THE EFFECT OF MINERAL SUPPLEMENTATION ON THE COLOR AND MYOGLOBIN CONCENTRATION OF PORK MUSCLE

Thesis for the Degree of M. S.

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Wayne Edward Henry

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By

Wayne Edward Henry

AN ABSTRACT

Submitted to the College of Agriculture
Michigan State University of Agriculture and
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Department of Animal Husbandry

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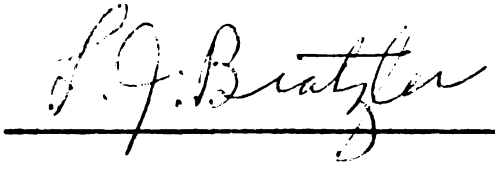
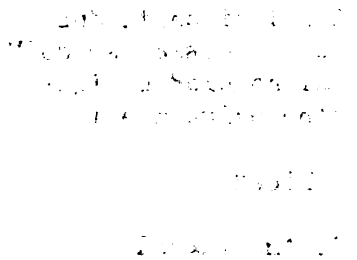
Approved: 

ABSTRACT

Two trials were conducted using 36 purebred Hampshire and 44 crossbred hogs to determine the effects of breed, and zinc, iron, and copper supplementation on color and myoglobin content of pork muscle using sections of the Longissimus dorsi. Surface color measurements were made by use of Munsell spinning disks and myoglobin values were determined spectrophotometrically.

Analysis of variance indicated no significant effect of mineral supplementation upon myoglobin concentration of the Longissimus dorsi. (Trial I. $F = 0.30$; Trial II, $F = 0.45$). In addition, non-significant relationships were found upon comparing breed to the myoglobin concentration of fresh sample ($F = 0.42$) and myoglobin concentration on fat-free, moisture-free basis ($F = 2.62$). The latter comparison was found to be approaching significance.

By use of the correlation coefficient, highly significant relationship was found between index of fading and myoglobin concentration ($r = -0.69$) indicating the feasibility of using the Munsell spinning disks as a method of predicting myoglobin content of pork muscle. Non-significant correlation coefficients were obtained upon comparing index of fading with ether extract (0.21) and total moisture (-0.16). Correlation coefficients obtained upon comparing myoglobin concentration to ether extract and total moisture were non-significant (-0.29 and -0.13, respectively). The results indicated a very low relationship between fat and moisture content of pork muscle and its color as measured spectrophotometrically or by disk colorimetry.



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INTRODUCTION

Desirable color of lean meat, although it does not affect the nutritive value, is recognized and demanded by the consumer. Wilson et al. (1959) stated that the ideal color of fresh pork is greyish-pink. Larzelere et al. (1956), in a consumer preference study, found that 61.8 percent of the dark colored pork chops were selected over greyish-pink pork chops.

Myoglobin, the pigment primarily responsible for meat color, is affected by many factors. Such factors as age, pH, breed, species, exercise, and the ration fed have been found to influence the concentration of myoglobin in the muscle. Bray et al. (1959) found that the supplementation of copper and iron in veal calf rations had a significant effect on the color and myoglobin content present of the muscle.

"Two-toned" hams and off-colored pork is a problem which has confronted the meat packer and retailer for years. Therefore, if a method could be found to produce a desirable and uniform color of fresh lean meat, one of the problems of merchandising could be eliminated.

The objectives of this investigation were to determine the effect of mineral supplementation of swine rations upon the color and myoglobin content of pork muscle. Surface color studies were made and the concentration of muscle pigment was determined spectrophotometrically. Moisture and fat contents were determined in order to study their relationship to surface color and myoglobin concentration.

REVIEW OF LITERATURE

A. Color of Fresh Muscle Tissue

Hoagland (1915), Brooks (1933) and Lavers (1948), stated that the red color of lean meat was due to oxyhemoglobin. Brooks (1937) reported that the color of lean meat was due primarily to the myoglobin present within the muscle fibers. In the same paper, Brooks reported the presence of hemoglobin in muscle fiber, but in quantities too small to have any significant effect on color. In 1932, Theorell (1947) isolated and crystallized the muscle pigment, myoglobin. Morgan (1936) confirmed Theorell's findings by crystallizing myoglobin from horse heart. Hill (1933) observed spectrophotometrically that there was a difference between myoglobin and hemoglobin. Hill also pointed out that myoglobin had a greater affinity for oxygen than did hemoglobin.

