5-2023

Effects of Sow Vocalization and Scent on complete blood cell count during early weaning period

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An Honors Thesis submitted in partial fulfillment of the requirements of Honors Studies in Biology

By

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Spring 2023

Biology
Fulbright College of Arts and Sciences
The University of Arkansas

Acknowledgements:

The author would like the thank Dr. Tsung Cheng Tsai and Dr. Yan Huang for being instrumental in the writing process, experimental setup, and data collection. He would like to thank Sarah Shelby, Robert Story, and Mattie Still for help with experiment set up and data collection.

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1. Abstract

Piglets are deprived of their sows' touch, sound, and smell after weaning which results in biological stress. The present study investigated continuing exposure of sow vocalization and scent during the early weaning period on complete blood cell count. A total of 12 litters, 8 piglets (4 male and 4 female) per litter were selected at weaning and assigned to 1 of 4 experimental treatments: control, O (olfactory group with scent and no vocal stimulation), V (non-olfactory with vocal stimulation), VO (vocal stimulation and olfactory stimulation) as a 2x2 factorial arrangement. Vocal stimulation was emulated by an audio recording of the sows, which was gathered between day-7 and day-14 of sow lactation. It was collected from 6 sows and compiled into a comprehensive audio file including all sows' vocal output. The VO group was exposed to sow vocal for 20 minutes, followed by an empty hour on loop for seven days postweaning. For olfactory stimulation, cotton fabric was rubbed on the sows' udders and vacuum sealed. The respective sows' scent was placed in a mesh basket outside of the pen for approximately 48 hours. Blood samples were collected on day-1, day-2, day-6, and day-28 into K2EDTA vacutainer tubes. The blood was processed within 6 hours of collection using an automatic hematological analyzer (Hemavet 950S). Data was analyzed using mixed procedure of SAS (Cary, NC) with vocal, scent and day as main effects. Interactions between day x vocal showed statistical significance ($P = 0.0215$) with both the vocal and non-vocal groups having a reduction on mean platelet volume (MPV) from d 0 to d 2 and while the non-vocal group continued an upward trend from d 2 to d 28, vocal group failed to restore MPV levels until d 28. As for Platelet (PLT) in the day x scent interaction, non-scent group increased PLT from d 0 to d 2 and a sharp decrease from d 2 to d 6. However, a similar level PLT from d 0 to d 2 observed in scent group ($P = 0.0433$). Neutrophil to lymphocyte ratio (NLR) decreased from d 0 to d 2 in all treatment groups. While control, V, and VO groups showed similar level of NLR from d 2 to d 6, O group had increased NLR. This response on NLR is mainly driven by percentage of neutrophil ($P = 0.0028$) since no statistically significant difference on lymphocyte percentage was found. Results of this study indicated that continuing exposing scent and sow vocal potentially influences complete blood cell of weaning pigs.

2. Introduction

Usually when piglets reach the age of 21 to 28 days, they are weaned from the sow. During the weaning, the piglets are separated from the sows and transferred to a new environment. By separating the piglets from the sow, they experience biological stress such as physiological, environmental, and social challenges when weaned from the sow (Campbell et al., 2013). To accommodate the growth of the piglets, researchers often transition from liquid to solid feed. The piglets are deprived of the sows' touch, image, sound, and smell during weaning. In addition to the nutritional needs that the sow provides for the piglets, their removal from the sow induces the deprival of the sows' touch, image, sound, and smell during weaning. Pigs have a highly developed sense of smell, hearing, and vocal communication (Parfet & Gonyou, 1991), and sound is a vital sensory system for piglets when communicating with the sow (Weary et al., 1996). There is a characteristic 'oink' produced from the sow during her lactation period that is known as an infant-direct vocalization. Infant-direct vocalization can prompt the piglets to latch to the nipple for feeding (Whittemore & Fraser, 1974). It also is a soothing sound to the piglets and directs them to the sow for comfort if they are roaming freely. Studies have shown that mammal infants have significant response to the infant-direct vocalization of their mothers (Trainor et al., 1997). Losing infant-direct vocalization between the sow and her piglets can lead to a decrease in water intake and time spent feeding (Petrie & Gonyou, 1988). Additionally, sows secrete pheromones during the lactation period to calm the piglets (Sankarganesh, 2021). These pheromones can also help direct the piglet to the sow's nipples to feed. Piglets also have shown preferences for other sows smells in the first 7 days of life, such as their own mother's fecal matter and the smell of their own litter mates.

The purpose of this study is to investigate the effects of the smell and vocalizations of sows on the growth performance and weaning stress of piglets. If weaning stress may be reduced by hearing and smelling the sow though artificial means after weaning, the piglets are potentially less prone to sickness, will resume normal feeding and behavior more quickly. They may also gain more and thereby promoting animal welfare and animal performance in the first nursery stage. Hematology profiles, including complete blood cell count, can contribute to early identification of disease and poor growth performance in pigs (Perri et al., 2017; Klem et al., 2010; Zhang et al, 2022).

The response of different immune cell counts in weaning pigs may vary based on various factors such as diet, environment, stress, and exposure to pathogens. The normal range of leukocyte cell counts for pigs are as follows: white blood cell (11.0 - 22.0 K/µL), neutrophils (3.1 – 11.2 K/µL), lymphocytes (4.3 – 13.6 K/µL), monocytes (0.2 – 2.2 K/µL), eosinophils (0.1 – 2.4 K/µL), and basophils (0.0 – 0.4 K/µL). The normal range of erythrocytes are as follows: red blood cells (5.00 – 8.00 M/µL), hemoglobin (10.0 - 16.0 g/dL), hematocrit (32.0 – 50.0 %), mean corpuscular volume (MCV) (50.0 – 68.0 %), mean corpuscular hemoglobin (MCH) (17.0 – 21.0 pg), mean corpuscular hemoglobin concentration (MCHC) (30.0 – 34.0 g/dL), red cell distribution width (RDW) (12.0- 27.0 %). The expected range of thrombocytes in pigs is as follows: platelet (325. – 715. K/µL), and mean platelet volume (MPV) (5.0 – 20.0 fL). These normal ranges of complete blood cell count are from the MASCOT hematology profile of pigs. All abbreviations and definitions used throughout this experiment are defined in Table 1.

A study using mice confirm that neutrophil to lymphocyte ratio can be a valid indicator of chronic stress (Hickman, D., 2017). It showed that neutrophil to lymphocyte ratio was significantly increased in the mice during stressful periods. This is due to an increase in neutrophils and a decrease in lymphocytes (Tang et al., 2022). Erythrocytes, including red blood cells, hemoglobin, and hematocrit have positive associations with average daily gain (ADG) and average daily feed intake (ADFI) (Bhattarai & Nielsen, 2015). ADG and ADFI are both factors in growth performance of pigs. There is not expected to be a difference in immune response based on gender in weaning pigs. A study by (Humann-Ziehank and Ganter, 2012) found no significant differences in immune response between male and female piglets during the weaning period.

