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An Analytical Approach for Differentiation of Meat from Slaughtered and Dead Chicken at Different Periods of Time

Rohit Kumar Jaiswal¹, Aseem Kumar Anshu¹, Sushma Kumari¹, Gargi Mahapatra¹, Anjay², Sanjay Kumar³, Kaushlendra Kumar³, Ajeet Kumar⁴, Himalaya Bhardwaj⁴

¹Department of Livestock Products Technology, Bihar Veterinary College, Bihar Animal Sciences University, Patna, India

²Department of Veterinary Public Health and Epidemiology, Bihar Veterinary College, Bihar Animal Sciences University, Patna, India

³Department of Animal Nutrition, Bihar Veterinary College, Bihar Animal Sciences University, Patna, India

⁴Department of Veterinary Biochemistry, Bihar Veterinary College, Bihar Animal Sciences University, Patna, India

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*Corresponding author:

*E-mailaddress: rohit@basu.org.in

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ABSTRACT

Meat shops in developing countries are often located along roadsides, where animals are slaughtered in front of consumers and fresh meat is supplied regularly to institutions such as restaurants and defence establishments. This practice creates opportunities for unethical substitution of meat from slaughtered animals with that from dead animals, particularly in poultry. Scientifically, meat reaches its ultimate pH within approximately 6 hours post-slaughter, a period during which the risk of such substitution is higher. Limited research has been conducted to differentiate meat from slaughtered and dead chickens at various post-mortem intervals. Therefore, a pilot study was undertaken to evaluate physicochemical, microbiological, and proximate parameters for distinguishing meat from slaughtered and dead chickens at 1, 3, and 6 hours after slaughter or death. The results revealed that cooking loss, myoglobin content, moisture, and microbial counts were significantly higher ($P < 0.05$) in meat from dead chickens. In contrast, pH, extract release volume, water holding capacity, protein, and fat content were lower compared to meat from slaughtered chickens. Over time, most quality parameters decreased, while cooking loss and microbial load increased. These findings indicate that such parameters can effectively differentiate meat sources.

Keywords Differentiation, Dead chicken, Meat, Quality Control, Slaughtered chicken, Time period

INTRODUCTION

Poultry meat constitutes the highest share of worldwide meat trade, and in developing nations, it is the most preferred meat, contributing to more than half of the total meat production. However, there is a large gap between consumer demand and supply (OECD FAO 2021). In developing countries, consumers mostly demand fresh poultry meat, and most of the poultry birds are slaughtered in front of them and allow them to choose their choice of bird and their cut-up parts

while directly purchasing. Apart from this, the meat retailers or suppliers are supplying fresh meat after the slaughter of poultry birds to various food processing establishments, such as restaurants, hotels, canteens, and military and institutional mess, as per their price and agreement.

Meat obtained after death of poultry birds is identified as cold-slaughtered meat, which denotes cutting and dressing next to death from unidentified causes or anomalies which is not suitable for eating as spoilage/pathogenic microbes and antibiotic/chemical residues persist (Belore et al. 2023). The mortality percentage in poultry birds while reared on

farms and during transportation in cages, including dead on arrivals at slaughter houses and lairages, ranges from 1 to 5% (Cockram et al. 2018). In meat retailing, the chances of mortality during transportation are higher, and dead-on-arrival birds are produced in greater numbers, affecting the cost-benefit ratio of retailers. Therefore, the dead birds that arrive during transportation and the death of birds before slaughter in lairages are often sold by meat retailers to increase their profit by mislabeling/misrepresenting them as freshly slaughtered birds. This practice also encourages meat traders to replace slaughtered meat with those from dead poultry in wholesale supply or to unlawfully vend the dead poultry meat after processing into comminuted meat products, which significantly affect the ethical and safety apprehensions of customers (Si et al. 2021). The unauthorized sale of poultry birds that succumb to disease, toxin exposure, heat stress, or natural calamities like flooding adversely affects meat safety and contributes to the occurrence of foodborne and zoonotic diseases. This situation causes severe food safety risks among meat-eating consumers (Sohaib et al. 2020).

