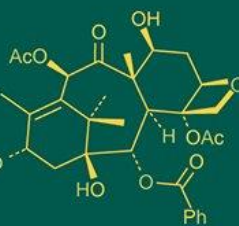
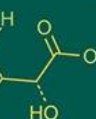
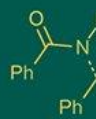


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(8): 01-10
www.biochemjournal.com
 Received: 06-04-2026
 Accepted: 09-05-2026

All authors' names and affiliations are given below, after the references.

Post-mortem physicochemical and biochemical changes in scientifically slaughtered and cold slaughtered broiler chicken meat

Hridhya Vijay C, Irshad A, Vasudevan VN, Suresh N Nair, Sathu T, Geetha N, Chinnu MV, Silpa S, Akhila VV and Thulasi K

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i8a.9384>

Abstract

Cold slaughtered broiler chicken meat is a food safety and meat authenticity concern because it is obtained from birds that die before slaughter and are processed without proper exsanguination. The present study compared early post-mortem physicochemical and biochemical changes in scientifically slaughtered (SS) and cold slaughtered (CS) broiler chicken meat. A total of 100 broiler chickens, comprising 50 SS and 50 CS birds, were evaluated. Breast and leg muscle samples were collected during 0-2, 2-4 and 4-6 h post-mortem intervals and analysed for carcass temperature, pH, R-value, lactic acid concentration and malachite green test reaction. Muscle temperature declined significantly during the first 6 h post-mortem in both groups. The pH of breast and leg muscles decreased progressively with time, with CS samples showing lower pH than SS samples. At 4-6 h, breast muscle pH was 5.83 ± 0.04 in SS and 5.63 ± 0.04 in CS, while leg muscle pH was 6.02 ± 0.02 in SS and 5.81 ± 0.02 in CS. R-value increased significantly with post-mortem time in both groups, indicating progressive ATP degradation. Lactic acid concentration also increased with time and was higher in CS meat, particularly during the early post-mortem period. The malachite green test revealed imperfect bleeding in 68 per cent of breast and 70 per cent of leg muscle samples from CS carcasses, whereas all SS samples showed normal bleeding. The findings indicate that cold slaughtered meat undergoes altered post-mortem biochemical changes and shows greater residual blood retention. Combined assessment of pH, R-value, lactic acid and malachite green test may provide useful supportive indicators for detecting cold slaughtered broiler chicken meat.

Keywords: Broiler chicken, Cold slaughter, Scientific slaughter, Post-mortem glycolysis, Lactic acid, R-value, Meat quality

Introduction

Poultry meat is one of the most widely consumed animal protein sources because of its high nutritional value, affordability, consumer acceptance, rapid production cycle and absence of major religious restrictions. After slaughter, muscle undergoes a series of post-mortem biochemical and physicochemical changes, including ATP depletion, anaerobic glycolysis, lactic acid accumulation, pH decline and rigor mortis development. These changes directly influence important meat quality attributes such as colour, tenderness, water-holding capacity and oxidative stability^[1-4].

Scientific slaughter involves proper handling, stunning and effective exsanguination, which helps in efficient blood removal and normal progression of post-mortem metabolism^[5]. In contrast, cold slaughtered meat is obtained from birds that die before slaughter due to transport stress, disease, suffocation, heat stress or other causes. Such birds may be classified as dead-on-farm or dead-on-arrival, depending on the stage at which death occurs. Since these carcasses are not subjected to proper exsanguination, residual blood remains within the tissues, which can alter biochemical reactions, affect meat quality and accelerate deterioration.

The illegal processing and marketing of cold slaughtered birds as fresh poultry meat, particularly in unorganised retail sectors, is an important concern related to food safety, meat authenticity and consumer protection. Previous studies have reported that meat from dead or improperly bled birds differs from properly slaughtered meat in pH, residual blood content,

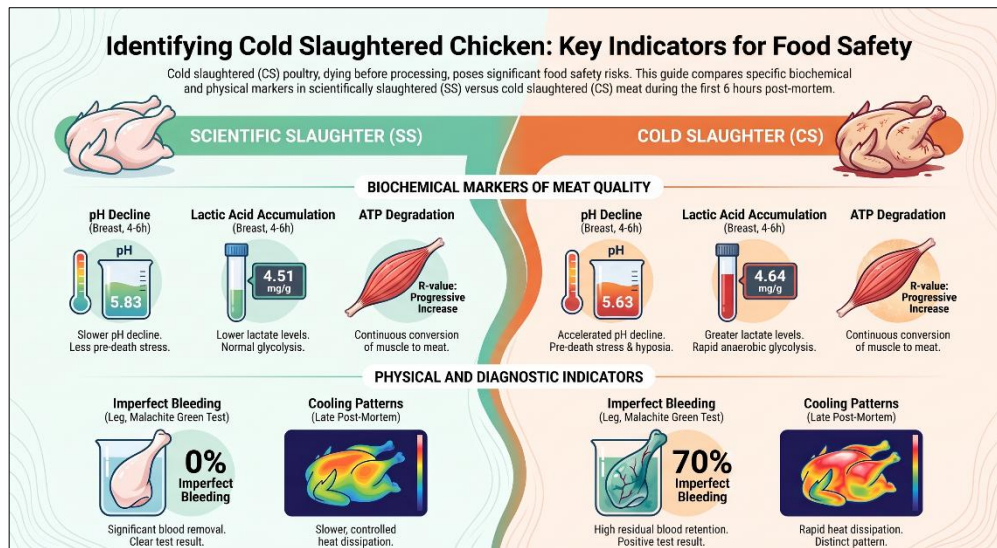
Corresponding Author:

Dr. Irshad A

Assistant Professor
 Department of Livestock
 Products Technology, College
 of Veterinary and Animal
 Sciences, Kerala Veterinary
 and Animal Sciences
 University (KVASU),
 Mannuthy, Thrissur, Kerala,
 India

biochemical changes and quality characteristics [6-9]. However, limited information is available on the comparative early post-mortem physicochemical and biochemical changes occurring in scientifically slaughtered and cold slaughtered broiler chicken meat.

Therefore, the present study was undertaken to compare carcass temperature, muscle pH, R-value, lactic acid concentration and bleeding efficiency in scientifically slaughtered and cold slaughtered broiler chicken meat during the first six hours post-mortem.



Materials And Methods

Experimental birds and sample collection

The study was conducted using 100 broiler chickens, comprising 50 scientifically slaughtered (SS) and 50 cold slaughtered (CS) birds. All birds had an approximate live weight of 1.5-2.5 kg.

Commercial broiler chickens weighing 1.5-2.5 kg were sourced from local markets for the SS group. The birds were provided a rest period of 4 h and subjected to pre-slaughter electrical stunning followed by exsanguination at the Meat Technology Unit, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy. After slaughter and dressing, the carcasses were kept at room temperature until collection of muscle samples at the specified post-mortem intervals.

