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The effect of genetically modified maize (MON 810) and soyabean meal (Roundup Ready) on rearing performance and transfer of transgenic DNA to calf tissues

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ABSTRACT. The experiment was performed to determine whether genetically modified maize (MON 810) and soyabean meal (Roundup Ready, MON 40-3-2) used as the main source of feed in a concentrate can affect the performance parameters, basal chemical composition of the *musculus thoracis* (MT), fatty-acid composition of intramuscular fat, and transfer of transgenic DNA (tDNA) to calf tissues, as well as affect the results of histological examination of calf organs and tissues. In the experiment, forty Polish Black-and-White HF bulls aged 10 days were allocated to 4 groups fed non-modified (traditional) maize and soyabean meal (group TMG/TS), non-modified maize and GM soyabean (group TMG/MS), GM maize and non-modified soyabean meal (group MMG/TS), or GM maize and GM soyabean meal (group MMG/MS). The experiment was terminated at the age of 90 days. Calves were housed in individual pens and fed *ad libitum* diets with balanced energy and protein according to the IZ-INRA (2009) system. All mixtures contained similar amounts (%) of maize (56), soyabean meal (25), oat (15), premix and limestone (1). There were no major differences in the feed value of Bt maize and RR soyabean meal and their non-modified isogenic counterparts and feed mixtures. There were no effects of GM components on final liveweight, average daily weight gain, MT chemical composition, or fatty-acid profile of intramuscular fat. The calf rumen fluid contained tDNA, but there was no tDNA in the intestinal content, blood, studied organs, or meat. Histological examination of the investigated organs and muscles found no differences among treatments.

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Introduction

In 2012 year, the surface area cultivated with GM plants resistant to various harmful factors such as insects or herbicides has rapidly increased worldwide 170 million hectares of transgenic crops were grown globally (James, 2013).

GM plants, mainly soyabean and maize containing tDNA originating from foreign organisms, are making up an increasingly larger part of the genetically modified crops available on the market in Poland.

The transgenic maize, MON 810, was modified by insertion of the *cry1Ab* gene isolated from the soil bacterium, *Bacillus thuringensis*. This gene encodes

the insecticidal Bt crystal protein, Cry1Ab, which is toxic to coleopteran insects, principally *Lepidoptera*, and causes resistance against the pest corn borer (*Ostrinia nubilalis*), in particular, and reduces mycotoxin contamination in Bt-maize, decreasing the *Fusarium* spp. content as shown in many studies (Flachowsky et al., 2005, 2007). The modified soyabean meal was derived from Roundup Ready (RR) genetically modified plants (Flachowsky et al., 2005). In the case of RR soyabean, the plant was endowed with the *epsps* gene (originating from *Agrobacterium* sp., strain CP4), conferring resistance to the glyphosate herbicide, Roundup.

In many studies, transgenic lines have been compared with corresponding isogenic foundation lines or commercial conventional lines. The modified plants, 'new plants' have been used as feed for decades in feeding food-producing animals with positive results of experiments. Notwithstanding this, doubts continue whether the transgenic lines correspond to their isogenic foundation lines or commercial conventional lines. They have been studied in relation to nutrient composition, the possibility of transferring DNA from the transgenic line to animal tissues, and the safety assessment of feed and food (McCann et al., 2005; Rossi et al., 2005; Flachowsky et al., 2007, 2012). A number of studies have now been carried out in which tDNA has not been detected in food products derived from livestock receiving GM feed ingredients, but some small fragments of plant chloroplast DNA have been detected in some animal tissues (Phipps et al., 2003; Flachowsky et al., 2012). The above findings have stimulated public discussion on the nutritional and safety assessment of feed and food-producing animals. The subject of concern is the influence of transgenic DNA and expressed proteins on animal and human health, as well as their environmental and socio-economic impact. There is an ongoing discussion whether to legally allow commercial GM crops in Poland (Lisowska and Cortez, 2013). Currently the interests of individuals or some companies dominate, and these are not always in agreement with public interests (Godfray et al., 2010; Foley et al., 2011; Flachowsky et al., 2012) and it is necessary that the studies on modified plants be continued. In reviews by Flachowsky et al. (2005, 2012) it was found that most of ingested DNA is degraded in the gastrointestinal tract, but some fragments have been found in animal tissues. No residues of tDNA, however, have been detected in any organ, tissue, or food product obtained from animals fed with feeds from GM plants. If, however, plant DNA fragments can be absorbed, it might be that tDNA fragments are also absorbed, although as shown by Mazza et al. (2005), the frequency is likely to be extremely low.

In cattle nutrition, young calves for the first 90 days of life are sensitive to ration composition, nutrient deficiency, or presence of harmful substances in feed. Until now there has been a lack of studies comparing the growth of calves fed feed mixtures containing glyphosate-tolerant (Roundup Ready) soyabean meal and Bt insect-resistant maize.

