Introduction

Pig diets have a high content of starch (40-55% on a dry matter basis; Bach Knudsen et al., 2006) and cereals represent the main starch source (Stevnebo et al., 2006). In Italy, corn is the most important cereal used in the feed mix industry (Assalzoo, 2010).

The Lepidopteran european corn borer (ECB, Ostrinia nubilalis) is one of the most important parasitic pests of corn. Lepidopterans larvae damage is a factor that predisposes corn to micotoxigenic Fusarium attack, because kernel wounds created by larvae encourage fungal colonization. Fusarium verticillioides (formerly F. moniliforme) and F. proliferatum (Thiel et al., 1991) are fungi producing fumonisin B1 (FB1), a toxin that inhibits sphingolipid synthesis, blocks folate transport and has been associated with increased incidences of human esophageal cancer and neural tube defects (Sadler et al., 2002, Voss et al., 2006). The higher content of FB1 in corn is related to the association between pest attacks following corn disease and the consequent host-insect-pathogen fungi interaction (Jarvis et al., 1984; Bruns and Abbas, 2006; Wu, 2006). In corn Fusarium spp are the most common toxigenic fungi and in the Po Valley more than 90% of corn is contaminated by FB1 (Reyneri, 2006).

In Bt corn the cry1A(b) gene, from the bacterium Bacillus thuringiensis (Bt), has been inserted. The gene encodes a plant insecticidal protein that is selectively toxic to Lepidopterans, while in animals the Bt protein is digested without detrimental effect (Kubena et al., 1995, 1997; Brake and Vlachos, 1998; Brake et al., 2003). One indirect benefit of the improved resistance of Bt corn to ECB attack is lower levels of FB1 contamination (Munckvold et al., 1997, 1999, Masoero et al., 1999; Hammond et al., 2004; Wu, 2006). In farm animals, FB1 can cause leukoencephalomalacia in horses (Marasas et al., 1998) and pulmonary edema in swine (Hascheck et al., 2001). Recently Fodor et al. (2008) reported that fumonins (36.6 mg/day) reduce GSH levels in blood plasma of piglets. FB1 was found to induce oxidative stress in humans, rat and mouse neural cell culture (Stockmann et al., 2004). Although many agencies recognize that Bt corn is safe, many European consumers are very reluctant to purchase genetically organisms, so additional data are needed to overcome the distrust of European consumers.

The objective of this study was to investigate and compare the effects of diet containing Bt corn or near conventional corn, both produced on Italian farms, on the performance of weaned piglets in the early weeks of life. Furthermore, the FB1 content of corn, which is typically affected by the expression of Bt protein, will be considered. Due to the widespread FB1 contamination of corn grown in Northern Italy (Reyneri, 2006), it was not possible to have a control group of isogenic corn without FB1 contamination.

Materials and methods

Bt and isogenic corn

The transgenic (Bt) corn seeds containing the cry1A(b) protein (a genetic event known as MON810) were produced by Dekalb Genetics Corp. (DeKalb, IL, USA) and imported to Italy by Dekalb Italy SpA (Lodi, Italy) with the authorization of the Italian Ministry of Agricultural Policy. The isogenic corn (isoline) was a conventional hybrid corn, derived from the same inbred parents used to produce the transgenic line. The Bt corn and the isogenic corn were both grown in field by Monsanto Italy (Milano, Italy) in Po valley in northern Italy on two farms located in the provinces of Lodi (farm A) and Venice (farm B). The environmental field conditions of growth and processing were identical in the two farms. Harvesting and storage were performed using standard procedures and conditions.
Experimental design
The study was conducted at the CERZOO (Research Centre for Livestock and Environment) experimental facility using Large White x Landrace commercial hybrid pigs. Piglets were weighed, selected from groups without disease, and administered typical vaccinations. After a pre-experimental period of 13 days, 128 animals with a 8.8±1.27 kg live weight (LW) were randomly allocated to four homogeneous groups of 32 animals (16 castrated males and 16 females) and fed the experimental diets: Bt-Farm A, Bt-Farm B, iso-Farm A, iso-Farm B for 35 consecutive days. During the pre-experimental period, the animals were fed medicated feed containing chlortetracycline (1000 mg/kg) and spiramycin (400 mg/kg). Each pen included 4 piglets of the same sex. The pens were assigned in a fully randomized block design using the Randomized Procedure of SAS software (2002).

Animals were housed in rooms at the same facility with controlled climate and natural daylight.

