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Effects of Maternal Infant-Directed Sensory Cues on Early Weaned Piglet Behavior

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Abstract

In the commercial pig production industry, weaning is a stressful time. Stress is related to loss in production due to increased incidence of illness, increased aggression, and reduced rate of gain, which results in economic losses for the producer. The purpose of this continuous behavior study was to explore the effects of provisional own-mother olfactory and auditory stimuli on early weaned piglet behavior and growth. During days 7-14 postpartum, the grunting of 12 individual sows was recorded while their piglets nursed. The audio files were put into a single loop with grunting occurring for 20 minutes followed by 60 minutes of silence. A cotton cloth was rubbed along the ventral midline of each sow and vacuum sealed to preserve scent. Eight piglets per litter (4 male and 4 female) for each of 12 litters were selected and randomly assigned to one of four treatment groups: control (CON, no scent, no vocal), scent (S), vocal (V), and vocal with scent (VS). Piglets were weighed and transported to an offsite nursery at 19 days of age. VS group was exposed to the vocalization loop for 7 days and cloths with their own mother's scent for 48 hours. V group was provided the vocalization loop for 7 days and cloths with no scent for 48 hours. S group was provided cloth with mother's scent for 48 hours. CON group was exposed to cloths with no scent for 48 hours. Behaviors deemed important to the study were approaching the scent towers curious, approaching the scent towers interested, approach feeder, feeding, and fighting. Behaviors were monitored with continual video recording for 7 days. Piglets weight was recorded on days 1, 3, 5, 7, 14, 21, 28, and 42. GLIMMEX Mixed Model Procedure SAS (Cary, NC) was used for data analysis. Results demonstrated that the scent groups had a significant reduction of fighting ($p<0.01$) and a significant increase in the interest of feeding and feeding behavior ($p<0.01$). The results suggest that providing scent shows a decrease in stress-induced behavior and were linked to an increase in feeding behaviors.

Further studies are needed to analyze the effects of vocalizations from other sow's and how scent affects return to normal behavior over a longer period.

Keywords: continuous behavior study, olfactory stimuli, auditory stimuli, weaning stress, piglet behavior

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Introduction

Background

According to USDA, in December of 2021, the United States reported having 74,201,100 hogs and pigs. Between September and November alone, there were 33.7 million pigs weaned on U.S. farms (Matlock et al. 2021). For commercial pigs, the weaning process involves an abrupt disruption of the mother-offspring bond by removing the piglet from the sow. This also includes shifting the piglets' diet to solid food and water only, and changing the piglets' environment, usually by moving to a collective nursery with many other piglets, comingling litters, thus, resulting in increased stressed early in life. Piglets in distress can undergo many physical changes, like intestinal and immune dysfunction, such as increased cortisol levels which cause a decrease in insulin with an increase in glucagon which affect metabolism. (Campbell 2013).

During periods of stress, feed intake may decrease, leading to reduced growth performance. Stress, even this early in life, is found to follow the piglet through the finishing stage, eventually affecting the price of how much the pig is sold for, meat quality, and carcass quality (Gatford et al. 2018). Physiologically, piglets can enter a hypermetabolic state which causes high temperatures and low pH's within the body. In result, muscle cells can be damaged, or heart failure may occur.

The effects of weaning are of particular interest in the animal management research field due to the fact that most domestic livestock are weaned in the first few months of life. Weaning affects swine, bovine, and ovine which goes on to affect the meat industry heavily.

Problem Statement

Weaning stress in piglets has affected the pork industry globally. Weaning stress can contribute to intestinal disease and immune dysfunction; therefore, causing a reduction in feed

intake and growth. In result, this affects pork quality, pricing, and pounds on the scale. There are currently limited continuous behavior studies on the weaning process of animals; many studies do “screen shots” which look at specific time intervals and record what happens within the interval. However, continuous behavior monitoring allows the recording of all behaviors exhibited through the entirety of the study. Lack of studies like this could be due to a lack of funding and time limitations. However, these types of studies allow for a more in depth investigation the types of behaviors expressed during a particular life stage.

Purpose Statement

The purpose of this study was to explore the effects of infant-directed vocalizations and scent of sows on piglets’ exhibited behaviors and weight gain. This study explored what behaviors were exhibited by the piglets when the infant-direct vocalizations were played on the recorder versus the control room where no recordings were played. In addition, the study explored what behavior the piglets exhibited when scent towers were presented versus the control when no scent was put onto the towers. This study focused on behaviors exhibited and weight gained with different treatments present versus when they were absent.

Research Objectives

1. Determine the effect of sows' scents on types of behavior exhibited by the piglets in the scent group when compared to the control group.
2. Determine the effect of infant-directed vocal communication of sows on types of behavior exhibited by piglets in the vocal group when compared to the control group.
3. Determine the effect of both infant-directed vocalizations and scents on piglets’ behavior in the vocal scent group when compared to the control group.

Hypotheses:

H_0: If own mother's scent and vocalization are present there will be no significant increase in interest and curiosity in scent towers, interest in feed tower, and actual feeding nor a significant decrease in fighting when compared to the control group.

H_A: If the own mother's scent is present then piglets will demonstrate a significant decrease in fighting incidence with significant increases in interest and curiosity in scent towers, interest in feed tower, and actual feeding when compared to control group.

H_2: If own mother's vocalization is heard then the piglets will demonstrate a significant decrease in fighting behaviors while exhibiting an significant increase in interest and curiosity in scent towers, interest in feed tower, and actual feeding when compared to the control group.

H_3: If own mother's scent and vocalization are present then interest and curiosity in scent towers, interest in feed tower, and actual feeding will increase significantly while agonistic behaviors (fighting) will decrease significantly when compared to the control group.

Literature Review

This section first discusses the importance of mother and infant communication as it contributes to newborn health. Next, it defines and explains the process of weaning. Proceeding this, it will outline the different types of behaviors typically found on ethograms in swine studies. Lastly, this section will dive into how continuous behavior monitoring studies work and examine past research.

Weaning:

Weaning of pigs is defined as the process in which the piglets are separated from their mother and shifted from milk and nursing to solid food and water (Oxford Dictionary et al.

