

Title: The effect of the hydrogen ion concentration evaluation mathematical methodology on genetic prediction and fixed effects estimation when assessing muscle quality in pork. **NPB # - 01-153**

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Abstract

An evaluation of pH and hydrogen ion concentration was conducted to determine if the mathematical conversion of hydrogen ion concentration to pH could affect 1. fixed and random effect inferences made when analyses of a study occurs and 2. prediction of genetic merit of animals when pH is used as an indicator in the assessment of pork quality. Data from 4,262 animals having complete three-generation pedigrees from National Barrow Show™ Progeny Test were utilized in this study. Existing muscle pH data was converted to its original hydrogen ion concentration. Existing fixed effects and their levels were not altered. Statistical analyses of the two dependent variables, pH and hydrogen ion concentration, were conducted using identical models using PROC MIXED of SAS (SAS, 2001). The model utilized in the analyses of each trait included the fixed effects of breed, test, gender, Hal 1843™ genotype, herd, and off test date within test group. Numerical changes in values occurred across all effects and in some cases, most notably breed and test, changes in ranking of subclasses occurred. These changes result in differing inferences that can be made depending on whether pH or H⁺ concentration is used as the dependent variable. Heritability estimates for pH and H⁺ concentration were 0.52 ± 0.074 and 0.62 ± 0.078 , respectively. Pearson coefficient of correlation between pH and H⁺ breeding values concentration was -0.92. Spearman Rank coefficient of correlation of -0.85 between pH and H⁺ breeding was calculated and indicates that animals do not rank the same when ordered by breeding values for each trait. Loss in H⁺ selection differential by selecting on pH instead of H⁺ ranges from 5% to 13% depending upon selection intensity utilized. The genetic correlation between pH and H⁺ concentration was -0.96. Changes in the absolute values of the genetic correlations between various pork quality indicator traits and pH or H⁺ concentration were 0.04 or less.

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Differences in the fixed effect results from the pH and H⁺ concentration and would likely impact decisions that swine breeders and meat processors who are concerned about pork quality. This combined with the greater heritability for H⁺ concentration indicate that H⁺ concentration rather than pH is the more appropriate trait breeders and processors should focus on when attempting to improve pork quality.

Introduction

The use of pH is becoming widely accepted as an indicator of pork quality. Additionally, selection programs used by seedstock suppliers to the U.S. pork industry have begun to emphasize pork quality and its indicators. An evaluation of pH and hydrogen ion concentration will allow breeders and researchers interested in pork quality and its indicators to properly evaluate data and make more accurate genetic predictions. Accurate evaluation of pH will allow the U.S. swine industry to improve U.S. pork quality more rapidly through the selection of animals that are more likely to improve muscle quality. Improved pork quality will enhance the position of U.S. pork in the global market place.

Biologically, pH is defined as the negative log of the hydrogen ion concentration ($\text{pH} = -\log [\text{H}^+]$) of an item being measured (Zubay, 1988). The use of pH has been widely accepted and used as an indicator of pork muscle quality when conducting genetic evaluations (Gibson et al., 1996; Goodwin and Burroughs, 1995; Goodwin, 1994) and studying various factors associated with and / or affecting pork quality (Stalder et al., 1998; Barton – Gade, 1990; Christian and Rothschild, 1981). Few investigations (Boutilier and Shelton, 1980) have examined the statistical differences and the effects on the inferences made when hydrogen ion concentration and pH are simultaneously evaluated. There are no studies that have investigated the effects of using pH or hydrogen ion concentration when estimating genetic parameters, conducting a genetic evaluation of lines or breeds, or when conducting an across-herd genetic evaluation. The U.S. pork industry is currently emphasizing pork quality in order to increase consumer acceptance and consumption of pork meat products. The U.S. swine breeding pork processing industries have been focusing on various genetic and environmental factors that affect the quality of pork produced from various pork harvesting and processing entities and by the offspring of animals derived from breeding programs of seedstock suppliers. Meat scientists and geneticists have focused on pork quality traits and their indicators in an attempt to improve the quality of pork that is produced on U.S. commercial pork operations today. One of the indicator traits receiving much attention has been pH. The U.S. pork harvesting and processing industry is concerned in identifying environmental factors that can improve the pH and other indicator traits so more of their pork products can be sold as premium products. The U.S. breeding stock industry is beginning to place selection emphasis through selection directly on pork quality traits and / or its indicators through index or direct breeding value selection. The U.S. seedstock and pork harvesting / processing industry need to know if the fixed and random effect inferences and breeding value estimates (selection decisions) are dependent on how hydrogen ion concentration is evaluated (directly or as pH).

