ORIGINAL RESEARCH PEER REVIEWED

Swine behavioral and physiological response to increasing sodium nitrite oral drench administration and resulting tissue residues

Brent J. Pepin, DVM, MS; Carissa Odland, DVM; Taylor Spronk, DVM; Roy Edler, MS; Todd Williams, DVM

Summary

Objectives: This study aimed to evaluate the physiological and behavioral responses of pigs administered sodium nitrite, determine an ideal dosing rate by oral drenching of sodium nitrite for depopulation events, and evaluate the nitrite residue present in the ocular fluid and skeletal muscle after sodium nitrite administration.

Materials and methods: Four groups of 10 market weight pigs (40 market weight pigs total) and 1 group of 10 sows were used. Each group of market weight animals received a different oral drench dose of sodium nitrite solution (1× [400-441 mg/kg], 2× [800-882 mg/kg], 2.5× [1000-1102 mg/kg], and 3× [1200-1323 mg/kg]) and was observed for distress behaviors. Two market weight animals in each treatment group were implanted with a monitor to measure body temperature, heart rate, and activity levels. The dosing rate with apparent best behavioral and physiological response was applied to the 10 sows and the same behaviors monitored. After death was confirmed, ocular fluid and skeletal muscle samples were collected from the sows.

Results: An increased dosage of sodium nitrite greatly reduced the time to distress with a significant linear relationship. A higher frequency of vocalizations and the most frequent spikes in activity levels were observed in the lowest dosing group. No correlation was found between ocular fluid nitrite and skeletal muscle sodium nitrite concentrations.

Implications: Oral drenching of sodium nitrite is a viable method for swine depopulation events. Higher doses of sodium nitrite have better welfare associations. Ocular fluid nitrite anion concentrations do not correlate with sodium nitrite skeletal muscle concentrations.

Keywords: swine, sodium nitrite, depopulation, oral drench, welfare

Received: November 25, 2020
Accepted: April 1, 2021

Resumen - Respuesta fisiológica y de comportamiento de los cerdos al aumento de la administración oral de nitrito de sodio y los residuos tisulares consiguientes

Objetivos: Este estudio tuvo como objetivo evaluar las respuestas fisiológicas y de comportamiento de los cerdos a los que se les administró nitrito de sodio, determinar una dosis ideal oral de nitrito de sodio en programas de despoblación y evaluar el residuo de nitrito presente en el líquido ocular y en el músculo esquelético después de la administración de nitrito de sodio.

Materiales y métodos: Se utilizaron cuatro grupos de 10 cerdos con peso de venta (40 cerdos en total, con peso de venta) y 1 grupo de 10 cerdas adultas. Cada grupo de animales con peso de venta recibió una dosis diferente de una solución de nitrito de sodio (1× [400-441 mg/kg], 2× [800-882 mg/kg], 2.5× [1000-1102 mg/kg], y 3× [1200-1323 mg/kg]) y se monitoreó sus conductas de ansiedad. En cada grupo, dos animales con peso de venta de cada grupo de tratamiento se les implantó un monitor para medir la temperatura corporal, la frecuencia cardíaca y los niveles de actividad. La dosis con la mejor respuesta fisiológica aparente y de comportamiento se utilizó en las 10 cerdas y se monitorearon los mismos comportamientos. Después de que se confirmó la muerte, se tomaron muestras de líquido ocular y músculo esquelético de las cerdas.

Resultados: Una dosis aumentada de nitrito de sodio redujo en gran medida el tiempo de ansiedad con una relación lineal significativa. En el grupo con la dosis más baja, se observó una mayor frecuencia de vocalizaciones y picos más frecuentes en los niveles de actividad. No se encontró correlación entre las concentraciones de nitrito en el líquido ocular y de nitrito de sodio en el músculo esquelético.

Implicaciones: La ingesta oral de nitrito de sodio es un método viable para programas de despoblación porcina. Las dosis más altas de nitrito de sodio tienen una mejor asociación al bienestar. Las concentraciones de aniones de nitrito en el fluido ocular no se correlacionan con las concentraciones de nitrito de sodio en el músculo esquelético.

Résumé - Réponse comportementale et physiologique du porc à l'administration orale de doses croissantes de nitrite de sodium et résidus tissulaires résultants

Objectifs: Cette étude visait à évaluer les réponses physiologiques et comportementales des porcs auxquels du nitrite de sodium a été administré, à

Pipestone Veterinary Services, Pipestone, Minnesota.

Corresponding author: Dr Brent J. Pepin, 1300 South Highway 75, Pipestone, MN 56164; Tel: 320-333-1401; Email: **brentpepin@gmail.com**.

This article is available online at **http://www.aasv.org/shap.html**.

Pepin BJ, Odland C, Spronk T, Edler R, Williams T. Swine behavioral and physiological response to increasing sodium nitrite oral drench administration and resulting tissue residues. *J Swine Health Prod*. 2021;29(6):310-318.

déterminer un taux de dosage idéal par administration orale de nitrite de sodium pour les procédures de dépeuplement et à évaluer le résidu de nitrite présent dans le liquide oculaire et le muscle squelettique après l'administration de nitrite de sodium.