Whether contacting the vocalization and smell of sows could reduce the weaning stress of piglets remains unknown. We hypothesized that exposing nursery piglets to the sows vocal and scent would decrease weaning stress and increase growth performance.

3. Methods

The study was conducted in two periods to reduce variability. Each period included 12 sows and 96 piglets, providing 6 replicates per treatment. The pigs were fed a typical isocaloric nursery diet that meet NRC 2012 requirements. To test for sow vocalization, the grunting of 6 sows were recorded between day-7 and day-14 of sow lactation. This vocalization was recorded by attaching a recording device near the sow's feeder, close to her head, in the farrowing crate. An audio file was created to include the vocalization of all the sows at weaning, a length of muslin cotton fabric was rubbed on the udder of each sow to allow for olfactory testing. The cotton fabric was vacuum sealed to preserve the scent.

Eight piglets per sow from the 12 sows were randomly divided into 4 groups based on their weaning body weight and gender. There were 2 piglets from the same litter per treatment group. The groups included a control group with no treatments applied; olfactory group with no sound stimulation (O); voice group with sound stimulation (V); and a voice group with olfactory stimulation (VO). Piglets were transported to the offsite nursery facility. The groups with and without sounds stimulation were placed at either end of the nursery, with an insolation wall in the middle to block the transduction of sound. All piglets will be blocked into 6 blocks for 42 days, until the end of the nursery stage. The audio recording was played at 21 days of age. The sow vocal was played for approximately 20 minutes, followed by an empty hour. This was on loop for 7 days. In the olfactory groups, the fabric with scent was wrapped on a wood holder, then placed inside a metal mesh basket. The basket was secured just outside of the pen, beside the feeder so the piglets may have access to the scent. Placed in this manner, the pigs were not able to bite or eat the fabric. The scent was provided for 48 hours. The groups without olfactory simulation had the same cloth without scent.

To determine the growth performance of the piglets, individual body wights were collected at day-0, day-1, day-3, day-5, day-7, day-28, and day-42 body weight. Ped feed was measured and used to calculate the average daily gain, average daily feed intake, and feed efficiency. To determine the stress response of the nursery pigs, blood samples were collected on day-0, day-2, day-6, and day-28 after weaning to test the serum cortisol level, immune, and antioxidant parameters. Salivary cortisol was collected on day-3 and day-7.

The blood was collected from each pig using a vacutainer system with K2EDTA tubes. Each pig was placed on their back in a V-shaped holder and restrained. The blood was collected from the external jugular vein and placed in a cooler for transport to lab. The blood was processed in the lab within six hours of collection using a veterinary hematology analyzer (Hemavet 950S). Each sample was analyzed, and the data was entered into an excel spread sheet for statistical analysis. Data was analyzed using mixed procedure of SAS (Cary, NC) with vocal, scent and day as main effects.

Ethical Note

Pigs used in this study were handled and managed following the Division of Agriculture Institutional Animal Care and Use Committee (Ag-IACUC) of the University of Arkansas guideline (#22025).

4. Results

Day effect:

The effect of age was statistically significant on all variables of the complete blood cell count (Table 2). Neutrophil percentage decreased from d 0 to d 2 followed by a slight increase from d 2 to d 6 then decreasing further from d 6 to d 28 while lymphocyte percentage followed an opposite trajectory (Figure 1). The neutrophil to lymphocyte ratio followed a similar trajectory as neutrophil percentage, decreasing from d 0 to d 2, and then increasing form d 2 to d 6 and a further decrease from d 6 to d 28 (Figure 2).

Vocal effect:

Most of the variables were not affected by sow vocal. However, the MCHC, being higher in the V group, trended towards significance ($P = 0.0866$) (Table 3).

Scent effect:

Scent alone did not have any statistically significant effect on peripheral blood cell count, but neutrophil to lymphocyte ratio and lymphocyte percentage trended towards statistical significance. Neutrophil to lymphocyte ratio was higher in the O group compared to the control group ($P = 0.0554$). This result was driven by a higher lymphocyte percentage in the control group ($P = 0.0730$) (Table 3).

Sex effect:

Gender did not have a statistical significance on most variables except monocyte percentage which trended towards significance (Table 3). Monocyte percentage was higher in females ($P = 0.0970$).

Day x Vocal effect:

Interactions between day x vocal showed statistical significance (*P* = 0.0215) with both the V and control groups having a reduction on MPV from d 0 to d 2 and while the control group continued an upward trend from d 2 to d 28, V group failed to restore MPV levels until d 28 (Figure 5) (Table 4). The monocyte to lymphocyte ratio trended towards significance (P = 0.0704) (Table 4). Monocyte to lymphocyte ratio followed similar trajectories in both V and control groups from day d 0 to d 6, increasing from d 0 to d 2 followed by a decrease from d 2 to d 6. However, the control group decreased form d 6 to d 28 while the V group increased.

Day x Scent effect:

As for Platelet (PLT) in the day x scent interaction, non-scent group increased PLT from d 0 to d 2 and a sharp decrease from d 2 to d 6. However, a similar level PLT from d 0 to d 2 observed in scent group (*P* = 0.0433) (Figure 6) (Table 5). No other variables were statistically significant.

Vocal x Scent effect:

There were no statistically significant variables in the vocal x scent interaction (Table 6).

Vocal x Sex effect:

Most of the variables were not affected by vocal x sex interaction expect neutrophil to lymphocyte ratio and lymphocyte percentage trended towards significance (Table 7). Neutrophil to lymphocyte ratio was highest in the non-vocal males (P = 0.0920) this result was driven by the non-vocal males having the lowest lymphocyte percentage (P = 0.0692).

Scent x Sex effect:

In the interaction between scent x sex, neutrophil percentage (P-Value = 0.0454) was highest in the no scent male group (Table 8) (Figure 7). The neutrophil to lymphocyte ratio, also highest in the no scent male group, trended towards significance (P = 0.0558). This result was heavily influenced by the neutrophil percentage. However, monocyte percentage was lowest in the no scent male group (P = 0.0857) (Table 8).

Day x Vocal x Scent effect:

The 3-way interaction between day x vocal x scent showed statistical significance in the neutrophil to lymphocyte ratio and neutrophil percentage. Neutrophil to lymphocyte ratio (NLR) decreased from d 0 to d 2 in all treatment groups. While control, V, and VO groups showed similar level of NLR from d 2 to d 6, O group had increased NLR (Figure 6). This response on NLR is mainly driven by percentage of neutrophil (*P* = 0.0028) (Figure 3) since no statistically significant difference on lymphocyte percentage was found. Red blood cell concentration and hematocrit percentage followed very similar trajectories (Figures 8 & 9). All groups increasing from d 0 to d 6, with VO group being highest on d 6 and O group being lowest on d 6. Both red blood cell concentration and hematocrit percentage decreased form d 6 to d 28. However, neither red blood cell concentration nor hematocrit percentage are statistically significant.

