INTEGRATED MULTIVARIATE ANALYSIS TO EVALUATE EFFECTS OF PRE-SLAUGHTER HANDLING ON PORK QUALITY

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ABSTRACT: The aim of this study was to investigate the effect of pre-slaughter handling on the occurrence of PSE (Pale, Soft, and Exudative) meat in swine slaughtered at a commercial slaughterhouse located in the metropolitan region of Dourados, Mato Grosso do Sul, Brazil. Based on the database (n=1,832 carcasses), it was possible to apply the integrated multivariate analysis for the purpose of identifying, among the selected variables, those of greatest relevance to this study. Results of the Principal Component Analysis showed that the first five components explained 89.28% of total variance. In the Factor Analysis, the first factor represented the thermal stress and fatiguing conditions for swine during pre-slaughter handling. In general, this study indicated the importance of the pre-slaughter handling stages, evidencing those of greatest stress and threat to animal welfare and pork quality, which are transport time, resting period, lairage time before unloading, unloading time, and ambience.

KEYWORDS: transport operation, ambience, PSE meat, multivariate analysis.

ANÁLISE MULTIVARIADA INTEGRADA PARA AVALIAÇÃO DOS EFEITOS DO MANEJO PRÉ-ABATE NA QUALIDADE DA CARNE SUÍNA

RESUMO: Este trabalho objetivou investigar a influência do manejo pré-abate na incidência de carne PSE (*Pale, Soft and Exudative*), em suínos abatidos em um abatedouro comercial, localizado na região metropolitana de Dourados-MS. A partir do banco de dados (n=1.832 carcaças), aplicou-se análise multivariada integrada, com a finalidade de identificar, dentre as variáveis selecionadas, quais representavam maior relevância para o estudo. Os resultados da Análise de Componentes Principais indicaram que os cinco primeiros componentes explicaram 89,28% da variância total. Na Análise de Fatores, o primeiro fator representou as condições fatigantes e de estresse térmico para os suínos, durante o manejo pré-abate. De modo geral, o estudo indicou a importância das etapas do manejo pré-abate, evidenciando aquelas que representam maior estresse e ameaça ao bem-estar animal e à qualidade da carne suína, quais sejam: o tempo de transporte, de descanso médio nas baias, de espera no pátio, de descarregamento e ambiência.

PALAVRAS-CHAVE: operação de transporte, ambiência, carne PSE, análise multivariada.

INTRODUCTION

Meat products obtainment is performed in three stages: breeding (productivity parameters), transformation (carcass traits), and commercialization (sensory perception of meat quality by consumers) (ZURITA-HERRERA et al., 2011). Pork quality is associated with several factors

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affecting the animal before muscle transformation into meat, including the transformation process itself (COSTA et al., 2002). Final pH control is decisive for meat quality, as its functional properties are a function of *post-mortem* glycolytic reactions, which affect meat pH and may result in PSE meat (Pale, Soft and Exudative) (MENDES & KOMIYAMA, 2011).

The concept of animal welfare during pre-slaughter handling operations should be applied at all stages, helping to reduce animal stress promoted by stimuli (hunger, thirst, and fear) that cause physiological and metabolic changes (MOTA-ROJAS et al., 2012). The sudden environment change is one the main pre-slaughter handling problems (LUDTKE et al., 2012). Other problems, such as fasting time; human-animal interaction; operations of handling, loading, density, transport, and unloading; transport time and distance from farm to slaughterhouse; climatic conditions; and resting period at the slaughterhouse, may significantly affect animal homeostasis (SANTIAGO et al., 2012).

Studies on food production are complex, and its quality is determined by the combination of a number of factors, such as chemical constituents and complex physical structures (NAES et al., 1996). In this sense, these authors recommend the use of multivariate analysis to evaluate food quality. Multivariate analysis deals with principles and methods developed with the aim to simultaneously analyze correlated experimental variables. Thus, multivariate methods involve reduction processes, optimization, and sorting and classification of multidimensional data (GONÇALVES & FRITSCHE-NETO, 2012).

In this context, the multivariate statistical analysis may help slaughterhouses and producers to detect the main factors that affect pork quality, as well as the relationship among associated variables. Thus, the aim of this study was to investigate the factors involved in pre-slaughter handling operations that most influence the drop rate of pH *post-mortem* and, hence, pork quality.

