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Transgenerational effects of feeding genetically modified maize to nulliparous sows and offspring on offspring growth and health[1]

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ABSTRACT: This study assessed the effect of feeding genetically modified maize expressing a truncated form of the Cry1Ab protein from *Bacillus thuringiensis* (Bt MON810 maize) to sows during gestation and lactation and their offspring from weaning to 115 d postweaning on offspring growth and health. After weaning at approximately 28 d of age (d 0), individually penned, mixed sex pigs (approximately 8 kg BW) from sows fed isogenic or Bt maize diets were blocked by sow treatment, sex, and BW and randomly assigned to Bt or isogenic maize diets as follows: i) isogenic maize-fed sow/isogenic maize-fed offspring (iso/iso); ii) isogenic maize-fed sow/Bt maize-fed offspring (iso/Bt); iii) Bt maize-fed sow/isogenic maize-fed offspring (Bt/iso); and iv) Bt maize-fed sow/Bt maize-fed offspring (Bt/Bt). Growth performance was recorded at intervals to harvest at approximately 105 kg BW (n = 15/treatment) and blood samples were taken for biochemical analysis on d 0, 30, 70, 100, and 115 postweaning (n = 10/treatment). Pigs were harvested on d 115 postweaning (n = 10/treatment), and carcass weight, backfat depth, and organ weights (heart, kidney, spleen, and liver) were recorded. Kidney, liver, lymph nodes, and small intestine were collected for histological analysis. Offspring from Bt maize-fed sows were heavier than offspring from isogenic maize-fed sows on d 30 ($P < 0.05$), 100 ($P < 0.05$), and 115 postweaning ($P < 0.05$) and had greater overall ADG ($P < 0.05$). Overall ADFI was greater for offspring from sows fed Bt maize ($P < 0.05$) and for Bt maize-fed pigs ($P < 0.05$). Offspring from Bt maize-fed sows had greater carcass ($P < 0.05$) and lighter spleen ($P < 0.05$) weights. Dressing percentage was greater for Bt maize-fed pigs than isogenic maize-fed pigs ($P < 0.05$), and livers were lighter for pigs in the Bt/Bt group than pigs in the iso/Bt or Bt/iso group ($P < 0.05$). Offspring from Bt maize-fed sows also had greater duodenal crypt depths ($P < 0.05$) and lower villus height/crypt depth ratios ($P < 0.05$). No pathology was observed in the organs, and serum biochemistry values generally remained within normal limits and no overall differences were observed, with the exception of overall γ glutamyltransferase, which was less for pigs on the Bt/Bt treatment than pigs on the iso/Bt and Bt/iso treatments. These results indicate that transgenerational consumption of Bt maize diets is not detrimental to pig growth and health.

Key words: *Bacillus thuringiensis*, Bt MON810 corn, Cry1Ab, swine, transgenic

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INTRODUCTION

Genetically modified (**GM**) insect-resistant maize expresses the truncated Cry1Ab protein from *Bacillus thuringiensis* (**Bt**), which confers resistance to certain maize pests. The Bt maize has been a matter of controversy from its introduction in 1995 (Hill et al., 1995; Wisniewski et al., 2002). This has lead to public concerns regarding the safety of GM crops destined for human consumption and the safety of GM crops for animals entering the human food chain (Aulrich et al., 2001; Gaskell, 2005; Costa-Font et al., 2008; Dona and Arvanitoyannis, 2009).

Studies researching the safety of Bt maize have revealed only minor changes in health and growth performance in healthy, nonpregnant animals (Piva et al., 2001; Domingo and Giné Bordonaba, 2011; Walsh et al., 2011; Zhang and Shi, 2011; Buzoianu et al., 2012; Walsh et al., 2012a). However, in pregnant females, even minor nutritional changes, which may not elicit any detectable symptoms in the mother, may alter fetal development in utero and postnatal growth performance of offspring (Ashworth et al., 2009). The stress of pregnancy itself, especially in nulliparous females, may increase sensitivity to dietary aggression. The safety of feeding GM Bt maize to pregnant females and the effect it may have on offspring growth and health needs to be established.

To our knowledge, to date, only 4 studies in mammals, 1 in sheep, 1 in rats, and 2 in mice have investigated the effect of feeding GM maize to pregnant females on offspring growth and health (Brake et al., 2004; Kilic and Akay, 2008; Trabalza-Marinucci et al., 2008; Haryu et al., 2009). The inclusion rate of GM maize in these diets ranged from 20 to 68%. The objective of this study was to address the issues by investigating the effect of feeding a GM maize diet (74.4 to 86.6% inclusion rate) to sows during gestation and lactation and subsequently to offspring from weaning to harvest at approximately 143 d of age on offspring growth performance and health.

MATERIALS AND METHODS

This pig study complied with European Union Council Directives 91/630/EEC (outlines minimum standards for the protection of pigs) and 98/58/EC (concerns the protection of animals kept for farming purposes) and was approved by, and an experimental license was obtained from, the Irish Department of Health and Children (License No. B100/4147). Ethical approval was obtained from Teagasc and Waterford Institute of Technology ethics committees.

Maize and Diets

Seeds derived from GM Bt MON810 and non-GM (isogenic) parent line control maize (PR34N44 and PR34N43, respectively; Pioneer Hi-Bred, Sevilla, Spain) were grown simultaneously side by side (Valtierra, Navarra, Spain) under similar management conditions by independent producers in 2007. This was done to avoid, insofar as possible, compositional differences between the isogenic and the Bt maize because of differences in environmental exposure, soil composition, and management practices.

The inclusion rate of isogenic and Bt maize in each diet at each pig growing phase was identical, and diets were formulated to exceed the National Research Council (1998) recommendations for pigs of a specific age. As a precautionary measure, a natural mycotoxin adsorbent (Mycosorb, Alltech, Dunboyne, Co. Meath, Ireland) was included in all diets. To prevent mold growth in the finisher diets over the summer months, a mold inhibitor (Myco Curb, Kemin Europa N. V. Herentals, Belgium) was included in the finisher diets. The Bt and isogenic maize lines were tested for proximate, AA, and carbohydrate composition, as well as for presence of the *cry1Ab* gene, pesticide contaminants, and mycotoxins as previously described by Walsh et al. (2012a). All diets were sampled in accordance with international guidelines (Hartnell et al., 2007) and analyzed for proximate and AA composition, as described by Walsh et al. (2012a).

Study Design and Animal Management

Twenty-four nulliparous sows (Large White × Landrace) were obtained (Hermitage A. I., Kilkenny, Ireland) at approximately 28 d of age and fed a non-GM diet until reaching approximately 165 kg BW. After AI, sows were blocked by BW and insemination date and randomly assigned to 1 of 2 dietary treatments: i) isogenic parent line maize from service to weaning (**iso**), and ii) GM Bt maize from service to weaning (Bt). Maize dietary inclusion rate was identical between treatments and ranged from 86.6% during gestation to 74.4% during lactation. The details of sow management during gestation and lactation were previously described by Walsh et al. (2012b).

