Animal welfare of barrows with different *antemortem* lairage times without food

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ABSTRACT: This study evaluated the effect of five different periods of *antemortem* lairage without food on the energy metabolism, gas exchange, mineral and blood acid-base balances of 1174 hybrid barrows, which were divided into six treatment groups according to the lairage period: 130 barrows were considered for the evaluation of the baseline levels (G_B); 214 had 0 h of lairage (R_0); 228 had 4 h of lairage (R_4); 204 had 8 h of lairage (R_8); 192 had 12 h of lairage (R_{12}); and 206 had 24 h (R_{24}). In all groups, increasing lairage periods triggered a significant reduction ($P < 0.05$) in pH, accumulation of lactic acid and percentage of hematocrit. These findings led to the conclusion that *antemortem* lairage periods longer than 4 h cause hyperglycaemia, hypercalcaemia, hyperlactataemia, hyperkalaemia, hyponatraemia, acidosis, and more severe dehydration in barrows.

Keywords: rest; hemodynamic response; stress; pig; lactate

The lairage or stabling of pigs before slaughtering allows the animals to recover the physiological conditions lost during the processes of loading, transport and unloading, while normalising such metabolic conditions as recuperation of levels of muscular glycogen and muscle tone, thus providing a certain relaxation to those animals most affected by the previous handling conditions (Fischer 1996). From the perspective of animal welfare and meet quality, transport and *antemortem* lairage seem to be the most important pre-slaughtering factors (Costa et al. 2002; Amtmann et al. 2006; Mota-Rojas et al. 2006; Gallo 2008; Smiecinska et al. 2011), given that they are as or more transcendent than genetic predisposition (Broom and Kirkden 2004). Numerous studies have been conducted to determine the ideal lairage period for the recovery of the pigs. Considering animal welfare, some authors have established minimum lairage periods of 6 to 8 h for pigs that are sensitive to stress while others consider that the ideal rest time is 2 to 4 h (Eikelenboom and Bolink 1991) because hogs slaughtered during the initial two hours of rest show aggressive behaviour, physical exhaustion and

physiological tension. Gispert et al. (2000) observed that increasing lairage time also increased the main blood indicators of stress (cortisol, lactate and creatine phosphokinase). Similarly, there are studies that assess the effect of rest on meat quality and yield. Their authors assume that certain groups of animals cannot recover from the energy loss that they have suffered, resulting in an increase of pH_{24} and the presence of Dry Firm and Dark (DFD) meat (Warriss 1998; Fortin 2002; Fabrega et al. 2007). Nonetheless, the different experiments designed to measure the degree of stress during this process have been based primarily on evaluations of the concentration of cortisol or catecholamines in plasma. The problem with this procedure is that cortisol manifests a wide variation and its half-life is very short; thus, results are often contradictory. At present, other indicators of stress include evaluating the packed cell volume, leukocyte count and the concentrations of glucose, lactate and ketone bodies, or the plasma concentration of proteins from the acute phase of animals in transit, and, recently, complete haemodynamic profiles carried out to assess the degree of animal stress and animal welfare from birth (Gonzalez-Lozano et al. 2009a,b; Orozco-Gregorio et al. 2008,2010; Mota-Rojas et al. 2011; Trujillo-Ortega et al. 2011) to death in the abattoir (Averos et al. 2007; Becerril-Herrera et al. 2009; 2010; Mota-Rojas et al. 2009; Tadich et al. 2009; Ondruska et al. 2011). In addition, there are few studies of pigs that have evaluated the degree of stress that the animals experience during their stay in the holding pens *antemortem* from the physiometabolic perspective (Skrlep et al. 2009; Smiecinska et al. 2011). Therefore, the objective of this study was to evaluate the energy metabolism, mineral and acid-base balances, and blood gas exchange of hogs during five different *antemortem* lairage periods without food in order to identify haemodynamic alterations.

MATERIAL AND METHODS

Experimental handling. The study was conducted at a Federal Inspection Type plant located in central Mexico. A total of 1174 castrated hybrid male pigs (barrows) from York × Landrace mothers and Pietrain father, all from the same farm, were assessed.

