Philippine Journal of Science
147 (4): 555-562, December 2018
ISSN 0031 - 7683
Date Received: 20 Feb 2018

Plasma Heat Shock Protein 70, Physiological and Behavioral Responses of Gilts to Varying Temperature Humidity Index in a Controlled Chamber

Carla Alilie A. Lontoc[1]*, **Pauline Joy R. Cajano**[2],
Antonio A. Rayos[1], and **Renato SA. Vega**[2]

[1]Animal Production Division, Institute of Animal Science, College of Agriculture
and Food Science, University of the Philippines Los Baños, College, Laguna 4031 Philippines
[2]Animal Physiology Division, Institute of Animal Science, College of Agriculture
and Food Science, University of the Philippines Los Baños, College, Laguna 4031 Philippines

The study aimed to determine physiological, behavioral, and extracellular heat shock protein 70 (HSP70) responses among gilts exposed to increasing temperature humidity index (THI). Individually caged, F1 gilts (N=3) were adjusted for a week given *ad libitum* commercial diet and water supply in an environmentally controlled chamber, after which the THI (65, 71, 77, and 83) was elevated every three days for a total of 12 days. Physiological responses [respiration rate (RR), pulse rate (PR), rectal temperature (RT)] and plasma HSP70 were all collected during their first day exposure at 65, 71, 77, and 83 THI. Behavioral responses – meal intake (MI), meal break (MB), standing, lying down, drinking, urination, and defecation – were recorded and counted for frequency and duration at three different periods of the day (08:00–15:00h, 15:01–23:00h, and 23:01–07:59h) using internet protocol cameras. Results showed that RR and plasma HSP70 concentration obtained significant differences at 83 and 71 THI, respectively. Frequency and duration of meal intake and meal break decreased, consistent with the reduction in voluntary feed intake ($P<0.05$). Among the responses, meal intake duration is the most visible heat stress response that significantly occurred at 77 THI (27 °C), showing meal intake duration reduced by 556 s *i.e.*, 25% of 2212 s (37 min) for the seven-hour period after feed is offered.

Key words: behavior, gilts, heat shock protein, heat stress, temperature humidity index

INTRODUCTION

Gilts lack sweat glands and are therefore highly sensitive to changes in temperature. Thus, they need to develop a wide range of thermoregulatory behaviors to maintain physiological homeostasis (Pedersen *et al.* 2003*,* Olczak 2015). Respiration rate, pulse rate, and rectal temperature have been reported to increase along with the increase in temperature (Huynh *et al.* 2005*,* Phuoc *et al.* 2005).

Behavioral responses such as maximizing contact with concrete, using dunging areas for lounging to cool themselves, creating wallows, decreasing feed intake, and increasing water consumption are coping mechanisms of pigs exposed to high temperature (Lammers *et al.* 2007). Meanwhile, at the cellular level, heat shock protein 70 (HSP70) is released in response to different stress stimuli – including heat stress – to protect cells from damage (Bloemhof 2008). Qu *et al.* (2015) observed greater levels of HSP27, HSP60, HSP70, and HSP90 on pigs in heat stress condition compared with control. Pearce *et al.* (2013) also reported that growing pigs exposed to high ambient temperature (35 °C) had greater HSP70 expression compared with those kept at thermoneutrality (24 °C).

---

*Corresponding author: calontoc@up.edu.ph; lontoccarla@gmail.com

Understanding these responses is important for successful implementation of strategies to ameliorate production level under warm climate (Renaudeau *et al.* 2012). However, there were limited studies on the effects of heat stress on gilts in relation to physiological and behavioral responses of pigs along with HSP70 concentration. This study, the first in the Philippines, aimed to establish responses that would serve as good indicators of heat stress and examined gilts' behavior and extracellular HSP70 expression.

MATERIALS AND METHODS

Experimental Animals

Three (3) F_1 (Landrace x Large White) gilts with 98±6 kg body weight were housed at the quarantine area for one week prior to transfer to the controlled chamber (5.89 m x 2.35 m x 2.39 m) where they were individually placed inside a metabolism cage (2 m x 1 m x 1.5 m) with slatted flooring. Each cage was provided with a nipple drinker for continuous water supply and an internet protocol (IP) camera for monitoring behavior. *Ad libitum* commercial breeder ration was given and feed intake was recorded everyday.