Matériels et méthodes: Quatre groupes de 10 porcs de poids de marché (40 porcs de poids de marché au total) et un groupe de 10 truies ont été utilisés. Chaque groupe d'animaux de poids commercial a reçu une dose différente de solution de nitrite de sodium (1× [400-441 mg/kg], 2× [800-882 mg/kg], 2.5× [1000-1102 mg/kg], et 3× [1200-1323 mg/kg]) et a été observé pour les comportements de détresse. Deux animaux de poids commercial dans chaque groupe de traitement ont été implantés avec un moniteur pour mesurer la température corporelle, la fréquence cardiaque et les niveaux d'activité. Le taux de dosage avec la meilleure réponse comportementale et physiologique apparente a été appliqué aux 10 truies et les mêmes comportements ont été surveillés. Une fois la mort confirmée, des échantillons de liquide oculaire et de muscle squelettique ont été prélevés sur les truies.

Résultats: Une dose accrue de nitrite de sodium a considérablement réduit le temps de détresse avec une relation linéaire significative. Une fréquence plus élevée de vocalisations et les pics d'activité les plus fréquents ont été observés dans le groupe recevant la dose la plus faible. Aucune corrélation n'a été trouvée entre les concentrations de nitrite dans le liquide oculaire et les concentrations de nitrite de sodium dans le muscle squelettique.

Implications: L'administration orale de nitrite de sodium est une méthode viable pour les procédures de dépeuplement porcin. Des doses plus élevées de nitrite de sodium ont de meilleures associations avec le bien-être. Les concentrations d'anions de nitrite dans le liquide oculaire ne sont pas corrélées avec les concentrations de nitrite de sodium dans les muscles squelettiques.

A foreign animal disease (FAD) diagnosis like African swine fever or foot-and-mouth disease in the United States will likely evoke a "stamping-out policy," implementing depopulation of all confirmed positive and exposed swine.[1,2] Depopulation is the first line of defense in eradicating an FAD to prevent further spread to at-risk animals.[2] For the most flexibility to fit different farm designs and practices, different depopulation options will be required. Research in depopulation methods is needed to develop these options.

Sodium nitrite ($NaNO_2$) has been researched in the United States and utilized by other countries to control feral swine (*Sus scrofa*) and other pest species.[3-6] Sodium nitrite ingestion causes a lethal rise in methemoglobin from the iron oxidation inside the oxygen-carrying red blood cells.[6,7] This oxidation prevents the release of oxygen into the animal's tissues leading to death at toxic levels.[6,7] The reported lethal oral dose of sodium nitrite is 400 mg/kg (181 mg/lb of body weight) for feral hog bait consumption. The lethal dose to kill 50% of the test population (LD_{50}) in the literature varies from 80 to 132.9 mg/kg, while the lethal dose for 95% is estimated to be 145 mg/kg.[3,4,6] Under trial conditions, hogs have been reported to die anywhere from 39 minutes to over 3 hours after oral consumption of a lethal dose of sodium nitrite.[3,6,8] The time-lapse between dosing and death is a potential advantage for depopulation events for allowing time to walk animals out of the barn before death occurs. However, getting animals to consume the product is problematic due to taste aversion to its bitter and salty properties.[3,9] This taste aversion is why bait is commonly used to mask the taste and encourage consumption.[3,9] To convince animals to drink nitrite solution freely, withholding water may be required. Withholding water can create welfare concerns and inefficiencies in time-sensitive events.

The knowledge for the potential use of sodium nitrite in domestic swine for depopulation events (eg, FAD outbreak) is limited.[6] Oral dosing through consumption and oral gavage (passing a tube down the throat to the stomach) of sodium nitrite has been explored in the literature, but not oral drenching of the product in solution. Oral drench provides a method for administration that does not depend on free choice consumption or passing of an oral tube for gavage. Convulsions, vomiting, gasping, and loss of coordination have all been reported as clinical signs of sodium nitrite intoxication in swine but not evaluated as a sign of distress under a predefined ethogram.[6] Sodium nitrite administration needs re-evaluation under specific ethogram definitions used in previous swine euthanasia studies to assess its impact on animal welfare.[10-12]

This study assesses the novel approach of oral drenching sodium nitrite to market weight animals (mean = 131 kg) at 4 different dosing rates. The starting dosing rate is based on the targeted oral dose for feral hog bait consumption from the literature (400 mg/kg).[4] This study is the first to measure behavioral responses of swine to sodium nitrite administration using a predefined ethogram. The first objective of this project was to establish the best dosing rate of sodium nitrite for animal welfare by observing market weight animals using the predefined ethogram. The best dose rate to achieve the shortest time to death was applied to adult swine (136-181 kg) for evaluation using the same ethogram. To assess the potential nitrite residue of the best dosing rate, ocular fluid and skeletal muscle samples were collected from the adult swine after death.

Animal care and use

Animal use was conducted under the guidance and approval of the Pipestone Research Institutional Animal Care and Use Committee (IACUC) protocol ID No. 2020-008.

Materials and Methods

Animals

Forty market weight pigs (mean weight = 131 kg) and 10 sows (mean weight = 174 kg) were used in this study. Of the 40 market weight pigs, 10 were assigned to each treatment group. The market animals selected were allocated conveniently by gate cut from healthy animals with no observable health issues or defects from a commercial finisher barn. The sows were cull sows from commercial facilities with no observable health or body condition issues. This sample size is similar to the number of animals used per treatment in previous swine euthanasia and behavior studies.[10-12] The study was conducted in the summer months at commercial barn locations in northwest Iowa. Animals were housed indoors until moved outside after each treatment was administered for observation.