Cold slaughtered birds consisted of dead-on-arrival broiler chickens obtained from local poultry retail outlets, farms and the Meat Technology Unit, Mannuthy. Information regarding transportation time was collected from suppliers, and the birds were reported to have been transported for approximately 1-2 h before arrival. Therefore, birds found dead during unloading were presumed to have died within the preceding 2 h. Immediately after collection, the carcasses were transported to the Meat Technology Unit and dressed. A detailed post-mortem examination was carried out, and carcasses showing evidence of infectious disease, fractures, external trauma or other pathological lesions were excluded. Only apparently healthy carcasses that died during transportation were included in the study.

Breast and leg muscle samples were collected during 0-2, 2-4 and 4-6 h post-mortem intervals for physicochemical and biochemical analyses. Core temperature was recorded at hourly intervals from 1 to 6 h post-mortem.

Carcass temperature

Core temperature of breast and leg muscles was measured using an HM Digital TM-1 industrial-grade digital thermometer. The probe was inserted into the geometric centre of each muscle until a stable reading was obtained. Temperature was recorded in degrees Celsius (°C).

Muscle PH

Muscle pH was determined according to O'Halloran *et al.* [10] using a calibrated digital pH meter (μ pH System, Systronics, India). Four readings were recorded from different locations of each muscle, and the mean value was used for statistical analysis. The pH meter was calibrated using pH 4.0 and pH 7.0 buffer solutions before measurement.

R-value

R-value was determined using a spectrophotometric absorbance ratio method for monitoring post-mortem ATP depletion [11]. Approximately 2 g of muscle sample was homogenised with 10 mL of 0.1 M perchloric acid and filtered through Whatman No. 1 filter paper. The filtrate was diluted with phosphate buffer (pH 7.0), and absorbance was measured at 250 and 260 nm using a UV-visible spectrophotometer (Systronics, India). The R-value was calculated as follows:

$$\text{R-value} = \frac{\text{Absorbance at 250 nm}}{\text{Absorbance at 260 nm}}$$

lactic acid estimation

Lactic acid concentration was determined using the modified Barker-Summerson method described by Pryce [12]. Muscle samples were homogenised with 20% trichloroacetic acid, and the extracts were reacted with p-hydroxybiphenyl reagent. Absorbance was measured at 565 nm using a UV-visible spectrophotometer (Systronics, India). Results were expressed as mg/g of muscle.

Malachite green test

Bleeding efficiency was assessed qualitatively using the malachite green test as described in previous studies [13, 14]. Meat extracts prepared from breast and leg muscles were treated with acid malachite green reagent followed by 3% hydrogen peroxide. The colour reaction was interpreted as follows:

Clear blue: normal bleeding

Cloudy green: imperfect bleeding

Olive green: unsatisfactory bleeding

Samples showing cloudy green or olive-green reactions were considered positive for residual blood.

Statistical analysis

Data were analysed using SPSS version 24.0. Temperature data were analysed using repeated-measures analysis of variance. The pH, R-value and lactic acid data were analysed using one-way analysis of variance followed by Duncan's multiple range test for comparison among post-mortem intervals. Differences between SS and CS groups at corresponding sampling intervals were evaluated using independent samples t-test. The proportion of samples showing imperfect bleeding in the malachite green test was compared using the Chi-square test, followed by pairwise comparison using the Z-test for two independent proportions where appropriate. Statistical significance was set at $p < 0.05$.

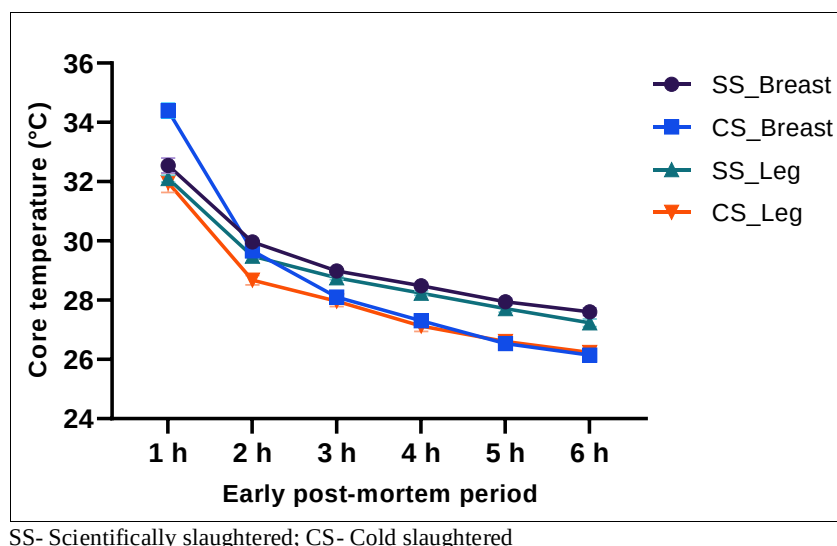
Results and discussion

Carcass temperature

Muscle temperature is an important post-mortem parameter because it influences the rate of glycolysis, ATP depletion, rigor mortis development and subsequent meat quality. In the present study, the temperature of both breast and leg muscles declined progressively and significantly ($p < 0.05$) during the first 6 h post-mortem in scientifically slaughtered (SS) and cold slaughtered (CS) broiler chicken meat (Table 1; Fig. 1). This decline reflected the gradual dissipation of body heat after death under room temperature holding conditions.

In breast muscle, the temperature of SS carcasses decreased from 32.55 ± 0.25 °C at 1 h to 27.61 ± 0.12 °C at 6 h, whereas CS carcasses showed a decline from 34.40 ± 0.25 °C to 26.15 ± 0.20 °C. Similarly, leg muscle temperature decreased from 32.10 ± 0.12 °C to 27.24 ± 0.13 °C in SS carcasses and from 31.96 ± 0.33 °C to 26.24 ± 0.19 °C in CS carcasses. From

the third hour onwards, CS carcasses showed significantly lower temperatures than SS carcasses in both muscles ($p < 0.01$). The progressive decline in carcass temperature observed in the present study represents normal post-mortem heat dissipation following cessation of circulation and metabolic activity. Temperature directly influences the rate of post-mortem biochemical reactions, including glycolysis and rigor mortis development. Under room temperature conditions, carcass cooling is slower than under refrigerated conditions, allowing biochemical changes to continue during the early post-mortem period. Teo *et al.* [15] reported that ambient temperature is one of the major factors influencing post-mortem body cooling. Similarly, Mohiddin *et al.* [16] observed that post-mortem carcass temperature generally decreases gradually until equilibrium with the surrounding environment is reached. Although CS breast muscle initially exhibited higher temperature, it cooled more rapidly after the second hour post-mortem. This rapid decline may be associated with the absence of physiological responses accompanying slaughter and exsanguination, leading to faster equilibration with ambient temperature [17]. The findings indicate that carcass cooling progressed normally in both groups but occurred more rapidly in cold slaughtered carcasses during the later post-mortem period. The declining temperature pattern observed in the present study is consistent with the normal process of algor mortis described during early post-mortem changes [18, 19]. The muscle temperature declined significantly with increasing post-mortem time in both SS and CS broiler chicken meat. Although carcass temperature alone cannot be considered a definitive indicator of cold slaughter, the different cooling pattern observed in CS carcasses may provide useful supportive information when interpreted along with pH, R-value, lactic acid concentration and bleeding efficiency.