After weaning at approximately 28 d of age, pigs (approximately 8 kg) from these sows (n = 36/sow treatment) were selected and blocked by sow treatment, sex, and BW and randomly assigned to dietary treatments. Offspring from each of the 2 sow treatments were assigned to either an isogenic maize (Pioneer PR34N43 event MON810) diet for 115 d or Bt maize (Pioneer PR34N44 event MON810) diet for 115 d, resulting in 4 dietary treatments: i) isogenic maize-fed sow/isogenic maize-fed offspring (**iso/iso)**; ii) isogenic maize-fed sow/Bt maize-fed offspring **(iso/Bt)**; iii) Bt maize-fed sow/isogenic maize-fed offspring **(Bt/iso)**; and iv) Bt maize-fed sow/Bt maize-fed offspring **(Bt/Bt)**.

Pigs were individually penned in 1 of 3 identical rooms containing 24 pens each (1.2 × 0.9 m) until d 70 of the study. Pens were fully slatted with plastic flooring (Faroex, Manitoba, Canada). Temperature was controlled by a hot air heating system and an exhaust fan drawing air from under slat level connected to a controller (Stienen Pcs 8400; Stienen BV, Nederweert, the Netherlands). Temperature was maintained at 28 to 30°C during the first week and reduced by 2°C per week to 22°C.

On d 70 of the study, 60 pigs (n = 15/treatment) were moved to 4 identical finisher rooms where the pigs remained until d 115 of the study. Pigs were individually penned in fully slatted pens (concrete slats: 75-mm solid width with 20-mm slots) measuring 1.81 × 1.18 m with plastic pen divisions. Ventilation was ensured by exhaust fans and wall mounted air inlets controlled by a controller (Stienen PCS 8200; Stienen BV) with air temperature maintained at 20 to 22°C.

Pigs were fed a sequence of diets in accordance with their growth stage. Both isogenic and Bt maize starter diets were fed from d 0 to 7 postweaning, link diets were fed from d 8 to 30, grower diets were fed from d 31 to 70, finisher 1 diets were fed from d 71 to 100, and finisher 2 diets were fed from d 101 to 115. In accordance with general recommendations regarding safety testing of GM feed ingredients (Hartnell et al., 2007; de Vendômois et al., 2010), animal growth was assessed and samples were taken at the beginning and end of the study, as well as at the end of each dietary phase.

At all stages, pigs were provided with ad libitum access to pelleted feed from a stainless steel, 30-cm-wide feeder (O'Donovan Engineering, Coachford, Co. Cork, Ireland). Water was supplied ad libitum from a nipple-in-bowl drinker (BALP, Charleville-Mezieres, Cedex, France). Pigs were inspected at least twice daily and any pig showing signs of ill health was treated appropriately. All veterinary treatments were recorded.

Pig Growth Performance and Sampling at Harvest

Body weight and feed disappearance were recorded at the time of each dietary change [weaning (d 0), on d 30, 70, and 100] and at harvest (d 115) for calculation of ADFI, ADG, and G:F. Water consumption by offspring was measured from d 70 to 115 of the study using individual water meters (GS D8; B Meters s.r.l., Udine, Italy) mounted on each drinker. On each sampling day, feed and water were available up to weighing.

On d 115 of the study, 10 pigs/treatment were harvested in a commercial abattoir by electrical stunning followed by exsanguination. After harvest, the heart, kidneys, spleen, and liver were removed, cleared of blood clots, connective tissue, and fat deposits, blotted dry, and weighed. Samples from the liver, kidney, and mesenteric lymph nodes were processed for histological examination as previously described (Walsh et al., 2012a). Samples from duodenum, jejunum, and ileum were also processed for histological examination of villus height, crypt depth, and the number of goblet cells per villus and per micrometer of villus as previously described (Walsh et al., 2012a). The internal organs and digestive tract were removed before measuring HCW and the head was left on the carcass. The HCW at harvest was multiplied by 0.98 to obtain the cold carcass weight. Dressing percentage was calculated by expressing cold carcass weight as a fraction of BW at harvest. Backfat depth (mm) was measured using a steel ruler on the split carcass at the last rib and between the third and fourth last ribs 1 d postharvest.

Serum Biochemistry

Blood was collected in 10-mL evacuated serum tubes containing a silica clot activator (BD Vacutainer Systems, Franklin Lakes, NJ) from 10 pigs/treatment on d 0, 30, 70, 100, and 115. Samples were collected from the cranial vena cava of pigs on d 0 and 30, from the external jugular vein on d 70 and 100, and during exsanguination on d 115. Blood was allowed to coagulate for 1 h and then centrifuged at 1,300 × g at room temperature for 10 min for serum collection. Serum was stored at –20°C before biochemical analysis. Serum samples were analyzed (ABX Pentra 400 Clinical Chemistry Analyzer; Horiba ABX, Northampton, UK) for aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), γ glutamyltransferase (**GGT**), alkaline phosphatase (**ALP**), creatinine, urea, and total protein (**TP**). The biochemistry analyzer was calibrated according to the manufacturer's instructions. Analyzer accuracy after calibration was assessed by analysis of samples of known concentration after every 10 experimental samples. All samples were analyzed in duplicate.

Statistical Analysis

Data were analyzed as a 2 by 2 factorial arrangement of treatments in a split-plot design using the MIXED procedure (SAS Inst. Inc., Cary, NC). Sow treatment was regarded as the main plot and offspring treatment was regarded as the subplot. Sow and offspring treatment and sampling day were included in the statistical model as fixed effects, and sow block, sow treatment × sow block interaction, and offspring block were included as random effects. Day was regarded as a repeated variable for the analysis of growth, serum biochemistry, and water consumption, and because of unequal spacing between sampling points, and as indicated by the model fit statistics, a first-order antedependence covari-

ance structure was fitted to the data. The denominator degrees of freedom were computed using the Satterthwaite approximation. The slice option was used to test for simple effects at each time point. To assess model suitability, data were examined using the influence diagnostics provided within PROC MIXED in SAS and by investigation of normality of scaled residuals using the Shapiro-Wilk test within the UNIVARIATE procedure of SAS. Least squares means were computed and *P*-values were adjusted for multiple comparisons using the Tukey-Kramer adjustment. For organ weights, final BW was included as a covariate in the statistical model. Day 0 values were used as a covariate in the analysis of serum biochemistry. Day 0 BW was used as a covariate in the analysis of growth performance data. For water consumption, ADFI was used as a covariate in the statistical model. Significance was reported for $P \leq 0.05$ and tendencies toward significance were reported for $P \leq 0.10$. For all response criteria, the individual pig was the experimental unit.

