Sex-related differences in the immune response of weanling piglets exposed to low doses of fumonisin extract

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(Received 22 July 2005 – Revised 24 January 2006 – Accepted 3 February 2006)

Fumonisin B₁ (FB₁) is a mycotoxin produced by *Fusarium verticillioides*, a fungus that commonly contaminates maize. Sex-related effects of FB₁ have been observed with respect to carcinogenicity in rodents, to performances in pigs and immunosuppression in mice. In the present study the sex-related effect of FB₁ on the pig immune response was determined. Female and castrated male piglets received for 28 d either control feed or feed contaminated with 8 mg FB₁/kg feed in the form of *F. verticillioides* culture material. At day 7 and day 21, animals were immunised subcutaneously with a *Mycoplasma agalactiae* vaccine. Ingestion of FB₁-contaminated feed significantly decreased weight gain in males but had no effect in females. No sex-related difference was observed in biochemical parameters, but a higher level of creatinine was noted in toxin-treated animals. FB₁ also altered the pig immune response in a sex-specific manner. In males, ingestion of FB₁-contaminated feed significantly decreased specific antibody levels after vaccination as well as the mRNA expression level of IL-10. In females, the toxin has no effect on specific antibodies or on cytokine mRNA levels. The results of the present study indicate that FB₁ is immunosuppressive in pigs. The magnitude of this FB₁-induced immunosuppression is highly dependent on sex, with males being more susceptible than females.

**Fumonisin: Mycotoxins: Immunosuppression: Food contamination: Pig: Gender-related effects**

Mycotoxins are secondary metabolites of fungi, which may contaminate animal feeds and human foodstuffs. The global occurrence of mycotoxins is considered an important risk factor for human and animal health, as up to 25 % of the world crop production may be contaminated (Fink-Gremmels, 1990; Bouhet & Oswald, 2005; Oswald et al. 2005).

Fumonisin B₁ (FB₁) belongs to the fumonisin family of toxins which are produced by *Fusarium verticillioides* and *F. proliferatum* fungi that commonly contaminate maize. Recent surveys of fumonisin contamination of food and feed in Europe and the USA also have raised concerns about the extent of FB₁ contamination of maize and its implications for food safety (Murphy et al. 1996; International Programme on Chemical Safety, 2000). FB₁ was found in up to 50 % of maize samples collected between 1988 and 1991 from the mid-Western USA (Murphy et al. 1993). In this survey, up to 10 % of the samples had toxin levels between 10 and 50 parts per million (Murphy et al. 1993). Similarly, another survey of fumonisins in maize gluten and other maize products in the UK found these mycotoxins in almost every sample at concentrations of up to 32 parts per million (Scudamore et al. 1990).

The mechanisms of FB₁ toxicity are complex and may involve several molecular sites (Riley et al. 1998). The primary biochemical effect of fumonisins is the inhibition of ceramide synthase leading to the accumulation of sphingoid bases and sphingoid base metabolites, and the depletion of more complex sphingolipids (Riley et al. 1998; Merrill et al. 2001). Ingestion of high doses of FB₁ induces different species-specific effects in domestic and laboratory animals including pulmonary oedema and cardiovascular changes in the pig, leukoencephalomalacia in horses and nephrotoxicity in rats, rabbits and lambs. It also causes hepatotoxicity in all species studied (Bolger et al. 2001; Haschek et al. 2001). This toxin has also been reported to be a contributing factor in human oesophageal cancers (International Agency for Research on Cancer, 2002). Ingestion of low doses of FB₁ increases intestinal and pulmonary infection in pigs (Oswald et al. 2003; Halloy et al. 2005) and alters immune responses in pigs and in mice (Bhandari et al. 2002; He et al. 2002; Bouhet et al. 2004; Taranu et al. 2005).

