

**EFFECT OF DIFFERENT ULTIMATE pH RANGE
ON MEAT QUALITY OF CROSSBRED POLISH
HOLSTEIN × LIMOUSIN HEIFERS**

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Key words: beef, pH_u value, meat quality.

Abstract

Fifty *longissimus thoracis* muscle samples were obtained from the chilled (48 h, 2–4°C) right carcass side of crossbred Polish Holstein x Limousin heifers (PHF x LIM). Vacuum-packaged samples were chill-stored (0–2°C) for five days, and then the proximate chemical composition, physicochemical properties and sensory attributes of meat were determined. In order to evaluate the influence of pH level on meat quality, the samples were divided into four groups based on their pH_u values: ≤ 5.4 , 5.4–5.7, 5.8–6.0 and > 6.0 . Meat with $pH_u > 6.0$ had the lowest dry matter and fat content, and meat with pH_u 5.5–5.7 had the highest dry matter and fat content. Meat with the highest pH_u (> 6.0) was characterized by the highest total water-soluble nitrogen content, the darkest color, the highest water-holding capacity and the highest scores in a sensory evaluation. No significant ($p > 0.05$) differences in the mean values of the analyzed physicochemical and sensory properties were found between samples of normal quality meat with pH_u of 5.5–5.7 and 5.8–6.0. It can be concluded that there is no need to divide the meat of crossbred PHF x LIM heifers with pH_u 5.5–6.0 into groups based on its technological quality for processing.

**WPLYW RÓŻNEJ WARTOŚCI pH KOŃCOWEGO NA JAKOŚĆ MIĘSA JAŁÓWEK
MIESZAŃCÓW POLSKA HOLSZTYŃSKO-FRYZYJSKA
ODMIANA CZARNO-BIAŁA × LIMOUSINE**

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Słowa kluczowe: wołowina, wartość pH_u , jakość mięsa.

A b s t r a k t

Materiał badawczy stanowiły próbki mięśnia *longissimus thoracis* pobrane z 50 losowo wybranych, wychłodzonych (48 h, 2–4°C) prawych półtuszy jałówek mieszańców uzyskanych z krzyżowania krów rasy polska holsztyńsko-fryzyjska odmiana czarno-biała z buhajami rasy limousine (PHF × LIM). Zapakowane próżniowo próbki przechowywano w warunkach chłodniczych (0–2°C) przez 5 dni, a następnie przeprowadzono analizę ich podstawowego składu chemicznego oraz ocenę właściwości fizykochemicznych i sensorycznych. W celu określenia wpływu wartości pH mięsa na jego jakość, próbki podzielono na cztery grupy, w zależności od wartości pH_u : $\leq 5,4$; 5,4–5,7; 5,8–6,0; $> 6,0$. W badaniach wykazano, że zdecydowanie najmniejszą zawartością suchej masy charakteryzowało się mięso o wartości $pH_u > 6,0$, natomiast największą – mięso o wartości pH_u 5,5–5,7. Zawartość tłuszczu w mięsie kształtowała się podobnie jak zawartość suchej masy. Największą całkowitą zawartość azotu związków rozpuszczalnych w wodzie stwierdzono w mięsie z najwyższą wartością pH_u ($> 6,0$). Mięso to odznaczało się także najciemniejszą barwą, największą wodochłonnością oraz zdecydowanie najlepszą jakością w ocenie sensorycznej. Nie stwierdzono istotnych różnic ($p > 0,05$) między średnimi wartościami analizowanych cech fizykochemicznych i sensorycznych mięsa „normalnego” z wartością pH_u 5,5–5,7 oraz 5,8–6,0. Tym samym uzyskane wyniki nie wskazują na potrzebę dzielenia mięsa jałówek mieszańców PHF x LIM o wartości pH_u 5,5–6,0 na dodatkowe grupy technologiczne o zróżnicowanej jakości przetwórczej surowca.

