The administration of diets contaminated with low to intermediate doses of deoxynivalenol and supplemented with antioxidants and binding agents slightly affects the growth, antioxidant status and vaccine response in weanling pigs [1]

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ABSTRACT

This study aimed to evaluate the impact of grading levels of deoxynivalenol (DON) in the diet of weaned pigs, as well as the effects of a supplementation with antioxidants (AOX), hydrated sodium calcium aluminosilicates (HSCAS) and their combination on the growth, antioxidant status, immune and vaccine response against the porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus 2 (PCV2). At weaning, 336 piglets were allocated to six dietary treatments according to a randomized complete block design. Treatments were as follows: basal diet (CTRL); basal diet containing DON at 1.2 mg/kg (DON1.2); basal diet containing DON at 2.4 mg/kg (DON2.4); DON2.4 diet + a mix of AOX which included vitamins A and E at 20,000 IU and 200 IU/kg feed respectively, selenized yeast at 0.3 mg/kg and a grape seed extracts at 100 mg/kg feed (DON2.4+AOX); DON2.4 diet + modified HSCAS at 1 g/kg (DON2.4+HSCAS); DON2.4+AOX+HSCAS. Pigs were vaccinated against PRRSV and PCV2 at 7 d; at 0, 14 and 35 d growth performance were recorded, and blood samples were collected in order to evaluate the oxidative status, inflammatory blood markers, lymphocyte blastogenic response and vaccine antibody response. Increasing intake of DON resulted in a quadratic effect at 35 d in the lymphocyte proliferative response to Concanavalin A and PCV2 as well as in the anti-PRRSV antibody response, whereas the catalase activity decreased in DON2.4 pigs compared to the CTRL and DON1.2 groups ($P \leq 0.05$). Compared to the DON2.4 diet, the AOX supplementation slightly reduced G:F ratio ($P = 0.026$) and increased the ferric reducing ability of plasma as well as α-tocopherol concentration ($P < 0.05$), whereas the association AOX+HSCAS increased the anti-PRRSV IgG ($P < 0.05$). Furthermore, the HSCAS supplement reduced haptoglobin

levels in serum at 14 d compared to the DON2.4 group; however, its concentration decreased in all the experimental treatments from 14 d to 35 d and particularly in the DON2.4+AOX pigs, whereas a different trend was evidenced in the DON2.4+HSCAS group, where over the same period haptoglobin concentration increased ($P < 0.05$). Overall, our results show that the addition of AOX and HSCAS in the diet may alleviate the negative effects due to DON contamination on the antioxidant status and immune response of vaccinated weanling pigs.

Keywords: antioxidants, binding agents, deoxynivalenol, weanling pig, vaccine response

List of abbreviations

Item	Term
ADFI	average daily feed intake
ADG	average daily gain
AOX	antioxidants
BrdU	5-bromo-2'-deoxy-uridine
CAT	catalase
CCAC	Canadian Council on Animal Care
ConA	concanavalin A
CTRL	control
DON	deoxynivalenol
FRAP	ferric reducing ability of plasma
G:F	gain to feed
GPx	glutathione peroxidase
HSCAS	hydrated sodium calcium aluminosilicates
IL	interleukin
L	linear
MDA	malondialdehyde
PBMC	peripheral blood mononuclear cells
PCV2	porcine circovirus type 2
Q	quadratic
PRRSV	porcine reproductive and respiratory syndrome virus
ROS	reactive oxygen species
SI	stimulation index
SOD	superoxide dismutase

INTRODUCTION

Deoxynivalenol (DON) is a mycotoxin mainly produced by *Fusarium* molds and belonging to the group of trichothecene toxins (Liao et al., 2018). It is considered as one of the most frequent mycotoxins in grains and grain by-products and a worldwide contaminant of food-crop (Kovalsky et al., 2016; Gruber-Dorninger et al., 2019; Khodaei et al., 2021). Among all the animal species evaluated to date, pigs are the more susceptible to DON, followed by mice, rats, poultry and finally ruminants (Pestka, 2007). Such distinct sensitivity among species has been ascribed to differences in DON metabolism, absorption, distribution, and elimination: pigs, and in particular piglets, are poorly tolerant to DON contamination, whereas ruminants are extremely resistant because of the major role played by the rumen microflora in DON detoxification (Pestka, 2007). For this reason, the Canadian Food Inspection Agency, United States Food and Drug Administration and the European Union limited its dietary inclusion in swine feed to under 1 mg/kg, 1 mg/kg, and 0.9 mg/kg of feed, respectively (EU-EFSA, 2006; US-FDA, 2010; Charmley and Trenholme, 2017).

Previous studies performed with pigs have already shown that DON may affect growth performance, decreasing average daily gain (ADG) and feed intake (Alizadeh et al., 2015; Wu et al., 2015; Reddy et al., 2018). However, such effect on the pig growth is controversial, and no effect has often been recorded after supplementation of low levels of DON (Accensi et al., 2006; Wellington et al., 2020; Wellington et al., 2021). Furthermore, DON exposure may also induce intestinal alterations by impairing intestinal barrier function and reshaping gut microbial structure (Alizadeh et al., 2015; Lessard et al., 2015; Liu et al., 2020). In addition, as reported in a review paper and in more recent research articles, DON is able to induce production of reactive oxygen species (ROS) in several cell types and to alter the antioxidant defense system, thus leading to DNA fragmentation, increased lipid peroxidation and protein damage (Mishra et al., 2014; Singh et al., 2015; Ren et al., 2020). Moreover,

DON may interfere with the innate and adaptive immune response and have either an immunostimulatory or immunosuppressing effect, depending on the dose, frequency and duration of exposure (Pierron et al., 2016): high doses promote leukocyte apoptosis and immunosuppression, whereas low concentrations may upregulate the expression of inflammation-related genes (Pestka, 2007; Pierron et al., 2016). Such effect of DON on the innate and adaptive immune system have been shown to have an impact on the immune response to vaccines and potentially affect the vaccination efficiency against porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) (Savard et al., 2014; Savard et al., 2015a; Savard et al., 2015b).

As summarised by many review papers, PRRSV and PCV2 are endemic in the majority of pig producing countries, with severe outbreaks that appear periodically worldwide and compromise global swine industry affecting mortality, reproduction and growth (Meng, 2012; Franzoni et al., 2019). Using published research between 2006–2016 as an indicator of research priorities, a recent study revealed that PRRSV and PCV2 are among the top researched viruses worldwide (VanderWaal and Deen, 2018). As a matter of fact, PRRSV infection has become one of the most important infectious diseases of pigs in terms of detrimental effect on productivity and economic impact for the producers, and in 2005 its cost in the only United States was estimated at over $560 million US dollars per year (Neumann et al., 2005; Holtkamp et al., 2013). Similarly, PCV2 has been associated with a number of disease syndromes and is now recognized as ‘one of the most important pathogens of the pig population worldwide’ (Karuppannan and Opriessnig, 2017), whose cost in England, prior to the introduction of PCV2 vaccines, has been estimated at £52.6 million per year (Alarcon et al., 2013). Both PCV2 and PRRSV target host immune cells, thus weakening host defenses and increasing the susceptibility to infections by primary and secondary pathogens (Eclercy et al., 2020). The control of these two viruses is based on management

strategies, control of co-infections, and mainly on vaccination (Martelli et al., 2013). For this reason, and as one of the consequences of the ingestion of mycotoxin-contaminated feed is a decreased vaccine efficacy (Pierron et al., 2016), minimizing the presence of DON in feed ingredients has become an important topic (Peng et al., 2018).