RESULTS

Maize and Diets

The proximate composition, AA, and carbohydrate content of the isogenic and Bt maize used in the present study were similar and have been presented by Walsh et al. (2012a). Mycotoxins were below the maximum allowable limits in animal feedstuffs as outlined by the European legislation (Office for Official Publications of the European Union, 2002, 2006) and are shown in Table 1. Analysis of the isogenic and Bt maize diets used in the present study revealed similar proximate composition and AA concentrations (Table 2).

Pig Growth Performance and Water Consumption

Twelve animals were removed from the study because of illness (1 from the iso/iso treatment, 1 from the iso/Bt treatment, and 1 from the Bt/iso treatment) or death (3 from the iso/iso treatment and 2 from each of the iso/Bt, Bt/iso and Bt/Bt treatments). Preweaning mortality was not different between the isogenic and Bt treatments (1.05 and 1.42 pigs/litter; pooled SEM = 0.25 pig/Litter; Walsh et al., 2012b).

The results of offspring growth performance are presented in Table 3. None of the growth performance measurements showed a sow treatment × offspring treatment × time interaction. There was a tendency for a sow treatment × offspring treatment interaction for BW on d 70 with pigs fed the Bt/Bt treatment tending to be heavier than pigs fed the iso/iso treatment ($P = 0.08$). Pigs fed the Bt/iso and Bt/Bt treatments tended to have a greater BW compared with pigs fed the iso/iso treatment on d 100 ($P = 0.06$) and 115 ($P = 0.06$). Offspring from sows fed Bt maize diets were heavier than offspring from sows fed isogenic maize diets on d 30 (22.4 and 20.5 kg; $P < 0.05$), 70 (56.3 and 53.8 kg; $P = 0.07$), 100 (84.1 and 79.1 kg; $P < 0.05$), and 115 (108.0 and 101.6 kg; $P < 0.05$). There was a tendency for pigs fed the Bt maize diets to be heavier than pigs fed the isogenic maize diets on d 70 (56.3 and 53.9 kg, respectively; $P = 0.06$). At weaning, a lighter BW was observed for offspring assigned to the Bt treatment compared with offspring assigned to the isogenic treatment. To account for this unexpected variability at d 0 of the study (weaning), d 0 values were included as a co-variates in the statistical model.

A sow treatment × offspring treatment interaction was observed for ADFI from d 71 to 100, with offspring fed the Bt/Bt treatment having greater ADFI than offspring fed the iso/iso and iso/Bt treatment and offspring fed the Bt/iso treatment having greater ADFI compared with offspring fed the iso/iso treatment ($P < 0.05$). From d 0 to 30, offspring from sows fed Bt maize diets tended to have greater ADFI than offspring from sows fed the isogenic maize diets (597 and 538 g/d; $P = 0.07$). Likewise, from d 71 to 100, offspring from sows fed the Bt maize diets had greater ADFI than offspring of sows fed the isogenic maize diets (2450 and 2216 g/d; $P < 0.05$). An overall sow treatment effect was also observed, with offspring from sows fed Bt maize diets having greater overall (d 0 to 115 postweaning) ADFI than offspring from sows fed the isogenic maize diets (1954 and 1828 g/d; $P < 0.05$). From d 101 to 115, offspring fed the Bt maize diets also tended to have greater ADFI than offspring fed the isogenic maize diets (3138 and 2956 g/d; $P = 0.06$). An overall offspring treatment effect was also

Table 1. Mycotoxin concentration in maize kernels and their maximum allowable limits in animal feedstuffs as regulated by the European legislation[1]

	Mycotoxin concentration		
Mycotoxin	Isogenic[2]	Bt[3]	Maximum allowable limits (reference)[4]
	μg/kg		
Aflatoxin	<2	<2	20 (2002/32/EC)
Ochratoxin	<2	<2	250 (2006/576/EC)
Zearalenone	<25	232	2000 (2006/576/EC)
Deoxynivalenol	<250	1230	8000 (2006/576/EC)
Fumonisin	<1000	<1000	6000 (2006/576/EC)

[1]As a preventive measure, a mycotoxin adsorbent (Alltech, Dunboyne, Co. Meath, Ireland) and a mold inhibitor (Kemin Europa N.V., Herentals, Belgium) were added to complete diets.

[2]Isogenic = isogenic parent line maize.

[3]Bt = *Bacillus thuringiensis*; genetically modified Bt MON810 maize.

[4]2002/32/EC, Office for Official Publications of the European Union, 2002; 2006/576/EC.

Table 2. Composition of experimental diets on an as-fed basis

	Starter (d 0 to 7)		Link (d 8 to 30)		Grower (d 31 to 70)		Finisher 1 (d 71 to 100)		Finisher 2 (d 101 to 115)	
Item	Isogenic[1]	Bt[2]	Isogenic	Bt	Isogenic	Bt	Isogenic	Bt	Isogenic	Bt
Ingredient	%									
Maize	27.33	27.33	38.88	38.88	65.31	65.31	73.33	73.33	79.05	79.05
Soybean meal (non-GM)	24.00	24.00	25.00	25.00	28.64	28.64	22.76	22.76	17.35	17.35
Lactofeed 70[3]	25.00	25.00	20.00	20.00	–	–	–	–	–	–
Immunopro 35[4]	12.50	12.50	9.00	9.00	–	–	–	–	–	–
Soybean oil	8.00	8.00	4.00	4.00	2.37	2.37	0.06	0.06	–	–
L-Lys · HCl	0.30	0.30	0.30	0.30	0.36	0.36	0.43	0.43	0.49	0.49
DL-Met	0.25	0.25	0.20	0.20	0.14	0.14	0.14	0.14	0.14	0.14
L-Thr	0.12	0.12	0.12	0.12	0.15	0.15	0.17	0.17	0.19	0.19
L-Trp	0.10	0.10	0.10	0.10	0.05	0.05	0.07	0.07	0.08	0.08
Vitamin and mineral premix	0.30[5]	0.30[5]	0.30[5]	0.30[5]	0.10[5]	0.10[5]	0.10[6]	0.10[6]	0.10[6]	0.10[6]
Mycosorb[7]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Dicalcium phosphate	0.50	0.50	0.50	0.50	1.19	1.19	1.13	1.13	0.90	0.90
Limestone flour	1.10	1.10	1.10	1.10	1.19	1.19	1.26	1.26	1.15	1.15
Myco Curb[8]	–	–	–	–	–	–	0.05	0.05	0.05	0.05
Analyzed chemical composition										
DM	91.4	91.0	90.9	90.5	89.2	88.7	87.5	87.7	87.4	88.2
CP	20.2	21.2	19.8	20.4	19.7	20.1	17.1	17.2	15.1	15.3
Oil, acid hydrolysis	10.1	9.9	6.8	7.0	5.4	5.2	3.1	3.4	2.9	3.0
Crude fiber	1.7	1.4	1.5	1.3	2.1	2.1	2.1	1.8	1.8	1.8
Ash	6.0	5.9	5.9	5.8	4.8	4.8	5.2	5.6	4.8	4.4
Lys	1.47	1.46	1.46	1.47	1.33	1.34	1.30	1.27	1.12	1.14
DE,[9] MJ/kg	16.33	16.33	15.38	15.38	14.50	14.50	14.00	14.00	13.99	13.99

[1]Isogenic = isogenic parent line maize.

