Journal of Animal and Feed Sciences, 23, 2014, 58–63
The Kielanowski Institute of Animal Physiology and Nutrition, PAS, Jabłonna

A four-generation feeding study with genetically modified (Bt) maize in laying hens

I. Halle[1] and G. Flachowsky

Institute of Animal Nutrition, Friedrich Loeffler Institute (FLI), Federal Research Institute of Animal Health
Bundesallee 50, 38116 Braunschweig, Germany

KEY WORDS: poultry, growth, laying performance, hatchability, GM-maize

Received: 13 September 2013
Revised: 12 Decenber 2013
Accepted: 4 March 2014

[1] Corresponding author:
e-mail: ingrid.halle@fli.bund.de

ABSTRACT. A four-generation study with at least 60 laying hens (LSL) and 10 cockerels (LSL) was carried out to investigate the influence of genetically modified maize (Bt 176) on animal health and feed intake, laying performance, feed efficiency, and hatchability of chickens and to compare GM-maize with its near isogenic counterpart. The chickens were divided into two groups (one pen/group) of at least 30 hens each and 3 cockerels. The diets contained 400 (chickens and pullets) or 500 g · kg^{-1} (laying hens) isogenic or genetically modified maize (Bt 176), respectively. Feed and water were provided *ad libitum*. Eggs for hatching were collected when the laying hen was aged 31 weeks. In the 31st week of life, brooding eggs were collected and brooded for every group. One-day-old chickens from each group were sex sorted and allocated to one pen per group. There were no significant differences in composition between the two maize varieties. For every generation, as well as the average of all four generations, there was no significant influence on the feed intake of chickens (32.2 and 32.0 g per day), pullets (68.4 and 70.4 g per day) and layers (114.9 and 112.9 g per hen per day for control and Bt-maize), body weight of chickens (652 and 636 g), pullets after 18 weeks (1316 and 1305 g), and laying hens after 31 weeks (1656 and 1626 g for control and Bt-maize), laying intensity (83.5% and 83.3%), fertility of eggs (96.6% and 97.5%), or hatchability of living chicks (86.8% and 88.0% for control and Bt-maize). In conclusion, feeding of 400 (grower) or 500 g · kg^{-1} (layer period) Bt-maize to chickens, pullets and laying hens for four generations did not significantly influence feed intake, growth, laying or breeding performance compared with an isogenic counterpart.

Introduction

Since 1996 the cultivation of genetically modified plants (GMP) has increased from 1.6 million ha to about 170 million ha in 2012 (James, 2013) or around 12% of arable land on a global scale. Soyabean, maize, cotton seed and rapeseed (canola) are the dominating cultures (see James 2013 for more details).

Safety and nutritional assessment of feed/food from GMP is one of the starting points for public acceptance (Chassy, 2010; Kleter and Kok, 2010). International agreed documents (e.g., OECD, 1993; EFSA, 2008) recommend compositional analysis and comparison of isogenic with transgenic products. Feeding studies with laboratory and/or target animal species are only recommended if the compositional analyses, as well *in vitro*, *in sacco*, *in silico*, or 'omics' measurements are not able to answer all questions concerning feed/food safety (ILSI, 2003, 2007; FDA, 2007; EFSA, 2008, 2011).

Recently, we (Flachowsky et al., 2012) described objectives of various types of feeding studies with laboratory and target animal species for safety and nutritional assessment of feeds from genetically modified plants, as shown in Table 1. In general, the costs of the studies mentioned in Table 1 increase from laboratory to target animals and from the top to the bottom of Table 1, but should be the highest for long-term and multi-generational studies with large target animal species.