Different nutritional strategies have been proposed in order to compensate the adverse impacts of mycotoxin-contaminated feed. Hydrated sodium calcium aluminosilicates (HSCAS) and their modified forms are used as binding agents able to block the absorption of different mycotoxins in the gastrointestinal tract (Peng et al., 2018). Their utilisation in weaning pig diets has been reported to decrease DON toxicity, mitigate the intestinal flora disorder and reduce the adverse effects on the growth performance (Liu et al., 2020; Zhang et al., 2020). Alternatively, the combined addition of different antioxidants (AOX) such as vitamins A, C and E, organic Se + glutathione, and plant extracts like milk thistle, rosemary, licorice, and boldo has been shown to reduce the pro-oxidative effect of DON-contaminated diets in pigs at weaning (Van Le Thanh et al., 2016; Holanda and Kim, 2020). However, there is no clear evidence that the acquired immune response, and particularly the vaccine response of pigs against PRRSV and PCV2, would benefit from the addition of such supplements in a DON-contaminated diet. Therefore, this study aims to determine the effects of different dietary levels of DON on the growth performance, oxidative status and vaccine response against PRRSV and PCV2 in weanling pigs. As the impact of high levels of DON on pigs is well documented, whereas effects of low levels of DON are little-known, we decided to use low to intermediate doses of DON in the present trial. Furthermore, the potential of an antioxidant-enriched dietary supplement and a HSCAS additive to reduce DON toxicity, alone or in combination, has been evaluated.

MATERIALS AND METHODS

Animals, housing and dietary treatments

All animal procedures were conducted according to the guidelines set by the CCAC (2009), and the experimental protocol was approved from the Laval University Animal Use and Care Committee. For this study, a total of 336 castrated male weanling pigs (5.64 ± 0.596 kg) were obtained from a commercial farm (Coop Seigneurie, St-Anselme, QC, Canada) and transferred to the Center for Animal Science Research (Deschambault, Quebec, Canada). Pigs were weighed and distributed in pens (0.30 m^2/pig) per group of seven according to their weanling weight, in order to form 8 blocks of 6 pens for a total of 48 pens. Within each block, the pens were randomly distributed to the 6 dietary treatments described below. Throughout the experiment, the photoperiod was fixed to 12 h light, while the temperature was adapted to the age of the pigs and gradually lowered from 29°C to 21°C. Furthermore, animals originated from a herd tested negative for PRRSV and PCV2.

For all the 336 pigs, a 6-day acclimation period was performed. During this period, animals were fed *ad libitum* with a commercial feed (Coop Fédérée, St-Hyacinthe, QC, Canada). Subsequently, animals received a phase 2 diet (days 0-14 of the trial) and then switched to a phase 3 diet (days 15-35 of the trial) (Table 1). All the diets were manufactured by Coop Fédérée; feed was provided *ad libitum* and formulated to meet the nutrient requirements for weaned pigs as recommended by the National Research Council (2012). After the acclimation period, each pen was randomly assigned to one of the following dietary treatments: 1) basal weanling diet (CTRL); 2) basal weanling diet containing DON at 1.2 mg/kg feed (DON1.2); 3) basal weanling diet containing DON at 2.4 mg/kg feed (DON2.4); 4) basal weanling diet containing DON at 2.4 g/kg feed + a mix of dietary supplements with antioxidant properties which included vitamins A and E (Dyets Inc., Bethlehem, PA, USA) at

20,000 IU and 200 IU/kg feed respectively, selenized yeast (Lallemand Animal Nutrition, Milwaukee, WI, USA) at 0.3 mg/kg in replacement of Na-selenite, and a grape seed extracts rich in polyphenols (Win'Ox, Phodé Laboratories, Terssac, France) at 100 mg/kg feed (DON2.4+AOX); 5) basal weanling diet containing DON at 2.4 g/kg feed + a supplement of modified HSCAS (Special Nutrients, Miami, FL, USA) at 1g/kg feed (DON2.4+HSCAS); 6) basal weanling diet containing DON at 2.4 g/kg feed + the two previous dietary supplements (DON2.4+AOX+HSCAS). The modified HSCAS and grape seed extract were added at level inclusion recommended by manufacturers. This design was repeated for each group of 42 pigs, according to a randomized complete block design. The DON1.2 and DON2.4 dietary treatments were respectively prepared by partially or totally replacing the uncontaminated wheat of the basal diet with DON naturally contaminated wheat. Samples from the wheat batches and the experimental diets were analysed for the mycotoxin content by Liquid Chromatography with tandem Mass Spectrometry (Actlabs, Ancaster, ON, Canada).

The experiment started immediately after the 6-day acclimation period (day 0) and lasted for 35 days. At day 7, animals were vaccinated with a commercial modified-live vaccine against PRRSV (Ingelvac PRRS MLV, Boehringer Ingelheim, Ingelheim, Germany) and a commercial inactivated vaccine against PCV2-Mycoplasma (Fostera PCV MH, Zoetis, Florham Park, NJ USA).

Sampling

Pig body weight was recorded at the beginning of the trial (day 0), and then at days 14 and 35; ADG was subsequently calculated. Feed consumption in the first (0-14 d) and second (15-35 d) phases of the trial was also measured in order to determine average daily feed intake (ADFI) and gain to feed (G:F) ratio. Two pigs within each pen were chosen at the beginning of the trial to evaluate DON and dietary treatment effects on the oxidative status, inflammatory blood markers, lymphocyte blastogenic response and vaccine antibody response. At 0, 14 and 35 d, blood samples were drawn from the jugular of the selected pigs by venipuncture using 10-mL Vacutainer tubes spray-coated with heparin and serum tubes (Becton Dickinson, Mississauga, ON, Canada). After sample centrifugation (1800 × g for 15 min at 4°C), plasma and serum were collected and stored at -20°C until further analysis. At day 35, an additional blood sample was collected in 10-mL Vacutainer tubes spray-coated with heparin from one of the two selected pigs within each pen, in order to evaluate lymphocyte proliferative response to concanavalin A (ConA), PRRSV and PCV2.