Fumonisin toxicity has not only been demonstrated to be species- and tissue-specific, but has also been shown to be sex-specific (Rotter et al. 1996; Bhandari et al. 2001;...
Howard et al. 2001; Johnson & Sharma, 2001). In a 2-year feeding study, hepatocellular adenomas and carcinomas were induced by FB1 in female mice but not in males. In the same study it was also revealed that male Fischer F344 rats developed renal tumours that were not seen in females (Howard et al. 2001). Sex-specific effects of FB1 have been described in the immune response of mice following subcutaneous injection (Bhandari et al. 2001; Johnson & Sharma, 2001). In females, FB1 treatment reduced relative spleen and thymus weights, splenic cellularity, thymocyte CD4+ / CD8+ double positive cells, lymphocyte proliferation, and IL-2 expression. These effects were not observed in male mice (Johnson & Sharma, 2001). In addition, FB1 administration caused increased expression of TNF-α, IL-12p40, interferon (IFN)-γ, IL-1β, IL-6 and IL-10 in male liver while female mice only showed an increased expression of IL-6 and a down modulation of IFN-γ (Bhandari et al. 2001). In pigs, males are more adversely affected by low doses of purified FB1 than females, as indicated by the effect of the toxin on average daily gain, serum biochemical parameters, pancreas and adrenal weight (Rotter et al. 1996).

In the present study the sex-specific effects of FB1 on the pig immune response was investigated. Ingestion of mycotoxin-contaminated feed altered the immune response of males but did not affect the immune response of females as indicated by the expression of T-helper (Th) 2 cytokines and by the production of specific antibodies upon vaccination.

Materials and methods

Experimental design

Twenty, 4-week-old, crossbred weanling piglets (ten females and ten castrated males) were studied for 28 d. The animals were from seven different litters and were balanced for litters across treatments. They were acclimatised for at least 1 week before being used in the experimental protocol and were given ad libitum access to water and feed. They were fed a maize-soyabean-meal-based diet (Marin et al. 2002) supplemented with or without a fumonisin-containing fungal extract. The extraction procedure following in vitro culture of the F. verticilloides strain NRRL 34 281 has been described (Oswald et al. 2003). Briefly, sterilised maize inoculated with the fungal strain was incubated for 4 weeks at 25°C. The culture was extracted with acetonitrile – water, filtered, and concentrated. The crude extract contained 54 % FB1, 8 % FB2 and 9 % FB3 (Tran et al. 2003). We verified that it did not contain detectable amounts of zearalenone, deoxynivalenol, fusaric, chromanone or trichothecenes (Oswald et al. 2003). The extract was incorporated into the pig basal diet to provide 8 mg FB1/kg feed. Considering the average feed consumption of the animals, this corresponded to doses of 0.99 and 1.49 mg/kg body weight per d for the first and the second halves of the experiment. Body weights and food consumption were recorded weekly throughout the experiment. Animals were cared for in accordance with the National Institute of Health Guide.

Immunisation and blood sample collection

On day 7 and day 21 of the experiment, all piglets were immunised by subcutaneous inoculation with a 1 ml suspension of Agavac® (Institute Pasteur, Bucharest, Romania) as previously described (Marin et al. 2002). This vaccine consists of a combination of formol-inactivated Mycoplasma agalactiae strains re-suspended in aluminium hydroxide. On day 0, day 20 and day 28 of the experiment, blood samples were aseptically collected by jugular vein puncture. Syrings without anticoagulant were used to collect serum for antibody and biochemical parameter measurements and syringes containing lithium-heparin were used to collect blood for measuring cytokine mRNA expression.

Measurement of blood biochemical parameters

Serum concentrations of Na, K, chloride, Ca, P, total proteins, urea, creatinine, glucose, cholesterol, triacylglycerols and bilirubin, and concentrations of alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, γ-glutamyl transferase and lactate dehydrogenase were determined on a Vitros 950 IRC analyser (Ortho Clinical Diagnostics, Raritan, NJ, USA) at the Laboratory of Biochemistry (Rangueil Hospital, Toulouse, France).

Measurement of total and specific antibody levels

Total concentrations of the different Ig subsets (IgG, IgA and IgM) were measured by ELISA (Bethyl, Interchim, Montluçon, France) as previously described (Taranu et al. 2005).

Antibodies against M. agalactiae were also measured by ELISA. Briefly, ELISA plates were coated with supernatant fraction from ultrasonicated M. agalactiae culture. Diluted serum samples (1/100) were then added to the plates and the anti-mycobacterial antibodies were detected with peroxidase-labelled anti-pig IgG (Marin et al. 2002). The absorbance at 405 nm was recorded using an ELISA plate reader.

