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# Variations of early postmortem pH in carcasses of grass-fed steers and its relationship with glycolytic potential and meat quality traits

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This study aimed to investigate the multivariate relationship between the glycolytic potential, and meat quality traits from grass-fed steers carcasses with variations in early *postmortem* pH. From a contemporary group of steers (n=70) from the same production unit and slaughtered under similar conditions, thirty carcasses (10/group) were selected based on pH values measured at 3h ($pH_{3h}$): Low (<6.2), Intermediate $pH_{3h}$ (6.2–6.5), and High (>6.5). Carcasses segregated by $pH_{3h}$ groups were different ($p < 0.05$) in muscular glycogen (MCG), glucose (G+G6P) content, glycolytic potential (GP) and $GP_{strict}$ (GP without lactate content). The interaction $pH_{3h}$ groups × sampling time was significant only for lactate content (LC) ($p < 0.05$). Quality traits, except redness did not vary ($p > 0.05$) among $pH_{3h}$ groups. Color variables had a positive and moderate correlation with MGC, G+G6P, LC, and GP. Results does not allow to recommend early carcass segregation by very early pH (3h *postmortem*); however, important bivariate and multivariate relationships between G+G6P, lactate content and instrumental color parameters in LL muscle from grass-fed cattle were demonstrated.

*Key words*: beef, color, tenderness, lactate, glycogen, glucose

## Introduction

The rate and extent of pH fall significantly influences meat quality traits, including color development, shelf life, water holding capacity, and tenderness (Young et al. 2004, Wu et al. 2014, Wang et al. 2021). These parameters are important for consumers and the meat processing industry.

Postmortem glycolytic potential allows to measure the potential lactate formation and it has been recognized to be highly correlated with ultimate pH (Wulf et al. 2002, England et al. 2016). The pH decline is correlated with the enzymatic degradation of glycogen to lactate and this process includes the metabolic coupling between glycogenolysis and glycolysis, and that are strongly upregulated by the AMPK (Strobel et al. 2021). The activities of various enzymes involved in glycogen synthesis and depletion play an important role in the rate and extent of glycolysis (Apaoblaza et al. 2017). Normal concentrations of muscle glycogen immediately at the time of slaughter and an adequate cooling managing of carcasses are relevant to let an efficient glycogenolytic/glycolytic flow required for lactate accumulation and pH decline (Strobel et al. 2021). Also, final meat quality is affected by the course of temperature in the postmortem period (Hamoen et al. 2013), specially at the onset of rigor (Hannula and Puolanne 2004). Immonen et al. (2000) studying the relationship between residual carbohydrate and ultimate pH, found that at low pH values (< 5.8), glycogen and glucose concentrations varied considerably, from 83 to 10 mmol $kg^{-1}$.

Skeletal muscle is composed of different types of fibers that affect the rate of energy metabolism in particular glycolysis (Hopkins et al. 2014). Each muscle fiber type has different glycolytic and oxidative capacities, contraction speed, fiber size, myoglobin, and glycogen content (Schiaffino and Reggiani 1994). Muscle fiber type I has slow-twitch, oxidative metabolic characteristics, and a low glycogen content. Type IIA is a fast oxidative–glycolytic fiber. On the other hand, type IIB has fast-twitch, glycolytic metabolic characteristics, and a high glycogen content (Schiaffino and Reggiani 1994, Karlsson et al. 1999). Type I (fast-twitch) fibers have low ATPase activity and are more prone to inactivation by a rapid pH decline (Bowker et al. 2004).

Many researchers have investigated the potential of using pH as a predictor variable for meat quality. Jones and Tatum (1994) studied the relationship between tenderness and the muscular pH measured at 3-hour *postmortem* ($pH_3$), finding that $pH_3$ values lower than 6.2 were strongly associated with shear force or tenderness. Smulders et al. (1990) also reported that $pH_{3h}$ values below 6.3 were associated with a lower incidence of undesirable ratings for tenderness. Lomiwes et al. (2014) reported and important relationship between meat tenderness and ultimate pH ($pH_u$); these authors found that meat with high (≥ 6.2) and low (≤ 5.79) $pH_u$ were less tender after ageing.

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Wu et al. (2014) studied the effect of $pH_u$ in beef on the proteolysis of large structural proteins related to tenderness and contributed with evidence of the strong relationship between $pH_u$ and meat quality. Hamoen et al. (2013) compared experimental data on pH decline in carcasses to develop a prediction model for ultimate pH and meat quality; and Young et al. (2004) patented a method for early determination of meat ultimate pH. More recently, Shulte et al. (2022) reported a proteomic and metabolome study in beef *longissimus thoracis* muscles from carcasses classified by pH values at 6 h *postmortem*, resulting in apoptotic, calcium homeostasis, and proteasome systems influencing pH classifications that could explain the observed pH, proteolysis, and beef tenderness differences.

Few studies (Smulders et al. 1990, Jones and Tatum 1994) had included the association between very early *postmortem* (3 h *postmortem*) pH and meat quality traits; however, none of these had evaluated the glycolytic potential during early *postmortem*. The objectives of this study were to investigate the multivariate relationship between glycolytic potential and meat quality traits from grass-fed steers carcasses with variations in early *postmortem* pH ($pH_{3h}$).

