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FEEDING SYSTEMS AND FRESH LAMB COLOR STABILITY

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THESIS

A thesis submitted in partial fulfillment of the
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ABSTRACT OF THESIS

FEEDING SYSTEMS AND FRESH LAMB COLOR STABILITY

Consumers consider the surface color of fresh lamb as the indicator of freshness and wholesomeness, which affects their purchase decisions. The redox chemistry of myoglobin (Mb) determines the color of fresh lamb. Artificial raising on milk replacer (a pre-weaning strategy) as well as red clover supplementation are management strategies used in lamb production. This thesis focuses on the effects of artificial raising on milk replacer and red clover supplementation on the color and oxidative stability of fresh lamb.

In the first experiment, the effect of pre-weaning with milk replacer on the carcass attributes, color stability and lipid oxidation of lamb longissimus lumborum (LL) muscle were evaluated during refrigerated storage. Polypay ram lambs were subjected to artificial raising on milk replacer (n = 10) or conventional raising with ewes (n = 10). Lambs were weaned at 60-d and then were wither fed *ad libitum* high-forage (50:50 concentrate:forage) or a high-concentrate (85:15 concentrate:forage) diet until reaching the target slaughter weight of 59 kg. Following harvest, fabricated 2.5-cm LL chops were placed on oxygen permeable polyvinyl chloride (PVC)-overwrap packaging and stored at refrigeration temperature (2°C) in darkness. Instrumental color, color stability (R630/580), pH, lipid oxidation, and metmyoglobin reducing activity of the chops were evaluated on 0, 3, and 6 days of storage. The carcass characteristics, surface redness (*a** value), yellowness (*b** value), hue angle, chroma, color stability (R630/580), pH, lipid oxidation, and metmyoglobin reducing activity (MRA) of LL chops were not affected ($P > 0.05$) by the pre-weaning management and finishing diet. During storage, lipid oxidation, yellowness (*b** value), and hue angle of the chops increased ($P < 0.05$), whereas color stability (R630/580) and MRA decreased ($P < 0.05$) in both treatments. The findings indicated that feeding milk replacer can be successfully utilized as an artificial raising strategy in lamb production without compromising fresh meat color.

In the second experiment, the influence of red clover (*Trifolium pratense* L.) supplementation on the carcass characteristics, color stability, and lipid oxidation of lamb LL muscle were examined during 6 days of storage. Polypay ram lambs were blocked by body weight and sire and then randomly assigned to 4 treatments: 85:15 concentrate:roughage with orchardgrass (*Dactylis glomerata* L.) without red clover (control; n = 6); 2.5% red clover with 12.5% orchardgrass (n = 6), 5% red clover with 10% orchardgrass (n = 6), and 7.5% red clover with 7.5% orchardgrass (n = 6) during the finishing phase. The lambs were fed *ad libitum* until reaching the target slaughter weight of 59 kg. Following harvest, LL muscles were fabricated, and 2.5-cm thick LL chops were placed on oxygen-permeable PVC packaging and assigned to refrigerated storage in darkness. Instrumental color, color stability (R630/580), pH, lipid oxidation, MRA, and

total color change (ΔE) were analyzed on 0, 3, and 6 days of storage. The hot carcass weight, cold carcass weight and lamb left shoulder increased in lambs supplemented with red clover diets (2.5% and 7.5%). Surface redness (*a**), yellowness (*b**), hue angle, chroma, color stability (R630/580), pH, lipid oxidation, and MRA were not affected ($P > 0.05$) by the red clover supplementation. During storage, meat pH, color stability (R630/580), redness (*a**) and chroma decreased ($P < 0.05$), whereas the yellowness (*b**), lipid oxidation, and hue angle increased ($P < 0.05$) in all samples. LL chops from red clover supplemented lambs (2.5%, 5%, and 7.5%) exhibited lower total color change (ΔE) than their control counterparts. These findings suggested that the supplementation of red clover can be a promising feeding strategy in lamb production without compromise of the fresh lamb color.

KEYWORDS: Artificial raising, Carcass characteristics, Color stability, Lipid oxidation, Milk replacer, Red clover

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CHAPTER 1

Review of Literature

1.1. Meat color

The per capita consumption of lamb in the United States increased since 2022 (USDA-ERS, 2023). Lamb is the fourth most consumed meat after pork, chicken, and beef (FAO, 2021; USDA, 2023). Fresh lamb has a characteristic light-red color (Calnan et al., 2014), and consumers associate its color as an indicator of freshness and wholesomeness (Suman et al., 2014; Mancini and Hunt, 2005). Meat discoloration leads to price discounts and/or converts high-value cuts into low-value ground products, resulting in a significant economic loss (Ramanathan et al., 2020a; Ramanathan et al., 2014).

Myoglobin (Mb) is a sarcoplasmic heme protein responsible for meat color (Suman and Joseph, 2013). Mb consists of a heme prosthetic group and a globin moiety, contains 153 amino acids, and has a molecular weight of approximately 17,000 Da. (Suman and Joseph, 2013). The globin peptide chain folds into eight helical segments that envelop the heme, allowing it to be soluble in water and shielding the heme iron from oxidation (Suman and Joseph, 2013). The chemistry and functions of Mb in live muscle and meat are different. In live animals, Mb acts as the oxygen binder and delivers oxygen to the mitochondria, enabling the tissue to maintain its physiological functions (Wittenberg & Wittenberg, 2003).

Myoglobin's chemical form and concentration play an important role in the customer's perception of fresh meat color (Faustman et al., 2023). Several factors can affect the variation in meat color stability. Preharvest factors such as live animal management and dietary strategy (Suman et al., 2014; McMillin, 2008), and post-harvest factors such as the meat pH, muscle source, aging, storage, and packaging (Mancini and

Hunt, 2005; Faustman and Cassens, 1990) can influence lamb color stability. Muscles in a carcass have specific anatomical locations and physiological functions in live animals, resulting in different metabolisms due to their fiber types (Hunt & Hedrick, 1977). Consequently, each muscle exhibits specific post-mortem biochemistry and color stability (Renerre & Labas, 1987). The main muscle fiber types in mammals are fiber type I (slow-twitch oxidative), type IIA (fast-twitch oxidative glycolytic), and type IIB (fast-twitch glycolytic) (Şirin et al., 2017). Muscles composed predominantly of fiber type IIB, such as longissimus lumborum (LL) (Ithurralde et al., 2018), exhibit greater content of glycogen and glycolytic potential than muscles composed of fiber I, such as psoas major (Ithurralde et al., 2018). The glycolytic metabolism of type IIB fiber promotes the use of glucose as an energy source, leading to the post-mortem accumulation of lactic acid and decreasing muscle pH (Patten et al., 2008).

1.2. Myoglobin chemistry

The mammalian myoglobin comprises an iron-based heme moiety encircled by a 153-amino acid globin peptide chain (Zhu, 2001; Pegg et al., 1997). The tertiary structure of Mb is determined by its primary structure, which governs the size of the heme cavity, net charge, oxidation-reduction properties, and interactions with other biomolecules, affecting meat color (Suman and Joseph, 2013). The Mb amino acid sequence differs between species, although the distal (position 64) and proximal (position 93) histidine are similar in livestock (mammalian) species (Livingston and Brown, 1981; Suman et al., 2009; Joseph et al., 2012). The iron atom can accept six electrons, resulting in the formation of

six-coordinate bonds. Four bonds are with pyrrole groups of the heme porphyrin ring, and one is with proximal histidine (position 93 in the globin chain), which connects heme to the globin chain. Another histidine residue (distal histidine at position 64 in the globin chain) is near the heme but not bonded with the heme. The sixth position of heme iron is available for binding with oxygen or other small ligands. The heme group contains an iron atom that can exist in a reduced (ferrous/Fe^{2+}) or oxidized (ferric/Fe^{3+}) form (Suman et al., 2014; Cornforth and Jayasingh, 2004).

Myoglobin can exist in three redox states in fresh meat, namely deoxymyoglobin (DMb), oxymyoglobin (OMb), and metmyoglobin (MMb), depending on the specific ligand at the sixth coordination site and the valence of the iron atom (Bekhit et al., 2019; Suman and Joseph, 2013; Watts et al., 1966). DMb occurs when no ligands are attached to the sixth coordination site of the heme, and the heme iron is in a ferrous state (Fe^{2+}). The meat becomes purplish red when DMb is formed and is associated with the color of fresh-cut and vacuum-packaged meat. Once air exposure, the DMb form converts to OMb through reversible oxygenation. An oxygen molecule occupies the sixth coordination site of OMb, where the heme iron is in a ferrous state (Fe^{2+}), resulting in a desirable cherry-red color. Oxidation of the ferrous iron (Fe^{2+}) of heme in DMb and OMb produces ferric MMb (Fe^{3+}), associated with brown meat color. A water molecule is bound to the sixth coordinate position of the ferric heme in MMb, which cannot carry oxygen.

Oxidation of Mb and subsequent accumulation of MMb at the meat surface during the storage and display period are the principal factors responsible for meat discoloration (Ledward et al., 1971). The reduction of ferric MMb to ferrous forms depends on the

metmyoglobin-reducing activity (MRA). MRA is the ability of muscle to reduce the MMb in DMb through enzymatic and non-enzymatic pathways, both dependent on the reducing equivalent NADH (Renerre and Labas, 1987; Echevarne et al., 1990). However, the enzyme activity, as well as the NADH pool, is continuously depleted in the post-mortem muscle (Mancini and Hunt, 2005), leading to meat discoloration (Kim et al., 2006; Bekhit and Faustman, 2005; Bekhit et al., 2003). Mitochondria can contribute to the metmyoglobin-reducing activity via regenerating the reducing equivalents and NADH through the electron transport chain (Mohan et al., 2010; Ramanathan et al., 2011; Tang et al., 2005) and cytochrome b5 reductase (Mohan, 2009; Ramanathan et al., 2019a; Tang et al., 2005; Suman and Joseph, 2013), respectively.

In living skeletal muscles, myoglobin delivers oxygen to mitochondria through the interaction between the oxygenated form of myoglobin (OMb) and the outer membrane of mitochondria, eventually generating energy by the mitochondria (Wittenberg and Wittenberg, 2003). On the other hand, in postmortem muscles, the mitochondria remain active up to 60 days and continue to consume and metabolize oxygen (Faustman and Cassens, 1990), affecting the color and oxidative stability of meat (Ramanathan et al., 2019a; Ramanathan et al., 2018).

1.3. Pre-harvest factors affecting fresh lamb color

1.3.1. Dietary strategies

1.3.1.1. Milk replacer

Milk replacer has been utilized as an artificial raising strategy instead of natural raising by ewes to raise triplets, surplus, or orphans and enhance lamb production efficiency regarding rearing the extra lambs and taking the stress off the ewe (Osorio et al., 2007; Campbell, 2019 (Notter et al., 2018). Despite the advantages, the use of milk replacer may negatively affect the lamb color. Lanza et al. (2006) documented a darker (lower lightness) longissimus dorsi from lambs raised with milk replacer. Chai et al. (2018) investigated the effect of rearing system (ewe-reared and reared with milk replacer) on color of longissimus thoracis (LT) from Hu lambs and observed greater lightness and yellowness in LT from artificially reared lambs, although no differences in redness, chroma and hue values were observed in both samples.

De Palo et al. (2015) investigated the effect of different milk replacers diets (goat milk, warm milk replacer or acidified milk replacer) on meat color of dairy goat kids and observed lower lightness and greater yellowness on meat from goats raised with warm milk replacer. No differences in redness were reported among all treatments. Ripoll et al. (2019a) evaluated the influence of the milk replacers on color of biceps femoris (BF), semimembranosus (SM), semitendinosus (ST), and longissimus thoracis (LT) muscles from Majorera goats and reported lower redness (*a** values) in BF, SM, ST but similar *a** values in LT, indicating that the influence of milk replacer on lamb color can be a muscle-specific effect.

1.3.1.2. Red clover supplementation

Red clover (*Trifolium pratense* L.) is a cool-season short-lived perennial legume utilized in lamb diet to improve feed efficiency (Weinert-Nelson et al., 2023). Despite the benefits for animal performance, the influence of red clover on lamb color is not completely understood. Previous investigations documented that the supplementation with red clover did influence lamb color (Girard et al., 2015; Luciano et al., 2019; Campbell et al., 2011). Girard et al. (2015) examined the effect of forage legumes on the color and sensory attributes of lamb and reported similar lightness and redness in the longissimus thoracis et lumborum muscles from lambs fed with red clover (*Trifolium pratense*), alfalfa (*Medicago sativa*), sainfoin (*Onobrychis viciifolia*), and birdsfoot trefoil (*Lotus corniculatus*) silages. Luciano et al. (2019) reported similar lightness, yellowness, chroma, hue, and lipid oxidation in muscle longissimus thoracis et lumborum from lambs fed with red clover (*Trifolium pratense*) and sainfoin (*Onobrychis viciifolia*). Moreover, Campbell et al. (2011) documented similar lightness and redness in loins from lambs finished on red clover (*Trifolium pratense*), brassica 'Goliath' (*Brassica napus, cv* Goliath), brassica 'Winfred' (*Brassica napus*, cv Winfred), turnip (*Brassica rapa*), radish (*Raphanus sativa*), pasture (*Lolium perenne*), and plantain (*Plantago lanceolata*) forages. Different results were reported by Kim et al. (2013), who documented greater redness, lightness, and chroma in longissimus dorsi from lambs fed with red clover, compared to those fed with ryegrass (*Lolium perenne*), lucerne (*Medicago sativa*), chicory (*Cichorium intubus*), and plantain (*Plantago lanceolata*).

1.4. Post-harvest factors affecting fresh lamb color

1.4.1. Storage

1.4.1.1. Temperature

Fresh lamb is a highly perishable product, and the use of low temperatures is the most common strategy utilized to preserve lamb during retail sales (Bellés et al., 2017a). The different storage methods include refrigerated storage (0 °C to 4 °C) (Fernandes et al., 2014), superchilled storage (-1 to -4 °C) (Bellés et al., 2017b), and frozen storage (-18 to -40°C) (Muela et al., 2012).

