# The dietary protein content slightly affects the body temperature of growing pigs exposed to heat stress

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**ABSTRACT:** Heat stress (HS) increases body temperature (BT) and reduces feed intake in pigs. Increasing the dietary protein content may correct the reduced amino acid intake provoked by HS, but it may further increase BT. The effect of dietary protein level on BT of HS pigs was analyzed with nine ileal cannulated pigs (61.7  $\pm$  2.5 kg body weight). A thermometer set to register BT at 5-min intervals was implanted into the ileum. There were two treatments: low-protein (10.8%) wheat-freeamino acid diet (LP); high-protein (21.6%), wheatsoybean-meal diet (HP). The study was conducted in two 10-d periods; in each period, d1 to d6 was for diet adaptation, d7 to d9 was for data analysis, and d10 for ileal sample collection. Pigs were fed at 0600 h (morning), 1400 h (midday), and 2200 h (evening), same amount each time. Following, the separate contribution of ambient temperature and thermal effect of feeding on the postprandial BT increment was analyzed in fed and fasted pigs. Ambient temperature ranged from 30.1 to 35.4 °C and relative humidity from 50% to 84%. Both ambient temperature and BT followed similar patterns. The BT of HP pigs after the morning and

midday meals was higher (P < 0.05) but size of the BT increments did not differ between HP and LP pigs. Midday and evening postprandial BT were higher than postprandial morning BT (P < 0.05). The BT increment was larger and longer after the midday than after the morning and evening meals (P < 0.05). The capacity of pigs to dissipate postprandial body heat depends on the accumulated thermal load received before their meals, because the thermal load before the morning meal was smaller than that before the evening meal. The estimated contribution of thermal effect of feeding (0.42 to 0.87 °C) on the total postprandial BT increment (0.69 to 1.53 °C) was larger (P < 0.05) than that of ambient temperature (0.27) to 0.66 °C). In conclusion, these data indicate that the dietary protein level has a small effect on the BT of HS pigs regardless of feeding time. Also both the thermal effect of feeding and ambient temperature impact the BT of HS pigs, although the former had a stronger effect. This information may be useful to design better feeding strategies for pigs exposed to HS conditions.

**Key words:** body temperature, dietary protein level, heat stress, pigs

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#### INTRODUCTION

Heat stress (HS) in pigs results from their exposure to ambient temperature above the thermo neutral zone. The first impact of HS is the rise in body temperature (BT) followed by a

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substantial reduction in the voluntary feed intake; the latter is aimed at reducing body heat production (Kerr et al., 2003; Morera et al., 2012) that otherwise would further increase BT. The reduced feed intake decreases the consumption of nutrients, especially amino acids (AA), that in turn significantly depresses the performance of HS pigs (Kerr et al., 2003; Pearce et al., 2013). The estimated economic loss associated with the depressed feed intake and performance of HS pigs is overwhelming. Thus, it is imperative to correct the deficient intake of AA.

The HS-related reduction of AA intake might be corrected by increasing the protein content in the diet. However, the thermal effect of feeding that includes the heat produced during the ingestion-digestion-metabolism process seems to be high for protein (Westerterp, 2004). Consuming high protein diets increases the thermal effect of feeding (Mikkelsen et al., 2000; Kerr et al., 2003), which might result in higher heat production. Pigs as homeothermic animals can maintain BT relatively constant (Rezende and Bacigalupe, 2015) but their exposure to HS increases up to 2.0 °C the BT (Pearce et al., 2014; Morales et al., 2016). Hence, increasing the diet protein content for HS pigs might further increase their BT, worsening the HS effects. There is no available report regarding the effect of the dietary protein level on the BT of HS pigs.

Our hypothesis was that pigs exposed to HS conditions may diminish their capacity to dissipate the additional heat produced because of the increased dietary protein content resulting in higher BT. In the present study, the effect of increasing the dietary protein level on the BT of growing pigs exposed to natural HS conditions was analyzed. The separate thermal effect of feeding on BT was also analyzed.