5. Discussion

During a stressful period, the NLR ratio typically increases due to an increase in neutrophils and a decrease in lymphocytes (Hickman, D., 2017). This could be caused by an increased level of cortisol, the molecular indicator of a stressed animal; this increase in cortisol can subsequently increase neutrophil count while decreasing the lymphocyte count (Onsrud & Thorsby 1981). This ratio being altered by the effect cortisol is why NLR can increase rapidly following acute physiological stress (Zahorec, 2001). Due to the general trends of the associations of stress to NLR, it was expected that during the early stages of the weaning period, a very stressful period for the piglets, the NLR would increase; however, this was not observed during this trial. Just based on age, the NLR decreased from d 0 to d 2, followed by a slight increase from d 2 to d 6, then a continual decrease to d 28 (Figure 2). Typically, the first 5 days of weaning are the most stressful (Yuan et al., 2018). The overall decrease of NLR from d 0 to d 6 could potentially indicate a decrease in stress during the this typically stressful period for the piglets.

A similar result was found in the interaction between day x vocal x scent. The NLR decreased from d 0 to d 2 in all treatment groups. The NLR increased form d 2 to d 6 in the O group, indicating a possible increase in stress, while control, V, and VO groups had similar or decreasing levels during the same period (Figure 4). The response of the decreased neutrophil percentage drove the results of the NLR. It followed a similar trajectory to NLR with a decrease from d 0 to d 2 (Figure 3). This potentially could be important because typically stress induces an increase in neutrophils (Tang et al., 2022). The expected increase in neutrophils was observed in the O group from d 2 to d 6, then decreasing again from d 6 to d 28. However, this increase was not observed in any other treatment groups. This interaction demonstrates that the vocalization of the sow may play a more important role in soothing the piglets than olfactory stimulation when they are distressed. While previous studies have tested the effects of mammillary gland-produced pigs appeasing pheromone (PAP) on weaning stress in piglets, stress-related behaviors such as relaxing postures were increased in the piglets in the PAP condition (Temple et al., 2016); however, the authors did not test any molecular indicators of stress, of which affects the NLR.

The interaction between day x vocal for MPV suggests that vocal stimuli may play a role in modulating platelet production or function during the weaning period. As increased MPV has been associated with cardiovascular disease and panic/anxiety disorders in humans (Vizioli, Muscari, & Muscari, 2009; Almis & Aksoy, 2017), it may be a general indicator of physiological stress within the context of thrombogenesis. The V group had reduced MPV in the weaning period and did not restore as the piglets grew; this data suggests that the vocalization of the sow may have had a role in decreasing the MPV, a potential indicator of physiological stress. These differences in MPV trends between the vocal and non-vocal groups highlight the potential importance of understanding how environmental factors, such as auditory stimuli, can impact the immune response and growth performance in piglets.

In the 3-way interaction between day x scent x vocal, red blood cell concentration and hematocrit percentage followed similar trajectories from d 0 to d 28. All groups increased RBC and hematocrit from d 0 to d 6 and remained the same or decreased from d 6 to d 28. However, these results were not significant. Increases in red blood cell parameters including red blood cell concentration and hematocrit have a positive correlation with ADG and ADFI, growth performance factors (Lindholm-Perry et al., 2021). Regardless, these effects were not bolstered by the vocalization, or the olfaction strategies employed. The effect of these strategies was most relevant for neutrophils, leukocyte, and platelet numbers – not RBCs.

This study has identified several trends and interactions that warrant further investigation to determine their impact on immune response and growth performance: NLR and MPV's association with vocalization and olfaction. Understanding these interactions is crucial for monitoring the health and well-being of pigs during this critical period in their development and could potentially inform the development of more effective strategies to optimize pig health and productivity during the weaning phase. The results of this study indicate that continuing exposing scent could potentially influence the complete blood cell count by increasing NLR during the early stages of the weaning phase. It also demonstrates that sow vocal has a role in influencing complete blood cell count by decreasing MPV, a potential indicator of physiological stress.

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Table 1. Abbreviations

MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
RDW	Red cell distribution width
PLT	Platelet
MPV	Mean platelet volume
NE/LY	Neutrophil: Lymphocyte ratio
MO/LY	Monocyte: Lymphocyte ratio
PLT/LY	Platelet: Lymphocyte ratio
Control	No treatments applied
O group	Olfactory stimulation
V group	Vocal stimulation
VO group	Vocal and Olfactory stimulation
NLR	Neutrophil: Lymphocyte ratio

Table 2. Effect of Day on complete blood cell count in nursery pigs (LSmeans)

	Day					P - Value
	0	2	6	28	SEM	Day
Concentration, k/µl						
WBC	14.97	9.24	14.42	15.40	0.47	<.0001*
Neutrophil (NEN)	7.95	4.37	7.15	6.76	0.33	<.0001*
Lymphocyte (LYN)	6.22	4.27	6.37	7.81	0.20	<.0001*
Monocyte (MON)	0.35	0.29	0.36	0.40	0.02	<.0001*
Eosinophil (EON)	0.44	0.18	0.46	0.38	0.05	0.0001*
Basophil (BAN)	0.04	0.04	0.08	0.02	0.01	<.0001*
NE/LY	1.31	0.99	1.12	0.88	0.05	<.0001*
MO/LY	0.06	0.07	0.06	0.05	0.00	<0.0001*
% over WBC						
Neutrophil (NEP)	51.29	45.67	48.36	43.51	1.08	<.0001*
Lymphocyte (LYP)	41.87	48.11	45.51	51.06	1.19	<.0001*
Monocyte (MOP)	2.39	3.13	2.59	2.69	0.13	0.0007*
Eosinophil (EOP)	3.54	1.85	3.05	2.46	0.39	0.0009*
Basophil (BAP)	0.29	0.39	0.48	0.16	0.05	<.0001*
RBC, M/µl	6.09	6.53	7.16	7.07	0.08	<.0001*
Hemoglobin, g/dL	8.68	9.04	10.56	10.60	0.15	<.0001*
Hematocrit, %	38.47	39.31	41.67	40.55	0.56	<.0001*
MCV	64.74	60.37	58.31	57.42	0.64	<.0001*
MCH, Pg	14.67	13.86	14.76	15.02	0.20	<.0001*
MCHC, g/dL	22.68	23.01	25.31	26.17	0.24	<.0001*
RDW, %	24.50	26.22	26.41	22.72	0.56	<.0001*
PLT, k/µl	627.9	698.7	445.8	460.2	19.6	<.0001*
MPV, fL	9.05	8.29	8.57	9.06	0.13	<.0001*