Experimental diets and water
Isonitrogenous and isoenergetic meal diets were formulated according to INRA (1989) requirements. The composition and the analytical characteristics of the diets are shown in Tables 1 and 2. The diets were produced in the CERZOO facility using a horizontal mixer (500 kg capacity) to mix the basal diet with Bt or isogenic corn, which represented 33% of the total diet; each diet was prepared twice during the study. To reduce cross-contamination, the mixer was cleaned between preparations of the diets using dry matter, crude protein (CP), ether extract (EE), ash, and starch according to the AOAC methods (1993), while NDF was determined according to Van Soest et al. (1991). The amino acid (AA) profiles of the transgenic corn, isogenic corn, and experimental diets were determined according to the method of Moore et al. (1971) using a Carlo Erba (Rodano, MI, Italy) (type 3A30) AA analyzer. Determination of aflatoxins was performed according to the recommendations of the European Commission (1992); fumonisin B1 was determined using the method of Shephard et al. (1996), and deoxynivalenol was determined using the method of the AOAC (1990, 1993). The analytical characteristics of Bt and isogenic corn are shown in Table 3.

Performance assessment
Fourteen and 35 days after the start of the study, any feed remaining in the feeders was weighed, the pen was the experimental unit. Of death.

Statistical analysis
The data for LW, feed intake, ADG, and F:G were statistically analyzed using a completely randomized block design with a factorial arrangement 2×2×2 (2 farms, 2 sex and 2 types of corn). Although animals were individually weighed, the pen was the experimental unit. Statistical analysis was performed according to the General Linear Model procedure of the SAS Institute software package 9.1 (2002).

The following experimental model was used:

\[ Y = \mu + \alpha + \beta + \gamma + \alpha \beta + \alpha \gamma + \beta \gamma + \epsilon \]

where:

- \( Y \) = items
- \( \mu \) = overall mean
- \( \alpha \) = farm
- \( \beta \) = sex
- \( \gamma \) = corn type

Table 1. Composition of the experimental diets (% as fed).

|               | Farm A°   | Farm B°   |
|---------------|-----------|-----------|
|               | Iso corn  | Bt corn   | Iso corn  | Bt corn   |
| Corn          | 33.00     | 20.72     | 33.00     | 20.72     |
| Soybean meal  | 44        | 20.72     | 44        | 20.72     |
| Soya oil      | 2.00      | 2.00      | 2.00      | 2.00      |
| Barley meal   | 7.00      | 7.00      | 7.00      | 7.00      |
| Barley flakes | 25.00     | 25.00     | 25.00     | 25.00     |
| Fish meal     | 3.00      | 3.00      | 3.00      | 3.00      |
| Dehydrated whey| 5.00   | 5.00      | 5.00      | 5.00      |
| L-lysine      | 0.50      | 0.50      | 0.50      | 0.50      |
| DL methionine | 0.10      | 0.10      | 0.10      | 0.10      |
| Limestone     | 0.30      | 0.30      | 0.30      | 0.30      |
| Dicalcium phosphate | 2.70 | 2.70      | 2.70      | 2.70      |
| Sodium chloride| 0.18     | 0.18      | 0.18      | 0.18      |
| Premix°       | 0.50      | 0.50      | 0.50      | 0.50      |

Table 2. Analyzed composition of the experimental diets.

|               | Farm A°   | Farm B°   |
|---------------|-----------|-----------|
|               | Iso corn  | Bt corn   | Iso corn  | Bt corn   |
| Dry matter, % | 89.19     | 89.19     | 89.03     | 89.27     |
| Crude protein, %DM | 20.70 | 20.49     | 21.18     | 20.52     |
| Ether extract, %DM | 4.97  | 5.26      | 5.02      | 5.22      |
| Neutral detergent fibre, %DM | 12.19 | 11.78     | 11.83     | 11.37     |
| Ash, %DM      | 6.88      | 7.20      | 7.13      | 7.12      |
| Starch, %DM   | 45.63     | 44.74     | 43.58     | 45.89     |
| Buffer capacity | 24.40  | 23.70     | 25.30     | 25.25     |
| Digestible energy, kcal/kgDM | 3786 | 3783      | 3790      | 3802      |
| Deoxynivalenol, μg/kgDM | 3810 | 40        | 61        | nd        |
| Fumonisin B1, μg/kgDM | 2519  | 3789      | 3783      | 3790      |
| AA, %DM       | 19.46     | 20.45     | 20.26     | 20.22     |
| Non essential AA, %DM | 8.55  | 9.07      | 8.77      | 8.32      |
| Essential AA/Total AA | 10.91 | 11.38     | 11.49     | 11.91     |

