Title: Validation and Demonstration of Utilizing High Expansion Nitrogen Foam for Large Scale Depopulation of Swine, **NPB Project #21-069.**

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Industry Summary:

The American Veterinary Medical Association (AVMA) defines depopulation as "the rapid destruction of a population of animals in response to urgent circumstances with as much consideration given to the welfare of the animals as practicable".[1] There is a multitude of causes that necessitate the depopulation of swine. These causes include an animal health emergency, natural disaster, animal welfare emergency, and industry disruption. The AVMA categorizes methods of depopulation as approved, permitted in constrained circumstances, and not recommended. For swine, the approved methods include gun shot, penetrating captive bolt, non-penetrating captive bolt, blunt force trauma (size dependent), electrocution, injectable anesthetic overdose, and inhalant gases. The permitted in constrained circumstances include ventilation shut down plus and sodium nitrite.[1]

The U.S. pork industry has continued to prepare for the introduction of a foreign animal disease (FAD), like African Swine Fever Virus (ASFV) which has recently been detected in the western hemisphere. Ongoing industry simulations of a FAD outbreak and the depopulations incurred in 2020 due to the Corona Virus Pandemic (Covid-19) have reaffirmed our lack of resources and infrastructure necessary to conduct large scale mass depopulation of swine in the U.S.[2]

The swine industry must continue to research and evaluate efficiently scalable methods of depopulation, providing a rapid loss of consciousness, and minimizing anxiety, pain, and distress of the animals. Further, these methods should consider the mental welfare and physical requirements of the caretakers performing these depopulations across the broad range of sizes of swine in our industry.

Nitrogen gas (N_2), as an approved method for euthanasia of swine, has not traditionally been used for gassing in open spaces or containers because of the practical difficulty to displace the oxygen to adequately low enough levels (<2%).[3] Livetec Systems has developed a Nitrogen Foam Delivery System (NFDS) that allows for the delivery of an inert gas (nitrogen) within a high expansion foam that envelopes the animal thereby displacing oxygen levels and resulting in death by anoxia. The objectives of this study were to validate the use of high expansion nitrogen foam technology for the large-scale depopulation of swine and demonstrate the Livetec Systems Nitrogen Foam Delivery

System (NFDS) for the large-scale depopulation of swine across multiple classes of swine to producers, veterinarians, researchers, and regulatory officials.

A 40 cubic yards roll-off dumpster and a portable cattle corral gating system were used for animal containment. The dimensions of the corral system were set to match that of the roll-off dumpster's internal measurements of 21' L x 7' W x 7' H. Further, the corral gating had ¼ inch plywood placed from the ground to a height of 4' and plastic bird netting extended to a height of 7' to contain the foam. Nitrogen gas was provided to the NFDS by a nitrogen pumper tanker and vaporizer; the gas was provided at a flowrate of 100,000 – 110,000 standard cubic ft/hour and a temperature of 70 – 80 F. The NFDS system then mixed this gas with water and a Class B high expansion (fluorine free) foam concentrate with the goal to produce a 20 mm bubble through a single foam generator at a rate of 1600 cu ft / min. All replicates had foam filled to a height of 7 ft to evaluate the fill time of a standard volume container. Filling a container to a height of at least two times that of the height of the animals being depopulated would be the recommended fill height. Once the container was filled, the nitrogen system was shut off, and a 20-minute dwell time was observed to ensure a minimum 7-minute exposure period to the Nitrogen gas and ample dewatering of the foam prior to using air lances (leaf blowers) to remove the foam and evaluate the animals.[3]

There was continuous monitoring and video recording during each replicate of depopulation. Prior to the trial, three (3) animals per replicate were anesthetized and had monitoring devices surgically implanted subcutaneously over the xiphoid process (sternum). These devices monitored temperature, heart rate, and activity. Ten (10) animals had accelerometers placed on a hind limb per replicate to monitor activity. Animals were also observed for escape attempts, grunts, squeals, and time to cessation of movement (COM). Death was confirmed, by a veterinarian, at the time of carcass removal. Ten pigs per replicate were then necropsied to evaluate for the presence of foam and if a total occlusion of the trachea occurred.

Phase 1 of this study utilized two (2) sedated feeder pigs to evaluate successful cessation of life at the end of the twenty (20) minute dwell time and lack of tracheal occlusion by the nitrogen foam. Phase 2 evaluated three replicates of feeder pigs utilizing 156 pigs averaging 73 lbs. The finishing pig replicate used 45 pigs averaging 240 lbs. The adult replicate utilized 25 animals averaging 390 lbs. The wean pig replicate utilized 325 pigs averaging 11.8 lbs. The adult and wean pig replicate also achieved the second objective. These were conducted with over 60 invited industry guests present to observe the depopulation. To reduce the time needed for observation, the wean pigs were contained within the corral system; this replicate was the only replicate to depopulate in the corral. All others utilized the 40 cubic yard roll-off container.

The time to fill the 40 cubic yard roll-off dumpster to 7 ft ranged from 100 to 140 seconds for complete container fills. The first two replicates of the phase 2 nursery evaluations utilized a divider wall to reduce the internal volume to one half the size. These two replicates took 80 and 89 seconds to fill. During the phase 2 observation of adult and wean pig groups, the wean pig group took 141 seconds to fill the corral, while the adult group took 100 seconds to fill the solid-sided roll-off container.

