**FULL PAPER** *Toxicology*

# The Effect of Naringenin on the Fate and Disposition of Deoxynivalenol in Piglets

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ABSTRACT. This research was conducted to evaluate the effect of naringenin (NAG) on fate and dispositions of deoxynivalenol (DON) in piglets following intravenous (i.v.) administration. Three piglets (Group 1) were pretreated orally with NAG at a dosage of 25 mg/kg bw, once a day for 3 consecutive days, followed by a single i.v. injection of DON at a dosage of 1 mg/kg bw. The other three piglets (Group 2) were intravenously administered with DON at the same dosage. The level of DON in the plasma and various piglets tissues were measured using liquid chromatography/tandem mass spectrometry. The plasma levels of DON were higher in the NAG-untreated piglets than in the NAG-pretreated piglets at each time point. However, the plasma DON concentrations in the piglets pretreated with NAG was lower than those of NAG-untreated piglets. The elimination half-life was longer in the NAG-untreated piglets than in the piglets pretreated with NAG. The initial peak concentration, area under the curve and mean residence time were higher in the NAG-untreated piglets than in the piglets pretreated with NAG. Plasma biomarker enzyme activities were also monitored and the levels of gamma glutamyltranspeptidase, aspartate aminotransferase, alanine aminotransferase, creatine phosphokinase, blood urea nitrogen, and creatinine were considerably lower in the piglets pretreated with NAG than in the NAG-untreated piglets. The toxicokinetic data and blood biochemical parameters indicate that NAG enhances the excretion of DON and reduces the opportunity for damage in piglets. Consequently, its toxicity is greater in NAG-untreated piglets than in piglets pretreated with NAG.

KEY WORDS: blood chemistry, deoxynivalenol, dispositions, naringenin, piglets.

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Deoxynivalenol (DON, vomitoxin) is a trichothecene mycotoxin produced by several plant pathogenic fungi, of which *Fusarium graminearum* and *Fusarium culmorum* are the most important sources. DON is the most abundant of the trichothecenes and has been found in a variety of foodstuffs worldwide, including wheat, maize, barley, oats, and rice [6, 7, 10, 26]. DON contamination has been associated with various adverse effects, such as feed refusal, anorexia, weight loss, vomiting and immunotoxic effects in animals [4, 5, 13, 18, 23, 28]. Consistent with this, DON is stable during the storage and processing of foods at high temperature [20, 22]. Swine are more sensitive to DON than mice, poultry, and ruminants because of species differences in the metabolism of DON, with males being more sensitive than females [5, 8, 23]. Human food poisoning has been reported after ingestion of DON. The symptoms described include abdominal pain or a feeling of fullness in the abdomen, dizziness, headache, throat irritation, nausea, vomiting, diarrhea, and blood in stools [21]. DON suppresses normal immune response to pathogens and simultaneously induces autoimmune-like effects, which are similar to human immunoglobulin A (IgA) nephropathy [3, 12, 17].

Naringenin (NAG; 4,5,7-trihydroxyflavanone), a aglycone of naringin, is a naturally occurring flavonone glycoside obtained from kino fruit (*Eucaliptus maculate*) and grapefruit (*Citrus paradisi*). It has been reported that citrus plants possess a wide range of pharmacological properties, including moderate diuretic, antioxidant, anti-inflammatory, free radical scavenging, and lipid peroxidation inhibitory effects [2, 16, 19, 27]. The flavanone naringenin and its glycosides are widespread in nature, and can reach significant concentrations in commonly consumed citrus juices [11].

This study is designed to evaluate the effect of naringenin on the absorption, distribution, and elimination of intravenously administered DON in piglets. To gain insight into the effect of NAG on DON toxicity, the sequential changes in biomarker enzymes were also studied in these animals. Due to its diuretic activity, NAG is considered to be able to accelerate excretion of DON from the body. If this hypothesis were proved, oral ingestion of NAG or NAG-containing fruits would be effective for reduction of DON toxicity in pig farming.

## MATERIALS AND METHODS

*Toxins and chemicals*: Naringenin was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Deoxynivalenol was purchased from Wako Chemical Co. (Tokyo, Japan). Other reagents and chemicals of an analytical grade were purchased from Sigma Chemical Co. Purified water was produced using the Milli-Q water purification system from Millipore, Inc. (Bedford, MA, U.S.A.).