Introduction

The pH value is an important indicator of meat quality. It is closely correlated with many other properties of meat, which affect its processing suitability and culinary uses, such as water-holding capacity, color, tenderness and shelf-life (JELENIKOVÁ et al. 2008, KNOX et al. 2008, HAMOEN et al. 2013, GLAMOCLIIJA et al. 2015). The meat pH is an easy-to-measure parameter that provides valuable information about post-mortem muscle glycolysis, thus enabling to detect quality defects of meat such as PSE (pale, soft, exudative meat) and DFD (dark, firm, dry meat) (RAMMOUZ et al. 2004).

The rate of post-mortem glycolysis may be too fast, leading to a rapid drop in pH (typical of PSE meat), or too slow, resulting in too high ultimate pH (typical of DFD meat) (KNOX et al. 2008). In both cases, abnormal physicochemical properties of meat (color, water-holding capacity) are developed. Such meat has limited processing suitability and low consumer acceptance, which generates vast economic losses (ADZITEY and NURUL 2011).

Consumer expectations regarding the quality of meat and meat products have risen over the years. Therefore, producers have to select raw materials characterized by the highest technological quality for processing. Differences in the quality of PSE, DFD and normal meat have been extensively documented in the literature (PARK et al. 2007, KNOX et al. 2008, WĘGLARZ 2010, ADZITEY and NURUL 2011, HOLDSTOCK et al. 2014). However, research findings (PURCHAS 1990, DEVINE et al. 1993, SILVA et al. 1999) have shown that the attributes of normal quality meat may also vary, although it remains unknown whether this is a general rule.

The objective of this study was to determine the effect of ultimate pH (pH_u) on the quality of meat from crossbred Polish Holstein-Friesian Black-and-White x Limousin heifers.

Materials and Methods

Materials. The experimental materials comprised samples of *longissimus thoracis* (LT) collected from 50 randomly selected half-carcasses of crossbred heifers produced by crossing Polish Holstein-Friesian (PHF) Black-and-White cows with Limousin (LIM) bulls. All animals were purchased by the same meat processing plant from the same producer. The identification of crossbreeds was based on mating certificates and their characteristic color. The animals rested in lairage for 20–24 hours before slaughter. Carcasses weighing 210 to 300 kg were analyzed.

The carcasses were chilled for approximately 48 hours at 2–4°C, and pH_u was measured in *longissimus dorsi* between 12–13 rib interfaces, on the right half-carcass. The pH of the muscle was measured with the use of a combination Double Pore electrode (Hamilton Bonaduz, Bonaduz, Switzerland) and a pH 340i pH-meter equipped with a TFK 150/E temperature sensor (WTW Wissenschaftlich-Technische Werkstätten, Weilheim, Germany) previously calibrated using two buffers (pH 4 and 7). LT samples were collected from right half-carcasses at the level of the last four thoracic vertebrae. The samples were vacuum-packaged in polyethylene bags polyamide/polyethylene (PA/PE) bags and were chill-stored (0–2°C). Five days post mortem, the samples were analyzed to determine meat quality.

Methods. The samples were taken out from bags, then stored for 0.5 hours at a temperature of 4°C, and meat color (1 point – light, 8 points – dark) and marbling (1 point – invisible, 5 points – very strong) were evaluated. Next some of the samples were used for a sensory analysis, and the remaining samples were put through a laboratory mincer with a 3 mm diameter mesh plate three times. Minced meat was mixed thoroughly, and the obtained samples were analyzed to determine the proximate chemical composition and physicochemical properties of meat.

