

ФУРАЖИ И ХРАНЕНЕ**GENETICALLY MODIFIED MAIZE IN MODEL NUTRITION**

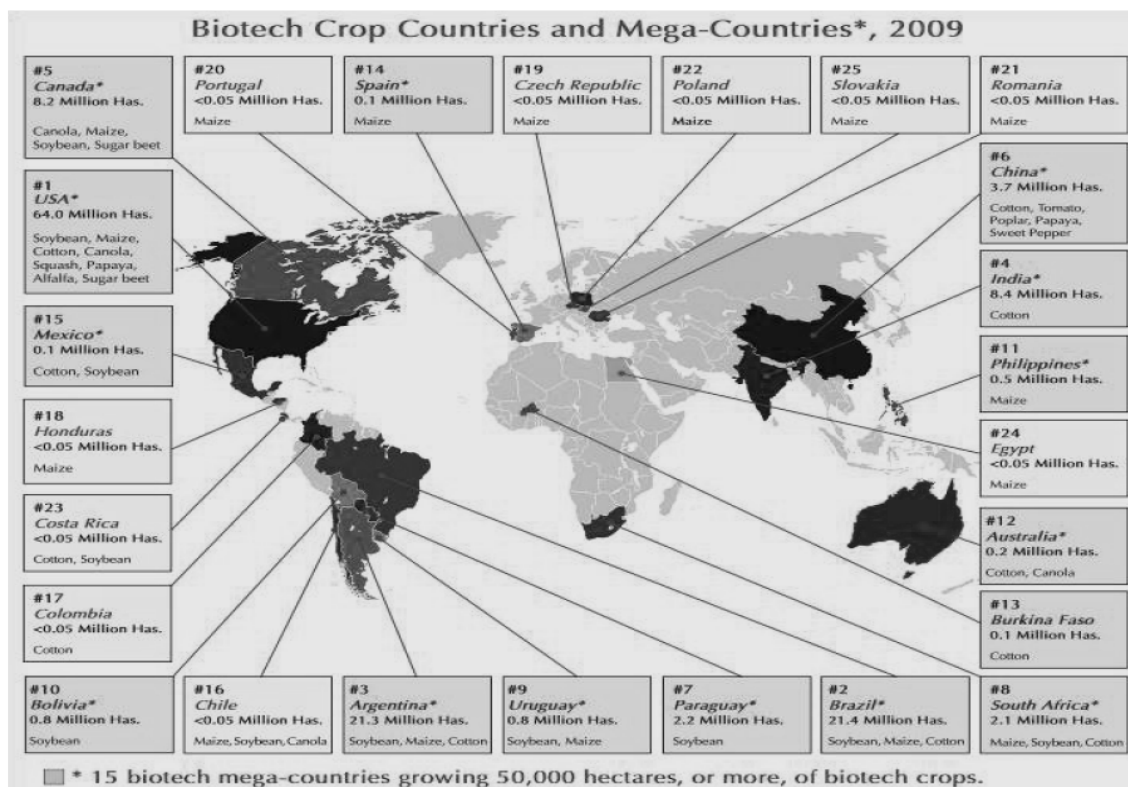
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In the European context, Slovakia ranks among other 7 EU countries which have practical experience in Bt maize cultivation; these are: Spain, France, Romania, Portugal, Germany, the Czech Republic and Poland (Kristková, 2010; Ervin et al, 2010). Here, Bt hybrids could represent a possible solution, as in terms of visual assessment of trials, a lower level of infection by fungal diseases was ascertained in comparison with conventional hybrids.

We tested genetically modified maize (Bt maize MON 88017) and isogenic maize (DKC5143) in the first experiment with rabbits and in the second experiment with Japanese quails. Bt maize contains bacterial gene from the bacteria *Bacillus thuringiensis*. This gene codes the protein which is toxic to some insects. We assessed substantial equivalence in nutrient contents between isogenic and Bt maize by means of chemical analyses.



