Genetically modified crops in a 10-generation feeding trial on Japanese quails — Evaluation of its influence on birds' performance and body composition

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ABSTRACT The effect of genetically modified (GM) feed components comprising soya bean meal and maize on the performance indices (reproduction, survival rate, growth, egg production, relative weight of chosen internal organs, and basic chemical composition of breast muscle and egg yolk) of Japanese quails was investigated during a 10-generation trial. A total number of 8,438 healthy quail chicks were used in the course of the trial. In each generation, birds were maintained in 3 experimental groups differing in the main feed components, i.e. 1) GM soya (Roundup Ready) and non-GM maize, 2) GM maize (MON810) and non-GM soya, and 3) non-GM soya and maize. The different feeds used did not influence any of the biological hatch indices, survival rate, or BW of young or adult quails. With regard to egg-laying performance, the GM maize group showed a better laying percentage and a higher egg mass production compared to the other groups; the GM soya group showed reduced average egg mass compared to the other groups, whereas the overall egg production level was the same as in the control group. Results showed a higher relative weight of breast muscle and gizzard in birds fed GM maize compared to the control group, whereas live BW and the relative weights of liver and heart were not different among groups. Meat from the GM soya group showed higher protein and lower fat levels compared to the control group. In the case of egg yolk, its chemical composition in the experimental groups did not differ from the control group. Even though some differences were found among the feeding groups, none could be judged as a negative influence of GM maize or GM soya in feed on the birds or final consumer products over 10 generations of Japanese quails.

Key words: genetically modified maize, genetically modified soya, Japanese quail, breeding, performance

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INTRODUCTION

Genetically modified organisms (**GMO**) and derived food and feed products are subject to a risk analysis and regulatory approval before they can enter the market. For example, in the European Union (**EU**) scientific advice on the safety of GMO is provided to the decision makers by the European Food Safety Authority's (**EFSA**) GMO Panel, which consists of experts with a broad range of expertise, covering, among others, molecular biology, biotechnology, and human and animal health. The panel is supported by additional experts and EFSA staff, as well as by EU member state authorities. (Devos et al., 2014). In the United States, which is the largest commercial grower of genetically modified (**GM**) crops in the world, a GMO must be approved for its release within the country, and it must be assessed for its safety by the Animal and Plant Health Inspection Service (**APHIS**) agency within the US Department of Agriculture (**USDA**). Depending on the intended use of the organism, it also may have to be assessed by the Food and Drug Administration (**FDA**) and the Environmental Protection Agency (**EPA**). Other countries have their own internal regulations or committees overlooking the release of GMO to the market or environment, and a regulatory safety assessment. Years before the first GM crops came to the market, international organizations such as the Food and Agriculture Organization (**FAO**), World Health Organization (**WHO**), and the Organization for Economic Cooperation and Development (**OECD**), had been building an international consensus on how to carry out the assessment of the safety of GMO. This led to the formulation of the comparative safety assessment approach, which later became enshrined in the guidelines for the safety assessment of foods derived from GM crops and from GM microorganisms in 2003 (Codex Alimentarius, 2003a,b; Kleter and Kok, 2010). Despite the strict regulations for regulatory approval, and the global consensus on the safety assessment of GMO, this technology still remains controversial in some parts of the globe for various reasons. Several critics have

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raised the issue of potential long-term health effects and the need to investigate these before market admission (Séralini et al., 2014), whereas a review on various long-term and multi-generation animal studies in different animal species with different GM crops concluded that the supplementary information thus provided by these studies did not indicate that shorter-term, 90-day rodent studies would be insufficient to test for specific hazards on a case-by-case basis (Snell et al., 2012).

Laboratory rodents are a commonly used model for testing the toxicity of substances. However, quails also have found an application in testing the toxicity of chemicals, including potential endocrine disrupting effects (Ottinger and Dean, 2011). Among the reasons for the popularity of quail are its well-known biology, its short reproductive cycle, and its use as a model for other avian species (both domestic and wild). In this study, to verify the safety and possible influence of GM feed ingredients on animals in the long term, a 10-generation experiment was conducted on Japanese quails as a model animal, evaluating bird performance indices, including reproduction, survival rate, growth, egg production, body composition (relative weight of chosen internal organs), and the basic chemical composition of breast muscle and egg yolk.