[2]Bt = *Bacillus thuringiensis*; genetically modified Bt MON810 maize.

[3]Lactofeed 70 contains 70% lactose, 11.5% protein, 0.5% oil, 7.5% ash, and 0.5% fiber (Volac, Cambridge, UK).

[4]Immunopro 35 contains whey protein powder with 35% protein (Volac, Cambridge, UK).

[5]Premix provided per kilogram of complete diet: Cu from copper sulfate, 155 mg; Fe from ferrous sulfate monohydrate, 90 mg; Mn from manganese oxide, 47 mg; Zn from zinc oxide, 120 mg; I from potassium iodate, 0.6 mg; Se from sodium selenite, 0.3 mg; retinyl acetate, 2.1 mg; cholecalciferol, 25 µg; DL-alpha-tocopheryl acetate, 100 mg; vitamin K, 4 mg; vitamin $B_{12,}$ 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 250 mg; vitamin $B_{1,}$ 2 mg; and vitamin $B_{6,}$ 3 mg.

[6]Premix provided per kilogram of complete diet: Cu from copper sulfate, 15 mg; Fe from ferrous sulfate monohydrate, 24 mg; Mn from manganese oxide, 31 mg; Zn from zinc oxide, 80 mg; I from potassium iodate, 0.3 mg; Se from sodium selenite, 0.2 mg; retinyl acetate 0.7 mg; cholecalciferol, 25 µg; DL-alpha-tocopheryl acetate, 40 mg; vitamin K, 4 mg; vitamin $B_{12,}$ 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin $B_{1,}$ 2 mg; and vitamin $B_{6,}$ 3 mg.

[7]Mycosorb is an organic mycotoxin adsorbent (Alltech, Dunboyne, Co. Meath, Ireland).

[8]Myco Curb is a mold inhibitor (Kemin Europa N.V., Herentals, Belgium).

[9]Calculated values.

observed for ADFI, with offspring fed the Bt maize diets having greater overall (d 0 to d 115 postweaning) ADFI compared with offspring fed the isogenic maize diets (1934 and 1848 g/d; $P < 0.05$).

There was a sow treatment × offspring treatment interaction for ADG from d 31 to 70, with pigs fed the iso/Bt and the Bt/Bt treatments having a greater growth rate than pigs fed the iso/iso and Bt/iso treatments ($P \leq 0.05$). From d 71 to 100, offspring fed the Bt/iso treatment had greater ADG than offspring fed the iso/iso and the iso/Bt treatments ($P \leq 0.05$). Similarly, offspring from sows fed Bt maize diets tended to have a greater ADG than offspring of sows fed the isogenic maize diets from d 0 to 30 (441 and 397 g/d; $P = 0.08$). From d 71 to 100, offspring from sows fed Bt maize diets had greater ADG than offspring from sows fed the isogenic maize diets (1001 and 902 g/d; $P < 0.05$). A sow treatment effect was observed for overall (d 0 to 115 postweaning) ADG, with offspring from sows fed the Bt maize diets having greater ADG than offspring from sows fed isogenic maize diets (877 and 822 g/d; $P < 0.05$). Offspring treatment also influenced ADG from d 31 to 70, with pigs

Table 3. Effect of feeding *Bacillus thuringiensis* (Bt) maize diets to sows during gestation and lactation and to offspring for 115 d on offspring growth performance[1]

	Treatment							
	Sow isogenic[2]		Sow Bt[2]			*P*-value		
Item	Offspring isogenic[3]	Offspring Bt[3]	Offspring Isogenic[3]	Offspring Bt[3]	Pooled SEM	Sow	Offspring	Sow × offspring
BW, kg								
d 0 (weaning)[4]	8.1	8.0	8.1	7.4	0.3	0.44	0.05	0.14
d 30	20.3	20.7	22.3	22.6	0.8	0.04	0.59	0.16
d 70	52.5^{y}	55.1^{xy}	55.3^{xy}	57.4^{x}	1.4	0.07	0.06	0.08
d 100	77.7^{y}	80.4^{xy}	83.8^{x}	84.4^{x}	2.0	0.01	0.37	0.06
d 115	100.6^{y}	102.5^{xy}	107.5^{x}	108.6^{x}	2.4	0.008	0.52	0.06
ADFI, g/d								
d 0 to 30	540	536	590	605	27	0.07	0.81	0.28
d 31 to 70	1524	1616	1631	1694	57	0.11	0.14	0.20
d 71 to 100	2158^{c}	2273^{bc}	2428^{ab}	2472^{a}	70	0.001	0.24	0.01
d 101 to 115	2883	3095	3030	3182	99	0.23	0.06	0.15
Overall	1776	1880	1920	1988	46	0.01	0.04	0.68
ADG, g/d								
d 0 to 30	390	403	434	448	24	0.08	0.54	0.32
d 31 to 70	912^{b}	990^{a}	918^{b}	998^{a}	30	0.82	0.006	0.05
d 71 to 100	894^{b}	909^{b}	1035^{a}	967^{ab}	39	0.01	0.48	0.05
d 101 to 115	1047	1029	1091	1125	43	0.11	0.84	0.37
Overall	811	833	870	885	21	0.02	0.32	0.85
G:F								
d 0 to 30	0.725	0.748	0.739	0.753	0.017	0.57	0.27	0.66
d 31 to 70	0.629	0.616	0.575	0.592	0.017	0.03	0.91	0.12
d 71 to 100	0.416^{ab}	0.400^{bc}	0.426^{a}	0.388^{c}	0.010	0.95	0.01	0.02
d 101 to 115	0.366	0.351	0.359	0.352	0.013	0.81	0.40	0.83
Overall	0.534	0.529	0.525	0.521	0.007	0.27	0.49	0.92

^{a,b}Within a row, means without a common superscript differ, $P \leq 0.05$.

^{x,y}Within a row, means without a common superscript differ, $P \leq 0.10$.

[1]Values presented as least squares means (n = 15/treatment).

[2]Sows were fed isogenic or Bt maize-based diets from service until 28 d postfarrowing.

[3]Offspring were fed isogenic or Bt maize-based diets from weaning for 115 d.

[4]Variability present in the data at weaning (d 0 of the study) has been accounted for by including d 0 values as covariates in the statistical model.

fed Bt maize diets having a greater ADG than pigs fed isogenic maize diets (994 and 915 g/d; $P < 0.05$).