1989). According to USDA, in December of 2021 the United States reported having 74,201,100 hogs and pigs. Between September and November alone, there were 33.7 million pigs weaned on U.S. farms (Matlock et al. 2021). For commercial pigs, the weaning process involves an abrupt disruption of the mother-offspring bond by removing the piglet from the sow. This also includes shifting the piglet's diet to solid food and water only, and changing the piglet's environment. This stage for "Early-weaned" (commercial) piglets begins around twenty-one days of age and continues for about two months (Taylor et al. 2006). Preceding weaning, piglets are typically in a farrowing crate with their mother to reduce mortality rate and optimize welfare. (Singh et al. 2016). The farrowing crate also promotes the nutrition of the piglets by making the teats more available (Singh et al. 2016). The first feeding period is the most important as it also allows for the intake of colostrum, which provide the piglets with energy and maternal antibodies to help protect them from infection (Devidich et al. 2005). Weaning is one of the most stressful events to occur in a piglet's life (Campbell et al. 2013), as it is the biggest change it undergoes; thus, making weaning stage an important event to understand.

Olfactory Stimuli:

Pigs use many senses, olfactory being included, to communicate with their conspecifics. A pig's nose is 2000 times more sensitive than that of a human. Pheromones from the mother allow pigs to find their specific mother and find the teats to suckle (Sommerville et al. 1998). The piglets are attracted to the udder due to mammary pheromones produced by the mammary tissue; the pheromones are picked up via the nasal cavity in the piglet and signal the hypothalamus and amygdala which then causes the piglet to suckle. McGlone and Anderson (2002) demonstrated that exploratory behavior, weight gain, and feed intake all increased when pigs were exposed to man-made version of the sows' pheromones. Another previous studied

done by Wells (2009) demonstrated that when the scent of maternal pheromones is removed from the environment then the piglets stop suckling. In addition, a study performed by Terry (2021) used a synthetic olfactory agonist (sows' scent) and found that it was correlated with reduced aggressive behaviors during weaning (Terry et al. 2021).

Auditory Stimuli:

Sows use auditory cues within the farrowing crates to communicate with their piglets. Sows vocalize before laying down to signal to their litter to move so they do not get crushed; one of the many causes of piglet death is due to the crushing of the piglet beneath to body of the mother (Kinane et al. 2021). Frequency and amplitude in sow vocalizations are important as different vocalizations hold different meanings (Murphy et al. 2014). In addition, different durations of the auditory cues while sows communicate with their litter can mean different things; there are currently about 20 different recognized sounds produced by pigs (Valla et al. 2019). One sound of a certain frequency and duration communicates play while the same vocalization at a different duration could indicate an alarming situation (Cordeiro et al. 2013). Previous experimental results have demonstrated that vocalization coming from a pig can affect the behavior of the other pigs surrounding it (Bensoussan et al. 2016). Nian (2023) performed a research study in which piglets were exposed to auditory enrichment, and in result, demonstrated that auditory stimuli were correlated to an increase in immune response, tail wagging, playing, and exploring. (Nian et al. 2023).

Behavior:

Piglets show a variety of behaviors depending on their physical, mental, and emotional state (Ridder et al. 2021). Pigs are curious creatures and tend to investigate the environment they are in. During weaning period, piglets demonstrate behavioral and psychological stress responses such as a reluctance to eat, which then leads to decreased body weight and decreased growth rate (Camerlink et al. 2018). Piglets in distress can undergo many physical changes, like intestinal and immune dysfunction, which causes pigs to demonstrate stress-induced behaviors (Campbell 2013). In order to adequately address the problem of stress during weaning one must increase animal welfare through promoting positive social interactions and offer exploratory material for piglets to induce positive behavior (Camerlink et al. 2018).

Economic Impact:

In nature, piglets are weaned very gradually from their mothers, usually around 10-12 weeks of age, though some may stay with their family unit several months later. To shorten generational intervals and increase sow production potential, commercial piglets are weaned much younger, around 2-4 weeks of age (Holman et al. 2021). Subsequently, sows typically return to estrus (heat) 5 to 6 days after weaning (Knox et al. 2022).

As a result, weaning commercial pigs early allows for more piglets weaned per sow per year. Thus, the earlier weaned the more efficient the sow is on producing more piglets for the industry. In addition, it has been found that earlier weaned pigs are less susceptible to harmful pathogens, such as Clostridium perfringens, Escherichia coli, Salmonella spp. (Holman et al. 2021). Due to the fact that the Veterinary Feed Directive (VFD) has restricted the type and amount of antibiotics commercial pigs receive, it has become more important to inhibit the transmission of harmful pathogens and disease. The goal of weaning pigs early and healthily is to

get them to an ideal marketing weight. Ultimately, market weight affects profitability thus, making weight an important economical factor (Wu et al. 2017).

Methods and Materials

Animals:

Ovine, PIC- 29: Pig Improvement Genetics, Study protocol was approved by the Agricultural Institutional Animal Care and Use of the University of Arkansas (Protocol # 22025).

Site:

University of Arkansas Savoy Swine Research and University of Arkansas off campus nursery.

Experimental Design:

Pre-Weaning:

Once approval of personnel, protocol, IACUC approval, and Citi training was conducted the work for the study began. All workers wore shoe covers, gloves, and disposable coveralls while working with animals. At the University of Arkansas Savoy Swine Research Unit, 12 sows and their litters were selected for the study. The sows were selected based on similar parity, age, and expected litter size. During weeks 2-3 of lactation, an audio recorder was placed on the outside of the sows' pens close enough to the snout to pick up grunting, but outside of the sow's reach. (Appendix L). The sows were each recorded for 2 hours, with a researcher monitoring the recording to ensure the vocalizations were collected while piglets were actively feeding. We then used GoldWave v6.80 by Softonic for audition analysis and editing (Appendix N) of all 12 sows vocalizations. We made an audio loop consisting of 20 minutes of grunting noises that was followed by 60 minutes of silence to mimic nursing in a natural environment. In addition, during weeks 2-3 of lactation my team and I collected scent from each sow by rubbing a 100% cotton

cloth down the ventral midline (Appendix M), between the teats, being careful not to get excess milk on it and then vacuum sealed them to preserve the smell. We also had 12 100% cotton cloths with no scent collected vacuum sealed to use for the non-scent groups. Eight (8) piglets from each of the 12 litters (4 females and 4 males) were weighed, blocked, and randomly assigned to one of the four experimental groups: control (CON, no scent, no vocal), scent (S), vocal (V), and vocal with scent (VS). Piglets were castrated prior to transportation. Preceding the transportation of the 96 piglets, eight cameras were set up at the off-site nursey, 4 per room, and positioned to record 3 pens each. Cameras were secured in position with duct tape and zip ties. The cameras were NightOwl security brand which allowed for continuous behavior monitoring for the first 7 days, that could be watched on a computer.