Analysis of the chemical composition of meat

The analysis of the proximate chemical composition of meat included the determination of dry matter content (samples were dried at 105°C to constant weight), total protein content by the Kjeldahl method (Kjeltec System 1026

Distilling Unit (Tecator AB, Hoganas, Sweden), fat content by Soxhlet extraction with diethyl ether as the solvent (Soxtec System HT2, Tecator AB, Hoganas, Sweden) and ash content (by incineration at 550°C to constant weight) (AOAC 1990). The content of nitrogen fractions in the water extracts of meat (total nitrogen and non-protein nitrogen) was determined by the Kjeldahl method. The protein nitrogen content of the water extracts of meat was calculated as the difference between total nitrogen and non-protein nitrogen. The water extracts of meat were prepared as described by HERRING et al. (1971).

Physicochemical properties of meat

An analysis of the physicochemical properties of meat included the determination of pH measured in the water homogenates of 10 g muscle tissue (muscle tissue to distilled water ratio of 1:1) using a combination Polilyte Lab electrode (Hamilton Bonaduz, Bonaduz, Switzerland) and a 340i pH-meter equipped with a TFK 325 temperature sensor (WTW Wissenschaftlich-Technische Werkstätten, Weilheim, Germany); color brightness – determined based on the percentage of light reflection against the surface of minced meat (Spekol spectrophotometer and remission attachment R45/0, 560 nm wavelength, VEB Carl Zeiss, Jena, Germany); water-holding capacity by the Grau and Hamm method (VAN OECKEL et al. 1999).

Sensory analysis of meat

The sensory attributes (aroma, taste, juiciness, tenderness) of meat were determined after heat treatment at a temperature of $96\pm 2^\circ\text{C}$, in a 0.6% solution of NaCl (the ratio between meat and solution was 1:2), which lasted until a temperature of 80°C was achieved inside the samples (BARYŁKO-PIKIELNA et al. 1964). Approximately 2 cm x 2 cm x 2 cm cubes of meat were cut from the middle of each cooked sample and wrapped in aluminium foil. Coded meat samples were presented to the panellists at room temperature. A taste-panel evaluation was made by five trained panelists (*Sensory analysis...* ISO 8586:1993). The panellists used a 5 – point hedonic scale (1 point – the worse, 5 points – the best) to express the intensity and desirability of traits (DASZKIEWICZ et al. 2012). Distilled water was made available to the panelists for mouth cleansing between samples. All sensory attributes of each sample were evaluated during a single session. A maximum of five meat samples were assessed per session.

Statistical analysis

In order to evaluate the influence of acidity on meat quality, the samples were divided into four groups based on their pH_u values (measured in LT 48 hours post mortem): ≤ 5.4 ($n=18$), $5.4-5.7$ ($n=20$), $5.8-6.0$ ($n=6$), > 6.0 ($n=6$). The results were processed statistically by one-way analysis of variance (ANOVA) for non-orthogonal designs in the Statistica ver. 10 program (Stat-Soft, Inc. 2011). The significance of differences between mean values in groups (at $P \leq 0.05$ and $P \leq 0.01$) was estimated by Duncan's test.

Results and Discussion

Chemical composition of meat and marbling

Meat with $\text{pH}_u > 6.0$ had the lowest dry matter content, and meat with pH_u 5.5–5.7 had the highest dry matter content (Table 1). The differences in average dry matter content observed in meat with various pH_u levels resulted from significant differences in fat content, which followed an identical pattern. Differences in the fat content of meat were reflected in marbling scores (Table 1). Marbling of LT samples with the highest pH_u was less than meat with $\text{pH}_u \leq 5.4$ ($P \leq 0.05$) and $5.5-5.7$ ($P \leq 0.01$).