Table 1. Important types of feeding studies with animals for nutritional and safety assessment of feed from GMP and animals recommended

Type of studies	Laboratory animals	Target animals
Testing of single substances (28 day study)	X	
90-day rodent feeding study	X	
Long-term feeding study	X	X
Multigeneration feeding study	X	X
Determination of digestibility/availability (including rumen fermentation and metabolism)	X	X
Tolerance study		X
Efficiency (performance) study		X
Product study (composition and quality of food of animal origin)		X

In the meantime, many feeding studies with laboratory animals, but also with food-producing animals (about 150) according the recommendations by EFSA (2008, 2011) and ILSI (2003, 2007) have been carried out (see summary by Flachowsky, 2013).

Long-term feeding studies and multigenerational experiments with target animals, especially pigs and ruminants, are very rare, as recently summarized by Snell et al. (2012) and Ricroch et al. (2013). High costs and limited feed amounts in earlier breeding stages may also restrict animal numbers and duration of such studies with large target animals. Recently, Sartowska et al. (2012) and Korwin-Kossakowska (2013) initiated an eight-generation project with Japanese quails and included soyabean meal (39.0% in grower; 29.5% in layer feed) and maize (25% in grower and layer feed) from GM-plants. In the publications cited above they reported the first results (animal performance, meat and egg quality after two generations, Sartowska et al., 2012; transfer of transgenic DNA-fragments in food of animal origin after four generations, Korwin-Kossakowska et al., 2013). To date, they did not find any significant effect on animal yields and no measurable transgenic DNA fragments in animal bodies or eggs.

Nevertheless, there are critical comments about the duration of feeding studies (e.g., Séralini et al. 2011, 2012). Although some of those studies and comments were not published in peer reviewed journals, they are nonetheless being considered in the public discussion (e.g., Velimirow et al. 2008; Antoniou et al., 2012).

Therefore, all long-term and multi-generational studies should be published in peer reviewed journals. In addition to our 10-generation study with Bt-maize in quails (Flachowsky et al., 2005), we carried out a four-generation study with Bt-maize in laying hens, which was only published as an abstract (Halle et al., 2006). Therefore, the objective of this paper is to examine the effects of genetically modified (Bt) maize on some traits in laying hens and to describe them in more detail.

Material and methods

Animals and experimental design

In the four-generation trial, a total of 64 laying hens (4 hens were reserves; Lohmann LSL) and 10 cocks (LSL) were purchased at the age of 18 weeks and randomly distributed to two groups (Control: Caesar maize; treatment: Bt 176 maize) and held in two pens (about 10 m^2 per pen, 0.3 m^2 per hen) with litter (at least 30 hens and 3 cocks per group). The laying hen diet was formulated to contain 500 $g \cdot kg^{-1}$ maize (isogen maize or Bt maize).

Feed (Table 3) and water were provided *ad libitum*. The lighting programme and climate corresponded to the specifications of the management guides. The number of laid eggs was recorded daily and the offered feed was weighed back weekly on a pen-basis. In the 28th and 29th weeks of life, the collected eggs were weighed for four days per group. In the 31st week of life, brooding eggs were collected and brooded for every group. In generation 1, more eggs were used for hatching because of the unknown fertility of the eggs. The one-day-old chicks from every group were sex sorted and allocated to one pen per group. Failure in sorting was eliminated after 8 or 18 weeks. Different animal numbers during the chicken and pullet periods result from the ignorance of breeding results of the hens, failures in sex sorting, and culling birds with low and nonstandard development of body weight.

The hatched female chicks were used as the next generation. Chicken and pullet diets were fed in the 2nd to 4th generations to 31–74 female chickens of each group (weeks 1 to 8 and 9 to 18; see Table 6), layer diets were fed to 31–37 hens and 3–5 cocks, each from weeks 19 to 31.

