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Brief communication

Evaluation of blood parameters as an early assessment of health status in nursery pigs

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Summary

Nursery pigs were categorized as healthy or unthrifty, and significant differences in certain blood gases and some ion concentrations were observed between health groups. However, differences between healthy and unthrifty pigs were not apparent upon necropsy. Assessment of hematological indicators may be useful in monitoring health of nursery pigs.

Keywords: swine, catecholamines, euthanasia, necropsy, unthrifty

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Resumen - Evaluación de parámetros sanguíneos como una valoración temprana del estado de salud de cerdos en el destete

Los cerdos en el destete fueron categorizados como saludables o desechos, y se observaron diferencias significativas en ciertos gases sanguíneos y algunas concentraciones de iones entre los grupos de salud. Sin embargo, las diferencias entre cerdos saludables y desechos no fueron aparentes en la necropsia. La valoración de indicadores hematológicos puede ser útil en el monitoreo de salud de cerdos en el destete.

Résumé - Évaluation des paramètres sanguins à titre d'indicateurs hâtifs du statut de santé de porcs en pouponnière

Des porcs en pouponnière ont été catégorisés comme étant en santé ou avec un retard de croissance, et des différences significatives dans certains gaz sanguins et quelques concentrations ioniques ont été observées entre les deux groupes. Toutefois, les différences entre les porcs en santé et ceux avec retard de croissance n'étaient pas apparentes lors de la nécropsie. L'évaluation d'indicateurs hématologiques pourrait être utile pour suivre la santé des porcs en pouponnière.

Unthrifty nursery pigs, which may account for approximately 1% of a nursery group,[1] may be weak, malnourished, or dull[2] and may be more susceptible to pathogens such as porcine reproductive and respiratory syndrome virus or *Streptococcus suis.* Additionally, lightweight or malnourished nursery pigs may also suffer from periweaning failure to thrive syndrome (PFTS) and therefore may show no signs of respiratory, systemic, or enteric diseases, but may have lower feed intake and become increasingly debilitated after weaning.[3] Some nursery pigs may suffer from other wasting diseases that cause loss of appetite, fever, dyspnea, and weight loss. Blood sampling for evaluation of hematological indicators may be highly valuable in the diagnosis, treatment, and prognosis of many diseases.[4] Additionally, monitoring herd health via hematological indicators may reveal adverse conditions, even though the animals may not be displaying clinical signs of disease.[5] Therefore, routine diagnostic sampling and evaluation of hematological indicators may aid in early identification of disease or disease-susceptible nursery pigs, which may prevent death loss from infection.

A deviation from normal hematological values can give an indication of how the environment is affecting the nursery pig's physiology[5] and can aid producers in better herd and health management. Therefore, the objective of this study, which was part of a larger study evaluating euthanasia methods in nursery pigs, was to determine if hematological indicators may be used to identify disease-susceptible (ie, unthrifty) nursery pigs.

Materials and methods

All animal use, handling, and sampling techniques described herein were approved by the Kansas State University Animal Care and Use Committee.

Fifty-eight nursery pigs (22 barrows and 36 gilts; Danbred × PIC 327; PIC USA, Hendersonville, Tennessee) with an average weight of 5.6 ± 1.3 kg were utilized in this experiment. Pigs were weaned and were approximately 21 to 28 days old during the study period. Weaned pigs were housed in environmentally controlled nursery barns in 2.74-m × 3.05-m pens, with approximately 27 to 29 pigs per pen. Pigs were selected arbitrarily from three nursery rooms and 14 pens. Pigs had ad libitum access to a single cup waterer and one double-sided feeder provided in each pen. The diet was based on corn, grain sorghum, and soybean meal. On the first day that blood samples were collected (Day 1), a veterinarian assigned the pigs to two health categories: healthy or unthrifty. Unthrifty pigs were free from abscesses or injury and were characterized via visible indicators of sickness, including, but not limited to, coughing, apparent weakness, distended abdomen, apparent dehydration or malnourishment, lethargy, and emaciation. Healthy pigs did not exhibit any of these signs. Feed was not withheld prior to blood sampling pigs.