Weaning:

When the piglets reached 19 days of age, they were weaned and transported from the swine birthing and nursing unit to an off-site nursery. There were 24 total pens, 12 pens in each room, and each pen initially held 4 piglets. There were no duplicate pigs, pigs from the same sow, in the same pen. The piglets were either placed in the East side room, which was used to test the effects of vocalizations or the West side room which played no auditory stimuli. Both rooms were set to 85 degrees Fahrenheit and stayed there the remainder of the study. Upon arrival, all pigs were weighed and had continuous access to water. After getting all 96 piglets into the correct pens we set up the scent towers (Appendix O). We used zip ties, duct tape, and the vacuum sealed towels that were collected at an earlier date. Each room had 6 pens with only one scent tower set up in front of it with the cotton cloth that had not been rubbed on any sow. The other 6 pens in the room had 4 scent towers set up in front of them, each tower consisted of the piglets' own mother's scent. Therefore, piglets in the East side with 4 scent towers were in the VS group. The piglets in the East side with 1 scent tower were in the V group. The piglets in

the West side room with 4 scent towers were in the S group. Lastly, the piglets in the West side room with 1 scent tower were the CON group.

Piglet weight was recorded on days 1, 3, 5, 7, 14, 21, 28, and 42 of weaning. During the weight recordings we also weighed the feed towers, then filled the feeder up and weighed it again to see how much feed was eaten since the previous weigh day. Both the S and VS group were exposed to own-mother's olfactory stimuli for 48 hours. After 2 days the scent towers were taken down and the vocalization loop began to play for the V and VS group. The vocalization loop played on for 20 minutes and off for 60 minutes for 24 hours a day for 5 days.

Post-Weaning:

Preceeding the study, the Night Owl camera system was used to watch continuous behavior. An ethogram was made based on extensive research of other studies and within limits for one person to observe at a time (Appendix E). Behaviors of interest in the study were approach scent tower curious, approach scent tower interested, approach feed tower, feeding, and fighting. Approach scent tower curious was defined as spending 2-4 seconds by the gate with nose pointed out toward the scent tower (Appendix I). Approach scent tower interested was defined by spending greater than or equal to 5 seconds by the gate with nose pointed out toward scent tower or laying down as close as possible to the tower (Appendix I). Approaching the feed tower was defined by going up to the feed tower for less than or equal to 2 seconds, but not actually feeding (Appendix G). Feeding was defined as nose and mouth inside the feeder greater than or equal to 3 seconds (Appendix H). Lastly, fighting was defined as ear/tail biting, chasing, and/or head butting for greater than or equal to 3 seconds (Appendix J). We recorded all these behaviors for 48 hours. The behaviors were sampled one hour at a time via a tally mark system. Each time a piglet in a pen would exhibit a behavior a tally mark was placed under that specific pen. After an hour's time, the data was put into excel to later be used for the data analysis. This

totaled to 4,032 hours, but we could watch 6 pens at a time and speed up time as necessary. The observational unit was the pen, as their behaviors were recorded under their pen number, and not specific to the individual piglet. The GLIMMEX Mixed Model Procedure SAS (Cary, NC) was used to analyze the data.

Results:

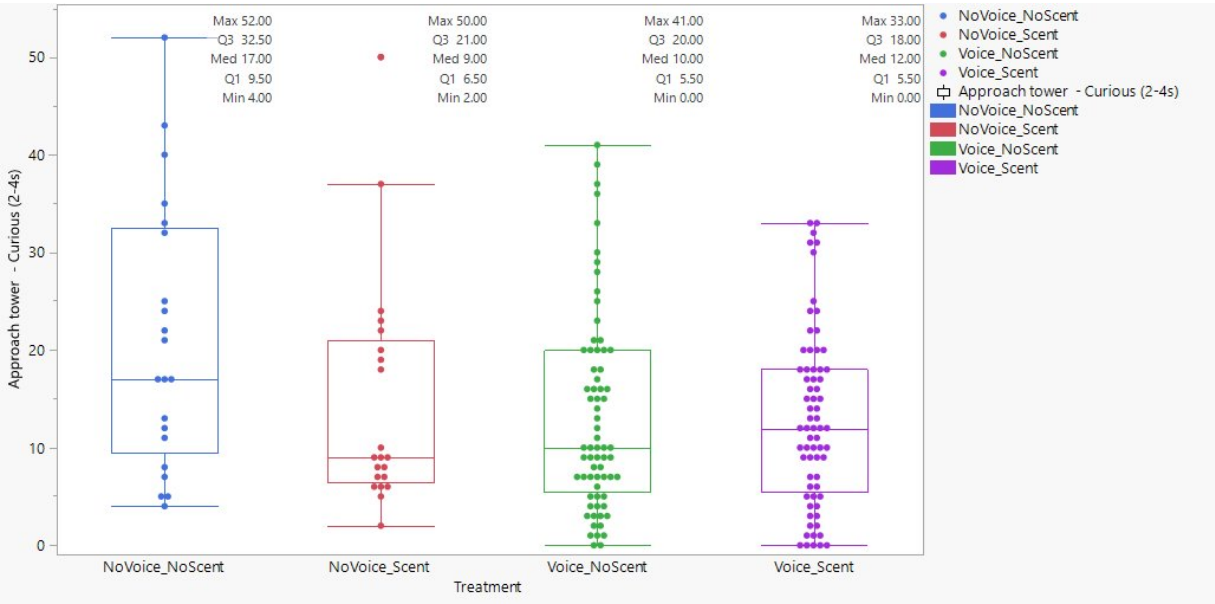
Subplot treatment factor was used for the scent data due to own-mother's scent being present along with others sows scent for other piglets in the pen, and scent treatment could not be absolutely randomized. Whole plot treatment factor was used for the vocalization data as the vocal room and non-vocal room was separated by a room in the middle dividing the sound vs. no sound treatments. In the previous section it was noted that weight of piglet and feed tower was recorded; however, this was analyzed in a different study.

Figure 1 (A) demonstrated that piglets showed no significant difference between treatment groups in approaching towers curiously with scent vs. no scent ($P>0.10$). Figure 1(B) exhibited that piglets showed no significant difference between treatment groups with approaching the towers interested ($P>0.10$). Figure 2 displayed that piglets exposed to scented cloth towers showed a significant reduction in agonistic behaviors when compared to groups with unscented cloth towers ($P<0.01$). Figure 3(A) revealed piglets exposed to scented cloth towers showed a significant increase in interest of feeders ($P<0.01$). Figure 3(B) demonstrated that piglets exposed to scented cloth towers demonstrated a significant increase in feeding ($P<0.01$). All of the figures exhibited that vocalizations had no significant effect on any of the behaviors deemed important to the study. However, Figure 2 displayed a trend (not a significant difference) that fighting behaviors tended to increase when vocalizations were played. Table 1 shows the total number of times each pen/ treatment performed each behavior within the first 48

hours. However, this is just an example of how we collected the data into the excel sheet and not all pens were included in table 1.