Table 2. Composition of Cesar (isogen) and BT 176 (transgenic maize), g · kg^{-1} dry matter (DM)

Nutrients	Isogenic (Cesar maize)	Transgenic (Bt 176 maize)
Dry matter, g · kg^{-1})	886.3	893.6
Crude protein	108.4	108.6
Ether extract	29.3	29.8
Crude fibre	23.3	25.4
Acid detergent fibre	32.9	36.5
Neutral detergent fibre	102.0	113.2
N-free extractives	826.0	822.6
Calcium	2.0	2.6
Total phosphorus	29.0	30.6
Starch	721.5	715.9
Sugars	16.6	18.2
Lysine	2.8	2.7
Methionine	2.0	2.0
Cystine	2.5	2.4
Threonine	4.4	4.3
Fatty acids, % ether extract		
C16:0	12.3	12.2
C18:0	2.2	2.1
C18:1	32.5	31.7
C18:2	47.7	47.3
C18:3	1.1	1.2
Mycotoxins, µg · kg^{-1} DM		
zearalenone	3.4	<1 (DL)
deoxynivalenol	390	<30 (DL)

DL – detection limit (Valenta and Oldenburg, 1995; Valenta et al., 2002)

Table 3. Composition of complete feeds for chickens, pullets and layers, g · kg^{-1}

Indices	Chicken (1–8 weeks)		Pullet (9–18 weeks)		Laying hen (19–31 weeks)	
	control	Bt 176	control	Bt 176	control	Bt 176
Maize	400	400	400	400	500	500
Soyabean meal	202.7	202.8	151.9	150.1	159.1	161.8
Peas	100.0	100.0	87.2	90.6	35.9	34.2
Grass meal	50.0	50.0	100.0	100.0	26.9	21.3
Wheat	200.0	200.0	21.8	18.8.	163.6	164.5
Barley	—	—	200.0	200.0	—	—
Methionine	0.8	0.8	1.0	1.0	1.0	1.0
Calcium carbonate	12.2	12.3	6.3	4.6	93.0	97.0
Di-calcium-phosphate	19.0	18.8	16.7	19.8	7.7	7.4
Sodium chloride	5.3	5.3	5.1	5.1	2.8	2.8
Vitamin-mineral premix[1]	10	10	10	10	10	10

[1] vitamin-mineral premix provided per kg of chicken and pullet diets: IU: vit. A 12000, vit. D_3 – 3500; µg: vit. B_{12} – 32, biotin – 50; mg: vit. E – 40, vit. K_3 – 4.5, thiamin – 2.5, riboflavin – 8, pyridoxin – 6, nicotinic acid – 45, pantothenic acid – 15, folic acid – 1.2; choline chlorid – 550, Fe – 32, Cu – 12, Mn – 100, Zn – 80, I – 1.6, Se – 0,4, Co – 0.4, BHT – 100; vitamin-mineral premix provided per kg of laying hen diet: IU: vit. A – 10000, vit. D_3 – 2500; µg: vit. B_{12} – 20, biotin – 25; mg: vit. E – 20, vit. K_3 – 4, thiamine – 2.5, riboflavin – 7, pyridoxine – 4, nicotinic acid – 40, pantothenic acid – 10, folic acid – 0.6, choline chloride – 400, Fe – 40, Cu – 10, Zn – 80, Mn – 100, Se – 0.25, I – 1.2, Co – 0.21

Composition of maize

For the whole study, isogenic maize of the conventional variety 'Cesar' and transgenic maize (Bt 176; *Bacillus thuringiensis* maize), which is able to express the Cry 1AB-protein to protect maize against the European corn borer, were used. The conventional maize and Bt maize were cultivated under similar agronomical conditions. Analytical data of both maize-varieties is shown in Table 2.

Composition of complete poultry feed

Three various complete feeds (for chickens, pullets and layers) were mixed according to the nutritive requirements (GfE, 1999) of the animals (Table 3). The feed for chickens/pullets contained 400 g · kg^{-1} of maize and the laying hen feed, 500 g · kg^{-1}. The complete feeds were produced in the feed mill of the Institute of Animal Nutrition.

The diets for chickens (0–8 weeks), pullets (9–18 weeks) and laying hens (after 18 weeks) were newly mixed for each generation and stored in containers.

Analytical methods

Feed samples were taken for each generation and for each age group. Before analyses the mixed feed was stored in glass at 4°C.

