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Effect of environmental enrichment on weaned piglets: physiological responses

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Abstract: The aim of this research consisted in assessing the effect of various kinds of environmental enrichment (EE) on the physiological responses of weaned piglets. The mean age of the 96 piglets that participated was 27 days. The piglets were weaned and then housed under two conditions: with no disruption of the social order (SO), and with disruption of the social order (DSO). After establishing the two experimental conditions, we proceeded to evaluate four different treatments; namely, control (C), suspended ropes (SR), aromatized bottles (AB) and pet toys and balls (PTB). The protocol required drawing three blood samples: at 30 (T_{30}), 60 (T_{60}) and 90 min (T_{90}) after weaning. The DSO piglets had higher pH and haematocrit levels than those weaned in the SO condition ($P < 0.05$). Also, pCO_2, potassium (K^+) and base excess (BE) concentrations were higher in the SO animals than in those in the DSO group ($P < 0.05$). The control piglets, which did not receive any type of EE, showed higher pCO_2 levels, but lower glucose and pH ($P < 0.05$) values, while the ones enriched with SR had increased lactate levels, but lower values for pH and $HCO_{3\_}$ compared to the piglets in the other EE treatment regimens ($P < 0.05$). The SR-enriched piglets had higher lactate and haematocrit levels, but lower values for pH and bicarbonate ($HCO_{3\_}$) than the animals in the other EE groups ($P < 0.005$). The piglets subjected to sensorial EE with AB had higher plasma glucose than the ones in the other groups ($P < 0.005$). Finally, the PTB-enriched subjects showed higher Na^+ levels than controls ($P < 0.005$). The alterations that were found to be related to the factor sampling time were more pronounced at T_{30} ($P < 0.05$) than T_{60} and T_{90}. These results indicate that the conditions (SO, DSO) and EE (C, SR, AB, PTB) under which the piglets were weaned influenced the blood variables measured in the study.

Keywords: blood variables; weaning; environmental conditions

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One particularly important area of research in the field of animal welfare norms deals with the problems provoked by conditions of intensive confinement. In swineproduction today, the weaning process is classified as a stressful event for piglets because it entails separation from the dam, living with unknown animals (Parratt et al. 2006; Pluske et al. 2007), a modified diet, a novel environment due to relocation to new pens and handling procedures not previously experienced (Orihuela et al. 2018). The results of changes of this magnitude can include physiological, endocrine and behavioural imbalances (Merlot et al. 2004; Stokes et al. 2004; Weary et al. 2008; Roldan-Santiago et al. 2013; Mota-Rojas et al. 2014; Lau et al. 2015; Mota-Rojas et al. 2015; Lezama-Garcia et al. 2019).

These effects emphasize the importance of designing strategies that will help to reduce the impact of these stress factors on piglets, and so improve their welfare as they go through this challenging phase of their development (Orihuela et al. 2018). The concept of environmental enrichment (EE) refers to the process of modifying a barren captive environment such that it improves the biological functioning of nonhuman animals (Newberry 1995; Orihuela et al. 2018).The aim of EE is to augment animal welfare by diminishing the effects of stress provoked by various factors (McGlone 2001). In the case of the wellbeing of pigs, EE is considered sufficiently important to be included among the requirements stipulated by the European Union (2009) in its Directive 2008/120/EC and Commission Recommendation (EU) (2016/336 82016). The latter document states that "pigs must have permanent access to a sufficient quantity of material to enable proper investigation and manipulation activities, such as straw, hay, wood, sawdust, mushroom compost, peat or a mixture of such, which does not compromise the health of the animals". The reality, however, is that some of the materials mentioned in this Directive are impractical under conditions of industrial production (Van de Weerd et al. 2003; Orihuela et al. 2018). One example is that placing excessive amounts of straw, hay or sawdust in normal pens that have partly or fully slatted floors can block liquid-slurry waste disposal systems (Van de Weerd and Day 2009; Westin et al. 2013). Also, these measures raise certain biosafety issues and could increase labour costs because they require additional handling. Hence, it is important to assess evidence which suggests that materials like balls and ropes, among others, can be used successfully to enrich an animal's environment, as long as they are sufficiently attractive to remain of interest over extended periods (Van de Weerd and Day 2009).

Jezek et al. (2018) argue that biochemical blood analyses can provide insight into an animal's metabolic and health condition, and it is important to keep in mind that when animals perceive stress – from either internal or external stimuli – adjustments to their physiology will occur (Eze et al. 2010; Brown and Vosloo 2017). On this topic, work by Becerril-Herrera et al. (2010), Mota-Rojas et al. (2012a), Mota-Rojas et al. (2012b), Mota-Rojas et al. (2012c) and Fazzio et al. (2015) have proven the usefulness of determining the levels of several physiological variables, especially, lactate, pH, glucose and blood gases (pCO_2, pO_2) as means of assessing stress and animal wellbeing. These physiological profiles have been shown to be effective for evaluating acute stress and animal welfare in the context of the survival rates of neonate and weaned piglets (Orozco-Gregorio et al. 2010; Trujillo-Ortega et al. 2011; Mota-Rojas et al. 2011, Mota-Rojas et al. 2012b; Martinez-Rodriguez et al. 2015; Mota-Rojas et al. 2018a; Islas-Fabila et al. 2018; Perez-Pedraza et al. 2018). Stress is a commonly used indicator of low animal welfare because it reveals the effects of nervous and emotional stimuli from the environment on physiological systems, e.g. nervous, endocrine, circulatory, digestive, as it modifies their functions in ways that can be measured precisely (Mota-Rojas et al. 2018b). Especially important is the fact that internal homeostasis induces changes in autonomic nervous system activity and the hypothalamus-pituitary-adrenocortical axis (HPA) (Manteca et al. 2013; Brown and Vosloo 2017; Lezama-Garcia et al. 2019).

