BLOOD CREATINE KINASE AS A PREDICTOR OF THE PORCINE STRESS SYNDROME


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SUMMARY

Gilts, barrows and boars of Pietrain (P), Yorkshire (Y) and P-Y-Hampshire (H) breeding, PxP (PHY), were used to study accuracy of prediction of susceptibility to the porcine stress syndrome (PSS) by the luciferase method for blood creatine kinase (CK). At 10 weeks of age, pigs were classified as PSS if they displayed muscle rigidity within 5 min after commencing anesthesia with 3% halothane in oxygen. At 28 weeks, blood was obtained 8 hr after exercise, CK determined, and the CK value used to retrospectively predict PSS classification (PSS+ or PSS−) as determined by the halothane test. For Yorkshire pigs (all PSS−) the mean CK level was 395 IU/liter. Therefore, for purposes of classifying pigs as PSS or normal via the blood CK level, 400 IU/liter was selected as the upper limit of classification of pigs as normal. A true positive outcome was defined as a PSS pig (halothane-induced rigidity) which displayed a CK value greater than 400 IU/liter blood. A normal pig (PSS− by halothane test) with greater than 400 IU/liter was considered a false positive. The number of true positive, false positive, true negative and false negative outcomes were as follows: for P-77, 6, 0 and 4; for Y-O, 8, 18 and 0; for PxP (PHY)-7, 5, 8 and 0; for all groups-84, 19, 26 and 4, respectively. Prevalence of PSS-susceptibility, based on response to halothane exposure, was 89, 0, 35 and 63% for P, Y, PxP (PHY) and all groups, respectively. Accuracy of prediction [(number correct ÷ total) × 100] of PSS-susceptibility by blood CK was 89, 69, 75 and 83% for P, Y, PxP (PHY) and all groups, respectively. The results of this study would suggest that the luciferase procedure for blood CK is a useful screening method for PSS. The efficacy of the method is influenced by prevalence of PSS in the breed being studied.

(Key Words: Malignant Hyperthermia, Blood Creatine Kinase, Pigs, Porcine Stress Syndrome.)

INTRODUCTION

The development of live-animal predictive tests for the porcine stress syndrome (PSS) and pale, soft, exudative (PSE) muscle has received much recent attention. Currently, the most actively studied diagnostic procedures are the use of halothane anesthesia (Eikelenboom and Minikema, 1974), immunological blood typing (Rasmussen and Christian, 1976) and creatine kinase (CK) in serum (Addis et al., 1974; Beermann et al., 1975) or in whole blood by the luciferase method (Hwang et al., 1977; Elizondo et al., 1977). The present study was conducted to investigate the use of blood CK as a predictor of PSS (as revealed by halothane anesthesia), including the statistical accuracy of the procedure.

EXPERIMENTAL PROCEDURES

Animals. Purebred Pietrain, purebred Yorkshire and crossbred PxP (PHY) pigs were used. Crossbred pigs consisted of offspring from mating Pietrain boars to PHY gilts (P=Pietrain, H=Hampshire and Y=Yorkshire). A total of 139 pigs was used, comprised of the following breeding and sexes, presented as boars, barrows and gilts, respectively: Pietrain = 25, 18, 50; Yorkshire = 22, 18, 41; P-Y-Hampshire = 30, 26, 43; and PxP (PHY) = 22, 18, 38. Purebred Yorkshire pigs were derived from a cross of Yorkshire boars with purebred Hampshire gilts. P-Y-Hampshire pigs were derived from the same cross and were crossbred to Pietrain boars. The crosses were made in order to increase the proportion of PSE muscle in the purebred Yorkshire. The purebred Pietrain were derived from a line of Yorkshire x Hampshire pigs, and the crosses were made to increase the proportion of PSE muscle in the purebred Yorkshire. The crosses were made to increase the proportion of PSE muscle in the purebred Yorkshire. The crosses were made to increase the proportion of PSE muscle in the purebred Yorkshire. The crosses were made to increase the proportion of PSE muscle in the purebred Yorkshire. The crosses were made to increase the proportion of PSE muscle in the purebred Yorkshire. The crosses were made to increase the proportion of PSE muscle in the purebred Yorkshire. The crosses were made to increase the proportion of PSE muscle in the purebred Yorkshire.
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PÆP (PHY) = 4, 7, 9; Yorkshire = 4, 6, 16. The application of the halothane anesthesia screening procedure to Pietrains resulted in the deaths of two boars, three barrows and one gilt. Therefore, 133 animals remained in the study.