#### MATERIALS AND METHODS

## Animals and Experimental Procedure

The experiment was conducted with nine crossbred pigs (Landrace  $\times$  Hampshire  $\times$  Duroc) with an initial body weight of 61.7  $\pm$  2.5 kg during the summer time, when natural ambient temperature rises up to 45 °C. A thermometer (Thermotracker BT; iButtonLink LLC, Whitewater, WI), set to register the temperature at 5 min intervals, was placed into the intestinal lumen of each pig through a cannulae surgically implanted at the end of the small intestine according to Sauer et al. (1983). All pigs were exposed to natural ambient temperature, and individually housed in metabolism pens (1.2)

m wide, 1.2 m long, and 1.0 m high) with elevated iron-mesh floor, equipped with a stainless-steel self-feeder and a nipple water drinker. The pigs had been exposed to HS conditions for 50 d before the experiment started. Ambient temperature and relative humidity inside the room was recorded with a higrothermograph (Thermotracker HIGRO; iButtonLink LLC) set to record those values every 15 min. The lights in the room were kept on all the time. All thermometers and higrothermographs were calibrated by the supplier before the start of the experiment. Ambient temperature and relative humidity data were used to calculate the heat index with the equation of Steadman (1979) modified by Rothfusz (1990). The pigs used in the present experiment were cared for in accordance with the guidelines established in the Official Mexican Regulations on Animal Care (NOM-062-Z00-1999, 2001).

The pigs were divided in two groups (four and five pigs each) randomly allotted to two dietary treatments, as follows: LP, low crude protein diet (10.8%) and HP, high crude protein diet (21.6%). The LP diet was based on wheat, and added crystalline L-Lys, L-Thr, vitamins, and minerals (Table

**Table 1.** Ingredient composition and calculated crude protein content of the experimental diets (%, as fed basis)

| Ingredient                              | LP    | HP    |
|---|-------|-------|
| Wheat                                   | 96.57 | 67.85 |
| Soybean meal, 48%                       | _     | 29.00 |
| L-Lys · HCl                             | 0.25  |       |
| L-Thr                                   | 0.03  |       |
| Calcium carbonate                       | 1.40  | 1.40  |
| Dicalcium phosphate                     | 1.00  | 1.00  |
| Iodized salt                            | 0.35  | 0.35  |
| Vitamin and mineral premix <sup>a</sup> | 0.40  | 0.40  |
| Calculated content                      |       |       |
| Crude protein                           | 10.80 | 21.60 |
| Arg                                     | 0.57  | 1.37  |
| His                                     | 0.29  | 0.54  |
| Ile                                     | 0.42  | 0.86  |
| Leu                                     | 0.82  | 1.52  |
| Lys                                     | 0.50  | 1.00  |
| Met                                     | 0.19  | 0.29  |
| Phe                                     | 0.55  | 1.03  |
| Thr                                     | 0.35  | 0.70  |
| Trp                                     | 0.14  | 0.27  |
| Val                                     | 0.51  | 0.95  |

"Supplied per kg of diet: vitamin A, 4,800 IU; vitamin  $D_3$ , 800 IU; vitamin E, 4.8 IU; vitamin  $K_3$ , 1.6 mg; riboflavin, 4 mg; D-pantothenic acid, 7.2 mg; niacin, 16 mg; vitamin  $B_{12}$ , 12.8 mg; Zn, 64 mg; Fe, 64 mg; Cu, 4 mg; Mn, 4 mg; I, 0.36 mg; Se, 0.13 mg. The premix was supplied by Nutrionix, S.A., Hermosillo, México.