Schweigert (1956) stated that myoglobin is a conjugated protein that contains a heme moiety (iron-containing porphyrin compound) attached to a globin molecule. Its function in the live animal is to accept oxygen from the hemoglobin of the blood for use in oxidative, energy-yielding reactions within the cell. Hawk et al. (1954) reported that myoglobin has a higher affinity for oxygen than has hemoglobin. Thus, the intracellular pigment (myoglobin) may be fully oxygenated at lower oxygen tensions than the blood pigment (hemoglobin). The relative amounts of hemoglobin and oxyhemoglobin (oxygenated hemoglobin) present in blood will depend upon the concentration of

oxygen present, which in turn is proportional to the oxygen tension (Henry's Law). Henry's law states that the dissociation of oxyhemoglobin per unit lowering of oxygen tension is greater at a low tension than at a high tension. Poel (1949) stated that myoglobin exists in the muscle as a distinct pigment and because of its high affinity for oxygen, may act as a store of oxygen for use in muscle contraction. Schweigert (1954) reported that the heme portion of the porphyrin compound gives rise to the color of myoglobin. However, the color of heme is modified considerably by the attachment of the protein globin and other colorless constituents.

Lewis (1954), using electrophoretic separation, reported that muscle pigment contains two compounds. He suggested that twenty percent of the muscle pigment was a hemoprotein. Husaini et al. (1950), extracting total pigment from beef muscle, found that ninety to ninety-five percent was myoglobin. Craig et al. (1956) found thirty-five percent of total muscle pigment to be hemoglobin and sixty-five percent to be myoglobin. Broumand (1953) concluded that the bright red color of beef is due to the presence of 90 to 100 percent oxymyoglobin and 10 to 0 percent metmyoglobin.

Currently, the opinion of many meat workers is that the myoglobin of fresh muscle tissue, which is purplish in color, exists in the reduced state. Upon exposure to air, the myoglobin becomes oxygenated, giving the desirable bright red color. However, upon extended exposure to air, the myoglobin is oxidized to metmyoglobin, which is

(1956), the effect of nutrition and age on color of beef muscle. The Hunter Color and Color Difference Meter is a tristimulus colorimeter that measures color on three scales, brightness, redness, and yellowness, which are read directly from the instrument.

Kennedy et al. (1926), in studying the identity of myoglobin, used a Bausch and Lomb spectrophotometer for color measurements. Bull et al. (1942) determined the color of beef muscle by use of a spectrophotometer. Hall et al. (1944) used color paddles to study dark-cutting cattle.

Disk colorimetry was developed in the laboratories of the United States Department of Agriculture for close tolerance measurements of color and for facilitating the observation of numerous samples (Nickerson, 1946). The Munsell renotations of hue, value, and chroma were applied to the data. The Munsell color system is commonly used with the spinning disks, as well as with other systems for naming different colors. Mackintosh (1932), in a study on dark-cutting beef, used the spinning disks in measuring the color of muscle tissue. He classified the color of beef muscle in terms of the percentage of red and black of the spinning disks used. Mackintosh et al. (1935), in another study of dark-cutting beef, explained the effect of finish on color. They found significant correlation coefficients of .610 and .685 between finish and brilliance (value), and between finish and chroma, respectively. In this study, Mackintosh et al. converted the percentages of red, yellow, black and white obtained from the spinning disks to Munsell renotations of hue, value and chroma. They suggested

findings in that no difference in myoglobin concentrations was found between exercised and unexercised beef steers. Craig et al. (1958) stated that the differences in color of lean were due to varying amounts of fat and moisture rather than the difference in pigment concentration. In contrast to these findings, Shenk et al. (1934), in a study of pasture versus dry lot fed cattle, reported that increased myoglobin concentration cannot be due to nutritional causes but may be explained by pasture fed animals having more exercise and hence a higher myoglobin content. Ginger et al. (1954) stated that the variation in muscle color may be attributed to differences in amount of exercise, however, they pointed out that it did not explain the variations observed within a particular muscle. According to Lawrie (1950), myoglobin increases with age and exercise. He concluded that activity is the fundamental factor responsible for controlling the amount of pigment found in any muscle.

