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Influence of pre-slaughter handling on stress responses, fresh and frozen pork quality, tenderness, and sensory attributes

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ABSTRACT

Pre-slaughter handling of pigs exerts a decisive influence on pork quality through modulation of physiological stress responses and post-mortem muscle metabolism. The present study evaluated the effects of standard handling, stressful handling and good management practices (GMP) on stress indicators, post-mortem pH decline, fresh and frozen pork quality, tenderness, oxidative stability and sensory attributes under Indian commercial conditions. Sixty market-weight pigs (100±5 Kg) were randomly allotted to three pre-slaughter handling treatments. Stress was assessed through behavioural indicators and serum cortisol concentration. *Longissimus dorsi* muscle samples were analysed for pH (45 min and 24 h), water-holding capacity, myofibrillar fragmentation index (MFI), Warner–Bratzler shear force (WBSF), lipid oxidation (TBARS), freeze–thaw loss and frozen storage stability up to 90 days at −18 °C. Sensory evaluation of fresh and frozen–thawed pork was conducted by a trained panel. Stressful handling significantly elevated cortisol concentration, accelerated post-mortem pH decline, reduced water-holding capacity, increased oxidative deterioration and lowered tenderness and sensory acceptability ($p < 0.05$). In contrast, GMP effectively minimized physiological stress, maintained desirable pH patterns and improved both fresh and frozen pork quality. The findings demonstrated that adoption of good pre-slaughter management practices is a practical, cost-effective and welfare-friendly strategy to enhance pork quality and frozen storage stability.

Keywords: Freeze–thaw stability, Good management practices, Pork quality, Post-mortem pH, Pre-slaughter stress, Sensory attributes

Pre-slaughter handling is one of the most critical determinants of pork quality and animal welfare in pig production systems. Stress associated with transport, handling, mixing, feed withdrawal and lairage activates the hypothalamic–pituitary–adrenal axis, resulting in elevated secretion of cortisol and catecholamines (Warriss 2000; Terlouw *et al*. 2008; Mohapatra *et al.* 2023). These physiological responses profoundly influence muscle energy metabolism, post-mortem glycolysis, and subsequent development of pork quality attributes (Ferguson and Warner 2008; Hambrecht *et al.* 2004).

The rate and extent of post-mortem pH decline are primary factors governing protein denaturation, proteolysis, water-holding capacity and oxidative stability of meat (Offer G 1988; Rosenvold and Andersen 2003; Huff-Lonergan and Lonergan 2005). Excessive pre-slaughter stress accelerates muscle glycogen depletion, leading to rapid pH decline and inferior meat quality characterized by higher drip loss, reduced tenderness and compromised sensory attributes. These effects are further exacerbated during freezing and frozen storage due to ice crystal formation, myofibrillar disruption and increased susceptibility to lipid oxidation (Leygonie *et al*. 2012, Bekhit *et al*. 2013).

In India, pork is increasingly marketed in frozen form to accommodate extended distribution chains and variable demand. Several studies have reported deficiencies in pre-slaughter handling practices and their adverse effects on meat quality under Indian conditions (Kandeepan *et al*. 2013, Nath *et al*. 2016, Biswas *et al*. 2020). However, integrated studies linking pre slaughter physiological stress responses with fresh meat quality, freeze–thaw stability and sensory attributes in pigs remain limited. The present investigation was therefore undertaken to provide a comprehensive evaluation of these interrelationships, with particular emphasis on frozen storage stability, which is highly relevant to emerging organized pork distribution and retail systems in India.

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MATERIALS AND METHODS

Experimental design and animals: Sixty crossbred pigs (Large White × Landrace), approximately six months of age and weighing 100 ± 5 kg, were randomly allocated to three pre-slaughter handling treatments (n = 20 per treatment). The experiment was conducted at the government piggery farm under the Animal Husbandry department during the winter season. The study was carried out over a defined experimental period, and all the animals were handled and slaughtered following the same standardized schedule to minimise day-to-day variation.