MATERIAL AND METHODS

Data of 1,832 carcasses from 33 swine lots were analyzed from the database of a commercial slaughterhouse located in Dourados, Mato Grosso do Sul State, Brazil, from May 2009 to July 2010.

Meteorological data concerning the dates of carcass evaluations at the slaughterhouse were obtained from UFGD meteorological station. Using daily mean values of temperature and relative humidity, the air dry bulb (DBT) and wet bulb (WBT) temperatures were obtained by Psicrom® program (RORIZ, 2003); subsequently, temperature-humidity indices (THI) were calculated using Equation 1 (ROLLER & GOLDMAN, 1969).

$$THI = 0.45\ WBT + 1.35\ DBT + 32 \quad (1)$$

Nine variables, divided into two groups, were evaluated. The first group, *ante-mortem*, comprised: temperature-humidity index (THI), and total operation time of pre-slaughter handling, in hours (T6), composed of the variables loading time (T1), transport time (T2), lairage time before unloading (T3), unloading time (T4), and mean resting time in the piggery slaughterhouse (T5); the second, *post-mortem*, consisted of carcass hydrogen potential (pH_{45}) and carcass temperature, in °C (CT). To determine total operation time of pre-slaughter handling (T6), the sum of each activity duration time was performed: loading, transport, lairage time before unloading, unloading, and mean resting time.

Integrated multivariate statistics was used for data analysis. Initially, the Principal Component Analysis (PCA) was applied, which aims to analyze structures of correlations among traits, and eliminate those that contribute little to the group variation of evaluated individuals (GONÇALVES & FRITSCHE-NETO, 2012). Thus, the PCA method consists in transforming a set of variables Z_1, Z_2, -..., Z_p into a new set of variables Y_1 (PC_1), Y_2 (PC_2), ..., Y_p (PC_p) non-correlated among each other, and arranged in a decreasing variance order, which the first principal components have the highest variability of the original data (PAIVA et al., 2010). Considering the fact that the original variables have different measurement units, it is necessary to standardize them to estimate the principal components (GONÇALVES & FRITSCHE-NETO, 2012). Furthermore, coefficients of the principal components are indicators of the correlation magnitude among the standardized variables and principal components.

The Factor Analysis (FA) was then performed assuming that each variable can be decomposed into two parts: common and specific. The first part is the variation shared with the other variables, and the second one, its own variation, generating factors that may explain the greatest variance of the original data (PREARO et al., 2011). In this sense, FA may be applied in this study, since it aims to generate factors using a large data amount, and verify the dependence degree among variables through commonalities among them.

Data were then submitted to Cluster Analysis (CA) seeking the formation of groups with homogeneous and heterogeneous properties among them; homogeneous groups were analyzed, as well as differences among them, which should be as great as possible (HÄRDLE & SIMAR, 2012). We used the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), employing Mahalanobis distance. In addition, the Canonical Correlation Analysis (CCA) was performed with the aim to express the existing linear multidimensional relationships between two groups or set of variables (BRUM et al., 2011).

Therefore, all variables of this study were evaluated by the four methods of multivariate analysis mentioned above, except the T6 variable, which was assessed by three methods (PCA, CA and CCA). Multivariate statistical analyses were performed using GENES software (BIOAGRO - INSTITUTE OF BIOTECHNOLOGY APPLIED TO AGRICULTURE, 2013).

RESULTS AND DISCUSSION

The first five principal components (PC), which presented higher variance than 0.7, explained 89.28% of the original data preserved variance. The nine original variables can be analyzed from the five PC, what ensures a variability reproduction of approximately 90%, thus representing greater influence on pork quality. The individual contribution of each component was: PC1 = 37.76%; PC2 = 19.48%; PC3 = 13.65%; PC4 = 9.51%, and PC5 = 8.86% (Table 1).

TABLE 1. Variation proportion explained and accumulated by the Principal Components, for pork quality, based on 1,832 carcasses.