Antioxidant status

Antioxidant status was assessed on the plasma samples collected from the two selected pigs within each pen on days 14 and 35. Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) plasma concentration as described by Jain et al. (1989). The results are expressed as micromoles per liter. The systemic antioxidant status was determined by the ferric reducing ability of plasma (FRAP) as proposed by Benzie and Strain (1996). Absorbance readings were then taken at 593 nm every 15 s during a 2-min monitoring period.

The results are expressed as micromoles per liter. The plasma activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were assessed with commercial kits (catalog numbers 706002, 703102 and 707002, respectively; Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions.

Furthermore, the blood samples collected from two pigs within each pen on the day 35 of the trial were also used to determine the plasma concentrations of total retinol (vitamin A), α-tocopherol (vitamin E) and DON. Vitamins A and E were analyzed by HPLC after saponification and extraction into hexane, as previously described (Jensen et al., 1999; Van Le Thanh et al., 2016). DON concentrations in the serum were also assessed by HPLC according to a method proposed by Valenta and Dänicke (2005).

Inflammatory blood markers and vaccine antibody response

Blood samples collected at 14 and 35 days were also used to determine the serum concentrations of haptoglobin, interleukin (IL) 8 and IL10. IL8 and IL10 were determined by ELISA with commercial kits according to the manufacturer's instructions (catalog numbers P8000 and P1000, respectively; R&D Systems, Minneapolis, MN, USA). The concentration of haptoglobin was also determined using a commercial kit (catalog number EPH2003-1, Assay Pro, St. Charles, MO, USA).

In addition, production of PCV2- and PRRSV- specific antibodies was also assessed on the blood samples collected at 0, 14 and 35 d. For PCV2, an immunofluorescence assay was performed according to the method proposed by Racine et al. (2004). The maximum level of dilution where serum sample gave a specific immunofluorescence was used to quantify the level of antibodies against PCV2. Serum sample was considered positive for PCV2 at 1/64. The dilution values were then logarithmically (Log10) transformed prior to statistical

analysis. The production of specific antibodies against PRRSV was evaluated by ELISA using the IDEXX PRRS X3 Ab Test commercial kit (IDEXX, Markham, ON, Canada), according to the manufacturer's instructions. A sample-to-positive ratio equal or greater than 0.4 was considered positive.

Lymphocyte proliferation

The additional whole blood sample collected at day 35 from one pig within each pen was used to evaluate the proliferative response of stimulated lymphocytes. First, peripheral blood mononuclear cells (PBMC) were isolated as previously described (Lo Verso et al., 2020). Subsequently, PBMC were resuspended in Roswell Park Memorial Institute 1640 (Wisent Bioproduct, St-Bruno, QC, Canada) supplemented with 10% fetal bovine serum (Wisent Bioproduct) and 1% of an antibiotic solution containing 5000 units/mL of penicillin and 5000 µg/mL of streptomycin (Wisent Bioproduct). PBMC were then plated in a 96-well cell culture plate (VWR international, Mississauga, ON, Canada) at a concentration of 2.5×10^6 cells/well and stimulated or not with 5 µg/mL ConA (Sigma-Aldrich, Oakville, ON, Canada), PRRSV and PCV2 (MOI 2 = 4×10^5 viruses). For each condition of each sample, cells were plated in triplicates and incubated for 48h (ConA stimulation) or 72h (virus stimulation) at 37°C in a 5% CO_2 humidified incubator. In order to evaluate cell proliferation, the Cell Proliferation ELISA kit (Roche Life Science, Laval, QC, Canada) was used, and 5-Bromo-2'-deoxy-uridine (BrdU) was added to the cells 16 h before the end of the incubation period. The absorbance reading was performed at 450 nm. The stimulation index (SI) was then calculated as follows: OD stimulated cells / OD unstimulated cells.

Statistical analysis

Data were analyzed according to a randomized complete block design with the pen of pigs as the experimental unit. The statistical model included the dietary treatment and block effects. When measurements were repeated over time, the factor day and its interaction with dietary treatments were added in the model and dietary treatments were compared after Tukey's adjustment. The value at day 0 was also added as covariable for statical analysis of SOD, CAT, MDA, FRAP, IL8, IL10, and haptoglobin. Results were analyzed using the MIXED procedure of SAS (Statistical Analysis System, Release 9.4, 2002–2012, SAS Institute Inc., Cary, NC, USA). *A priori* contrasts were designed to specifically examine the principal comparisons of the study: CTRL, DON1.2 and DON2.4: linear (L) and quadratic (Q) effects of DON; DON2.4 versus DON2.4+AOX: effect of the antioxidants; DON2.4 versus DON2.4+HSCAS: effect of the HSCAS; DON2.4 versus DON2.4+AOX+HSCAS: combined effect of the two additives.

RESULTS

Mycotoxin concentrations in the diet

DON concentrations in the uncontaminated and contaminated wheat batches were 1 and 8 mg/kg, respectively. Samples of the diets CTRL, DON 1.2, DON 2.4, DON2.4+AOX, DON2.4+HSCAS and DON2.4+AOX+HSCAS from phase 2 showed DON concentrations of 0.71, 1.16, 2.50, 2.15, 2.25 and 2.15 mg/kg, respectively (Table 1). Similar values were also recorded in the phase 3 diets of the same experimental groups (Table 1).

The concentrations of other mycotoxins, such as fumonisin, ochratoxin A, aflatoxin, T-2/HT-2 toxins and zearalenone were also measured, and for each of them values were under the detection limit (< 1 µg/kg for aflatoxins B1, B2, G1 and G2; < 0.1 mg/kg for fumonisins B1 and B2; < 0.003 mg/kg for ochratoxin A; < 0.06 mg/kg for T-2 and HT-2 toxins; < 0.09 mg/kg for zearalenone).

Growth performance

The growth performance of pigs receiving different experimental diets are shown in Table 2. There was no significant effect of DON contamination on pig weight at 14 d; however, after 35 days of feeding, results indicated a tendency for a linear decrease in pig body weight that was associated with increasing level of DON in the feed (P = 0.096). Conversely, no other significant effect due to DON contamination was noticed on the ADG, ADFI or G:F at any stage of rearing (P > 0.10).

Concerning the effects of the dietary treatments, the G:F ratio between days 0-14 of the trial was significantly reduced in pigs fed the DON2.4+AOX diet compared to the DON2.4 group (P = 0.026). Furthermore, DON2.4+HSCAS diet tended to reduce the ADFI during the last 3

weeks (14-35 d) compared to the DON2.4 group (P = 0.075). A tendency for an increased body weight at day 35 was also evidenced in pigs receiving the DON2.4+AOX+HSCAS diet when compared to the DON2.4 group (P = 0.075). No other dietary effect was observed on pig growth performance.