The adulteration or substitution of slaughtered chickens with meat from deceased birds is a serious problem prevalent in developing countries that can immensely reduce meat quality and negatively impact consumer well-being and religious principles (Bansal et al 2015). Furthermore, the consumption of dead animals is stringently prohibited in Islam and the Jewish community, and if such an act arises, it significantly affects the religious beliefs of consumers (Sohaib et al. 2020). Previous research has attempted to differentiate slaughtered chickens from dead chickens (Kar et al. 2025; Belore et al. 2023; Sohaib et al. 2020; Rahman et al. 2023; Swari et al. 2019). However, after the slaughter or death of poultry birds in field conditions, retailers keep the fresh meat in refrigeration for approximately five–six hours until it is sold on the same day. Additionally, when fresh meat is supplied in bulk to various meat processing units, it takes a minimum of five to six hours for transportation and processing and scientifically it is the time required to reach ultimate pH of meat during which chances of replacement is there. Therefore, considering the time to reach ultimate pH of meat and more chances of replacement of dead meat with slaughtered meat during initial 6 h, a pilot study aimed to differentiate slaughtered chicken meat (SCM) from dead chicken meat (DCM) at various time intervals after the slaughter / death.

MATERIALS AND METHODS

Place of research work

The Livestock Products Technology Department, Bihar Veterinary College, Bihar Animal Sciences University (BASU), Patna, India, was the place of the research. The study

was permitted by the Institute Animal Ethical Committee (IAEC), Bihar Veterinary College, BASU, Patna, regarding the slaughter of live poultry birds to be utilized for the conduction of research (Approval No.: IAEC/BVC/2023/03).

Slaughter and dressing of poultry birds

Live and dead poultry birds weighing approximately 1.5 kg reared at the Poultry Research Training Centre (PRTC), BASU, Patna were procured for the experiment. The poultry birds procured were separated into two categories: live and dead poultry birds. The first category consisted of live poultry birds that were halal slaughtered by severing the carotid arteries, jugular vein, trachea, and oesophagus while keeping the spinal cord intact and allowing them to bleed for 1.5 to 2.0 min. Further processing steps, such as scalding, de-feathering, evisceration, skin removal, cutting, and chilling, were performed using standard commercial practice. The breast meat obtained from slaughtered chickens was divided and packed into three different groups for analysis of parameters to be done at 1h, 3h, and 6h. The second category consisted of dead poultry birds in which no severing and blood loss was performed, immediately exposed to scalding, de-feathering, and removal of the organs and skin, trailed by cutting and chilling, alike to the first category. Similarly, meat obtained from dead chickens was divided and packed into three different groups for investigation of parameters to be done at 1h, 3h, and 6h.

Physicochemical parameters

Meat pH: pH of SCM and DCM were estimated at 1h, 3h, and 6h based on the methods defined by Troutt et al. (1992). 5.0 g meat was mixed with 25.0 mL of distilled water and homogenized with a tissue homogenizer for 1 min, and the pH was measured using a pH meter with an automatic temperature compensation probe (Hanna Instruments, HI200201, Woonsocket, RI-USA).

Water Holding Capacity (WHC): WHC of SCM and DCM samples were estimated at 1h, 3h and 6h as weight loss based on dimensions before and after pressing of meat in filter paper between glass plates, as per Wardlaw et al. (1973).

$$WHC (\%) = \frac{W1 - W2}{W1} \times 100$$

Where, W1 = Initial weight of meat before pressing; W2 = Final weight of meat after pressing.

Cooking Losses: The cooking losses of SCM and DCM were estimated at three-time interval as the loss of weight during cooking. A meat sample (5.0 ± 1.0 g) was weighed, packed and put at 80°C for 30 min in a water bath. The surface of sample was dried with tissue paper, weighed, and calculated with the help of following equation:

$$\text{Cooking losses (\%)} = \frac{(C2 - C3)}{C2} \times 100$$

Where, C2 = Meat weight before cooking (g) and C3 = Meat weight after cooking (g).

Extract Release Volume (ERV): ERV was determined for slaughtered and dead chicken meat samples at three-time intervals based on the protocol given by Strange et al. (1977). A 25.0 g meat sample was mixed with 100.0 mL distilled-water and homogenized for 2 minutes in a tissue homogenizer. The meat homogenate was transferred into glass funnel containing Whatman filter paper No.1 folded in the shape of funnel. The filtrate was collected in a graduated measuring cylinder for 15 minutes was measured as the ERV (mL).

Myoglobin content: Myoglobin content was determined by the protocol of Krzywicki (1982).

Microbiological parameters

The aerobic plate count (APC), *Staphylococcus* spp. and coliform count were determined as per procedure laid down by APHA (2001).