Data Figures:
Figure 1:

A



B

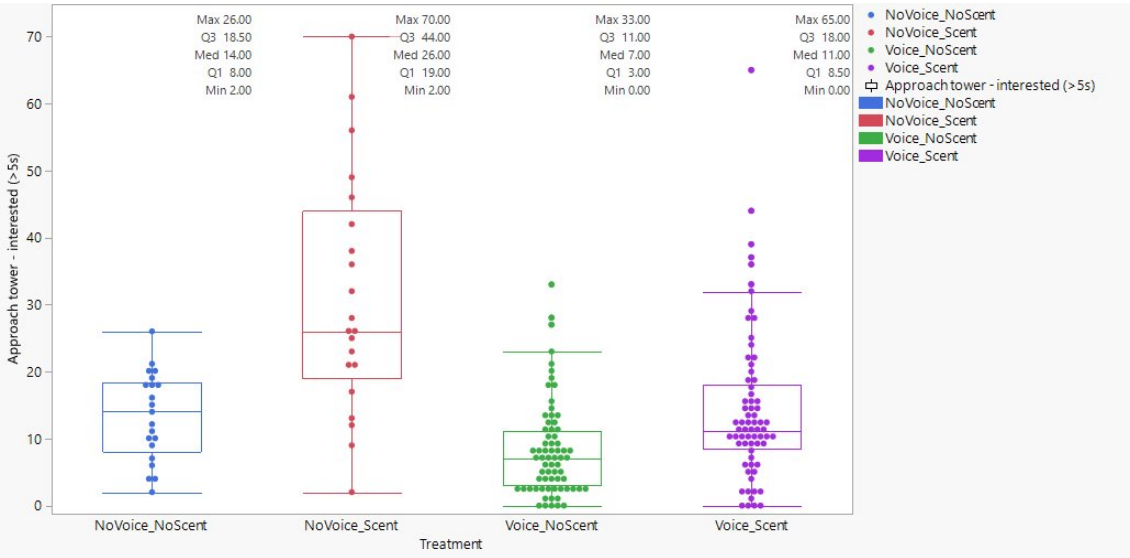


Figure 2:

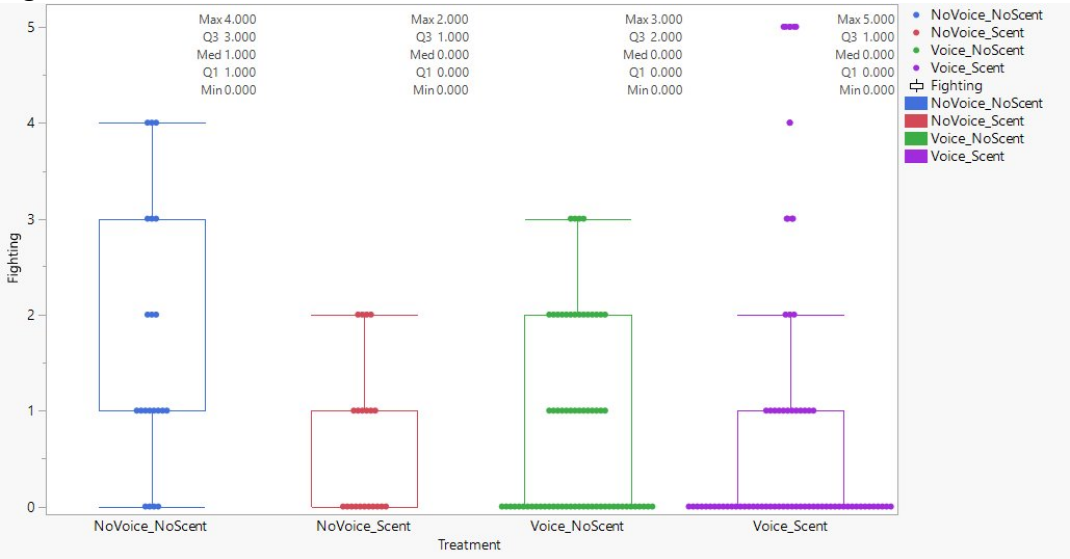
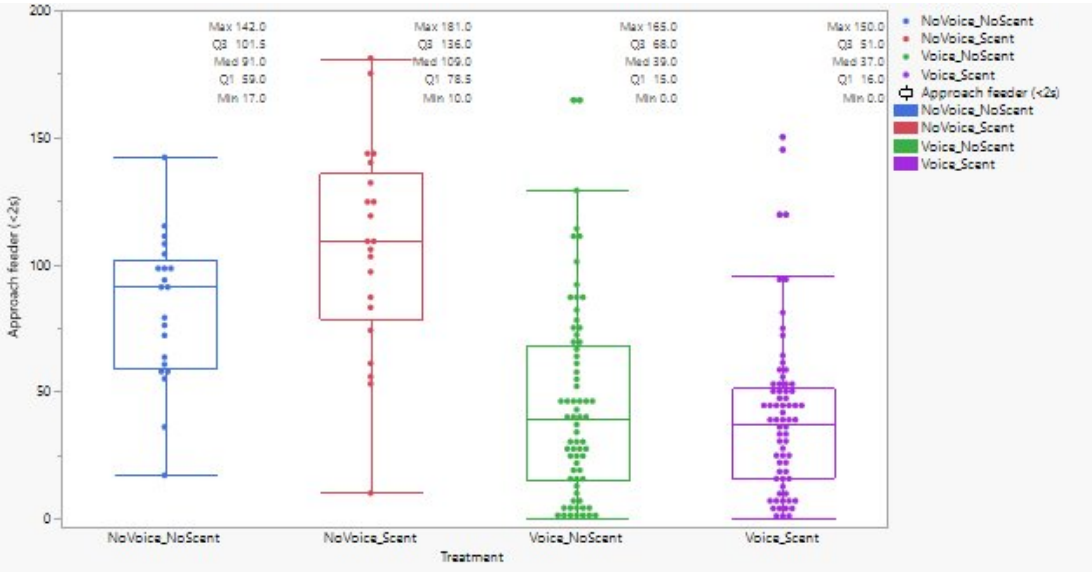


Figure 3:

A:



B:

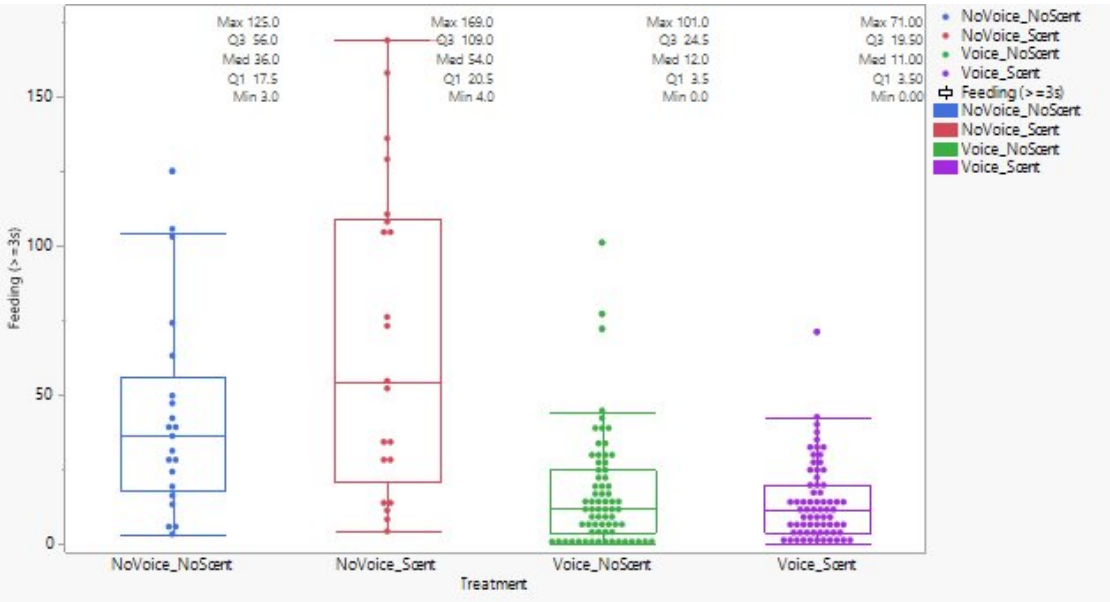


Table 1:

Pen	Treatment	Approach feeder (<2s)	Feeding (>=3s)	Approach tower - Curious (2-4s)	Approach tower - interested (>5s)	Fighting	ApproachFeeder/hr
W1	NoVoice_NoScent	578	263	149	100	4	205.619752
W2	NoVoice_NoScent	577	282	178	89	19	206.0001526
W4	NoVoice_NoScent	567	348	116	91	12	190.6122147
W6	NoVoice_Scent	645	526	83	127	5	236.7469945
W7	NoVoice_Scent	801	483	99	275	3	248.4362849
W8	NoVoice_Scent	781	438	123	251	6	278.0038274
E11	Voice_NoScent	738	117	125	64	8	445.4038855
E12	Voice_NoScent	471	137	112	93	13	266.8640082
E6	Voice_NoScent	332	191	124	69	5	280.2763804
E7	Voice_NoScent	679	367	274	173	12	580.5984105
E8	Voice_NoScent	419	179	136	95	8	382.2298361
E9	Voice_NoScent	460	138	142	74	8	291.9802318
E1	Voice_Scent	450	183	238	194	7	423.915315
E16	Voice_Scent	623	149	111	122	7	420.2239264
E17	Voice_Scent	534	118	122	168	7	262.4536469
E18	Voice_Scent	363	195	91	242	7	199.0773688
E3	Voice_Scent	520	150	224	187	13	441.7954567
E4	Voice_Scent	267	136	103	82	2	235.3727804

Discussion

Significance:

Piglets exposed to their own mother's scents demonstrated higher activity in behavioral observations of interest in feed towers and actual feeding ($P<0.01$). Results suggest that piglets

exposed to their sow's scent were able to return to feeding activity faster than the piglets that were not exposed to own mother's scent. These results were supported by McGlone and Anderson's study (2002), which demonstrated that feed intake increased when exposed to sow's pheromones. Wells (2009) study demonstrated piglets will stop suckling upon removal of maternal pheromones from lactating mothers, which supports how important olfactory senses are in feeding behavior. Piglets exposed to their own-mother's scent also demonstrated a significant decrease in fighting incidence ($P<0.01$). Our results aligned with Terry's study done in 2021 where they found that their olfactory agonist correlated with a reduction in agonistic behaviors. Future research may be extended to exposure of scent to 21 days, natural weaning time, to analyze the true effects on reducing stress and increasing feeding behavior. Hnull, H2, and H3 were all rejected, while H2 was the accepted hypothesis for our study based on results.

Additionally, own mother's vocalization also increased piglet activity. Although the vocalizations did not promote positive or affiliative behavior in pigs our results matched those of Bensoussan's 2016 study which demonstrated that sow vocalizations caused activity in the surrounding pigs. However, activity due to vocalizations had a trend in increasing stress-related behaviors such as fighting. Camerlink's study (2018), which discussed the importance of reducing stress in the weaning stage by exhibiting that piglets exhibiting stress response behaviors demonstrated a reluctance to feed. This reluctance to feed led to a decreased body weight. Their study illustrated that weaning stress can be reduced through promoting positive social interactions and offering exploratory material as pigs are curious animals. Further research is needed to determine the effects of unrelated sow's vocalization on feeding behavior and long-term effects of own mother scent exposure.

Issues:

Possible cofounding variables that may have impacted this study include: 4 pens full of piglets that were not in the study were dropped off at the same off-site nursery that the study was taking place. East pens 1 and 8 were next to the pigs not in the study. As a result, the potential for disease transmission was increased. The non-study pigs were also smaller and able to get out of their pens; thus, knocking over some of the scent towers. Lastly, on the day of arrival to the nursery, one of the piglets had been castrated in the truck. In result, this piglet may have had higher cortisol levels. This may have affected behavior recordings as the piglet was less mobile, possibly due to pain. Another piglet in the study had a hernia and was not castrated. This could also affect behavior due to an increase in testosterone when compared to the castrated piglets.