This experiment followed previous studies on the potential impacts of GM ingredients on quail health performed in our facility (Sartowska et al., 2012; Korwin-Kossakowska et al., 2013), which failed to find relevant health impacts of feeding these ingredients on quail health.

MATERIALS AND METHODS

Animals and Diets

The trial consisted of 10 generations of Japanese quails (*Coturnix cot. japonica*) bred in 3 parallel feeding groups in the yr 2010 to 2013 in the Experimental Farm of the Institute of Genetics and Animal Breeding in Jastrzebiec, Poland. Eggs used for incubation were examined for egg cracks, proper size, shape, and shiny surface. At the d of hatch (d 17) chicks and unhatched eggs were examined. Eggs were opened and divided into 2 groups: dead-in-shell and unhatched (if the chick was still alive). Crippled chicks were culled. The first generation of birds was obtained from chicks of the institute's own flock of Japanese quails and divided randomly into 3 feeding groups since their first day of life. In the 10 generations, a total of 10,947 eggs were incubated and 8,438 healthy chicks entered the trial A detailed description of the rearing of the chicks is described in a previous publication by the same authors, Sartowska et al., 2012. Healthy birds were transferred to cages. Chicks from each of the 3 groups were raised in separate cages with infrared heaters and 24-hr light until 2 wk of age. Afterwards, young birds were moved to temporary cages (5 repetitions per group) until they grew up. Then, at the age of 6 wk, part of the healthy birds of a proper body composition were chosen and randomly divided into the final selection flocks of 17 females and 5 males in each. Each group consisted of 6 repetitions of such flocks, resulting in 102 females and 30 males in each of 3 groups in each of 10 generations. In total 3,960 adult birds were used in the trial (396 per generation). Birds were housed according to the procedure existing on the Experimental Farm. They remained in stainless-steel wire battery cages (in accordance with applicable standards), equipped according to their age with a mesh floor, infrared heating lamps, and manual drinking and feeding appliances, or later with a sloped wire floor and with automated drinking and manual feeding appliances. During the early laying period (from age 7 wk to 16 wk), egg production was evaluated, and at the age of 16 wk eggs were collected for incubation in order to form the next generation.

The following feeds were used: A - GM soya bean meal included; B - GM maize included; C - non-GMO materials. The GM soya bean meal used was produced from: Roundup Ready soya beans (Glycine max. L cv A 5403, line 40–3–2), GM MON 810 maize, and their conventional counterparts. Birds received 2 types of diet: grower (1 to 6 wk) and layer (7 to 16 wk), with the same pattern in all 10 generations. Feeds were prepared by the feed company Agro-Kocieba (Bogdan Kocieba, Czarnocin, Poland). Details of feed composition are presented in Table 1. The level of basic nutrients in the feeds was analyzed in each generation according to the methods of A.O.A.C. (2005), and it was confirmed to be as planned. The content of modified DNA in feeds was examined by a reference GMO laboratory in the Plant Breeding and Acclimatization Institute - National Research Institute (Radzików, Poland). Detection, identification, and quantitative determination of the GMO were done by quantitative PCR and Real-Time PCR methods. Results confirmed the presence of MON 810 maize DNA in group B feed, but not in other feeds, as well as the presence of RR soya DNA in group A feed, but not in other feeds. Group C feed was proven to be free from the above modifications.

Birds in each cage received the same amount of feed, and leftovers were monitored weekly.

Sample Collection and Analyses

Basic production performance was recorded in the course of the trial. Biological hatch analyses were performed each time new birds hatched, including candling eggs on d 14 of incubation (when the number of infertile eggs were noted), and a more detailed analysis on d 18 (including chicks dead-in-shell, chicks unhatched, crippled chicks, and healthy chicks). Quails hatched on d 17 of incubation, but they were maintained for 24 more hr in the incubator, according to standard procedures for breeding flocks. The results of hatch analyses were calculated into the percentage of eggs set for incubation

Table 1. Composition and nutritional value of feeds: experimental diets A, B, and C Groups: A-GM soya, B-GM maize, C-non-GMO.

