of α1 globulin slightly decreased with age in both groups of animals. At the age of 8 months its concentration in the control and the experimental group was 5.60 and 4.95 g / l respectively, which is 24.84 and 24.43% less than in the initial period.

The concentration of α2 globulin in the blood of experimental animals varied depending on their age and composition of the diet. With age, it dropped by 48.42% in the control group and by 63.08% (p = 0.0005) in the experimental group. Essentially but unreliably due to high variability, the concentration of specified globulin in the control group decreased between 4 and 8 months, when it was equal to 15,80 g / l. In 8-month-old pigs that were fed with GM soy, the amount of α2 globulin was 21.54% lower (p = 0.001) than controls. Such change, in our view, can indirectly indicate a decrease in estrogen levels in the blood of pigs from research group, which will further negatively affect their reproductive ability.

Fraction of β globulin has also undergone a significant decrease in concentration with age, with a 37.20% drop in the control group (p = 0.002) and 32.29% drop in the experimental group (P = 0.01), but the factor of feeding didn't affect the specified index. The content of γ globulin also decreased from 4 to 8 months by 28.67% and 44.77% (p = 0.00001), respectively. Given that the concentration of γ globulin was 39.90% (p = 0.006) higher in the experimental group compared to the control animals at 4 months, its reduction at the age of 8 months is much more significant. Nevertheless, in the blood of 8-month experimental animals the number of γ globulin was significantly (p = 0.0005) higher compared to controls by 24.33% and was equal to 18.65 g / l.

Age-related changes in the concentration of albumin (growth) and globulin (decrease) affected the albumin-globulin ratio in the blood of pigs from control and experimental groups: it grew by 128.57% (p = 0.00003) and 184.38% (p = 0.004) respectively.

Conclusions

Significant changes in the concentration of protein fractions of blood in 8-month pigs receiving GM soy were observed: the amount of α2 globulin was 21.54% less (p = 0.001) and the number of γ globulin was 24.33% higher (p = 0 , 0005) than in controls, which indirectly indicates a decrease in estrogen level in the blood of pigs.

Changes of some indicators of the resistance may further adversely affect the functional activity of the reproductive system of the pig's body. When using genetically modified soy in the diets of pigs, especially those intended for reproduction, must maintain control of the biochemical composition of the pigs' blood, including its protein profile.

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