# Materials and methods

## Animals, slaughter, carcass handling, and sampling

From a contemporary group (age approximately 24 months) of Angus and Friesian crossbred steers (n=70) raised on the same farm and fattened system (pasture, and supplementation with silage and minerals). Animals were transported in a single group in the same truck to the slaughterhouse. Total lairage time was 16 h. Animals had free access to drinking water, except during transportation. Animals were not mixed before or after transport. They were slaughtered on the same day, according to national regulations (INN 2002) (slaughter line speed is approximately 50 cattle/h). All carcasses were kept in the same cold chamber (temperature ranged between +0.4 to 2.8 °C) during 48 h postmortem. Carcass average weights were 275.4 ± 21.4 kg.

The muscular pH was measured with a using a pH meter (MPI pH meter, Meat probes Inc. USA) (at the level of the 12$^{th}$ rib) at 3 h ($pH_{3h}$) and a sample of approximately 1 g were taken from the same location and time using a Bergstrom biopsy needle. Samples were placed in Eppendorf tubes, identified, and immediately frozen in liquid nitrogen (–196 °C) and the maintained in ultra-freezer (–80 °C).

Carcasses were segregated according to Jones and Tatum (1994) as follows: Low $pH_{3h}$ (<6.29), Intermediate $pH_{3h}$ (6.3 to 6.49), and High $pH_{3h}$ (>6.5). A total of 10 carcasses per each $pH_{3h}$-group were selected for the study and muscular pH and sampling were accomplished (for selected carcasses) at 6, and 24 h *postmortem*. At 24 h *postmortem*, the entire LL muscle was removed from each left carcass side. Steaks of 2.5 cm-thick of each muscle were taken, and vacuum packed individually, one steak was used immediately to evaluate color and drip loss and two steaks were frozen at –20 °C for 30 days for further analysis.

## Glycogen, glucose, lactate contents and glycolytic potential

Muscular glycogen (MGC), glucose+glucose-6 phosphate (G+G6P), and lactate contents (LC) were determined as described in Apaoblaza et al. (2015). Briefly, muscle samples (100 mg) were homogenized in ice-cold phosphate buffer (pH 7). Ten µl of homogenate was hydrolyzed in 200 µl of 0.1M HCl at 100 °C for 2 h, after which pH was adjusted to 6.5–7.5. MCG and glucose (G+G6P) were determined via NADP reduction with a linked assay involving hexokinase and glucose-6-phosphate dehydrogenase (Glucose HK 16-50 Sigma). The LC was determined from the same homogenate via NAD reduction with a linked assay involving lactate dehydrogenase and glutamate pyruvate transaminase (Boehringer Mannheim). Glycolytic potential (GP) was calculated with the following formula GP = (LC) + 2([MGC] + [G+G6P]) (Monin and Sellier 1985). Also, the GP was calculated using the same equation excluding the LC (called $GP_{strict}$). Values were expressed as mmol $kg^{-1}$ of muscle.

## Meat quality traits

Instrumental color and drip loss (DL) were measured in fresh samples immediately (after 24 h *postmortem*). DL was measured according to Honikel (1998), briefly, samples ranging 80 and 100 g were used, each portion was identified and separately placed in an air-tight plastic bag and stored at a refrigeration temperature of 2 °C for 48 h; after which, the weight of the meat sample was recorded. The DL was expressed in the percentage of water released from the original sample. A Hunter Lab Mini Scan XE Plus (Hunter Associates, Reston, VA) was used with a 2.5-cm open port, Illuminant D65, and 10° standard observer to evaluate color objectively. Three readings will be

obtained from the muscle surface and the mean will be calculated. Readings were obtained after exposing muscles to air for 30 min (bloom). The Hunter L, a, and b values were taken as indicators of lightness, redness, and yellowness, respectively. For Warner-Bratzler Shear force (WBSF) and cooking loss (CL) evaluation, steaks were cooked in a convection oven (Albin Trotter model E-EMB Digital) to a final internal temperature of 70 °C following the guidelines of the American Meat Science Association (AMSA 2015). The temperature was monitored using an oven thermometer (–10 – +110 °C ± 1 °C) inserted into the geometric center of each steak. The cooked steaks were chilled for 2 h at 2 °C, and then between eight to ten cores (1.27 cm in diameter) were removed parallel to the muscle fiber orientation. Cores were sheared once each on the Warner-Bratzler Meat Shear apparatus (GR Manufacturing Co., Manhattan, USA) to get WBSF values. Cooking loss (CL) was determined by weight loss during exposure to high temperature expressed as percentage compared to the original weight of the sample.

## Statistical analysis

Analysis of variance was used with a linear mixed model consisting of $pH_{3h}$ groups as a fixed effect, sampling time as a repeated measure, and fat thickness as a covariate. The statistical significance threshold will be set at $p < 0.05$. Values were expressed as the mean ± standard error (SE). Tukey-Kramer multiple comparison test was used for the comparison of means at a 5% level of significance. The Bonferroni correction was also performed to adjust the probability of *p* values.

To analyze the relationship among the subgroup of the variables: meat quality traits with respect to the subgroups of glycolytic components traits and pH, a canonical correlation analysis (CCA) was carried out with meat quality data and glycolytic related data measured at 24 h *postmortem*. Wilk´s Lambda and Bartlett tests were used to determine the significance of canonical correlations. Additionally, bivariate correlations analyses were performed among all traits evaluated at 3, 6, and 24 h *postmortem*.