SS- Scientifically slaughtered; CS- Cold slaughtered

Fig 1: Mean (±SE) Core temperature (°C) of breast and leg muscles of scientifically slaughtered (SS) and cold slaughtered (CS) broiler chicken during the early post-mortem period

Muscle pH

Muscle pH is one of the most important indicators of post-mortem biochemical change, as it reflects the extent of anaerobic glycolysis, ATP degradation, lactic acid accumulation and the buffering capacity of muscle tissue. In the present study, the pH of both breast and leg muscles declined progressively from 0-2 h to 4-6 h post-mortem in

scientifically slaughtered (SS) and cold slaughtered (CS) broiler chicken meat (Table 2). In breast muscle, pH decreased from 6.05 ± 0.03 to 5.83 ± 0.04 in SS meat and from 5.86 ± 0.03 to 5.63 ± 0.04 in CS meat. In leg muscle, pH decreased from 6.17 ± 0.02 to 6.02 ± 0.02 in SS meat and from 6.09 ± 0.03 to 5.81 ± 0.02 in CS meat. Cold slaughtered meat generally showed lower pH values than SS meat during the

early post-mortem period, while breast muscle maintained lower pH than leg muscle throughout the study.

The post-mortem decline in pH is mainly associated with the continuation of anaerobic glycolysis after cessation of blood circulation and oxygen supply. During this process, muscle glycogen is degraded and lactate accumulates, while ATP breakdown also contributes to hydrogen ion accumulation and reduction in muscle pH. This process continues until glycogen reserves are depleted or glycolytic enzyme activity is inhibited by the acidic environment. The progressive pH decline observed in the present study is therefore consistent with the normal conversion of muscle to meat. Similar post-mortem acidification patterns in poultry meat have been reported by Mir *et al.* [20] and Ramanathan *et al.* [3], who related pH decline to glycogen degradation, lactate accumulation and post-mortem energy metabolism.

The lower pH observed in CS meat may be attributed to altered pre-death and post-mortem metabolic conditions. Birds that die before slaughter may undergo stress, struggling, hypoxia, exhaustion or prolonged transport-related physiological disturbance before death. Such conditions can modify muscle energy metabolism and accelerate anaerobic glycolysis after death. In the absence of effective exsanguination and controlled slaughter, post-mortem biochemical changes may proceed in an irregular and accelerated manner. In the present study, the lower pH recorded in CS samples was also associated with higher lactic acid concentration and increased R-values, supporting the view that CS meat underwent faster glycolytic activity and ATP degradation during the early post-mortem period.

Similar observations were reported by Sohaib *et al.* [6], Belore *et al.* [7] and Kar *et al.* [8], who found lower pH values in meat from dead or cold slaughtered broiler chickens than in properly slaughtered birds.

The difference in pH decline between breast and leg muscles may be explained by variation in muscle fibre composition and metabolic characteristics. Breast muscle is predominantly composed of glycolytic fibres and generally has higher glycogen reserves, which favour faster post-mortem glycolysis and greater pH decline. In contrast, leg muscle contains a higher proportion of oxidative fibres and therefore shows a comparatively slower decline in pH. The present findings agree with Fletcher [21], Van Laack *et al.* [22] and Schreurs [23], who reported that post-mortem pH decline is closely related to glycogen content, glycolytic activity and muscle fibre type. Baldi *et al.* [24] also observed that chicken breast muscle undergoes faster post-mortem acidification than leg muscle due to differences in metabolic properties. Rapid pH decline, particularly when associated with relatively high carcass temperature, may promote denaturation of myofibrillar and sarcoplasmic proteins, resulting in reduced water-holding capacity, altered colour and soft texture. Therefore, the lower pH observed in CS meat indicates accelerated post-mortem acidification and suggests possible deterioration in meat quality. However, pH alone may not be sufficient for definitive identification of cold slaughtered meat. It should be interpreted along with lactic acid concentration, R-value, carcass temperature and malachite green test for more reliable differentiation between SS and CS broiler chicken meat.

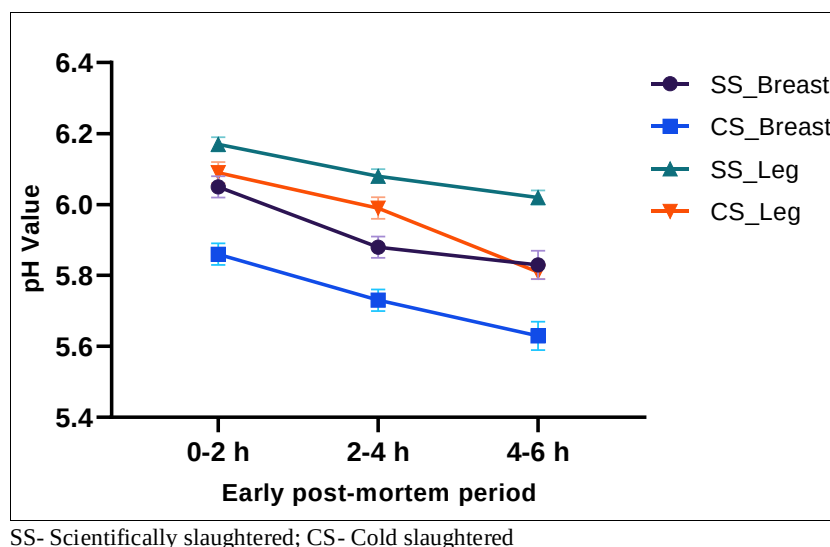


Fig 2: Mean ($\pm$ SE) pH of breast and leg muscles of scientifically slaughtered and cold slaughtered broiler chicken during the early post-mortem period

R-value

R-value is an important biochemical indicator of post-mortem ATP degradation and rigor mortis development in muscle. It represents the absorbance ratio at 250 nm and 260 nm and reflects the conversion of ATP into degradation products such as inosine monophosphate, inosine and hypoxanthine. In the present study, R-value increased significantly ($P < 0.001$) with increasing post-mortem time in both breast and leg muscles of scientifically slaughtered (SS) and cold slaughtered (CS) broiler chicken meat (Table 3; Fig 3). This progressive increase indicated continuous degradation of high-energy phosphate compounds during the early post-mortem period.