1.4.1.1.1. Refrigerated storage

Storage temperature can affect lamb color (Muela et al., 2010; Li et al., 2017; Rosenvold and Wiklund, 2011). Muela et al. (2010) evaluated the effect of cooling temperature (CT) (0–2°C, 2–4°C, or 4–6°C) on the color of lamb longissimus thoracis et lumborum and reported that the decrease of CT leads to the decrease of lightness (*L** values), and an increase of redness (*a**), yellowness (*b**), hue angle (*h**), and chroma (*C**) values. The decrease of lamb temperature decreases the oxygen consumption rate (OCR) and MMb accumulation due to the increase of the depth of oxygen penetration (O'Keefe and Hood, 1982). Less oxygen consumption rate causes deeper oxygen penetration and a deeper MMb layer within the meat (O'Keeffe & Hood, 1982), resulting in a lower color deterioration (Madhavi & Carpenter, 1993).

Similar results were observed by Li et al. (2017), who investigated the effect of controlled freezing point storage (CFPS, 0.8 °C) and storage at 4 °C (control) on color

stability of lamb longissimus thoracis et lumborum during 10 days of storage. The authors observed greater a^*, b^*, R630/580 and chroma in samples stored in CFPS compared to the control samples, from day 2 to 10. Additionally, greater OMb content, NADH content, and metmyoglobin reductase activity (MRA) were observed in samples stored in CFPS than those in control over 10 days of storage, indicating a more color stable sample in CFPS group.

Rosenvold and Wiklund (2011) documented that the retail color of chilled lamb is affected by the storage temperature. The authors evaluated the effect of storage temperature, prior to retail display, on the color stability of vacuum packaged lamb longissimus dorsi and observed a decrease in lightness and yellowness with the decrease of pre rigor temperatures (5 °C, 15 °C, 25 °C and 42 °C). For redness, the greater *a** values were observed in loins at 25 °C compared to the other temperatures on day 3; whereas at 42 °C the loins exhibited a pronounced decrease in redness from day 6 to day 8. Additionally, the hue was greater in loins at 42 °C than in the other temperatures.

1.4.1.1.2. Frozen storage

Freezing is a widely used preservation method to extend lamb shelf life (Kim et al., 2024; Pinheiro et al., 2019; Rosenvold and Wiklund, 2011), and allows the use of processing techniques, such as aging (Kim et al., 2024; Rosenvold and Wiklund, 2011). Frozen lamb products are deboned, vacuum-packed, stored and transported at temperature of −1.5 °C. The period of storage and transportation, which could be up to ten weeks, ensures that the product tenderness and quality are good and acceptable

(Rosenvold and Wiklund, 2011). Despite the benefits, frozen storage can also negatively affect lamb color, enhancing the discoloration (Kim et al., 2018). These quality deteriorations of frozen meat are primarily attributed to the formation of ice crystals. The rate of freezing will determine the location, distribution, size, and number of ice crystals, and therefore the impact on meat quality (Leygonie et al., 2012). Slow-freezing rates favor the generation of large extracellular ice crystals, increasing muscle and structures cells damage upon thawing. This in turn, results in an increase of water loss and protein denaturation in frozen/thawed meat (Chabela and Oyague, 2004). On the other hand, fast freezing allows the formation of fine and uniform intracellular ice crystals, which minimize the negative impacts of freezing on lamb quality attributes.

Recent investigations (Kim et al., 2024) evaluated the effects of aging and fast freezing on lamb longissimus dorsi (LD), gluteus medius (GM), quadriceps femoris (QF), semimembranosus (SM), and biceps femoris (BF) color during prolonged frozen storage. To determine the effect of aging then fast-freezing, the muscles were vacuum-packaged and assigned to: (1) non-frozen control (aged only for 5 weeks at −1.5°C); (2) aging for 4 weeks, fast-frozen in a −18°C glycol immersion chamber, and frozen storage in −18°C for 1 week; or (3) aging for 4 weeks, fast-frozen, and frozen storage for 24 wk.

Regarding lamb color attributes, the freezing/thawing resulted in a decrease in *L** values (lightness), especially in the LD, SM, and BF, which exhibited a pronounced decrease in lightness compared to the aged-only treatment (Kim et al., 2024). A decrease in lightness of frozen/thawed lamb loins can be attributed to a decrease in myoglobin

oxygenation (blooming) ability of aged/frozen/thawed meat compared to never-frozen (aged-only) counterparts (Kim et al., 2011).

Additionally, the *a** values (redness) and chroma values (color intensity) of the lamb cuts were influenced by freezing and frozen storage treatments (Kim et al., 2024). A slight decrease in redness and color intensity of lamb cuts was observed during freezing/thawing. Also, color stability is a muscle specific trait and the redness of LD remained unaffected by the freezing/thawing treatment. This observation could be attributed to aging prior to freezing (Kim et al., 2011), that decreases meat oxygen consumption (OC), increasing the oxygen available to Mb oxygenation as well as the maintenance of redness (Kim et al., 2011).

In addition, a pre rigor temperature has been reported to affect lamb color (Rosenvold and Wiklund, 2011). Rosenvold and Wiklund (2011) investigated the impact of storage temperature prior to retail display on the color stability of vacuum packaged lamb longissimus dorsi and reported that a high pre rigor temperature (42 °C) reduced color stability while differences in color stability between pre rigor temperatures of 5 °C, 15 °C and 25 °C were limited. The redness of the 42 °C loins was only substantially lower after 7 days retail display. In contrast, the impact of pre rigor temperature was significantly greater on lightness, yellowness, and hue value, with high values achieved for all three in the 42 °C loins, intermediate values achieved in the 25 °C loins (lightness and yellowness) and identical values in the 25 °C and 15 °C loins. The greater lightness, yellowness and hue value can be explained by protein denaturation caused by the low

pH, at the high pre rigor temperatures, which results in less metmyoglobin reducing ability and inferior color stability (Hertog-Meischke et al., 1997).

1.4.1.1.3. Influence of storage period

The color of frozen lamb can be affected by the storage period (Pinheiro et al., 2019). Pinheiro et al. (2019) evaluated the color of lamb longissimus lumborum frozen stored for 0, 3, 6, 9, and 12 months and reported that lightness (*L** values) was not influenced by the different periods of frozen storage. On the other hand, redness exhibited a decrease with the increase of frozen storage period, probably due to the Mb and lipid oxidation (Faustman et al., 2010; Setyabrata and Kim, 2019). Setyabrata and Kim (2019) attributed the decrease in color stability to the denaturation of myoglobin and reduced myoglobin redox system caused by ice crystal damage. Additionally, the meat structural changes induced by ice crystallization increase meat susceptibility to oxidative damage, due to the rupture of cell membranes, and consequently release of prooxidant compounds into the muscle, enhancing oxidation.

Daszkiewicz et al. (2017) evaluated the color changes of lamb longissimus thoracis et lumborum, vacuum-packaged and frozen-stored (-26°C) for 6 and 12 months and did not observe differences on lightness, redness, yellowness, chroma and hue value. Frozen storage can decrease meat lightness (darker color), mainly after long term frozen storage. Myoglobin denaturation and its susceptibility to auto-oxidation can contribute to the undesirable changes in color stability (Leygonie et al., 2012).

1.4.1.1.4. Influence of freezing rate

The freezing rate is a determining to the final quality of frozen foods. A slow rate results in the formation of large ice crystals that might damage the microstructure of meat. A fast-freezing rate prevents the migration of water and produces fine and numerous ice crystals (Le Bail, 2004). Intercellular ice crystals promote a pressure separating the fibers, whereas the intracellular ice crystals generate a pressure from within the fibers, promoting the rupture of the muscle cells (Lui et al. 2010).

Jacob and Thomson (2012) compared the effect of two conventional (minimum of 2.75 °C in 14 h, and 15 min on day 1 and 1.25 °C in 22 h) and fast (−10.2 ± 0.29 °C and the minimum temperature was reached in 45 min) chilling rates on color of lamb longissimus dorsi, semimembranosus, and semitendinosus muscles during simulated retail display, and observed lower hue angle for conventional frozen samples during 4 days of retail display. Additionally, the color stability was greater for convention than fast frozen samples until 3 days of retail display. This effect may be due to the blooming (oxygenation) at the beginning of retail display, as well as differences in the rate of metmyoglobin formation (oxidation).

1.4.1.1.5. Microwave assisted freezing (MAF)

As discussed above, the final quality of the frozen lamb depends on the size of the ice crystals since it can cause irreversible damage to the cellular structure and degrade the texture and color of the product. For this reason, emerging technologies have been developed to control the process of ice crystallization, and to improve the rate of ice

crystal formation and size (Xanthakis et al., 2014). Microwave assisted freezing (MAF) consists in applying microwave radiation during freezing and has been demonstrating to decrease the size of ice crystals and improve the quality of frozen lamb (Atani et al., 2022). Atani et al. (2022) investigated the effect of power levels of MAF (0%, 40%, 50%, and 60%) on ice crystal size and it effect on color of lamb longissimus dorsi (LD) and observed that the increase of power level of MAF improve the microstructure of frozen meat, by reducing the size of ice crystals, and consequently reduce the amount of drip loss and color change.

Regarding color, the frozen lamb LD exhibited the lower lightness after thawing, under microwave power levels of 0% and 40%, whereas the microwave-assisted freezing at the power levels of 50% and 60% improved the lightness of thawed lamb samples. Additionally, the microwave-assisted freezing at the power levels of 50% and 60% decreased the color change of thawed meat samples. Myoglobin is the main protein responsible for the fresh meat color (Mancini and Hunt, 2005). During thawing Mb can be lost due to the drip, affecting the thawed meat color. As the microstructure of meat was improved by increasing the power level of MAF, and the drip loss decreased, may have occurred a preservation of Mb and consequently a color preservation of frozen lamb loins.

1.4.1.1.6. Freezing under electrostatic field

Numerous studies have been performed to determine the best method for reducing the size of ice crystals. These can be achieved either by increasing the freezing rate (as discussed previously) or applying new emerging technologies, such as

electrostatic field (ESF) (Dalvi-Isfahan et al., 2016). ESF are constant fields, which do not change in intensity or direction over time. In the presence of an electric field, the water molecules tend to align with the field, and the application of electrostatic field in meat can decrease the ice of ice crystals, reduce the degree of supercooling, and improve the cellular structure (Xanthakis et al., 2014).

In this regard, Dalvi-Isfahan et al. (2016) evaluated the effects of electrostatic field (intensity of E=0–5.8 × 10^4 V/m) on the size of ice crystals and color attributes of frozen lamb (−20 °C), followed by thawed and color assessment. The authors reported that the size of ice crystal in the frozen lamb samples under ESF decreased up to 60% compared to the conventional freezing process. No significant effect of the different treatment on color changes was observed in frozen lamb subject to ESF.

1.4.2. Modified atmosphere packaging (MAP)

1.4.2.1. Carbon dioxide MAP (CO_2 MAP)

Modified atmosphere packaging (MAP) in combination with storage at low temperatures is an important strategy to maintain the quality and extend the shelf life of lamb. MAP can delay the spoilage microorganism development and contribute to promote attractive cherry-red color (Zhao et al., 2023; Kim et al., 2012a). To do that, a proper gas mixture should be applied. The main gases used for the MAP of red meat are carbon dioxide (CO_2), oxygen (O_2), and nitrogen (N_2), whose effect in lamb preservation

have been extensively reported (Vergara and Gallego et al., 2001; Bellés et al., 2017; Rodrigues et al., 2018; Stahlke et al., 2019; Zhao et al., 2023).

CO_2 is one of the most widely used compounds in MAP due to its antimicrobial activity, and it is partially soluble in water and fat within the food until the solubility equilibrium is reached (Gill, 1988). The O_2 is responsible for the desirable bright red color (OMb) at the time of purchase. However, O_2 can promote lipid and myoglobin oxidation, resulting in the MMb formation and lamb discoloration (Bórnez et al., 2010). Usually, a MAP with high levels of O_2 (70–80%) and 20–30% of CO_2 are used in lamb due to their effectiveness in reducing microbial growth and maintenance of red meat color desired by global consumers (Fernandes et al., 2014).

Recently investigations have evaluated the influence of modified atmosphere packaging (MAP) on fresh lamb quality (Zhao et al., 2023; Stahlke et al., 2019; Rodrigues et al., 2018). Zhao et al. (2023) compared the color of lamb longissimus thoracis lumborum (LTL) chops packed with air (AP) or MAP, with gas mixtures of 75%O_2 + 25%CO_2, or 50%O_2 + 50%CO_2 at 1, 6, and 24 h post-mortem and then for 6, 12, 24, 72, and 144 h post-mortem. The authors reported an increase in redness (a^* values) in lamb LTL packed in both MAPs 75%O_2 + 25%CO_2, and 50%O_2 + 50%CO_2 compared to the AP chops. The results may be attributed to the increase of O_2 concentration in MAP, which promotes myoglobin oxygenation and meat redness (Ripoll et al., 2013). Redness (a^* values) is more strongly related to a consumer's perception of fresh and displayed lamb (Khliji et al., 2010) and may be the best measure to predict consumers preferences (McLean et al., 2009). The color stability (R630/R580) of 75%O_2 + 25%CO_2 chops were greater than in the other

treatments at 6, 12, and 24 h of storage. The ratio R630/R580 estimates the meat color stability, and greater ratios and differences indicate more redness, whereas ratios close to 1.0 indicate essentially 100% MMb (King et al., 2023). When compared to the AP group, the greater O_2 level in the MAP group increased the R630/R580, due to the increase of OMb content during retail display. The MAP with 75% O_2 atmosphere promoted the formation of OMb with a greater R630/R580 and *a** values than in 50%O_2. However, lamb packed in high O_2 atmospheres for more than 7 days can exhibit a decrease in the Mb oxygenation capacity (and formation of OMb), and consequently do not exhibit a bright red color (Kim et al., 2012a).

In this regard, Rodrigues et al. (2018) evaluated the effect of vacuum (control), and MAP (15%O_2 + 85% CO_2 (O15); 30%O_2 + 70%CO_2 (O30); 45%O_2 + 55%CO_2 (O45); and 60%O_2 + 40% CO_2 (O60)) on lamb color and consumers acceptance during 21 days of refrigerated (1 ± 1 ºC) storage. The authors observed that vacuum and O45 samples exhibited a darker color (lower *L** values) at the 21 days of storage. A constant decrease in red color intensity (*a** values) was observed over time, for all treatments, after 7 days of storage, except for vacuum. However, before 7 days of storage the loins exposed to high concentrations of oxygen (O60 and O45) exhibited greater redness than vacuum-packed loins.