Evaluating the surgically implanted sensors, the average time to COM, from the start of foam filling, for the first two replicates of the nursery pigs were 100.3 and 89.0 seconds. The third replicate took 151.3 seconds, which was similar to the 44 to 54 seconds of additional fill time required to fill the container. For the market weight group, the average time to COM was 63.3 seconds. The adults took an average of 169.25 seconds to achieve COM.

There were 28 nursery pigs evaluated for the presence of foam in the trachea. A total of 13 animals had the presence of foam. Of the ten market animals evaluated, seven had the presence of foam. Six of the twelve adult animals evaluated had the presence of foam. The wean pigs had six of the ten pigs evaluated show the presence of foam. Of these 60 animals evaluated, none showed signs of occlusion of the trachea. Thus, presuming that death was achieved by anoxia from exposure to inert gas.

In summary, a total of 6 replicates utilizing a total of 551 pigs from wean age to adult were successfully depopulated using high density expansion foam with the Livetec Nitrogen Foam Depopulation System. The Livetec Systems NFDS was demonstrated to over 60 pertinent industry representatives.

Key Findings:

- High expansion water based foam as a delivery system for Nitrogen gas (N_2), an inert gas, successfully depopulated all classes of swine. No animals needed to be euthanized. (post 20 minute dwell time.)
- Although foam was present in some of the tracheas, there was no observed tracheal obstruction by the foam under the conditions of this study. Thus, presuming that death was achieved by anoxia from exposure to inert gas.
- As evaluated via the implanted sensor, the average time of COM, ranged from 63.33 to 169.25 seconds post start of foam filling, for feeder pig, market, and adult animals.

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Keywords: Nitrogen, N_2, inert gas, depopulation, high density expansion foam, nitrogen foam

Scientific Abstract:

The American Veterinary Medical Association (AVMA) defines depopulation as “the rapid destruction of a population of animals in response to urgent circumstances with as much consideration given to the welfare of the animals as practicable”.[1] There are a multitude of causes that necessitate the depopulation of swine including an animal health emergency, natural disaster, animal welfare emergency, and industry disruption. The AVMA categorizes methods of depopulation as approved, permitted in constrained circumstances, and not recommended. For swine, inhalant gases are an approved method which lead to death by hypoxia. The utilization of CO2 gas has recently been studied and utilized in the United States. However, the use of CO2 requires a modified airtight container that allows for the displacement of oxygen with the introduction of CO2 over a 5-minute period.[4] Nitrogen gas (N_2) has not been traditionally used for euthanasia or depopulation. It has been reported that nitrogen-based anoxia was the most promising method of euthanasia when oxygen concentrations were reduced to less than 2%.[5] Further, it has been shown that “N2-filled foam was effective at quickly purging the air of a containment box to create and maintain stable anoxic conditions.”[6] The objectives of this study were to validate the use of high expansion nitrogen foam for the large-scale depopulation of swine and demonstration of Livetec Systems Nitrogen Foam Delivery System (NFDS) across multiple classes of swine. A standard roll off dumpster and portable cattle corral gating system were used for animal containment, with matched dimensions. Nitrogen

foam was filled to a height of 7 feet to evaluate fill time of a standard volume container. A 20-minute dwell time was observed prior to using air lances to remove the foam and evaluate the animals. A total of 6 replicates were performed, with 3 replicates using feeder pigs (n=156), 1 replicate each of market animals (n=45), adult animals (n=25), and wean pigs (n=325). There was continuous monitoring and video recording through each replicate of depopulation. Prior to the trial, three (3) animals per replicate were anesthetized and had monitoring devices surgically implanted over the xiphoid, subcutaneously (excluding wean pigs). These monitored temperature, heart rate, and activity. Ten (10) animals per replicate had accelerometers placed on a hind limb to monitor activity. Animals were also observed for escape attempts, grunts, squeals, and time to cessation of movement (COM). Insensibility was confirmed by a veterinarian. Ten pigs per replicate were necropsied to evaluate presence of and if full occlusion of the trachea occurred from the foam. The time to fill the roll off container had a range of 80 to 140 seconds. Wean pigs in the corral containment took 141 seconds to fill. Evaluating the implanted sensors, the average time to cessation of movement, from the start of foam filling, for the three replicates of nursery pigs were 100.33, 89.00, and 151.33 seconds. For the market weight group the average time to COM was 63.33 seconds. The adults took an average of 169.25 seconds. For evaluation of the presence of foam in the trachea thirteen of twenty-eight nursery pigs, seven of ten market animals, six of twelve adults, and six of ten wean pigs all had presence of foam in the trachea. Of these 60 animals evaluated, none showed signs of occlusion of the trachea. In summary, a total of 6 replicates utilizing a total of 551 pigs from wean age to adult were successfully depopulated, with none displaying occlusion of the trachea, utilizing high expansion foam with the Livetec Nitrogen Foam Depopulation System.