**Table 2.** Calculated SID amino acid:SID Lys ratio in the experimental diet (as fed basis)

| Amino acid:Lys ratio | LP  | HP  |
|----------------------|-----|-----|
| Arg:Lys              | 138 | 115 |
| His:Lys              | 55  | 58  |
| Ile:Lys              | 86  | 84  |
| Leu:Lys              | 153 | 164 |
| Met:Lys              | 29  | 38  |
| Phe:Lys              | 103 | 110 |
| Thr:Lys              | 70  | 71  |
| Trp:Lys              | 27  | 28  |
| Val:Lys              | 95  | 103 |

1). The HP diet was based on wheat and soybean meal to meet the NRC (2012) requirements. The analyzed content (method 982.30; AOAC, 2006) and the standardized ileal digestible (SID) coefficients of AA for wheat and soybean meal (Stein et al., 2001) were used to calculate the SID contents of AA in the diets. Both diets were formulated to contain similar SID AA:SID Lys ratios (Table 2) to prevent potential variations in body heat production that might result from differences in the SID AA:SID Lys ratios. All pigs were fed 1.20 kg/d. Heat stress pigs reduce 20 to 40% the voluntary feed intake compared with thermal neutral pigs (Collin et al., 2001a; Cervantes et al., 2017); 1.2 kg/d is approximately 70% of the voluntary feed intake these pigs might have if exposed to thermal neutral conditions. The feed was provided three times a day, at 0600 h (morning), 1400 h (midday), and 2200 h (evening), same amount of feed each time. The pigs were trained to consume their daily meals within 30 min or less; water was available to all pigs during the study. The experiment was conducted in two 10-d periods; in period 1, a group of four pigs was assigned to LP diet and a group of five pigs to HP diet, in period 2, dietary treatments were inverted. In each period, d1 to d6 was for diet adaptation and d7 to d9 for BT analysis. Total ileal content was collected consecutively during 5 h after the morning and midday meal of d10 to calculate the intestinal disappearance of dry matter. All pigs were weighed at the beginning and finish of each experimental period, and the average daily gain and, gain:feed ratio were determined.

The thermometer was removed from the small intestine the day after each experimental period ended and the BT information was recovered. The average of the four BT recordings right before each feeding time (0540 to 0555 h for the morning meal, 1340 to 1355 h for the midday meal, and 2140 to 2155 h for the evening meal) was calculated and defined as basal BT. Because the digestion of a meal in the small

**Table 3.** Performance of heat stress pigs fed a low (LP) or high protein (HP) diet

|                        | Treatment   |              |      |         |
|------------------------|-------------|--------------|------|---------|
| Item                   | Low protein | High protein | SEM  | P value |
| Daily intake, g/d      | '           |              |      |         |
| Feed                   | 1,200       | 1,200        | 0    |         |
| Crude protein          | 129.6       | 259.2        | 0    | < 0.001 |
| SID Lys                | 6.0         | 12.0         | 0    | < 0.001 |
| Daily weight gain, g/d | 51          | 725          | 72   | < 0.001 |
| Gain:feed              | 0.043       | 0.604        | 0.05 | < 0.001 |

intestine appears to occur within 5 h (Wilfart et al., 2007), the magnitude of the BT change during the first 5 h postprandial was calculated by subtracting the basal BT from each individual postprandial BT recorded at each specific time point.

Additional BT measurements were made to calculate the separate contribution of ambient temperature and that of the thermal effect of feeding on the total BT increase recorded after the morning feeding. Immediately after the end of the second experimental period, all pigs received the HP diet during 5 d at 0700 and 1900 h, 600 g/pig each time. The following day, four pigs received their morning meal but the other five pigs were fasted. The difference in BT between the fed and fasted pigs at each BT recording point, from 0800 to 1600 h, was calculated.

#### Statistical Analyses

The BT and performance data were analyzed as a crossover design with repeated measures, where treatment and sampling time were considered as subject and within subject factors, respectively. The model considered dietary CP level as fixed term and pig and period as random terms; the interaction between CP level and experimental period was analyzed. The effect of CP level on the BT at each time point was compared by using the LSD method. The significance of the magnitude of BT changes at each time point as compared with the basal BT was tested by using the Dunnett's test (Bretz et al., 2011). Regression analyses between ambient temperature and BT were also performed. Probability levels of P < 0.05, and  $0.05 \le P < 0.10$  were defined as significant and tendencies, respectively.