Antioxidant status and plasma concentrations of DON, retinol and α-tocopherol

The effects of different dietary supplements and levels of DON contamination on pig antioxidant status on days 14 and 35 are shown in Table 3. First, results revealed an important time effect for all the tested parameters (P < 0.05), due to a significant increase of the plasma activity of SOD, GPx and FRAP, as well as to a reduction of MDA concentration from day 14 to day 35. Furthermore, a significant day × DON interaction was also recorded for the CAT concentration (P = 0.020) indicating a marked reduction of the CAT activity on day 35 in pigs receiving the DON2.4 diets compared to the CTRL and DON1.2 groups (P < 0.05).

Concerning the effects of the mycotoxin contamination, pigs fed with grading levels of DON in the diet showed on one hand a tendency for a linear reduction of FRAP and GPx plasma values, and on the other hand a tendency for a linear increase in the total retinol concentration on day 35 (P < 0.1, Table 3). However, the supplementation of the DON2.4 diet with AOX as well as with the mix of AOX and HSCAS was associated with an increase of the FRAP activity (P = 0.044 and P = 0.067, respectively), as well as an increase in plasma α-tocopherol concentration when compared to the DON2.4 group (P = 0.032 and P = 0.040, respectively).

Finally, results revealed that serum DON concentration increased linearly with increasing levels of DON in the feed (P = 0.045, Table 3). However, when DON2.4 diet was

supplemented with AOX+HSCAS, DON increase in pigs' serum was limited compared to the DON2.4 group ($P = 0.064$).

Systemic immune functions and vaccine response

Serum concentrations of IL8, IL10 and haptoglobin are shown in Table 4. The results showed that the IL8 and IL10 concentrations significantly decreased from day 14 to day 35, regardless of the dietary treatment or DON contamination in the diet (day effect: $P < 0.05$). Furthermore, serum haptoglobin showed a significant day × dietary treatment interaction ($P = 0.010$), indicating that its concentration was higher in the DON2.4 group compared to pigs receiving the DON2.4+HSCAS diet at 14 d; however, its values massively decreased in all the experimental treatments from 14 d to 35 d and particularly in the DON2.4+AOX group, where it reduced to a quarter of its former level. Conversely, a different trend was evidenced in the DON2.4+HSCAS group, where over the same period haptoglobin concentration increased. For this reason, at 35 d its values were shown to be greater in the latter group when compared to DON2.4+AOX pigs ($P < 0.05$). No other effect due to DON contamination or dietary supplements was recorded.

The impact of the different DON levels and dietary supplementations on the lymphocyte proliferation are shown in Table 4. Feeding pigs with diets containing graded levels of DON resulted in a quadratic response in the proliferation of ConA- and PCV2- stimulated PBMC, with a peak at 1.2 and 2.4 mg/kg of DON, respectively ($P = 0.005$ and $P = 0.052$, respectively). However, no other significant effect due to DON contamination or dietary supplements was evidenced.

Serum concentrations of specific antibodies against PRRSV and PCV2 are also shown in Table 4. As antibody response to both vaccines was under the detection limit at 0 and 14 d,

only the results at 35 d (28 days after vaccination) are further described and discussed. These results showed that the antibody response against PRRSV vaccine was modulated in a quadratic manner by the DON levels in the diet, indicating a higher IgG concentration in pigs fed the DON1.2 diet (quadratic effect: P = 0.017). Moreover, the addition of the AOX+HSCAS supplements in the diet significantly increased the antibody concentration against PRRSV when compared to the DON2.4 group (P = 0.031). Concerning the response against PCV2, anti-PCV2 IgG were detected only in 50 to 65% of the samples for each experimental group, and no significant effect due to DON contamination or dietary supplements was evidenced.

DISCUSSION

DON (or vomitoxin) is the most common occurring trichothecene which can be found in wheat, barley, and corn (Liao et al., 2018; Khodaei et al., 2021). Its presence in the feedstuffs has been shown to cause a range of effects starting from feed refusal to immunosuppression and productivity loss of the livestock (CAST, 2003). Swine is particularly sensitive to DON, and its exposure to feed naturally contaminated with this mycotoxin may have various impacts on health and production (Alizadeh et al., 2015; Lessard et al., 2015; Liao et al., 2018). Therefore, it is important to find alternatives to mitigate these adverse effects. In the present research, the aim was not only to evaluate the impacts low to intermediate doses of DON in the diet, but also the potential of antioxidant additives and modified hydrated sodium calcium aluminosilicates, alone or in combination, to reduce DON negative effects on the growth, antioxidant status and immunity in newly weaned pigs vaccinated against PRRSV and PCV2.

Effects of DON on pig growth, antioxidant status and immunity

Deoxynivalenol is among the mycotoxins with the greater impact on the pig growth, and it may cause a reduction in feed consumption and in the efficiency of protein deposition (Andretta et al., 2012; Knutsen et al., 2017). However, in our experimental conditions only limited effects due to DON contamination were observed on the growth performance, similarly to previous studies where DON was administered at doses ranging from 2.2 up to 3 mg/kg feed (Pinton et al., 2008; Grenier et al., 2011). This is not surprising, as the impact of DON on the growth has not been always reported: some authors show an effect on the weight gain, even if transient, with dietary concentrations of DON at 1–2 mg/kg feed whereas in other studies no effect is observed for higher doses (Waché et al., 2009; Grenier et al., 2011). In addition, pigs seem to adapt to DON intake, and even when a severe depression in the growth performance is reported at the beginning after exposure to DON, this is usually transient and animals can recover rapidly (Serviento et al., 2018; Wellington et al., 2020). Furthermore, it is important to point out that in the present trial DON level in the CTRL diet was 0.7 mg/kg feed, meaning that the differences in the supplementation levels compared to the CTRL group were <1 and <2 mg/kg feed for DON1.2 and DON 2.4 dietary treatments, respectively. Such relatively low doses of mycotoxin supplementation may be responsible for the lack of effects recorded on pig growth, as reported in other studies where DON was administered at 1 mg/kg feed or lower (Accensi et al., 2006; Wellington et al., 2020; Wellington et al., 2021). Furthermore, along with the dose there are also other factors influencing the severity of these effects; the DON-dependent reduction in the feed intake and growth rate may vary according to sex, age, nutritional and health status, duration of exposure and interaction with other mycotoxins (Andretta et al., 2012; Knutsen et al., 2017). Moreover, even changes in the physical activity and feed behavior after DON ingestion may alter the response of the growth performance (Serviento et al., 2018; Shen et al., 2021).

Therefore, it is possible that the dose used as well as a combination of the other mentioned factors may have caused the lack of effects on the growth performance recorded in our study.

Concerning the effects on the oxidative stress, in the present study the administration of DON naturally contaminated diets affected GPx and FRAP plasma values, as well as CAT activity. Similarly, previous studies have already reported an impairment of the antioxidant defenses after exposure to DON (Mishra et al., 2014). As a matter of fact, trichothecenes are able to induce cell damages via the release of ROS (Wu et al., 2014) or by direct action (Frankič et al., 2008), which may explain the lack of effect on the MDA concentration recorded in our study.