PC	$\lambda_{(1)*}$	p1%$_{(2)}$	p2%$_{(3)}$
PC_1	**3.39**	37.76	37.76
PC_2	**1.75**	19.48	57.24
PC_3	**1.22**	13.65	70.90
PC_4	**0.85**	9.51	80.42
PC_5	**0.79**	8.86	89.28
PC_6	0.68	7.61	96.90
PC_7	0.27	3.09	99.98
PC_8	<0.01	<0.01	99.99
PC_9	<0.01	<001	100

(1)Eigenvalues. *Values in **bold** indicate eigenvalues higher than or equal to 0.7. (2)p1 - Proportion of the explained variation. (3)p2 - Proportion of the explained and accumulated variation.

The four variables that showed higher coefficients from the last PC, that is, T6, T4, THI and CT, were excluded from the analysis due to the fact that they presented eigenvalues lower than 0.7, what indicates little contribution to the total variation. PAIVA et al. (2010), when studying production characteristics of laying hens, used the PCA technique and observed that, from 11 variables, only three (77%) were selected as the most important for the study. BARBOSA et al.

(2006), when assessing 10 pork quality characteristics (*post-mortem*), concluded that four variables (40%) could be discarded.

In this study, the variables that most contributed to total variation of pork quality were: transport time (T2), mean resting time (T5), hydrogen potential (pH_{45}), lairage time at the slaughterhouse (T3), and loading time (T1) (Table 2). Therefore, we may infer that variation in the first five PC is associated with pre-slaughter handling, which affects physical conditions of animals, altering their behavior during such operation; this is due to several factors, such as animal handling, loading, collection time, density, and transport (MOTA-ROJAS et al., 2012). Concepts of welfare and ambiance should be considered at all stages of pre-slaughter handling operations, as swine longer exposure to transport time, climatic factors, and poor road conditions increases animal stress, causing greater muscle glycogen depletion and influence on pH levels (OCHOVE et al., 2010). Despite the fact that ambience presents little contribution to total sample variation by PCA, it directly influences the animal thermal comfort, especially in Mato Grosso do Sul State, which is located in the Cerrado where climate is predominantly hot and dry. Swine welfare is influenced by climatic conditions that, at certain times of the year, exceed the limits of animal comfort (SARUBBI et al., 2012).

The pre-slaughter handling is considered one of the most stressful stages to animals, resulting in chain losses that occur due to multifactorial problems that involve swine, management, handling, transport, processing industry, and environmental factors (RITTER et al., 2009; SILVEIRA, 2010). In this study, total time of pre-slaughter handling (T6) considered values between 5.7 and 19 hours, with emphasis to transport time, mean resting time, lairage time at the slaughterhouse, and loading time, with greater weight among the other activities.

The PCA was important for variable selection because it enabled the identification of variables that most contribute to total variation of pork quality, which were T2, T5, pH_{45}, T3 and T1. In this study, PCA results indicated that 10.72% of the variables were redundant and may be discarded.

TABLE 2. Weighting coefficients of nine principal components extracted according the variables pH_{45}, CT, T1, T2, T3, T4, T5, T6 and THI.

Principal	Variables(2)								
Component	pH_{45}	CT	T1	T2	T3	T4	T5	T6	THI
PC1	0.0099	-0.0309	-0.2638	0.3658	0.4000	0.4952	0.3172	0.4652	0.2707
PC2	0.0186	0.2002	0.3655	0.3768	-0.0057	0.2336	-0.5225	-0.2727	0.531
PC3	0.6997	-0.662	0.2102	0.0204	-0.073	-0.0354	0.0294	0.0468	0.1338
PC4	0.525	0.3323	-0.2129	-0.2691	0.5265	0.1795	-0.2831	-0.1978	-0.2597
PC5	0.0632	0.3252	0.6693	-0.3940	0.214	-0.1064	0.2927	0.3243	0.1978
PC6	-0.4799	**-0.5518**	0.1678	-0.2968	0.5059	0.1485	-0.2418	-0.1070	-0.0328
PC7	-0.0027	0.0076	0.4622	0.4211	-0.0217	0.2517	-0.0663	0.1468	**-0.7205**
PC8	0.0000	0.0001	-0.0182	-0.4769	-0.5017	**0.7096**	-0.0849	0.0984	0.0004
PC9	0.0001	0.0001	-0.1313	-0.0561	-0.0593	-0.2578	-0.6242	**0.7211**	-0.0009

(1)In **bold**, the four variables subject to discard for presenting minor importance in the total variance explanation. (2)pH_{45} - hydrogen potential, 45 minutes after slaughter, CT - Carcass Temperature, T1 - Loading Time, T2 - Transport Time, T3 - Lairage Time at the slaughterhouse, T4 - Unloading Time, T5 - Mean Resting Time, T6 - Total Time of pre-slaughter handling, THI - Temperature-Humidity Index.

