RESEARCH REPORT

ANIMAL WELFARE

Title: Evaluation of Sodium Nitrite for mass euthanasia of commercial pigs – **NPB #20-118**

Investigator: Aaron Lower, DVM

Institution: Carthage Veterinary Service, Ltd.

Co-Investigators: Youngsoo Lee, Ph.D., Beau Peterson, Ph.D, Gustavo Silva, Ph.D
Joseph Connor, MS, DVM

Date Received: November 4, 2020

Industry Summary:
It is critical to develop feasible mass euthanasia technology that is humane, economical, safe, and less labor intensive. Microencapsulated sodium nitrite (meSN) feral swine bait has been developed and marketed by Animal Control Technologies Australia (ACTA) as a feed-based toxicant for feral swine control. This technology has not been applied to commercial swine in confinement barns, just feral swine. Sodium nitrite is a chemical commonly used in low concentrations as a preservative in processed meats. If consumed at high doses, sodium nitrite can convert hemoglobin to methemoglobin that is unable to transport oxygen in the blood. The reduced oxygen carrying capacity of the blood depletes the brain and tissues of oxygen, causing unconsciousness and death. Previous pilot trials showed that SN can effectively euthanize swine, however, has been difficult to achieve reliable ingestion when SN is added to commercial feed due to palatability issues.

110 early nursery weight pigs (6.2 kg average weight) were randomly allotted to one of the 11 treatments (one treatment per pen, 10 pigs per pen). Granular form SN (un-encapsulated) salt and microencapsulated SN were the two sources of SN used in the feeds. There were three different feed flavors or taste suppressor combinations. Dried molasses at 4.4% inclusion was the first feed flavor. Vanilla at 0.1% inclusion was utilized as the second flavor. A bitterness taste suppressor at 0.2% inclusion was utilized as the third treatment. The dosage of SN was targeted at 20 grams of SN/100kg of pig at 2 lbs offered per pig. The resulting concentration was 2.2% SN per kg of feed. The dosage of SN in the water was targeted for an intake of 20g of SN per pig with an intake estimation of 0.25 gallons per pig.

1	Feed
2	Feed + SN
3	Feed + SN + Molasses
4	Feed + SN + Taste suppressor
5	Feed + SN + Flavor
6	Feed + meSN
7	Feed + meSN + Molasses
8	Feed + meSN + Taste suppressor
9	Feed + meSN + Flavor
10	Water
11	Water + SN

Feed was removed 24 hours prior to offering treatments. The water treatment pens had water removed also 24 hours to trial start. Pigs were offered their respective treatments for 3 hours of ab libitum consumption.

Of the feed treatment groups, 53 of 80 pigs (66%) were euthanized by SN. Each feed treatment resulted in a range of 50-80% mortality regardless of treatment. 63% of the pigs vomited during the feed treatment. The average time to death was 2 hours and 12 minutes. The earliest pig died at 1 hour and 13 minutes and latest pig at 3 hours. The water treatment group (Group 11) failed to induce clinical signs or mortality. Average feed consumption was 0.14 kg/pig. Pigs on averaged consumed 0.49 g of SN per kg of body weight with a range of 0.20 to 1.09 g of SN per kg.

General timeline of clinical signs:

- 0-45 minutes: good feed intake
- 45-75 minutes: stop eating and huddle
- 75 minutes: start to observe vomiting. Progresses to ataxia and palor and then lateral recumbency.
- 90 minutes: earliest mortality. Most were 90-180 minutes.

All feed formulations performed similarly, with only a 30% mortality rate range between treatments. This method of euthanasia is promising for use in constrained circumstances for depopulation, however, it did not achieve 100% mortality in this trial. It is imperative that pigs receive a bolus of SN for a lethal amount. Increasing the inclusion rate may increase the mortality rate but at the risk of pigs being averse to consume the product. It would also be recommended that pigs have uncompetitive access to consume feed when SN feed is administered.

There were minor differences in intake and mortality between the following groups (meSN versus SN, molasses versus vanilla versus taste blocker groups, unflavored meSN and SN versus flavored and taste blocker). The flavorings and taste blockers did not improve intake and mortality in comparison to feed with meSN and SN by itself.

Pigs were averse to consuming water with SN solubilized. There was minimal intake and no clinical signs.

Sodium nitrite is a viable option for mass depopulation in constrained circumstances. It euthanized between 50-80% of the pigs when offered to commercial swine in this study. Further development is needed to increase the euthanasia rate of pigs, speed up time to death, and decrease the percentage of pigs that vomit.