Controlled Chamber Set-up

Four (4) temperature regimes with 4 °C interval (19, 23, 27, and 31 °C) were set. Temperature values were adjusted every three days for 12 days. The relative humidity (RH) was fixed at 70% (±10%). The temperature humidity index (THI) was computed using the formula suggested by NOAA (1976).

$$\text{THI} = 0.8\text{Ta} + (\text{RH}/100) \times (\text{Ta}-14.3) + 46 \qquad (1)$$

Where:

T_a – ambient temperature (°C)

RH – relative humidity (%)

THI values were calculated using the formula ***i.e.***, 65 (19 °C), 71 (23 °C), 77 (27 °C), and 83 (31 °C).

Time and Place of Study

The study was conducted at the Institute of Animal Science, College of Agriculture & Food Science, UP Los Baños from 1 May 2015 to 20 May 2015.

Experiment 1: Physiological and Plasma HSP70 Responses to THI

Pulse rate (PR), respiration rate (RR), and rectal temperature (RT) were collected every 07:00h and 15:00h prior to blood collection. The gilts were at rest while RR was collected by counting the frequency of the rise and fall movements of the hind flank in 60 s. PR was measured by counting the heartbeat (systole) every 60 s, done using a stethoscope placed on the left side of the thorax. RT was taken using a digital thermometer inserted in the rectum.

For plasma assay, blood was collected using vacu-container tubes with ethylene diamine tetraacetic acid (EDTA) through the gilt's ear vein while being restrained using a snout rope. All the procedures were conducted in compliance with the Republic Act No. 8485 under the supervision of a licensed veterinarian. The blood samples were centrifuged for 15 min at 1000 x g at 2–8 °C within 30 min of collection. The aliquot plasma samples were stored at –40 °C until the time of assay. The study utilized the HSP70 enzyme ligand immunoassay (HSP70 ELISA; Cusabio®, USA). The ELISA procedure follows the manufacturer's protocol.

To minimize imposed stress, collection of blood and physiological parameters were only done in the morning (07:30h) and afternoon (15:30h) of the gilt's first day exposure to each THI values.

The experimental design used was completely randomized design (CRD). The programmed THI values were 65 (T1), 71 (T2), 77 (T3), and 83 (T4); if ANOVA showed significant differences at $P<0.05$, least significant difference (LSD) was used to compare treatment means. Meanwhile, the statistical model used was:

$$Yij = \mu + \tau\, i + \varepsilon ij \qquad (2)$$

where:
Yij = j^{th} observation of the i^{th} temperature humidity index
μ = general average of response variable
τi = effect of i^{th} temperature humidity index (i=4)
εij = random error

Experiment 2: Behavioral Responses to Gradual Rise in THI

Voluntary feed intake (VFI) was based on the feed consumed in 24 hr during the first day of exposure to each THI setup. The data gathered was subsequently analyzed using the same experimental design and statistical model of Experiment 1.

Meanwhile, for the detailed observation on the behavioral responses, there was continuous recording of the IP cameras throughout the experiment – with videos automatically saved every 10 min for a total of 144 observations per day. Days were divided into three eight-hour periods, namely 08:00–15:00h (P1), 15:01–22:00h (P2), and 22:01–07:59h (P3). The behavior of each gilt was observed through the footages and the frequency and duration of their activities were tabulated. Feed intake was based on the feed consumed in 24 hr during the first day of exposure to each THI set-up.

To annotate the behavior, the following were observed: meal intake (MI), meal breaks (MB), drinking, lying down and standing, urination (URI), and defecation (DEF).

This experiment utilized 4x3 factorial in CRD. There were four programmed THI values (65, 71, 77, and 83) used. The video observation for gilts' behavioral responses was divided into three periods. Comparison of means between and within different factors was done using LSD. The statistical model is:

$$Yijk= \mu+ \alpha i + \beta j+ (\alpha\beta)ij+ \varepsilon ijk \tag{3}$$

where:
Yijk = value of the response variable
μ = general average of response variable
αi = effect of i^{th} temperature humidity index (i=4)
βj = effect of j^{th} period of observation (j=3)
(αβ)ij = interaction effect of the i^{th} temperature humidity index and j^{th} period of observation
εijk = random error associated with the k^{th} gilt of the i^{th} temperature humidity index and j^{th} period of observation.

