Final Report:

Utilization of an advanced computer vision platform to identify changes in the physiological and behavioral changes associated with illness and aggressive/damaging behavior during the nursery and finisher phase. (NPB Project #19-122)

PI: Ty Schmidt

University of Nebraska – Lincoln

22 December, 2023

Industry Summary:

Rapid identification and treatment of morbid/injured pigs is essential for swine producers to ensure the health and wellbeing of each individual animal. However, in the modern commercial swine facilities, a single caretaker may have to access the health/wellness of ~4000 pigs/day (500 pigs/hour). This can be an insurmountable task to make individualized diagnoses using the daily "snapshots" obtained through periodic visual observation. Within each phase of production, pigs are exposed to a multitude of stressors that increase rates of illness and/or injury. One of the most challenging times for a piglet, as indicated by increased morbidity and mortality rates, is the pig's transition to the nursery and the resulting stress of weaning. Stressors experienced during this early phase of production influence the competence of the developing immune system. Such alterations during the early development of the acquired immune system and can render pigs more vulnerable to "subclinical sickness" – morbidity that cannot be identified using traditional single-point human observations due to a lack of visible clinical symptoms. To overcome the limitations of human observations, we utilized an advanced computer vision platform (NU*track* Livestock Monitoring System) to identify physiological and behavioral changes associated with illness and aggressive/damaging behavior during the nursery and finisher phase. One hundred and ninety-two newly weaned pigs were utilized for this trial in a nursery study and a finisher study. For the nursery phases, pigs were randomly assigned to one of three treatments, a control group, and one of two endotoxin challenge groups. The two endotoxin challenge groups consisted of 100% of the pigs within a pen receiving an endotoxin challenge and the other group consisted of only 50% of the pigs receiving the endotoxin challenge. Following the nursery period, pigs were then assigned to two treatments groups within the finisher phase: mixed population pens or cohort pens. Pigs for the mixed population were randomly selected across the three nursery groups and assigned to a finisher pen. Pigs in the cohort pens comprised pigs from the original nursery pens. Results from this study provided significant insight into the capabilities of using precision livestock technology to assist caretakers in identifying compromised pigs. When compared to human observations, the

NU*track* system provided greater sensitivity and specificity for identifying passive and active behaviors compared to trained human observation. In addition, using machine learning and tracking the activities of individual pigs provided insight into changes in active and passive behaviors of immune challenged nursery pigs. Using changes in behaviors, results suggest that tracking subtle changes behaviors has the potential to identify compromised pigs prior to the presentation of indicators visible to caretakers. Overall, results of this study indicate the precision livestock technology NU*track* has the potential to be more accurate at identifying changes in behaviors of immune challenged pigs and has the potential to caretakers with a reliable alert system to assist in the identification of compromised pigs.

Key Findings:

1. The NU*track* system could identify changes in passive and active behaviors of pigs exposed to an immune challenge.
2. Use of precision livestock technology with the capabilities to track the changes in behavior of nursery pigs has to be more accurate for identification of compromised nursery pigs, when compared to human observations.
3. Using subtle changes in passive and active behaviors has the ability to identify immune challenged nursery pigs, and potentially identify compromised pigs prior to human observations.

Keywords:
Computer vision, immune-challenged, behavior changes, precision livestock technology, NU*track*

Scientific Abstract:

Rapid identification and treatment of morbid/injured pigs is essential for swine producers to ensure the health and wellbeing of each individual animal. However, in the modern commercial swine facilities, a single caretaker may have to access the health/wellness of ~4000 pigs/day (500 pigs/hour). This can be an insurmountable task to make individualized diagnoses using the daily "snapshots" obtained through periodic visual observation. Within each phase of production, pigs are exposed to a multitude of stressors that increase rates of illness and/or injury. One of the most challenging times for a piglet, as indicated by increased morbidity and mortality rates, is the pig's transition to the nursery and the resulting stress of weaning. Stressors experienced during this early phase of production influence the competence of the developing immune system. Such alterations during the early development of the acquired immune system and can render pigs more vulnerable to "subclinical sickness" – morbidity that cannot be identified using traditional single-point human observations due to a lack of visible clinical symptoms. To overcome the limitations of human observations, we utilized an advanced computer vision platform (NU*track* Livestock Monitoring System) to identify changes in the physiological and behavioral changes associated with illness and aggressive/damaging behavior during the nursery and finisher phase. One hundred and ninety-two newly weaned pigs were utilized for this trial in a nursery study and a finisher study. For the nursery phases, pigs were randomly assigned to one of three treatments, a control group, and one of two endotoxin challenge groups. The two endotoxin challenge groups consisted of 100% of the pigs within a pen receiving an endotoxin challenge and the other group consisted of only 50% of the pigs receiving the endotoxin challenge. Following the nursery period, pigs were then assigned to two treatments groups within the finisher phase: mixed population pens or cohort pens. Pigs for the mixed population were randomly selected across the three nursery groups and assigned to a finisher pen. Pigs in the cohort pens were comprised of pigs from the original nursery pens. Results indicated that human observation presented acceptable rates for identification of compromised pigs on day 0 and 1 (0.85 AUC, >70% Sensitivity, >85% Specificity), however by day 2 human observations declined to undesirable rates (<0.57 AUC, <33% Specificity). While NU*track* had superior AUC, true positive, and true negative compared to human observations on d 0, 1, and 3 (>0.98 AUC, >79% Sensitivity, >94% Specificity). When compared to human observation, false positive identification was less frequent (P<0.05) for NU*track* identification. Overall, when compared to human observations, NU*track* had more days with adequate sensitivity and specificity and human observations created more false positives and false negatives. For early alert identification, results indicated that daily total values for each behavior provided greater accuracy (accuracy of 0.915 and 0.989) when compared to changes in daily behaviors (~0.70 and 0.63). As for model, logistic regression model did not serve as an acceptable model for identification of compromised pigs (produced a large number of control pigs classified as immune challenged pigs. B-Spline model provided an acceptable model, when using daily total values. The B-Spline model was not only capable of identifying LPS-challenged pigs (85 of 96 pigs), but also manager identified pigs during the post-challenged period (17 of 44 pigs). Overall results indicate that precision livestock technology has the ability to provide accurate identification of compromised pigs and there is potential for precision livestock technology to provide caretakers with an early-alert system for the identification of compromised pigs.

Materials and Methods

Materials and Methods

Animals and Housing

All experimental procedures adhered to the Guide for the Care and Use of Agricultural Animals in Research and Teaching. All procedures were reviewed and approved by the University of Nebraska—Lincoln Institutional Animal and Care and Use Committee (IACUC #1409).

One hundred and ninety-two newly weaned pigs (gilts and barrows) were sourced from the

University of Nebraska—Lincoln Eastern Nebraska Research and Extension Center's swine unit and housed in 12 nursery pens (16 pigs/pen) within one nursery room. Ad libitum water was provided through water nipples, and feed was offered through an 8-hole feeder. Diet was formulated to meet the NRC requirements for nursery pigs [23]. At the time of processing (0-2 days after birth), pigs had a generic button tag added to both ears. Pigs were weaned between 21-25 days of age.

Treatments after Weaning

At weaning, a unique color and alpha-numeric ear replaced the generic tag, which allowed the visual tracking system to auto-correct and recovers lost individual identification [22]. A day before treatment (d -1; Figure 1), all pigs were weighed to calculate dosages of treatments appropriately.

This experiment was a portion of a larger project that included commingling or remaining with groupmates at the finishing stage. First, pigs were randomly assigned pens, stratifying gender (barrows and gilts) across 24 pens. Then, finishing pens were randomly assigned commingling or no-commingling. Then, those pens were randomly assigned one of three nursery treatments (this report) so that nursery pigs began in pens of 16.

At the nursery phase, the 16 pigs were placed in 12 pens. Nine days after weaning, pigs were administered treatments. The nursery had 3 types of pens, 4 pens each: 1)In control pens, pigs were sham-handled (Sham; 3 mL of sterile saline); 2) In all-challenged pens, all 16 pigs were administered lipopolysaccharide (LPS; isolated from cell walls of heat-killed Escherichia coli O111:B4; Sigma-Aldrich, St. Louis MO, USA) at a dose of 300 µg/kg body weight, dissolved in 3 mL of sterile saline, and; 3) In half-and-half pens, 8 pigs were administered LPS, and the other 8 were sham-treated. All injections were administered subcutaneously in the left medial inguinal area using a syringe with a ½ inch long 21-gauge needle. The IACUC leading veterinarian required rescue interventions for LPS-treated pigs that became completely unresponsive to external stimuli on the day of treatment. To improve cardio-respiratory function, the protocol included epinephrine (0.5 mL/pig) and dexamethasone (0.5 mL/pig) intramuscularly.

Precision Monitoring System

The NU*track* system's cameras were installed at a 90-degree angle above the 12 nursery pens before pigs' placement. This deep learning-based, multi-object tracking system can achieve greater than 92.5% precision and recall when tracking individual pigs' long-term individual identity, location, and individual posture in group-housed settings [20]. The hardware component of the system was FLIR/Lorex NVR System (Lorex Corporation, Linthicum, Maryland). The IP-based cameras had 4K (8MP) and infrared capability for low-light recording. Visual data were collected continuously at a rate of 5 frames per second. Visual data were then pushed to a Dell-

Alienware GPU-equipped desktop computer for processing. The software used fully convolutional networks to detect the location and orientation of pigs and their ear tags. The software aspect of the NU*track* system was based upon a Bayesian multi-object tracking approach, as reported previously [19–22]. This method combined visual classification of ear tags with frame-to-frame movement probabilities, which allowed for correct identification, even when ear tags were obscured. Measures for this report (Figure 2) included distance each pig traveled (m), angle (radius, pivot behavior), as well as the durations of stand, walk, and lie (lie-sternal, lie-lateral, sit).

Human Derived Data

Live time point data were methodically collected by two expert human observers (veterinarian and trained technician) with interobserver reliability > 95%. Human observers were blinded to the treatments. After reliability was established, each observer stood in front of the pen and classified the animals according to visual sickness (Figure 3) and hide score (Figure 4) for three minutes.

The sickness scores were adapted from established calf health scores [27]. The scores ranged from 0 to 2: normal and alert were scored as 0; sleepy or drowsy was scored as 1, and non-responsive to stimuli was scored as 2. The score of 2 included lateral lying and open mouth respiration. Hide scores (Figure 3) and emesis events were recorded but were not used in the final analyses because hides could not be assessed when pigs piled on each. Emesis events only occurred the day of the challenge and were not frequent enough to be included in the analyses.

Statistical Analysis

The behavioral data collected from the precision technology were analyzed using the receiver operator characteristic curve (ROC) analysis on RStudio (RStudio: Integrated Development for R. RStudio, PBC, Boston, MA). The first analyses included the pens with all-sham treated pigs, all-LPS treated pigs and the half-and-half pigs. Then, the half-and-half pens were analyzed separately. From these analyses, the area under the curve (AUC) was measured to evaluate the predictive ability of the precision data, which statisticians consider a more superior measure than accuracy. The AUC ranges from 0 to 1, an outcome of 0.5 means that the measure cannot discriminate between LPS and sham pigs, and a 1 means that the measure is perfect at identifying the treated animals (Figure 3). The human observation data were used as a contrast reference. Human data and precision data were fitted to a logistic regression equation, using the binomial family option to predict the known outcome of sick (received LPS) or not sick (did not receive LPS) individuals. ROC curves were plotted, and AUC values for the regression, pivot behavior, rest behavior, distance traveled, and human sick scores were calculated starting on treatment day (d 0) and moving individually to each day post-treatment (d 1 to d 7). Both precision and the human data were tested for the validity (sensitivity and specificity) of screening pens to find sickness in pigs as they recover from their treatments.

The precision data cutoff values were calculated on RStudio based on Youden's index. The optimal cutoff point was then chosen by the maximum Youden index, maximizing the sum of sensitivity and specificity [28,29]. Optimal cutoff points were calculated for precision data only because human derived data are considered categorical. In contrast, the precision data collected continuous variables based on the pigs' behaviors. Once the optimal cutoffs were established, data could then be converted to a binomial categorical variable with two factors: LPS or Sham treated. Pigs' initial human-derived data and precision-derived data were included in the dataset for treatment day (d 0).

At the conclusion of the nursery study (42 days), the remaining 183 pigs were moved into the finisher facility and randomly assigned to one of four treatments: LPS challenged pigs that remained with cohorts (**NoMix-LPS**), pigs that did not receive the LPS challenge and remained with cohorts (**NoMix-SAL**), LPS challenged pigs that were placed into mixed population pens (**Mix-LPS**), and pigs that did not receive the LPS challenged and placed into mixed population pens (**Mix-SAL**). Pigs were placed into finisher pens, 8 pigs/pen equipped with the NU*track* system The NU*track* system was programmed to start the collection of videos as soon as pigs were placed into the pens. At the point of placement, until the completion of the finisher phase, continuous video was captured and analyzed. Data generated by the NU*track* system included active behaviors (meters walked/day, velocity of meters traveled, pivot/radius of head, and time at the feeder) and passive behaviors (time lying lateral, time lying sternal, time standing, and time sitting). Passive and active behavior during the last 7 days of the nursery phases were averaged and used for a baseline comparison. On day 1, 45, and 132 pigs were weighed and on day 132 ultrasounds were collected.

Performance data were analyzed as a completely randomized design using the PROC MIX procedures of SAS. Day, week, and sex were included as fixed effects, pen as a random effect, and individual pig served as the experimental unit. Active and Passive traits were analyzed using the PROC GLIMMIX procedure for repeated measures of SAS (SAS Inst. Inc., Cary, NC). Day, week, and sex were included as fixed effects, pen as a random effect, and individual pig served as the experimental unit. When main effects or interactions were significant ($P < 0.05$), specific comparisons were made using the PDIFF option in SAS, 0.06 – 0.10 was considered a tendency. Data is presented as LSMeans ± SEM.

Objective 1 and 4

Objective 1: *Train the Deep Feature-based Detection and Tracking Platform (DF-DTP) to* *distinguish endotoxin-challenged nursery pigs from their healthy cohorts and healthy-pens using measures from* <u>*validated*</u> *behavior outcomes and disruption in biorhythms.*

Objective 4: *Refine a data management system to identify compromised pigs well past the sickness bout using predictive modeling.*

Results for Objectives 1 and 4:

Behavioral variables used in these analyses included locomotion-spatial measures (angle and distance), animal-structural measures such as lying (on side or sternum), sitting (upright front legs, but back legs and rump down on floor), standing (without locomotion, all four legs upright), and structural-spatial measure feeding (pig is in feeder area, head is angled down into a feeder hole. Each behavioral variable data was summarized daily. The recorded data from the system were transformed into values that captured the total length for each time slot, on a per-day basis. This recorded data was notated as the daily total value $y_i(t)$ at Study Day t for the ith behavioral variable. Moreover, the daily change values were defined as that captured the difference of y_i between two days

$$y_i^*(t) = y_i(t) - y_i(t-1)$$

where $t = -5, -4, \ldots, 0, 1, \ldots 34$. The daily changes value for all behavioral variables on Study Day -5 was set up to zero as the baseline.

Statistical Analysis

Continuous behavioral variables were expressed as mean ± SE, according to the normality of distribution. Bonferroni multiplicity adjustment was applied when comparing behavioral variables among four treatments from Study Day 0 (the day before injection) to Study Day 6. Probability values of $P < 0.05$ (2-tailed) were considered statistically significant for all comparisons.
Subsequently, behavioral parameters significantly associated with LPS were evaluated for LPS-compromised prediction from Study Day 1 (treatment day) to Study Day 6. For each measurement approach (daily total values or daily changes value), behavioral variables (except sitting) were compared with each other on a per-day basis by computing Pearson's correlation coefficient. A Pearson correlation coefficient > 0.8 or < -0.8 was taken to indicate a strong correlation between the two measures.

Logistic regression

The association of each predictor variable (angle, distance, lying, feeding, and standing) and LPS was then assessed by simple logistic regression and compared to Study Day 1 and Day 2, respectively. For *ith* predictor variable, assume $y_i(t) = 1$ if a piglet is LPS-challenged at Day

$t\ (= 1 \text{ or } 2)$, and $y_i(t) = 0$ otherwise. Denote by $E(Y_i\,(t)) = \pi_i(t)$ the probability that the pig is LPS-challenged at day t. A linear logistic model has the form.

$$\text{logit}(\pi_i(t)) \equiv \log\left(\frac{\pi_i(t)}{1-\pi_i(t)}\right) = \alpha_i(t) + x_i(t)\beta_i(t)$$

Where $\alpha_i(t)$ was the intercept parameter and $\beta_i(t)$ was the slope parameter for *ith* predictor variable (lying, feeding, standing, or moving) at day $t\ (= 1 \text{ or } 2)$.

To obtain the results, 60 percent of the data was used for model training and the remaining 40 percent as testing data. For each measurement approach, to establish unbiased cut-off values for single behavior to differentiate between sham and LPS-compromised animals, several logistic regression analyses were performed using significant single behavioral data as a predictor variable. Predictive performance was quantified by the area under the curve (AUC) of the receiver operating characteristic (ROC) curve and accuracy. The range of AUCROC is 0 to 1, and if AUCROC is close to 0.5 then the model corresponds to random chance. The more close to 1 means higher accuracy. (Zou et al., 2007). The cut-off point was established by the statistical function using the ROC curve based on the Youden index (*J*), and calculated from the following threshold (Youden, 1950):

$$J = \text{Sensitivity} + \text{Specificity} - 1$$

where Sensitivity (called true positive rate) measures the probability that a study subject is classified as diseased given that the subject is truly diseased, and Specificity (i.e. true negative rate) measures the probability that a study subject is classified as not diseased given that the subject is truly not diseased (Magder and Hughes, 1997).

For each parameter in two different measurement approaches (daily total values or daily changes value), all values were evaluated as potential decision thresholds to predict the behavioral outcomes of LPS. The optimal cut-off point was then determined by selecting the value which achieved the highest *J* value (J_M), and then the optimal decision threshold for each predictor was determined for a given animal and was used to define that animal as sham or LPS-challenged. This allowed a cut-off value with high sensitivity and high specificity to be defined.

Penalized B-spline regression

The Penalized B-spline regression model that we propose for the *pth* predictor variable (PV_p) of sham piglets was based on the assumption that the time variation can be modeled and minimized using cubic spline functions (Eilers and Marx, 1996)

$$\sum_{p=1}^{m}\left\{PV_p(=\mu_p) - \sum_{j=1}^{k}\beta_j B_j(x_p)\right\}^2$$

where m represents data points (x_p, μ_p) in the regression model on a set of k B-splines B_j denoting the cubic B-spline basis functions of degree $d = 3$ with coefficients β_j and $B_j(x_p)$ is obtained from equidistant knots x_{pj} ($j \in \{1, \dots, k\}$) representing locations between j and $j-1$. The first-order difference for the jth element of β is defined by $\Delta\beta_j = \beta_j - \beta_{j-1}$. Higher-order differences are defined by $\Delta^h\beta_j = \Delta(\Delta^{h-1}\beta_j)$, e.g., for $h = 2$, $\Delta^2\beta_j = \Delta(\Delta^1\beta_j) = \Delta(\beta_j - \beta_{j-1}) = \beta_j - 2\beta_{j-1} + \beta_{j-2}$. For a B-spline expansion with degree d and k interior knots, the penalized B-spline fit is obtained by solving the following penalty expressions on (higher-order) finite differences of the coefficients of adjacent B-splines:

$$\hat{\beta}_j = \underset{\beta_j}{\operatorname{argmin}}\left(\frac{1}{2}\sum_{p=1}^{n}\omega_p\left(PV_p - \sum_{j=1}^{k}\beta_j B_j(x_p)\right)^2 + \frac{\lambda}{2}\sum_{j=h+1}^{d+k+1}\left(\Delta^h\beta_j\right)^2\right)$$

where h is the order of the penalty, λ is a nonnegative smoothing parameter and ω_p are observation weights, for default value they can be set to 1, ($\omega_i = 1$). In this model, the nonzero elements of tridiagonal or tetra-diagonal matrix D with order $h = 3$ are given by $\Delta^3\beta_j = \beta_j - 3\beta_{j-1} + 3\beta_{j-2} - \beta_{j-3}$. Also, here with large sample size, the number of knots k is not a crucial parameter, and small k may cause over-fitting (Eilers and Marx, 2010). To make certain that k is sufficiently large to fit the data, we used the default option $k = 100$ was used. Furthermore, with $k = 100$, order $h = 2{,}3$, and degree $d = 3$, D is the difference matrix with order h which had dimension $(d + 1 + k - h) \times (d + 1 + k)$. Since observation weights ω_i were 1, W which is a diagonal matrix of ω_i will be identity matrix I. Therefore, the penalized B-spline transformation $\hat{u}$ of u is a linear smoother $\hat{u} = H_j u$, where matrix H_j is simply defined by

$$H = B \times \left(B\,\widetilde{W}\,B + \lambda\,D_h^T\,D_h\right)^{-1} \times B^T\,\widetilde{W}$$

where D_h is the matrix representation of the difference operator Δ^h , and the elements of B are given by $b_{ij} = B_j(x_i)$. Also, $\widetilde{W}$ is a diagonal matrix of weights with the elements w_{ii} that can be calculated from $w_{ii} = \frac{1}{v_i}\left(\frac{\partial\mu_i}{\partial\eta_i}\right)^2$, where v_i is the variance of PV_i , given μ_i. When $\lambda = 0$, we have the standard normal equations of linear regression with a B-spline basis, and with $k = 0$, a special case of ridge regression occurs (Eilers and Marx, 2010). The role of the smoothing parameter in penalized smoothing is to control the smoothness of the fitted curve. The trace of H will approach h as λ increases. For the optimal smoothing parameter λ, AIC is considered as the selection criteria for all models (Eilers and Marx, 1996).

All statistical analyses were performed using the MIXED, TRANSREG, and LOGISTIC procedures of SAS/STAT software, Version 9.4 (SAS Institute Inc., Cary, NC, USA), and Python 3 programming language (Van et al., 1995).

Results

Due to LPS challenge, which is used to mimic septic shock, six piglets in LPS groups died after injection. Meanwhile, this study aims for an alert system of potential morbidity rather than mortality; hence, these piglets were not used for the following behavioral and logistic or penalized B-spline regression analysis.

Automated Behavior Measurement

The NU*track* System identifies changes in multiple pigs' structural, temporal, and spatial behaviors. For this experiment, 16 pigs in each pen were used, but the number of pigs within an area can be much larger. This technology has an over 99% precision and over 96% recall (Psota et al., 2019). First, the temporal pattern of precision measures was visualized for the nursery phase (Figure 1). Regardless of the treatment group, there were no significant differences ($P > 0.05$) observed in all behavioral measures evaluated (Figure 1l days -5 to -1 relative to the treatment day). For 5 days after application of treatments (Study Day 1), all LPS-challenged piglets (mixed or non-mixed pens) had increased lying duration, decreased feeding, standing duration, and decreased locomotion measures (i.e., angle and distance; Figure 1; $P > 0.05$) compared to their baseline measures (days -5 to -1) and all sham treated pigs. These changes in spatial, locomotor, and posture behaviors mostly remained until Study Day 5 (Table 1). However, there was no significant difference observed in Sitting no matter whether piglets got LPS injection or not. In a further study, sitting duration was not considered a predictor for the logistic or penalized B-spline regression model.

Selection of optimal predictor and decision threshold

Including independent predictors is essential for fitting the logistic or penalized B-spline regression model (e.g., variables that are not highly correlated). Two different measurement approaches (daily total values and daily changed values) were used to show the correlations among potential predictors on days 1 to 6 relative to treatment (Figure 2). When using daily total values of each behavioral variable as a predictor, distance, angle, standing, and lying strongly correlated with Study Day 1 to Day 6 (Figure 2A and Supplemental 1 A). While feeding showed a high association with other predictor variables in the first two days after injection (Days 1-2; Figure 2 and Supplemental 1A) but this association decreased as the day increased (Days 3-6 (Supplemental 1B).

Similar results for relationships among distance, angle, standing, and lying variables, when using daily changed values were found (shown in Fig. 2B and Supplemental 1B). That is, they had a strong correlation with each other on Study Day 1. However, distance and angle show a less positive correlation with standing and lying on the following days (Study Days 2-6). Meanwhile, Feeding shows a decreased association with distance and angle across Study days 1-6, while it has a moderate positive correlation with standing and lying.

Therefore, to avoid multicollinearity issues in our logistic or penalized B-spline regression models regarding overall daily behavior relationships among these predictors, it could be appropriate to select each behavioral variable as the predictor for health and LPS-challenged piglets.

Logistic regression

The optimal LPS predictor and its corresponding decision threshold were determined on Study Days 1 and 2. In Fig. 3, the depiction of the ROC curve and decision threshold values are shown for each evaluated parameter for two different measurement approaches (daily total values or daily changes value). In Table 2, accuracy measures are listed as AUCROC values, the corresponding decision thresholds based on the max Youden J_M achieved, and equations for the logistic regression models.