For further questions or information on this study, please contact:
Aaron Lower, DVM
alower@hogvet.com

Key Findings:

- Sodium nitrite euthanized 50-80% of the pigs per treatment regardless SN formulation used (microencapsulated SN or free form SN) or feed treatment (molasses flavor, vanilla flavor, bitterness taste blocker)
- Administration of sodium nitrite through the water did not achieve sufficient intake to induce mortality

Keywords: Euthanasia, depopulation, sodium nitrite, methemoglobinemia, feed

Scientific Abstract:
It is critical to develop feasible mass euthanasia technology that is humane, economical, safe, and less labor intensive. Microencapsulated sodium nitrite (meSN) feral swine bait has been developed and marketed by Animal Control Technologies Australia (ACTA) as a feed-based toxicant for feral swine control. This technology has not been applied to commercial swine in confinement barns, just feral swine.

110 early nursery weight pigs (6.2 kg average weight) were randomly allotted to one of the 11 treatments. Granular form SN (un-encapsulated) salt and microencapsulated SN were included at a dosage of SN targeted at 20 grams of SN/100kg of pig at 2 lbs offered per pig. The resulting concentration was 2.2% SN per kg of feed. Three different feed flavors or taste suppressor combinations were utilized including dried molasses at 4.4% inclusion, vanilla at 0.1% inclusion, and a bitterness taste suppressor at 0.2% inclusion. The dosage of SN in the water was targeted for an intake of 20g of SN per pig with an intake estimation of 0.25 gallons per pig.

Of the feed treatment groups, 53 of 80 pigs (66%) were euthanized by SN. Each feed treatment resulted in a range of 50-80% mortality regardless of treatment. 63% of the pigs vomited during the feed treatment. The average time to death was 2 hours and 12 minutes. The earliest pig died at 1 hour and 13 minutes and latest pig at 3 hours. Average feed consumption was 0.14 kg/pig. Pigs on averaged consumed 0.49 g of SN per kg of body weight with a range of 0.20 to 1.09 g of SN per kg.

All feed formulations performed similarly, with only a 30% mortality rate range between treatments. The flavorings and taste blockers did not improve intake and mortality in

comparison to feed with meSN and SN by itself. Pigs were averse to consuming water with SN solubilized.

Sodium nitrite is a viable option for mass depopulation in constrained circumstances. It euthanized between 50-80% of the pigs when offered to commercial swine in this study. Further development is needed to increase the euthanasia rate of pigs, speed up time to death, and decrease the percentage of pigs that vomit.

Introduction:

There is an urgent need for mass euthanasia technology in the US swine industry. In the spring of 2020, COVID19 disrupted pig production flows resulting in growing pig space shortages. Additionally, foreign animal diseases like African Swine Fever, if introduced domestically, will similarly disrupt market channels or require depopulation of infected premises. It is critical to develop feasible mass euthanasia technology that is humane, economical, safe, and less labor intensive. Microencapsulated sodium nitrite (meSN) feral swine bait has been developed and marketed by Animal Control Technologies Australia (ACTA) as a feed-based toxicant for feral swine control. This technology has not been applied to commercial swine in confinement barns, just feral swine. Sodium nitrite is a chemical commonly used in low concentrations as a preservative in processed meats. If consumed at high doses, sodium nitrite can convert hemoglobin to methemoglobin that is unable to transport oxygen in the blood. The reduced oxygen carrying capacity of the blood depletes the brain and tissues of oxygen, causing unconsciousness and death. Pigs are highly susceptible to methemoglobinemia as they have low levels of methemoglobin reductase (Smith et al., 1966), an enzyme that can reverse methemoglobin formation.

SN has several attractive features as a euthanasia method in depopulation strategies. The AVMA has previously approved SN as a mass euthanasia technique in constrained circumstances (AVMA Guidelines for the Depopulation of Animals: 2019 Edition). SN breaks down quickly, making it environmentally safe (USDA, 2017). There are low carcass residues limiting the risk to scavengers and allowing multiple carcass disposal options (Snow et al., 2018).

Little research has been conducted utilizing SN as a euthanasia method for domestic swine. The researchers involved in this study have completed in 2018, multiple pilot studies with commercial swine (unpublished). The results of those pilot trials showed that SN can effectively euthanize swine, however, has been difficult to achieve reliable ingestion when SN is added to commercial feed due to palatability issues. Flavoring or taste blocking agents may improve the ingestion by commercial swine when SN is added, resulting in predictable intake and death.

The aim is to identify the hog feed blend(s) including SN to encourage sufficient voluntary ingestion by commercial swine for a humane euthanasia technology.

Objectives:

1. Evaluate consumption and mortality of pigs consuming meSN (micro-encapsulated sodium nitrite) versus non-encapsulated SN without and with the addition of flavoring and bitterness taste blockers.