Table 3. Effect of Vocal, Scent, and Sex on complete blood cell counts of nursery pigs (LSmeans)

	Vocal			Scent			Sex			P - Value		
	Yes	no	SEM	Yes	no	SEM	F	M	SEM	Vocal	Scent	sex
Concentration, k/µl												
WBC	13.84	13.18	0.42	13.82	13.19	0.42	13.06	13.95	0.42	0.2747	0.2988	0.1436
Neutrophil (NEN)	6.71	6.41	0.28	6.79	6.33	0.28	6.34	6.77	0.28	0.4620	0.2605	0.2902
Lymphocyte (LYN)	6.35	5.98	0.21	6.14	6.20	0.21	5.99	6.34	0.21	0.2087	0.8472	0.2351
Monocyte (MON)	0.35	0.35	0.02	0.36	0.35	0.02	0.36	0.34	0.02	0.8997	0.7712	0.3705
Eosinophil (EON)	0.37	0.36	0.06	0.38	0.35	0.06	0.36	0.37	0.06	0.8945	0.7234	0.9201
Basophil (BAN)	0.04	0.05	0.01	0.05	0.04	0.01	0.04	0.05	0.01	0.6816	0.2353	0.1987
NE/LY	1.10	1.05	0.04	1.13	1.02	0.04	1.05	1.10	0.04	0.4088	0.0554*	0.3578
MO/LY	0.06	0.06	0.00	0.06	0.06	0.00	0.06	0.06	0.00	0.8247	0.8599	0.2216
% over WBC												
Neutrophil (NEP)	47.12	47.30	0.94	48.18	46.24	0.94	46.79	47.63	0.94	0.8910	0.1514	0.5287
Lymphocyte (LYP)	46.58	46.69	1.04	45.28	47.99	1.04	46.89	46.38	1.04	0.9426	0.0730*	0.7339
Monocyte (MOP)	2.68	2.72	0.09	2.65	2.74	0.09	2.80	2.59	0.09	0.7234	0.4647	0.0970*
Eosinophil (EOP)	2.79	2.66	0.45	2.77	2.68	0.45	2.66	2.79	0.45	0.8408	0.8856	0.8481
Basophil (BAP)	0.31	0.34	0.05	0.36	0.30	0.05	0.30	0.36	0.05	0.6754	0.3955	0.4006
RBC, M/µl	6.70	6.73	0.08	6.69	6.73	0.08	6.77	6.65	0.08	0.8022	0.7232	0.2994
Hemoglobin, g/dL	9.73	9.71	0.14	9.67	9.77	0.14	9.74	9.70	0.14	0.9054	0.6288	0.8176
Hematocrit, %	39.87	40.13	0.50	39.70	40.31	0.50	40.04	39.96	0.50	0.7104	0.3942	0.9159
MCV	60.08	60.34	0.80	60.28	60.14	0.80	59.51	60.91	0.80	0.8121	0.9064	0.2191
MCH, Pg	14.66	14.50	0.23	14.63	14.52	0.23	14.42	14.73	0.23	0.6098	0.7362	0.3389
MCHC, g/dL	24.49	24.10	0.16	24.36	24.23	0.16	24.33	24.26	0.16	0.0866*	0.5793	0.7769
RDW, %	24.48	25.45	0.77	24.71	25.21	0.77	25.61	24.31	0.77	0.3804	0.6523	0.2424
PLT, k/µl	545.1	571.2	19.8	568.8	547.5	19.8	579.5	536.7	19.8	0.3586	0.4519	0.1346
MPV, fL	8.82	8.67	0.17	8.84	8.64	0.17	8.69	8.80	0.17	0.5404	0.4051	0.6299

Table 4. Effect of Day x Vocal interaction on complete blood cell count in nursery pigs (LSmeans)

	d 0		d 2		d 6		d 28			P - Value
	No Vocal	Vocal	No Vocal	Vocal	No Vocal	Vocal	No Vocal	Vocal	SEM	Day*Vocal
Concentration, k/µl										
WBC	15.74	14.20	8.96	9.51	14.88	13.96	15.77	15.04	0.67	0.2524
Neutrophil (NEN)	8.52	7.39	4.03	4.71	7.53	6.76	6.75	6.77	0.47	0.1093
Lymphocyte (LYN)	6.51	5.94	4.29	4.26	6.43	6.30	8.19	7.43	0.28	0.2692
Monocyte (MON)	0.36	0.34	0.28	0.30	0.38	0.35	0.39	0.42	0.03	0.4491
Eosinophil (EON)	0.49	0.39	0.16	0.20	0.47	0.46	0.37	0.39	0.08	0.7340
Basophil (BAN)	0.04	0.04	0.04	0.05	0.07	0.08	0.03	0.02	0.01	0.4421
NE/LY	1.36	1.27	1.01	0.97	1.20	1.04	0.82	0.93	0.07	0.1151
MO/LY	0.06	0.05	0.07	0.07	0.06	0.06	0.05	0.06	0.00	0.0704*
% over WBC										
Neutrophil (NEP)	51.73	50.86	45.04	46.29	49.40	47.32	42.30	44.73	1.52	0.2453
Lymphocyte (LYP)	41.10	42.64	48.25	47.97	44.69	46.32	52.29	49.84	1.68	0.3728
Monocyte (MOP)	2.37	2.40	3.21	3.04	2.60	2.58	2.52	2.87	0.18	0.5249
Eosinophil (EOP)	4.03	3.05	1.75	1.94	2.88	3.21	2.49	2.43	0.55	0.5746
Basophil (BAP)	0.32	0.26	0.33	0.44	0.43	0.54	0.18	0.14	0.07	0.2541
RBC, M/µl	6.13	6.04	6.49	6.57	7.10	7.21	7.07	7.08	0.11	0.7491
Hemoglobin, g/dL	8.71	8.65	9.07	9.01	10.53	10.58	10.61	10.59	0.22	0.9889
Hematocrit, %	38.69	38.25	38.96	39.67	41.25	42.10	40.59	40.51	0.79	0.8442
MCV	64.46	65.01	60.14	60.60	58.17	58.46	57.53	57.31	0.90	0.9065
MCH, Pg	14.76	14.59	13.99	13.73	14.85	14.68	15.04	14.99	0.28	0.9608
MCHC, g/dL	22.92	22.44	23.34	22.69	25.53	25.09	26.17	26.18	0.34	0.6439
RDW, %	23.88	25.13	25.54	26.89	25.74	27.07	22.76	22.69	0.79	0.2240
PLT, k/µl	642.0	613.8	695.2	702.2	419.7	472.0	423.7	496.7	27.8	0.1271
MPV, fL	9.16	8.95	8.28	8.30	8.73	8.41	9.10	9.03	0.19	0.0215*

