

Effect of feeding genetically modified maize and soybean meal to sows on their reproductive traits, haematological indices and offspring performance

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Received: April 2, 2013

Accepted: August 17, 2013

Abstract

The effect of Roundup Ready MON-40-3-2 soybean meal and *Bt* maize MON810 on sows performance and haematological indices, including parameters of erythrocytes, leukocytes, and trombocytes, as well as the piglets rearing indices were evaluated in the experiment carried out on 24 sows and their progeny. After mating sows were divided into groups: I – control, conventional soybean meal and conventional maize; II – genetically modified (GM) soybean meal and conventional maize; III – conventional soybean meal and GM maize; IV – GM soybean meal and GM maize. All used feed mixtures were isonitrogenous and isoenergetic, however differed in presence or absence of genetically modified maize MON810 (5% for pregnant and 8% for lactating sows) and/or soybean meal MON-40-3-2 (4% for pregnant and 14% for lactating sows). Born piglets were allotted to the same group as their mothers. The study showed that feeding pregnant and lactating sows with mixtures containing genetically modified RR soybean or/and meal *Bt* maize did not significantly affect their reproductive characteristics and offspring performance. There was no effect of dietary treatment on haematological indices. Transfer of transgenic DNA from GM feed to blood was not confirmed.

Key words: sows, piglets, genetically modified feed, animal feeding, blood indices, reproductive performance.

Introduction

Genetically modified (GM) crops have become more and more popular. Since the time, when the first GM plants appeared in 1996, the area of world GM crops increased about 94-fold (8). The investigation of Polish feed market in recent years indicated that the majority of soybean is imported in processed form as soybean meal and about 98% originated from GM plants (7, 19). Beside soybean meal, also maize is a very important material for producing compound feeds for farm animals raised for meat, milk or eggs. Modification of Roundup Ready (RR) soybean, used in the experiment, was developed by inserting a gene from *Agrobacterium* sp. (CP4 strain), that confers tolerance to glyphosate, an ingredient of herbicides.

GM maize contains the gene originating from soil bacterium *Bacillus thuringiensis* (*Bt*) encoding protein Cry1A(b), which has a specific activity against certain corn pests, such as the European corn borer. The mentioned modifications have some important and beneficial agricultural attributes – resistance to insect or herbicides without any influence on their nutritive value (1, 6, 14, 21).

Despite the fact that both feed materials are economically indispensable in farm animal feeding, the inclusion of GM products in animal and human diet induces some consumers concerns related to health: transfer of transgenic DNA, transfer of antibiotic resistance, potential toxicity in next generations, allergenicity of transgenic proteins or possibility of inflammatory reaction induction. Nevertheless, the

number of domestic studies connected with the influence of GM feeds on animal performance and health is still insufficient. It is worth noting, that in recent years, pig has become a well recognised model for research in physiology and immunology, due to a comparable reactions to humans, therefore it seems to be the best animal model for human health study (16). The aim of this study was to evaluate the effect of Roundup Ready MON-40-3-2 soybean meal and *Bt* maize MON810 on sows performance and haematological parameters as well as the piglets rearing indices.

Material and Methods

All procedures included in the experiment, relating to the use of live animals, were in agreement with the Local Ethic Committee for Experiments with Animals. During the entire research all animals were humanely cared for and remained under the monitoring of veterinary surgeon.

Animals management, feeding, and housing. The experiment was conducted on 24 sows (PL x WLP) mated with boar (Du x Pi). After mating sows were divided into four groups, kept in individuals pens and fed restricted amounts of compound feeds, according to the phase of reproductive cycle. All feeds were isonitrogenous and isoenergetic and were made of barley, wheat, wheat bran, maize, soybean meal, rapeseeds oil, crystalline amino acids, and vitamin-mineral premix. They differed, however, in the presence or absence of soybean meal MON-40-3-2 (4% for pregnant sows and 14% for lactating) and/or genetically modified maize MON810 (5% for pregnant sows and 8% for lactating). Feeds for pregnant sows (1-100th d of pregnancy) contained 11.8 MJ ME, 132 g crude protein, 6.8 g Lys, and 4.5 g Met + Cys. Feeds for pregnant (from 100th d of pregnancy to farrowing) and lactating sows contained 12.5 MJ EM, 162 g crude protein, 8.2 g Lys, and 5.5 g Met + Cys. Body weight was controlled at mating, 100th d of pregnancy, after farrowing, and after the weaning of piglets (28th d of lactation).

