



# The Ability of an Algoclay-Based Mycotoxin Decontaminant to Decrease the Serum Levels of Zearalenone and Its Metabolites in Lactating Sows

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This study evaluated the effect of an algoclay-based mycotoxin decontaminant on the levels of ZEN, DON, and their derivatives in the colostrum, milk, and serum of sows, as well as in the serum of weaned piglets after maternal mycotoxin exposure during the last week of gestation and during lactation of sows (26 days). For this, sows (n = 5) were fed diets artificially contaminated with 100 (LoZEN) or 300 (HiZEN) ppb ZEN, with or without an algoclay-based mycotoxin decontaminant in the highly contaminated diet. All diets contained 250 ppb deoxynivalenol (DON). Dietary treatments did not affect the performance of the sows and piglets. Only a-ZEL was significantly increased in the colostrum of sows fed the HiZEN diet, and this increase was even higher in the colostrum of the sows fed the HiZEN diet supplemented with the test decontaminant. However, no differences in milk mycotoxin levels were observed at weaning. The highest levels of ZEN,  $\alpha$ -ZEL, and  $\beta$ -ZEL were observed in the serum of sows fed the HiZEN diet. When the HiZEN diet was supplemented with the tested algoclay-based mycotoxin decontaminant the levels of ZEN and its metabolites were significantly decreased in the serum of sows. Although all sows were fed the same levels of DON, the serum level of de-epoxy-DON was increased only in the serum of piglets from the sows fed a diet with the non-supplemented HiZEN diet. In conclusion, the tested algoclay-based mycotoxin decontaminant can decrease the levels of ZEN and its metabolites in the serum of sows and the level of de-DON in the serum of piglets.

Keywords: milk, colostrum, biological samples, pig, fusarium, mycotoxins

## INTRODUCTION

Mycotoxins are toxic compounds produced by different fungal species, *Fusarium* being responsible for most contamination of feedstuffs in the field. Among more than 400 identified mycotoxins, deoxynivalenol (DON) and zearalenone (ZEN) are of most concern for the pig industry. Pigs are very sensitive to both DON and ZEN. When acute dietary exposure occurs, DON can lead to vomiting and feed refusal (1), whereas ZEN has oestrogenic effects (2). However, in practice, acute toxicity occurs occasionally, and chronic dietary exposure plays the main role in economic losses,

especially when young piglets are exposed to these mycotoxins. Such exposure may even occur via the placenta and/or during lactation (3). The presence of DON and its metabolite de-epoxy-DON (de-DON) was detected in the colostrum of sows (4, 5). Recently, DON, ZEN, and their metabolites were identified in the colostrum, milk, and serum of sows fed diets containing 250 ppb DON and 100 or 300 ppb ZEN (6). In this earlier study, we also confirmed the transmission of these mycotoxins during the last week of gestation and lactation by measuring mycotoxin levels in the serum of suckling piglets. The gestation and lactation exposure did not impair the performance of the piglets but an inflammatory process was taking place.

To limit exposure, diets should be prepared with grains containing low levels of mycotoxins. However, feed is prepared with different feedstuffs that will present variable levels of different mycotoxins. Therefore, feed additives capable of inactivating these mycotoxins or preventing their absorption by the animals may help prevent outbreaks and financial losses. Bentonites are widely used for this purpose and registered in the EU for their capacity to bind aflatoxin B1 (7). Bentonites are phyllosilicates with variable physicochemical properties (8, 9) concluded there are significant correlations between in vitro ZEN adsorption and both the d-spacing (interlayer space) and mineral fraction at different pH levels. Another study indicated that increasing the bentonite interlayer space thanks to alkyl groups may improve the adsorption of mycotoxins (10). However, the use of alkyl groups in animal feed is prohibited in Europe. The polysaccharide ulvan, present in the cell wall of green seaweed (Chlorophyceae) of the genus Ulva, is a natural compound able to increase the bentonite interlayer space (11). An algoclay technology composed of seaweed extract and bentonite has therefore been developed (12).

The aim of the present study was to evaluate the efficacy of the algoclay-based mycotoxin decontaminant against the transmission of ZEN and DON from sows to piglets during the last week of gestation and lactation. For this, sows were fed diets naturally contaminated with ZEN (300 ppb) and DON (250 ppb).