MATERIAL AND METHODS

A total of 48 weaned rabbits (35th day of age, male sex, Hycole hybrid, housed individually in cages) were divided into 2 experimental groups. The rabbits in the 1st group were fed granulated mixture including 12% transgenic maize (MON 88017). The rabbits in the 2nd group were fed granulated mixture including 12% isogenic maize (DKC 5143).

The samples of individual feeds were analyzed for content of nutrients (Table 1; Table 2 Table 3) according to procedures of the AOAC (1995), and starch according to the alpha-amylglucosidase method. The ME content was calculated by the equation of **Wiseman et al.** (1992). Rabbits were fed *ad libitum* and they had free access to drinking water from nipple drinkers during the experiment. The diet formulation (complete granulated mixture, pellets of 3 mm diameter) for all groups is presented in Table 2. The experiment lasted for 42 days. Body weight and feed consumption were registered weekly. In fattening experiment were studied the growth of live weight and consumption of feed mixtures per unit of live weight growth. Between 65 and 70 days of age, 5 rabbits from each group were selected for digestibility tests using the balance method. The digestibility test was performed in

accordance with the recommended methodology (**Maertens and Lebas**, 1989).

Five animals from each group were slaughtered at 42nd day of experiment by cutting the jugularis and the carotid artery after electro-anaesthesia (90 V for 5 s). The samples of *Musculus longissimus dorsi* (MLD) were collected immediately after death and stored at 5°C for 24 h and physical-chemical analyses (according to STN 57 0185) were made. The content of proteins, fat, ash and water holding capacity were determined by standard method (estimated using an INFRATEC 1265 spectroscope and expressed in g.100g⁻¹ original matter from these values). Water holding capacity was determined by compress method at constant pressure (**Hašek and Palanská**, 1976). The content of gross energy value of MLD meat was calculated:

$$\text{Energy value in kJ.100 g}^{-1} = (16.75 \times \text{protein content}) + (37.68 \times \text{fat content}).$$

The ultimate pH 24 hours *post mortem* was measured by portable pH-meter mod. Radelkis OP-109 with a combined glass-gel electrode penetrating 3 mm into the MLD. Blood samples for biochemical and haematological analyses were obtained from the marginal ear vein (*Vena auricularis*) into dry non-heparinized glass tubes and blood serum was separated by centrifugation at 3000 x G for 10 min. In

Table 1. Chemical composition of transgenic maize and isogenic maize

Investigated parameters	Unit	1- transgenic maize MON 88017	2-isogenic maize DKC 5143
Dry matter	g.kg ⁻¹	872.94	870.02
Crude protein	g.kg ⁻¹	71.5	71.24
Crude fibre	g.kg ⁻¹	17.17	17.96
Fat	g.kg ⁻¹	33.08	36.1
Ash	g.kg ⁻¹	11.82	12.22
Nitrogen-Free Extract	g.kg ⁻¹	739.38	732.5
Organic matter	g.kg ⁻¹	861.12	857.71
Starch	g.kg ⁻¹	645.66	641.12
Sugar total	g.kg ⁻¹	20.5	20.74
Calcium total	g.kg ⁻¹	0.34	0.43
Phosphorus total	g.kg ⁻¹	2.28	2.32
ME	MJ.kg ⁻¹	13.32	13.37

Table 2. **Ingredients and chemical analysis of the experimental diets for rabbits**

Feed ingredients	in %	Chemical analysis (g. kg ⁻¹)	Experimental group	
			1-with transgenic maize MON88017	2- with isogenic maize DKC 5143
Lucerne meal	41	Dry matter	901.8	895.8
Dried beet pulp	10	Crude protein	172.8	168.1
Rape extr. meal	20	Crude fibre	179.2	183
Wheat	3	Fat	38.4	35.6
Apple pomace	9	N free extract	432.2	438.8
Maize	12	Organic matter	822.6	821.6
Carob meal	0.4	Starch	154.2	157.6
Minerals & Vitamins*	3.2	Calcium	9.3	6.7
Rape oil	1	Phosphorus	6.9	4.1
Limestone, pulverized	0.4	ME (MJ. kg ⁻¹)	9.42	9.16