We hypothesized that it is possible for transgenic plants to affect growing calves in the rearing period, which could be demonstrated by using transgenic Bt maize grain (event MON 810) and glyphosate-tolerant soyabean meal (Roundup Ready) as the main (81%) feed components of concentrates for calves.

The objective was to determine the effect of GM feed on the growth of new-born calves, feed and meat chemical composition, fatty-acid composition of intramuscular fat of *musculus thoracis*, and the histology of tissues and organs. In addition, ruminal fluid, ileum digesta, blood and meat were tested for the presence of transgenic and endogenous plant DNA.

Material and methods

Animal management

Calves were kept in individual cages (Alfa Laval) with slatted wooden floors covered with straw and equipped with automatic watering bowls and feeding troughs. During the entire study, all animals were treated humanely and were under veterinary supervision. The Local Krakow Ethics Committee for Experiments with Animals approved all of the experimental procedures relating to the use of live animals.

Tested feeds

The genetically modified (M) maize grain (MG) and modified soyabean meal were compared with non-modified feeds further referred to as traditional (T). The T and M maize was produced in Poland under similar conditions. The soyabean meal (T and M) was imported from Brazil (by the company, Agsol, Poland). The GM maize grains were obtained from plants containing the Bt gene and expressing the Cry1Ab protein (YieldGard, MON 810), genetically modified for protection against the European corn borer (*Ostrinia nubilalis*). The tested soyabean meal was produced from glyphosate-tolerant plants (Roundup Ready, MON-40-3-2). The non-modified, isogenic, parental line of maize grain (DKC 3420) and non-modified commercial soyabean served as control feeds.

Experimental design and animal feeding

The experiment was carried out on 40 Polish Black and White HF bull calves from 10 ± 3 days of age at the beginning to 90 days at the end of the experiment. The calves were divided into 4 groups of 10 animals according to calf condition and an analogue method based on liveweight. The calves were allocated successively to appropriate groups as they were born. All groups were completed within six months. The calves were offered liquid feed and concentrate. Before the experiment the calves received only colostrum and whole milk. From the beginning of the trial, all of the calves were offered liquid feed (to 56 days of age). Liquid feed was prepared from milk replacer powder (SanoRot) that contained, according to the manufacturer's specification, sweet dried whey, soya protein concentrate, refined vegetable and animal oils, vitamins and probiotics. The concentration of solid milk replacer in liquid feed was 125 g per litre. Liquid feed was provided from plastic buckets with nipples. All concentrates were fed individually *ad libitum* and contained similar amounts of each feed ingredient (Table 1). The concentrates for individual groups did not contain, or did contain genetically modified feeds. The concentrates used for groups contained: traditional (non-modified) maize grain and soyabean meal (TMG/TS, control group), or traditional maize grain and modified soyabean meal (TMG/MS group), or modified maize grain and traditional soyabean meal (group MMG/TS), or modified maize grain and modified soyabean meal (group MMG/MS).

Table 1. The composition of concentrates, %

Ingredient	Groups[1]			
	TMG/TS	TMG/MS	MMG/TS	MMG/MS
Maize MON810, ground	—	—	56.0	56.0
Traditional maize, ground	56.0	56.0	—	—
Soyabean meal RR	—	25.0	—	25.0
Traditional soyabean meal	25.0	—	25.0	—
Oat, ground	15.0	15.0	15.0	15.0
Premix CJ Komplet[2]	3.0	3.0	3.0	3.0
Limestone	1.0	1.0	1.0	1.0

[1] TMG/TS – non-modified maize and soyabean meal (control group), TMG/MS – traditional maize and modified soyabean meal, MMG/TS – modified maize and traditional soyabean meal, MMG/MS – modified maize and modified soyabean meal; [2] BASF mineral, g · kg^{-1}: Ca – 212.8, P – 60, Na – 88, Mg – 25, Zn – 4, Mn – 2.5, Fe – 1.5, vit. E – 0.8; IU · kg^{-1}: vit. A – 4500000, vit. D_3 – 100000

The calves were fed milk replacer solution according to IZ-PIB-INRA (2009) recommendations. The protein and energy value of feeds, and proportion of ingredients in concentrates were formulated using INRAtion-PrévAlim version 3x (2005) software based on our own chemical analysis of feeds and using our own coefficients of rumen protein degradability (deg_P) and intestinal protein digestibility (dsi_P) for concentrate components. For milk replacer the values of $deg_P = 0.10$ (due to the function of reticular groove) and $dsi_P = 0.95$ were assumed.

The calves were observed regularly, on a once-daily basis, in terms of general health. Feed intake was monitored daily by weighing feed refusals and representative samples were analysed at one-month intervals. The liveweight of calves was monitored over two successive days at the beginning of the experiment (d 10 ± 3), at weaning (d 56) and at the end of the experiment (d 90). At the end of the experiment, 6 calves from each group were slaughtered after 24-h feed withdrawal to take samples from different body parts.