2. Evaluate the mortality of pigs consuming non-encapsulated SN through the water.

Materials & Methods

Animals:

A total of 110 early nursery weight pigs (6.2 kg average weight) were sourced and housed at the Carthage Innovative Swine Solutions, Veterinary Research Farm. Pigs were randomly allotted to one of the 11 treatments listed above in Tables 1 and 2 (one treatment per pen, 10 pigs per pen).

Materials:

A commercial feed composed of corn and soy was used as a base feed. Granular form SN (un-encapsulated) salt (Duda Energy, 99.9% pure food grade SN) and microencapsulated SN (Animal Control Technologies (Australia) Pty Ltd 46-50 Freight Drive, Somerton, VIC, 3062, Australia +61 3 9308 9688) were the two sources of SN used in the feeds.

There were three different feed flavors or taste suppressor combinations. Dried molasses at 4.4% inclusion was the first feed flavor. Vanilla at 0.1% inclusion (Lucta, Luctarom 32619Z) was utilized as the second flavor. A bitterness taste suppressor (Lucta, Luctarom Bitteroff "S" 5413Z) at 0.2% inclusion was utilized as the third treatment. The vanilla flavor and taste suppressor inclusion rate were added per the recommended by the manufacturer (Lucta). The molasses inclusion rate was based on recommendation from Dr. Youngsoo Lee, University of Illinois, Dept of Food Science and Human Nutrition, and targeted to be two times the inclusion rate of SN.

The dosage of SN was targeted at 20 grams of SN/100kg of pig at 2 lbs offered per pig. The resulting concentration as 2.2% SN per kg of feed. The sample preparation was completed at Integrated Bioprocessing Research Laboratory (IBRL, https://ibrl.aces.illinois.edu/) pilot plant at the University of Illinois, Urbana-Champaign.

Table 1:

In Feed — Weight in *grams* for 10 pigs

	Sample	Sample codes	Feed	SN	meSN	Molasses	Taste Supressor	Flavor	%SN
1	Feed	Con	9091						
2	Feed + SN	SN	8891	200					2.2%
3	Feed + SN + Molasses	SN+ML	8491	200		400			2.2%
4	Feed + SN + Taste suppressor	SN+TS	8891	200			18.2		2.2%
5	Feed + SN + Flavor	SN+FL	8891	200				9.1	2.2%
6	Feed + meSN	meSN	8871		220				2.2%
7	Feed + meSN + Molasses	meSN+ML	8471		220	400			2.2%
8	Feed + meSN + Taste suppressor	meSN+TS	8871		220		18.2		2.2%
9	Feed + meSN + Flavor	meSN+FL	8871		220			9.1	2.2%
		Total	79338	800	880	800	36	18	

Number of pigs		10
	lb/pig	g/pig
Feed intake	2	909.1
	lb	g
Base feed	20	9091
		g SN/pig
SN dose		20
		g meSN/pig
meSN dose		22
Molasses (82 Brix)	x2 of SN	
		% of feed
Taste supressor		0.2
		% of feed
Flavor		0.1

90% SN in meSN

*SN – free sodium nitrite; meSN – microencapsulated sodium nitrite; Taste suppressor – taste suppressor from a commercial partner; Flavor – vanilla flavored feed additive

The dosage of SN in the water was targeted for an intake of 20g of SN per pig with an intake estimation of 0.25 gallons per pig.

Table 2:

	In Drinking Water		For 10 pigs		
	Sample	Sample codes	Water (gal)	SN (g)	Water intake per pig estimation
10	Water	W Con	2.5		0.25 gal/pig
11	Water + SN	WSN	2.5	200	

This concentration is base on 20g SN/pig.
The test done by Swine Vet Center, P.A. used 1800g SN/ gal water with either 24 or 72 hours of offered water.
Soluble up to 84g SN/100g water (3000 g SN/1 gal water) at 25 C (77 F).

Administration:
Nursery feed and water were offered to all pens on a continuous basis for 48 hours to allow for acclimation. At 24 hours prior to offering treatment diets and water, feeders were emptied for all groups. The feed treatment groups were offered water, the water treatment pens had water removed. At the start of the trial, pigs were offered their respective treatments for 3 hours of ab libitum consumption. 20 lbs of feed were offered per treatment in a two hole feeder with 14'' wide holes. Water was offered through 1 cup per pen.

Measurements:
Pigs were individually numbered and weighed in each treatment. Treatments were monitored for feed intake at a group level, death, time to death, and vomiting

Results:
Control pigs (Group 1 – feed control and Group 10 – water control) did not show any clinical signs or mortality during the treatment.