*Provided per kg diet: vit. A 12000 IU; vit. D₂ 2500 IU; vit. E 20 mg; vit. B₁ 1.5 mg; vit. B₂ 7.5 mg; vit. B₆ 4.5 mg; vit. B₁₂ 30 µg; vit. K 3 mg; nicotic acid 45 mg; folic acid 0.8 mg; biotin 0.08 mg; Choline chloride 450 mg; Premix minerals (per kg diet) Ca 9.25 g; P 6.2 g; Na 1.6 g; Mg 1.0 g; K 10.8 g; Fe 327.5 mg; Mn 80 mg; Zn 0.7 mg

Table 3. **Ingredients and chemical analysis of the experimental diets of Japanese quails**

Feed ingredients	in %	Chemical analysis (g. kg ⁻¹)	Experimental group	
			1-with transgenic maize MON88017	2- with isogenic maize DKC 5143
Maize	40	Dry matter	901.8	895.8
Soybean meal (49%)	21	Crude protein	198.8	198.1
Rape meal (35%)	7	Crude fibre	35.2	35
Wheat	14.3	Fat	47.7	47.6
Sunflower meal (36%)	4.5	Starch	388.8	388.8
Malt sprouts (27%)	3	Sugar	37.8	37.6
Sodium chloride	0.3	Lysine	11.2	11.1
Minerals & Vitamins*	2.5	Calcium	9.3	9.7
Rape oil + animal fat	3	Phosphorus	4.9	4.7
Limestone, pulverized	4.4	ME (MJ. kg ⁻¹)	11.72	11.68

*1 kg premix contains: lysine 10 g, methionine 50 g, Ca 140 g, P 60 g, Na 35 g, Vit. A 400 000 IU, Vit. D₃ 100 000 IU, Vit. E 430 g, Vit. B₁ 80 mg, Vit. B₂ 175 mg, Vit. B₆ 110 mg, B₁₂ 600 mg, Vit. K₃ 80 mg, Folic acid 20 mg, Biotin 890 mg, Choline 12 000 mg, Calcium pantothenate 200 mg, Cu 480 mg, Zn 3 000 mg, Mn 48 000 mg, Fe 3 600 mg, KI 50 mg, Endox 5 000 mg.

blood serum were examined levels of proteins and lipids (g.l⁻¹), cholesterol (mmol.l⁻¹), glucose (mmol.l⁻¹), calcium (mmol.l⁻¹). Biochemical parameters were determined by an enzymatic colorimetric procedure using commercial set of Randox (United Kingdom). The activity of blood glutathione-peroxidase (GSH-Px; U.gHb⁻¹) was determined by a RANSEL stan-

dard set from Randox (United Kingdom). Moreover, the phagocytic activity (PA) was monitored and expressed as percentage of bacteria ingested per phagocyte (100 neutrophils) during a limited period of incubation of particles suspension and phagocytes in serum (**Hrubisko et al.**, 1981).

A total of 184 (1day-old) Japanese quails

(*Coturnix coturnix japonica*) were divided into four experimental groups and were housed in cages (46 per cage).