Various studies have observed that when applied post-weaning, EE can impact behaviour indirectly, perhaps by easing stress or providing distractions (Oostindjer et al. 2011; Orihuela et al. 2018). To be successful, EE measures need to reduce the frequency of abnormal behaviour patterns (for pigs: stereotypies, belly-nosing, ear- and tail-biting) but raise the incidence of species-specific behaviours like social interaction, foraging end exploration (Petersen et al. 1995; Kelly et al. 2000; Van de Weerd et al. 2003; Nowicki et al. 2007; Trickett et al. 2009; Van de Weerd and Day 2009; Nowicki and Klocek 2012; Telkanranta et al. 2014; Nowicki et al. 2015;

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Orihuela et al. 2018). Despite these findings, however, few studies have set out to assess the effects of EE on the physiological indices of weaned piglets, and some of those limited their evaluations to immune activity or hormone levels (Rodarte et al. 2004; Nannoni et al. 2016). In light of this, our work was conceived to precisely identify the physiological modifications that occur in weaned piglets in relation to gas exchange, acid-base balance, energy metabolism and hydric equilibrium. The experiment involved two conditions: with and without disruption of the social order and with the application of four distinct kinds of EE.

MATERIAL AND METHODS

This research was conducted in June and July 2016 in the weaning-fattening area of a commercial farm located in central Mexico. There, 720 sows were handled in a full-cycle (intensive) production system, with a mean of 330 piglets being weaned weekly.

Animals and housing conditions. The study evaluated 96 weaned piglets (12 per treatment regimen in each condition). All piglets were Yorkshire × Landrace hybrids. Their mean age at weaning was 27 ± 1.6 days, while average body weight was 7.01 ± 0.20 kg. During their time in the farrowing barns they remained with their mothers in individual crates with flat-iron flooring (no plastic cover). Mean relative humidity and temperature in the barn were 60% and 30.5 °C, respectively. The crates held a moulded plastic box (1.45 m long × 0.52 m wide × 0.57 m high) and had a solid, integrated plastic floor in the frontal area. Before beginning the experiment, all piglets were given water and standard commercial feed in pellet form with 1.25% lysine and 20% raw protein *ad libitum*. This diet was introduced at seven days of age and was continued after weaning. The piglets were weaned during the study period and then moved to crates raised 55 cm off the floor. The surface area of the crates was 6 m^2 (3 m long × 2 m wide), so the vital space available to each animal was 0.5 m^2. The crates had a nipple-type water dispenser and a stainless-steel bowl for food (91 cm high × 63.5 cm wide).

Treatments. As mentioned above, once weaned, the piglets were housed under one of two conditions (Figure 1):

1. Without disruption of the social order (SO): this group included piglets from the same litter born to multiparous dams with no mixing after weaning.
2. With disruption of the social order (DSO): these were the offspring of multiparous sows, but piglets from 30 distinct litters were mixed (i.e. non-siblings). The final distribution of the recently-weaned piglets was based on size, while the subjects were divided evenly by gender into the different treatment groups. This protocol respected the handling routines used on the farm, so the piglets were mixed by a veterinarian and the handler responsible for the area.

After defining the conditions, four treatment regimens were analysed (Figure 1):

- Control treatment (C): piglets without environmental enrichment (EE).
- Suspended ropes (SR): the piglets were provided with occupational enrichment by placing four braided nylon ropes in the crates (red with blue colour). The ropes were 5 mm in diameter and hung from the roof of the crate to a height of 25 cm from the floor so that they were within reach of the piglet's snout and could be pulled, twisted or bitten.
- Aromatised bottles (AB): here, sensorial enrichment took the form of six variably sized, high-density polyethylene (HDPE) bottles. Before being introduced into the crates, the bottles were aromatised with a synthetic strawberry essence. This scent was select-

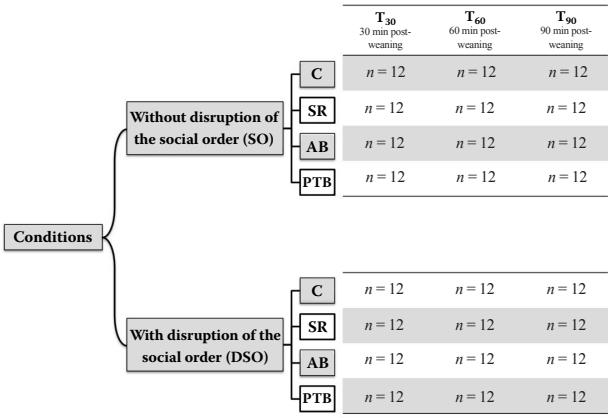

Figure 1. Experimental design for treatment of weaned piglets incorporating two conditions, four treatments and three blood sampling times

AB = aromatised bottles; C = control; *n* = number of weaned piglets sampled; PTB = pet toys and balls; SR = suspended ropes

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ed in light of previous work by Nowicki et al. (2015) with 35-day-old weaned piglets, which found that strawberry was the most popular aroma.

- Pet toys and balls (PTB): these piglets also received occupational enrichment, but the stimuli were three natural rubber pet toys and three latex balls of different sizes.

Blood sampling. Three blood samples were taken from each piglet in the different experimental groups, first at 30 min post-weaning (T_{30}), then at 60 (T_{60}), and 90 min post-weaning (T_{90}) (Figure 1). Lithium heparin was applied to the hypodermic syringes to prevent effects on blood gas values. All samples were drawn from the vena cava according to the relevant Mexican norms. For this procedure, piglets were placed in a supine position with the head and neck stretched out and the front limbs pulled backwards. Handlers located the right jugular groove and inserted the needle just cranial to the thoracic inlet. Ideally, the needle entered the top of the opposite shoulder at an angle of approximately 30° from the median and 90° from the neck line (i.e. from the thoracic inlet to the head). The experimenters who took the samples succeeded in drawing blood on the first try in < 12 seconds. It is important to minimise handling to avoid altering the blood values, so those individuals were first trained. Sample size was ~2 ml.

Immediately after being drawn, the samples were placed in the blood gas and electrolyte parameter analyser to assess the following factors: glucose (mmol/l), lactate (mmol/l), calcium (Ca^{2+}, mmol/l), sodium (Na^{+}, mmol/l), potassium (K^{+}, mmol/l), haematocrit (HTC, %), partial carbon dioxide (pCO_2) (mmHg) and oxygen pressures (pO_2) (mmHg), bicarbonate ($HCO_{3\_}$) (mmol/l), base excess (BE) (mmol/l) and pH. All measurements were made with a critical blood variable analyser (GEM Premier 3000, Instrumentation Laboratory Diagnostics, Milano, Italy/Lexington, USA), and the changes in these biomarkers identified during the experiment were used to evaluate the welfare of the piglets in the four EE treatment groups, housed as described previously.