Blood cell culture for cytokine mRNA expression analysis

The mRNA expression of five different cytokines was analysed in the blood samples obtained from the control and FB1-treated animals. Whole blood was cultured as previously described (Marin et al. 2002). Briefly, blood was diluted 10-fold in RPMI 1640 supplemented with 2 mM-L-glutamine, penicillin (100 U/ml), streptomycin (0.1 mg/ml; Sigma, St Louis, MO, USA), 10% fetal calf serum (Hyclone, Perbio, Brebieres, France) and then 2 ml of diluted blood was stimulated with phytohaemagglutinin (10 μg/ml). After 24 h of culture, cell pellets were harvested and re-suspended in 1 ml Trizol (Gibco BRL Life Technologies, Cergy Pontoise, France) then frozen at −80°C until used.

Determination of cytokine mRNA expression by semi-quantitative reverse transcriptase polymerase chain reaction

Total RNA was extracted following the manufacturer’s recommendations and quantified by spectrophotometry. Semi-quantitative determination of IL-4, IL-10, IL-6, IL-2 and IFN-γ and cyclophilin, chosen as a housekeeping gene, was carried out using RT-PCR performed as previously described (Dozois et al. 1997; Fournout et al. 2000). Briefly, mRNA was reverse transcribed with Moloney leukemia virus RT (Promega, Charbonnières, France) and amplified with DNA
Taq polymerase enzyme (Invitrogen, Life Technology, Cergy Pontoise, France) using the already published primer sequences (Dozois et al. 1997; Fournout et al. 2000). Semi-quantitative analysis of PCR products was done by hybridisation of 32P-labelled specific oligonucleotide probes to PCR products immobilised on nitrocellulose membranes by dot blotting (Pié et al. 2004). The DNA probes used for hybridisation of the different cytokines have already been described (Darwich et al. 2003; Pié et al. 2004). The relative amounts of each product were determined by measuring radioactivity with a Phosphor Imager (Molecular Dynamics, Sunnyvale, CA, USA). For each cytokine, the amounts of RT-PCR products were normalised to the values obtained with cyclophilin, which was used as an internal standard for each sample.

Statistical analysis

All data are expressed as mean values and standard errors of the mean. Statistical differences between groups for feed consumption, biochemical parameters, serum immunoglobulin subsets and cytokine expression levels were determined using an ANOVA two-way analysis; the measurements for these parameters were done once at the end of the experiment. A two-way ANOVA with replications was used to analyse the antibody and the average weight gain during the experiment. Further differences between means were determined by the least square difference Fisher procedure. Values of $P<0.05$ were considered significant.

Results

Sex-related effect of fumonisin on animal performance

We first investigated the sex-related effect of FB$_1$ treatment on clinical signs and animal weight. The feeding of 8 mg FB$_1$/kg feed for 28 d did not produce any detectable alteration in the general status of either male or female piglets. As shown in Fig. 1, in females the feeding of fumonisin had no significant effect on the average daily weight gain ($P=0.821$). By contrast, in males, throughout the 4 weeks of the experiment, ingestion of fumonisin-contaminated feed decreased ($P=0.001$) the average daily weight gain by up to 23% when compared with control. Further differences between control and fumonisin male groups at specific time points were determined by the least square difference Fisher procedure. A significantly treatment effect began after the 3rd ($P=0.021$) and the 4th ($P=0.049$) week of intoxication. This lower weight gain was not due to reduced feed intake. The experimental protocol did not allow measurement of individual feed ingestion but group data indicated that feed consumption was 2.6 and 10.6% higher for males compared with females during the first and the second halves of the experiment respectively.

Sex-related effect of fumonisin on biochemical parameters

At the end of the experiment, blood samples were collected from the piglets to investigate the sex-related effect of fumonisin on biochemical parameters. The presence of FB$_1$ in the diet did not alter the activities of serum enzymes including alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, γ-glutamyl transferase and lactate dehydrogenase (Table 1). The contamination of pig feed with fumonisin also did not have any influence on the serum concentration of Na, K, chloride, bicarbonate, Ca, P, protein, urea, glucose, bilirubin, cholesterol and triacylglycerol. By contrast, the feeding of fumonisin-contaminated diet significantly increased the creatinine concentrations in the serum of treated animals ($P=0.035$).

