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Protective Role of Selenium in Immune-Relevant Cytokine and Immunoglobulin Production by Piglet Splenic Lymphocytes Exposed to Deoxynivalenol

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Abstract Deoxynivalenol (DON) is a mycotoxin that causes immunosuppression, especially in swine. Selenium (Se) is essential for proper functioning of the immune system in animals. However, little is known about the effects of DON and Se on cytokine or immunoglobulin production in piglets. Here, we addressed this gap by examining piglet splenic lymphocyte responses in vitro. Cells were stimulated with concanavalin A, a T cell stimulatory lectin, in the absence or presence of DON (0.1, 0.2, 0.4, and 0.8 μg/mL), Se (Na_2SeO_3, 2 μM), or combinations of Se 2 μM and DON 0.1–0.8 μg/mL for 12, 24, or 48 h. At each time point, supernatants and cells were collected and the expression of cytokine and immunoglobulin protein and mRNA was examined. Compared with control and Se-alone treatments, DON exposure significantly and dose dependently decreased the expression levels of IL-2, IL-4, IL-6, IL-10, IFN-γ, IgG, and IgM mRNA and protein. By contrast, co-treatment with DON + Se significantly increased the mRNA and protein levels of all factors examined, except IL-4 and IL-6, compared with DON treatment alone. The results of this investigation demonstrate that Se has the potential to counteract DON-induced immunosuppression in piglets and is a promising treatment for DON-mediated toxicity.

Keywords Deoxynivalenol · Selenium · Cytokine · Immunoglobulin · Splenic lymphocyte

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Abbreviations

DON	Deoxynivalenol
Se	Selenium
IL-2	Interleukin-2
IFN-γ	Interferon gamma
IgM	Immunoglobulin M
ELISA	Enzyme-linked immuneabsorbent assays
ConA	Concanavalin A

Introduction

Deoxynivalenol (DON) is one of the most common *Fusarium* mycotoxins and is found predominantly in grains. DON causes reproductive toxicity and is genotoxic, immunotoxic, and carcinogenic to varying extents in humans and livestock [1, 2]. In the past three decades, a number of studies have reported that almost all animal species investigated to date are susceptible to DON toxicity, and pigs are much more sensitive than poultry, mice, or ruminants [3, 4]. DON has damaging effects on various organs [5–7], especially those of the immune system [8, 9]. Moreover, lymphocytes are one of the main targets of DON [10, 11]. DON contamination most commonly occurs through oral consumption, and addition of DON to pig feed is an effective administration method for research. Nevertheless, in vitro systems have often been used to investigate the effects of low doses of toxins on cellular responses, and they are reliable alternatives to the use of animals in research.

Cytokines are regulatory proteins that play critical roles in modulating immune responses. Among their many functions, cytokines mediate intercellular communication and promote the activation, maturation, and differentiation of immune cells [12, 13]. There is substantial evidence that DON inhibits the secretion of interleukin 2 (IL-2), IL-4, IL-6, and IL-10 cytokines and of various immunoglobulins [14–16]. Therefore, in this study, we chose to examine the effects of DON on the production of selected cytokines and immunoglobulins by porcine spleen lymphocytes.

Selenium (Se) is a trace mineral element that is essential for the health of humans and other animals. Many studies have shown that Se promotes the immune function of cells [17–19], and Se supplementation has been shown to increase production of interferon-γ (IFN-γ), IL-2 [20–23], and immunoglobulins M and G (IgM and IgG) [24, 25] in vivo and in vitro. Se has also been shown to play a pivotal role in protecting T and B lymphocytes against the toxic effects of the *Fusarium* T-2 toxin [26, 27]. Moreover, Se improves cellular immune function in chickens exposed to aflatoxin B1 by increasing cytokine mRNA and protein levels [28–30]. Collectively, these data suggest that Se could serve as a potential therapy for mycotoxin-induced lesions in some animals.

To date, there have been few studies on the protective effects of Se against DON-induced damage in porcine spleen lymphocytes. Prompted by the previous findings, we sought to address this gap using specific enzyme-linked immunosorbent assays (ELISAs) and quantitative and real-time PCR to measure protein and mRNA expression levels of the cytokines IL-2, IL-4, IL-6, IL-10, and IFN-γ and of IgG and IgM by activated porcine spleen lymphocytes treated with DON in the presence or absence of Se.