Overall, the daily total value of all significant behaviors on Days 1 and 2 achieved the highest 0.960 < AUCROC < 1.000 and the greatest accuracy of [0.915, 0.987]. The J_M corresponded to the optimal decision threshold for Study Day 1 of distance = 782.07 m, angle = 2613.41 rad, feeding = 2.18 h, standing = 20.90%, and lying = 78.31% (Fig. 3A). When using daily change values, the capability for distinguishing healthy and LPS-challenged piglets was not as good as using the daily total values in the dataset, especially for Study Day 2. Models for daily changed values had great performance on Study Day 1 with an 0.975 < AUCROC < 0.990 and 0.900 < accuracy < 0.929 but were not acceptable on Study Day 2 with an AUCROC ~ 0.70 and accuracy of ~ 0.63. For daily changes, the J_M corresponding to the optimal decision threshold for Study Day 1 calculated distance = -137.21 m, angle = -77.03 rad, feeding = -0.99 h standing = -6.58%, and lying = 6.76% (Figure. 3B).

Penalized B-spline regression

Fitted with associated 95% predictive limits based on Sham piglets when using a penalized B-spline model for two measurement approaches (daily total values and daily changes value) of each predictor variable were shown in Fig. 4 and Fig. 5. The authors based this method on the assumption that the slow time variations in each predictor variable could be modeled by a set of splines. The optimal smoothing parameters λ (selected by minimizing the AIC) for daily total values are 1.86 (angle), 4617.4 (feeding), 1.77 (distance), 4.04 (standing), and 3.08 (lying), respectively. Meanwhile, the optimal smoothing parameters for daily change values are 0.25 (angle), 4.62 (feeding), 0.22 (distance), 0.52 (standing), and 0.22 (lying), respectively.

Performance comparison for compromised pig alert system

A one-level early alert system could be developed based on logistic or penalized B-spline regression analyses for two different measurement approaches of data. In this case, the threshold line could be determined by the logistic or penalized B-spline regression analyses, and then it would assign piglets to one of two statuses: healthy or compromised.

In this work, the raw recorded data from the system for each behavioral or transformed variable into values that daily changes would lead to the following classifications:

1) *Healthy*:

a) For the logistic regression model, distance $> d_1$; angle $> a_1$; feeding $> f_1$; standing $> s_1$; lying $< l_1$. The corresponding decision threshold parameters d_1, a_1, f_1, s_1 and l_1 are shown in Table 2.

b) For penalized B-spline regression model, distance $> d(t)$; angle $> a(t)$; feeding $> f(t)$; standing $> s(t)$; lying $< l(t)$. $d(t), a(t), f(t),$ and $s(t)$denote 95% predictive interval lower bound at Day t. Meanwhile, $l(t)$ is a 95% predictive interval upper bound at Day t.

2) *Compromised*

a) For the logistic regression model, distance $< d_1$; angle$< a_1$; feeding $< f_1$; standing $< s_1$; lying $> l_1$.

b) For the penalized-spline regression model, distance $< d(t)$; angle $< a(t)$; feeding $< f(t)$; standing $< s(t)$; lying $> l(t)$.

Based on these considerations, a one-level alert system was used for assessing the risk of injury or illness from the LPS dataset. Fig.6 and Fig.7 are compared among the count of piglets outside their alert line for daily total or daily change values across all study days and predictor variables for Logistic regression based on Study Day 1 and penalized B-spline regression.

Raw data evaluated with logistic regression may not be appropriate to set up the one-level alert system for assessing the risk of morbidity. In this model, no matter what predictor variable was used, there were large numbers of sham pigs but classified as compromised in the post-challenged phase. The misclassification in this situation is called false positive detection. However, after transforming raw data (daily changes values), the performance of the one-level alert based on the logistic regression model improved considerably for some predictor variables (e.g., Feeding, Standing, and Lying), especially using data from Day 1.

The performance of the penalized B-spline regression model was superior to logistic regression, whether data were transformed or not. More specifically, penalized B-spline regression modeling could detect changes in behavior at day 1 and also detect pigs that managers identified (Day 1, right after injection) and highly (~85 out of 96) compromised piglets without the issues in the late nursery phase.

Additional Performance comparison for compromised pig monitoring system

After injection on Study Day 1, challenged pigs reduced activity drastically. However, activity increased in 5- or 6-days post challenge. The authors assumed all piglets would be recovered from the LPS challenge by Study Days 6 to 34. After the nursery phase was completed, all the piglets were supposed to be transferred into the finisher building. However, fourteen piglets observed by managers as either lame or severe tail destruction. They had to be isolated or treated before moving into the finisher building (Table 3). The statisticians were blinded to this information until after the models were applied, and the out-of-bound pigs were identified late in the nursery phase. Therefore. reporting of each predictor variable results was assessed (marked as challenged piglets) for Study Days 23 to 34, based on the appropriate proposed models (Fig. 8 and Supplemental 4). To understand the out-of-range values across predictor variables and on each Study Day, the individuals that were outside the one-level alert line for all predictor variables were compared. At the individual level, ten out of fourteen animal care taker compromised piglets were detected early by the proposed model based on penalized B-spline for daily total value (Table 3 and Fig. 8A). Most of these pigs' data kept triggering the one-level alert line on multiple predictor variables of consecutive days, except Pigs 412 and 454. For instance, Pig 257 started to be marked as compromised by angle, distance, lying, and standing out-of-bound measures since Study Day 23. These same pigs' data continued to trigger the proposed alert line for lying until the end of the nursery phase. Although there exist some false-positive cases, most of them only showed once being the outlier of one predictor variable (except Piglet 303, shown in Supplemental 4A). However, the other two proposed models (Penalized B-spline and Logistic regression) based on daily changes values seems that they could not reliably report these compromised piglets (Fig. 8B and 8C). Some of the compromised piglets could be detected by them, but also there exist extremely too more false-positive than expected (Supplemental 4B and 4C). Therefore, it will be a challenge to distinguish true abnormal piglets and false-positive detected piglets.

Discussion

Observational ethograms and behavior activity budgets of piglets are associated with fulfilling specific requirements for swine survival and growth. In addition to moving, standing, lying, feeding, and drinking, these behaviors include social or aggressive (Maselyne et al., 2014). Several studies have demonstrated significant differences in daily activity budgets in compromised pigs, particularly those following an infection (Matthews et al., 2016). Reiner et al. (2009) and

Escobar et al., (2007) reported that distinct behavioral changes in pigs (such as standing, feeding, lying, or walking) were observed following the LPS challenge, which was consistent with our results. Field also found (Fernández-Carrión et al., 2017) that lying time increased substantially and feeding or walking duration decreased during which animals were in motion decreased substantially across the following post-infection phases.

Furthermore, the development of continuous monitoring and rapidly and accurately automated identifying compromised (sick or injured) pigs by changes in behavioral patterns in commercial systems is becoming increasingly important for timely intervention to enhance treatment success at the individual level, reduce the impact on welfare, and promote sustainable pig production. Video processing of livestock provided us with the possibility of non-intrusive animal monitoring in real time. Over the past two decades, it has been proven that a computer vision system could potentially provide an efficient precision livestock technology for behavioral monitoring of animals (Alameer et al, 2020; Shao and Xin, 2008). However, when using automated image analysis to assess movement within groups of animals, some of the methods have the issue of following individual animals, especially for pigs because they can be very homogenous in appearance (Ahrendt et al., 2011; Robie et al., 2017). In the real-world setting, individual pig identification is essential to the classification of individual pig activities and behaviors and the identification of sick animals even in group housing. In earlier version of NU*track*, the capability to track pigs individually has been proven to be able to accurately identify, maintain identification, and continuously track the activities of newly weaned nursery pigs, and the detection of changes in activity over time can be beneficial as a baseline to detect illness or injury (Lancaster, 2018; Psota et al., 2019; Schmidt et al., 2022)

In this study, the first aim was to utilize a controlled immune challenge to create compromised pigs to compare to sham-handled pigs housed in homogenous treatments. There is concern that compromised animals may influence the behaviors of healthy animals, therefore, a second aim was to compare pigs within the same pen that were sham-handled or challenged with LPS. The results of this study showed that the decline in activity and increase in resting (Lying behavior) of piglets after the LPS challenge was visible in overall data evaluation even before any alteration was noticed by any healthy-compromised signs. Then, the modelling identified out-of-bounds pigs late in the nursery phase. These pigs should have recovered from the LPS challenge. After further investigation, these were the pigs that the animal caretakers (blinded to treatments) needed to treat or isolate. This evidence provides further support for the idea of using this technology as an early warning sign of a compromised pig. Süli et al., (2017) stated that their. online monitor systems could detect a significant difference between motion values before and after the challenge with the virus.

Here we describe the results of a study of a remote monitoring system for the early detection of immune-compromised piglets. The results suggest that whenever spatial behavior, locomotor, and posture activities data from the NU*track* system are considered together or individually, remote monitoring can reliably identify the challenged piglets the day received the injection, even in pens with both challenged and non-challenged pigs. Therefore, this precision technology provided an inexpensive alternative to direct motion measurement. The system could provide a feedback system to alert animal caretakers in semi-real time, considerably reducing the financial and logistical costs of periodic sampling and increasing the individualized care of group housed animals. Therefore, our second aim was to propose a one-level alert for distinguishing healthy and healthy-compromised piglets, based on longitudinal data of behavioral variables.

Although there has been increasing concern over the early detection of compromised pigs on computer vision systems, a specific alert system in the real production systems is not fully established. However, some researchers (Süli et al., 2017) established their online monitoring system on sixteen pigs for early disease detection based on body temperature and an average of 24-hourly motion data. Martínez-Avilés et al., (2017) also established their two-stage alert system based on discriminant analyses using RFID temperature and motion data for differentiating healthy and infected animals. Vranken and Berckmans (2017) listed some examples of the development of early warnings with precision livestock farming systems, such as pig water intake and cough monitoring systems. However, for longitudinal data, there always exists a time dependence issue; most of the aforementioned studies were only based on a few days, while the current study was based on the entire nursery phase. For the current study as piglets grew, their active behaviors (feeding, moving and standing) gradually decreased and less active behaviors increased (lying or sitting). If temporal data in rapidly growing animals are not considered, then a fixed alert line for system recorded raw data (such as daily total values) may increase false positive detections, especially is logistic regression model for daily total values on raw data are used. Therefore, this project provides an optimal method by constructing a non-linear alert curve based on piglet behavioral patterns in the nursery phase.

Since smoothing methods have been known for their flexibility in complex patterns, modeling longitudinal data with smooth curves has gained much attention in many fields' research (Djeundje and Currie, 2010; Muggeo and Hajat, 2009; Stoklosa and Huggins, 2012). Our one-level alert using penalized B-spline for daily total values performed very well, even for pigs identified by the animal caretakers as compromised at the end of the nursery phase, thus achieving a minimum of one-week early detection.

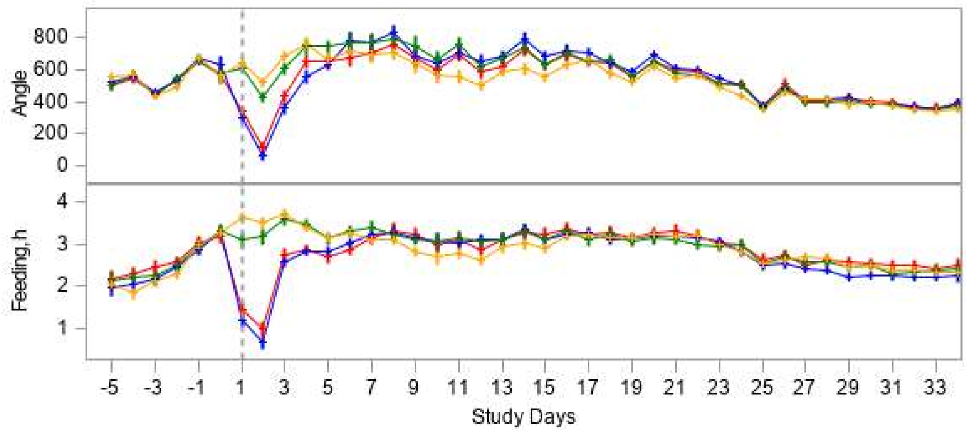
An alternative method is using transformed data, such as daily changes value. After raw recorded data was transformed, behavioral patterns for nursery piglets could be eliminated. Also, changes in activity caused by out-of-bounds behavior due to health status could be captured immediately. The results of the current experiment suggest that when using daily change values, models for some predictor variables (such as standing, feeding, and lying) are improved, and they could reliably detect LPS-compromised piglets, even for logistic regression methods. Since transformed data already removed this time dependence pattern, the performance of the threshold line based on the logistic model may improve. The penalized B-spline model performed consistently well, no matter what measurement approaches were used. Clearly, for a sudden and big change due to some acute diseases, alert lines need to appear. However, if pigs with preclinical chronic diseases or injuries exist, this modeling may not capture these minor changes. For instance, these abnormal piglets were removed at the end of the study. Human observers may not be able to notice small or non-consecutive changes.

Based on our results the online monitoring system, The one-level alert strategy could provide animal caretakers with usable and effective information, so that they take proper actions. When an animal's data triggers the alert line, an alert system could provide the caretaker within semi-real time that there may be something wrong. It makes it easier to spot problems before they get too big to fix.

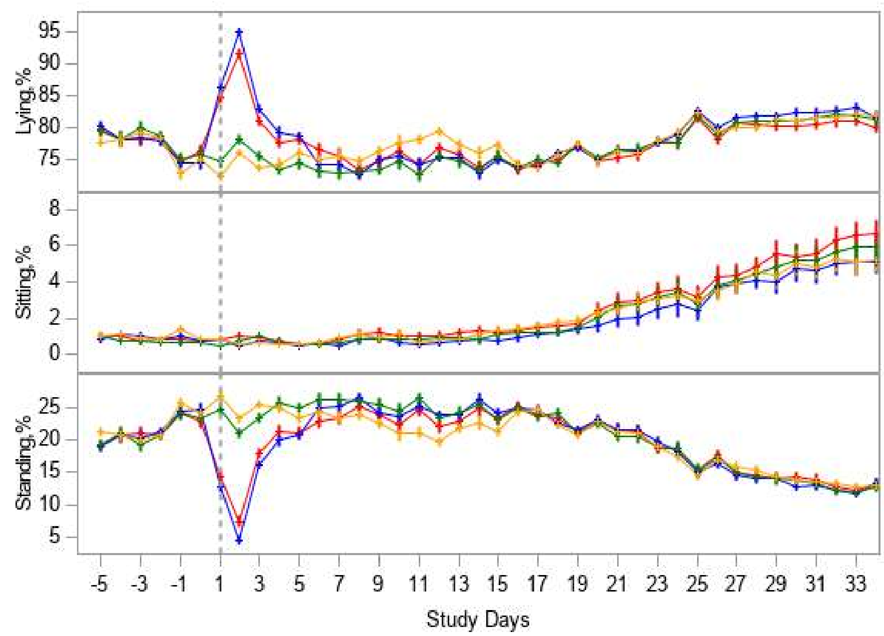
In conclusion, the findings of this study demonstrate the potential for detecting infection early through monitoring behavioral changes in piglets. The results suggest that the NUtrack system can detect potential health problems as fast as sensors installed on drinking devices or room indicators such as video cameras. The one-level alert based on the penalized B-spline regression model provides much more reliability than logistic regression. Our system may be handy for the early detection of health-compromised nursery pigs that follow behavioral changes soon. It would be interesting to test it in future experiments or commercial scales.

Limitation: our objective is to provide a reliable one-level alert for distinguishing healthy and healthy-compromised piglets during the nursery phase. However, due to some noisy data in the acclimation period, our models only used the last 39 days of 43 days. Future work should be conducted (using new experimental data to test our model). Also, add the first 4 days of the nursery phase data to complete the model.

A



B



C

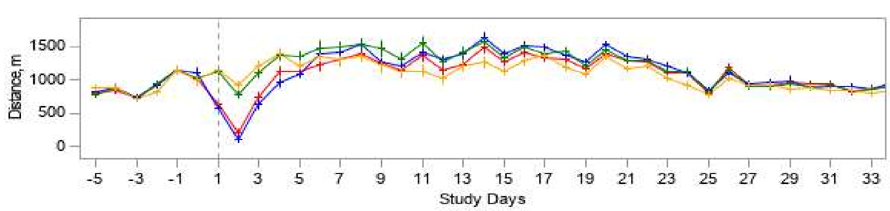


Figure 1. Automated measurements of behaviors duration for 43-day (Study days: -5 to 34) nursery phase (Treatment groups: All Sham, orange; Mixed Sham, green; Mixed challenged, red; All challenged, blue), where the lipopolysaccharide challenge was provided on Study Day 1 (Grey vertical reference dash line). Each data point is the mean of 4 pens of piglets. (A) Spatial behaviors: Angle (rad, ± SE), and Feeding (h, ± SE). (B) Posture activities (%, ± SE): Lying, Sitting, and Standing. (C) Distance travelled (m, ± SE).

Table 1 Pairwise comparisons of behavior variables for four treatments on Study Days 0 - 6[1]

Items	SD[2]	All Sham	Mixed (Half sham – Half Challenged)		All Challenged	*P*-value
			Sham	Challenged		
Angle	0	571.53 ± 55.26	573.76 ± 57.02	568.84 ± 57.02	631.83 ± 55.26	0.83
	1	636.82 ± 32.28[b]	609.82 ± 33.77 [b]	342.86± 33.77[a]	304.69 ± 32.28[a]	<0.01
	2	520.91 ± 27.81[a]	429.45± 29.64[a]	123.54± 30.17[b]	62.60 ± 27.94[b]	<0.01
	3	685.38 ± 36.06[a]	605.34 ± 38.27[a]	434.24± 38.91[b]	358.79 ± 36.22[b]	<0.01
	4	759.44 ± 41.08[b]	744.05± 43.79[ab]	655.40± 44.58[ab]	559.59 ± 41.32[a]	0.02
	5	664.79 ± 36.52	744.73 ± 39.62	651.78 ± 40.51	633.03 ± 36.80	0.24
	6	715.17 ± 58.81	767.75 ± 60.81	677.83 ± 61.41	779.91 ± 58.98	0.62
Feeding, h	0	3.27± 0.12	3.31 ± 0.17	3.20 ± 0.17	3.29 ± 0.12	0.97
	1	3.62 ± 0.15[a]	3.09 ± 0.18[a]	1.43 ± 0.18[b]	1.21 ± 0.15[b]	< 0.01
	2	3.50 ± 0.14[a]	3.18 ± 0.18[a]	0.98 ± 0.19[b]	0.65 ± 0.15[b]	<0.01
	3	3.71 ± 0.11[a]	3.59 ± 0.15[a]	2.75 ± 0.17[b]	2.58 ± 0.11[b]	<0.01
	4	3.41 ± 0.09[a]	3.47 ± 0.13[a]	2.86 ± 0.14[b]	2.84 ± 0.10[b]	<0.01
	5	3.14 ± 0.10	3.14 ± 0.14	2.69 ± 0.15	2.83 ± 0.10	0.05
	6	3.25 ± 0.09	3.29 ± 0.13	2.88 ± 0.14	3.03 ± 0.09	0.10
Lying, %	0	75.11 ± 0.98	75.93 ± 1.11	76.39 ± 1.11	74.58 ± 0.98	0.63
	1	72.48 ± 0.81[a]	74.80 ± 0.92[a]	84.85 ± 0.92[b]	86.33 ± 0.81[b]	< 0.01
	2	76.08 ± 0.57[a]	78.26 ± 0.79[a]	91.61 ± 0.84[b]	94.86 ± 0.59[c]	<0.01
	3	73.89 ± 0.53[a]	75.65 ± 0.73[a]	80.99 ± 0.78[b]	82.99 ± 0.55[b]	<0.01
	4	74.39 ± 0.84[ac]	73.63 ± 0.95[ac]	77.78 ± 0.98[bc]	79.39 ± 0.85[b]	<0.01
	5	76.17 ± 0.69	74.47 ± 0.83	78.33 ± 0.87	78.68 ± 0.70	<0.01
	6	75.07 ± 0.88	73.12 ± 0.98	76.64 ± 1.01	74.32 ± 0.89	0.14
Sitting, %	0	0.84 ± 0.08	0.69 ± 0.09	0.74 ± 0.09	0.77 ± 0.08	0.65
	1	0.80 ± 0.08	0.47 ± 0.12	0.88 ± 0.12	0.81 ± 0.82	0.09
	2	0.57 ± 0.15	0.75 ± 0.17	1.06 ± 0.17	0.51 ± 0.15	0.12
	3	0.64 ± 0.10	1.01 ± 0.11	0.96 ± 0.10	0.76 ± 0.11	0.09
	4	0.56 ± 0.13	0.69 ± 0.15	0.77 ± 0.15	0.69 ± 0.14	0.78
	5	0.56 ± 0.08	0.60 ± 0.09	0.52 ± 0.09	0.60 ± 0.08	0.90
	6	0.64 ± 0.12	0.53 ± 0.13	0.60 ± 0.14	0.62 ± 0.12	0.93
Standing, %	0	24.04 ± 1.01	23.37 ± 1.13	22.87 ± 1.14	24.65 ± 1.01	0.68
	1	26.72 ± 0.83[a]	24.73 ± 0.94[a]	14.27 ± 0.94[b]	12.86 ± 0.83[b]	<0.01
	2	23.35 ± 0.64[a]	20.98 ± 0.83[a]	7.32 ± 0.88[b]	4.63 ± 0.66[b]	<0.01
	3	25.47 ± 0.51[a]	23.34 ± 0.72[a]	18.04 ± 0.76[b]	16.25 ± 0.53[b]	<0.01
	4	25.04 ± 0.86[ac]	25.68 ± 0.96[ac]	21.45 ± 0.99[bc]	19.92 ± 0.86[b]	<0.01
	5	23.27 ± 0.73	24.27 ± 0.87	21.07 ± 0.90	20.80 ± 0.74	0.01
	6	24.30 ± 0.90	26.34 ± 1.00	22.75 ± 1.03	25.05 ± 0.91	0.14
Distance, m	0	1001.34 ± 88.97	1024.78 ± 92.58	1017.94 ± 92.58	1103.79 ± 88.97	0.85
	1	1157.01 ± 66.97[a]	1120.79 ± 69.81[a]	644.82 ± 69.81[b]	570.48 ± 66.97[b]	< 0.01
	2	921.37 ± 54.56[a]	783.37 ± 58.03[a]	198.93 ± 58.03[b]	114.29 ± 54.56[b]	<0.01
	3	1210.67 ± 75.38[a]	1115.34 ± 79.39[a]	737.50 ± 80.56[b]	631.31 ± 75.67[b]	<0.01
	4	1399.77 ± 71.01[b]	1372.11 ± 76.38[b]	1134.53 ± 77.93[ab]	954.61 ± 71.48[a]	<0.01
	5	1214.86 ± 63.49	1357.37 ± 69.49	1127.57 ± 71.20	1094.68 ± 64.02	0.07
	6	1349.77 ± 104.18	1483.37 ± 108.63	1234.44 ± 109.54	1391.56 ± 104.57	0.47

[a, b, c]Means in the same row with different superscript letters differ significantly ($P < 0.05$).

[1]All Sham, all piglets received saline injections (2mL/kg body weight) on Day 10; Mixed Sham, fifty percent pigs

of each pen were randomly assigned saline injections (2mL/kg body weight or 2.0 mL/pig) and grouped with Mixed Challenged; Mixed Challenged, fifty percent pigs of each pen were randomly assigned LPS injections (2mL/kg body weight or 2.0 mL/pig) and grouped with Mixed Sham; All Challenged, all pigs were randomly assigned LPS injections (2mL/kg body weight).

[2]SD: Study Days. Study Day 1 was the day the lipopolysaccharide challenge was provided.