The need to investigate the effect of pH and H⁺ concentration on fixed estimates and random predictions arises because of the mathematical properties of log functions. This might best be shown by the following example.

Example 1. Two sires with three progeny and each having identical pH averages, but differing hydrogen ion concentration¹.

	Phenotypic pH values			Average pH	Mean H	Mean H (pH units)
Sire A	5.6	6.2	6.2	6.00	1.2579E-06	5.90
Sire B	5.7	6.0	6.3	6.00	1.1655E-06	5.93

¹Mean hydrogen ion concentration values have been converted to pH values (taking the negative log of the original value) in order to compare them on the same scale.

This example shows how two different sires having three progeny with pH data can have identical average pH values, but differing average hydrogen ion concentration values. Herein lays a potential problem.

Lastly, hydrogen ion concentration may be the better value to attempt to make breed improvement because it is the biologically active component. The reason pH was developed was to get around the extremely small numbers when measuring hydrogen ion concentration as shown in Example 1 under the Mean H column. When evaluating meat or any other substance, a pH value does not exist, but a hydrogen ion concentration does. Hence, the true value swine breeders are attempting to improve through selection is hydrogen ion concentration and not pH. A similar situation has been described by Johnson et al. (1984), who selected for components of litter size to improve litter size at birth in swine.

Objectives

The objectives of this study are to determine if the mathematical conversion of hydrogen ion concentration to pH can affect 1. fixed and random effect inferences made when analyses of a study occurs and 2. prediction of genetic merit of animals when pH is used as an indicator in the assessment of pork quality.

Procedures

The project utilized the National Barrow Show TM Progeny Test data. Complete three-generation pedigrees, and fixed and random classifications used for analyses of various traits, and pertinent muscle quality information (pH) were obtained from the National Pork Board (Genetics Program Committee, Dr. Rodney Goodwin). The number of animals and fixed effect classes are indicated in Table 1. For the purposes of this study, it was assumed that all pedigree, fixed and random classification, and dependent variable data were accurate.

Existing muscle pH data was converted to its original hydrogen ion concentration. Statistical analyses of the two dependent variables, pH and hydrogen ion concentration, were conducted implementing identical models using PROC MIXED of SAS (SAS, 2001). The model utilized in the analyses of each trait included the fixed effects of breed, test group, off-test date within test group, gender, and Hal 1843TM genotype. The notation for the model used in this analysis is as follows:

$$Y_{ijklmno} = \mu + B_i + H_j + T_k + OD_{l(k)} + S_m + P_n + e_{ijklmno}, \text{ where}$$

$$Y_{ijklmno} = \text{the pH or } H^+ \text{ concentration of } o^{\text{th}} \text{ individual with the } n^{\text{th}} \text{ Hal 1843}^{\text{TM}} \text{ genotype of } m^{\text{th}} \text{ sex from the } l^{\text{th}}(k^{\text{th}}) \text{ off-test date within test- group from the } k^{\text{th}} \text{ test group from } j^{\text{th}} \text{ herd and from the } i^{\text{th}} \text{ breed,}$$

μ	= overall mean,
B_i	= fixed effect common to the i^{th} breed,
H_j	= fixed effect common to the j^{th} herd,
T_k	= fixed effect common to the k^{th} test group,
$OD_{k(j)}$	= fixed effect common to the l^{th} (k^{th}) off-test date within test group,
S_l	= fixed effect common to the m^{th} sex,
P_n	= fixed effect common to the n^{th} Hal 1843™ genotype, and
$e_{ijklmno}$	= random residual error.

Inferential fixed and random effect differences and associated P value changes were evaluated for biological interpretation and accuracy. These analyses accomplished objective 1 of the study.