- *Control (C):* Standard commercial handling and transport (approximately 30 min; distance 20 Km)
- *Stressful (T1)*: Extended transport (approximately 90 min; distance 60 Km), rough handling, mixing of unfamiliar animals and prolonged lairage (6–8 h)
- *Good management practices (GMP) (T2)*: Short transport (20–30 min; distance 15 Km), gentle handling, minimal mixing, adequate lairage space with rest and access to water

All the animals were handled uniformly, electrically stunned, and slaughtered following the same standardized schedule under hygienic conditions following standard commercial procedures. Samples of *Longissimus dorsi* muscle were collected at 45 min and 24 h post-mortem.

Assessment of stress indicators: Behavioural stress indicators including vocalization, slipping, falling, and escape attempts were recorded during pre-slaughter handling and scored using a standardized scale. (Terlouw *et al*. 2008). Blood samples were collected during exsanguination and serum cortisol concentration was determined using a commercial ELISA kit.

Meat quality and water-holding capacity: Post-mortem pH of *Longissimus dorsi* muscle was measured at 45 min and 24 h using a calibrated microcontroller-based digital pH meter, (brand Systronics). Water-holding capacity was assessed by measuring drip loss, cooking loss and expressible moisture using standard procedures (Huff Lonergan and Lonergan 2005). Protein solubility was determined to assess the extent of protein denaturation.

Tenderness and proteolysis: Myofibrillar fragmentation index (MFI) was determined as an indicator of post-mortem proteolysis (Koohmaraie and Geesink G H. 2006). Instrumental tenderness was measured using Warner–Bratzler shear force (WBSF) on cooked samples. (Lawrie and Ledward 2006)

Freeze–thaw stability and oxidative changes: Pork samples were frozen and stored at −18 °C for up to 90 days. Freeze–thaw loss was calculated after thawing at refrigerated temperature (4 ºC). Lipid oxidation was quantified as thiobarbituric acid reactive substances (TBARS) and expressed as mg malondialdehyde (MDA)/kg meat (Lawrie and Ledward 2006).

Sensory evaluation: Fresh and frozen–thawed pork samples were evaluated after cooking. Pork curry was prepared using a standardized cooking procedure, and the sensory evaluation was carried out on the cooked curry samples by a trained sensory panel using an eight-point descriptive scale for appearance, tenderness, juiciness, flavour and overall acceptability. The sensory panel consisted of eight trained members drawn from the Department of Livestock Products Technology. Panelists underwent training sessions for two weeks to familiarize them with the sensory descriptors and evaluation procedure. Informed consent was obtained from all panelists prior to participation.

Statistical analysis: Data were analysed using one-way analysis of variance (ANOVA). Differences among treatment means were evaluated using Tukey's post hoc test and considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Stress indicators: The marked elevation in behavioural stress scores and serum cortisol concentrations observed in pigs subjected to stressful pre-slaughter handling clearly reflected activation of the hypothalamic–pituitary–adrenal axis (Table 1). Cortisol-mediated stress responses are known to accelerate muscle glycogen depletion before slaughter, thereby altering substrate availability for post-mortem glycolysis. Similar associations between pre-slaughter stress, muscle glycogen depletion and inferior pork quality have also been documented under commercial processing conditions (Hambrecht *et al.* 2004). Reduced glycogen reserves limit the muscle's buffering capacity and predispose carcasses to rapid early pH decline, a key biochemical driver of pork quality deterioration (Warriss 2000; Terlouw *et al*. 2008).

In contrast, pigs managed under good management practices (GMP) exhibited significantly lower cortisol concentrations, indicating reduced physiological stress. Lower stress load before slaughter plausibly preserved muscle energy reserves, enabling a more controlled post-mortem metabolic progression. These findings underscored the pivotal role of pre-slaughter handling in modulating endocrine stress responses that initiate downstream biochemical events influencing meat quality. These findings are consistent with previous reports highlighting the beneficial effects of improved handling practices on animal welfare and meat quality (Nath *et al*. 2016, Sharma *et al.* 2021).

