2.4 Performance recordings and sample collection

Body weight was measured for each quail at 6 wk of age when being feather-sexed, and at study termination at 16 wk of age. About 2 h after start of the light phase in the morning, the animals were removed from their cages in groups of 10 animals and were then transferred in boxes to a dissection room. They were weighed, sacrificed by cervical dislocation and were subsequently decapitated. Blood samples were collected immediately thereafter. All blood samples were obtained within a time interval of 8 to 12 min following removal of the birds from cages and feed. Contamination of blood samples with crop content was avoided by accurate conduction of the sampling procedure. In the following, the livers were carefully removed and immediately weighed. Organ weight was recorded and calculated relative to body weight. Laying intensity in female quail (7–10 wk of age) and the feed intake combined for males and females were recorded during starter and grower period (wk 1-6) and in adult animals (wk 7-10).

2.5 Preparation of liver sections and histological evaluation

Immediately after removal from the body, three small fragments (2 x 2 mm) from the medial lobe of the liver were dissected. For conventional ultrastructural morphology, these samples were fixed in Karnovsky's fixative (Karnovsky, 1965), and then postfixed in 1 % osmium tetroxide, dehydrated with degraded series of ethanol concentrations, and embedded in Spurr resin according to Spurr (1969). From each liver fragment, five sections (0.5 µm in thickness) were cut with glass knives on a Reichert Ultracut E ultramicrotome (Reichert; C., Optische Werke AG, Vienna, Austria), collected on glass slides and stained with 1 % toluidine blue. After staining, the slides were cleared in Xylol (Applechem Inc. Newark, New Jersey, USA), covered under coverslips using Entellan (Merck, Darmstadt, Germany) and were evaluated with a Leica DML light microscope (Leica Microsystems GmbH, Wetzlar, Germany) and a JVC KY-F75U digital camera (JVC, Tokyo, Japan). Histological analyses were performed at light microscopic level on Sparr resin-embedded liver samples by using a computerised image analysis system (Discus, C. H. Hilgers, Königswinter, Germany). Hepatocyte nuclear areas were measured with a grid within a total area of 7.1 mm^2 at a final magnification of 100x in all 15 sections from each animal.

2.7 Serum chemistry

After overnight clotting at 4°C, the blood samples were centrifuged for 20 min at 4,000 x *g*. The separated serum was transported to a commercial laboratory (Synlab Vet Laboratory, Cologne, Germany) and was analyzed for albumin, total protein, glucose, cholesterol, and triglyceride concentration and the enzyme activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (γ-GT) using a clinical chemistry analyzer Olympus AU 640 (Olympus Deutschland GmbH, Hamburg, Germany).

2.8 Statistical analyses

All analyses were carried out using SPSS 17.0 software for Windows (SPSS Inc., Chicago, Illinois, USA). An univariate analysis of variance (ANOVA) with a post hoc Bonferroni correction was performed for each biological variable; generation, feeding group, sex, and the respective two and three-way interactions were considered to be fixed factors. Differences were considered significant, if both the ANOVA and the Bonferroni adjustment were significant ≤($p0.05$). For analyses of mycotoxin concentrations in the diets, as well as laying intensity, and feed intake no individual animal data, but only pooled data were available, and thus comparison between Bt, ISO, and REF groups were conducted by using the Student's t-test.

3. Results

3.1 Mycotoxin and Cry1Ab protein contents in the diets

The mycotoxin concentrations of DON and ZON in the diets were below acceptable limits for animal feed according to guidance of the European Commission concerning levels of mycotoxins in animal feeds (EU-Kommission, 2006), and did not differ between diets containing Bt-maize in comparison to diets containing isogenic or reference control maize, respectively (Table 3).

Using a highly specific ELISA, Cry1Ab protein (46.9 ng/g diet) was detectable only in one of the transgenic Bt-maize diets.

3.2 Performance

All variables recorded were analysed and are presented in the following order:

- comparison of data in quail from generation 0 and generations F17 to F20 fed on Bt or isogenic control maize, respectively (Table 4);
- comparison of data in quail from generation 0 and generations F17 to F20 fed on Bt or isogenic control maize vs. two commercial reference diets;
- comparison of data in quail from generation 0 fed on Bt or isogenic control maize vs. two commercial reference diets (Table 5)

Most variables recorded were significantly influenced by generation throughout the study, though these differences were not consistent and showed irregular alterations. Comparing male and female animals, most performance variables showed sex differences. In contrast, only few variables were affected by the maize variety fed in the different groups.

Bt-maize fed male and female quail from generation 0 and generations F17 to F20 had significantly lower ($p < 0.001$) body weights at 6 wk of age when compared to isogenic control animals (Table 4). Comparing these animals with quail fed the reference control diets, differences between Bt-maize fed animals and the REF1 group occurred. Analogous differences were recorded when comparing solely generation 0 vs. the reference controls (Table 5). In contrast, final body weight ranging from 154 to 166 g in adult males and from 180 to 188 g in females did not differ between feeding groups in either comparison (Tables 4, 5).

No macroscopic alterations of liver were observed; the absolute liver weight and relative liver weight was not different between diet groups with the only exception of generation 0, in which both male and female Bt-maize fed quail as well as isogenic fed male animals had higher relative liver weights than the REF2 animals. In contrast female quail from the isogenic feeding group had lower relative liver weight than the REF2 animals (Table 5).

On a g/animal/day basis, feed intake ranged from 15.7 to 16.3 g during the grower phase from hatch until 6 wk of age, and from 21.4 to 24.6 g during 7 to 10 wk of age without differences between feeding groups. Similarly, there were no differences in

the laying intensity in female quail, ranging from 69.4 to 79.0 % during the time of observation from 7–10 wk of age.

3.2 Histological examinations

Light microscopic examinations of Sparr resin-embedded liver samples showed similar hepatocyte nuclear size in both male and female Japanese quail when comparing Bt-maize fed animals from generation 0 and generations F17 to F20 with isogenic control animals (Table 4), as well as in comparison to different REF diets. Differences in hepatocyte nuclear size were observed for the Bt vs. ISO and for Bt vs. REF2 comparison in generation 0 with increased sizes in Bt-maize fed quail (Table 5).

3.3 Serum chemistry parameters

Most serum chemistry values were physiological influenced by sex. Observing the influence of different diets only few differences were recorded, when comparing Bt-maize fed animals from generation 0 and generations F17 to F20 with isogenic control animals (Table 4), and with different REF diets. Differences occurred solely in the enzyme activity of γ-GT between Bt- vs. ISO fed animals (Table 4), Bt-maize fed animals vs. REF1 and 2, as well as between isogenic control animals vs. REF1 and 2. Comparing serum chemistry parameters in male and female quail from generation 0 with both REF diets, differences between the enzyme activities of AST, ALT, and γ-GT, as well as glucose were observed (Table 5). AST and ALT values were higher in Bt and isogenic fed quail in comparison to the REF1 feeding group. The enzyme activity of and γ-GT was higher in male and female Bt-maize fed animals in comparison to both the isogenic group and the REF2 feeding group. Differences in serum chemistry parameters were not limited to Bt vs. ISO comparisons, but also occurred between Bt and isogenic control groups vs. REF groups; no differences were observed between the different REF groups (Table 5). Furthermore, no feeding effect was found for serum values of albumin, total protein, total cholesterol, and triglycerides in either comparison.