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# Effect of Different Muscles on the Quality and Color Stability During Aging Of Hot-Boned Sheep Meat

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## ABSTRACT

This study investigated the impact of Aging on muscle-specific meat quality in various muscle types, including *Vastus lateralis* (VL), *Gluteo biceps femoris* (GBF), *Gluteomedius* (GM), *Longissimus dorsi et lumborum* (LL), *Psoas major* (PM), and *Semitendinosus* (ST) in sheep. Meat quality parameters such as water holding capacity (WHC), thiobarbituric acid reactive substance (TBARS), drip loss, and color stability myoglobin (Mb), met-myoglobin (met-Mb), instrumental color [*L*, *a** and *b**]) were assessed under refrigeration conditions (4±1 ° C) on the 0$^{th}$, 5$^{th}$, 10$^{th}$, and 15$^{th}$ day post-mortem. The findings of this study indicated a significant decrease ($P$<0.05) in myoglobin concentration on the 10$^{th}$ day for VL and GBF muscles, while GM, LD, and ST muscles exhibited this decrease on the 15$^{th}$ day. Notably, met-Mb percentage showed considerable variation ($P$<0.05) during different post-mortem aging periods among the muscles. TBARS values were significantly higher ($P$<0.05) on the 5$^{th}$ and 10$^{th}$ days, while water holding capacity and drip loss exhibited a substantial increase ($P$<0.05), reaching their highest ($P$<0.05) values on the 15$^{th}$ day post-mortem. Furthermore, the lightness value (*L*) increased significantly ($P$<0.05) in VL, GBF, LL, PM, and ST as the post-mortem aging period progressed. The redness (*a**) and yellowness (*b**) also increased significantly ($P$<0.05) with advancing post-mortem aging period. These results provide valuable insights into the effect of Aging on the meat quality of different muscle types in sheep, shedding light on changes in color stability, myoglobin content, lipid oxidation, and water holding capacity during the post-mortem aging process.

**Key words:** Meat quality; Aging; Myoglobin; Water holding capacity; Color stability.

# INTRODUCTION

Sheep serve as a valuable source of high-quality protein for human consumption. Over the past two decades, there has been a consistent global increase in sheep meat production, a trend that correlates with the rise in per capita GDP (Sen et al., 2021). This pattern suggests that heightened mutton consumption can be considered, to some extent, an indicator of an improved standard of living. In 2023, India alone consumed nearly 743 thousand metric tons of sheep meat, marking an increase compared to the previous year (STATISTA, 2023). India stands as the largest exporter of sheep and goat meat worldwide, having exported 9,592.31 MT of sheep andgoat meat worth Rs. 537.18 Crores/66.92 Millions USD in the year 2022-23 (APEDA, 2023).

Sheep meat stands out as an exceptional source of essential amino acids, omega-3 fatty acids, as well as vitamins B6 and B12, along with essential minerals like phosphorus, iron, and zinc (Williams, 2007). Consumer preference tilts significantly towards fresh and high-quality red meat, as opposed to pale, discoloured, or darker variations, which are considered of lower quality (Naveena et al., 2019; Corlett et al., 2021). The transition of muscle to meat involves substantial; biochemical and biophysical changes, directly influencing meat quality attributes (Kim et al., 2014; Kiran et al., 2020). Post-mortem aging represents an established method for enhancing tenderness, involving both physical and biochemical changes at the cellular level (Bhat et al., 2018). The acceptance of meat products by consumers and the profitability of the meat industry critically hinge on meat quality and color stability (Garmyn, 2020), where color, water holding capacity (WHC), and flavour play pivotal roles (Huang et al., 2020). In this context, lamb meats emerge as a preferred choice for consumers, who are willing to invest in a high-quality product. However, sheep meat often struggles to find its market niche due to a lack of standardization and quality when it reaches consumers (Thorne et al., 2021).The color of meat is intrinsically tied to the myoglobin content of the muscle. Generally, higher myoglobin content leads to a more pronounced reddish hue due to increased iron content, rendering the meat more visually appealing (Listrat et al., 2016). Upon exposure to air and subsequent oxidation, myoglobin undergoes oxidative changes, transforming into oxymyoglobin, resulting in the more desirable bright red color(Bjelanović et al., 2015). Consumers gravitate towards sheep meat products due to their distinct flavour, color, and texture, which are influenced by a multitude of factors spanning from the farm to the dinner table. Post-mortem aging significantly impacts the initial color and color stability of meat (Vitale et al., 2014; Kiran et al., 2020). However, prolonged aging can have adverse effects on the meat's shelf life, particularly for retail display, resulting in a rapid decline in color stability and pronounced lipid oxidation during storage (Yu et al., 2021).