Sampling

Samples of feeds were taken twice during the experiment. Representative samples were prepared from feed refusals. Immediately after slaughter, samples of digesta were collected from the rumen, duodenum, jejunum and caecum as well as samples of tissues such as blood and skeletal muscles, *musculus thoracis* (MT) and *musculus gracilis* (MG), and organs (lungs, liver, kidney, spleen, pancreas). Those samples were placed individually in plastic containers and frozen at –18°C and stored until DNA analysis. In addition, samples of tested feeds were collected to determine the percentage of genetically modified maize grain and soyabean. Digesta from different sections of the digestive tract was sampled to determine the possibility of transfer of transgenic DNA to selected microorganisms. Segments of liver, kidney, spleen, pancreas, duodenum, jejunum, MT and MG were collected for histopathological examination and fixed in 10% neutral buffered formalin.

Analysis

The nutrient content of feed and feed refusals, and chemical composition of MT were determined according to AOAC (2005) standard methods. The composition of fatty acids in MT was determined with a VARIAN 3400 chromatograph using a capillary column Rtx 2330 (105 m, 0.32 mm, 0.2 μm), injection and detector temperature of 250°C, and helium as the carrier gas (3 ml · min^{-1}). Temperature programme was: 60°C (10 min), up to 120°C (20°C · min^{-1}), up to 240°C (3°C · min^{-1}). The presence of tDNA of GM soyabean meal and GM maize grain in the concentrate mixture and transfer of tDNA into bacteria and biological materials were determined by PCR (polymerase chain reaction) analysis.

Table 2. Primers used in the study for the detection of maize and soyabean genes

Primer	Sequence 5' – 3'	Target element	Amplicon size, bp	References
35s-f2 Petu-r1	TGA TGT GAT ATC TCC ACT GAC G TGT ATC CCT TGA GCC ATG TTG T	transition site of 35S promoter sequence to the chloroplast-transit-signal sequence in Roundup Ready soyabean	172	QL-CON 00-001
VW01 VW03	TCG AAG GAC GAA GGA CTC TAA CG TCC ATC TTT GGG ACC ACT GTC G	transition site of the genomic DNA into the 35S promoter in MON810 maize	170	QL-EVE-ZM 001
p35s-cf3 p35s-cf4	CCA CGT CTT CAA AGC AAG TGG TCC TCT CCA AAT GAA ATG AAC TTC C	CaMV 35s promoter of RR soyabean and MON810 maize	123	QL-ELE-00-004
HA-NOS 118-f HA-NOS 118-r	GCA TGA CGT TAT TTA TGA GAT GGG GAC ACC GCG CGC GAT AAT TTA TCC	NOS terminator of RR soyabean	118	QL-ELE 00-009
GM03 GM04	GCC CTC TAC TCC ACC CCC ATC C GCC CAT CTG CAA GCC TTT TTG TG	soyabean lectin gene (endogenous)	118	QL-CON 00-001
IVR1-F IVR1-R	CCG CTG TAT CAC AAG GGG TGG TAC C GGA GCC CGT GTA GAG CAT GAC GAT	maize invertase gene (endogenous)	226	QL-EVE-ZM 001

The transfer of transgenic DNA into rumen digesta and bacteria species selected from the ileum was analysed. Selective media were used to culture microorganisms. TBX medium (tryptone bile agar with X-glucuronide) was used for *E. coli* and Slanetz-Bartley medium containing sodium azide for *Enterococcus faecalis* and *Enterococcus faecium*. BHI (brain heart infusion) medium was used to multiply these microorganisms.

DNA from homogenized samples of gastrointestinal tract digesta and tissue samples was extracted using CTAB methods (PN-EN ISO/IEC 21571:2007); from heparinized blood, with a commercial extraction kit (Blood Genomic AX Kit by DNA, Poland), according to the manufacturer's instructions.

PCR reactions were performed according to JRC (Joint Research Center) GMO methods ('GMOMETHODS: The European Union Database of Reference Methods for GMO Analysis'; gmo-crl.jrc.ec.europa.eu/gmomethods). The list of the primers used in the study together with information relating to their base sequences (5'–3'), target element and amplicon size is given in Table 2.

Material for histopathological examination was processed by routine histological methods. Paraffin sections (5 μm) were stained with haematoxylin and eosin (HE) and examined under a Zeiss Axioskop 2 light microscope.

Statistical analysis of calf rearing performance and meat composition data were subjected to one-way factorial analysis of variance. The significance of differences between the means of treatment was determined using Duncan's multiple range test, with the differences being considered significant at $P \leq 0.05$. The statistical analyses were performed with SAS (2001).

The histopathology results were analysed using multiple comparison procedures (Kruskal-Wallis test, Dunnett's test and Spearman rank correlation coefficients). For comparison of the fraction of positive results between experimental groups, one-way ANOVA with Dunnett's test was applied, with the differences being considered significant at $P \leq 0.05$. The calculations were performed using STATISTICA software, version 10, StatSoft.