The experiment was carried out in accordance with the guidelines for the ethical use of animals (Sherwin et al. 2003). All procedures related to the use and care of the animals were in accordance with the agreement NOM-062-ZOO (1999). During herding of the pigs from the pen at the farm to the truck (shipping), and descent from the truck to the holding pens at the abattoir (unloading), the pigs were herded gently with no shouting or blows, and without using electric prods or goads.

On the day before transport, blood samples were taken randomly from 130 pigs to serve as reference values. All the pigs fasted for 8 h prior to transport, and were transported to the slaughter house with no water or food available during an average time period of 5 h. The distance from the farm to the slaughter house averaged around 300 km; the average transport speed was 70 km/h. The five groups of pigs left the farm at 5 am at an average temperature and relative humidity (RH) of 15 °C and 62%, respectively. The arrival time of the pigs in the five journeys at the abattoir was recorded between 9:45 and 10:15 am. Average ambient temperature and RH upon arrival were 19 °C and 61%, respectively

Distribution of treatments. The distribution of treatments was arranged according to the different lairage periods; the zero hour was considered as the moment at which the pigs entered the holding pens at the abattoir, as shown in Table 1. During the rest period at the abattoir, all pigs were food restricted and only had access to water *ad libitum*. Barrows used a space allowance of 0.75 m^2/100 kg during lairage time.

Energy metabolism, acid-base balance and blood gasometry. Once the pigs arrived in the holding pens at the slaughter house, their ear temperature was recorded (ThermoScan Braun, Germany), and blood samples were taken from the jugular vein using a 0.25 ml heparinised syringe. The investigators who sampled the pigs were able to collect blood at the first attempt in < 15 s. Lithium

Table 1. Distribution of treatments according to lairage period without food in the holding pens of the abattoir, showing the number of animals per treatment

Treatment	Number of castrated males and lairage period without food
G_B	130 barrows (baseline levels)
R_0	214 barrows, 0 h
R_4	228 barrows, 4 h
R_8	204 barrows, 8 h
R_{12}	192 barrows, 12 h
R_{24}	206 barrows, 24 h

heparine was used to impede any modification of the blood gas values. Obtained samples were placed in a blood gas and electrolyte parameter analyser (GEM Premier 3000, Instrumentation, Laboratory Diagnostics USA/Italy). The physio-metabolic profile included the critical blood variables: hematocrit (%), glucose (mg/dl), electrolytes,[Na^+, K^+ and Ca^{2+} (mmol/l)], lactate levels (mg/dl), and the partial pressure of carbon dioxide [pCO_2 (mmHg)] and oxygen [pO_2 (mmHg)]. Cell phones, radio, televisions or other sources of noise were not allowed in the post-unloading at the plant in order to decrease sources of stress. Animals were treated humanely throughout the study.

Statistical analysis. Descriptive statistics were obtained and tests of normality were conducted (PROC UNIVARIATE, SAS 9.0 (SAS 9.0, 2004) for all the variables under study at all lairage periods (0, 4, 8, 12 and 24 h). To determine the effect of rest time on the physiometabolic variables, including body temperature, a variance analysis for a general linear model was performed (PROC GLM), (SAS 9.0, 2004), using the physiometabolic variables and body temperature as dependant variables and lairage time as the independent variable. The statistical differences among the least square means of the different lairage times were evaluated (alpha = 0.05). In the case of variable pH, a Kruskal-Wallis analysis was conducted to compare medians (alpha = 0.05). Pearson correlation coefficients (PROC CORR) (SAS 9.0, 2004) were calculated among the following physio-metabolic variables pCO_2, pO_2, Ca^{2+}, glucose and lactate. The investigators who performed the evaluation and collected the study outcomes were not aware of the treatments and did not participate in the selection of animals or in the data analysis. The investigator who carried out the analyses was not aware of the treatments.