Sex-related effects of fumonisin on total and specific antibody responses

To investigate the sex-related effect of fumonisin on the immune responses, piglets were immunised with *Mycoplasma*. Serum antibody levels were measured by ELISA after the primary (day 21) and the secondary injections (day 28). As expected, the vaccinal injections increased the antibody levels (Fig. 2). This increase was observed in both males and females receiving either the control or the fumonisin-contaminated diet. Nevertheless a sex-related effect of fumonisin was observed on mycoplasma-specific antibody levels. Repeated-measures ANOVA used for data statistical analysis showed that both ingestion of fumonisin-contaminated feed and the time of exposure significantly decreased the specific antibody synthesis ($P=0.022$) in males. The further pairwise comparison between the control and FB$_1$ group at each specific time point resulted in a 38% decrease in specific antibody levels ($P=0.045$) at day 28 of the experiment whereas in females, ingestion of the contaminated feed did not have any

Fig. 1. Sex-related effect of fumonisin on animal daily weight gain. Male (a) or female (b) piglets received a control diet (– – –) or a fumonisin B$_1$-contaminated diet ( – – –). Animals were weighed weekly and results are expressed as the average daily weight gain; values are means for five animals, with the vertical bars representing standard errors. Comparison of the daily weight gain observed in the control and fumonisin B$_1$-treated animals was done using a repeated-measures ANOVA ($P=0.82$ for females and $P=0.001$ for males). * At specific time points, mean value was significantly different between control and treated groups ($P<0.005$, determined by the least square difference Fisher procedure).
significant effect \( P=0.186 \). This sex-related effect was only observed for specific antibody level and no effect of FB1 was observed on total Ig levels, irrespective of the subsets considered (Table 2).

**Sex-related effect of fumonisin on cytokine mRNA expression**

The ability of fumonisin to modulate the cytokine expression in a sex-dependent manner was then investigated on whole-blood samples stimulated with mitogen. The mRNA expression of both Th1 (IL-2, IFN-\( \gamma \)) and Th2 (IL-4, IL-10 and IL-6) cytokines was measured by RT-PCR at the end of the experiment. The mRNA synthesis of Th1 cytokines (IL-2 and IFN-\( \gamma \)) and Th2 (IL-4, IL-10) was investigated on whole-blood samples stimulated with mitogen. The mRNA synthesis of Th1 cytokines (IL-2 and IFN-\( \gamma \)) and Th2 (IL-4, IL-10) was observed on total Ig levels, irrespective of the subsets considered (Table 2).

**Table 1. Effect of dietary fumonisin administration on blood biochemical parameters in piglets†**

(Mean values with their standard errors)

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Males</th>
<th>Females</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control feed</td>
<td>Fumonisin-containing feed</td>
</tr>
<tr>
<td>Na (mM)</td>
<td>141·0 1·0</td>
<td>145·2 0·7</td>
</tr>
<tr>
<td>K (mM)</td>
<td>8·43 0·55</td>
<td>7·83 0·20</td>
</tr>
<tr>
<td>Chloride (mM)</td>
<td>99·8 1·2</td>
<td>100·2 1·3</td>
</tr>
<tr>
<td>Bicarbonate (mM)</td>
<td>16·2 1·6</td>
<td>17·7 1·0</td>
</tr>
<tr>
<td>Ca (mM)</td>
<td>2·53 0·03</td>
<td>2·53 0·09</td>
</tr>
<tr>
<td>P (mM)</td>
<td>4·24 0·51</td>
<td>4·44 0·19</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>69·3 1·4</td>
<td>73·7 3·9</td>
</tr>
<tr>
<td>Urea (mM)</td>
<td>5·21 0·70</td>
<td>4·46 0·73</td>
</tr>
<tr>
<td>Creatinine (( \mu )M)</td>
<td>85·2 4·2</td>
<td>97·7* 4·8</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>4·26 0·51</td>
<td>4·05 0·36</td>
</tr>
<tr>
<td>Bilirubin (( \mu )M)</td>
<td>2·9 0·3</td>
<td>3·3 0·3</td>
</tr>
<tr>
<td>Cholesterol (mM)</td>
<td>2·77 0·19</td>
<td>3·11 0·14</td>
</tr>
<tr>
<td>Triacylglycerols (mM)</td>
<td>0·50 0·12</td>
<td>0·40 0·01</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/l)</td>
<td>205·2 28·7</td>
<td>283·0 88·3</td>
</tr>
<tr>
<td>( \gamma )-Glutamyl transferase (IU/l)</td>
<td>180·8 20·9</td>
<td>140·2 5·1</td>
</tr>
<tr>
<td>Aspartate aminotransferase (IU/l)</td>
<td>76·5 1·0</td>
<td>60·5 3·6</td>
</tr>
<tr>
<td>Alanine aminotransferase (IU/l)</td>
<td>48·4 9·8</td>
<td>49·0 8·3</td>
</tr>
<tr>
<td>Lactate dehydrogenase (IU/l)</td>
<td>1877 198</td>
<td>1363 164</td>
</tr>
</tbody>
</table>

*Mean value was significantly different from that of the animals of the same sex fed the control feed \((P<0.05)\).