Proximate Chemical analysis

Proximate Analysis: The proximate parameters (moisture, protein and fat) were assessed using an INSTALAB 700 NIR Analyzer (Dicky-John, Auburn, Lee County, Alabama, United States). After removing tendons and connective tissues, the meat samples were chopped, finely homogenized using a high-speed crusher, and placed in sample cups for determination of moisture, fat, and protein contents (Anderson 2007).

Mineral Analysis: The mineral content (iron, magnesium, copper, zinc, and manganese) contents were measured using a Graphite Furnace Atomic Absorption Spectrophotometer (Pinnacle 900T, Perkin Elmer, Singapore) in dead and slaughtered meat samples at 1h only. A 5.0 g dried meat sample was digested using 7.0 mL HNO₃ and 3.0 mL HClO₄ on a hot plate until a clear solution formed. The digest was diluted to 10.0 mL with deionized water, and mineral content was reported in µg/kg

Statistical analysis

The experimental data obtained from fifteen replicates per treatment were subjected to statistical analysis using SPSS software (version 26.0 for Windows; SPSS, Chicago, IL, USA). One-way ANOVA was performed to examine the effect of slaughtered and dead conditions on meat quality attributes. Additionally, two-way ANOVA was used to evaluate the effects of carcass condition and post-mortem storage duration (1, 3, and 6 h), including their interaction. Post hoc comparisons among treatment means were conducted using Tukey's Honest Significant Difference test, considering $P < 0.05$ as statistically significant.

RESULTS AND DISCUSSION

Comparison of physicochemical parameters between meat from slaughtered chicken and dead chicken at different time intervals: Meat pH, WHC, cooking loss, ERV, and myoglobin content were measured to compare the physicochemical parameters (Table 1) between meat from slaughtered and dead chickens at 1h, 3h, and 6h after slaughter/death. The values differed significantly ($P < 0.05$) among the physicochemical parameters in the meat samples derived from slaughtered and dead chickens, and all the parameters differed significantly among groups measured at different time intervals after death/slaughter.

Meat pH: The meat pH of SCM at 1h was 6.27, which was significantly ($P < 0.05$) reduced to 5.64 at 6h, whereas in DCM, the pH at 1h was 5.81, which reduced to 5.69. The pH of SCM and DCM differed significantly ($P < 0.05$) at 1 and 3 h, whereas no significant difference was noted at 6 h post-slaughter/death. Muscle pH is one of the dimensions of sensory quality, and the final pH is suggestively impacted by the slaughter practices utilized at slaughter houses (Belore et al.2023). Although microbes do not grow during frozen storage, the action of spoilage bacteria fermenting the carbohydrates available in the meat at refrigeration temperatures results in the decline in pH attributed to lactic acid formation. The usual pH of meat obtained from poultry may vary from 5.9 to 6.2, while dark, firm, and dry (DFD) meat is described by a pH>6.4, and PSE meat by a pH<5.7 after 15 min of slaughter (Qiao et al. 2002). The reason for the lowest pH at 6 h, followed by 1 and 3 h after slaughter of poultry was elevated lactic acid levels and depletion of adenosine triphosphate (ATP) in muscle tissues resulting from post-mortem glycolytic phenomenon (Jaiswal et al. 2020). The ineffective bleeding produced due to death causes more residual blood content in DCM, which maintain a significantly low pH compared to SCM. According to earlier reports, dead chickens have the lowest pH values when compared to halal slaughtered chicken meat (Belore et al. 2023; Sohaib et al. 2020). Contrary to the above findings, Hafiz et al. (2015) found that samples of dead meat had a higher pH than slaughtered meat.

WHC: The WHC of SCM was 70.04 % at 1h and it reduced significantly ($P < 0.05$) to 64.92 % at 6h showing a decrease in WHC with time after the slaughter of poultry, whereas the WHC of DCM varied between 38.16– 41.96 %. A significant difference ($P < 0.05$) in WHC was observed between SCM and DCM at all evaluated time points (1, 3, and 6 h) following slaughter/death. The association between muscle pH and rigor stage may be the reason for the higher WHC in the SCM than in the DCM. After slaughter, the WHC of muscle reaches its maximum, and once the rigor is achieved with the ultimate pH, it falls to its final value (Lawrie et al 1998). Consequently, WHC of SCM at 1 h was higher than that of DCM, and the WHC of both sample groups decreased

between 1 and 6 h. These WHC trends are associated with a decline in the rigor state and pH (Hannula and Puolanne 2004; Savell et al. 2005). The increased loss of water from the muscles, along with soluble proteins and flavour producing chemicals, may be the reason for the reduced WHC in DCM, which eventually lowers the quality of the meat (Lawrie et al. 1998).