Table 1
Proximate chemical composition [g kg^{-1}] and marbling (points) of meat in relation to pH_u (means \pm SD)

Trait	pH_u value of meat			
	≤ 5.4 ($n = 18$)	$5.5-5.7$ ($n = 20$)	$5.8-6.0$ ($n = 6$)	> 6.0 ($n = 6$)
Dry matter	277.53 ± 22.4^A	280.42 ± 21.40^A	269.45 ± 11.72^a	244.95 ± 5.67^{Bb}
Fat	36.39 ± 17.87^a	43.68 ± 24.85^A	36.38 ± 18.68^a	14.65 ± 6.63^{Bb}
Total protein	217.95 ± 8.03	217.76 ± 9.31	221.53 ± 6.64	219.85 ± 1.53
Ash	11.91 ± 1.16	11.50 ± 1.23	11.37 ± 1.35	11.58 ± 1.06
Water/protein ratio (W/B)	3.32 ± 0.13^a	3.31 ± 0.14^a	3.30 ± 0.08^a	3.43 ± 0.04^b
Marbling	2.92 ± 1.13^a	3.33 ± 1.34^A	2.33 ± 0.41	1.67 ± 0.61^{Bb}

Values in the same row with different letters are significantly different, $^{AB} - P \leq 0.01$; $^{ab} - P \leq 0.05$

No significant ($P > 0.05$) differences in the percentages of total protein and ash were found between meat samples with different pH_u values (Table 1).

The results of studies investigating relationships between the proximate chemical composition and pH of meat are inconclusive and contradictory. HOLDSTOCK et al. (2014) observed no significant differences in the content

of dry matter, protein and fat between muscles (*longissimus thoracis*) with different average pH_u values (5.57, 5.83, 6.62). In contrast, LAWRIE and GATHERUM (1962), SOBINA (1998), AASLYNG et al. (2003) and DASZKIEWICZ et al. (2009), noted higher dry matter content in meat with low pH. The lowest fat content in meat with the highest pH was observed in our study. It is consistent with the findings of MELLER et al. (1998) and SOBINA (1998), whereas it does not support the previous research of AASLYNG et al. (2003) who noted the opposite relationship. The cited authors (MELLER et al. 1998, SOBINA 1998, AASLYNG et al. 2003,) demonstrated that meat characterized by low acidity had the lowest total protein content, which was not observed in our study.

An analysis of water-soluble nitrogen fractions revealed that meat with the highest pH_u (> 6.0) had the highest content of water-soluble protein and non-protein nitrogen and, consequently, the highest total water-soluble nitrogen content (Table 2). Meat with pH_u 5.8–6.0 contained the lowest concentrations of the above nitrogen fractions.

Table 2

Nitrogen fractions in meat in relation to pH_u (means \pm SD)

Trait	pH_u value of meat			
	≤ 5.4 ($n = 18$)	5.5–5.7 ($n = 20$)	5.8–6.0 ($n = 6$)	> 6.0 ($n = 6$)
The ratio between total N of water-soluble compounds and total N in meat [%]	27.58 \pm 1.42 ^{aa}	26.55 \pm 1.59 ^A	25.83 \pm 0.82 ^{ab}	31.61 \pm 2.25 ^B
The ratio between N of water-soluble non-protein compounds and total N in meat [%]	12.27 \pm 1.38	12.03 \pm 0.77	11.41 \pm 1.57 ^a	12.62 \pm 0.75 ^{kb}
The ratio between N of water-soluble protein compounds and total N in meat [%]	15.30 \pm 1.20 ^A	14.52 \pm 1.66 ^A	14.41 \pm 1.75 ^A	18.99 \pm 2.52 ^B

Values in the same row with different letters are significantly different, ^{AB} – $P \leq 0.01$; ^{ab} – $P \leq 0.05$

The differences in the content of water-soluble nitrogen in meat with various pH_u values were probably due to different activities of proteolytic enzymes in the early post-mortem period (KEMP et al. 2010, WU et al. 2014). In the initial stage of meat aging during the normal course of glycolysis, non-lysosomal enzymes (calpains) are activated at high pH values. As post-mortem glycolysis progresses, meat acidity increases, the activity of calpains decreases and the activity of lysosomal enzymes (cathepsins) increases as they become involved in autolysis initiated by calpains (LOMIWES et al. 2014). Lower concentrations of nitrogen compounds were noted in the water extracts of meat with pH of 5.8–6.0, because such pH values do not promote the activity of calpains or cathepsins.