There was a sow treatment × offspring treatment interaction for G:F from d 71 to 100 of the study, with offspring on the Bt/Bt treatment having a lower G:F than offspring on the iso/iso and Bt/iso diets and pigs fed the Bt/iso treatment having a greater G:F than pigs fed the iso/Bt treatment ($P < 0.05$). From d 31 to 70, offspring from sows fed Bt maize diets had poorer G:F than offspring of sows fed isogenic maize (0.584 and 0.622, respectively; $P < 0.05$). An offspring treatment effect was also observed for G:F from d 71 to 100, with pigs fed Bt maize diets having a poorer G:F than pigs fed isogenic maize (0.394 and 0.421; $P < 0.05$). Overall (d 70 to 115) offspring water consumption was not affected by feeding Bt maize, with similar values observed for the iso/iso, iso/Bt, Bt/iso and Bt/Bt treatments (6.1, 6.4, 6.3, and 5.7 L/d, respectively; pooled SEM = 0.6 L/d).

Harvest Characteristics

The data obtained at harvest are presented in Table 4. Offspring from sows fed Bt maize diets had heavier carcasses than offspring from sows fed the isogenic maize diets (82.5 and 78.6 kg; $P < 0.05$). Carcass weight tended to be heavier for pigs fed the Bt maize than pigs fed the isogenic maize (82.1 and 79.0 kg; $P = 0.07$). Likewise, dressing percentage was greater for pigs fed the Bt maize compared with pigs fed the isogenic maize (77.3 and 76.5%; $P < 0.05$). There was a tendency toward a sow treatment × offspring treatment interaction, with pigs fed the Bt/Bt treatment having greater backfat depth at the last rib than pigs fed the iso/Bt treatment ($P = 0.06$). No differences were observed between treatments for backfat depth measured between the third and fourth last ribs or for kidney and heart weight at harvest. Offspring from sows fed Bt maize diets had lower spleen weights than offspring from sows fed isogenic

Table 4. Effect of feeding *Bacillus thuringiensis* (Bt) maize diets to sows during gestation and lactation and to offspring for 115 d on offspring carcass characteristics and organ weights[1]

	Treatment							
	Sow isogenic[2]		Sow Bt[2]			*P*-value		
Item	Offspring isogenic[3]	Offspring Bt[3]	Offspring isogenic[3]	Offspring Bt[3]	Pooled SEM	Sow	Offspring	Sow × offspring
Carcass, kg	76.3	80.9	81.8	93.2	1.7	0.02	0.07	0.33
Dressing percentage[4]	76.2	77.3	76.8	77.2	0.4	0.47	0.04	0.44
Backfat last rib, mm	21^{xy}	20^{y}	21^{xy}	23^{x}	1	0.20	0.55	0.06
Backfat 3 to 4,[5] mm	19	19	19	20	1	0.83	0.40	0.44
Organ weights,[6] g								
Heart	404.3	422.8	414.0	403.9	14.3	0.75	0.76	0.33
Kidneys	408.0	363.9	382.7	386.1	15.6	0.92	0.19	0.14
Spleen	171.1	181.2	165.5	160.0	9.9	0.04	0.71	0.26
Liver	1868^{ab}	1925^{a}	1955^{a}	1761^{b}	63.4	0.47	0.19	0.04

^{a,b}Within a row, means without a common superscript differ, $P \leq 0.05$.

^{x,y}Within a row, means without a common superscript differ, $P \leq 0.10$.

[1]Values presented as least squares means ($n = 10$)/treatment.

[2]Sows were fed isogenic or Bt maize-based diets during gestation and lactation.

[3]Offspring were fed isogenic or Bt maize-based diets for 115 d.

[4]Dressing percentage is the ratio of the cold carcass weight to final BW.

[5]Backfat depth measured between third and fourth last ribs.

[6]Organ weights were computed with final BW as a covariate in the statistical model.

maize (163 and 176 g; $P < 0.05$). There was also a sow treatment × offspring treatment interaction for liver weight, with pigs fed the Bt/Bt treatment having lighter liver weights compared with pigs fed the iso/Bt and the Bt/iso treatments ($P < 0.05$).

Intestinal Morphology

Duodenum. Offspring from sows fed the Bt maize diets had deeper duodenal crypts compared with offspring from sows fed the isogenic maize diets (523 and 453 μm; $P < 0.05$; Table 5). Likewise, offspring from sows fed the Bt maize diets had a lower duodenal villus height to crypt depth ratio than offspring from sows fed the isogenic maize diets (0.88 and 1.03; $P < 0.05$). No treatment effects were observed for duodenal villus height or the number of goblet cells per villus or per micrometer of villus.

Jejunum. Offspring from sows fed Bt maize tended to have fewer goblet cells per villus than offspring from sows fed isogenic maize (7.8 and 10.6; $P = 0.06$). No treatment effects were observed for any of the other traits measured in the jejunum.

Ileum. Pigs fed Bt maize tended to have reduced ileal villus height compared with pigs fed the isogenic maize diets (354 and 383 μm; $P = 0.10$). This lead to a tendency toward a lower villus height to crypt depth ratio for pigs fed the Bt maize diets compared with pigs fed the isogenic maize diets (1.27 and 1.50; $P = 0.10$). No sow or offspring treatment effects were observed for ileal crypt depth, number of goblet cells per villus, and per micrometer of villus.

Organ Histopathology

No signs of histopathology were observed in the liver or kidney for any of the treatments. A mild eosinophilic infiltration was observed in the mesenteric lymph nodes of pigs from all dietary treatments. None of the tissues examined showed signs of cellular membrane disruption, nuclear fragmentation, shrinkage, or vacuolar changes. Likewise, hemorrhage, edema, fibrosis, or inflammation was not present in the samples of liver, kidney, and spleen examined. Neither abnormal cell growth nor excessive cell pigmentation were observed.

Serum Biochemistry

Serum biochemistry results are presented in Table 6. With the exception of a sow treatment × offspring treatment × time interaction for ALT ($P < 0.05$) and a tendency toward a sow treatment × offspring treatment × time interaction for serum urea ($P = 0.10$), no other such interactions were observed. On d 30, there was a tendency toward a sow treatment × offspring treatment interaction for serum ALP, with pigs fed the iso/Bt treatment tending to have reduced serum ALP compared with all other treatments ($P = 0.08$). This resulted in a tendency toward decreased serum ALP for pigs fed the Bt treatment (267 and 288 units/L; $P = 0.07$). Overall (d 0 to 115), ALP tended to be less for pigs fed the Bt maize di-

Table 5. The effect of feeding *Bacillus thuringiensis* (Bt) maize-based diets to sows during gestation and lactation and to offspring for 115 d on offspring intestinal morphology[1]

