RESEARCH REPORT

ANIMAL WELFARE

Title: Effect of short term high tryptophan diet fed to sows on subsequent piglet behavior; #15-066

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Date Submitted: July 10th 2017

Industry Summary:

Laws have been passed in several states requiring group housing for pregnant sows. However, the predominant problem with group housing sows is aggression. Different approaches, such as changing group size, space per sow, advanced technologies (such as electronic sow feeding), and change in diet and feeding pattern etc., are still being evaluated to reduce aggression and improve sow welfare. One of the approaches taken to reduce aggression and improve welfare of the pregnant sows could be feeding high-tryptophan diets. A high tryptophan diet has been shown to reduce aggression and fights and thus improve welfare of grow-finish gilts and nursery pigs. A diet high in tryptophan could also be used to decrease aggression in pregnant sows, however, the impact of such a diet on her subsequent offspring has not been studied. This research examined the effect of feeding high tryptophan diets during the fourth week of pregnancy on production, behavior, performance, and welfare of the piglets. During gestational days 28 to 35, York x Landrace multiparous sows were fed 1 of 3 diets: Control (0.14% tryptophan), Medium (0.28% tryptophan), or High (0.42% tryptophan). Blood samples were taken prior to and after tryptophan supplementation. Sows were gestated in standard gestations stalls and then were transferred to farrowing stalls at 112 days of gestation. Time budgets of the sows and pigs were recorded for 24 hours, during three separate days prior to weaning. To determine how their subsequent piglets responded to novelty, fear, and social situations, starting at 14 days of age the piglets were challenged with an isolation test, a human approach test, and a social challenge test. Behavior and vocalizations were recorded. Blood samples were taken from the pigs prior to the tests and immediately after the social challenge test. Productivity of the sows proved to be equal, with sows producing similar sized litters and weaned pigs. Teat aggression during nursing bouts did not differ among treatments. High pigs performed fewer nursing bouts than did pigs in the other two treatments. The Isolation Test, Human Approach Test, and Social Challenge Test were all used to determine how the pig coped with stress and novelty. The first two tests found that pigs from all three treatments reacted similarly to these challenges. The Social Challenge test found that High pigs confronted the head of another pig more often, with Medium pigs in between. The time budget data indicated similar amounts of activity for pigs in all three treatments, showing that the nervous system in general had not been altered. In conclusion, feeding high concentrations of tryptophan for a short duration early in gestation does not have a negative impact on sows' subsequent offspring.

Keywords: aggression, tryptophan, piglet, sow, prenatal, behavior, welfare

Scientific Abstract:

The move to house sows in groups creates the challenge of decreasing fighting amongst sows. One proposed method is to feed a high tryptophan diet at the time of mixing of pregnant sows. However, the effect of high tryptophan on the developing fetus and subsequent piglet is unknown. Thus, 66 sows were fed 1 of 3 diets: Control (0.14% SID tryptophan), Medium (0.28% SID tryptophan), or High (0.42% SID tryptophan), from d 28 to 35 of gestation. Sows gestated in standard gestation stalls. Blood samples were taken prior to and after tryptophan supplementation. Day of birth was considered d 0. On d 1 and 2, 3 nursing bouts were observed from each sow to record disputes and displacements from teat competition. The pigs' time active, inactive, and fighting were recorded on d 3, 7, and 11 from 0700 h to 1700 h. On d 12, 4 pigs per litter (2 females, 2 males) were blood sampled. On day 14, 2 pigs per litter (1 female, 1 male) were subjected to a 10-min Isolation Test and 5-min Human Approach Test. On d 15, 2 pigs (same sex) from different litters were subjected to a 10-min Social Challenge test and then immediately blood sampled. On d 18 ± 1.5 pigs were weaned. There were no differences ($P > 0.10$) for number born (12.7 ± 0.4), born alive (11.7 ± 0.4), or mortality (1.1 ± 0.2) among treatments. Pigs from all 3 treatments had equal amounts of disputes and displacements during nursing ($P > 0.10$). Nursing bout duration was similar among treatments (198.8 ± 4.8 s, $P > 0.10$). High pigs performed fewer nursing bouts than did pigs in the other two treatments ($P < 0.02$). No differences were detected for any of the variables for Isolation Test or the Human Approach Test ($P > 0.10$). During the Social Challenge test, High pigs had more contacts approaching the head of the companion pig than did either Medium or Control pigs (14.3 ± 1.1, 10.7 ± 1.1, and 9.69 ± 0.8 respectively, $P < 0.007$). Total number of aggressive interactions during the test tended to be greater for Medium pigs compared to High pigs (9.3 ± 1.5 vs 5.1 ± 0.9, $P < 0.06$), with Control pigs (7.1 ± 1.5) being intermediate and not different than Medium pigs ($P > 0.10$). Time budget data of the litter indicate that pigs from all 3 treatments spent equal amounts of time active and inactive ($P > 0.10$). Aggression was low with .3 ± .04 % of the observation periods recording aggression for all 3 treatments. Feeding high concentrations of tryptophan for a short duration early in gestation does not have a negative impact on sows' subsequent offspring.