Fernandes et al. (2014) evaluated the effect of different MAP systems (vacuum, 75% O_2 + 25% CO_2, and 100% CO_2) on the color stability of lamb longissimus lumborum refrigerated stored for 28 days and observed a lower redness in samples with 100% CO_2, resulting in a greater discoloration and in a gray appearance. The presence of low levels

of residual O_2 (above 0.1%) favors Mb oxidation, resulting in the MMb formation and the consequent brown appearance of meat during storage (Insausti et al., 2001). Additionally, CO_2 concentrations above 30% are related to increase the degree of discoloration in red meat (Luño et al., 1998).

Vergara and Gallego (2001) evaluated the effect of MAP with 20% CO_2 + 10% O_2 + 70% N_2 and 80% CO_2 + 20% O_2 in color of lamb longissimus dorsi and reported greater redness in loin samples packed with 80% CO_2 + 20% O_2 on days 7 and 13 of storage.

1.4.2.2. Vacuum packaging (VP) and vacuum-skin packaging (VSP)

Vacuum packaging (VP) refers to the practice of removing air from a pack, prior to sealing, by pulling the packaging material into intimate contact with the product (Emblem et al., 2013). VP lamb usually exhibits a purple-red color due to DMb formation (Frank et al., 2017). Once removed from the vacuum, the Mb oxygenates and forms OMb, resulting in a light red color. In this sense, VP lamb products are usually re-packaged into a retail display form, either oxygen permeable polyvinyl chloride overwrap (PVC) or high oxygen modified atmosphere packaging (HiOx-MAP; 80% oxygen, 20% carbon dioxide).

Vacuum-skin packaging (VSP) is an alternative for retail packaging formats. VSP is a flexible skin polymer, with a low oxygen transmission rate, that is shrink-wrapped over the meat. VSP is an improvement of conventional VP once minimizes the formation of air pockets and wrinkles in the packaging creating a visually appealing format (Aaslyng et al., 2010). VSP also decreases purge loss compared to conventional VP. A disadvantage of VP/VSP systems is the purplish DMb color that could not be attractive to consumers.

However, after removal from vacuum and re-exposure to air, the meat develops desirable bright red color (Frank et al., 2017).

Previous investigation reported a stable redness (*a** values) in VP samples compared to the MAP. Berruga et al. (2005) evaluated the effect of vacuum package (VP) and MAP (gas mixtures of 40% CO_2 + 60% N_2; 80% CO_2 + 20% O_2, and 80% CO_2 + 20%N_2) on color of lamb longissimus dorsi and reported a greater redness (*a** values) in VP samples compared to the MAP ones for 28 days of storage.

1.4.3. Aging

Meat aging is an important strategy for adding value to the products and increasing the marketing value (Gürbüz et al., 2022). Postmortem carcass processes during the first 24 h impact the quality attributes such as tenderness, water holding capacity and color (Savell et al., 2005).

Vacuum packaging aging (VP; or wet aging) is the most common aging system utilized by the meat industry. VP meat is normally aged for several days or weeks at retail stores or butcheries. Posteriorly, the meat is removed from the packaging, cut as chops or ground, and displayed under aerobic atmosphere. Following vacuum packaging, the surface color of fresh meat changes from bright red to purplish red due to DMb formation (Kim et al., 2012b). When exposed to oxygen, a bright red OMb is rapidly formed on the surface, allowing the meat to bloom and exhibit the color of fresh meat.

Dry aging involves keeping the meat at temperatures between 1 – 3ºC, humidity around 70 – 85%, for approximately 21 - 28 days. In this process, occurs the development

of flavor and tenderness due to enzymatic and biochemical changes in the meat (Warren et al., 1992).

Aging can increase lamb redness (*a** values) (Gürbüz et al., 2022; Callejas-Cárdenas et al., 2014; Ponnampalam et al., 2013). Gürbüz et al. (2022) investigated the effect of dry aging on the meat quality characteristics of longissimus thoracis (LT) and longissimus lumborum (LL) muscles from Akkaraman lambs and observed greater redness (*a** values) in LT samples aged for 7 and 14 days. The initial color development (OMb formation) of LT increase, with the increase of postmortem aging time, could be due to the decrease in oxygen consumption (OC) (Lee et al., 2008). OC is the ability of muscle to consume oxygen, primarily by mitochondria, to a low partial pressure of oxygen, when naturally forms MMb or DMb, according to the reducing activity. The decrease in OC results in more oxygen available to bind to Mb oxygenation and thus form OMb (English et al., 2016).

Callejas-Cárdenas et al. (2014) investigated the effects of vacuum aging on color of lamb biceps femoris and quadriceps femoris muscles for 14 days and reported greater redness and lower hue angle compared to the non-aged ones. Rant et al. (2019) documented an aging time effect on lamb color chances. The authors evaluated the color of lamb longissimus lumborum (LL) and gluteus medius (GM) aged for 14 days and documented an increase in redness (*a** values) and yellowness (*b** values), and a decrease in lightness (*L** values) in GM muscle after the 14^{th} day of aging period. Conversely, aging had no effect on lightness, redness and yellowness, as well as chroma

and hue angle in LL muscle, suggesting that the influence of aging in lamb color is muscle specific effect.

The type of aging (dry or wet) also impacts lamb color (Zhang et al., 2021). Zhang et al. (2021) compared the color of in-bag dry-aged and wet aged lamb and observed that dry-aged lamb exhibited lower color attributes (L^*, a^* and b^*) than the wet-aged ones. Overall, in-bag dry-aging resulted in a darker and brown color, which could be attributed to the loss of moisture content after aging resulting in lower light reflection (Kim et al., 2011; Kim et al., 2016).

1.4.4. Meat pH

Meat pH plays an important role in determining the color stability in fresh lamb. Both the ultimate pH and the rate of pH decline can affect lamb color (Rosenvold and Wiklund, 2011). Abril et al. (2001) reported that the pH had a significant effect on all the color variables (L^*, a^*, b^*, chroma, and hue). Meat with low pH values exhibited greater yellowness (b^* values) and hue values. On the other hand, an increase of pH decreases the lightness (L^* values; meat became darker), and the muscle reflectance (Abril et al., 2001). The lower L^* values can be attributed to the lower light reflectance and greater absorption at all wavelengths. A greater pH allows meat to hold more water, resulting in cell swelling and lower light reflectance (Tang et al., 2005; Ramanathan et al., 2019b). Additionally, meat with a greater pH is a more conducive environment for oxygen-consuming enzymes, leading to greater content of DMb (Tang et al., 2005) and a darker color.

The formation of MMb is pH dependent (Mancini & Hunt, 2005; Ramanathan et al., 2020a). At neutral pH, myoglobin's distal histidine forms a hydrogen bond with oxygen which hinders autoxidation (Brantleyet al., 1993). However, lower pH conditions can affect bonding and orientation of proximal and distal histidines and limit their protective effects against heme oxidation (Ramanathan et al., 2020b). An ultimate pH below 5.80 is reported to minimize the autoxidation of Mb in lamb (Rosenvold and Wiklund, 2011).

The oxygen consumption (OC) and metmyoglobin reducing activity (MRA) affect the lamb color and are influenced by pH (Ramanathan et al., 2019a). Both are inherent biochemical properties that affect the proportion of Mb forms on the meat surface and therefore influence the meat color (Madhavi and Carpenter, 1993). MRA represents the ability of the postmortem muscle to donate an electron to MMb (Fe^{3+}) to form DMb (Fe^{2+}). In postmortem muscle, there is competition for the available oxygen between mitochondria and Mb. If mitochondria are active, there will be limited Mb oxygenation, resulting in darker meat, due to DMb form (English et al., 2016). In this sense, a great ultimate meat pH can limit the OMb formation due to greater mitochondrial and oxygen consuming enzyme activity, that reduces OMb formation and the actual OC (Ramanathan et al., 2019b).

The oxidation rate of OMb is affected by pH (Hoa et al., 2021), and OMb is more stable in neutral and basic environments (Tatiyaborworntham et al., 2012). Hoa et al. (2021) investigated the effect of pH (5.3, 5.8, 6.4 and 7.4) on OMb oxidation for 3h and 48h, and observed that after 3 h, approximately 80–85% OMb remained in all pH conditions. When prolonging the storage time to 24h and 48h, the residual OMb was

significantly lower at pH 5.3 compared to those at pH 5.8, 6.4 or 7.4. Calnan et al. (2016) evaluated the impact of ultimate pH (pH at 24h) in lamb longissimus thoracis color (*L**, *a**, *b**, hue and chroma), and reported that the increase of pH decreased the *L**, *a**, *b**, hue and chroma values. Increasing the ultimate pH from 5.4 to 6 reduced lamb loin *L** by 1.9 units, *a** by 2.5 units, *b** by 2.1 units, hue by 4.9 units and chroma by 2.8 units.

1.4.5. Muscle source

Fresh lamb color is a muscle-specific trait, and the differences on lamb color are related to the differences of muscle fiber types (Tschirhart-Hoelscher et al., 2006) as well as to the oxidative and reductive capacities of the post-mortem muscles (Calnan et al., 2014; Huang et al., 2023). In lamb, muscle fiber types are classified as type I, and types IIA, IIB, IIC according to the energy source utilized, metabolic pathways, and contraction rate (Peinado et al., 2004). Muscles with greater proportions of oxidative fibers, such as psoas major and semimembranosus (Ithurralde et al., 2015), exhibited greater OCR, redness (Tschirhart-Hoelscher et al., 2006), Mb and iron concentrations, and are characterized as color-labile (Gao et al., 2013). Glycolytic muscles, such as longissimus lumborum and longissimus thoracis (Ithurralde et al., 2015), exhibited lower ultimate pH and OCR, greater lightness (Tschirhart-Hoelscher et al., 2006) and MRA, and are classified as color-stable muscles (Gao et al., 2013).

Ithurralde et al. (2015) described the contractile and metabolic fiber types in 16 heavy lamb muscles and observed greater proportion of type I (oxidative) fibers in muscle *psoas* compared to longissimus lumborum and longissimus thoracis, whose exhibited

greater amount of type II (glycolytic) fibers. Tschirhart-Hoelscher et al. (2006) analyzed the physical, chemical, and histological characteristics of 18 lamb muscles and reported greater lightness in muscle latissimus dorsi and tensor fasciae latae, whereas the muscle adductor and semimembranosus, exhibited darker lean color (lower lightness), compared to all other muscles. Regarding redness, the authors reported greater redness in muscle supraspinatus and psoas major among all the muscles studied.

Gao et al. (2013) evaluated the effect of postmortem time on the MRA, OC, and color stability of lamb longissimus dorsi, semitendinosus, and psoas major muscles and reported greater redness in LL, followed by ST and PM, which exhibited lower *a** values. MRA is a muscle (Ramanathan et al., 2021) and a species-specific trait (Elroy et al., 2015). MRA is the ability of the post-mortem muscle to regenerate MMb (Fe^{3+}) into OMb (Fe^{2+}) or DMb (Fe^{2+}) but is not the primary determinant of color stability of ovine longissimus muscle (Bekhit et al., 2001). Bekhit et al. (2001) determined the characteristics of MRA and its effect on color stability of ovine longissimus and documented that the presence of MRA in the ovine LD muscle was evident from the decrease in the peaks at 505 and 630 nm (MMb), and the increase of peaks at 540 and 580 nm (OMb). However, the MRA was only stable in the temperature range of 5 ± 25ºC, and negatively affected by temperatures above 30ºC. Chroma was significantly increased by incubation above 25ºC, and this increase was related to an increase in redness and yellowness.

The iron concentration also influences the color stability of lamb (Warner et al., 2017). Warner et al. (2017) evaluated the iron content of longissimus lumborum (LL) and semimembranosus (SM) muscles from 391 lamb carcasses and documented greater

muscle iron content and lower color stability in SM than in LL muscle. The lamb SM has a greater percent of type I muscle fibers, less percent of type IIB and a greater oxidative score compared to the LL muscle (Ithurralde et al., 2015, 2018).

Lamb color can also be influenced by metabolic pathways and biochemical changes that occur in muscle during conversion of muscle to meat. Huang et al. (2023) investigated the ability of 8 potential biomarkers, such as phosphoglycerate kinase-1 (PGK1), pyruvate kinase-M2 (PKM2), phosphoglucomutase-1 (PGM1), β-enolase (ENO3, myosin-binding protein-C (MYBPC1), myosin regulatory light chain-2 (MYLPF), troponin C-1 (TNNC1) and troponin I-1 (TNNI1) to characterize meat lamb color in muscle quadriceps femoris (QF), and longissimus thoracis (LT) muscles. The authors reported greater abundance of glycolytic enzymes such as PKM2, PGK1, PGM1 and ENO3 in the LT, a glycolytic muscle, compared to QF muscle.

In addition, the amount of isocitrate dehydrogenase activity can be an indicator of oxidative metabolism, which abundance is related with the decrease of lamb *a** values and color stability (Calnan et al., 2014). This enzyme is crucial in the oxygen-dependent citric acid cycle of mitochondria, which are more abundant in oxidative fibers (Hoppeler, 1985). Calnan et al. (2014) utilized spectrophotometric measures to determine the color stability (R630/R580) of 4,238 lamb longissimus lumborum (LL) and reported that the increase of isocitrate dehydrogenase activity in the LL muscle was associated to a reduction in R630/R580. This observation supports the decrease of lamb color stability with the increase of muscle oxidative capacity during display (Calnan et al., 2014).

CHAPTER 2

Artificial raising on milk replacer did not affect the color and oxidative stability of longissimus lumborum muscles from ram lambs

Abstract:

Lamb growth and production traits can be affected by the pre-weaning management strategy, including artificial raising on milk replacer. The objective of the current study was to examine the effect of pre-weaning management on the color and oxidative stability of longissimus lumborum (LL) muscle from ram lambs during refrigerated storage. Polypay ram lambs were raised conventionally with ewes (CR; n = 10) or artificially on milk replacer (AR; n = 10). After weaning at 60 days, rams were fed *ad libitum* and finished on a high-forage diet (50:50 forage:concentrate) (HF; n = 10) containing 50% forage (orchardgrass pellet) and 50% concentrate (76% cracked corn and 24% pre-mixed protein pellet), or a high-concentrate diet (85:15 concentrate:forage) (HC; n = 10) containing 85% concentrate (78% cracked corn and 22% pre-mixed protein pellet) and 15% forage (orchardgrass pellet). until reaching the target slaughter weight of 59 kg. The lambs were harvested, and the LL muscles from both sides of the carcasses (24 h post-mortem) were fabricated into 2.5-cm thick chops. Carcass characteristics were evaluated while harvesting and fabricating. The chops were placed on polystyrene trays, overwrapped with oxygen-permeable polyvinyl chloride film, and randomly assigned to refrigerated storage (2°C) in the darkness for either 0, 3, or 6 days. Instrumental color, color stability (R630/580), pH, lipid oxidation, and MRA of the chops were evaluated at the end of storage periods. Pre-weaning management and finishing system had no influence ($P > 0.05$) on the carcass characteristics, surface redness (a^* value), yellowness (b^* value), hue, chroma, color stability (R630/580), pH, and MRA of LL chops. Color stability (R630/580) and MRA decreased ($P < 0.05$) during storage in both CR and AR

chops, whereas lipid oxidation, yellowness (*b** value), and hue value of the chops increased ($P < 0.05$) in both treatments during the storage period. These findings suggested that milk replacer could be employed as a practical approach for successful artificial raising strategy in lamb production without compromising fresh meat color.