Results

The composition and nutritional value of Bt maize and RR soyabean meal compared with isogenic, traditional lines did not differ from the standard range (Table 3). The feed value of the four treatment concentrates offered throughout the study contained similar levels of protein and energy (per kg DM): an average of 206 ± 2 g crude protein, 137 ± 1.0 g PDI and 1.07 ± 0.03 UFL.

Analysis of compound feed samples showed that the feed given to groups TMG/TS and MMG/TS contained a very small level of GM soyabean meal, while the feeds for groups TMG/MS and MMG/MS contained 100% GM soyabean (Table 4). The content of MON 810 maize grain in compound feeds for calves from groups TMG/TS and TMG/MS points to trace amounts of MON 810. An almost 33% content of MON 810 maize grain was found in the concentrate for groups MMG/TS and MMG/MS.

In milk replacer, for all experimental groups, the content of RR soyabean meal was 0.2%.

Bacterial DNA showed no presence of transgenic elements of the 35S promoter and NOS terminator. There were no significant differences between the groups in the quantitative composition of different types of rumen microorganisms.

The differences between the groups of calves in daily intake of feed and nutrients in all periods of the experiment (Table 5) were not statistically significant ($P > 0.05$).

Table 3. Chemical composition, % of DM, and nutritive value (according IZ-PIB INRA2009) of the concentrates, kg DM

Indices	Dry matter	Crude protein	Ether extract	Crude fibre	Ash	PDIN[1], g	PDIE[2], g	UFL[3]
Feeds								
maize MON 810, ground	88.32	8.77	3.91	2.12	1.40	68	93	1.21
traditional maize, ground	86.18	8.90	3.50	2.18	1.51	70	97	1.20
oat, rolled	87.20	11.01	5.30	13.8	2.87	69	70	0.85
soyabean meal RR	88.69	51.50	3.63	4.87	7.12	387	282	1.25
traditional soyabean meal	87.89	56.01	2.85	3.89	6.34	411	280	1.26
milk replacer, powder	97.30	20.50	20.40	1.03	8.29	195	239	1.69
Concentrates for groups								
TMG/TS[4]	88.67	20.78	3.97	4.13	6.82	156	138	1.08
TMG/MS[4]	88.87	20.34	3.51	3.73	7.09	153	138	1.08
MMG/TS[4]	88.25	20.84	4.09	4.35	7.00	156	136	1.08
MMG/MS[4]	88.41	20.37	3.52	4.09	7.02	153	137	1.07

[1] PDIN – protein digestible in the intestine corresponds to the amount of microbial protein syntheised in the rumen which depends on level of nitrogen originating from protein in the rumen plus dietary protein undegraded (PDIN for milk replacer correspnds to digested crude protein); [2] PDIE – protein digestible in the intestine corresponds to the amount of microbial protein synthesised in the rumen which depends on energy available plus dietary protein undegraded; PDI – total protrin digestibility in the intestine meeting the requirement of animal which corresponds to lower value of PDIN or PDIE; [3] UFL – feed unit for milk production, 1 UFL = 1.7 Mcal; [4] see Table 1

Table 4. Content of GM plants in concentrates

GM plants	Concentrates for groups[1]			
	TMG/TS	TMG/MS	MMG/TS	MMG/MS
Soyabean meal RR, %	0.1	100	0.5	100
Maize grain MON 810, %	0.06	0.04	32.3	32.9

[1] see Table 1

The differences in liveweight (LW) and daily liveweight gains (LWG) before and after weaning and in the entire period of the experiment (Table 6) were not for the most part significant ($P > 0.05$). The difference between liveweight at weaning of calves in the MMG/MS group compared with the other groups was statistically significant ($P < 0.03$) and the final liveweights and daily weight gains in all periods of the experiment were numerically greater.

No statistically significant differences between groups of calves in feed conversion (Table 7) were noted.

The differences between results of meat chemical composition (Table 8) were not statistically significant ($P > 0.05$).

There were no significant differences ($P > 0.05$) in the fatty-acid content of the intramuscular fat of MT (Table 9).

Table 5. Daily intake of feed and nutrients during different periods of the experiment