Ethical note. Veterinary supervision was constant during the entire experimental procedure. Handling of the piglets and all experimental regimens complied with Mexico's norms (NOM-062-ZOO-1999) for experimental animals (a publication of the Department of Agriculture, Rural Development, Fisheries and Alimentation). The study protocol was approved by the Doctoral Commission of Biological Sciences and Health (Code number: DCB.072.15) of the Universidad Autónoma Metropolitana Iztapalapa-Xochimilco in Mexico City, and obeyed the guidelines for the ethical use of animals in applied ethological studies (Sherwin et al. 2003).

Statistical analysis. A total of 12 replicates were examined for each experimental group (Model × Treatment × Time), so three-factor ANOVAs were run for each physiological variable to compare the effects of the fixed factors – i.e., conditions (SO, DSO), treatments (C, SR, AB, PTB) and blood-sampling times (T_{30}, T_{60}, T_{90}) – including their interactions. This ANOVA model considered individual variation in each piglet (manifested in the replicates) using the "Mean Sum-of-square within-groups" technique (i.e., error MS) to calculate F-values. Because certain physiological variables (e.g. pH) barely satisfied the parametric model assumptions, all data were subjected to a log10 transformation (Gotelli and Ellison 2004). The next step was to verify the assumptions that the data achieved normality (D'Agostino-Pearson omnibus test) and homoscedasticity (Levene test) (Zar 2010). When a significant difference was observed, a multiple comparison Tukey test was applied ($\alpha = 0.05$) to compare the means of the levels identified for the different factors. All analyses were run with SAS 9.2 (SAS 2004).

The experimenters who conducted the assessment and gathered the study outcomes were unaware of the treatments and took no part in choosing the animals or analysing the data. Likewise, the researcher who carried out the analyses was unaware of the treatments.

RESULTS

Environmental enrichment was found to elicit alterations in the blood variables analysed ($P < 0.05$), as these assessments revealed significant differences for the effects of condition, the four treatments and the blood sampling times. Table 1 presents means + standard errors for the blood variables in the piglets under two conditions; that is, with and without +on of the social order (DSO, SO). For the effect of condition our findings show that the animals weaned in the DSO condition had higher

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Table 1. Physiological responses (mean ± s.e.) of piglets weaned under two conditions: without disruption of the social order, and with disruption of the social order

Blood variables	Conditions		Significance (*P*-value)
	SO (*n* = 144) mean ± SE	DSO (*n* = 144) mean ± SE	model
pH	7.42 ± 0.0043	7.44 ± 0.0043	0.0017
pCO_2 (mmHg)	42.15 ± 0.497	38.59 ± 0.497	0.0001
pO_2 (mmHg)	37.91 ± 1.48	38.54 ± 1.48	0.9842
Na^+ (mmol/l)	140.43 ± 0.276	139.82 ± 0.276	0.1411
K^+(mmol/l)	5.39 ± 0.0756	4.93 ± 0.0756	0.0001
Ca^{++}(mmol/l)	1.19 ± 0.0091	1.20 ± 0.0091	0.3528
Glucose (mmol/l)	5.52 ± 0.07	5.49 ± 0.07	0.6317
Lactate (mmol/l)	2.31 ± 0.12	2.62 ± 0.12	0.2983
Haematocrit (%)	31.76 ± 0.2616	32.56 ± 0.2616	0.0434
Bicarbonate $HCO_{3\_}$ (mmol/l)	26.86 ± 0.1870	26.52 ± 0.1870	0.1487
Base Excess (mmol/l)	2.34 ± 0.3671	1.23 ± 0.3671	0.0343

DSO = disruption of the social order; *n* = number of weaned piglets sampled; SO = social order

pH (7.44) ($P < 0.005$) and haematocrit (32.56%) ($P < 0.05$) levels than the ones in the SO group (7.42 and 31.76%, respectively). The concentrations of pCO_2 (42.15 mmHg) ($P < 0.005$), potassium (K^+) (5.39 mmol/l) ($P < 0.005$) and base excess (BE) (2.34 mmol/l) ($P < 0.05$) were all higher in the SO subjects than in the piglets weaned under the DSO condition (38.59 mmHg, 4.93 mmol/l, 1.23 mmol/l, respectively).

Table 2 displays the means + standard errors for the piglets' blood variables in relation to the four treatment regimens. The analysis of the effect of

Table 2. Physiological responses (mean ± s.e.) of weaned piglets subjected to four different treatments: C = control; SR = suspended ropes; AB = aromatised bottles; PTB = pet toys and balls

Blood variables	Treatments				Significance (*P*-value)	
	C (*n* = 72) mean ± SE	SR (*n* = 72) mean ± SE	AB (*n* = 72) mean ± SE	PTB (*n* = 72) mean ± SE	Treatment	Cond X Tr
pH	7.42 ± 0.006^{c}	7.42 ± 0.006^{c}	7.44 ± 0.006^{a}	7.43 ± 0.006^{b}	0.0377	0.7738
pCO_2 (mmHg)	42.02 ± 0.70^{a}	39.84 ± 0.70^{a,b}	38.80 ± 0.70^{b}	40.80 ± 0.70^{a,b}	0.0105	0.8170
pO_2 (mmHg)	35.83 ± 2.09^{a}	37.76 ± 2.09^{a}	40.38 ± 2.09^{a}	38.94 ± 2.09^{a}	0.4183	0.2386
Na^+ (mmol/l)	139.33 ± 0.39^{b}	140.05 ± 0.39^{a,b}	139.90 ± 0.39^{a,b}	141.22 ± 0.39^{a}	0.0085	0.4005
K^+ (mmol/l)	5.18 ± 0.10^{a}	5.36 ± 0.10^{a}	5.07 ± 0.10^{a}	5.01 ± 0.10^{a}	0.0856	0.1479
Ca^{++} (mmol/l)	1.20 ± 0.01^{a}	1.20 ± 0.01^{a}	1.18 ± 0.01^{a}	1.20 ± 0.01^{a}	0.7563	0.3539
Glucose (mmol/l)	5.30 ± 0.11^{b}	5.43 ± 0.11^{b}	5.87 ± 0.11^{a}	5.41 ± 0.11^{b}	0.0043	0.1536
Lactate (mmol/l)	2.40 ± 0.17^{a,b}	2.90 ± 0.17^{a}	2.22 ± 0.17^{b}	2.35 ± 0.17^{a,b}	0.0077	0.6248
Haematocrit (%)	31.75 ± 0.37^{a}	32.66 ± 0.37^{a}	31.90 ± 0.37^{a}	32.33 ± 0.37^{a}	0.3181	0.0408
Bicarbonate $HCO_{3\_}$ (mmol/l)	26.87 ± 0.26^{a}	25.86 ± 0.26^{b}	26.85 ± 0.26^{a}	27.17 ± 0.26^{a}	0.0067	0.3819
Base Excess (mmol/l)	2.33 ± 0.51^{a}	0.91 ± 0.51^{a}	2.04 ± 0.51^{a}	1.86± 0.51^{a}	0.2403	0.5994

