AN APPROACH TO DETERMINE THE RELATIONSHIPS AMONG BREED COMPOSITION, SKELETAL MUSCLE PROPERTIES AND CARCASS QUANTITATIVE TRAITS

G. Elizondo, P. B. Addis, W. E. Rempel, C. Madero and A. Antonik

University of Minnesota, St. Paul 55108
Antonik Laboratories, Elk Grove Village, IL 60007

SUMMARY

Breed composition effects on blood creatine kinase (CK), longissimus muscle transverse area, percentage ham plus loin and postmortem longissimus characteristics were studied by crossbreeding between contrasting purebreds. The experiment utilized pigs of Pietrain (P), which are lean, muscular and a high percentage of which are stress susceptible, and Minn. No. 1 (M), which have the opposite characteristics, and seven cross-bred groups. Resulting composition for the 62 barrows ranged from 100% P to 0% P in 12.5% increments. At approximately 60 kg live weight pigs were subjected to heat stress (10 min, 43 C). Ear-blood samples were taken immediately prior to and following stress and 2- and 24-hr following stress. Blood CK was determined by the luciferin-luciferase procedure. Pigs were sacrificed at 90 kg live weight. No significant differences were noted among breed groups for blood CK prior to and 24 hr after stress. Purebred M displayed the highest CK immediately after stress (840 light units); purebred P the highest CK 2 hr after stress (2,650 light units). CK 2 hr after stress tended to increase with increasing percentage P in the genotype. Longissimus muscle color, structure, marbling and 20-min postmortem pH generally tended to decrease as percentage P in the genotype increased. Longissimus muscle transverse area and percentage ham plus loin increased as percentage P increased. Important exceptions were noted, however, as the 87.5% P group exhibited normal values (close to 0% P) for 20-min postmortem pH, color, structure, marbling and solubility, yet also displayed longissimus muscle area and percentage ham plus loin nearly equal to 100% P. Blood CK screened animals which exhibited low (<6.0) 20 min postmortem pH; such animals exceeded log CK = 2.9.

(Key Words: Breed Composition, Blood Creatine Kinase, Stress, Muscularity, Muscle Properties.)

INTRODUCTION

Hall (1972) reviewed the significant monetary impact of the Porcine Stress Syndrome (PSS) and pale, soft, exudative (PSE) muscle. It is generally believed that selection for increased muscularity and decreased fat thickness has also selected for PSE and PSS (Judge, 1968; Judge et al., 1968; Dildey et al., 1970). A fundamental question therefore is, to what extent is it possible to combine leanness, muscularity and stress resistance? Data are extremely scarce with respect to this question. In contrast to the foregoing reports on the relationship of muscularity to PSE, the studies of Bekaert et al. (1968) on Pietrain pigs and Charpentier et al. (1971) for the Large White breed demonstrated no consistently significant correlations between muscularity and postmortem muscle quality. Charpentier et al. (1971) concluded that muscularity and meat quality are not necessarily mutually exclusive traits. The conclusions of Bekaert et al. (1968) and Charpentier et al. (1971) were derived from observations of quantitative-qualitative correlations on a within-

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Department of Food Science and Nutrition, University of Minnesota, St. Paul.
Person from whom reprints should be requested.
Department of Animal Science, University of Minnesota, St. Paul.
Antonik Laboratories, Elk Grove Village, IL 60007.

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breed basis. The present study utilized crosses of two contrasting purebreds so that the proportion of each breed is known and the degree to which genes from a particular breed influenced different traits could be evaluated.

A diagnostic test for PSE and/or PSS could provide an important criterion to add to already available indices for selection of swine. Studies which have noted relationships between postmortem muscle characteristics or visible indications of stress-sensitivity and blood levels of non-plasma specific enzymes include Addis (1969), Addis and Kallweit (1969), Addis et al. (1974) and Beermann et al. (1975). In contrast, Schmidt et al. (1974) and Wax et al. (1975) noted that serum creatine (CK) kinase levels were not significantly correlated to meat quality parameters in pigs which were not intentionally stressed. Recently, Hwang (1976) adapted the bioluminescence (luciferase) blood CK method used for human myopathies (Antonik, 1977) to determination of porcine CK. The present study was undertaken to (1) determine if cross-breeding between contrasting purebreds is a useful technique in studying the relationship between qualitative and quantitative traits and the inheritance of PSE muscle; (2) study the effects of breed composition on blood CK prior to and after acute heat stress; and (3) study the applicability of blood CK, determined by luciferase, as a screening procedure for PSE muscle.