In sheep, the first four days of aging play a pivotal role in the physical and biochemical responses of muscles, ultimately enhancing meat quality. While leg and shoulder cuts are preferred by sheep meat consumers, further research exploring other major meat cuts is warranted. In light of this, the present study was designed to assess the impact of aging on hot-boned sheep (*Ovis aries*) meat quality and color stability in various muscles during refrigeration storage.

# MATERIALS AND METHODS

## Materials

Skeletal muscle samples were procured from sheep of market age (12-18 months) that were considered to be in apparent good health. These sheep were slaughtered using the conventional halal technique at a local slaughterhouse located in Bidar, Karnataka, India. Subsequent to the slaughter, the hindquarters, which included the back portions, were carefully transported in ice boxes to the laboratory immediately. From the heterogenous group of muscles available, specific muscles under consideration namely *Vastus lateralis* (VL), *Gluteo biceps* femoris (GBF), *Gluteo medius* (GM), *Longissimus thoracis et lumborum* (LL), *Psoas major* (PM), and *Semitendinosus* (ST), were chosen for study. The selected muscle was separated carefully from other Muscles and made free of fascia and other connective tissues carefully. The separated muscles were stored in HDPE film till evaluation. The selection of these muscles was guided by their known variability in tenderness, as established in previous research in beef (Belew et al., 2003), as there is no such published work in Sheep.

## Analytical procedure

## Water Holding Capacity

Water holding capacity (WHC) was determined following method described by Wardlaw et al. (1973). Ten grams of minced meat were mixed with 15 ml 0.6 N NaCl, refrigerated for 15 minutes, centrifuged at 5000 rpm for 15 minutes, and the retained water was measured and expressed as a percentage.

## Drip loss

Drip loss was assessed following a modified method based on Honikel and Hamm (1994). Muscle samples were enclosed in low-density polyethylene bags and kept at 4°C.

Weights were measured after 1, 5, 10, and 15 days of storage, and drip loss was calculated as equation 1.

$$Drip\ loss\left(\%\right)=\left(\frac{Final\ weight}{Initial\ weight}\right)\times 100 \quad (1)$$

## Myoglobin and met-myoglobin Content

Myoglobin (Mb) extraction from the muscle was carried out using a modified approach based on Warris (1979). In this method, 5 grams of muscle sample were blended with 25 ml of cold 0.04M phosphate buffer at pH 6.8 for 10 seconds using homogenizer (Model: Z742486, Benchmark, D1000 Handheld Homogenizer, Malaysia). The mixture was then centrifuged at 5000 rpm in a refrigerated centrifuge (Model: Eppendorf Centrifuge 5804 R, Germany) for 30 minutes. The absorbance of the resulting filtrate was measured at 525, 572, and 700 nm employing a UV-VIS spectrophotometer (Model: Agilent Cary 60 UV-VIS, DE, Germany). The myoglobin concentration and percentage of MetMb were determined following Trout (1989) using formulas 2 and 3, respectively.

$$Myoglobin\left(mg/g\right)=\left(A525-A700\right)\times 2.303\times Dilution\ factor \quad (2)$$

$$Met-myoglobin\left(\%\right)=1.395-\left[\frac{A572-A700}{A525-A700}\right]\times 100 \quad (3)$$