As expected, the ingestion of graded levels of DON also linearly increased DON blood concentrations at 35 d, as previously reported (Van Le Thanh et al., 2015). More surprisingly, a tendency for a linear increase of retinol blood concentrations was also observed in pigs fed the DON naturally contaminated diets. It is not clear how DON may increase retinol concentrations in plasma; however, other Authors have shown that low doses of mycotoxins, even when applied for a short period, may affect the liver (Skiepko et al., 2020). As most of the body's vitamin A is stored in the liver (Mora et al., 2008), it is possible that DON contamination has altered its functionality, thus limiting vitamin A storage and leading to an increase in blood retinol. However, further research is required in order to confirm these results.

In the present study, DON ingestion also partially affected both general and specific, as well as humoral and cell-mediated immune responses, as shown by the proliferative response of ConA- and PCV2- stimulated PBMC, as well as the antibody response against PRRSV. Such impact of DON on different aspects of the immune response may be an issue, considering that immunity against PRRSV and PCV2 requires the activation of all the different branches of the immune system. In fact, after infection with PRRSV and PCV2, not only the sole

induction of a humoral response, but also the cell-mediated immunity and innate immunity are believed to play an important role (Martelli et al., 2011; Sinkora et al., 2014; Zhou et al., 2021).

As already mentioned, DON is known to strongly affect the immune system, which can either be stimulated or suppressed (Liao et al., 2018): low level DON exposure may lead to immune stimulation and inflammation, whereas high doses may cause immunosuppression (Pestka, 2007). As a matter of fact, once inside the cell DON binds to active ribosomes and, besides causing translational inhibition, it can also rapidly trigger some mitogen-activated protein kinases (MAPKs) in a process known as "ribotoxic stress response" (Pestka, 2007). The consequent signaling cascades may then promote the expression of immune and pro-inflammatory factors, when the mycotoxin doses are closed to those found naturally; alternatively, at the highest concentrations it may lead to the rapid onset of leukocyte apoptosis and immunosuppression (Pestka, 2007; Payros et al., 2016). In addition, a previous murine study showed that the different cells of the immune system may be distinctively affected by DON, and divergent effects on antigen presenting cells, T and B cells have been reported (Islam et al., 2013).

Such DON-induced regulation of the immune and inflammatory responses may also affect the immunoglobulin production (Pestka, 2007). However, once again DON effects on the humoral response seem to be controversial, and both increased and reduced antibody pruduction have been reported after DON ingestion (Goyarts et al., 2006; Pinton et al., 2008; Grenier et al., 2011; Lessard et al., 2015; Zhang et al., 2020). In the present study, the results showed that the level of anti-PRRSV IgG was higher in the DON1.2, but not in the DON2.4, group when compared to the CTRL pigs. This finding is quite interesting, as there is still scarce research on the effects of DON on the specific humoral response to vaccines. Previous studies have shown a DON negative effect on PRRSV-specific humoral responses in pigs

receiving diets contaminated with DON at concentrations of at least 2.5 mg/kg feed (Savard et al., 2014; Savard et al., 2015a). However, *in vitro* results have also shown that the overexpression of proinflammatory cytokines following the exposition to low concentrations of DON (140 ng/mL) could potentially amplify PCV2 replication and the production of PCV2-specific antibodies (Savard et al., 2015b). However, in our study no effect of DON on the anti-PCV2 antibody response was evidenced. Deoxynivalenol seems to selectively compromise pig immune response, suggesting that the effects of DON on acquired immunity after vaccination may be dependent on the characteristics of the injected vaccines (Yunus et al., 2012; Zhang et al., 2020).

As previously stated, the effects of DON on the immune response seem to be dose-dependent. Indeed, results revealed a DON quadratic effect on the lymphocyte proliferation, particularly after ConA stimulation. *In vitro* results have already provided evidence that low concentrations of mycotoxins (0.1–1 μg/mL) may enhance cellular proliferation of human and porcine PBMC, while higher concentrations caused a decrease in a dose-dependent manner (Taranu et al., 2010). Furthermore, also the time of exposure and the technique of analysis have to be considered (Goyarts et al., 2006; Pinton et al., 2008; Lessard et al., 2015). Further *in vivo* studies are still needed in order to fill critical data gaps regarding the dose-dependent effects of DON on the immune system and health status.

Effects of supplementing DON-contaminated diets with AOX and HSCAS on pig growth, antioxidant status and immunity

Different post-harvest measures have been developed to protect the livestock from the harmful effects of mycotoxins (Peng et al., 2018). One such measure is using feed additives like binding agents able to reduce the absorption of mycotoxins by promoting their excretion in the feces (Jans et al., 2014). In particular, adsorbents like HSCAS are designed to behave like a "chemical sponge", thereby preventing the blood and target organ absorption and later distribution of mycotoxins as well as the carryover of these fungal metabolites into animal products (Jans et al., 2014; Čolović et al., 2019). Despite this, as resumed by many review papers, aluminosilicates are not considered good binders for all mycotoxins and, while possessing a special chemisorption for aflatoxins, they don't efficiently adsorb low polar and hydrophobic mycotoxins, such as trichothecenes (Li et al., 2018; Čolović et al., 2019). This selective activity, however, may be overcome by chemical modifications such as changes in the surface characteristics, that may enhance the hydrophobicity of these adsorbents (Li et al., 2018; Čolović et al., 2019), thus enabling them to bind other mycotoxins and be effective in mitigate their toxicity *in vivo* (Wei et al., 2019; Zhang et al., 2020; Holanda and Kim, 2021). Furthermore, in order to counteract the adverse effects of mycotoxins other feed additives may be used, including components able to promote gut health, stimulate the immune system, and provide sources of functional and conditionally essential nutrients, thus improving the detoxification ability (Holanda and Kim, 2020). Among them, antioxidant agents as vitamins, quercetin, selenium, glucomannans, nucleotides, antimicrobial peptides, bacteria, polyunsaturated fatty acids, oligosaccharides, and plant extracts have gained increasing

interest, because of their potential to inhibit trichothecene-induced oxidative stress (Wu et al., 2017).

First, an interesting finding from the present study is the trend for a reduced DON concentration in the blood only when AOX and HSCAS were administered together, showing a possible synergistic action of these two additives. As a matter of fact, even if they are not strictly considered as binding agents, many plant extracts and polyphenols are known to improve the detoxification abilities and reduce mycotoxin intestinal absorption in humans and broilers (Abd El-Hack et al., 2018). Even if such results show just a tendency, it is possible that the plant extract contained in the AOX supplement has helped enhancing the sequestering ability of the modified HSCAS. Further studies are required in order to better understand this mechanism of action. Furthermore, in the present research the supplementation of DON-contaminated diets with AOX, alone or in combination with HSCAS, partially affected pig antioxidant status, as evidenced by the increases in the FRAP activity and blood α-tocopherol concentration. This is not surprising, as the beneficial effects of AOX on the antioxidant defenses are well known (Lauridsen, 2010; Van Le Thanh et al., 2016; Rey et al., 2020).