Introduction:

The swine industry is a cornerstone of agriculture in the United States. As an industry, U.S. swine contributes $39 billion to the GDP annually.[7] A recent study has demonstrated the significant economic impact an outbreak of African Swine Fever (ASF) would have on our industry. This economic impact ranges from a $15 billion loss in 2 years if ASF is eradicated to a $50 billion loss and losses of 140,000 jobs at the end of a 10-year period, if ASF is not contained and able to be eradicated.[8]

Today, significant efforts have been put forth to prevent the introduction of ASF to the United States. The U.S. pork industry has continued to prepare for the introduction of a foreign animal disease (FAD), like African Swine Fever Virus (ASFV) which has recently been detected in the western hemisphere. Mass depopulation is a tool that would be utilized in a herd with a positive diagnosis of a FAD. The ability to rapidly depopulate known positive populations is a pillar to successful reduction of spread and further eradication of a FAD. Ongoing industry simulations of a FAD outbreak and the depopulations incurred in 2020 due to the Corona Virus Pandemic (Covid-19) have reaffirmed our lack of resources and infrastructure necessary to conduct large scale mass depopulation of swine in the U.S.[2]

The American Veterinary Medical Association (AVMA) defines depopulation as "the rapid destruction of a population of animals in response to urgent circumstances with as much consideration given to the welfare of the animals as practicable".[1] The AVMA provides Guidelines for the Depopulation of Animals. These guidelines categorize methods of depopulation as approved, permitted in constrained circumstances, and not recommended. For swine, inhalant gases are an approved method which lead to death by hypoxia. The utilization of CO_2 gas has recently been studied and utilized in the US.[2] However, the use of CO_2 requires a modified airtight container that allows for the displacement of oxygen with the introduction of CO_2 over a 5-minute period.[4] Nitrogen gas (N_2), has not been traditionally used for euthanasia or depopulation. However, Zhang, et al. reported that nitrogen-based anoxia was the most promising method of euthanasia when oxygen concentrations were reduced to less than 2% as quickly as possible.[5] Further, Lindahl et al. has shown that "N2-filled foam was effective at quickly purging the air of a containment box to create and maintain stable anoxic conditions."[6] This study evaluates nitrogen gas delivered in a high expansion foam to multiple classes of swine utilizing Livetec Systems Nitrogen Foam Delivery System.

Objectives:

1. Validation of high expansion Nitrogen foam for the large-scale depopulation of swine
2. Demonstration of Livetec Systems Nitrogen Foam Delivery System (NFDS) for the large-scale depopulation of swine to producers, veterinarians, researchers, and regulatory agencies

Materials & Methods:

A 40 cubic yards roll off dumpster and portable cattle corral gating system were used for animal containment, with matched dimensions of 21' L x 7' W x 7' H. Further the corral gating had ¼ inch plywood placed from the ground to a height of 4' and plastic bird netting extended to a height of 7' to contain the foam. Within each container type, graduated markings were placed at one-foot intervals to evaluate fill time. These containment units were set up in a field in Southwest Minnesota, in Late July 2021.

Nitrogen gas was provided to the NFDS by a nitrogen pumper tanker and vaporizer; the gas was provided at a flowrate of 100,000 – 110,000 standard cubic ft/hour and a temperature of 70 – 80 F. The NFDS system then mixed this gas with water and a Class B high expansion foam concentrate (Fomtec LS xMax) with the goal to produce a 20mm bubble through a single foam generator at a flow rate of 1600 cu ft / min. All replicates had foam filled to a height of 7 ft to evaluate the fill time of a standard volume container.

Once the container was filled, the nitrogen system was shut off, and a 20-minute dwell time was observed to ensure a 7-minute exposure period to the Nitrogen gas and ample dewatering of the foam prior to using air lances (leaf blowers) to remove the foam and evaluate the animals.

There was continuous monitoring and video recording through each replicate of depopulation. Video of the container was taken to evaluate fill time and ethogram behaviors including escape attempts, observed (in person auditory) COM, grunting, and squealing. Three (3) animals per replicate were anesthetized and had monitoring devices (DST centi-HRT ACT; Star-Oddi) surgically implanted subcutaneously, over the xiphoid process, 48 hours prior to depopulation. (excluding wean replicate) These monitored temperature, heart rate (electrical activity), and activity (external acceleration > 1 standard gravity) every 30 seconds. Body temperature was evaluated over time. Heart rate, measured in beats per minute is a measurement of electrical activity across the heart. The Star-Oddi sensors provide a quality index (QI) measurement for each heart rate data point ranging from 0 to 3. A rating of 0 is the best reading. Heart rate results only represent those measurements with the QI equal to 0. A visual analysis of activity graphed over time was used to determine the time of COM. Upon arrival to the study site, Ten (10) animals per replicate had accelerometers (X16-5 and X16-4; Gulf Coast Data Concepts) placed on a hind limb and secured in place with Vet Flex bandage wrap and 1" athletic sports tape. These monitored activity over time at a frequency of 10 readings per second. The veterinarian at time of carcass removal confirmed death by checking for the absence of a corneal reflex response and absence of respiration or movement.

Ten pigs per replicate where necropsied to evaluate presence of and if full occlusion of the trachea occurred from the foam.

Phase 1 of this study utilized two (2) sedated feeder pigs to evaluate successful cessation of life at the end of the twenty (20) minute dwell time and lack of tracheal occlusion by the nitrogen foam. Phase 2 evaluated three (3) replicates of feeder pigs totaling 156 pigs averaging 73 lbs. The finishing pig replicate used 45 pigs averaging 240 lbs. The adult replicate utilized 25 animals averaging 390 lbs. The wean pig replicate utilized 325 pigs averaging 11.8 lbs.

The adult and wean pig replicates were conducted with invited industry guests present to observe the depopulation. The wean pig replicate was performed in the corral. All others utilized the 40 cubic yards roll-off container.