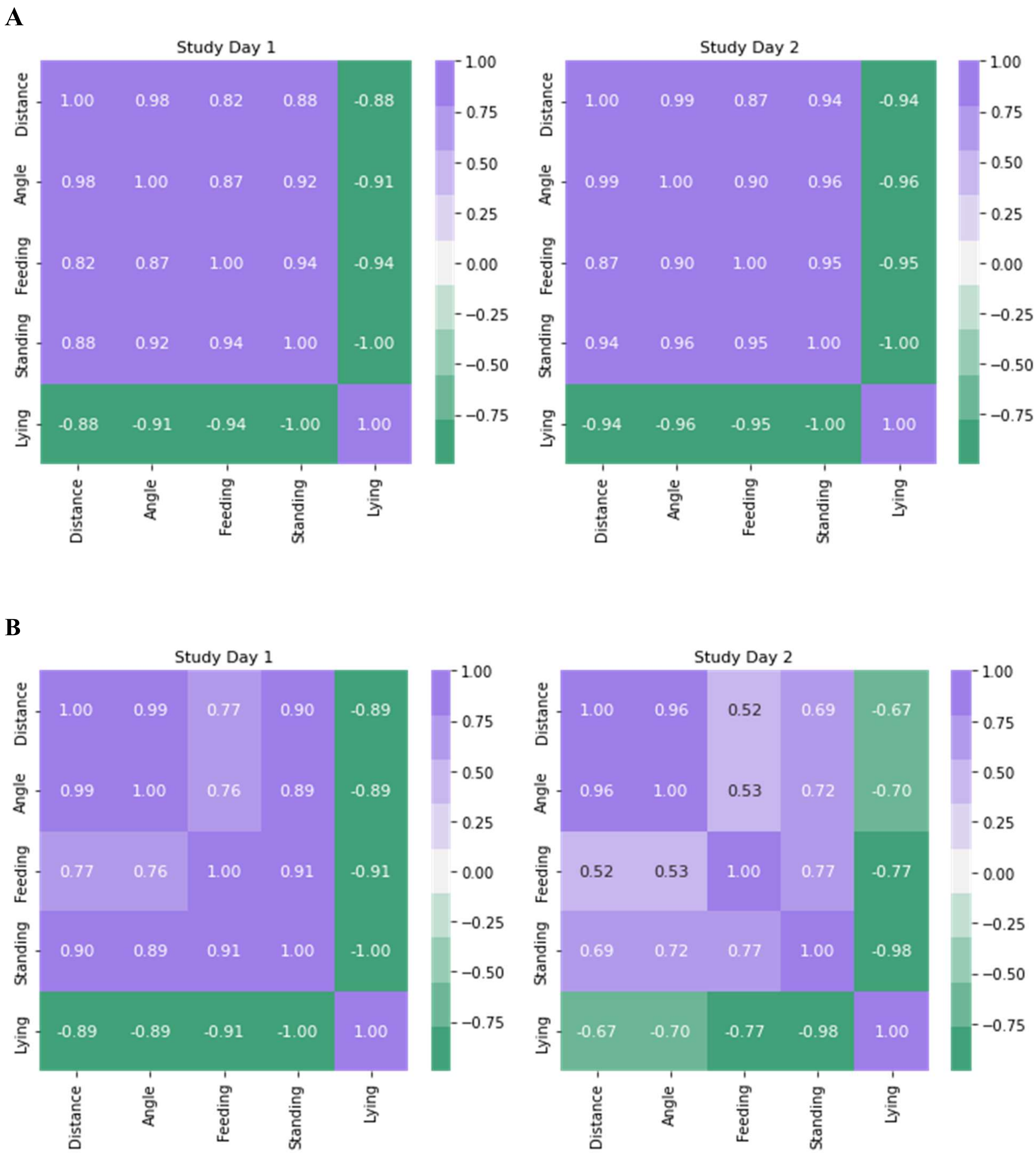
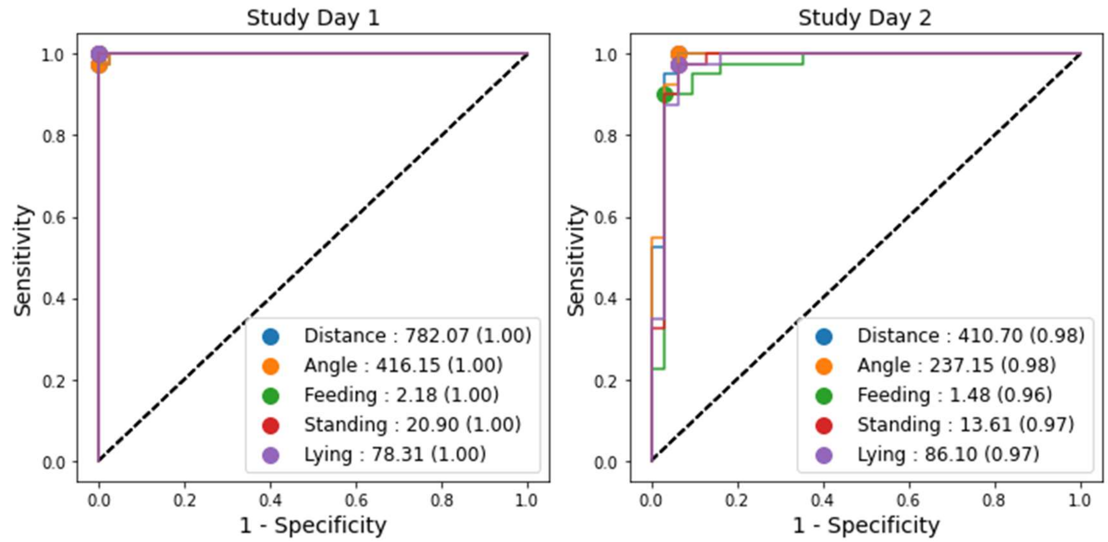


Figure 2. Correlations among five behaviors (not including Sitting) for Study Day 1 and 2, respectively. (A) Daily total values (B) Daily changes started on Study Day -5.

A



B

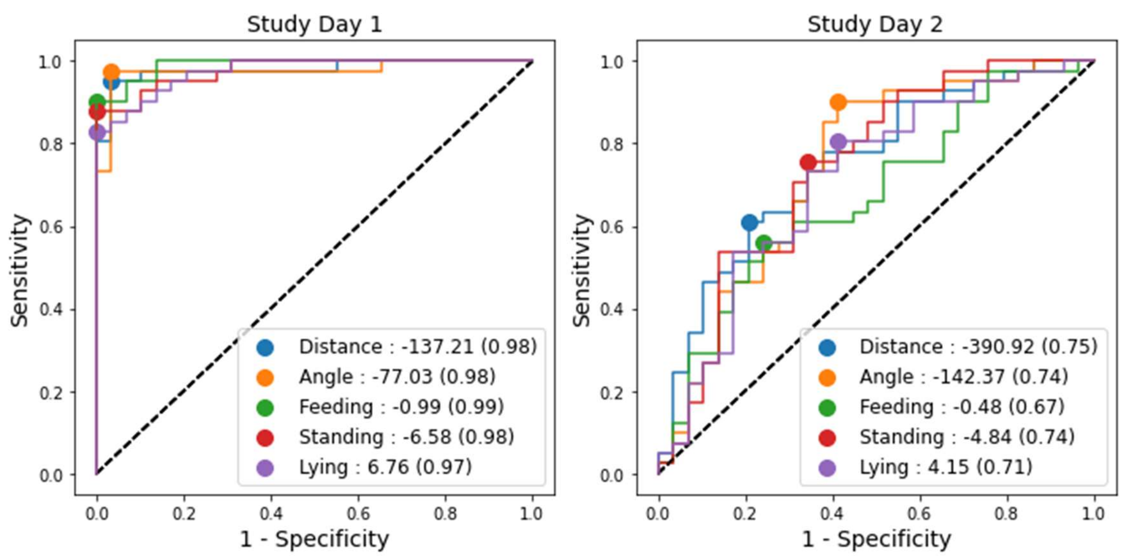


Figure 3. Receiver operating characteristic (ROC) curves for prediction of lipopolysaccharide (LPS) challenge: ROC curves for the prediction of LPS challenge for days (Study Day 1 and 2) after injection are depicted in individual sub-panels for five behaviors (five models). The dot values on the ROC curves are the cutpoints chosen using the maximum Youden index (J_M) for each behavior variable, respectively. These values are listed within the sub-panel alongside the corresponding decision threshold with the area under the curve of the receiver operating characteristic (AUC) for each behavior variable. (A) Daily total values (B) Daily changes started on Study Day -5.

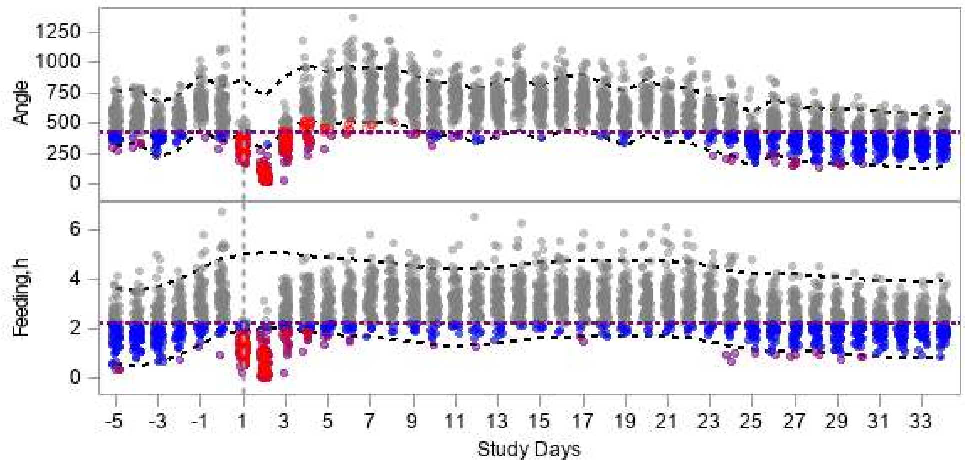
Table 2. Predictive performance, optimal decision threshold, and equations of five predictor models[1].

Item	SD[2]	Variables	Index			
			Accuracy	AUC	Decision threshold	Equation
Daily total values	1	Distance	0.974	0.999	782.075, m	10.323 - 0.012x
		Angle	0.987	0.999	416.148	12.801 - 0.028x
		Feeding	0.987	1.000	2.179, h	6.657 – 3.096x
		Standing	0.987	1.000	20.898, %	18.779 – 1.005x
		Lying	0.987	1.000	78.310, %	-80.197 + 0.998x
	2	Distance	0.972	0.983	410.698, m	7.959 - 0.018x
		Angle	0.972	0.983	237.149	8.472 - 0.033x
		Feeding	0.915	0.960	1.481, h	4.941 – 2.521x
		Standing	0.958	0.973	13.606, %	7.527 - 0.535x
		Lying	0.958	0.972	86.096, %	-48.083 + 0.565x
Daily changes	1	Distance	0.900	0.979	-137.213, m	-2.499 - 0.013x
		Angle	0.929	0.976	-77.031	-2.669 - 0.022x
		Feeding	0.914	0.990	-0.989, h	-2.601 – 2.560x
		Standing	0.914	0.977	-6.581, %	-3.173 - 0.644x
		Lying	0.900	0.975	6.758, %	-2.961 + 0.625x
	2	Distance	0.686	0.748	-390.923, m	-2.719 - 0.007x
		Angle	0.629	0.740	-142.375	-3.115 - 0.016x
		Feeding	0.614	0.671	-0.483, h	-0.468 - 0.739x
		Standing	0.629	0.741	-4.843, %	-1.982 - 0.306x
		Lying	0.614	0.711	4.151, %	-1.986+ 0.302x

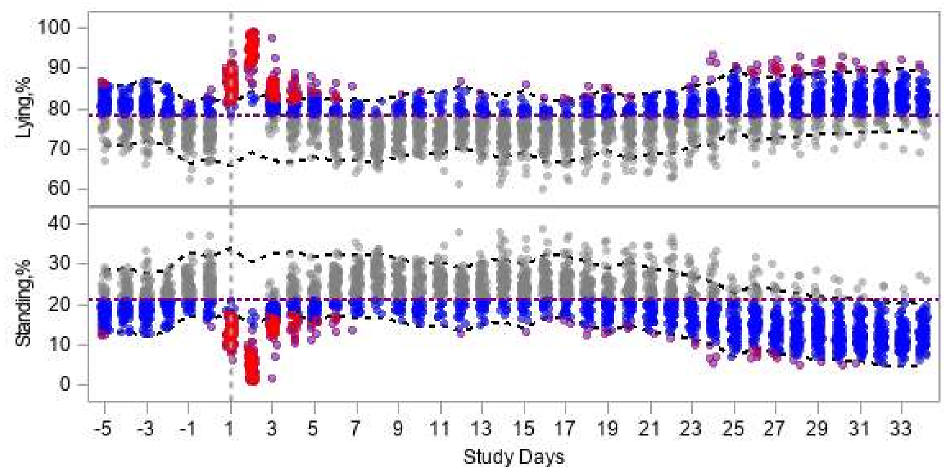
[1]AUC = area under the curve of the receiver operating characteristic; the Decision threshold was based on Cutpoints which were chosen using the maximum Youden index (J_M).

[2]SD: Study Days. Study Day 1 was the day the lipopolysaccharide challenge was provided.

A



B



C

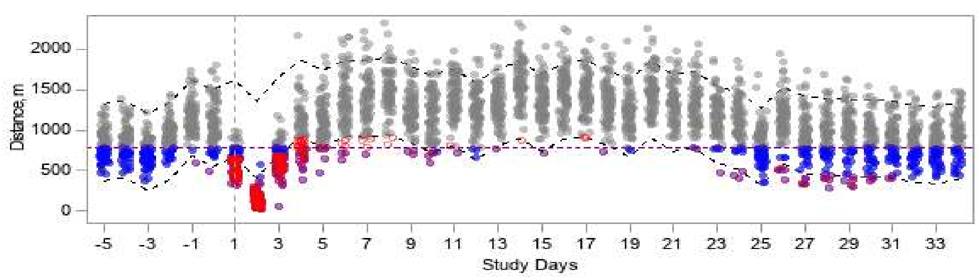


Figure 4. Scatter plot for Challenged piglets with marked outliers of two regression models (penalized B-spline: red; Logistic: blue) for daily total values. The fitted smooth curves (two black lines) represent associated 95% predictive interval bounds using penalized B-spline model based on Sham piglets. The purple horizontal line represents the threshold line using a logistic regression model based on Study Day 1 (grey vertical dash line). The optimal smoothing parameter (λ) for penalized B-spline is selected by minimizing the AIC. (A) Spatial behaviors: λ=1.86 for Angle and λ=4617.4 for Feeding. (B) Posture activities: λ=4.04 for Standing and λ=3.08 for Lying. (C) Distance traveled: λ=1.77.

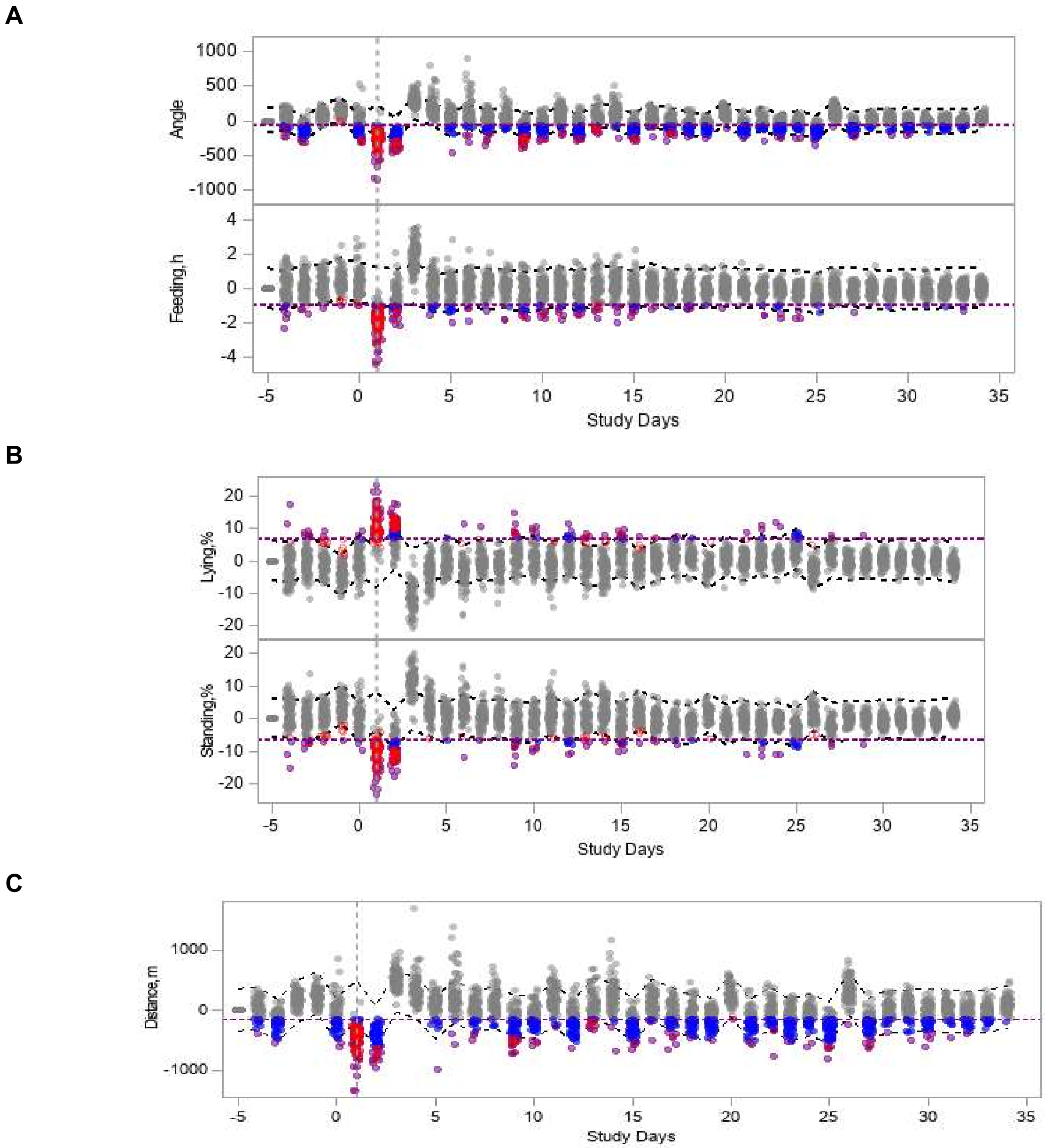


Figure 5. Scatter plot for Challenged piglets with marked outliers of two regression models (penalized B-spline: red; Logistic: blue) for daily changes value. The fitted smooth curves (two black lines) represent associated 95% predictive interval bounds using penalized B-spline model based on Sham piglets. The purple horizontal line represents the threshold line using a logistic regression model based on Study Day 1 (grey vertical dash line). The optimal smoothing parameter (λ) for penalized B-spline is selected by minimizing the AIC. (A) Spatial behaviors: λ=0.25 for Angle and λ=4.62 for Feeding. (B) Posture activities: λ=0.52 for Standing and λ=0.46 for Lying. (C) Distance traveled: λ=0.22.

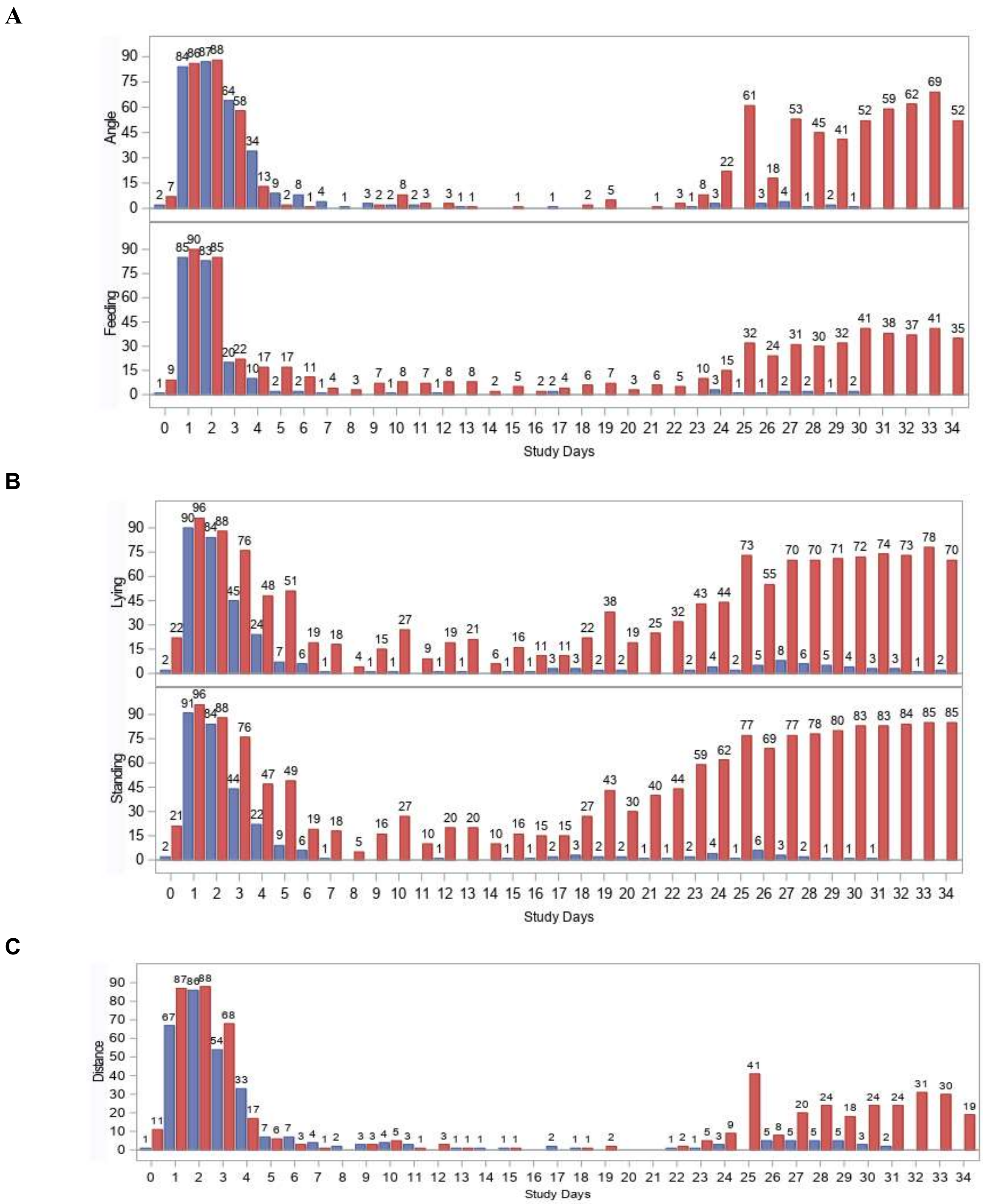
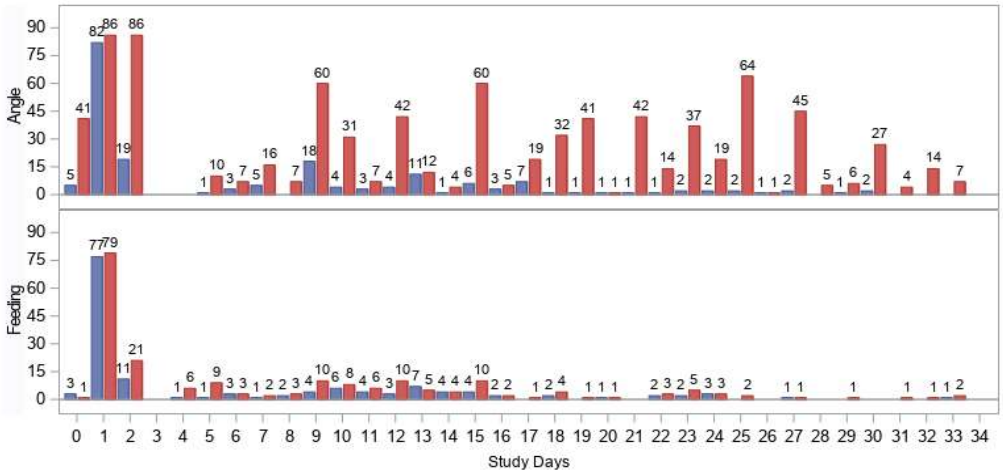
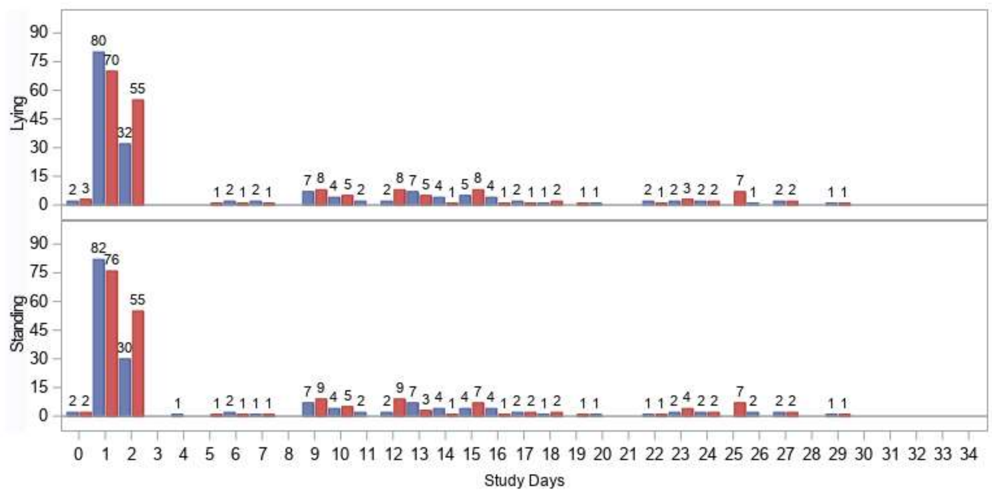


Figure 6. Comparison among the count of Challenged piglets outside their alert line for daily total values across all study days for Logistic regression based on Study Day 1 and penalized B-spline regression. (A) Spatial behaviors: Angle and Feeding. (B) Posture activities: Lying and Standing. (C) Distance traveled.