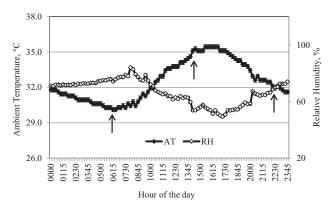
#### RESULTS

Regardless of HS and the very low protein content in the LP diet, all pigs remained healthy during the study. The performance results are presented in Table 3. By design, all pigs had the same feed

intake but pigs fed the HP diet consumed twice as much CP and SID Lys than pigs fed the LP diet (P < 0.001), thus as expected the daily weight gain was higher and the gain:feed was better in the HP pigs (P < 0.001).

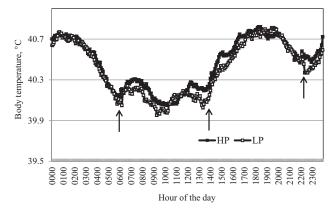
The average ambient temperature and relative humidity registered at 15-min intervals during d7 to d9 of both periods as well as during the additional trial varied in cyclical manner (Figure 1). The lowest ambient temperature (30.1 °C) was recorded at around 0630 h, and reached the highest value (35.4 °C) at around 1630 h. The relative humidity followed a pattern opposite to the ambient temperature pattern. The lowest relative humidity values (50% to 54%) were recorded between 1600 and 1800 h and the highest values (74% to 84%) were recorded between 0630 and 0930 h. The average of the calculated heat index was 109 (101 to 123).

Figure 2 shows the average BT of pigs fed either the LP or the HP diet, registered at 5-min intervals during d7 to d9 of both periods. There was no interaction between period and CP level (P > 0.10). The BT of pigs followed a pattern similar to that of ambient temperature regardless of the diet protein level, except for the BT rises observed right after each feeding time. The highest morning BT value was reached at approximately 80 min postprandial and lasted about 60 min in both groups of pigs, after which it decreased to basal values (preprandial time). Immediately after pigs received the meal at 1400 h their BT increased, reaching the highest value at about 1810 and 1815 h for pigs fed the LP and HP diet, respectively, followed by a gradual decrease until the next feeding time. After the evening meal, all pigs showed a rapid BT increase; this BT peak lasted between 90 and 105 min (from 0015 to 0155 h) followed also by a rapid decrease to values below the evening preprandial BT. There was a

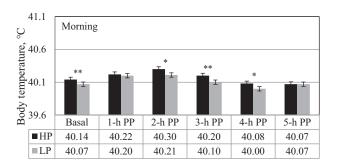


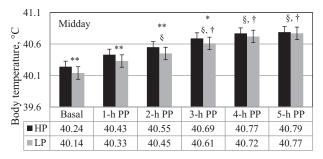
**Figure 1.** Average ambient temperature (AT) and relative humidity (RH) registered at 15-min intervals during d 7 to 9 of each period. Each data point is the mean of 27 records. The arrows show the time when pigs received their meals (0600, 1400, and 2200 h).

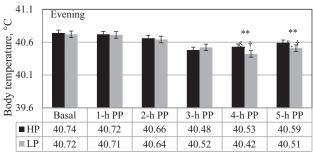
rapid BT decrease during the night from around 0130 h until 0600 h, right before the pigs received the morning meal.



**Figure 2.** Average body temperature (BT) of pigs fed a low (LP) or high protein (HP) diet, recorded at 5-min intervals during d 7 to 9 of each period. Each data point is the mean of 27 records. The arrows show the time when pigs received their meals (0600, 1400, and 2200 h).