Results:

The anesthetized feeder pigs evaluated in phase 1 were successfully depopulated, confirmed dead at 20 minutes post foaming, and had no tracheal occlusions.

In phase 2, the environmental conditions and time to fill the container are described in Table 1.

The use of the NFDS was successful for depopulation of all of classes of swine as confirmed by the veterinarian at the end of the 20-minute dwell time. This evaluation included 6 replicates utilizing a total of 551 swine. (Table 2) All six replicates were observed to be successful as death of all animals was confirmed at removal and no animals required secondary methods of euthanasia. (Table 3) Further, under the conditions of this study, presence of foam was noted in the trachea of 32 of the 60 animals evaluated. However, there were no animals observed to have tracheal occlusion (Table 4). The mean time to COM, as evaluated by the Star-Oddi sensors ranged from 63.33 seconds to 169.25 seconds. The observed (in person auditory) time to COM ranged from 95 seconds to 248 seconds. These results are listed in Table 5.

The ethogram results are listed in Table 6. There were no squeals or grunts observed under the conditions of this study. Escape attempts are reported with a start, stop, and duration of time observed. Escape attempts were observed as early as 7 seconds post start of foam filling and lasted up to 81 seconds post start of foam filling. The durations of escape attempts ranged from 38 to 68 seconds. Figure 1 shows the mean activity of animals with sensors implanted, reported as external acceleration > 1 standard gravity over time. Mean body temperature fluctuated less than +/- 1.1C across the observed time periods of all replicates and is shown in Figure 2. Heart rate (electrical activity) measured as beats per minute are shown in figures 3a.,b.,c. All animals demonstrated a loss of normal sinus rhythm prior to the conclusion of the twenty-minute dwell time. However, persistent electrical activity (PEA) is noted in the figures.

Due to the low success rate of retrieval of information from the Gulf Coast Accelerometers, no data was reported from these sensors. Several factors contributed to this low success rate including the high moisture environment these sensors were placed in, becoming dislodged from the leg and wrap during the foaming process, and concern over proper synchronizing of time of the sensors.

Discussion:

The results of this study show that high expansion nitrogen foam was successful as a method of mass depopulation of swine under the conditions of this study. There was a rapid COM post application of foam. This COM was followed by a rapid onset of bradycardia with apparent cessation of sinus rhythm by 8 – 12 minutes post foam application. All animals were confirmed dead at the conclusion of a twenty-minute dwell time, although foam was present in some tracheas, no occlusion was observed in evaluated animals. With lack of occlusion of the tracheas, it can be presumed that death occurred by anoxia from exposure to an inert gas (N_2). The presence of foam ranged from small amounts to moderate amounts. Also, its location within the trachea ranged from the upper trachea lower to the tracheal bifurcation. It was hard to determine if the foam at the lower tracheal bifurcation was from the Nitrogen foam or postmortem changes. The success rate and the time observed to COM appear to meet the objectives of the AVMA depopulation guidelines of bringing about a rapid loss of consciousness and minimize anxiety, pain, and distress. This evaluation demonstrated that animals of all sizes could be relocated to and held in a containment device that can be quickly set up and moved. Further, human and animal interaction was minimal during the depopulation process and the containment methods allow for readily available machinery to move carcasses. These should be beneficial to minimize emotional and physical distress on care takers during mass depopulation events.

Several observations were noted during the study. When gas was initially forced through the foam generator nozzle the sound was very loud and led to the excitation of the animals. The excitement subsided and animals returned to normal behavior prior to proper foam being formed and the foam being directed into the container. The noise of the NFDS-associated equipment and potentially the foam itself may have obscured our ability to hear squeals and grunts from the animals. However, noise associated with animal movements could be heard during the foaming process.

As the foam was being applied, it would achieve a higher height at site of application and then spread outward to the edges of the container. This allowed observation of the animal's reaction to the foam, which was of curiosity, until the foam reached the height of the pig's snout, at which point some animals tilted their heads upward to keep it out of the foam and escape attempts began shortly thereafter. Secondary to this observation is the realization of animals standing closest to where the foam is dumping into the container achieving the height of snout coverage quicker and those further away having a delay. Thus, leading to the corresponding range of escape attempt time. This may also account for variation in time to COM. These observations are similar to those reported by Lindahl, et al.[6]

The objectives were met by validating the success of utilization of high expansion nitrogen foam for the large-scale depopulation of swine. This study was conducted under conditions which would be encountered in the field if the need arose for mass depopulation. Further, key industry personnel including producers, veterinarians, researchers, and regulatory agencies were able to observe and learn about the capabilities of high expansion nitrogen foam utilizing the Livetec Systems NFDS. This study also highlighted the ability of the NFDS to work across various weather conditions including heat, rain, and wind. The wind impacted the fill time across containment type as well as within containment type.

This study was conducted under field conditions. These conditions hindered some data that was hoped to be collected. Including auditory assessment of COM and accelerometer data from Gulf Coast Loggers.

The swine industry needs to continue to research and evaluate efficiently scalable methods of depopulation, providing a rapid loss of consciousness, and minimizing anxiety, pain, and distress of the animals. Further, these methods need to consider the mental welfare and physical requirements of the caretakers performing these depopulations across the broad range of sizes of swine in our industry. The outcomes of this study support further evaluation of high expansion nitrogen foam's use as a method of depopulation of swine.