Introduction:

Several states in the U.S. have already banned individual gestation stalls for pregnant sows, and other states are considering regulating sow gestational housing. This will require housing sows in groups. Pigs have a natural behavior to maintain social hierarchies in groups. The order of hierarchy is maintained by fighting which usually settles in 72 hours after they are mixed. Various approaches have been studied to reduce aggression in sows and improve welfare. One method is to increase tryptophan in the diet. Tryptophan is an essential amino acid which serves as a precursor for serotonin (5-hydroxytryptamine, 5-HT). Serotonin regulates behavioral and physiological processes such as elevation of mood, appetite, immunity, stress hormone secretion, and reduction of aggressive behavior. Poletto et al. (2010) has shown that aggression in young pigs while mixing could be reduced by short-term high tryptophan dietary supplementation. Warner et al. (1998) also reported similar results of reduced number of aggressive acts and a lower number of mounts in lairage after transportation. However, whether feeding higher amounts of tryptophan to pregnant sows will impact welfare and behavior of subsequent piglets has not been studied.

Arevola et al. (1990) reported that tryptophan concentrations increased in different fetal organs, including the brain, when pregnant rats were orally administered high doses of tryptophan. In the same study, tryptophan concentration in fetal brain tissue increased with an oral dose of high concentration of tryptophan to the mother, suggesting that high levels of tryptophan can cross the placental barrier. Studies conducted in pregnant rats have shown that a tryptophan enriched diet fed throughout pregnancy and lactation diminished serotonin and decreased activity of tryptophan hydroxylase in the cortex and in the brain stem of 5-day old rat pups (Huether et al., 1992). A recent study by Dennis et al. (2013) demonstrated that chick hens hatched after being given excess embryonic serotonin exhibited significantly less aggressive behavior. However, no such studies have been conducted in pigs.

Objective:

To evaluate the behavior, physiology, and welfare of piglets born to sows fed high-tryptophan gestation diets from day 28 to 35 post-breeding.

Materials & Methods:

This study was conducted at the Swine Farm at Purdue University, West Lafayette, IN and approved by the Purdue University IACUC (# 1402001024). Sixty-six, Yorkshire x Landrace multiparous sows were assigned to 1 of 3 treatments: Control (0.14 % SID tryptophan), Medium (0.28 % SID tryptophan, MED), or High (0.42 % SID tryptophan, HIGH). Treatments and diets are outlined in Table 1. Details of the diets are presented in Table 2. The concentration of tryptophan in the diets is based on previous research (Poletto et al., 2010) which showed that a 0.42 % digestible tryptophan diet raised blood tryptophan level, reduced time spent standing, and increased lying behavior in gilts. The high tryptophan diet also reduced agonistic interactions and aggressiveness.