# Results

## Glycolytic potential and its components in LL muscles from carcasses grouped by $pH_{3h}$

The interaction $pH_{3h}$ group × sampling time was significantly different for LC ($p < 0.05$). The interaction $pH_{3h}$ × sampling time was not significant ($p > 0.05$) for the rest of the variable, therefore means were presented by main factors ($pH_{3h}$ group and sampling time) for MCG, G+G6P, GP and $GP_{strict}$.

Carcasses that were segregated by $pH_{3h}$ as Low (< 6.29), Intermediate (6.3 to 6.49), and High (> 6.5) were different in the MCG ($p = 0.03$), and G+G6P ($p = 0.01$). Carcasses classified as Intermediate $pH_{3h}$ had less MGC and G+G6P than those classified as High or Low $pH_{3h}$; consequently, Intermediate $pH_{3h}$ carcasses also showed the lowest value of Gpot and $GP_{strict}$ ($p = 0.02$; Table 1).

Table 1. Means and standard errors of the glycolytic potential and its components by $pH_{3h}$ groups

| Variables* | $pH_{3h}$ groups | | | *p*-value |
|---|---|---|---|---|
| | High (>6.5) | Intermediate (6.2 to 6.49) | Low (< 6.2) | |
| MGC | 12.32 ± 1.37 $^{b}$ | 5.97 ± 1.44 $^{a}$ | 11.77 ± 1.14 $^{b}$ | 0.03 |
| G+G6P | 4.93 ± 0.52 $^{ab}$ | 3.69 ± 0.54 $^{a}$ | 5.86 ± 0.58 $^{b}$ | 0.01 |
| LC | 38.34 ± 1.76 | 38.34 ± 1.42 | 41.82 ± 2.05 | 0.20 |
| GP | 72.84 ± 6.02 $^{ab}$ | 57.67 ± 4.70 $^{a}$ | 77.08 ± 4.07 $^{b}$ | 0.02 |
| $GP_{strict}$ | 34.50 ± 4.38 $^{b}$ | 19.32 ± 3.64 $^{a}$ | 35.27 ± 3.78 | 0.02 |

MGC = Muscular glycogen content; G+G6P = Glucose + Glucose 6-phosphate; LC = Lactate content; GP = Glycolytic Potential. $GP_{strict}$ = GP without LC; * mmoles $kg^{-1}$; n = 30

At 3 h *postmortem*, carcasses from the Intermediate and High groups exhibited the lowest content of lactate compared to carcasses from the Low $pH_{3h}$ group (Table 2; $p < 0.05$). A similar trend was observed at 24 h, but without being statistically different ($p > 0.05$). The LC increased with time, resulting in exhibiting the highest values of LC, the carcasses categorized as Low $pH_{3h}$.

Sampling times were statistically different ($p < 0.05$) for all traits except G+G6P. The MGC, and $GP_{strict}$ significantly decreased with time ($p < 0.05$), and LC increased ($p = 0.01$) with time (Table 3). On the other hand, G+G6P and GP were not affected by sampling time ($p > 0.05$).

Table 2. Effect of the interaction $pH_{3h}$ groups × sampling time on lactate content

| Sampling time | $pH_{3h}$ groups | | |
|---|---|---|---|
| | High (>6.5) | Intermediate (6.2 to 6.49) | Low (< 6.2) |
| 3 h | 31.22 ± 0.86 $^{a/d}$ | 33.87 ± 1.15 $^{a/d}$ | 40.55 ± 1.01 $^{b/d}$ |
| 6 h | 42.24 ± 0.85 $^{a/e}$ | 39.53± 0.94 $^{a/e}$ | 42.01 ± 0.95 $^{a/de}$ |
| 24 h | 41.54 ± 0.98 $^{a/e}$ | 39.88 ± 0.05 $^{ab/e}$ | 45.34 ± 0.09 $^{a/e}$ |

$^{a,b,c}$: different letters within the same row indicate $p < 0.05$; $^{d,e,f}$: different letters within the same column indicate $p < 0.05$. Values are presented as means ± SE in mmoles $kg^{-1}$. n = 10/group

Table 3. Means and SE of the glycolytic potential and its components by sampling time

| Variables* | Sampling time | | | *p*-value |
|---|---|---|---|---|
| | 3 h | 6 h | 24 h | |
| MGC | 15.00 ± 1.19 $^{b}$ | 9.17 ± 1.47 $^{ab}$ | 5.61 ± 1.47 $^{a}$ | 0.001 |
| G+G6P | 4.94 ± 0.56 | 4.46 ± 0.49 | 5.13 ± 0.63 | 0.85 |
| LC | 35.21 ± 1.38 $^{a}$ | 41.21 ± 1.87 $^{b}$ | 42.26 ± 1.89 $^{b}$ | 0.01 |
| GP | 75.11± 4.85 | 69.54 ± 4.48 | 63.73 ± 5.87 | 0.48 |
| $GP_{strict}$ | 39.90 ± 4.38 $^{b}$ | 28.31 ± 3.72 $^{ab}$ | 21.48 ± 3.62 $^{a}$ | 0.01 |

MGC = Muscular glycogen conten; G+G6P = Glucose + Glucose 6-phosphate; LC = Lactate content; GP = Glycolytic Potential; $GP_{strict:}$ = GP without LC; n = 30/sampling time. * expressed as mmoles $kg^{-1}$

Figure 1 shows the dynamic of decreasing pH values for Low (< 6.2), Intermediate (6.2–6.5), and High (> 6.5) pH3h from 3 h to 24 h postmortem. The pH3h groups were significantly ($p < 0.05$) different at 3 h and 6 h. The highest ultimate pH values were consistently from the High pH3h group, also noticeable that the drastic decline occurred between 3 and 6 h postmortem. The highest decreased variation of pH from 24 to 3 h was observed in the High pH3h carcasses (–10.41%) compared to the Intermediate pH3h (–6.69%) and the Low pH3h ones (–5.58%), respectively.