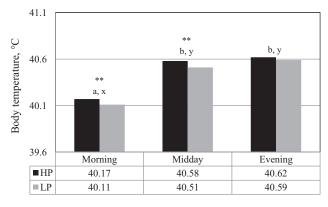




**Figure 3.** Basal and hourly means of postprandial (PP) body temperature recorded in pigs fed a high protein (HP) or low protein (LP) diet during the 5 h (1, 2, 3, 4, 5 h) after the morning midday and evening meal. At each feeding time, \* indicates tendency (P < 0.10) and \*\* indicates difference (P < 0.05) between HP and LP; \$Basal vs. x-h PP: P < 0.05 for the HP pigs; †Basal vs. x-h PP: P < 0.05 for the LP pigs.

The basal and the hourly BT means after each meal are presented in Figure 3. The morning postprandial BT did not differ from the basal BT (P > 0.10); also, the basal BT and that at 3-h postprandial was higher (P < 0.05), and at 2 and 4-h posrprandial tended to be higher (P < 0.10) in the HP pigs. As for the midday meal, compared to the basal, the BT was higher at 2-, 3-, 4-, and 5-h postprandial in the HP pigs, and at 3-, 4-, and 5-h postprandial in the LP pigs (P < 0.05). The basal and the BT mean at 1- and 2-h postprandial was higher in pigs fed the HP diet than in pigs fed the LP diet (P < 0.05); at 3-h postprandial, the BT tended to be higher in the HP pigs (P < 0.10). After the evening meal, the BT recorded in both groups of pigs at 4and 5-h postprandial was lower than the basal BT (P < 0.05). Also the BT means recorded at 4- and 5-h postprandial were higher in the HP pigs than in the LP pigs (P < 0.05). On average, the 5-h postprandial midday and evening meal BT was higher (P < 0.05) than the postprandial morning meal without regards of the dietary protein level (Figure 4); also, after the morning and midday meals, the BT was higher in the HP pigs than in the LP pigs (P < 0.05).

The results of the lineal regression analyses between ambient temperature and BT of HS pigs fed the LP and HP diet, during a 5-h period after each of the three feeding times are presented in Figure 5. After the morning meal, there was a linear decrease (P < 0.001) in the BT of pigs fed the HP ( $r^2 = 0.707$ ) or LP ( $r^2 = 0.493$ ) diet, as the ambient temperature increased, despite the postprandial BT increase. After the evening meal, the BT linearle decreased (P < 0.001) as the ambient temperature increased (P < 0.001) as the ambient temperature increase



**Figure 4.** Means of postprandial body temperatures recorded in pigs fed a high protein (HP) or low protein (LP) diet during the 5 h after the each meal. \*\*At each feeding time indicates difference between HP and LP (P < 0.05). <sup>a,b</sup>Difference among feeding times for HP pigs. <sup>x,y</sup>Difference among feeding times for LP pigs (P < 0.05). SEM = 0.018.

no relationship between ambient temperature and BT in pigs fed either the HP ( $r^2 = 0.020$ ) or the LP ( $r^2 = 0.250$ ) diet (P > 0.10).

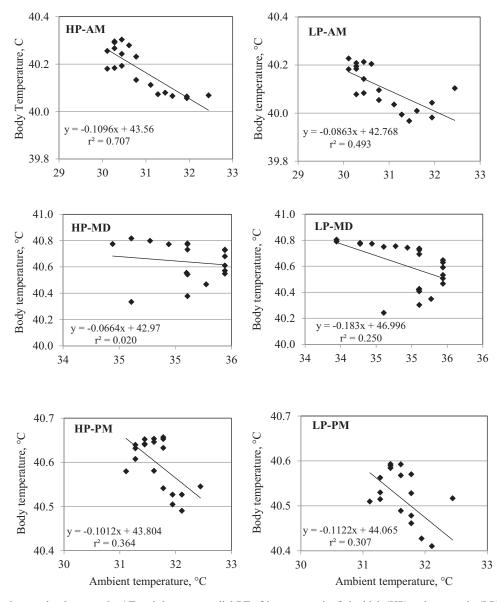
The magnitude of the BT change registered at 5-min intervals after each meal compared with its respective basal BT is presented in Figure 6. After the morning meal, the BT of pigs fed the LP diet was 0.16 to 0.18 °C higher from 0740 to 0755 h, and that of the HP pigs was 0.16 to 0.17 °C higher from 0705 to 0755 h (P < 0.05). As for the midday meal, the postprandial BT in pigs fed the LP or the HP diet was 0.15 to 0.64 °C higher as soon as 20 min postprandial and remained high for the following 4.5 h (P < 0.05). Regarding the evening meal, the BT of pigs fed the LP diet was 0.20 to 0.24 °C higher from 2435 to 2450 h, and that of the HP pigs was 0.20 to 0.26 °C higher from 2355 to 0255 h of the following day (P < 0.05).