On d 27 after sows were bred, pregnancy was diagnosed by ultrasound. Treatment diets were fed to the sows from d 28 to 35. On d 27 and d 35 of gestation, 5 mL i.v. blood samples were collected from sows using EDTA tubes. Plasma was separated and stored at -80 °C until analyzed to evaluate the change in peripheral blood serotonin and tryptophan concentrations before and after supplementation. Throughout the study, sows were housed individually in 0.61 m x 2.13 m (2’ x 7’) stalls and moved into farrowing crates on d 112 of gestation. Piglets were weaned at 18 ± 1.5 d. The study was conducted over 4 farrowing cycles with each treatment represented in every month.

When the sows farrowed, data were collected to record number of piglets born, weight of piglets at approximately 24 hours of age and at weaning, number of live piglets, and pre-weaning mortality rate and reason. Behavior of the litters was recorded (Nuvico CB-HD2N-L IR Bullet Camera, Nuvico Inc., Englewood, NJ) for 10 h, when piglets were approximately 3, 7, and 11 d old. A 5-min scan sample was used to evaluate the time-budget of behaviors and postures of sows and the piglets. The ethogram (Table 3) used for time-budget was based on that as described by Poletto et al. (2010). A schematic of the timing and sequence of all procedures is presented in Figure 1.

On d 12 of age, 3 mL i.v. blood samples were collected from 2 male and 2 female piglets for analysis of serotonin and cortisol. Blood was allowed to coagulate at room temperature for less than 2 hours, at which time serum was separated and stored at -80 °C until analyzed. Piglets were selected to represent the average weight of the litters, 2 males and 2 females. One male and one female piglet were then used for the following behavior tests, while the other male and female were not behavior tested to act as controls for cortisol levels before and after behavior testing. At d 14 of age, an isolation test was performed. For each isolation test, the piglet was taken from its litter and placed into a 4’x 4’ pen in a room absent of other pigs and people. The test lasted 10 min during which time behavior (Canon Vixia HFR 700 camcorder, Canon USA Inc., Melville, NY) and vocalizations were recorded (Song Meter SM3, Wildlife Acoustics, Maynard, MA). To conduct a human approach test, a human entered the pen immediately after the isolation test, extended the pen’s length to 8 ft, and stood motionless at the far end of the pen from the pig for 2 min (Figure 2). An observer stood outside the area, out of view, to record the time the pig approached the human, contacted the human, and the duration of contacts. At the end of the 2 min the human would kneel motionless in the same location. At this time the observer recorded the pig's response: neutral, which included no reaction or walk away, or fear, which included vocalization, freeze, startled jump, or run away. Once the pig again approached the now kneeling human, the test was stopped. The maximum length of the total human approach test was 5 min. The pen had visually designated areas (Figure 2) to allow the observer to record when the pig crossed the 'isolation line', the 'approach line', and when it touched the observer. Total number of contacts with the human and duration of all contacts were recorded. Refer to Table 4 for an ethogram for all behavior tests.

To conduct a Social Challenge Test, at 15 d of age, the same pigs used for the isolation test were paired by sex and treatment (for example, a male Medium pig paired with another male Medium pig from a different crate) and each pair was placed in the 4'x 8' pen for 10 min. During the test, escape attempts, social interactions, agonistic encounters, and vocalizations were recorded. Refer to Table 4 for an ethogram for all behavior tests. After the test, 3 mL i.v. blood samples were collected and the pigs were returned to their dam. Blood was allowed to coagulate at room temperature for less than 2 hours, at which time serum was separated and stored at -80 °C until being analyzed for cortisol.

Statistics

Analysis of variance using mixed models (treatment was a fixed effect and rep served as a random effect) was used to analyze most data. Data that were not normal were transformed, and if normality could not be achieved data were analyzed using the Wilcoxon-Mann-Whitney test. Sow was considered the statistical unit. Observance of nursing bouts during the time budget analysis was analyzed using chi-square tests. Significance was set at $P < 0.05$.