## MATERIALS AND METHODS

#### **Animals and Housing**

This study was performed alongside another trial evaluating the effect of ZEN transmission from sows to piglets during lactation (6). To reduce the number of experimental animals, part of the control data from the present trial was shared with this former study. A total of 15 clinically healthy sows (Landrace x Large White; parity 1 to 6) with normal body condition were used in this experiment. Sows had an average parity of three and parity was balanced across treatments as much as possible. During gestation, sows were housed in groups of approximately 150 sows with four feeding stations available per group. Sows were fed a commercial diet (Supplementary Table 1) during gestation with marginal levels of mycotoxins. Sows were transferred from the gestation unit to the farrowing rooms at day 109 of gestation and fed the experimental diets (Supplementary Table 2). The farrowing pens (0.60 x 2.50 m for the sow; 2.25 x 2.50 m total area) were equipped with a feeding bin and the sows were TABLE 1 | Experimental treatments.

Treatments	Day 109 of gestation to 26 days of lactation
1	LoZEN
2	HIZEN
3	HiZEN + 0.15% algoclay-based mycotoxin decontaminant

All diets also contained  ${\sim}250$  ppb DON, regardless of the treatment. LoZEN:  ${\sim}100$  ppb; HiZEN:  ${\sim}300$  ppb.

able to fill the feeder by pushing a metal bar in the feeder. Sows did not have access to straw or other bedding material. Pens had a plastic slatted floor, including a heated section for piglets programmed to reach  $40^{\circ}$ C at farrowing, reducing to  $30^{\circ}$ C at 3 weeks after birth. The room temperature was scheduled to decrease from  $24^{\circ}$ C at farrowing to  $20^{\circ}$ C at 5 days after farrowing. Artificial light was provided from 6:00 to 22:00 hours. From day 14 after birth and until weaning, all the piglets received creep feed (**Supplementary Table 3**), which was not contaminated with mycotoxins.

#### **Diets and Experimental Design**

The experimental diets were prepared with naturally contaminated feedstuffs and, as a consequence, the Fusarium mycotoxin DON was present in all of them at the same level ( $\sim$ 250 ppb). Two batches of sugar beet pulp were used as a source of ZEN in the present study. One had negligible contamination, while the other was highly contaminated with ZEN. All other main feedstuffs (corn, soybean meal, wheat, sunflower seed meal, soybean hulls, and clean beet pulp batch) were present at the same inclusion levels in all diets. As a result, the experiment had two main ZEN levels in the diets (low:  $\sim 100$  ppb or LoZEN; high: ~300 ppb or HiZEN). The HiZEN diet was divided into two sub-batches were one of them was supplemented with the tested mycotoxin decontaminant, which consisted of an algoclay technology using the water-soluble polysaccharide ulvan, present in the cell wall of green seaweed (Chlorophyceae) of the genus Ulva, (13) and montmorillonite (layer clay) was used in this study. This material was prepared following the patent EP2674397 with at least 60-90% montmorillonite and 40-10% seaweed on dry matter basis and providing an interlayer space up to 3 nm, manufactured by Olmix, S.A. The recommended maximum level of ZEN in sow diets is 250 ppb (14).

The experiment comprised three dietary treatments and five replicates per treatment, where each sow was a replicate. Treatments were randomly allocated to the sows. 1 week before farrowing, sows were moved to the lactation unit and the gestation diet was replaced by the respective experimental diets (**Table 1**). From day 14 after birth until weaning (day 26) all piglets received an uncontaminated commercial creep feed. Sows and piglets were monitored daily for abnormalities, such as abnormal behavior, clinical signs of illness, and mortality throughout the experiment.

	Diet				
Mycotoxin (ppb)	LoZEN (T1)	HiZEN (T2)	HiZEN + decontaminant (T3)		
ZEN	118	318	318		
DON	259	255	245		
Fumonisin B1 + B2	83.1	84.0	119		
AOH	26.9	29.5	39.4		
AME	68.5	68.6	69.3		
Beauvericin	19.8	27.7	28.9		
Enniatin A1	3.5	-	-		
Enniatin B	32.6	28.9	31.5		
Enniatin B1	9.2	8.6	9.6		

Below detection level in all diets, Aflatoxin B1, B2, G1, and G2, 3 + 15 Acetyl deoxynivaloenol (Ac-DON), Deoxynivalenol 3-Glucoside (DON-3-G), Nivalenol, Ochratoxin A, T2 & HT2 Toxin, Diacetoxyscirpenol, Cytochalasine E, Sterigmatocystin, Alternariol ME, Citrinin, Roquefortine C, Enniatin A, A1, B, and B1, Moniliformin. ZEN, zearalenone; DON, Deoxynivalenol; AOH, Alternariol; AME, Alternariol monomethyl ether.