## Thiobarbituric acid reactive substance (TBARS)

The TBARS was estimated using modified extraction technique as outlined by Witte et al. (1970). Four grams of the sample were homogenized with 20 ml of a 20% trichloroacetic acid solution, and the resulting homogenate was subjected to centrifugation for 10 minutes at 3000 rpm. The obtained centrifugate was subsequently filtered. Approximately 2 ml of the filtrate were mixed with an equal volume of freshly prepared 0.1% thiobarbituric acid and boiled for 30 minutes in a water bath at 100°C. The absorbance was measured at 532 nm using a UV-VIS spectrophotometer (Model: Cary 60 UV-VIS, DE, Germany), and the TBARS values were expressed in mg malondialdehyde per kilogram of the sample.

## Instrumental color

Color values (CIE *L, a*, b**) of the muscle samples were assessed using a handheld colorimeter (Model: CR10 Plus, Konica Minolta Limited Inc, Japan). The instrument, which had been pre-calibrated, was configured by aligning the sample surface aperture with the sample for measurement. Subsequently, the measurement was carried out, and the outcomes were presented in terms of lightness (*L*), redness (*a*), and yellowness (*b*) on the device's screen, following the Commission Internationale de l'Eclairage standards.

## Statistical Analysis

The entire experiment was replicated three times, with each replication involving distinct specific muscle samples obtained from different animals. Statistical analysis was conducted through analysis of variance (ANOVA) using SPSS (SPSS version 26.0 for Windows; SPSS, Chicago, IL, USA). A 6x4 factorial design with three replicates was implemented, with muscle type and aging period serving as the primary factors. This analysis was carried out using a General Linear Model two-way ANOVA. Least square means for F-tests were determined using Duncan's multiple range tests, and significance was assessed at a level of $P<0.05$.

# RESULTS AND DISCUSSION

## Meat quality parameters

The Water Holding Capacity (WHC) plays a crucial role in determining various quality attributes of meat, encompassing aspects such as color, texture, freshness, firmness in raw meat, and juiciness and tenderness in cooked meat (Honikel, 1987). In the context of this experiment, it was observed that the WHC among different skeletal muscles in sheep did not exhibit significant variations (Table 1). However, an interesting trend emerged as the post-mortem aging period advanced the WHC demonstrated a statistically significant increase (P<0.05) in WHC with ageing. Aging was found to be a factor contributing to the loss of water in meat, primarily due to the expansion or contraction of myofibrils stemming from changes in the constituent myofilament lattices (Offer and Trinick, 1983). It's important to note that the range of WHC observed in our study aligns with the findings of other researchers (Govindaiah et al., 2023; Kiran et al., 2016; Naveena et al., 2011; Sen and Karim, 2011). The improvement in water-holding capacity, as seen in our study, is thought to result from post-mortem proteolysis affecting structural and cytoskeleton proteins, such as desmin, titin, nebulin, and integrin (Zhang et al., 2006; Devatkal, 2020).

Drip loss serves as a metric for assessing a fresh meat's ability to retain water during the aging process. In our study, a statistically significant difference (P<0.05) in drip loss was evident among fresh samples from different

**Table 1:** Comparative Analysis of Meat Quality Parameters across Various Muscles in Hot-Boned Sheep Meat during Post-Mortem Storage Period