Lawrie (1950) reported that pork muscle can vary in color and still have the same myoglobin content. He observed that muscles appear much darker at the pH 7.0 than at pH 6.3. Briskey (1958b), studying exercised hogs versus unexercised hogs, found that exercise increased pH and produced a darker color. However, the difference in myoglobin concentration between the control and treated lots was not significant. This is in agreement with Rongey (1958) who found that exercise was associated with a slight increase in pH which resulted darker colored ham muscle. However, Jacobson et al. (1956) stated

that color variations could not be explained on the basis of pH. Briskey et al. (1958c), in a later study, observed the effect of exercise and high sucrose rations on the chemical characteristics of pork ham muscle. The gluteus medius (outer ham muscle) was studied. Exhaustive exercise with normal rations were found to produce dark muscles, whereas high sucrose rations and no exercise produced muscles that were pale in color. The basal ration (no exercise) and high sucrose ration (exhaustive exercise) produced muscles which were similar in color. Muscle color was determined by the Hunter Color and Color Difference Meter. The average L values were 37.85, 39.45, 39.75, and 40.18 for the basal ration plus exercise, high sucrose ration plus exercise, basal ration, and high sucrose ration respectively. The higher the L values the lighter the color. Myoglobin concentration was determined but no significant differences were found between treatments.

Wilson et al. (1959) in a study of "two-toned" hams, postulated that the concentration of myoglobin and the degree of "two-toning" are heritable characteristics. The degree of "two-toning" was observed to be more closely related to the amount of myoglobin in the dark muscles than the amount of myoglobin in the light muscles.

According to Niedermeier et al. (1959), iron and copper supplementation greatly influence the color and myoglobin concentration of veal muscle. It was also pointed out that an iron deficient ration would reduce the muscle myoglobin concentration. Bray et al. (1959) have shown that iron and copper supplementation has a definite effect

on the color and amount of myoglobin in veal muscle. Calves were fed a basal ration supplemented with 240 mg. of iron as ferric pyrophosphate and 7 mg. of copper as copper sulfate per day. It was found that the iron and copper-supplemented animals had a significantly larger quantity of myoglobin, or 0.54 mg. per gram of lean tissue. The authors hypothesized that the increased iron intake accounted for a greater production of myoglobin. In the same study it was observed that the animals having the highest quantity of myoglobin produced a darker colored muscle as indicated by the Hunter Color and Color Difference Meter. The dark and light samples had an average L value of 29.47 and 27.3, respectively. A significant correlation coefficient of -0.91 was found between the myoglobin concentrations and L values.

In view of the studies reviewed above, the present study was initiated to determine what effect, if any, mineral supplementation of swine rations would have on the color of pork muscle. The observations were made by use of Munsell spinning disks and spectrophotometric estimations of myoglobin.

EXPERIMENTAL PROCEDURE

A. Source of Animals

Animals used in this study were obtained from an experiment at Michigan State University designed to determine the relationship of zinc, iron and copper supplementation to the incidence of parakeratosis in swine. Previous workers (Bray et al., 1959) have indicated a possible relationship existing between high level mineral supplementation and the color and myoglobin content of muscle tissue. Therefore, this study was conducted to determine the effect of supplementing swine rations with zinc, iron and copper at various levels of calcium on the color and myoglobin content of pork muscle. After the initial parakeratosis experiment was concluded, the animals were fed to approximately 200 lbs., slaughtered, and physical and chemical determinations made. In addition, since animals of known breeding were used, it was possible to study the possible affect of breed on color.

Two separate trials involving a total of 80 pigs were conducted. Trial I consisted of 35 purebred Hampshire hogs and Trial II consisted of 43 crossbred hogs of known breeding. Two additional animals were used in the study of the relationship between myoglobin and color.

The basic ration was a typical fattening ration consisting of corn, soybean meal, meat and bone scraps, fish meal, alfalfa meal, limestone, dicalcium phosphate, iodized salt, B-vitamin mix, A and D concentrate, B_{12}, and Aurofac 10.

C. Color Measurements

Munsell spinning disks were used to determine the surface color of the lean meat, and hue, value, and chroma renotations were calculated. Essentially the same technique was employed as described by Voegeli (1952) and Saffle (1958). A sample consisting of a 2 cm. slice of the Longissimus dorsi, taken at the 10th rib, was placed in a Cryovac bag and then placed in a 36-38°F. cooler. Color measurements were taken at the end of two hours. The average Munsell renotations for each of the lots were calculated as described by Saffle (1958).