Results:

To verify that diets were formulated as prescribed, a pooled sample (batches and replicates) of each dietary treatment were taken for later tryptophan analysis. Diet samples were analyzed by an independent laboratory (Experiment Station Chemical Laboratories, Univ. MO, Columbia MO) and found to contain 0.16% (Control), 0.29% (Medium), and 0.37% (High) tryptophan respectively (Table 2). These concentrations were considered to be close enough to the targeted concentrations of 0.14, 0.28, and 0.42 % tryptophan respectively.

Sows delivered approximately 12 pigs per litter with 1 stillborn (Table 5), and did not differ by treatment ($P > 0.10$). Mortality was also similar among treatments with approximately 1 pig per litter dying prior to weaning. Birth weight, weaning weight and average daily gain were similar among treatments (Figure 3.)

When pigs were observed during 3 nursing bouts it was found that Control pigs tended to have more disputes than Medium pigs ($P < 0.08$, Table 6), but not High pigs ($P > 0.10$). Although High pigs showed numerically more disputes than Control and Medium pigs this was not statistically significant. High pigs had more displacements than Medium pigs ($P < 0.05$) but not Control pigs (Table 6). Nursing bout duration was similar among treatments lasting approximately 3 min.

Examining the time budget data of the litter it was found that pigs from all 3 treatments spent equal amounts of time active and inactive (Figure 4). For the most part pigs were inactive, spending about 50 % of their time resting, and 15% of their time exploring their pen, dam or littermates. Aggression was low with $.3 \pm .04$ % of the observation periods recording aggression for all 3 treatments. High pigs performed fewer nursing bouts than did Control or Medium pigs ($P < 0.02$).

No differences were detected for any of the variables for Isolation Test ($P > 0.10$, Table 7). Pigs tried to escape the enclosure approximately 10 times during the test. And they rested only about 15 seconds. Similarly, when the human entered the enclosure the pigs responded similarly with no differences detected among treatments ($P > 0.10$, Table 8). During the Human Approach Test, pigs contacted the human within approximately 40 seconds, and stayed in contact with the human for only 10 seconds (Table 8).

During the Social Challenge test, High pigs had more contacts approaching the head of the companion pig than did either Medium or Control pigs ($P < 0.007$, Table 9). Total number of aggressive interactions during the test tended to be greater for Medium pigs compared to High pigs ($P < 0.06$), with Control pigs being intermediate and not different than Medium pigs ($P > 0.10$).

Discussion:

This research was conducted to determine if feeding a high tryptophan diet to sows during gestation could negatively affect their subsequent litter. Previous research has shown that feeding pregnant rats a high tryptophan diet during their entire pregnancy caused retardation of the serotonergic neurons in their offspring (Huether et al, 1992). Tryptophan is the precursor to serotonin which has been shown to be associated with decreased levels of aggression. Thus a producer, who may want to decrease aggression when mixing sows after breeding, may want to explore the use of feeding increased concentrations of tryptophan to do so.

Overall, this research shows that feeding high concentrations of tryptophan to pregnant sows from gestational d 28 to 35 has very little effect on their subsequent offspring. Productivity of the sows proved to be equal, with sows producing similar sized litters and weaned pigs. Several tests were conducted to test the temperament of the piglets. Examining teat aggression during nursing bouts found that piglets from all 3 treatments had equal amounts of displacements and disputes. The Isolation Test, Human Approach Test, and Social Challenge Test were all used to determine how the pigs coped with stress and novelty. The first two tests found that pigs from all three treatments react similarly to these challenges. The Social Challenge test did find that High pigs approached the head of another pig more often, with Medium pigs in between. This measure was taken as a sign of boldness, but this behavior was not followed by increased aggression, and thus could indicate less fear in these pigs. However, other measures of fear taken in this study were not different, indicating any effect of maternal tryptophan is slight. The time budget data also indicate similar amounts of activity for pigs in all three treatments showing that the nervous system in general has not been altered.

In conclusion, feeding high concentrations of tryptophan for a short duration early in gestation does not have a negative impact on sows' subsequent offspring.

Literature Cited:

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Table 1. Treatments, diets and number of litters used for the study.