Cond = conditions evaluated (OS, DSO); *n* = number of weaned piglets sampled; Tr = treatments evaluated (C, SR, AB, PTB)
The superscript letters $^{a, b}$ and ^{c} in the same row indicate statistically significant differences ($P < 0.05$) between treatments (Tukey test)

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treatment showed clearly that the piglets without EE (the control group, C) had higher pCO_2 concentrations (42.02 mmHg) than those that were given sensorial enrichment with AB (38.80 mmHg) ($P < 0.05$). Measurements of Na^+ found that the PTB-enriched piglets showed higher levels (141.22 mmol/l) than controls (139.33 mmol/l) ($P < 0.005$). Results for plasma glucose levels showed that the AB-enriched piglets presented higher concentrations (5.87 mmol/l) than all other animals (C: 5.30, SR: 5.43 and PTB: 5.41 mmol/l) ($P < 0.005$). Turning to lactate values, data showed that the piglets enriched with SR had higher values (2.90 mmol/l) than the AB-enriched piglets (2.22 mmol/l) ($P < 0.005$). The interaction ANOVA (condition × treatment) proved statistical significance for the percentage of haematocrit ($P < 0.05$), while for bicarbonate levels (HCO_{3-}) it is clear that the SR-enriched piglets had lower concentrations (25.86 mmol/l) than the subjects in the other treatment groups (C: 26.87, AB: 26.85 and PTB: 27.17 mmol/l) ($P < 0.01$). Generally speaking, model × treatment interactions did not reach the level of significance ($P > 0.14$), which means that the factor model did not impact the treatment factor patterns, except in the case of haematocrit ($P < 0.05$). This finding indicates that the mean values of the treatments (C, SR and AB) in the DSO model exceeded those of the SO model, while the PTB treatment revealed the inverse pattern.

Table 3 presents the means + standard errors found for the blood variables in the piglets at the three sampling times: 30 (T_{30}), 60 (T_{60}) and 90 min after weaning (T_{90}). Observations of Ca^{++} indicate lower concentrations at T_{90} (1.17 mmol/l) ($P < 0.005$) than T_{30} (1.22 mmol/l) and T_{60} (1.20 mmol/l). Findings for blood glucose levels revealed differences ($P < 0.005$) related to the effect of sampling time, since the readings taken at T_{30} (5.85 mmol/l) were higher (5.85 mmol/l) than those ascertained at T_{90} (5.24 mmol/l).

Similarly, the interaction treatment × time had only a marginal effect on blood glucose levels ($P = 0.0537$), though these differences did prove to be significant in all treatment groups, except PTB. Turning to plasma lactate levels, the differences found ($P < 0.005$) are attributable to the effect of sampling time, since the piglets had higher concentrations at T_{30} (3.04 mmol/l) than T_{60} (2.20 mmol/l) and T_{90} (2.16 mmol/l).

These analyses further indicated that the interaction treatment × time affected lactate levels

Table 3. Physiological responses (mean ± s.e.) of weaned piglets at the three blood sampling times: 30 (T_{30}), 60 (T_{60}), and 90 (T_{90}) min post-weaning

Blood variables	Sampling times (T)			Significance (*P*-value)		
	T_{30} ($n = 96$) mean ± SE	T_{60} ($n = 96$) mean ± SE	T_{90} ($n = 96$) mean ± SE	time (T)	interaction Cond[1] XT	interaction Tr[2] × T
pH	7.42 ± 0.005[a]	7.44 ± 0.005[a]	7.43 ± 0.005[a]	0.0863	0.0217	0.8735
pCO_2 (mmHg)	40.65 ± 0.60[a]	40.43 ± 0.60[a]	40.02 ± 0.60[a]	0.7567	0.1315	0.7936
pO_2 (mmHg)	39.21 ± 1.81[a]	37.43 ± 1.81[a]	38.04 ± 1.81[a]	0.6958	0.1126	0.4338
Na^+ (mmol/l)	140.39 ± 0.33[a]	140.07 ± 0.33[a]	139.91 ± 0.33[a]	0.5536	0.7725	0.1536
K^+ (mmol/l)	5.21 ± 0.09[a]	5.08 ± 09[a]	5.18 ± 09[a]	0.5040	0.2914	0.6549
Ca^{++} (mmol/l)	1.22 ± 0.01[a]	1.20 ± 0.01[a]	1.17 ± 0.011[b]	0.0028	0.2997	0.3422
Glucose (mmol/l)	5.85 ± 0.09[a]	5.41 ± 0.09[b]	5.24 ± 0.09[b]	0.0001	0.1130	0.0537
Lactate (mmol/l)	3.04 ± 0.15[a]	2.20 ± 0.15[b]	2.16 ± 0.15[b]	0.0001	0.2763	0.0146
Haematocrit (%)	33.16 ± 0.32[a]	31.78 ± 0.32[b]	31.54 ± 0.32[b]	0.0017	0.7174	0.9148
Bicarbonate HCO_{3-} (mmol/l)	26.36 ± 0.22[b]	27.22 ± 0.22[a]	26.49 ± 0.22[a, b]	0.0158	0.0132	0.6646
Base Excess (mmol/l)	1.05 ± 0.44[b]	2.62 ± 0.44[a]	1.69 ± 0.44[a, b]	0.0487	0.0374	0.5457

Cond = conditions evaluated (without (SO) and with (DSO) disruption of the social order (SO); *n* = number of weaned piglets sampled; T = times of blood sampling (T_{30}, T_{60}, T_{90}); Tr = treatments evaluated: control (C), suspended ropes (SR), aromatised bottles (AB), and pet toys and balls (PTB)

The superscript letters [a] and [b] in the same row indicate statistically significant differences ($P < 0.05$) among blood samples (Tukey test)

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($P < 0.05$), since the sequence of the values for the SR and AB treatments was $T_{30} > T_{60} > T_{90}$, while the measurements for C and PTB showed a return to average values at T_{90}.