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*Animals*: Six 4-week-old male piglets (Landrace × Large Yorkshire) were purchased from Kadoi Co., Ltd. (Ibaraki, Japan) and acclimatized for one week under controlled conditions (temperature: 23 ± 2°C; 12-hr light and 12-hr dark cycle) in a nursery complete with a diet corresponding to their age. Drinking water was available *ad libitum* via automatic drinkers. The animals were placed in individual stainless-steel cages at the Laboratory Animal Facility, Animal Research Science Center, the University of Tokyo. All experimental procedures involving animals in this study were ethically approved by the University of Tokyo Animal Care and Use Committee.

*Experimental design*: To evaluate the effect of naringenin on the toxicokinetic and biochemical parameters of DON in piglets, the experimental piglets were weighed and then divided into two groups (n=3). Three piglets (Group 1) were orally (p.o.) pretreated with NAG at a dosage of 25 mg/kg bw, once a day for 3 consecutive days, followed by single intravenous (i.v.) administration of DON at a dosage of 1 mg/kg bw. The other three piglets (Group 2) were not treated with NAG, but they were intravenously administered with DON at a dosage of 1 mg/kg bw. The dose level of NAG and DON used in this study was selected based on studies by Alarcon de la Lastra, *et al.* [1] and Prelusky and Trenholm [15], respectively. Because the oral administration was selected for NAG, intravenous administration was selected for DON. The regimen of single bolus injection of DON is well-established for analysis of acute effect of this toxin [9]. Blood samples were taken from jugular veins using heparinized syringes at 0, 0.5, 1, 2, 4, 8, 12, and 20 hr after DON administration. Plasma were separated by centrifugation at 1,968 × *g* for 15 min. After DON administration at 20 hr, all experimental animals were intravenously administered with ketamine (10 mg/kg bw) and medetomidine (80 $\mu$g/kg bw) and were euthanized by i.v. injection of 3M potassium chloride. The livers, kidneys, spleen, muscle, bile, urine, and feces were immediately collected and stored at –20°C until analysis.

Plasma chemical parameters including alkaline phosphatase (ALP), aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), gamma glutamyltranspeptidase (GGT), creatine phosphokinase (CPK), lactic acid dehydrogenase (LDH), total bilirubin (TBIL), glucose (GLU), blood urea nitrogen (BUN), creatinine (CRE), calcium (Ca), sodium (Na), potassium (K) and chloride (Cl) were measured using a reagent kit (FDC Laminate Series 7000, FUJIFILM, Tokyo, Japan).

*Sample extraction*: The extraction of DON from piglet plasma, bile, urine, and feces was carried out as described previously, with some modifications [25]. Briefly, 1 m*l* of piglet plasma, diluted with 0.5 m*l* of phosphate buffer (PB, pH 6.8) was applied to a ChemElut column (Varian, 5 m*l*). The column was eluted with 50 m*l* of ethyl acetate. The eluate was evaporated to dryness under vacuum at 40°C.

One milliliter of urine and bile, diluted with 1.5 and 3 m*l* with PB was added to 8,000 and 4,000 units of $\beta$-glucuronidase, respectively. The mixture was incubated overnight at room temperature. The samples were applied to a ChemElut column (Varian, 5 m*l*). The column was eluted with 50 m*l* of ethyl acetate. The elulate was evaporated to dryness under a vacuum at 40°C. Additionally, bile residues, dissolved with 20 m*l* of acetonitrile (ACN)/water (84/16, v/v) were defatted with petroleum ether by shaking them with 2 g of a mixture of charcoal, alumina, and celite (7/5/3, w/w) for 15 min. After filtration (Whatman No. 1, Whatman International Ltd., Tokyo, Japan), an aliquot of 5 m*l* was evaporated to dryness with the addition of ethanol under nitrogen stream at 50°C.

Five grams of feces were mixed with 24 m*l* of water and left standing overnight at room temperature. The mixture was added to 126 m*l* of ACN and shaken for 1 hr. After filtration, 30 m*l* of the extraction were defatted with petroleum ether by shaking it with 3 g of a mixture of charcoal, alumina, and celite (7/5/3, w/w) for 15 min and filtrated. An aliquot of 10 m*l* was evaporated to dryness under a nitrogen stream at 50°C.