In breast muscle, R-value increased from 1.24 ± 0.01 to 1.34 ± 0.01 in SS meat and from 1.25 ± 0.01 to 1.33 ± 0.01 in CS meat. In leg muscle, the values increased from 1.19 ± 0.01 to 1.31 ± 0.01 in SS meat and from 1.17 ± 0.02 to 1.33 ± 0.01 in CS meat. Breast muscle generally recorded higher R-values than leg muscle during the early post-mortem period, indicating faster ATP degradation in breast muscle.

The progressive increase in R-value observed in both slaughter groups reflects the normal depletion of ATP after death or slaughter. Following cessation of circulation, oxygen supply to muscle tissue stops and ATP regeneration becomes limited. As post-mortem metabolism proceeds,

ATP is degraded into adenine nucleotide breakdown products, leading to an increase in R-value. Therefore, the increasing R-value recorded in the present study confirms the progression of post-mortem energy metabolism and rigor mortis development. Similar observations were reported by Sams and Janky^[25], who observed increasing R-values in broiler muscles during early post-mortem storage. Roncalés *et al.*^[11] also reported that R-value is a rapid and useful indicator for monitoring ATP depletion because of its close association with adenine nucleotide degradation products.

The higher R-values observed in breast muscle may be attributed to its predominantly glycolytic fibre composition. Glycolytic muscles usually undergo faster post-mortem ATP depletion and glycolysis than oxidative muscles. In contrast, leg muscle contains a higher proportion of oxidative fibres and therefore may show comparatively slower ATP degradation during the early post-mortem period. Similar findings were reported by Honikel and Fischer^[26], Honikel and Hamm^[27] and Smith *et al.*^[28], who stated that muscle fibre type, metabolic activity and glycogen utilisation influence ATP depletion and post-mortem biochemical changes.

The relationship between R-value, pH and carcass temperature is also important while interpreting the present findings. Rapid glycolysis and elevated post-mortem temperature can accelerate ATP breakdown and advance rigor mortis development. McKee and Sams^[29] reported that higher carcass holding temperature accelerates post-mortem pH decline and promotes earlier rigor development. In the present study, the simultaneous increase in R-value, decline in pH and increase in lactic acid concentration clearly indicates continued post-mortem glycolysis and ATP degradation in both SS and CS meat.

Although R-value increased significantly with post-mortem time in both slaughter groups, the difference between SS and CS meat was less consistent than that observed for pH, lactic acid concentration and malachite green test. Therefore, R-value may be considered a useful supportive indicator of post-mortem biochemical progression, but it should be interpreted along with pH, lactic acid concentration, carcass temperature and bleeding efficiency for more reliable differentiation of scientifically slaughtered and cold slaughtered broiler chicken meat.

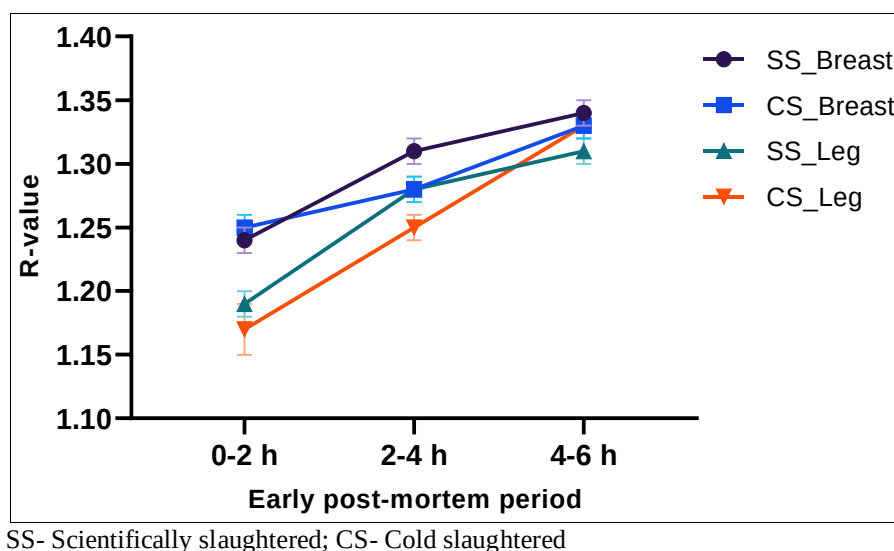


Fig 3: Mean ($\pm$ SE) R-value of breast and leg muscles of scientifically slaughtered and cold slaughtered broiler chicken during the early post-mortem period

Lactic acid concentration

Lactic acid is an important biochemical indicator of post-mortem anaerobic glycolysis and is closely associated with the decline in muscle pH during the conversion of muscle to meat. In the present study, lactic acid concentration increased significantly ($p < 0.001$) with advancing post-mortem time in both breast and leg muscles of scientifically slaughtered (SS) and cold slaughtered (CS) broiler chicken meat (Table 4; Fig 4). This increase indicated continued glycogen breakdown and lactate accumulation during the early post-mortem period.

In breast muscle, lactic acid concentration increased from 2.16 ± 0.06 to 4.51 ± 0.11 mg/g in SS meat and from 2.87 ± 0.13 to 4.64 ± 0.24 mg/g in CS meat. Similarly, in leg muscle, the values increased from 1.93 ± 0.08 to 3.15 ± 0.09 mg/g in SS meat and from 2.13 ± 0.08 to 3.25 ± 0.20 mg/g in CS meat. Cold slaughtered meat generally recorded higher lactic acid concentrations than SS meat, particularly during the early post-mortem period.