Materials and Methods

Reagents

Fetal bovine serum was purchased from Gibco/Life Technologies (USA). Na_2SeO_3 powder was purchased from Xiya Reagent (China). DON and concanavalin A (ConA) were obtained from Sigma-Aldrich (USA). RPMI-1640 medium was obtained from Boster Biological Technology Co., Ltd. (China). Hank's solution and lymphocyte separation medium were obtained from Tianjin HaoYang Biological Institute (China). Porcine IL-2, IL-4, IL-6, IL-10, IFN-γ, IgM, and IgG ELISA kits were obtained from the Nanjing Jiancheng Bioengineering Institute (China). TRIzol reagent was purchased from Invitrogen Biotechnology Co., Ltd. (Shanghai, China). PrimeScript™ RT Reagent Kit and SYBR® Premix Ex Taq™ II were purchased from Takara (Shiga, Japan).

Preparation and Treatment of Porcine Splenic Lymphocytes

All study procedures were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University. Twelve 30–40-day-old healthy weaned piglets (Duroc × Large White × Landrace) were purchased from New Hope Group (China).

Piglets in good condition were anesthetized with an intramuscular injection of ketamine (20 mg/kg) [31] and tranquilizers (0.2 mg/kg). Approximately 5–15 min after injection, the piglet was positioned on its side to facilitate breathing and intravenous cannulation of an ear vein. After laparotomy, spleen samples were removed aseptically, soaked in 75% alcohol for 5 min, and washed three times with Hank's solution. The surrounding connective tissue and fat was removed, and the splenic tissue was cut into fragments and dispersed into a single-cell suspension using a 200-mesh gauze sieve. The cell suspension was gently added to a centrifuge tube containing an equal volume of lymphocyte separation medium. After centrifugation for 20 min at 400×*g*, the second layer of cells was collected and washed twice by addition of RPMI-1640

medium followed by centrifugation at 400×*g* for 5 min at room temperature. More than 95% of cells were viable based on trypan blue dye exclusion. Finally, the density of spleen lymphocytes was adjusted to 3 × 10^6 cells/mL [32] in RPMI-1640 medium containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin, and the cells were incubated in a humidified 5% CO_2 in air atmosphere.

The prepared spleen cells were cultured in triplicate in 12-well tissue culture plates at 3 × 10^6 cells/mL. All wells contained ConA at 5 μg/mL to induce T cell proliferation. Ten groups of cells received medium, DON, or Se in the following combinations: group C (control, cells with medium alone); Se (Na_2SeO_3 2 μM); D1, D2, D3, and D4 (DON at 0.1, 0.2, 0.4, or 0.8 μg/mL, respectively); and SD1, SD2, SD3, and SD4 (combinations of Na_2SeO_3 2 μM [33, 34] and DON 0.1, 0.2, 0.4, and 0.8 μg/mL, respectively). Previous studies had identified 0.82 ± 0.35 μg/mL DON as the 50% inhibitory concentration for [33]. Cells were incubated for 12, 24, or 48 h, and the supernatants and cells were collected for cytokine analysis and RNA isolation, respectively.

Detection of Cytokines and Immunoglobulins

The cell supernatants were analyzed by ELISA to quantify secreted IL-2, IL-4, IL-6, IL-10, IFN-γ, IgG, and IgM.

Quantitative RT-PCR

Total RNA was isolated from the harvested cells using TRIzol reagent according to the manufacturer's protocol. RNA concentration and quality were determined using NanoDrop 2000 and 1% agarose gel electrophoresis, respectively [35]. The A260/A280 was between 1.80 and 2.00, and three bands were visualized on the gels.

The RNA samples were reverse transcribed to cDNA using PrimeScript™ RT Reagent Kit with gDNA Eraser. The cDNA was then used as a template for quantitative PCR analysis. PCR reactions were performed in a total volume of 25 μL containing 12.5 μL SYBR Premix Ex Taq II, 1.0 μL of forward primer, 1.0 μL of reverse primer, 2 μL cDNA, and 8.5 μL RNase-free water. The reaction conditions were dwell temperature of 95 °C for 3 min followed by 40 cycles of 95 °C for 5 s, annealing for 30 s, and a final step for fluorescence measurement. A melting curve was then generated by increasing the temperature from 65 to 95 °C in increments of 0.5 °C. Fluorescence was recorded after each step. β-Actin was measured as an internal control for normalization [34]. Primer sequences were obtained from GenBank (National Center for Biotechnology Information). Primers (listed in Table 1) were designed with Primer 5 and were synthesized by BGI Tech (Shenzhen, China). Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta CT}$ method [36].

Statistical Analysis

All data were analyzed using SPSS Statistics version 20 (IBM, Armonk, NY, USA). Differences in cytokine and mRNA levels were analyzed using one-way ANOVA followed by Dunnett's post hoc test. All values are expressed as the mean ± standard deviation (SD). $P < 0.05$ was considered statistically significant.