**PSS Classification.** Pigs were classified as stress susceptible (PSS+) or stress resistant (PSS−) depending upon the reaction they displayed when subjected to halothane (fluothane) anesthesia (Eikelenboom and Minkema, 1974) at approximately 10 weeks of age. Each pig was placed upon a table, manually restrained and given a mixture of 3% halothane in oxygen at a flow rate of 2 liters/min using a semi-closed circle system rebreathing apparatus. The anesthetic was delivered to the pigs using a Fluotec Mark III Precision Vaporizer (Cyprane Ltd., England). The animal was considered to be in a light surgical plane of anesthesia when voluntary muscular activity ceased and medial rotation of the eyes occurred. Time was recorded from the commencement of anesthesia to the onset of PSS symptoms or 5 min, whichever occurred first. The primary symptom used for PSS classification was muscle rigidity (Eikelenboom and Minkema, 1974), although cutaneous erythema, mottled blanching and cyanosis were also frequently observed in rigid pigs. Anesthesia was stopped immediately at the onset of rigidity and the animal was allowed to recover (or die) without subsequent intervention. Rigid animals were classified as PSS+. Animals which were non-rigid at the end of the 5-min test period were classified as stress resistant (PSS−).

**Blood CK.** Blood samples, in the form of blood spots collected on sample cards, were obtained from the ear following exercise stress at 28 ± 1 weeks of age. CK was determined by the method of Hwang et al. (1977) using an unpurified luciferase preparation (Antonik, 1977). This study provided a comparison of the efficacy of the luciferase test for blood CK (Antonik, 1977; Elizondo et al., 1977; Hwang et al., 1977) as a predictor of PSS in breed groups exhibiting high, intermediate and low (zero) incidences of PSS. Figure 1 illustrates a diagram for the screening method used. A log scale plot of blood CK value vs stress susceptibility status (PSS+ or PSS−) is presented for each animal by breed and sex. Two horizontal lines represent hypothetical values for the upper limit of normal for blood CK value. To calculate the efficacy statistics for the blood CK screening test in the present study, the lower line (400 IU/liter) was used. It is recognized that the screening value may be somewhat arbitrary. By comparison, the stress-resistant Minnesota No. 1 pigs studied by Elizondo et al. (1976) displayed serum CK values of 624 IU/liter after heat stress. However, due to differences in breeds and methods, a single valid comparison is difficult to make. Since the Yorkshires were all negative, they were used to establish a “normal” CK level. The pooled mean was 395 IU/liter or close to the 400 IU/liter used for screening. Furthermore, the overall pooled mean for PSS− pigs was 432 IU/liter and for PSS− pigs of the PÆP (PHY) genotype the mean value was 388.

**RESULTS AND DISCUSSION**

The pooled mean for blood CK of pigs classified as PSS+ was 1899 IU/liter compared to 432 IU/liter for PSS− pigs. Pooled means for the three breed groups were Pietrain, 1750; PÆP (PHY), 1608; and Yorkshire, 395 IU/liter. Pooled means for PÆP (PHY) separated according to response to anesthesia, were PSS+, 4281 and PSS−, 388 IU/liter. The large difference between the PSS+ and PSS− pigs in the PÆP (PHY) breed was in part due to one PSS+ pig which displayed an extraordinarily high CK value (>12,000 IU/liter).

**Figure 1.** Scatter diagram of blood CK levels in PSS+ (●) and PSS− (X) pigs, with horizontal screening lines set at 400 and 500 IU.
TABLE 1. THE POSSIBLE OUTCOMES OF SCREENING FOR PSS BY BLOOD CK

<table>
<thead>
<tr>
<th>Blood CK result</th>
<th>Anesthesia screen result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>TP^a</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>FN^c</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>TP + FN</td>
<td></td>
</tr>
</tbody>
</table>

^a TP = the number of pigs correctly identified as PSS+ by blood CK test.
^b FP = the number of PSS- pigs classified as PSS+ by blood CK test.
^c FN = the number of PSS+ pigs classified as PSS- by blood CK test.
^d TN = the number of PSS- pigs correctly identified as PSS- by blood CK test.
^e Total (T) = (TP + FP + TN + FN).