The BT of fed and fasted pigs did not differ before feed was provided (0700 h) to the fed pigs (Figure 7A). However, in both groups of pigs the BT gradually increased after that time. The BT increment of fed pigs started to become larger than that of fasted pigs at about 3 h after meal time. At about 1530 h, when the ambient temperature reached its highest value the BT of fasted pigs rose up to 0.66 °C whereas in the fed pigs it increased up to 1.53 °C. Based on calculations, from 1100 to 1530 h, ambient temperature contributed with 0.27 to 0.66 °C of the BT increments whereas the thermal effect of feeding contributed with 0.42 to 0.87 °C. Indeed, there was a linear ( $r^2 = 0.92$ ) and highly significant (P < 0.001) relationship between ambient temperature and BT (Figure 7B).

The flow of ileal digesta (total and dry matter) collected during five consecutive hours after the morning and midday meals did not differ (P > 0.10) between the HP and LP pigs (total, 307 and 293 g; dry matter, 32.1 and 23.7 g, respectively). Hence, the intestinal dry matter disappearance between HP (92.0%) and LP (94.1%) diets and did not differ (P > 0.10).

# **DISCUSSION**

The impact of dietary protein level on the BT of HS pigs was analyzed in the present experiment. Pigs were exposed to ambient temperature and relative humidity that varied daily from 30.1 to 35.4 °C and 50% to 84%, respectively. The thermal neutral zone of growing pigs ranges from 23 to 25 °C (Quiniou et al., 2001). Pigs exposed to ambient temperature above 25 °C are heat stressed (Collin et al., 2001a); at 33 °C or above, pigs show marked signs of HS (Collin et al., 2001b; Kerr et al., 2003).

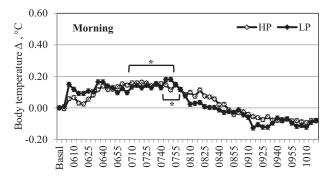


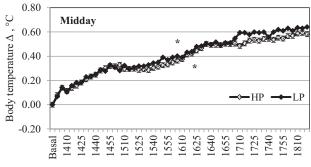
**Figure 5.** Lineal regression between the AT and the postprandial BT of heat stress pigs fed a high (HP) or low protein (LP) diet, during a 5-h period after each of the three different feeding times (morning, AM; midday, MD; and evening, PM).

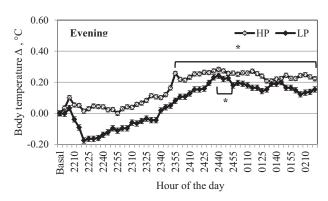
Hence, all pigs in the present report were exposed to HS most of the time.