Table 1 Real-time PCR primer sequences and polymerase chain reaction amplification efficiencies

Gene name	Serial number	Primer sequence (5′-3′)	Melting temperature (°C)	Amplification efficiency (%)	R^2
β-Actin	DQ845171.1	F:CTGCGGCATCCACGAAACT R:AGGGCCGTGATCTCCTTCTG	59.8	103.3	0.998
IL-2	NM_213861.1	F:TGCCCAAGCAGGCTACAGAA R:CTTGTTTCAGATCCCTTTAGTTCCA	57.4	99.8	0.997
IL-4	NM_214123.1	F:TCGGCACATCTACAGACACCAC R:TCTCCTTCATAATCGTCTTTAGCCT	58.7	95.4	0.994
IL-6	NM_00125249	F:GCTTCCAATCTGGGTTCAATCA R:CTTTGGTACTAATCTGCACGGCC	58.3	96.3	0.998
IL-10	NM_214041.1	F:TGCTCTATTGCCTGATCTTCCTG R:AGTCGCCCATCTGGTCCTTC	59.0	101.2	0.983
IFN-γ	NM_213948	F:AATGGTAGCTCTGGGAAACTGAA R:TCTGGCCTTGGAACATAGTCTGA	57.6	105.8	0.997
IgM	NM_213948	F: TTCGCTGGCATCTTCCTCAC R:TCTTCAGAACCTCGCCGTCC	58.5	100.4	0.997
IgG	NM_213828	F:CTGTACAGCAAGCTCGCGGT R:TGGACTTCTGGGTGTAGTGGTTGT	60.0	102.2	0.997

Table 2 The concentrations of IL-2 and IFN-γ in supernatants of porcine splenic lymphocytes

Groups	IL-2 level (ng/L)			IFN-γ level (ng/L)		
	12 h	24 h	48 h	12 h	24 h	48 h
C	158.95 ± 5.15[a]	137.28 ± 8.98[a]	138.44 ± 9.89[a]	29.42 ± 0.98[b]	31.29 ± 1.24[ab]	35.33 ± 1.40[b]
S	158.63 ± 10.54[a]	146.50 ± 1.59[b]	147.95 ± 3.68[a]	34.15 ± 0.46[a]	31.79 ± 2.91[a]	44.64 ± 1.99[a]
D1	130.78 ± 10.46[b]	136.60 ± 3.83[a]	104.54 ± 2.47[b]	19.53 ± 0.59[e]	27.58 ± 0.71[cd]	30.34 ± 0.48[d]
D2	56.68 ± 3.70[d]	72.82 ± 3.58[d]	92.39 ± 2.39[c]	19.33 ± 0.69[e]	25.06 ± 0.91[e]	27.60 ± 0.23[e]
D3	53.56 ± 6.41[d]	63.71 ± 2.67[d]	69.88 ± 10.90[d]	14.79 ± 0.11[f]	20.78 ± 1.04[f]	26.91 ± 1.95[ef]
D4	42.60 ± 2.68[e]	72.50 ± 2.97[d]	58.32 ± 6.59[e]	11.32 ± 0.56[h]	16.64 ± 0.18[g]	21.52 ± 0.79[g]
SD1	153.23 ± 10.54[a]	142.77 ± 4.60[b]	107.09 ± 3.87[b]	25.01 ± 0.17[c]	29.31 ± 1.26[bc]	32.97 ± 0.08[c]
SD2	99.00 ± 6.55[c]	86.34 ± 2.29[d]	77.39 ± 5.90[d]	22.86 ± 0.81[d]	29.02 ± 1.17[bc]	32.98 ± 1.46[c]
SD3	58.69 ± 1.99[d]	66.72 ± 4.24[e]	72.50 ± 10.20[d]	15.68 ± 0.35[f]	26.13 ± 0.97[de]	44.64 ± 1.99[a]
SD4	41.25 ± 2.57[e]	55.57 ± 3.26[f]	75.46 ± 7.69[d]	13.48 ± 0.77[g]	19.08 ± 0.34[f]	24.93 ± 0.25[f]

Data are presented with the means ± standard deviation ($n = 6$). In the same column, the different case letters (e.g., a and b) represent significant difference ($P < 0.05$) between groups

Results

Secretion of Cytokines

The concentrations of IL-2, IL-4, IL-6, IL-10, and IFN-γ in the culture supernatants are shown in Tables 2, 3, and 4. Se treatment alone had significant effect ($P < 0.05$) on cytokine production compared with the control group (ConA and medium). In contrast, DON at 0.1–0.8 μg/mL significantly reduced the secretion of IL-2, IL-4, IL-6, IL-10, and IFN-γ ($P < 0.05$) at all incubation times, except for IL-4 secretion at 12 h. The effect was also dose dependent, with higher concentrations of DON showing greater inhibitory effects. Cells incubated with DON + Se showed increased cytokine secretion compared with DON treatment at all time points examined, and the increases were significant for the lower two concentration groups (SD1 and SD2; $P < 0.05$). The kinetics of the protective effect of Se against DON-induced suppression differed for the various cytokines. Thus, Se had the greatest effects on IL-2 and IL-4 levels at 24 h incubation, on IL-6 at 12 h, and on IL-10 and IFN-γ at 48 h.

