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Comparison of pig restraint, sampling methods, and analysis on blood lactate concentration

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Comparison of Pig Restraint, Sampling Methods, and Analysis on Blood Lactate Concentration

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Summary

The objective of the study was to examine the effects of restraint and blood sampling method on blood lactate concentration (LAC) in pigs. Restraint methods used were snaring or restraint with sorting boards. Blood was sampled from 120 pigs at approximately 165 d of age (278.0 ± 6.4 lb) over 2 consecutive days. Each day, 30 pigs were sampled per method. All pigs were housed in one barn, and pigs in adjacent pens were not sampled simultaneously. Snaring consisted of a trained handler snaring each pig while blood was collected via jugular venipuncture (approximately 7 mL). Restraint with sorting boards consisted of a trained handler restraining each pig with two sorting boards and the side of the pen to form a three-sided barrier to reduce pig movement. The distal ear vein was pricked with a 20-gauge needle to obtain several drops of blood for LAC analysis. Lactate concentration was measured using a handheld lactate analyzer. The duration of restraint and a behavior score (1 to 4; 1 = no vocalization or movement and 4 = constant movement, vocalization, and struggle) for each pig were recorded during sampling. Blood lactate was compared between the 2 sampling methods and duration of restraint was used as a covariate in the analysis.

Results indicated that snared pigs had greater ($P = 0.04$) LAC than pigs restrained using the sorting board method, 2.4 ± 0.1 and 2.1 ± 0.1 mM, respectively. Both measurements of LAC were considerably lower than the baseline LAC reported in published literature. A positive correlation ($r = 0.42$, $P = 0.001$) was observed between duration and LAC for pigs that were restrained by snaring; the longer the restraint duration, the greater the LAC. Positive correlations were observed between duration and behavior score ($r = 0.41$, $P = 0.001$), duration and LAC ($r = 0.64$, $P = 0.001$) and behavior score and LAC ($r = 0.26$, $P = 0.05$) in pigs restrained with sorting boards. In the boarded group, longer durations and higher behavior scores were related to increased LAC. In addition to analyzing behavior, duration of restraint, and LAC, different methods of blood analysis were measured to determine whether the analysis method affected LAC. Samples for this trial were collected from exsanguination blood from a separate set of 56 market-weight pigs to the same specifications as restraint blood samples. Both serum and plasma were analyzed using 3 methods — YSI analyzer, handheld lactate analyzer, and ELISA plate reader — to compare the differences in LAC. Results showed significant variation in values obtained from the three different methods of analysis ($P = 0.001$). Additionally, values obtained from serum differed significantly from values obtained from plasma ($P < 0.001$). When comparing LAC values across studies, attention should be given to the medium of measurement and the method of analysis to make reliable comparisons.

Key words: blood, laboratory, lactate, nursery pig, restraint, stress

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Introduction

Understanding animal well-being is a vital component in the production equation. An animal that is not being cared for properly will not be efficient. Well-being can be affected by stress, for which there are several physiological indicators such as epinephrine, cortisol, and lactate. Blood lactate concentration (LAC) has been used as a determinant of stress in pigs (Benjamin et al., 2001²; Hambrecht et al., 2004³; Edwards, 2010⁴) because it delivers a quick value and does not require a large blood sample. Through experimentation and measurement of such indicators, animal scientists have been able to determine what practices and situations are stressful to an animal (Hamilton et al. 2004⁵; Hambrecht et al. 2005⁶; Grandin, 2010⁷).

Experimental procedures have drawbacks; for instance, collecting samples from animals is often stressful in itself, thereby affecting the measurement obtained from the sample. Furthermore, opinions vary about what method of sampling is the least stressful to the animal. The objective of this study was to compare two different methods of restraint, snout snaring and sorting boards, while evaluating behavior and measuring LAC.

A wide variety of methods are used to analyze LAC, which can increase the complexity of comparing studies that utilize differing methods of analysis. LAC can be measured in both serum and plasma, but it is not known which medium provides a more accurate measurement. Moreover, researchers do not use the same method of analysis for every experiment, which renders comparisons between research studies difficult. An additional objective of this study was to analyze serum and plasma with three different methods and compare the results to evaluate which method provides a more precise and accurate value of LAC.

Procedures

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

One hundred-twenty cross-bred pigs (58 barrows and 62 gilts) were used during this study (TR 4 × 1050, PIC, USA, Hendersonville, TN) with an average weight of 278.0 ± 6.4 lb. Pigs were housed and observed in the finishing facility at the K-State Swine Teaching Research Unit. The pigs were kept in two different sizes of pens with slatted

² Benjamin, M. E., H. W. Gonyou, D. J. Ivers, L. F. Richardson, D. J. Jones, J. R. Wagner, R. Seneriz, and D. B. Anderson. 2001. Effect of animal handling method on the incidence of stress responses in market swine in a model system. *J. Anim. Sci.* 79(1):279.