| Meat quality parameters | Storage period | *Vastus lateralis* (VL) | *Gluteo biceps femoris* (GBF) | *Gluteomedi-us*(GM) | *Longissimus dorsi et lumborum* (LL) | *Psoas major* (PM) | *Semitendinosus* (ST) |
|---|---|---|---|---|---|---|---|
| **Water Holding Capacity (%)** | 0 day | 19.00±0.58[1] | 19.33±1.76[1] | 18.67±1.76[1] | 20.00±1.15[1] | 20.67±0.88[1] | 18.67±0.67[1] |
| | 5 days | 21.67±0.88[a1] | 25.67±0.88 [bc1] | 28.67±1.76 [c2] | 25.67±0.88 [bc2] | 26.67±0.88 [bc2] | 23.67±0.88 [ab2] |
| | 10 days | 32.33±1.76 [2] | 33.33±2.40 [2] | 33.00±1.53 [23] | 30.33±0.88 [3] | 34.67±0.88 [3] | 34.00±2.31 [2] |
| | 15 days | 34.67±1.45 [a2] | 43.33±2.40 [b3] | 37.33±.88 [a3] | 35.67±.88 [a4] | 37.67±.88 [ab4] | 38.67±2.91 [ab3] |
| **Drip loss (%)** | 0 day | 1.17±0.06[a1] | 1.28±0.04[ab1] | 1.26±0.03[c1] | 1.42±0.02[c1] | 1.41±0.03[c1] | 1.36±0.05[bc1] |
| | 5 days | 1.53±0.26[a1] | 2.45±0.16[b2] | 1.77±0.09[a2] | 1.75±0.09[a1] | 1.80±0.07[a1] | 1.63±0.20[a1] |
| | 10 days | 4.89±0.19 [cd2] | 5.13±0.16 [d3] | 2.96±0.04 [a3] | 3.92±0.17 [b2] | 4.46±0.33 [bc2] | 4.81±0.09 [cd2] |
| | 15 days | 5.24±0.31 [ab2] | 4.73±0.18 [a3] | 5.13±0.25 [ab4] | 5.24±0.06 [ab3] | 5.67±0.05 [b3] | 5.07±0.04 [ab2] |
| **TBARS values (mg/g)** | 0 day | 0.18±0.03[12] | 0.19±0.01[1] | 0.22±0.01[2] | 0.19±0.01[1] | 0.20±0.00[1] | 0.20±0.00[1] |
| | 5 days | 0.23±0.01[a2] | 0.24±0.02 [a2] | 0.34±0.01 [b3] | 0.34±0.01 [b4] | 0.31±0.01 [b2] | 0.26±0.02 [a2] |
| | 10 days | 0.21±0.01 [2] | 0.26±0.01 [b2] | 0.23±0.01 [a2] | 0.29±0.01 [c3] | 0.31±0.01 [c2] | 0.21±0.01 [a1] |
| | 15 days | 0.14±0.01 [a1] | 0.25±0.01 [d2] | 0.17±0.00 [b1] | 0.24±0.01 [d2] | 0.21±0.01 [c1] | 0.17±0.01 [b1] |

[a–d] Means ± standard deviation without a common superscript were determined to be significantly different between muscles ($P< 0.05$).
[1–4] Means ± standard deviation without a common superscript were determined to be significantly different between storage period ($P< 0.05$).

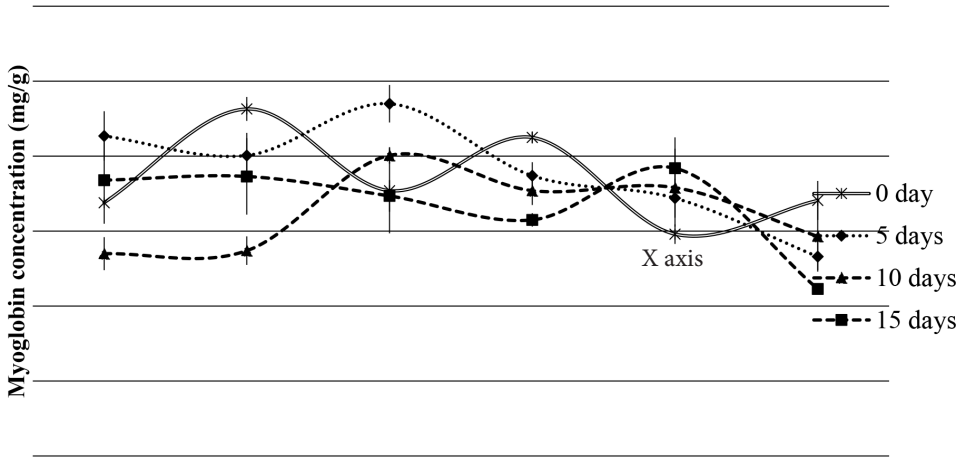


**Fig 1:** A Graphical Representation of Influence of Muscle Types on Myoglobin Content during Aging of Hot-Boned Sheep Meat
*VL-Vastus lateralis, GBF-Gluteo biceps femoris, GM-Gluteomedius, LL-Longissimus thoracis et lumborum, PM- Psoas majo, ST-Semitendinosus*