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Blood sampling and assays

Pigs in this study ranged from approximately 21 to 28 days old on Day 1, but precise ages were not recorded, as date of birth for some pigs was unknown. Each pig was selected arbitrarily by the veterinarian for blood sampling on one of three consecutive days, designated as Day 1 (n = 20), Day 2 (n = 22), or Day 3 (n = 16), and pigs were euthanized approximately 24 to 32 hours after samples were collected. Approximately 15 mL of blood was collected into two tubes from each pig via jugular venipuncture: a 9-mL K_3EDTA tube (Fisher Scientific, Pittsburgh, Pennsylvania) for analysis of stress hormones and a 6-mL lithium heparin tube (Fisher Scientific) for analysis of blood gases and ion concentrations. After blood sampling, pigs were weighed, ear tagged, and marked with a livestock grease marker.

Prior to centrifugation, a subsample of the blood collected into the lithium heparin tube was used for analysis of lactate concentration (Lactate Scout; EKF Diagnostic GmbH, Magdeburg, Germany), and glucose, ionized calcium (iCa), potassium, sodium, hemoglobin, hematocrit, pH, and partial pressures of oxygen (P_{O2}) and carbon dioxide (P_{CO2}) (iSTAT with CG8+ cartridge; Abaxis, Union City, California).

After lactate and iSTAT analyses were completed, blood samples were centrifuged on-site for 15 minutes at 1000*g* at room temperature, and in each case the resulting plasma was removed, transferred to a polypropylene storage tube, and stored on ice during transport to the laboratory. Plasma samples were stored at -20°C until analysis of cortisol and catecholamines.

Plasma cortisol was analyzed using a Coat-A-Count Kit (Diagnostic Products Corporation, Los Angeles, California) and a Packard Cobra Gamma Counter (PerkinElmer, Waltham, Massachusetts). Recovery for the cortisol assay was 100.14% and parallelism was 90.45%.

Plasma epinephrine and norepinephrine were isolated using activated alumina and 0.1M $HClO_4$ and quantified in duplicate using high-performance liquid chromatography as described by Holladay and Edens.[6] A plasma sample of 0.5 mL was combined with 250 ng of the internal standard (3,4-dihydroxybenzylamine hydrobromide [DHBA]). Catecholamine:DHBA peak height ratios for samples and standards were determined and sample catecholamine concentrations were calculated using the regression equation generated from each catecholamine standard. Recovery of the internal standard ranged from 83% to 87%, and duplicate samples were averaged when coefficients of variation (CVs) were ≤ 5%. Duplicate samples with CV > 5% were re-analyzed until variation was within the acceptable limits.

Euthanasia and necropsy

Pigs were euthanized either by slow ascent hypobaric hypoxia (approximately 36.9 m per second) or carbon dioxide gas (CO_2; induction of approximately 20% of the chamber volume [1.0 m^3] per minute). This trial was part of a larger trial comparing euthanasia methods, and therefore the euthanasia treatment was assigned alternately to every other pig. Approximately 3 minutes after cessation of cardiac and brain electrical activity (as monitored via electrocardiograph and electroencephalograph, respectively), necropsies were performed by a certified veterinary pathologist (not blinded to euthanasia method) to evaluate the effect of the two euthanasia methods on the presence of pulmonary lesions. Only pulmonary lesions were recorded for this trial. After necropsy, pigs were classified into three categories: pigs with pre-existing lesions (gross evidence of disease not related to euthanasia method), pigs with significant pulmonary lesions (related to the euthanasia process, localized in the lungs, excluding cyanosis), and pigs with no significant lesions. For the purposes of this paper, we will be discussing pre-existing lesions and their relationship to health status.

Statistical analysis

Statistical analysis was performed using a general linear mixed model and chi-square analysis. Data were analyzed using PROC MIXED in a completely randomized design in SAS 8.2 (SAS Institute Inc, Cary, North Carolina), with health status serving as the fixed effect. Day was initially used as a blocking parameter in the statistical analysis of blood parameters. As no block interactions were observed, it was removed from the analysis. The Kendwardroger approximation was used to calculate denominator degrees of freedom. Incidences of pulmonary lesions were analyzed using the PROC FREQ CHISQ function. Pig was the random effect in all analyses. Pigs were euthanized in the chamber two at a time. Each use of the euthanasia chamber, signified as a "run," was the experimental unit. A value of $P < .05$ was considered statistically significant in all analyses.

Results

Values for blood parameters are reported in Table 1. Healthy nursery pigs had higher concentrations of glucose and sodium than unthrifty pigs. Hemoglobin concentrations and hematocrits were higher in unthrifty pigs than in healthy pigs. No significant differences between healthy and unthrifty nursery pigs were detected in epinephrine, norepinephrine, cortisol, potassium, lactate, pH, or P_{O2}. There was a trend for healthy pigs to have higher concentrations of ionized calcium and greater P_{CO2} than unthrifty pigs ($P < .10$).