Preparation of various tissue samples for DON analyses was performed according to the method described previously, with some modifications [15, 24]. Various tissues including the liver, kidney, spleen, and muscle (4 g for each tissue) were homogenized in 8 m*l* of water. Then, the homogenate was added to 16 m*l* of ACN, and the mixture was blended with a high-speed blender for 10 min. The supernatant was separated by centrifugation at 1,968 × *g* for 15 min. For cleanup step, 5.6 m*l* of supernatant were used.

*Cleanup with immunoaffinity (IAC) and alumina column*: The extracts from plasma, urine, bile, and feces were dissolved in 2 m*l* of 5% polyethylene glycol (PEG), filtrated with a Minisart® RC filter (pore size: 0.45 $\mu$m, Sartorius AG, Goettingen, Germany). The dissolved solution was applied to an IAC (DONPrep®, R-Biopharm Rhone Ltd., Dmstadt, Germany). The column was washed twice with 2.5 m*l* of water. After flushing with air, DON was finally eluted 3 times with 1 m*l* of methanol. The eluate was then evaporated to dryness under a nitrogen stream at 50°C. The residue was reconstituted in 1 m*l* of methanol/water (10/90, v/v). After passing it through a Minisart® RC filter (pore size: 0.45 $\mu$m, Sartorius AG), the sample was subjected to liquid chromatography/tandem mass spectrometry (LC/MS/MS).

Aliquot samples of 5.6 m*l* per each tissue sample were passed through an alumina column (Varian, Inc., Palo Alto, CA, U.S.A.). DON was eluted with 10 m*l* of ACN/water (21/4, v/v) and evaporated to dryness under a nitrogen stream at 50°C. The sample was subjected to LC/MS/MS.

*LC parameters*: The LC analysis was carried out using an Agilent 1100 series system consisting of a binary high-pressure gradient pump, a vacuum solvent degassing unit, an automatic sample injector, a column thermostat, and a photodiode array detector (Agilent Technologies, Waldbronn, Germany). Separation was achieved by an Inertsil® ODS-3 column (3 $\mu$m, 2.1 × 150 mm) with an Insertsil® ODS-3 guard column (3 $\mu$m, 1.5 × 10 mm) (GL Sciences Inc., Tokyo, Japan). The column was maintained at a tempera-

ture of 40°C. The LC mobile phase program consisted of a binary gradient of water and methanol. The composition started out at 10% methanol and increased linearly to 90% methanol by 32 min. The mobile phase then returned to 10% methanol by 40 min, and the column was equilibrated for 5 min. The flow rate was 200 $\mu l$/min, the injection volume was 10 $\mu l$, and the needle was flushed with 200 $\mu l$ of 100% methanol between samples. The detection limit of DON was 1 *n*g/m*l* (*n*g/g) in piglet plasma and various tissues.

*MS parameters*: Mass spectrometry was performed using an API 3000 MS/MS spectrometer equipped with a TurboSpray® electrospray ionization (ESI) source and Analyst® Software version 1.4.2 (Applied Biosystems, Foster City, CA, U.S.A.). ESI-MS/MS was operated at unit mass resolution in multiple reaction monitoring (MRM) positive ion mode with the following settings: nebulizer gas pressure (NEB): 12 psi, curtain gas pressure (CUR): 12 psi, collision gas pressure (CAD): 8 psi, ion spray voltage (IS): –3,500V, and source temperature (TEM): 400°C. The molecular ions and fragments employed for DON were as follows: Q1: *m/z* 295.2 $[M-H]^+$, Q3(1): *m/z* 265.1, CE(1): –16 eV, Q3(2): *m/z* 247.1, CE(2): –16 eV.

*Fortification procedure*: To evaluate recovery, DON was added to samples of blank plasma and tissues to yield final drug concentrations of 1, 5, 10, 50, 100, and 500 *n*g/m*l* (*n*g/g), respectively. The spiked samples were then analyzed in duplicate as described in the extraction/cleanup procedures. The mean (± SD) recoveries of DON were 90.78 ± 4.33, 80.75 ± 3.72, 84.40 ± 2.88, 82.18 ± 3.67, and 77.63 ± 5.12% in the plasma, liver, kidney, spleen, and muscle, respectively.