At the selection of retained factors, we used those that presented highest total variance proportions, from 60% and 80% as the ideal, or considered only eigenvalues higher than or equal to 1.0, which represent, at least, the variance information of an original variable (GONÇALVES & FRITSCHE-NETO, 2012). Commonalities among factors were higher than 0.70, what amounts to a correlation higher than or equal to 0.80 between the standardized variable and common part; this evidences great efficiency on the variable representation by a common part. In case of commonalities lower than these values, the variable must be excluded and factor analysis performed again (FIGUEIREDO & SILVA JÚNIOR, 2010), as indicated in Table 3.

The first factor (F1) grouped three variables, which were transport time (T2), temperature-humidity index (THI), and unloading time (T4), presenting factor loadings higher than 0.7; thus, it represents the fatiguing conditions and thermal stress to which the animal is submitted. Since these variables have similar effects on meat quality, they should be jointly analyzed. The swine, after certain transport time, may suffer serious physical consequences, such as dehydration, fatigue, breathing difficulty, and increased stress (YU et al., 2009; SILVEIRA, 2010); furthermore, the unloading time also affects the final meat quality, as animals undergo great physical efforts during such operation (SCHWARTZKOPF-GENSWEIN et al., 2012).

TABLE 3. Proportion of the explained variation and commonalities extracted by Factor Analysis after Varimax rotation with four factors, for pork quality, based on 1,832 carcasses.

$\lambda_{(1)*}$	p1%(2)	p2%(3)	Variable	Factor				$\varphi_{(4)}$
				F1	F2	F3	F4	
2.70	33.79	33.79	T2	0.904	-0.209	0.043	0.139	0.883
1.84	23.01	56.81	CT	0.070	-0.249	-0.876	0.105	0.846
1.32	16.56	73.37	pH_{45}	-0.063	0.911	0.143	0.086	0.863
0.87	10.97	84.35	T3	0.230	0.220	0.039	0.892	0.899
0.50	6.30	90.66	T1	0.150	0.487	-0.232	-0.655	0.743
0.41	5.21	95.88	T5	-0.013	-0.122	0.801	0.321	0.761
0.32	4.11	99.99	THI	0.856	0.155	-0.168	-0.034	0.786
0.00	0.00	100	T4	0.733	0.005	0.059	0.650	0.963

(1)Eigenvalues. *Values in **bold** indicate eigenvalues higher than or equal to 1.0. (2)p1 - Proportion of the explained variation. (3)p2 - Proportion of the explained and accumulated variation. (4)Commonalities. Underlined values indicate correlations higher than 0.7.

Therefore, longer transport time implies greater exposure of animals to climatic conditions, increasing animal thermal stress, especially in cold or hot regions. According to FIETZ & FISCH (2008), climate in Dourados consists of hot summers and dry winters, and may be divided into two periods: the first presents temperature means higher than 20 °C (from September to April), and the second, lower than that value (from May to August); high temperatures occur especially in December and January (summer), and low temperatures, between June and July (winter). This reflects on the animal transport period, and may be the reason for association of the variables T2, T4 and THI in the first factor (F1).

The distance between production and processing points justifies the animal transport, which may impact on animal welfare in three ways: the first, handling management, vehicle loading and unloading, and new environment lead to psychological stress (SILVEIRA, 2010); the second, fasting time and transport density comprise physiological and fatiguing challenges for animals (YU et al., 2009; MOTA-ROJAS et al., 2012); and the third, thermal and physical conditions of the vehicle represent a risk to the physical integrity of the transported animals (DALLA COSTA et al., 2009; OCHOVE et al., 2010). According to OWADA et al. (2007), although the animal physical health is accepted as a welfare estimator, it is still considered controversial whether only this measure would be enough, as these physiological indicators may be an animal natural response to activities or excitations.