Grossly observable pulmonary lesions that could be attributed to health status and not euthanasia method were observed in one healthy and two unthrifty pigs. These lesions were abnormal for any pig of any stage of life or health status, and it was determined by the pathologist that the lesions were not caused by euthanasia method. No significant difference in number of pulmonary lesions was found between healthy and unthrifty pigs ($P = .88$).

Discussion

The blood parameters measured in the present experiment included markers for stress, energy or nutritional status, and blood composition. Alterations in these parameters may indicate a disruption in homeostasis, and therefore closely monitoring their levels may aid in early detection of diseases or conditions that may be subclinical or simply lead to unthriftiness.

Results from this trial seem to indicate dehydration or malnourishment in the unthrifty nursery pigs, yet the parameters assessed do not directly provide information as to why the unthrifty pigs may not have been eating or drinking. Anderson et al[7] found that normal hematocrit values in 5-day-old weaned miniature pigs ranged from 29.8% to 32.8%. In the present study, average hematocrit was significantly higher in the unthrifty pigs than in the healthy pigs. Abnormally elevated hematocrit can influence cardiac function,[8] and may be evidence of dehydration or anorexia. For example, Xin et al[9] fasted pigs 22 to 26 days of age for 72 hours and found that hematocrit values started to increase at the onset of the fasting period and continued to increase throughout the fasting period (33.5% to 40.1%). By comparison, the present results, in which pigs were

Table 1: Blood values in unthrifty (n = 32) and healthy (n = 26) nursery pigs*

Parameter	Unthrifty	Healthy	*P*†
Lactate (mmol/L)	3.5 ± 0.28	3.8 ± 0.32	.47
Glucose (mg/dL)	78.0 ± 2.82	95.0 ± 3.12	< .001
Hemoglobin (g/dL)	11.7 ± 0.29	9.9 ± 0.31	< .001
Hematocrit (% PCV)	35.0 ± 0.85	29.0 ± 0.92	< .001
iCa (mmol/L)	1.3 ± 0.02	1.4 ± 0.02	.05
Sodium (mmol/L)	134.0 ± 0.66	137.0 ± 0.73	< .01
Potassium (mmol/L)	5.6 ± 0.14	5.4 ± 0.15	.44
P_{CO2} (mm Hg)	41.2 ± 1.56	45.8 ± 1.73	.05
P_{O2} (mm Hg)	49.0 ± 3.90	41.0 ± 4.33	.17
pH	7.4 ± 0.01	7.4 ± 0.02	.45
Cortisol (ng/mL)	22.3 ± 8.85	16.0 ± 9.08	.34
Epinephrine (ng/mL)	0.1 ± 0.02	0.1 ± 0.02	.86
Norepinephrine (ng/mL)	0.2 ± 0.01	0.2 ± 0.01	.43

* Pigs were weaned at approximately 21 days of age (5.6 ± 1.3 kg) and categorized into study groups by a veterinarian. Blood samples were collected from arbitrarily selected pigs on 3 consecutive days (Day 1, n = 20; Day 2, n = 22; day 3, n = 16). Pigs ranged in age from 21 to 28 days of age on each sample day; precise ages were not recorded as date of birth for some pigs was unknown. Values represented are the mean ± the standard deviation.

† Data were analyzed using a general linear mixed model in a completely randomized design. Differences are considered statistically significant at $P < .05$.

approximately 28 days old yet had not been fasted, may indicate that pigs categorized as unthrifty were fairly early in the "fasted" stage, because their average hematocrit of 35% is at the lower end of the range found by Xin et al.[9]

Biologically adequate levels of hemoglobin in 21- to 28-day-old nursery pigs are reported to range from 9.2 to 10.5 g per dL.[10] In this study, healthy nursery pigs had mean hemoglobin values within the biologically normal range, and the greater hemoglobin values of the unthrifty pigs may be attributed to dehydration or malnourishment.

Our results show that unthrifty nursery pigs exhibited lower concentrations of glucose than healthy pigs, which may be an additional indicator of depressed feed intake, dehydration, or both. Normal blood glucose concentrations for nursery pigs range from 65 to 95 mg per dL.[11] Unthrifty nursery pigs in this study exhibited glucose concentrations within the reference range but lower than that of their healthy contemporaries. The present results parallel those of Gentz et al,[12] who fasted piglets for up to 120 hours and found that blood glucose concentrations in newborn, 1-, 3-, 9-, and 16-day-old piglets decreased over the treatment period. Although Gentz et al measured glucose using a different method than the one used in the present study, the approximate values reported by these authors do show a decrease over the treatment period, which affirms the results from the present study, in which unthrifty pigs that may have been malnourished had significantly lower glucose concentrations than those of their healthy contemporaries.