For evaluation of desirable pork muscle color, a pork loin sample was selected by members of the Michigan State Animal Husbandry department. The sample had a renotation of 4.3YR 4.9/3.6 which was considered as an "ideal" pork color. This color was considered as the standard for calculating the index of fading by the formula of Nickerson (1946).

D. Myoglobin Determination

The quantitative determination of myoglobin as outlined by Ginger et al. (1954) was used in this study with the exception of a few modifications. The method used in its entirety follows.

Duplicate 25 gram aliquots of the ground sample were minced in a Waring Blender for two minutes with 100 ml. of cold distilled water. This was contrary to the procedure of Ginger et al. (1954) of overnight extraction in a refrigerator by mixing 10 grams of ground meat

with 10 ml. of water. After blending, the solution was transferred to a 250 ml. centrifuge bottle and centrifuged for 20 minutes at 2500 revolutions per minute. All centrifugation was carried out at temperatures of 34 to 38°F.

The supernatant was then filtered through cotton which retained some of the fat that had risen to the top of the solution. After filtering, the red supernatant was adjusted to pH 7.0 with 1 N sodium hydroxide. Foreign proteins were precipitated by the addition of 0.25 volumes of saturated basic lead acetate. Lead acetate was added at room temperature because at lower temperatures the protein precipitation is incomplete and at high temperatures (38°C.), the myoglobin will also precipitate (Schweigert, 1954). The lead acetate solution was allowed to stand for 20 minutes to allow complete precipitation of the foreign proteins, followed by 20 mintues of centrifugation (2500 rpms). The resulting supernatant was brought to pH 6.6 and the phosphate concentration to 3 M by the addition of mono and dibasic potassium phosphate in the solid form. The phosphates facilitate the precipitation of hemoglobin, leaving myoglobin in solution (Ginger et al., 1954). Following the third centrifugation of 20 minutes, the supernatant was filtered through Whatman number 41H filter paper into a 50 ml. volumetric flask. Two milliliters of the solution were removed by a volumetric pipette and potassium ferricyanide and sodium cyanide were added to the filtrate to final concentrations of 0.6 mM and 0.8 mM per liter, respectively. The potassium ferricyanide oxidizes all of the myoglobin into metmyoglobin; and the sodium cyanide

E. Fat and Moisture Determination

Approximately 5 gram duplicate samples were taken from the ground Longissimus dorsi, placed in disposable aluminum dishes and dried at 100°C. for 24 hours for moisture determination. Ether extract was determined from the same samples used in moisture analysis. The fat was extracted with anhydrous ether for 4 hours in a Goldfish Fat Extractor. All samples were weighed to the nearest .0001 gram. Formulae for calculating the percent moisture and fat were as follows:

$$\frac{\text{wt. of dried sample}}{\text{wt. of fresh sample}} \times 100 = \% \text{ moisture}$$

$$\frac{\text{wt. of ether extract}}{\text{wt. of fresh sample}} \times 100 = \% \text{ fat}$$

F. Statistical Analysis

Statistical analysis of data included means, analysis of variance, standard error of estimate, simple correlation coefficients, and predicting formulae.

The following formulae used in analyzing these data are in accordance with Snedecor (1958):

Analysis of variance

$$\Sigma X^2 - \frac{(\Sigma X)^2}{N} = \text{Total sum of squares}$$

$$\frac{(\Sigma X_1)^2}{N_1} + \frac{(\Sigma X_2)^2}{N_2} + \text{----------} \frac{(\Sigma X_N)^2}{N_n} - \frac{(\Sigma X)^2}{N} = \text{Between treatment sum of squares}$$

Total sum of squares - between treatment sum of squares = Error

Correlation coefficient

$$"r" = \frac{\Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{N}}{\sqrt{\left(\Sigma X^2 - \frac{(\Sigma X)^2}{N}\right) \times \left(\Sigma Y^2 - \frac{(\Sigma Y)^2}{N}\right)}}$$

Slope of regression line

$$B = \frac{\Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{N}}{\Sigma X^2 - \frac{(\Sigma X)^2}{N}}$$