The progressive increase in lactic acid observed in the present study is consistent with the normal biochemical events occurring after death or slaughter. Following cessation of circulation, oxygen supply to the muscle is interrupted and ATP production through oxidative metabolism declines. Muscle cells then depend mainly on anaerobic glycolysis, during which glycogen is degraded and lactate accumulates. This metabolic change contributes to the gradual decline in muscle pH. Puolanne *et al.*^[30] reported that post-mortem pH decline is closely related to lactic acid accumulation in muscle. Ramanathan *et al.*^[3] also stated that anaerobic glycolysis continues after slaughter until glycogen reserves are depleted or enzyme activity is inhibited by the acidic environment. The inverse relationship between lactic acid concentration and pH was clearly evident in the present study. As lactic acid concentration increased from 0-2 h to 4-6 h, pH values decreased correspondingly in both breast and leg muscles. This confirms that post-mortem acidification in the samples

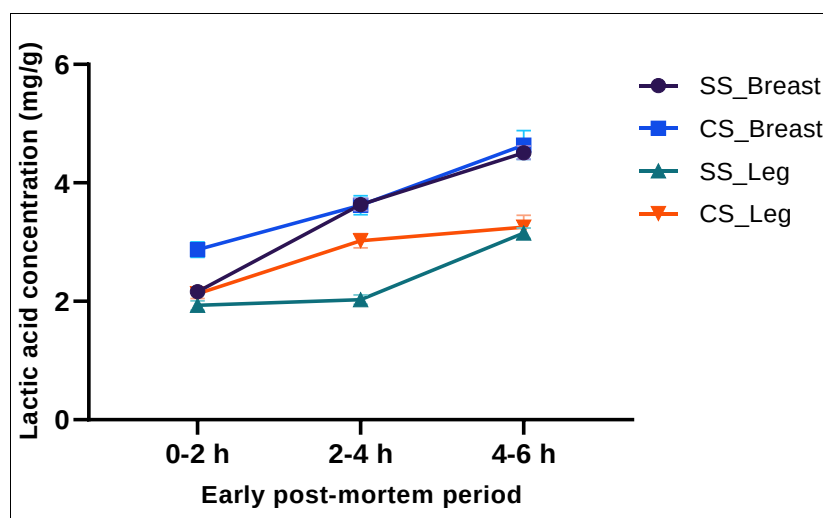
was mainly associated with lactate accumulation and ATP degradation. Similar observations were reported by Bodwell *et al.* [31] and Simek *et al.* [32], who observed progressive increases in lactic acid concentration along with a decline in muscle pH during the post-mortem period.

The higher lactic acid concentration observed in CS meat may be attributed to pre-death stress, hypoxia, struggling and physiological exhaustion before death. These conditions can accelerate anaerobic metabolism before dressing, resulting in greater early lactate accumulation. The lower pH and higher R-values recorded in CS samples further support this interpretation, indicating faster glycolytic activity and ATP degradation. Similar findings were reported by Kar *et al.* [8], who observed higher L-lactate levels in dead-on-farm and dead-on-arrival broiler chicken meat than in properly slaughtered birds.

Breast muscle consistently recorded higher lactic acid concentrations than leg muscle. This may be due to the

predominance of glycolytic fibres and higher glycogen reserves in breast muscle, which favour faster post-mortem glycolysis. In contrast, leg muscle contains a higher proportion of oxidative fibres and therefore showed comparatively lower lactate accumulation. Zhang *et al.* [33] similarly reported higher lactate production in broiler breast muscle than in thigh muscle.

The progressive increase in lactic acid concentration, together with declining pH and increasing R-values, confirms the continuation of post-mortem glycolysis in both SS and CS broiler chicken meat. The higher lactate accumulation observed in CS meat indicates altered post-mortem metabolism associated with death before exsanguination. Therefore, lactic acid concentration may serve as a useful supportive indicator for differentiating cold slaughtered meat when interpreted along with pH, R-value, carcass temperature and malachite green test.



SS- Scientifically slaughtered; CS- Cold slaughtered

Fig 4: Mean ($\pm$ SE) Lactic acid concentration (mg/g) of breast and leg muscles of scientifically slaughtered and cold slaughtered broiler chicken during the early post-mortem period

Malachite green test

The malachite green test is a simple qualitative test used to assess bleeding efficiency in meat by detecting residual haemoglobin in muscle tissue. The test is based on the colour reaction produced when haemoglobin-containing meat extract reacts with acidified malachite green reagent and hydrogen peroxide. A clear blue colour indicates normal or satisfactory bleeding, whereas cloudy green or olive-green reactions indicate imperfect or unsatisfactory bleeding. Therefore, the test provides a rapid indirect indication of residual blood retention in meat samples.

In the present study, the malachite green test clearly differentiated scientifically slaughtered (SS) and cold slaughtered (CS) broiler chicken meat (Table 5). None of the SS samples showed evidence of imperfect bleeding, whereas 34 out of 50 breast muscle samples and 35 out of 50 leg muscle samples from CS carcasses produced positive reactions indicative of residual blood retention. Thus, imperfect bleeding was detected in 68% of breast and 70% of leg muscle samples from CS carcasses, and the difference between SS and CS groups was highly significant ($P < 0.001$). The predominance of cloudy green reaction in CS samples can be attributed to the absence of effective exsanguination. In scientifically slaughtered birds, the neck cut and active cardiac pumping facilitate proper drainage of

blood from the carcass. In contrast, birds that die before slaughter are not subjected to effective bleeding because cardiac activity and circulation have already ceased. As a result, blood remains within the vascular system and muscle tissues, leading to higher residual haemoglobin content. The colour reaction observed in CS samples in the present study is therefore consistent with the basic principle of the malachite green test and confirms poor bleeding efficiency in cold slaughtered carcasses. The present findings are in agreement with Ahmad *et al.* [13], who reported better bleeding efficiency in scientifically slaughtered poultry meat than in imperfectly bled meat. Sasidharan *et al.* [14] also reported that the malachite green test is useful for assessing residual

blood in meat samples, although it is more suitable as a screening test than as a definitive confirmatory method. Similar observations were made by Quawsar *et al.* [34], who reported that meat from dead or inadequately bled birds produced cloudy or greenish reactions due to increased residual haemoglobin, whereas properly slaughtered meat generally produced a clear blue reaction.

The high proportion of positive reactions observed in CS carcasses demonstrates the usefulness of the malachite green test as a simple, rapid and low-cost screening method for identifying inadequately bled poultry meat. Residual blood

in meat is undesirable because it may affect colour, promote oxidative deterioration and support microbial growth, thereby reducing meat quality and shelf life. However, the test may not always provide complete discrimination because residual blood distribution can vary among carcasses and muscle sites. Factors such as sampling location, dilution of haemoglobin in the extract, time elapsed between death and testing, and visual subjectivity in interpreting colour reactions may influence the result.

Overall, the malachite green test proved to be a practical supportive tool for differentiating SS and CS broiler chicken meat in the present study. However, it should be interpreted along with other physicochemical and biochemical indicators such as pH, lactic acid concentration, R-value and carcass temperature for more reliable identification of cold slaughtered meat.

