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

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Fumonisin B_1 (FB_1) is a mycotoxin produced by *Fusarium verticillioides*, a fungus that commonly contaminates maize. Sex-related effects of FB_1 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 FB_1 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 FB_1/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 FB_1-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. FB_1 also altered the pig immune response in a sex-specific manner. In males, ingestion of FB_1-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 FB_1 is immunosuppressive in pigs. The magnitude of this FB_1-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, 1999; Bouhet & Oswald, 2005; Oswald *et al.* 2005).

Fumonisin B_1 (FB_1) 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 FB_1 contamination of maize and its implications for food safety (Murphy *et al.* 1996; International Programme on Chemical Safety, 2000). FB_1 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 FB_1 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 FB_1 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 FB_1 increases intestinal and pulmonary infection in piglets (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;

Abbreviations: FB_1, fumonisin B_1; IFN, interferon; Th, T-helper.

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Howard *et al.* 2001; Johnson & Sharma, 2001). In a 2-year feeding study, hepatocellular adenomas and carcinomas were induced by FB_1 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 FB_1 have been described in the immune response of mice following subcutaneous injection (Bhandari *et al.* 2001; Johnson & Sharma, 2001). In females, FB_1 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, FB_1 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 FB_1 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 FB_1 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 % FB_1, 8 % FB_2 and 9 % FB_3 (Tran *et al.* 2003). We verified that it did not contain detectable amounts of zearalenone, deoxynivalenol, fusarochromanone or trichothecenes (Oswald *et al.* 2003). The extract was incorporated into the pig basal diet to provide 8 mg FB_1/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. Syringes 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, Montlucon, 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 FB_1-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 leukaemia 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 ^{33}P-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 $P<0{\cdot}05$ 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 ($P=0{\cdot}821$). By contrast, in males, throughout the 4 weeks of the experiment, ingestion of fumonisin-contaminated feed decreased ($P=0{\cdot}001$) 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 ($P=0{\cdot}021$) and the 4th ($P=0{\cdot}049$) 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 ($P=0{\cdot}035$).

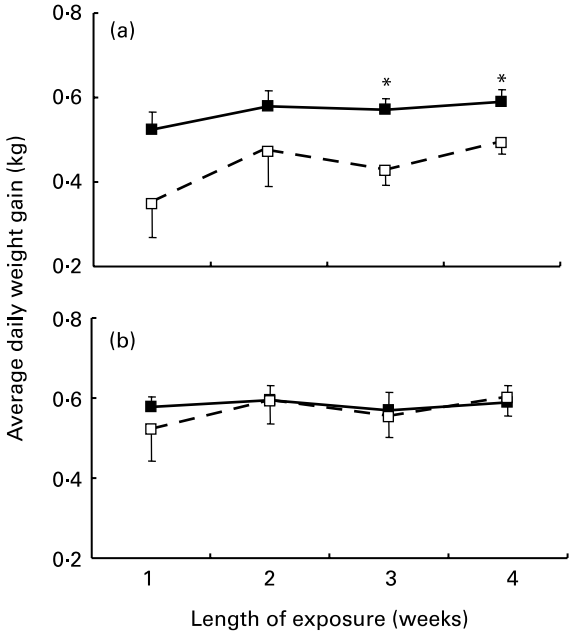

Fig. 1. Sex-related effect of fumonisin on animal daily weight gain. Male (a) or female (b) piglets received a control diet (–■–) or a fumonisin B_1-contaminated diet (–□–). Animals were weighed weekly and results are expressed as the average daily weight gain; values are means for five animals, with 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 ($P=0{\cdot}82$ for females and $P=0{\cdot}001$ for males). * At specific time points, mean value was signinficantly different between control and treated groups ($P<0{\cdot}005$; determined by the least square difference Fisher procedure).