Indices	Groups[1]				*P*	SE
	TMG/TS	TMG/MS	MMG/TS	MMG/MS		
Before weaning						
milk replacer powder, kg day^{-1}	0.83	0.83	0.82	0.85	0.29	0.01
concentrate, kg day^{-1}	0.40	0.37	0.38	0.38	0.90	0.01
dry matter, kg day^{-1}	1.17	1.13	1.14	1.17	0.79	0.01
crude protein, g day^{-1}	254	245	250	254	0.77	3.13
PDIN, g day^{-1}	224	217	221	225	0.62	2.47
PDIE, g day^{-1}	218	213	213	218	0.75	2.24
UFL, day^{-1}	1.84	1.80	1.81	1.85	0.71	0.02
After weaning						
concentrate, kg day^{-1}	3.47	3.29	3.32	3.46	0.57	0.06
dry matter, kg day^{-1}	3.07	2.93	2.93	3.06	0.59	0.05
crude protein, g day^{-1}	721	670	692	706	0.47	11.7
PDIN, g day^{-1}	540	504	518	530	0.48	8.80
PDIE, g day^{-1}	479	454	452	471	0.55	7.76
UFL, day^{-1}	3.74	3.55	3.58	3.74	0.58	0.06
Entire period of experiment						
concentrate, kg day^{-1}	1.69	1.60	1.62	1.69	0.55	0.03
dry matter, kg day^{-1}	1.95	1.87	1.87	1.96	0.51	0.03
crude protein, g day^{-1}	450	423	435	446	0.44	6.28
PDIN, g day^{-1}	358	338	345	355	0.44	4.73
PDIE, g day^{-1}	327	314	313	328	0.44	4.21
UFL, day^{-1}	2.64	2.54	2.55	2.66	0.48	0.03

[1] see Table 1

Table 6. Liveweight and daily liveweight gains

Indices	Groups[1]				*P*	SE
	TMG/TS	TMG/MS	MMG/TS	MMG/MS		
Liveweight, kg						
initial	45.25	42.75	42.85	47.22	0.19	0.84
at weaning (56th day of age)	73.75^{ab}	68.95^{a}	70.05^{a}	76.00^{b}	0.03	0.96
final (90th day of age)	113.0	109.15	107.65	119.50	0.11	1.85
Daily liveweight gains, g day^{-1}						
from beginning of experiment to weaning	606	559	577	626	0.51	16.42
from weaning to the end of experiment	1154	1182	1106	1279	0.40	39.35
from beginning to theend of experiment	837	820	800	903	0.49	21.72

[1] see Table 1; ^{a,b,c} values within a row with different letter differ significantly at $P \leq 0.05$

Table 7. Feed conversion, per kg liveweight gain

Indices	Groups[1]				*P*	SE
	TMG/TS	TMG/MS	MMG/TS	MMG/MS		
Concentrates, kg	2.02	1.95	2.02	1.87	0.86	0.03
Dry matter, kg	2.36	2.29	2.30	2.28	0.92	0.04
Crude protein, g	545	519	534	520	0.77	10.01
PDI, g	397	385	384	383	0.90	7.42
UFL	3.20	3.11	3.14	3.10	0.94	0.06

[1] see Table 1

Table 8. Chemical composition of *musculus thoracis*, % DM

Nutrients	Groups[1]				*P*	SE
	TMG/TS	TMG/MS	MMG/TS	MMG/MS		
Dry matter	22.79	22.19	22.15	22.42	0.15	0.11
Crude protein	92.42	91.47	91.80	90.49	0.30	0.36
Crude fat	3.92	3.88	3.99	3.54	0.72	0.14
Ash	4.90	4.74	4.84	4.68	0.60	0.06

[1] see Table 1

Table 9. Composition of fatty acids in *musculus thoracis,* per 100 g of all estimated acids

Fatty acids	Groups[1]				*P*	SE
	TMG/TS	TMG/MS	MMG/TS	MMG/MS		
$C_{8:0}$	0.04	0.03	0.17	0.02	0.37	0.03
$C_{10:0}$	—	—	0.09	0.08	0.58	0.03
$C_{12:0}$	0.66	0.60	0.58	0.61	0.99	0.14
$C_{14:0}$	1.80	1.86	1.49	1.54	0.95	0.25
$C_{16:0}$	23.90	21.83	20.94	20.95	0.75	1.03
$C_{16:1}$	1.26	1.10	0.85	0.85	0.25	0.09
$C_{18:0}$	11.30	12.10	12.01	12.52	0.67	0.34
$C_{18:1}$	27.67	25.04	22.98	21.82	0.53	1.45
$C_{18:2^{\cdot}\ n\text{-}6}$	23.33	25.28	27.67	28.29	0.45	1.18
$C_{18:3\text{-}}$ γ	0.04	0.09	0.12	0.09	0.59	0.02
$C_{18:3\text{-}}$ α	0.433	0.66	0.54	0.63	0.29	0.04
$C_{20:0}$	0.26	0.21	0.17	0.19	0.85	0.04
CLA c-9-t 11	0.34	0.51	0.39	0.38	0.68	0.05
CLA t10-c12	0.23	0.19	0.21	0.15	0.78	0.03
CLA c-9-c 11	0.07	0.16	0.13	0.10	0.66	0.03
CLA t9-t11	0.09	0.11	0.18	0.10	0.30	0.02
$C_{22:0}$	0.01	0.18	0.26	0.26	0.43	0.06
$C_{20:4}$	7.76	9.10	10.02	10.16	0.57	0.65
$C_{22:1}$	0.36	0.30	0.37	0.39	0.91	0.04
$C_{20:5}$. n-3. EPA	0.22	0.29	0.37	0.32	0.84	0.06
$C_{22:6}$. n-3. DHA	0.23	0.36	0.48	0.55	0.49	0.07
SFA	37.97	36.80	35.70	36.17	0.96	1.44
UFA	62.03	63.19	64.30	63.83	0.96	1.43
MUFA	29.29	26.44	24.20	23.06	0.49	1.48
PUFA	32.74	36.75	40.10	40.77	0.43	1.87
n-6	31.13	34.47	37.81	38.55	0.45	1.75
n-3	0.88	1.31	1.39	1.50	0.50	0.14
DFA	73.33	75.30	76.31	76.36	0.86	1.33
OFA	26.67	24.70	23.69	23.64	0.86	1.33
UFA/SFA	1.70	1.79	1.85	1.84	0.94	0.09
DFA/OFA	2.88	3.26	3.44	3.42	0.76	0.20
MUFA/SFA	0.80	0.74	0.70	0.66	0.80	0.05
PUFA/SFA	0.90	1.05	1.16	1.19	0.62	0.08
n-6/n-3	47.84	29.06	37.88	30.83	0.55	4.90
CLA	0.73	0.97	0.90	0.72	0.47	0.06