³ Hambrecht E., J. J. Eissen, R. I. J. Nooijen, B. J. Ducro, C. H. M. Smits, L. A. den Hartog, and M. W. A. Verstegen. 2004. Preslaughter stress and muscle energy largely determine pork quality at two commercial processing plants. *J. Anim. Sci.* 82:1401–1409.

⁴ Edwards, L. N, T. E. Engle, J. A. Correa, M. A. Paradis, T. Grandin, and D. B. Anderson. 2010. The relationship between exsanguinations blood lactate concentration and carcass quality in slaughter pigs. *Meat Sci.* 85:435–440).

⁵ Hamilton, D. N. 2004. Effects of handling intensity and live weight on blood acid-base status in finishing pigs. *J. Anim. Sci.* 82:8: 2405.

⁶ Hambrecht E., J.J. Eissen, D.J. Newman, C. H. M. Smits, L. A. den Hartog, and M. W. A. Verstegen. 2005. Negative effects of stress immediately before slaughter on pork quality are aggravated by suboptimal transport and lairage conditions. *J. Anim. Sci.* 83:440–448.

⁷ Grandin, T. 2010. Electric prodding or jamming of pigs during pre-slaughter handling increases stress and raises lactate levels. Abstract.

floors; both sizes of pens allotted 8 ft²/pig. The larger pen was constructed by removing gates between two smaller pens. Both pens contained one feeder and cup waterer per pen; in the large pens, 16 pigs were allotted 1.75 in. of feeder space, and in the small pens, 8 pigs were allotted 3.5 in. of feeder space. The facility was climate controlled with an average temperature of 59.9°F during the study. Each pig was identified with a unique ear notch. Pigs were provided with ad libitum feed and water; their diet was corn-soy-based with 20% dried distillers grains with solubles (DDGS), fed in meal form, and manufactured at the K-State Animal Science Feed Mill.

Samples for the laboratory method analysis portion of the trial were collected from 56 market weight pigs raised and housed at the K-State Swine Teaching and Research Center. Pigs had previously been part of a trial examining the effects of supplementing Astaxanthin to pigs. Pigs were slaughtered in the K-State Meat Laboratory.

Two different methods of blood sampling and restraint methods were used in this experiment: (1) restraint with sorting boards and blood sampling from a distal ear vein and (2) restraint with a standard snout snare and blood sampling from the jugular vein. Sixty animals per method were used in this trial, which took place over the course of 2 days. During the experiment, no pig was sampled twice, but due to the different sizes of pens, the larger pens were entered twice to obtain samples from different pigs. Restraint with the sorting boards was performed by a trained researcher forming a 3-sided barrier with the boards and the pen to restrict pig movement while another researcher pricked one of the pig's distal ear veins with a retractable 20-gauge needle. A sample strip was inserted into a handheld lactate analyzer (Lactate Scout; EKF Diagnostic GmbH, Magdeburg, Germany), and a drop of blood from the pig's ear was immediately administered to the sample strip. The analyzer provided LAC in approximately 15 s and the value was recorded. After blood was collected, the pig was marked with a livestock chalk marker and released. The snaring method was executed by a trained handler who snared the pig by the snout. Another trained researcher collected approximately 7 ml of blood via jugular venipuncture. Blood was collected into sodium fluoride potassium oxalate tubes (Catalog #: 02-688-48, Fisher Scientific, Pittsburgh, PA) to inhibit further glycolytic metabolism and also into polypropylene serum tubes (Catalog #: 2328, Perfection Scientific, Atascadero, CA). After collection, the plasma in the sodium fluoride potassium oxalate tubes was used to determine LAC of the snared pigs using the same handheld lactate analyzer as used for the sorting board group. After pigs were snared and sampled, they were marked with a livestock chalk marker and released back into the pen. The lactate analyzer was tested with a standard solution to ensure accuracy (CV was 2.8%). The CV reported by the analyzer manufacturer is 3 to 8% depending on the concentration measured.

During blood sampling for both treatment groups, a behavior score of 1 to 4 (1 = no vocalization or movement; 2 = initial vocalization upon boarding; 3 = intermittent movement and vocalization; 4 = constant vocalization and movement, rearing) was assigned to each animal as it was handled. The duration of restraint of the animal was also recorded. To measure duration, time was started when the animal was first touched by the handler and time was stopped upon marking the animal with the livestock chalk marker.