In the individual groups were tested:

1. quail fed mixture including 40 % transgenic maize (MON 88017);

2. quail fed mixture including 40 % isogenic maize (DKC 5143);

All quails were fed with experimental diet *ad libitum* and had free access to drinking water bell drinkers during the experiment. The growing experiment lasted for 42 days. In fattening experiment were studied the growth of live weight and consumption of feed mixtures per unit of live weight growth. We compared and observed the influence of Bt maize and isogenic maize on live weight growth, feed conversion and health status. For meat production is the slaughtering time at the age of 7 weeks. Six males from each of group were slaughtered and blood was collected to examine biochemical and mineral parameters in blood, and meat quality from these animals was tested. The quails were divided into 4 lines of meat type and three laying hybrids (line 11 was selected for low content and line 12 for high content of yolk cholesterol, and line 13 is control non-selected line). The evaluated egg performance of the meat type and the 3 laying hybrids Japanese quail during 12 months of laying period (100 Japanese quails in total) until 58th week of layers age. During the laying period all quails were weighed monthly three times; during the experiment the health status of each animal was reported. Eggs were co-

llected and weighed daily for the determination of laying-%; egg weight, egg mass and feed conversion rate and analyses of the egg content were done. Blood samples for biochemical parameters were collected after slaughter. Biochemical parameters were determined by an enzymatic colorimetric procedure using commercial set of Randox (United Kingdom). We evaluated 20 traits in total. The results were quoted as mean \pm standard deviation (SD); statistical evaluation of the results was performed by the one-way ANOVA and Tukey test.

RESULTS AND DISCUSSION

The study was carried out in the Animal Production Research Centre, Nitra, in the Institute of Nutrition. The performance of feed conversion in two mixtures with 12% of transgenic maize and isogenic maize intended for broiler rabbits were studied.

After weaning at 35 days, the rabbits were fattened until they were 77 days old (pelleted mixture, *ad libitum*). We did not find large differences among experimental groups in feed intake, body weight and carcass value in the fattening experiment (Table 4). The coefficients of digestibility for fat were within the interval from 72.50 to 78.22 %, which were similar to the data of **Battaglini and Grandi** (1988).

The values of blood parameters were changed in the framework of the physiological level (Post Graduate Committee in Veterinary Science). Some authors presented a wide range of blood parameters, mainly of cholesterol (**Canzi et al.**, 2000). Increased

Table 4. Test results of complete feed mixture in feeding and balance experiments with rabbits

Investigated parameters (<i>n</i> =24)	Experimental group	
	1- with MON 88017 transgenic maize	2- with DKC 5143 isogenic maize
Daily weight gain g/day	36.88	36.95
Feed conversion ratio in g/g	3	3.08
Carcass yield in %	57.27	57.78
	<i>Coefficient of nutrients digestibility (n=5)</i>	
Crude protein	65.72	63.97
Fat	72.5	78.22*
Crude fibre	25.25	25.29
Nitrogen-Free Extract	75.57	75.3
Organic matter	61.34	62.16

P < 0.05

levels of the biochemical parameters in blood serum could be explained as a result of better resorption and utilization of these nutrients from the gastrointestinal tract, which was also described in our previous studies (Sommer et al., 2004; Chrastinová et al., 2006; Chrenková et al., 2007; Po-gány Simonová et al., 2009).

At day 42 immunostimulative effect of maize was

noted in both experimental groups. Phagocytic activity was measured in the range from 43.7 to 44.8 %. Feeding of both kinds of maize to rabbits did not influence biochemical and zootechnical parameters, and it had no negative effect on health status and growth performance of rabbits.

In the experiment on Japanese quails was tested the effect 40% proportion of GM maize in mixture

Table 5. **Meat quality traits of longissimus dorsi muscle of broiler rabbits 24 h post mortem (x ± SD)**

Investigated parameters (g.100g ⁻¹ MLD)	Experimental group	
	1- with MON 88017 transgenic maize	2- with DKC 5143 isogenic maize
Content of water	75.37±0.06	75.6±0.46
Total proteins	21.83±0.15	21.9±0.20
Content of fat	1.8±0.10	1.47±0.31
Energetic value (kJ.100g ⁻¹)	433.53±1.34	423.34±14.86
pH ₂₄	5.56±0.02	5.57±0.04
Electrical conductivity (μS)	0.96±0.04	1.36±0.83
Water holding capacity	29.49±6.66	29.30±5.94