[1] see Table 1

Table 10. Presence of transgenic and endogenous DNA in digesta and tissues of calves fed concentrates with conventional or GM soyabean meal and maize

Items	Groups[1]															
	TMG/TS				TMG/MS				MMG/TS				MMG/MS			
	NOS terminator	35 S promoter	Lectin	Invertase	NOS terminator	35 S promoter	Lectin	Invertase	NOS terminator	35 S promoter	Lectin	Invertase	NOS terminator	35 S promoter	Lectin	Invertase
Blood	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Kidney	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Liver	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Spleen	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Skeletal muscle	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lungs	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pancreas	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Rumen digesta	–	–	+	+	+	+	+	+	–	+	+	+	+	+	+	+
Duodenum digesta	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Jejunum digesta	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ileum digesta	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[1] see Table 1

The results of investigations determining the fate of transgenic DNA in the gastrointestinal tract and organs of calves fed concentrates containing Bt maize (MON 810) and RR soyabean meal revealed that small fragments of single-copy RR and Bt (172 and 170 bp) were present only in the rumen digesta (Table 10).

Endogenous lectin and invertase genes of conventional soyabean and maize were detectable in the rumen digesta of calves from the TMG/TS group. Bt or RR transgenes were not detected in the digesta of the distal intestinal parts, blood, liver, spleen, lung, or skeletal muscle.

Histopathological examination of the liver, kidneys, spleen, pancreas, duodenum, jejunum and skeletal muscle showed no significant differences between the group fed non-modified maize and

Table 11. Results of histopathological examination of internal organs and muscles of calves fed genetically modified and non-modified maize and soyabean meal

Organs	Histopathological changes in organs
Liver	lymphoid cell infiltrates, foamy structure of a slight degree in hepatocytes, congestion of the parenchyma from a slight to moderate degree
Kidney	lymphoid cell infiltrates
Spleen	no observed histopathological change in organ
Pancreas	no observed histopathological change in organ
Duodenum	no observed histopathological change in organ
Jejunum	no observed histopathological change in organ
Skeletal muscle	no observed histopathological change in organ

soyabean meal (group TMG/TS) and the experimental groups (Table 11). Occasionally, regardless of the use of GM feed, changes in the liver and kidneys were observed. Lymphoid cell infiltrates, foamy structure of a slight degree in hepatocytes, congestion of liver parenchyma from a slight to moderate degree, and lymphoid cell infiltrates in kidneys were found. These changes were obseved in a similar range in all groups, regardless of the presence of GM components in the concentrates.

Discussion

The chemical composition and feed value of glyphosate-tolerant soyabean meal and the Bt maize, MON 810, in relation to conventional (traditional) soyabean and maize grain used in the present study did not differ. Although numerically small differences were observed in feed values, they were within the normal expected range and were comparable to the feeds used in Poland. Such GM plants are characterized as being without substantial changes in composition and/or nutritive value (GM plants of the first generation) and can be considered as being overall equivalent to their isogenic counterpart (Flachowsky et al., 2012). Some studies show that the composition of commercial glyphosate-tolerant soyabeans over three years of breeding and into multiple varieties remains equiavalent to that of non-modified conventional soyabeans (McCann et al., 2005). Rossi et al. (2005) compared Bt maize with the corresponding near isogenic line used in feeding broilers and showed that the chemical composition did not differ between both forms of maize grain. Phipps et al. (2003) fed cows total mixed rations (TMR) containing either maize silage from a genetically modified (GM) variety that was tolerant to the herbicide, gluphosinate ammonium, or its near isogenic non-GM counterpart and found a similar composition and nutritive value of both feeds.

The results of many animal experiments already completed have mostly revealed no significant differences between isogenic and transgenic hybrids (Flachowsky et al., 2005). Results confirming that there are no significant differences between Bt maize MON 810 and glyphosate-tolerant soyabean meal in relation to traditionally non-modified components are presented in a study on broilers by Świątkiewicz et al. (2010).