A



B



C

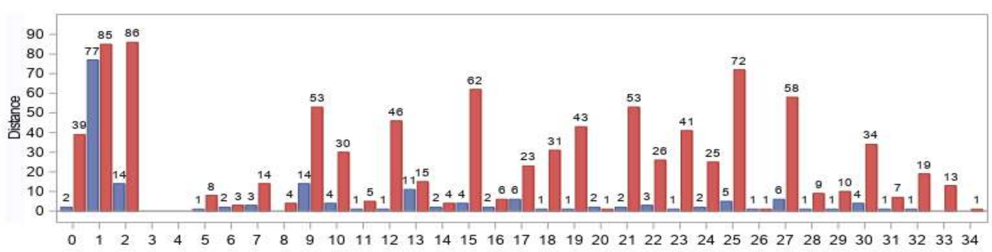


Figure 7. Comparison among the count of Challenged piglets outside their alert line for daily change values across all study days for Logistic regression based on Study Day 1 and penalized B-spline regression. (A) Spatial behaviors: Angle and Feeding. (B) Posture activities: Lying and Standing. (C) Distance traveled.

Table 3. Observed healthy compromised Piglets at end of Study days[1].

Piglet IDs[2]	Removal Index		
	Post-SD	Date	Condition
212	+1	10-Oct	Tail Bite
240*	+2	11-Oct	Lameness
257	+2	11-Oct	Lameness
270	+3	12-Oct	Lameness
332	+3	12-Oct	Lameness
343*	+4	13-Oct	Tail Bite
348	+3	12-Oct	Lameness
412	+2	11-Oct	Lameness
414	+4	13-Oct	Lameness
426*	+2	11-Oct	Lameness
432*	+3	12-Oct	Lameness
434	+2	11-Oct	Lameness
436	+2	11-Oct	Lameness
454	+4	13-Oct	Tail Bite/Lameness

[1]SD: Study Days. Study Day 1 was the day the lipopolysaccharide challenge was provided.

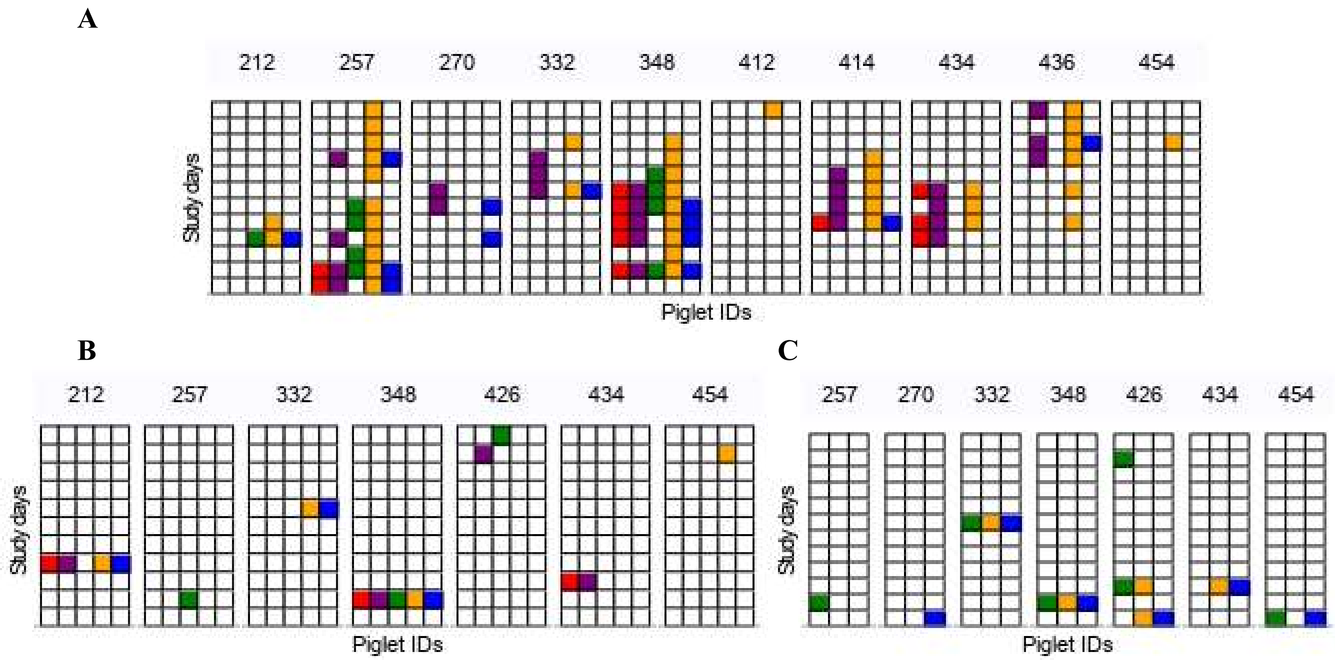
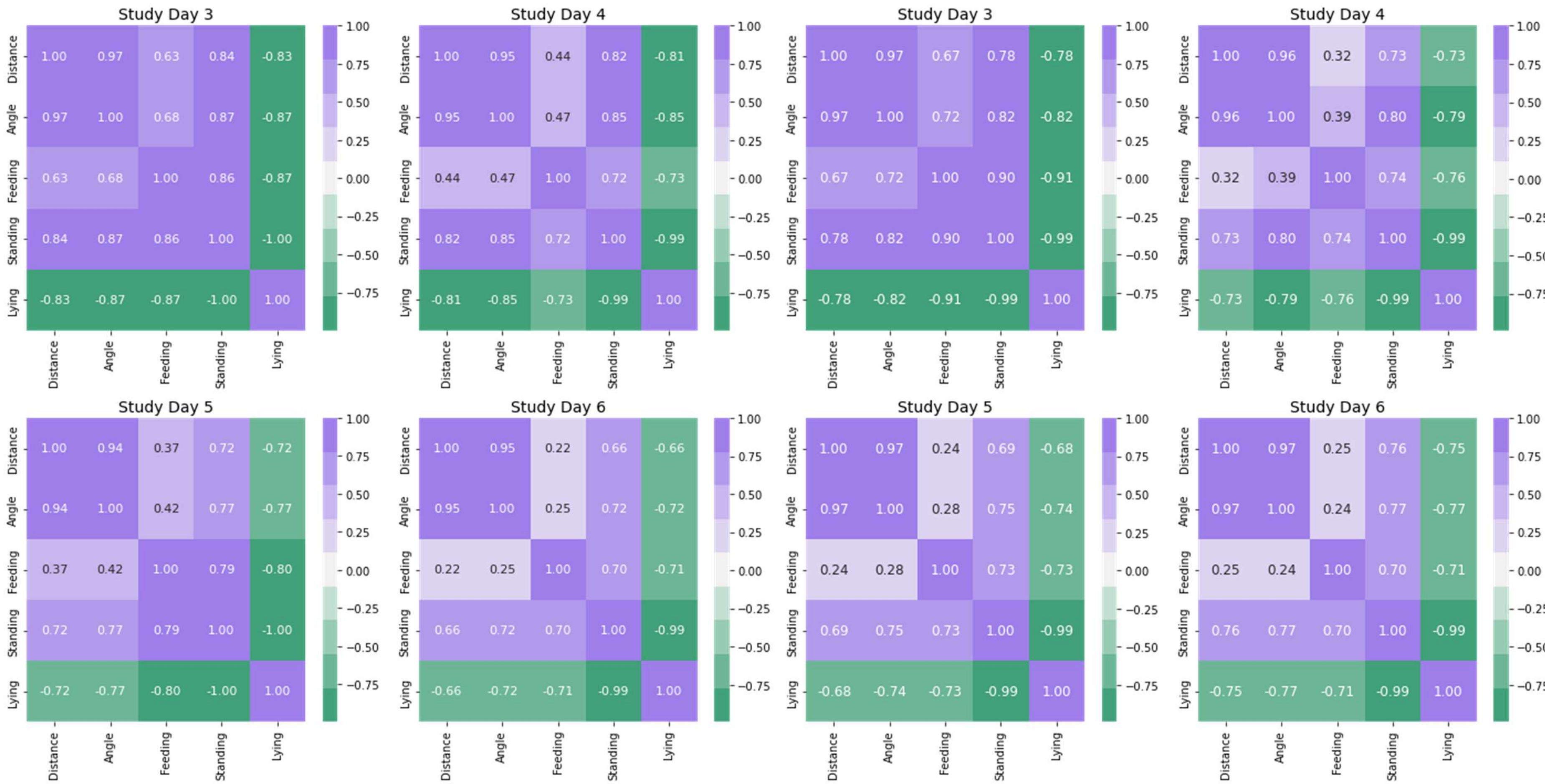


Figure 8. The panel of abnormal Piglet IDs results is displayed by a 2-dimensional heatmap. Each row represents one of the Study days (22-34, from bottom to top), and the columns aggregate the automated measurements for each study day and predictor variables. The column for each predictor variable is ordered from left to right by Angle, Distance, Feeding, Lying, and Standing. Colored squares indicate if the measurement is outside the one-level alert line (Angle, red; Distance, purple; Feeding, green; Lying, orange; Standing, blue). (A) The one-level alert line, based on penalized B-spline for daily total values (including false positives). (B) The one-level alert line, based on penalized B-spline for daily changes value (only including true positive). (C) The one-level alert line, based on logistic regression for daily changes value on Study Day 1 (only including true positive).

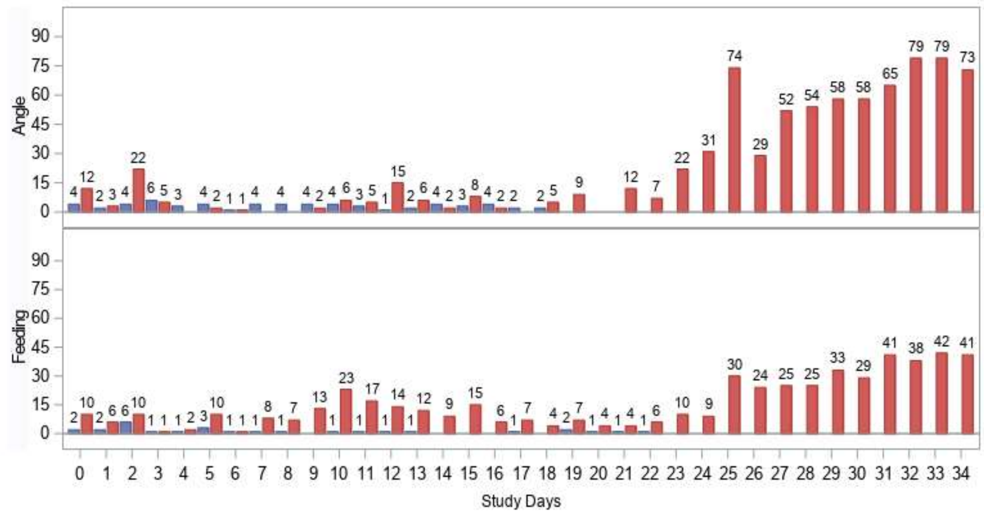
Supplemental

A B

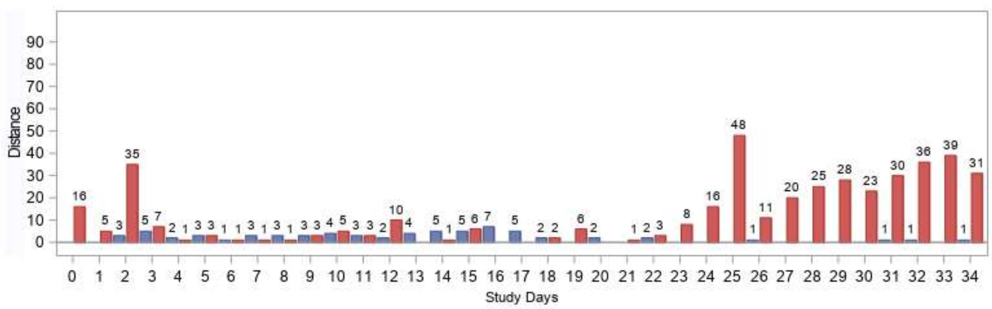


Supplemental 1. Correlations among five behaviors (not including Sitting) for Study Days 3 to 6, respectively. (A) Daily total values (B) Daily changes started on Study Day -5.

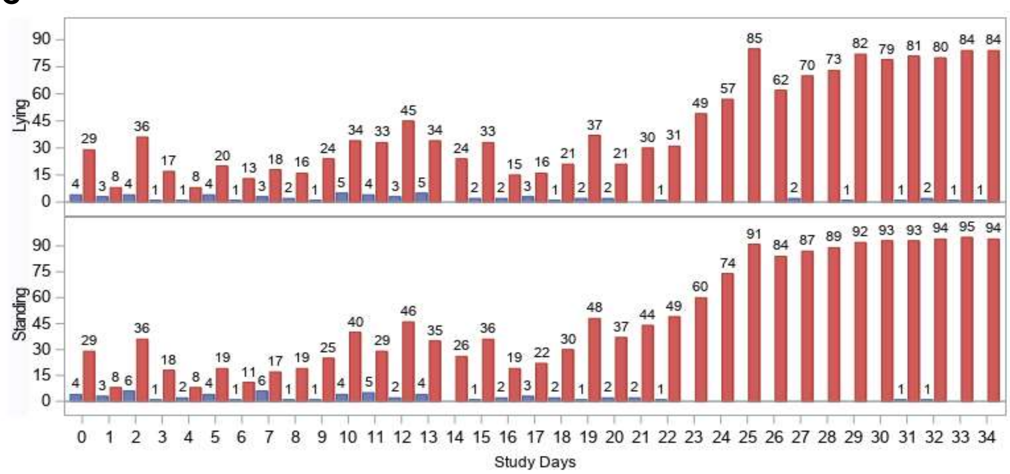
A



B

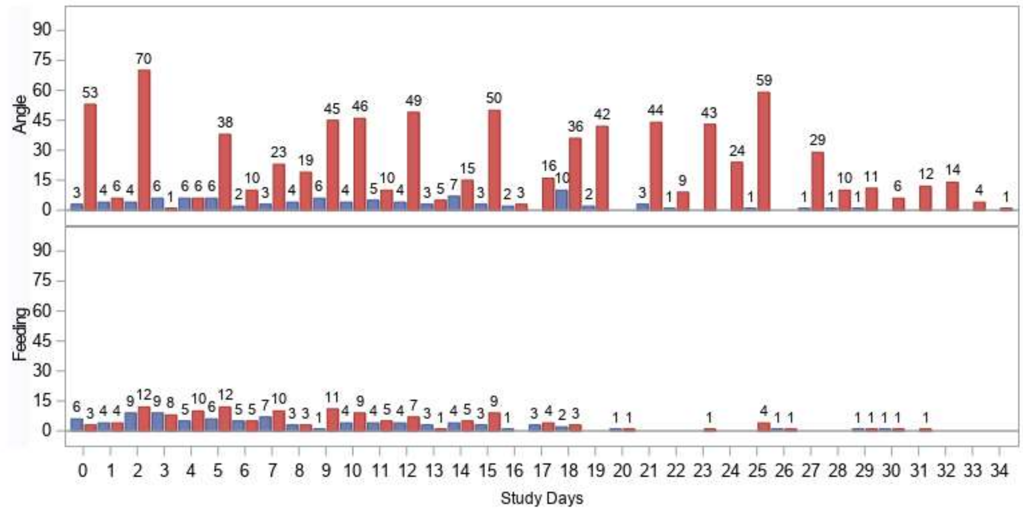


C

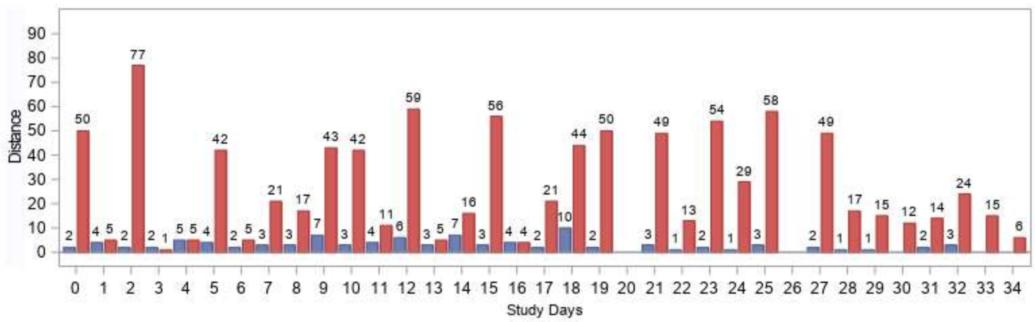


Supplemental 2. Comparison among the count of Sham piglets outside their alert line for daily total values across all study days for Logistic regression based on Study Day 1 and P-spline regression. (A) Spatial behaviors: Angle and Feeding. (B) Distance traveled. (C) Posture activities: Standing and Lying.

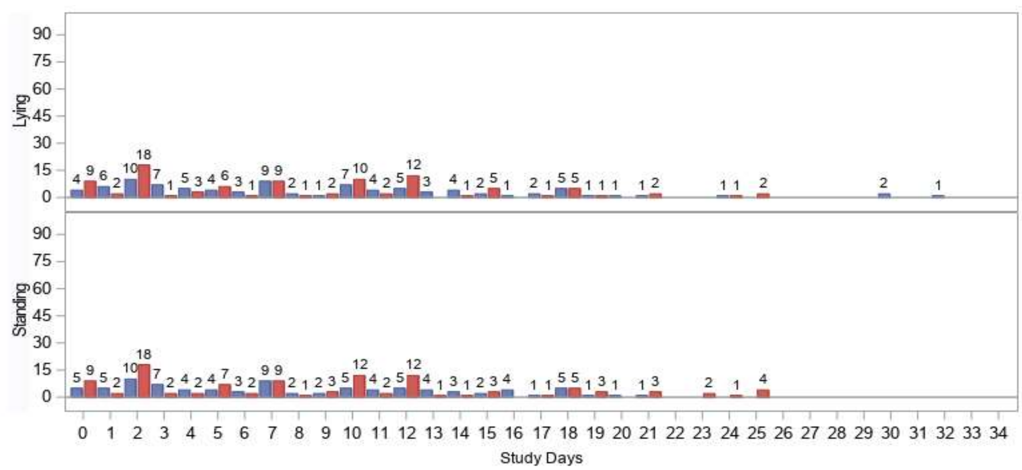
A



B

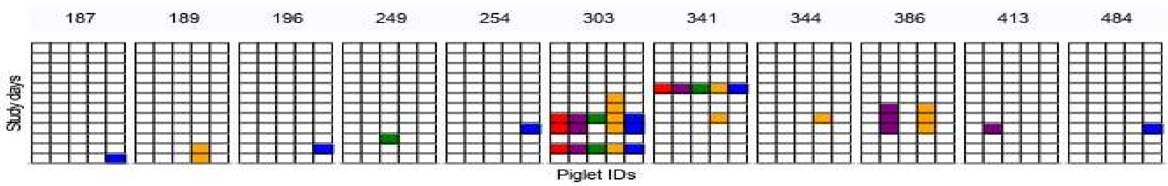


C

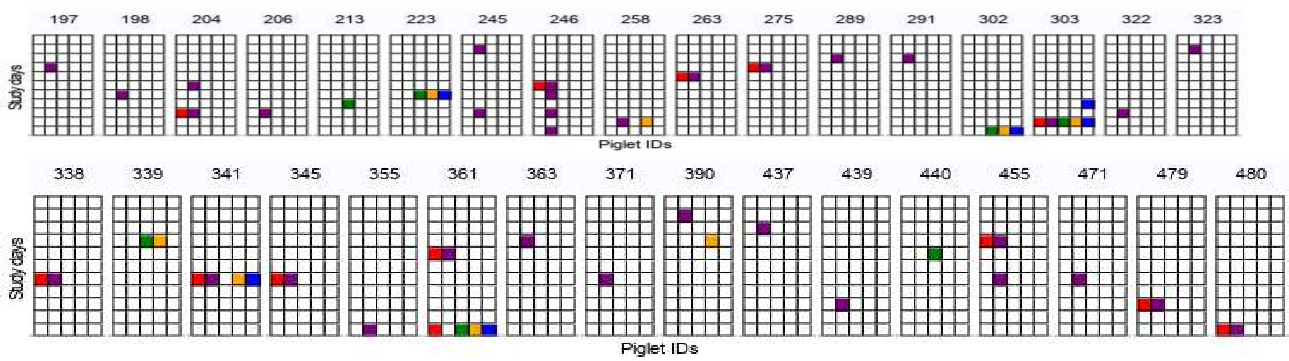


Supplemental 3. Comparison among the count of Sham piglets outside their alert line for daily total values across all study days for Logistic regression based on Study Day 1 and P-spline regression. (A) Spatial behaviors: Angle and Feeding. (B) Distance traveled. (C) Posture activities: Standing and Lying.

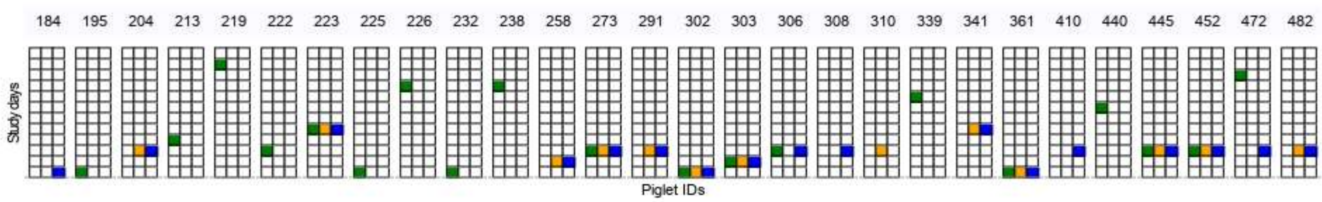
A



B



C



Supplemental 4. The panel of false-positive Piglet IDs results is displayed by a 2-dimensional heatmap. Each row represents one of the Study days (23-34, from bottom to top), and the columns aggregate the automated measurements for each study day and predictor variables. The column for each predictor variable is ordered from left to right by Angle, Distance, Feeding, Lying, and Standing. Colored squares indicate if the measurement is outside the one-level alert line (Angle, red; Distance, purple; Feeding, green; Lying, orange; Standing, blue). (A) The one-level alert line, based on penalized B-spline for daily total values (including false positives). (B) The one-level alert line, based on penalized B-spline for daily changes value (only including false positive). (C) The one-level alert line, based on logistic regression for daily changes value on Study Day 1 (only including false positive).

Objective 2: *Disrupt social hierarchy at finishing to train the DF-DTP to identify agonistic interactions, play-behaviors, nose bellying, ear- and tail-biting behavior.*

Results

General Results

Nine pigs received the rescue protocol intervention, however, those pigs did not recover and died from the endotoxin challenge, translating into a case-fatality of 9.37%. Hide scores were not included in the analyses because pigs rest and pile on top of each other, and human observers were not permitted to influence behavior unless there was an intervention event. Emesis events were also not included in the analyses because pigs ingested the vomit prior to data collection, and these records were infrequent.

Human-data for Entire Population

When all pens were considered, human derived data presented acceptable AUC, true positive rates, and false positive rates (0.85 AUC; > 0.70 Sensitivity; > 85 Specificity; Table 1) on days 0 and 1. By day 2, human data AUC, true positive, and false positive rates declined to less desirable AUC, true positive, and false positive rates (Table 1). Days 3 and 7 human observations were not conducted because of extenuating circumstances. For days 4, 5, and 6, AUC and false positives were at less desirables rates (< 0.57 AUC; < 33% Specificity; Table 1), but true positives remained acceptable (> 83% Sensitivity; Table 1. However, true positive rates declined again for the last human observation time point, day 6 (Table 1).

Area under the curve (AUC) was calculated using the linear regression model and provides the validity measures for each behavior computed by NUtrack, with a 0 to 1 range (0.5 = no discrimination; 1 = perfect performance); Optimal Cutoffs were based on maximum Youden's index, maximizing the sum of Sensitivity and Specificity [28–30].

Precision Data for Entire Population

For the entire population of pigs, all precision measures had superior AUC values, true positive rates, and false positive rates than human data on days 0, 1, and 3 (>0.98 AUC; > 79% sensitivity; > 94% specificity). Sensitivity value from human data was greater than distance- and pivot-precision data for day 4 (84.4% vs. 72.9% and 80.2%, respectively). On day 4, only lying duration (both positions) had a marginally acceptable AUC (0.715). Human data had greater sensitivity (84.4 %) than precision-data on days 4 (Table 1). Nonetheless, false positives were less frequent among precision data for days 4 and 5 compared with human-data (Table 1), especially for lie-duration (80.2% specificity for day 5; Table 1). Area under the curve can serve as a reliable prediction analyses toolset, and all precision data behaviors had excellent AUC for precision data for days 0-2 (> 0.90; Table 1), except for day 4 feed duration (0.787 AUC). Nonetheless, precision data AUC declines steadily after day 3.

Half LPS and Half Sham Data

When only data from pens that had half of the pigs treated with LPS and the other half sham-treated were evaluated, human data had lower AUC for all comparable precision data AUC (Table 1). However, the false-positive rate for human scores was remarkably better on days 4-6 on half-and-half pens than when the entire population was scored (Tables 1 and 2). Precision measures of pigs in the half-and-half pens resulted in AUC that were comparable (>0.99) to the entire population for days 0 and 1, but AUC declined starting on day 2 (Tables 1 and 2). The sensitivity of precision data for half-and-half pigs were greater than the comparable human-derived sensitivity (Table 2), with 100% sensitivity on day 1 for distance moved and feeding duration. A noteworthy observation is that feeding duration sensitivity increased from 73.9% to 100% when the entire population vs. half-and-a-half pigs were observed (Tables 1 and 2).