Indices	Grower feed			Layer feed		
	Group			Group		
	A	B	C	A	B	C
Ingredients, g/kg						
RR soya bean meal GMO	390.0	0.0	0.0	295.0	0.0	0.0
Maize grain MON 810 GMO	0.0	250.0	0.0	0.0	250.0	0.0
Maize grain non-GMO	250.0	0.0	250.0	250.0	0.0	250.0
Soya bean meal non-GMO	0.0	390.0	390.0	0.0	295.0	295.0
Wheat	196.7	222.0	196.7	233.3	233.8	233.8
Triticale	25.0	0.00	25.0	50.0	50.0	50.0
Rapeseed meal	41.7	41.7	41.7	0.0	0.0	0.0
Rapeseed	0.0	0.0	0.0	55.6	55.6	55.6
Soya oil	22.3	21.9	22.3	29.8	30.0	30.0
Potato protein	25.3	23.1	25.3	0.0	0.0	0.0
Maize gluten	15.5	17.8	15.5	3.1	0.0	0.0
Limestone	13.4	13.4	13.4	62.6	62.6	62.6
Monocalcium phosphate additives[1]	9.0	9.0	9.0	9.5	9.5	9.5
Nutritional value (calculated)						
Energy[2], x10^3 kcal	2.95	2.95	2.95	2.80	2.80	2.80
Crude protein, %	26.5	26.5	26.5	20.9	20.8	20.8
Crude fat, %	6.1	6.0	6.1	5.5	5.6	5.6
Crude fibre, %	2.8	2.8	2.8	3.0	3.0	3.0
Ash, %	5.8	5.8	5.8	10.4	10.4	10.4
Ca total, %	0.97	0.97	0.97	2.78	2.77	2.77
P available, %	0.45	0.45	0.45	0.45	0.45	0.45

[1] The following additives were included in the diets in grower/layer feed (g/kg): vitamin-mineral premix 3.2/5, NaCl 2.5/2.5, sodium bicarbonate 1.4/2.1, phyzyme 0.1/0.1, ronozyme 0/0.2, methionine 1.8/1, L-lysine 0/0.4 and threonine 0/2.3.

[2] AME = Apparent metabolizable energy.

or the percentage of fertile eggs (in the case of healthy chicks).

Weekly BW was evaluated as average for birds in each cage: wk 0 to 4 (brooding period) all chicks together; wk 7, 11, and 15 (laying period) separately for females and males. Weekly laying performance was measured by the number of eggs laid divided by the number of females in each cage, and calculated into the laying percentage. Average egg weight was measured on a chosen d in the middle of the wk. Egg production was calculated as a product of the egg-laying percentage and average egg mass.

Each case of a bird's death or culling during the experiment was noted, and mortality was then calculated for both the brooding and laying phases (1 to 6 wk and 7 to 16 wk, respectively).

At wk 17 of each generation, experimental dissection took place, and measurements were taken of live BW and weight of breast muscle, liver, gizzard, and heart of each bird. Birds were sacrificed according to the *Guiding Principles for the Care and Use of Experimental Animals*, in a separate room, by cervical dislocation. Dissection comprised 12 females and 12 males randomly chosen from each nutritional group in each generation, resulting in 720 birds sacrificed and 720 samples of each organ collected. The relative weight of organs was calculated into the percentage of the live BW of each respective bird.

The chemical composition of fresh minced meat from the *musculus pectorialis major* samples was evaluated by NIR spectrometer (percentage of water, protein, fat, and ash) with the use of the near-infrared radiation spectrometer (NIR Flex Solids N500, Buchi, Switzerland). Dry matter was then calculated from the water content. During wk 17 samples of egg yolk were also obtained—12 samples (pooled from 5 egg yolks) from each nutritional group in each of 10 generations, resulting in 360 total samples. The collected egg yolk samples were lyophilized in order to evaluate the percentage of dry matter. The obtained dry samples were analyzed with the use of the near-infrared radiation spectrometer (NIR Flex Solids N500, Buchi, Switzerland), for protein, fat, ash, and residues of water content. Indices were then calculated, taking into account water losses during the lyophilization step.