For both experiments, all the values were analyzed using PROC GLM of SAS. All values obtained were presented in mean±SEM.

RESULTS AND DISCUSSION

Experiment 1: Physiological and Plasma HSP70 Responses to THI

Physiological responses were observed under different THIs, with results presented in Figures 1 and 2.

Respiration Rate (RR). A pig's normal RR ranges from 15 to 25 breaths per min, with risk of heat stress detected at 40 breaths per min above and heat stress at 60 breaths per min above (Rozeboom *et al.* 2000). Increased RR is a result of a pig's tendency for frequent panting to dissipate heat (Pearce 2014). Significant increase in RR is expected at 27–31 °C (Renaudeau 2004), which mirrored the study's results (Figure 1) with the sudden increase of RR from 35 breaths per min to 81 breaths per min ($P<0.001$). Evaporative critical temperature (ECT) is the temperature where RR was expected to increase significantly, which in the study's case is at 83 THI. Therefore, since the RR values from 65 to 77 THI (T1 to T3) showed no significant difference, it is suggested that RR could be an indicator of heat stress at 83 THI.

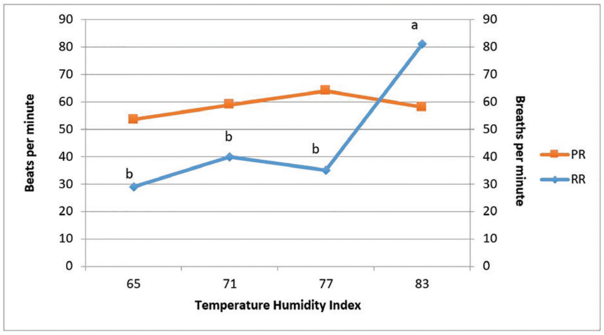

Figure 1. Mean±SEM of respiraton rate (RR) and pulse rate (PR) in different temperature humidity index (THI).

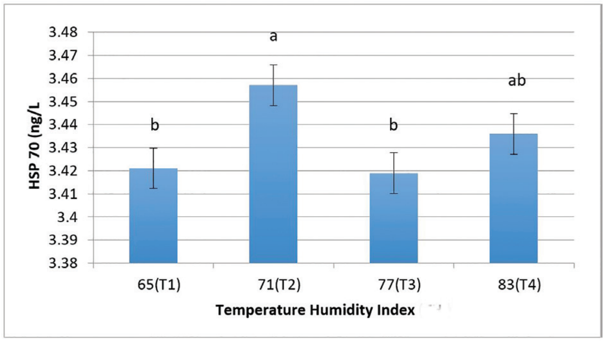

Figure 2. Mean±SEM of plasma HSP70 (ng/L) concentration in different temperature humidity index.

Pulse Rate (PR). The consistent increase of PR of gilts from 53.65 to 64 beats per minute (bpm) after being exposed from 65 THI (19 °C) to 77 THI (27 °C) is not significant ($P=0.336$). Meanwhile, Phuoc *et al.* (2005) reported a positive strong linear relationship between air temperature and pulse rate of two breeds of pigs, on which the rapid increase was observed at air temperature above 25 °C.

Rectal Temperature (RT). The normal rectal temperature of pigs ranges from 38.7 to 39.8 °C (Robertshaw 2004). Results showed no significant difference ($p=0.105$) among the RT values observed (data not shown). The highest RT was 39.57 °C at THI 83, which still falls within the normal range. Various studies suggested that RT was expected to increase at temperatures higher than 31 °C. Renaudeau (2004) also concluded that RT remains constant between 26 and 30 °C at 38.3 °C and foreseen to increase beyond the critical threshold temperature (30–32 °C). Likewise, results of the study indicate comparable RT values between 65 THI (19 °C) and 83 THI (31 °C).

Relationship between Increased THI and HSP70. The release of HSPs is triggered when there is an environmental change that causes stress to the physiological state of an organism (Oksala *et al.* 2014). It could be reckoned that the significant increase in HSP70 concentration ($p=0.\ 0238$) from 65 to 71 THI (3.421–3.4571 ng/L) was prompted by the increase in temperature when heat stress was detected (Figure 2). The increase was followed by comparable values from 77 to 83 THI (3.419–3.436 ng/L). These values imply that the prior release of HSP70 was sufficient to cope with the increasing temperature, possibly indicating adaptation. Based on the initial significant increase, it could be concluded that HSP70 concentration is a valid indicator of heat stress.