Results for the percentage of haematocrit include differences ($P < 0.005$) also attributable to a sampling time effect. In this case, the percentage in the piglets at T_{30} was 1.62% higher than at T_{90}. Finally, for bicarbonate (HCO_{3-}) ($P < 0.05$) and base excess (BE) ($P < 0.05$) values, higher levels were found at T_{60} (HCO_{3-}: 27.22 mmol/l; BE: 2.62 mmol/l) than T_{30} (HCO_{3-}: 26.36 mmol/l; BE: 1.05 mmol/l) or T_{90} (HCO_{3-}: 26.49 mmol/l; BE: 1.69 mmol/l). In this case, however, a significant effect of model × time interaction on bicarbonate ($P < 0.05$) and base excess ($P < 0.05$) was determined, since in both cases the sequence of the mean values for the SO model was $T_{60} > T_{30} > T_{90}$, whereas for the DSO model it was $T_{60} > T_{90} > T_{30}$.

DISCUSSION

Our results show that piglets weaned and housed under two experimental conditions and four means of environmental enrichment experienced a variety of physiological imbalances. Observations on the impact of the two conditions included lower pH and higher pCO_2, K^+ and BE levels ($P < 0.05$) in the piglets in the SO condition compared to their DSO counterparts. Metabolic acidosis is caused by an increase in the production of metabolic acid, the inability to eliminate the excess acid and/or the kidneys' capacity to reabsorb excess base. Metabolic acidosis decreases blood pH (Ruffin et al. 2014) but raises the concentration of K^+ (Aronson and Giebisch 2011; DiBartola 2012b; Klein 2014). In the context of our study, this result could be due to hypoventilation (i.e., reduced alveolar ventilation) in the piglets in this experimental condition, which increased pCO_2, K^+ and BE levels, but lowered pH. A higher percentage of haematocrit was found in the piglets in the DSO experimental group compared to the SO animals ($P < 0.05$). A particularly important aspect is that this increase was larger through the first 30 min post-weaning (T_{30}) than at T_{60} and T_{90} ($P<0.05$). Swine production systems that are intensive in nature often mix unfamiliar piglets in the weaning period, a measure that obliges the animals to form new hierarchies, but often results in more fights among the piglets involved. This aggression tends to be more intense in the first 1–2 h, and usually decreases steadily to low levels around 24–72 h after the formation of the new groups (Berry and Lewis 2001; Gonyou and Keeling 2001; Fels et al. 2014). This finding clearly shows that mixing triggered a stress response in the piglets (Main et al. 2004; Mota-Rojas et al. 2014). On this topic, Becerril-Herrera et al. (2010) have stated that higher catecholamine levels cause splenic contractions that, in turn, raise percentages of haematocrit.

In terms of glucose levels in weaned piglets, Rootwelt et al. (2012) found base glucose levels of 2.6 ± 0.11 mmol/l, values that are below those reported here. In all our treatment groups, we found higher plasma glucose concentrations, though the piglets that experienced AB sensorial enrichment had the highest values of all treatment groups ($P < 0.005$). Another significant observation is that this increase was greater ($P < 0.05$) in the first 30 min after weaning (T_{30}) than at T_{60} and T_{90}. These results can be attributed to catecholamine secretion, because this regulates metabolism during processes of stress. Mota-Rojas et al. (2011) have argued that glucose values should only be taken as indirect indicators of stress. Epinephrine secretion raises glucose concentrations through glycogenolysis, but this newly formed glucose tends to be released into the bloodstream, where it increases glucose levels (Becerril-Herrera et al. 2009; Orozco-Gregorio et al. 2010; Mota-Rojas et al. 2011; Orozco-Gregorio et al. 2011; Wijtten et al. 2011; Mota-Rojas et al. 2012a; Mota-Rojas et al. 2012b; Mota-Rojas et al. 2012c). The greater glucose values seen in our work suggest that the act of weaning itself may alter metabolic indices, apart from the changes caused by the possible contact of the piglets with this kind of EE. These results could also be related to greater physical activity, perhaps because the AB are more manipulatable or, at least, can be chewed and moved more easily, a fact that increases expenditures of energy reserves. On this aspect, Van de Weerd et al. (2003) affirmed that scent is a particularly important property of objects that significantly affects their attractiveness at the first moment of contact. This concurs with Nannoni et al.'s (2016) findings, since they reported higher glucose levels in piglets weaned at 25 days that received EE in the form of wooden logs (6.84 mmol/l) and edible blocks (7.38 mmol/l) placed in their crates, than in animals that had access to hanging metal chains and wooden briquettes.

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Similar to our observations of glucose levels, a larger increase in lactate concentrations, but lower pH and bicarbonate (HCO_{3-}) values, were observed in the piglets in the group that was given occupational enrichment with SR, compared to the ones that received sensorial enrichment with AB ($P < 0.05$). In a recent study, Roldan-Santiago et al. (2015) used physiological profiles to identify the impact of transportation on weaned piglets. Their results included an increase in blood lactate above reference values in piglets aged 8, 15 and 22 days when transported with no straw bedding in the vehicle. Roldan-Santiago et al. (2015) registered basal values of piglets at weaning (22 days of age), finding a value for base lactate levels of 1.24 mmol/l. In our findings, meanwhile, plasma lactate concentrations increased at T_{30} (3.04 mmol/l), which was 0.86 mmol/l more than the readings taken at T_{60} (2.20 mmol/l) and T_{90} (2.16 mmol/l). Barth et al. (2007), in turn, observed that epinephrine-induced hyperglycaemia coincided with hyperlactataemia, while Gladden (2004) stated that blood lactate levels offer a more exact indication of sympathoadrenal activity, because adrenaline activates phosphorylase, and so increases anaerobic glycolysis and lactate production as a result of insufficient O_2 delivery. Those effects might account for the higher lactate levels seen in the piglets in our study. Lactate and pCO_2 values combine with water to produce carbonic acid (H_2CO_3) in organisms, but this reduces pH, as seen in our SR piglets, which experienced alterations in the acid-base balance. Blood pH levels usually remain in a narrow range thanks to the action of various buffer systems in the body, such as bicarbonate (HCO_{3-}) (Orozco-Gregorio et al. 2008), which react to an increase in the production/concentration of hydrogen ions by acting to capture more hydrogen ions from the environment. This leads to the formation of carbonic acid (H_2CO_3), which unfolds in water, and carbonic anhydride, which is released by respiration. In our findings, the lower level of bicarbonate (HCO_{3-}) at T_{30} ($P < 0.05$) may have buffered the lactate-elicited decrease in pH. In terms of Ca^{++} concentrations, the present work found higher levels at T_{30} than T_{60} and T_{90} ($P < 0.05$). DiBartola (2012a,b) reported that hypercalcaemia results from a dehydration process that is attributable to haemoconcentration, which signals the kidneys to increase calcium re-absorption. This finding agrees with the higher haematocrit percentage found in our study at T_{30}, since the retention of Ca^{++} and water can be caused by a larger release of the antidiuretic hormone, greater cortisol secretion and activation of the renin-angiotensin system.