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 ($P=0{\cdot}022$) 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 ($P=0{\cdot}045$) at day 28 of the experiment whereas in females, ingestion of the contaminated feed did not have any

Table 1. Effect of dietary fumonisin administration on blood biochemical parameters in piglets†
(Mean values with their standard errors)

	Males				Females			
	Control feed		Fumonisin-containing feed		Control feed		Fumonisin-containing feed	
Biochemical parameters	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Na (mM)	141·0	1·0	145·2	0·7	141·8	1·3	146·5	3·0
K (mM)	8·43	0·55	7·83	0·20	6·68	0·32	8·04	0·54
Chloride (mM)	99·8	1·2	100·2	1·3	97·6	0·6	100·2	2·7
Bicarbonate (mM)	16·2	1·6	17·7	1·0	18·8	1·3	18·4	1·5
Ca (mM)	2·53	0·03	2·53	0·09	2·52	0·07	2·40	0·06
P (mM)	4·24	0·51	4·44	0·19	4·03	0·23	4·96	0·82
Total protein (g/l)	69·3	1·4	73·7	3·9	65·2	1·5	66·6	1·4
Urea (mM)	5·21	0·70	4·46	0·73	4·13	0·48	4·46	0·71
Creatinine (μM)	85·2	4·2	97·7*	4·8	86·0	3·6	93·6*	3·3
Glucose (mM)	4·26	0·51	4·05	0·36	3·89	0·25	4·05	0·17
Bilirubin (μM)	2·9	0·3	3·3	0·3	2·8	0·4	3·6	0·9
Cholesterol (mM)	2·77	0·19	3·11	0·14	2·81	0·24	2·90	0·23
Triacylglycerols (mM)	0·50	0·12	0·40	0·01	0·40	0·03	0·32	0·03
Alkaline phosphatase (IU/l)	205·2	28·7	283·0	88·3	198·0	39·9	266·4	61·8
γ-Glutamyl transferase (IU/l)	180·8	20·9	140·2	5·1	135·2	23·8	144·4	30·5
Aspartate aminotransferase (IU/l)	76·5	1·0	60·5	3·6	52·0	8·2	60·6	2·2
Alanine aminotransferase (IU/l)	48·4	9·8	49·0	8·3	52·0	8·2	46·8	7·0
Lactate dehydrogenase (IU/l)	1677	198	1363	164	1535	80	1483	103

* 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.

significant effect ($P=0·186$). This sex-related effect was only observed for specific antibody level and no effect of FB_1 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-γ) 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-γ) was not modified by the contamination with fumonisin in either male or female piglets (Fig. 3). Ingestion of fumonisin-contaminated feed induced a sex-dependent decrease in the expression of Th2 cytokines. In males, fumonisin treatment decreased the expression level of mRNA encoding for IL-4, IL-6 and IL-10 (by 31, 51 and 45 % respectively). However, the difference was only significant for IL-10 ($P=0·040$, $P=0·268$, $P=0·159$ for IL-10, IL-6 and IL-4 respectively), due to the small number of animals in each group. By contrast, in female piglets, fumonisin treatment did not induce any changes in Th2 cytokine expression.

Discussion

We have studied the sex-related effects of a sub-chronic exposure (28 d) to fumonisin on body-weight gain, blood biochemical parameters and immune response of piglets. In general, males were more adversely affected by the presence of fumonisin in the diet than females. Average daily gain decreased by 23 % in males upon fumonisin treatment, for example, whereas it was not affected in females. Similar results were obtained by Rotter *et al.* (1996) who demonstrated that castrated pigs were more sensitive to 1–10 mg purified FB_1 in the diet than females. Zomborszky *et al.* (2000), in contrast, did not observe any significant effect on body-weight gain or feed consumption when feeding weanling piglets with 10–40 mg FB_1/kg feed for 4 weeks.

The present results indicate that low doses of FB_1 had limited effects on the blood biochemical parameters of the piglets (Table 1). In a previous study with purified toxin, biochemical changes were only observed at early (2 weeks) or late (8 weeks) time points (Rotter *et al.* 1996). No significant effect of FB_1 from fungal culture material was observed on blood parameters (Zomborszky-Kovacs *et al.* 2002).

Sex differences after chronic exposure to FB_1 toxicity have been reported for carcinogenic effects in rodents, performance changes in pigs and immunosuppression in mice (Rotter *et al.* 1996; Bhandari *et al.* 2001; Howard *et al.* 2001; Johnson & Sharma, 2001).