	Treatment							
	Sow isogenic[2]		Sow Bt[2]			*P*-value		
Item	Offspring isogenic[3]	Offspring Bt[3]	Offspring isogenic[3]	Offspring Bt[3]	Pooled SEM	Sow	Offspring	Sow × offspring
Duodenum								
Villus height, μm	467	456	412	471	33	0.46	0.35	0.22
Crypt depth, μm	460	446	530	515	33	0.03	0.63	0.98
Villus height: crypt depth	1.03	1.01	0.82	0.93	0.08	0.04	0.57	0.40
GC[4]/villus	16.1	17.6	13.3	14.7	2.3	0.17	0.47	0.99
GC/μm villus	0.034	0.039	0.032	0.034	0.005	0.56	0.46	0.80
Jejunum								
Villus height, μm	435	412	407	390	35	0.46	0.56	0.92
Crypt depth, μm	328	332	366	353	22	0.14	0.84	0.67
Villus height: crypt depth	1.41	1.26	1.16	1.18	0.11	0.11	0.52	0.38
GC/villus	10.1	11.0	7.2	8.4	1.4	0.06	0.42	0.90
GC/μm villus	0.024	0.032	0.028	0.022	0.005	0.64	0.83	0.12
Ileum								
Villus height, μm	393	348	373	360	21	0.81	0.10	0.34
Crypt depth, μm	273	299	272	275	19	0.44	0.39	0.48
Villus height: crypt depth	1.56	1.18	1.45	1.36	0.16	0.80	0.10	0.32
GC/villus	20.8	24.2	23.1	20.4	2.7	0.79	0.91	0.25
GC/μm villus	0.057	0.072	0.060	0.058	0.009	0.55	0.48	0.34

[a,b]Within a row, means without a common superscript differ, $P \leq 0.05$.

[x,y]Within a row, means without a common superscript differ, $P \leq 0.10$.

[1]Values presented as least squares means ($n = 10$)/treatment.

[2]Sows were fed isogenic or Bt maize-based diets from service until approximately 28 d postfarrowing.

[3]Offspring were fed isogenic or Bt maize-based diets from weaning for 115 d.

[4]GC = goblet cells.

ets compared with pigs fed the isogenic maize diets (198 and 214 units/L; $P = 0.06$). The ALP decreased up to d 100 of the study and increased thereafter ($P < 0.05$; data not shown). For serum ALT, there was a sow treatment × offspring treatment interaction on d 30, with pigs fed the iso/Bt treatment having less serum ALT than pigs fed the iso/iso and the Bt/Bt treatments ($P \leq 0.05$). The ALT changed over time, decreasing until d 70 and increasing thereafter ($P < 0.05$; data not shown). For serum AST, a sow treatment × offspring treatment interaction was observed at d 115, with pigs fed the Bt/Bt treatment having greater serum AST than pigs on all other treatments ($P < 0.05$). On d 0, offspring from sows fed Bt maize diets had greater AST than offspring of sows fed isogenic maize diets (39.5 and 35.2 units/L; $P < 0.05$). Likewise, on d 115, offspring from sows fed the Bt maize diet had greater AST than offspring from sows fed the isogenic maize diet (56.2 and 47.4 units/L; $P < 0.05$). There was an effect of time on serum AST, with values decreasing from d 30 to 100 and increasing thereafter ($P < 0.05$; data not shown). An overall sow treatment × offspring treatment interaction was observed for serum GGT, with pigs fed the Bt/Bt treatment having less serum GGT than pigs fed the Bt/iso and the iso/Bt treatments ($P < 0.05$). A change over time was also observed, with GGT increasing from d 30 to 70, decreasing from d 70 to 100 and increasing again thereafter ($P < 0.05$; data not shown). For serum TP, no treatment effect was observed. However, a tendency toward a sow treatment × offspring treatment was observed at d 0, with offspring on the iso/Bt treatment having reduced TP compared with the iso/iso treatment ($P = 0.07$). Serum TP increased over time ($P < 0.05$; data not shown). A sow treatment effect was observed for d 0 serum urea, with offspring of Bt maize-fed sows having greater values than offspring of isogenic maize-fed sows (3.4 and 2.6 mmol/L; $P < 0.05$). On d 115, serum urea tended to be less in pigs fed the Bt maize diets compared with pigs fed isogenic maize diets (4.8 and 5.5 mmol/L; $P = 0.08$). An effect of time was also observed for serum urea, with values increasing from d 30 to 70, decreasing to d 100, and increasing thereafter ($P < 0.05$; data not shown).

Table 6. Effect of feeding *Bacillus thuringiensis* (Bt) maize-based diets to sows during gestation and lactation and to offspring for 115 d on offspring serum biochemistry[1]

	Treatment							
	Sow isogenic[2]		Sow Bt[2]			*P*-value		
Item	Offspring isogenic[3]	Offspring Bt[3]	Offspring isogenic[3]	Offspring Bt[3]	Pooled SEM	Sow	Offspring	Sow × offspring
Alkaline phosphatase, units/L								
d 0[4]	465	429	440	432	54	0.85	0.63	0.77
d 30	293[x]	248[y]	284[x]	285[x]	16	0.24	0.07	0.08
d 70	206	193	194	194	15	0.63	0.50	0.81
d 100	173	164	175	140	18	0.47	0.16	0.33
d 115	194	173	195	183	17	0.69	0.23	0.62
Overall	216	195	212	201	12	0.93	0.06	0.59
Alanine aminotransferase, units/L								
d 0[4]	41.4	39.1	40.6	40.8	2.8	0.87	0.65	0.62
d 30	48.6[a]	39.6[b]	45.2[ab]	50.1[a]	3.6	0.23	0.43	0.05
d 70	44.9	42.1	43.7	47.9	4.0	0.47	0.82	0.58
d 100	50.0	46.9	51.9	49.9	4.5	0.54	0.51	0.84
d 115	52.3	55.9	58.0	53.0	3.5	0.62	0.80	0.39
Overall	49.0	46.1	49.7	50.2	2.8	0.27	0.49	0.32
Aspartate aminotransferase, units/L								
d 0[4]	34.6	35.8	37.4	41.7	2.7	0.05	0.17	0.51
d 30	40.8	36.5	39.2	39.7	2.9	0.77	0.49	0.73
d 70	34.9	35.9	35.3	33.9	2.9	0.77	0.94	0.96
d 100	29.8	27.3	28.4	29.8	2.5	0.81	0.81	0.84
d 115	48.0[b]	46.9[b]	51.1[b]	61.3[a]	3.9	0.02	0.21	0.02
Overall	38.4	36.6	38.5	41.2	1.8	0.24	0.77	0.17
Gamma glutamyltransferase, units/L								
d 0[4]	48.1	43.9	46.2	45.9	5.0	0.99	0.58	0.65
d 30	41.6	45.1	43.8	41.2	3.2	0.82	0.86	0.73
d 70	61.9	73.4	68.8	57.3	6.1	0.46	0.99	0.22
d 100	48.3	50.1	47.7	48.9	3.6	0.82	0.62	0.95
d 115	54.4	55.1	58.1	46.7	4.5	0.60	0.19	0.21
Overall	51.5[ab]	55.9[a]	54.6[a]	48.5[b]	2.8	0.50	0.72	0.03
Total protein, g/L								
d 0[4]	55.8[x]	52.2[y]	54.4[xy]	54.8[xy]	1.5	0.62	0.12	0.07
d 30	56.2	54.0	54.9	56.8	1.6	0.65	0.94	0.58
d 70	62.1	60.5	61.5	63.0	1.1	0.37	0.96	0.37
d 100	64.7	64.3	65.8	66.1	1.3	0.27	0.98	0.71
d 115	72.3	75.2	71.9	71.3	1.9	0.24	0.52	0.43
Overall	63.8	63.5	63.5	64.3	0.9	0.78	0.77	0.51
Urea, mmol/L								
d 0[4]	2.8	2.5	3.5	3.3	0.3	0.01	0.23	0.83
d 30	2.9	3.0	2.6	2.8	0.3	0.44	0.42	0.70
d 70	4.1	4.0	3.5	4.3	0.3	0.55	0.18	0.21
d 100	3.6	4.1	3.7	3.5	0.3	0.41	0.53	0.50
d 115	5.0	4.9	6.0	4.7	0.4	0.38	0.08	0.13
Overall	3.9	4.0	3.9	3.8	0.2	0.71	0.94	0.60
Creatinine, μmol/L								
d 0[4]	100.8	100.9	94.4	99.3	3.9	0.28	0.42	0.43
d 30	81.9[a]	81.3[a]	78.1[ab]	72.4[b]	3.0	0.02	0.20	0.05
d 70	94.0	98.7	97.4	95.4	2.7	0.97	0.53	0.46
d 100	109.4	108.4	108.3	104.5	3.0	0.35	0.35	0.54
d 115	136.8	140.8	142.6	147.5	5.8	0.26	0.42	0.55
Overall	105.5	107.3	106.6	105.0	2.5	0.76	0.98	0.41