The results of this experiment show that providing weaned piglets with environmental results in variations in blood variables, because the animals handled in the SO condition proved to have greater susceptibility to acid-base, hydric and gas exchange imbalances. It is possible that the SO piglets required less time to re-establish social hierarchies, which could entail more physical activity (i.e., exploring the new surroundings) than that undertaken by the DSO piglets. Regarding the alterations attributed to the impact of the four treatment regimens, the piglets without EE (controls) were more prone to acid-base and gas exchange imbalances, while the SR piglets had higher lactate and haematocrit indices, but lower pH and bicarbonate (HCO_{3-}) values than the ones in the other treatment groups. These piglets were also more susceptible to acid-base and metabolic imbalances. Alterations caused by the effect of sampling time were found to affect Ca^{2+}, glucose, lactate and haematocrit levels, since higher concentrations were seen at T_{30} than T_{60} and T_{90}. The conditions and EE under which weaning takes place are thus key aspects of animal welfare, since only 90 min sufficed to cause physiological imbalances. Finally, further studies are necessary due to the importance of exploring behavioural and other physiological indicators that could complement the results of this study, and because of the potential importance of these aspects for the field of swine production.

REFERENCES

Aronson PS, Giebisch G (2011): Effects of pH on potassium: New explanations for old observations. Journal of the American Society of Nephrology 22, 1981–1989.

Barth E, Albuszies G, Baumgart K, Matejovic M, Wachter U, Vogt J, Radermacher P, Calzia E (2007): Glucose metabolism and catecholamines. Critical Care Medicine 35, S508–S518.

Becerril-Herrera M, Alonso-Spilsbury M, Lemus-Flores C, Guerrero-Legarreta I, Olmos-Hernandez A, Ramirez-Necoechea R, Mota-Rojas D (2009): CO_2 stunning may compromise swine welfare compared with electrical stunning. Meat Science 81, 233–237.

https://doi.org/10.17221/104/2018-VETMED

Becerril-Herrera M, Alonso-Spilsbury M, Ortega MET, Guerrero-Legarreta I, Ramirez-Necoechea R, Roldan-Santiago P, Perez-Sato M, Soni-Guillermo E, Mota-Rojas D (2010): Changes in blood constituents of swine transported for 8 or 16 h to an Abattoir. Meat Science 86, 945–948.

Berry RJ, Lewis NJ (2001): The effect of duration and temperature of simulated transport on the performance of early-weaned piglets. Canadian Journal of Animal Science 81, 199–204.

Brown EJ, Vosloo A (2017): The involvement of the hypothalamo-pituitary-adrenocortical axis in stress physiology and its significance in the assessment of animal welfare in cattle. Onderstepoort Journal of Veterinary Research 84, 1–9.

Commission Recommendation (EU) 2016/336 of 8 March 2016 on the application of Council Directive 2008/120/EC laying down minimum standards for the protection of pigs as regards measures to reduce the need for tail-docking.

DiBartola SP (2012a): Disorders of sodium and water: hypernatremia and hyponatremia. In: DiBartola SP (ed.): Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice. 4th edn. St Louis. Elsevier Health Sciences. 45–79 p.

DiBartola SP (2012b): Disorders of potassium: Hyperkalemia and hypokalemia. In: DiBartola SP (ed.): Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice. 4th edn. St Louis. Elsevier Health Sciences. 92–119 p.

European Union (2009): Council Directive 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs. Official Journal of the European Union. Chapter 1, point 4, L47/5.

Eze JI, Onunkwo JI, Shoyinka SVO, Chah FK, Ngene AA, Okolinta N, Nwanta JA, Onyenwe IW (2010): Haematological profiles of pigs raised under intensive management system in south-eastern Nigeria. Nigerian Veterinary Journal 31, 115–123.

Fazio F, Casella S, Giudice E, Giannetto C, Piccione G (2015): Evaluation of secondary stress biomarkers during road transport in rabbit. Livestock Science 173, 106–110.

Fels M, Hartung J, Hoy S (2014): Social hierarchy formation in piglets mixed in different group compositions after weaning. Applied Animal Behaviour Science 152, 17–22.

Gladden LB (2004): Lactate metabolism: a new paradigm for the third millennium. Journal of Physiology 558, 5–30.

Gonyou HW, Keeling LJ (2001): Social Behavior in Farm Animals. CABI Pub, New York. 406 pp.

Gotelli NJ, Ellison AM (2004): A Primer of Ecological Statistics. Sinauer Associates Publishers, Sunderland. 510 pp.

Islas-Fabila P, Mota-Rojas D, Martinez-Burnes J, Mora-Medina P, Gonzalez-Lozano M, Roldan-Santiago P (2018): Physiological and metabolic responses in newborn piglets associated with the birth order. Animal Reproduction Science 197, 247–256.

Jezek J, Staric J, Nemec M, Plut J, Golinar OI, Klinkin M, Stukelj M (2018): The influence of age, farm, and physiological status on pig hematological profiles. Journal of Swine Health and Production 26, 72–78.

Kelly HRC, Bruce JM, English PR, Fowler VR, Edwards SA (2000): Behaviour of 3-week weaned pigs in Straw-Flow (R), deep straw and flatdeck housing systems. Applied Animal Behaviour Science 68, 269–280.

