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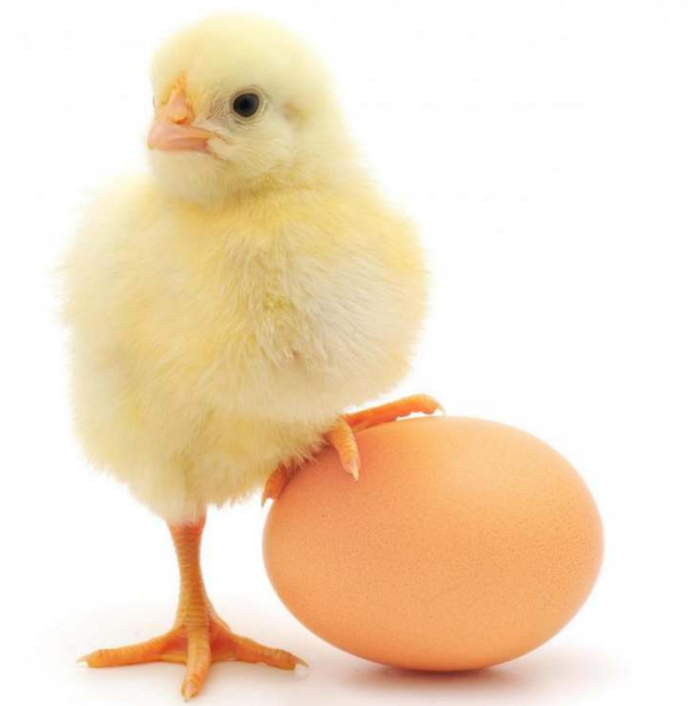

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Effects of Bt (*Bacillus thuringiensis*) Corn on Reproductive Performance in Adult Laying Hens

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Abstract: Genetically Modified (GM) crops are widely used. Research has focused on examining differences between GM and conventional corn feeding on broiler performance and carcass composition. However, relatively little attention has been focused on reproductive effects on the recipient animals. To address this, the present study employed laying hens as a model due to their rapid reproductive development and rate of egg production. This study investigated the putative effects of a diet consisting of Starlink corn (expressing the Bacillus thuringiensis gene Cry9C) on the fecundity of the laying hen. Twenty-week-old point of lay pullets were fed a diet containing corn positive for the Cry9C (Starlink) gene (not a current variety) for 3 weeks, paired with a corn-based diet known not to contain the gene. During the study, the number of eggs collected, egg shell thickness, number of yolks and egg weight and, upon necropsy, body, ovary and ovary weight, together with the number of yolky (yellow) follicles were recorded. There were no negative effects observed on any parameter measured. The present data do not suggest that Bt corn is a cause of concern to the poultry or livestock industry.

Keywords: Bt corn, reproduction, egg production

Introduction

Bacillus thuringiensis (Bt) Cry proteins are commonly employed as insecticidal controls in agricultural crops. Chickens fed genetically modified corn expressing (Bt) Cry proteins exhibit normal growth whether receiving corn expressing Cry1A (b) protein (Bt 176) (Brake and Vlachos, 1998; Brake *et al.*, 2003; Taylor *et al.*, 2003; Aeschbacher *et al.*, 2005) or a variant of the wild type Cry 3Bb1 protein (Taylor *et al.*, 2005a,b). Feeding a genetically modified Bt176 corn hybrid or non-modified counterpart did not affect egg production in hens (Aeschbacher *et al.*, 2005). Moreover, Bt-gene specific constructs were not detectible in the meat or eggs from the poultry (Aeschbacher *et al.*, 2005). A ten-generation study was conducted with quail fed diet containing either isogenic or transgenic (Bt 176) corn. There were no effects on quail health and performance (Flachowsky *et al.*, 2005). The reproductive safety of Bt (*Bacillus thuringiensis*) corn has also been evaluated using mouse testes as a sensitive biomonitor. Pregnant mice were fed a Bt corn or a nontransgenic (conventional) diet during gestation/lactation and for up to 12 weeks of age for the resulting male offspring. There were no effects on testicular cell populations (Brake *et al.*, 2004). Brake *et al.* (2004) concluded based on mouse studies that "Bt corn is not harmful to human reproductive development" and by extrapolation to other species.