*Toxicokinetic calculation*: The DON levels in the plasma of experimental piglets with respect to time were pharmacokinetically analyzed using a non-compartmental pharmacokinetic model with the PK Solutions 2.0™ Program including $C_p^0$ (peak concentration at initial time), $AUC_{0-\infty}$ (area under the curve), $t_{1/2\beta}$ (elimination half-life), $t_{1/2\alpha}$ (distribution half-life), $V_{d(area)}$ (volume of distribution), *Cl* (clearance), and *MRT* (mean residence time).

*Statistical analysis*: The plasma concentration curve of DON in piglets pretreated or not with NAG is shown as the mean (± SD) of 3 piglets. The plasma levels of DON at each time point and toxicokinetic parameters are shown as means (± SD). Statistical analysis was generally performed according to the Student's *t*-test. When the individual differences were large, Welch's *t*-test was performed. A value of $P<0.05$ was judged to be significant, and $P<0.01$ was judged to be highly significant.

## RESULTS

*Clinical findings*: DON-treatment produced vomiting and diarrhea in both sets of experimental piglets. Two piglets that were not treated with NAG died at 16 and 18 hr, respectively; whereas, no death occurred after the administration of DON to piglets pretreated with NAG.

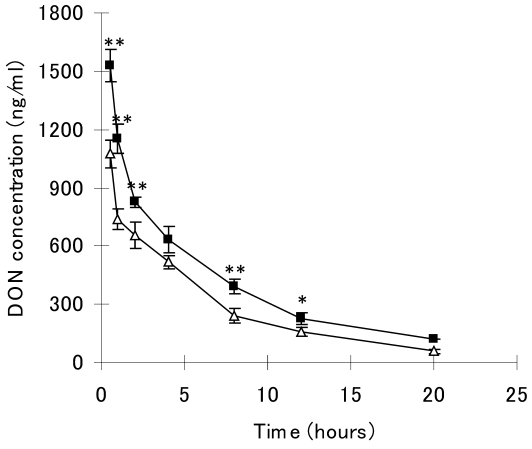


Fig. 1. Mean (± SD) deoxynivalenol levels in the plasma of piglets pretreated with naringenin (△) and piglets not treated with naringenin (■) after single intravenous administration of deoxynivalenol at a dosage of 1 mg/kg bw. (n=3). *=$P<0.05$; **=$P<0.01$: significantly different between NAG-untreated group and NAG-pretreated group.

*Toxicokinetic and residue analyses*: DON was detected in the plasma, urine, feces, and various tissues of experimental piglets following i.v. administration of DON. The plasma concentration-time profiles of DON at a dosage of 1 mg/kg bw in piglets either pretreated or not with NAG are illustrated in Fig. 1. The toxin was detectable from 30 min to 20 hr after i.v. administration of DON in both groups. The plasma profile displayed a rapid decrease in the DON concentration with time in both groups, as was to be expected following i.v. administration. However, the plasma concentration of DON was lower in the piglets pretreated with NAG than that in the untreated piglets at every time point (Fig. 1). Administration of naringenin resulted in significantly smaller $C_p^0$, $t_{1/2\beta}$, $V_{d(area)}$, $AUC_{0-\infty}$ and *MRT* values compared with those in the NAG-untreated piglets (Table 1). The *Cl* value was higher in the piglets pretreated with NAG than in those not treated with NAG (Table 1).

At 20 hr after the administration of DON, the kidney contained the highest concentration of DON, followed by the spleen, liver, and muscle (Table 2). The DON concentrations in urine were higher in the piglets that were not treated with NAG than in those pretreated with NAG; whereas, they were higher in the feces of the piglets pretreated with NAG than in the piglets that were not treated with NAG (Table 2).

*Changes in biomarker enzyme activities*: In the present study, several enzymes were used as biochemical markers for early acute hepatic and renal damage after DON administration. AST, GGT, TBIL, LDH, CPK, ALP, GLU, CPK, Ca, Na and Cl activities were markedly higher in NAG-untreated piglets than in those pretreated with NAG (Table 3).