Next Steps:

Since this was a pilot study, another study just like this one is expected to be performed. In this next study a few adjustments will be made to get more accurate results. In future studies researchers will make sure that only piglets within the study are present, all piglets are castrated before transport to the facility, feed and water and towers ready upon arrival, ensure all piglets stay within their pens, build sturdier scent towers, and minimize the amount of time piglets are exposed to humans. In the next study we want to examine the effects of unrelated sow's vocalization on indication of stress-related behaviors versus own mother's vocalizations. In addition, scent stimuli will be present for 21 days to mimic natural weaning time. The future study will exercise more caution, use more materials, and use information gathered from this pilot study. Continuous studies are hard and rare to come by; our hope is to find more information with each study we perform. Our goal is to figure out how to cause the least amount of stress to piglets during the weaning phase of their life, as this phase affects all other phases.

Conclusion

Overall, the results suggested that piglets exposed to mother's own scent had a faster transition in adapting to new feeding behavior. These results demonstrate that scent stimuli from piglet's own mother help reduce stress during the weaning phase and own mother vocalization induces stress during the weaning phase. If producers provide olfactory stimuli from the mother's mammary pheromones their piglets should have a significant increase in interest in feeding and actual feeding behavior. More interest in feeding and more intake of feed is correlated with more weight gain in the piglet. Heavier weights are correlated with higher selling prices of piglets to feed lot. The higher selling price allows the producer to have more money in their pocket.

The results also demonstrated that providing own-mother olfactory stimuli resulted in a significant decrease in stress-induced behaviors (fighting). Although, vocalizations did not demonstrate a positive affect on piglet behavior there was a trend of increased agonistic behavior when mother's own vocalization was played. These results demonstrate that own mother vocalizations may induce stress during the weaning phase, while providing own mother scent may help reduce stress during the weaning phase. The least amount of stress during weaning is ideal as weaning effects all other stages to come in a piglets' life. Less stress is correlated with less disease, higher carcass weight, and more money in the producer's pocket.

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Appendix A

Funding Grant

DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

Arkansas Agricultural Experiment Station
RESEARCH INCENTIVE GRANTS

Purpose: The Research Incentive Grants (RIG) program is designed to give early career investigators an opportunity to generate proof-of-concept, preliminary data, or explore emerging areas of research. Funds will be used to leverage extramural grants and contracts. Participants must identify further funding opportunities to be competitive for RIG.

Eligibility: All early career faculty with an appointment in the Agricultural Experiment Station will be eligible to apply for RIG funding. Early Career is defined as faculty in the first 7 years of employment who have a research appointment with the Arkansas Agricultural Experiment Station. A faculty member can only be awarded RIG funding once.

Source of Funds: A portion of indirect costs collected by AAES from extramural grants and contracts will be dedicated each year to the RIG. RIG funding from AAES will require a 1:1 match from Departmental RIF accounts. The amount of funding available for the RIG program will be determined annually by the Senior Associate Vice President-Research.

Amount and Duration: Awards will be made for a maximum of $30,000 including the departmental match. Awards will be a one-time allocation and are not eligible for renewal. Funds must be expended within 24 months of the initiation date.

Annual Process: Investigators are invited to submit one proposal electronically in PDF format following the proposal template included below. The call for proposals will be issued from the Director's office on February 1 each year, with a submission deadline of March 15. Proposals must be submitted by email to the department assistant, who will upload to the RIG Box file.
(1) All Files | Powered by Box

Submissions are limited to 3 pages excluding citations, budget, and current and pending support. Multidisciplinary projects are encouraged. Senior faculty members (i.e. not fitting the early career criteria defined above) cannot serve as co-PIs but can serve the role of collaborator on the project. Companion proposals submitted by two PIs to work on a common and collaborative project may be considered.

A committee of peers appointed by the Director will individually and anonymously review and rank each proposal according to scientific merit, potential for leveraging of additional funds, and likelihood of accomplishing objectives. All RIG recipients agree to serve on the peer review committee in subsequent years.

Deliverables: An interim report will be due on March 1 following the award. A Final Report will be

submitted upon completion of the project and will include detailed information regarding leveraged funding opportunities the investigator is pursuing including the funding agency and the specific program targeted and/or grants that have been awarded as a result of RIG funding.

As recipients of RIG funding, faculty members commit to complete a trip to visit with federal program officers sometime during the span of the funded project. The federal agency trips will be organized and funded by AAES. In addition, investigators receiving RIG funding are encouraged to volunteer to serve on a grant review panel.

Research Incentive Grant Proposal Format:

The proposal (item 1-6) is limited to 3-pages. Budget format should follow the format on the next page. A blank Current and Pending Support Form – CSRRES 2005 in PDF will be provided with the RFP call.

Please submit a single document in PDF format and name the file:

PI-LastName_DEPT_RIG_2022.

The proposal should follow the format below:

1. **Project Title**
2. **Principle Investigator** (Name, title and Department/Unit)
    a. Co-PIs
    b. Collaborators
3. **Project performance dates**
4. **Project Summary/Abstract**
5. **Project Narrative**
    a. Introduction (including goals and objectives)
    b. Rationale and Significance
    c. Approach (including methods and timetable)
6. **How will this project be leveraged to apply for extramural funding?**
7. **Citations**
8. **Budget**
9. **Current and Pending Support**

Appendix B

Approval of Protocol

DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

To: Yan Huang
Fr: Billy Hargis - Ag-IACUC Chair
Date: April 22nd, 2022
Subject: IACUC Approval
Expiration Date: April 20th, 2025

The Division of Agriculture Institutional Animal Care and Use Committee (Ag-IACUC) has APPROVED your protocol # 22025 *Effects of Infant-direct Vocalization of Sows on the Growth Performance and Weaning Stress of Piglets*.

In granting its approval, the Ag-IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the Ag-IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond April 20th, 2025 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy, the Ag-IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Yan Huang, Tsung-Cheng Tsai, Sarah Shelby, and Yongii Wang. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The Ag-IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

BMH/tmp

22025

Appendix C

Approval of Personnel

DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

To:	Yan Huang
Fr:	Billy Hargis - Ag-IACUC Chair
Date:	May 20th, 2022
Subject:	IACUC Approval
Expiration Date:	April 20th, 2025

The Division of Agriculture Institutional Animal Care and Use Committee (Ag-IACUC) has APPROVED your personnel addition(s) of Mattie Still, Mason Paladino, Palika Morse, and Robert Story to protocol # **22025:** *Effects of Infant-direct Vocalization of Sows on the Growth Performance and Weaning Stress of Piglets*.

In granting its approval, the Ag-IACUC has approved only the addition of the personnel listed. Should there be any further changes to the protocol during the research, please notify the Ag-IACUC in writing (via the Modification form) prior to initiating the change.By policy the Ag-IACUC cannot approve a study for more than 3 years at a time.