References:

1. AVMA Guidelines for the Depopulation of Animals: 2019 Edition. Schaumburg, IL: AVMA, 2019.
2. Arruda, A.G. et. al. A systematic Literature Review on Depopulation Methods for Swine. Animals 2020,10,2161
3. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Schaumburg, IL: AVMA, 2019.
4. Pepin, B.J., et al. Modification of a standard dump trailer into a mobile carbon dioxide depopulation unit for swine. J Swine Health Prod.2022;30(1):31-38
5. Zhang, Q. Euthanasia of Pigs Using Nitrogen Gas and Decompression. In Proceedings of the 2017 International Symposium on Animal Environment and Welfare, Chongqing, China, 23–25 October 2017; Volume 279, p279-286
6. Lindahl, C. et. al. Responses of pigs to stunning with nitrogen filled high-expansion foam. Animals 2020, 10,2210
7. https://nppc.org/pork-facts/ (Accessed March 27,2022)
8. Carriquiry M, Elobeid A, Swenson D, Hayes D. Impacts of African swine fever in Iowa and the United States. 2020 Mar. https://www.card.iastate.edu/products/publications/synopsis/?p=1300

Table 1. Time of day, weather, and fill time of container (7 ft)

Production Stage	Containment Type	Replicate	Time of Day	Temperature (F)	Wind Speed (mph)	Wind Direction (degrees)	Precipitation	Time to Fill (sec)
Nursery	Roll off	Nursery 1	15:18	89.10	3.55	115.10	No	80
Nursery	Roll off	Nursery 2	17:14	90.00	4.07	145.10	No	89
Nursery	Roll off	Nursery 3*	19:12	89.20	3.31	274.50	No	133
Market	Roll off	Market RO1	10:08	80.30	7.48	121.70	No	140
Adult	Roll off	Adult	11:32	64.84	4.75	243.00	Yes	100
Wean	Corral	Wean	11:29	64.84	4.75	243.00	Yes	141

* The roll off container divider was removed for the third replicate to accommodate size and number of animals

Table 2. Descriptive statistics (BODY WEIGHT of evaluated animals)

Production Stage	N	Mean	Median	Min	Max	Std Dev	CV
Adult*, lbs.	25	390	350	350	450	-	-
Market, lbs.	45	240	240	231	249	9.08	3.78
Nursery, lbs.	156	73.46	76	44	100	16.17	22.01
Wean**, lbs.	325	11.8	11.8	11.8	11.8	-	-

* 15 sows with an average weight of 350 lbs. and 10 boars averaging 450 lbs.
** A trailer weight of wean pigs was utilized

Table 3. Evaluation of replicates for success of depopulation of all animals

Phase	# Replicates	# Animals	Successful	Not Successful
Nursery	3	156	3	0
Market	1	45	1	0
Adult	1	25	1	0
Wean	1	325	1	0

Table 4. Tracheal evaluation for presence of foam and occlusion

Production Stage	Containment Type	Replicate	N	Presence	No Occlusion	Occlusion
Nursery	Roll off	Nursery 1	7	3	7	0
Nursery	Roll off	Nursery 2	11	5	11	0
Nursery	Roll off	Nursery 3	10	5	10	0
Market	Roll off	Market RO1	10	7	10	0
Adult	Roll off	Adult	12	6	12	0
Wean	Corral	Wean	10	6	10	0
		Total	60	32	60	0

Table 5. Descriptive statistics for Star-Oddi observed time to Cessation of Movement (sec) from start of foam filling

Production Stage	Containment Type	Replicate	N	Mean	Median	Min	Max	Std Dev	CV	Observed COM
Nursery	Roll off	Nursery 1	3	100.33	91	91	119	16.17	16.11	190
Nursery	Roll off	Nursery 2	2	89	89	75	103	19.8	22.25	229
Nursery	Roll off	Nursery 3*	3	151.33	142	142	170	16.17	10.68	248
Market	Roll off	Market RO1	3	63.33	54	54	82	16.17	25.52	95

Adult	Roll off	Adult	4	169.25	162	162	191	14.50	8.57	210
Wean	Corral	Wean	0	-	-	-	-	-	-	226

* Replicate Nursery 3 utilized the full dimensions of the container

Table 6. Ethogram evaluation by replicate

Production Stage	Containment Type	Replicate	Number grunts	Number squeals	Start of Escape Attempts* (Sec)	Stop of Escape Attempts* (Sec)	Duration of Escape Attempts (Sec)
Nursery	Roll off	Nursery 1	0	0	7	45	38
Nursery	Roll off	Nursery 2	0	0	7	56	49
Nursery	Roll off	Nursery 3	0	0	18	72	54
Market	Roll off	Market RO1	0	0	15	81	66
Adult	Roll off	Adult	0	0	21	73	52
Wean	Corral	Wean	0	0	13	81	68

* Time is recorded as seconds post start of foam filling

Figure 1. Mean activity of Market (n=3), Adult (n=4), Nursery 1 (n=3), Nursery 2 (n=2), Nursery 3 (n=3) evaluated animals from thirty minutes prior to foaming to 21 minutes post foaming.