StarLink corn, expresses an insecticidal protein Bt gene Cry9C. It was registered for domestic animal feed and non-food, industrial use in the USA between 1998 and 2000 (Reviewed Buccini and Goldman, 2002). In 2002, an Iowa farmer was reported as stating that a specific corn (Starlink) fed to pigs was negatively impacting their reproductive performance including increased incidence of pseudopregnancy (Organic Consumers, 2003). There is further anecdotal evidence of other farmers reporting reproductive problems associated with feeding Bt corn (Organic Consumers, 2003). Concerns included possible mycotoxin contamination and/or the presence of endogenous phytochemicals in corn. There is evidence that corn contains reproductive inhibiting phytochemicals (Markaverich *et al.*, 2002a,b; 2005; 2007). For instance, furan fatty acids have been reported to impair reproduction in rodents (Markaverich *et al.*, 2002a,b). Another constituent of corn, 9,10-dihydroxy-12-octadecenoic acid (leukotoxin diol) is a leukotoxin; oral administration of which at very low doses disrupts estrous cyclicity in female rats (Markaverich *et al.*, 2005). It should be noted that furan fatty acids were without effect on reproductive indices in laying hens (Wilheims *et al.*, 2006).

The present study examines the ability of the suspect Bt-corn to influence reproductive development, egg production and ovarian functioning in the hen. It was important to use chickens because of the high corn content in many feeds. Moreover, as the supply of the

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Table 1: Effect of Suspect Bt corn on Reproductive and other Functions in Laying Hens. Pullets (20 week old) were fed control diet or a diet containing suspect Bt corn for three weeks

Parameter	Control diet	Bt corn diet
Ovary weight (g)	42.6±3.03	45.8±1.81
Oviduct weight (g)	52.4±1.89	54.9±1.84
Weight of large yolky follicles(F1 to F5) (g)	33.8±2.12	34.8±1.33
Number of Large follicle >0.8 mm diameter	6.0±0.41	6.3±0.29
Number of follicles 0.4-0.8 mm diameter	13.0±1.65	14.9±2.14
Feed intake per day (g)	92.0±4.82	99.0±0.87
Increase in body weight (g)	69.7±24.2	39.4±20.6

Table 2: Effect of Suspect Bt corn on Egg Production and Egg Quality in Laying Hens. Pullets (20 week old) were fed either control diet or a diet containing suspect Bt corn for three weeks

Parameter	Control diet	Bt corn diet
Egg number	19.0±0.33	18.3±0.62
Week 1 Egg number per week	6.56±0.18	5.89±0.45
Week 2 Egg number per week	6.78±0.15	6.67±0.33
Week 3 Egg number per week	5.67±0.24	5.77±0.15
Mean Egg weight (g)	50.6±0.56	52.0±0.49†
Week 1Egg weight (g)	48.5±0.78	49.0±0.78
Week 2 Egg weight (g)	50.7±0.78	54.0±1.07*
Week 3 Egg weight (g)	52.6±1.01	53.0±0.99

† P = 0.0776; * P = 0.0264 compared to control

suspect Bt-corn was limited, poultry offered a useful and sensitive biological model for livestock.