To accomplish objective 2, genetic prediction and heritability estimates were individually estimated for pH and hydrogen ion concentration using ASREML software (Gilmour et al., 2001). A sire model that incorporated the full relationship matrix of individuals and the fixed effects from objective one was implemented. The notation of the model used in this analysis is as follows:

y	= $X_1b_1 + X_2b_2 + X_3b_3 + X_4b_4 + X_5b_5 + Zu + e$, where,
y	= vector of pH and H^+ concentration phenotypic values,
$X_{1,2,3,4,5, \text{ and } 6}$	= are incidence matrices for fixed effects related to the phenotypic values,
b_1	= unknown vector of a fixed breed effect associated with the record in y ,
b_2	= unknown vector of a fixed herd effect associated with the record in y ,
b_3	= unknown vector of a fixed test group effect associated with the record in y ,
b_4	= unknown vector of a off-test date within a test group fixed effect associated with the record in y ,
b_5	= unknown vector of a fixed sex effect associated with the record in y ,
b_6	= unknown vector of a fixed Hal 1843™ effect associated with the record in y ,
Z	= incidence matrix for random effects related to the phenotypic performance values (pH and H^+ concentration),
u	= unknown vector of random sire effects (using full relationship) associated with the records in y , and
e	= vector of random residual effects.

Spearman rank coefficients of correlation were calculated to evaluate breeding value estimates obtained for two traits. Relative difference among estimated breeding values differences and the ranking order of individuals were evaluated.

Results

Distribution of animals by breed and test group is indicated in Table 2. In total, data from 4,262 animals were utilized in this study.

Objective 1:

The results for the fixed effect model for breed, test, sex and Hal 1843™ genotype are shown in Table 3. All model effects were significant ($P < 0.03$ or smaller) sources of variation in the analysis of pH and H⁺ concentration. The hydrogen ion concentration has been converted back to pH in order to make comparisons between the two methods of analysis easier. Numerical changes in values occurred across all effects and in some cases, most notably breed and test, changes in ranking of subclasses occurred. These changes result in differing inferences that can be made depending on whether pH or H⁺ concentration is used in the analysis. The relative gilt – barrow and Hal 1843™ NN - Nn differences are the same in the analysis of both pH and H⁺ concentration (Table 3).

Objective 2:

The heritability estimates for pH and H⁺ concentration were 0.52 ± 0.074 and 0.62 ± 0.078 , respectively. Both estimates would be considered highly heritable, however greater genetic progress would be expected if selection were based upon H⁺ concentration rather than its transformed pH value when attempting to improve pork quality. The Pearson coefficient of correlation between pH and H⁺ breeding values concentration was -0.92. The highly negative correlation between the two traits was expected, as pH is a transformed value of H⁺ concentration.

To better understand the impact of selection based upon pH or H⁺ concentration, a Spearman Rank coefficient of correlation was calculated. While still considered a high correlation, the -0.85 rank correlation between pH and H⁺ breeding values indicates that some differences in ranking are likely to occur when comparing the breeding values obtained for pH and H⁺ concentration. Selection programs attempting to improve pork quality could be impacted by selecting on pH or H⁺ concentration as shown in an example of truncation selection on the two traits is shown in Figure 1. In this example, loss in H⁺ selection differential by selecting on pH instead of H⁺ ranges from 5% to 13% depending upon selection intensity utilized. This is a consequence of not selecting individuals in the lower left quadrant of the graph, instead choosing those in the upper right. The graph (Figure 1) shows 5% selection intensity. Additionally, the companion table associated with Figure 1 shows the loss in H⁺ in selection differential by selection on pH instead of H⁺ at the top 1, 5 and 25 percent truncation levels. It should be noted that the selection differential loss increases as selection intensity increases.

To gain an understanding of the impact of selection on pH or H⁺ concentration on various other pork quality indicator traits, genetic correlations were estimated (Table 5). The genetic correlation between pH and H⁺ concentration was -0.96. This was expected because the traits are one in the same only pH is a transformed value of the composite genetic effects on H⁺ concentration. Changes in the absolute values of the genetic correlations between various pork quality indicator traits and pH or H⁺ concentration were 0.04 or less. These indicate that selection on pH or H⁺ concentration would yield similar correlated responses in other pork quality traits.