Sodium nitrite solution

Granular, free-flowing 99% food grade sodium nitrite (Chemtrade Logistics Inc) was used for the solution preparation. Solutions were prepared by pre-weighing milligrams of sodium nitrite combined with 3.79 L of water using the assumed solubility of approximately 70 to 85 g/100 mL with 20°C to 25°C water.[13] For every 3.79 L of solution prepared for dosing, 2649.5 g of sodium nitrite powder

was added, providing a final sodium nitrite concentration of 0.7g/mL of solution. The solution was made fresh immediately before each treatment group dosing procedure began.

Sodium nitrite dosing

A starting dosing range of 400 to 441 mg/kg of body weight was targeted to provide 400 mg/kg of sodium nitrite solution to a market weight pig. This extended range tries to account for the volume of solution that the pig may not swallow during dosing. The treatment groups of 1× (400-441 mg/kg), 2× (800-882 mg/kg), 2.5× (1000-1102 mg/kg), and 3× (1200-1323 mg/kg) were used in this study. The 1× group received 80 mL of sodium nitrite solution by oral drench, the 2× group received 150 mL, the 2.5× group received 200 mL, and the 3× group received 238 mL of sodium nitrite solution.

Five animals from each treatment group (n = 20) were withheld from feed for 24 hours with *ad libitum* access to water. The other 5 animals in each treatment group were allowed *ad libitum* feed and water until the sodium nitrite administration.

The dosing level used for the sows was determined by observations of the market weight animals. The dosing level selected for use provided market animals the shortest time to death while still allowing adequate time to walk them out of a building. The same solution and dosing method were used for administration to both sows and market weight animals. Sows were dosed at 1200 to 1323 mg/kg based on their individual weight (range, 141-202 kg), and therefore received a dose ranging from 250 mL to 350 mL.

An air-compressor powered hooked drench gun designed for liquid dewormer administration to cattle was used (Valbazen; Zoetis) to administer the oral drench. Pigs were restrained individually in the corner of a pen using a hinged sort panel. Each animal had the drench hook placed in its mouth and was administered their calculated dose. Immediately after administration, pigs were numbered on their backs with livestock marker spray (Prima Tech Prima Glo Fluorescent Marking Spray) and walked into a corralled outdoor area for monitoring.

Behavior observations and death confirmation

The behavioral response of each animal after sodium nitrite administration was recorded according to the ethogram in Table 1. Each behavior was selected as an indicator of distress in swine based on previous studies except for the definition of retching, which was derived from the vomiting description for the conditions of this study.[10-12] Prior to the start of the study, a team of 5 individuals were familiarized with the ethogram. Each group of 10 pigs had 1 person recording and a minimum of 2 people always providing continuous observations. The observers would call out the pig's number visible on their back and the behavior being expressed. The recorder would then write the time observed, the pig number, and the behavior expressed. As confirmation, the recorder would repeat the pig number and observed behavior back to the observer. The time oral drench was administered was also recorded in the same manner, with the person administering the sodium nitrite calling out the pig's given number. Behaviors were recorded until the time of death of the individual animal was confirmed. For this study, death was equated with the observation of respiratory arrest as defined in the ethogram. Death was confirmed by the absence of a corneal reflex when touching the pig's eye immediately after observing respiratory arrest.

Any pig alive 2 hours after sodium nitrite dosing was euthanized via a penetrative captive bolt. The 2-hour timepoint was selected to prevent unnecessary and prolonged stress due to pigs being confined outdoors in warm summer weather during the observation period.

Heart rate, activity, and body temperature monitoring

Forty-eight hours before sodium nitrite administration, one fasted pig and one *ad libitum* fed pig in each dosing category (n = 8) were sedated for installation of an internal implant monitor (DST centri-HRT ACT; Star-Oddi) to record the animal's heart rate (beats per minute [bpm] derived from ECG), activity (measured as external acceleration > 1 standard gravity), and body temperature. Activity was measured with the implant by calculating the external acceleration of the *g*-force above the standard gravity from a 3-axis accelerometer. The implant was installed subcutaneously over the xiphoid process of the sternum. Implant readings were taken once every 30 minutes until the day of sodium nitrite administration, when readings were taken every 13 seconds.

Ocular fluid and skeletal muscle residues

Residue testing only occurred in the 10 sows. After death was confirmed in the sows, ocular fluid and skeletal muscle samples were collected from each animal. The ocular fluid was collected by inserting an 18-gauge needle into the eye with vacuum pressure from the attached syringe. Ocular fluids were kept cold until testing. Ocular fluid nitrite anion concentrations were measured under standard diagnostic laboratory procedures at Iowa State University Veterinary Diagnostic Laboratory by high pressure liquid chromatography. Skeletal muscle was collected by dissection of the animal's ham to attain a 10.16 cm × 10.16 cm × 2.54 cm section of skeletal muscle. Skeletal muscle samples were kept frozen until testing. Sodium nitrite skeletal muscle concentrations were tested at Eurofins Microbiology Laboratory under standard diagnostic laboratory procedures for sodium nitrite concentration by ion-exchange chromatographic analysis.