The following were determined in the feed: dry matter (DM), crude protein, ether extract, crude fibre, further fibre fractions (neutral detergent fibre, NDF, acid detergent fibre, ADF), and ash content according to the methods of VDLUFA (2012). Amino acids and fatty acids were analysed using an amino acid analyzer (Beckmann 6300 amino acid analyzer) and gas chromatography. The mycotoxins zearalenone and dioxynivalenol were determined according to Valenta and Oldenburg (1995) and Valenta et al. (2002). Transgenic DNA was determined as described by Reuter and Aulrich (2003).

Statistical methods

Data from the growing, laying and hatching trials were analysed by one-way analysis of variance (GLM procedure): $y_i = \mu + a_i + e_i$ (y_i = growing, laying, hatching parameters of chickens or hens per generation (1 to 4) and growing, laying, hatching parameters of chickens or hens per treatment (isogen maize, Bt maize), µ – mean, a_i – treatment (isogen maize, Bt maize), e_i – error term). All statistics were carried out using SAS software (2002/03).

Results and discussion

The composition of isogenic maize and Bt-maize is shown in Table 2. There were no significant differences between the maize hybrids in crude nutrients, fibre fractions, starch, some minerals, amino acids and fatty acids. The content of zearalenon and deoxynivalenol of transgenic maize was below the detection limit; the isogenic hybrid contained small amounts of both mycotoxins (Table 2). All of the results agree with previous findings as summarized by Flachowsky (2013).

The content of some nutrients and metabolizable energy of complete feed for chickens, pullets and layers are given in Table 4. Only small differences were found or calculated between mixtures based on control or Bt-maize. The protein and amino acid contents, as well as the P-content, decreased from chicken to pullets and laying hen mixtures, but the Ca content of the feed for laying hens increased markedly.

Table 4. Nutrient composition (as fed) of complete feeds for chicken (n = 3)[1], pullets (n = 3)[1] and layers (n = 4)[1]

Indices	Chicken (1–8 weeks)		Pullets (9–18 weeks)		Laying hens (19–31 weeks)	
	control	Bt 176	control	Bt 176	control	Bt 176
DM, g · kg^{-1} [2]	874	872	875	873	883	880
Crude protein[2]	189	189	175	175	155	155
Starch [2]	422	421	416	415	435	434
Lysine [3]	9.0	9.0	7.9	7.9	6.8	6.8
Methionine + cystine[3]	6.5	6.5	6.3	6.3	6.0	6.0
Ca[2]	11.0	11.0	9.0	9.0	38.4	39.8
P[2]	7.0	7.0	6.5	7.0	4.5	4.5
ME, MJ · kg^{-1} [4]	11.1	11.1	10.8	10.8	10.7	10.7

[1] number of analysed samples; [2] analysed values; [3] calculated values; [4] N-corrected metabolizable energy, calculated according to WPSA-formula (1984)

The mortality of animals was very low in all age groups and was not treatment- or generation-related (Tables 5 and 6).

The daily feed intake of chickens was between 29.7 and 33.7 g, of pullets – between 64.6 and 76.8 g (Table 5). No significant differences in feed intake or body weight of chickens and pullets after 8 and 18 weeks were registered for any generation fed with control or Bt-maize (Table 5).

The hens of generation 1 (purchased hens) consumed less feed and were lighter at the beginning of the study and after 31 weeks of life than hens of all other generations (Table 6). Animal losses in the laying period (weeks 19–31) were very low (1.5%). No significant differences in feed intake (weeks 19–22 and 23–31) were found among hens of any generation fed with control or Bt-maize. Feed intake of hens fed with control maize or Bt-maize varied in some generations. Laying intensity in hens of generation 1 was lower, and feed intake per kilogram egg mass (feed efficacy) was higher than those of all other generations.

No significant differences in fertility and hatches of living chickens were found for any of the four generations (Table 6).