Meat quality is intrinsically linked to animal production management, slaughter techniques, and final product industrialization. The second factor (F2) indicated the pH_{45} variable as the measure of pork quality. Therefore, we can infer that F2 is associated with responses from the chemical processes of muscle transformation into meat, which may be affected by *post-mortem* glycolytic reactions. Pork characteristics such as texture, juiciness, color, flavor, and aroma may be influenced by the biochemical changes that occur during muscle conversion into meat; its quality comprises decisive inherent properties that ensure success of the industrialization of meat products and, also, *in natura* meat market (SANTIAGO et al., 2012). According to LAVILLE et al. (2005), PSE meat represents a major quality problem in the pork industry due to low water-holding capacity, flaccid texture, pale color, and high water losses during processing, what is undesirable for both consumers and industry; it occurs more frequently in swine genetically susceptible to stress when submitted to stressful factors before slaughter.

The third factor (F3) grouped T5 and CT variables, indicating that it is associated with carcass temperature and resting time at the slaughterhouse. Thus, resting pens should be a peaceful environment, minimizing animal stress during pre-slaughter handling (DALLA COSTA et al., 2009). According to BAPTISTA et al. (2011), swine metabolic mechanisms maintain homeothermy when animals are submitted to high temperatures, so the ambient temperature influences both the surface and body temperatures, which may act as the animal physiological response to inadequate and adaptive environmental conditions (NASCIMENTO et al., 2011).

Therefore, the adequate resting time associated with pen conditions at slaughterhouses (easy access to water, sprinkler system, and fans) contribute to thermal stress reduction and animal recovery, thus better carcass temperature (SANTIAGO et al., 2012). In this study, the resting time varied between 3.5 and 14.5 h, so highest carcass temperatures (> 40 °C) occurred in swine that rested from 3.5 to 10 h; swine resting more than 10 h presented temperature decrease. SANTIAGO et al. (2012) found that resting times under than 6 h and over 8 h influenced the pH_{45} variable, which was associated with carcass temperature and PSE meat incidence.

Cluster analysis was performed by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), using Mahalanobis distance as a dissimilarity measure to assess whether there were differences among lots regarding the expression of the analyzed variables (Figure 1).