"Y" intercept

$$A = \bar{Y} - B\bar{X}$$

Standard error of estimate

$$\sigma_e = \sqrt{\frac{\Sigma Y^2 - A\Sigma Y - B\Sigma XY}{N-2}}$$

Predicting formula

$$\hat{Y} = A + B\,(X)$$

Standard deviation of B (slope of line)

$$\sigma_B = \frac{\sigma_e}{\sqrt{\Sigma X^2 - \frac{(\Sigma X)^2}{N}}}$$

RESULTS AND DISCUSSION

A. Preliminary Studies

Several preliminary studies were conducted in order to become familiar with myoglobin determination and the use of the Munsell spinning disks. Color renotations and myoglobin concentrations were determined on pork muscle using sections of the Longissimus dorsi. The technique and apparatus used were as outlined by Voegeli (1952) and Saffle (1958). The preliminary studies indicated that a thorough understanding of the three-dimensional concept of color was necessary to make rapid and accurate measurements of fresh lean tissue. The three color qualities, hue, value, and chroma, are defined by Munsell (1916) as follows:

Hue - is the quality by which we distinguish one color from another, as a red from a yellow, a green, a blue, or a purple.

Value - is the quality by which we distinguish a light color from a dark one.

Chroma - is the quality by which we distinguish a strong color from a weak one.

The three dimensional concept of color required the operator using the Munsell spinning disk apparatus to make three distinct comparisons when matching a sample.

the disk mixture from darker than the sample to lighter than the sample. In order to facilitate the use of whole units of disk area in calculating renotation values, it was decided to use the lighter readings for purposes of standardization.

A method of expressing color differences is useful in many cases. Several workers have derived formulae for expressing small color differences, however, Nickerson's (1946) index of fading, which is based upon the Munsell scale of hue, value, and chroma is applicable for expressing color tolerance, color fading, etc. The formula is as follows:

$$I \text{ (index of fading)} = \frac{C}{5}(2\Delta H) + 6\Delta V + 3\Delta C$$

C = chroma of sample

Δ H = difference in hue between sample and standard

Δ V = difference in value between sample and standard

Δ C = difference in chroma between sample and standard

Butler (1953b) indicated that there is a slight error in the application of this formula. The formula assumes that at a chroma of 5, a one unit change in value has the same effect on the overall color as do two units change in chroma or three units change in hue.

Myoglobin determination is a time consuming and tedious procedure. Therefore, if disk colorimetry could be applied as a suitable method for estimating muscle pigment concentration, studies involving myoglobin content of muscle tissue would be markedly accelerated.

Upon comparing myoglobin, fat, and moisture content and index of fading of all samples studied, the following relationships were found: index of fading versus myoglobin content produced the highly significant correlation coefficient of -0.69 (Figure I) which supports the concept of myoglobin being highly associated with muscle tissue color. Although over one-half of the variability ($1-r^2$) between the two methods was unaccounted for, the predicting formula of $Y = 1.01 - .023\ (X)$ appears to be a useful estimator for predicting myoglobin content from disk colorimetry readings of fresh pork loin muscle.

Comparing the fat content with index of fading and myoglobin concentration, the low and insignificant correlation coefficients of 0.21 and -0.29, respectively, were obtained, indicating that little relationships exist between fat content and muscle color. Such appears to be true whether color is measured as a surface phenomenom by disk colorimetry or as total myoglobin content determined spectrophotometrically.

The effect of moisture content upon index of fading and myoglobin was found to be low and in a negative direction as indicated by the non-significant correlation coefficients of -0.16 and -0.13, respectively. The slight effect upon color due to variations in fat and moisture content appears to be somewhat of a lightening effect (increase in value) with an increase in fat, and a slight darkening of muscle color (decrease in value) with an increase in moisture (Appendix Tables A and B). The usual high relationship between fat

and moisture content was found as indicated by the highly significant correlation coefficient of -0.88.

These findings are not in accordance with Craig et al. (1958), who indicated that differences in color of lean meat were due to varying amounts of fat and moisture rather than differences in pigment concentration. However, the results of the present study would indicate that color differences cannot be explained on the basis of fat and moisture concentration.

C. Effect of Zinc, Iron, and Copper Supplementation on Myoglobin Concentration. Trial I.

Myoglobin values and levels of mineral supplementation are presented in Table III. It will be noted that greater variations exists between animals than between treatments. The loin eyes of animals receiving iron or iron plus zinc (treatments II & III) contained slightly more myoglobin (3.55 mg./g.) than did those of animals receiving zinc, copper, or copper plus zinc (3.52, 3.44, and 3.27 mg./g., respectively). However, analysis of variance indicated no significant differences between treatments (Table IV). The results of this experiment are not in agreement with Bray et al. (1959) who indicated that supplementation of iron and copper to veal calf rations increases myoglobin concentration. In their work, 240 mg. iron and 7 mg. of copper were fed daily per animal. In the present study, minerals were mixed into the ration prior to feeding, and estimation of mineral intake per animal was based on daily feed consumption.

maturity than veal calves that are dressed at about the same weight. Myoglobin concentration increases with age and maturity, therefore, at the degree of maturity of the hogs studied the treatment difference in myoglobin content may have been minimized.