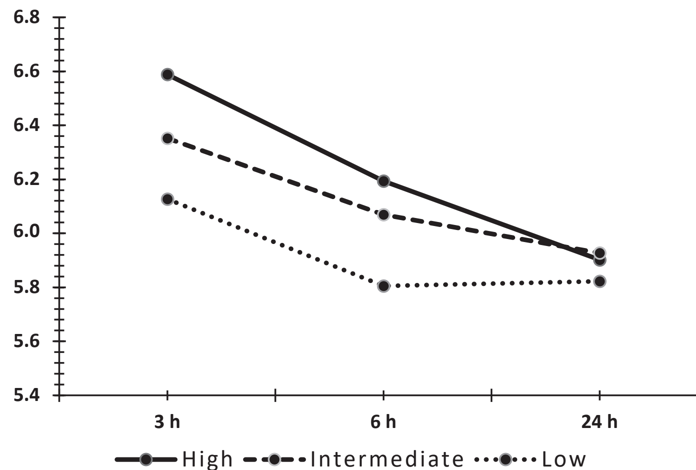


Fig. 1. Dynamic decreasing of pH values in carcasses by $pH_{3h}$ categories (Low:< 6.2; Intermediate: 6.2 to 6.5; and High:> 6.5). n = 10 per group

## Effects of early pH on meat quality traits

Means for WBSF, CL, DL, pH, L (lightness), b (yellowness) evaluated after 24 h *postmortem* were not statistically different ($p > 0.05$) when comparing $pH_{3h}$ groups (Table 4). However, early pH affected ($p = 0.01$) the redness (a values); meat samples from carcasses classified as Intermediate $pH_{3h}$ resulted in fewer redness intensities than the other $pH_{3h}$ groups.

The LL muscle from carcasses categorized as High and Low $pH_{3h}$ tended to have lower WBSF values than the Intermediate group (Table 4). Carcasses from the Intermediate $pH_{3h}$ group showed the highest DL values (0.92 ± 0.16), cooking loss (16.21 ± 0.45), ultimate pH (5.93 ± 0.05,) and WBSF (2.20 ± 0.09), without being statistically different from the other two groups ($p > 0.05$).

Table 4. Means and standard errors of beef traits according to $pH_{3h}$ groups evaluated after 24 h *postmortem*

| Variable* | $pH_{3h}$ groups | | | *p*-value |
|---|---|---|---|---|
| | High (>6.5) | Intermediate (6.2–6.49) | Low (<6.2) | |
| L | 24.06 ± 1.20 | 23.21 ± 0.69 | 23.84 ± 0.69 | 0.80 |
| a | 12.57 ± 0.35 [a] | 11.69 ± 0.64[b] | 13.56 ± 0.62 [a] | 0.01 |
| b | 7.84 ± 0.35 | 7.37 ± 0.40 | 8.18 ± 0.37 | 0.88 |
| CL, % | 14.81 ± 0.41 | 16.21 ± 0.45 | 14.91 ± 0.55 | 0.24 |
| DL, % | 0.77 ± 0.18 | 0.92 ± 0.16 | 0.75 ± 0.10 | 0.68 |
| WBSF, kg. | 1.82 ± 0.11 | 2.20 ± 0.09 | 1.80 ± 0.07 | 0.35 |
| beef pH | 5.90 ± 0.06 | 5.93 ± 0.05 | 5.82 ± 0.04 | 0.08 |

L = lightness; a = redness; b: yellowness; DL = drip loss (%); CL = cooking loss (%); WBSF = Warner Bratzler shear force (kg). n = 10 per group

## Canonical correlation and bivariate correlation

The canonical correlation analysis between the meat quality traits (U) and glycolytic related variables (V) revealed four canonical correlations resulted to be significant ($p < 0.05$). The first two canonical correlation resulted with an eigenvalue >1. Canonical redundancy analysis revealed that the first canonical correlation represents 76.80% of the explained variance, with a high degree of association between U and V (0.897). The second one represents 20.79% of the explained variance with lower degree of correlation (0.726). The total explained variance of two canonical correlation was 97.59%. The standardized canonical coefficient or canonical weight of original variables represent their relative contribution to the corresponding canonical variates. The largest canonical weights for the canonical variate U1 were detected in color related traits (a and L; Table 5) and the variable yellowness resulted with the largest correlation coefficient (0.959), followed by redness (0.942) and lightness values (0.812). These correlation coefficients also indicates that these variables exert an important contribution to the constitution of the canonical variate U1. Canonical variate U2 is represented mainly by CL (0.827), followed by WBSF (0.493) and L (–0.407); with a moderate correlation coefficient 0.623, and 0.729 for CL and WBSF, respectively. The variance of the canonical variate U1 associated with the variables of its own group represented 49.6% of the total data variation which could be attributed to the high loading values of the variables related to color. However, the variance of the canonical variate U2 only represented 17% both canonical variates sum up 66.6%.