Statistical Analyses

An analysis of variance (GLM procedure of SAS/STAT 2010, SAS Institute Inc., Cary, NC) was performed to evaluate the differences in the level of the traits among the 3 feeding groups. The analysis was made on data referring to generations one to 10. The significance of differences between the values of the traits was estimated by Duncan's test. Means were regarded as different at the level of $P < 0.01$.

The models for analysis of variance included fixed effects as follows:

1. for the biological hatch analyses and mortality: feeding group and subsequent generation;

Table 2. Biological hatch analyses (%) (LSMean, ± SE), average of 10 generations.

Group	Eggs not fertilized	Chicks dead-in-shell	Chicks unhatched	Chicks crippled	Chicks healthy	Healthy Healthy
A	5.8	10.9	4.1	1.9	77.2	82.1
B	5.8	12.3	4.5	2.7	74.7	79.2
C	6.1	13.5	5.0	2.4	73.1	77.7
SE	±1.05	±3.61	±0.92	±0.38	±4.00	±4.06

Groups: A-GM soya, B-GM maize, C-non GMO.

LSMean = Last Square Means; SE = Standard Error.

n = 10,947 incubated eggs.

2. for the BW in the brooding period: the feeding group, subsequent generation, age, feeding group-by-age interaction;
3. for the BW in the laying period: the effects of the feeding group, subsequent generation, sex and age of birds, the interaction of feeding group-by-age-by-sex;
4. for the laying rate effects of the feeding group, subsequent generation, age and feeding group-by-generation, group-by-age, generation-by-age;
5. for the egg weight effects of the feeding group, subsequent generation, age and feeding group-by-generation, group-by-age interactions;
6. for the carcass and meat composition, including fixed effects as follows: feeding group, sex, subsequent generation and group-by-sex and generation-by-sex interaction;
7. for egg yolk composition: this same effect was included in the model, except the sex of birds.

All procedures were approved by the third Local Ethics Committee for Animal Experimentation in Warsaw, Poland, in Resolution No 27/2009, and performed in accordance with the *Guiding Principles for the Care and Use of Experimental Animals*.

RESULTS AND DISCUSSION

Hatching

Biological hatch analyses revealed no differences among the feeding groups (Table 2). Fertility was maintained at an exceptionally high level (around 94%) as compared to standard values presented by Shanaway (1994) and Szczerbińska et al. (2012). The biggest losses were caused by chicks dead-in-shell, which are understood to have died in earlier stages of development. Along with the duration of the trial, in the hatch of generations 3 and 9, an increased number of dead-in-shell chicks was observed (19.5 and 38.0% respectively), which is explained by an incidental electricity outage during one night. However, it influenced the control and experimental groups in the same manner. Otherwise, in the other generations, the level of dead-in-shell chicks fluctuated between 1 and 11%, with an average of 6.75%. The percentage of unhatched and crippled chicks was reasonably low and, as a result, the percentage of healthy chicks was satisfactory compared to the results achieved by another research team in Poland (Szczerbińska et al., 2012).

Table 3. BW of young quails (LSMean, ± SE), average of 10 generations.

Age (wk)	Group			SE
	A	B	C	
0 (*n = 30)	7.6	7.5	7.4	±0.73
1 (*n = 30)	23.7	24.7	24.5	±0.73
2 (*n = 30)	42.1	43.2	43.5	±0.72
3 (**n = 40)	77.2	77.9	77.0	±0.70
4 (**n = 40)	109.8^B	114.5^A	112.2	±0.62

Groups: A-GM soya, B-GM maize, C-non GMO.

LSMean = Last Square Means; SE = Standard Error.

^{A,B}Means with different superscripts in lines differ at $P \leq 0.01$. Letters indicate differences between feeding groups.

*n = 10 generations x 3 repetitions (cages) (about 94 chicks in one cage in each group).

**n = 10 generations x 4 repetitions (cages) (about 70 chick in one cage in each group).

Feed Consumption

The level of feed consumption (based on the evaluation of leftovers) was similar in all groups. The average weekly feed consumption of the adult quails was 220 g/bird.