Table 5. Daily feed intake (g per bird) of chickens and pullets and animal numbers per treatment and age group and body weight (g per bird) of chickens and pullets of generations 1–4 (body weight: P = 0.3–0.7) (mortality: 1st wk 0–5 per group, wk 2nd to 18th 0–2 per group)

Age	Generation 1		Generation 2		Generation 3		Generation 4	
	control	Bt 176	control	Bt 176	control	Bt 176	control	Bt 176
Feed intake, g per chicken, pullet								
wk 1–8	[1]	[1]	33.4	32.4	30.9	29.7	32.4	33.7
wk 9–18	[1]	[1]	67.6	68.4	72.9	76.8	64.6	66.0
Numbers and body weight, g per chicken, pullet								
1st day	—	—	127 41 ±4	131 40 ±3	88 39 ±3	85 40 ±2	89 42 ±3	95 42 ±3
8 wk	—	—	62 621 ±91	76 600 ±77	40 668 ±91	38 659 ±58	53 676 ±79	47 677±68
18 wk	31 1227±93	32 1233±102	61 1309 ±213	74 1280 ±185	40 1325 ±160	38 1359 ±135	53 1369 ±145	47 1324 ±218

[1] animals were purchased with an age of 18 weeks and distributed to two groups

Table 6. Body weight, laying performance and hatchability, %, of hens per generation (P = 0.09–0.9)

Age	Generation 1		Generation 2		Generation 3		Generation 4	
	control	Bt 176	control	Bt 176	control	Bt 176	control	Bt 176
Number of hens/body weight, g per hen								
19 wk	31 1227 ±93	32 1233 ±102	31 1282 ±124	31 1278 ±94	37 1336 ±148	36 1367 ±134	32 1424 ±130	33 1377 ±99
31 wk	31 1577 ±143	32 1523 ±102	31 1706 ±221	31 1670 ±148	37 1601 ±173	34 1606 ±131	31 1753 ±177	32 1707 ±171
Feed intake, g per laying hen								
wk 19–22	54.1	45.3	78.6	88.2	88.6	87.1	96.2	79.3
wk 23–30	106.8	99.2	113.1	113.0	120.6	120.9	119.0	118.4
Laying performance, wk 23–30								
laying intensity, %,	71.2	64.5	81.9	89.8	89.5	90.2	91.3	88.7
mean egg weight, g	57.0	55.4	55.7	58.0	55.8	57.6	59.6	57.9
feed to egg mass production, kg · kg^{-1}	2.631	2.778	2.479	2.170	2.413	2.327	2.188	2.317
hatched eggs, number	154	152	100	100	104	104	100	100
fertile eggs, %	97.4	98.0	95.0	97.0	96.2	100	98.0	95.0
hatched living chickens, % [1)]	82.5	86.5	88.0	85.0	85.6	91.4	85.0	85.0

[1] hatched living chicks of all hatched eggs

Table 7. Means over four generations

Group	Control	Bt 176	*P* value
Feed intake, g per bird per day			
wk 1–8	32.2 ±1.2	31.9 ±2.1	0.8
wk 9–18	68.4 ±4.2	70.4 ±5.7	0.6
wk 19–22	79.4 ±18.3	75.0 ±20.2	0.8
wk 23–30	114.9 ±6.3	112.9 ±9.7	0.7
Body mass, g per bird			
1st day	40 ±3	40 ±3	1.0
8 wk	652 ±90	636 ±79	0.8
18 wk	1316 ±173	1305 ±152	0.8
19 wk	1320 ±145	1316 ±124	0.9
31 wk	1656 ±192	1626 ±155	0.6
Laying performance, wk 23–30			
laying intensity, %	83.5 ±9.1	83.3 ±12.5	0.9
mean egg weight, g	57.0 ±1.8	57.2 ±1.2	0.9
feed to egg mass production, kg · kg^{-1}	2.43 ±0.18	2.40 ±0.26	0.8
Breeding results (4 broods)			
fertility eggs, %	96.6 ±1.3	97.5 ±2.0	0.5
hatched living chicks, %	85.3 ±2.2	86.9 ±3.0	0.4

Table 7 summarizes the most important results over all four generations. No significant effects ($P > 0.05$) were registered between control and Bt-maize for any of the parameters under study.