EXPERIMENTAL PROCEDURE

This experiment utilized 62 barrows including purebred Pietrain (P) pigs which are lean and muscular and Minnesota No. 1 (M), which represent the reverse of these characteristics (Elizondo et al., 1976) and seven intermediate genotypes. Breed composition varied from 100% P to 100% M with 12.5% breed increments (table 1). Breed designations in table 1 list breed of sire first, followed by breed of dam. Purebred Minnesota No. 1 and purebred Pietrain pigs are listed as MxM and PxP, respectively. Similarly, MxM(PxM) designates pigs sired by a Minnesota No. 1 boar and out of a crossbred M(PxM) dam. The 50% P pigs were out of F2 sires and dams of PxM crosses. F1 pigs of PxM crosses were previously compared to purebred PxP and MxM pigs (Elizondo et al., 1976). No F1 pigs were available for this study. The objective of the experiment was to compare different levels of breed composition per se and not attempt to identify specific genotypic effects at this time. For example, the seven pigs in the 87.5% P group were composed of two genotypes: 6 P(PxM)xP and 1 (PxM)xP.

At approximately 60 kg live weight, blood samples were obtained to determine CK activity per se and not attempt to identify specific genotypic effects at this time. For example, the seven pigs in the 87.5% P group were composed of two genotypes: 6 P(PxM)xP and 1 (PxM)xP.

Although the pigs used in this experiment were not screened for halothane sensitivity, the Pietrains were selected from a herd which, by the most recent estimates, is 88% sensitive (Hwang, 1976). No Minn. No. 1 pigs screened for halothane sensitivity were positive reactors (unpublished). Halothane sensitivity was determined by halothane challenge at a mean age of 15 weeks by the method of Eikelenboom and Minkema (1974).

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**TABLE 1. BREED COMPOSITION AND GENOTYPE OF EXPERIMENTAL PIGS**

<table>
<thead>
<tr>
<th>Breed Composition</th>
<th>Genotype</th>
<th>No. Barrows</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>PX P</td>
<td>4</td>
</tr>
<tr>
<td>87.5</td>
<td>P(PxM)xP, (PxM)xP</td>
<td>6, 1</td>
</tr>
<tr>
<td>75</td>
<td>(PxM)PxM</td>
<td>8</td>
</tr>
<tr>
<td>62.5</td>
<td>P(MxM)</td>
<td>4</td>
</tr>
<tr>
<td>50</td>
<td>(PxM)FxM</td>
<td>8</td>
</tr>
<tr>
<td>37.5</td>
<td>(PxM)xM</td>
<td>8</td>
</tr>
<tr>
<td>25</td>
<td>Mx(MxM)</td>
<td>8</td>
</tr>
<tr>
<td>12.5</td>
<td>Mx(MxM)</td>
<td>7</td>
</tr>
<tr>
<td>0</td>
<td>MxM</td>
<td>8</td>
</tr>
</tbody>
</table>

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a. Expressed as percentage pietrain in the genotype.

b. Total=62.
the same 24-hr period. A total of four samples was obtained: (1) before stress—animals bled in their pens with a minimum of handling and physical activity; (2) immediately post-thermal-stress (43 C for 10 min) in a stress chamber (Addis et al., 1974); (3) 2-hr post-stress and (4) 24-hr post-stress. Animals experienced, in addition to heat stress, some degree of physical exertion while walking to and from the thermal stress chamber (a distance of 10 to 15 m) and also with respect to the avoidance reaction they displayed when confronted with the chamber (Elizondo et al., 1976). These factors (exertion, etc.) may also elevate blood CK levels (Thomson et al., 1975).

Blood samples were obtained by lancing the ear (Addis et al., 1974) and allowing drops of blood to fall onto phenylketonuria (PKU) cards (Schleicher & Schuell No. 903). The ear-lance method prevents direct CK contamination from injured skeletal muscle since the part of the ear lanced is devoid of skeletal muscle. Blood spots were air-dried and sent to the laboratory for CK analysis by the luciferin-luciferase method (Antonik, 1977). Hwang (1976) verified the applicability of the luciferase procedure to the determination of porcine blood CK. Blood CK was transformed to log CK to normalize variability of the luciferase procedure to the extreme variation encountered.