Table 5. Effect of Day x Scent interaction on complete blood cell count in nursery pigs (LSmeans)

	d 0		d 2		d 6		d 28			P - Value
	No Scent	Scent	No Scent	Scent	No Scent	Scent	No Scent	Scent	SEM	Day*Scent
Concentration, k/µl										
WBC	15.81	14.13	9.78	8.69	13.97	14.86	15.72	15.09	0.67	0.1736
Neutrophil (NEN)	8.47	7.44	4.80	3.93	6.88	7.41	6.99	6.53	0.47	0.4162
Lymphocyte (LYN)	6.25	6.20	4.28	4.26	6.20	6.54	7.83	7.79	0.28	0.7030
Monocyte (MON)	0.37	0.33	0.29	0.29	0.35	0.38	0.41	0.39	0.03	0.3925
Eosinophil (EON)	0.47	0.41	0.18	0.17	0.46	0.47	0.40	0.36	0.08	0.9609
Basophil (BAN)	0.04	0.03	0.05	0.03	0.09	0.06	0.02	0.03	0.01	0.2195
NE/LY	1.40	1.22	1.11	0.87	1.10	1.13	0.91	0.85	0.07	0.2081
MO/LY	0.06	0.06	0.07	0.07	0.06	0.06	0.06	0.05	0.00	0.5951
% over WBC										
Neutrophil (NEP)	52.71	49.88	48.09	43.25	47.82	48.91	44.11	42.92	1.52	0.1566
Lymphocyte (LYP)	40.10	43.64	44.95	51.26	45.98	45.04	50.09	52.03	1.68	0.1511
Monocyte (MOP)	2.41	2.36	2.89	3.36	2.51	2.67	2.79	2.59	0.18	0.2593
Eosinophil (EOP)	3.43	3.65	1.90	1.79	3.10	2.99	2.64	2.28	0.55	0.7592
Basophil (BAP)	0.33	0.25	0.41	0.36	0.57	0.40	0.13	0.18	0.07	0.2607
RBC, M/µl	6.08	6.10	6.47	6.60	7.15	7.16	7.07	7.07	0.11	0.8184
Hemoglobin, g/dL	8.52	8.85	8.96	9.12	10.60	10.51	10.60	10.60	0.22	0.8300
Hematocrit, %	37.53	39.41	38.98	39.64	41.59	41.76	40.69	40.41	0.79	0.6491
MCV	64.69	64.78	60.51	60.22	58.27	58.36	57.63	57.21	0.90	0.7092
MCH, Pg	14.80	14.55	13.88	13.84	14.84	14.69	15.02	15.01	0.28	0.9481
MCHC, g/dL	22.88	22.49	22.98	23.04	25.48	25.14	26.09	26.26	0.34	0.5559
RDW, %	24.10	24.90	26.01	26.42	26.04	26.77	22.70	22.74	0.79	0.3967
PLT, k/µl	629.2	626.5	746.2	651.1	427.8	463.8	471.9	448.6	27.8	0.0433*
MPV, fL	9.15	8.95	8.33	8.25	8.73	8.41	9.17	8.96	0.19	0.3023

Table 6. Effect of Vocal x Scent on complete blood cell count in nursery pigs (LSmeans)

	None		Vocal			P - Value
	None	Scent	None	Scent	SEM	Vocal*Scent
Concentration, k/µl						
WBC	14.08	13.59	13.56	12.79	0.60	0.8154
Neutrophil (NEN)	6.90	6.52	6.68	6.14	0.40	0.8466
Lymphocyte (LYN)	6.28	6.43	6.00	5.96	0.29	0.7350
Monocyte (MON)	0.34	0.37	0.37	0.33	0.02	0.1485
Eosinophil (EON)	0.37	0.37	0.39	0.33	0.08	0.6664
Basophil (BAN)	0.05	0.04	0.06	0.04	0.01	0.5867
NE/LY	1.16	1.03	1.10	1.00	0.06	0.7438
MO/LY	0.06	0.06	0.06	0.06	0.00	0.3017
% over WBC						
Neutrophil (NEP)	48.10	46.14	48.26	46.34	1.33	0.9880
Lymphocyte(LYP)	45.47	47.69	45.09	48.30	1.48	0.7384
Monocyte (MOP)	2.53	2.82	2.77	2.67	0.13	0.1228
Eosinophil (EOP)	2.74	2.83	2.80	2.52	0.64	0.7794
Basophil (BAP)	0.33	0.30	0.39	0.30	0.08	0.6825
RBC, M/µl	6.72	6.68	6.67	6.78	0.11	0.5091
Hemoglobin, g/dL	9.65	9.82	9.69	9.72	0.20	0.7289
Hematocrit, %	39.67	40.07	39.73	40.54	0.71	0.7706
MCV	60.00	60.15	60.55	60.14	1.13	0.8012
MCH, Pg	14.64	14.68	14.63	14.37	0.32	0.6439
MCHC, g/dL	24.48	24.50	24.24	23.96	0.22	0.4996
RDW, %	24.30	24.66	25.13	25.76	1.09	0.9027
PLT, k/µl	565.0	525.2	572.6	569.8	28.0	0.5135
MPV, fL	8.91	8.73	8.78	8.56	0.24	0.9306

Table 7. Effect of Vocal x Sex interaction on complete blood cell count in nursery pigs (LSmeans)

	No Vocal		Vocal			P - Value
	F	M	F	M	SEM	Vocal*sex
Concentration, k/µl						
WBC	13.10	14.57	13.02	13.33	0.60	0.3343
Neutrophil (NEN)	6.23	7.18	6.45	6.36	0.40	0.2072
Lymphocyte (LYN)	6.27	6.44	5.72	6.25	0.29	0.5427
Monocyte (MON)	0.36	0.35	0.37	0.33	0.02	0.5442
Eosinophil (EON)	0.34	0.40	0.38	0.34	0.08	0.5155
Basophil (BAN)	0.03	0.05	0.04	0.05	0.01	0.4179
NE/LY	1.02	1.17	1.07	1.03	0.06	0.0920*
MO/LY	0.06	0.06	0.06	0.06	0.00	0.3636
% over WBC						
Neutrophil (NEP)	45.76	48.48	47.82	46.79	1.33	0.1657
Lymphocyte (LYP)	48.21	44.95	45.57	47.81	1.48	0.0692*
Monocyte (MOP)	2.78	2.57	2.83	2.61	0.13	0.9428
Eosinophil (EOP)	2.44	3.14	2.89	2.43	0.64	0.3693
Basophil (BAP)	0.27	0.36	0.33	0.36	0.08	0.7150
RBC, M/µl	6.78	6.62	6.76	6.69	0.11	0.7063
Hemoglobin, g/dL	9.83	9.64	9.66	9.76	0.20	0.4781
Hematocrit, %	39.94	39.80	40.14	40.13	0.70	0.9296
MCV	59.41	60.74	59.61	61.08	1.12	0.9503
MCH, Pg	14.55	14.77	14.29	14.70	0.32	0.7783
MCHC, g/dL	24.59	24.39	24.06	24.14	0.22	0.5338
RDW, %	25.08	23.87	26.14	24.76	1.09	0.9373
PLT, k/µl	578.7	511.5	580.4	562.0	28.0	0.3897
MPV, fL	8.66	8.97	8.71	8.63	0.24	0.4195