Scheme of the experiment for sows and piglets was as followed: Group I – control, conventional soybean meal and conventional maize; Group II – GM soybean meal and conventional maize; Group III – conventional soybean meal and GM maize; Group IV – GM soybean meal and GM maize. The progeny of all sows was also included in the experiment. After birth piglets remained in the same experimental group as their mother. Since 7th d of age, the piglets were fed diets differing in presence or absence of genetically modified soybean meal MON-40-3-2 (26% in diet) and/or maize MON810 (10% in diet). Feeds for all groups of piglets were similar in metabolic energy (13.3 MJ), content of crude protein (214 g), and amino acids level (Lys-13.2 g, Met+Cys- 7.8 g). On the 28th d

of age, all piglets were weaned and kept in group pens (every litter in separate pen) until 84th d of age.

Sampling procedures and analysis. Blood samples for haematological analysis collected taken from the jugular vein, from six sows from each group. The samples were collected twice: before the experimental feeding (0) and at the end of the trial (1). The following haematological indices were determined: red blood cell (RBC) count, mean cell volume (MCV), haemoglobin concentration (HGB), mean amount of cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), haematocrit value (HCT), platelet cell (PLT) count, mean platelet volume (MPV), white blood cell (WBC) count, and lymphocyte percentage (LYM). The haematological indices were evaluated with Celoscope-AutoCounter AC 920 (Swelab Instrument AB, Sweden).

The transfer of transgenic DNA, from tested GM feed materials to sows' organism, was also estimated. DNA was extracted from heparinised blood with commercial extraction kit (Blood Genomic AX Kit by DNA, Poland), according to the manufacturer's instruction. PCR was performed in 20 µL mixtures (Applied Biosystem, Inc., USA) containing: 1 x buffer for PCR; 0.2 mM dNTP; 2.5 mM MgCl₂; 0.5 µM forward and reverse primers; 0.025 U/µL of Amplitaq Gold polymerase, and genomic DNA templates. For detection of corn gene sequences, DNA was incubated in thermocycler under the following programme: 12 min denaturation at 95°C, and 40 cycles: 30 s at 95°C, 30 s at 64°C, and 30 s at 72°C, with final extention step of 3 min at 72°C. For detection of soybean gene sequences, DNA was incubated in thermocycler under the following programme: 10 min denaturation at 95°C, and 40 cycles: 30 s at 95°C, 30 s at 60°C, and 25 s at 72°C, with final extention step of 3 min at 72°C. In case of MON810 maize, the fragment of 170 bp, and for RR soybean, the fragment of 172 bp were amplified. The PCR products were analysed by electrophoresis in 2.0% agarose gels containing SYBRGOLD dye.

Statistical analysis of treatment effects was conducted by the one-way analysis of variance with comparison of means by Duncan's multiple range test at $P \leq 0.05$ and $P \leq 0.01$ levels of significance using the Statistica package.

Results

The experimental use of GM feeds did not significantly affect the sows and offspring performance. During the whole experiment, the body weight of sows did not significantly differ among groups (Table 1). The average body weight of sows at mating was similar in all groups and amounted to 206–225 kg, and at the end of reproductive cycle, body weight of all sows was higher by 19.1–22.3 kg, in comparison to the body weight at the beginning of cycle. During lactation sows lost weight: 9.9 kg

(group II) and 12.6 kg (group IV). There was no significant difference among groups in feed intake during the whole reproductive cycle (Table 1).

Body weight gains and feed utilisation of piglets were similar in all groups (Table 2). In the experiment, 265 piglets were born and their average body weight at 1st d of age was 1.46 kg and at weaning 7.21 kg. During the nursing period (1-28 d of age), the average daily weight gains amounted to 214 g and after weaning (28-84 d of age) – 346 g. The average number of piglets born in the litter was: 10.8 in groups I and III, 11.0 in group II, and 11.5 in group IV.