Treatments	Tryptophan level	N =	Days on treatment
Control	0.14%	19 litters	7 days (Gestational day 28 to 35)
Medium (MED)	0.28%	20 litters	7 days (Gestational day 28 to 35)
High (HIGH)	0.42%	21 litters	7 days (Gestational day 28 to 35)

Table 2. Diet composition for sows on the Control diet, Medium diet (2X Tryp, 0.28% inclusion rate), and the High diet (3X Tryp, 0.42% inclusion rate).

Ingredients, %	Gestation Control	2X Tryp 2X	3X Tryp 3X
Corn	43.575	43.500	43.280
Soybean Meal, 48%	11.47	11.47	11.47
DDGS	40.00	40.00	40.00
Monocal. Phosphate	0.51	0.51	0.51
Limestone	1.79	1.79	1.79
Salt	0.50	0.50	0.50
Choice white grease	1.00	1.00	1.00
Lysine -HCl	--	--	--
DL-Methionine	--	--	--
L-Threonine	--	--	--
L-Tryptophan	--	0.075	0.295
Swine Vit. Premix	0.25	0.25	0.25
Swine TM Premix	0.125	0.125	0.125
Sow Vitamin Premix	0.25	0.25	0.25
Selenium premix	0.05	0.05	0.05
Phytase (600 PU/g)	0.10	0.10	0.10
Rabon Larvacide	0.13	0.13	0.13
Defusion Plus	0.25	0.25	0.25
ME, kcal/kg	1492.1	1492.1	1492.1
Crude Protein, %	19.59	19.59	19.59
Tot. Lysine, %	0.83	0.83	0.83
SID Lys, %	0.60	0.60	0.60
SID Thre, %	0.56	0.56	0.56
SID Tryp, %	0.14	0.28	0.42
SID Meth + Cys, %	0.59	0.59	0.59
Ca, %	0.85	0.85	0.85
P, %	0.63	0.63	0.63
Phytase avail. P, %	0.45	0.45	0.45

Table 3. Ethogram for time-budget behaviors and postures observed in the piglets (adapted from Poletto et al., 2010).

Variable	Description
Pig Behavior	
Active	Physically mobile, standing, walking or running
Inactive	Physically immobile, without activity
Aggressive interaction	Engaging in agonistic interaction-pushing, biting, and head-knocking with another pig
Nursing	Mouth on the teat
Sow Posture	
Upright	Standing on all four legs or dog-sitting with rump on the floor and shoulders raised up with front legs extended
Lying Lateral	Lying on side
Lying Sternal	Lying on sternum

Table 4. Ethogram for the Isolation, Human Approach, and Social Challenge behavior tests.

Variable	Description
Isolation Test	
Activity start	Time the pig starts walking after being placed in the pen
Resting	Lying or sitting
Escape attempt	Jumping or climbing against pen wall
Human Approach	
Contact	Sniffing the human
Social Challenge	
Activity start	Time the pig starts walking after being placed in the pen
Social contact	Approaching to sniff the penmate, either head-on or approaching the body
Nudge	Using its snout to push the penmate
Agonistic encounters	Biting, head tossing, mounting, and shoving the penmate
Duration in proximity	Being within one body length of the penmate

Table 5. Production data per sow.

Variable	Control	Medium	High
Born (#)	13.6 ± 0.7	11.5 ± 0.6	13.2 ± 0.7
Born Alive (#)	12.3 ± 0.7	10.7 ± 0.6	12.2 ± 0.6
Mortality (#)	1.5 ± 0.4	0.8 ± 0.3	1.0 ± 0.2
Scours (#)	0.4 ± 0.1	0.3 ± 0.1	0.1 ± 0.1

Table 6. Nursing bout duration and percentage of disputes and displacements during nursing.