RESULTS

Table 2 shows the mean and standard error of the energy profile, acid-base balance and blood gases of the pigs in the five different *antemortem* lairage periods without food. The results obtained reveal differences ($P < 0.05$) in the physiometabolic variables between each lairage period and the baseline levels. With respect to the acid-base balance, it is clear that the pH value was significantly lower ($P < 0.05$) in all the groups of pigs that arrived at the slaughterhouse compared to the reference sample. However, in the group that rested for 4 h (R_4) a pH level similar to that of the baseline group (G_B) was observed. With respect to lactate concentrations (Figure 1), an increase ($P < 0.05$) was seen in all groups when compared to the baseline values, showing that lactate doubled in the groups R_0, R_8 and R_{12}, and almost tripled in the group R_{24} in which the observed values were compatible with a state of hyperlactataemia [Lactate (87.98 ± 1.63 mg/dl)]. Nonetheless, pigs in group R_4 were the only ones

Table 2. Mean and standard error of energy metabolism, acid-base balance and blood gases in five different *antemortem* lairage periods without food in barrows

Variables	Treatment					
	G_B (n = 130)	R_0 (n = 214)	R_4 (n = 228)	R_8 (n = 204)	R_{12} (n = 192)	R_{24} (n = 206)
	mean ± SE					
Temperature (°C)	38.35 ± 0.03^a	39.21 ± 0.06^b	38.83 ± 0.05^c	38.39 ± 0.01^a	37.98 ± 0.07^d	37.91 ± 0-09^d
pH*	7.45 ± 0.007^a	7.29 ± 0.01^b	7.38 ± 0.009^c	7.34 ± 0.009^d	7.29 ± 0.01^b	7.14 ± 0.01^e
pCO_2 (mmHg)	57.97 ± 0.47^a	36.83 ± 0.39^b	49.81 ± 0.66^c	54.29 ± 0.71^d	55.13 ± 0.69^d	74.70 ± 1.21^e
pO_2 (mmHg)	32.39 ± 0.50^a	25.67 ± 0.45^b	27.60 ± 0.56^c	26.64 ± 0.53b^c	27.86 ± 0.51^c	27.90 ± 0.64^c
Na^+ (mmol/l)	141.61 ± 0.18^a	148.20 ± 0.23^b	144.57 ± 0.28^c	139.31 ± 0.56^d	139.43 ± 0.60^d	136.34 ± 0.71^e
K^+ (mmol/l)	5.43 ± 0.03^a	5.26 ± 0.04^a	5.34 ± 0.03^a	9.61 ± 0.18^b	9.55 ± 0.18^b	12.12 ± 0.22^c
Glucose (mg/dl)	76.42 ± 0.58^a	101.15 ± 1.06^b	88.06 ± 1.09^c	103.47 ± 3.19^{bd}	96.72 ± 3.18^b	57.87 ± 1.76^e
Hematocrit (%)	30.96 ± 0.49^a	40.61 ± 0.29^b	34.78 ± 0.45^c	46.90 ± 0.38^d	46.79 ± 0.37^d	51.01 ± 0.35^e

*Kruskal-Wallis analysis ($P < 0.05$)

^{a,b,c,d,e}different superscripts in the same row indicate significant differences, Tukey test ($P < 0.05$)

n = number of pigs sampled; SE = standard error

Treatment: G_B= baseline group levels; R_0, R_4, R_8, R_{12} and R_{24} = groups with 0, 4, 8, 12 and 24 h of lairage without food