Area under the curve (AUC) was calculated using the linear regression model and provides the validity measures for each behavior computed by NU*track*, with a 0 to 1 range (0.5 = no discrimination; 1 = perfect performance); Cutoffs were based on Youden's index, maximizing the sum of Sensitivity and Specificity [28–30].

Discussion

The use of endotoxin challenge to replicate sickness without risking infection is a well-established controlled immune challenge in both human studies and animal models [31]. Lipopolysaccharide is harvested from the outer membrane of heat-killed gram-negative bacteria, and researchers can control the amount and location administered. Administration of greater doses can replicate a cytokine and febrile response that is comparable to septic shock and low doses can be used to replicate preclinical sickness. In this experiment, LPS was used to create positive and negative sick subjects, and better understand a precision technology's ability to identify these subjects whether all of them were challenged, not challenged, or mixed. The aforementioned pen treatment would not be possible to control if a live pathogen was used.

One of the goals of the NU*track* system is to provide support to the workforce as a means to improve health and welfare of livestock. Animal technicians in large swine operations do not have the time needed to observe animals continuously. Instead, they rely on previous experience and used timepoint sampling to identify compromised pigs from non-compromised pigs. The authors used a more methodical score system for live, time-point observations for the purpose of comparing the capabilities to precision livestock technology. Humans and this technology both rely on visual cues from the pigs; the human compares the structural changes of a compromised pigs to pen mates, whereas precision data rely on both individual animal structural changes, and spatial relationships using temporal data. Behaviors that are valid in indicating illness are those with high sensitivity and high specificity when compared with a “golden standard”. Measures of sensitivity and specificity have been previously used to assess the validity of precision livestock technology in diverse animal production systems. The behaviors selected in this study were measured as continuous variables, so optimal cutoff values were necessary to maximize sensitivity and specificity.

The ability of precision technology to correctly identify pigs based on their behavior reflects the pig’s physiology. In general, immune compromised pigs will have decreased intake of substrates (water and feed) and increased resting time to conserve energy while the immunological insult is resolved.

The ability of a human to identify an immune challenged pig on day of challenge (d 0) was adequate; however, when both the entire population and the half-and-half pigs were examined, precision technology had more days with adequate sensitivities and specificities. Human observations created more false positives and false negatives than precision-measures when the entire population was considered. In commercial swine operations, false positive pigs may be treated with antibiotics, which can impact the rate of antibiotic resistant pathogens. A more likely challenge in production is false negative pigs. False negative pigs may go undetected, even by experienced technicians; these pigs can potentially serve as vectors, especially as they are commingled into the finishing phase.

Some behaviors can be tracked by humans (e.g., lie, and spatial location at a timepoint), but precision technology provides additional measures that the human cannot assess without technology. For example, pivot behavior is challenging to assess with human eyes. Pivot behavior captured by the current technology had good sensitivity and specificity to detect challenged pigs, and excellent AUC. Nordgreen et al. (2018) studied the effects of a low dose LPS challenge on the behavior and brain neurotransmitters in pigs. They harvested pigs 72 h after challenge, and found that neurotransmitters and markers of inflammation in the brain (e.g., hippocampus, hypothalamus, amygdala) were still elevated. This same research group and other conducted follow-up studies with group housed pigs; where they reported that the most significant change in social behavior was an increase in LPS challenged pigs ear biting their pen mates [42–44]. Ear biting is an active, social behavior, rather than a classic lethargic behavior, that would not be intuitive for animal technician identification of compromised pigs. This active-state of social interaction may explain why human-data had unacceptable sensitivity and specificity just 48 h after the challenge, while precision data had acceptable sensitivity and specificity 72 h later for the current experiment. During this interaction, the pig that is being bitten will likely turn away (pivot) away from the offender, therefore more research is needed regarding pivot measures and social interactions after immune challenges.

The usage of precision livestock technology does not substitute work force for swine operations. Instead, it serves as an additional tool to help decision-making. The presence of caretakers to check pigs' health is necessary because it causes a moderate disruption of behaviors, providing pigs with a chance of showing exploratory behaviors that will facilitate the identification of sick individuals by precision technology. For example, in the current experiment, the human observations from the half-and-half pens had lower false positives than the entire population data, especially as pen mates recovered (i.e., days 4-6). This finding was not surprising to the authors because previous experiments using continuous measures from repeated human approach tests indicated that compromised pigs will be less responsive and move at a slower pace when a human is standing in front of the pen [45,46]. In the pens that had a mix of compromised pigs and healthy pigs, technicians can directly compare each pig's behavior with their pen mates. This finding indicates that a human timepoint sample in conjunction with precision technology may be more ideal than expecting precision technology to completely take away human observation. For example, a technician could spend a few minutes observing each pen, record the obvious sick pigs, and then precision technology can provide the granular measures of behaviors within a pen. Precision data provide granularity because optimal cutoff values can be extrapolated for maintenance and social behaviors. The AUC from precision data can serve as a better predictor for other endpoints such as performance, or risk of morbidity and mortality.

Conclusions

Precision technology has great AUC, sensitivity, and specificity when compared with human observations, especially during the first 72 h after observation. Sensitivity and specificity are expected to decrease as pigs recover, but AUC may be an important measure for determining the risk of morbidity or need for medical intervention later in the pigs' life. Further experiments are needed for determining the precision's ability to detect compromised pigs among a greater number of healthy pigs. Nonetheless, this technology in combination with timepoint human observations may serve as an optimum system for semi-real time identification of sick pigs.

Tables and Figures: Objectives 2.

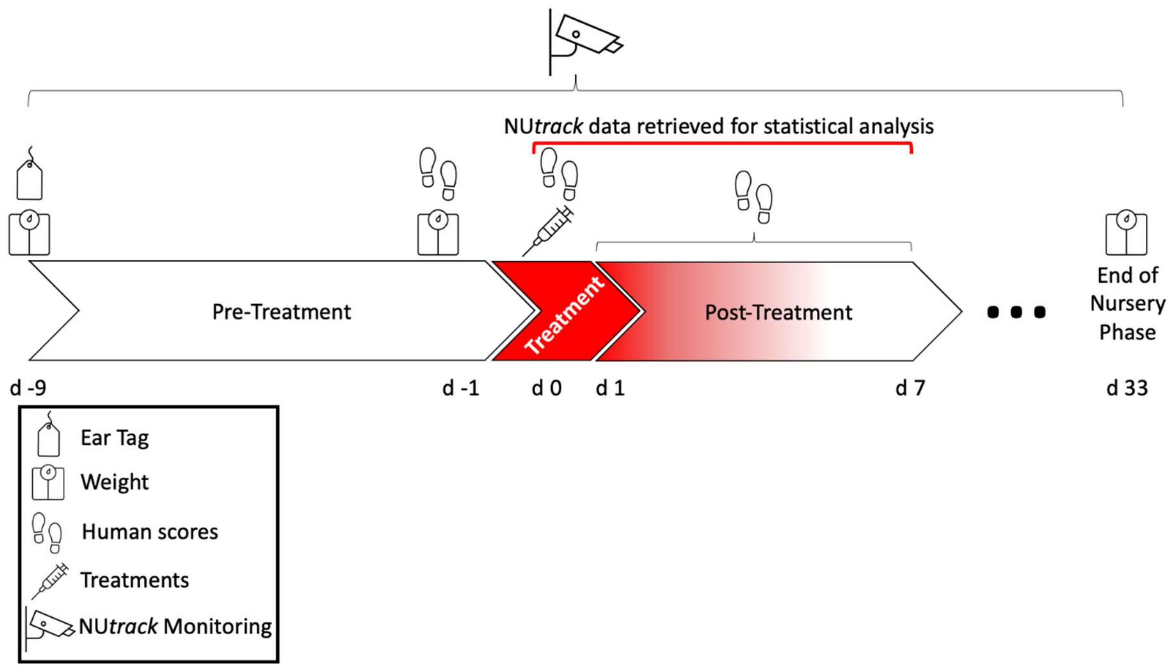


Figure 1. Timeline of experiment and data collection. Newly weaned pigs (n = 192) were randomly assigned to one of 12 pens (4 pens per treatment). Generic eartags were replaced with NU*track* ear tags. Body weights were recorded on days -9 and -1 relative to immune challenge (day 0). At 0900-0930 h on treatment day (d 0), pigs received one of the treatments assigned to pens: 1) all sham-handled, controls (injection of 3 mL saline solution); entire pen challenged with a single subcutaneous dose of lipopolysaccharide (LPS from E. coli O111:B ; 300 μg/kg of body weight in a total of 3 mL saline), and; 3) one-half of the pigs were sham-handled, and one-half of the pigs were treated with the same amounts as described earlier (half-and-half). Human data were collected starting on days -1, 0, and once per day during seven days post treatment (d 1 to d 7). A precision livestock technology (NU*track*) was utilized to continuously capture behavior measures (distance traveled, pivot behavior, feed, and total time lying) during the entirety of this timeline (d -9 to d 7). The 24-h data from the precision technology (NU*track*) data were used for this experiment.

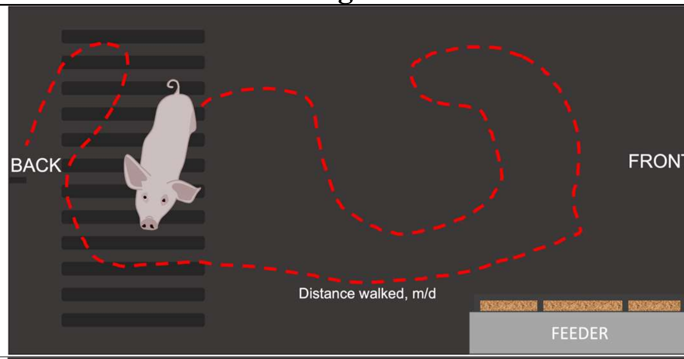
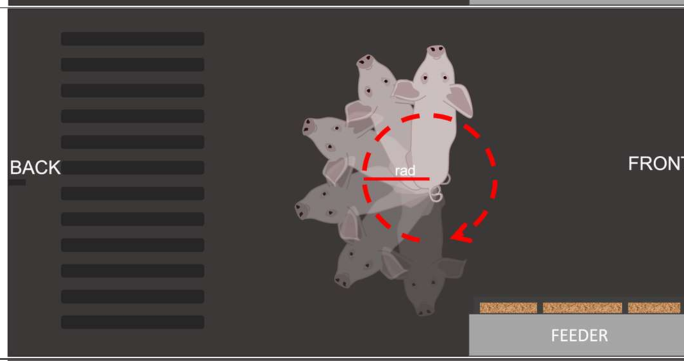
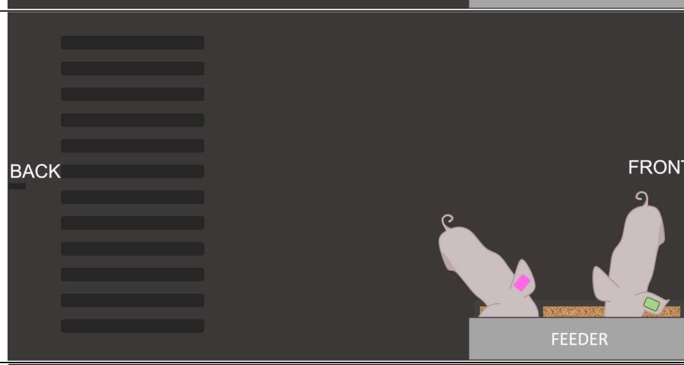
Behavior Definition	Diagram
Distance traveled, m/day- a spatial measure of locomotion	
Pivot, rad/day- structural turning of head plus movement of front limbs while back limbs are still [24]	
Feed, min/day- Total duration over head over feeder [25,26]	
Lie, min/day- total duration of Sternal or recumbent rest. The legs are legs straight, bent, or tucked under pig's body [22]	

Figure 2. Ethogram used by the precision technology (*NUtrack*) to automatically track nursery pig behavioral data.

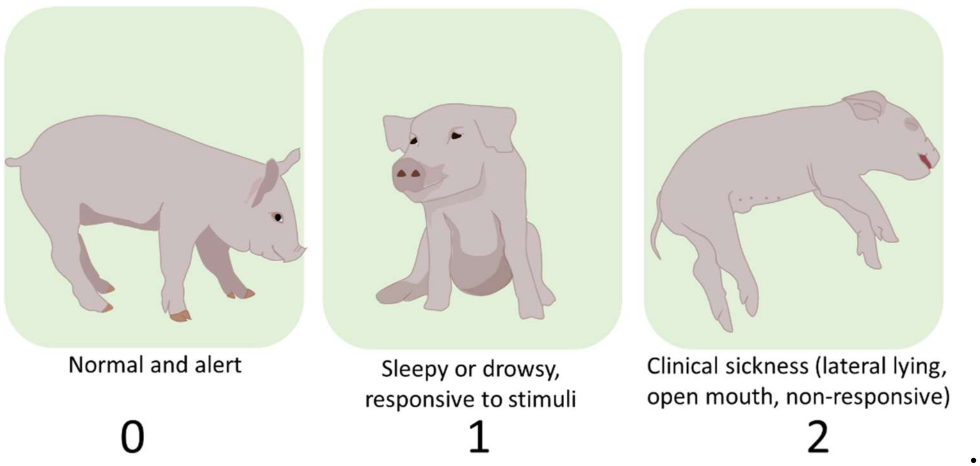


Figure 3. Human data: Posture and alertness categories and score used by trained observers to classify pigs according to their clinical signs. Humans collected data for each pig in a pen, while standing in front of the pen for 3 min.

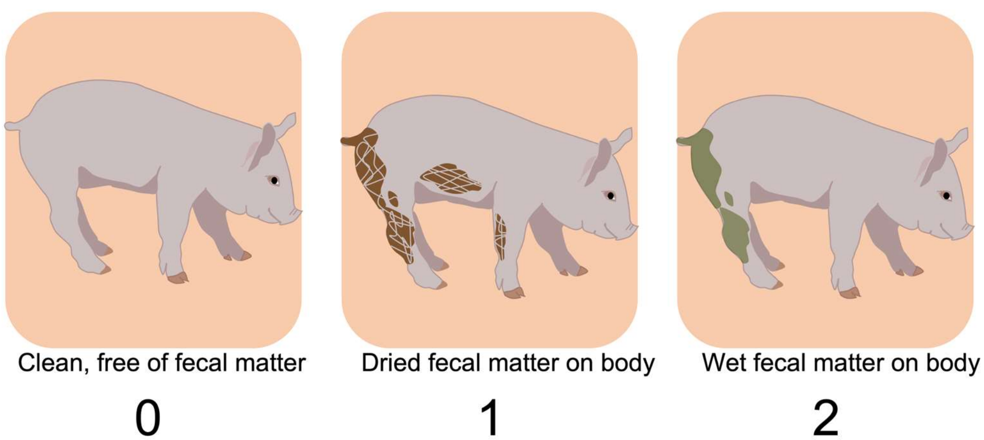


Figure 4. Human data- Hide score used by observers to classify pigs according to their clinical signs. In addition, emesis events were recorded. Humans collected data for each pig in a pen, while standing in front of the pen for 3 min.

Table 1. Entire population of nursery pig optimal cutoff values, the area under the curve (AUC), sensitivity, and specificity for human data and precision data (NU*track*). Pigs received one of the treatments randomly assigned to pens: 1) all sham-handled, controls (injection of 3 mL saline solution); entire pen challenged with a single subcutaneous dose of lipopolysaccharide (LPS from E. coli O111:B; 300 μg/kg of body weight in a total of 3 mL saline), and; 3) one-half of the pigs were sham-handled, and one-half of the pigs were treated with the same amounts as described earlier (half-and-half). Human real-time live scoring (human data) and data from a precision livestock technology (NU*track*; precision data) were evaluated.

	Day							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Human Data								
Sick Score								
Cutoff	-	-	-	-	-	-	-	-
AUC, 0-1	0.871	0.849	0.614	-	0.570	0.503	0.510	-
Sensitivity, %	88.5	70.7	53.4	-	84.4	83.3	68.8	-
Specificity, %	85.4	97.7	68.8	-	28.7	17.2	33.3	-
Precision Data								
Distance traveled								
Cutoff, m/day	784.6	365.7	958.7	1177.8	1132.1	1078.2	1008.4	1870.0
AUC, 0-1	0.981	0.999	0.929	0.846	0.686	0.542	0.481	0.391
Sensitivity, %	94.7	98.9	79.1	81.2	72.9	89.5	94.7	12.2
Specificity, %	91.6	98.8	96.2	76.7	60.9	21.8	12.6	93.1
Pivot behavior								
Cutoff, rad/d	2668	1384	3317	4151	3859	3761	4073	7583
AUC, 0-1	0.999	0.998	0.935	0.798	0.627	0.479	0.440	0.324
Sensitivity, %	96.8	98.9	82.2	83.3	80.2	86.4	73.3	10.0
Specificity, %	92.7	97.7	95.4	70.9	49.4	24.1	28.7	10.0
Feed								
Cutoff, sec/d	7681	6885	10922	9148	11376	11489	9029	18136
AUC, 0-1	0.992	0.987	0.787	0.695	0.628	0.588	0.490	0.428
Sensitivity (%)	95.8	96.8	73.9	92.7	48.9	51.0	84.3	4.1
Specificity (%)	93.7	94.3	70.4	39.5	74.7	64.3	20.6	9.8
Total lie								
Cutoff, sec/d	69390	74387	67042	65262	67750	68346	66379	56058
AUC, 0-1	0.993	0.996	0.903	0.800	0.715	0.518	0.487	0.381
Sensitivity, %	97.9	96.5	90.9	81.3	57.7	19.5	33.1	10.0
Specificity, %	94.8	97.9	77.0	69.7	80.2	88.5	75.0	20.8

Table 2. Half-and-Half nursery pig optimal cutoff values, the area under the curve (AUC), sensitivity, and specificity for human data and precision data (NU*track*). Four pens out of 16 were randomly assigned the half-and-half treatment, then one-half of the pigs randomly assigned treatments of either sham-handling, (controls; 3 mL saline solution subcutaneous) or single subcutaneous dose of lipopolysaccharide (LPS from E. coli O111:B ; 300 µg/kg of body weight in a total of 3 mL saline). Human real-time live scoring (human data) and data from a precision livestock technology (NU*track* precision data) were evaluated.

	Day							
	0	1	2	3	4	5	6	7
Human Data								
Sick Score								
Cutoff	-	-	-	-	-	-	-	-
AUC	0.703	0.738	0.569	-	0.662	0.525	0.513	-
Sensitivity, %	75.0	92.9	50.0	-	46.4	14.3	21.4	-
Specificity, %	65.6	53.1	62.5	-	84.4	90.6	81.2	-
Precision Data								
Distance traveled								
Cutoff, m/day	784.6	304.3	803.3	1191.1	1251.1	1449.5	1478.0	1578.7
AUC	0.970	0.992	0.799	0.729	0.710	0.680	0.648	0.580
Sensitivity, %	90.6	100.0	81.2	75.0	65.6	59.3	50.0	43.7
Specificity, %	96.8	96.4	75.0	67.8	75.0	82.1	78.5	78.5
Pivot behavior								
Cutoff, rad/d	2863	1489	2891	4610	4199	4578	4341	5572
AUC	0.988	0.985	0.792	0.676	0.663	0.629	0.612	0.517
Sensitivity, %	90.6	96.9	84.3	59.3	71.8	65.6	75.0	25.0
Specificity, %	100	92.8	75.0	82.1	57.1	67.8	50.0	89.2
Feed								
Cutoff, sec/d	7856	5554	10922	9358	8667	10405	11955	18136
AUC	0.976	0.967	0.685	0.643	0.641	0.612	0.557	0.422
Sensitivity, %	87.5	100.0	71.8	93.7	90.6	81.2	53.1	62.5
Specificity, %	96.8	89.2	64.2	46.6	42.8	42.8	64.2	100.0
Total lie								
Cutoff, sec/d	67660	74387	68184	65262	67442	65125	65280	64283
AUC	0.988	0.980	0.787	0.762	0.775	0.703	0.665	0.487
Sensitivity, %	100.0	89.2	75.0	78.5	67.8	64.2	60.7	50.0
Specificity, %	87.5	96.8	75.0	78.1	87.5	75.0	81.2	59.9

Objective 3: *Test the hypothesis that remixing (commingling) of cohorts with previously sick pen-mates will increase unwanted behaviors compared to pigs left in original cohorts.*

Results:

Performance:

Performance data for At the time of placement of pigs into the finisher phase, there was a tendency for pigs in the NoMIX treatment group to have greater (P = 0.07) body weight than pigs in the MIX treatment group, 25.8 kg and 24.7 kg, respectively. At the conclusion of the finisher period there was no difference between the treatment groups in regard to final body weight (P = 0.60), total weight gain (P = 0.83), ADG (P = 0.82), and 10^{th} rib back fat (P = 0.26.). There was a difference (P = 0.009) in loin eye area. Pigs in the NoMIX had a larger loin eye area, when compared to pigs in the MIX treatment group (52.9 cm^2 and 51.1 cm^2, respectively). For the duration of the finisher phase only five tail biting events were observed, three events in the NoMIX treatment group and two events in the MIX treatment groups.

Passive and Active Behavior:

For the first seven days in the finisher phase there was a finisher treatment effect (P = 0.004), day effect (P = <0.001), and LPS challenge x finisher treatment x day interaction (P = 0.01) daily distance traveled. For the first three days in the finisher phase there was no difference between the treatment groups. On the fourth day, pigs from the NoMIX-LPS treatment group walked more than pigs in the MIX-LPS and MIX-SAL group; NoMIX-SAL was intermediate (Figure 1). Starting on day 5 and continuing to day 7 there was no difference between the treatment groups. There was a finisher treatment x week interaction (P = <0.001) for weeks during the finisher phases. The distance traveled by pigs in the NoMIX treatment groups walked more (P = 0.04) during the first week of the finisher phase when compared to pigs in the MIX treatment group. Following this difference during the first week, there was no difference between the treatment groups (Figure 2).

For the velocity of meters traveled, there was a finisher treatment effect (P = 0.01), a day effect (P =<0.001), and a finisher treatment x day interaction (P =<0.001). For the first three days of the finisher phase, there was no difference between the treatment groups. On day 4 of the finisher phase the velocity for meters traveled by pigs in the NoMIX treatment group was greater (P =<0.001) than the velocity of pigs in the MIX treatment group. Following this difference, the velocity of meters traveled was similar between the treatment groups for the remainder of the first week in the finisher phase (Figure 3). For weeks of the finisher phase, there was no finisher treatment effect (P = 0.06) and no finisher treatment x week interaction (P = 0.42). Regardless of the treatment groups, there was no difference in the velocity of meters traveled for the treatment groups during the 13-week finisher phase (Figure 4).

For the first seven days of the finisher periods there was a finisher treatment effect (P = 0.009), day effect (P = <0.001), and LPS challenge x finisher treatment x day interaction (P = <0.001) for head/body angle and rotation. For the first three days of the finisher phase there was no difference (P = >0.05) between the treatment groups. On day 4, pigs in the NoMIX-LPS had a greater head/body angle and rotation, when compared to pigs in the MIX-Chall and MIX-NoChall groups, pigs in the NoMIX-NoChall was intermediate **(Figure 5)**. Starting on day 5 and continuing to day 7, there was no difference between the treatment groups (P = >0.05). In regards to weeks of the finisher phase, there was a LPS challenge x finisher treatment x week interaction P = <0.001) for head/body angle and rotation. During the first week of the finisher phase, head/body angle and rotation was greater (P = <0.05) for pigs in the NoMIX-Chall group, when compared to pigs in the MIX-Chall group, pigs in both the NoMIX-NoChall and MIX-NoChall treatment groups were intermediate **(Figure 6)**. Following the first week, there was no difference in head/body angle and rotation between the treatment groups.

For time spent at the feeder, there was no effect for LPS challenge (P = 0.97) and finisher treatment (P = 0.09), and no interaction between finisher treatment x day interaction (P = 0.49). Across the first seven days of the finisher phase the time spent at the feeder was similar between the treatment groups **(Figure 7)**. For the weeks of the finisher phase there was no finisher treatment effect (P = 0.23), but there was a finisher treatment x week interaction (P = <0.001). For the first six weeks for the finisher phase there was no difference (P = > 0.05) between the treatment groups. However, during the seventh week in the finisher phase there was a difference between the treatment groups. Time spent at the feeder was greater for pigs in the NoMIX treatment group, when compared to pigs in the MIX treatment group (Figure 8). Following this difference at 7 weeks, for the remainder of the finisher phase there was no difference (P = > 0.05) between the treatment groups **(Figure 8)**.