Table 5. Standardized canonical coefficient (canonical weights) and canonical correlation coefficient (CCC) between the original variables and its canonical variate "Meat Quality" (U)

| Original variables | Canonical variate $U_1$ | | Canonical variate $U_2$ | |
|---|---|---|---|---|
| | Canonical weight | CCC | Canonical weight | CCC |
| L | –0.515 | 0.812 | –0.407 | –0.275 |
| a | –0.864 | 0.942 | –0.162 | 0.022 |
| b | 0.286 | 0.959 | –0.178 | –0.107 |
| DL | 0.032 | –0.116 | 0.033 | 0.127 |
| CL | –0.015 | 0.660 | 0.817 | 0.623 |
| WBSF | –0.120 | 0.241 | 0.493 | 0.729 |

L = lightness; a = redness; b = yellowness; DL = drip loss (%); CL = Cooking loss (%); WBSF = Warner Bratzler shear force (kg); n = 30

Table 6 shows the standardized coefficients and correlations coefficients between the original glycolytic components and their canonical variates (V1 and V2). The variables G+G6P and LC were the largest contributors to the formation of the canonical variate V1, mainly represented by G+G6P (0.542) and LC (0.384) with a high correlation coefficient (r= 0.92 and 0.90, respectively; Table 6). Regarding canonical variate V2, LC showed the highest canonical coefficient (1.775); however, only the variable $pH_u$ resulted with high correlation (r= 0.72);

therefore, this canonical variate (U2) showed an irregular conformation and canonical variate V1 is represented mainly by G+G6P and LC. The variance of the canonical variate V1 associated with the variables of its own group represented 54.3% of the total data variation which could be attributed to the high loading values of the variables G+G6P and LC; the variance of the canonical variate V2 is the lowest one (8.5%). Both canonical variates covered 62.8% of the total variation.

Table 6. Standardized canonical coefficient (canonical weight), and canonical correlation coefficient (CCC) between the original variables and the canonical variate "Glycolytic components" (V)

| Original variables | Canonical variate $V_1$ | | Canonical variate $V_2$ | |
|---|---|---|---|---|
| | Canonical weight | CCC | Canonical weight | CCC |
| MGC | –0.091 | 0.413 | –0.039 | –0.436 |
| G+G6P | 0.542 | 0.920 | –0.184 | –0.291 |
| LC | 0.384 | 0.900 | 1.775 | 0.245 |
| pH | –0.329 | –0.587 | –0.190 | –0.720 |

MGC = Muscular glycogen content; G+G6P = Glucose +Glucose 6-phosphate; LC = Lactate content; n = 30

Canonical cross correlation describes the correlation between variables and the opposite canonical variate (Tables 7 and 8). Color instrumental parameters (L, a, and b) showed the highest canonical cross loading with the canonical variate V1; meanwhile, for the V2, the cross correlations were significant for CL and WBSF with low to medium coefficients (Table 7). This result indicates a strong and significant linear correlation between these variables. In Table 8 is presented the canonical cross-correlation coefficients between original glycolytic related traits and canonical variates U1 and U2. As expected, G+G6P and LC showed the highest canonical cross correlation with U1 and all cross-correlation coefficient with U2 were very low, verifying that this canonical variate is not applicable.

Table 7. Cross correlations (canonical cross-loadings) between the meat quality traits and canonical variates V1 and V2

| Original variables | Canonical Variate V1 | Canonical Variate V2 |
|---|---|---|
| L | 0.729 | –0.200 |
| a | 0.845 | 0.016 |
| b | 0.861 | –0.078 |
| Drip loss | –0.104 | 0.092 |
| Cooking loss | 0.592 | 0.452 |
| WBSF | 0.217 | 0.529 |
| Total variance, % | 43.7 | 4.5 |

WBSF = Warner Bratzler shear force; n = 30

The cross variance between meat quality traits (canonical variate U) and glycolytic related traits (V) accounted for 48.2% (Table 7) and the cross variance between V and U accounted for 48.9% (Table 8). The cross variance for the first canonical variate (U1 = 43.7% and V1 = 39.9%) confirmed that only the first canonical variates (U1 and V1) were representative of each group of variables based also of the fact that high correlation coefficients were detected between some original variables and the opposite canonical variate.

Table 8. Cross correlations (canonical cross-loadings) between the glycogenic related traits and canonical variates U1 and U2 (n = 30)

| Original variables | Canonical variate U1 | Canonical variate U2 |
|---|---|---|
| MGC | 0.371 | –0.317 |
| G+G6P | 0.826 | –0.211 |
| LC | 0.808 | 0.178 |
| $pH_u$ | –0.527 | –0.052 |
| Total variance, % | 39.9 | 9.0 |

The canonical correlation between meat quality traits and glycolytic related parameters (G+G6P, and LC) aligns with the results obtained from the linear correlation between these variables (Table 9). A bivariate correlation analysis was performed including data from all sampling times (3, 6, and 24 h) for glycolytic related traits. Results

showed that $pH_{3h}$ was not correlated ($p > 0.05$) with any of the meat quality characteristics; however, $pH_u$ showed a significant and negative ($p < 0.05$) correlation with L (r= –0.55), a (r= –0.59) and b (r= –0.59) values. Correlation coefficients indicate that, the L, a, and b values decrease as the pH increases. At all sampling times, MGC, G+G6P, LC, and GP showed significant ($p < 0.05$) and moderate correlation with colored-related variables: L, a, and b values (Table 8). The CL was correlated (P < 0.05) with G+G6P (at 3, 6, and 34 h *postmortem*), with LC and GP (at 6 and 24 h *postmortem*), and with pH at 6 h *postmortem*. DL and WBSF were not significantly correlated with any variable measured.