Table 3 presents the general haematological parameters in sows. In addition, Figs. 1 and 2 demon-

strate the mean volume of RBC and PLT. The received results, widely known as routine health parameters, point to the lack of significant influence of GM soybean meal MON-40-3-2 and maize MON810. The number of erythrocytes, leukocytes, and trombocytes, their volume and content of haemoglobin did not differ among groups. The mean level of blood parameters, estimated at the beginning of experiment (0) and at the end (1), were similar in both probes.

No fragments of transgenic DNA, typical for genetically modified soybean or maize, were detected in sows' blood samples (Table 4).

Table 1. Feed intake and changes in body weight of sows during the reproductive cycle

	Experimental group*				SEM
	I	II	III	IV	
Average body weight of sows (kg):					
at mating	225.5	206.5	215.7	225.0	5.514
100 d of pregnancy	266.3	248.0	255.8	267.7	4.938
farrowing	256.8	238.7	246.2	259.3	5.061
weaning piglets	245.2	228.8	234.8	246.7	4.915
Changes in body weight (kg):					
mating – 100 d of pregnancy	40.8	41.5	40.1	42.7	1.671
100 d of pregnancy – farrowing	9.5	9.3	9.6	8.4	0.739
mating – farrowing	31.3	32.2	30.5	34.3	2.005
lactation	11.6	9.9	11.4	12.6	1.037
total reproductive cycle	19.7	22.3	19.1	21.7	1.967
Average feed intake per sow (kg):					
mating – 100 d of pregnancy	244.2	242.8	243.8	241.7	0.991
100 d of pregnancy – farrowing	63.6	62.4	57.2	63.6	1.295
mating – farrowing	307.8	305.2	301.0	305.3	1.800
lactation	155.2	155.2	156.8	160.0	3.802
total reproductive cycle	463.0	460.4	457.8	465.3	3.918

*/ I – control, non-modified soybean meal and corn, II – GM soybean meal and non-modified corn, III – non-modified soybean meal and GM corn, IV – GM soybean meal and GM corn

Table 2. Piglets rearing indices

	Experimental group*				SEM
	I	II	III	IV	
Total number of piglets born	65	66	65	69	-
Total of weaned piglets	58	58	59	61	-
Average number of piglets born in litter	10.8	11.0	10.8	11.5	-
Average number of piglets weaned in litter	9.7	9.7	9.8	10.2	-
Lost piglets (%)	10.8	12.1	9.2	11.6	-
Average individual body weigh of piglet (kg):					
1d of age	1.47	1.48	1.50	1.40	0.020
28 d of age	7.16	7.18	7.32	7.20	0.099
84 d of age	26.27	26.47	27.04	26.85	0.341
Average daily body weight gains in estimated periods (g):					
1–28 d of age	211	211	221	215	3.426
28–84 d of age	341	344	349	351	5.329
1–84 d of age	299	301	308	307	4.045
Average feed utilization per 1 kg of body weight gain (kg):					
1–28 d of age	0.12	0.12	0.11	0.12	0.002
28–84 d of age	2.13	2.08	2.06	2.01	0.054
1–84 d of age	1.64	1.62	1.59	1.57	0.033

*/ I – control, non-modified soybean meal and corn, II – GM soybean meal and non-modified corn, III – non-modified soybean meal and GM corn, IV – GM soybean meal and GM corn

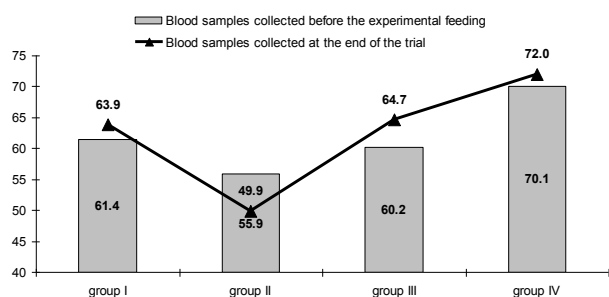


Fig. 1. Mean values of red blood cell volume (fl)