Table 1. Noncompartmental toxicokinetic parameters of deoxynivalenol (DON) after intravenous administration at 1 mg/kg bw DON in piglets pretreated (n=3) or not (n=3) with naringenin (NAG) at a dosage of 25 mg/kg bw, once a day for three consecutive days

| Toxicokinetic Parameters (units) | Piglets | | *P*-value |
|---|---|---|---|
| | Pretreated with NAG | Untreated with NAG | |
| $C_p^0$ (ng/m*l*) | 1,157 ± 83.46 | 1,624 ± 92.09 | <0.01 |
| $t_{1/2\beta}$ (hr) | 5.50 ± 0.98 | 8.80 ± 1.69 | <0.05 |
| $t_{1/2\alpha}$ (hr) | 2.97 ± 0.45 | 1.73 ± 0.37 | <0.05 |
| *Kel* ($hr^{-1}$) | 0.126 ± 0.01 | 0.079 ± 0.005 | <0.01 |
| $V_{d(area)}$ (*l*/kg) | 1.22 ± 0.24 | 1.27 ± 0.22 | 0.80 |
| *Cl* (*l*/kg/hr) | 0.21 ± 0.02 | 0.10 ± 0.01 | <0.01 |
| *MRT* (hr) | 10.4 ± 3.24 | 17.0 ± 2.45 | <0.05 |
| $AUC_{0-\infty}$ (*n*g-hr/m*l*) | 6,494.3 ± 753.4 | 9,989.6 ± 937.8 | <0.01 |

$C_p^0$=plasma concentration at initial time; $t_{1/2\beta}$=elimination half-life; $t_{1/2\alpha}$=distribution half-life; *Kel*=elimination rate constant; $V_{d(area)}$=volume of distribution; *Cl*=clearance; $AUC_{0-\infty}$=area under the curve extrapolated to infinity; *MRT* = mean residence time.

Table 2. Mean deoxynivalenol (DON) residues (*n*g/g) in pig tissues at 20 hr following intravenous injection at 1 mg/kg bw DON in piglets pretreated (n=3) or not (n=1) with naringenin (NAG) at a dosage of 25 mg/kg bw, once a day for three consecutive days

| Tissues | Deoxynivalenol levels (*n*g/g) | |
|---|---|---|
| | NAG-untreated piglets | NAG-pretreated piglets |
| Spleen | 24.17 | 12.88 |
| Liver | 19.83 | 18.73 |
| Kidney | 42.25 | 33.92 |
| Muscle | 9.75 | ND |
| Bile | 384 | 389.6 |
| Urine | 5,648 | 2,072 |
| Feces | 906 | 1,342 |

ND = not detected.

## DISCUSSION

Deoxynivalenol, a Fusarium toxin, is a major contaminant in food and feedstuffs. There have been extensive reports that pigs are the species most susceptible to DON [11]. The reason why pigs are very sensitive to the toxin is not readily apparent, although there are distinct differences in the toxicokinetic behaviors among species. NAG is a selected component of grapefruit juice and has been reported to possess a variety of pharmacological activities. Due to the beneficial effects of NAG and its very low toxicity, it is considered to belong to a group of various studied pharmacological agents with moderate diuretic, antioxidant, anti-inflammatory, free radical scavenging, and lipid peroxidation inhibitory actions [2, 16, 19, 27]. In the present

Table 3. Mean values (± SD) of plasma chemical parameters in the control and piglets untreated or pretreated with naringenin (NAG) followed by single intravenous administration of deoxynivalenol (DON)