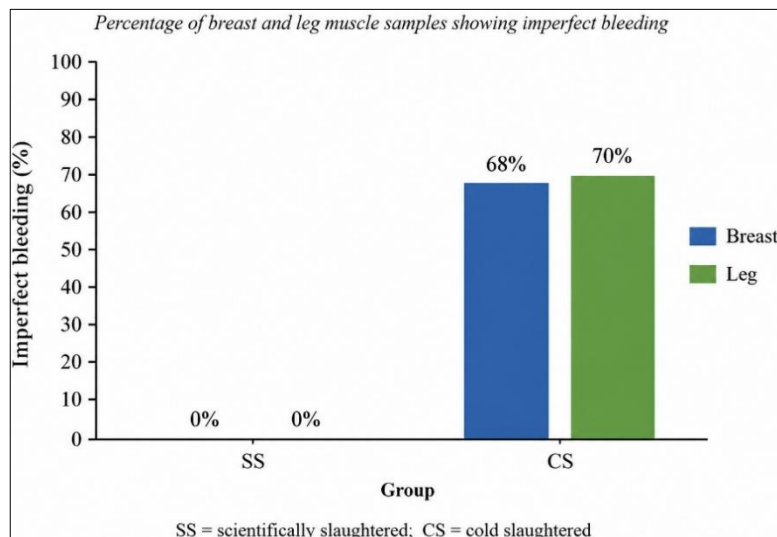


Fig 5: Percentage of breast and leg muscle samples showing imperfect bleeding in scientifically slaughtered (SS) and cold slaughtered (CS) broiler chicken meat as detected by malachite green test

Table 1: Mean ($\pm$ SE) core temperature ($^{\circ}$ C) of breast and leg muscles of scientifically slaughtered (SS) and cold slaughtered (CS) broiler chicken during the early post-mortem period

Muscle	Time interval after death	SS	CS	F-value (P-value)
Breast	1 h	32.55 ^{aAa} $\pm$ 0.25	34.40 ^{aAa} $\pm$ 0.25	0.039 (0.844)ns
	2 h	29.96 ^{aBa} $\pm$ 0.14	29.66 ^{aBa} $\pm$ 0.19	5.854 (0.017)*
	3 h	28.98 ^{aCa} $\pm$ 0.08	28.10 ^{bCa} $\pm$ 0.18	49.084 (0.000)**
	4 h	28.49 ^{aDa} $\pm$ 0.08	27.31 ^{cDa} $\pm$ 0.17	42.546 (0.000)**
	5 h	27.95 ^{aEa} $\pm$ 0.11	26.54 ^{cEa} $\pm$ 0.20	23.933 (0.000)**
	6 h	27.61 ^{aFa} $\pm$ 0.12	26.15 ^{bFa} $\pm$ 0.20	13.850 (0.000)**
	F-value (P-value)	327.77** (<0.001)	445.98** (<0.001)	
Leg	1 h	32.10 ^{aAa} $\pm$ 0.12	31.96 ^{aAa} $\pm$ 0.33	28.821 (0.000)**
	2 h	29.48 ^{aBa} $\pm$ 0.06	28.67 ^{bBa} $\pm$ 0.16	37.555 (0.000)**
	3 h	28.75 ^{aCa} $\pm$ 0.05	27.97 ^{bCa} $\pm$ 0.18	89.506 (0.000)**
	4 h	28.24 ^{aDa} $\pm$ 0.08	27.12 ^{bDa} $\pm$ 0.17	41.733 (0.000)**
	5 h	27.71 ^{aEa} $\pm$ 0.12	26.61 ^{cEa} $\pm$ 0.17	6.918 (0.010)**
	6 h	27.24 ^{aFa} $\pm$ 0.13	26.24 ^{bFa} $\pm$ 0.19	9.361 (0.003)**
	F-value (P-value)	420.13** (<0.001)	176.71** (<0.001)	

** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant. SS: Scientifically slaughtered; CS: Cold slaughtered

Means having different small letter superscripts differ significantly within a row. Means having different capital letter superscripts differ significantly within a column for

each muscle. Means having different Greek letter superscripts differ significantly between muscles within each group and time interval.

Table 2: Mean ($\pm$ SE) pH of breast and leg muscles of scientifically slaughtered and cold slaughtered broiler chicken during the early post-mortem period

Muscle	Time interval after death (h)	SS	CS	F-value (P-value)
Breast	0-2	6.05 ^{Aa} $\pm$ 0.03	5.86 ^{Aa} $\pm$ 0.03	0.382 (0.538)ns
	2-4	5.88 ^{Ba} $\pm$ 0.03	5.73 ^{Ba} $\pm$ 0.03	1.989 (0.162)ns
	4-6	5.83 ^{Ba} $\pm$ 0.04	5.63 ^{Ba} $\pm$ 0.04	5.628 (0.020)*
	F-value (P-value)	40.100** (<0.001)	26.000** (<0.001)	
Leg	0-2	6.17 ^{aAa} $\pm$ 0.02	6.09 ^{bAa} $\pm$ 0.03	5.769 (0.018)*
	2-4	6.08 ^{aBa} $\pm$ 0.02	5.99 ^{bBa} $\pm$ 0.03	28.624 (0.000)**
	4-6	6.02 ^{aCa} $\pm$ 0.02	5.81 ^{cCa} $\pm$ 0.02	0.266 (0.607)ns
	F-value (P-value)	24.219** (<0.001)	46.470** (<0.001)	

** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant. SS: Scientifically slaughtered; CS: Cold slaughtered

Means having different small letter superscripts differ significantly within a row. Means having different capital letter superscripts differ significantly within a column for

each muscle. Means having different Greek letter superscripts differ significantly between muscles within each group and time interval.

Table 3: Mean ($\pm$ SE) R-value of breast and leg muscles of scientifically slaughtered and cold slaughtered broiler chicken during the early post-mortem period

Muscle	Time interval after death (H)	SS	CS	F-value (P-value)
Breast	0-2	1.24 ^{bCa} $\pm$ 0.01	1.25 ^{bBa} $\pm$ 0.01	2.336 (0.013)*
	2-4	1.31 ^{Ba} $\pm$ 0.01	1.28 ^{Ba} $\pm$ 0.01	0.014 (0.905)ns
	4-6	1.34 ^{Aa} $\pm$ 0.01	1.33 ^{Aa} $\pm$ 0.01	0.219 (0.641)ns
	F-value (P-value)	31.522** (<0.001)	16.477** (<0.001)	
Leg	0-2	1.19 ^{aCb} $\pm$ 0.01	1.17 ^{bCb} $\pm$ 0.02	23.819 (0.000)**
	2-4	1.28 ^{Bb} $\pm$ 0.01	1.25 ^{Bb} $\pm$ 0.01	0.266 (0.607)ns
	4-6	1.31 ^{Ab} $\pm$ 0.01	1.33 ^{Aa} $\pm$ 0.01	0.003 (0.957)ns
	F-value (P-value)	61.451** (<0.001)	46.939** (<0.001)	

** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant. SS: Scientifically slaughtered; CS: Cold slaughtered

Means having different small letter superscripts differ significantly within a row. Means having different capital letter superscripts differ significantly within a column for

each muscle. Means having different Greek letter superscripts differ significantly between muscles within each group and time interval.