Statistical analysis

Behavior observations were analyzed using a generalized linear model where dose, feed, and dose × feed interaction were held as fixed effects. Polynomial contrasts were used to determine linear and quadratic effects on increasing sodium nitrite dosage. A student *t* test was used to look for differences in response between the market weight pigs and sows to sodium nitrite administration. Nitrite anion concentration (ppm) between ocular fluid and sodium nitrite level in skeletal muscle were compared using Pearson's correlation analysis.

Results

Death rate by dosage

Table 2 shows the death rate and mean time to death after sodium nitrite administration by dosage rate. Not all the animals died from sodium nitrite administration under the confines of this study and 7 had to be euthanized by captive bolt. The animals in this study that were euthanized by captive bolt did not express any distress behaviors within 10 minutes of the end of the 2-hour observation period and were of normal mentation and activity at the time of euthanasia.

Table 1: Ethogram of pig behaviors indicating distress recorded after sodium nitrite administration

Behavior	Definition	Variables recorded
Convulsions	Involuntary contraction of skeletal muscles (tonic, clonic, or both) and paddling*	Latency to onset and frequency
Gasping	Low frequency, very deep breathing through the wide-open mouth with large abdominal movements and stretching of the neck	Latency to onset
Head shaking	Vigorous, rapid, and purposeful movements of the head from side to side (at least two consecutive movements)	Frequency
Loss of coordination	Loss of balance, stumbling, or diminished muscle control	Latency to onset
Loss of posture	Animal collapses into recumbent position with no evidence of posture control and does not regain posture or show further evidence of awareness	Latency to onset
Respiratory arrest (death†)	Permanent cessation of respiratory movements (minimum of 60 seconds without a breath)	Latency to onset
Vocalization	Pig emits an audible bout of a squeal or grunt‡	Frequency of bouts
Vomiting	Ejection of gastrointestinal contents through the mouth	Latency to onset and frequency
Retching	Making the sounds and movements of vomiting but not ejecting gastrointestinal contents from the mouth§	Frequency

* Tonic defined as prolonged generalized contraction. Clonic defined as alternating contraction/relaxation in quick succession. Paddling defined as involuntary walking/running/galloping motion of the limbs.

† Following respiratory arrest, death was confirmed by verifying the absence of a corneal reflex.

‡ A bout is defined as a single discreet event or a period of a continuous event with a < 1-second pause. A pause > 1-second is the end of the bout.

§ Definition for the purposes of our study was derived from the description of vomiting.

Table 2: Percent death rate and time to death after sodium nitrite administration

Group	Dose rate, mg/kg	Death rate, No. (%)	Time to death, mean, min	Time to death, range, min
1× dose	400-441	6 (60)	83	52-101
2× dose	800-882	10 (100)	47	24-92
2.5× dose	1000-1102	9 (90)	42	24-100
3× dose	1200-1323	9 (90)	34	17-58
Sows*	1200-1323	9 (90)	31	23-49

* Sows were administered the 3× dose rate

Repeated behavior results

Repeated behaviors can occur more than once in an individual animal and frequency was recorded. Table 3 shows the number of pigs in each treatment group that expressed repeated distress behaviors after sodium nitrite administration. Convulsions were observed in most pigs across the treatments, while head shaking was expressed the least. The sows showed the largest number of pigs vomiting compared to the market weight treatment groups. As shown in Table 4, the total frequency of vomiting events in sows was 17 as compared to the next highest of 6 events in the 2.5× group. The total frequency of vocalizations was highest in the 1× group with 50 vocalizations, and the next closest group being 2.5× with 16 vocalizations. The frequency and number of animals expressing convulsions, head shaking, and retching were comparable among all treatment groups.

Time to behavior onset results

Table 5 presents the linear relationship between the dosing rate and the time to onset of distress behaviors in the market weight pigs. All times are given as least squares means that reflect the best fit for the data points in the model rather than the observed values, which are presented in Table 2. Time to onset of convulsions, gasping, loss of coordination, loss of posture, respiratory arrest, and vocalization were all found to have a linear relationship. Thus, as the sodium nitrite dose increased, the time to onset of that behavior parameter decreased. Head shaking and retching did not have enough frequency among the pigs to statistically model a response. Vomiting was not found to have a linear relationship with the sodium nitrite dosing rate ($P = .38$).

Market weight pigs withheld from feed for 24 hours before sodium nitrite administration expressed convulsions ($P = .01$), loss of posture ($P = .049$), respiratory arrest ($P = .02$), and vocalization ($P = .02$) sooner than the non-fasted pigs. Fasting did not appear to affect time to onset of gasping, loss of coordination, or vomiting. First sign of distress to death is defined as the length of time in minutes from the first expression of any distress behaviors defined in Table 1 to respiratory arrest. A linear relationship of shorter time to death as the dose increased was found ($P < .001$). Animals fasted for 24 hours also displayed a decreased time to death after the first sign of distress compared to non-fasted animals ($P = .005$).

A comparison of the 3× market weight group to the adult sow group dosed at the same rate (Table 6) revealed a difference in time to onset of vomiting ($P = .004$) and the first sign of distress to death ($P = .02$). Sows experienced a longer time from the first sign of distress to death than the 3× market group.