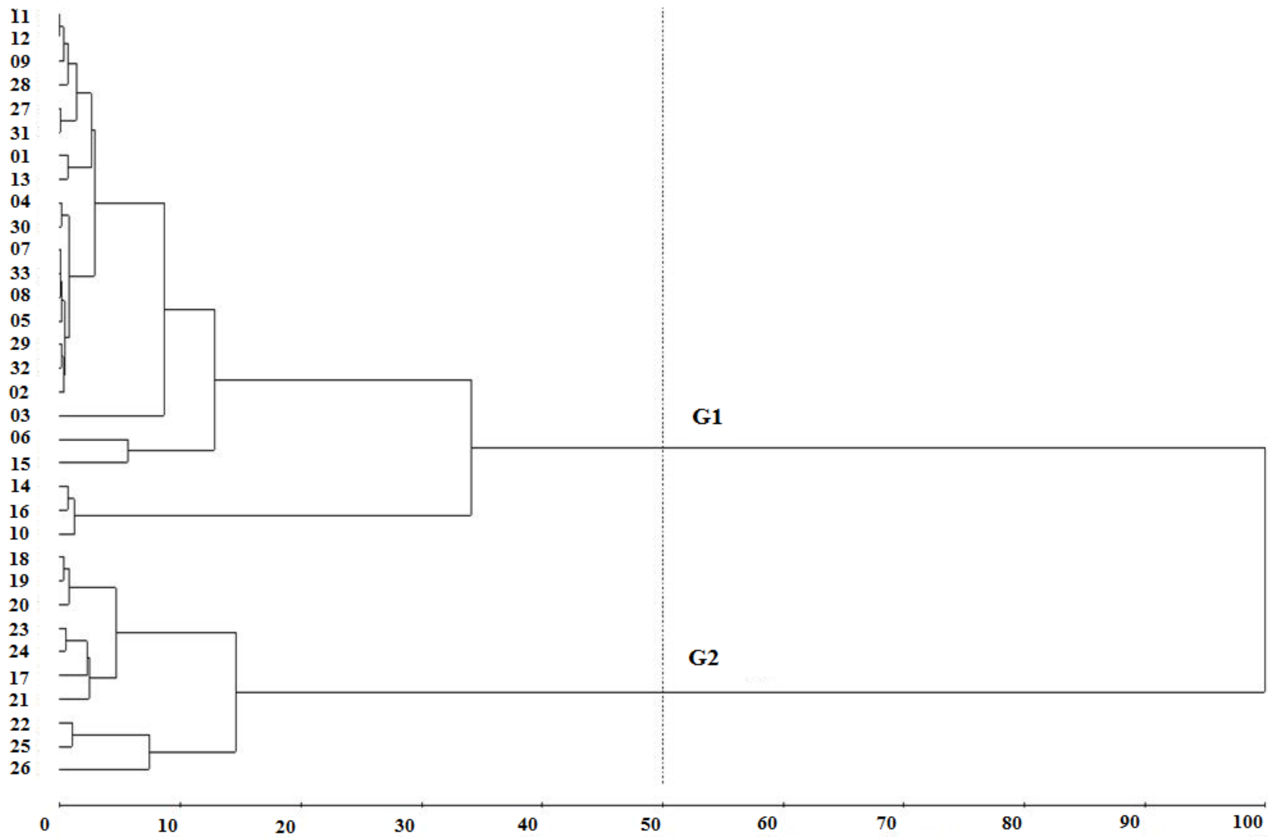

FIGURE 1. Dendrogram representing the dissimilarity among 33 studied lots. Cophenetic correlation (0.915**).

From a dendrogram standard cut in 50% dissimilarity, we may observe that only two groups are formed, with little difference between lots. This result allows us to infer that the lots have no influence on the analyzed variables; thus, pork quality does not depend on the assessed lots. Therefore, the standardization of pre-slaughter handling operations and temperature control, aiming at animal welfare and thermal comfort, may be similarly implemented for several lots. The most striking difference between G1 and G2 was probably caused by the difference of transport time, which is associated with the distance from farm to slaughterhouse; this is in agreement with SILVEIRA (2010) since the lots of the first group presented higher variation, so that variable is the most important one among samples.

Evaluation of groups formed by the variables showed that a 60% cut in the dendrogram generated two groups. The G1 grouped the pH_{45} variable and all those related to times comprising the pre-slaughter handling, including total time (T6); G2 grouped carcass temperature and temperature-humidity index (FIGURE 2).

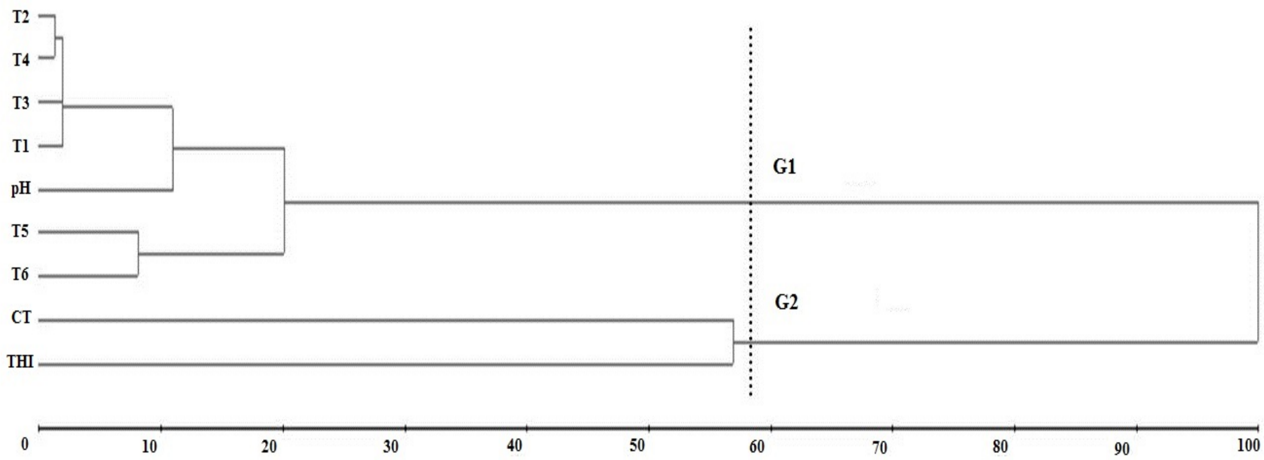

FIGURE 2. Dendrogram representing the dissimilarity among the studied variables, obtained by Unweighted Pair Group Method with Arithmetic Mean (UPGMA), using Mahalanobis distance as a dissimilarity measure. Cophenetic correlation (0.915**).