Table 6. **Biochemical parameters in the blood of rabbits (x ± SD)**

Investigated parameters (n=6)	Experimental group	
	1- with MON 88017 transgenic maize	1- with DKC5143 isogenic maize
Total proteins TP (g.l ⁻¹)	61.44± 3.87	54.77 ± 3.80
Cholesterol CHOL (mmol.l ⁻¹)	2.08 ± 0.24	1.71± 0.29
Triglycerides TRIGS (mmol.l ⁻¹)	1.10 ±0.45	0.98± 0.35
Glucose GLU (mmol.l ⁻¹)	8.10±0.37	7.96 ± 0.36
Alaninaminotransferaze ALT (U.l ⁻¹)	10.03± 2.78	8.61 ± 1.47
Calcium Ca (mmol.l-1)	3.24 ± 0.14	3.07 ± 0.15

(Crumlin, UK) kits (TP 245, CH 200, AL 100, GL 2623, CA 590) a Bio-La-Test (Lachema Brno) TG L 250 S

Table 7. **The value of PA and IPA in rabbits blood and the activity of blood GSH-Px**

Experimental group	Phagocytic Activity (%)	Index PA	The activity of blood enzyme glutathione peroxidase GSH-Px (U/g Hb)
1-Mon 88017	43.7±0.5	1.8±0.05	125.34±31.58
2-DKC5143	44.8±0.8	1.8±0.06	121.54±24.89

x ± SD; P > 0.05

on performance from hatching till sexual maturity (on day 42 of age). The average feed consumption of Japanese quails ($\sigma + \rho$) was 2.01 g on 1 g live weight. The average weight of meat line 09 (Pharaon) at the

Table 8. Effect of feeding mixtures with 40% content of transgenic and isogenic maize on the quality characteristics in eggs of Japanese quail

Observed parameters (%)		Line 9		Line 11		Line 12		Line 13	
		1	2	1	2	1	2	1	2
Yolk	Proportion out of total egg weight	32.5	30.5	33	32.1	31.5	29.1	31.2	31.3
	Dry mater	49.3	49.4	42.4	44.9	44.7	49.4	49.4	49.4
	Total protein	15.2	15.6	13.1	14.1	14	15.8	16	15.2
	Fat	25.9	25.6	22.5	23.9	21.1	25.9	23.9	26.5
Egg-white	Proportion out of total egg weight	53.7	53.3	53.8	54.3	49.8	56	54.1	54.5
	Dry mater	8.5	13.4	9.8	10.4	11.1	9.9	13.4	13.4
	Total protein	7.2	11.5	8.4	8.9	9.4	8.5	11.4	11.5
Egg shell	Proportion out of total egg weight	13.8	16.2	13.2	13.6	18.7	14.9	14.7	14.2
Total egg weight in g ($x \pm SD$)		12.56 \pm 1.2	12.49 \pm 1.9	10.52 \pm 0.9	10.51 \pm 0.9	11.2 \pm 1.2*	10.7 \pm 0.9	10.8 \pm 0.9	9.31 \pm 1.5

$P < 0.05$

Table 9. Content of nutrients of egg yolk in meat line and layer lines of Japanese quail at the end of laying period