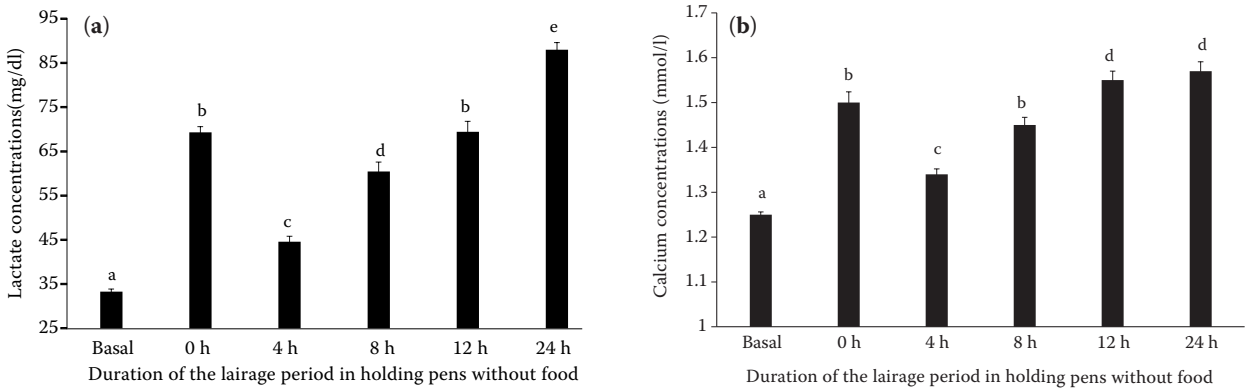

Figure 1. Effect of antemortem lairage time without food on the concentrations of lactate (**a**) and Ca^{++} (**b**) in barrows. Data are presented as means ± standard error. Different superscripts, a, b, c, d, e, between columns indicate significant differences according to the Tukey test ($P < 0.05$). GB = baseline levels in the group of barrows at rest, with access to food and water *ad libitum*. During the rest period at the abattoir, all pigs were food restricted and only had access to water *ad libitum*. Barrows used a space allowance of 0.75 m^2/100 kg during lairage time

whose blood level of lactate was similar to that of the reference group. In terms of gas exchange, a condition of hypercapnia [pCO_2 (74.70 ± 1.21 mmHg)] was detected in group R_{24}, with a significant increase ($P < 0.05$) of 11 mg/dl, when compared to all other groups. In contrast, and with respect to the energy profile, glucose rose significantly ($P < 0.05$) in all groups when compared to the baseline group (G_B), except for the group R_{24}, which showed a marked reduction ($P < 0.05$) with values compatible with a state of hypoglycaemia.

Once again, the R_4 group of pigs had glucose levels comparable to the baseline group (76.42 ± 0.58 vs. 88.06 ± 1.09 mg/dl, respectively). With respect to the mineral balance, K^+ increased significantly ($P < 0.05$) in the groups of pigs R_8, R_{12} and R_{24}. The group R_{24} presented a more marked condition of hyperpotassaemia, with an average of 7 mmol/l above the reference value. The R_{24} group (Figure 1) also presented a difference ($P < 0.05$) in blood levels of Ca^{++} in comparison to the blood levels of the baseline group (1.57 ± 0.02 vs 1.25 ± 0.006 mmol/l, respectively). In terms of the percentage of hematocrit, it should be noted that this increased ($P < 0.05$), in all groups compared to the BG pigs, except for the group R_{24}, in which the pigs suffered greater dehydration (20% above the reference value).

Pigs in group R_0, showed significant correlations ($P < 0.0001$) between the variables pCO_2 and pO_2 ($r = -0.51$), and between pCO_2 and glucose levels ($r = 0.26$). Pigs in group R_4 showed significant correlations ($P < 0.0001$) between pO_2 and blood levels of Na^+ ($r = -0.47$), glucose ($r = -0.51$), lactate ($r = -0.42$), and hematocrit ($r = -0.51$). Pigs in group R_{24} showed significant correlations ($P < 0.0001$) between pO_2 and Ca^{2+} blood levels ($r = -0.41$) and pCO_2 ($r = -0.33$), as well as between lactate and Na^+ blood levels ($r = -0.26$).

DISCUSSION

This study found that the *antemortem* lairage time without food among hybrid barrows has an effect on their metabolic acid-base balance, mineral balance, and degree of dehydration. It is important to point out that the values for the critical blood variables are similar to the reference values when the pigs are at rest for 4 h. Lairage periods longer than 4 h alter the metabolic, mineral and acid-base balances and gas exchange. In addition, lairage periods greater than 4 h cause hyperglycaemia, hypercalcaemia, hyperlactataemia, hyperkalaemia, hyponatraemia, a marked decrease in blood pH, and a more severe degree of dehydration, even though the pigs had access to water.

The alterations observed in the physiometabolic blood profile of the pigs exposed to different *antemortem* lairage periods can be explained by the fasting they undergo, which triggers a process of stress that elicits an increase in basal metabolism that, in turn, leads to an increase in heart rate, oxygen consumption and body temperature, but a reduction in pH and an accumulation of lactic acid (Hambrecht et al. 2004), together with an increase in gluconeogenesis (Mota-Rojas et al. 2005, 2009).