Experiment 2: Behavioral Responses in Gradual Rise in THI

Increased THI and Voluntary Feed intake (VFI). Reduced VFI is the most common thermoregulatory mechanism of pigs. Although it minimizes heat loads in their body, it causes negative effects on their growth performances (Quiniou & Noblet 1999*,* Martin 2012).

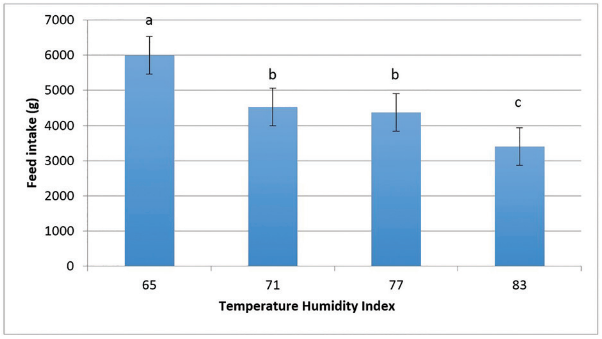

Figure 3. Mean±SEM voluntary feed intake (VFI) of gilts under different temperature humidity index set-up.

The study observed that there was a significant decline in VFI (P<0.001) as the temperature increased (Figure 3). The highest VFI observed was 6000 g, while the lowest was 3403 g. The results of Lammers *et al.* (2007) and Olczak *et al.* (2015) supported the current study wherein reduction in VFI with the gradual increase in heat stress was observed.

Interaction between THI and Period on Meal Intake (MI) Duration. Interaction effects (p=0.0200) were observed for temperature and period on MI duration at 08:00–15:00h (P1) and 15:01–22:00h (P2) (see Table 1). Longest MI duration was at 65 THI and 71 THI for P1 and P2, respectively (2642.67 s *vs*. 2212 s), while the shortest at 83 THI for both periods (763.00 s *vs*. 1044.33 s). Debrecéni *et al.* (2014) also found interaction effects of temperature and time on eating behavior.

Pigs are most active in the morning and afternoon (Simonsen *et al.* 1990), hence, P1 should have longer MI duration than P2. However, as the temperature increased – or as it reached 77 THI in this study's case – the MI duration became longer for P2. This implied that the effect of period on MI duration became less with the increase in THI. Since THI and period had interaction effects on MI duration, it could be a reliable indicator for heat stress. It was calculated that the cumulative average MI duration shorter than 2250.66 s (37 min) during P1 and P2 at 71 THI is a sign of heat stress for gilts.

Increased THI and Thermoregulatory Behaviors. Pigs tend to rest more and they become inactive and uncomfortable with the increase in temperature (Olczak 2015). Huynh *et al.* (2005) stated that for each degree Celsius increase in temperature, there was a 0.2% increase in the lying percentage, with high temperature causing higher proportions of lying activity (72.7%) compared with thermoneutral temperature (65.8%). Similar effects on the gilts' activities resulting in a constant decrease in standing duration and increase on lying duration were observed in this study (Table 3).

Based on previous studies (Collin *et al.* 2001*,* Quinou *et al.* 2000), shorter meal duration was expected with increasing temperature. Results of the study (Table 2) reflect the same findings of a constant decreased MI duration and increased meal break (MB) duration. Additionally, frequencies of

Table 1. Mean±SEM of duration (s) of meal intake (MI) of gilts at different time period under different temperature humidity index (THI).

	Period			
THI	**08:00–15:00h**	**15:01–23:00h**	**23:01–07:59h**	**Mean±SEM**
65 (19 °C)	2642.67^{ax}	1841.33^{aby}	217.00^{z}	1567.00±405.50^{a}
71(23 °C)	2289.33^{ax}	2212.00^{ax}	206.67^{y}	1569.30±345.56^{a}
77(27 °C)	1407.33^{bx}	1656.00^{bx}	575.67^{y}	1213.00±219.72^{a}
83 (31 °C)	763.00^{cx}	1044.33^{cx}	262.67^{y}	690.00±161.17^{b}
Mean±SEM	1775.58±264.10^{x}	1688.42±189.27^{x}	315.50±61.66^{y}	

In a column, values with different superscripts (^{abc}) are significantly different at 5% level.
In a row, values with different superscripts (^{xyz}) are significantly different at 5% level.