We may predict that the first group is associated with pre-slaughter handling (human-animal interaction, loading, transport time, and distance, among others), while the second one, with climate conditions, such as temperature and humidity, which may be the main reason for the difference between variables. The pH results allowed us to suppose that there is greater association of pre-slaughter handling variables (T1, T3, T4 and T2), followed by T5 and T6; also, that the other variables are little related to pH. Corroborating this study, LUDTKE et al. (2012), after analyzing pH, color, water-holding capacity, and drip loss, observed that the appropriate pre-slaughter handling did not influence meat quality, and that the resting time reduced the level of animal stress.

The phenotypic correlation matrix was used in the canonical correlation analysis to verify the existing associations between the variables *post-mortem* (group 3) and *ante-mortem* (group 4) (TABLE 4).

The first two canonical pairs were significant by the chi-square test, demonstrating that variables are not independent of each other. In other words, the *post-mortem* variables related to carcass temperature and pH_{45}, defined in group 3, indicate a strong correlation with the *ante-mortem* variables related to the times, comprising pre-slaughter handling, and THI (group 4). Therefore, groups 3 and 4 are of study interest.

Based on the canonical loads, we can observe that the first canonical pair, with canonical correlation rc = 0.6179 ($p<0.01$), associates carcasses with higher temperature (CT) and lower pH with carcasses with shorter mean resting time (T5) and total handling time (T6), as well as with better ambience (THI). Thus, to obtain carcasses with better pH and CT, both pre-slaughter handling and mean resting times must be adapted to the ambience. The ambient temperature may affect animal behavior and physiology, thus reducing animal capacity, production efficiency, welfare, and health (BANHAZI et al., 2008). This corroborates studies by KIEFER et al. (2010), who found that the thermal environment influences swine behavior ($p<0.01$). Pens with adequate ambiance associated with proper handling will contribute to better meat quality.

TABLE 4. Canonical pairs and loads of the variables in Groups 3 and 4.

GROUP	VARIABLE	CANONICAL PAIRS		CANONICAL LOADS	
		1st	2nd	1st	2nd
	pH	0.2028	1.0259	-0.1016	0.9948
3	CT	1.0403	0.1063	0.9810	-0.1939
	T1	1.78142	0.5089	0.1643	0.5634
	T2	15.1904	-5.4053	0.1158	-0.3690
	T3	14.8508	-3.8871	0.0137	0.3940
4	T4	-18.4171	6.8709	0.0657	0.0137
	T5	8.2237	-0.6049	-0.7719	-0.0431
	T6	-10.5639	0.7864	-0.5966	0.0639
	THI	0.1066	0.4141	0.3274	0.1987
$\hat{\rho}$		0.6179	0.3891		
χ^2		209.08	53.20		
D.F.		14	6		
α (%)		0.00	<0.001		

$\hat{\rho}$ - Canonical correlation, χ^2 - Chi-square, D.F. - Degrees of freedom, α (%) - Significance level.

Observing the canonical loads, we can infer that the second canonical pair, with canonical correlation rc = 0.3891 ($p<0.01$), associates carcasses with lower temperature and higher pH_{45} with carcasses with longer loading time (T1) and shorter transport time (T2), as well as with longer lairage time at the slaughterhouse (T3). During swine loading, principles of welfare must be respected, using management boards and slow animal movement towards the vehicle, with three to five animals at a time, under mild temperatures to avoid animal stress (SILVEIRA, 2010). The integrated use of several multivariate analysis methods improved the interpretation of the influence of the evaluated variables on pork quality. Similar results were found by PAIVA et al. (2010), who used the multivariate analysis combining multiple information originated from the sampling unit.

CONCLUSIONS

This study confirmed the importance of pre-slaughter handling operations to obtain higher pork quality. Among handling activities, transport, resting, and ambience should be managed by producers and slaughterhouses to minimize the impact on animal stress and incidence of PSE meat.

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