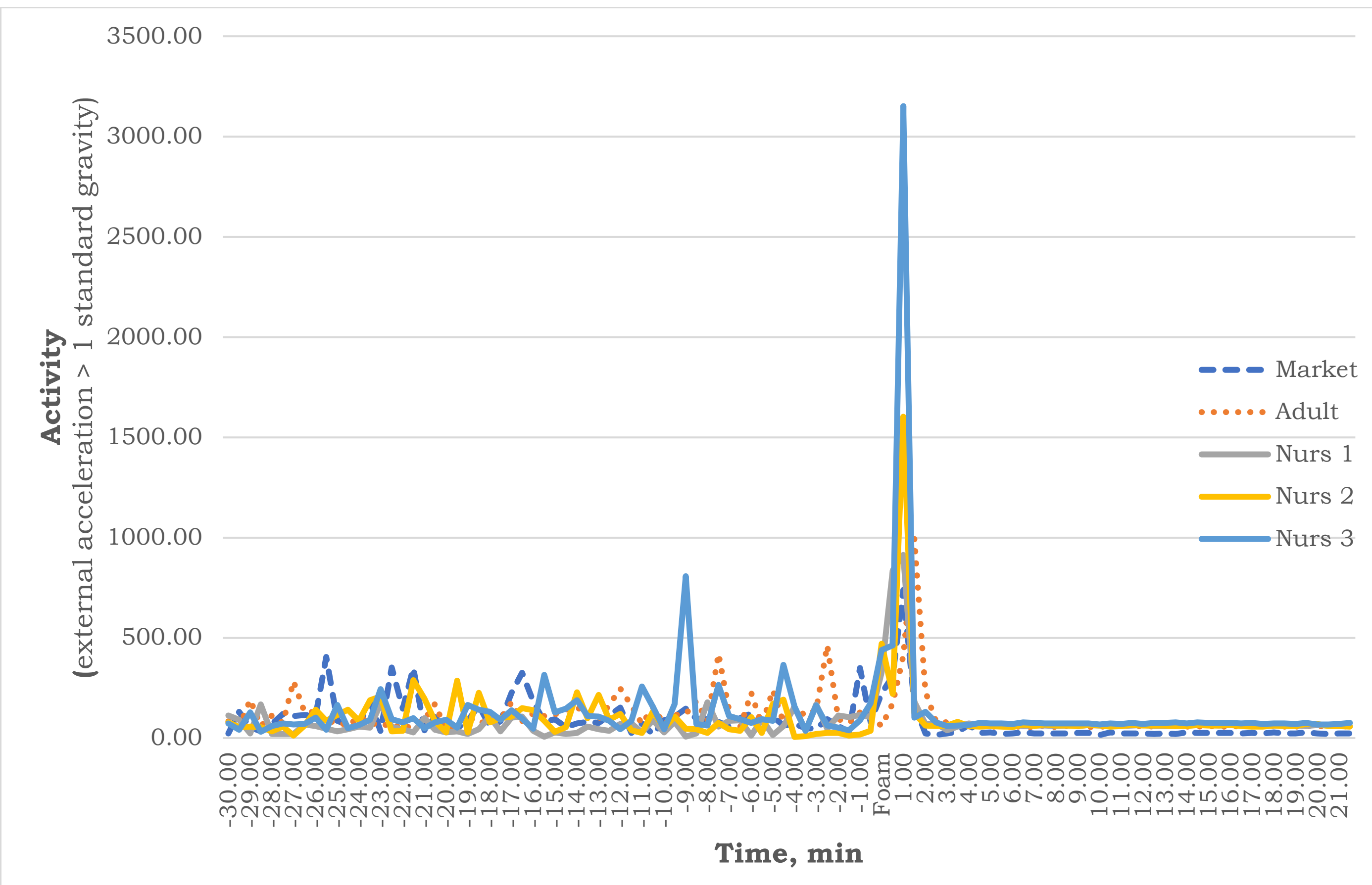

Figure 2. Mean body temperature (C) of Market (n=3), Adult (n=4), Nursery 1 (n=3), Nursery 2 (n=2), and Nursery 3 (n=3) evaluated animals from thirty minutes prior to foaming to 21 minutes post foaming.