Biologically normal serum sodium concentrations in 3-week-old piglets are 144.9 ± 2 mmol per L.[13] In this study, both healthy and unthrifty nursery pigs showed lower than normal sodium concentrations. Accensi et al[14] fed rations containing increasing levels (280, 560, and 840 μg per kg) of deoxynivalenol (DON), a mycotoxin found in cereal grains such as wheat, barley, and corn, to three different groups of weanling pigs (approximately 42 day of age) and found that sodium values for the three groups fed DON did not differ significantly from those of pigs on the control diet (142.9 mmol per L). Results of Accensi et al[14] indicate that although feed intake decreased, sodium levels did not necessarily decrease. Other hematological factors in the present study indicate that pigs categorized as unthrifty were likely dehydrated, malnourished, or both. However, taken together with the results of Accensi et al,[14] it is possible that some other factor may be responsible for the lower sodium levels seen here.

Normal blood serum concentrations of ionized or free calcium (iCa) for 14- to 35-day-old nursery pigs fall between 10.9 mg per dL (2.7 mmol per L) and approximately 11.5 mg per dL (2.9 mmol per L).[13] Our results showed a trend for healthy pigs to have higher concentrations of ionized calcium than unthrifty pigs; however, in this study, iCa concentrations of both healthy and unthrifty nursery pigs were lower than the reference range. Calcium concentrations in our study were lower than those reported by Tuchscherer et al,[15] who found that piglets that died within 10 days of birth had higher calcium values (3.02 mmol per L; $P = .04$) than piglets that lived ≥ 10 days post birth (2.95 mmol per L).

Normal partial pressures of carbon dioxide (P_{CO2}) in pigs are 40 ± 3 mm Hg.[11] While present results showed a trend for healthy pigs to have greater P_{CO2} than unthrifty pigs, P_{CO2} in the unthrifty pigs was within the reference range. This finding is surprising, as P_{CO2} may be an indicator of stress and was positively correlated with epinephrine levels in newborn pigs.[16] The unthrifty nursery pigs would be expected to be at least slightly stressed. However, micropigs that exhibited more avoidance behaviors after exposure to a stressful stimulus had a lower P_{CO2} ($P < .05$) when the stimulus was present than when it was not present (values not reported),[17,18] and thus P_{CO2} may vary too much over the short term to serve as a reliable indicator of stress. It is possible in this study that our assay values for P_{CO2} were slightly higher than those of Bollen et al[11] because of the difference in the assays used. Thus, the P_{CO2} values reported for the unthrifty pigs might have represented levels slightly below the normal values reported in studies such as that of Bollen et al.[11] It is likely that P_{CO2} could be altered in unthrifty nursery pigs due to stress or anxiety caused by any number of factors, including disease, anorexia, or aphagia.

Neither group of nursery pigs exhibited significantly increased epinephrine or norepinephrine levels. Significant results for other blood parameters, such as sodium, glucose, and hematocrit, may have indicated that some pigs were experiencing weakness, hyperpnea, dehydration, or loss of appetite. However, the effects of the stimuli that caused these signs may not have been intense or specific enough to elicit the fight-or-flight response that would cause an increase in stress hormones.

Pulmonary lesions may have been caused by injuries sustained from a rough interaction with another pig or from a previous illness and may have been the primary reason the unthrifty pigs appeared to be emaciated.

On the basis of the results of this study, it is possible that unthrifty nursery pigs may be identified by blood chemistry analysis at a time when they are showing no outward signs of disease. Early detection of unthriftiness may therefore allow for intervention strategies or early culling by producers. In addition, by assessing blood parameters such as blood glucose, serum sodium, hemoglobin, and hematocrit, it may be possible for researchers to identify and therefore study nursery pigs before they would normally be diagnosed with signs of PFTS (or other wasting diseases), providing the opportunity to assess early causative factors in this economically important but poorly understood syndrome.

Implications

- Assessment of blood glucose and sodium, hematocrit, and hemoglobin may be useful in early detection or study of nursery-pig wasting diseases.
- Blood parameters suggest that unthrifty nursery pigs may be experiencing anorexia and weakness.

Conflict of interest

None reported.

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