muscle groups (Table 1). As the post-mortem aging period advanced, we observed a significant increase ($p<0.05$) in drip loss for all muscle groups, with the highest values occurring around the 15th day post-mortem. This increase in drip loss aligns with the trend observed in WHC during aging, indicating that, regardless of the initial WHC or drip loss, the aging process tends to disrupt the myofibrillar integrity of skeletal muscle as it transitions into meat. It's worth noting that this phenomenon of drip loss occurring at a similar rate during post-mortem aging has also been reported in goats (Nagaraj et al., 2005), sheep (Sen and Karim, 2011) and poultry (Kiran et al., 2013).

The levels of TBARS serve as an indicator of the degree of lipid peroxidation within muscle tissue. Notably, when examining fresh samples from various muscles such as VL, GBF, GM, LL, PM, and ST, no statistically significant difference ($p<0.05$) was observed in TBARS levels (Table 1). However, an increase in TBARS values was observed on

**Table 2:** Instrumental color attributes of different muscles under post-mortem storage period in hot boned sheep meat

| Instrumental color | Storage period | *Vastus lateralis* (VL) | *Gluteo biceps femoris* (GBF) | *Gluteomedius*(GM) | *Longissimus dorsi et lumborum* (LL) | *Psoas major* (PM) | *Semitendinosus* (ST) |
|---|---|---|---|---|---|---|---|
| ***L*** | 0 day | 35.57±0.38$^{b12}$ | 34.70±0.32 $^{b1}$ | 36.20±0.58 $^{b12}$ | 31.37±0.56 $^{a1}$ | 35.43±0.75 $^{b1}$ | 35.70±0.49 $^{b1}$ |
| | 5 days | 38.23±0.66 $^{bc2}$ | 36.43±0.38 $^{ab3}$ | 36.37±1.30 $^{ab1}$ | 33.83±0.84 $^{ab}$ | 38.47±0.38 $^{bc2}$ | 40.57±1.01 $^{c2}$ |
| | 10 days | 38.40±0.46 $^{ab2}$ | 36.03±0.38 $^{ab3}$ | 36.37±0.71 $^{a1}$ | 35.27±0.64 $^{a2}$ | 39.67±0.61 $^{b2}$ | 39.93±1.99 $^{b12}$ |
| | 15 days | 36.83±0.61 $^{abc1}$ | 34.93±0.27 $^{a12}$ | 35.60±0.83 $^{ab1}$ | 34.43±0.49 $^{a2}$ | 38.47±1.07 $^{c2}$ | 37.80±1.03 $^{bc12}$ |
| ***a**** | 0 day | 17.83±0.15$^{a1}$ | 18.77±0.23 $^{b1}$ | 17.23±0.27 $^{a1}$ | 17.17±0.19 $^{a1}$ | 17.60±0.29 $^{a1}$ | 18.70±0.23 $^{b1}$ |
| | 5 days | 19.13±0.26 $^{a3}$ | 20.00±0.15 $^{b2}$ | 20.23±0.30 $^{b3}$ | 18.70±0.23 $^{a2}$ | 20.67±0.39 $^{b3}$ | 18.50±0.21 $^{a1}$ |
| | 10 days | 18.53±0.19 $^{a2}$ | 19.67±0.24 $^{cd2}$ | 20.13±0.18 $^{cd3}$ | 18.73±0.19 $^{ab2}$ | 19.40±0.36 $^{bc23}$ | 20.30±0.26 $^{d2}$ |
| | 15 days | 18.17±0.07 $^{a12}$ | 18.90±0.25 $^{ab1}$ | 18.83±0.19 $^{ab2}$ | 18.17±0.19 $^{a2}$ | 19.13±0.58 $^{bc2}$ | 19.97±0.09 $^{c2}$ |
| ***b**** | 0 day | 7.73±0.19$^{a1}$ | 8.77±0.23 $^{b1}$ | 7.23±0.27 $^{a1}$ | 7.17±0.19 $^{a1}$ | 7.60±0.29 $^{a1}$ | 8.70±0.23 $^{b1}$ |
| | 5 days | 8.83±0.23 $^{ab2}$ | 9.90±0.12 $^{c2}$ | 8.93±0.41 $^{ab2}$ | 8.10±0.12 $^{a2}$ | 9.80±0.36 $^{c2}$ | 9.63±0.20 $^{bc2}$ |
| | 10 days | 9.33±0.12 $^{bc2}$ | 10.50±0.17 $^{e3}$ | 9.10±0.17 $^{b2}$ | 8.57±0.12 $^{ab3}$ | 9.67±0.15 $^{cd2}$ | 9.87±0.12 $^{d2}$ |
| | 15 days | 9.03±0.34 $^{2}$ | 9.43±0.12 $^{2}$ | 8.83±0.15 $^{2}$ | 8.80±0.15 $^{3}$ | 9.30±0.15 $^{2}$ | 8.93±0.19 $^{1}$ |