Summary

Statistical analyses of the two dependent variables, pH and hydrogen ion concentration, were conducted using identical models. Numerical changes in values occurred across all effects and in some cases, most notably breed and test, changes in ranking of subclasses occurred. These changes result in differing inferences that can be made depending on whether pH or H⁺ concentration is used as the dependent variable. Heritability estimates for pH and H⁺ concentration were 0.52 ± 0.074 and 0.62 ± 0.078 , respectively. Spearman Rank coefficient of correlation of -0.85 between pH and H⁺ breeding was calculated and indicates that animals do not rank the same when ordered by breeding values for each trait. Loss in H⁺ selection differential by selecting on pH instead of H⁺ ranges from 5% to 13% depending upon selection intensity utilized.

The H⁺ concentration heritability is greater than that for pH and the genetic correlations between the two values and other pork quality indicator traits are nearly identical. Differences in the fixed effect results from the pH and H⁺ concentration and would likely impact decisions that swine breeders and meat processors who are concerned about pork quality. These two facts indicate that H⁺ concentration rather than pH is the more appropriate trait breeders and processors should focus on when attempting to improve pork quality.

Table 1. Descriptive statistics for the random and fixed effects included in the model used to evaluate pH and H⁺.

Descriptive Statistics		
Item	No.	
Pigs	4262	
Breeds	8	
Test groups	11	
Herds	217	
Contemporary groups	87	(2410, 1852)
Gender	2	(barrow, gilt)
Stress gene status	2	(NN & Nn)

Table 2. Distribution of animals by breed and test group from the National Barrow Show TM Progeny Test.

Breed	Test Group											Total
	1	2	3	4	5	6	7	8	9	12	13	
Berkshire	30	36	23	22	23	8	104	91	185	97	181	800
Chester White	56	8	37	0	16	8	38	6	99	47	44	359
Duroc	50	83	90	55	47	47	71	39	78	92	63	715
Hampshire	72	53	54	24	56	53	54	23	23	31	0	443
Landrace	18	66	28	7	64	86	76	48	55	30	31	509
Poland China	25	32	20	15	31	15	39	39	23	44	47	330
Spotted	57	45	60	0	32	16	39	15	13	0	8	285
Yorkshire	94	110	114	24	101	47	86	63	71	55	56	821
Total	402	433	426	147	370	280	507	324	547	396	430	4262

Table 3. Hydrogen ion concentration and pH least square means¹ (\pm SE) for breed, test, sex, and Hal 1843™.

	pH		SE	Hydrogen ion concentration ²		SE
Breed						
Berkshire	5.82 ^a	\pm	0.024	5.76 ^a	\pm	0.027
Chester White	5.78 ^a	\pm	0.029	5.71 ^a	\pm	0.029
Duroc	5.69 ^b	\pm	0.014	5.65 ^b	\pm	0.012
Hampshire	5.53 ^e	\pm	0.018	5.51 ^f	\pm	0.011
Landrace	5.59 ^d	\pm	0.015	5.55 ^e	\pm	0.010
Poland China	5.67 ^{b,c}	\pm	0.027	5.63 ^{b,c}	\pm	0.023
Spotted	5.62 ^{c,d}	\pm	0.024	5.58 ^{c,d,e}	\pm	0.017
Yorkshire	5.62 ^{c,d}	\pm	0.013	5.57 ^c	\pm	0.010
Test						
1	5.79 ^a	\pm	0.013	5.74 ^{a,b}	\pm	0.013
2	5.66 ^c	\pm	0.013	5.61 ^d	\pm	0.010
3	5.61 ^d	\pm	0.013	5.56 ^{e,f}	\pm	0.009
4	5.74 ^{a,b}	\pm	0.030	5.68 ^{b,c}	\pm	0.028
5	5.78 ^a	\pm	0.013	5.75 ^a	\pm	0.015
6	5.58 ^{d,e}	\pm	0.024	5.54 ^{f,g}	\pm	0.016
7	5.65 ^c	\pm	0.012	5.57 ^e	\pm	0.009
8	5.57 ^d	\pm	0.014	5.55 ^f	\pm	0.009
9	5.54 ^e	\pm	0.012	5.51 ^g	\pm	0.008
10	5.66 ^c	\pm	0.013	5.62 ^c	\pm	0.010
11	5.73 ^b	\pm	0.013	5.67 ^c	\pm	0.012
Sex						
Barrow	5.67 ^a	\pm	0.006	5.62 ^a	\pm	0.006
Gilt	5.65 ^b	\pm	0.007	5.60 ^b	\pm	0.006
Hal 1843™ Genotype						
Nn	5.68 ^a	\pm	0.006	5.63 ^a	\pm	0.005
Nn	5.65 ^b	\pm	0.011	5.60 ^b	\pm	0.009

¹ Column means within an effect with different superscripts differ ($P < 0.05$).