† At the end of the experiment, serum from the piglets (four or five per group) was used to measure the blood biochemical parameters.

In the present study, sex differences in the immune response of pigs after exposure to fumonisin were observed for both antibody and cytokine responses. The feeding of fumonisin was shown to decrease the specific antibody response developed after immunisation with an anti-Mycoplasma vaccine. This effect was only observed in males and was specific for the acquired immune responses. Immunosuppressive effects of FB1 on humoral immune response have been described after immunisation with sheep erythrocytes in male rats receiving 25 mg FB1/kg body weight per d (decreased specific IgM; Tryphonas et al. 1997) and in male mice receiving 0·25–5 mg FB1/kg body weight in one dose (reduced number of specific plaque-forming cells; Martinova & Merrill, 1995). Turkeys treated with 200 mg FB1 for 4 weeks also had significantly lower antibody responses during vaccination against Newcastle disease virus (Li et al. 2000).
They were immunised with a Mycoplasma vaccine on day 7 and day 21 of the experiment. Serum samples were collected on day 0, day 21 and day 28 and levels of vaccine-specific antibody were determined by ELISA. Results are expressed as optical density (405 nm); values are means determined by a repeated-measures ANOVA (P = 0.022 for males and P = 0.18 for females). *Mean value was significantly different from that of the animals fed the control feed at the same time point (P = 0.05; determined by the least square difference Fisher procedure).

By contrast, ingestion of a high dose of fumonisin-contaminated feed for 8 d, or a low dose of toxin for 3–4 months, did not alter antibody titres against Aujeszky virus (Tornyos et al. 2003). In the present study the total level of Ig (IgM, IgG or IgA) was not affected by the fumonisin treatment (Table 2). The presence of fumonisin in the diet, however, significantly decreased the mRNA expression level of IL-10, a Th2 cytokine, in male peripheral blood cells. A tendency for a decrease of other Th2 cytokines, IL-6 (P = 0.68) and IL-4 (P = 0.159), were also observed in males. The lack of significance of these parameters may be due to the small number of animals present in each group (n = 5).

IL-4, IL-6 and IL-10, are implicated in the development of the humoral immune response and antibody production (Abbas et al. 1996). IL-4 is involved in the stimulation of the antibody production by B cells, promotion of growth and survival of T cells (Nelms et al. 1998). It is a major factor for the differentiation of B lymphocytes that promotes the Ig switch and favours synthesis of IgG. Moreover, IL-4 and IFN-γ play a key role in the regulation of immune response by their mutually antagonistic mechanisms on cytokine synthesis (Abbas et al. 1996). The development of antibody response is also sustained by IL-6, which in addition to its role as activator of the T cells is involved in the final differentiation of the B cells in plasmocyte cells which regulate antibody synthesis (Diehl & Rincon, 2002). IL-10 performs a complex role in the immune response. In cooperation with IL-4, IL-10 stimulates B cells by increasing the expression of class two molecules of the major histocompatibility complex (Abbas et al. 1996) and by stimulating their proliferation and their differentiation to express and produce IgM, IgG and IgA. IL-10 also plays an important role in the cytokine network, by inhibiting cytokine production by Th1 cells.

Table 2.Effect of dietary fumonisin administration on serum immunoglobulin G, immunoglobulin A and immunoglobulin M in piglets at the end of the experiment* (Mean values with their standard errors)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Fumonisin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>IgG (mg/ml)</td>
<td>21·1</td>
<td>4·39</td>
</tr>
<tr>
<td>IgA (mg/ml)</td>
<td>2·45</td>
<td>0·37</td>
</tr>
<tr>
<td>IgM (mg/ml)</td>
<td>3·49</td>
<td>0·46</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>IgG (mg/ml)</td>
<td>20·2</td>
<td>1·22</td>
</tr>
<tr>
<td>IgA (mg/ml)</td>
<td>1·56</td>
<td>0·17</td>
</tr>
<tr>
<td>IgM (mg/ml)</td>
<td>3·38</td>
<td>0·56</td>
</tr>
</tbody>
</table>

* Piglets (five per group), fed a contaminated or control diet, were bled at the end of the experiment. IgG, IgA and IgM concentrations were determined by ELISA. Two-way ANOVA (animal sex and FB1 treatment) did not reveal any effect of either factor on any serum Ig subset (IgG, IgA or IgM).
lymphocytes and monocytes and macrophages (Mocellin et al. 2003). In the present study, the decreased expression of Th2 cytokines (IL-10, IL-4 and IL-6) correlates with a decreased production of specific antibodies (Fig. 2 and Fig. 3). The lower specific antibody synthesis observed in males may, therefore, be a consequence of a lower Th2 cytokine expression.