D. Effect of Zinc and Copper Supplementation of Calcium Levels of 0.55 and 1.31 Percent. Trial II.

In Trial II the average daily mineral intake per animal was: 114 mg. of zinc, 295 mg. of copper, 104 mg. of zinc and 218 mg. of copper for treatments I, II, III, and IV, respectively. As indicated in Table V, there is little variation between treatments. However, the loin eyes from the control animals generally contained less myoglobin per gram than did those of treated animals. These data (Trial II) are very similar to Trial I in that copper or zinc had little effect on total myoglobin concentration. In addition, greater variation was observed between animals within treatments than between treatments. Analysis of variance (Table VI) indicates no significant differences between treatments.

TABLE V

CONCENTRATION OF MYOGLOBIN OF PORK LONGISSIMUS DORSI MUSCLE. TRIAL II

Animal Number	Myoglobin (mg./g.)	Animal Number	Myoglobin (mg./g.)
*Control		***Treatment II	
**X-33-4	3.46	X-33-3	4.24
X-61-8	3.74	X-59-3	5.03
X-60-9	3.71	X-62-5	2.88
X-58-10	3.34	X-33-9	4.64
X-59-6	3.65	X-61-7	3.02
X-37-11	4.13	X-65-1	3.19
X-66-1	3.09	X-35-4	3.99
X-61-11	2.31	X-34-12	3.84
X-77-1	3.93	X-61-5	2.73
X-36-6	3.82	X-60-7	4.07
Av.	3.52	Av.	3.76
Treatment I		Treatment III	
X-61-1	4.09	X-33-10	3.69
X-59-9	4.76	X-61-4	2.47
X-33-1	3.46	X-59-4	3.60
X-58-9	3.65	X-59-8	3.99
X-33-2	3.02	X-39-3	4.03
X-58-1	3.54	X-61-1	3.52
X-77-2	4.16	X-58-4	3.44
X-64-1	3.71	X-34-7	4.31
X-33-1	4.54	X-66-10	2.90
X-62-3	3.43	Av.	3.56
Av.	3.84	Treatment IV	
		X-59-2	4.02
		X-33-5	2.96
		X-61-2	4.66
		X-34-11	3.12
		Av.	3.69

*Control received only .55% calcium
**X = crossbred hogs
***Treatment I - Zinc 75 ppm + .55% calcium
Treatment II - Copper 125 ppm + .55% calcium
Treatment III - Zinc 75 ppm + 1.31% calcium
Treatment IV - Copper 125 ppm + 1.31% calcium

TABLE VI

ANALYSIS OF VARIANCE OF MYOGLOBIN VALUES. TRIAL II

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	42	15.78		
Between Treatments	4	.71	.178	*.45 N.S.
Within Treatments	38	15.07	.397	

*N.S. indicates non-significance of the variance ratio, F, at the 0.05 level.

E. Effect of Breed on Myoglobin Concentration.

Wilson et al. (1959) postulated that the concentration of myoglobin found within a particular muscle may be a heritable characteristic. Studying the light and dark colored muscles of "two-toned" hams from three breeds of hogs (Poland China, Duroc, and Chester White), a distinct difference in myoglobin concentration of the dark muscles was noted between breeds. The Poland China had the highest myoglobin content with 2.35 mg./g., followed by the Duroc with 1.80 mg./g. and the Chester White with 1.58 mg./g. of fresh muscle tissue. However, the differences between breeds in myoglobin concentration of the lighter muscles were found to be non-significant.

Table VII summarizes the myoglobin content of the Longissimus dorsi muscles from the breeds of hogs represented in this study.

By examination of Table VII, it can be seen that when myoglobin is expressed in mg./g. of fresh muscle tissue, there is less difference between breed means than when myoglobin is expressed on a moisture-

cance at the (p = .05) level. However, the F - ratio of 2.62 approaches significance.