The content of GM plants in experimental concentrates suggests that the compound feeds had the appropriate composition. The percentage content of GM feeds in concentrates with traditional feeds (0.04%–0.5%) show that non-modified soyabean meal and maize grain were conventional lines without RR soyabean meal and maize MON 810. Generally, a 0.9% content of GM components in conventional isogenic feeds is accepted. In context of mark the GM plants the level of 0.09% level is admissible if detection of GM component was accidental.

In the present study, the nutritional value of transgenic Bt maize and glyphosate-tolerant soyabean meal did not demonstrate any negative effect of these feeds on calf rearing results, i.e. feed and nutrient intake, body weight gains and feed conversion. The significantly greater LWG at weaning and numerically somewhat higher LW in the remaining periods and LWG in each period of the experiment when the calves were offered both GM maize grain and GM soyabean meal (group MMG/MS) could be due to the smaller content of mycotoxins in genetically modified plants. Folmer et al. (2002) evaluated the efficacy of corn borer-protected maize silage for growing beef steers and observed significantly greater LWG than for steers fed a non-transgenic maize silage diet. According to Piva et al. (2001), the reduced presence of mycotoxins could explain the higher body weight gains of piglets receiving the Bt maize in feed mixture.

Insect-resistant GM plants contain a lower amount of mycotoxins as a result of reduced damage by the corn borer and *Fusarium* infection level. *Fusarium* spp. are often related to accumulation of mycotoxins, e.g., fumonisins, deoxynivalenol and zearalenone, in maize kernels. Flachowsky et al. (2007) stated that many authors have reported lower contents of *Fusarium* spp. toxin in Bt maize. Ostry et al. (2010) revealed that in 19 out of 23 studies, Bt maize was less contaminated with mycotoxins than the conventional control variety in each case.

The number of studies comparing the performance of newborn calves fed a diet formulated with transgenic glyphosate-tolerant (Roundup Ready) and those fed non-modified soyabean meal, as well as with Bt maize MON 810 is limited. The presented study lasted only 90 days and may have been insufficient to reveal the presence of late effects in animals. Notwithstanding, in their systematic review Snell et al. (2012) discussed experiments on long-term feeding studies (of more than 90 days) and a multigenerational study showed that the differences among the examined parameters using biochemical analysis, histological analysis of specific organs, haematology, and the detection of transgenic DNA were also not generally statistically significant and did not show any health hazard. They examined the results of 2 to 5 generations of experiments, mainly

on rodents and laying hens (4 generations), but also with sheep (44 months). Flachowsky et al. (2005) described in a review article the results of long-term experiments on fattening bulls fed isogenic or transgenic maize made into silage and compared experiments with high portions of Bt maize in the concentrate of finishing diets of feedlot steers. Almost no significant differences were observed between animals fed nontransgenic and transgenic feed in dry matter intake, LW, daily LWG, carcass weight, area of *muscullus longissimus dorsi* and marbling score.

The tested plants also did not affect the chemical and fatty-acid composition of the *musculus thoracis* of the calves. Kerley et al. (2001) reported no differences in performance and carcass characteristics of bulls (yield and quality grades) fed borer-protected maize and its isogenic counterpart in a feedlot finishing diet. Similar results were observed by Vander Pol et al. (2005) for beef cattle grazing maize residues or feedlot cattle fed Bt maize grain, and by Erickson et al. (2003) in an experiment with Roundup Ready maize for feedlot steers. Flachowsky et al. (2012) reported that changed fatty-acids patterns for soyabeans and derived feed can enrich soyabean oil in stearidonic acid (SDA-oil). It can be transferred into the body fat of lactating cows after duodenal infusion of SDA-soyabean oil (Bernal-Santos et al., 2010), or after feeding ruminal protected SDA-oil to dairy cows (Kitessa and Young, 2011).

The highest priority for the present is the safety assessment of food-producing animals offered GM feeds in terms of horizontal transfer of foreign DNA to animal tissue and organs and its unintended effect on animal health and human consumers.

The presence of transgenic DNA fragments in rumen contents suggests that they originated from ruminally undigested particles of genetically modified feed. These fragments were not found in the duodenum and further along the digestive tract, nor in tissues and examined organs. It can be assumed that they had been completely digested.

PCR detected short tDNA fragments of RR soyabean meal (172bp) and Bt maize (170bp) only in rumen samples, but not in other samples of the digestive tract, tissues, or organs. Similarly, the presence of DNA elements of the CMV35s promoter found in both examined GM feeds (123 bp), the NOS terminator of RR soyabean, the endogenous soyabean lectin gene (each 118 bp), as well as the endogenous maize invertase gene (226 bp) was found only in rumen digesta. Similarly, in a study with laying hens fed a diet containing GM RR soyabean meal and Bt maize, fragments of soyabean and maize transgenic DNA were not detected in the digesta of the jejunum, ileum and ceaca, excreta, tissues, or in eggs (Świątkiewicz et al., 2011).