Table 4: Mean ($\pm$ SE) lactic acid concentration (mg/g) of breast and leg muscles of scientifically slaughtered and cold slaughtered broiler chicken during the early post-mortem period

Muscle	Time interval after death (H)	SS	CS	F-value (P-value)
Breast	0-2	2.16 ^{bCa} $\pm$ 0.06	2.87 ^{aCa} $\pm$ 0.13	11.289 (0.001)**
	2-4	3.63 ^{aBa} $\pm$ 0.10	3.62 ^{bBa} $\pm$ 0.16	20.150 (0.000)**
	4-6	4.51 ^{bAa} $\pm$ 0.11	4.64 ^{aAa} $\pm$ 0.24	24.238 (0.000)**
	F-value (P-value)	161.02** (<0.001)	23.973** (<0.001)	
Leg	0-2	1.93 ^{bCb} $\pm$ 0.08	2.13 ^{aCb} $\pm$ 0.08	5.882 (0.017)*
	2-4	2.03 ^{bBb} $\pm$ 0.08	3.02 ^{aBb} $\pm$ 0.12	9.235 (0.003)*
	4-6	3.15 ^{bAb} $\pm$ 0.09	3.25 ^{aAb} $\pm$ 0.20	88.834 (0.000)**
	F-value (P-value)	89.687** (<0.001)	21.103** (<0.001)	

** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant. SS: Scientifically slaughtered; CS: Cold slaughtered

Means having different small letter superscripts differ significantly within a row. Means having different capital letter superscripts differ significantly within a column for

each muscle. Means having different Greek letter superscripts differ significantly between muscles within each group and time interval.

Table 5: Malachite green test reaction of breast and leg muscles of scientifically slaughtered and cold slaughtered broiler chicken

Group	Breast number	Breast per cent	Leg number	Leg per cent
SS	0	0 ^b	0	0 ^b
CS	34	68.0 ^a	35	70.0 ^a
χ^2 value (P-value)	73.026** (<0.001)		79.582** (<0.001)	

** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant. SS: Scientifically slaughtered; CS: Cold slaughtered. Breast n=50; Leg n=50.

Means having different superscript letters differ significantly within a column

Conclusion

The present study demonstrated clear differences in early post-mortem physicochemical and biochemical changes between scientifically slaughtered and cold slaughtered broiler chicken meat. Muscle temperature declined progressively in both groups during the first 6 h post-mortem, but cold slaughtered carcasses showed a comparatively faster reduction during the later post-mortem period. Cold slaughtered meat recorded lower pH and higher lactic acid concentration than scientifically slaughtered meat, indicating accelerated post-mortem glycolysis associated with death before exsanguination. R-value increased with post-mortem time in both groups, confirming progressive ATP degradation and rigor mortis development. The malachite green test detected imperfect bleeding in 68% of breast and 70% of leg muscle samples from cold slaughtered carcasses, while all scientifically slaughtered samples showed normal bleeding reaction. These findings

indicate that cold slaughtered broiler chicken meat undergoes altered post-mortem biochemical changes and shows greater residual blood retention. The combined assessment of muscle pH, lactic acid concentration, R-value, carcass temperature and malachite green test may serve as a simple and practical supportive approach for differentiating cold slaughtered broiler chicken meat from scientifically slaughtered meat during the early post-mortem period.