Investigated parameters ($n=6$)	1- with MON 88017 transgenic maize		2-with KC5143 isogenic maize	
	Meat type	Layer type	Meat type	Layer type
Total water, (g.100g ⁻¹)	50.63	55.27	50.65	55.08
Total proteins, (g.100g ⁻¹)	15.76	14.01	15.59	14.08
Proportion EAA, %	55.35	53.63	54.39	53.81
Proportion NEAA, %	44.65	46.37	45.61	46.19
Total fat, (g.100g ⁻¹)	25.89	21.05	25.61	23.95
Energy value, (kJ.100g ⁻¹)	1239.7	1028	1226.3	1140.3
<i>Inorganic matter</i>				
Calcium, (g.1000g ⁻¹)	0.99	0.84	0.99	0.89
Phosphorus, (g.1000g ⁻¹)	5.55	5.15	5.58	5.07
Magnesium, (g.1000g ⁻¹)	0.11	0.1	0.1	0.09
Natrium, (g.1000g ⁻¹)	0.37	0.25	0.3	0.29
Potassium, (g.1000g ⁻¹)	1.12	1.14	1.07	1.02
Iron, (mg.1000g ⁻¹)	75.78	68.92	70.96	65.31
Manganese, (mg.1000g ⁻¹)	1.83	1.92	1.3	1.32
Zinc, (mg.1000g ⁻¹)	34.43	29.18	28.3	29.57
Copper, (mg.1000g ⁻¹)	1.65	1.6	1.51	1.54

age of 35 d has been 160 -165 g, weight of carcass varied from 117g to 120 g. The proportion of breast of the Japanese quail carcass was 30%.

The average live weight in one day old Japanese quails from lines 11, 12 and 13 was 6.5 ± 0.58 g and in meat line L9 (Pharaon) it was 8.46 ± 0.08 g. At the beginning of laying period was the average weight of layer lines (L11, L12 and L13) very similar 120 ± 2.28 g and live weight of layer after 20 weeks of laying increased to 150 ± 13.05 g, which is in accordance with results of other authors (Konèeková and Baumgartner, 2004; Baumgartner et al.,

2004). The average feed consumption of experimental diet (from birth to 42 days of age) was 400g per quail. The level of laying intensity in quails to 20 weeks age was 65.23 %.

Weight of eggs, egg-white and yolk was higher in meat line compared with eggs from quails of laying type, however, weight of shell was higher in laying lines. If the proportion of individual components in egg is concerned, higher proportion of white and lower proportion of yolk and shell out of the egg weight were in meat line quails compared with laying line quails (Table 9).

Table 10. Biochemical parameters of blood in Japanese quails at the age 7 weeks

Investigated parameters (n=6)	Units	Meat type		Layer type	
		Line 9	Line 11	Line 12	Line 13
Total proteins, (CB)	g.l ⁻¹	35.10	34.09	37.89	35.26
Glucose, (GLU)	mmol. l ⁻¹	14.85	14.20	14.40	13.71
Triglyceride, (TRIGS)	mmol. l ⁻¹	1.62	1.15	1.34	1.36
Cholesterol, (CHOL)	mmol. l ⁻¹	7.11	6.89	6.27	6.53
Calcium, (Ca)	mmol. l ⁻¹	2.00	2.30	1.60	2.40
Phosphorus, (P)	mmol. l ⁻¹	2.21	2.43	2.74	2.35
Magnesium, (Mg)	mmol. l ⁻¹	1.33	1.23	1.25	1.33
Sodium, (Na)	mmol. l ⁻¹	150.50	146.30	142.30	146.80
Potassium, (K)	mmol. l ⁻¹	3.10	3.90	5.50	4.80
Chloride, (Cl)	mmol. L ⁻¹	112.40	111.60	108.30	112.10

Table 11. Physico-chemical characteristics of breast muscles in meat line and layer lines of Japanese quail of 7 weeks age

Investigated parameters (n=6)	Meat type		Layer type	
	Line 9	Line 11	Line 12	Line 13
<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>
pH ₂₄	6.20	6.19	6.08	5.96
Total of water (g.100g ⁻¹)	73.80	74.70	74.10	73.90
Total proteins, (g.100g ⁻¹)	22.95	22.70	22.80	22.60
Proportion EAA, %	56.61	55.31	56.47	55.62
Proportion NEAA, %	43.39	44.69	43.53	44.38
Total fat, (g.100g ⁻¹)	2.25	1.60	2.10	2.50
Water holding capacity, (g.100g ⁻¹)	24.85	26.25	26.77	34.67
Energy value, (kJ.100g ⁻¹)	469.19	440.51	461.03	472.75