 Iso, isogenic corn; Bt, transgenic corn; °farm A was located in the province of Lodi and the Farm B in the province of Venezia; °farm A° Farm B°; °the study was carried out prior to the ban on use of meat and bone meal in animal diets; °the premix provided (kg–1 feed) in the 1st growing period: Vit. A, 15,500 U; Vit. D3, 1200 U; Vit. E, 25 mg; Vit. B6, 1.5 mg; Vit. B12, 0.03 mg; Vit. H, 0.13 mg; Vit. K3, 3.5 mg; Vit. PP, 17 mg; Choline chloride, 300 mg; Mn, 42 mg; Fe, 250 mg; Cu, 95 mg; Zn, 105 mg; I, 1.2 mg; Se, 0.17 mg; Co, 0.33 mg; and in the 2nd growing period: Vit. A, 10,500 U; Vit. D3, 1200 U; Vit. E, 25 mg; Vit. B6, 1.5 mg; Vit. B12, 0.03 mg; Vit. H, 0.13 mg; Vit. K3, 3.5 mg; Vit. PP, 17 mg; Choline chloride, 300 mg; Mn, 42 mg; Fe, 250 mg; Cu, 95 mg; Zn, 105 mg; I, 1.2 mg; Se, 0.17 mg; Co, 0.33 mg; mEq NaOH/100 g;消化エネルギー（DE）はWhittemore（1980）の式で評価した：DE（kcal/kg）= 238.8 + 0.015 NDF（g/kg）+ 0.016 EE（g/kg）+ 0.008 CP（g/kg）– 0.033 Ash（g/kg）．AA、アミノ酸、nd、未検出（値が検出下限以下）．
different diets are reported in Table 4. The ADG with the Bt corn was higher for the entire study period (Bt: 396.4 g/d, isogenic: 374.1 g/d, P<0.05) and between day 15 and 35 (Bt: 464.1 g/d, isogenic: 429.1 g/d, P<0.05). At the end of the study, the live weight was higher in piglets

Table 3. Analyzed composition of transgenic and isogenic corn from farm A and farm B.

| Item                        | Farm A  | Bt  | Farm B  |
|-----------------------------|---------|-----|---------|
| Dry matter, %               | 87.43   | 87.22 | 88.21   | 87.63   |
| Crude protein, %DM          | 8.50    | 8.93 | 8.84    | 9.68    |
| Ether extract, %DM          | 4.41    | 4.04 | 4.29    | 4.55    |
| Neutral detergent fibre, %DM| 8.84    | 7.36 | 7.75    | 8.91    |
| Ash, %DM                    | 1.42    | 1.30 | 1.51    | 1.43    |
| Starch, %DM                 | 74.36   | 72.67 | 74.58   | 72.94   |
| Mycotoxins                  |         |     |         |
| Deoxynivalenol, μg/kgDM     | 123     | 184  | 93      | nd      |
| Fumonisin B1, μg/kgDM       | 6458    | 922  | 13,166  | 5,212   |
| Lysine, %DM                 | 0.24    | 0.23 | 0.27    | 0.29    |
| Histidine, %DM              | 0.16    | 0.19 | 0.25    | 0.26    |
| Arginine, %DM               | 0.39    | 0.39 | 0.45    | 0.45    |
| Aspartic acid, %DM          | 0.59    | 0.60 | 0.66    | 0.65    |
| Threonine, %DM              | 0.29    | 0.29 | 0.32    | 0.32    |
| Serine, %DM                 | 0.42    | 0.45 | 0.46    | 0.47    |
| Glutamic acid, %DM          | 1.42    | 1.55 | 1.61    | 1.62    |
| Proline, %DM                | 0.75    | 0.81 | 0.80    | 0.81    |
| Glycine, %DM                | 0.32    | 0.34 | 0.34    | 0.34    |
| Alanine, %DM                | 0.57    | 0.62 | 0.60    | 0.60    |
| Valine, %DM                 | 0.37    | 0.34 | 0.41    | 0.41    |
| Methionine + cysteine, %DM  | 0.35    | 0.36 | 0.38    | 0.39    |
| Isoleucine, %DM             | 0.28    | 0.32 | 0.33    | 0.33    |
| Leucine, %DM                | 0.95    | 1.07 | 1.05    | 1.06    |
| Phenylalanine + tyrosine, %DM| 0.65  | 0.67 | 0.70    | 0.71    |
| AA, %DM                     | 7.75    | 8.23 | 8.63    | 8.71    |
| Essential AA, %DM           | 3.25    | 3.42 | 3.70    | 3.75    |
| Non essential AA, %DM       | 4.50    | 4.81 | 4.93    | 4.96    |
| Essential AA/total AA       | 41.94   | 41.56 | 42.87   | 43.05   |