The state of hyperlactataemia shown in different groups of pigs reflects a period of acute stress

that leads to greater physical activity (Warriss et al. 1994) and a subsequent excessive expenditure of energy (Werner and Gallo 2008). Under these conditions, the oxygen demand of the muscles for aerobic glucose breakdown is not satisfied, thus leading anaerobic glycolysis and the formation of lactic acid (lactate ion) (Fuente et al. 2005). In addition, Werner and Gallo (2008) consider lactate to be an indicator of the stress that results from the rapid production of energy or a lactic acidosis due to the action of catecholamines. Similarly, Warriss et al. (1994) established that lactate indicates the level of activity to which the animals are being subjected in the new environment. In this way, when the animals rest for only a short time (30 min or 1 h), it is probable that they may still be suffering the consequences of the processes of loading, transport and unloading. This seems to be sufficient to provide the necessary conditions for the pigs to suffer a state of acidosis (Eikelenboom and Bolink 1991). On the other hand, the state of hypercapnia observed in the group of pigs that had a lairage time of 24 h (R_{24}) underlines the importance of the respiratory apparatus in supplying O_2 required to maintain tissue metabolism and eliminate CO_2 (Hanna et al. 1995). In animals at rest, carbon dioxide is an important stimulus for the ventilation that later increases central ventilatory conduction to block any potential increase in blood levels of CO_2. However, animals that show marked reductions in their respiratory conduction fail to respond to these stimuli and suffer hypercapnia (Dibartola 2007). Oxygen consumption and carbon dioxide production vary as a function of the metabolic index that, in turn, depends on the animal's level of activity. When the pig exercises its muscles require an increased consumption of oxygen, which leads to a lower capacity to liberate CO_2 (Cunningham 2007). In this aspect, Mota-Rojas et al. (2005) reports that a metabolic and respiratory imbalance occurs, affecting the glycolytic potential up a certain degree, depending on the stress level. Therefore, the hyperglycaemia shown by pigs in all groups is considered an indirect indicator of stress, because in response to that condition glucose levels rise due to the secretion of catecholamines and glucocorticoids (Pollard et al. 2002). The hypoglycaemia seen in the R_{24} group of pigs indicates the possible utilisation of other important sources of glucose, such as renal gluconeogenesis, due to prolonged fasting. If the fasting condition persists, glycaemia decreases gradually, as does the utilisation of glucose, producing a change towards an energy economy at the expense of a process of triglyceride lipolysis of the adipose tissue with the formation of glycerol and free fatty acids that are transformed into the primary fuel for diverse tissues. This has the effect of reducing even further glucose uptake by the brain (Jean-Marc Guettier and Phillip Gorden 1998).

Our study confirms the findings described previously by Gispert et al. (2000), who pointed out that lairage periods greater than 10 h or 12 h are inappropriate for the animals due to the long hours of fasting. This leads the pigs to suffer a state of progressive fatigue because it reduces their reserves of muscle glycogen and the possibility of recovering normal muscle tone and physiological conditions. Moreover, the condition of hyperpotassaemia present in all the groups of pigs has been associated with a response to stress due primarily to the fact that catecholamines exercise a biphasic effect on the serum concentration of potassium, which initially causes a transitory increase in the level of potassium by stimulating α-adrenergic (Peinado et al. 1993).

The condition of hypercalcaemia present in the group R_{24} may be explained by an interruption of normal homeostatic controls brought on by a chronic process of acidosis due to renal insufficiency. The result of this process is an excessive amount of calcium passing into the extracellular space due, mainly, to states of fatigue (Carrengher et al. 1997). With respect to the increase of hematocrit in all groups, but more accentuated in the group R_{24}, the explanation may lie in the splenic contraction caused by the liberation of catecholamines during sympathetic stimulation (Jain 1993). This may also play a direct role in dehydration as a result of such factors as water deprivation, lost of liquids through urination, excessive sweating and tachypnea (Tadich et al. 2000).

CONCLUSION

According to the physiometabolic responses evaluated in this study, lairage periods greater than 4 h without food cause an increase in the levels of glucose, hypercalcaemia, hyperlactataemia, hyperkalaemia, hyponatraemia, acidosis, and a more severe degree of dehydration. Prolonged periods of rest without food represent an additional source of stress in barrows which is reflected in haemodynamic alterations.

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