



Effect of heat stress on performance and expression of selected amino acid and glucose transporters, HSP90, leptin and ghrelin in growing pigs

Miguel Cervantes, Margarita Cota, Néstor Arce, Gilberto Castillo, Ernesto Avelar, Salvador Espinoza, Adriana Morales*

Instituto de Ciencias Agrícolas, Universidad Autónoma de Baja California, Álvaro Obregón S/N, Colonia Nueva, CP 21100 Mexicali, BC, México

ARTICLE INFO

Article history:

Received 13 August 2015

Received in revised form

5 December 2015

Accepted 17 April 2016

Available online 4 May 2016

Keywords:

Heat stress

Pig

Nutrient transporter

HSP90

ABSTRACT

Exposing animals to high ambient temperature provokes heat stress (HS) that may affect cellular function and reduced productive performance. The effect of chronic exposure (21d) of pigs to high ambient temperature on expression of amino acid ($b^{0,+}$ AT, CAT1) and glucose (SGLT1, GLUT4) transporters, ghrelin, leptin and HSP90 was evaluated. Eighteen pigs (32.6 kg body weight) were distributed into 3 groups: (1) pigs housed under natural high ambient temperature conditions, and fed *ad libitum* (HS); (2) pigs housed in an air-conditioned room at 24 °C (thermo-neutral) fed *ad libitum* (TN_{ad}); (3) pigs housed as in (2), but pair-fed with the HS pigs (TN_{pf}). Body temperature, respiratory frequency, weight gain, feed intake, and feed conversion ratio were measured. At d-21 pigs were euthanized and samples from stomach, duodenum, jejunum, liver, *longissimus* and *semitendinosus* muscles, and white adipose tissue were collected for mRNA analysis. In the HS room ambient temperature fluctuated every day (23.6–37.6 °C). Respiratory frequency and body temperature were higher in HS pigs ($P < 0.001$). Weight gain and feed intake of TN_{ad} were higher ($P < 0.001$) than TN_{pf} and HS; gain: feed ratio was not affected by ambient temperature. Expression of HSP90 was higher in duodenum and *longissimus* ($P \leq 0.038$) of HS compared to TN_{pf}. Expression of ghrelin, leptin and $b^{0,+}$ AT were not affected by ambient temperature ($P > 0.050$). CAT1 expression in liver was higher ($P = 0.050$) but in *longissimus* was lower ($P = 0.017$) in HS than in TN_{pf} pigs. Expression of SGLT1 was higher ($P = 0.045$) in duodenum of HS than in TN_{pf} but it was not different in jejunum ($P = 0.545$); GLUT4 tended to be higher in liver and *semitendinosus* of HS pigs ($P = 0.063$). In conclusion, feed intake remains low whereas respiratory frequency and body temperature remain higher; and expression of HSP90, CAT1, SGLT1 and GLUT4 increases in some tissues in pigs under chronic HS conditions.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The pig production in the world takes place mostly in areas where the ambient temperature during the summer months is above the thermo-neutral zone. In México, more than 80% of pork is produced under temporary heat stress (HS) conditions. Based on the models by St-Pierre et al. (2003), there is an estimated growth loss of 5 kg/head per year, and 0.5% deaths of pigs associated with

HS in the Mexican swine industry. Accordingly, the estimated HS-associated economic loss is close to US\$ 100 million/year.

Exposing animals to HS conditions affects their physiology, metabolism and behavior. Heart rate, peripheral blood flow and body temperature increase (Wilson and Crandall, 2011), but voluntary feed intake reduces (Huynh et al., 2005; Renaudeau et al., 2010) in HS pigs. These changes are aimed to promote loss of heat and to reduce heat produced within the body (Bernabucci et al., 2009). The biological impact appears to be stronger during the first 8–10 days of exposure to HS conditions, including increased synthesis of heat shock proteins (HSP; Pearce et al., 2013a), alterations in cell membrane function, cellular proliferation (Sonna et al., 2002) and reduced intestinal villus height in pigs (Yu et al., 2010; Pearce et al., 2014). We hypothesize that HS pigs have less absorptive cells and nutrient transporters that may affect nutrient absorption. Although the HS impact seems to decrease as the