All diets were analyzed in an independent and accredited (BELAC 057-TEST/ISO17025) laboratory (Primoris Holding, Gent, Belgium) via liquid chromatography with tandem mass spectrometry (LC-MS/MS). This multi-mycotoxin analysis confirmed that ZEN was the main contaminant, followed by DON, while other mycotoxins were found at low to negligible levels. Importantly, although all diets also contained low levels of DON, this mycotoxin was at a constant background level in all three diets. Values of all detected mycotoxins in the diets are presented in **Table 2**.

# Measurements

#### Performance

Sows were individually weighed at day 109 of gestation, on the day of farrowing, and at weaning. Backfat thickness was also measured at these time points. The sow's feed intake was calculated by the difference in feed allowance and feed refusals during the experimental period. After this, the average feed intake and body weight gain during the whole experimental period (day 109 of gestation until weaning) were calculated. The gestation length and number of piglets born (alive and still) were also recorded.

Piglets were weighed at birth and at weaning. The intake of creep feed was also recorded from day 14 up to day 26. Average feed intake and growth were calculated. Mortality rate was recorded in sows and piglets.

# Levels of ZEN, DON, and Their Derivatives in Colostrum, Milk, and Serum Samples

Serum samples were collected from the sows before starting the feeding trial at day 109 of gestation. Colostrum samples were collected from the sows at farrowing for mycotoxin analysis. At weaning (day 26 after farrowing), milk and serum samples were collected from the sows and serum samples were collected from 10 piglets per sow. The method of analysis has previously

been validated and described (15, 16). In brief, the analysis was performed on a 4,000 QTrap mass spectrometer equipped with an ESI source (Applied Biosystems, Darmstadt, Germany) and a 1,200 series LC system (Agilent Technologies, Böblingen, Germany). The analytical column was a Pursuit XRs Ultra C18 column (100  $\times$  2 mm, 2.8  $\mu$ m; Agilent Technologies). A binary gradient of LC-MS grade water as eluent A and MeOH-ACN (70:30) as eluent B was used to separate ZEN, DON, and their metabolites. The ESI-MS/MS was performed in negative mode using a multiple reaction monitoring (MRM) technique. The serum samples were prepared as described by (15). A slightly modified sample preparation method was used for milk and colostrum (16). LOD and LOQ values were calculated from low spiked milk and serum samples based on signal-to-noise ratios of 3:1 and 10:1 using the Analyst Software tool and the quantifier transition (16). Samples were not corrected for recovery, which was calculated as the ratio of the concentration obtained from the calibration curve and the known spiking level.

Transfer of ZEN and DON to colostrum and milk was calculated as previously described with a slight modification (17). In the present study, instead of calculating the carry-over factor, the percentage of transfer was calculated as mycotoxin concentration in the colostrum or milk divided by the mycotoxin exposure via feed. The toxin exposure was calculated by multiplying the toxin concentration of the diet with the feed intake of each sow and dividing it by the mean body weight (18) between the start of the trial and farrowing for the colostrum period, and between farrowing and weaning for the milk period.

#### Statistical Analysis

To determine the minimum number of replicates, a power analysis was performed based on a study from (19) where sows were fed diets contaminated with deoxynivalenol. This calculation was based on a probability of 5% and a power of 80%, with a SEM of 0.09 and a relevant difference of 0.64 kg of feed intake/day. The experimental data were analyzed with ANOVA (GenStat Version 20.0, 2020). Each sow was an experimental unit. Data collected from piglets (10 per sow) were used as a mean value per sow. Treatment means were compared by least significant difference (LSD). Values with  $P \leq 0.05$  were considered statistically significant. For all parameters, parity was used as a covariate to minimize the effect of age. Furthermore, the body weight and backfat thickness of the sow at the start of the experiment was used as a covariate for body weight and backfat development. The percentage of stillborn piglets, individual piglet weight at birth, and coefficient of variance at birth were corrected for the number of total born piglets. Preweaning litter growth and pre-weaning mortality were corrected for the number of piglets at standardization and weaning age.