Table 8. Effect of Scent x Sex interaction on complete blood cell count in nursery pigs (LSmeans)

	No Scent		Scent			P - Value
	F	M	F	M	SEM	Scent*sex
Concentration, k/µl						
WBC	13.44	14.20	12.69	13.70	0.60	0.8348
Neutrophil (NEN)	6.38	7.19	6.30	6.35	0.40	0.3516
Lymphocyte (LYN)	6.13	6.14	5.85	6.54	0.29	0.2480
Monocyte (MON)	0.39	0.32	0.34	0.36	0.02	0.0857*
Eosinophil (EON)	0.38	0.38	0.34	0.36	0.08	0.8616
Basophil (BAN)	0.05	0.06	0.03	0.05	0.01	0.7254
NE/LY	1.05	1.21	1.05	0.99	0.06	0.0558*
MO/LY	0.06	0.06	0.06	0.06	0.00	0.7481
% over WBC						
Neutrophil (NEP)	46.39	49.98	47.19	45.29	1.33	0.0454*
Lymphocyte (LYP)	46.71	43.85	47.07	48.92	1.48	0.1183
Monocyte (MOP)	2.86	2.44	2.75	2.74	0.13	0.1074
Eosinophil (EOP)	2.68	2.86	2.64	2.71	0.64	0.9385
Basophil (BAP)	0.34	0.38	0.25	0.34	0.08	0.7950
RBC, M/µl	6.76	6.62	6.78	6.69	0.11	0.7987
Hemoglobin, g/dL	9.75	9.59	9.74	9.80	0.20	0.6009
Hematocrit, %	39.92	39.47	40.16	40.46	0.70	0.5981
MCV	59.46	61.09	59.56	60.73	1.12	0.8379
MCH, Pg	14.46	14.81	14.39	14.66	0.32	0.9011
MCHC, g/dL	24.39	24.32	24.26	24.21	0.22	0.9489
RDW, %	25.24	24.19	25.98	24.44	1.09	0.8283
PLT, k/µl	598.5	539.1	560.6	534.4	28.0	0.5566
MPV, fL	8.61	9.08	8.76	8.53	0.24	0.1593

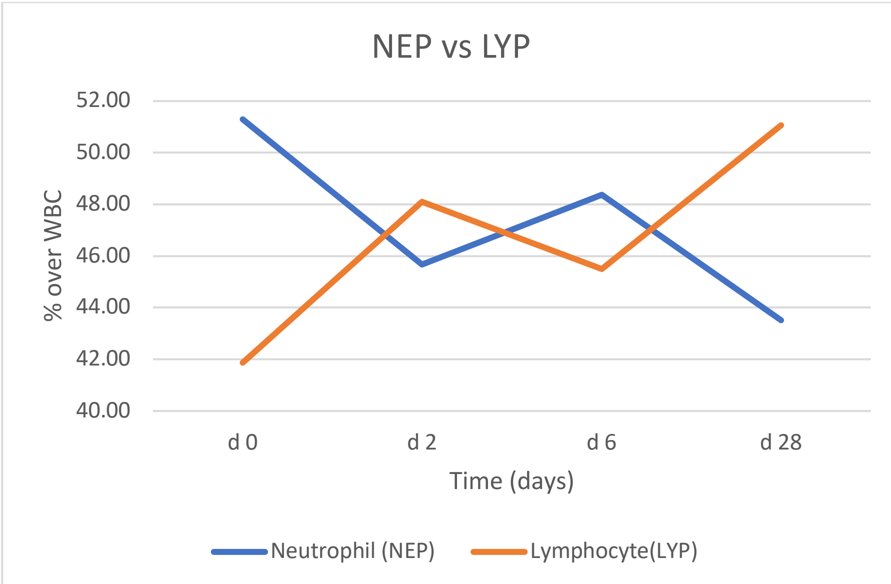

Figure 1. Day effect on neutrophil percentage compared to the lymphocyte percentage with time in days on the x-axis.

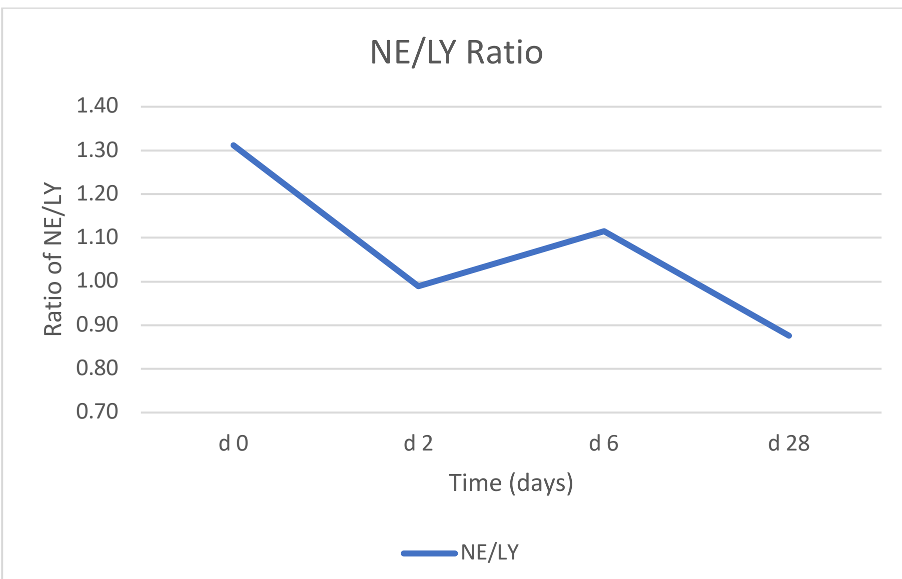

Figure 2. Day effect on neutrophil to lymphocyte ratio through the duration of the trial with time in days on the x-axis.