Secretion of IgM and IgG

IgG and IgM concentrations in the supernatants of ConA-stimulated cells incubated with and without DON and/or Se are shown in Table 5. DON addition at 0.2–0.8 μg/mL, but not at 0.1 μg/mL, caused a significant ($P < 0.05$) reduction in IgG and IgM secretion compared with the control or Se-alone groups. Moreover, the addition of Se to DON-exposed cells increased IgM and IgG production compared with the corresponding amount of DON alone. This increase was significant

Table 3 The concentrations of IL-4 and IL-6 in supernatants of porcine splenic lymphocytes

Groups	IL-4 level (ng/L)			IL-6 level (ng/L)		
	12 h	24 h	48 h	12 h	24 h	48 h
C	3.35 ± 0.18[dc]	6.86 ± 0.20[b]	11.58 ± 0.19[b]	38.05 ± 1.09[b]	41.41 ± 0.54[b]	44.37 ± 0.29[b]
S	3.95 ± 0.15[b]	7.36 ± 0.17[a]	12.45 ± 0.08[a]	40.88 ± 0.69[a]	46.00 ± 1.16[a]	51.27 ± 0.94[a]
D1	5.13 ± 0.13[a]	5.60 ± 0.35[dc]	8.76 ± 0.30[e]	20.59 ± 1.71[d]	24.26 ± 1.43[d]	28.78 ± 0.03[d]
D2	3.79 ± 0.17[b]	5.23 ± 0.32[de]	8.03 ± 0.29[f]	16.98 ± 0.23[e]	19.74 ± 0.27[f]	22.27 ± 2.26[e]
D3	3.09 ± 0.05[d]	4.32 ± 0.24[f]	6.83 ± 0.35[g]	12.49 ± 0.92[f]	15.44 ± 0.16[gh]	20.01 ± 0.31[f]
D4	2.78 ± 0.09[e]	3.85 ± 0.13[g]	6.25 ± 0.12[h]	9.15 ± 2.48[g]	13.77 ± 1.78[h]	15.42 ± 0.40[g]
SD1	5.40 ± 0.33[a]	6.68 ± 0.21[b]	10.19 ± 0.15[c]	30.70 ± 0.38[c]	32.26 ± 0.85[c]	33.09 ± 0.33[c]
SD2	3.83 ± 0.12[b]	5.75 ± 0.27[c]	9.33 ± 0.11[d]	19.23 ± 1.41[d]	22.12 ± 2.00[e]	29.78 ± 0.35[d]
SD3	3.41 ± 0.29[c]	5.01 ± 0.12[e]	7.77 ± 0.18[f]	16.17 ± 0.07[d]	17.13 ± 0.38[g]	21.03 ± 0.07[ef]
SD4	3.43 ± 0.12[c]	4.31 ± 0.16[f]	6.83 ± 0.17[g]	12.57 ± 0.40[f]	15.25 ± 0.25[gh]	17.01 ± 0.74[h]

Data are presented with the means ± standard deviation ($n = 6$). In the same column, the different case letters (e.g., a and b) represent significant difference ($P < 0.05$) between groups

Table 4 The concentrations of IL-10 in supernatants of porcine splenic lymphocytes

Groups	IL-10 level (ng/L)		
	12 h	24 h	48 h
C	78.07 ± 1.74^{b}	123.67 ± 2.89^{b}	134.51 ± 11.31^{b}
S	144.15 ± 8.54^{a}	150.57 ± 4.00^{a}	150.23 ± 4.45^{a}
D1	75.09 ± 0.92^{b}	70.10 ± 3.93^{e}	97.70 ± 5.59^{d}
D2	56.54 ± 0.96^{d}	48.68 ± 2.46^{fg}	81.48 ± 3.61^{e}
D3	38.65 ± 6.82^{e}	52.79 ± 5.14^{g}	59.15 ± 0.83^{f}
D4	21.00 ± 0.61^{f}	36.77 ± 0.46^{h}	45.59 ± 1.16^{g}
SD1	81.61 ± 3.37^{b}	103.30 ± 0.86^{c}	111.08 ± 3.74^{c}
SD2	65.59 ± 1.94^{c}	87.63 ± 4.50^{d}	92.14 ± 2.51^{d}
SD3	53.49 ± 8.84^{d}	63.76 ± 2.28^{ef}	76.58 ± 0.95^{e}
SD4	27.77 ± 3.32^{f}	39.30 ± 4.64^{h}	62.07 ± 1.21^{f}

Data are presented with the means ± standard deviation (n = 6). In the same column, the different case letters (e.g., a and b) represent significant difference ($P < 0.05$) between groups

($P < 0.05$) for low-concentration groups (SD1 and SD2). The protective effects of Se on IgM and IgG production were most significant after 12 and 24 h, respectively.