## RESULTS

#### Sows and Litter Performance

The feed intake, body weight, and backfat development of the sows are shown in **Table 3**. Average individual feed intake during the whole experimental period (day 109 of gestation until weaning) was 4.9 kg/d. At the start of the experiment, sows had an

	<b>T</b> 4	70	TO	1.00	Durahua
	11	12	13	LSD	P-value
	(n = 5)	( <i>n</i> = 5)	( <i>n</i> = 5)		
ADFI (kg/d)					
d109-weaning	5.1	4.8	4.9	1.30	0.87
Farrowing-weaning	5.5	5.4	5.6	1.40	0.95
BW development (kg)					
BW d109	267	269	269	42.6	> 0.99
BW farrowing	253	247	249	8.88	0.38
BW weaning	220	218	224	26.4	0.87
BWG d109-farrowing	-15.4	-21.1	-19.5	8.88	0.38
BWG farrowing-weaning	-33.4	-29.3	-24.6	23.6	0.73
BWG d109-weaning	-48.8	-50.3	-44.1	26.4	0.87
BF development (mm)					
BF farrowing	15.2	14.7	14.7	1.08	0.55
BF weaning	12.7	13.3	12.2	1.90	0.48
BFG d109-farrowing	-0.14	-0.67	-0.59	1.08	0.55
BFG farrowing-weaning	-2.54	-1.36	-2.51	2.08	0.44
BFG d109-weaning	-2.67	-2.03	-3.10	1.90	0.48

TABLE 3   Effect of dietary treatments on average	daily feed intake (ADFI), body weight (BW), I	BW gain (BWG), backfat (BF), and BF gain (BFG) <sup>a</sup> .
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<sup>a</sup> All values for body weight and backfat thickness were corrected for body weight and backfat thickness of the sow at the start of the experiment; T1, LoZEN from d109 gestation until d26 of lactation; T2, HiZEN from d109 gestation until d26 of lactation; T3, HiZEN + algoclay-based mycotoxin decontaminant from d109 gestation until d26 of lactation. No effects of dietary treatment on litter size, litter weight, or individual piglet.

TABLE 4   Effect of dietary treatment on performance of the sow at farrowing.						
	T1	T2	ТЗ	LSD	P-value	
Gestation length (d)	114.8	114.2	114.6	2.68	0.87	
Number of piglets (n)						
Total born	14.8	17.0	17.0	4.89	0.55	
Live born (LB)	14.0	16.0	14.6	4.04	0.56	
Still born	0.8	1.0	2.4	2.11	0.24	
Birth weight (LB)						
Litter (kg)	20.4	20.9	18.8	6.03	0.74	
Individual (kg) <sup>a</sup>	1.4	1.3	1.3	0.39	0.95	

<sup>a</sup> Values are corrected for the number of total born piglets. T1, LoZEN from d109 gestation until d26 of lactation; T2, HiZEN from d109 gestation until d26 of lactation; T3, HiZEN + algoclay-based mycotoxin decontaminant from d109 gestation until d26 of lactation.

average body weight of 269 kg. No effect of the dietary treatments was observed regarding sow's body weight gain or average daily feed intake (**Table 3**).

No effects of dietary treatment on litter size, litter weight, or individual piglet weight were observed (**Table 4**). On average, sows in the experiment had 16.3 total born (TB). Litters were standardized between 24 and 48 h after birth at 12–14 piglets per litter, resulting in an average of 13.3 piglets per sow. For this, cross fostering was not necessary because of the average 14.9 live born (LB) piglets with an average birth weight of 1,355. Instead, some litters had more than 14 piglets. In this case, the extra LB piglets were transferred to sows outside the experiment.

Dietary treatments did not affect litter/piglet pre-weaning growth, nor did it affect pre-weaning mortality (**Table 5**). In this experiment, the piglets had an average weaning weight of 8.1 kg, which means an average daily gain of 245 g/d until weaning.

No significant differences in weaning weight were observed among treatments.