The Ag-IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

BMH/tmp

Appendix D

CITI Training Certificate

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI PROGRAM)
COMPLETION REPORT - PART 1 OF 2
COURSEWORK REQUIREMENTS*

* NOTE: Scores on this Requirements Report reflect quiz completions at the time all requirements for the course were met. See list below for details. See separate Transcript Report for more recent quiz scores, including those on optional (supplemental) course elements.

- **Name:** Mattie Still (ID: 11154455)
- **Institution Affiliation:** University of Arkansas Fayetteville (ID: 1277)
- **Institution Email:** mmstill@uark.edu
- **Phone:** 4794668684

- **Curriculum Group:** Working with the IACUC
- **Course Learner Group:** Same as Curriculum Group
- **Stage:** Stage 1 - Basic Course
- **Description:** The CITI Basic Course in Laboratory Animal Welfare for Investigators, Staff and Students.

- **Record ID:** 48737035
- **Completion Date:** 09-May-2022
- **Expiration Date:** 08-May-2025
- **Minimum Passing:** 80
- **Reported Score*:** 100

REQUIRED AND ELECTIVE MODULES ONLY	DATE COMPLETED	SCORE
Working with the IACUC: Introduction (ID: 17660)	02-May-2022	No Quiz
About the IACUC (ID: 17661)	02-May-2022	5/5 (100%)
Federal Laws, Policies, and Guidelines (ID: 17662)	02-May-2022	5/5 (100%)
Planning Research and Completing the Protocol Form (ID: 17663)	02-May-2022	5/5 (100%)
Procedures: Surgery, Antibody Production, and Blood Collection (ID: 17664)	02-May-2022	5/5 (100%)
Personnel and Their Welfare (ID: 17665)	09-May-2022	5/5 (100%)
Special Animal Welfare Considerations (ID: 17666)	09-May-2022	4/4 (100%)
Making Changes to an Approved Animal Use Protocol (ID: 17667)	09-May-2022	3/3 (100%)
Reporting Animal Use Concerns (ID: 17668)	09-May-2022	3/3 (100%)

For this Report to be valid, the learner identified above must have had a valid affiliation with the CITI Program subscribing institution identified above or have been a paid Independent Learner.

Verify at: www.citiprogram.org/verify/?k630a165c-360d-43cc-9d84-16d5870e8896-48737035

Collaborative Institutional Training Initiative (CITI Program)
Email: support@citiprogram.org
Phone: 888-529-5929
Web: https://www.citiprogram.org

Appendix E

Swine Ethogram

Response to New Environment Behaviors		
Agonistic behaviors		
Fights	Mutual pushing of opponent with the head, lifting opponent with snout, could be with or without biting	Fights that last <3 is scored as mild. Fights that last 3-5 seconds are scored as moderate. Fights that last >5 seconds are scored as severe
Head-Butting	Knocks head against the head of opponent with a vigorous upward motion	3 or more knocks between 2 pigs in retaliation is considered a fight
Biting	Biting with manipulation can be ear or tail directed	<3 seconds can be considered play or a notion to move. 3-6 seconds can be scored as moderate. >6 can be scored as severe
Locomotion		
Pacing	Walking back and forth and/or around the pen at a brisk speed	Anything under 20 seconds is considered negligible. <30 seconds of pacing is scored as mild pacing. 30-45 seconds of pacing is scored as moderate pacing. 45< seconds of pacing is scored as severe pacing
Attempting Escape	Head-butting gate, squeezing through the gate, attempting to jump over gate	>5 seconds is considered an attempt at escape. * If pig gets out of the pen attempt was successful
Resuming Normal Behavior		
Position		
Lying/ Sleeping	Sternum to floor without support of the legs; eyes open or closed	Lying on floor with legs tucked under body or in huddles indicates pigs are cold. Lying on side with legs out indicates comfortable. Lying away from other pigs on a cold fence, panting and/or pigs are covered in feces indicates pigs are hot
Curiosity		
Walking around	Walking around environment at a normal pace	Walk up to front of pen and doesn't stay. Biting the material in the pen
Checking out feed tower	Walk up to feed tower; sniff the tower, lay right by the tower	Standing and/or sniffing feed tower for 3+ seconds
Playing	One pig chasing another, tugging loosely on an object, reciprocal	<5 seconds= slight 5-10 seconds= adequate

	non-aggressive biting, nudging of another pig	>10 seconds= extreme
Foraging	Walk up to feeder; put head in the feeder; eat	Head inside feeder for 3+ seconds
Olfactory Cues		
Curiosity		
Checking out scent towers	Snout on or out of gate closest to the feed tower	<2 seconds= negligible 3-5 seconds= curious >5 seconds= interested

Vocalization Cues	
Walks to feeder Y/N	Note how many pigs out of 4 walks to feeder when sow noises play
Foraging Y/N	Note how many pigs out of 4 walks to feeder and eat when sow noises play
Remains still Y/N	Note how many pigs out of 4 don't respond to the sow noise playing