Physiological measures

Body temperature increased after sodium nitrite administration and plateaued between 40.0°C and 41.0°C. Figure 1 shows the activity level and heart rate (bpm) by dosage group over time. In the 1× group, one of the implanted pigs did not die from sodium nitrite so was euthanized by penetrative captive bolt and, therefore, not included in Figure 1. The more frequent and highest spikes of activity were seen

Table 3: Number of pigs that expressed repeated distress behaviors following sodium nitrite administration

Behavior parameter	Treatment group, No. of pigs (%)					
	1× dose*	2× dose*	2.5× dose*	3× dose*	Sows†	Total (N = 50)
Convulsions	7 (70)	10 (100)	9 (90)	9 (90)	8 (80)	43 (86)
Head shaking	2 (20)	0 (0)	0 (0)	0 (0)	1 (10)	3 (6)
Retching	1 (10)	1 (10)	0 (0)	2 (20)	3 (30)	7 (14)
Vocalization	9 (90)	5 (50)	5 (50)	6 (60)	4 (40)	29 (58)
Vomiting	1 (10)	2 (20)	4 (40)	4 (40)	8 (80)	19 (38)

* The dosages of sodium nitrite were: 1× = 400-441 mg/kg; 2× = 800-882 mg/kg; 2.5× = 1000-1102 mg/kg; and 3× = 1200-1323 mg/kg.

† Sows were administered the 3× dose of 1200-1323 mg/kg.

Table 4: Frequency of repeated distress behaviors expressed following sodium nitrite administration

Behavior parameter	Event frequency, No.				
	1× dose*	2× dose*	2.5× dose*	3× dose*	Sows†
Convulsions	19	21	25	24	19
Head shaking	3	0	0	0	1
Retching	1	2	0	2	5
Vocalization	50	8	16	8	5
Vomiting	1	4	6	4	17

* The dosages of sodium nitrite by body weight were: 1× = 400-441 mg/kg; 2× = 800-882 mg/kg; 2.5× = 1000-1102 mg/kg; and 3× = 1200-1323 mg/kg.

† Sows were administered the 3× dose of 1200-1323 mg/kg of body weight.

Table 5: Least squares means for time to onset of observed behavior by dose rate and feed-fasting status in market weight pigs

	Time to onset, min							
Behavior parameter	**1× dose***	**2× dose***	**2.5× dose***	**3× dose***	***P*‡**	**On feed†**	**Off feed†**	***P***
Convulsions	80.2	44.6	38.03	29.88	< .001	57.49	38.86	.01
Gasping	89.5	34.38	39.38	34.5	< .001	56.38	42.5	.19
Head shaking	Not enough frequency to calculate							
Loss of coordination	75.17	40.67	35.83	26	.005	52.29	36.54	.1
Loss of posture	83.96	34.75	46.5	23	< .001	55.44	38.67	.049
Respiratory arrest (death)	83.12	47.3	41.08	35.78	< .001	61.44	43.58	.02
Retching	Not enough frequency to calculate							
Vocalization	76.48	49	43.5	31.67	.002	62.05	38.27	.02
Vomiting	33.9	47.9	34.04	27.2	.38	39.69	31.85	.36
First sign distress to death§	53.88	25.2	16.88	11.98	< .001	35.39	18.58	.005

* The dosages of sodium nitrite by body weight were: 1× = 400-441 mg/kg; 2× = 800-882 mg/kg; 2.5× = 1000-1102 mg/kg; and 3× = 1200-1323 mg/kg.

† No interaction found between sodium nitrite dosage and the 24-hour fasting status before sodium nitrite administration. The fasting status *P* value compares on and off feed effects from the generalized linear model. Values are considered significant when $P < .05$

‡ Generalized linear model with fixed effects for dosage and feed status, where the *P* value represents a linear response for the main effect of dosage. Values were considered significant when $P < .05$.

§ First sign of distress to death defined as the length of time from the first expression of any distress behaviors defined in Table 1 to respiratory arrest.

Table 6: Least squares means for time to onset of observed behaviors of market weight pigs vs sows* after sodium nitrite administration at 1200-1323 mg/kg of body weight

	Time to onset, min		
Behavior parameter	**Market weight pigs**	**Sows**	***P*†**
Convulsions	29.10	29.00	.98
Gasping	36.33	39.00	.86
Head shaking	Not enough frequency to calculate		
Loss of coordination	26.00	18.17	.23
Loss of posture	28.40	24.20	.43
Respiratory arrest (death)	34.87	31.22	.50
Retching	16.00	12.67	.64
Vocalization	31.67	27.50	.64
Vomiting	25.25	12.38	.004
First sign distress to death‡	11.56	22.22	.02

* Market pigs (n = 10) averaged 131 kg in body weight. Sows (n = 10) averaged 174 kg in body weight.

† Student *t* test for differences between groups. Values are considered significant when $P < .05$.

‡ First sign of distress to death is defined as the length of time from the first expression of any distress behaviors defined in Table 1 to respiratory arrest.

in the 1× dose group. Heart rates over 250 bpm were observed most frequently in the 2.5× and 3× dosage groups.

Nitrite ocular fluid anion and sodium nitrite tissue concentrations

Table 7 shows the nitrite anion concentrations present in the ocular fluid and the sodium nitrite concentration in the skeletal muscle after death. The mean (SD) ocular fluid anion and skeletal muscle sodium nitrite concentrations were 5.68 (2.88) and 25.95 (4.40), respectively. Pearson's correlation coefficient between the two concentrations revealed no correlation ($r = .331$; $P = .35$).