Physicochemical properties of meat

An evaluation of the physicochemical properties of meat revealed that meat with the highest pH_u (> 6.0) was characterized by the significantly ($P \leq 0.05$) darkest color and the highest water-holding capacity in comparison with other groups (Table 3). Presented results confirm the literature data. No significant differences in the mean values of the evaluated physicochemical parameters (color and WHC) were noted between samples of normal quality meat and meat with $\text{pH}_u \leq 5.4$. However, despite the absence of significant ($P > 0.05$) differences, the values of water-holding capacity varied across samples of normal quality meat. A tendency towards lower water-holding capacity was observed in meat with pH_u 5.8–6.0 relative to meat with pH_u 5.5–5.7.

Table 3
Physicochemical properties of meat in relation to pH_u (means \pm SD)

Trait	pH_u value of meat			
	≤ 5.4 ($n = 18$)	5.5–5.7 ($n = 20$)	5.8–6.0 ($n = 6$)	> 6.0 ($n = 6$)
$\text{pH}_{48 \text{ h}}$	5.32 ± 0.06^A	5.54 ± 0.05^B	5.81 ± 0.02^C	6.27 ± 0.12^D
$\text{pH}_{168 \text{ h}}$	5.32 ± 0.06^A	5.60 ± 0.08^B	5.82 ± 0.04^C	6.35 ± 0.15^D
Color brightness [%]	12.67 ± 1.68^A	12.70 ± 2.05^A	12.33 ± 1.37^A	9.67 ± 1.03^B
Color (points)	4.72 ± 0.67^A	5.23 ± 0.99^a	5.08 ± 0.97^a	6.17 ± 0.61^{Bb}
Water-holding capacity [cm^2]	7.21 ± 1.31^A	6.92 ± 1.47^a	7.65 ± 0.80^A	5.22 ± 1.49^{Bb}

Values in the same row with different letters are significantly different, $^{ABCD} - P \leq 0.01$; $^{ab} - P \leq 0.05$

The correlations between the pH and color of meat have been widely described in the literature. In meat with high pH_u , fibers are tightly packed and meat structure is closed. Such meat is dark because its surface does not scatter light to the same extent as the more open surface of meat with lower pH_u (SEIDEMAN et al. 1984, LI et al. 2014). In addition, the closed structure of meat reduces the diffusion of oxygen into the muscle from the surface, and any oxygen reaching the interior is used up by the high activity of the cytochrome encouraged by the high pH. This results in a thin surface layer of bright red oxygenated myoglobin (MbO_2) allowing the purple color of the underlying reduced myoglobin (Mb) to show through (WARRISS 2000, ABRIL et al. 2001, MIN et al. 2002, LI et al. 2014).

The differences in the water-holding capacity of meat with various acidity levels result from the fact that at high pH_u , proteins far from their isoelectric points can bind more water and the water-holding capacity of meat increases (PARK et al. 2007). The water-holding capacity of meat is also related to the autolytic degradation of cytoskeletal proteins (HUFF-LONERGAN and LONERGAN

2005, ZHANG et al. 2006, PEARCE et al. 2011). The degradation of cytoskeletal proteins (desmin, talin and vinculin) reduces the connections between the myofibrils and the sarcolemma, and between the myofibrils, which are involved in the transmission of longitudinal and lateral shrinkage of the myofibrils to the entire muscle cell during rigor mortis. Thus, water can move into the extracellular space where it is lost as drip. Proteolysis of the cytoskeletal framework of the muscle cell leads to the weakening of the sarcolemma, followed by expulsion of water from the extracellular space to the muscle cell. As a result, the water-holding capacity of meat increases. Nevertheless, LI et al. (2014) demonstrated that the degradation of muscle proteins (mostly desmin) may be slower at pH 5.8–6.2 than at higher pH values, which can be associated with lower water-holding capacity of meat, as confirmed by our study.