Materials and Methods

Animals: All procedures had been approved by the Iowa State University Committee on Animal Care (protocol 8-03-5498-G). White Leghorn pullets (20 weeks old) were obtained from Sparboe Farms (Litchfield, Minnesota). Birds were weighed and randomly placed individually into cages (30.5W×40.5L×44H in cm) with a feeder supplying feed to three adjacent cages. The composition of the control diet was as follows: ground corn 566.7 g/kg; soybean meal (48% crude protein) 265 g/kg; animal and vegetable fat blend 36.5 g/kg; DL methionine 2.5 g/kg; dicalcium phosphate 22.3 g/kg; limestone 81.0 g/kg; ground oyster shell 15 g/kg; sodium chloride (iodized) 5.0 g/kg; trace mineral premix 3.0 g/kg (supplying to the diet: manganese 80 mg/kg as manganese sulfate, zinc 90 mg/kg as zinc oxide, iron 60 mg/kg as ferrous sulfate, copper 12 mg/kg as cupric sulfate, selenium 147 µg/kg as selenium premix); vitamin premix 3.0 g/kg (supplying to the diet: vitamin A 8065 IU per kg, vitamin $D_3$1580 IU/kg, 25 hydroxy-vitamin D_3 31.5 µg/kg, vitamin E 15 IU/kg, riboflavin 7.8 mg/kg, pantothenic acid 12.8 mg/kg, niacin 75 mg/kg, choline chloride 509 mg/kg, biotin 270 µg/kg, menadione 4 mg/kg). This was formulated to meet NRC requirements (National Research Council, 1994) and mixed at the Iowa State University Poultry Research Center. The concentrations of calcium and non-phytate phosphorus were, respectively, 4.22 and 0.52%. The treatment diet replaced commercial corn with ground suspect Bt-corn. Unless stated otherwise, all ingredients were obtained from Archer-Daniels-Midland Company or local suppliers. Feed and water were available *ad libitum*. A long day length (14L:10D) was imposed to bring the birds into full reproduction.

Experimental design and treatments: It was hypothesized that the suspect Bt-corn would inhibit reproduction in female poultry. There were two treatments: the control diet and the test diet. Test diet was formulated in an identical manner but commercial blended corn substituted for the suspect Bt-corn [Cry 9c (Starlink) gene] purchased from an Iowa farmer.

Blocks of three cages, each containing one pullet, were randomly assigned to one of three treatments with nine replicate cages/birds per treatment or three replicate blocks of cages per treatment.

Experimental protocol: At the start of the study (48 hours after the arrival of the pullets), body weights were recorded. The chickens were then fed treatment diets *ad libitum* for 21 days. During this period, feed intake, number of eggs laid, egg weight and any overt abnormalities were recorded. Shell thickness was also determined (measured at four sites on the egg and the mean used in analysis).

On day 21, the hens were weighed and sacrificed by decapitation. Ovary and oviduct weights were determined as were any reproductive overt abnormalities (atretic follicles, internal eggs). The ovarian follicles were dissected. The weight of the F1 – F5 largest follicles were determined as were the number of large yolky follicles (>8.0 mm diameter) and of the small yellow follicles (4-8 mm diameter).

Statistics: The study was analyzed as a randomized design with sub-sampling. All data were analyzed using PROC MIXED in SAS version 9.0 (SAS Institute, Cary, NC) with the exception of egg lay. Egg lay was analyzed by binomial and multinomial logistic regression. Values found different were separated using Dunnett's method. Significance was determined at p = 0.05.

Results and Discussion

The study examined the effect of a suspect sample of Bt-corn on reproduction in hens. The Bt-corn exhibited no acute toxicity as indicated by the absence of any mortality (0/9 birds) and any changes in feed consumption (Table 2). Moreover, there were no differences in ovarian or oviductal weights or the number or weights of large yolky follicles (Table 1).

Table 2 summarizes the effects of the diet containing the suspect corn on egg production and egg weight. There were no differences in the number of egg produced between the hens on the control and Bt corn diets over the three-week treatment period (egg number over 20 days: Control diet 19.0±0.33; Bt corn diet 18.3±0.62) or each of the three weeks at the beginning of egg production (Table 3). There was a tendency (P = 0.0776) for the egg weights to be higher on the Bt corn diet over the three-week period (mean egg weight: control diet 50.6±0.56g; Bt corn diet 52.0±0.49) and this difference compared to the control diet was significant ($p<0.05$) on the second week of treatment. There were no effects of dietary treatment on egg-shell thickness (data not shown).

Contrary to the possibility of impaired reproduction, there were no negative effects of suspect Bt-corn on reproductive performance on reproductive development in the layer hen. The present report is consistent with the reports of an absence of negative effect of other transgenic Bt-corn containing different Cry proteins on growth in broiler chickens (Brake and Vlachos, 1998; Brake *et al.*, 2003; Taylor *et al.*, 2003; 2005a,b; Rossi *et al.*, 2005) and on reproduction in male mice (Brake *et al.*, 2004), quail (Flachowsky *et al.*, 2005) and laying hens (Aeschbacher *et al.*, 2005).

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