The results of the four-generation laying hen study agree with the previous ten generation study with laying quails fed with control and Bt-maize (Flachowsky et al. 2005).

Presently, we do not know of other generation studies with poultry. Multigeneration studies with Bt-maize in mice (e.g., Brake et al., 2004; Haryu et al., 2009) and rats (e.g., Kiliç and Akay, 2008), but also in pigs (Buzoianu et al., 2012a,b) showed no relevant biological effects or influence on animal performance or reproduction parameters. Some studies have been published on other animal species. For 44 months, Trabalza-Marinucci et al. (2008) fed rations with Bt 176 maize to sheep and observed some differences in cytosolic activities in liver and pancreas cell nuclei and in the immune response to *Salmonella* vaccination. According to the authors, the significance and the reproducibility of these phenomena is unclear.

Other authors tested gluphosinate ammonium-tolerant maize in rats (2 generations; Tyshko et al., 2010), glyphosate-tolerant soyabeans in rats (4 generations; Brake and Evenson, 2004; 2 generations, Daleprane et al., 2009) or goats (2 generations, Tudisco et al., 2010), gluphosinate ammonium-tolerant triticale in mice (5 generations, Baranowski et al., 2006; 5 generations, Krzyzowska et al., 2010), gluphosinate ammonium-tolerant potatoes in rats (5 generations, Rhee et al., 2005) or lysine-rich rice in rats (3 generations, Zhou et al., 2012). No authors observed biologically relevant effects of GM-feed on animal health and welfare, feed intake, animal performance, or reproduction indices.

Conclusions

Feeding of Bt 176 maize to growing (400 g/kg diet) and laying hens (500 g · kg^{-1} diet) over four generations did not significantly influence the growth, laying or breeding performance of hens compared with the isogenic counterpart. Our results are in agreement with other generation studies using various GM-feeds in different animal species as recently summarized by Snell et al. (2012) and Ricroch et al. (2013).

References

Antoniou M., Robinson C., Fagan J., 2012. GMO Myths and Truths. Earth Open Source. London, pp. 123

Baranowski A., Rosochacki S., Parada R., Jaszczak K., Zimny J., Połoszynowicz J., 2006. The effect of diet containing genetically modified triticale on growth and transgenic DNA fate in selected tissues of mice. Anim. Sci. Pap. Rep. 24, 129–142

Brake D.G., Evenson D.P., 2004. A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development. Food Chem. Toxicol. 42, 29–36

Brake D.G., Thaler R., Evenson D.P., 2004. Evaluation of Bt (*Bacillus thuringiensis*) corn on mouse testicular development by dual parameter flow cytometry. J. Agr. Food Chem. 52, 2097–2102

Buzoianu S.G., Walsh M.C., Rea M.C. et al., 2012a. Effect of feeding Bt maize to sows during gestation and lactation on maternal and offspring immunity and fate of transgenic material. PLoS One 7, 10:e47851

Buzoianu S.G., Walsh M.C., Rea M.C., Cassidy J.P., Ryan T.P., Ross R.P., Gardiner G.E., Lawlor P.G., 2012b. Trans-generational effects of feeding genetically modified maize to nulliparous sows and offspring on offspring growth and health. J. Anim. Sci., published ahead of print October 24, 2012, doi:10.2527/jas.2012–5360

Chassy B.M., 2010. Food safety and consumer health. New Biotechnol. 27, 534–544

Daleprane J.B., Pacheco J.T., Boaventura G.T., 2009b. Evaluation of protein quality from genetically modified and organic soybean in two consecutives generations of Wistar rats. Brazil Arch. Biol. Technol. 52, 841–847