Plasma samples were stored on ice upon collection. After experimentation had concluded, plasma samples were centrifuged for 20 min at $1,200 \times g$, then stored at -4°F . Serum samples were refrigerated overnight, then centrifuged and stored at the same specifications as plasma.

Blood samples for the laboratory method analysis were collected during exsanguination in the K-State Meat Laboratory. Blood was collected into sodium fluoride potassium oxalate tubes and into polypropylene serum tubes following the same specifications as the restraint methods trial.

Plasma and serum samples collected during exsanguinations were centrifuged at $1,200 \times g$ for 20 min, then stored at -4°F . Blood lactate was determined using three different methods: (1) YSI 2300 Stat Plus Analyzer (YSI Life Sciences, Yellow Springs, OH), which immobilizes lactate oxidase between a polycarbonate membrane and a cellulose membrane, yielding hydrogen peroxide that is measured by a platinum electrode (the amount of hydrogen peroxide corresponds to the LAC amount); (2) a hand-held lactate analyzer (Lactate Scout, EKF Diagnostic GmbH, Magdeburg, Germany), which holds a disposable strip that measures LAC in a blood sample $\geq 0.5 \mu\text{l}$ and takes up the blood and provides a LAC value in 10 sec; and (3) a lactate assay kit (Eton Bioscience Inc., San Diego, CA) used in an ELISA plate reader (Wallac Victor² 1420 multilabel counter, International Trading Equipment LTD., Vernon Hills, IL).

Data for blood sampling and restraint were analyzed using a MIXED model procedure in SAS 9.2 (SAS Institute, Inc., Cary, NC) with restraint/sampling treatment as a fixed effect and duration of restraint as a covariate. Individual pig was included as a random effect, and the Kenwardroger approximation was used to calculate denominator degrees of freedom. Pearson correlations also were performed to determine relationships between duration of restraint, behavior score, and LAC. Pig was the experimental unit in all analyses.

Data for laboratory assays were analyzed using a MIXED model procedure in SAS (9.2). Individual pig was included as a random effect, and the Kenwardroger approximation was used to calculate degrees of freedom. Pig was the experimental unit during analyses.

Results and Discussion

The LAC for both blood sampling and restraint methods are shown in Table 1. Also shown are the summary statistics for behavior scores and restraint duration for both methods. Results indicated that pigs restrained with the snaring method had greater ($P = 0.04$) LAC than pigs restrained with the sorting board method. Baseline LAC can range from 2.8 mM (Hamilton et al. 2004⁸) to approximately 4 mM (Benjamin et al. 2001²). Baseline LAC is the term used to describe LAC that can be compared across similar treatments. Hamilton et al (2004⁸) reported baseline LAC after snout snaring was 2.8 mM in hogs whose blood was collected via jugular venipuncture. Benjamin et al. (2001²) measured LAC after aversive handling of pigs. Baldi et al (1989⁸) suggests that different sampling techniques do not affect plasma parameters because blood metabolites are influenced more by presampling activities than the sampling period itself.

⁸ Baldi, A., et al. 1989. Effects of blood sampling procedures, grouping and adrenal stimulation on stress responses in the growing pig. *Reproduction, Nutrition, Développement* 29.1:95.

Relationships were observed between duration of restraint and LAC and behavior in both test groups. A positive correlation was seen between duration of restraint and lactate ($r = 0.42$, $P = 0.001$) in pigs restrained with a snare; the longer the pig was restrained, the greater the LAC. Positive correlations were observed between duration of restraint and behavior ($r = 0.41$, $P = 0.001$), duration of restraint and LAC ($r = 0.65$, $P < 0.001$), and behavior score and LAC ($r = 0.26$, $P = 0.05$) in the group restrained with sorting boards. Pigs in the sorting board group had higher values of LAC because of longer restraint times. Duration of restraint is a contributor to increased LAC and higher behavior scores in both the sorting board and snaring groups. These results indicate that restraint time should be minimal. Panepinto et al. (1983⁹) evaluated observational stress of a portable sling manufactured from cotton and nylon on Yucatan Miniature Swine. Matte (1999¹⁰) and Baldi et al. (1989⁸) collected blood samples by catheterization of the jugular vein and jugular venipuncture, respectively, while piglets were in dorsal recumbence; Matte (1999¹⁰) also utilized the snaring method to collect blood samples from pigs weighing in range from 66 to 220 lb and found that pigs restrained via snout snare exhibited decreased ADG, thereby questioning the efficiency of snaring. These studies encourage simple, quick restraint and sampling methods that reduce the amount of stress placed on the animal. As shown in the present study, when duration of restraint increases, LAC increases in both the snaring and boarding procedures. Longer restraint times and more stressful sampling procedures can significantly affect the outcome of the blood parameter measurements.⁹