Keywords: artificial raising, color stability, lamb quality, milk replacer

2.1. Introduction

The global demand for the meat from small ruminants (i.e., sheep and goats) is increasing (Ke et al., 2023). Over the last two years (2022-24), the per capita consumption of lamb and mutton meat demonstrated an upward trend in United States (ERS USDA, 2023). The sheep industry strives to improve the quality attributes of fresh lamb, which are dependent on pre-harvest (e.g., feeding systems, live animal management, muscle source) and post-harvest (e.g., packaging, aging, and storage conditions, pH, mitochondrial activity) factors (Suman et al., 2014; Faustman and Cassens, 1990; Mancini and Hunt, 2005; McMillin, 2008). Among these factors, diet (pre-weaning management, forage and concentrate finishing) is the most important factor (Karaca et al., 2016; Cabiddu et al., 2022) influencing the quality of lamb (Zervas et al., 2011; Watkins et al., 2013).

Fresh meat color is the first quality attribute considered by consumers at the point of sale. The color of fresh lamb is light red, and its discoloration is a limiting factor to product retail display resulting in a significant economic loss for the lamb industry (Calnan et al., 2014). In this sense, the preservation of fresh meat color is crucial to avoid consumer rejection and discounts on product price (Suman and Joseph, 2013; Mancini and Hunt, 2005).

To enhance lamb production efficiency and live weight gains, milk replacer has been utilized as an artificial raising strategy (Napolitano et al., 2002a, 2002b; Osorio et al., 2007; Campbell, 2019) to raise triplets, surplus, or orphan lambs to improve their growth and survival (Notter et al., 2018). Artificial weaning is also used in commercial dairy

systems (Napolitano et al., 2008; McCoard et al., 2021). Despite the advantages, the use of milk replacer may affect the lamb color, and investigations in this aspect have demonstrated conflicting results. Chai et al. (2018) reported a brighter meat color in lambs raised on milk replacer compared to those exclusively fed on ewe's milk. Ward et al. (2017) reported similar redness and muscle pH in meat from both naturally (control) and artificially raised lambs. De Palo et al. (2015) documented lower lightness and greater yellowness and hue angle in meat from goats fed with milk replacer. Moreover, Ripoll et al. (2019b) reported lower redness in meat from goats raised with milk replacer than their counterparts naturally fed.

During the fattening period, the lambs can be subject to different finishing systems, such as pasture and concentrate feeding. Pasture-based finishing systems are low-cost and are associated with improved animal welfare, whereas may result in a less efficient production performance, carcass grade and conformation compared to concentrated-finished lambs in confinement (Aguayo-Ulloa et al., 2013). The meat of pasture-fed lambs is associated with a high ultimate pH and darker color (Calnan et al., 2016). Although previous investigations evaluated the influence of feeding milk replacers on the color of lamb, it's influence on lamb color and oxidative stability is yet to be investigated. Therefore, the aim of the present study was to evaluate the effect of milk replacer as a pre-weaning strategy on color stability and lipid oxidation of lamb longissimus lumborum muscle during 6 days of refrigerated storage.

2.2. Materials and methods

2.2.1. Lamb production

All protocols were approved by the Institutional Animal Care and Use Committee of the University of Kentucky (Protocol #2021-3772). Twenty (n = 20) Polypay ram lambs were used for this experiment. The lambs were born at the University of Kentucky C. Oran Little Research Center Sheep Unit (Versailles, KY; geographic coordinates: 38°4'36"N, 84°44'22"W) and were either conventionally raised (CR; n = 10 with ewes for 60-day of pre-weaning period) or raised under artificial milk replacer (AR; n = 10) raising. After weaning, the lambs were finished in confinement with a high-forage (HF) (50:50 forage: concentrate) or a high-concentrate (HC) diet (85:15 concentrate: forage) (Table 2.1 and Table 2.2). All diets were made to be isonitrogenous. The lambs were fed ad libitum until they reached the target slaughter weight (SW) of 59 kg (84.0 ± 0.8 days-of-age).

2.2.2. Lamb harvest, fabrication, and carcass characteristics

After achieving the SW, the lambs were humanely harvested at the USDA-FSIS Inspected Meat Laboratory of the University of Kentucky. The lambs were humanely harvested at live weights of 59.06 ± 0.52 kg for CR-HC; 58.97 ± 1.04 kg for CR-HF; 58.79 ± 1.17 kg for AR-HC; 58.33 ± 0.42 kg for AR-HF. Hot carcass weights were recorded, and the carcasses were chilled for 24 h at 2°C. Following a 24-h chilling (2°C), conformation, quality grade (flank fat streaking), overall grade, cold carcass weight, dressing percentage, rib eye area, fat thickness, and body wall thickness were measured of each carcass. All carcass grading and measurements were performed according to the methods described in Meat

Evaluation Handbook of American Meat Science Association (AMSA, 2001). The neck, foreshank (IMPS #210), shoulder (IMPS # 207), rack (IMPS # 204), loin (IMPS # 232), and leg (IMPS # 233A), were removed and weighed individually according to Institutional Meat Purchasing Specifications (North American Meat Processors Association, 2010). Primal cuts were recorded in absolute weight (weight in kg of the primal).

The LL muscles from both sides of the carcasses were excised. The muscles were fabricated into 2.5-cm thick chops, placed into Styrofoam trays, and overwrapped with oxygen permeable polyvinyl chloride film (15,500-16,275 cm3/m2/24 h oxygen transmission rate at 23°C) and randomly assigned to storage (2°C) in darkness. Instrumental color, color stability (R630/580), pH, lipid oxidation, and metmyoglobin reducing activity (MRA) were evaluated on days 0, 3, and 6, whereas myoglobin concentration was determined on day 0.

2.2.3. Myoglobin concentration

Duplicate 5 g muscle samples were homogenized with 45 mL ice-cold sodium phosphate buffer (40 mM, pH 6.8) for 45 seconds and centrifuged at 1500 x g for 30 min at 4°C (Faustman and Phillips, 2001). The supernatant was filtered through Whatman No. 1 paper, and the absorbance of the filtrate was measured at 525 nm (A525) utilizing a UV-241PC spectrophotometer (Shimadzu Inc., Columbia, MD) with 40 mM sodium phosphate buffer as a blank. The Mb concentration was calculated using the following equation:

Mb (mg/g) = [A525 / (7.6 mM-1 cm-1 × 1 cm)] × (17,000/1000) × 10

where 7.6 mM-1 cm-1 = mM absorptivity coefficient of Mb at 525 nm; 1 cm = length of the light path in the cuvette; 17,000 Da = average molecular weight of Mb; and 10 = dilution factor.

2.2.4. Meat pH

The pH value of raw samples was determined according to the method of Strange et al. (1977). Five grams of samples were homogenized with 30 mL of distilled deionized water (at 25°C), and the pH was measured utilizing an Accumet AR25 pH meter (Fisher Scientific, Pittsburg, PA).

2.2.5. Instrumental color

The surface color of chops was measured using a HunterLab LabScan XE colorimeter (Hunter Associates Laboratory, Reston, VA) with 1.27-cm-diameter aperture, illuminant A, and 10° standard observer. The colorimeter was calibrated with standard black and white plates. On day 0 of storage, the oxygen permeable film was removed from the packages, and the chops were allowed to bloom for 2 h at 2°C before the instrumental color attributes were evaluated. CIE (1976) *L** (Lightness), *a** (redness) and *b** (yellowness) values were measured at 3 random locations on the oxygen-exposed surface of each chop (King et al., 2023). Additionally, the reflectance was measured from 700 to 400 nm, and the ratio of reflectance at 630 nm and 580 nm (R630/580) was determined as an indirect estimate of surface color stability (King et al., 2023)

2.2.6. Lipid oxidation (TBARS)

Lipid oxidation was measured using the thiobarbituric acid assay (Yin et al., 1993). Duplicate 5-g of sample was homogenized with 22.5 mL of 11% trichloroacetic acid (TCA) solution and filtered through Whatman No. 1 filter paper (GE healthcare, Little Chalfront, UK). Subsequently, 1.5 milliliters of the resulting aqueous filtrate were added with an equal volume of 20 mM thiobarbituric acid (TBA) solution and incubated at 25°C for 20 hours. A blank of 20 mM TBA and 11% TCA was simultaneously incubated with the other samples. The absorbance value at 532 nm was measured utilizing a UV-2401PC spectrophotometer (Shimadzu Inc., Columbia, MD) and the results were presented as thiobarbituric acid reactive substances (TBARS).

2.2.7. Metmyoglobin reducing activity (MRA)

The metmyoglobin reducing activity (MRA) was evaluated according to Sammel et al. (2002). The lamb chops were submerged in 0.3% sodium nitrite (Sigma-Aldrich Co., St. Louis, MO) solution for 30 min at room temperature to facilitate metmyoglobin formation. After 30 min, the samples were removed from the solution, blotted dry, and vacuum packaged (99% vacuum; Sipromac Model 600A; Drummondville, Quebec, Canada) in Prime Source vacuum pouches (3 mil, Bunzl Koch Supplies Inc., Kansas City MO). The reflectance spectra were measured from 700 to 400 nm on the light-exposed surface using a HunterLab LabScan XE colorimeter immediately after vacuum packaging in order to calculate pre-incubation surface metmyoglobin values (King et al., 2023). The samples were then incubated at 30°C for 2h allowing for metmyoglobin reduction, and

then the surface reflectance was rescanned to calculate post-incubation metmyoglobin values (King et al, 2023). MRA was calculated using the following equation:

MRA = 100 × [(% pre-incubation surface metmyoglobin - % post-incubation surface metmyoglobin)/% pre-incubation surface metmyoglobin]

2.2.8. Statistical analysis

The experimental design was a split-plot, with the pre-weaning management and finishing system as a whole plot and storage days as a sub-plot. The LL muscles from twenty (n = 20) lamb carcasses were utilized in this study. The LL muscles from each lamb carcass served as the experimental unit, whereas the subplot experimental units consisted of chops fabricated from each lamb carcass and assigned for 0, 3, or 6 days of refrigerated storage. The analysis of variance was determined utilizing the PROC GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). The Least Square means were separated using the PDIFF option with a Tukey-Kramer adjustment, and the differences among means were considered statistically different at a 5% level.

2.3. Results and discussion

2.3.1. Carcass characteristics

A weaning x finishing system interaction ($P > 0.05$) was observed for right shoulder cuts (Table 2.3). The results of live weight, hot carcass weight, carcass conformation, quality grade (flank fat streaking), overall grade, cold carcass weight, dressing percentage, ribeye area, fat thickness, body wall thickness, neck, foreshank, left and right shoulder,

rack, loin, left, and right leg are presented in Table 2.3. There was no effect ($P > 0.05$) of finishing system on rack and loin cuts, but an effect of weaning was observed ($P < 0.05$). The rack from artificially raised lambs exhibited greater ($P < 0.05$) weights compared to those from the conventionally raised lambs. On the other hand, the loins from artificially raised lambs exhibited lighter weight ($P < 0.05$) than their counterparts conventionally raised.

Similarly, Chai et al. (2018) examined the carcass traits of artificially and naturally raised Hu lambs and reported similarities in hot dressing percentage, and back fat thickness in both groups. Napolitano et al. (2002a) evaluated the effect of artificial raising on meat production and behavioral response of lambs and reported no differences in the percentages of carcass yield and secondary cuts between the groups, whereas lower loin percentages were found in artificially raised lambs. On contrary, previous investigations reported no effect of weaning in lamb carcass characteristics. Napolitano et al. (2002b) evaluated the effect of conventional and artificial raising on the lamb carcass characteristics and reported no differences in slaughter weight, hot carcass weight, first grade wholesale cuts (rack, loin, and leg) percentage, leg lean percentage, and leg bone percentage. Lanza et al. (2006) found no differences in slaughter weight and carcass yield percentages between the naturally and artificially raised lambs. Rodríguez et al. (2008) examined the effect of artificial and natural raising system on the carcass and meat characteristics of lambs and reported no differences in the cold carcass weight and dressing percentage of lambs raised conventionally and artificially.

Napolitano et al. (2002b) examined the carcass traits and meat quality of artificially and naturally raised lambs and reported that the milk replacer fed lambs produced greater carcass yield, greater second grade wholesale cuts (shoulder, neck, breast) percentage, and lower leg fat percentage than their naturally fed counterparts. Girolami et al. (1994) reported lower dressing percentage in artificially raised lambs. Napolitano et al. (2002a) reported greater slaughter weight and percentages of rack in ewe-raised lambs.

2.3.2. Myoglobin Concentration

There was no weaning x finishing system interaction (P = 0.69) for Mb concentration in lamb chops (7.8 ± 0.83 mg/g in CR-HC; 7.9 ± 0.35 mg/g in CR-HF; 7.7 ± 0.74 mg/g in AR-HC; 8.5 ± 0.49 mg/g in AR-HF). Additionally, there was no effect of weaning (P = 0.788) and finishing system (P = 0.6064) for Mb concentration. Supporting our results, Osorio et al. (2008) evaluated the effect of artificial raising on the color and lipid oxidation in the M. longissimus dorsi muscle from suckling lambs and observed similar myoglobin content between the conventionally and artificially raised animals.

2.3.3. Meat pH

There was no weaning x finishing system x storage interaction (P = 0.8744) for pH (Table 2.4). Also, there was no effect of weaning (P = 0.5923), finishing system (P = 0.0807), and storage (P = 0.0936) for pH.

The observed similarity in pH could be attributed to the similar energy intake provided by milk replacer and ewe's milk (Belanche et al., 2019). Belanche et al. (2019) documented that artificially and naturally raised lambs exhibit a similar concentration of plasma glucose due to high digestibility of milk replacer. Plasma glucose is taken up by muscle cells as a result of insulin action and could be stored as glycogen or oxidized for energy (Immonen et al., 2000). Glycogen plays an important role in post-mortem anaerobic glycolysis, and is the primary substrate for lactic acid production, decreasing the ultimate muscle pH (Immonen et al., 2000). In this sense, the similarity in energy intake of both milk replacer and ewe's milk may have contributed to similar muscle glycogen storage as well as similar postmortem pH decrease in both treatments.