² Hydrogen ion concentration means have been converted back to pH values after being analyzed in order to facilitate comparisons between the two analysis methods.

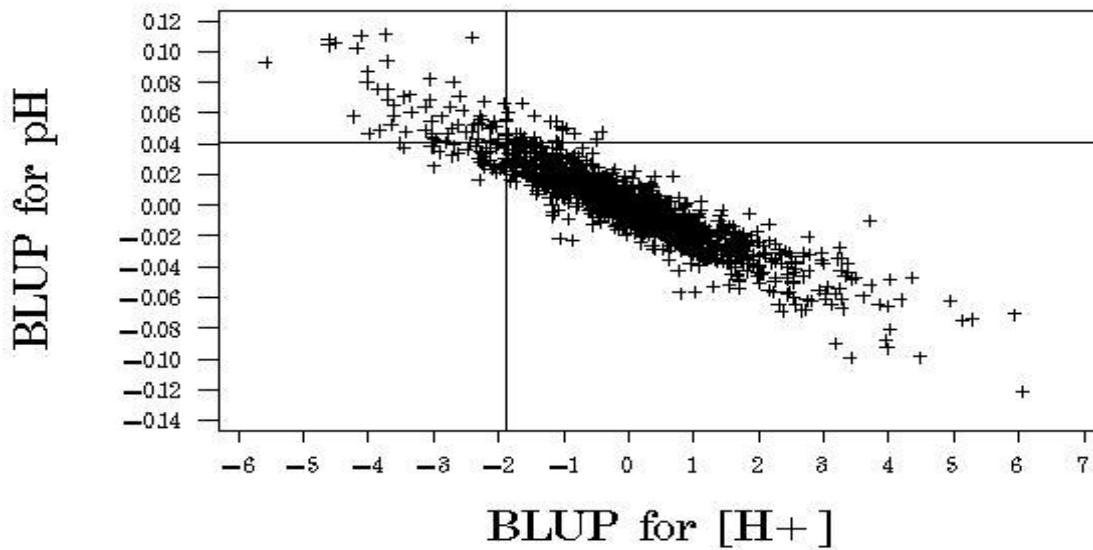
Table 4. Heritability Estimates (\pm SE) & Correlations of pH and H⁺ of pork meat from the National Barrow Show™ Progeny Test.

Trait	$H^2 \pm SE$	Correlation of BLUP Breeding Values
pH	0.52 \pm 0.074	
Hydrogen ion concentration	0.62 \pm 0.078	
Pearson Correlation Coefficient		-0.92
Spearman Rank Correlation Coefficient		-0.85

Table 5. Genetic correlations (\pm SE) between hydrogen ion concentration, pH and indicators of pork quality from the National Barrow Show TM Progeny

Trait	pH	Hydrogen ion Concentration
H concentration	-0.9676 \pm 0.0124	
Minolta L*	-0.5425 \pm 0.0818	0.5088 \pm 0.0803
Lipid	-0.0021 \pm 0.1075	0.0068 \pm 0.1015
InstronTM	-0.1257 \pm 0.1275	0.1235 \pm 0.1222
Hunter Color Test.	-0.6262 \pm 0.0958	0.6241 \pm 0.0925

Figure 2. Example truncation selection for pH and Hydrogen ion concentration based on top 5 percent breeding values.



Selection Intensity	pH Selection Differential			H+ Selection Differential			
	Select on pH	Select on H+	Selection Differential	Select on pH	Select on H+	Selection Differential	Selection Differential Loss, %
25%	0.016	0.015	0.01	-0.78	-0.82	-0.04	4.8
5%	0.034	0.036	0.02	-1.80	-1.93	-0.13	6.7
1%	0.062	0.054	0.08	-2.53	-2.91	-0.38	13.1

Loss in H+ selection differential by selecting on pH instead of H+ ranges from 5% to 13% depending upon selection intensity utilized. This is a consequence of not selecting individuals in the lower left quadrant of the graph, instead choosing those in the upper right. Graph shows 5% selection intensity.

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