Abbreviations: $b^{0,+}$ AT, Cationic amino acid transporter, SLC7A9; CAT1, Cationic amino acid transporter 1, SLC7A1; GLUT4, Facilitated glucose transporter, SLC2A4; HS, Heat stress; HSP90, Heat-shock protein 90-kDa; mRNA, Messenger RNA; SGLT1, Sodium/glucose co-transporter, SLC5A1; TN, Thermo-neutral; TN_{ad}, Thermo-neutral *ad libitum*; TN_{pf}, Thermo-neutral pair fed

* Corresponding author.

E-mail address: adriana_morales@uabc.edu.mx (A. Morales).

animal becomes acclimated (Renaudeau et al., 2010), it is not clear whether these alterations remain after its long term (chronic) exposure to HS.

The absorption of lysine and glucose is critical in HS pigs because lysine is first limiting amino acid and glucose is the main energy source in most feeds. Lysine is transported by systems $b^{0,+}AT$ and CAT1 (Majumder et al., 2009); $b^{0,+}AT$ is mainly expressed in epithelial cells (Torrás-Llort et al., 2001) and CAT1 is ubiquitously expressed (Hatzoglou et al., 2004). Glucose is mostly transported by systems SGLT1 and GLUT4 in epithelial and non-epithelial cells, respectively. Except for one study reported by Morales et al. (2014), regarding $b^{0,+}AT$ expression and serum lysine in pigs exposed to HS conditions, the chronic response to HS has not been reported. On the other hand, leptin and ghrelin participate in the voluntary feed intake regulation in pigs under thermo neutral conditions (Carroll and Allee, 2009). However, it is unknown whether these hormones are involved in the feed intake reduction during HS conditions, and if the molecular alterations because of HS remain in pigs apparently acclimated to high ambient temperature.

The majority of HS related studies were conducted with pigs exposed to artificial and constant high ambient temperature, and immediately after being maintained under thermo-neutral conditions (e.g., Renaudeau et al., 2010; Yu et al., 2010; Pearce et al., 2013a, 2013b, 2014). However, pigs in production farms are exposed to natural and daily variations in ambient temperature. A better understanding of the metabolic and physiological changes resulting from exposure of pigs to natural high ambient temperature seems necessary to design more efficient feeding and housing strategies. The objectives of this study were: (a) to evaluate the response of pigs to chronic exposure (21 d) to high ambient temperature on body temperature, respiratory frequency, and the expression of genes coding for the transporters of cationic amino acids ($b^{0,+}AT$ and CAT1) and glucose (SGLT1 and GLUT4), as well as ghrelin, leptin and HSP90 in several tissues of growing pigs, and (b) to determine if the performance depression of pigs exposed to high ambient temperature is also provoked by factors other than the lowered voluntary feed intake.

2. Materials and methods

2.1. Location, animals and diets

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). An experiment was conducted in the North western part of Mexico during summer time when the ambient temperature fluctuates from 25 (average low) to 42 °C (average high). Eighteen crossbred (Landrace x Hampshire x Duroc) pigs with an initial body weight of 32.6 ± 3.2 kg were randomly distributed into 3 groups of 6 pigs each, based on sex, litter and initial body weight, according to a Randomized Complete Block design. One group was housed in a room with no temperature control, exposed to natural high ambient temperature, and were fed *ad libitum* (HS). The other two groups were housed inside an air-conditioned room (thermo-neutral, TN) with the thermostat set at $24 (\pm 2)$ °C; one of these groups was fed *ad libitum* (TN_{ad}) whereas the other group was pair-fed with the HS pigs (TN_{pf}). Pigs were individually housed in raised floor metabolism pens (1.2 m wide, 1.2 m long and 1.0 m high) equipped with a stainless-steel self-feeder, a nipple water drinker, and iron mesh floor. All pigs were weighed on a weekly basis; average daily weight gain, feed intake and the feed conversion ratio were calculated with the same frequency. The average body weight of the pigs at the end of the 21-d study was 46.1 ± 4.5 kg.