Variable	Control	Medium	High
Disputes (%)	32.5 ± 3.6[a]	26.5 ± 3.9[b]	36.9 ± 4.6[a,b]
Displacements (%)	7.5 ± 1.4[c,d]	6.8 ± 1.5[c]	11.9 ± 1.8[d]
Nursing Bout (s)	189 ± 9.4	194.7 ± 7.6	210.0 ± 8.0

[a,b]Means with different superscripts tend to differ ($P < 0.08$).
[c,d]Means with different superscripts differ ($P < 0.05$).

Table 7. Response of pigs when subjected to a 10-min Isolation Test.

Variable	Control	Medium	High
Activity latency (s)	20.63 ± 4.66	16.72 ± 2.51	15.42 ± 2.54
Latency to investigate pen (s)	14.17 ± 4.20	9.06 ± 2.22	9.16 ± 2.24
Latency to attempt escape (s)	307.04 ± 13.47	310.15 ± 36.43	313.83 ± 34.12
Number of escape attempts	12.79 ± 2.82	8.39 ± 1.51	11.19 ± 1.62
Resting duration (s)	11.92 ± 5.35	21.81 ± 9.64	19.35 ± 6.87
Number of resting bouts	0.50 ± 0.18	0.45 ± 0.16	0.80 ± 0.26

Table 8. Response of pigs when subjected to a 5-min Human approach Test.

Variable	Control	Medium	High
Latency to exit isolation are (s)	33.81 ± 8.60	31.71 ± 7.19	27.86 ± 5.50
Approach latency (s)	37.00 ± 6.62	42.43 ± 7.92	33.80 ± 6.07
Contact latency (s)	45.56 ± 6.46	41.89 ± 6.01	38.18 ± 4.57
Number of contacts	4.96 ± 0.48	4.75 ± 0.52	5.03 ± 0.44
Duration in contact (s)	9.69 ± 1.09	10.33 ± 1.28	12.50 ± 1.38
Latency to contact after human movement (s)	28.27 ± 6.31	35.68 ± 8.52	23.26 ± 3.27
Duration of contact after human movement	12.85 ± 3.50	11.91 ± 3.16	11.00 ± 2.47

Table 9. Response of pigs when subjected to a Social Challenge Test.

Variable	Control	Medium	High
Activity latency (s)	12.62 ± 2.70	26.47 ± 8.89	17.95 ± 3.61
Social interaction latency (s)	27.46 ± 3.92	42.67 ± 11.46	36.05 ± 3.61
Number of contacts approaching the head	9.69 ± 0.84[a]	10.67 ± 1.09[a]	14.26 ± 1.11[b]
Number of contacts not approaching the head	21.85 ± 2.37	16.27 ± 2.05	18.42 ± 1.53
Number of nudges	2.46 ± 0.58	3.67 ± 0.87	4.37 ± 0.67
Latency to aggressive interactions (s)	186.42 ± 26.42	194.73 ± 31.34	271.17 ± 42.72
Number of aggressive interactions	7.08 ± 1.53[c,d]	9.27 ± 1.45[d]	5.05 ± 0.92[c]
Total duration of aggressive interactions (s)	42.62 ± 10.30	60.47 ± 13.78	32.68 ± 8.09
Time spent in proximity (s)	571.23 ± 8.20	570.13 ± 5.60	569.21 ± 6.13
Number of escape attempts	0.08 ± 0.08	0.73 ± 0.36	0.53 ± 0.25

[a,b]Means with different superscripts differ (P < 0.007).
[c,d]Means with different superscripts tend to differ (P < 0.06).

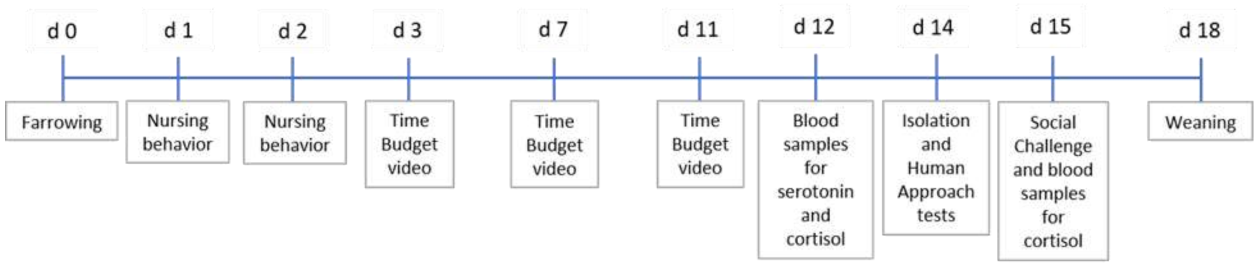

Figure 1. Time and sequence of data collection relative to day of age.