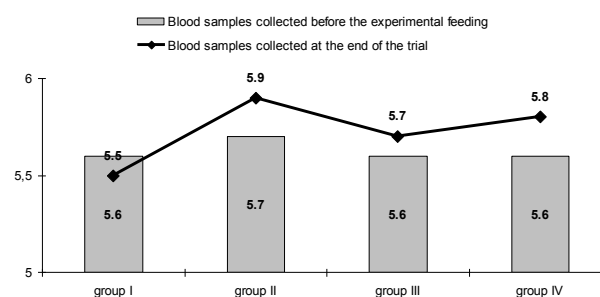


Fig. 2. Mean values of platelet volume (fl)

Table 3. Parameters of peripheral blood characterising haematological status in sows

		Experimental groups *			
		I	II	III	IV
RBC (x 10 ¹² /L)	0**	6.13 ± 0.49	5.91 ± 0.60	5.67 ± 0.4	7.05 ± 0.2
	1	6.41 ± 0.36	5.69 ± 0.31	5.89 ± 0.5	6.96 ± 0.4
HCT (l/l)	0	0.65 ± 0.10	0.67 ± 0.10	0.63 ± 0.22	0.64 ± 0.21
	1	0.66 ± 0.14	0.65 ± 0.32	0.60 ± 0.24	0.62 ± 0.14
HGB (mmol/L)	0	10.01 ± 1.59	10.11 ± 0.51	9.14 ± 1.16	11.9 ± 2.06
	1	10.65 ± 1.08	9.50 ± 1.10	9.63 ± 2.10	11.2 ± 1.46
MCH (fmol)	0	1.8 ± 0.01	1.20 ± 0.04	1.15 ± 0.07	1.91 ± 0.04
	1	1.7 ± 0.04	1.75 ± 0.07	1.35 ± 0.04	2.10 ± 0.09
MCHC (mmol/L)	0	28.2 ± 0.2	26.5 ± 0.27	28.7 ± 0.37	29.2 ± 0.14
	1	29.1 ± 0.3	28.5 ± 0.21	27.5 ± 0.71	24.5 ± 0.17
PLT (x 10 ⁹ /L)	0	230.2 ± 110.0	321.9 ± 122.7	218.0 ± 58.9	299.5 ± 54.09
	1	299.8 ± 77.8	310.5 ± 66.96	230.0 ± 78.33	305.5 ± 94.11
WBC (x 10 ⁹ /L)	0	11.59 ± 5.10	12.5 ± 5.13	9.4 ± 3.05	10.9 ± 1.22
	1	10.93 ± 2.04	13.5 ± 4.15	10.5 ± 4.11	11.5 ± 2.98
LYM (%)	0	48.1 ± 11.5	50.3 ± 5.11	49.9 ± 4.13	48.9 ± 4.35
	1	49.0 ± 3.14	53.1 ± 9.01	48.9 ± 10.1	51.0 ± 5.13

*/ I – control, non-modified soybean meal and corn, II – GM soybean meal and non-modified corn, III – non-modified soybean meal and GM corn, IV – GM soybean meal and GM corn

**/ 0 – blood samples collected before the experimental feeding; 1 – blood samples collected at the end of the trial

Table 4. Results of detection of the transgenic DNA fate in blood of sows

	Experimental group*							
	I		II		III		IV	
	RR	MON 810	RR	MON 810	RR	MON 810	R	MON 810
Peripheral blood	0	0	0	n/a**	n/a	0	0	0

*/ I – control, non-modified soybean meal and corn, II – GM soybean meal and non-modified corn, III – non-modified soybean meal, and GM corn, IV – GM soybean meal and GM corn

**/ n/a – not assessed

Discussion

Results of the study indicate that feeding genetically modified RR soybean meal and *Bt* maize to pregnant and lactating sows did not significantly affect their condition during reproductive cycle and the quality of their litters. Piglets rearing indices also remained unchanged. The studies conducted by Walsh *et al.* (27) on sows receiving mixtures with GM or not-