Cytokine mRNA Expression

As shown in Tables 6, 7, and 8, levels of IL-2, IL-4, IL-6, and IFN-γ mRNA were significantly decreased ($P < 0.05$) in the DON-treated groups compared with the control and Se-alone groups at 12, 24, and 48 h incubation times. Moreover, co-treatment with Se increased the mRNA expression levels compared with DON alone, especially in SD1 group.

Immunoglobulin mRNA Expression Levels

Table 9 shows that IgG and IgM mRNA levels were significantly decreased ($P < 0.05$) in the DON-treated group compared with the control and Se-alone groups at 12, 24, and 48 h. As also observed for the IgM and IgG protein expression, co-treatment with Se protected the cells from DON-mediated suppression. The maximal effects of Se were observed at 48 h for IgG mRNA and at 12 and 24 h for IgM mRNA.

Discussion

Several studies have documented the suppressive effects of DON on multiple immune cells [3, 37–39], especially splenic lymphocytes [40]. Many studies have sought to develop methods for the detoxification of DON in feed materials [41, 42]. However, relatively few investigations have focused on counteracting DON-inflicted damage by using nutrition to improve the animals' physiology. In this study, we examined the effects of Na_2SeO_3 on DON-induced impairment of immune-relevant cytokine production by porcine splenic lymphocytes. Our results demonstrate that mRNA and protein levels of IL-2, IL-4, IL-6, IL-10, IFN-γ, IgG, and IgM were decreased by DON exposure for 12, 24, and 48 h in a dose-dependent manner. Importantly, we also found that Se effectively alleviated the DON-mediated immunosuppression.

IL-2 and IFN-γ are mainly produced by Th1 cells and primarily promote cell-mediated immune responses [43, 44]. IL-2 is widely recognized to be a T cell growth factor and to promote a range of responses, including replication, maturation, and differentiation of lymphocytes [45–47]. In addition to promoting T and B cell proliferation, IL-2 increases

Table 5 The concentrations of IgG and IgM in supernatants of porcine splenic lymphocytes

Groups	IgG level(μg/mL)			IgM level (ng/L)		
	12 h	24 h	48 h	12 h	24 h	48 h
C	1.87 ± 0.01^{b}	2.52 ± 0.05^{b}	3.11 ± 0.07^{a}	358.87 ± 12.37^{b}	294.73 ± 7.08^{b}	257.53 ± 14.53^{b}
S	2.01 ± 0.02^{a}	2.78 ± 0.04^{a}	3.12 ± 0.32^{a}	384.94 ± 11.96^{a}	315.36 ± 3.91^{a}	287.35 ± 7.93^{a}
D1	1.79 ± 0.14^{b}	2.15 ± 0.11^{d}	2.35 ± 0.17^{c}	263.69 ± 5.41^{d}	242.52 ± 10.07^{d}	212.30 ± 2.51^{d}
D2	1.09 ± 0.09^{e}	1.56 ± 0.08^{f}	1.94 ± 0.04^{d}	178.39 ± 4.59^{f}	163.11 ± 5.95^{ef}	142.28 ± 1.15^{e}
D3	1.12 ± 0.03^{e}	1.3 ± 0.13^{h}	1.81 ± 0.12^{de}	152.47 ± 1.94^{g}	139.65 ± 8.71^{g}	99.08 ± 11.34^{g}
D4	0.85 ± 0.12^{f}	1.05 ± 0.04^{i}	1.65 ± 0.15^{e}	108.39 ± 12.93^{i}	86.69 ± 7.13^{h}	42.43 ± 7.42^{h}
SD1	1.78 ± 0.05^{b}	2.25 ± 0.06^{c}	2.69 ± 0.13^{b}	302.59 ± 7.79^{c}	261.05 ± 6.09^{c}	226.14 ± 1.46^{c}
SD2	1.56 ± 0.13^{c}	1.91 ± 0.13^{e}	2.24 ± 0.16^{c}	224.85 ± 1.28^{e}	174.84 ± 2.89^{e}	155.68 ± 7.86^{e}
SD3	1.28 ± 0.11^{d}	1.44 ± 0.14^{g}	1.85 ± 0.09^{de}	166.28 ± 4.77^{f}	156.25 ± 6.50^{f}	120.25 ± 9.75^{f}
SD4	0.5 ± 0.12^{g}	1.03 ± 0.12^{i}	1.66 ± 0.09^{de}	134.41 ± 1.68^{h}	98.54 ± 10.55^{h}	89.71 ± 2.38^{g}