$^{a–d}$ Means ± standard deviation without a common superscript were determined to be significantly different between muscles ($P< 0.05$).

$^{1–3}$ Means ± standard deviation without a common superscript were determined to be significantly different between storage period ($P< 0.05$).

the 5th and 10th days of postmortem aging, suggesting an augmented fat degradation process. Kim et al. (2012) proposed that extended aging may be linked to intrinsic factors like the accumulation of pro-oxidants (e.g., heme and non-heme iron) or the depletion of endogenous reducing compounds and antioxidants. In contrast, Ma et al. (2017) discovered that oxidative stability could be differentially influenced by muscle type and aging duration. Their research indicated that aged *psoas major* steaks exhibited higher levels of lipid oxidation when compared to steaks from aged longissimus lumborum and semimembranosus muscles.

## Color stability attributes

The role of meat color as a decisive factor in consumers' meat purchasing choices is well-established (Howes et al., 2015). In the current study, the color parameters *L* (Lightness), *a** (Redness), and *b** (Yellowness) for sheep skeletal muscles during various postmortem periods were observed to fall within an acceptable range (Table 2). As the post-mortem aging period advanced, the Lightness (*L*) values significantly increased (P<0.05) in VL, GBF, LL, PM, and ST muscles (Table 2). Concurrently, both Redness (*a**) and Yellowness (*b**) also exhibited significant increases (P<0.05) as the post-mortem aging progressed (Table 2). These variations in the color of different skeletal muscles of sheep likely stem from the composition of distinct muscle fibers, which impact meat color due to differences in myoglobin content. Changes in the color characteristics of goat meat are strongly influenced by postmortem pH (Simela et al., 2004). Postmortem aging notably affects both the initial color and the color stability of meat (Vitale et al., 2014). It's worth noting that aged meat demonstrated improved surface redness compared to non-aged meat, a phenomenon attributed to the reduced oxygen consumption of respiratory enzymes within the mitochondria of aged meat (Abdullah and Qudsieh, 2009). Similar to our findings, prior research has reported a reduction in the *a** value due to the conversion of oxymyoglobin to metmyoglobin (Sañudo et al., 2007), along with an increase in the *b** value (Cetin et al., 2012) during the aging of sheep meat and beef (Sasidharan et al., 2022).