#### Analysis of Biological Samples Mycotoxins in Colostrum and Milk

A significant increase in  $\alpha$ -ZEL levels was observed in the colostrum of sows from T2, i.e., those fed a HiZEN diet from d109 until weaning. These levels were even higher when the sows were fed the HiZEN diet supplemented with a decontaminant. However, no differences were observed among the treatments when evaluating the milk samples at weaning (**Table 6**). The percentage transfer of ZEN from feed to colostrum in the ZEN form was 0.004%, 0.002%, and 0.002% for T1, T2, and T3, respectively. The percentage transfer of ZEN from feed to milk in the ZEN form was 0.004%, 0.002%, and 0.002% for T1, T2, and

	T1	T2	Т3	LSD	P-value
Weaning age (d)	27.2	27.0	27.0	2.60	0.99
Number of piglets					
Start <sup>a</sup>	12.8	13.6	13.2	1.31	0.44
Weaning	11.6	12.8	12.2	1.98	0.45
Weaning weight					
Litter (kg) <sup>b</sup>	107.0	91.7	95.7	22.8	0.39
Piglet (kg) <sup>b</sup>	8.7	7.5	7.9	1.81	0.36
CV (%) <sup>b</sup>	12.3	18.3	18.2	9.66	0.35
Growth					
Piglet (g/d) <sup>b</sup>	267	225	242	0.61	0.35
Litter (kg/d) <sup>b</sup>	3.3	2.7	2.9	0.78	0.35
Mortality					
Pre-weaning <sup>a</sup> (%)	6.5	8.4	7.9	10.16	0.92
Creep feed intake					
Piglet (g/d)	18.1	26.7	30.3	19.92	0.41

#### **TABLE 5** | Effect of dietary treatment on piglet performance.

<sup>a</sup> After standardization. <sup>b</sup> Values are corrected for the number of piglets at standardization and weaning age. T1, LoZEN from d109 gestation until d26 of lactation; T2, HiZEN from d109 gestation until d26 of lactation; T3, HiZEN + algoclay-based mycotoxin decontaminant from d109 gestation until d26 of lactation. CV, Coefficient of variation.

**TABLE 6** | Effect of dietary treatment on mycotoxin levels (ng/ml) in colostrum and milk.

	T1	T2	Т3	LSD	P-value
Colostrum					
ZEN	0.053	0.075	0.059	0.0535	0.67
α-ZEL	0.094ª	0.218 <sup>b</sup>	0.299 <sup>c</sup>	0.0643	< 0.01
DON	0.75	0.76	0.71	1.183	> 0.99
Milk					
ZEN	0.120	0.115	0.110	0.0870	0.97
α-ZEL	0.062	0.156	0.121	0.1340	0.34
DON	2.98	2.37	1.61	1.893	0.32

<sup>a-c</sup> Different superscripts indicate significant differences among treatments ( $P \le 0.05$ ). T1, LoZEN from d109 gestation until d26 of lactation; T2, HiZEN from d109 gestation until d26 of lactation; T3, HiZEN + algoclay-based mycotoxin decontaminant from d109 gestation until d26 of lactation.  $\beta$ -ZEL, ZAN,  $\alpha$ -ZAL,  $\beta$ -Zearalanol, and de-DON were not detected in the samples. DON, Deoxynivalenol; de-DON, De-epoxy-deoxynivalenol; ZEN, Zearalenone;  $\alpha$ -ZEL,  $\alpha$ -Zearalenol;  $\beta$ -ZEL,  $\beta$ -Zearalenol; ZAN, Zearalanone;  $\alpha$ -ZAL,  $\alpha$ -Zearalanol;  $\beta$ -ZAL,  $\beta$ -Zearalanol.

T3, respectively. The percentage transfer of the sum ZEN and  $\alpha$ -ZEL relative to ZEN intake was 0.011%, 0.008%, and 0.010% in the colostrum from T1, T2, and T3, respectively. For milk, the transfer of the sum ZEN and  $\alpha$ -ZEL relative to ZEN intake was 0.006%, 0.003%, and 0.003% in the colostrum from T1, T2, and T3, respectively. Likewise, the percentage transfer of DON from feed to colostrum was low, being 0.03%, 0.03%, and 0.02% for T1, T2, and T3, respectively. The percentage transfer of DON from feed to milk was 0.04%, 0.05%, and 0.03% for T1, T2, and T3, respectively. These values did not differ significantly.