Results

Corn and diet analysis
In our study, the Bt-corn and near conventional corn had the same analytical characteristics (Table 3). The content of CP, EE, NDF, ash, starch, were in the normally accepted range. The AA composition was the same for the two types of corn grown in the same provinces. In our experiment the FB1 concentration was lower in Bt than in isogenic corn, independent of the farm where it was produced (Table 3) (86% for corn produced at farm A and 60% for corn produced at farm B). On average the FB1 concentration in Bt corn was reduced by 73%. Aflatoxin B1 was not detected in our samples, while deoxynivalenol was present in all samples, even if at low levels (Table 3).

Consistent with expectation, the starter and grower/finisher diets were isonitrogenous and levels (Table 3). The content of CP, EE, NDF, ash, starch, were in the normally accepted range. The AA composition was the same for the two types of corn grown in the same provinces. In our experiment the FB1 concentration was lower in Bt than in isogenic corn, independent of the farm where it was produced (Table 3) (86% for corn produced at farm A and 60% for corn produced at farm B). On average the FB1 concentration in Bt corn was reduced by 73%. Aflatoxin B1 was not detected in our samples, while deoxynivalenol was present in all samples, even if at low levels (Table 3).

Performance
The performances of animals fed the four different diets are reported in Table 4. The ADG with the Bt corn was higher for the entire study period (Bt: 396.4 g/d, isogenic: 374.1 g/d, P<0.05) and between day 15 and 35 (Bt: 464.1 g/d, isogenic: 429.1 g/d, P<0.05). At the end of the study, the live weight was higher in piglets
fed the diet containing Bt corn (Bt: 22.68 kg, isogenic: 21.83 kg; P<0.05). As reported in Table 4, there was a negative relationship between the piglets’ growth and the FB1 content of the corn.

The feed was well accepted by the animals, and we detected no negative effect of Bt corn on feed intake. In fact, between days 15 and 35, the female piglets fed Bt corn ate more feed than did their counterparts fed isogenic corn. The animals grown in Farm B (with the highest level of FB1 contamination) showed a higher feed intake than the piglets of Farm A. However this increment in feed intake did not result in a better weight gain and, as a consequence, the F:G ratio was made worse. In particular the F:G ratio decreased linearly (P<0.05) with the FB1 content of the diet.

A sex effect was observed for ADG, with females growing more than castrated males during the first 14 days of the trial (females: 303 g/d, males: 283 g/d) but eating less feed during the second feeding phase (females: 798.5 g/d, males: 884.5 g/d). Also, feed intake for the whole study was smaller for females versus males. As a result, the global feed efficiency was better in female piglets than in castrated male piglets.

**Discussion**

**Corn and diet analysis**

Previous studies on Bt-corn show no difference in the analytical composition of genetically modified and near isogenic corn (Brake and Vlachos, 1998; Masoero et al., 1999; Barrière et al., 2001; Rossi et al., 2003). Masoero et al. (1999) reported a difference in nutritional value, a reduction in the structured carbohydrate components of Bt-corn, with consequent improvement in the in vitro nutrient digestibility and energy content. In contrast, the results obtained by Aulrich et al. (2001) in pigs show no significant difference in the digestibility of organic matter and CP between different corn varieties. Spencer et al. (2000) showed that genetically modified corn in pig diets had a lower level of phytate and therefore more available phosphorus content than did conventional corn. The small differences in CP, EE, and starch content were not substantial enough to affect the LW and FG.

There was no reason to expect a large difference in AA content due to the expression of cry1A(b) protein, and in fact no differences in AA composition were found between Bt and isogenic corn. In a previous work (Rossi et al., 2003) we observed a higher Phe content in Bt versus isogenic corn, but this was due to a lower lignin content that spared the AA from the lignin synthesis pathway.