Galen (1975) developed mathematical definitions useful in screening humans for myocardial infarction. A modification of the method of Galen (1975) was used to analyze screening data of the present study. A number of useful statistics are calculated from four possible outcomes: true positivity (TP), false positivity (FP), true negativity (TN), and false negativity (FN), as shown in table 1. These statistics are sensitivity, specificity, predictive value, and accuracy, as defined in table 2. Sensitivity indicates frequency of positive blood CK test results in PSS+ pigs, whereas specificity indicates frequency of negative blood CK test results in PSS- pigs. Predictive value denotes the frequency of PSS+ pigs among all pigs with positive blood CK test results, and accuracy denotes the sum of the frequency of PSS+ pigs with positive test results and that of PSS- pigs with negative CK test results.

In the screening method used here, it is obvious that the level of blood CK that is used as the screening value (400 IU/liter in this study) will directly influence the numbers of TP, FN, FP and TN. Due to the overlapping distribution of blood CK in PSS+ and PSS- pigs (figure 1), it is impossible to select a screening value for blood CK that would afford complete discrimination between the two groups (PSS+ and PSS-). At any screening value one must sacrifice sensitivity for specificity and vice versa.

In swine testing for genetic screening, FN values are a problem of greater severity than FP values because a FN test results in the retention in the breeding herd of animals which have a genetic predisposition toward PSS. Therefore, it would appear advantageous to use a rigorous (low) screening value so as to reduce the num-

TABLE 2. SUMMARY OF BLOOD CK SCREENING EFFICACY IN DIAGNOSIS OF PSS^a

<table>
<thead>
<tr>
<th>Item</th>
<th>Test result^b</th>
<th>Efficacy statistics, %^c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>TP</td>
</tr>
<tr>
<td>Pietrain</td>
<td>87</td>
<td>77</td>
</tr>
<tr>
<td>Yorkshire</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>P X P (PHY)</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>133</td>
<td>84</td>
</tr>
</tbody>
</table>

^a See table 1 for definitions of T, TP, FP, TN and FN.
^b Based on blood CK prediction of halothane response (PSS), with the outcomes calculated on the lower screening line (CK = 400 IU/liter).
^c P = (TP + FN) (100)/T; S = TP(100)/(TP + FN); Sp= TN(100)/(FP + TN); PV = TP(100)/(TP + FP); and A = (TP + TN) (100)/T, where T = TP + TN + FP + FN, P = prevalence, S = sensitivity, Sp = specificity, PV = predictive value and A = accuracy.
^d Not applicable since TP = FN = 0 and undefined value (0/0) results.
number of FN values. The effect of lowering the screening value is to increase the number of FP animals. The percentage of FN was also calculated because of the importance of this in breeding programs. The data in table 2 are the summary of the data in figure 1 in which all 133 pigs are plotted by breed and sex. The results indicate that the Pietrain group had an extremely high prevalence of PSS (89%). A lower incidence was reported for Dutch Landrace pigs (17% in gilts, 13% for barrows) by Eikelenboom and Minkema (1974).

An interesting effect of prevalence on predictive value was seen as the test was applied on high, intermediate and low prevalence breed groups (table 2) which gave high, intermediate and low predictive values, respectively. Likewise, although the overall accuracy and efficacy values are good, it is revealing to consider sensitivity, specificity, predictive value and accuracy together as a function of prevalence. For example, in the Pietrain, four FN pigs were found (5% of total Pietrain pigs). Yet no TN pigs were observed, although the reasons for this remain obscure at the present time.

In the Yorkshire group, blood CK testing may not be useful since no PSS occurred. Blood CK data could perhaps be used as a certification method to demonstrate the fact that the incidence of PSS is extremely low in this group of Yorkshires. However, in the P×P (PHY) group the efficacy of this test was very high as sensitivity=100% and FN=0%. Previous studies from this laboratory have demonstrated that crossbreeding between Pietrain animals and a contrasting breed (Minnesota No. 1) was useful in that it provided a breeding design to develop lean, muscular animals with a low incidence of PSE muscle (Elizondo et al., 1976; 1977; 1978). The present study, therefore, gives further support to the effectiveness of such a crossbreeding program since it may provide breed groups in which the PSS problem is predicted with greater sensitivity and fewer false negative outcomes than in purebreds.

**LITERATURE CITED**