Because HS increases BT (Pearce et al., 2014; Morales et al., 2016), pigs reduce the voluntary feed intake in an attempt to prevent further BT increments. The voluntary feed intake of 75 kg pigs was reduced 77 g/d for each 1 °C of ambient temperature increment from 19 to 29 °C (Quiniou et al., 2000), or 616 g/d when 30 kg pigs were exposed to 33°C compared with pigs at 23 °C (Collin et al., 2001b). The reduction in voluntary feed intake is expected to decrease the consumption of AA, among other nutrients, thus increasing the protein content of the diet may help to correct the lowered AA consumption. However, it was speculated that the high heat increment of diet proteins (Pesta and Samuel, 2014) might further increase the BT of HS pigs. The BT

of pigs depends on the balance between body heat load and body heat release; ambient temperature and body heat production account for most of body heat load. Heat production in healthy animals results from the basal metabolic rate, physical activity, and the thermal effect of feeding (van Milgen et al., 1997). Any heat produced above the basal metabolic rate in resting healthy animals comes from the thermal effect of feeding (Musharaf and Latshaw, 1999; Westerterp, 2004). At similar ambient temperature, production of body heat by healthy and resting pigs of similar body weight and genetics is expected to depend only on the thermal effect of feeding, which includes ingestion and digestion of feed, metabolism of absorbed nutrients, and hypothetically the protein content and AA profile of the diet.

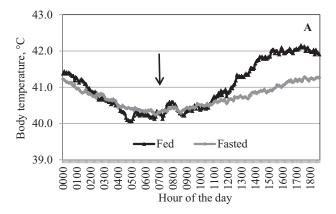


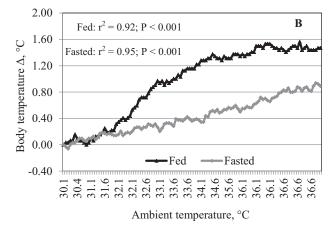




**Figure 6.** Changes ( $\Delta$ ) in BT of HS pigs fed a high (HP) or a low protein (LP) diet, during a 5-h period after the morning, midday, and evening meals, compared with the preprandial (basal) BT. The basal was the average of the four BT recordings right before each feeding time, from 0540 to 0555 h for the morning meal, from 1340 to 1355 h for the midday meal, and from 2140 to 2155 h for the evening meal (n = 9 pigs). Each data point was calculated by subtracting the basal from each BT registered every 5 min; data points enclosed within the brackets differ (P < 0.05) from the basal BT.

In the present experiment, all pigs were healthy, exposed to same ambient temperature, had similar low physical activity, consumed the same amount of the same diet, and as given in Table 2, the diets had similar SID AA:SID Lys ratios. Diet protein content was the only difference between the two groups of pigs. However, the BT of pigs fed the HP diet increased only 0.07 °C compared with that of pigs fed the LP diet. Morales et al. (2018b) recently reported no difference in the BT of thermal neutral pigs fed a LP or a HP diet. Thus, what is the implication of this small postprandial BT difference in HS pigs? In recent studies, we (Morales





**Figure 7.** Body temperature of fed and fasted pigs recorded at 5-min intervals from 0000 to 1800 h on d 5 of trial 2 (A), and the relationship between BT increments in fed and fasted pigs and ambient temperature analyzed between 0700 h (feeding time of fed pigs) and 1700 h on the same day (B). The arrow shows the feeding time of fed pigs; fast pigs received their last meal the day before at 1900 h.

et al., 2014; Morales et al., 2016; Cervantes et al., 2017) and others (i.e., Yu et al., 2010) found that HS pigs increased 1.5 to 2.8 °C the BT compared with thermal neutral pigs, which was associated with substantial reductions (20% to 40%) in the voluntary feed intake. The BT difference between HS and thermal neutral pigs is more than 20× larger than the BT difference between the LP and HP pigs (0.07 °C) of the present study. The thermal effect of feeding contributed with 0.42 °C (57%) to 0.87 °C (61%) of the total postprandial BT increment (0.69 and 1.53 °C) thus the estimated BT increment related to the thermal effect of feeding of HP pigs was only 0.04 °C higher than that of LP pigs. Moreover, the BT increment (0.60 °C) observed during the 5-h following the midday meal (highest ambient temperature) was 4× larger than that (0.15 °C) after the morning meal (lowest ambient temperature). Hence the small BT increment in HS pigs fed the HP diet may have no significant impact on the voluntary feed intake. Actually, we did not see any difference in the voluntary feed

intake of HS pigs fed low or high protein diets (Morales et al., 2018a).