During the first seven days of the finisher phase, there was no finisher treatment effect (P = 0.14) and no finisher treatment x day interaction (P = 0.75) for time spent lying lateral. Regardless of the day, there was no difference (P = > 0.05) between the treatment groups (**Figure 9**). In terms of time spent lying lateral for the weeks in the finisher phase there was no finisher treatment effect (P = 0.14), but there was a LPS challenge x finisher treatment x day interaction (P = <0.001). For the first four weeks of the finisher there was no difference between the treatment groups (P = >0.05). However, during the fifth week pigs in the NoMIX-NoChall group spent less (P = <0.05) time lying lateral, when compared to pigs in the NoMIX-Chall group, with MIX-NoChall and MIX-Chall being intermediate (**Figure 10**). During the sixth week of the finisher phase pigs in the NoMIX-NoChall treatment group spent less (P = <0.05) time lying lateral, when compared to pigs in the MIX-NoChall treatment group, with NoMIX-Chall and MIX-Chall being intermediate. A similar pattern was also observed during the final two weeks of the finisher phase (weeks 12 and 13). During week 12, pigs in the NoMIX-NoChall treatment group spent less (P = <0.05) time lying lateral, when compared to the other treatment groups. Furthermore, during the 13^{th} week, pigs in the NoMIX-NoChall treatment group spent less time lying lateral than pigs in the MIX-Chall treatment group, pigs in the MIX-NoChall and NoMIX-Chall treatment groups were intermediate (**Figure 10**).

For time spent lying sternal, there was no finisher treatment effect (P = 0.74) or finisher treatment x week interaction (P = 0.66). During the first seven days of the finisher phase the amount of time spent lying sternal was similar between the treatment groups (**Figure 11**). In regards to time spent lying sternal, there was no finish treatment effect (P = 0.60), but there was finisher treatment x week interaction (P = <0.001). For the first three weeks there was no difference (P = >0.05) in the time spent lying sternal between the treatment groups. However, during the fifth week of the finisher phase, pigs in the NoMIX treatment group spent more (P = <0.05) lying sternal, when compared to pigs in the MIX treatment group (**Figure 12**). Following this difference in time spent lying sternal during the fifth week, there was no difference (P = >0.05) between the treatment groups for the remainder of the finisher phase (weeks 6 – 13).

There was a finisher treatment effect (P = 0.005) and a finisher treatment x day interaction (P = 0.004) for the total time spent lying (lying lateral and sternal). For the first two days of the finisher phase there was no difference between the treatment groups. By the third day of the finisher phase, pigs in the MIX spent more total time lying, when compared to pigs in the NoMIX treatment groups (**Figure 13**). Following day 3, there was no difference between the treatment groups for the remaining days of the first week (days 4 – 7). For total time spent lying (lateral and sternal) by weeks in the finisher phase, there was no finisher treatment effect (P = 0.10), but there was a finisher treatment x week interaction (P = <0.001). For the first two weeks of the finisher phase there was no difference between the treatment groups. However, during the third week of the finisher phase, pigs in the NoMIX treatment group spent more (P = <0.05) time lying, when compared to pigs in the MIX treatment group (**Figure 14**). Following this difference during the third week, there was no difference (P = <0.05) between the treatment groups in regard to the total time spent lying.

During the first seven days of the finisher period there was a finisher treatment effect (P = 0.01), but there was no finisher treatment x day interaction (P = 0.18). Overall, pigs in the NoMIX treatment group spent more (P = 0.04) spent more time lying, when compared to pigs in the MIX treatment group (234.9 min/d and 249.6 min/day, respectively). As for time spent standing across the 13 weeks of the finisher phase, there was no finisher treatment effect (P = 0.25) or finisher treatment x week interaction (P = 0.64). Across the 13 weeks, on average pigs in the NoMIX treatment group spent 232.9 min/d standing and the pigs in the MIX treatment group spent 226.4 min/d standing.

For the amount of time spent standing, there was no finisher treatment effect (P = 0.28) nor a finisher treatment x day interaction (P = 0.65; **Figure 17**). During the first seven days in the finisher phase, pigs in the NoMIX treatment group spent 30.8 min/d standing and pigs in the NoMIX treatment group spent 33.2 min/d sitting. A similar trend was observed for time spent sitting across the 13 weeks of the finisher phase. For time spent sitting, there was no finisher treatment effect (P = 0.77) nor was there a finisher treatment x week interaction (P = 0.25; **Figure 18**). Overall, pigs in the NoMIX treatment group spent on average 35.8 min/d sitting and pigs in the MIX treatment group spent 36.7 min/d sitting.

For the total amount of time spent resting (lying and sitting) there was a finisher treatment effect (P = 0.02) and a finisher treatment x day effect (P = 0.007). The time spent resting was similar between the treatment groups for the first two days of the finisher phase. On the third day of the finisher phase, pigs in the NoMIX treatment group spent more time resting, when compared to pigs in the MIX treatment group (**Figure 19**). Aside from this difference on day 3, there was no difference (P = >0.05) between the treatment groups for the remaining four days of the first week in the finisher phase. There was no finisher treatment effect (P = 0.10) or finisher treatment x week interaction (P = 0.21). Across the 13 weeks, on average the pigs in the NoMIX treatment group spent 1204.7 min/d resting and pigs in the MIX treatment group spent 1214.5 min/d.

Discussion:

Performance results suggest that penning pigs into pens with unfamiliar pen mates did not have an impact. For all measurements of production performance (body weight gain or ADG), there was no difference between pigs that remained with cohorts from the nursery pen and pigs that were placed into mixed pens with unfamiliar pen mates. For ADG, the of a difference in contrasts with results reported by Montoro et al. (2021) and Hyun and Johnson (1998). Montoro et al. (2021) reported that non-mixed pigs had a greater ADG (78 g/d more), when compared to pigs within mixed pens. During the first four weeks of the nursery phase, Hyun and Johnson (1998) reported that non-mixed gained 52 g/d more than pigs in mixed pens. A lack of difference in weight gain is also in contrast to that of Montoro et al. (2022). Montoro et al. (2022) reported that non-mixed pigs were ~5.3 kg heavier than pigs in mixed population pens. For the current study, there was only a 0.4 kg difference between the two treatment groups.

In terms of changes in passive and active behaviors between pigs penned with nursery pen cohorts and pigs penned with unfamiliar pen mates, results suggest that mixing pigs into unfamiliar population pens in the finisher phase had a minimal impact on behaviors. In terms of the meters walked/day and the speed at which pigs traveled, the only difference was observed during the first week of the nursery phase. Pigs that did not receive the LPS challenge during the nursery phase and then remained with cohorts in the finisher walked more meters/day on the fourth day of the finisher period than pigs that were in mixed population pens. Following this difference during the first week of the finisher phase, there was no difference for the remaining 12 weeks. A similar result was observed for speed that pigs walked during the first week of the finisher phase. Pigs that remained with cohorts from the nursery pen had a greater velocity when compared to pigs placed into mixed population pens. Following this difference during the first week, for the remaining 12 weeks there was no difference in velocity between the mixed and non-mixed pigs.

To evaluate potential changes in aggressive behaviors during the mixing of nursery pigs into unfamiliar finisher pigs the NU*track* system was programmed to determine the changes in the head/body angle and rotation. Our hypothesis being that greater changes in the head/body angle and rotation would indicate the aggressive behavior of fighting. However, across the treatment groups, placing nursery pigs into unfamiliar populations had limited impact on the head/body angle and rotation. During the first week of the finisher phase, there was no difference in head/body angle and rotation for the first three days. There was a difference in head/body angle and rotation on day 4. However, this difference was not what we would have expected. On day 4, head/body angle and rotation for pigs that received an LPS challenge during the nursery phase and then placed into pens with cohorts from the nursery pen was greater than pigs that were placed into mix population pens. Following this difference on day 4 of the first week, there was no difference between the treatment groups for the remaining 12 weeks of the finisher phase. Of note is the results that indicated that there was no difference between the treatment groups during the first 48 hours of the finisher phase. It was anticipated that the greatest change and potential differences in the change in head/body angle and rotation would have been observed during the first 48 hours, as this was the time that unfamiliar pigs in the mixed pigs would be more aggressive toward pen mates to re-establish social hierarchy. While the greatest change in head/body angle and rotation was observed on day 2, when compared to the baseline head/body angle and rotation, but no difference between the pigs in mixed pens and pigs in cohort pens.

During the transition from the nursery to finisher phase it is vital that pigs overcome the stress of this transition and re-establish eating behaviors as soon as possible. Results of this study suggest that compounding the stress of transitioning to the finisher by mixing pigs into pen with unfamiliar pen mates did not impact the re-establishment of eating behavior. During the first week of the finisher phase the time spent at the feeder for mixed and non-mixed pigs was similar. For the first day of the finisher phase, regardless of treatment the time spent at the feeder was similar to the average time pigs spent at the feeder during the last week of the nursery phase. This similarity did not continue on day 2 of the finisher phase. Regardless of treatment group, the time spent at the feeder was lower than baseline and day 1 of the finisher phase. Time at the feeder did not return an amount of time similar to the baseline until day 5 of the first week. Following this decrease in time following the first week, the greatest amount of time spent at the feeder was observed during the third week of the finisher phase, regardless of treatment groups. As time in the finisher phase increased the amount of time pigs spent at the feeder indicated a linear decrease throughout the weeks in the finisher phase. This decrease in time at the feeder somewhat similar to previous work from our team, which reported that time at the feeder steadily decreased as the number of days in the nursery phase increased. During a 42 day nursery study, time at the feeder gradually increased until the fourth week, after which the time at the feeder gradually declined during the fifth and sixth week.

Transitioning pigs into the finisher phase as cohorts or mixed into unfamiliar pens appear to have only a minor impact on the total amount of time pigs spent lying (lateral and sternal). Aside from a difference on day 3 of the finisher phase, in which pigs in mixed population pens spent more total time lying, when compared to non-mixed pigs, there was no difference between the treatment groups for the duration of the finisher phase. As days in the finisher phase increased, the amount of time spent lying displayed a linear increase. This linear increase in time spent lying is like results from our previous research. In a study in which the NU*track* system was utilized to monitor the changes in behavior of group housed nursery pigs. In the previous study, as days in the nursery phase increased, time spent lying increased. The linear increase in time spent lying coincides with the linear increase in total time spent resting (sum of time lying and sitting).

Conclusion:

Overall, using the NU*track* system to track the passive and active behaviors of group-housed pigs during the finisher phased suggest that the mixing of nursery pigs into pens with unfamiliar pen mates resulted in only minor changes in the passive and active behaviors. These minor changes in behaviors are backed up with performance data that also suggest that the mixing of pigs with unfamiliar pen mates, when compared to pigs that remained with nursery pen cohorts within the finisher phase.

Table 1. Performance data finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.

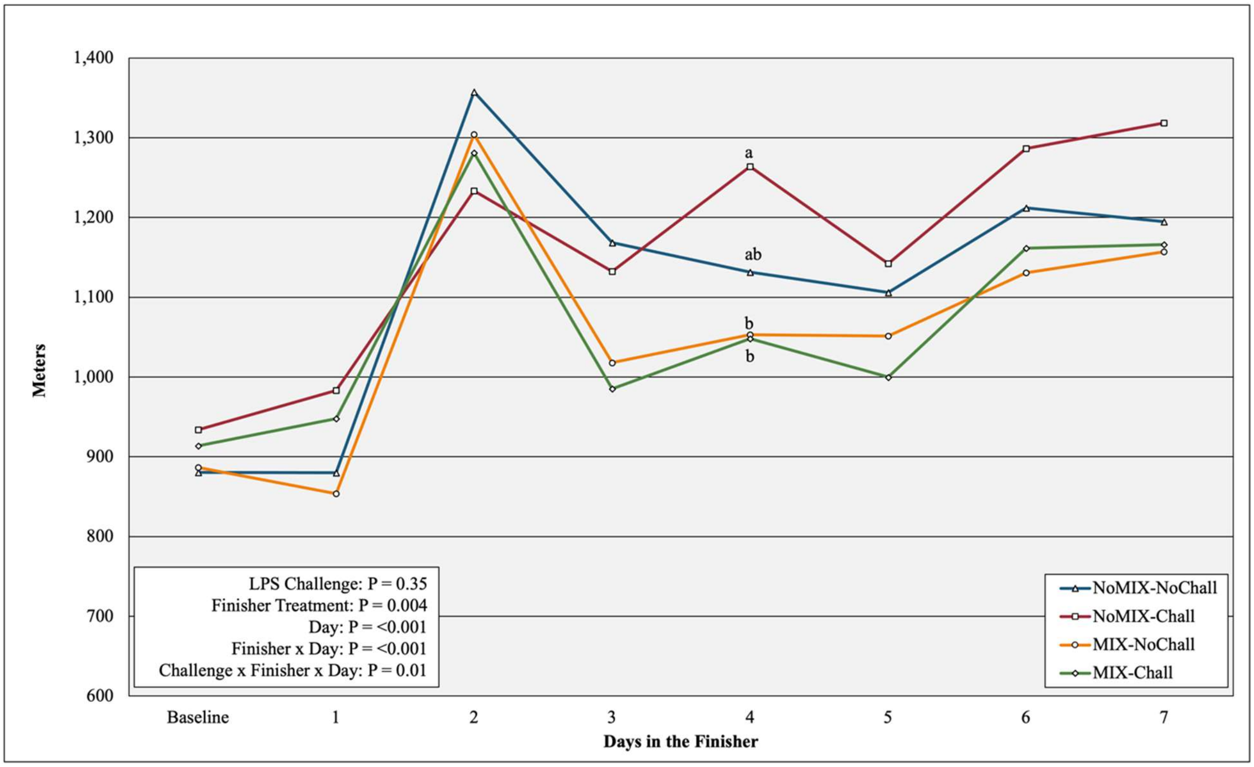
	NoMIX[1]	MIX[2]	P – Value[3]
Entry weight (kg)	25.8	24.7	0.07
Final weight (kg)	124.2	123.7	0.60
Finisher Gain (kg)	98.4	98.7	0.83
Finisher ADG (kg)	1.11	1.12	0.82
10^{th} Rib Back Fat (cm)	16.4	17.1	0.26
Loin Eye Area (cm^2)	52.9	51.1	0.009
Tail Biting Events	3	2	-

[1] Pigs remained penned with cohorts during the finisher phase.

[2] Pigs were penned in mixed population pens during the finisher phase.

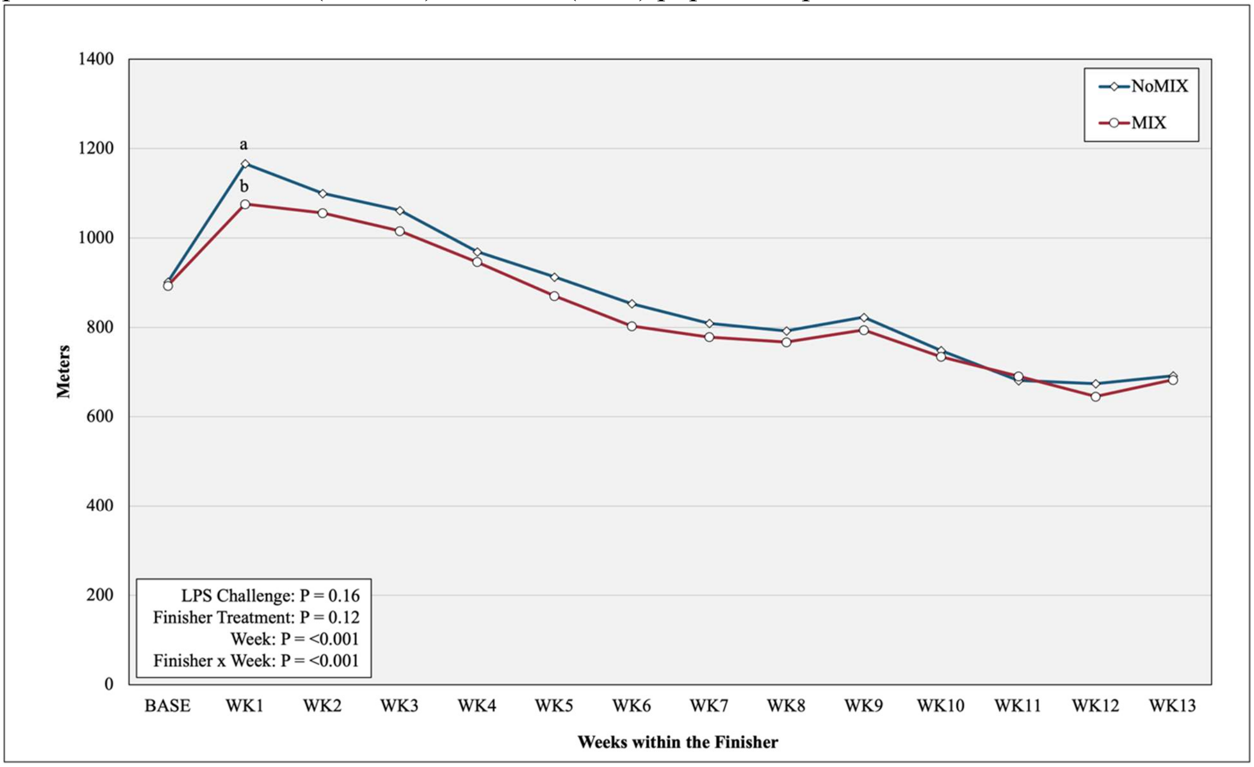
[3] There was no LPS challenge x finisher treatment interaction (P = >0.21) for any measure of production performance.

Figure 1. Daily distance traveled (meters) during the first seven days of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



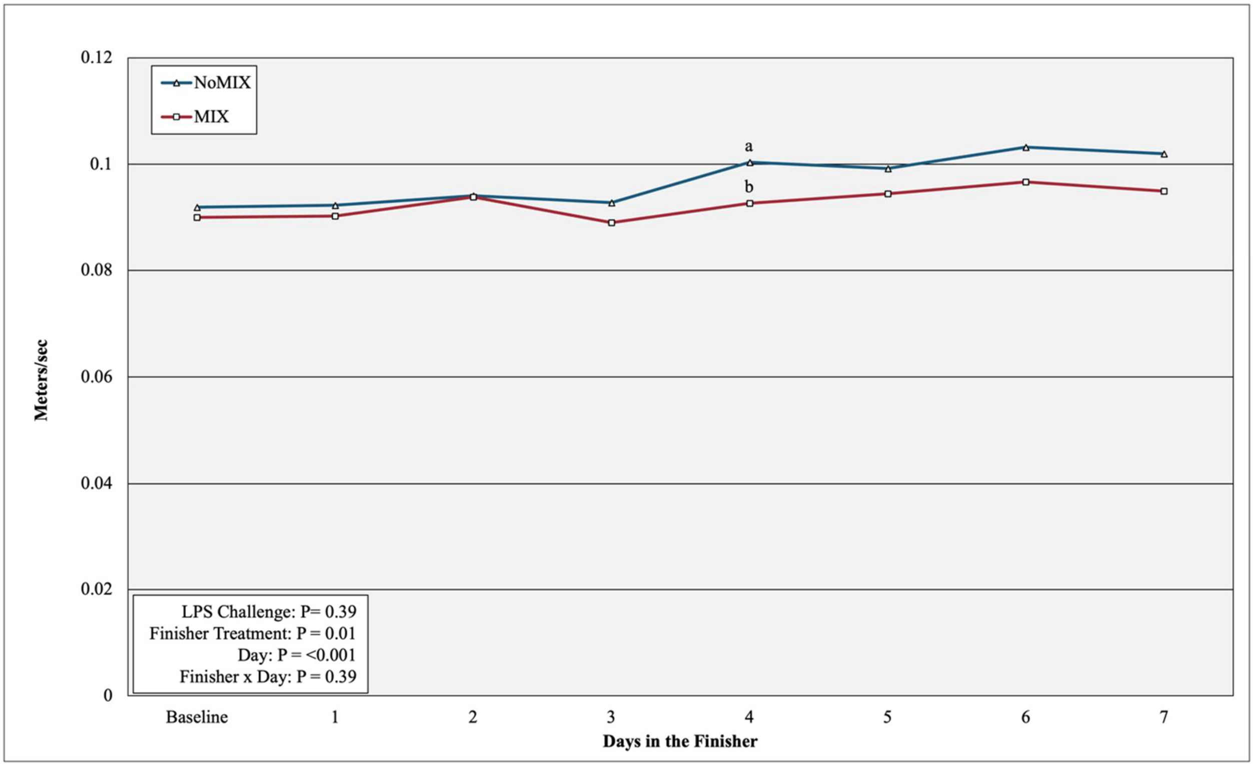
[1]Treatment groups consisted of NoMIX-NoChall (no LPS challenge in the nursery phase and penned as cohorts during the finisher phase), NoMIX-Chall (LPS challenged in the nursery phase and penned as cohorts during the finisher phase), MIX-NoChall (no LPS challenge in the nursery phase and penned as mixed population during the finisher phase), and MIX-Chall (LPS challenged in the nursery phase and penned as mixed population during the finisher phase).

Figure 2. Daily distance traveled (meters) across the 13 week finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



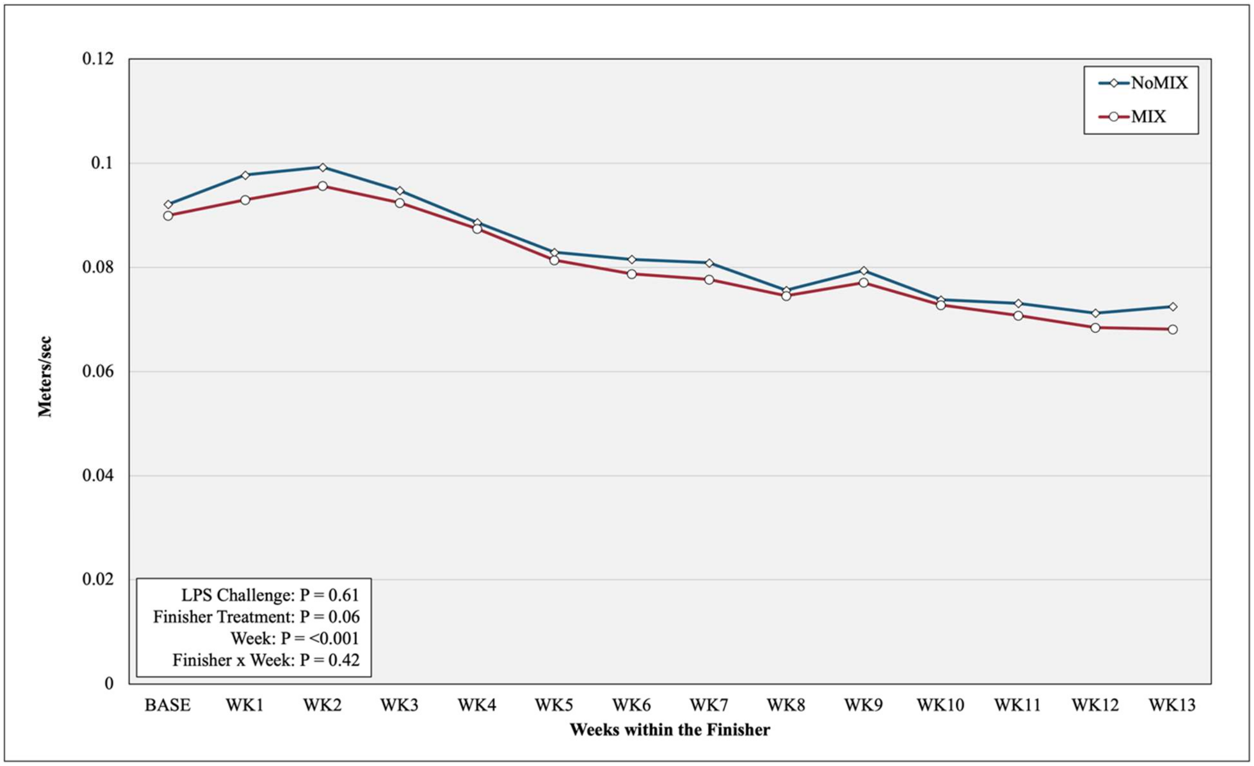
[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 3. Velocity of meters traveled (meters/sec) during the first seven days of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



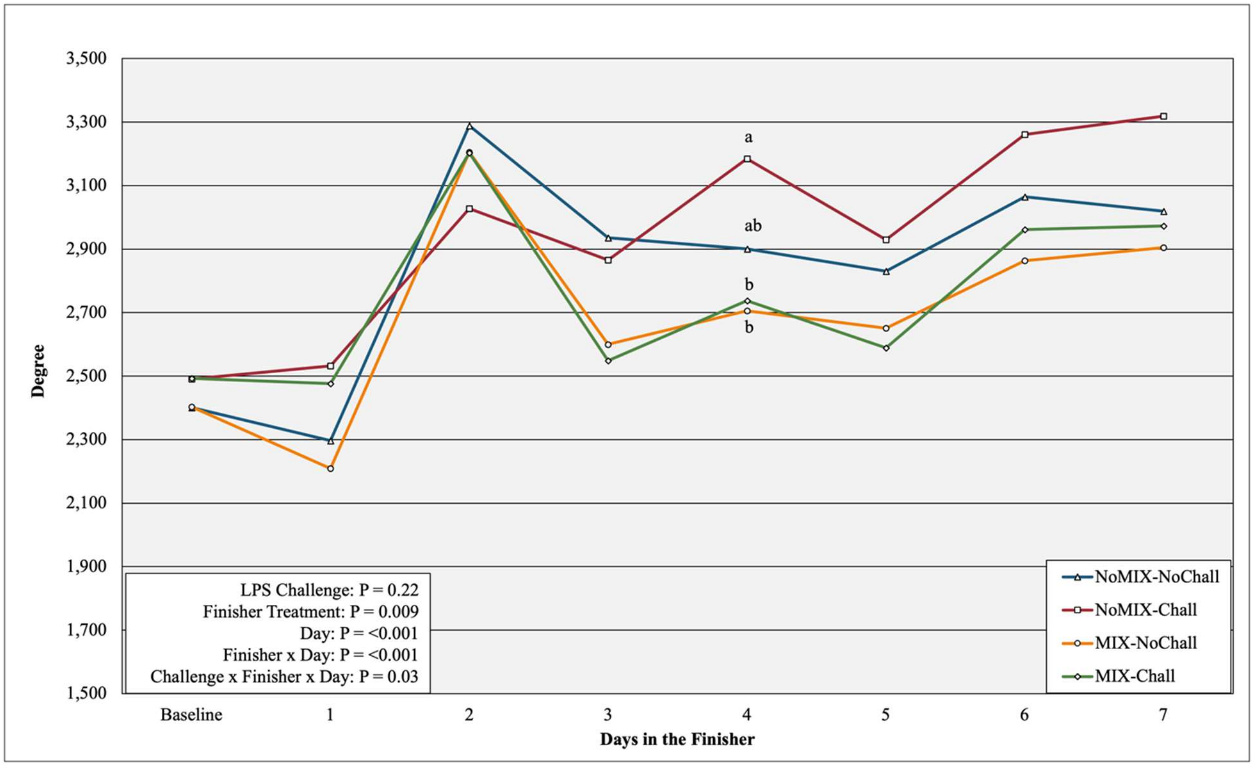
[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 4. Velocity of meters traveled (meters/sec) across the 13 weeks of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