Table 1. Behavioural stress score and serum cortisol concentration of pigs subjected to different pre-slaughter handling practices

Parameter	C	T1	T2
Behavioural stress score	5.30 ± 1.0^{b}	13.50 ± 1.6^{a}	3.10 ± 0.8^{c}
Serum cortisol (ng mL^{-1})	29.00 ± 4.1^{b}	56.20 ± 5.4^{a}	21.90 ± 3.6^{c}

Values are mean ± standard error (n = 20).

Means within a row with different superscripts (a–c) differ significantly ($p < 0.05$).

Table 2. Post-mortem pH values of *Longissimus dorsi* muscle from pigs subjected to different pre-slaughter handling practices

Parameter	C	T1	T2
pH (45 min)	6.34 ± 0.05^{b}	5.92 ± 0.06^{c}	6.46 ± 0.04^{a}
pH (24 h)	5.71 ± 0.04^{b}	5.54 ± 0.05^{c}	5.76 ± 0.03^{a}

Values are mean ± standard error (n = 20).

Different superscripts within a row indicate significant differences ($p < 0.05$).

Post-mortem pH and fresh meat quality: The accelerated pH decline observed in stressed pigs at both 45 min and 24 h post-mortem reflected intensified glycolytic flux resulting from stress-induced metabolic disruption (Table 2). Rapid early pH declines with muscle temperature remaining high promote denaturation of myofibrillar and sarcoplasmic proteins, particularly myosin, leading to reduced protein functionality and compromised water-holding capacity (Huff-Lonergan and Lonergan 2005; Scheffler and Gerrard 2007).

Conversely, the more gradual and higher ultimate pH observed in T2 pork indicated preservation of muscle metabolic integrity. Controlled pH declines favoured the maintenance of myofibrillar spacing and limit protein denaturation, thereby supporting superior water retention, tenderness and oxidative stability (Huff-Lonergan and Lonergan 2005; Scheffler and Gerrard 2007). These results reinforced the concept that post-mortem pH kinetics, rather than ultimate pH alone, are critical determinants of pork quality.

Water-holding capacity: Water-holding capacity is closely linked to the net charge and structural organization of myofibrillar proteins (Offer G 1988). The higher drip loss, cooking loss, and expressible moisture observed (Table 3) in T1 can be attributed to pH-induced protein denaturation and shrinkage of the myofibrillar lattice, which expelled immobilized water from the muscle structure. Stress-induced alterations in sarcoplasmic protein solubility might have further impaired the ability of muscle to retain water during processing and storage.

In T2 pork, improved water-holding capacity possibly indicated reflects preservation of myofibrillar architecture and reduced extent of protein denaturation. Enhanced water retention not only improved fresh meat juiciness and yield but also played a critical role in protecting muscle fibres from mechanical damage during freezing by limiting ice crystal growth and migration.

Table 3. Water-holding capacity parameters of pork as affected by pre-slaughter handling practices

Parameter	C1	T1	T2
Drip loss (%)	2.60 ± 0.3^{b}	4.20 ± 0.4^{a}	2.10 ± 0.2^{c}
Cooking loss (%)	24.50 ± 1.2^{b}	31.60 ± 1.5^{a}	22.30 ± 1.1^{c}
Expressible moisture (%)	17.80 ± 0.9^{b}	24.90 ± 1.2^{a}	15.60 ± 0.8^{c}

Values are mean ± standard error (n = 20).

Means with different superscripts within a row differ significantly ($p < 0.05$).

Freeze–thaw stability and oxidative changes: The significantly higher freeze–thaw losses and TBARS values observed (Table 4) in T1 pork during frozen storage highlighted the long-term consequences of pre-slaughter stress on meat stability. Poor initial muscle quality characterized by low pH and denatured proteins increased the susceptibility to ice crystal-induced disruption of muscle fibres, resulting in greater moisture exudation upon thawing. Additionally, stress-related depletion of endogenous antioxidant defences might accelerate lipid oxidation during frozen storage (Zhang *et al.* 2013).