Means and standard errors for blood CK activity are presented in table 2. No significant differences were noted among breed groups prior to heat stress, a finding which disagrees with those of Garcia (1974) who noted a positive relationship in human subjects between muscularity and resting CK values. It is difficult to know for certain if present values are true "resting" values from completely unstressed animals. Yet, after heat stress, several breed groups displayed large increases over pre-stress levels. From CK values obtained before stress it would appear that none of the tested animals displayed levels indicative of a specific muscle disease and/or trauma (Zellweger and Antonik, 1975). It is also significant that pre-stressed breed means (table 2) were not related to variations in postmortem muscle glycolytic rate (figure 1) or protein solubility (figure 2).

Differences among breeds were noted for CK immediately after stress in which MxM barrows (0% P) exhibited slightly higher values than several groups including 100% P. However, at 2 hr after stress the 100% P group was higher than all others. At 24 hr after stress, means for 50%, 75%, 87.5% and 100% P remained high, although the values were quite variable as indicated by large standard errors.

The reason for the variable responses noted among breed groups, with respect to the rate of CK efflux, is unclear. Two factors which may
Figure 1. Effect of breed composition on 20 min (a) and 24 hr (b) postmortem longissimus muscle pH.

be involved are size of muscles (and myofiber diameter) and stress-induced vasoconstriction. Addis et al. (1976) noted that P×P pigs exhibited significantly (P<.05) greater myofiber diameter than M×M pigs. Thus, the myofiber-capillary diffusional distance may be longer in P×P pigs than in M×M pigs. Williams et al. (1975) reported that PSS pigs exhibit a severe peripheral vasoconstriction during halothane anesthesia and during stress which would delay clearance of CK into the circulation. Nevertheless, the delay in CK efflux after stress in P×P pigs is parallel to the lag which occurs in CK efflux from human cardiac tissue following myocardial infarction (Galen, 1975). The results are consistent with the findings of Max-

Figure 2. Effect of breed composition on post-mortem longissimus muscle protein solubility as measured by transmission value.
well et al. (1976) and Addis et al. (1976) which demonstrated that in stress-susceptible pigs, blood sampling for CK should be delayed until 8 hr after stress.

Figures 1 to 6 graphically depict trends in the variables vs breed composition. Analysis of variance demonstrated that significant (P<.05) effects due to breed composition occurred for all nine variables summarized in figures 1 to 6.

Figure 1 shows a general tendency of 20 min postmortem longissimus muscle pH to decrease as percentage P increased. However, the 87.5% P group exhibited slower glycolysis than 100% P group.

No definite pattern could be discerned for the graph of ultimate pH (figure 1) except that the 87.5% P group exhibited lower pH than all other breed groups. The 0% P group exhibited higher pH than all other groups. High transmission value (low sarcoplasmic muscle protein solubility) occurred only in the 100% P group (figure 2). Researchers who have used the transmission value method (Hart, 1962; Ockerman and Cahill, 1968; Burroughs, 1971) generally regard percentage transmission values of 40 to 100% as indicating PSE muscle. Therefore, it would appear reasonable to conclude that only the 100% Pietrain group had PSE musculature.

Subjective muscle color, structure, and marbling score (figure 3) display very little tendency to decrease with increasing percentage P until >75% P, although a slight downward trend occurs at 25% P. All three measures follow a pattern similar to each other and all three tend to corroborate the findings for pH and protein solubility in that the 87.5% P group was relatively normal. Marbling showed a general tendency to decrease with increasing percentage P with minima at 25, 87.5 and 100% P. The findings of high incidence of PSE muscle in Pietrain pigs and superior muscle quality in Minn. No. 1 pigs are in agreement with Elizondo et al. (1976, 1977) and Aberle et al. (1976).

The longissimus muscle area (figure 4) displayed a constant tendency to increase with increasing percentage P, except for 25% P and 37.5% P. The difference noted between the 87.5 and 100% P breed groups was slight.

Percentage ham plus loin (figure 5) displayed the same pattern as longissimus muscle area but plateaus occur in the curve from 12.5 to 50 and 62.5 to 75% P.

Backfat thickness (data not presented) decreased as percentage P increased but the differences between 87.5% and 100% P were small.