GM maize showed a similar body weight at the beginning and at the end of pregnancy, as well as a similar backfat thickness for both treatments. Moreover, the average daily body weight gains of their nursing progeny were not affected by maternal feeding. Similar results were observed by Buzoianu *et al.* (3), when *Bt* maize was given to sows and offspring (from weaning to 115th d of age). In case of male pigs receiving GM feed from weaning until 110th d of age, Buzoianu *et al.* (2) also did not note any effect of the

feeding on growth performance, carcass weight, and intestinal histology. Other studies on weaned piglets fed MON810 maize also did not confirm any influence of this feed on the body weight gains, feed conversion, intestinal morphology, and organs weight (25, 26). On the contrary, Piva *et al.* (15) observed higher weight gains in piglets receiving *Bt* maize. However, the authors considered that the reason for improved performance was probably the quality of *Bt* maize grain - less damaged by European corn borer and less infected with *Fusarium* mycotoxins. The effect of GM feeds was also tested on females of other species. Feeding *Bt* maize to sheep for three years had no effect on females condition, growth rate, and mortality of lambs (24). In case of rats, there was no influence of *Bt* maize offered to animals for three generations on birth rate and survival of offspring (10). No significant differences in growth performance, in comparison to control group, were observed in the experiment on poultry fed mixtures containing genetically modified *Bt* maize or/and glyphosate tolerant RR soybean meal (23).

Data on the effect of GM feeds on swine health and blood parameters is still insufficiently published in scientific literature, although concerns regarding potential toxicity of protein expressed by transgenic DNA are still growing. Using pigs as an experimental animal model is especially important because of the similarity of porcine intestinal physiology, microbiota, and immune function to those of humans (16). Therefore, the results obtained in the trials carried out on pigs could be potentially more related to humans than those from trials using less adequate animal models. In the study, no significant influence of GM feeds on haematological indices of sows was noted. According to Winnicka (29), average values of estimated blood indices are located in the range typical for healthy adult swine. These results remain in agreement with findings of Walsh's *et al.* (27), who showed that feed mixture containing MON810 maize did not influence the biochemistry and haematological indices in sows and their offspring. Another experiment by Walsh *et al.* (28), carried out on weaned piglets receiving GM maize for 80 or 110 d, did not confirm any effect of the diet on basic haematological indices (erythrocyte count, erythrocyte parameters, haemoglobin concentration, and haematocrit). The leukocyte count was also not altered by the GM material occurring in feeds. Similarly, in the experiment with GM rice (expressing Cry1Ab protein) offered to rats, the haematological indices were not affected by the treatment (17).

One of the most important part of the study on genetically modified feeds is the evaluation of transgenic DNA fate in the organism. Results of such tests provide the answer to the consumers' concerns about the possible transfer of the transgene to human tissues or to products of animal origin. Analysis performed in the trial on sows proved no transgenic

DNA, typical for RR soybean (172 bp) or *Bt* maize (170 bp), in sow blood. In the previous experiments, the presence of GM DNA fragments was not confirmed in any of the examined organs and muscles in fattened pigs or laying hens (20, 22). Results of the experiment by Buzoianu *et al.* (4), conducted on sows fed *Bt* maize diets and their offspring, did not show any transgenic fragments (149 and 211 bp) in blood and faeces of the sows as well as in blood and tissues of piglets. No transgenic GM maize gene (211 bp) and Cry1Ab protein were detected in white blood cells and other examined tissues of organs (heart, liver, spleen, kidneys) and muscles of pigs in the research of Walsh *et al.* (25). The lack of transgenic DNA was confirmed in numerous animal studies: in blood, liver, and muscles of pigs (30); in blood and milk of cows and in beef (11, 13); in blood, meat, skin, liver, spleen, and lungs of broiler chickens (9); in meat, liver, spleen, stomach, kidneys, heart, and eggs of quails (5). On the contrary, Sharma *et al.* (18) observed 278 bp transgene fragment in one liver and one kidney sample (out of 36 samples tested) of pigs, likewise Mazza *et al.* (12) who detected 519 bp transgene fragment in the blood, liver, kidneys, and spleen of piglets. However, such short particles do not show biological activity while full length of active transgene of RR soybean includes 3500 bp and *Bt* maize 1800 bp, and those were never detected. It could be stated that transgenic DNA and expressed protein are effectively digested along the alimentary tract reducing the possibility of its translocation to organs or excretion to the environment.

Summing up the obtained results it can be concluded that feeding pregnant and lactating sows feeds containing genetically modified RR soybean meal or/and *Bt* maize did not significantly affect their reproductive characteristics and offspring performance. There was no effect of dietary treatment on sow haematological status. The lack of transgenic DNA in sow blood was confirmed.

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