Table 2. Mean±SEM and total duration and frequency of meal intake and meal break of gilts at different temperature humidity index (THI) and periods of observation.

	Behavioral Activities			
	Meal Intake		Meal Break	
THI	Duration (s)	Frequency	Duration (s)	Frequency
65(T1=19 °C)	4701.00^{a}	10.22^{a}	429.00^{b}	34.22
71(T2=23 °C)	4708.00^{a}	11.11^{a}	550.00^{ab}	26.33
77(T3=27 °C)	3639.00^{a}	5.78^{b}	364.67^{b}	22.44
83(T4=31 °C)	2070.00^{b}	7.78^{ab}	722.33^{a}	23.44
Mean±SEM	3779.5±282.99	8.73±1.79	516±42.08	26.61±6.62
p-value	*0.0017*	*<0.0001*	*0.0308*	*0.4264*
Period				
08:00–15:00h (P1)	1775.58^{a}	11.25^{a}	241.58^{a}	37.58^{a}
15:01–23:00h (P2)	1688.42^{a}	12.00^{a}	242.67^{a}	34.50^{a}
23:01–07:59h (P3)	315.50^{b}	2.92^{b}	32.42^{b}	7.76^{b}
Total	3779.50	26.17	516.67	79.84
p-value	*<0.0001*	*<0.0001*	*0.0173*	*0.0002*

In a column, values with different superscripts (^{abc}) are significantly different at 5% level.

Table 3. Mean±SEM and total duration and frequency of standing and lying of gilts at different temperature humidity index (THI) and periods of observation.

	Behavioral Activities			
	Standing		Lying	
THI	Duration (s)	Frequency	Duration (s)	Frequency
65 (19 °C)	6696.00	16.56	22304.00	42.11
71(23 °C)	5957.00	17.00	23043.00	44.22
77(27 °C)	3917.00	11.33	24953.00	46.67
83(31 °C)	3490.00	16.00	25495.00	48.89
Mean±SEM	4993.25±1110.28	71.42±2.29	17747.5±2185.27	35.20±4.36
p-value	*0.0727*	*0.0834*	*0.0726*	*0.1892*
Period				
08:00–15:00h (P1)	6179.00^{a}	17.58^{a}	1985.00^{b}	38.00^{b}
15:01–23:00h (P2)	7036.00^{a}	20.00^{a}	18067.00^{b}	37.50^{b}
23:01–07:59h (P3)	1830.00^{b}	8.08^{b}	34195.00^{a}	60.92^{a}
Total	15045.00	45.66	54256.00	136.42
p-value	*0.0003*	*<.0001*	*<0.0001*	*<0.0001*

In a column, values with different superscripts (^{abc}) are significantly different at 5% level.

MB and MI both significantly decreased.

The current study did not find any significant effect of the increase in temperature in the drinking behavior of gilts (Table 4). On the other hand, Waltz *et al.* (2014) found a significant increase of water intake after temperature increased from 24 °C to 32 °C. For excretion behaviors, urination and defecation were observed. But there were no significant differences on the duration and frequency of these activities (Table 4).

Effect of Different Observation Periods on the Thermoregulatory Behaviors. Each day was divided into three periods to identify the behavioral patterns exhibited by the gilts. Results showed significant effects on all of the behaviors observed, except for urination and

Table 4. Mean ± SEM of duration and frequency of urination, defecation, and drinking of gilts at different temperature humidity index (THI) and periods of observation.