In agreement with our results, Osorio et al. (2008) examined the effect of conventional and artificial raising on lamb quality and documented similar pH in the longissimus dorsi muscles in both naturally and artificially raised animals. Additionally, Rodríguez et al. (2008) reported no differences in pH in longissimus thoracis and longissimus dorsi muscles from lambs raised with mother's milk, milk replacer *ad libitum* twice a day, and milk replacer at 70% of *ad libitum*. Lanza et al. (2006) evaluated the meat quality of lambs fed with milk replacer or maternal milk and reported similarities in pH in longissimus dorsi muscle in both raising systems. Napolitano et al. (2006) examined the meat quality of lambs raised artificially and conventionally and reported no differences in pH in longissimus dorsi, semimembranosus, and semitendinosus muscles from both treatments. Zurita-Herrera et al. (2013) reported similar pH in the longissimus thoracis et lumborum, semimembranosus, and triceps brachii muscles from both artificially and

naturally raised dairy breed goat kids. Ripoll et al. (2019a) evaluated the effect of feeding with natural milk or milk replacer to the texture profile of meat from suckling kids and documented similar pH in the longissimus thoracis muscle from kids raised with milk replacer or natural dam milk. Ripoll et al. (2019b) also evaluated the impacts of the raising system on the meat color of suckling kids and reported a similar pH in longissimus thoracis muscle. Argüello et al. (2005) documented no differences in pH values in the longissimus dorsi, semimembranosus, and triceps brachii muscles of both the artificially and conventionally raised goat kids. In contrast, Zullo et al. (2006) reported greater pH values *in* longissimus dorsi, semimembranosus, rectus femoris, and gluteobiceps muscles from naturally raised lambs.

2.3.4. Lightness (L values)*

There was no weaning x finishing system x storage interaction (*P* = 0.666) for lightness (Table 2.4). Also, there was no effect of weaning (*P* = 0.1657), finishing system (P = 0.5379), and storage (*P* = 0.1028) for *L** values. Artificial weaning and finishing system (forage- and concentrate-based) did not affect (*P* > 0.05) *L** values during 6 days of storage (Table 2.4). The observed similarity in *L** values could be attributed to the meat pH (Abril et al., 2001). Similar meat pH (Table 2.4) in the treatments may have contributed to a similar light reflectance as well as similar lightness on the samples.

In agreement, Santos-Silva et al. (2002) evaluated the meat quality of lambs raised on three different feeding systems – pasture with dams, pasture with dams plus concentrate *ad libitum*, weaning and concentrate *ad libitum* – and reported similar

lightness in longissimus thoracis muscle. Napolitano et al. (2006) evaluated the effects of artificial raising on meat quality of lambs and reported similar lightness in semitendinosus muscles from conventionally and artificially raised lambs. Rodriguez et al. (2008) examined the impact of natural and artificial raising system on carcass and meat characteristics of Assaf milk fed lambs and reported no differences in lightness in the longissimus lumborum muscles between natural raised, artificial raised as well as restricted artificial raised animals. Additionally, Zurita-Herrera et al. (2013) evaluated the impact of three different management systems (extensive, intensive with natural raising and intensive with artificial raising) on meat quality of dairy breed goat kids and reported similar lightness in the longissimus thoracis et lumborum, semimembranosus, and triceps brachii muscles from both artificially and naturally raised animals. Similarly, Argüello et al. (2005) reported no differences in lightness in the longissimus dorsi, semimembranosus, and triceps brachii muscles of both the artificially and naturally raised goat kids. In contrast, Lanza et al. (2006) evaluated the influence of natural or artificial milk feeding regime and reported that the meat from naturally raised lambs exhibited greater *L** when compared to their artificially raised counterparts. Chai et al. (2018) evaluated the effect of raising system on meat quality of Hu lambs and reported greater lightness (*L** values) in the longissimus thoracis muscle from artificially raised lambs. Osorio et al. (2008) evaluated the quality of meat from artificially and naturally raised suckling lambs and reported greater *L** values in longissimus dorsi muscle of suckling lambs raised naturally (maternal milk) than those artificially fed. De Palo et al. (2015) evaluated the effect of diet (goat milk, milk replacer, and acidified milk replacer) on the

meat color of suckling kids and observed lower lightness in the meat from kids fed with milk replacer.

2.3.5. Redness (a values)*

There was no weaning x finishing system x storage interaction (P = 0.6617) for redness (Figure 2.1). Additionally, there was no effect of weaning (P = 0.5775), finishing system (P = 0.8734), and storage (P = 0.6784) for *a** values. Weaning and finishing system (forage- and concentrate-based) did not affect (P > 0.05) *a** values during 6 days of storage (Figure 2.1). The similarities in *a** values could be attributed to the meat pH (Calnan et al., 2016). Mitochondria remain active in postmortem muscle, influencing beef color by oxygen consumption (OC) and the pH plays an important role in OC rate. When pH increases, it enhances mitochondria activity, increasing OC and dark muscle due to decreased oxygen partial pressure (Tang et al., 2005; Ramanathan et al., 2018). The similarities in pH in present study may have contributed to similar mitochondrial activity as well as the similarity in redness.

Supporting our results, Napolitano et al. (2006) reported no differences in redness (*a** values) in semitendinosus muscle from lambs raised either with ewe's milk or on milk replacer. Lanza et al. (2006) documented similar redness in longissimus dorsi muscle from artificially and naturally raised lambs. Chai et al. (2018) reported similar redness in the longissimus thoracis muscle from both naturally and artificially raised lambs. Rodríguez et al. (2008) examined the impact of natural and artificial raising system on carcass and meat characteristics of Assaf milk fed lambs and reported no differences in redness in the

longissimus lumborum muscle between natural and artificial-raised lambs. De Palo et al. (2015) reported no differences in redness in longissimus thoracis muscle of suckling kids raised on goat milk, milk replacer, and acidified milk replacer. Zurita-Herrera et al. (2013) evaluated the impact of three different management systems (extensive, intensive with natural raising, and intensive with artificial raising) on meat quality of dairy breed goat kids and documented similar redness in triceps brachii muscle. In contrast, Osorio et al. (2008) evaluated the influence of raising system (raising with maternal milk or with milk replacer) on quality traits of suckling lamb and documented greater *a** values in longissimus dorsi muscle from artificially raised lambs compared to their naturally (raising with maternal milk) raised counterparts. Zullo et al. (2006) evaluated the effect of raising system on the lamb quality and documented greater *a** values in longissimus dorsi, semimembranosus, rectus femoris, and gluteobiceps muscles from naturally raised animals than their artificially raised counterparts. Santos-Silva et al. (2002) evaluated the meat quality of lambs raised on three different feeding systems – pasture with dams, pasture with dams plus concentrate *ad libitum*, and weaning and concentrate *ad libitum* and documented lower redness in longissimus dorsi muscle of animals from pasture-fed systems.

2.3.6. Yellowness (b values)*

There were no weaning x finishing system x storage interaction ($P = 0.6405$) for yellowness (Table 2.4). Additionally, there was no effect of weaning ($P = 0.0636$) and finishing system ($P = 0.3617$) on *b** values. However, an effect of storage ($P < 0.0001$) was

found for the *b** values (Table 2.4). The samples exhibited an increase ($P < 0.05$) in yellowness from day 0 to 6 of storage.

In support of our results, Rodríguez et al. (2008) evaluated the effect of natural and artificial raising on the carcass and quality characteristics of lamb and documented similarities in yellowness in longissimus thoracis and longissimus dorsi muscles in both feeding systems. Lanza et al. (2006) documented similar yellowness in longissimus dorsi muscle from lambs raised with artificial and natural milk. Additionally, Napolitano et al. (2006) reported no differences in yellowness in semitendinosus muscles from ewe-raised and artificially raised lambs. Zurita-Herrera et al. (2013) evaluated the meat quality of dairy breed goat kids raised extensively, intensively with natural milk, and intensively with milk replacer and documented similar yellowness in longissimus thoracis et lumborum and triceps brachii muscles in all the three raising systems. In contrast, Osorio et al. (2008) evaluated the meat quality from lambs raised conventionally and artificially and reported greater yellowness in the longissimus lumborum muscle from lambs fed with maternal milk compared to their milk replacer-fed counterparts. Ripoll et al. (2018) examined the effects of feeding with milk replacer and maternal milk on the shelf life of leg chops from suckling kids and reported greater *b** values in semimembranosus, semitendinosus, and biceps femoris muscles from kids raised with milk replacer compared to the conventional raised counterparts. Chai et al. (2018) evaluated the effect of raising system on meat quality of Hu lambs and documented greater *b** values in longissimus thoracis muscle from artificially raised lambs. Zullo et al. (2006) documented greater *b** values in longissimus dorsi, semimembranosus, rectus femoris, and gluteobiceps muscles from

lambs raised with maternal milk, in comparison with the artificially raised ones (reconstituted milk). Additionally, De Palo et al. (2015) examined the effect of goat milk, warm milk replacer and acidified milk replacer on the carcass traits and meat color of suckling kids and reported greater yellowness in the longissimus thoracis muscle of kids fed on warm milk replacer.

Regarding storage, Osorio et al. (2008) documented an increase of yellowness in longissimus lumborum muscles from conventionally (maternal milk) and artificially (milk replacer) raised lambs during 21 days of storage. In contrast, Ripoll et al. (2018) reported similar yellowness of semimembranosus, semitendinosus, and biceps femoris muscles from kids raised with mother's milk or with milk replacer during 6 days of storage.

2.3.7. Color stability (R630/580)

No weaning x finishing system x storage interaction was found ($P = 0.9704$) for color stability (Figure 2.2). Additionally, there was no effect of weaning ($P = 0.2021$) and finishing system ($P = 0.865$), but an effect of storage ($P < 0.0001$) in R630/580. All samples exhibited a decrease ($P < 0.05$) in color stability from day 0 to day 6 of storage ($P < 0.05$).

The observed decrease in color stability could be attributed to the myoglobin oxidation and metmyoglobin accumulation (Faustman et al., 2010). The R630/580 (redness indicator) estimates surface discoloration, where greater ratios indicate lower surface metmyoglobin accumulation and consequently greater redness and color stability (King et al., 2023). Lipid oxidation affects myoglobin oxidation due to reactivity of primary and secondary products derived from unsaturated fatty acids. These products act

triggering oxidative reactions, contributing to the myoglobin oxidation and to the decrease of color stability (Faustman et al., 2010). In agreement with our results, Alvarenga et al. (2019) reported a decrease in color stability (R630/580) of lamb longissimus thoracis during 4 days of retail display. Ponnampalam et al. (2017) documented a decrease in R30/580 in lamb longissimus lumborum and semimembranosus muscles during 5 and 60 days of storage period. Coombs et al. (2017) evaluated the effect of chilled-then-frozen storage in lamb quality and reported a decrease in color stability of longissimus lumborum muscles up to 52 weeks of frozen storage. Jian-zeng et al. (2017) observed decreased color stability in mutton longissimus dorsi muscles over 8 days of refrigerated storage. In contrast, Li et al. (2017) reported stable values of R630/580 in lamb longissimus lumborum muscles from day 4 to 10 of storage.

2.3.8. Hue angle

There was no weaning x finishing system x storage interaction ($P = 0.8766$) for hue angle (Figure 2.3). Artificial weaning and finishing system (forage- and concentrate-based) did not affect ($P > 0.05$) the hue values. However, there was an effect of storage ($P < 0.0001$) for hue angle (Figure 2.3). All samples exhibited an increase ($P < 0.05$) in hue angle from day 0 to 6 of storage.

The observed increase of hue angle could be attributed to the metmyoglobin formation and accumulation onto meat surface during storage (Luciano et al., 2009a; Luciano et al., 2011; King et al., 2023). Hue angle is associated with meat discoloration,

where greater values denote less red and greater metmyoglobin content (King et al., 2023). The rate of metmyoglobin accumulation in meat over storage could also be related to oxidative processes, such as lipid oxidation. The increase of lipid oxidation (as observed in our results) generates secondary oxidation products, such as malondialdehyde, capable to accelerate metmyoglobin formation (Suman et al., 2007; Faustman et al., 2010), contributing to the increase of hue angle. A positive correlation between metmyoglobin accumulation and increase of hue angle has been reported previously in beef (Ijaz et al., 2020; and Aroeira et al., 2017).

In agreement with our results, Napolitano et al. (2006) evaluated the effects of conventional and artificial raising on lamb quality and reported no differences in hue values in semitendinosus muscle. Lanza et al. (2006) evaluated the influence of natural or artificial milk feeding regime on lamb quality and reported similar hue angle in the longissimus dorsi muscle of both treatments. Chai et al. (2018) evaluated the effect of raising system on meat quality of Hu lambs and documented no differences in hue angle in the longissimus thoracis muscle of lambs raised with maternal milk or on milk replacer. Zullo et al. (2006) documented no effect of raising system on hue values of lamb. Argüello et al. (2005) evaluated the effects of natural and milk replacer feeding on the meat quality of goat kids and reported no differences in hue values in the longissimus dorsi, semimembranosus, and triceps brachii muscles from kids of both treatments. Ripoll et al. (2018) reported similar hue values in semimembranosus, semitendinosus, and biceps femoris muscles from kids fed with milk replacer and with maternal milk. In contrast, De Palo et al. (2015) evaluated the effect of milk diet (goat milk, milk replacer, and acidified

milk replacer) on the meat color of suckling kids and observed greater hue values in the longissimus thoracis muscle of kids fed on warm milk replacer than their maternal milk and acid milk replacer-fed counterparts.

The increase of hue angle values during storage are in contrast with Ripoll et al. (2018) that reported similar hue values in semimembranosus, semitendinosus, and biceps femoris muscles from kids raised with mother's milk or with milk replacer during up to 8 days of storage. This difference in the observation could be due to differences between species (Young and West, 2001).

2.3.9. Chroma

There was no weaning x finishing system x storage interaction (P = 0.5434) for chroma (Figure 2.4). Also, there was no effect of weaning (P = 0.3618), finishing system (P = 0.8488), and storage (P = 0.6493) for this parameter (Figure 2.4). Chroma or saturation index is an indicator of color intensity, and high values indicate more intense or vivid color. Chroma calculation utilizes the a^* and b^* values following the equation $(a^2 + b^2)^{½}$ (King et al., 2023) and as discussed previously there was no effect of weaning and diet on redness (a^* values) and yellowness (b^* values), which may have contributed to the similarity of chroma values.