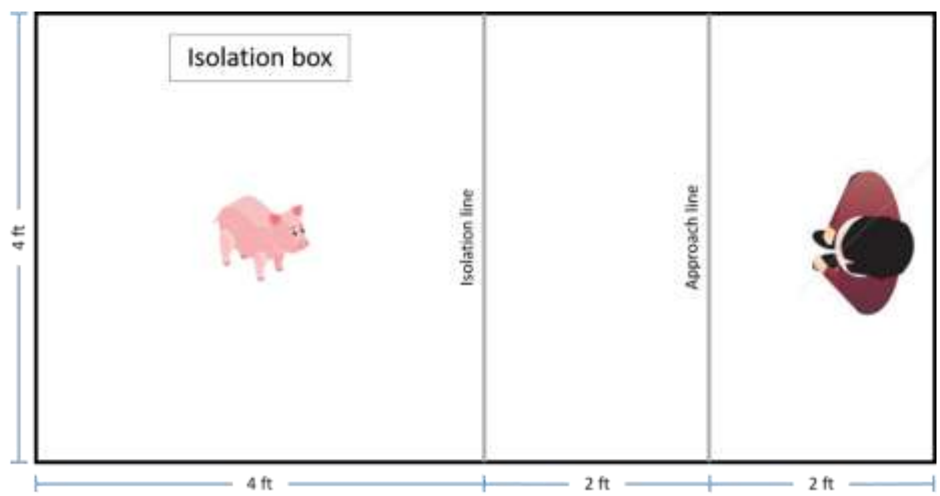

Figure 2. Testing pen for the Human Approach Test. The latency for the pig to explore and cross the Isolation line, cross the Approach Line, and Contact the Human was recorded.