Data are presented with the means ± standard deviation (n = 6). In the same column, the different case letters (e.g., a and b) represent significant difference ($P < 0.05$) between groups

Table 6 The IL-2 and IFN-γ mRNA levels in porcine splenic lymphocytes

Groups	IL-2 mRNA level			IFN-γ mRNA level		
	12 h	24 h	48 h	12 h	24 h	48 h
C	1.000 ± 0.051^{b}	1.000 ± 0.057^{b}	1.000 ± 0.055^{b}	1.000 ± 0.026^{b}	1.000 ± 0.083^{c}	1.000 ± 0.021^{b}
S	1.210 ± 0.088^{a}	1.134 ± 0.131^{a}	1.210 ± 0.120^{a}	1.225 ± 0.077^{a}	1.466 ± 0.117^{a}	1.544 ± 0.143^{a}
D1	0.464 ± 0.047^{d}	0.512 ± 0.072^{d}	0.363 ± 0.011^{de}	0.760 ± 0.066^{c}	0.805 ± 0.136^{de}	0.884 ± 0.110^{bc}
D2	0.183 ± 0.051^{ef}	0.455 ± 0.039^{d}	0.564 ± 0.100^{c}	0.745 ± 0.108^{c}	0.647 ± 0.090^{ef}	0.871 ± 0.221^{bc}
D3	0.130 ± 0.019^{ef}	0.298 ± 0.082^{e}	0.494 ± 0.043^{d}	0.376 ± 0.023^{e}	0.305 ± 0.071^{e}	0.730 ± 0.041^{c}
D4	0.112 ± 0.024^{f}	0.183 ± 0.034^{e}	0.252 ± 0.012^{de}	0.172 ± 0.017^{f}	0.183 ± 0.026^{g}	0.534 ± 0.073^{cd}
SD1	0.575 ± 0.072^{c}	0.693 ± 0.065^{c}	0.731 ± 0.102^{c}	0.804 ± 0.037^{c}	1.188 ± 0.098^{b}	1.471 ± 0.032^{a}
SD2	0.224 ± 0.045^{e}	0.492 ± 0.030^{d}	0.732 ± 0.181^{c}	0.795 ± 0.067^{c}	0.844 ± 0.033^{cd}	0.892 ± 0.151^{bc}
SD3	0.202 ± 0.044^{ef}	0.489 ± 0.053^{d}	0.684 ± 0.162^{c}	0.571 ± 0.009^{d}	0.531 ± 0.193^{f}	0.814 ± 0.034^{bc}
SD4	0.173 ± 0.032^{ef}	0.264 ± 0.032^{e}	0.425 ± 0.044^{de}	0.325 ± 0.045^{e}	0.233 ± 0.100^{g}	0.570 ± 0.111^{c}

Data are presented with the means ± standard deviation ($n = 6$). In the same column, the different case letters (e.g., a and b) represent significant difference ($P < 0.05$) between groups

cytokine production. IFN-γ prevents the development of Th2 cells by inhibiting the production of IL-4, which is required for Th2 cell differentiation [48–50]. IL-2 and IFN-γ protein and mRNA production were decreased in cells treated with DON alone compared with the control group, which is similar to previous findings in mice [51, 52]. However, we also showed that addition of Se to DON-treated groups reduced the effects of DON on IFN-γ and IL-2; the latter effect is consistent with a previous study by Yang et al. [29]. There are two potential explanations for the alleviation of DON-induced inhibition by Se. First, Se may alleviate the immune suppression toxicity of DON by increasing the activities of antioxidant enzymes [53], especially glutathione peroxidase. Second, Se may have further activated ConA-stimulated splenocytes [34] by enhancing the secretion of IL-2 and IFN-γ by DON-treated cells.