#### Mycotoxins in Serum

At the start of the trial, no differences were observed in the serum levels of mycotoxins. However, at the end of the trial, i.e., day 26 of lactation, a significant increase was observed in the serum levels of ZEN,  $\alpha$ -ZEL, and  $\beta$ -ZEL in sows fed diet T2 (HiZEN). Sows fed a HiZEN diet supplemented with a decontaminant (T3)

showed a significant decrease in the serum levels of ZEN,  $\alpha$ -ZEL, and  $\beta$ -ZEL, the latter to a level similar to the control (T1). Although there were no significant differences in the levels of ZEN and its derivatives in the serum of piglets, the serum levels of de-DON were significantly higher in the piglets from T2, but similar to the control in the piglets from T3 (**Table 7**).

## DISCUSSION

In the present study, we evaluated the ability of an algoclay-based mycotoxin decontaminant to limit the transmission of ZEN and DON to piglets during the last week of the sows' gestation and lactation. Exposure to ZEN and DON at the tested levels did not affect the performance of the sows and piglets. Importantly, the algoclay-based mycotoxin decontaminant did not impair the performance of the sows and piglets either. It is known that mycotoxin-adsorbing agents may interact with dietary nutrients TABLE 7 | Effect of treatments on the mycotoxin levels (ng/ml) in the serum of sows and piglets.

	T1	T2	тз	LSD	P-value
Sows					
Day 109 of gestation					
ZEN	0.066	0.066	0.050	0.0353	0.54
α-ZEL	0.148	0.258	0.155	0.1470	0.24
β-ZEL	0.057	0.081	0.063	0.0523	0.61
DON	0.445	0.929	0.445	0.5080	0.37
de-DON	-	-	-	-	-
Day 26 weaning					
ZEN	0.43 <sup>a</sup>	1.11°	0.74 <sup>b</sup>	0.295	< 0.001
α-ZEL	0.94 <sup>a</sup>	3.42 <sup>c</sup>	2.40 <sup>b</sup>	0.735	< 0.001
β-ZEL	0.23 <sup>a</sup>	0.53 <sup>b</sup>	0.27 <sup>a</sup>	0.111	< 0.001
DON	3.07	2.10	3.26	1.817	0.36
de-DON	0.11	0.15	0.21	0.325	0.83
Piglets at weaning					
ZEN	0.024	0.024	0.017	0.0152	0.49
α-ZEL	0.011	0.040	0.015	0.0279	0.09
β-ZEL	0.042	0.041	0.040	0.0345	0.59
DON	0.045	0.099	0.062	0.0572	0.15
de-DON	0.040 <sup>a</sup>	0.058 <sup>b</sup>	0.040 <sup>a</sup>	0.0160	0.05

<sup>a-c</sup> Different superscripts indicate significant differences among treatments ( $P \le 0.05$ ). T1: LoZEN from d109 gestation until d26 of lactation. T2: HiZEN from d109 gestation until d26 of lactation. T3: HiZEN + algoclay-based mycotoxin decontaminant from d109 gestation until d26 of lactation. ZAN,  $\alpha$ -ZAL,  $\beta$ -ZAL were not detected in the samples. DON, Deoxynivalenol; de-DON, De-epoxy-deoxynivalenol; ZEN, Zearalenone;  $\alpha$ -ZEL,  $\alpha$ -Zearalenol;  $\beta$ -ZEL,  $\beta$ -Zearalenol; ZAN, Zearalanone;  $\alpha$ -ZAL,  $\alpha$ -Zearalanol.

(e.g., vitamins or minerals), impairing animal performance (20). Nevertheless, in 2006 it was demonstrated that the algoclay technology does not affect the bioavailability of nutrients (21).

Dietary exposure during the last week of gestation significantly increased the levels of  $\alpha$ -ZEL in the colostrum of sows fed a diet containing 300 ppb ZEN when compared to a diet with 100 ppb ZEN. When the diet was supplemented with the test mycotoxin decontaminant, the levels of  $\alpha$ -ZEL in the colostrum were even higher. However, when the milk was evaluated at weaning, no differences were observed among the dietary treatments. This may indicate a temporary effect of the mycotoxin decontaminant in enhancing the conversion of ZEN to α-ZEL, which probably occurred at the level of intestinal microbiota as an important site of ZEN metabolism (22) as the decontaminant is assumed to act in chyme through adsorption of mycotoxins. However, an additional interaction between the decontaminant and the physiological state of the sow probably contributed to the observed differences in the metabolization of ZEN between the colostrum and milk period. The nature of this interaction cannot be answered by the present experimental findings but might include differences in feed intake and consequently the mean retention time of ingesta, resulting in different periods of time for interactions between decontaminant, microbiota, ZEN and mucosal contact. Other explanations could include simple dilution/concentration effects mediated through varying colostrum/milk yields. However, there was no correlation between the number and weight of suckling piglets and the mycotoxin residues in colostrum and milk.