Acknowledgements

The authors acknowledge the Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University, for providing the facilities required to conduct this study.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Maltin CA, Balcerzak D, Tilley R, Delday MI. Determinants of meat quality: tenderness. *Proc Nutr Soc.* 2003;62(2):337-347. DOI:10.1079/PNS2003248.
- Matarneh SK, Silva SL, Gerrard DE. New insights in muscle biology that alter meat quality. *Annu Rev Anim Biosci.* 2021;9:355-377. DOI:10.1146/annurev-animal-021419-083902.
- Ramanathan R, Hunt MC, Mancini RA, Nair MN, Denzer ML, Suman SP, *et al.* Recent updates in meat color research: Integrating traditional and high-throughput approaches. *Meat Muscle Biol.* 2020;4(2). DOI:10.22175/mmb.9598.
- Hwang YH, Lee EY, Lim H, Joo ST. Multi-omics approaches to improve meat quality and taste characteristics. *Food Sci Anim Resour.* 2023;43(6):1067-1086. DOI:10.5851/kosfa. 2023.e63.
- Sabow AB, Zulkifli I, Goh YM, Kadir MZAA, Kaka U, Imlan JC, *et al.* Bleeding efficiency, microbiological quality and oxidative stability of meat from goats subjected to slaughter without stunning in comparison with different methods of pre-slaughter electrical stunning. *PLoS One.* 2016;11(4). DOI:10.1371/journal.pone.0152661.
- Sohaib M, Zafar MS, Arshad MS, Nauman K, Malhi IY. Evaluation of quality and safety attributes of slaughtered versus dead chicken birds meat. *Braz J Poult Sci.* 2020;22:1-10.
- Belore BM, Maheswarappa NB, Kulkarni VV, Banerjee R, Hazarika P, Dasoju S, *et al.* Biomarker discovery and authentication of cold-slaughtered chicken through classical analytical procedures and mass spectrometry based proteomic approaches. *Br Poult Sci.* 2023;64(5):605-613. DOI:10.1080/00071668.2023.2239168.
- Kar P, Talukder S, Biswas AK, Sen AR, Agrawal RK, Kumar P. Classical laboratory techniques to distinguish broiler chicken meat from slaughtered and dead birds for effective detection of meat adulteration. *Food Anal Methods.* 2025; 18:2291-2303. DOI:10.1007/s12161-025-02865-0.
- Talukder S, Kar P, Mishra A, Sen AR. Development of tests to differentiate meat from unfit dead and properly slaughtered broiler chickens. *Food Addit Contam A.* 2026;43(1):2-14. DOI:10.1080/19440049.2025.2588668.
- O'Halloran GR, Troy DJ, Buckley DJ. The relationship between early post-mortem pH and the tenderisation of beef muscles. *Meat Sci.* 1997;45(2):239-251. DOI:10.1016/S0309-1740(96)00074-5.
- Roncalés P, Beltran JA, Jaime I, López-Lorenzo P. A simple method for following postmortem ATP depletion in lamb muscle. *J Food Sci.* 1989;54(5):1365-1366. DOI:10.1111/j.1365-2621.1989.tb05995.x.
- Pryce JD. A modification of the Barker-Summerson method for the determination of lactic acid. *Analyst.* 1969; 94:1151-1152. DOI:10.1039/AN9699401151.
- Ahmad T, Kumar Y, Gaikwad N, Raj D. Correlation between some quality parameters and bleeding status of poultry meat. *Asian J Dairy Food Res.* 2015;34(4). DOI:10.18805/ajdr.v34i4.6889.
- Sasidharan V, Vasudevan V, Sunil B, Sathu T, Ramnath V, Sankar KJA, *et al.* Evaluation of colour and bleeding efficiency of imperfectly bled, scientifically slaughtered and cold slaughtered beef carcasses. *J Vet Anim Sci.* 2022;53(3):385-391. DOI:10.51966/jvas.2022.53.3.385-391.
- Teo CH, Hamzah NH, Hing HL, Hamzah SPAA. Decomposition process and post mortem changes: review. *Sains Malays.* 2014;43(12):1873-1882. DOI:10.17576/jsm-2014-4312-08.
- Mohiddin SK, Hussain AJ, Kumar V, Subhedar A, Khan MT. Analysis of cooling curves obtained from the human corpses where time since death is known. *J Indian Acad Forensic Med.* 2019;41(1):34-38. DOI:10.5958/0974-0848.2019.00008.3.
- Riggs MR, Hauck R, Baker-Cook B, Osborne RC, Pal A, Terra MTB, *et al.* Meat quality of broiler chickens processed using electrical and controlled atmosphere stunning systems. *Poult Sci.* 2023;102(3):102422. DOI:10.1016/j.psj.2022.102422.
- Kaliszan M, Wujtewicz M. Eye temperature measured after death in human bodies as an alternative method of time of death estimation in the early post mortem period. *Legal Med.* 2019;38:10-13. DOI:10.1016/j.legalmed.2019.03.004.
- Listos P, Gryzińska M, Panasiuk-Flak K, Ciesielka M, Teresiński G. Assessment of temperature changes in carcasses in the early post-mortem period using the spectrum of a thermal imaging camera. *Med Weter.* 2021;77(5):253-257. DOI:10.21521/mw.6535.
- Mir NA, Rafiq A, Kumar F, Singh VK, Shukla V. Determinants of broiler chicken meat quality and factors affecting them: a review. *J Food Sci Technol.* 2017;54(10):2997-3009. DOI:10.1007/s13197-017-2789-z.
- Fletcher DL. Broiler breast meat color variation, pH, and texture. *Poult Sci.* 1999;78(9):1323-1327. DOI:10.1093/ps/78.9.1323.
- Van Laack RLJM, Liu CH, Smith MO, Loveday HD. Characteristics of pale, soft, exudative broiler breast meat. *Poult Sci.* 2000;79(7):1057-1061. DOI:10.1093/ps/79.7.1057.
- Schreurs FJG. Post-mortem changes in chicken muscle. *Worlds Poult Sci J.* 2000;56(4):319-346. DOI:10.1079/WPS20000023.
- Baldi G, Soglia F, Laghi L, Meluzzi A, Petracci M. The role of histidine dipeptides on postmortem acidification of broiler muscles with different energy metabolism. *Poult Sci.* 2021;100(2):1299-1307. DOI: 10.1016/j.psj.2020.11.032.
- Sams AR, Janky DM. Characterization of rigor mortis development in four broiler muscles. *Poult Sci.* 1991;70(4):1003-1009. DOI:10.3382/ps.0701003.
- Honikel KO, Fischer C. A rapid method for the detection of PSE and DFD porcine muscles. *J Food Sci.* 1977;42(6):1633-1636. DOI:10.1111/j.1365-2621.1977.tb08444.x.
- Honikel KO, Hamm R. Influence of cooling and freezing of minced pre-rigor muscle on the breakdown of ATP and glycogen. *Meat Sci.* 1978;2(3):181-188. DOI:10.1016/0309-1740(78)90003-7.
- Smith DP, Fletcher DL, Papa CM. Post-mortem biochemistry of Pekin duckling and broiler chicken pectoralis muscle. *Poult Sci.* 1992;71(10):1768-1772. DOI:10.3382/ps.0711768.
- McKee SR, Sams AR. Rigor mortis development at elevated temperatures induces pale exudative turkey

- meat characteristics. *Poult Sci.* 1998;77(1):169-174. DOI:10.1093/ps/77.1.169.
30. Puolanne EJ, Pösö AR, Ruusunen MH, Sepponen KV, Kylä-Puhju MS. Lactic acid in muscle and its effects on meat quality. *Proc 55th Reciprocal Meat Conf.* 2002; 55:57-62.
 31. Bodwell CE, Pearson AM, Spooner ME. Post-mortem changes in muscle. I. Chemical changes in beef. *J Food Sci.* 1965;30(5):766-772. DOI:10.1111/j.1365-2621.1965.tb01838.x.
 32. Simek J, Vorlova L, Malota L, Steinhäuserova I, Steinhäuser L. Post-mortal changes of pH value and lactic acid content in the muscles of pigs and bulls. *Czech J Anim Sci.* 2003;48:295-299.
 33. Zhang ZY, Jia GQ, Zuo JJ, Zhang Y, Lei J, Ren L, *et al.* Effects of constant and cyclic heat stress on muscle metabolism and meat quality of broiler breast fillet and thigh meat. *Poult Sci.* 2012;91(11):2931-2937. DOI:10.3382/ps.2012-02255.
 34. Quawsar AM, Afrin F, Begum MI, Haider M, Rauf SMDA, Golbar HM. Differentiation of meat from dead and slaughtered animals based on hemoglobin content. *J Bio-Sci.* 2024;32(1):31-39. DOI:10.3329/jbs.v32i1.74986.
- Management, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University (KVASU), Pookode, Wayanad, Kerala, India
7. **Dr. Chinnu MV**
Assistant Professor, Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University (KVASU), Mannuthy, Thrissur, Kerala, India.
 8. **Dr. Silpa S**
Assistant Professor, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University (KVASU), Mannuthy, Thrissur, Kerala, India.
 9. **Dr. Akhila VV**
Senior Research Fellow, NLM Project, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University (KVASU), Mannuthy, Thrissur, Kerala, India.
 10. **Thulasi K**
Young Professional-II, NLM Project, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University (KVASU), Mannuthy, Thrissur, Kerala, India

ALL AUTHORS

1. **Dr. Hridhya Vijay C**
PhD Scholar, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University (KVASU), Mannuthy, Thrissur, Kerala, India
2. **Dr. Irshad A**
Assistant Professor, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University (KVASU), Mannuthy, Thrissur, Kerala, India
3. **Dr. Vasudevan VN**
Professor and Head, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University (KVASU), Mannuthy, Thrissur, Kerala, India
4. **Dr. Suresh N Nair**
Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University (KVASU), Pookode, Wayanad, Kerala, India
5. **Dr. Sathu T**
Professor, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University (KVASU), Mannuthy, Thrissur, Kerala, India
6. **Dr. Geetha N**
Professor, Department of Livestock Production