[a,b]Within a row, means without a common superscript differ, $P \leq 0.05$.

[x,y]Within a row, means without a common superscript differ, $P \leq 0.10$.

[1]Values presented as least squares means (n = 10/treatment).

[2]Sows were fed isogenic or Bt maize-based diets from service until approximately 28 d postfarrowing.

[3]Offspring were fed isogenic or Bt maize-based diets from weaning for 115 d.

[4]Variability present in the data at weaning (d 0 of the study) has been accounted for by including d 0 values as covariates in the statistical model.

A sow treatment × offspring treatment interaction was observed for serum creatinine on d 30 of the study when pigs fed the Bt/Bt treatment had decreased serum creatinine than pigs fed the iso/iso and the iso/Bt treatments ($P \leq 0.05$). A sow treatment effect was also observed for serum creatinine on d 30, with offspring from sows fed Bt maize diets having decreased serum creatinine than offspring from sows fed isogenic maize diets (75.3 and 81.6 μmol/L; $P < 0.05$). An overall time effect was also observed for serum creatinine with concentrations increasing throughout the study ($P < 0.05$; data not shown).

DISCUSSION

To our knowledge, this is the first transgenerational study in pigs to investigate the effect of feeding GM Bt maize to sows during gestation and lactation on offspring growth and health. In accordance with international guidelines (Hartnell et al., 2007; European Food Safety Authority, 2008), Bt maize was included in the present study at the maximum possible rate of inclusion without unbalancing the diet.

Growth Performance

Offspring of sows fed Bt maize had improved growth throughout their productive life compared with offspring of sows fed isogenic maize, regardless of the maize line fed between weaning and harvest. This was not thought to be a result of differing diet composition, as similar nutrient content was found for the Bt and isogenic maize diets fed to sows (Walsh et al., 2012b). Our study was not designed to examine reproductive performance and, therefore, lacked the statistical power needed to make definitive conclusions regarding this aspect. Despite this, the average number of piglets born from sows fed Bt maize was numerically greater than sows fed the isogenic maize (total born, 15.8 and 12.7 piglets, respectively; live born, 14.3 and 11.8 piglets, respectively) and this resulted in a numerically lighterbirth weight for offspring from Bt maize-fed sows compared with offspring of isogenic maize-fed sows (1.33 and 1.45 kg, respectively; Walsh et al., 2012b). This difference is thought to be unrelated to treatment. The numerically greater litter size in sows fed Bt maize would have resulted in more severe in utero crowding for their offspring than for offspring of sows fed isogenic maize. We hypothesize that this could have resulted in reduced nutrient supply to fetuses of Bt maize-fed sows (Wu et al., 2006). Reduced nutrient supply to the fetus in utero has been shown to induce leptin resistance, leading to increased appetite (Ross and Beall, 2008) and accelerated postnatal compensatory growth (Attig et al., 2008). This may account for the improved lifetime growth in offspring observed in the present study.

Previously, we found that feeding Bt maize diets increased feed intake, but not growth rate, in a 31-d study in weanling pigs (Walsh et al., 2012a). This was thought to have occurred as a result of a decreased level of enzyme resistant starch found in the Bt maize (Walsh et al., 2012a). In addition, no differences in growth performance or harvest characteristics were found in a 110-d pig-feeding study when the same Bt and isogenic maize were compared (Buzoianu et al., 2012). Although increased feed intake and reduced G:F were previously observed in grower-finisher pigs fed Bt maize (Custodio et al., 2006), this is likely to have been more indicative of feed wastage than an effect of treatment. Greater BW gain was observed as a result of feeding Bt maize in transgenerational mice studies (Brake et al., 2004; Haryu et al., 2009). However, most studies found no growth differences as a result of feeding Bt maize as was the case in grower-finisher pigs (Reuter et al., 2002) and in a transgenerational sheep study (Trabalza-Marinucci et al., 2008).

These conflicting data make it difficult to reach a definitive conclusion on the influence of Bt maize on growth performance. The greater ADG and BW in weaner pigs fed Bt maize observed by Piva et al. (2001) occurred because of greater mycotoxin content of the isogenic maize. However, in the present study, mycotoxin concentrations in maize samples were below the European maximum acceptable limits (Office for Official Publications of the European Union, 2002, 2006). However, even though the sampling procedure before analysis was thorough and the mycotoxin analysis was repeated on a number of occasions, it is possible that pockets of mycotoxin contaminated maize may have existed, as when present, they are not evenly distributed within any batch of feedstuff (Lawlor and Lynch, 2001). To avoid this possibility, a mycotoxin binder was added to all diets in the current study and, for this reason, we do not believe that growth differences observed can be attributed to mycotoxin contamination.

Harvest Characteristics

The increased carcass weight and dressing percentage observed in the present study, as a result of feeding Bt maize, is in contrast with previous work, which found no effect of feeding Bt maize throughout the grower-finisher period on harvest characteristics (Reuter et al., 2002; Custodio et al., 2006; Buzoianu et al., 2012). The growth benefits observed in response to feeding Bt maize in this study indicate a greater nutritional value for the Bt maize compared with its isogenic counterpart. Nonetheless, growth performance is merely a gross indicator of health and more specific health indicators must also be evaluated.