IL-4, IL-6, and IL-10 are produced by Th2 cells and are involved in antibody-mediated immunity. Each of these cytokines can promote B lymphocyte proliferation and immunoglobulin secretion [54]. In this study, we found that DON treatment dose dependently suppressed the expression of IL-4, IL-6, and IL-10 mRNA and protein compared with the control group, which is in line with the conclusions of several earlier studies [44, 55, 56]. However, other studies have reported that DON promotes the expression of IL-4, IL-6, and IL-10 mRNA and protein [57, 58]. The discrepancy between those studies and ours may be at least partly due to the different experimental conditions used, such as different doses and duration of DON treatment or different cell models. Interestingly, our experimental results also revealed that although Se improved cellular immune function, different

Table 7 The IL-4 and IL-6 mRNA levels in porcine splenic lymphocytes

Groups	IL-4 mRNA level			IL-6 mRNA level		
	12 h	24 h	48 h	12 h	24 h	48 h
C	1.000 ± 0.080^{b}	1.000 ± 0.013^{b}	1.000 ± 0.041^{b}	1.000 ± 0.080^{a}	1.000 ± 0.013^{a}	1.000 ± 0.041^{a}
S	1.127 ± 0.100^{a}	1.147 ± 0.106^{a}	1.148 ± 0.030^{a}	1.003 ± 0.084^{a}	1.039 ± 0.067^{a}	0.974 ± 0.032^{a}
D1	0.246 ± 0.010^{d}	0.347 ± 0.011^{cd}	0.350 ± 0.048^{cd}	0.296 ± 0.049^{c}	0.509 ± 0.020^{c}	0.572 ± 0.040^{c}
D2	0.229 ± 0.029^{de}	0.201 ± 0.005^{e}	0.214 ± 0.004^{e}	0.203 ± 0.053^{de}	0.493 ± 0.102^{c}	0.394 ± 0.052^{cd}
D3	0.160 ± 0.008^{ef}	0.177 ± 0.033^{e}	0.180 ± 0.010^{e}	0.160 ± 0.020^{e}	0.203 ± 0.028^{d}	0.302 ± 0.031^{cd}
D4	0.140 ± 0.024^{f}	0.149 ± 0.030^{e}	0.151 ± 0.038^{e}	0.155 ± 0.019^{e}	0.178 ± 0.013^{d}	0.193 ± 0.063^{d}
SD1	0.411 ± 0.041^{c}	0.437 ± 0.080^{c}	0.445 ± 0.023^{c}	0.427 ± 0.032^{b}	0.606 ± 0.029^{b}	0.801 ± 0.091^{b}
SD2	0.254 ± 0.017^{d}	0.257 ± 0.072^{de}	0.269 ± 0.041^{de}	0.255 ± 0.032^{cd}	0.542 ± 0.091^{c}	0.594 ± 0.130^{c}
SD3	0.236 ± 0.013^{de}	0.210 ± 0.030^{e}	0.239 ± 0.029^{e}	0.241 ± 0.057^{cde}	0.468 ± 0.066^{c}	0.572 ± 0.062^{c}
SD4	0.154 ± 0.013^{ef}	0.175 ± 0.023^{e}	0.182 ± 0.051^{e}	0.174 ± 0.021^{de}	0.199 ± 0.017^{d}	0.344 ± 0.060^{cd}

Data are presented with the means ± standard deviation ($n = 6$). In the same column, the different case letters (e.g., a and b) represent significant difference ($P < 0.05$) between groups

Table 8 The IL-10 mRNA level in porcine splenic lymphocytes

Groups	IL-10 mRNA level		
	12 h	24 h	48 h
C	1.000 ± 0.05^{b}	1.000 ± 0.087^{b}	1.000 ± 0.087^{d}
S	1.614 ± 0.169^{a}	1.871 ± 0.061^{a}	1.927 ± 0.054^{a}
D1	0.249 ± 0.021^{f}	0.401 ± 0.102^{e}	0.733 ± 0.080^{f}
D2	0.379 ± 0.013^{e}	0.423 ± 0.094^{e}	0.817 ± 0.209^{e}
D3	0.407 ± 0.022^{e}	0.526 ± 0.016^{d}	1.410 ± 0.081^{c}
D4	0.741 ± 0.070^{d}	0.745 ± 0.023^{c}	1.493 ± 0.229^{c}
SD1	0.359 ± 0.064^{e}	0.423 ± 0.055^{e}	0.775 ± 0.172^{ef}
SD2	0.418 ± 0.062^{d}	0.439 ± 0.047^{e}	0.875 ± 0.032^{e}
SD3	0.797 ± 0.243^{c}	0.702 ± 0.079^{c}	1.714 ± 0.375^{b}
SD4	0.826 ± 0.110^{c}	0.920 ± 0.459^{b}	1.740 ± 0.250^{b}

Data are presented with the means ± standard deviation (n = 6). In the same column, the different case letters (e.g., a and b) represent significant difference (P < 0.05) between groups

effects were observed on distinct cytokines. Se has been reported to effectively reduce oxidative damage, cell apoptosis, and immune damage caused by aflatoxin B1 [30, 59, 60]; however, we are the first to report the ability of Se to counter DON-induced immunosuppression. Oxidative stress resulting from elevated levels of reactive oxygen species can damage proteins and nucleic acids [61]. We speculate that Se may act by inhibiting reactive oxygen species production and mitochondrial dysfunction caused by DON [62, 63], thereby improving cell viability and function [64, 65] and antagonizing the inhibitory effects of DON on cytokine secretion. It has also been suggested that Se may affect mRNA expression, since selenoprotein transcripts are affected via nonsense-mediated mRNA decay mechanism [66].