Of the feed treatment groups, 53 of 80 pigs (66%) were euthanized by SN. Each feed treatment resulted in a range of 50-80% mortality regardless of treatment. 63% of the pigs vomited during the feed treatment. The average time to death was 2 hours and 12 minutes. The earliest pig died at 1 hour and 13 minutes and latest pig at 3 hours.

The water treatment group (Group 11) failed to induce clinical signs or mortality.

Table 3:

Treatment	Sample	Vomit	Mortality	Feed Consumed per pig (kg)	SN consumed per pig (g)	SN/kg	Average Time to Death	Range
1	Feed	0%	0%	Invalid Data				
2	Feed + SN	70%	60%	0.21	4.57	0.76	1:57	1:37 - 2:39
3	Feed + SN + Molasses	70%	60%	0.06	1.26	0.20	2:08	1:38 - 2:18
4	Feed + SN + Taste suppressor	70%	80%	0.31	6.77	1.09	2:01	1:13 - 2:49
5	Feed + SN + Flavor	60%	60%	Invalid Data			2:11	1:53 - 2:51
6	Feed + meSN	40%	60%	0.11	2.36	0.37	2:32	1:50 - 3:00
7	Feed + meSN + Molasses	70%	50%	0.06	1.26	0.21	1:59	1:40 - 2:25
8	Feed + meSN + Taste suppressor	60%	80%	0.11	2.36	0.36	2:19	1:40 - 3:00
9	Feed + meSN + Flavor	60%	80%	0.11	2.36	0.40	2:27	2:01 - 2:48
10	Water	0%	0%					
11	Water + SN	0%	0%					

*SN – free sodium nitrite; meSN – microencapsulated sodium nitrite; Taste suppressor – taste suppressor from a commercial partner; Flavor – vanilla flavored feed additive

Treatment 1 (Control) did not have feed offered during the trial period, so no consumption data is available. Treatment 5 (Feed + SN + Flavor) had invalid data from the feeder weights taken to determine pen consumption. Average feed consumption was 0.14 kg/pig. Pigs on averaged consumed 0.49 g of SN per kg of body weight with a range of 0.20 to 1.09 g of SN per kg.

Discussion:

The timeline of clinical signs is predictable with ab libitum feed intake of SN in commercial swine.

Timeline:

- 0-45 minutes: good feed intake
- 45-75 minutes: stop eating and huddle
- 75 minutes: start to observe vomiting. Progresses to ataxia and palor and then lateral recumbency.
- 90 minutes: earliest mortality. Most were 90-180 minutes.

In previous pilot projects, intake of meSN was low and inconsistent resulting in a mortality rate of less than 10%. The feed treatments of flavoring and taste blocking were to improve consumption of SN. Feed intake during this trial was much improved for all treatments, even those with just SN added to the feeds. All feed formulations performed similarly, with only a 30% mortality rate range between treatments. The rate of vomiting and retching is high (63%). An antiemetic, in combination with SN, should be evaluated.

This method of euthanasia is promising for use in constrained circumstances for depopulation, however, it did not achieve 100% mortality in this trial. It is imperative that pigs receive a bolus of SN for a lethal amount. When pigs begin to experience clinical signs, there will be no further consumption of SN feed. Dosing in this study was at 2.2% inclusion rate. In an effort to

overcome the intake concerns in previous work, we used a lower inclusion rate. The feral swine product has used formulations of 10% and moved to 5% inclusion rate. Increasing the inclusion rate may increase the mortality rate but at the risk of pigs being averse to consume the product. Work needs to be done to determine correct dose titration. Current information is based on group intake and weights to determine dosage. Additionally, increasing the feeder space will also have a positive impact on the bolus of SN consumed. In this trial, there were 5 pigs per feed space hole (14''). Effectively, there were 2 pigs eating out of the feeder space at a time. It would be recommended that pigs have uncompetitive access to consume feed when SN feed is administered.

There were minor differences in intake and mortality between the following groups:

- meSN versus SN
- Molasses versus vanilla versus taste blocker groups
- Unflavored meSN and SN versus flavored and taste blocker

It is interesting that there was not an appreciable difference in mortality rate between any of these groups. The flavorings and taste blockers did not improve intake and mortality in comparison to feed with meSN and SN by itself.

Pigs were averse to consuming water with SN solubilized. There was minimal intake and no clinical signs.

Sodium nitrite is a viable option for mass depopulation in constrained circumstances. It euthanized between 50-80% of the pigs when offered to commercial swine in this study. Further development is needed to increase the euthanasia rate of pigs, speed up time to death, and decrease the percentage of pigs that vomit.

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