Growth

Changes in the BW of hatched quail chicks and within their first wk of life were very fast—typical for this species (Table 3). No differences between feeding groups were noted on the d of hatching and in most of the brooding period. However, at the age of 4 wk quails from group B (GM maize) were significantly heavier compared to birds from group A (GM soya). Birds from the control group had a BW in-between.

Even though 4-week-old chicks were of different weight, adult quails showed no differentiation in BW among the groups (Table 4). During the trial adult birds slightly increased their BW, but no differences were found among the feeding groups. Marked differences were noted between males and females, as quails are a species showing dimorphism in size and color (Rutkowski, 2012).

Table 4. BW of adult quails (LSMean, ± SE) average of 10 generations.

Age (wk)		Group			SE
		A *n = 60	B *n = 60	C *n = 60	
7	Female	201.7	206.8	201.6	±1.36
	Male	169.0	169.6	169.0	
11	Female	219.9	226.3	220.1	±1.36
	Male	177.5	178.3	176.9	
15	Female	226.9	232.1	229.5	±1.36
	Male	182.8	182.7	180.8	

Groups: A-GM soya, B-GM maize, C-non GMO.

LSMean = Last Square Means; SE = Standard Error.

*n = 10 generations x 6 repetitions (cages):17 adult females, 5 adult males in each cage.

Table 5. Mortality of young and adult quails, average of 10 generations in different feeding groups (in%; LSMean, ± SE).

Group	1 to 6 wk *n = 30	7 to 16 wk **n = 60	1 to 16 wk ***$\sum$
A	16.5	2.4	17.8
B	19.4	5.4	24.8
C	18.3	3.4	21.7
SE	±3.40	±0.92	±3.50

Groups: A-GM soya, B-GM maize, C-non GMO.

LSMean = Last Square Means; SE = Standard Error.

*n = 10 generations x 3 repetitions (cages).

**n = 10 generations x 6 repetitions (cages).

*** $\sum$ Sum of mortality from wk 1 to 6 and 7 to 16.

Survival Rate

The mortality of young and adult birds in the 3 nutritional groups was at the same level—no significant differences were revealed by the statistical analyses. (Table 5). Mortality in the brooding period seems quite high, although the average was increased by the weak results of the first generation. There were no lesions in birds at this time, and the reason for mortality is not certain. Presumably, this may be too high density of chicks, too low room temperature, or cannibalism of young animals (Shanaway, 1994). It was considered of no influence on the birds' subsequent performance, as the growth and laying performance did not differ much among generations. Otherwise, in the other generations, the mortality in this period was below 15%. This number includes necessary culling. Mortality in the laying period was maintained at a reasonable level—below 4% on average.

Egg Production

The laying percentage as well as the daily egg production achieved by birds from group A (GM soya) was the same as in the control group, whereas the results from group B (GM maize) were significantly ($P < 0.01$) higher. Average egg mass was the same in group B as compared to the control group, but was reduced in group A ($P < 0.01$), as presented in Table 6. Laying percentage changes observed with time on the laying curve show an advantage in group B during the early wk of the laying period (Figure 1).

Carcass Composition

The BW of birds randomly selected for dissection did not differ among groups, as presented in Table 7. The relative weight of heart and liver was the same in all 3 nutritional groups. The relative weight of breast muscle was significantly higher in group B (GM maize) compared to groups A (GM soya) and C (non-GMO) ($P < 0.01$). Similarly, the relative weight of gizzard was significantly higher in group B compared to group C ($P < 0.01$), whereas group A had a weight in-between. As quails are characterized by sexual dimorphism, both the BW of live birds and all internal organ weights were significantly different between males and females. As discussed in our previous article (Sartowska et al., 2014), the differences are related to the hormonal system of males and females.

Chemical Composition

The basic chemical composition of breast muscle meat was the same, whether obtained from male or female quails (Table 8). However, meat obtained from group A (GM soya) had significantly higher levels of protein and significantly lower levels of fat compared to the control group C (non-GMO) ($P < 0.01$). Group B (GM maize) showed levels in-between. From the viewpoint of human nutrition, these differences are not of great importance. In general, the values were in line

Table 6. Egg production indices of quails, average of 10 generations in different feeding groups (in%; LSMean ± SE).