IgG and IgM are secreted by B lymphocytes and are critical mediators of humoral immunity [67, 68]. We observed that IgG and IgM protein and mRNA production by porcine lymphocytes were significantly and dose dependently inhibited by DON, which is consistent with a report that IgA, IgM, and IgG secretion by cultured murine lymphocytes is impaired by DON [69, 70]. In our experiments, IgG and IgM production was higher in the DON + Se-treated group than the DON-treated group. Since T cells provide help for B cell antibody synthesis, and Se upregulates T cell responses, including IL-2, IL-4, and Il-6 production [71], this may explain the stimulatory effects of Se on antibody production. Therefore, we speculate that Se alleviates the toxic effects of DON on IgG and IgM secretion mainly by improving cytokine secretion by T cells.

In summary, our results indicate that DON inhibits the expression of cytokine and immunoglobulin mRNA and protein by porcine splenic lymphocytes, but the DON effects are attenuated by co-treatment with Se, especially at low doses. These data shed light on the immunotoxic effects of DON, and potentially other trichothecene mycotoxins, and show that Se protects against DON toxicity. However, further in-depth studies will be necessary to understand the underlying molecular mechanisms. In addition, our results suggest that improved nutrition may be a novel approach to mitigating mycotoxin contamination in animal production.

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Table 9 The IgG and IgM mRNA levels in porcine splenic lymphocytes

Groups	IgG mRNA level			IgM mRNA level		
	12 h	24 h	48 h	12 h	24 h	48 h
C	1.000 ± 0.087^{b}	1.000 ± 0.122^{b}	1.000 ± 0.021^{b}	1.000 ± 0.039^{b}	1.004 ± 0.015^{b}	1.000 ± 0.010^{b}
S	1.161 ± 0.139^{a}	1.330 ± 0.328^{a}	1.533 ± 0.091^{a}	1.596 ± 0.091^{a}	1.329 ± 0.031^{a}	1.214 ± 0.221^{a}
D1	0.565 ± 0.055^{d}	0.707 ± 0.180^{d}	0.594 ± 0.071^{f}	0.869 ± 0.170^{c}	0.820 ± 0.073^{c}	0.692 ± 0.111^{b}
D2	0.400 ± 0.061^{e}	0.521 ± 0.094^{e}	0.513 ± 0.032^{g}	0.757 ± 0.029^{d}	0.682 ± 0.007^{d}	0.585 ± 0.022^{d}
D3	0.307 ± 0.011^{e}	0.491 ± 0.050^{e}	0.504 ± 0.034^{g}	0.661 ± 0.046^{e}	0.573 ± 0.060^{e}	0.531 ± 0.040^{d}
D4	0.283 ± 0.071^{e}	0.452 ± 0.076^{f}	0.461 ± 0.024^{h}	0.565 ± 0.055^{d}	0.407 ± 0.180^{e}	0.594 ± 0.071^{f}
SD1	0.708 ± 0.069^{c}	0.848 ± 0.028^{c}	0.971 ± 0.033^{c}	0.988 ± 0.140^{b}	0.972 ± 0.036^{b}	1.034 ± 0.101^{b}
SD2	0.406 ± 0.065^{e}	0.573 ± 0.041^{e}	0.794 ± 0.102^{d}	0.869 ± 0.146^{c}	0.842 ± 0.008^{c}	0.660 ± 0.052^{c}
SD3	0.339 ± 0.055^{e}	0.539 ± 0.092^{e}	0.734 ± 0.130^{e}	0.611 ± 0.188^{e}	0.583 ± 0.035^{e}	0.554 ± 0.031^{d}
SD4	0.288 ± 0.014^{e}	0.467 ± 0.011^{f}	0.601 ± 0.023^{f}	0.573 ± 0.070^{ef}	0.479 ± 0.058^{f}	0.510 ± 0.024^{d}

Data are presented with the means ± standard deviation (n = 6). In the same column, the different case letters (e.g., a and b) represent significant difference (P < 0.05) between groups

Compliance with Ethical Standards The authors confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with submission to *FEBS Letters*.

Conflict of Interest The authors declare that they no conflict of interest.

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