Sensory attributes of meat

Meat with $\text{pH}_u > 6.0$ received the highest scores for taste, tenderness and juiciness (Table 4). Meat with pH_u 5.8–6.0 was characterized by the lowest values of the above sensory attributes. The differences between mean values in these groups were significant. There were no significant ($P > 0.05$) differences in the mean values of the analyzed sensory properties between samples of meat with pH_u 5.5–5.7 and 5.8–6.0. However, a tendency towards lower sensory quality was noted in meat with pH_u 5.8–6.0.

Table 4
Sensory properties (points) of meat in relation to pH_u (means \pm SD)

Trait	pH_u value of meat			
	≤ 5.4 ($n = 18$)	5.5–5.7 ($n = 20$)	5.8–6.0 ($n = 6$)	> 6.0 ($n = 6$)
Aroma – intensity	5.00 \pm 0.00	4.93 \pm 0.24	5.00 \pm 0.00	5.00 \pm 0.00
Aroma – desirability	4.97 \pm 0.11	4.90 \pm 0.26	5.00 \pm 0.00	5.00 \pm 0.00
Taste – intensity	4.17 \pm 0.49 ^a	4.33 \pm 0.63	3.83 \pm 0.75 ^A	4.83 \pm 0.26 ^{Bb}
Taste – desirability	4.25 \pm 0.46	4.33 \pm 0.65	3.83 \pm 0.75 ^A	4.67 \pm 0.52 ^B
Tenderness	4.19 \pm 0.75	4.08 \pm 0.71	3.83 \pm 0.68 ^a	4.67 \pm 0.26 ^b
Juiciness	4.06 \pm 0.48	3.83 \pm 0.63 ^a	3.67 \pm 0.82 ^A	4.58 \pm 0.49 ^{Bb}

Values in the same row with different letters are significantly different, ^{AB} – $P \leq 0.01$; ^{ab} – $P \leq 0.05$

Our results, which point to higher tenderness of beef with high pH (> 6.0) in comparison with meat with intermediate pH values (5.8–6.0), corroborate the findings of other authors (PURCHAS 1990, DEVINE et al. 1993, JELENIKOVÁ

et al. 2008, DASZKIEWICZ et al. 2009, PULFORD et al. 2009, WU et al. 2014). According to PURCHAS (1990), meat with pH of around 6.0 is characterized by the lowest tenderness.

The results of the sensory evaluation are consistent with the previously described post-mortem changes in meat, affected by endogenous proteolytic enzymes (calpains and cathepsins). Their lower activity at pH 5.8–6.3 as well as slower degradation of myofibrillar proteins decrease meat tenderness (SILVA et al. 1999). According to TAKAHASHI (1996), changes in tenderness during post-mortem meat aging result from the direct effect of calcium ions on myofibrils, which is determined by pH values, similarly to the activity of the calpain system. It should be noted that the sensory impressions or sensations experienced during meat consumption are interrelated. For instance, tenderness and juiciness are closely related. The more tender the meat, the more quickly the juices are released by chewing and the more juicy the meat appears, which was observed in our study and reported by other authors (SILVA et al. 1999, BINDER et al. 2004, JAWORSKA and PRZYBYLSKI 2014). High juiciness of meat with high pH results also from water binding which contributes to lower drip loss during thermal processing.

Conclusions

1. The results of the present study revealed differences in the proximate chemical composition, physicochemical properties, sensory attributes and processing suitability of normal quality meat, PSE meat ($\text{pH} \leq 5.4$) and DFD meat ($\text{pH} > 6.0$).

2. No significant differences were found for the physicochemical properties and sensory attributes of normal quality meat with pH_u in the ranges of 5.5–5.7 and 5.8–6.0. It can be concluded that there is no need to divide the meat of crossbred PHF \times LIM heifers with pH_u 5.5–6.0 into groups based on its technological quality for processing.

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