In agreement, Napolitano et al. (2006) examined the effects of artificial and conventional raising on meat quality of lambs and reported similar chroma values in semitendinosus muscles in both treatments. Lanza et al. (2006) documented similar chroma values in longissimus dorsi muscles in both naturally (maternal milk) and

artificially (milk replacer) raised lambs. Chai et al. (2018) documented similar chroma values in the longissimus thoracis muscles from Hu lambs raised with conventional and artificial milk. De Palo et al. (2015) examined the effect of feeding regime (goat milk, warm milk replacer, and acid milk replacer) on the meat color of suckling kids and documented no differences in chroma values in longissimus thoracis muscles from lambs subjected to the three treatments. Ripoll et al. (2018) reported similar chroma values in semimembranosus, semitendinosus, and biceps femoris muscles from kids naturally and artificially raised. In contrast, Zullo et al. (2006) evaluated the effect of raising system on the lamb quality and documented lower chroma values in longissimus dorsi, semimembranosus, rectus femoris, and gluteobiceps muscles from artificially raised animals compared to their naturally raised counterparts. Argüello et al. (2005) reported greater chroma values in the longissimus dorsi, semimembranosus, and triceps brachii muscles of kids raised on milk replacer.

2.3.10. Lipid oxidation (TBARS)

There was no weaning x finishing system x storage interaction (P = 0.2911) for TBARS. Additionally, there was no effect of weaning (P = 0.7703) and finishing system (P = 0.3349) for TBARS values. However, there was an effect of storage (P < 0.0001) for lipid oxidation (Figure 2.5). All samples exhibited an increase (P < 0.05) in lipid oxidation from day 0 to 6 of storage (Figure 2.5).

The increase in lipid oxidation during storage could be associated to the decrease of meat redox capacity and generation of free radicals, responsible for triggering the

reactions of lipid oxidation (Min and Ahn, 2005). Additionally, iron content has great influence on the rate of oxidation (Warner et al., 2017). Myoglobin is a heme protein with a central iron atom that can be oxidized generating reactive intermediates (e.g., hydrogen peroxide) able to further increase myoglobin and lipid oxidation (Faustman et al., 2010).

An increase in lipid oxidation of lamb during the storage period was reported previously. Berruga et al. (2005) observed an increase of lipid oxidation in longissimus dorsi muscles during 28 days of storage. Coombs et al. (2018) evaluated the effect of storage on the fatty acid profile and lipid oxidation in lamb M. longissimus lumborum and reported an increase of TBARS during 8 weeks of frozen storage. In addition, Ponnampalam et al. (2017) documented an increase of lipid oxidation in frozen lamb longissimus lumborum and semimembranosus muscles up to 60 days of storage. Soldatou et al. (2009) reported an increase of lipid oxidation of refrigerated Souvlaki-type lamb during 13 days of storage. Pinheiro et al. (2019) reported an increase of lipid oxidation in frozen lamb longissimus lumborum over 12 months of storage. Gonzales-Barron et al. (2021) evaluated the lipid oxidation of lamb in longissimus lumborum muscles of European breeds and reported an increase of lipid oxidation during 15 days of storage. Furthermore, Inserra et al. (2014) increase of lipid oxidation in lamb longissimus thoracis et lumborum muscles over 6 days of aerobic storage. On contrary, Baltar et al. (2019) evaluated the oxidative stability of lamb patties stored under refrigeration (4° C) and reported stable TBARS values during five days of storage. Luciano et al. (2012) observed similar TBARS in longissimus dorsi muscles from pasture-fed lambs during 7 days of

refrigerated storage. Luciano et al. (2013) reported similar TBARS values in longissimus dorsi muscles from pasture-fed lambs during 8 days of storage.

2.3.11. Metmyoglobin reducing activity (MRA)

There was no weaning x finishing system x storage interaction (P = 0.6289) for MRA (Figure 2.6). Additionally, there was no effect of weaning (P = 0.5784) and finishing system (P = 0.082); whereas an effect of storage (P < 0.0001) was observed for MRA. All samples exhibited a decrease (P < 0.05) in MRA from day 0 to day 6 of storage (Figure 2.6).

The observed decrease in MRA could be attributed to the decrease of nicotinamide adenine dinucleotide (NADH) content during storage (Kim et al., 2009). Metmyoglobin reducing activity is the ability of meat to delay discoloration, through an electron addition to metmyoglobin, through enzymatic or nonenzymatic processes (Ramanathan et al., 2014). The NADH plays an important role in MRA since it acts as a coenzyme and electron carrier in the reduction of metmyoglobin (Renerre, 1990) and can be regenerated by lactate dehydrogenase (LDH) activity (Ramanathan et al., 2014). During storage, there is a reduction of LDH activity as well as of the NADH content, which may reduce the MRA of the beef (Kim et al., 2009).

A decrease of MRA during storage in lamb and goat meat was reported previously. Li et al. (2017) reported a decrease in MRA of lamb longissimus lumborum muscles during 10 days of storage period. Gao et al. (2013) reported a decrease of MRA in lamb longissimus dorsi, semitendinosus, and psoas major muscles over 6 days of storage. Gao

et al. (2014) examined the impacts of different feeding strategies on MRA of lamb semitendinosus muscle and reported a decrease in MRA during 9 days of storage. Gao et al. (2016) reported a decrease in MRA of lamb cardiac muscles during 5 days of refrigerated storage. Adeyemi et al. (2016 a,b) documented a decrease in MRA in goat gluteus medius, infraspinatus, and gluteus medius muscles during 7 days of storage. Further work (Adeyemi et al., 2017) documented a decrease in goat longissimus thoracis MRA throughout 10 days of storage.

2.4. Conclusions

The findings of the present study indicate that the artificial raising of ram lambs with milk replacer did not influence the color and oxidative stability of meat. The meat from lambs raised conventionally and artificially (with milk replacer) as well as finished with forage and concentrate exhibited similar carcass characteristics, color (*L**, *a**, *b**, R630/580, hue angle, chroma), and oxidative stability (MRA and lipid oxidation). Additionally, there was an effect of storage, increasing *b** values, hue angle, and lipid oxidation and decreasing R630/580, and MRA. These findings suggest that milk replacer can be successfully exploited as an artificial raising strategy in lamb production without negative impact on fresh meat color.

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Table 2.1: Ingredient composition of high-concentrate (HC) and high-forage (HF) diets.

Ingredient, %	**Diet**[1]	
	HC	**HF**
Cracked Corn	78	76
Protein Pellet[2]	22	24
Orchardgrass Pellet	15	50

[1] HC = 85:15 concentrate:forage; HF = 50:50 concentrate:forage.

[2] Composition: 63.33% soybean meal (48% CP), 21.25% distillers dried grains with solubles, 4.38% ground limestone, 3.13% salt, 2.50% ammonium chloride, 1.50% sheep premix, 0.50% Vitamin E (20,000 IU/lb), 0.25% Vitamin A (10,000 IU/lb), 0.25% Vitamin D3 (15,000 IU/lb).

Table 2.2: Chemical composition of high-concentrate (HC) and high-forage (HF) diets.

Component, %[2]	**Diet[1]**	
	HC	**HF**
Dry Matter	89.6	91.5
Crude Protein	16.1	15.6
Acid Detergent Fiber	8.0	23.0
Neutral Detergent Fiber	16.6	33.4
Starch	44.2	19.0
TDN[b3]	84	79

[1] HC = 85:15 concentrate:forage, HF = 50:50 concentrate:forage.

[2]All components excluding dry matter on a 100% DM basis.

[3] TDN[b] = total digestible nutrients.

Table 2.3: Carcass characteristics of Polypay ram lambs raised conventionally (CR) or artificially with milk replacer (AR) and finished with high-forage (HF; 50:50 forage:concentrate) or a high-concentrate (HC; 85:15 concentrate:forage) diet in confinement[1].

Carcass Characteristics	Raising system				*P*-values		
	Conventional raising		Artificial raising				
	HF	HC	HF	HC	Pre-weaning	Finishing system	Pre-weaning x Finishing system
Live Weight (kg)	58.97 ± 1.04	59.06 ± 0.52	58.33 ± 0.42	58.79 ± 1.17	0.6213	0.7385	0.8267
Hot Carcass Weight (kg)	27.44 ± 0.73	28.17 ± 0.64	27.31 ± 0.36	26.72 ± 0.55	0.1958	0.933	0.2831
Cold Carcass Weight (kg)	27.31 ± 0.68	28.08 ± 0.60	27.13 ± 0.36	26.81 ± 0.59	0.2082	0.7183	0.3679
Ribeye Area (cm^2)	15.87 ± 0.99	15.39 ± 0.69	15.23 ± 0.49	16.23 ± 1.57	0.9277	0.8006	0.4757
Fat Thickness (cm)	0.38 ± 0.06	0.52 ± 0.08	0.41 ± 0.06	0.41 ± 0.04	0.4916	0.289	0.2495
Body Wall Thickness (cm)	2.12 ± 0.17	2.17 ± 0.15	2.59 ± 0.13	2.45 ± 0.28	0.0702	0.8224	0.6253
Neck (kg)	0.92 ± 0.07	0.89 ± 0.07	0.95 ± 0.02	0.93 ± 0.04	0.4884	0.6084	1.0000
Foreshank; IMPS # 210 (kg)	1.44 ± 0.03	1.48 ± 0.10	1.30 ± 0.03	1.41 ± 0.06	0.0968	0.2126	0.6016
Left Shoulder; IMPS # 207 (kg)	2.72 ± 0.11	2.79 ± 0.06	2.74 ± 0.13	2.65 ± 0.04	0.5382	0.8981	0.4219
Right Shoulder; IMPS # 207 (kg)	2.72 ± 0.09^{c}	2.99 ± 0.10^{a}	2.87 ± 0.05^{b}	2.70 ± 0.12^{c}	0.4972	0.5803	0.0284
Rack; IMPS # 204 (kg)	2.64 ± 0.12^{b}	2.70 ± 0.09^{b}	3.11 ± 0.05^{a}	2.93 ± 0.15^{a}	0.0056	0.5769	0.2866
Loin; IMPS # 232 (kg)	2.47 ± 0.12^{a}	2.43 ± 0.16^{a}	2.15 ± 0.12^{b}	2.14 ± 0.15^{b}	0.0421	0.875	0.9202
Left leg; IMPS # 233A (kg)	4.27 ± 0.14	4.42 ± 0.13	4.19 ± 0.06	4.11 ± 0.10	0.0904	0.7609	0.3231
Right leg; IMPS # 233A (kg)	4.33 ± 0.11	4.43 ± 0.05	4.17 ± 0.10	4.26 ± 0.13	0.1236	0.3653	1.0000
Dressing Percentage (%)	46.53 ± 0.81	47.69 ± 0.85	46.82 ± 0.68	45.46 ± 0.56	0.2058	0.8900	0.1058
Carcass Conformation[2]	(C^0) 580.00 ± 37.42	(C^0) 560.00 ± 40.00	(C^+) 620.00 ± 37.42	(C^+) 620.00 ± 37.42	0.2077	0.7962	0.7962

Quality Grade (FFS)[3]	(Sm) 332.00 ± 63.51	(Sm) 354.00 ± 58.10	(Sm) 396.00 ± 56.36	(Sm) 376.00 ± 43.08	0.4519	0.9859	0.7115
Overall Grade[2]	(C^+) 600.00 ± 31.62	(C^0) 580.00 ± 20.00	(C^+) 660.00 ± 50.99	(C^+) 640.00 ± 60.00	0.1876	0.6525	1.0000

CR = conventionally raised with ewes, AR = artificially raised on milk replacer, HF = finished on high-forage, HC = finished on high-concentrate.

[1] Results are expressed as mean ± standard error of the mean (SEM).

[2] 400 = low choice (C^-), 500 = average choice (C^0), 600 = high choice (C^+), 700 = low prime (P^-), 800 = average prime (P^0), 900 = high prime (P^+).

[3] FFS = flank fat streaking. 100 = traces (Tr), 200 = slight (Sl), 300 = small (Sm), 400 = modest (Mt), 500 = moderate (Md), 600 = slightly abundant (Sa), 700 = moderately abundant (Ma), 800 = abundant (Ab).

Table 2.4: Meat pH, lightness (*L**) and yellowness (*b**) values of longissimus lumborum chops from Polypay ram lambs conventionally raised (CR) or artificial raised with milk replacer (AR) and finished with high-forage (HF; 50:50 forage:concentrate) or a high-concentrate (HC; 85:15 concentrate:forage) diet during 6 days of refrigerated storage.

Parameter	Pre-weaning x finishing system	Days of storage		
		0	3	6
Meat pH	CR-HF	5.63 ± 0.07	5.53 ± 0.05	5.60 ± 0.05
	AR-HF	5.62 ± 0.04	5.58 ± 0.04	5.59 ± 0.07
	CR-HC	5.69 ± 0.04	5.61 ± 0.03	5.66 ± 0.03
	AR-HC	5.68 ± 0.04	5.71 ± 0.06	5.68 ± 0.02
*L** value	CR-HF	39.89 ± 1.09	42.37 ± 0.81	40.93 ± 1.14
	AR-HF	38.28 ± 0.61	39.66 ± 0.53	40.65 ± 0.91
	CR-HC	38.95 ± 0.49	49.13 ± 0.70	40.76 ± 1.29
	AR-HC	38.24 ± 1.12	40.84 ± 1.67	40.07 ± 0.86
*b** value	CR-HF	11.92 ± 0.54[b]	14.40 ± 0.42[a]	14.40 ± 0.39[a]
	AR-HF	12.80 ± 0.20[b]	14.36 ± 0.26[a]	15.04 ± 0.27[a]
	CR-HC	12.79 ± 0.26[b]	14.47 ± 0.36[a]	14.02 ± 0.29[a]
	AR-HC	13.60 ± 0.61[b]	15.01 ± 0.18[a]	14.69 ± 0.23[a]

CR = conventionally raised with ewes, AR = artificially raised on milk replacer, HF = finished on high-forage, HC = finished on high-concentrate.

[a-b]Means without common superscripts are different ($P < 0.05$).

[1]Results are expressed as mean ± standard error of the mean (SEM).