| Blood chemical parameters (units) | Control[a)] | Piglets | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pretreated with NAG | | | | | | | Untreated with NAG [b)] | | | | | | |
| | | 0.5 | 1 | 2 | 4 | 8 | 12 | 20 | 0.5 | 1 | 2 | 4 | 8 | 12 | 20 |
| GGT (u/*l*) | 26.7 ± 9.5 | 18.7 ± 3.1 | 15.7 ± 0.5 | 14.7 ± 0.9 | 21.7 ± 3.1 | 26.0 ± 8.3 | 15.3 ± 2.1 | 16.7 ± 1.2 | 32 ± 7.9* | 28.7 ± 6.9* | 25 ± 2.9** | 38 ± 0.8** | 30.3 ± 3.4 | 29 ± 7.8* | 20 |
| AST (u/*l*) | 86.7 ± 23.9 | 46.7 ± 3.1 | 53.3 ± 16.5 | 39.3 ± 7.4 | 76.0 ± 13.3 | 89 ± 22.3 | 116 ± 39.7 | 104 ± 34.5 | 106 ± 2.2** | 92.3 ± 32 | 56 ± 9.3 | 82 ± 15.7 | 144 ± 28 | 151 ± 22 | 124 |
| ALT (u/*l*) | 44.3 ± 4.9 | 39.0 ± 2.9 | 36.3 ± 3.4 | 34.0 ± 2.2 | 40.3 ± 1.7 | 40.7 ± 8.7 | 39.0 ± 2.2 | 45.0 ± 5.1 | 38.0 ± 2.9 | 41 ± 4.5 | 38.7 ± 3.1 | 40 ± 2.2 | 45.3 ± 4.2 | 50.3 ± 12 | 46 |
| TBIL (mg/d*l*) | 0.67 ± 0.2 | 0.6 ± 0.1 | 0.6 ± 0.05 | 0.4 ± 0.08 | 0.9 ± 0.3 | 1.03 ± 0.3 | 0.5 ± 0.15 | 0.7 ± 0.2 | 1.03 ± 0.4 | 0.8 ± 0.3 | 0.8 ± 0.2* | 1.3 ± 0.1 | 0.93 ± 0.4 | 0.8 ± 0.3 | 0.8 |
| ALP (u/*l*) | 901.0 ± 94.9 | 621.7 ± 98.8 | 634.3 ± 96.9 | 648.3 ± 105.9 | 605.7 ± 75.9 | 524 ± 99.8 | 624 ± 113 | 778 ± 82.1 | 754 ± 114 | 663 ± 119 | 684 ± 130 | 546 ± 162 | 641 ± 178 | 933 ± 97* | 670 |
| LDH (u/*l*) | 829.0 ± 25.7 | 703.3 ± 72.5 | 695.0 ± 36.7 | 586.3 ± 50 | 783.3 ± 94.7 | 806 ± 92 | 744 ± 50 | 681 ± 23 | 649 ± 92 | 766 ± 85 | 710 ± 15** | 863 ± 25 | 857 ± 8.3 | 763 ± 91 | 934 |
| BUN (mg/d*l*) | 5.4 ± 0.1 | 3.20 ± 0.2 | 5.57 ± 1.9 | 6.27 ± 2.3 | 10.9 ± 3.3 | 21.2 ± 5.4 | 30.8 ± 6.9 | 47.5 ± 9.5 | 8.1 ± 3.3 | 8.8 ± 3.4 | 10.8 ± 3.7 | 13.9± ± 4.0 | 29.3 ± 9.6 | 31 ± 4.7 | 50 |
| CRE (mg/d*l*) | 1.0 ± 0.05 | 1.07 ± 0.05 | 1.07 ± 0.05 | 1.10 ± 0.01 | 1.23 ± 0.05 | 2.0 ± 0.2 | 2.6 ± 0.7 | 2.3 ± 0.8 | 1.1 ± 0.2 | 1.07 ± 0.2 | 1.07 ± 0.1 | 1.3 ± 0.2 | 2.2 ± 0.2 | 2.6 ± 0.04 | 3 |
| GLU (mg/d*l*) | 118.3 ± 14.0 | 181.3 ± 5.9 | 198 ± 41.8 | 218 ± 33.9 | 130 ± 20.5 | 87 ± 4.9 | 70.7 ± 8.4 | 79 ± 14.7 | 146 ± 12** | 187 ± 22 | 246 ± 20 | 137 ± 10 | 79.3 ± 21 | 70 ± 8 | 70 |
| CPK (u/*l*) | 2,913.7 ± 817 | 1,031.7 ± 225 | 876.5 ± 78.5 | 748.3 ± 102 | 1,195 ± 247 | 2,010 ± 410 | 3,155 ± 745 | 763 ± 145 | 637 ± 84** | 830 ± 41 | 1,510 ± 286** | 4,100 ± 280** | 5,925 ± 105** | 9,210 ± 1,549* | 2,268 |
| Ca (mg/d*l*) | 11.8 ± 0.5 | 11.2 ± 0.5 | 11.5 ± 0.2 | 11.3 ± 0.4 | 11.8 ± 0.9 | 8.73 ± 0.7 | 8.97 ± 1.2 | 6.07 ± 1.9 | 10.7 ± 0.9 | 11.5 ± 1.1 | 12.2 ± 0.2* | 11.1 ± 0.9 | 10.1 ± 0.3* | 7.3 ± 1.3 | 6.8 |
| Na (mEq/*l*) | 154.0 ± 5.9 | 146.3 ± 1.9 | 146.3 ± 3.7 | 145.0 ± 5.9 | 152. ± 5.2 | 150 ± 1.6 | 151 ± 6.5 | 144 ± 4.8 | 147 ± 8.1 | 146 ± 4.1 | 145 ± 5.1 | 142 ±1.7* | 157 ± 11 | 145 ± 3.1 | 151 |
| K (mEq/*l*) | 6.60 ± 0.1 | 4.43 ± 0.5 | 3.83 ± 0.4 | 3.57 ± 0.4 | 5.80 ± 0.3 | 6.23 ± 0.7 | 6.37 ± 0.7 | 5.13 ± 1.1 | 4.23 ± 0.9 | 4.3 ± 0.2 | 4.1 ± 0.3 | 5.4 ± 0.3 | 6.1 ± 0.4 | 6.9 ± 0.5 | 6.2 |
| Cl (mEq/*l*) | 119.7 ± 12.1 | 108.0 ± 1.4 | 107.7 ± 2.5 | 107.3 ± 4.5 | 111.3 ± 5.4 | 109 ± 3.6 | 111 ± 6.9 | 111 ± 8.8 | 108 ± 5.4 | 107 ± 2.9 | 107 ± 4.2 | 103 ± 2.5* | 105 ± 3.6 | 109 ± 5.1 | 124 |