Table 9. Significant bivariate correlations between pH, glycolytic potential and its components, and meat quality traits at different sampling times

| Variable | Sampling times | L | a | b | DL | CL | WBSF |
|---|---|---|---|---|---|---|---|
| MGC | 3 h | 0.433 | 0.597 | 0.586 | – | – | – |
| | 6 h | 0.636 | 0.622 | 0.700 | – | – | – |
| | 24 h | 0.383 | – | 0.396 | – | – | – |
| G+G6P | 3 h | 0.619 | 0.725 | 0.764 | – | 0.514 | – |
| | 6 h | 0.685 | 0870 | 0.852 | – | 0.529 | – |
| | 24 h | 0.721 | 0.785 | 0.829 | – | 0.417 | – |
| LC | 3 h | 0.529 | 0.426 | 0.525 | – | – | – |
| | 6 h | 0.395 | 0.590 | 0.508 | – | 0.665 | – |
| | 24 h | 0.597 | 0.781 | 0.776 | – | 0.648 | – |
| GP | 3 h | 0.617 | 0. 89 | 0.310 | – | – | – |
| | 6 h | 0.708 | 0.781 | 0.817 | – | 0.459 | – |
| | 24 h | 0.661 | 0.760 | 0.786 | – | 0.467 | – |
| $GP_{strict}$ | 3 h | 0.515 | 0.666 | 0.667 | – | – | – |
| | 6 h | 0.674 | 0.701 | 0.763 | – | – | – |
| | 24 h | 0.608 | 0.625 | 0.772 | – | – | – |
| pH | 3 h | – | – | – | – | – | – |
| | 6 h | – | -0.351 | -0.351 | – | -0.413 | – |
| | 24 h | -0.550 | -0.591 | -0.591 | – | – | – |

Only significant r correlation coefficients ($p < 0.05$) are shown. MGC = Muscular glycogen content; G+G6P = Glucose +Glucose 6-phosphate; LC = Lactate content; GP = Glycolytic Potential; $GP_{strict}$ = GP without LC; L = lightness; a = redness; b = yellowness; DL: drip loss (%); CL = Cooking loss (%); WBSF = Warner Bratzler shear force (kg); n = 30

# Discussion

*Postmortem* factors, like temperature, pH rate of decline and aging time have a great impact on meat quality traits (Pogorzelski et al. 2022). At the slaughterhouse the ultimate pH (measured between 24 and 48 h *postmortem*) is a widely-used indicator of beef carcass quality (Hopkins et al. 2014). Few studies (Smulders et al. 1990, Jones and Tatum 1994, Shulte et al. 2022) had investigated the association between early *postmortem* pH and meat quality traits, but none of those had included the glycolytic potential and their compounds during the early *postmortem*.

The results from this study showed that carcasses classified by pH measured at 3h *postmortem* significantly varied in glycolytic potential, MGC and G+G6P content; however, there were not variation in meat quality traits except in redness (a value). The carcasses with an intermediate $pH_{3h}$ (6.2 to 6.49) exhibited less amount of muscular glycogen and G+G6P resulting with the lowest glycolytic potential when comparing to High or Low $pH_{3h}$ carcasses. Interaction $pH_{3h}$ category × sampling time was only significant for LC; which increased with postmortem time; however, lower values were found in Intermediate $pH_{3h}$ carcasses.

Immonen et al. (2000) investigating the relationship between glycogen and ultimate pH, found that in muscle with low pH values, residual muscle glycogen varied from 83 to 10 mmol $kg^{-1}$. In this study, MGC were below 45 mmol $kg^{-1}$ at 3h *postmortem,* and after 24 h MGC values ranged between 16.19 and 4.14 mmol $kg^{-1}$. Steers selected for this study were grass-fed without energy supplementation during the fattening phase; also they were fasted 16 h

before slaughtering, this condition could explain the very low levels of MGC detected in this study. Previous studies performed in the same region also reported very low levels of *postmortem* MGC. Amtmann et al. (2006) found that samples from *longissimus thoracis* muscles in grass-fed carcasses with pH >5.8 contained 14.9 ± 13.0 mmol $kg^{-1}$ of glycogen and in carcass with pH <5.8 found 35.5 ± 15.7 mmol $kg^{-1}$. Also, Apaoblaza et al. (2015) compared carcasses with high (> 5.9) and normal (<5.8) muscle pH at 24 h *postmortem* and reported MGC at 30 min post-slaughter of 65.5 ± 5.6 mmol $kg^{-1}$ in normal pH carcasses and 29.5 ± 7.22 mmol $kg^{-1}$ in high pH carcasses leading to 5.71 ± 0.01 and 6.34 ± 0.05 at 24 h, respectively. In this study, we did not take samples at 30 min *postmortem*.

*Postmortem* pH is influenced by the rates of ATP hydrolysis and glycolysis, phosphofructokinase activity, AMPK activity and to a much lesser extent, pH buffering capacity (Apaoblaza et al. 2015, Strobel et al. 2021, Wang et al. 2021). Chauhan and England (2018) indicated that the role of MGC in elevated $pH_u$ is quite clearly defined; however, the role in normal $pH_u$ is less recognized when MGC is not reduced. This discrepancy in variation in $pH_u$ in the presence of residual glycogen has been partially explained in an *in vitro* study utilizing a reconstituted muscle glycolytic buffer system (England et al. 2016). These authors proved that in the presence of glycogen excess, oxidative muscle still produces high ultimate pH, suggesting that the glycolytic capacity of muscle dictates ultimate pH instead of glycogen content. A rapid early-*postmortem* glycolytic metabolism in carcasses categorized as Intermediate $pH_{3h}$ suggests that initial MGC is not directly associated with early pH and that there is a variation on the rate of glycolysis which is not directly related to the initial pH.