The reduced FB1 content in Bt corn is consistent with other reports, where application of transgenic technology to corn indirectly reduced the mean FB1 concentration (Clements et al., 2003; Munkvold et al., 1997, 1999). This is due to an improvement in corn resistance against the European corn borer larvae (Ostrinia nubilalis) (Mason et al., 1996; Magg et al., 2001) that damage the stalk and facilitate the plant colonization by FB1 producing Fusarium. This is also a cause of yield reduction and loss of grain quality (Mason et al., 1996).

**Performance**

The most important result of this experiment was the absence of detrimental effect of Bt-corn on performances, confirming the results of Hyun et al. (2005) who used heavier piglets (from an initial weight of 23 kg to a final weight of 44 kg), while Custódio et al. (2006) observed a worsening on feed efficiency, but not on weight gain, when feeding Bt corn to older pigs (initial weight 60 kg). However in these experiments the mycotoxin content of the diet was not determined. In our trial the inclusion of Bt corn in the diet improved the ADG of animals. Because control corn showed higher levels of FB1 than Bt maize, the reduction of growth in control group might be related to the immunosuppressive effect of FB1 (Taranu et al., 2005; Bouhet et al., 2006), to its impairment of the barrier function of porcine intestinal epithelial cells (Oswald et al., 2003; Bouhet et al., 2004) and to its reduction of cardiac output and heart rate (Constable et al., 2000; Smith et al., 2000). However, no significant difference in the health status of the two groups of piglets was observed. The highest observed values of FB1 contamination (2.52 and 5.53 mg/kg, both for isogenic diets) were lower than the levels demonstrated by Fernandez-Surumay et al. (2005) to exert an immunosuppressive effect (72 mg/kg of diet). However, Zomborszky-Kovács et al. (2002) were able to show a toxic effect of FB1 in piglets with contamination in the range of 4.2 to 7 mg/kg of diet. Thus, it is possible that the relatively low levels of contamination observed in our experiment negatively affected growth, and this might account for the superior growth of piglets fed the Bt-diet, which was less contaminated by FB1. Rotter et al. (1996) reported an 8% reduction of growth in castrated male piglets that received 1 mg of FB1 for kg of diet.

The better growth of female piglets could be related to the lower sensitivity of gilts to FB1, as reported by Rotter et al. (1996) and Marin et al. (2006). In particular, female pigs are less sensitive than males to the immunosuppressive effect of FB1. In our study, there was also a strong difference in FB1 contamination between not only Bt and isogenic corn, but between farm A and B. Although the level of contamination was low compared to the level producing acute toxicity, the inverse linear response between ADG and FB1 content supports the hypothesis that the sex effect was mediated by differences in responsiveness to FB1. Unlike deoxynivalenol, which sharply reduces feed intake in pigs (Morgavi and Riley, 2007), the effect of FB1 on feed consumption is less clear. Feeding a heavily contaminated feed (381 mg/kg of FB1) resulted in a 60% decrease of initial value with the feed intake piglets (Fernandez-Surumay et al., 2005). A slight decrease in feed intake (~8.3%) was observed by Dilkin et al. (2003) when feeding weaned piglets with 30 mg/kg of FB1, but the same group reported an increase in feed intake when animals were fed a less contaminated diet (10 mg/kg of FB1). In our experiment we observed a higher dry matter intake in animals grown in farm B with a fumonisin content higher than Farm A. The level of FB1 in diet B was lower than 10 mg/kg and this confirms the results of Dilkin et al. (2003) where a low contamination with FB1 can increase feed intake.

A sex effect was also observed on feed:gain ratio through out the entire time and this could be due to the lower sensitivity of gilts to FB1 (Rotter et al., 1996; Marin et al., 2006). A significant interaction between corn and sex on the feed intake in the first 14 days of the experiment was detected. Feed intake resulted higher in gilts compared to male piglets, but this difference increased in animals fed with isogenic compared to transgenic
corn (Figure 1). Isogenic corn had a higher content of FB1 compared to Bt maize and since females are less sensitive than males to FB1, this could explain the corn and sex interaction. In this period, gilts showed a trend for a better growth (P 0.06) than male piglets and this could partially justify the finding.

Conclusions

Weaned piglets fed Bt corn performed better than piglets fed near conventional corn. Our findings suggest that this better performance is due to the lower FB1 content of Bt corn. Our data and the results of previous studies (Brake et al. 2003 in broilers, Barrière et al. 2001 in sheep and dairy cows, and Aulrich et al. 2001 in pigs) suggest that including Bt corn in diets for weaned piglets does not have any detrimental effect and can improve performances due to lower FB1 levels.

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