In addition, supplementing the diet of weanling pigs with AOX, HSCAS or both partially affected the systemic immune response. First, our results showed that the dietary treatments differently affected the haptoglobin concentration. Haptoglobin, as a member of the acute phase proteins, is released by the liver as part of the body's innate immune response and stimulates phagocytosis, thus facilitating the elimination of pathogens and toxic factors (Petersen et al., 2002; Piñeiro et al., 2009; Zielonka et al., 2010). Its production may increase after DON ingestion and has even been proposed as a diagnostic biomarker for DON intoxication (Kim et al., 2008). In our study, the HSCAS supplementation was able to partially mitigate the inflammatory stimuli caused by DON ingestion and the consequent

activation of the systemic immune response, as shown by the marked reduction of the haptoglobin concentration at 14 d when compared to the DON2.4 group. However, at 35 d the haptoglobin concentration in the DON2.4+HSCAS group slightly increased and turned out to be higher when compared to DON2.4+AOX pigs, whereas a reduction was evidenced for all the other dietary treatments. The reason for such difference is not clear. However, antioxidants such as vitamins and plant extracts are also known to reduce haptoglobin concentration in pigs challenged with different pathogens (Liu et al., 2013a; Liu et al., 2013b; Kim et al., 2016). Further studies are needed to elucidate the neutralizing effects of AOX and HSCAS on the DON-induced activation of the innate immune response. Moreover, our results also evidenced that the PRRSV-specific antibody response was modulated by the AOX+HSCAS supplementation. Studies reporting the potential of such dietary treatments to neutralize the impact of DON on the acquired immune response are rare. However, Zhang et al. (2020) showed that dietary supplementation of 0.05% modified HSCAS successfully alleviated the DON-induced reduction of anti-PCV2 antibody titers. This suggests that mycotoxin detoxifiers could overcome deoxynivalenol immunosuppressor effect; however, further studies are needed in order to confirm the potential of these feed additives in counteracting DON-induced effects on pig immune response to vaccines.

Finally, in our experimental conditions the supplementation of DON naturally contaminated diets with AOX and HSCAS did not have important repercussions on pig growth performance. Similarly, previous studies failed to show a significant effect of HSCAS or AOX on pig growth (Döll et al., 2005; Van Le Thanh et al., 2016). However, it is important to point out that almost no effect of the DON-contaminated diets on pig growth was evidenced in our study, thus causing the lack of important counteracting effects of the HSCAS and AOX dietary supplementations.

CONCLUSIONS

Overall, the results obtained in the present study provide evidence that DON ingestion at 1.2-2.4 mg/kg feed did not exert a significant impact on pig growth but was still able to alter the plasma antioxidant status and to affect the systemic immune response, particularly when administered at the lowest concentration. Furthermore, the addition of AOX+HSCAS in pig diets naturally contaminated with the highest level of DON could partially overcome DON toxicity, boosting the vaccine response against PRRSV and reducing DON plasma concentration. The scarcity of effects on the growth performance may be probably ascribed to the DON doses that were used. Nevertheless, our study showed the possible impact of DON ingestion on the immune response mounted during a vaccination protocol, as well as the potential of the tested feed additives to, at least partially, counteract such effects. As the vaccination remains a fundamental instrument in the control of many diseases, such as the ones PRRSV and PCV2 are associated to, our results highlight the importance of a better understanding of how DON affects the immune system: the current state of research would benefit from further investigations to determine precisely at what concentration DON starts impairing the immune response, as well as what nutritional strategy may fully overcome its toxic effects.

CONFLICTS OF INTEREST

None of the authors of this manuscript has a financial or personal relationship with individuals or organizations that could exercise an inappropriate influence or bias on the contents of this manuscript. We have full control of all primary data and we agree to allow the journal to review our data if requested.

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Table 1. Composition of the experimental diets[1] for the phases 2 and 3 (as-fed basis)

Item	Phase 2						Phase 3					
	CTRL	DON1.2	DON2.4				CTRL	DON1.2	DON2.4			
			---	AOX	HSCAS	AOX+HSCAS			---	AOX	HSCAS	AOX+HSCAS
Ingredients, %:												
Corn	31.9	31.9	31.9	31.8	31.8	31.7	34.0	34.0	34.0	34.0	34.0	34.0
Soybean meal	15.4	15.4	15.4	15.4	15.4	15.4	27.5	27.5	27.5	27.5	27.5	27.5
Soy protein (HP 300)	8.4	8.4	8.4	8.4	8.4	8.4	---	---	---	---	---	---
Control wheat[2]	33.3	16.7	---	---	---	---	33.3	16.7	---	---	---	---
Contaminated wheat[3]	---	16.7	33.3	33.3	33.3	33.3	---	16.7	33.3	33.30	33.3	33.3
Whey	5.1	5.1	5.1	5.1	5.1	5.1	---	---	---	---	---	---
L-lysine HCl	0.50	0.50	0.50	0.50	0.50	0.50	0.45	0.45	0.45	0.45	0.45	0.45
DL-methionine	0.16	0.16	0.16	0.16	0.16	0.16	0.14	0.14	0.14	0.14	0.14	0.14
L-threonine	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.16	0.16
Dicalcium phosphate	1.41	1.41	1.41	1.41	1.41	1.41	1.35	1.35	1.35	1.35	1.35	1.35
Limestone	1.25	1.25	1.25	1.25	1.25	1.25	0.90	0.90	0.90	0.90	0.90	0.90
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
White fat	1.60	1.60	1.60	1.60	1.60	1.60	1.35	1.35	1.35	1.35	1.35	1.35
Vitamin premix[4]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[5]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
HSCAS	---	---	---	---	0.10	0.10	---	---	---	---	0.10	0.10
Antioxidants[6]	---	---	---	0.10	---	0.10	---	---	---	0.10	---	0.10
Calculated chemical												