Figure 2.1: Redness (*a** values) of longissimus lumborum chops from Polypay ram lambs conventionally raised (CR) or artificially raised with milk replacer (AR) and finished with high-forage (HF; 50:50 forage:concentrate) or a high-concentrate (HC; 85:15 concentrate:forage) diet during 6 days of refrigerated storage. Standard error bars are indicated. [a-c]Means within a weaning-finishing system or day of storage with different letters are different ($P < 0.05$).

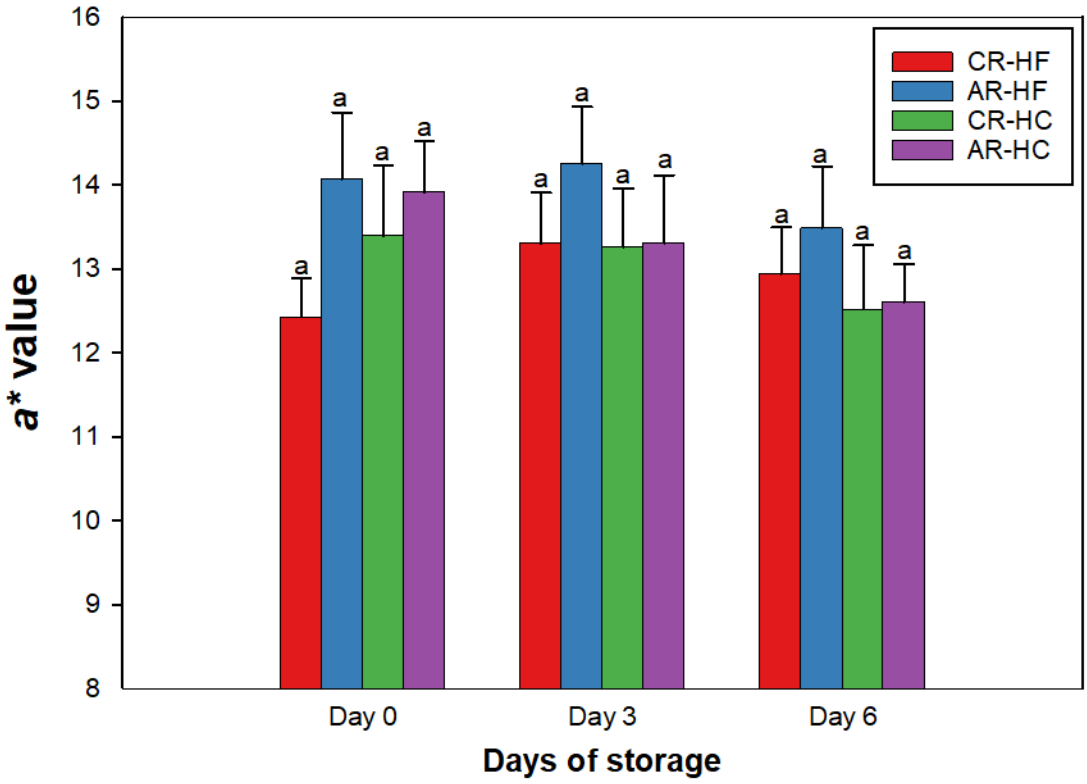


Figure 2.2: Color stability (R630/580) of longissimus lumborum chops from Polypay ram lambs conventionally raised (CR) or artificially raised with milk replacer (AR) and finished with high-forage (HF; 50:50 forage:concentrate) or a high-concentrate (HC; 85:15 concentrate:forage) diet during 6 days of refrigerated storage. Standard error bars are indicated. [a-c]Means within a weaning-finishing system or day of storage with different letters are different ($P < 0.05$).

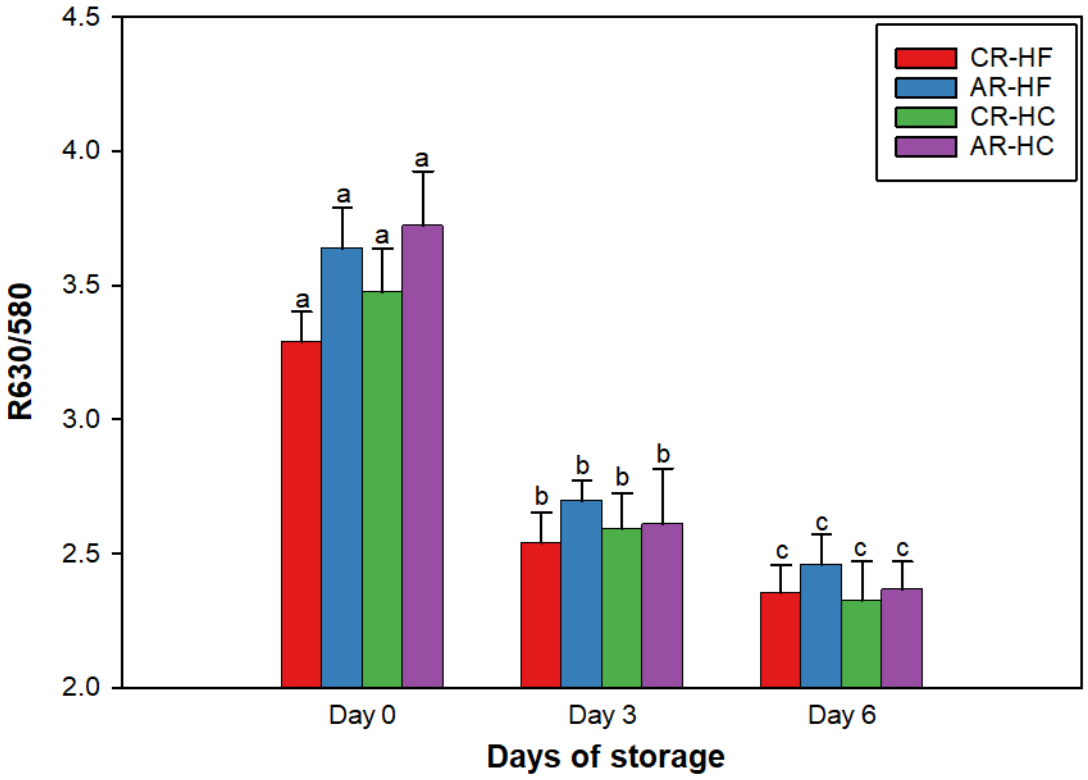


Figure 2.3: Hue angle of longissimus lumborum chops from Polypay ram lambs conventionally raised (CR) or artificially raised with milk replacer (AR) and finished with high-forage (HF; 50:50 forage:concentrate) or a high-concentrate (HC; 85:15 concentrate:forage) diet during 6 days of refrigerated storage. Standard error bars are indicated. [a-b]Means within a weaning-finishing system or day of storage with different letters are different ($P < 0.05$).

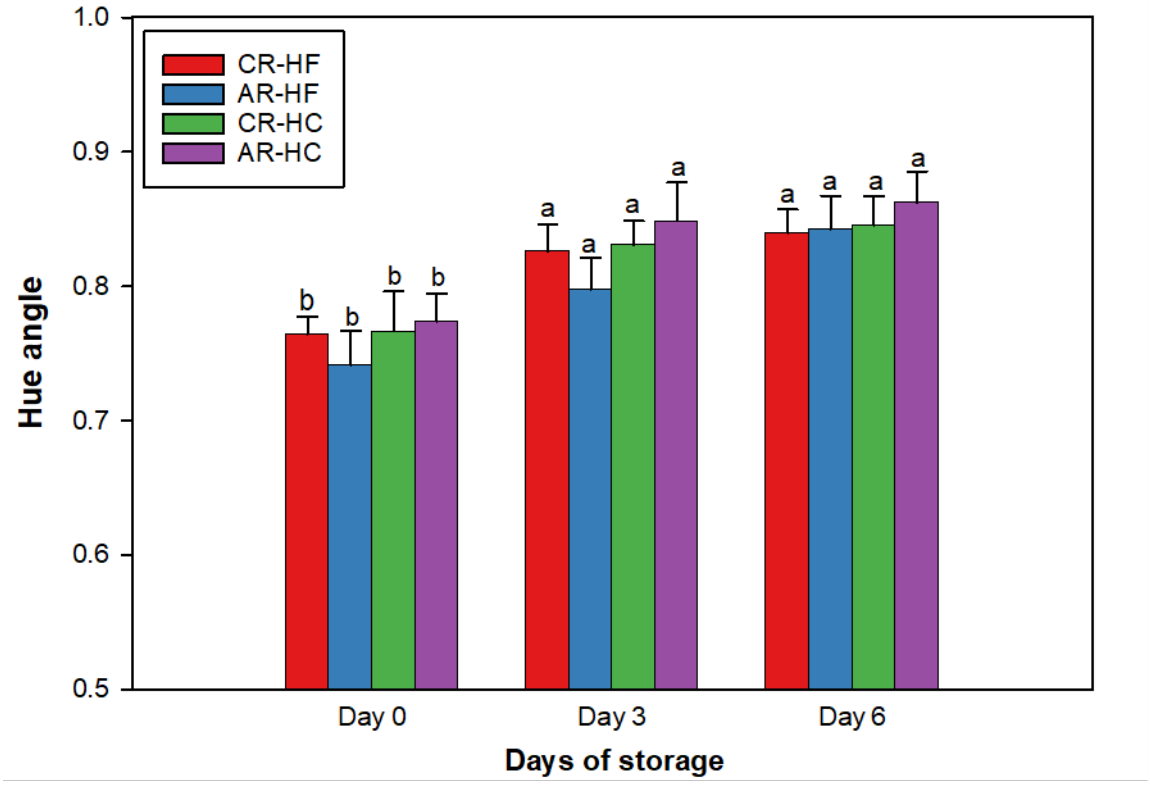


Figure 2.4: Chroma of longissimus lumborum chops from Polypay ram lambs conventionally raised (CR) or artificially raised with milk replacer (AR) and finished with high-forage (HF; 50:50 forage:concentrate) or a high-concentrate (HC; 85:15 concentrate:forage) diet during 6 days of refrigerated storage. Standard error bars are indicated. [a-b]Means within a weaning-finishing system or day of storage with different letters are different ($P < 0.05$).

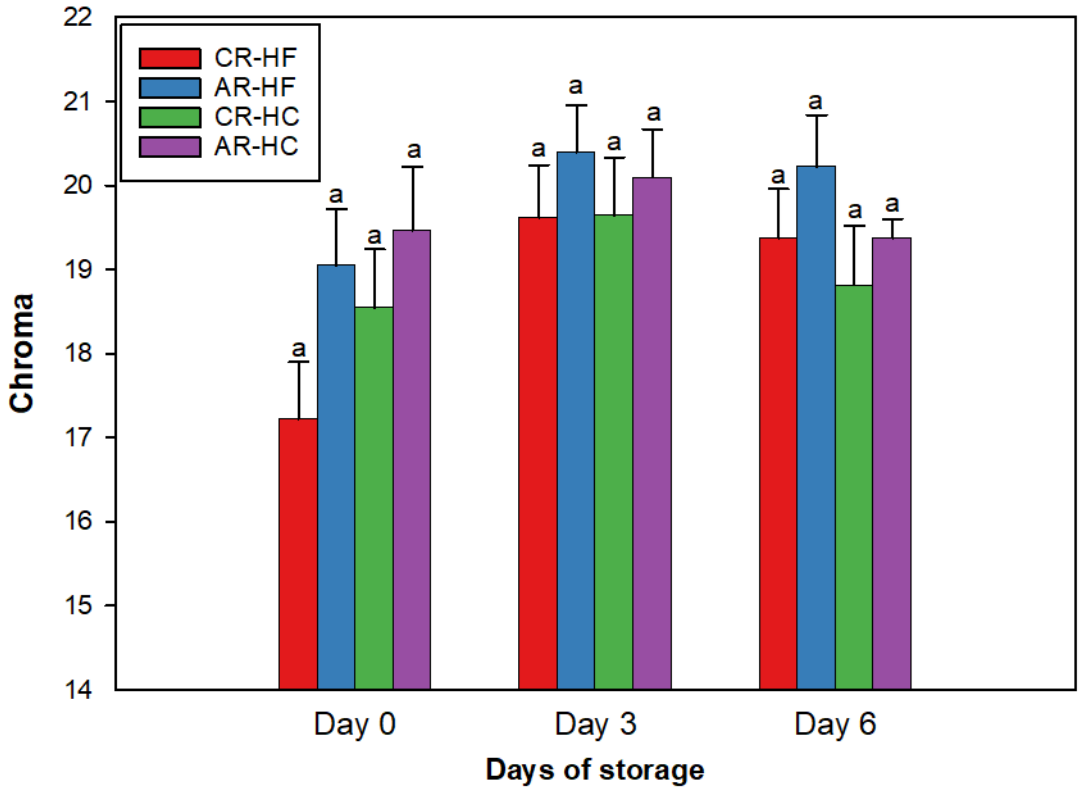


Figure 2.5: Lipid oxidation (TBARS) of longissimus lumborum chops from Polypay ram lambs conventionally raised (CR) or artificially raised with milk replacer (AR) and finished with high-forage (HF; 50:50 forage:concentrate) or a high-concentrate (HC; 85:15 concentrate:forage) diet during 6 days of refrigerated storage. Standard error bars are indicated. [a-b]Means within a weaning-finishing system or day of storage with different letters are different ($P < 0.05$).