Transgenic DNA and newly expressed proteins show comparable properties to normal plant DNA and proteins and are mostly degraded in digestive tract of an animal (Alexander et al., 2007; Flachowsky et al., 2012). Some authors have shown the possible survival of recombinant plant DNA fragments after digestion. Small transgenic DNA fragments and fragments of newly expressed protein may be detected in trace amounts in animal tissues (Tudisco, 2010).

Endogenous plant genes have been detected in several animal tissues and products, but no recombinant DNA sequences have been found in any organ or tissue sample from animals fed genetically modified plants, probably because the amount of transgenic protein ingested by livestock depends on the concentration of the protein in the feed and the feed intake (Tudisco, 2006; Alexander et al., 2007). The transgenic protein concentration varies with the transgenic event and type of plant tissue in which it is expressed (Stave, 2002). The proportion of tDNA in total DNA fragments of animal diets is not high. The results of many experiments reviewed by Flachowsky et al. (2005) showed that cows fed rations containing 40% silage and 20% grain from transgenic Bt maize had an intake of DNA originating from the transgenic DNA of 54 µg per day, which amounted to 0.00094% of total DNA intake. Assuming an intake of 10 kg MON 810 grain per day, the total daily Cry1Ab intake by cows will be approximately 3.1 mg (Alexander et al., 2007). The risk of DNA transfer from GM plants to animal organisms is not higher than transfer of DNA from conventional plants.

The protein products of transgenes introduced into current commercial crops and recombinant DNA are degraded in the digestive tract in a similar manner as their protein and DNA counterparts in the ration.

The digestion of DNA, which is a component of each ration for ruminants, initiates in the mouth where nucleases are secreted in saliva, and then the dietary nucleic acids are degraded by microbial nucleases in the rumen. There is, however, extensive synthesis of microbial nucleic acid in the rumen. All nucleic acids entering the ileum are degraded by pancreatic nucleases to shorter monomers, apparently prior to reaching the large intestine. Rossi et al. (2005) reported that the minimal functional unit of the RR soyabean transgene has a length of 3500 bp, of Bt maize, 1800 bp, but the fragments detected in the presented experiment and in others were a magnitude shorter. Small transgenic DNA fragments and fragments of newly expressed pro-

tein may be detected in the animal body as shown by Flachowsky et al. (2012), who summarized the results of earlier experiments and normal physiological processes. They did not discuss, however, biological activity and influence on animal health, performance, composition, and quality of animal products. The results of our experiment suggest that fragments of the transgenic *Bt* maize characteristic gene could not be detected in the animal body because calves intake probably very little transgenic Bt DNA. Instead, plant DNA fragments can be detected in body samples such as meat, milk, or eggs and can occur as a result of passing from the gastrointestinal tract into the blood because their frequency is usually higher (Flachowsky et al., 2005). Phipps et al. (2003) show that fragments of rubisco DNA (189 bp) were detected in the blood and milk of dairy cows, and 1176 bp and 850 bp fragments were detected in ruminal fluid and duodenal digesta where 351 bp fragments were also detected; these fragments appear in faeces, too.

In the present study, the feeds containing genetically modified plants given to calves did not change the quantitative composition of gastrointestinal microflora and did not transfer transgenic DNA to bacteria colonizing different segments of the digestive tract.

An *in vitro* study (Sung et al., 2006) has shown that transgenic maize (MON 810 and Event 176) did not affect the major cellulolytic and amylolytic bacterial species of the rumen (*F. succinogenes* and *S. bovis*).

The histological examination of the liver, kidney, spleen, pancreas, duodenum, jejunum and skeletal muscles showed that GM feed did not cause any negative histopathological changes in these organs. Some changes in the liver and kidneys of calves were not related to feeding GM feeds. Reichert et al. (2012) discussed in detail the effect of Roundup Ready soyabean meal and Bt maize MON 810 on histopathological changes in some organs of broiler chickens, lying hens, pigs and calves (fed the same GM feeds as in our experiment) and suggested that they can be similar to results obtained in intensive feeding of animals. They concluded that the foamy structure of hepatocytes, observed depending on the group, can be associated with leached glycogen that was hydrolysed during histological processing.

Conclusions

In summary, it can be said that insect-resistant MON 810 maize and glyphosate-tolerant soyabean meal (Roundup Ready) compared with non-modified feeds did not significantly affect performance parameters or chemical composition of meat, or the fatty-acid composition of intramuscular fat of calves. The transgenic DNA fragments of the studied GM feeds were not absorbed into the organs and tissues, but were observed in the rumen. The studied GM plants did not affect histopathological changes in the investigated samples of calf organs and muscles. The obtained results show that genetically modified feeds used in feeding calves for 90 days do not show a negative influence on animal health and food quality, even when the calves were fed the concentrate that contained 81% GM feeds in the group provided modified maize MON 810 and modified RR soyabean meal.

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