Klein BG (2014): Homeostasis ácido-básica. In: Klein BG (ed): Cunningham: Fisiología Veterinaria. 5th edn. Elsevier, Barcelona. 607 pp.

Lau YYW, Pluske JR, Fleming PA (2015): Does the environmental background (intensive v. outdoor systems) influence the behaviour of piglets at weaning? Animal 9, 1361–1372.

Lezama-Garcia K, Orihuela A, Olmos-Hernandez A, Reyes-Long S, Mota-Rojas D (2019): Invited review: Facial expressions and emotions in domestic animals. CAB Reviews 14, 1–12.

Main RG, Dritz SS, Tokach MD, Goodband RD, Nelssen JL (2004): Increasing weaning age improves pig performance in a multisite production system. Journal of Animal Science 82, 1499–1507.

Manteca X, Mainau E, Temple D (2013): Stress in farm animals and effect on performance. The Farm Animal Fact Welfare Sheet 6, 1–2.

Martinez-Rodriguez R, Roldan P, Orozco H, Trujillo-Ortega M, Mora-Medina P, Gonzalez-Lozano M, Sanchez M, Garcia-Herrera R, Hernandez-Trujillo ME, Mota-Rojas D (2015): Physiological responses and blood gas exchange following long distance transport of piglets weaned at different ages over unpaved or paved roads. Livestock Science 172, 69–78.

McGlone JJ (2001): Farm animal welfare in the context of other society issues: toward sustainable systems. Livestock Production Science 72, 75–81.

Merlot E, Meunier-Salaun MC, Prunier A (2004): Behavioural, endocrine and immune consequences of mixing in weaned piglets. Applied Animal Behaviour Science 85, 247–257.

Mota-Rojas D, Orozco-Gregorio H, Villanueva-Garcia D, Suarez-Bonilla X, Hernandez-Gonzalez R, Roldan-Santiago P, Trujillo-Ortega ME (2011): Foetal and neonatal energy metabolism in pigs and humans: a review. Veterinarni Medicina 56, 215–225.

Mota-Rojas D, Becerril-Herrera M, Roldan-Santiago P, Alonso-Spilsbury M, Flores-Peinado S, Ramirez-Necoechea R, Ramirez-Telles JA, Mora-Medina P, Perez M, Molina E, Soni E, Trujillo-Ortega ME (2012a): Effects of

https://doi.org/10.17221/104/2018-VETMED

long-distance transportation and CO_2 stunning on critical blood values in pigs. Meat Science 90, 893–898.

Mota-Rojas D, Martinez-Burnes J, Villanueva-Garcia D, Roldan-Santiago P, Trujillo-Ortega ME, Orozco-Gregorio H, Lopez-Mayagoitia A (2012b): Animal welfare in the newborn piglet: a review. Veterinarni Medicina 57, 338–349.

Mota-Rojas D, Villanueva-Garcia D, Hernandez-Gonzalez R, Roldan-Santiago P, Martinez-Rodriguez R, Mora-Medina P, Gonzalez-Meneses B, Sanchez-Hernandez M, Trujillo-Ortega M (2012c): Assessment of the vitality of the newborn: an overview. Scientific Research and Essays 7, 712–718.

Mota-Rojas D, Roldan-Santiago P, Perez-Pedraza E, Martinez-Rodriguez R, Hernandez-Trujillo E, Ortega MET (2014): Stress factors in weaned piglet. Veterinaria Mexico 45, 37–51.

Mota-Rojas D, Fierro R, Orozco-Gregorio H, Gonzalez-Lozano M, Bonilla H, Martinez-Rodriguez R, Garcia-Herrera R, Mora-Medina P, Flores-Peinado S, Sanchez M, Ramirez-Necoechea R (2015): Outcomes of gestation length in relation to farrowing performance in sows and daily weight gain and metabolic profiles in piglets. Animal Production Science 55, 93–100.

Mota-Rojas D, Lopez A, Martinez-Burnes J, Muns R, Villanueva-Garcia D, Mora-Medina P, Gonzalez-Lozano M, Olmos-Hernandez A, Ramirez-Necoechea R (2018a): Invited review: Is vitality assessment important in neonatal animals? CAB Reviews 13, 1–13. doi: 10.1079/PAVSNNR13036.

Mota-Rojas D, Orihuela A, Napolitano F, Mora-Medina P, Orozco-Gregorio H, Alonso-Spilsbury M (2018b): Invited review: Olfaction in animal behaviour and welfare. CAB Reviews 13, 1–13. doi: 10.1079/PAVSNNR201813030.

Nannoni E, Sardi L, Vitali M, Trevisi E, Ferrari A, Barone F, Bacci ML, Barbieri S, Martelli G (2016): Effects of different enrichment devices on some welfare indicators of post-weaned undocked piglets. Applied Animal Behaviour Science 184, 25–34.

Newberry RC (1995): Environmental enrichment: Increasing the biological relevance of captive environments. Applied Animal Behaviour Science 44, 229–243.

NOM-062-ZOO-1999 (1999): Norma Oficial Mexicana. Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. Secretaría de Agricultura, Ganadería. Desarrollo Rural, Pesca y Alimentación. Comité Consultivo Nacional de Normalización de Protección Zoosanitaria. Diario oficial. México. 107–165 p.

Nowicki J, Klocek C (2012): The effect of aromatized environmental enrichment in pen on social relations and behavioural profile of newly mixed weaners. Annals of Animal Science 12, 403–412.

Nowicki J, Kopyra M, Klocek C (2007): The behavioural reaction of weaners to hanging toys: wooden ball and aromatized wooden ball – way to reduce aggression after mixing. Journal of Central European Agriculture 8, 447–452.

Nowicki J, Swierkosz S, Tuz R, Schwarz T (2015): The influence of aromatized environmental enrichment objects with changeable aromas on the behaviour of weaned piglets. Veterinarski Arhiv 85, 425–435.

Orihuela A, Mota-Rojas D, Velarde A, Strappini-Asteggiano A, de la Vega LT, Borderas-Tordesillas F, Alonso-Spilsbury M (2018): Invited review: Environmental enrichment to improve behaviour in farm animals. CAB Reviews 13, 1–25.