Discussion

Sodium nitrite is "Permitted in Constrained Circumstances" by the American Veterinary Medical Association (AVMA).[2] The most significant limitations to the effectiveness of its use in depopulation events are the lack of research in commercial pigs and the taste aversion of the product for quick ingestion.[2,6] The novel method of oral drenching explored in this study provides a way to ensure appropriate dosage ingestion in domestic pigs. As seen in Table 2, all dose rates used in this study provided enough time for animals to be walked outside the barn after dosing before death. Sodium nitrite toxicity also provides no observed blood loss, unlike other depopulation methods like penetrative captive bolt or gunshot. No blood loss is a considerable benefit as diseases like African swine fever can spread readily by blood contact.[14]

The literature suggests the sodium nitrite LD_{50} be 80 to 132.9 mg/kg for oral consumption.[3,4,6] The results of this study suggest an LD_{50} for oral drench to be closer to the 400 mg/kg dose rate (Table 2). This may be partly due to the solution not swallowed in the administration process. Under the confines of the study, the pigs were only observed up to 2 hours before being humanely euthanized. It is possible that more pigs would have died from sodium nitrite under an extended observation period as documented in the literature, with some deaths taking over 3 hours post administration.[3,6] Sodium nitrite is also documented as unstable in water solutions, requiring fresh preparation or being kept on ice before use.[15] The instability of sodium nitrite in solution is greatly influenced by the acidity of the water, where higher acidity increases the rate of breakdown.[16] Although the current study solution was prepared immediately before administration, this instability may have affected the observed death rate. Water used was from the barn onsite, and the water's pH was not measured before the solution was prepared.

The current study suggests an improvement in animal welfare as the dose increased. Table 3 reveals that more pigs expressed vocalization in the 1× group compared to all other treatments. The dose effect is further supported by the pigs in 1× treatment having 50 recorded vocalization events compared with the next closest of 16 vocalization events in the 2.5× treatment group (Table 4). As seen in Table 5, the time between the first sign of distress (expression of any behavior from the ethogram) and death decreased as the dose increased. The time from administration to death also significantly decreased as the dose rate increased (Table 5). This quicker time interval may be more beneficial to the pig as the higher rate shortens the time the animal experienced discomfort. Despite the dosage rate applied, the body temperature of pigs all increased until the time of death. Pigs fasted for 24 hours also had a quicker death after administration (Table 5). When able, the fasting of pigs before sodium nitrite administration may also improve animal welfare by quickening sodium nitrite absorption. It is also important to note that even if an animal did not die from sodium nitrite administration, all animals except one in the 2.5× treatment group expressed at least one behavior indicating distress during the observation period.

As the time to death post administration decreased with the increased dose rate, the time to onset of distress behaviors also decreased except for vomiting, head shaking, and retching. Head shaking and retching in this study did not appear to be common observable distress behaviors with sodium nitrite administration. The number of animals expressing vomiting behavior was numerically similar among the market weight dosing groups (Table 3). The frequency of vomiting events was numerically lowest in the 1× dosing group among the market weight animals (Table 4). The low frequency in the 1× group implies that increased sodium nitrite dose may influence observed vomiting frequency, but a larger study would be needed to confirm. However, vomiting occurred in greater frequency in sows with 17 individual events from 8 of the 10 sows compared to only 4 individual vomiting events in 4 of the 10 market weight pigs at the same dosing rate (Tables 3 and 4). This difference in vomiting frequency is further supported by the significant difference between market weight pigs and sows in time to onset (Table 6). The other significant difference between market weight animals and sows administered the same dose rate was the first sign of distress to death, which was longer in sows. However, the time to respiratory arrest and the confirmation of death after dosing was not statistically different. The observed difference between sows and market pigs suggests age or animal size may affect the response to sodium nitrite toxicity. These differences in response may reveal different welfare outcomes as the size and age of the animal changes even when the dosing rate by weight remains the same. A weakness of the current study is that sex of the market weight pigs selected were not recorded; therefore, potential differences between barrows and gilts could not be examined.

The concern of secondary toxicity from sodium nitrite may limit how the carcasses can be disposed of after administration.[17] The literature documents no risk of secondary toxicity in pigs dosed at the oral dosing rate in bait stations. However, this study favored using a higher dosing rate than most bait stations target.[17] Table 7 shows sodium nitrite presence in the skeletal muscle after 3× oral dose administration to range from 18.4 to 29.9 ppm. Previous research in swine consuming the 1× dose rate revealed only 2 to 3 ppm in the skeletal muscle.[17] Sodium nitrite is a common food additive in cured meats due to its ability to prevent the growth and toxin formation of *Clostridium botulinum*.[18] During the curing process, the United States Department of Agriculture limits ingoing sodium nitrite to 200 ppm for immersion and massaged curing methods, 156 ppm for comminuted methods, 625 ppm for dry cured, and only 120 ppm specifically for bacon.[19] The detected sodium nitrite in the current study is well below the ingoing allowed amounts. The current study also revealed that ocular fluid nitrite anion concentration does not predict skeletal muscle concentration. The lack of prediction prevents the easy to collect ocular fluid nitrite anion testing from being used to estimate skeletal muscle nitrite concentrations. Further research on the residue of sodium nitrite at different dose rates in different tissues is needed.