In contrast, T2 pork exhibited superior freeze–thaw stability and lower lipid oxidation (Table 4), indicating that reduced pre-slaughter stress enhanced both structural resilience and oxidative stability of muscle tissue (Xia *et al.* 2009). These findings demonstrated that pre-slaughter handling indirectly governs frozen meat quality by modulating early post-mortem biochemical events, emphasizing upon the importance of welfare-oriented practices in frozen meat production systems.

Tenderness, proteolysis and sensory attributes: Post-mortem tenderization is primarily driven by calpain-mediated proteolysis of key myofibrillar proteins (Geesink and Koohmaraie 1999; Lonergan *et al.* 2010). The lower myofibrillar fragmentation index and higher shear force values observed in stressed pork (Table 5) suggested impaired proteolytic activity, potentially due to stress-induced alterations in calpain–calpastatin balance and unfavourable pH conditions. Rapid pH decline might have inhibited optimal calpain activity, limiting degradation of structural proteins essential for tenderization.

T2 pork exhibited enhanced proteolysis, reflected by higher MFI and lower shear force values, even after frozen storage. Improved tenderness was corroborated by sensory evaluation, with higher scores for tenderness, juiciness and overall acceptability (Lonergan *et al.* 2010). These sensory responses are mechanistically linked to favourable pH decline, improved water retention and sustained proteolytic activity, reinforcing the central role of pre-slaughter handling in shaping consumer-perceived meat quality.

The present study demonstrated that pre-slaughter

Table 4. Freeze–thaw loss and lipid oxidation (TBARS) of pork during frozen storage (−18 °C) as influenced by pre-slaughter handling

Parameter	C	T1	T2
Freeze–thaw loss (%)	4.70± 0.4^{b}	9.00 ± 0.7^{a}	3.80 ± 0.3^{c}
TBARS (mg MDA kg^{-1} meat) at 90 d	0.83 ± 0.07^{b}	1.48 ± 0.12^{a}	0.60 ± 0.06^{c}

Values are mean ± standard error (n = 20).

TBARS = Thiobarbituric acid reactive substances. Different superscripts within a row indicate significant differences ($p < 0.05$).

Table 5. Tenderness-related parameters and sensory scores of frozen–thawed pork from pigs subjected to different pre-slaughter handling practices

Parameter	C	T1	T2
Myofibrillar fragmentation index	78.60 ± 3.4[b]	62.10 ± 2.9[c]	85.40 ± 3.1[a]
Warner–Bratzler shear force (N)	39.50 ± 2.1[b]	52.80 ± 2.6[a]	34.70 ± 1.9[c]
Appearance	5.80 ± 0.4[b]	3.70 ± 0.4[c]	6.50 ± 0.4[a]
Tenderness score	5.90 ± 0.4[b]	4.10 ± 0.3[c]	6.70 ± 0.4[a]
Juiciness score	5.70 ± 0.3[b]	3.80 ± 0.4[c]	6.50 ± 0.3[a]
Flavour	5.60± 0.3[b]	3.90 ± 0.4[c]	6.40 ± 0.3[a]
Overall acceptability	5.80 ± 0.3[b]	4.20 ± 0.4[c]	6.90 ± 0.3[a]

Values are mean ± standard error (n = 20). Sensory scores were evaluated using an eight-point descriptive scale (1 = extremely poor; 8 = extremely desirable). Means with different superscripts within a row differ significantly ($p < 0.05$).

handling significantly affects pork quality through modulation of stress responses and post-mortem muscle metabolism. Stressful handling increased cortisol levels, accelerated pH decline, and resulted in poor water-holding capacity, higher lipid oxidation, reduced freeze–thaw stability, and inferior tenderness and sensory attributes. In contrast, good management practices (GMP) effectively minimized stress and improved both fresh and frozen pork quality. Adoption of scientifically designed pre-slaughter SOPs based on GMP can serve as a practical, low-cost strategy to enhance meat quality, ensure product consistency, and improve economic returns for the meat industry.

However, the study was limited by its sample size, specific handling conditions, and focus on a single muscle without molecular-level analysis. Future research needs to address diverse production systems, environmental factors, and underlying biochemical mechanisms.

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