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 FB_1 on humoral immune response have been described after immunisation with sheep erythrocytes in male rats receiving 25 mg FB_1/kg body weight per d (decreased specific IgM; Tryphonas *et al.* 1997) and in male mice receiving 0·25–5 mg FB_1/kg body weight in one dose (reduced number of specific plaque-forming cells; Martinova & Merrill, 1995). Turkeys treated with 200 mg FB_1 for 4 weeks also had significantly lower antibody responses during vaccination against Newcastle disease virus (Li *et al.* 2000).

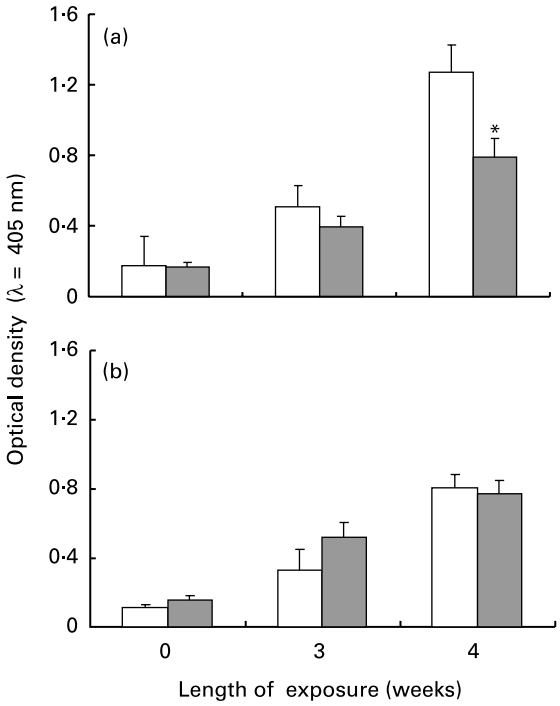

Fig. 2. Sex-related effect of fumonisin on specific antibody production. Male (a) or female (b) piglets received a control diet (□) or a fumonisin B_1-contaminated diet (■). They were immunised with a *Mycoplasma* vaccine on day 7 and day 21 of the experiment. Serum samples were collected on day 0, day 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 for five animals, with the vertical bars representing standard errors. Comparison of the specific antibody production in the control and fumonisin B_1-treated animals was done using a repeated-measures ANOVA ($P=0{\cdot}022$ for males and $P=0{\cdot}18$ for females). * Mean value was significantly different from that of the animals fed the control feed at the same time point ($P<0{\cdot}05$; 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 animals received feed contaminated with 8 mg FB_1/kg, and considering their body weight and their feed consumption, this represented an exposure of 1·24 mg FB_1/kg body weight per d. It seems that higher concentrations of FB_1 are necessary to affect the total antibody synthesis. In male Sprague–Dawley rats, 7·5 mg FB_1/kg body weight injected intraperitoneally for 4 consecutive days increased serum concentrations of IgM and IgG (Bondy *et al.* 1995). Similarly, white Leghorn Cornell chicks receiving feed containing *F. proliferatum* culture material with 61 mg FB_1/kg showed a significant suppression in total Ig and IgG levels (Qureshi *et al.* 1995).

Few investigations have examined the sex-related effect of FB_1 on cytokine synthesis (Bhandari *et al.* 2001; Johnson & Sharma, 2001), with no studies on pigs. In male mice, FB_1 treatment increased the expression of TNF-α, IL-12 p40, IFN-γ, IL-1β, IL-6 and IL-10, while female mice showed only an increased expression of IL-6, and a down modulation of IFN-γ and IL-2 (Bhandari *et al.* 2001; Johnson & Sharma, 2001). In the present study, no effect of fumonisin was observed on Th1 cytokines (IL-2 and IFN-γ). 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 ($P=0{\cdot}68$) and IL-4 ($P=0{\cdot}159$), 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).