Trait	Group			SE
	A *n = 600	B *n = 600	C *n = 600	
Laying percentage	75.7^B	77.7^A	75.5^B	±0.31
Egg weight (g/egg)	11.4^B	11.6^A	11.6^A	±0.02
Egg production (g/bird per day)	8.9^B	9.3^A	9.1^B	±0.05

Groups: A-GM soya, B-GM maize, C-non GMO.

LSMean = Last Square Means; SE = Standard Error.

^{A,B}Means with different superscripts in lines differ at $P \leq 0.01$. Letters indicate differences between feeding groups.

*n = 10 generations x 10 wk x 6 repetitions (cages) (17 females in each cage).

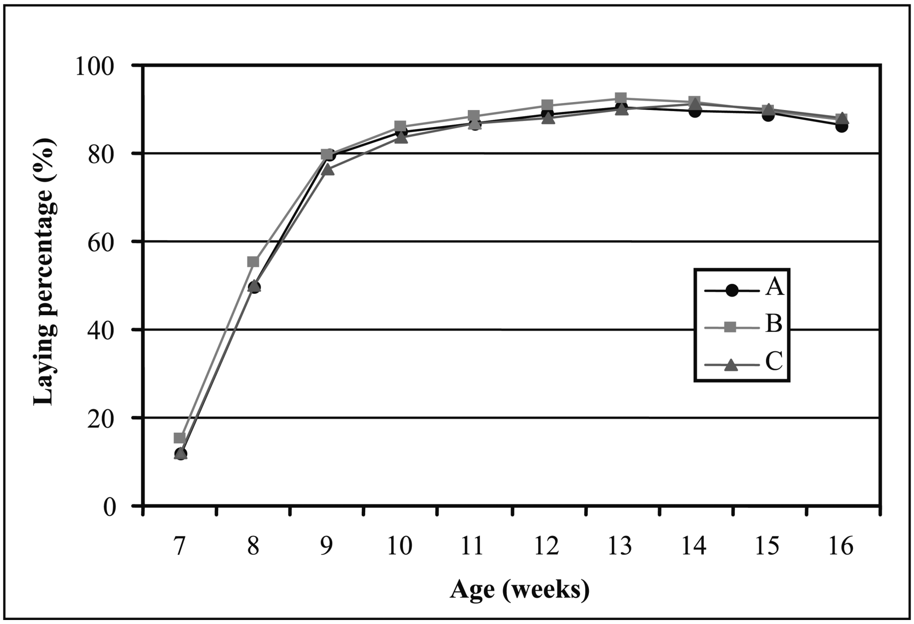

Figure 1. Early laying performance of quails in the 3 feeding groups, average of 10 generations (n = 600). A - animals fed GM soy; B - animals fed GM maize; C - non-GM ingredients in feed.

Table 7. BW and percentage of edible organs weight in relation to BW of birds randomly selected for dissection in different feeding groups, results from 10 generations (as LSMean ± SE).

Trait	Group			SE	Mean of all groups (**n = 360)
	A	B	C		
BW (g)					
Male	188.8	187.8	184.1	±1.57	186.9^{Y}
Female	221.9	225.8	226.6	±1.57	224.77^{X}
Mean (*n = 240)	205.4	206.8	205.4	±1.11	SE±0.90
Breast muscle (% of BW)					
Male	18.0	18.6	17.9	±0.15	18.2^{X}
Female	17.2	17.4	16.9	±0.15	17.2^{Y}
Mean (*n = 240)	17.6^{B}	18.0^{A}	17.4^{B}	±0.10	SE±0.08
Liver (% of BW)					
Male	2.1	2.1	2.04	±0.04	2.1^{Y}
Female	3.2	3.2	3.14	±0.04	3.2^{X}
Mean (*n = 240)	2.6	2.6	2.59	±0.03	SE±0.02
Gizzard (% of BW)					
Male	1.4	1.4	1.4	±0.02	1.4^{X}
Female	1.3	1.4	1.3	±0.02	1.3^{Y}
Mean (*n = 240)	1.4	1.4^{A}	1.3^{B}	±0.01	SE±0.01
Heart (% of BW)					
Male	0.9	0.9	0.9	±0.01	0.9^{X}
Female	0.8	0.8	0.8	±0.01	0.8^{Y}
Mean (*n = 240)	0.8	0.8	0.8	±0.01	SE±0.01

Groups: A-GM soya, B-GM maize, C-non GMO.