TABLE IX

ANALYSIS OF MYOGLOBIN CONCENTRATION BETWEEN BREEDS (Fat-free, Moisture-free Basis)

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	77	26.94		
Breeds	1	.89	.89	*2.62 N.S.
Error	76	26.05	.34	

*N.S. - non-significant at p = .05 level.

Although no significant difference in myoglobin concentration was found between breeds, the data suggest that there may be a relationship between breeds and the amount of muscle pigment present within the muscle. An F - ratio of 3.96 is required for significance. It is recognized, however, that variations in myoglobin content are found within breeds as well as between breeds. Therefore, in order to draw any valid conclusions, additional studies would be required.

SUMMARY AND CONCLUSIONS

The levels of zinc, iron and copper used to supplement swine rations in this study had no significant effect upon myoglobin concentration of the Longissimus dorsi muscle. In addition, the effect of breed upon myoglobin concentration of pork muscle on both fresh tissue basis and fat-free, moisture-free basis, was found to be non-significant. However, on the fat-free, moisture-free basis, the relationship between myoglobin content and breed approached significance, indicating that a sufficiently strong relationship exists between breed and myoglobin content to warrant further investigation.

A highly significant relationship was found between the index of fading and myoglobin concentration. From this, it may be concluded that disk colorimetry might be used as a method for estimating myoglobin content of pork muscle.

The effect of fat and total moisture content upon color renotations and myoglobin concentrations was found to be non-significant. This indicates that little relationship exists between the fat and moisture content of pork muscle and its color, whether it be measured as a surface phenomenon by disk colorimetry, or, as a pigment concentration by spectrophotometry.

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APPENDIX A

DATA COLLECTED FROM TRIAL I
(Purebred Hampshire Hogs)

Animal Number	Moisture (%)	Fat (%)	Myoglobin[a] Content (mg./g.)	Myoglobin[b] Content (mg./g.)	Munsell Renotation	Index of[c] Fading
Lot 1						
H-83-3	71.28	5.86	.816	3.57	7.9 YR 5.5/3.0	9.7
H-81-2	75.29	1.63	.919	3.98	5.6 YR 5.5/3.0	7.0
H-87-2	74.85	1.03	.728	3.02	8.6 YR 6.0/2.5	14.2
H-86-6	74.28	2.13	.867	3.68	5.4 YR 5.2/2.7	5.7
H-82-4	73.07	4.90	.844	3.83	7.5 YR 5.4/2.8	9.0
H-80-7	74.35	2.17	.793	3.38	5.8 YR 5.3/3.0	6.6
H-87-4	72.26	4.70	.737	3.20	6.9 YR 5.5/2.9	8.7
Mean	73.62	3.20	.815	3.52	6.8 YR 5.5/2.8	8.7
Lot 2						
H-87-6	75.45	1.89	.718	3.17	7.2 YR 5.6/2.8	9.8
H-86-2	75.73	1.09	.760	3.28	5.9 YR 5.8/2.5	10.1
H-86-5	75.50	1.82	1.041	4.59	5.0 YR 5.8/2.5	9.4
H-80-9	72.82	6.18	.611	2.91	6.8 YR 5.6/2.6	9.8
H-81-4	74.96	2.62	.849	3.79	7.0 YR 5.4/3.3	7.5
Mean	74.89	2.72	.796	3.55	6.4 YR 5.6/2.7	9.3
Lot 3						
H-83-5	75.04	1.47	.924	3.93	6.7 YR 5.8/2.5	11.1
H-84-3	73.26	4.21	.840	3.73	6.3 YR 5.0/3.0	4.8
H-81-6	72.32	3.78	.788	3.30	7.6 YR 5.9/2.9	11.9
H-85-2	73.64	4.54	.751	3.44	8.5 YR 5.8/2.7	12.6
H-80-6	74.59	1.63	.779	3.28	5.1 YR 5.2/2.7	5.4
H-87-1	75.10	.93	.872	3.64	8.2 YR 5.7/2.7	11.7
H-86-8	74.60	2.21	.826	3.56	8.6 YR 5.4/3.1	9.8
Mean	74.08	2.68	.826	3.55	7.4 YR 5.7/2.9	9.6
Lot 4						
H-86-4	75.34	.76	.802	3.36	8.0 YR 5.7/2.5	11.8
H-87-9	75.17	.34	1.230	5.02	3.9 YR 5.1/3.1	-3.4
H-80-3	71.05	6.08	.658	2.88	6.4 YR 6.0/3.4	10.3
H-80-5	74.85	1.45	.840	3.54	8.4 YR 5.6/2.8	11.2
H-81-1	74.84	1.47	.793	3.35	8.4 YR 6.3/2.9	11.8
H-84-5	72.55	4.67	.732	3.21	6.2 YR 5.3/3.4	5.6
H-83-4	73.78	3.07	.723	3.12	8.4 YR 6.3/2.9	15.3
H-82-1	74.45	1.16	.746	3.06	4.7 YR 5.2/3.0	4.1
Mean	74.00	2.37	.816	3.44	6.8 YR 5.6/3.0	8.3