In addition, pre-slaughter factors, such as feeding, transportation time, and environmental conditions affect both MGC and $pH_u$. Apaoblaza et al. (2017) evaluating the effect of the time of year and supplementation with flacked corn on MGC, LC and GP; found that the energy supplementation four weeks before slaughter reduced the depletion of muscle glycogen concentration but did not affect $pH_u$. Other factors, such as the type of muscle, its histology, and biochemical properties are also related to growth rate and the number and fiber types affect *postmortem* decline of pH (Onopiuk et al. 2016). Muscle fiber type I has a slow-twitch, with a higher oxidative metabolism and low glycogen content and Type II is a fast-twitch glycolytic metabolic manner with higher content of MGC (Schiaffino and Reggiani 1994). Variations in metabolite content, especially glycogen and lactate content can explain variations in meat quality (Choe et al. 2008). In this study, the $pH_u$ was similar ($p$ > 0.05) for all $pH_{3h}$ categories (Fig. 1) showing a more drastic decline between 3 and 6 h *postmortem,* especially in High $pH_{3h}$ carcasses. This result indicates that most of the biochemical changes occurred early in the very early *postmortem (*during the first six hours *postmortem)*. We could not measure pH and take muscle samples at 9, 12, and 18 h due to slaughter operative plant restrictions. Further studies should be focus on the first 6 h *postmortem*, since differences between 6 and 24 h *postmortem* were not significant ($p$ > 0.05).

In this study, GP and $GP_{strict}$ were significantly different ($p$ = 0.02) when comparing $pH_{3h}$ groups. Low and High $pH_{3h}$ carcasses resulted with similar ($p$ > 0.05) glycolytic potential and the Intermediate $pH_{3h}$ group of carcasses resulted with 15.17 and 19.42 mmol $kg^{-1}$ of lactate less that High and low $pH_{3h}$ groups, respectively. For decades, GP has been used as a good approximation to the total compounds transformable to lactic acid present in the muscle at slaughter (Monin et al. 1981, Monin and Sellier 1985). A curvilinear relationship was found between GP and ultimate pH, which described a threshold of 100 µmol $g^{-1}$ of GP, below which lower GP was associated with higher ultimate pH and above which GP had no effect on $pH_u$ (Wulf et al. 2002). However, more recently several studies (Holdstock et al. 2014, England et al. 2016) have reported that the rate of depletion of muscular pH is not only dependent on glycogen concentration and their metabolites. In this study, the $GP_{strict}$ represented 32% of the sum of all glycolytic compounds at 24 h *postmortem*, leaving the rest (68%) to LC; similar values were found at 6 h *postmortem*. This result agreed with Maribo et al. (1999), who stated that LC represents between 70 and 80% of the GP measured at 24 h *postmortem.* However, at 3 h *postmortem*, the amount of MCG + G+G6P and LC were close to 50%. The $GP_{strict}$ was studied considering that under *postmortem* conditions, gluconeogenesis is not possible, and lactate has not the potential to convert into glucose (unlike *in vivo* condition). *Postmortem* glycolytic potential expresses the potential of lactate formation (Monin and Sellier 1985), and it has been recognized to be highly correlated with ultimate pH (Wulf et al. 2002); however, results from another study (England et al. 2016) also had indicated that although glycolytic potential is correlated with the extent of metabolism, $pH_u$ is not necessarily substrate driven. Przbylski et al. (1994) reported a quadratic relationship between glycolytic potential and ultimate pH, considering only direct glycogenolysis products; highlighting that this relationship might be useful to predict the potential meat ultimate pH from muscle obtained from live animals. Other authors (Van Laack and Kauffman 1999, Immonen and Puolanne 2000, Copenhafer et al. 2006) stated that *postmortem* glycolysis may stop in the presence of residual glycogen. Immonen et al. (2000) demonstrated that muscle with pH < 5.8 could have a wide range of glycogen content, indicating that other factors influencing the rate and extent of glycolysis like fiber type and the activity of various enzymes related to glycogen metabolism affect *postmortem* pH decline. Our results

suggest that for GP determination in *postmortem* muscles is not necessary to include LC in the formula proposed by Monin and Sellier (1985), and therefore GP could be determined using only MGC and residual glucose contents.

The pH represents one of the most important characteristics of meat because it is highly related to meat quality (Young et al. 2004). In this study, meat quality traits like WBSF, lightness (L), yellowness (b) DL, and CL were not affected by early pH variation, but redness (a; $p$ = 0.01).