Figure 1: Pig heart rate and activity by sodium nitrite dosing group over time of administration to death. The red lines in each graph represent heart rate and black lines represent activity measurement. Implants recorded measurements every 13 seconds. One of the two implanted pigs in the 1× group was euthanized by a captive bolt and not included in the figure. All other dose groups display two pigs, where the solid line and dotted line represent different animals. The dosages of sodium nitrite by body weight were: 1× = 400-441 mg/kg; 2× = 800-882 mg/kg; 2.5× = 1000-1102 mg/kg; and 3× = 1200-1323 mg/kg. Activity was a measured value of external acceleration > 1 standard gravity.

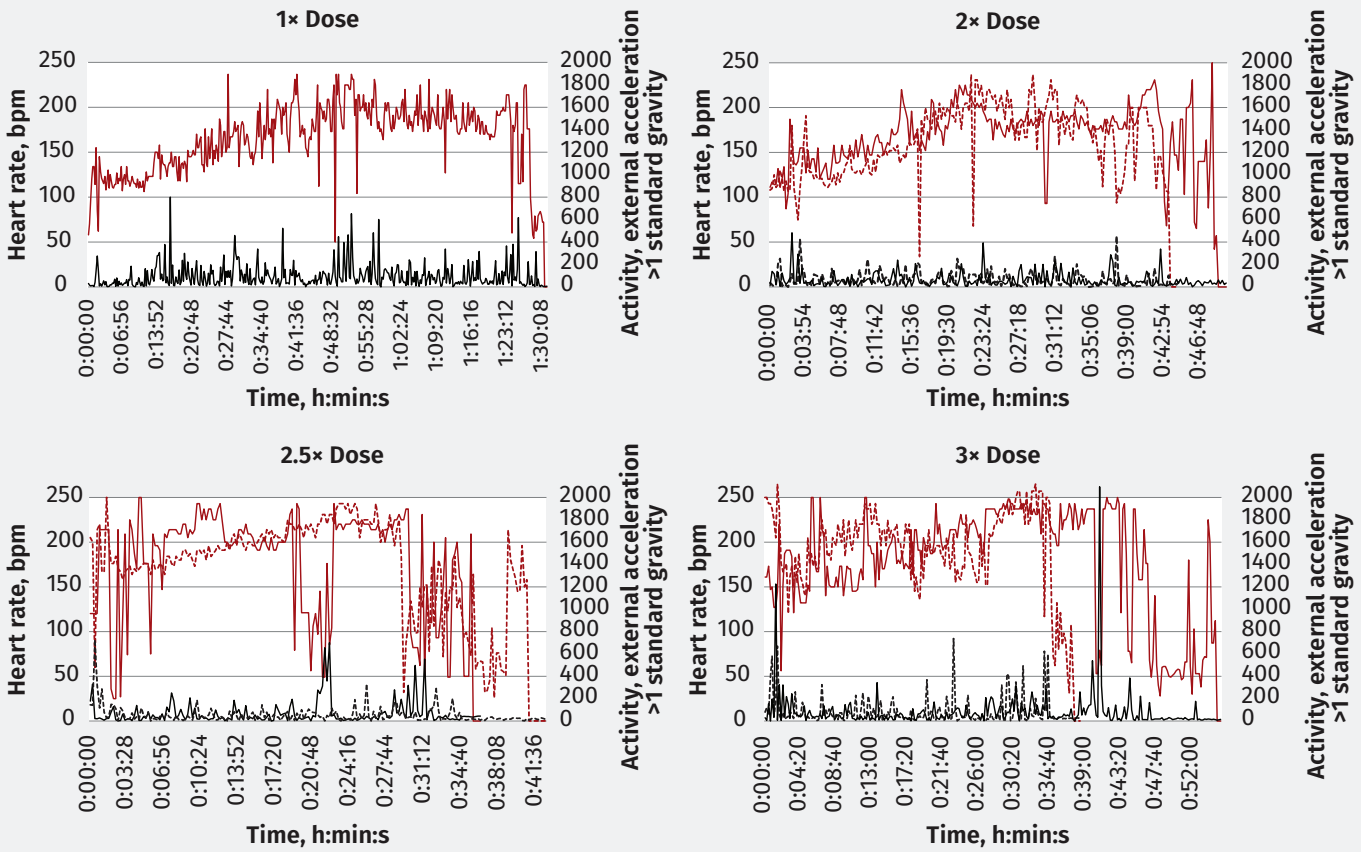

Table 7: Nitrite anion in ocular fluid and sodium nitrite concentration in skeletal muscle after death by sodium nitrite intoxication*

Individual Animal	Ocular fluid nitrite anion, ppm	Skeletal muscle sodium nitrite, ppm
Sow 1	4.1	22.0
Sow 2	3.3	28.1
Sow 3	5.6	33.5
Sow 4	5.7	18.4
Sow 5	13.5	29.9
Sow 6	3.4	27.0
Sow 7	5.6	28.6
Sow 8	7.3	26.1
Sow 9	3.5	20.0
Sow 10	4.8	25.9

* Oral drench of sodium nitrite at 1200-1323 mg/kg of body weight.

Based on the current study results, sodium nitrite by oral drench is a viable option for the depopulation of swine. However, the signs of distress experienced by swine administered sodium nitrite, including those who did not die from the administration, support the AVMA's current classification of "Permitted in Constrained Circumstances" for depopulation events.[2] Further research with sodium nitrite is needed on different application methods, how different sexes, ages, and sizes of pigs may be affected, and dosages beyond those looked at in the current study.