Orozco-Gregorio H, Mota-Rojas D, Alonso-Spilsbury M, Olmos-Hernandez, A, Ramirez-Necoechea R, Velazquez-Armenta EY, Nava-Ocampo AA, Hernandez-Gonzalez R, Trujillo-Ortega ME, Villanueva-Garcia D (2008): Short-term neurophysiologic consequences of intrapartum asphyxia in piglets born by spontaneous parturition. International Journal of Neuroscience 118, 1299–1315.

Oostindjer M, Munoz JM, Van den Brand H, Kemp B, Bolhuis JE (2011): Maternal presence and environmental enrichment affect food neophobia of piglets. Biology Letters 7, 19–22.

Orozco-Gregorio H, Mota-Rojas D, Bonilla-Jaime H, Trujillo-Ortega ME, Becerril-Herrera M, Hernandez-Gonzalez R, Villanueva-Garcia D (2010): Effects of administration of caffeine on metabolic variables in neonatal pigs with peripartum asphyxia. American Journal of Veterinary Research 71, 1214–1219.

Orozco-Gregorio H, Mota-Rojas D, Villanueva D, Suarez-Bonilla X, Torres-Gonzalez L, Bolanos D, Gonzalez RH, Martinez-Rodriguez R, Trujillo-Ortega ME (2011): Caffeine therapy for apnoea of prematurity: Pharmacological treatment. African Journal of Pharmacy and Pharmacology 5, 564–571.

Parratt CA, Chapman KJ, Turner C, Jones PH, Mendl MT, Miller BG (2006): The fighting behaviour of piglets mixed before and after weaning in the presence or absence of a sow. Applied Animal Behaviour Science 101, 54–67.

Perez-Pedraza E, Mota-Rojas D, Gonzalez-Lozano M, Guerrero-Legarreta I, Martinez-Burnes J, Mora-Medina P, Ramirez-Necoechea R (2018): Infrared thermography and metabolic changes in castrated piglets due to the effects of age and the number of incisions in the testicles. American Journal of Animal and Veterinary Science 13, 104–114.

Petersen V, Simonsen HB, Lawson LG (1995): The effect of environmental stimulation on the development of behaviour in pigs. Applied Animal Behaviour Science 45, 215–224.

Pluske JR, Durmic Z, Payne HG, Mansfield J, Mullan BP, Hampson DJ, Vercoe PE (2007): Microbial diversity in

https://doi.org/10.17221/104/2018-VETMED

the large intestine of pigs born and reared in different environments. Livestock Science 108, 113–116.

Rodarte LF, Ducoing A, Galindo F, Romano MC, Valdez RA (2004): The effect of environmental manipulation on behavior, salivary cortisol, and growth of piglets weaned at 14 days of age. Journal of Applied Animal Welfare Science 7, 171–179.

Roldan-Santiago P, Martinez-Rodriguez R, Yanez-Pizana A, Trujillo-Ortega ME, Sanchez-Hernandez M, Perez-Pedraza E, Mota-Rojas D (2013): Stressor factors in the transport of weaned piglets: a review. Veterinarni Medicina 58, 241–251.

Roldan-Santiago P, Mota-Rojas D, Orozco-Gregorio H, Borderas-Tordesillas F, Martinez-Rodriguez R, Mora-Medina P, Flores-Peinado S, Sanchez-Hernandez M, Trujillo-Ortega ME (2015): Welfare of recently weaned piglets transported on unpaved roads: the effect of age and the use of straw bedding. Animal Production Science 55, 648–653.

Rootwelt V, Reksen O, Farstad W, Framstad T (2012): Blood variables and body weight gain on the first day of life in crossbred pigs and importance for survival. Journal of Animal Science 90, 1134–1141.

Ruffin VA, Salameh AI, Boron WF, Parker MD (2014): Intracellular pH regulation by acid-base transporters in mammalian neurons. Frontiers in Physiology 5, 1–11.

SAS (2004): Statistical Analysis System. Institute, Inc.: Cary, NC.

Sherwin CM, Christiansen SB, Duncan IJ, Erhard HW, Lay DC, Mench JA, O'Connor CE, Petherick JC (2003): Guidelines for the ethical use of animals in applied ethology studies. Applied Animal Behaviour Science 81, 291–305.

Stokes CR, Bailey M, Haverson K, Harris C, Jones P, Inman C, Pie S, Oswald IP, Williams BA, Akkermans ADL, Sowa E, Rothkotter HJ, Miller BG (2004): Postnatal development of intestinal immune system in piglets: implications for the process of weaning. Animal Research 53, 325–334.

Telkanranta H, Bracke MBM, Valros A (2014): Fresh wood reduces tail and ear biting and increases exploratory behaviour in finishing pigs. Applied Animal Behaviour Science 161, 51–59.

Trickett SL, Guy JH, Edwards SA (2009): The role of novelty in environmental enrichment for the weaned pig. Applied Animal Behaviour Science 116, 45–51.

Trujillo-Ortega ME, Mota-Rojas D, Juarez O, Villanueva-Garcia D, Roldan-Santiago P, Becerril-Herrera M, Hernandez-Gonzalez R, Mora-Medina P, Alonso-Spilsbury M, Rosales AM, Martinez-Rodriguez R, Ramirez- Necoechea R (2011): Porcine neonates failing vitality score: physiometabolic profile and latency to the first teat contact. Czech Journal of Animal Science 56, 499–508.

Van de Weerd HA, Day JEL (2009): A review of environmental enrichment for pigs housed in intensive housing systems. Applied Animal Behaviour Science 116, 1–20.

Van de Weerd HA, Docking CM, Day JEL, Avery PJ, Edwards SA (2003): A systematic approach towards developing environmental enrichment for pigs. Applied Animal Behaviour Science 84, 101–118.

Weary DM, Jasper J, Hotzel MJ (2008): Understanding weaning distress. Applied Animal Behaviour Science 110, 24–41.

Westin R, Holmgren N, Mattsson B, Algers B (2013): Throughput capacity of large quantities of chopped straw in partly slatted farrowing pens for loose housed sows. Acta Agriculturae Scandinavica Section A. Animal Science 63, 18–27.

Wijtten PJA, van der Meulen J, Verstegen MWA (2011): Intestinal barrier function and absorption in pigs after weaning: a review. British Journal of Nutrition 105, 967–981.

Zar JH (2010): Biostatistical Analysis. Prentice Hall, New Jersey. 944 pp.

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