Th2 cytokines, especially 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

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)

	Males				Females			
	Control		Fumonisin		Control		Fumonisin	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
IgG (mg/ml)	21·1	4·39	17·5	2·02	20·2	1·22	21·2	1·36
IgA (mg/ml)	2·45	0·37	1·00	0·30	1·56	0·17	1·63	0·37
IgM (mg/ml)	3·49	0·46	3·62	0·43	3·38	0·56	3·60	0·54

* 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 FB_1 treatment) did not reveal any effect of either factor on any serum Ig subset (IgG, IgA or IgM).

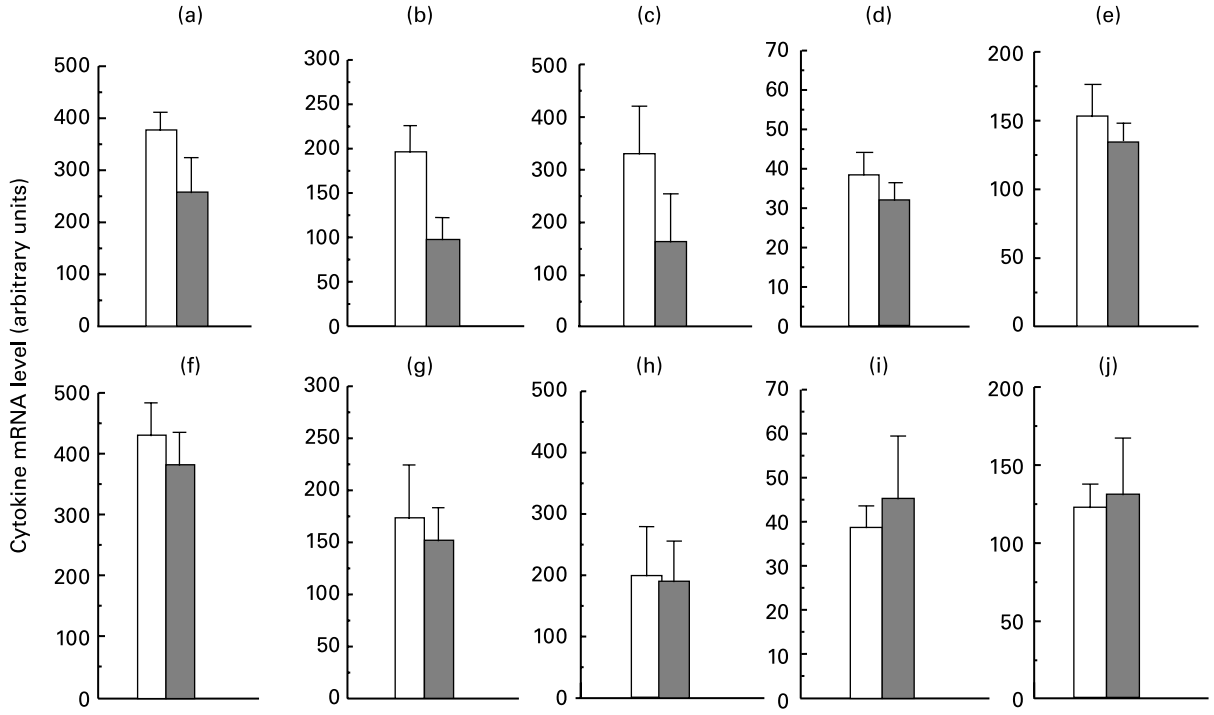

Fig. 3. Sex-related effect of fumonisin on T-helper (Th) 1 and Th2 cytokine mRNA expression. Male (a, b, c, d, e) or female (f, g, h, i, j) piglets were fed a control diet (□) or a fumonisin B_1-contaminated diet (■). Blood samples were taken on day 28 of treatment and cultured for 24 h with phytohaemagglutinin (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 ($P<0{\cdot}05$).

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, FB_1 is a potent inhibitor of ceramide synthase, an enzyme critical to sphingolipid biosynthesis. The sex-related effect of FB_1 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 FB_1 when compared with males (Bhandari *et al.* 2001). In pigs, however, after consumption of diet contaminated with 10 mg FB_1/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 Cζ selectively suppressed IL-10 production (Foey & Brennan, 2002). Thus, the higher inhibition of IL-10 expression observed in male pigs upon FB_1 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 FB_1 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 FB_1 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, FB_1 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 FB_1 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 that 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.

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