Cooking losses: The cooking losses results for SCM and DCM showed a trend opposite to that of WHC. The cooking losses increased significantly for SCM at 6h from 1h whereas DCM showed non-significant changes from 1h to 6h whereas SCM and DCM showed non-significant differences in terms

of cooking losses at 1h, 3h, and 6h after death/slaughter of poultry. As WHC and cooking loss are variables that rely on water binding capacity of meat proteins, the results for the cooking loss of SCM and DCM exhibited a reverse trend compared to WHC. Similar trends in cooking losses were detected by D'Agata et al. (2009) and Addeen et al. (2014) in poultry meat samples slaughtered using various techniques. Samples of meat taken after halal slaughter demonstrated reduced cooking loss, which increased with storage duration. Conversely, Hafiz et al. (2015) found no discernible difference between halal and Chinese butchered meat in terms of thawing and cooking losses.

Table 1 Physicochemical parameters of slaughtered and dead chicken meat at different time interval after slaughter and death of poultry birds

Parameters	Time after slaughter/death (in h)	Slaughtered Chicken Meat	Dead chicken meat
pH	1	6.27±0.02 ^{aA}	5.81±0.01 ^{aB}
	3	5.86±0.02 ^{bA}	5.70±0.01 ^{bB}
	6	5.64±0.02 ^c	5.69±0.01 ^b
Water Holding Capacity (%)	1	70.04±0.64 ^{aA}	41.96±0.75 ^{aB}
	3	66.41±0.28 ^{abA}	38.45±0.47 ^{bB}
	6	64.92±0.35 ^{bA}	38.16±0.31 ^{bB}
Cooking loss (%)	1	21.08±0.20 ^{bB}	23.89±0.37 ^A
	3	25.05±0.35 ^{aB}	24.69±0.22 ^A
	6	26.33±0.42 ^{aB}	24.67±0.28 ^A
Myoglobin content (mg/g)	1	2.00±0.02 ^{aB}	2.75±0.02 ^{aA}
	3	1.87±0.01 ^{bB}	2.25±0.01 ^{bA}
	6	1.80±0.01 ^{bB}	2.14±0.01 ^{cA}
Extract Release Volume (mL)	1	28.01±0.26 ^{aA}	24.26±0.24 ^{aB}
	3	26.87±0.34 ^{aA}	21.64±0.26 ^{bB}
	6	26.18±0.18 ^{bA}	19.46±0.16 ^{cB}

n = 15; mean ± S.E. Values with different superscripts differ significantly (P < 0.05), with capital letters denoting row-wise and small letters denoting column-wise comparisons

ERV: The volume of extract produced by homogenized meat after it has been permitted to pass through the filter paper for a specific amount of time is known as the extract release volume, which is inversely correlated with the degree of protein hydrolysis and spoilage (Jaiswal et al. 2025). The ERV of SCM was 28.01 mL at 1h and it reduced significantly to 26.18 mL after 6h whereas DCM showed ERV less than 25.0 mL from 1h to 6h. The ERV for SCM and DCM differed significantly (P < 0.05), but the value for ERV was less than 20 mL for DCM after 6h, suggesting poor quality. ERV is a crucial factor in assessing the quality of fresh meat as it is a sensitive indicator of spoilage and microbial activity (Omar et al. 2025). The ERVs of SCM and DCM differed significantly in the current investigation. An odd finding was that the ERV for DCM was less than 20 mL, and for SCM, it was more than 20 mL. This is below the recommended meat safety level (>20 mL), as recommended by the Food Safety and Standards of India, suggesting that SCM may be free of