composition, %:												
Dry matter	88.1	88.1	88.1	88.1	88.1	88.1	87.4	87.4	87.4	87.4	87.4	87.4
Net energy, MJ/kg	10.3	10.3	10.3	10.3	10.3	10.3	10.0	10.0	10.0	10.0	10.0	10.0
Crude protein	19.0	19.0	19.0	19.0	19.0	19.0	19.4	19.4	19.4	19.4	19.4	19.4
Fat	3.9	3.9	3.9	3.9	3.9	3.9	3.6	3.6	3.6	3.6	3.6	3.6
Ca	0.91	0.91	0.91	0.91	0.91	0.91	0.75	0.75	0.75	0.75	0.75	0.75
P	0.63	0.63	0.63	0.63	0.63	0.63	0.60	0.60	0.60	0.60	0.60	0.60
Standardized ileal digestible, %:												
Lys	1.17	1.17	1.17	1.17	1.17	1.17	1.19	1.19	1.19	1.19	1.19	1.19
Thr	0.74	0.74	0.74	0.74	0.74	0.74	0.75	0.75	0.75	0.75	0.75	0.75
Met	0.42	0.42	0.42	0.42	0.42	0.42	0.41	0.41	0.41	0.41	0.41	0.41
Met/Cys	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Trp	0.18	0.18	0.18	0.18	0.18	0.18	0.20	0.20	0.20	0.20	0.20	0.20
Analyzed chemical composition :												
Vitamin A, IU/kg	6,909	7,260	6,141	29,550	8,007	36,480	5,582	4,559	7,217	28,730	12,344	26,500
Vitamin E, IU/kg	65	43	49	241	67	319	52	46	43	235	55	304
Se, mg/kg	0.70	0.75	0.78	0.70	0.70	0.75	0.75	0.78	0.80	0.78	0.75	0.80
Crude protein, %	20.6	21.2	20.1	19.8	19.4	20.1	18.2	17.5	18.1	18.1	17.8	17.5
P, %	0.71	0.77	0.72	0.78	0.74	0.71	0.70	0.65	0.66	0.65	0.68	0.68
Ca, %	1.07	1.06	0.99	0.99	1.03	1.08	0.87	0.89	0.89	0.90	0.90	0.89
DON, mg/kg	0.71	1.16	2.50	2.15	2.25	2.15	0.70	1.20	2.50	2.15	2.32	2.17

[1] CTRL: basal weanling diet; DON1.2: basal weanling diet containing DON at 1.2 mg/kg feed; DON2.4: basal weanling diet containing DON at 2.4 mg/kg feed; DON2.4+AOX: basal weanling diet containing DON at 2.4 g/kg feed + vitamins A and E (20,000 IU and 200 IU/kg feed, respectively), selenized yeast (0.3 mg/kg of Se) in replacement of Na-selenite, and a grape seed extract (100

mg/kg feed); DON2.4+HSCAS: basal weanling diet containing DON at 2.4 g/kg feed + HSCAS (1g/kg feed); DON2.4+AOX+HSCAS: basal weanling diet containing DON at 2.4 g/kg feed + the two previous dietary supplements.

[2] Control wheat: wheat containing no more than 1 mg / kg of DON.

[3] Contaminated wheat: wheat containing DON at 8 mg / kg.

[4] Supplied per kg of diet: vitamin A: 2,250 IU; vitamin D_3: 250 IU; vitamin E: 16 IU; vitamin K as menadione: 3.25 mg; thiamine: 1.25 mg; riboflavin: 4 mg; niacin: 30 mg; pantothenic acid: 25 mg; pyridoxine: 8.75 mg; biotin: 0.1 mg; choline: 625 mg; vitamin B_{12}: 25 µg.

[5] Provided per kg of diet: Zn (as zinc carbonate): 100 mg; Fe (as iron citrate): 100 mg; Cu (as copper carbonate): 6 mg; I (as potassium iodate): 0.15 mg; Mn (as manganese carbonate): 4 mg; Se (as sodium selenite or selenized yeast): 0.30 mg.

[6] Mix of dietary supplements containing vitamins A and E (20,000 IU and 200 IU/kg feed, respectively), selenized yeast (0.3 mg/kg of Se) in replacement of Na-selenite, and a grape seed extract (100 mg/kg feed).

Table 2. Effects of the administration of DON naturally contaminated diets and dietary supplements on the growth performance of weanling pigs

Growth Performance	Dietary treatments[1]						SEM	Contrasts (*P* value)[2]				
	CTRL	DON1.2	DON2.4					DON		Supplement[3]		
			---	AOX	HSCAS	AOX+ HSCAS		L	Q	AOX	HSCAS	AOX +HSCAS
Weight, kg:												
0 d	7.3	7.4	7.3	7.4	7.6	7.5	0.4	ns[4]	ns	ns	ns	ns
14 d	13.5	13.6	13.5	13.6	13.8	13.7	0.6	ns	ns	ns	ns	ns
35 d	27.9	27.5	27.4	27.2	27.5	28.0	0.5	0.096	ns	ns	ns	0.075
ADG, g/d:												
0-14 d	441	446	436	439	448	444	20	ns	ns	ns	ns	ns
14-35 d	721	698	698	682	694	714	22	ns	ns	ns	ns	ns
0-35 d	606	594	589	582	587	603	11	ns	ns	ns	ns	ns
ADFI, g/d:												
0-14 d	569	554	548	577	569	566	24	ns	ns	ns	ns	ns
14-35 d	1241	1208	1223	1203	1175	1214	27	ns	ns	ns	0.075	ns
0-35 d	963	938	945	945	926	945	24	ns	ns	ns	ns	ns
G:F ratio:												
0-14 d	0.78	0.80	0.80	0.76	0.79	0.79	0.01	ns	ns	0.026	ns	ns
14-35 d	0.58	0.58	0.57	0.57	0.58	0.59	0.03	ns	ns	ns	ns	ns
0-35 d	0.63	0.64	0.63	0.62	0.64	0.64	0.02	ns	ns	ns	ns	ns

[1] CTRL: basal weanling diet; DON1.2: basal weanling diet containing DON at 1.2 mg/kg feed; DON2.4: basal weanling diet containing DON at 2.4 mg/kg feed; DON2.4+AOX: basal weanling diet containing DON at 2.4 g/kg feed + vitamins A and E (20,000 IU and 200 IU/kg feed, respectively), selenized yeast (0.3 mg/kg of Se) in replacement of Na-selenite, and a grape seed extract (100

mg/kg feed); DON2.4+HSCAS: basal weanling diet containing DON at 2.4 g/kg feed + HSCAS (1g/kg feed); DON2.4+AOX+HSCAS: basal weanling diet containing DON at 2.4 g/kg feed + the two previous dietary supplements.

[2] Results of the *a priori* contrasts designed to examine the principal comparisons of the study: linear (L) and quadratic (Q) effects of DON concentration; effect of the antioxidant supplementation (AOX), the HSCAS supplementation (HSCAS) and combined effect of the two additives (AOX+HSCAS) in a DON-contaminated diet.

[3] AOX: AOX vs. DON2.4 group; HSCAS: HSCAS vs. DON2.4 group; AOX+HSCAS: AOX+HSCAS vs. DON2.4 group.

[4] ns: not significant.