Lomiwes et al. (2014) reported a curvilinear relationship between tenderness and ultimate pH (24 h *postmortem*), explaining that the mechanisms that lead to meat tenderization were not the same for a high final pH (≥ 6.2) and a low pH (≤ 5.79) muscle. Some researchers reported that tenderness had a linear relationship with $pH_u$ in Friesan-Holstein calves (Guignot et al. 1994). Pulford et al. (2008) reported a curvilinear relationship between these traits with samples form 22-month-old Angus or Angus × Limousin bulls. On the other hand, Wu et al. (2014) in a study to investigate the effect of ultimate pH on meat tenderness using Angus bulls between 18 to 24 months of age, found that the lowest shear force was observed in the high $pH_u$ meat, followed by meat from the low $pH_u$ group, the meat with intermediate $pH_u$ showed the highest shear force values. Jones and Tatum (1994) used $pH_{3h}$ as a predictor parameter from carcasses produced under commercial conditions (n=240), finding that values lower than 6.2 were associated with a reduction in the variability of shear force. Also, Smulders et al. (1990) reported that $pH_3$ values below 6.3 were associated with a lower incidence of undesirable ratings for tenderness.

In this study, color-related parameters exhibited a positive and moderate correlation with MGC, G+G6P, LC, GP, and $GP_{strict}$ at any sampling time. Jeremiah et al. (1991) reported that colorimeter readings have a higher coefficient correlation with muscle $pH_u$ than color scores (evaluated by panelists). The results from this study confirm a significant relationship between MGC and meat quality traits such as pH and meat color. Also, Przybylski et al. (2016) reported evidence of a significant relationship between glucose and lactate measured in drip loss with muscle glycogen, lactate, glycolytic potential, and meat quality traits.

There is a consensus that the use of a CCA as a multivariate approach is appropriate for evaluating the interrelations among complex traits such meat quality and carcass traits (Vargas et al. 2018, 2021). For this study, CCA was suitable because it measures the magnitude of interrelations between sets of multiple variables (Ventura et al. 2011). In this study, CCA stablished that glycolytic related traits were represented by LC and G+G6P; on the other hand, meat quality traits were mostly represented with color instrumental parameter. The cross-correlation coefficients confirmed a strong association between these variables. Canonical weights are important parameters for defining the contribution of original variables to the canonical variates. However, the understanding of canonical loadings and cross-loadings is critical because these values describe the correlation between original and canonical variates. The CCA was useful in describing the relation between meat quality traits and glycolytic metabolites, avowing the multicollinearity bias providing insights that may contribute to the beef industry to get better early *postmortem* predictors for meat quality. Also, it would allow to characterize meat quality considering glycolytic metabolites measured at a few hours *postmortem*. Our findings represent the first evidence of a strong multivariate relationship of meat quality traits with glycolytic components in *postmortem* muscle.

Ultimate pH is a very common parameter used to evaluate carcass quality and as a predictor of meat quality. This technique is simple, but it is measured 24 h after refrigeration and it is not possible to take a decision before that time. Also, in the industries with a high rate of carcass production, conventional pH testing in chillers is a difficult task (Young et al. 2004). This study did not offer strong evidence to recommend the measurement of early pH (measure at 3 h *postmortem*) in beef carcass instead of ultimate pH as an indicator of meat quality, since variation in meat quality traits evaluated were not correlated with meat quality traits or glycolytic metabolites, but LC. However, the results from this study provided a better understanding of early *postmortem* pH and its relationship with *postmortem* glycolysis. Rather than using early pH to predict meat quality, it would be possible to predict the potential meat ultimate pH from glycogen muscle, residual glucose, or lactate content from early *postmortem* samples. Edwards et al. (2010) reported a significant relationship between blood lactate concentration and pH measured at 1 h *postmortem*. Przybylski et al. (2016) concluded that simple measurement of glucose and lactate by applications of biosensors can be a good predictor of glycolytic potential and pH. A patent (WO 001112844) online method of predicting ultimate pH from a rapid measurement of glycogen potential was reported by Young et al. (2004) suitable to be used in slaughterhouses.

The results also suggest that to identify changes in metabolites and enzymes, the muscle samplings should be carried out more often than just at 0.5 and 24 h, at least within the first six hours *postmortem,* since we observed that most of the *postmortem* variation in MGC, LC, GP and pH occurred during the first six hours *postmortem*.

Apparently, a 24-hour period would be too long to find differences in some parameters of muscle metabolisms that could finally affect meat quality. The determination of glycolytic enzymes and AMPK activity will be improved knowledge of how pH and temperature affect the biochemical and physical conversion of muscle to meat in beef will provide an understanding of how to optimize control of *postmortem* metabolism to ensure uniformity of carcass quality and reduce meat quality defects like dark cutting meat.

# Conclusions

Results from this study indicate that the measurement of early pH (at 3 h *postmortem*) does not allow to recommend to segregate carcasses by pH, since the evolution of pH and the glycolytic potential differ in carcasses with an intermediate pH compared to the others and meat quality traits were not affected by $pH_{3h}$.

Canonical correlation analysis is an optimized multivariate technique for evaluating the existence or non-existence of relationships between complex groups of variables. In this study it proves to be a powerful tool to study the relationship between the selected set of meat quality traits and glycolytic potential components. An important relationship between glucose and lactate content and instrumental color parameters in beef LL muscle from grass fed cattle were demonstrated.

Considering that under *postmortem* conditions, gluconeogenesis is not possible, and lactate has not the potential to convert into glucose, the measurement of $GP_{strict}$ calculated with glycogen and glucose content resulted as a suggested way to measure the glycolytic potential in *postmortem* muscles, without considering the lactate content. This recommendation deserves further validation.

The results strongly suggest that to investigate early postmortem metabolism, the muscle samplings should be concentrated within the first six hours *postmortem,* since we observed that most of the *postmortem* variation glycolytic related traits and pH occurred during the first six hours *postmortem*.

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