As mentioned earlier, FB1 is a potent inhibitor of ceramide synthase, an enzyme critical to sphingolipid biosynthesis. The sex-related effect of FB1 could be due to a sex-related difference in sphingolipid metabolism. A higher accumulation of free sphingoid bases was observed in the liver of female mice treated with FB1 when compared with males (Bhandari et al. 2001). In pigs, however, after consumption of diet contaminated with 10 mg FB1/kg, the increase in free sphingoid bases in the lung was more pronounced in males than in females (Rotter et al. 1996). Sphingosine is a potent competitive inhibitor of protein kinase C (Gopee & Sharma, 2003) and, recently, it has been shown that protein kinases are involved in the regulation of cytokine synthesis. In human macrophages, for example, inhibition of protein kinase C selectively suppressed IL-10 production (Foey & Brennan, 2002). Thus, the higher inhibition of IL-10 expression observed in male pigs upon FB1 exposure (Fig. 3) may be due to a higher increase of sphingosine and a greater suppression of protein kinase C.

The species-specific effect of FB1 on sphingolipid metabolism may also explain the contrasting effects on cytokine synthesis observed in mice and pigs. Free sphingoid bases inhibit lymphocyte growth, especially the Th2 subtype (Tokura et al. 1996; Desai et al. 2002). Exposure to FB1 increases free sphingoid bases in male pigs and in female mice (Rotter et al. 1996; Bhandari et al. 2001). By selectively acting on Th2 lymphocytes, these sphingoid bases could decrease synthesis of cytokines (IL-4, IL-6 and IL-10) in a sex- and species-dependent manner. Through its effect on sphingolipid metabolism, FB1 may also modulate the concentration of the different subclass of gangliosides, known to regulate cytokine production. GD1b, GT1b and GQ1b, for example, enhanced IL-2 and IFN-γ production but suppressed IL-4 and IL-5, IL-6 and IL-10 synthesis. GD1a and GM3, however, stimulate IL-10 production (Kanda, 1999; Kanda & Watanabe, 2000, 2001). Further studies are needed to determine the implication of species and sex on lipid metabolism and its modulation by FB1 on the observed variation of cytokine expression.

In conclusion, it has been shown that fumonisin has toxic effects on the immune response and that these effects are more pronounced in males than in females. Due to the possible risk and implications for pig production and human health, this sex specificity requires further investigation, especially in the areas highly contaminated with Fusarium species.

Acknowledgements

D. E. M. was supported by a doctoral fellowship from a bilateral project ‘Réseau Formation-Recherche’ between INRA, France and IBNA, Romania, granted by the ‘Ministère de l’Éducation Nationale’ and the ‘Ministère de la Recherche’.

Fig. 3. Sex-related effect of fumonisin on T-helper (Th) 1 and Th2 cytokine mRNA expression. Male (a, b, c, d, e) or female (f, g, h, i, j) piglets were fed a control diet (C) or a fumonisin B1-contaminated diet (F). Blood samples were taken on day 28 of treatment and cultured for 24 h with phytohaemagglutinin (10 μg/ml). Total RNA was isolated and assayed for expression of Th1 cytokines (IL-2 (e and j); interferon-γ (d and i)) and Th2-type cytokines (IL-4 (a and f); IL-6 (c and h); IL-10 (b and g)). Results are expressed in arbitrary units as the ratio between the cytokine-specific and the cyclophilin RT-PCR values. Values are means for three to five animals, with the vertical bars representing standard errors. * Mean value was significantly different from that of the animals fed the control feed (P<0.05).
Effects of fumonisin B\textsubscript{1} on immune responses

(Paris, France). I. T. was the recipient of an INRA post-doc-toral fellowship. Thanks are also due to Dr Grossu, General Manager of IBNA, for her constant support and to Dr Trevor Smith for his editorial assistance.

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