LSMean = Last Square Means; SE = Standard Error.

^{A,B}Means with different superscripts in lines differ at $P \leq 0.01$. Letters indicate differences between feeding groups.

^{X,Y}Means with different superscripts in columns differ at $P \leq 0.01$. Letters indicate differences between males and females.

*n = number of birds (12 females and 12 males) in each generation in each group.

**n = number of birds in all 3 groups (36 females and 36 males).

with an earlier trial conducted by Jakubowska et al. (2013).

In the case of egg yolk basic chemical composition, groups A and B differed between each other, but they did not differ from the control group (Table 9).

Summarizing, based on the 10-generation experiment, GM maize or soya used in Japanese quails' feed did not negatively influence their hatch parameters, growth, egg production, or body composition indices. Results presented in the current article also indicate no negative effect on the chemical composition of meat or eggs obtained from quails under trial. Despite the reported small differences in some of the evaluated traits in different feeding groups, the birds showed no trends that would indicate any negative effect of the modifications used. These results are in line with

Table 8. Chemical composition of *musculus pectorialis major* samples obtained from males and females in different feeding groups (in%, as LSMean ± SE), average of 10 generations.

Trait	Group A	 B	 C	SE	Mean of all groups (**n = 360)
Dry matter					
Male	25.8	26.0	25.9	±0.07	25.9
Female	25.9	25.9	25.9	±0.07	25.9
Mean (*n = 240)	25.9	25.9	25.9	±0.05	SE±0.04
Protein					
Male	22.9	22.9	22.7	±0.04	22.9
Female	22.9	22.9	22.8	±0.04	22.9
Mean (*n = 240)	22.9^{A}	22.9	22.8^{B}	±0.03	SE±0.02
Fat					
Male	1.9	2.1	2.4	±0.07	2.2
Female	2.2	2.3	2.3	±0.07	2.3
Mean (*n = 240)	2.1^{B}	2.2	2.4^{A}	±0.05	SE±0.04
Ash					
Male	1.6	1.6	1.6	±0.02	1.6
Female	1.5	1.6	1.6	±0.02	1.6
Mean (*n = 240)	1.5	1.6	1.6	±0.01	SE±0.01

Groups: A-GM soya, B-GM maize, C-non GMO.

LSMean = Last Square Means; SE = Standard Error.

^{A,B}Means with different superscripts in lines differ at $P \leq 0.01$. Letters indicate differences between feeding.

*n = number of birds (12 females and 12 males) in each generation in each group.

**n = number of birds in all 3 groups (36 females and 36 males).

Table 9. Chemical composition of egg yolk (in%, LSMean ± SE), pooled samples of 5 egg yolks, average of 10 generations.

Trait	A *n = 120	Group B *n = 120	C *n = 120	SE
Dry matter	51.3^{B}	51.6^{A}	51.4	±5.59
Crude fat	32.1^{B}	32.4^{A}	32.2	±6.60
Crude protein	13.7	13.5	13.4	±7.81
Ash	1.6	1.7	1.7	±0.01

Groups: A-GM soya, B-GM maize, C-non GMO.

LSMean = Last Square Means; SE = Standard Error.

^{A,B}Means with different superscripts in lines differ at $P \leq 0.01$. Letters indicate differences between feeding groups.

*n = 10 generations x 12 samples from each group.

previous findings reported by Flachowsky et al. (2005) on a multi-generational quail feeding study with GM maize (Bt176). The results of the current trial also answer consumers' demands with regard to GMO safety, when used as feed ingredients at relatively high dosage, over a prolonged period of time, and over multiple generations.

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