	Behavioral Activities					
	Drinking		**Urination**		**Defecation**	
THI	Duration (s)	Frequency	Duration (s)	Frequency	Duration (s)	Frequency
65 (19 °C)	147.11	8.33	24.22	1.78	19.78	2.00
71(23 °C)	118.11	7.22	23.00	1.78	18.44	2.00
77(27 °C)	52.22	4.56	29.33	1.56	12.11	1.44
83(31 °C)	184.00	10.11	37.56	2.67	8.88	1.44
Mean ± SEM	125.36±41.83	7.56±1.97	28.53±11.54	1.95±0.68	14.80±5.20	1.72±0.44
p-value	*0.2768*	*0.8786*	*0.445*	*0.4069*	*0.8263*	*0.664*
Period						
08:00–15:00h (P1)	139.50	8.50^{a}	24.08	1.17	13.33	1.58
15:01–23:00h (P2)	160.83	11.0^{a}	30.17	2.33	20.08	2.42
23:01–07:59h (P3)	75.75	3.17^{b}	31.33	2.33	10.92	1.17
Total	376.08	22.67	85.58	5.83	44.33	5.17
p-value	*0.3334*	*0.0041*	*0.3753*	*0.902*	*0.3825*	*0.0793*

In a column, values with different superscripts (abc) are significantly different at 5% level.

defecation. Debrecéni *et al.* (2014) claimed that pigs eat longer and more frequently in the morning, which was demonstrated accordingly with the results of the study. Duration and frequencies of MI, MB (see Table 2), and drinking behavior (see Table 4) during P1 and P2 are significantly longer and more frequent compared with observations during P3. Standing was also more frequent and longer during the first two periods since they had to stand in order to eat the feeds in the trough. On the other hand, lying down was most frequent and longest at P3 (see Table 3) where gilts spent time sleeping.

CONCLUSION

The study aimed to identify physiological, behavioral, and cellular responses that will detect heat stress among gilts. Results showed that only RR and HSP70 significantly differ at increasing THI. These responses were sensitive to the sudden increase in THI, causing immediate manifestations as their values significantly increased along with the THI levels. However, for HSP70 level, after the sudden increase at 71 THI it returned to baseline level. This sudden excitation in extracelluar HSP70 indicates the gilts' response to heat stress, hence the return to baseline level suggests that the animal becomes adapted to the prevailing condition *i.e.*, THI 77 and 83. On the other hand, other physiological responses such as RT and PR lack the considered adequate set-up of factors to manifest like longer heat exposure, which led to insignificant results.

For behavioral responses, the increasing THI levels resulted to significant decrease on VFI, MI, and MB, which was observed at 77 THI (27 °C). Meanwhile, duration and frequencies of MI, MB, standing, drinking, and lying down behavior during the last period are significantly different with observations during the first two periods. Noticeable compensatory night meal was also observed at 77 (27 °C) but not at 83 (31 °C) THI. Furthermore, the consistent proportional decrease in meal intake was observed from 71 to 83 THI, indicative of the effect of heat stress on the voluntary feed intake. The interaction effects of THI and period were observed, which revealed that significant decrease in meal intake duration occurred at 77 THI (27 °C) – showing meal duration reduced by 556 s *i.e.*, 25% of 2212 s (37 min) for the seven-hour period after feed is offered. Meanwhile, validation of the results of the study in an actual commercial farm is recommended.

ACKNOWLEDGMENT

The authors would like to extend their sincerest gratitude to the Department of Science and Technology – Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (DOST-PCAARRD) for funding the project "Development of Computer Aided Remote Expert (CARE) System Model for enhancing the productivity and efficiency in swine farms," in which this research is part of. They would also like to thank the project support staff for the assistance during the conduct of the study.

REFERENCES

BLOEMHOF EH, VAN DER WAAJ EH, MERKS JWM, KNOL EF. 2008. Sow line differences in heat stress tolerance expressed in reproductive performance traits. J Anim Sci 86: 3330–37

COLLIN A, VAN MILGEN J, DUBOIS S, NOBLET J. 2001. Effect of high temperature on feeding behaviour and heat production in group-housed young pigs. British Journal of Nutrition 86: 63–70.

CUSABIO. 2013. ELISA Kit: User Manual (USA) for Pig Heat Shock Protein 70 (HSP-70). CSB–E08317p

DEBRECÉNI O, LEHOTAYOVÁ A, BUCKO O, PETRAK J. 2014. The Behaviour of the Pigs Housed in Hot Climatic Conditions. Journal of Central European Agriculture 15(1): 64–75.

HUYNH T, AARNINK AJA, GERRITS WJJ, HEETKAMP MJH, CANH TT, SPOOLDER HAM, KEMP B, VERSTEGEN MWA. 2005. Thermal behaviour of growing pigs in response to high temperature and humidity. Applied Animal Behaviour Science 91(1–2): 1–16.