GGT=gamma glutamyltranspeptidase; AST=aspartate aminotransferase; ALT=alanine aminotransferase; TBIL=total bilirubin; ALP=alkaline phosphatase; LDH=lactic acid dehydrogenase; BUN=blood urea nitrogen; CRE=creatinine; GLU=glucose; CPK=creatine phosphokinase; Ca=calcium; Na=sodium; K=potassium; Cl=chloride; *=$P$<0.05; **=$P$<0.01; The significant difference were compared between NAG-untreated piglets and NAG-pretreated piglets.

a) Control values represent those of animals before treatment with DON with or without co-administration of NAG (n=6).

b) The values of parameters in piglets at 20 hr untreated with NAG represent those of one animal because two piglets in this group died at 16 and 18 hr after DON administration

study, we examined the hypothesis that NAG affects on the fate of DON by calculating its pharmacokinetic parameters using a non-compartmental pharmacokinetic model to examine the effects of the absence and presence of NAG in piglets. In our study, deepoxydeoxynivalenol (DOM-1), a metabolite of DON formed in pigs, was not investigated because Prelusky, *et al.* [14] reported that 95% was presented as the parent toxin, metabolic conversion to DON conjugate was estimated at less than 5%, and the nontoxic DOM-1 was not detected after oral administration of DON in pigs.

The DON in plasma and various tissues was analyzed using LC/MS/MS after i.v. administration. The plasma concentration-time profiles of DON showed the curves to be parallel in both sets of piglets, but the level of DON was markedly lower in piglets pretreated with NAG than in those not treated with NAG. The initial plasma level of DON of the piglets not treated with NAG was also higher than that of those treated with NAG. The elimination half-life ($t_{1/2\beta}$) of DON was also longer in the NAG-untreated piglets than that in the piglets pretreated with NAG. The area under the curve (*AUC*) of DON was higher in the NAG-untreated piglets than that of those treated with NAG; whereas, clearance (*Cl*) and elimination rate constant (*Kel*) were higher in the piglets treated with NAG than in those that were not. These findings clarify that DON is absorbed more efficiently in untreated piglets than those pretreated with NAG and that DON is more rapidly excreted from piglets pretreated with NAG than those not treated with NAG. The level of DON in various tissues in piglets corresponded well to the plasma level profile of DON in the two groups of piglets. It appears that AUC data represent the length of time the compounds were detectable, higher AUC numbers longer tissue tenure in NAG-untreated piglets, indicating less efficient excretion and enhanced chance for damage. Regarding the biomarker enzymes of hepatic and renal functions, the level of almost all the enzymes studied including AST, ALT, GGT, TBIL, LDH, CPK, ALP, BUN, and CRE were markedly decreased in piglets pretreated with NAG. In addition, the death of piglets not treated with NAG occurred at 16 and 18 hr after DON administration.

In conclusion, the results demonstrated that DON is absorbed more efficiently in piglets that have not been treated with NAG. It appears that AUC data represent the length of time that the compounds can be detectable. The higher AUC values thus would reflect longer tissue tenure of DON, indicating less efficient excretion to result in higher risk for DON-induced damage in NAG-untreated piglets than those pretreated. Therefore, the toxicity is greater in piglets not treated with NAG than in piglets pretreated with NAG, followed by DON administration.

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