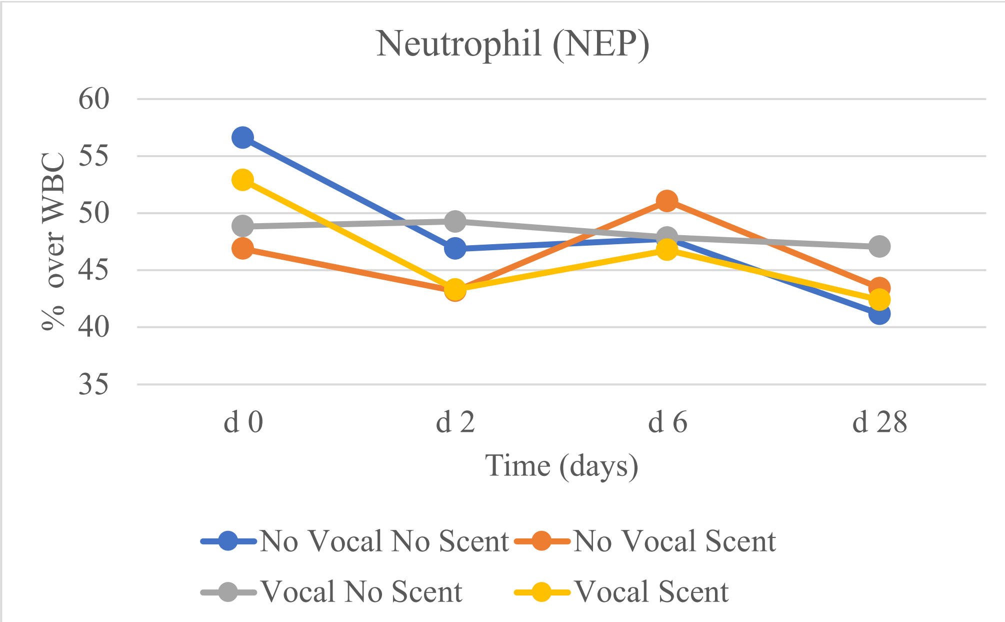

Figure 3. Neutrophil percentage based on each group (day x scent x vocal). Time in days on the x-axis.

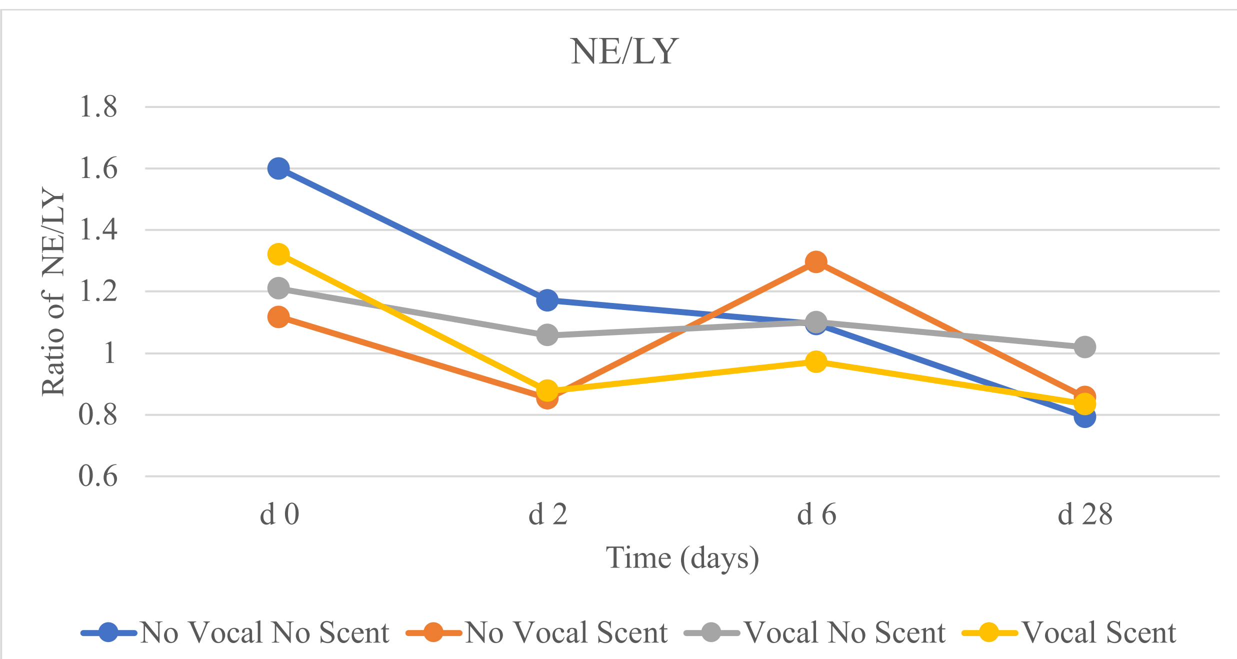

Figure 4. Neutrophil to lymphocyte percentage with respect to day x scent x vocal. Time in days on the x-axis.

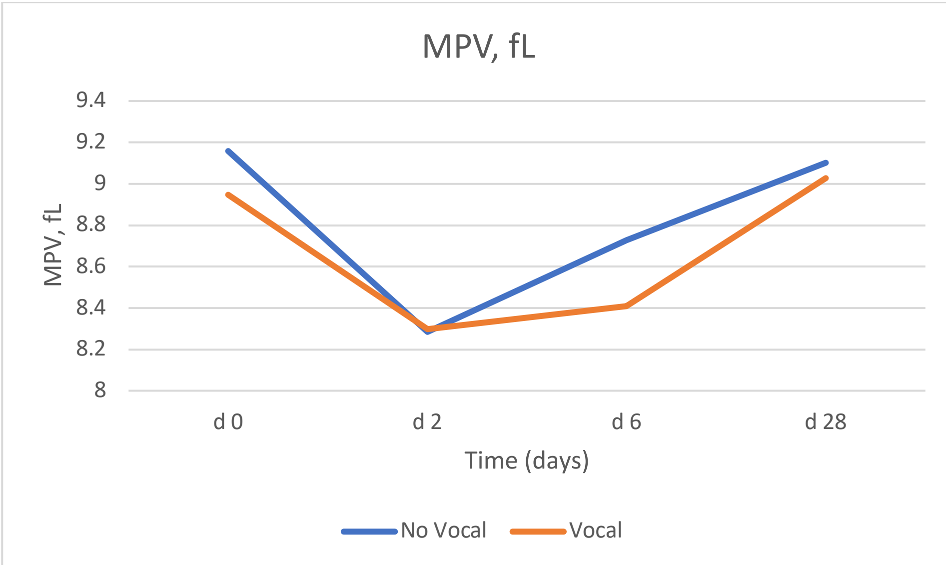

Figure 5. MPV, mean platelet volume, with respect to day*vocal interaction.