The mean transit time of digesta through stomach and small intestine of pigs fed typical cereal-soybean meal diets is about 5 h (Wilfart et al., 2007), thus it is reasonable to believe that the digestion of feed occurred entirely within this period of time. Indeed, 92% to 94% of the ingested dry matter apparently disappeared from the stomach and small intestine in 5 h after the morning and midday meals. This coincides with the BT increase sustained for about 5 h after the morning meal of thermal neutral pigs reported by Morales et al. (2018b). However, in the present study the morning and evening postprandial BT rise in HS pigs lasted less than in thermal neutral pigs. It has been reported (Adair and Black, 2003) that pigs can maintain BT relatively constant within certain range of variable heat load through an efficient thermoregulatory mechanism. Moreover, pigs exposed to high ambient temperature for 8 or more days may become acclimated to hot environments (Renaudeau et al., 2010; Yu et al., 2010). In the present experiment, pigs had been exposed to HS for 50 d before the experiment started suggesting that they were acclimated to high ambient temperature. Hence, the small and short lasting BT increment recorded after the morning and evening meals may be explained as a result of the activation of the thermoregulatory mechanism of pigs acclimated to HS. This activated mechanism might partially explain the small impact of dietary protein content on the BT of HS pigs. The negative relationship between ambient temperature and BT after the morning and evening meals may indicate that HS pigs developed a more efficient machinery to dissipate body heat resulting from the thermal effect of feeding in comparison with thermal neutral pigs. In contrast, the high ambient temperature combined with the thermal effect of feeding of the midday meal might have exceeded the capacity of HS pigs to dissipate the postprandial heat because the BT increased continuously for about 6 h after the midday meal despite the fact that ambient temperature started to decline about 2 h after this meal. Interestingly, as shown in Figure 7, the contribution of the thermal effect of feeding on the total postprandial BT increment was greater than that of ambient temperature, which explains the continued BT increase observed even after ambient temperature decreased. Nonetheless, the negative balance between body heat dissipation and thermal load of these pigs was not large enough to affect their health. Histologic (Yu et al., 2010) and biochemical (heat shock proteins; Pearce et al., 2014; Morales

et al., 2016) variables may account for those adaptive changes of pigs to HS conditions.

The lack of difference in BT between the postprandial midday and evening meal suggests a body heat overload after the midday meal that prevented pigs from removing the postprandial evening meal heat; the heat dissipation capacity of HS appeared to be exceeded after the evening meal. It should be mentioned that, when the midday and evening meals were offered, the BT of both LP and HP pigs was already higher (0.11 and 0.42 °C, respectively) than at the morning meal. These results seem to indicate that the capacity to dissipate body heat related to the thermal effect of feeding depends on the thermal load received by the pigs before and after ingesting their meals; the ambient temperature-related thermal load received during the 3 h preceding the morning meal (ambient temperature < 32 °C) was lower than that during the 3 h before the midday meal (ambient temperature > 32 °C). Hence, a stronger negative impact of HS on the voluntary feed intake can be expected during the afternoon and evening hours, compared with that during the early morning hours. In general, these results may be useful when designing feeding times for pigs exposed to HS conditions. This is the first report showing the effect of dietary protein level on the BT of HS pigs.

In summary, the results of the present study indicate that, although statistically significant, the dietary protein level had only a small effect on the BT of HS pigs. The impact of AT on the BT of pigs is stronger than that of the dietary protein level, but the contribution of the thermal effect of feeding on the BT increment is larger than that of ambient temperature. Feeding pigs during the warmest time of the day further affects their body heat dissipation capacity, which in turn might affect the voluntary feed intake. These data may be useful to design better feeding strategies for HS pigs.

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