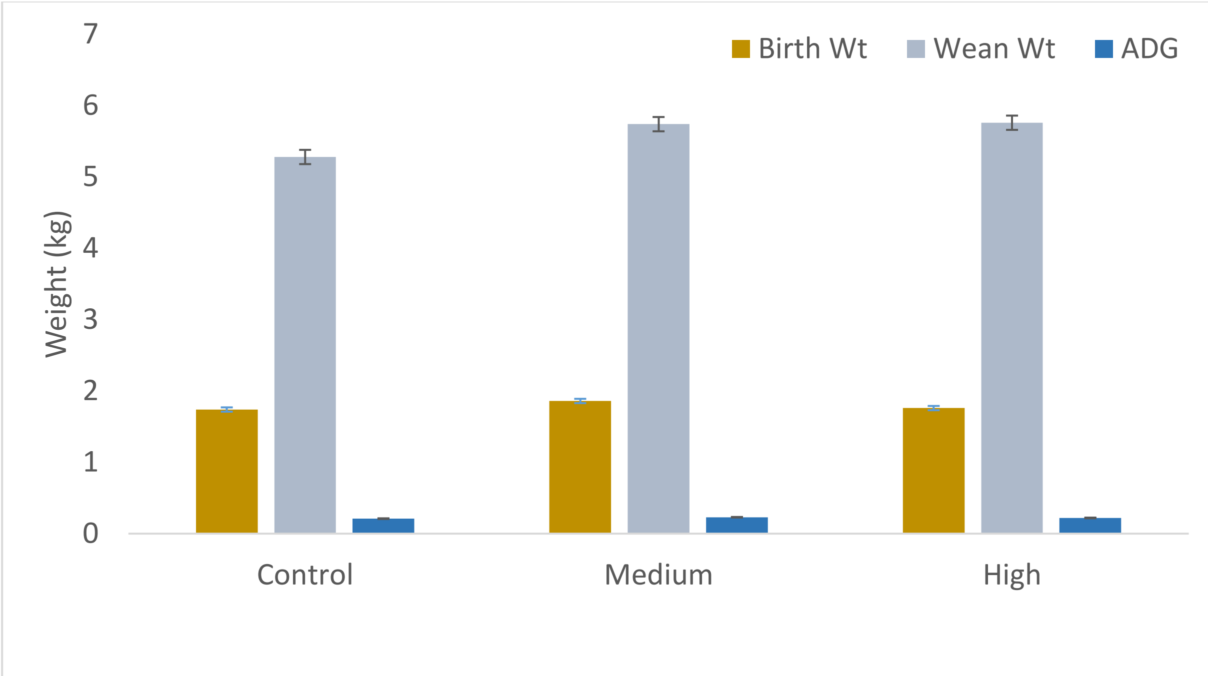

Figure 3. Birth weight (Birth Wt), Weaning (Wean Wt), and average daily gain (ADG).

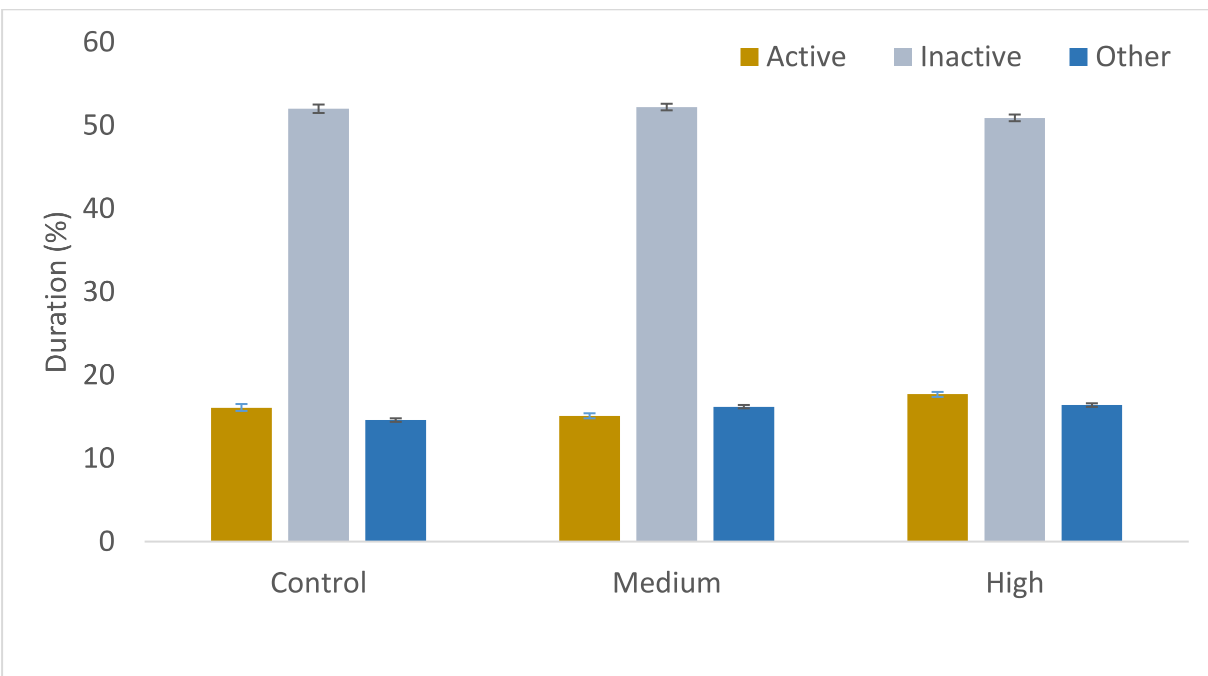

Figure 4. Time pigs spent active and inactive from 0700 h to 1700 h, over 3 days from birth to weaning.