EFSA (European Food Safety Agency), 2008. Safety and nutritional assessment of GM plants and derived food and feed: the role of animal feeding trials. Food Chem. Toxicol. 46, 2–70

EFSA, 2011. Guidance for risk assessment of food and feed from genetically modified plants. EFSA J. 9(5), 2150, pp. 37

FDA (Food and Drug Administration; Redbook), 2007. Guidance for Industry and other Stakeholders, Toxicological Principles for the Safety Assessment of Food Ingredients. July 2000; Revised July 2007; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition. http://www.cfsan.fda.gov/guidance.html

Flachowsky G. (Editor), 2013. Animal Nutrition with Transgenic Plants. CAB International. Wallingford (UK), pp. 234

Flachowsky G., Halle I., Aulrich K., 2005. Long term feeding of Bt-corn – a 10 generation study with quails. Arch. Anim. Nutr. 59, 449–451

Flachowsky G., Schafft H., Meyer U., 2012. Animal feeding studies for nutritional and safety assessments of feeds from genetically modified plants: a review. J. Consum. Prot. Food Safety 7, 179–194

GfE (Gesellschaft für Ernährungsphysiologie), 1999. Recommendations for Energy and Nutrient Requirements of Laying Hens and Broilers (in German). DLG-Verlag,Frankfurt, pp. 185

Halle I., Aulrich K., Flachowsky G., 2006. Four generations feeding GMO-corn to laying hens. Proc. Soc. Nutr. Physiol. 15, 114

Haryu Y., Taguchi Y., Itakura E., Mikami O., Miura K., Saeki T., Nakajima Y., 2009. Long term biosafety assessment of a genetically modified (GM) plant: the genetically modified (GM) insect-resistant Bt11 corn does not affect the performance of multi-generations or life span of mice. Plant Sci. J. 3, 49–53

ILSI, 2003. Best Practices for the Conduct of Animal Studies to Evaluate Crops Genetically Modified for Input Traits. International Life Sciences Institute. Washington, DC, pp. 62

ILSI, 2007. Best Practices for the Conduct of Animal Studies to Evaluate Crops Genetically Modified for Output Traits. International Life Sciences Institute. Washington, DC, pp. 194

James C., 2013. Global Status of Commercial Transgenic Crops: 2012. ISAAA, Ithaca, NY, http://www.isaaa.org

Kiliç A., Akay M.T., 2008. A three-generation study with genetically modified Bt corn in rats: biochemical and histopathological investigation. Food Chem. Toxicol. 46, 1164–1170

Kleter G.A., Kok E.J., 2010. Safety assessment of biotechnology used in animal production, including genetically modified (GM) feed and GM animals – a review. Anim. Sci. Pap. Rep. 28, 105–114

Korwin-Kossakowska A., Sartowska K., Linkiewicz A., Tomczyk G., Prusak B., Sender G., 2013. Evaluation of the effect of genetically modified RR soya bean and MON 810 maize in the diet of Japanese quail on chosen aspects of their productivity and retention of transgenic DNA in tissues. Arch. Tierzucht 56, 60–70

Krzyzowska M., Wincenciak M., Winnicka A., Baranowski A., Jaszczak K., Zimny J., Niemiałtowski M., 2010. The effect of multigenerational diet containing genetically modified triticale on immune system in mice. Pol. J. Vet. Sci. 13, 423–430

OECD, 1993. Safety Evaluation of Foods Derived by Modern Biotechnology. Concepts and Principles. OECD, Paris (France)

Reuter T., Aulrich K., 2003. Investigations on genetically modified maize (Bt-maize) in pig nutrition: Fate of feed-ingested foreign DNA in pig bodies. Eur. Food Res. Technol. 216, 185–192

Rhee G.S., Cho D.H., Won Y.H. et al., 2005. Multigeneration reproductive and developmental toxicity study of bar gene inserted into genetically modified potato on rats. J. Toxicol. Env. Health A 68, 2263–2276