In this study, a significant (P<0.05) distinction was evident in the myoglobin (Mb) and metmyoglobin (Met-Mb) content across the VL, GBF, GM, LL, PM, and ST muscles (Figure 1 and 2). Specifically, the PM muscle exhibited the lowest myoglobin content at 2.96 mg/g, while the GBF muscle had the highest content at 4.63 mg/g. The higher myoglobin content corresponds to a greater iron content, resulting in a redder appearance of the meat (Listrat et al., 2016). Similarly, reported myoglobin levels ranging from 2.7 to 9.4 mg/g, dependent on the muscle type and age (Valin et al., 1984). Throughout various time intervals within the postmortem period, we observed significant fluctuations in myoglobin concentration across these muscles. The myoglobin content reached its lowest point on the 10thday in VL and GBF muscles and on the 15thday in GM, LL, and ST muscles. These findings suggest that myoglobin content in skeletal muscle decreases during the postmortem phase, a phenomenon likely attributed to the proteolysis that occurs during aging. Prolonged oxidation leads to the formation of metmyoglobin, which imparts a brown hue to the meat (Bjelanović et al., 2015). Notably, there was a wide variation in the percentage of Met-Mb during different postmortem aging periods for various muscles, and no consistent trend was observed in these postmortem changes. This variability may be attributed to the differences in muscle location within the body, as deeper muscles may experience limited oxygen penetration.

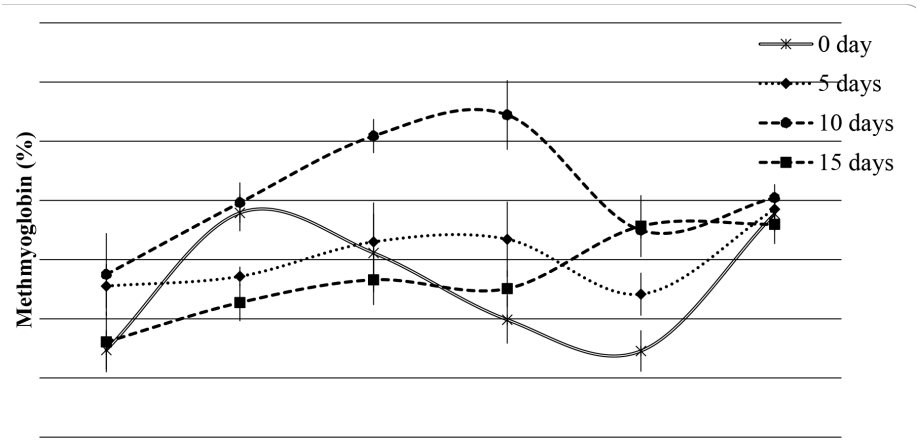


**Fig 2:** A Graphical Representation of Influence of Muscle types on MetMyoglobin content during Aging of Hot-Boned Sheep Meat

VL-*Vastus lateralis*, GBF-*Gluteo biceps femoris*, GM-*Gluteomedius*, LL-*Longissimus thoracis et lumborum*, PM- *Psoas major*, ST-*Semitendinosus*

# CONCLUSION

In conclusion, our research sheds light on key meat quality parameters and color stability attributes in various skeletal muscles of sheep during distinct post-mortem periods. Water Holding Capacity (WHC) exhibited a significant increase with aging, underscoring the role of post-mortem proteolysis and structural protein interactions. Drip loss showed initial variations among muscle groups but consistently increased during aging, indicating the impact of aging on myofibrillar integrity. Myoglobin content decreased with aging, influencing meat color from bright red to a brownish hue, and variations in myoglobin levels were observed across different muscles. Thiobarbituric Acid Reactive Substances (TBARS) levels increased during aging, indicating lipid peroxidation and fat degradation. Meat color stability, assessed through $L$, $a^*$, and $b^*$ values, displayed fluctuations during aging, reflecting differences in muscle fibre composition and protein denaturation rates. These findings research sheds light on the influence of post-mortem aging on meat quality, aiding the meat industry in refining product attributes to meet consumer preferences. We observed significant variations in color stability across different muscle types, offering valuable insights for targeted product formulation. By leveraging these findings, the industry can enhance its offerings and competitiveness while meeting diverse consumer demands effectively.

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**COMPETING INTEREST:** The authors have no known competing interests either financial or personal between themselves and others that might bias the work.

**ETHICS STATEMENT:** Not Applicable

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