LAMMERS PJ, STENDER DR, HONEYMAN MS. 2007. Environmental Needs of the Pig. Niche Pork Production. IPIC NPP110 2007.

MARTIN WR. 2012. Effects of heat stress on thermoregulation, reproduction and performance of different parity sow [Ph.D. Thesis]. University of Missouri.

[NOAA] National Oceanic and Atmospheric Administration. 1976. Livestock Hot Weather Stress. Operations Manual Letter C-31-76. Kansas City.

OLCZAK K, NOWICKI J, KLOCEK C. 2015. Pig behaviour in relation to weather conditions – A review. Ann. Anim. Sci. 15(3): 601–610.

OKSALA KJ, EKMEKCI FG, OZSOY E, KIRANKAYA S, KOKKOLA T, EMECEN G, LAPPALAINEN J, KAARNIRANTA K, ATALAY M. 2014. Natural thermal adaptation increases heat shock protein levels and decreases oxidative stress. Redox Biology 3: 25–28.

PEARCE SC, GABLER NK. ROSS, JW, ESCOBAR J, PATIENCE JF, RHOADS RP BAUMGARD LH. 2013. The effects of heat stress and plane of nutrition on metabolism in growing pigs. Journal of Animal Science 91: 2108–18.

PEARCE S. 2014. Evaluation of the chronological impact heat stress has on swine intestinal function and integrity [Ph.D. Thesis]. Paper 14010. Digital Repository at Iowa State University.

PEDERSEN S, SOUSA P, ANDERSEN L, JENKEN KH. 2003. Thermoregulatory behaviour of growing-finishing pigs in pens with access to outdoor area. Agricultural Engineering International: the CIGR Journal of Scientific Research and Development. Manuscript BC 03 002.

PHUOC LV, NGOAN LD. 2005. Effect of the environmental factors on physiological parameters, feed intake and growth of Mongcai and Landrace pigs in central Vietnam. Workshop-seminar "Making better use of local feed resources." MEKARN–CTU: Cantho. Retrieved on 29 Oct 2015.

QUINIOU N, NOBLET J. 1999. Influence of high ambient temperatures on performance of multiparous lactating sows. J. Anim Sci. 77: 2124–34.

QUINIOU N, DUBOIS S, NOBLET J. 2000. Voluntary feed intake and feeding behaviour of group-housed growing pigs are affected by ambient temperature and body weight. Livestock Production Science 63(1): 245–253.

QU H, DONKIN SS, AJUWON KM. 2015. Heat stress enhances adipogenic differentiation of subcutaneous fat depot-derived porcine stromovascular cells. J Anim Sci. 93(8): 3832–42. doi:10.2527/jas.2015-9074

RENAUDEAU D. 2004. Effects of short term-exposure to high ambient temperature and relative humidity on thermoregulatory responses of European (Large White) and Caribbean (Creole) restrictively-fed growing pigs. Anim. Res. 54: 81–93.

RENAUDEAU D, COLLIN A, YAHAV S, DE BASILIO V, GOURDINE JL' COLLIER RJ. 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Animal 6(5): 707–728. doi:10.1017/S1751731111002448

ROBERTSHAW D. 2004. Temperature regulation and the thermal environment. Duke's Physiology of Domestic Animals. p. 962–973.

ROZEBOOM J, TODD S, FLOWERS W. 2000. Management Practices to Reduce the Impact of Seasonal Infertility on Sow Herd Productivity. ANS00-813S. North Carolina Cooperative Extension Service. Retrieved from https://projects.ncsu.edu. Retrieved on 15 Jun 2015.

SIMONSEN HB. 1990. Behaviour and distribution of fattening pigs in the multi-activity pen. Applied Animal Behaviour Science 27(4): 311–324.

WALTZ X, BAILLOT M, CONNES P, BOCAGE B, RENAUDEAU D. 2014. Effects of Hydration Level and Heat Stress on Thermoregulatory Responses, Hematological and Blood Rheological Properties in Growing Pigs. PLoS ONE 9(7): 102537. Retrieved from https://doi.org/10.1371/journal.pone.0102537 on 15 Jun 2015.