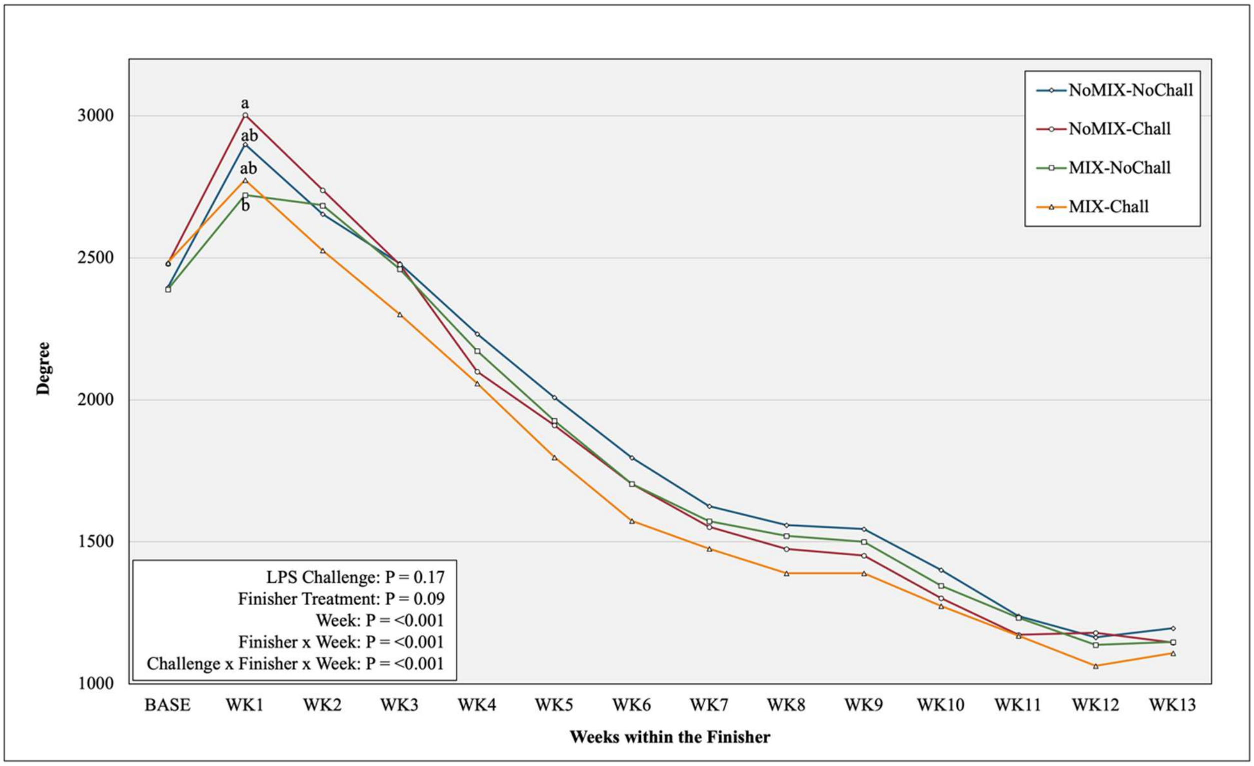
[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 5. Daily change in head/body angle and rotation during the first seven days of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.
[1]Treatment groups consisted of NoMIX-NoChall (no LPS challenge in the nursery phase and



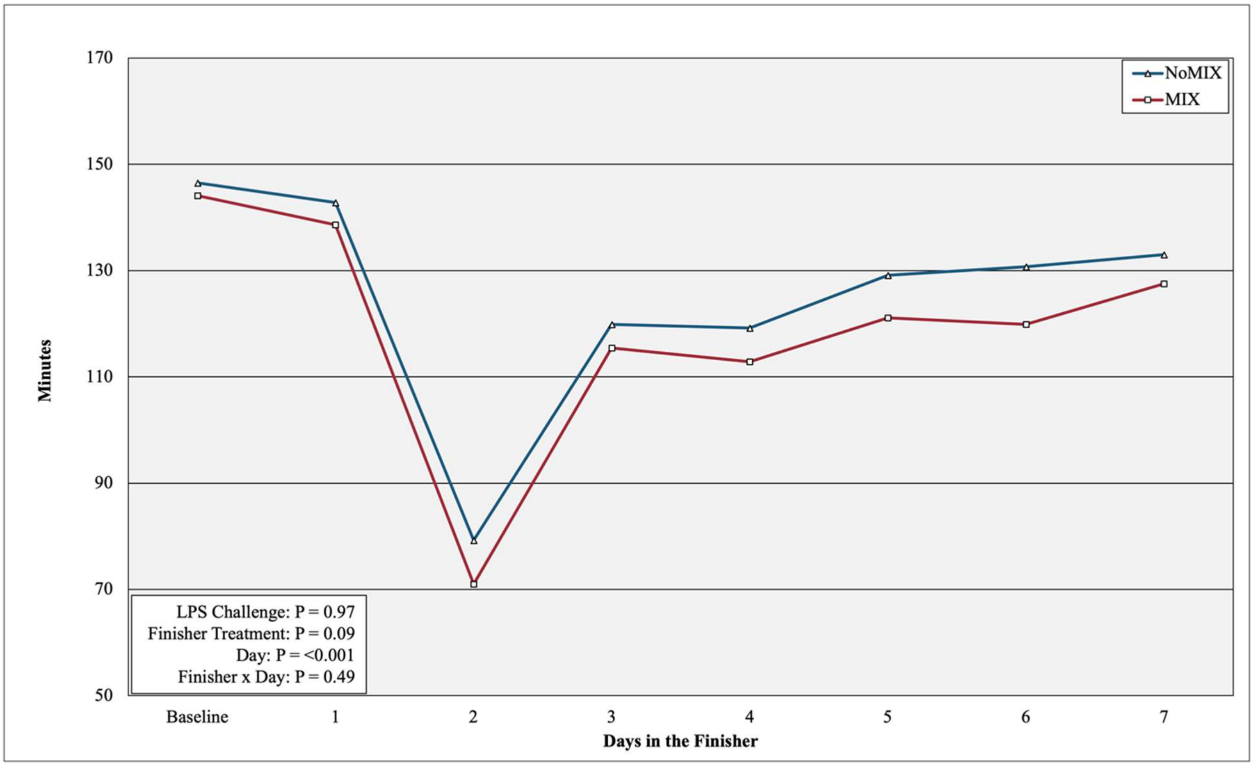
penned as cohorts during the finisher phase), NoMIX-Chall (LPS challenged in the nursery phase and penned as cohorts during the finisher phase), MIX-NoChall (no LPS challenge in the nursery phase and penned as mixed population during the finisher phase), and MIX-Chall (LPS challenged in the nursery phase and penned as mixed population during the finisher phase).

Figure 6. Daily change in head/body angle and rotation across the 13 weeks of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



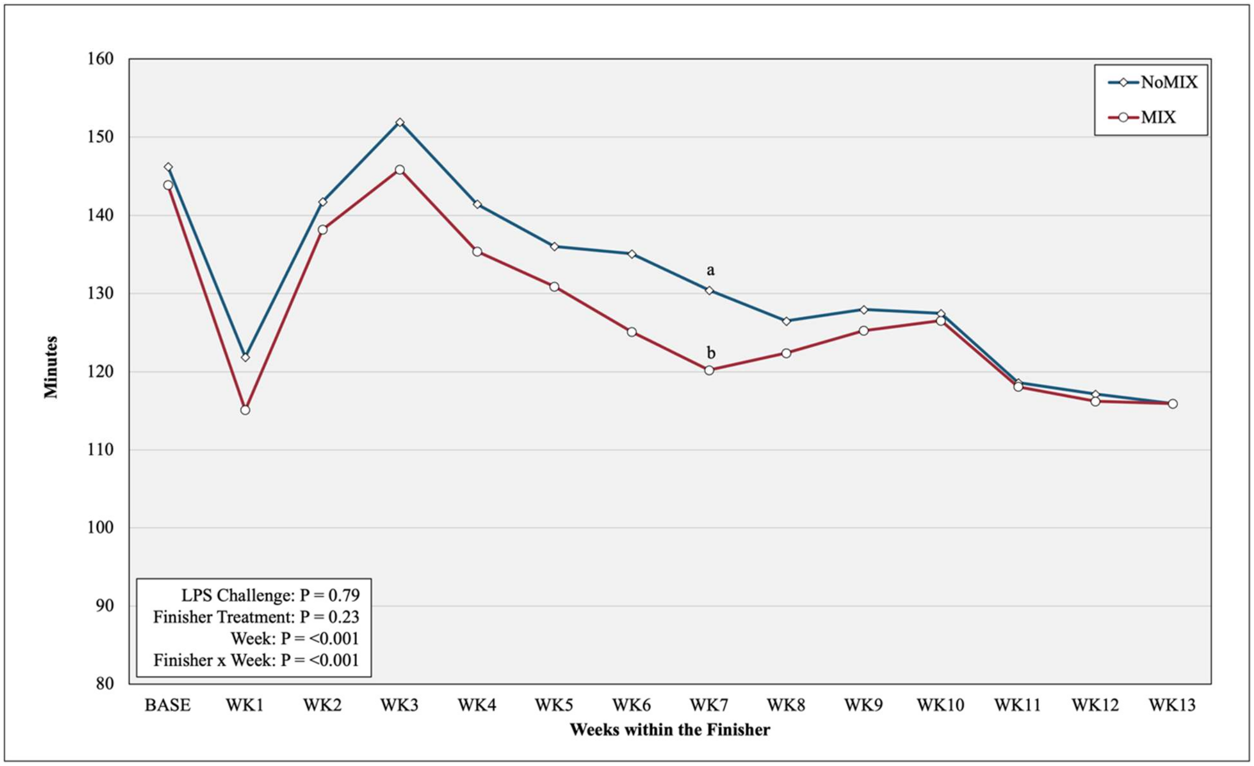
[1]Treatment groups consisted of NoMIX-NoChall (no LPS challenge in the nursery phase and penned as cohorts during the finisher phase), NoMIX-Chall (LPS challenged in the nursery phase and penned as cohorts during the finisher phase), MIX-NoChall (no LPS challenge in the nursery phase and penned as mixed population during the finisher phase), and MIX-Chall (LPS challenged in the nursery phase and penned as mixed population during the finisher phase).

Figure 7. Daily time spent at the feeder during the first seven days of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



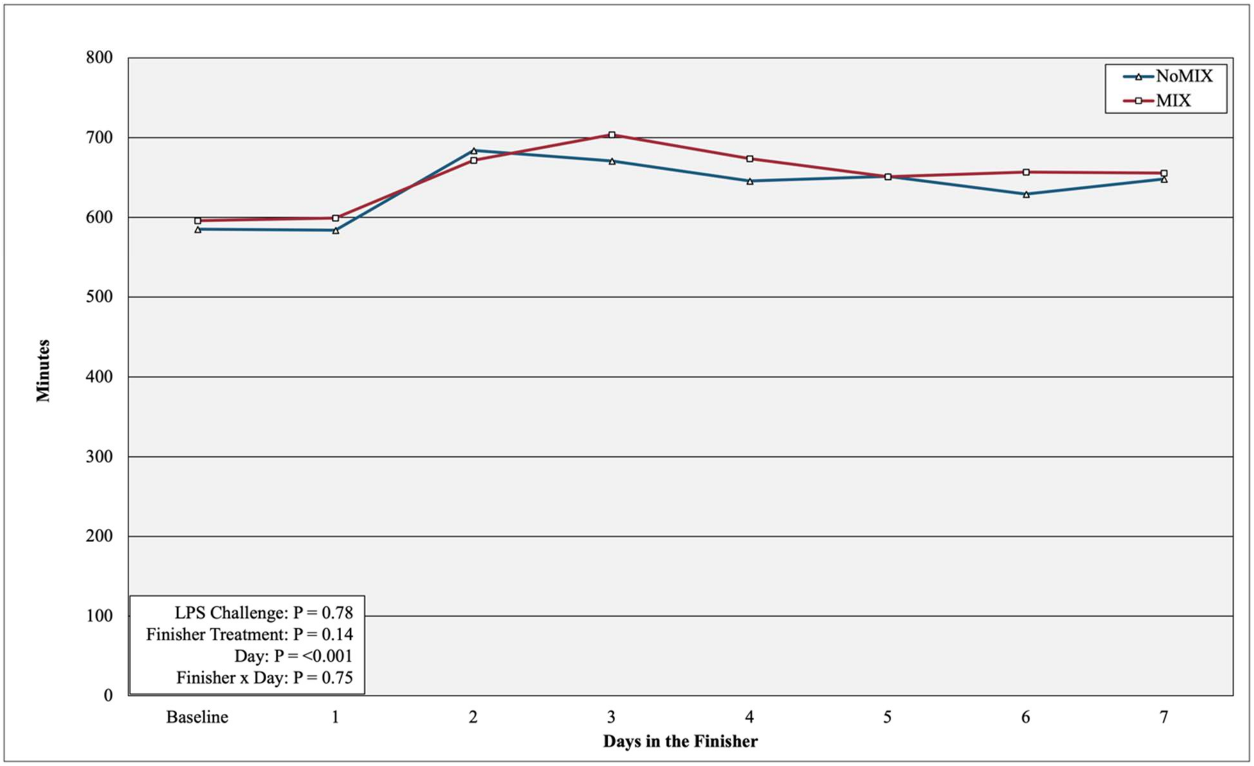
[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 8. Daily time spent at the feeder (minutes/day) across the 13 weeks of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



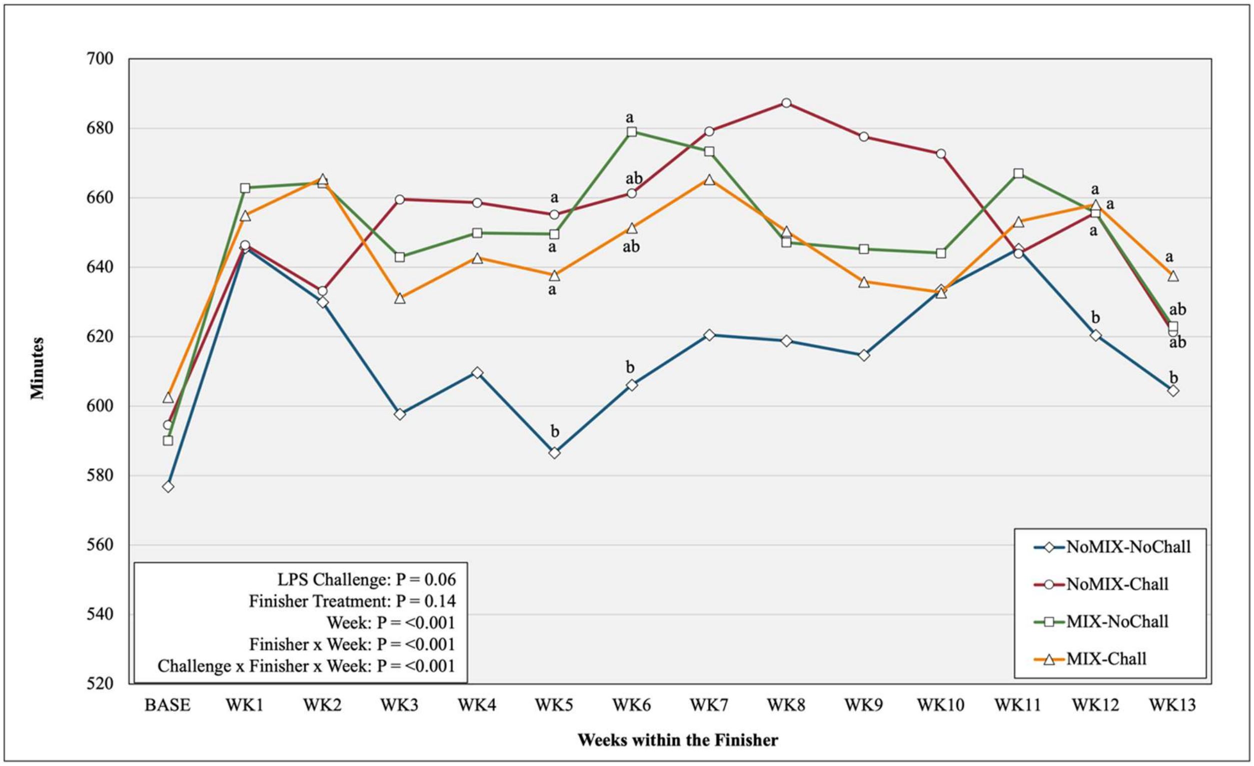
[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 9. Daily time spent lying lateral (minutes) during the first seven days of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



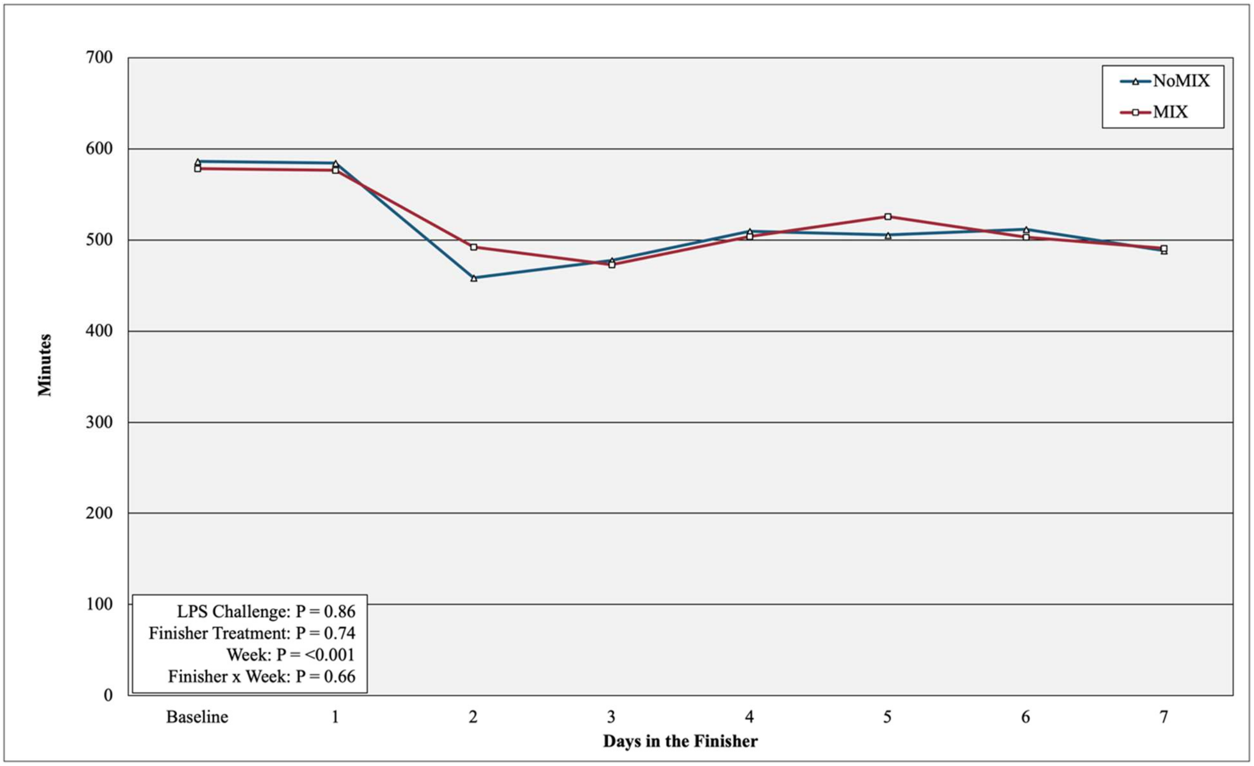
[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 10. Daily time spent lying lateral (minutes) across the 13 weeks of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



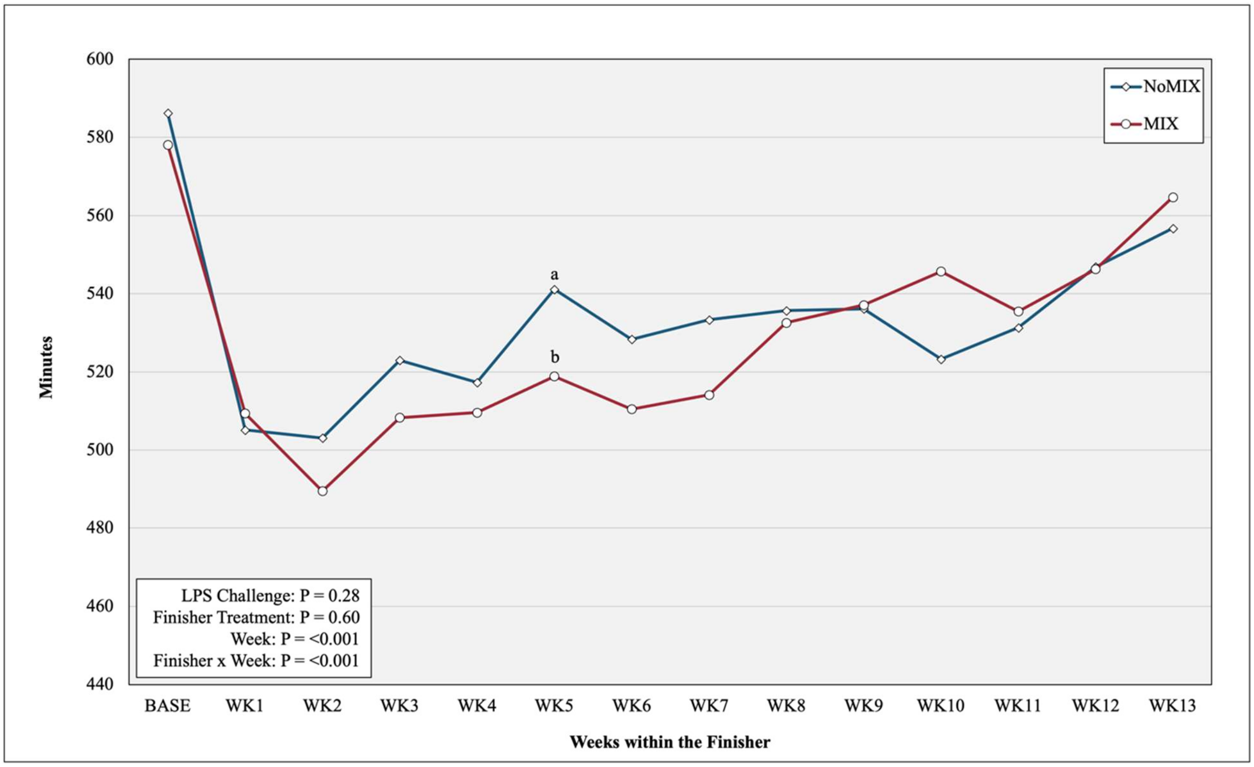
[1]Treatment groups consisted of NoMIX-NoChall (no LPS challenge in the nursery phase and penned as cohorts during the finisher phase), NoMIX-Chall (LPS challenged in the nursery phase and penned as cohorts during the finisher phase), MIX-NoChall (no LPS challenge in the nursery phase and penned as mixed population during the finisher phase), and MIX-Chall (LPS challenged in the nursery phase and penned as mixed population during the finisher phase).