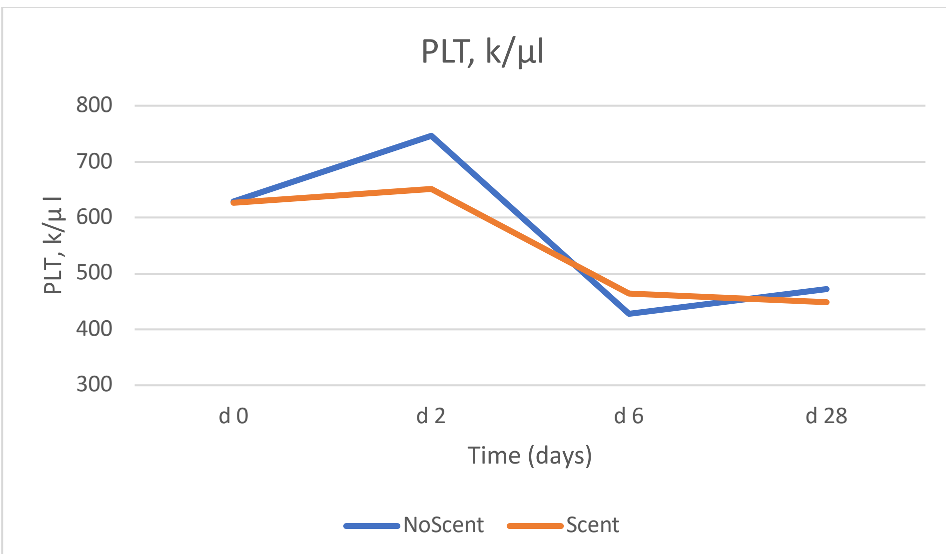

Figure 6. Platelet values based on day*scent.

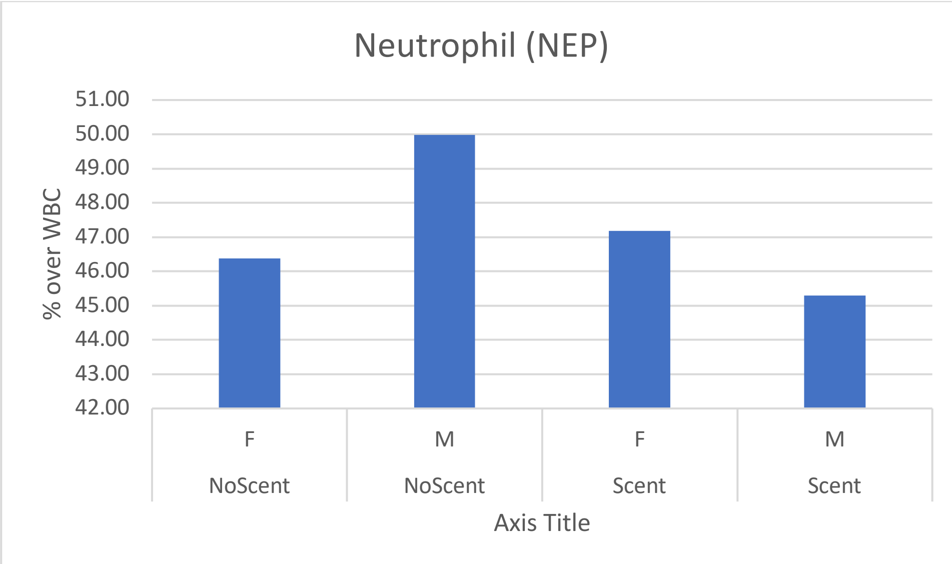

Figure 7. Neutrophil percentage regarding scent x sex interaction.

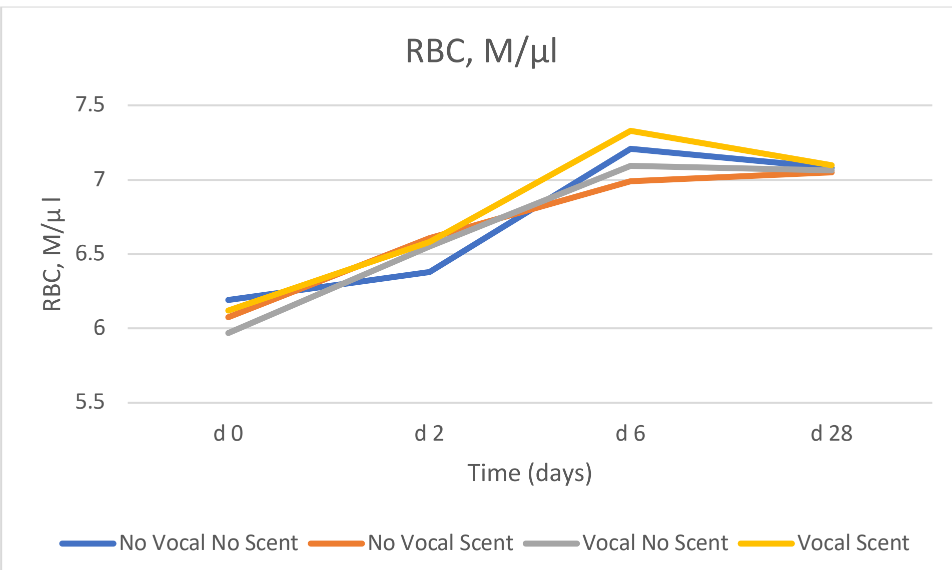

Figure 8. Red blood cell concentration in day x vocal x scent interaction.

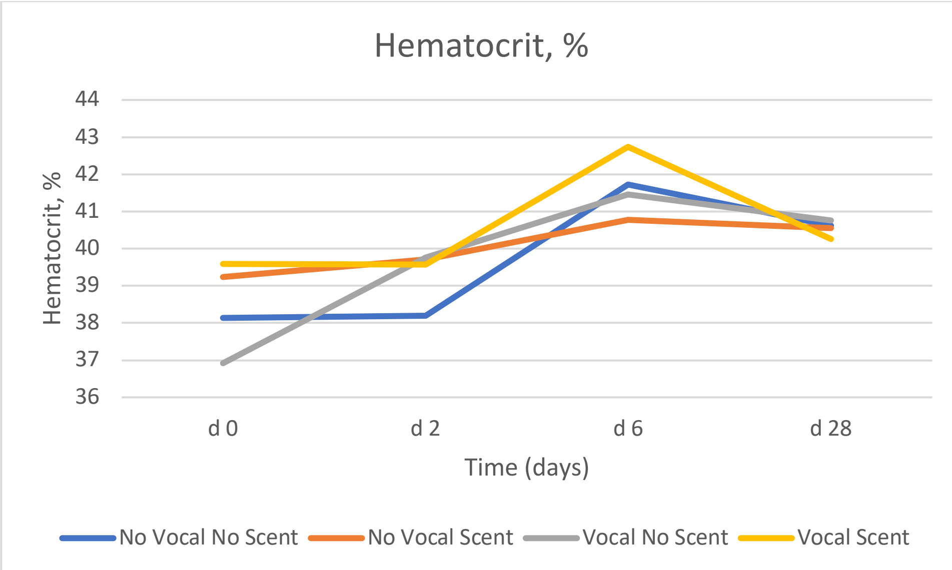

Figure 9. Hematocrit percentage in day x vocal x scent interaction.