Carcass length (figure 6) tended to decrease...
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with increasing percentages of P with exceptions at 12.5% and 75% P. The differences noted between the 87.5 and 100% P groups were slight. The results of the quantitative carcass data are in general agreement with Elizondo et al. (1976).

Results of the present study appear to agree with the studies of Judge, 1968; Judge et al. (1968); Topel et al. (1968) and Dildey et al. (1970)—all of which suggest that as muscularity increases the degree of stress susceptibility increases and meat quality decreases. However, in the 62.5, 75 and 87.5% P groups, a fairly high degree of combination of meat quantity and quality was achieved. Thus, the results are also consistent with the suggestions of Skelly and Handlin (1971) and Charpentier et al. (1971) that selection for thin backfat and high percentage lean should not result in reduced quality. Although a valid comparison is difficult to make, it appears that the M×M and P×P pigs approximately bracket most populations used by Skelly and Handlin (1971). With respect to Charpentier et al. (1971) it would appear that the breed that they used (Large White) was not particularly muscular since they appeared to be less muscular than the M×M pigs of the present study. Therefore, the conclusion that muscularity is not related in any way to meat quality is incorrect.

It must be cautioned that variation between two genotypes at the same level of breed composition could occur and, furthermore, specific sire and dam effects could produce variable results within a given genotype. Therefore the results obtained with the 87.5% P group are of biological significance, but do not necessarily represent a specific breeding system which would consistently produce a meritorious breed group. Recently, Elizondo et al. (1978) combined ideal muscle quality with a high degree of leanness-muscularity by using a simple, terminal cross of Pietrain x (Hampshire x Minn. No. 1).

Most of the variables studied in this experiment appeared to be inherited in a quantitative manner as revealed by the strong breed composition effects noted. These results are consistent with findings on PSE porcine muscle by Elizondo et al. (1976) and Aberle et al. (1976) and human malignant hyperthermia by Kelsrup et al. (1974) and are suggestive of a multigenic (quantitative) form of inheritance. Earlier studies had suggested autosomal dominance as the mechanism of inheritance for human (Karlow and Britt, 1973) and porcine (Woolf et al., 1970) malignant hyperthermia. It is recognized, therefore, that single-gene effects are important in PSS. However, there is considerable variation in traits related to PSS which are not accounted for by a single gene pair. While multigenic inheritance is complex to study, it affords the possibility of developing genotypes with superior leanness and muscle quantity without PSE muscle. This advantageous combination of traits, which was suggested as being possible in the present study, would not appear to be possible if the PSE trait is determined by a single pair of genes and was related to leanness-muscularity only by pleiotropic effects.

Previous studies have noted a significant statistical relationship between blood CK and stress susceptibility (Addis and Kallweit, 1969; Addis et al., 1974; Beermann et al., 1975). Blood CK, as determined by luciferase, has been shown by Hwang (1976) to be an accurate

![Figure 7. Scatter diagram for log CK in blood vs 20 min postmortem longissimus muscle pH before and 24 hr after stress.](image-url)
predictor of halothane sensitivity. In the present study, a screening method was used in place of correlation analysis. The values for pH at 20 min postmortem vs log CK values (unstressed) were plotted and compared to a plot of log CK 24 hr after stress vs 20 min pH (figure 7). None of the values before stress exceeded CK higher than log 3.0, while at 24 hr after stress some of them exceeded 3.0. Logarithm transformation of blood CK values was used to normalize the data (Richter et al., 1973; Elizondo et al., 1976). This approach is a screening type analysis used in human medicine and may have application in genetic screening of swine for PSE muscle.

The values with pH >6.0 and log CK >2.9 are considered false positives (+CK, –PSE). The high CK levels noted in the false positives may be related to direct muscle trauma, bruises, et cetera. The vertical line was placed so that no false negatives occurred using this approach (–CK, +PSE). False negative results are far more detrimental to a genetic screening procedure than are false positives. The approach outlined here should be considered only as an illustration of one screening method. Considerable experimentation would be necessary to determine accurately where the vertical line should be placed for a given population of pigs. Hwang (1976) noted an incidence of false negative results of 3%, using the luciferase-CK procedure employed herein, in prediction of halothane sensitivity; the overall accuracy of prediction was 83%.

LITERATURE CITED