Figure 11. Daily amount of time (minutes/day) spent lying sternal during the first seven days of the finishing phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



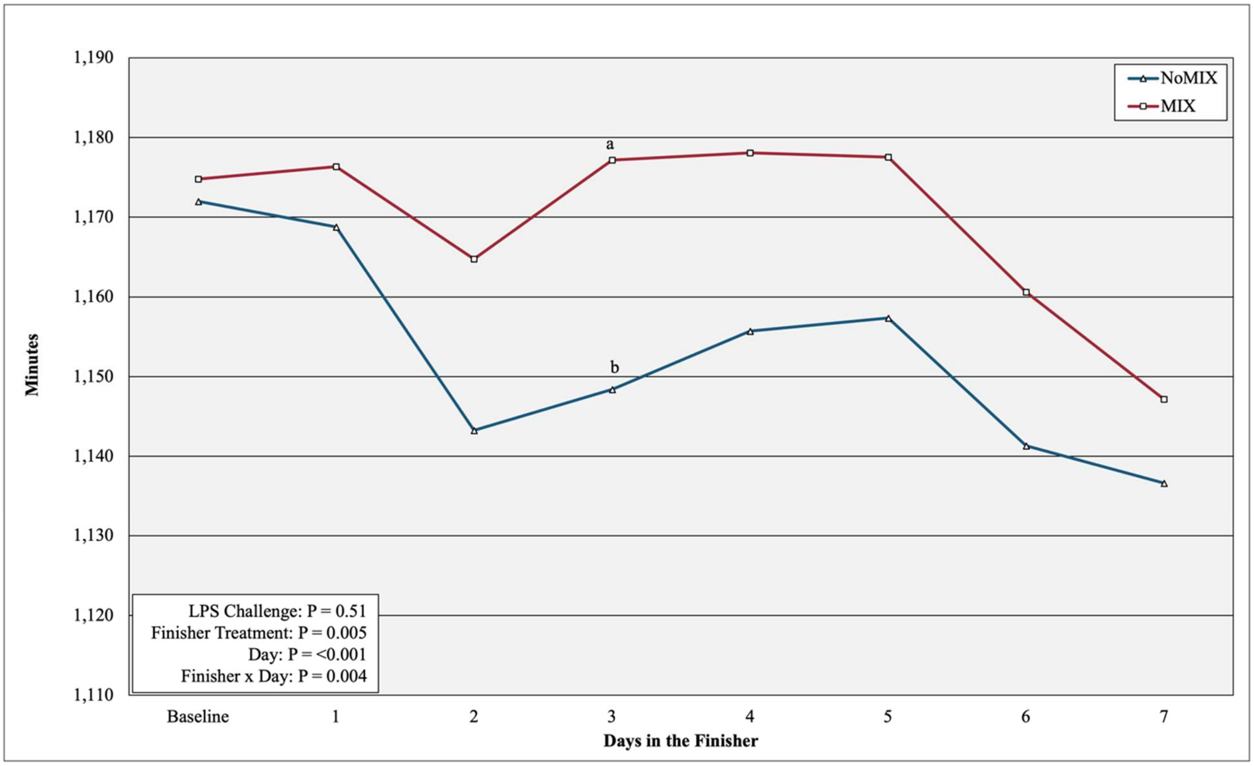
[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 12. Daily amount of time (minutes/day) spent lying sternal across the 13 weeks of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



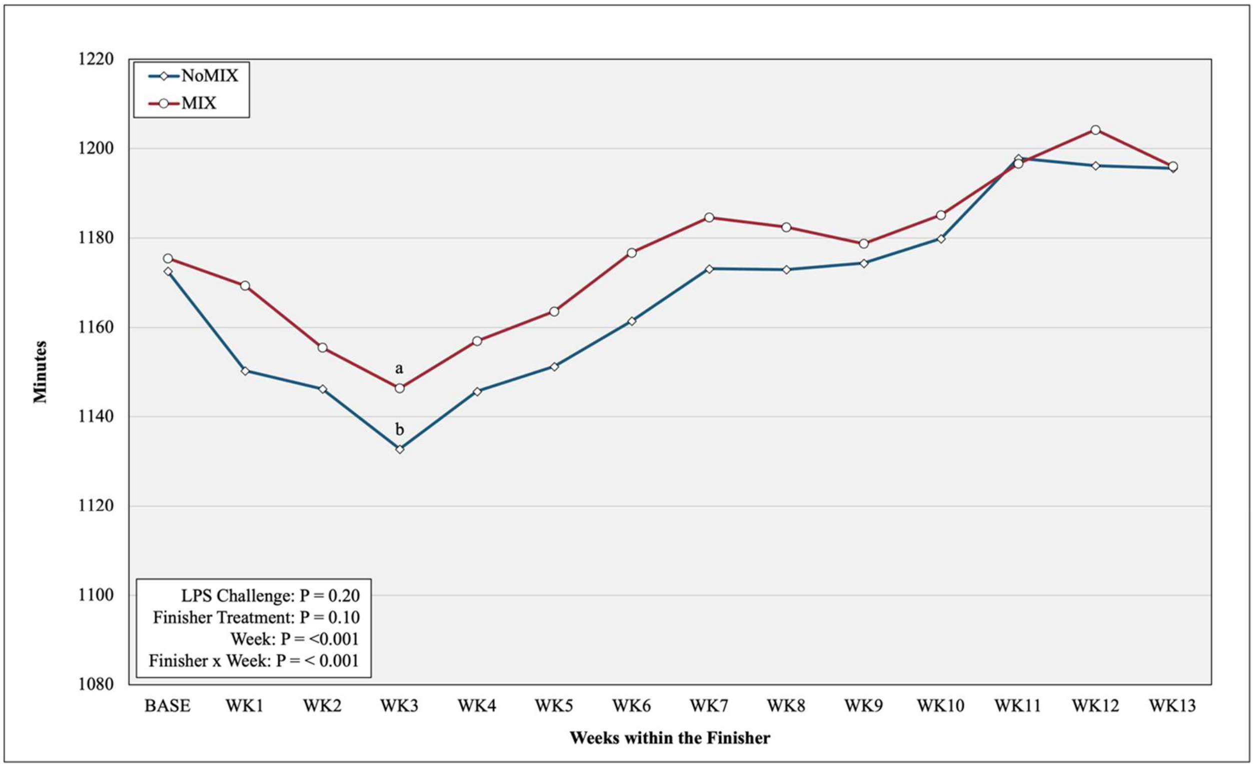
[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 13. Daily amount of time (minutes/day) spent lying (lateral and sternal) during the first seven days of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



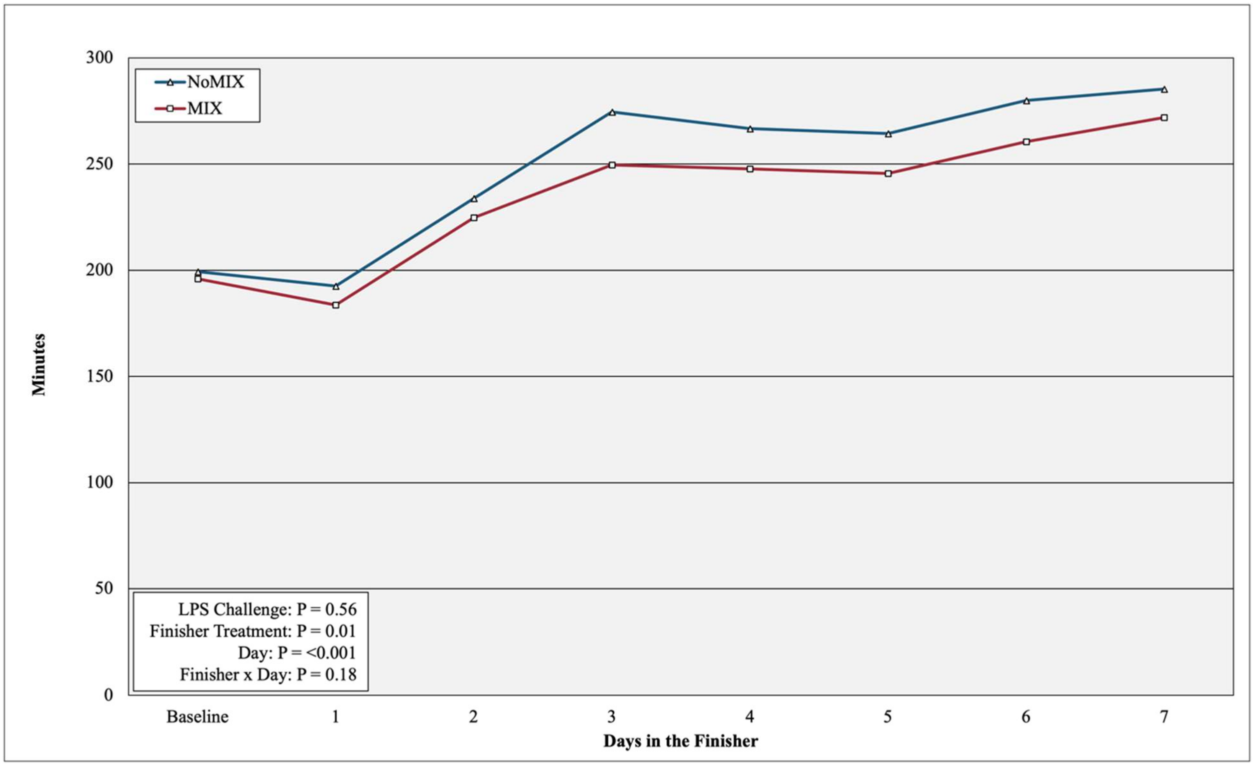
[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 14. Daily amount of time (minutes/day) spent lying (lateral and sternal) across the 13 weeks of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



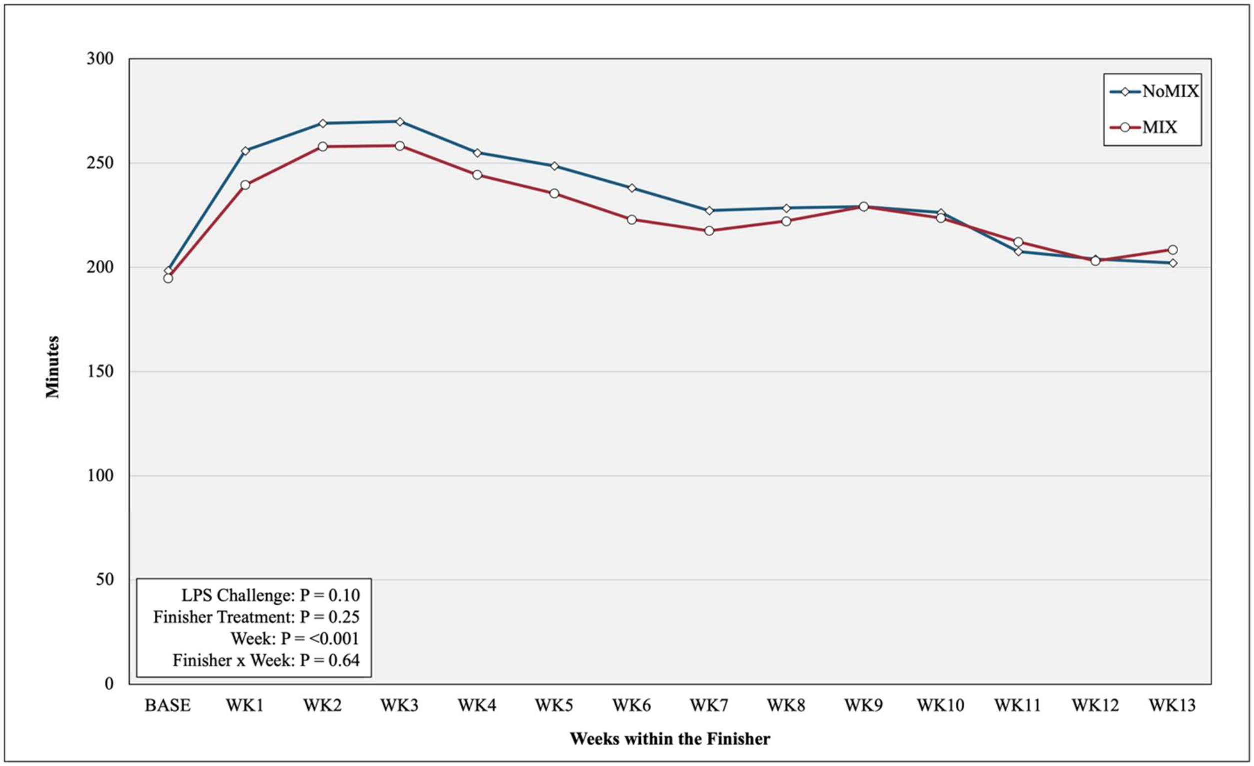
[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 15. Daily amount of time (minutes/day) spent standing during the first seven days of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



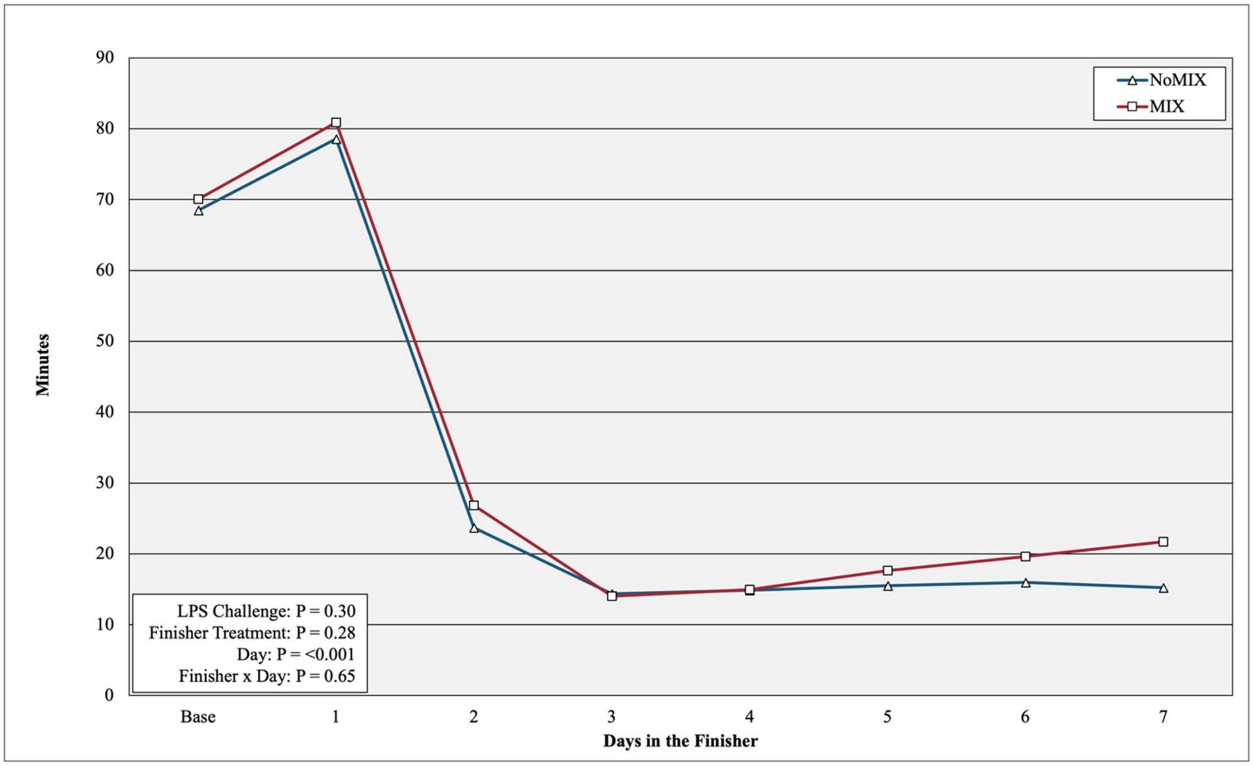
[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 16. Daily amount of time (minutes/day) spent standing across the 13 weeks of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



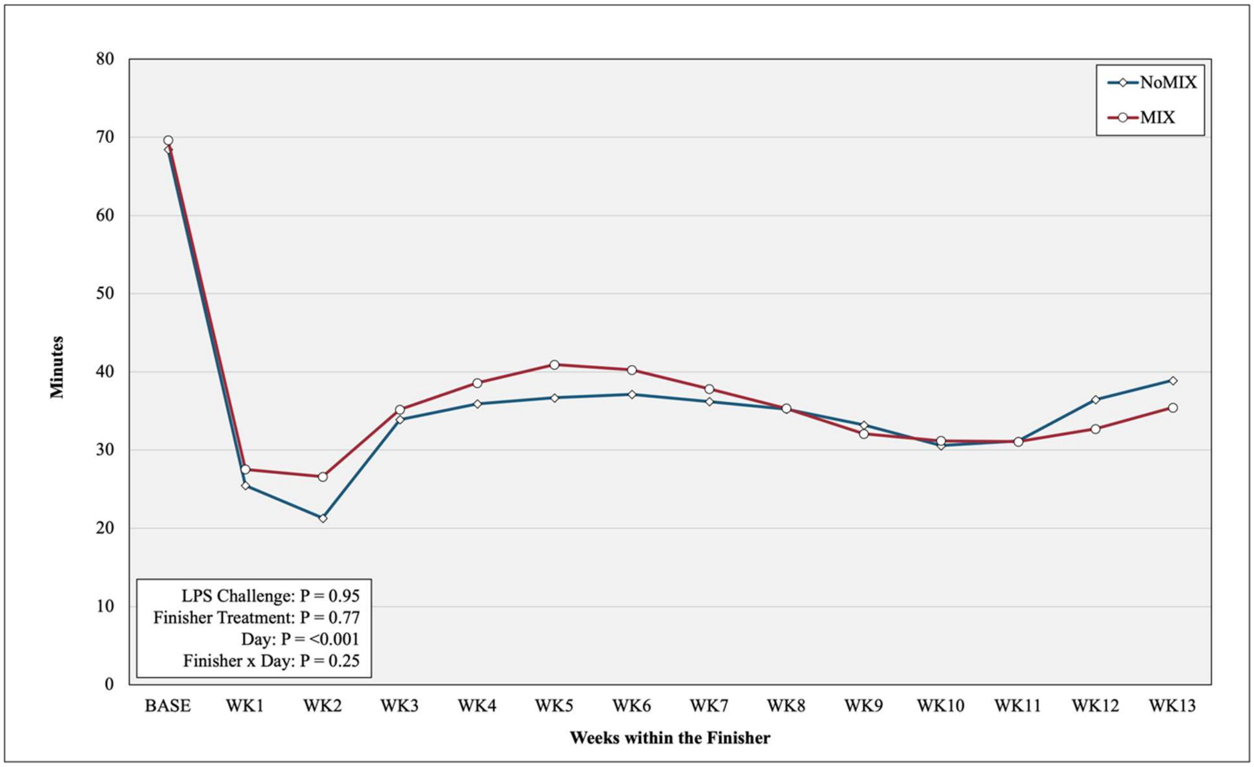
[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 17. Daily amount of time (minutes/day) during the first seven days of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



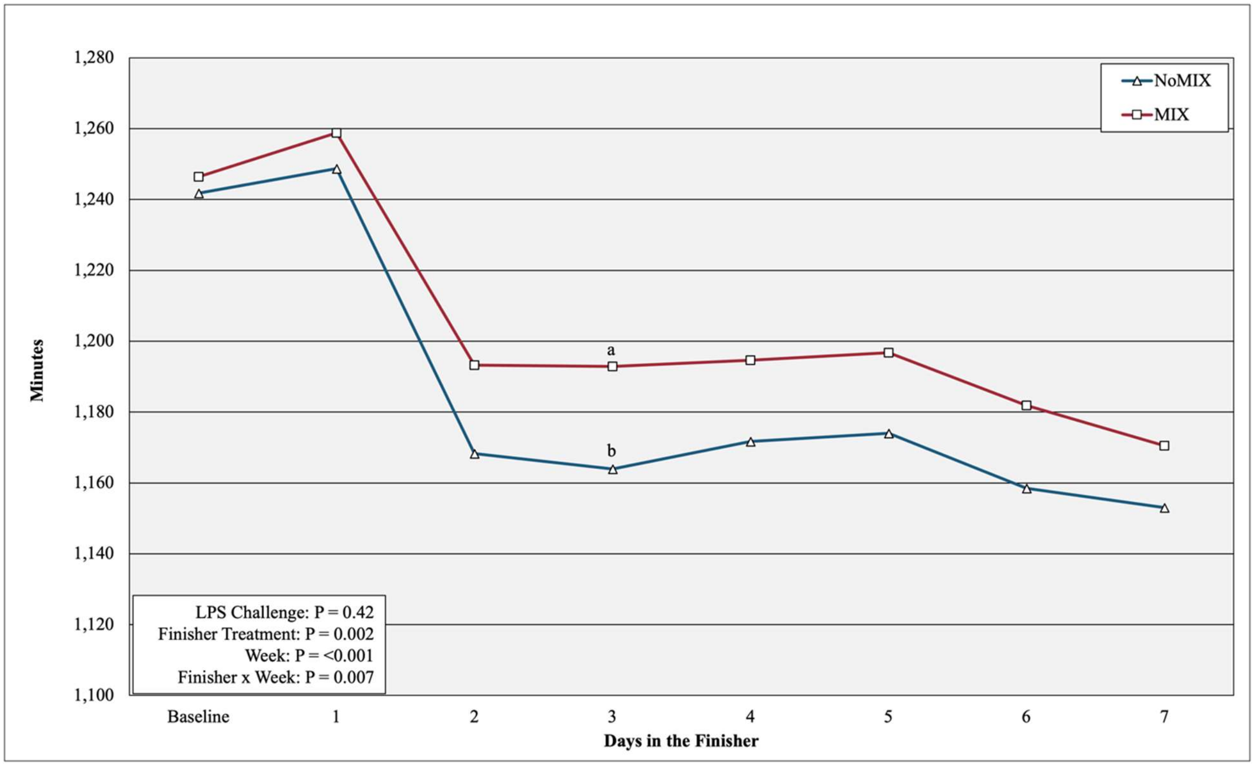
[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 18. Daily amount of time (minutes/day) across the 13 weeks of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



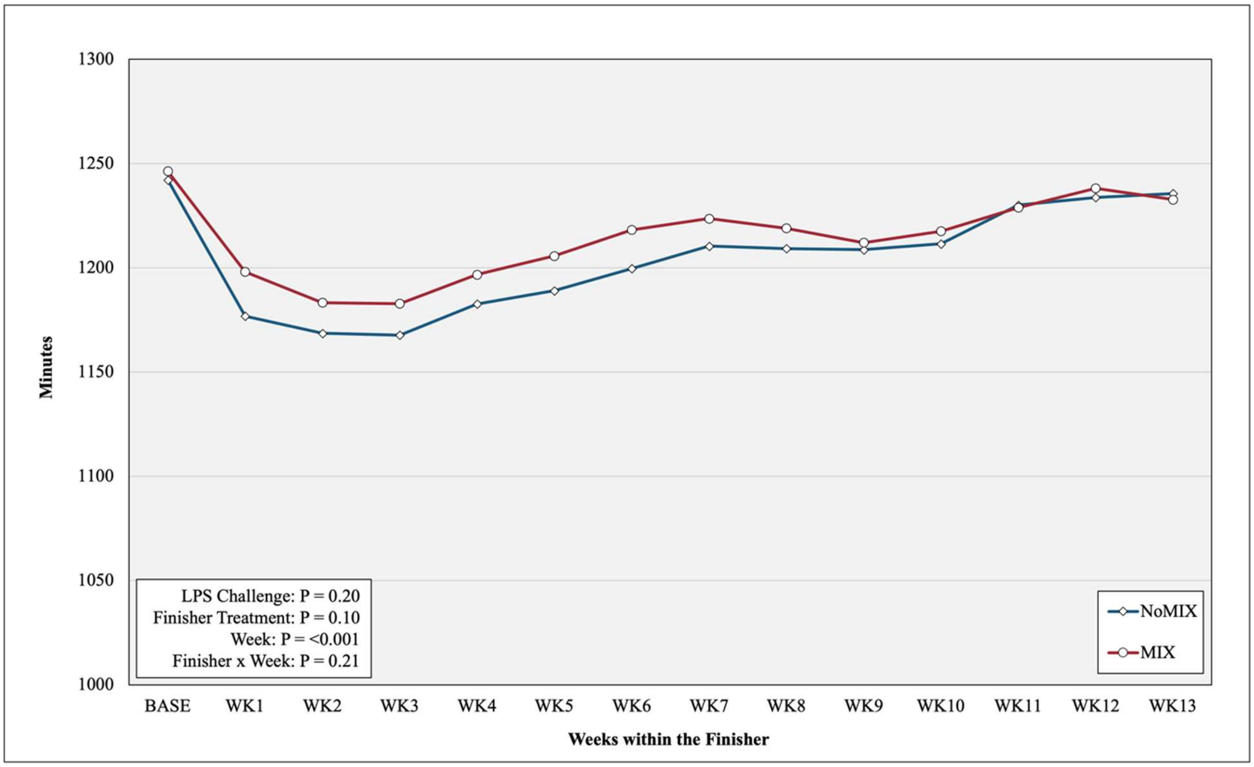
[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 19. Daily amount of time (minutes/day) spent resting (lying and sitting) during the first seven days of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)

Figure 20. Daily amount of time (minutes/day) spent resting (lying and sitting) across the 13 weeks of the finisher phase for pigs that received an endotoxin challenge (NoChall or Chall) in the nursery phase and then penned in the finisher phases as either cohorts (NoMIX) or mixed (MIX) population pens.



[1]Treatment groups consisted of NoMIX (penned as cohorts in the finisher phase) and MIX (penned as mixed population during the finisher phase)