Appendix F

Scent Tower

Appendix G

Approach Feeder

Appendix H

Feeding

Appendix I

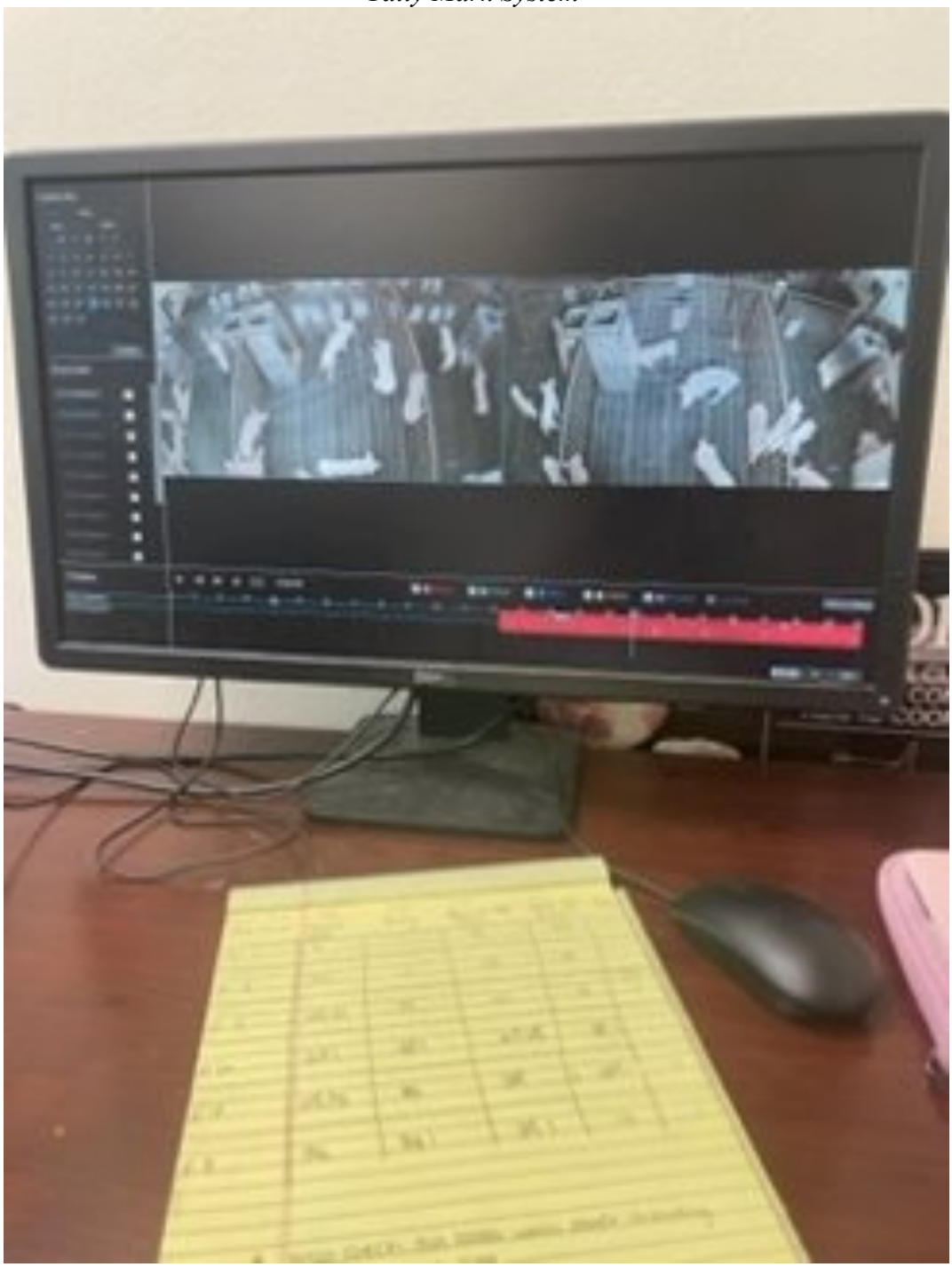
Approach Scent Tower Curios (first) and Interested (second)

Appendix J

Fighting

Appendix K

Tally Mark System



Appendix L

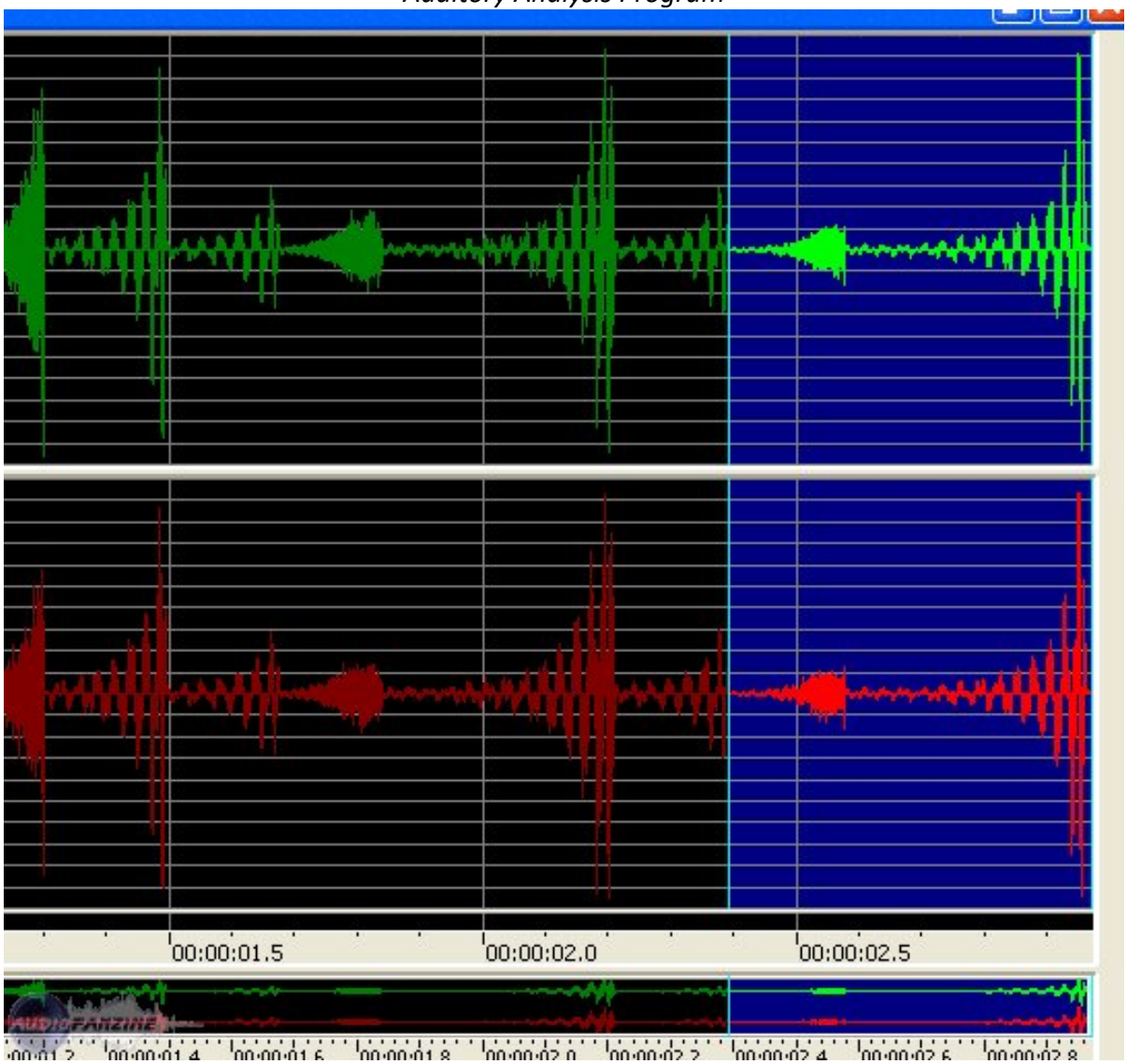
Recording Sows

Appendix M

Ventral Midline (where cloth was rubbed)

Appendix N

Auditory Analysis Program



Appendix O

Scent Tower