incipient spoilage. When meat was kept at room temperature, endogenous enzymes and microbiological activity may have hydrolyzed the meat proteins, resulting in a decrease in the ERV of both SCM and DCM as the time went from 1 to 6 h. **Myoglobin content:** The myoglobin content for SCM at 1h, 3h, and 6h was 2.0 ± 0.02, 1.87 ± 0.01, and 1.80 ± 0.01 mg/g, respectively, whereas DCM showed 2.75 ± 0.02, 2.25 ± 0.01 and 2.14 ± 0.01 mg/g, respectively. A significantly elevated myoglobin level (P < 0.05) was observed in DCM relative to SCM, possibly resulting from incomplete bleeding and higher residual blood content in dead poultry birds. Iron is a crucial component of myoglobin, which imparts color to meat and has a positive impact on sensory qualities (Lucke et al. 2017). The concentration of meat pigments, primarily myoglobin, as well as their chemical state and enzymes, determine the color of the flesh (Rosenvold and Anderson 2003). Because dead poultry birds have a greater residual blood content, the myoglobin content differs greatly between SCM and DCM

(Sohaib et al. 2020). Our results corroborate the findings of Addeen et al. (2014) indicating reduced myoglobin content in slaughtered chicken meat as a result of effective blood loss, while dead birds exhibited elevated myoglobin levels.

Comparison of microbiological parameters between meat from slaughtered chicken and dead chicken at different time intervals

Aerobic plate count (APC), Staphylococcal, and Coliform counts were measured to compare the microbiological load (Figure 1) between SCM and DCM at 1h, 3h, and 6h after slaughter/death. The APC for SCM at 1h, 3h, and 6h was 2.32 ± 0.01 , 2.44 ± 0.01 and $2.56 \pm 0.02 \log_{10}$ CFU/g, respectively whereas DCM showed 4.18 ± 0.08 , 4.41 ± 0.07 and $4.82 \pm 0.04 \log_{10}$ CFU/g, respectively. The DCM showed a significant ($P < 0.05$) difference in APC at 1h, 3h, and 6h due to the presence of residual blood and spoilage microbes. The Staphylococcal and Coliform counts for SCM showed a numerical increase,

whereas DCM displayed a significant ($P < 0.05$) increase from 1h to 6h after slaughter/death of poultry.

The volume of blood remaining in a carcass following the slaughter or death of food animals is closely correlated with the microbial burden (Hafiz et al. 2015). The remaining blood in DCM serves as a nutritional supply and supports the development of microorganisms. Variable amounts of blood loss at slaughter or death determine microbial growth, and DCM had a noticeably larger microbial load (aerobic plate, staphylococcal, and coliform counts) than SCM. As the period increased from one to six hours, the microbial load of both SCM and DCM increased, with DCM showing greater growth. The retention of more blood inside the blood arteries and the presence of bacteria in DCM are likely causes of the elevated microbial load in DCM. The present results corroborate earlier studies by Addeen et al. (2014), Rahman et al. (2019) and Belore et al. (2023) which reported a positive correlation between microbial counts and residual blood content in meat. Thus, a low microbial burden in meat depends on efficient blood clearance during slaughtering.