Serum levels of ZEN and  $\alpha$ -ZEL of sows fed the HiZEN diet were much higher than those measured in the milk, i.e., 1.11 vs. 0.12 ng/ml and 3.42 vs. 0.16 ng/ml, respectively. The levels of DON in serum were similar to those detected in milk. The low levels of ZEN in milk were expected due to its low carryover (23), which might be affected by the animal health status as previously shown in cattle (24). In the present trial, only healthy sows without reproductive disorders were selected for dietary exposure to the mycotoxin.

A significant increase in ZEN,  $\alpha$ -ZEL, and  $\beta$ -ZEL levels was observed in the serum of sows fed the HiZEN diet, whereas dietary supplementation with the tested mycotoxin decontaminant significantly decreased the levels of ZEN and its metabolites in serum. In 2006, it was demonstrated that the intestinal absorption of mycotoxins (DON and fumonisins) was decreased in the presence of the algoclay technology used in this study (21). Thus, a reduction in intestinal absorption in the presence of the algoclay-based mycotoxin decontaminant is likely to be responsible for the decrease in the levels of ZEN and its metabolites in serum.

When the HiZEN diet was supplemented with the algoclaybased mycotoxin decontaminant, the de-DON levels were similar to those of control piglets. Furthermore, there was a trend toward increased  $\alpha$ -ZEL levels in the serum of piglets from the HiZEN diets, and this effect was counteracted by the test algoclay-based mycotoxin decontaminant. In a previously published study (6) we demonstrated that piglets from the HiZEN-exposed sows had an increased serum level of  $\alpha$ -ZEL together with a decrease in serum oestradiol levels, showing that this low exposure was sufficient to influence the oestrogenic activity in piglets. Although the  $\alpha$ -ZEL level in the serum of piglets was only numerically decreased when the algoclay-based mycotoxin decontaminant was present in the HiZEN diet, the levels of this mycotoxin were significantly decreased in the serum of sows, indicating that the exposure of piglets should also be minimized.

### CONCLUSIONS

In a previous study, it was demonstrated that dietary exposure of sows to 300 ppb ZEN will result in increased milk transmission of this mycotoxins and metabolites to piglets, which will experience an inflammatory process (6). In the present study, the tested algoclay-based mycotoxin decontaminant was able to decrease the levels of ZEN and its metabolites in the serum of sows and decrease de-DON in the serum of piglets. The temporal increase in  $\alpha$ -ZEL in the colostrum of decontaminant-supplemented sows requires further clarification. Although no effects on performance were observed and the transfer of ZEN from feed to milk is low (0.002%), it is important to note that piglets often experience stress immediately after weaning. Therefore, methods to minimize the exposure to mycotoxins during lactation may avoid secondary diseases or other factors leading to impaired performance of piglets during growth. The present test algoclay-based mycotoxin decontaminant did not affect performance. Whether the decontaminant-mediated reduction in the bioavailability of ZEN and DON has long-term effects on the reproductive performance of both the sows and their offspring needs to be clarified further.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**,

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further inquiries can be directed to the corresponding authors.

# ETHICS STATEMENT

This study was conducted according to the guidelines of the Animal and Human Welfare Codes/Laboratory practice codes in the Netherlands. The protocol was approved by the Ethics Review Committee: Body of Animal Welfare at SFR (AVD246002015280), approval date: 8 January 2019.

## **AUTHOR CONTRIBUTIONS**

FM and RS conceived and designed this study. XB and RS performed the experiments. XB, RS, and JS conducted the analysis. FM, JL, and MR acquired the funding. FM, SD, and RS supervised the study. XB, JS, and RS wrote the original draft. XB, JS, JL, MR, FM, SD, and RS revised and edited the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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#### SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets. 2021.704796/full#supplementary-material

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