APPENDIX A (Continued)

Trial I - Continued

Animal Number	Moisture (%)	Fat (%)	Myoglobin[a] Content (mg./g.)	Myoglobin[b] Content (mg./g.)	Munsell Renotation	Index of[c] Fading
Lot 5						
H-83-9	73.83	3.09	.770	3.34	9.7 YR 6.0/2.8	14.0
H-83-6	74.44	2.34	.672	2.89	5.8 YR 5.3/3.0	6.0
H-85-1	73.48	3.96	.732	3.24	6.3 YR 5.2/2.9	6.2
H-84-6	75.16	1.94	1.130	4.93	5.4 YR 5.1/3.1	4.1
H-80-8	75.26	.44	.597	2.46	8.2 YR 5.1/2.7	11.7
H-86-3	73.88	3.24	.676	2.95	7.6 YR 5.6/3.0	10.0
H-80-2	74.30	1.60	.849	3.52	7.0 YR 5.4/3.0	8.0
H-87-8	73.20	3.63	.658	2.84	6.4 YR 5.6/2.3	10.0
Mean	74.19	2.53	.760	3.27	7.1 YR 5.5/2.9	8.8
H-87-3[d]	74.02	2.57	.672	2.87	7.9 YR 5.9/2.5	12.9

[a]Myoglobin expressed in mg./g. of fresh muscle tissue
[b]Myoglobin expressed in mg./g. on a moisture-free, fat-free basis
[c]Standard = Hue 4.3 Yellow-Red, Value 4.9, Chroma 3.6
[d]Unassigned animal used in computing correlation coefficients

APPENDIX B (Continued)

Trial II Continued

Animal Number	Moisture (%)	Fat (%)	Myoglobin[a] Content (mg./g.)	Myoglobin[b] Content (mg./g.)	Munsell Renotation	Index of[c] Fading
Lot 4						
X-33-10	72.81	2.44	.914	3.69	4.3 YR 5.5/2.6	7.6
X-61-4	71.96	7.26	.513	2.47	9.4 YR 6.5/2.7	17.8
X-59-4	73.73	2.92	.840	3.60	6.4 YR 6.0/3.2	12.1
X-59-8	73.59	1.85	.980	3.99	3.7 YR 4.9/3.7	1.2
X-39-3	72.47	6.94	.830	4.03	5.7 YR 6.0/3.0	10.1
X-61-12	73.52	2.38	.849	3.52	6.9 YR 5.4/3.1	7.7
X-58-4	71.82	6.50	.746	3.44	7.6 YR 5.6/3.0	10.0
X-34-7	71.89	6.68	.924	4.31	5.2 YR 5.5/3.1	6.2
X-66-10	73.77	3.72	.653	2.90	8.8 YR 6.0/3.2	13.6
Mean	72.84	4.52	.805	3.56	6.8 YR 5.7/3.1	8.6
Lot 5						
X-59-2	73.89	2.90	.933	4.02	6.9 YR 5.6/2.5	7.7
X-33-5	74.30	1.95	.704	2.96	8.5 YR 5.7/2.7	12.0
X-61-2	74.01	4.11	1.020	4.66	6.5 YR 5.5/2.8	8.5
X-34-11	71.65	7.43	.653	3.12	6.9 YR 5.6/3.2	8.7
Mean	73.46	4.10	.828	3.69	7.2 YR 5.7/2.8	9.2
X-58-3[d]	74.20	3.75	.849	3.85	7.4 YR 5.5/3.1	9.2

[a]Myoglobin expressed in mg./g. of fresh muscle tissue
[b]Myoglobin expressed in mg./g. on a moisture-free, fat-free basis
[c]Standard - Hue 4.3 Yellow-Red, Value 4.9, Chroma 3.6
[d]Unassigned animal used in computing correlation coefficients