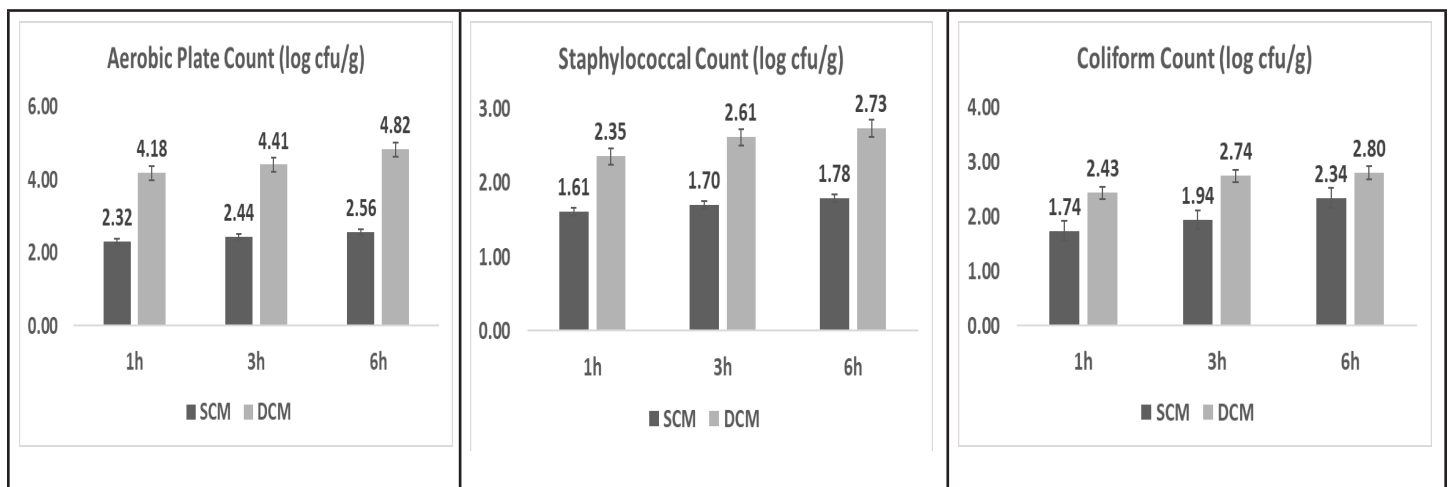


Figure 1 Microbiological load of slaughtered (SCM) and dead chicken meat (DCM) at different time interval after slaughter and death of poultry birds (n=15; Results are presented in Mean \pm S.E. and differ significantly ($P < 0.05$))

Comparison of proximate parameters and minerals between meat from slaughtered chicken and dead chicken at different time intervals

To evaluate differences in chemical composition between SCM and DCM, moisture, protein, fat (Figure 2), and mineral contents including Fe, Cu, Mg, Zn, and Mn (Table 2) were assessed at 1, 3, and 6 h. The moisture content of SCM at 1h, 3h, and 6h was 74.09 ± 0.18 , 73.41 ± 0.13 , and 73.05 ± 0.21 %, respectively, and it was highest after the slaughter of poultry and decreased significantly as time passed. At 1, 3, and 6 h post-mortem, the moisture content of DCM was recorded as $77.23 \pm 0.17\%$, $76.43 \pm 0.20\%$, and $75.55 \pm 0.16\%$, respectively. The consistently higher moisture content observed in DCM compared with SCM can be attributed to increased residual

blood content and fluid deposition resulting from post-mortem physiological changes. Meat type, pH level, drip loss, and butchering techniques all affect the water content of the meat. Dipole pressures cause the meat moisture content to decrease from the tissues throughout the slaughtering process. The moisture values for SCM and DCM were more in line with those published by Rahman et al. (2019) and Aksit et al. (2006). Contrary to these results, Bostami et al. (2018) observed moisture levels between 61.21% and 59.51% in meat from animals subjected to captive bolt stunning with pithing (non-halal, death state; CSNHS) and without pithing (halal, live state; CSHS). According to Rahman et al. (2019) the moisture content of Halal slaughtered meat samples is marginally lower than that of other slaughtering procedures because more blood spills from the body during Halal slaughtering than during other slaughtering methods.

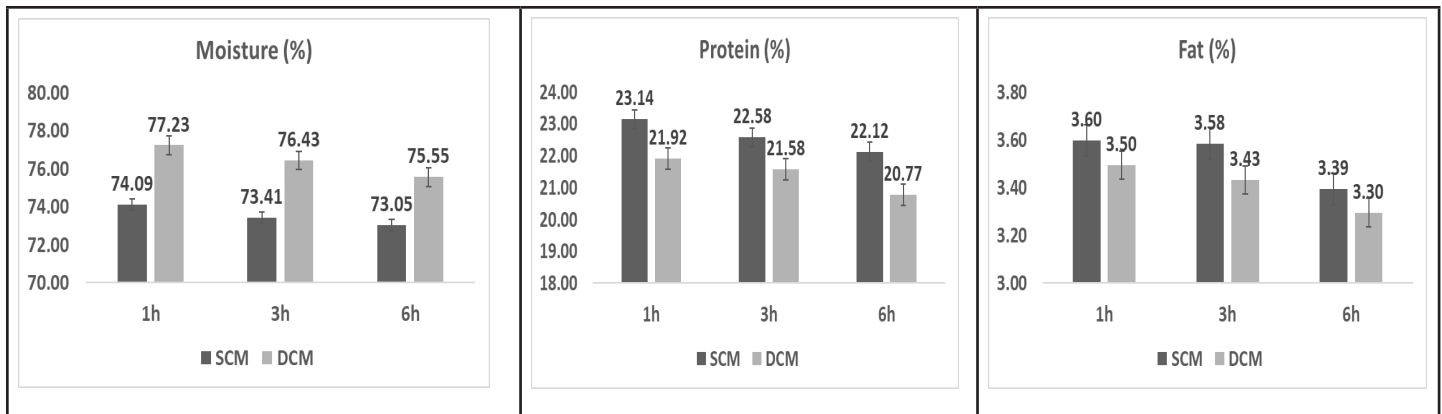


Figure 2 Proximate analysis of slaughtered (SCM) and dead chicken meat (DCM) at different time interval after slaughter and death of poultry birds (n=15; Results are presented in Mean ± S.E. and differ significantly (P<0.05)