Table 3. Effects of the administration of DON naturally contaminated diets and dietary supplements on the antioxidant status and blood concentrations of DON and vitamins A and E of weanling pigs

Item[1]	Day	Dietary treatments[2]						SEM	*P* value[3]						
		CTRL	DON1.2	DON2.4					Day	Treatment × Day	DON		Supplement[4]		
				--	AOX	HSCAS	AOX+HSCAS				L	Q	AOX	HSCAS	AOX+HSCAS
SOD, U/mL	14	2.48	3.05	3.11	2.51	3.18	3.69	0.29	0.015	ns[5]	ns	ns	ns	ns	ns
	35	2.78	3.39	3.98	3.02	3.49	3.75								
CAT, nmol/min/mL	14	2.50	1.36	3.98	3.76	3.49	3.83	1.59	0.001	0.020	ns	ns	ns	ns	ns
	35	9.96[a]	11.15[a]	5.55[b]	2.37[b]	3.10[b]	3.73[b]								
GPx, nmol/min/mL	14	955	956	794	797	811	856	60	0.001	ns	0.074	ns	ns	ns	ns
	35	1127	1118	1130	1008	955	1171								
MDA, μmol/L	14	1.39	1.48	1.26	1.19	1.46	1.24	0.11	0.001	ns	ns	ns	ns	ns	ns
	35	0.54	0.59	0.55	0.49	0.46	0.60								
FRAP, μmol/L	14	102	113	93	115	111	105	10	0.023	ns	0.073	ns	0.044	ns	0.067
	35	138	128	117	127	111	131								
Retinol, μg/mL	35	0.62	0.70	0.76	0.74	0.68	0.71	0.06	--	--	0.076	ns	ns	ns	ns
α-Tocopherol, μg/mL	35	5.17	6.14	5.19	6.66	5.80	6.65	0.85	--	--	ns	ns	0.032	ns	0.040
DON, ng/mL	35	1.84	8.75	9.80	7.20	8.80	5.10	1.82	--	--	0.045	ns	ns	ns	0.064

[a-b] Within a row, means without a common superscript differ (P<0.05). Dietary treatments were compared after Tukey's adjustment.

[1] SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; MDA: malondialdehyde; FRAP: ferric reducing ability of plasma.

[2] CTRL: basal weanling diet; DON1.2: basal weanling diet containing DON at 1.2 mg/kg feed; DON2.4: basal weanling diet containing DON at 2.4 mg/kg feed; DON2.4+AOX: basal weanling diet containing DON at 2.4 g/kg feed + vitamins A and E (20,000 IU and 200 IU/kg feed, respectively), selenized yeast (0.3 mg/kg of Se) in replacement of Na-selenite, and a grape seed extract (100 mg/kg feed); DON2.4+HSCAS: basal weanling diet containing DON at 2.4 g/kg feed + HSCAS (1g/kg feed); DON2.4+AOX+HSCAS: basal weanling diet containing DON at 2.4 g/kg feed + the two previous dietary supplements.

[3] Values for the DON and supplement effects are the results of *a priori* contrasts: linear (L) and quadratic (Q) effects of DON concentration; effect of the antioxidant supplementation (AOX), the HSCAS supplementation (HSCAS) and combined effect of the two additives (AOX+HSCAS) in a DON-contaminated diet.

[4] AOX: AOX vs. DON2.4 group; HSCAS: HSCAS vs. DON2.4 group; AOX+HSCAS: AOX+HSCAS vs. DON2.4 group.

[5] ns: not significant.

Table 4. Effects of the administration of DON naturally contaminated diets and dietary supplements on the systemic immune functions and vaccine response of weanling pigs

Item	Day	Dietary treatments[1]						SEM	*P* value[2]						
		CTRL	DON1.2	DON2.4					Day	Treatment × Day	DON		Supplement[3]		
				--	AOX	HSCAS	AOX+HSCAS				L	Q	AOX	HSCAS	AOX+HSCAS
IL8, ng/mL	14	426	510	529	507	303	454	64	0.004	ns[4]	ns	ns	ns	ns	ns
	35	204	208	200	242	242	234								
IL10, ng/mL	14	5.35	6.77	9.26	4.95	10.84	7.98	1.54	0.013	ns	ns	ns	ns	ns	ns
	35	1.84	3.97	1.63	4.04	8.97	3.42								
Haptoglobin, μg/L	14	274[ab]	202[ab]	304[a]	253[ab]	109[b]	281[ab]	50	0.005	0.010	ns	ns	ns	ns	ns
	35	139[ab]	168[ab]	147[ab]	60[b]	183[a]	135[ab]								
Proliferation index[5]:															
ConA	35	33	77	40	51	51	60	13	--	--	ns	0.005	ns	ns	ns
PRRSV	35	1.87	2.41	1.85	1.62	1.12	3.20	0.60	--	--	ns	ns	ns	ns	ns
PCV2	35	1.14	3.11	3.59	2.04	2.34	2.24	1.15	--	--	0.081	0.052	ns	ns	ns
Antibody response[6]:															
PRRSV	35	1.86	2.16	1.67	1.90	1.67	2.08	0.13	--	--	ns	0.017	ns	ns	0.031
PCV2	35	1.31	1.24	2.06	1.43	1.35	2.52	0.49	--	--	ns	ns	ns	ns	ns

[a-b] Within a row, means without a common superscript differ (P<0.05).

[1] CTRL: basal weanling diet; DON1.2: basal weanling diet containing DON at 1.2 mg/kg feed; DON2.4: basal weanling diet containing DON at 2.4 mg/kg feed; DON2.4+AOX: basal weanling diet containing DON at 2.4 g/kg feed + vitamins A and E (20,000 IU and 200 IU/kg feed, respectively), selenized yeast (0.3 mg/kg of Se) in replacement of Na-selenite, and a grape seed extract (100 mg/kg feed); DON2.4+HSCAS: basal weanling diet containing DON at 2.4 g/kg feed + HSCAS (1g/kg feed); DON2.4+AOX+HSCAS: basal weanling diet containing DON at 2.4 g/kg feed + the two previous dietary supplements.

[2] Values for the DON and supplement effects are the results of *a priori* contrasts: linear (L) and quadratic (Q) effects of DON concentration; effect of the antioxidant supplementation (AOX), the HSCAS supplementation (HSCAS) and combined effect of the two additives (AOX+HSCAS) in a DON-contaminated diet.

[3] AOX: AOX vs. DON2.4 group; HSCAS: HSCAS vs. DON2.4 group; AOX+HSCAS: AOX+HSCAS vs. DON2.4 group.

[4] ns: not significant.

[5] Proliferation index: OD stimulated cells / OD unstimulated cells; ConA: concanavalin A.

[6] Serum IgG against PRRSV and PCV2 were not detected at 0 and 14 d; values relative to PRRSV are expressed as sample-to-positive ratio (a sample-to-positive ratio equal or greater than 0.4 was considered positive); values relative to PCV2 are expressed as log of highest dilution where the serum sample gave a specific immunofluorescence.