Implications

Under the conditions of this study:

- Sodium nitrite is a viable depopulation method for constrained circumstances.
- Higher sodium nitrite dose improved pig welfare.
- Swine age and size may affect reaction to sodium nitrite.

Acknowledgments

The authors would like to acknowledge the National Pork Board for funding this study and support of the work presented.

Conflict of interest

None reported.

Disclaimer

Scientific manuscripts published in the *Journal of Swine Health and Production* are peer-reviewed. However, information on medications, feed, and management techniques may be specific to the research or commercial situation presented in the manuscript. It is the responsibility of the reader to use information responsibly and in accordance with the rules and regulations governing research or the practice of veterinary medicine in their country or region.

References

*1. World Organization for Animal Health. Article 15.1. Infection with African swine fever virus. In: *Terrestrial Animal Health Code*. 28th ed. OIE; 2019. Accessed August 2, 2020. **https://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/current/chapitre_asf.pdf**

*2. American Veterinary Medical Association. *Guidelines for the Depopulation of Animals: 2019 Edition*. Schaumburg, Illinois: American Veterinary Medical Association; 2019. Accessed 14 October 2020. **https://www.avma.org/sites/default/files/resources/AVMA-Guidelines-for-the-Depopulation-of-Animals.pdf**

3. Shapiro L, Eason C, Bunt C, Hix S, Aylett P, MacMorran D. Efficacy of encapsulated sodium nitrite as a new tool for feral pig management. *J Pest Sci*. 2016;89:489-495. **doi:10.1007/s10340-015-0706-7**

4. Snow NP, Halseth JM, Lavelle MJ, Hanson TE, Blass CR, Foster JA, Humphrys ST, Staples LD, Hewitt DG, VerCauteren KC. Bait preference of free-ranging feral swine for delivery of a novel toxicant. *PLoS One*. 2016;11(1):e0146712. **doi:10.1371/journal.pone.0146712**

5. Shapiro L, Eason C, Bunt C, Hix S, Aylett P, MacMorran D. Encapsulated sodium nitrite as a new toxicant for possum control in New Zealand. *N Z J Ecol*. 2016;40(3):381-385. **doi:10.2307/26198772**

6. Cowled BD, Elsworth P, Lapidge SJ. Additional toxins for feral pig (*Sus scrofa*) control: Identifying and testing Achilles' heels. *Wildl Res*. 2008;35:651-662. **doi:10.1071/WR07072**

7. Bradberry SM, Aw TC, Williams NR, Vale JA. Occupational methaemoglobinaemia. *Occup Environ Med*. 2001;58(9):611-618.

*8. Institute of Medical and Veterinary Science. *Assessing the humaneness and efficacy of a new feral pig bait in domestic pigs*. Veterinary Services Division, Institute of Medical and Veterinary Science. 2010. Study PC0409 Report.

9. Lewis RJ. *Sax's Dangerous Properties of Industrial Materials*. 10th ed. John-Wiley & Sons, Inc; 1999.

10. Kells N, Beausoleil N, Johnson C, Sutherland M. Evaluation of different gases and gas combinations for on-farm euthanasia of pre-weaned pigs. *Animals*. 2018;8:40. **doi:10.3390/ani8030040**

11. Sadler LJ, Karriker LA, Johnson AK, Schwartz KJ, Widowski TM, Wang C, Millman ST. Swine respiratory disease minimally affects responses of nursery pigs to gas euthanasia. *J Swine Health Prod*. 2014;22(3):125-133.

12. Sutherland MA, Bryer PJ, Backus BL. The effect of age and method of gas delivery on carbon dioxide euthanasia of pigs. *Anim Welf*. 2017;26:293-299. **doi:10.7120/09627286.26.3.293**

13. Lide DR. *CRC Handbook of Chemistry and Physics*. 86th ed. CRC Press, Taylor & Francis; 2005.

14. Chenais E, Depner K, Guberti V, Dietze K, Viltrop A, Ståhl K. Epidemiological considerations on African swine fever in Europe 2014-2018. *Porcine Health Manag*. 2019;5:6. **doi:10.1186/s40813-018-0109-2**

15. Misko TP, Schilling RJ, Salvemini D, Moore WM, Currie MG. A fluorometric assay for the measurement of nitrite in biological samples. *Anal Biochem*. 1993;214(1):11-16. **doi:10.1006/abio.1993.1449**

16. Remington JP. *Remington's Pharmaceutical Sciences*. 16th ed. Mack Publishing Co; 1980.

17. Snow NP, Foster JA, VanNatta EH, Horak KE, Humphrys ST, Staples LD, Hewitt DG, VerCauteren KC. Potential secondary poisoning risks to nontargets from a sodium nitrite toxic bait for invasive wild pigs. *Pest Manag Sci*. 2018;74:181-188.

18. Devine C, Dikeman M. *Encyclopedia of Meat Sciences*. 2nd ed. Elsevier; 2014.

19. Sindelar JJ, Milkowski AL. Sodium nitrite in processed meat and poultry meats: A review of curing and examining risk/benefit of its use. *Am Meat Sci Assoc White Paper Series*. 2011;3:1-14.

* Non-refereed references.