Ricroch A.E., Bernheim A., Snell C., Pascal G., Paris A., Kuntz M., 2013. Long-term and multigenerational feeding studies. In: G. Flachowsky G. (Editor) Animal Nutrition with Transgenic Plants. CAB International, pp.112–129

Sartowska K., Korwin-Kossakowska A., Sender G., Jozwik A., Prokopiuk M., 2012. The impact of genetically modified plants in the diet of Japanese qails on performance traits and the nutritional value of meat and eggs – preliminary results. Arch. Geflügelk. 76, 140–144

SAS, 2002–2008. Institute Inc., SAS 9.2, Cary, NC

Séralini G.E., Clair E., Mesnage R., Gress S., Defarge N., Malatesta M., Hennequin D., Spiroux de Vendômois J., 2012. Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food Chem. Toxicol. http://dx.doi.org/10.1016/j.fct.2012.08.005 (visited on November 6, 2012)

Séralini G.-E., Mesnage R., Clair E., Gress S., Vendomois J.S.de, Cellier D., 2011. Genetically modified crops safety assessments: present limits and possible improvements. Environ. Sci. Eur. 23, 10

Snell C., Berheim A., Bergé J.B., Kuntz M., Pascal G., Paris A., Ricroch A., 2012. Assessment of the Health Impact of GE Plant Diets in Long Term and Multigenerational Animal Feeding Trials: a Literature Review. Food Chem. Toxicol. 50, 1134–1148

Trabalza-Marinucci M., Brandi G., Rondini C. et al., 2008. A three-year longitudinal study on the effects of a diet containing genetically modified Bt176 maize on the health status and performance of sheep. Livest. Sci. 113, 178–190

Tudisco R., Mastellone V., Cutrignelli M.I., Lombardi P., Bovera F., Mirabella N., Piccolo G., Calabro S., Avallone L., Infascelli F., 2010. Fate of transgenic DNA and evaluation of metabolic effects in goats fed genetically modified soybean and in their offsprings. Animal 4, 1662–1671

Tyshko N.V., Zhminchenko V.M., Pashorina V.A., Selyaskin K.E., Saprykin V.P., Utembaeva N.T., Tutelyan V.A., 2010. Assessment of the impact of GMO of plant origin on rat progeny development in 3 generations (in Russian). Probl. Nutr. 80(1), 14–28

Valenta H., Dänicke S., Wolff J., 2002. Comparison of a HPLC- and an ELISA-method for determination of deoxynivalenol in dust of mills, brans and cereals (in German), 114. VDLUFA-Kongressband, p. 255

Valenta H., Oldenburg E., 1995. Determination of zearalenon, alpha-zearalenon and alpha-zerealenol in maize silage with HPLC and GC/MS (in German). VDLUFA-Schriftenreihe 40/1995, 107 VDLUFA-Kongress, Garmisch-Partenkirchen, pp. 957–960

VDLUFA-Methodenbuch (Editor), 2012. Band III, 1976 bis 2007. Die chemische Untersuchung von Futtermitteln. 3. Auflage. Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten. VDLUFA (Editor), 3.–8.

Velimirow A., Binter C., Zentek J., 2008. Biological effects of transgenic maize NK 603 × MON 810 fed in long term reproduction studies in mice. Research Report of Section IV, Vol. §/2008 of the Bundesministerium für Gesundheit, Familie und Jugend. Vienna (Austria)

WPSA (World Poultry Science Association), 1984. The prediction of apparent metabolizable energy values for poultry in compound feeds. Working Group No. 2 – Nutrition. World Poultry Sci. J. 40, 181–182

Zhou X.H., Dong Y., Wang,Y., Xiao X., Xu Y., Xu B., Li X., Wei X.S., Liu Q.Q., 2012. A three generation study with high-lysine transgenic rice in Sprague–Dawley rats. Food Chem. Toxicol. 50, 1902–1910