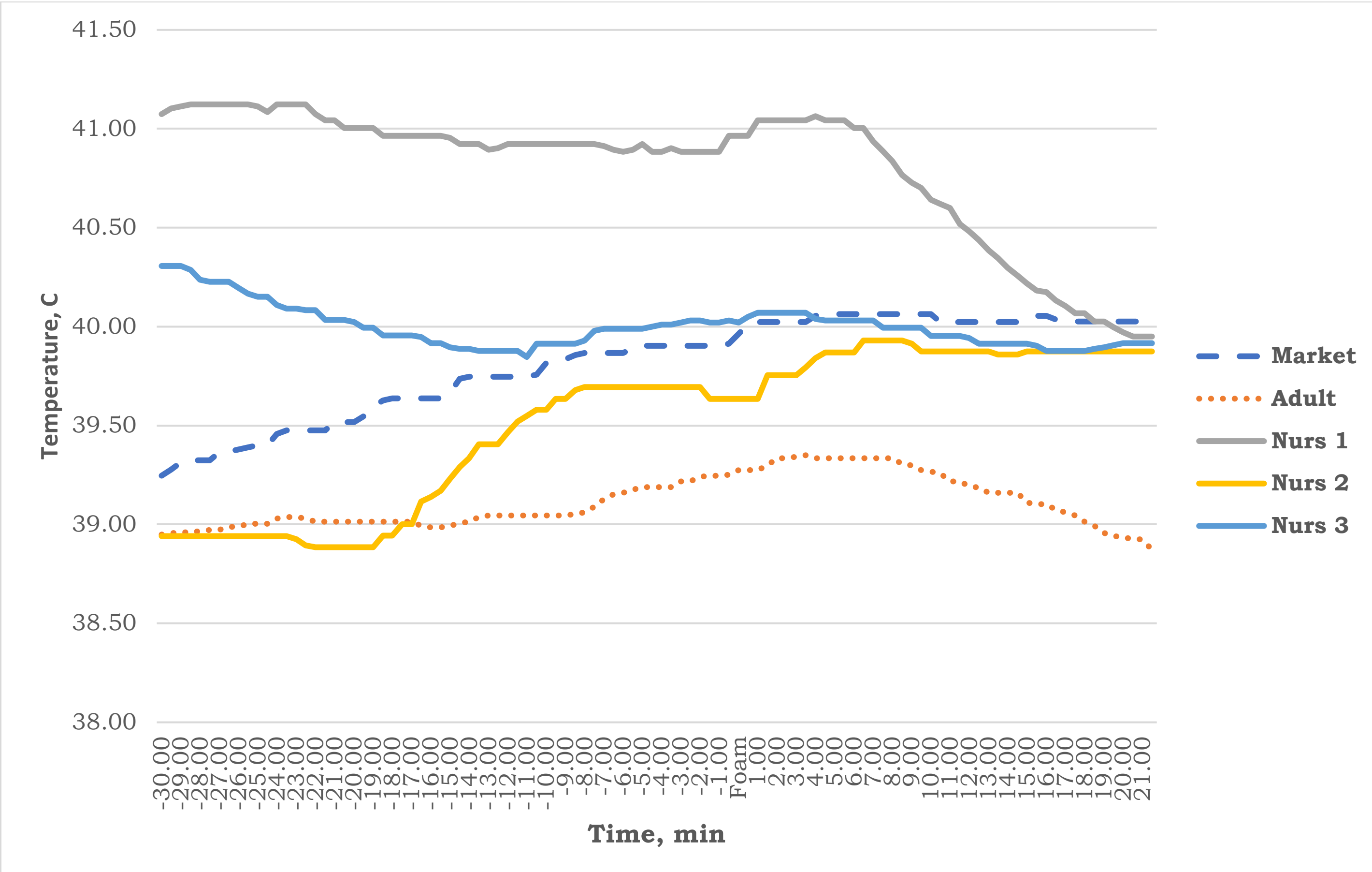

Figure 3a. Minimum, mean, and maximum heart rate of Market (n=3) evaluated animals from thirty minutes prior to foaming to 21 minutes post foaming