Organ Health

A more specific health indicator is organ weight. Spleen was lighter at harvest in offspring from sows fed Bt maize diets compared with those from sows fed isogenic maize. A reduction in spleen weight can result from starvation or aging (Feldman et al., 2006), but neither were encountered in this study. On the contrary, offspring of Bt maize-fed sows had increased feed intake and growth. As discussed earlier, these pigs may have experienced growth retardation in utero, which has been shown to result in reduced organ weight (Attig et al., 2008). Numerically lighter spleens at birth were observed in littermates of the pigs used in this study (Walsh et al., 2012b). Previous work by our group found no differences in spleen weight or spleen histopathology in pigs after 31 (Walsh et al., 2012a) or 110 d of feeding Bt maize (Buzoianu et al., 2012). Therefore, lighter spleens observed in this study were unexpected and not consistent with our previous findings.

Although liver weight was lighter at harvest for the Bt/Bt treatment compared with the iso/Bt and the Bt/iso treatments, it was not different between pigs on the Bt/Bt treatment and those on the iso/iso treatment. Furthermore, no indicators of liver dysfunction were observed after histopathological examination. Liver dysfunction is initially characterized by an increase, rather than a decrease, in liver size and a concomitant increase in the liver enzymes AST, ALT, GGT, and ALP (Boone et al., 2005; Schneider et al., 2006; Kaneko et al., 2008). It is only if an aggression persists that the liver loses its ability to regenerate, with a reduction in weight and hepatic tissue being replaced by fibrous tissue, leading to changes in serum-detectable liver enzymes. Only on d 115 was the pattern of differences between treatments consistent for 1 of the liver enzymes (AST) and liver weight. With the exception of a transiently greater AST on d 0 for offspring of Bt maize-fed sows, differences in AST between treatments were not observed at any other time point and values remained within the normal range for pigs (Odink et al., 1990; Radostits et al., 2007; Kaneko et al., 2008). This is in accordance with previous work from our group, which showed an absence of liver pathology in pigs fed Bt maize for 31 (Walsh et al., 2012a) or 110 d (Buzoianu et al., 2012). Similar to the present study, low magnitude changes in serum biochemistry were observed in sheep (Trabalza-Marinucci et al., 2008) and rats (Kilic and Akay, 2008) as a result of Bt maize feeding, and, as is the case in the present study, it was concluded that such differences are unlikely to be of biological significance.

With the exception of the overall decreased GGT found for pigs fed the Bt/Bt treatment compared with the iso/Bt and Bt/iso treatments, no other differences for serum GGT were observed. In addition, it is an increase in GGT that would be associated with liver injury (Kaneko et al., 2008) rather than the decrease observed in the present study. Therefore, the differences in overall GGT are not believed to be of biological significance and are not indicative of liver dysfunction. Serum ALP was unchanged by treatment throughout the study.

Differences in serum ALT were observed only at d 30 of the study but, even then, no differences between the iso/iso and the Bt/Bt treatment were observed. A twofold increase in serum ALT above its normal upper limit is indicative of liver dysfunction, and, where such an increase occurs, it is likely to be accompanied by a similar increase in serum AST (Boone et al., 2005). However, no such differences were observed at d 30 in the present study. On d 115, however, an increase in serum AST was observed for offspring from sows fed Bt maize and for pigs fed the Bt/Bt treatment compared with all other treatments. However, this increase was not accompanied by an increase in other liver-associated enzymes and no evidence of liver dysfunction was observed after histopathological examination. Furthermore, values generally remained within the normal range for pigs (Odink et al., 1990; Radostits et al., 2007; Kaneko et al., 2008). Therefore, the differences observed are not believed to be of biological importance.

Although kidney dysfunction is characterized by changes in kidney weight, increased serum creatinine, and a concomitant rise in serum urea (Kaneko et al., 2008), such changes were not observed in the present study. A transiently greater serum urea was found at d 0 in offspring of Bt maize-fed sows compared with isogenic maize-fed sows. Serum creatinine transiently decreased at d 30 for offspring of sows fed Bt maize and in pigs fed Bt/Bt treatment compared with pigs fed the iso/iso and iso/Bt treatments. However, these differences did not persist and kidney weights and histological structure at harvest were similar for all treatments. This absence of kidney pathology after Bt maize consumption is in agreement with our previous findings in pigs fed Bt maize diets for 31 (Walsh et al., 2012a) or 110 d (Buzoianu et al., 2012). In contrast, Kilic and Akay (2008) observed decreased serum TP in rats fed Bt maize for 3 generations and this was associated with kidney histopathology. Unlike the present study, Kilic and Akay (2008) did not investigate the presence of mycotoxin or pesticide contaminants, which could have affected kidney function. As the differences observed in the present study were neither consistently detected nor associated with organ histopathology, they are unlikely to be of biological significance.

Intestinal Morphology

Duodenal crypt depth was greater in the offspring of sows fed Bt maize diets. Deepening of the intestinal crypts may be a sign of intestinal inflammation (Stevens et al., 2002). However, inflammation of the intestine is also accompanied by shortening or even disappearance of the villi, an increase in the number of goblet cells, malabsorption, and diarrhea (Stevens et al., 2002); none of which were observed in the present study. Deeper crypts indicate increased intestinal metabolism and cell turnover rate (Pluske et al., 1997) and feed intake is known to be positively correlated with intestinal development and crypt depth (Kelly et al., 1991). Therefore, a greater feed intake in offspring from sows fed Bt maize diets may have increased the metabolic activity of duodenal enterocytes, leading to an increased rate of epithelial renewal. This may have resulted in a greater demand for and a more intense production of epithelial cells in the duodenal crypts, and consequently, a deepening of the latter. As no differences were observed between treatments for duodenal goblet cell numbers, villus height, or other intestinal morphological features at the other small intestinal sites examined, and no signs of histopathology were observed in the mesenteric lymph nodes, the differences observed in the duodenum are not considered to be of major biological significance.

In conclusion, feeding Bt maize to sows and to offspring from weaning to harvest did not adversely influence offspring growth, and carcass characteristics and organ weights at harvest. On the contrary, feeding Bt maize to sows increased growth performance of offspring. However, we speculate that this could have been because of the numerically greater litter size found for Bt maize-fed sows, rather than being an effect of feeding Bt maize per se. No intestinal health abnormalities were observed and organ histology and organ health indicators did not reveal adverse effects after Bt maize consumption for 2 generations. Although differences were observed for liver and spleen weight at harvest and for some serum measurements, values generally remained within normal ranges for pigs and no pattern indicating organ dysfunction was observed. These results indicate that consumption of Bt maize for 2 generations is not detrimental to pig health.

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