In our study, a short restraint duration using the sorting board method could be less stressful than snout snaring if blood sampling and replication are needed. The sorting board method may serve as a replacement for catheterization of the vena cava and jugular venipuncture when repeated lactate sampling is necessary⁴. Regardless of the restraint and sampling methods utilized, duration of restraint should be kept to a minimum to provide for the well-being of the animal. Variation in LAC is possible between swine serum and plasma, so researchers must be aware of the potential drawbacks of analyzing only one medium with one specific method if they desire to compare results.

Blood lactate and its relationship to stress can be measured using a variety of methods. Handheld lactate analyzers have recently been used by researchers to measure LAC because of the speed of results and ease of operation (Edwards, 2010⁴). Results of the laboratory methods analyses indicated that significant variation in LAC values among the three different methods of analysis ($P = 0.001$). A significant difference between LAC also was observed between serum and plasma ($P < 0.0001$). The average values for each method, for both plasma and serum, can be viewed in Table 2. Our data indicate that the value of the concentration can vary with the method of measurement and illustrate a need for further research into what methods are most dependable for measuring LAC in swine. Comparing LAC across studies can be difficult because of the variation in methods of analysis. Studies measuring LAC in swine have utilized handheld lactate analyzers (Edwards, unpublished data; Grandin, 2010⁷), spectrophotometry^{3,6} and I-STAT clinical analyzers (Ritter et al. 2009¹¹). Several forms of stationary LAC analyz-

⁹ Panepinto, L., et al. 1983. A comfortable, minimum stress method of restraint for yucatan Miniature Swine. *Laboratory Animal Science* 33.1:95.

¹⁰ Matte, J. 1999. A Rapid and non-surgical procedure for jugular catheterization of pigs. *Laboratory Animals* 33.3:258.

ers also have been utilized (Hambrecht et al. 2004³; Chai et al. 2009¹²; Geesink et al. 2004¹³). In these cases, plasma has been the medium analyzed for LAC, which provides for research to be conducted on LAC in swine serum. In this study, glycolytic potential was inhibited in the tubes from which plasma data were collected. These differences could play a large part in the variations between LAC values in plasma and serum.

Table 1. Mean lactate concentration (LAC), behavior score, and duration of restraint¹

Item	Mean	Standard deviation	Minimum	Maximum
LAC, mmol				
Snare	2.4	0.73	1.2	5.1
Boarding	2.1	0.79	1.1	6.9
Behavior score ²				
Snare	2.58	0.50	2	3
Boarding	1.97	0.97	1	4
Restraint duration, sec				
Snare	64.4	36.4	21	192
Boarding	40.6	8.4	28	150

¹ Values represent the mean of 60 pigs per treatment at approximately 165 d of age (278.0 ± 6.4 lb). Pigs were restrained with one of two different methods: snout snare or sorting boards. Blood was drawn and analyzed for LAC; duration of restraint was recorded in seconds.

² Behavior score: 1 = no vocalization or movement and 4 = constant movement, vocalization, and struggle

Table 2. Mean lactate concentration (LAC, mmol) in plasma and serum for three different methods of laboratory analysis¹

Medium	YSI	Handheld	ELISA
Plasma	5.2	6.0	1.8
Serum	6.0	7.6	7.7

¹ Blood samples collected from 120 pigs at approximately 165 d of age (278.0 ± 6.4 lb; n=120 for each method).

¹¹ Ritter, M. J., M. Ellis, D. B. Anderson, S. E. Curtis, K. K. Keffaber, J. Killefer, F. K. McKeith, C.M. Murphy and B. A. Peterson. 2009. Effects of multiple concurrent stressors on rectal temperature, blood acid-base status, and longissimus muscle glycolytic potential in market-weight pigs. *J. Anim. Sci.* 87:351–362.

¹² Chai, J., Q. Xiong, C. X. Zhang, W. Miao, F. E. Li, R. Zheng, J. Peng and S. W. Jiang. 2010. Effect of pre-slaughter transport on blood constituents and meat quality in halothane genotype of NN Large White x Landrace pigs. *Livestock Sci.* 127:211–217.

¹³ Geesink, G. H., R. G. C. van Buren, B. Savenije, M. W. A. Verstegen, B. J. Ducro, J. G. P. van der Palen, and G. Hemke. 2004. Short-term feeding strategies and pork quality. *Meat Sci.* 67:1–6.