All pigs were fed a diet (10.4% crude protein) based on wheat (905 g/kg) and soybean meal (65 g/kg) supplemented with 0.48% L-lysine and 0.13% L-threonine. The dietary ingredients were analyzed for amino acids content (method 982.30; AOAC, 2006). The calculated SID content of the essential amino acids in the diet (g/kg) was: arginine, 7.1; histidine, 4.3; isoleucine, 4.7; leucine, 9.1; lysine, 8.3; methionine, 2.5; phenylalanine, 7.1; threonine, 5.2; tryptophan, 1.6; valine, 5.8. The diet contained 3.0% crude fiber and 10. MJ of NE per kg. The feed intake of HS pigs was restricted to 95% of their *ad libitum* feed intake recorded the previous week, which was approximately 20% lower than the voluntary feed intake of TN_{ad} pigs. This amount of feed was adjusted every week during the study. The TN_{pf} pigs were pair-fed with their counterparts HS pigs; two equal meals were offered daily, at 0700 and 1900 h. All pigs were adapted to the metabolism pens and trained to consume their daily meals within 30 min or less, during 7 days previous to the start of the trial. Purified water was available to all pigs during the whole study.

The body temperature and respiratory frequency were measured in all pigs on the 5th and 6th day of every week at 0800, 1200, 1600 and 2000 h. The body temperature was measured with the aid of an otic thermometer, at the same time the respiratory frequency was measured by counting the number of thorax expansions resulted of inspirations during 1 min. Also, the ambient temperature and relative humidity inside each room was recorded during the whole study with the aid of a Higrothermograph (Thermotracker HIGRO; iButtonLink LLC, Whitewater, WI, USA) set to record those values every 15 min. The ambient temperature and relative humidity data were used to calculate the heat index according to the equation of Steadman (1979) modified by Rothfus (1990).

2.2. Collection of tissue samples

At the end of the 21-d trial, 2.5 h after the last meal, all pigs were euthanized by electrical stunning and exsanguination using a stunner (Best & Donovan, Cincinnati, OH, USA) with an output voltage of 620 V during 2–3 s. Before the stunning, water was sprayed over the body surface (neck and head) where the electric current was applied. Then, the pigs were immediately bled until death and the carcasses were quickly eviscerated. Mucosal samples (approximately 1 g) scratched from stomach (fundus region), duodenum (about 30 cm from pylorus) and middle jejunum were collected into 2-ml micro tubes after flushing them with physiological solutions. Samples from liver, *longissimus* and white *semi-tendinosus* muscles, and subcutaneous white adipose tissue (dorsal fat at the 10th and 11th ribs) were also collected. All samples were immediately frozen in liquid nitrogen and stored at -82 °C until analysis.

2.3. RNA extraction and reverse transcription

All tissue samples were treated to extract total RNA by the Trizol reagent (Invitrogen, Corp., Carlsbad, CA, USA), as reported by Méndez et al. (2011). Purified RNA was then eluted with 30 μ l of RNase-free water and stored at -82 °C. The concentration of total RNA was determined spectrophotometrically at 260 nm (Helios β , Thermo Electron Co., Rochester, NY, USA), and purity of RNA was assessed by using the A260/A280 ratio, which ranged from 1.8 to 2.0 (Sambrook and Russell, 2001). The integrity of total RNA was evaluated by gel electrophoresis on 1% agarose gels. All RNA samples had good quality with a 28S:18S rRNA ratio around 2.0:1 (Sambrook and Russell, 2001). Approximately 2 μ g of total RNA were treated with 1 U of DNase I (1 U μ l⁻¹; Invitrogen) and reverse transcription was performed using random hexamers as previously described by (García-Villalobos et al., 2012). The

Download English Version:

<https://daneshyari.com/en/article/2842680>

Download Persian Version:

<https://daneshyari.com/article/2842680>

[Daneshyari.com](https://daneshyari.com)