Continuation of Table 11

<i>I</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>
	<i>Inorganic matter</i>			
Calcium, (g.1000g ⁻¹)	0.26	0.20	0.27	0.17
Phosphorus, (g.1000g ⁻¹)	2.54	2.68	2.67	2.64
Magnesium, (g.1000g ⁻¹)	0.28	0.30	0.30	0.31
Natrium, (g.1000g ⁻¹)	0.37	0.40	0.43	0.40
Potassium, (g.1000g ⁻¹)	4.01	4.09	4.16	4.06
Iron, (mg.1000g ⁻¹)	15.53	19.09	21.61	20.77
Manganese, (mg.1000g ⁻¹)	0.33	0.47	0.50	0.30
Zinc, (mg.1000g ⁻¹)	18.53	20.49	17.60	25.71
Copper, (mg.1000g ⁻¹)	1.44	1.82	2.13	2.28

There were almost no differences in physico-chemical characteristics of meat and eggs qualities in Japanese quails fed feed mixture with 40 % proportion of Bt maize compared with values in control groups. The changes were not recorded in biochemical indicator of blood till the end of experiment either (Table 11).

The values of selected biochemical parameters in blood ranged within the physiological limits. Physico-chemical characteristics of breast muscles meat are presented in Table 12. Additional maize in complete feed mixtures did not significantly influence the physico-chemical composition of meat; higher values of water holding capacity, fat and energy value were detected in layer line 13 of Japanese quail fed with DKC 5143 isogenic maize.

The results of this experiment showed that the incorporation of Bt maize had no significant influence on followed parameters of Japanese quails.

CONCLUSION

The health status of animals was good. Feeding of transgenic maize and isogenic maize to rabbits and Japanese quails did not negatively influence biochemical parameters of meat and zootechnical parameters and biochemical parameters of blood. As well as it had no negative effect on growth performance of rabbits. It had no negative influence on body weight and eggs quality parameters during laying period In Japanese quails.

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SUMMARY

The objective of this work was to determine the effect of genetically modified Bt maize (MON 88017) in animal nutrition. Bt maize was tested in two experiments and two animal species. In the first Bt maize was included in rabbit (12%) and in the second in Japanese quail (40%) diets. We compared and observed the influence of Bt maize and isogenic maize on live weight growth, feed conversion and health status, digestibility of nutrients, biochemical and mineral parameters in blood and meat quality of the tested animals. We evaluated

the egg performance of the meat type and of the three laying hybrids of Japanese quail during 12 months of laying period (100 Japanese quails in total) from the 58th week of layers age. We evaluated 20 traits in total. Weight of eggs, egg-white and yolk was higher in meat line compared with those of eggs from quails of laying type, however, weight of shell was higher in the laying lines. As far as the proportion of individual components in egg is concerned, higher proportion of white and lower proportion of yolk and shell out of the egg weight were found in meat quails compared with laying quails. For the test of meat production the slaughtering was made at the quail age of 7 weeks. The quails had a very well developed breast part reached in the mentioned time more than 30% of the males' carcass yield. Also the technological quality and nutritive value of quail meat is very high. In the rabbit part of the experiment were tested complete feed mixtures with 12 % proportion of Bt (MON 88017) and isogenic maize (DKC5143) on 48 broiler rabbits (Hycola). Significant differences between Bt and isogenic maize were not recorded in any of the observed parameters.

In the experiment with Japanese quails 40 % of Bt maize were included into an experimental diet and this experimental group was compared with the control group with diet with 40% ration of isogenic maize. The results of this experiment showed that the incorporation of Bt maize had not significant influence on the followed parameters of Japanese quails.

Key words: *rabbit; nutrients in meat; biochemical and mineral parameters in blood; Bt maize (MON 80817) Japanese quail; quality of eggs*

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