Protein content in DCM remained unchanged across time intervals, while SCM showed significantly higher values ($P < 0.05$) at 1 and 3 h than at 6 h. Overall protein levels differed significantly between SCM and DCM, likely due to residual blood in DCM. In mineral analysis, Fe and Mn were significantly lower in SCM, whereas Cu and Mg were significantly higher ($P < 0.05$) than in DCM.

The measurement of protein content showed that meat from dead chickens had more protein than meat from slaughtered birds. The lower protein content of dead meat in the current study might be due to the presence of more blood residues, and there was no appreciable difference in protein content between SCM and DCM at one, three, and six hours. This is in line with earlier research by Bostami et al. (2018) who found that the crude protein concentration varied between 22.62% and 23.66% depending on the slaughter technique. Poultry birds experience severe stress at the time of slaughter or death, which triggers the release of muscle glycogen into the bloodstream and encourages muscle production of lactic acid. This phenomenon results in muscle acidification and triggers various biochemical alterations in postmortem muscle tissue (Fraser 2008). These alterations reduce the extractability of

proteins, leading to increased nitrogen loss from muscles and subsequent protein degradation. Furthermore, muscle proteins begin to break down immediately after death (in the absence of bleeding) due to various microbial and enzymatic processes. Substantial protein degradation was evident in meat from deceased chickens, whereas slaughtering live birds ensured effective blood removal and resulted in reduced protein degradation (Hafiz et al. 2015).

The fat content between SCM and DCM showed no significant difference due to the stability of visceral fat, which does not respond to hydrolytic and microbial rancidity. Regarding mineral content, DCM exhibited higher levels of iron and manganese than SCM, likely due to the presence of residual blood in DCM. The iron found in this residual blood serves as a pro-oxidant and provides a nutrient source for iron-reducing bacteria and protein degradation (Rahman et al. 2019). Manganese is a vital component of manganese-containing superoxide dismutase (MnSOD), which acts as an antioxidant in animal systems and helps reduce visceral fat and plays a role in enhancing meat quality through its association with MnSOD (Lu et al. 2006).

Table 2 Mineral estimation of slaughtered and dead chicken meat (µg/Kg)

Meat sample	Fe	Cu	Mg	Zn	Mn
Slaughtered Chicken	1678±14.46	1856.33±10.97 ^a	160.07±2.48 ^a	BDL	317.1±2.26 ^b
Dead chicken	2006.67±5.81 ^a	1601.67±13.02 ^b	145.57±2.41 ^b	BDL	532.73±1.46 ^a

n=3; # Mean ± S.E., bearing different superscripts column wise (small alphabet) differ significantly ($P < 0.05$), BDL-Below determined level

In contrast to the previously mentioned findings, magnesium and copper levels were greater in SCM than in DCM. Magnesium serves as a cofactor in various enzymatic reactions within the body and in the synthesis of hydrogen transporters, particularly in the conversion of muscle into meat and also plays a crucial role in the metabolism of glucose, lipids, proteins, and nucleic acids, as well as cellular defence mechanisms (Lin et al. 2015). This may explain

the higher Mg content in SCM than in DCM. Conversely, copper functions as an antioxidant by neutralizing free radicals and preventing oxidative damage to cellular and organelle proteins, membrane lipids, and nucleic acids; also, it functions as a cofactor for specific enzymes involved in energy metabolism, connective tissue synthesis, and iron utilization (Bost et al. 2016). Therefore, the reduced copper levels in DCM may be attributed to the increased

concentration of iron in DCM, resulting from ineffective iron metabolism.

CONCLUSION

This study demonstrates that slaughtered chicken meat (SCM) and dead chicken meat (DCM) can be clearly differentiated using physicochemical, microbiological, and proximate parameters across postmortem time intervals. It is essential as it provides a scientific basis for meat quality assessment and food safety, supporting effective detection and regulation of inferior or unsafe poultry meat.

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