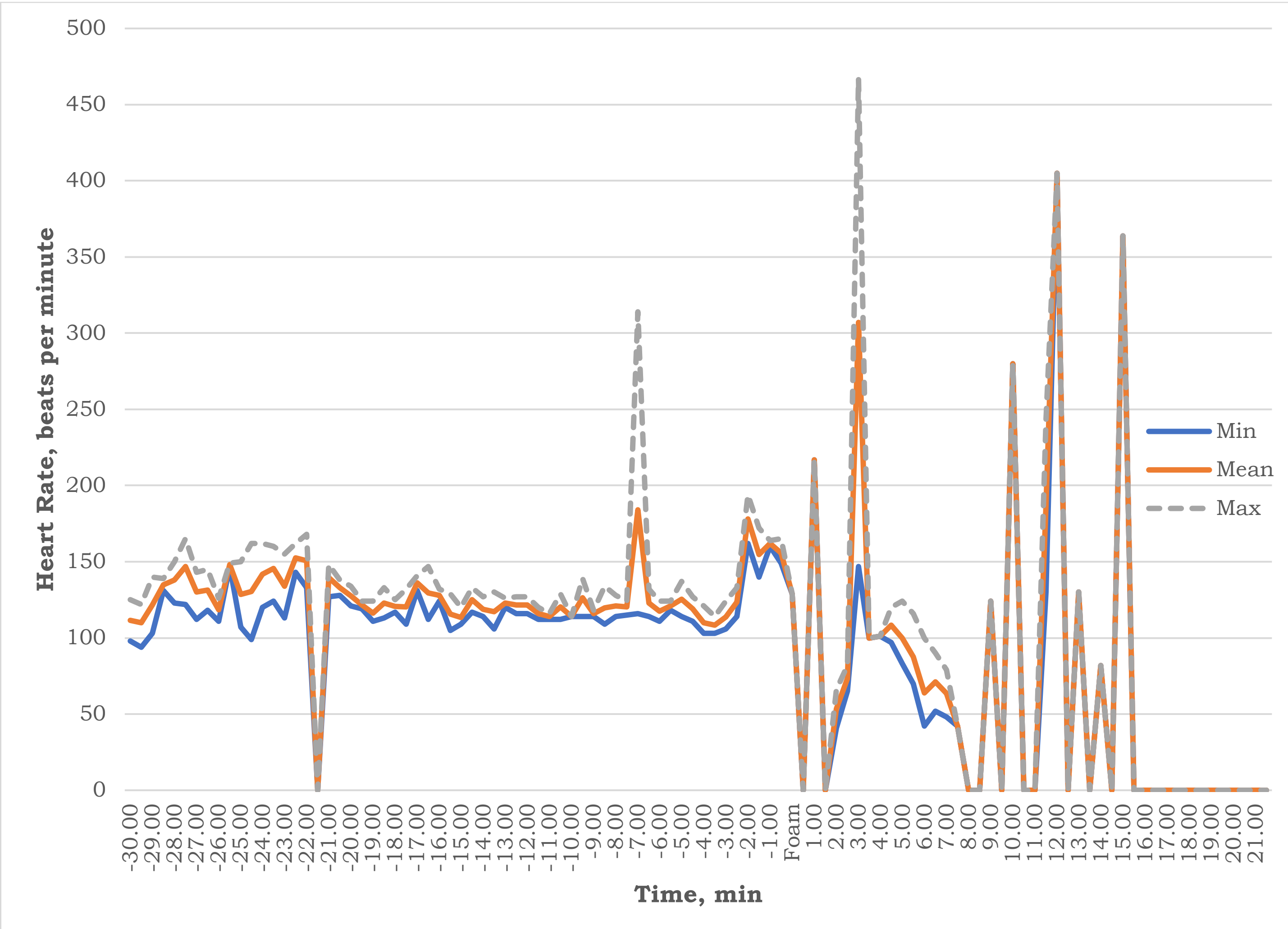

Figure 3b. Minimum, mean, and maximum heart rate of Adult (n=4) evaluated animals from thirty minutes prior to foaming to 21 minutes post foaming

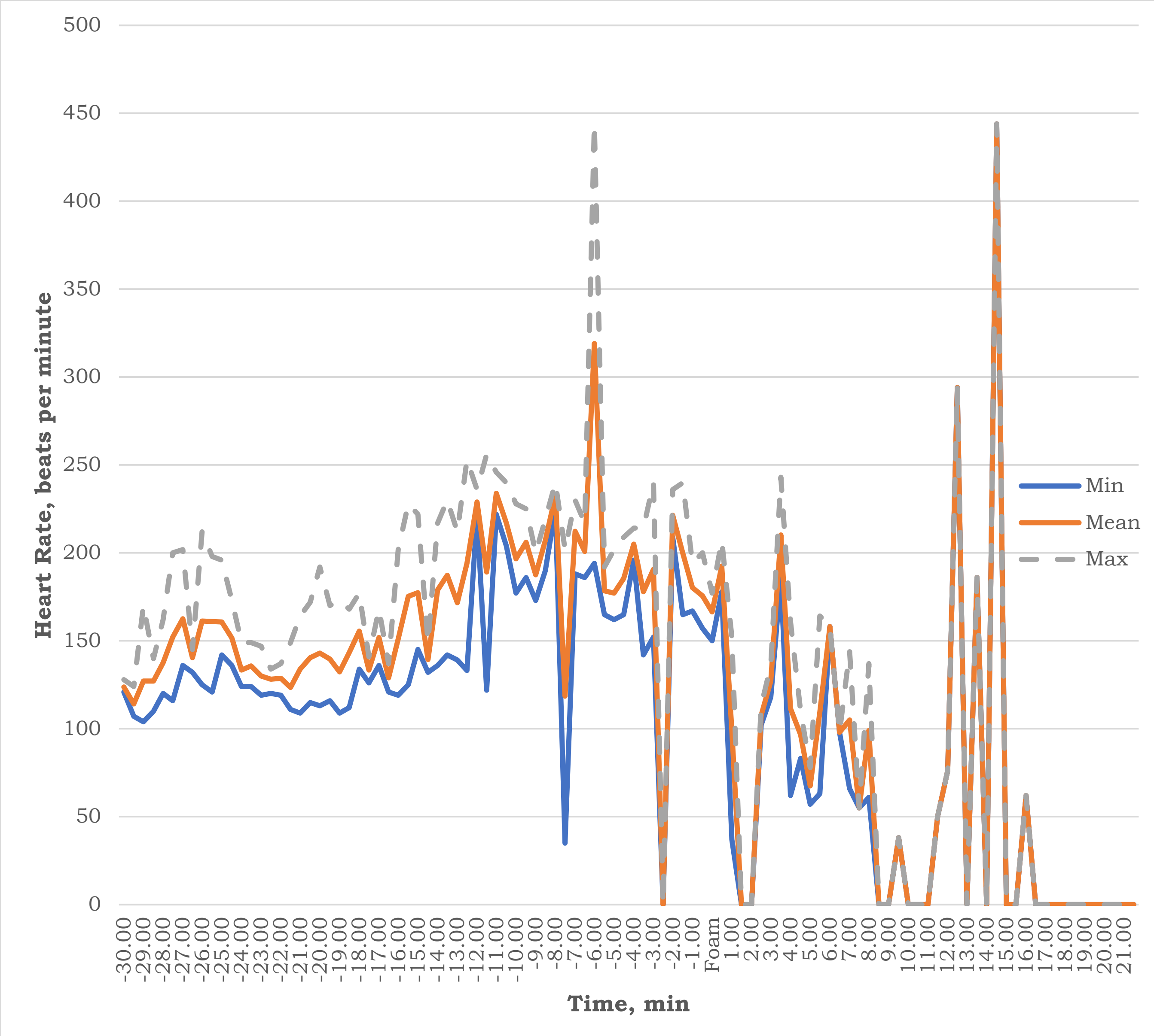

Figure 3c. Minimum, mean, and maximum heart rate of Nursery 1-3 (n=9) evaluated animals from thirty minutes prior to foaming to 21 minutes post foaming