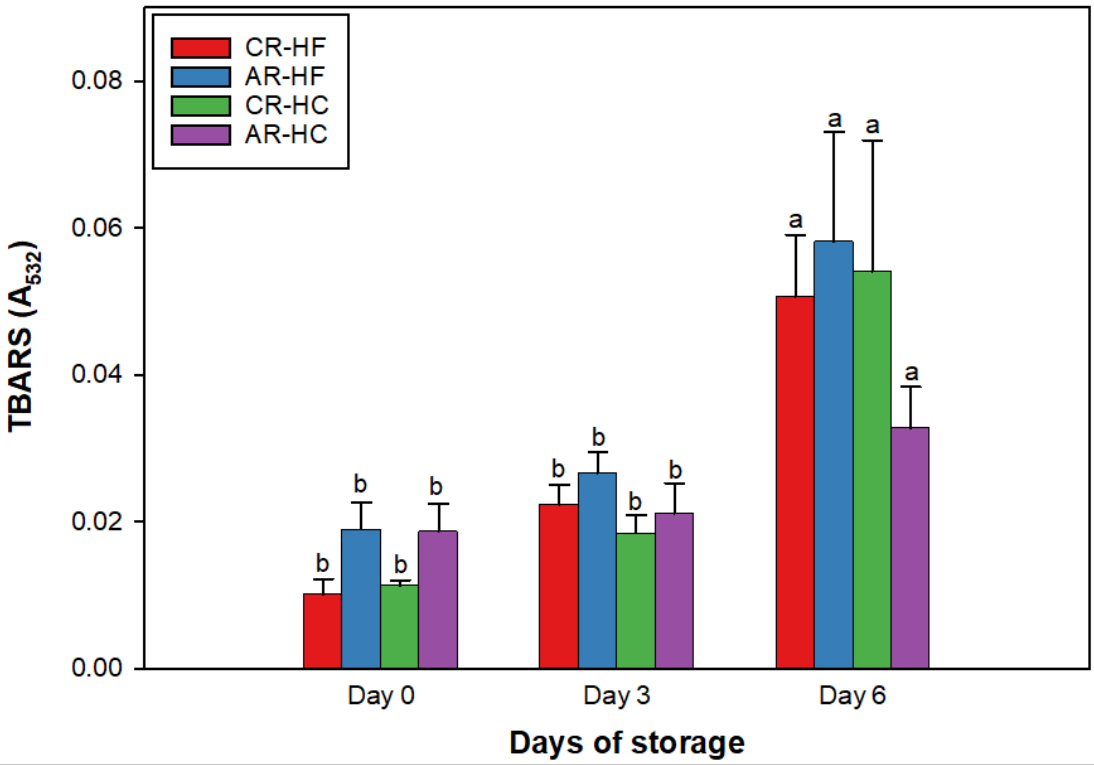
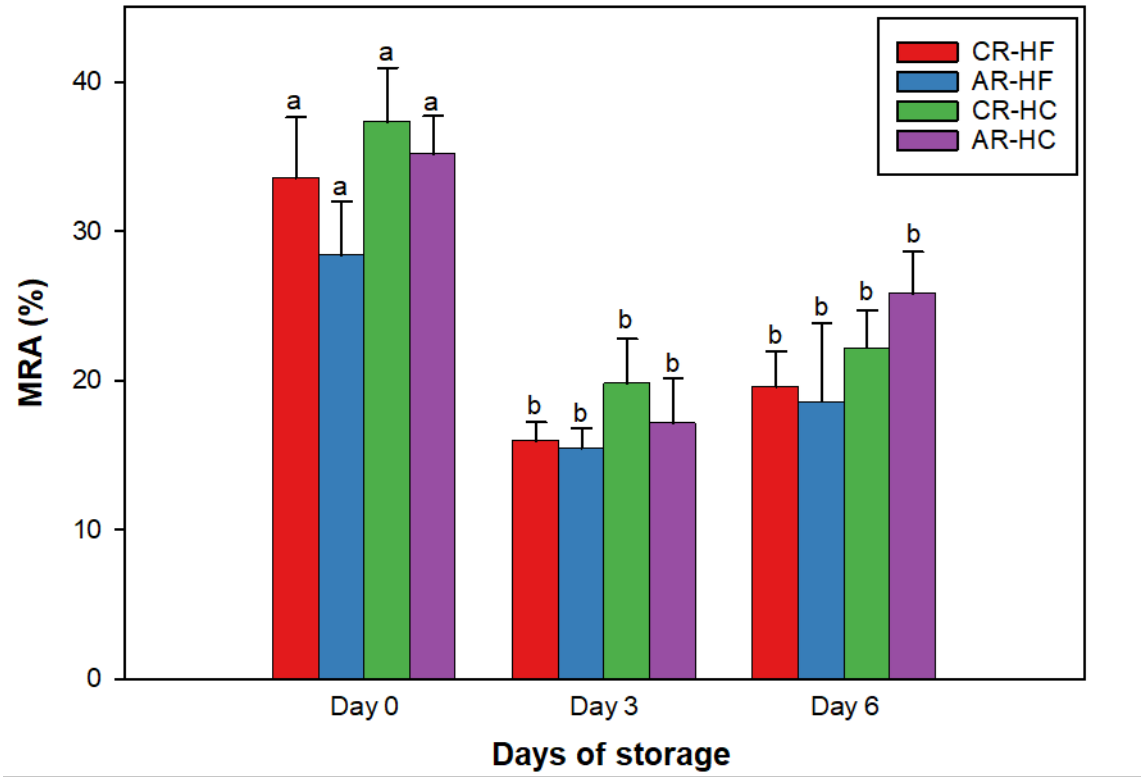


Figure 2.6: Metmyoglobin reducing activity (MRA) of longissimus lumborum chops from Polypay ram lambs conventionally raised (CR) or artificially raised with milk replacer (AR) and finished with high-forage (HF; 50:50 forage:concentrate) or a high-concentrate (HC; 85:15 concentrate:forage) diet during 6 days of refrigerated storage. Standard error bars are indicated. [a-b]Means within a weaning-finishing system or day of storage with different letters are different ($P < 0.05$).



CHAPTER 3

Red clover supplementation does not affect carcass characteristics, color stability and lipid oxidation of lamb longissimus lumborum muscles

Abstract:

This study aimed to investigate the effect of red clover supplementation on the carcass attributes, color stability, and lipid oxidation of longissimus lumborum (LL) muscle from ram lambs during 6 days of storage. Twenty-four (n = 24) Polypay ram lambs were blocked by body weight and sire ID and then randomly assigned to either: control (CON; n = 6) consisted of concentrate:roughage (85:15) with orchardgrass (*Dactylis glomerata* L.) and no red clover (*Trifolium pratense* L.); 2.5% w/w red clover with 12.5% w/w orchardgrass (2.5-RC; n = 6); 5% w/w red clover with 10% w/w orchardgrass (5-RC, n = 6); and 7.5% w/w red clover with 7.5% w/w orchardgrass (7.5-RC; n = 6) diets. All diets were made to be isocaloric and isonitrogenous. The lambs were fed *ad libitum* until reaching the target slaughter weight of 59 kg, and then harvested under USDA inspection. After 24 h post-mortem, the LL muscles from both sides of the carcasses were excised and fabricated into 2.5-cm thick chops. The chops were packaged in aerobic conditions, and randomly assigned to 0, 3, and 6 days of storage (2°C) in the darkness. Instrumental color, color stability (R630/580), pH, lipid oxidation, metmyoglobin reducing activity (MRA), and total color change (ΔE) were assessed. Red clover diets (2.5% and 7.5%) increased ($P < 0.05$) hot carcass weight, cold carcass weight, and lamb left shoulder. No effect of diet ($P > 0.05$) was observed in surface redness, yellowness, hue, chroma, color stability (R630/580), pH, lipid oxidation, and MRA. Regarding storage, muscle pH, color stability (R630/580), redness, and chroma decreased ($P < 0.05$) from day 0 to 6; whereas an increase ($P < 0.05$) of yellowness, lipid oxidation, and hue value was observed in all samples. The LL chops from lambs fed with red clover (2.5%, 5%, and 7.5%) exhibited

lower total color change (ΔE) ($P < 0.05$) than their control counterparts. These findings suggested that red clover supplementation can be a promising feeding strategy, improving carcass characteristics without compromising fresh meat color.

Keywords: biochanin A, carcass characteristics, color stability, lamb quality, lipid oxidation, red clover

3.1. Introduction

The United States has an inventory of 1.36 million market lambs, of which meat production is the most common use (USDA Animal and Plant Health Inspection Service, 2014; USDA-NASS, 2024). Lamb is the fourth most consumed meat after pork, poultry, and beef (FAO, 2021; USDA, 2023), and the per capita consumption of sheep meat demonstrated a tendency of increase since 2022, in addition to the increase of market pricing for choice/prime lamb carcasses (USDA-ERS, 2023). Many pre-harvest (e.g., feeding system, muscle source, live animal management) and post-harvest (e.g., pH, packaging and storage conditions) factors affect the carcass traits and lamb quality (Suman et al., 2014; Faustman and Cassens, 1990; Mancini and Hunt, 2005). Among them, feeding system is known to influence lamb color (Karaca et al., 2016; Cabiddu et al., 2022), which is the primary quality attribute considered by the consumers in purchasing decisions. Fresh lamb has a light red color, and any deviation in this aspect results in economic losses (Calnan et al., 2014).

A variety of grass and legume forage types are utilized in the small ruminant production in United States, such as red clover (*Trifolium pratense* L.), orchardgrass (*Dactylis glomerata* L.), and white clover (*Trifolium repens* L.). Those forages differ in yield potential, seasonality, and nutritive value (Turner et al., 2014). Red clover is a cool-season short-lived perennial legume mostly grown in the Appalachian region (eastern USA), and the use of red clover in lamb diet have demonstrated to improve lamb feed efficiency, increasing growth rate, and reducing feed intake (Weinert-Nelson et al., 2023). This effect on animal performance is due to the capacity of red clover to enhance protein

degradability and lipolysis in the gastrointestinal tract (Cassida et al., 2000; Lee et al., 2004).

Despite the benefits for animal performance, the influence of red clover in lamb color is not completely understood. Kim et al. (2013) evaluated the effect of forage-feeding regimes with ryegrass (*Lolium perenne*), lucerne (*Medicago sativa*), chicory (*Cichorium intubus*), plantain (*Plantago lanceolata*) and red clover (*Trifolium pratense*) on the color and oxidative stability of chilled lamb longissimus dorsi muscles and documented greater redness, lightness, and chroma in meat from lambs fed with red clover. Girard et al. (2015) examined the effect of forage legumes on the color and sensory attributes of lamb and reported similar lightness and redness in the longissimus thoracis et lumborum muscles from lambs fed with red clover (*Trifolium pratense*), alfalfa (*Medicago sativa*), sainfoin (*Onobrychis viciifolia*), and birdsfoot trefoil (*Lotus corniculatus*) silages. Luciano et al. (2019) evaluated the impacts of red clover (*Trifolium pratense*) and sainfoin (*Onobrychis viciifolia*) feeding on color and lipid oxidation of lamb longissimus thoracis et lumborum muscles and reported similar lightness, yellowness, chroma, hue, lipid oxidation in lambs fed with both silages. Moreover, Campbell et al. (2011) examined the effect of forage treatments on carcass attributes and lamb color and documented similar lightness and redness in short loins from lambs finished either on red clover (*Trifolium pratense*), brassica 'Goliath' (*Brassica napus, cv* Goliath), brassica 'Winfred' (*Brassica napus*, cv Winfred), turnip (*Brassica rapa*), radish (*Raphanus sativa*), pasture (*Lolium perenne*), or plantain (*Plantago lanceolata*) forages. Previous investigations reported the effect of different forages on lamb color and oxidation.

However, the influence of different concentrations of red clover (*Trifolium pratense* L.) on color and oxidative stability of lamb is yet to be understood. Therefore, this study investigated the effect of red clover supplementation (0, 2.5, 5, and 7.5%) on carcass traits, color stability, and lipid oxidation of the longissimus lumborum (LL) muscle from Polypay ram lambs during 6 days of refrigerated storage.

3.2. Materials and methods

3.2.1. Lamb production

All protocols were approved by the Institutional Animal Care and Use Committee of the University of Kentucky (Protocol #2021-3968). Twenty-four (n = 24) Polypay ram lambs were used for this experiment. The lambs were born and raised at the University of Kentucky C. Oran Little Research Center Sheep Unit (Versailles, KY; geographic coordinates: 38°4'36"N, 84°44'22"W). The ram lambs (initial age: 98.12 ± 1.71 days; initial weight: 32.55 ± 0.64 kg) were blocked by sire and initial body weight, and randomly assigned to one of four experimental diets: orchardgrass (*Dactylis glomerata* L.) hay without red clover (*Trifolium pratense* L.) hay (CON; n = 6); 2.5% red clover diet with 12.5% orchardgrass hay (2.5-RC; n = 6); 5% red clover diet with 10% orchardgrass hay (5-RC, n = 6); and 7.5% red clover diet with 7.5% orchardgrass hay (7.5-RC; n = 6) (Table 3.1). All experimental diets consisted of 85:15 concentrate:roughage ratio (85%, w/w, of the total diet). For all the diets, ground corn and corn-based dried distillers' grains with solubles (DDGS) were used as the concentrate source. All diets were made to be isocaloric and isonitrogenous. The ram lambs were then randomly assigned to adjacent blocks of

individual pens (n = 6 per block). Two consecutive periods were maintained: a 14-day adaptation period to allow the adjustment to the control diet and housing; and a finishing period with a target slaughter weight of 59 kg when the assigned four experimental diets were provided to the ram lambs. The lambs were housed together in indoor group pens for the first 7 d of the adaptation period. They were housed in individual pens (1.2 m × 1.5 m) for the remainder to facilitate monitoring feed intake. The lambs were fed *ad libitum* with experimental diets until reaching the target slaughter weight (SW) of 59 kg (164.42 ± 3.39 days).

3.2.2. Lamb harvest, fabrication, and carcass characteristics

After achieving the SW, the lambs were humanely harvested at the USDA-FSIS inspected Meat Laboratory of the University of Kentucky at live weights of 57.43 ± 0.19 kg for CON; 59.17 ± 0.68 kg for 2.5-RC; 59.40 ± 0.79 kg for 5-RC; and 60.08 ± 1.29 kg for 7.5-RC. Hot carcass weights were recorded, and the carcasses were chilled for 24 h at 2°C. Following a 24-h chilling (2°C), the conformation, quality grade (flank fat streaking), overall grade, cold carcass weight, dressing percentage, ribeye area, fat thickness, and body wall thickness of each carcass were measured. Neck, foreshank (IMPS #210), shoulder (IMPS #207), rack (IMPS #204), loin (IMPS #232), and leg (IMPS #233A) were individually weighed according to Institutional Meat Purchasing Specifications (North American Meat Processors Association, 2010). The weights of the primal cuts were recorded in absolute weight (weight in kg of the primal). All carcass grading and

measurements were conducted according to the methods outlined in the Meat Evaluation Handbook of American Meat Science Association (AMSA, 2001).

The LL muscles from both sides of the carcasses were excised. The muscles were fabricated into 2.5-cm thick chops, placed in styrofoam trays, overwrapped with oxygen-permeable polyvinyl chloride film (with an oxygen transmission rate of 15,500-16,275 $cm^3/m^2/24$ h at 23°C) and randomly assigned to storage (2°C) in darkness. Instrumental color, color stability (R630/580), pH, lipid oxidation, metmyoglobin reducing activity (MRA), and total color change or Delta E (ΔE) were evaluated on days 0, 3, and 6.

3.2.3. Meat pH

The pH of the raw meat samples was assessed following the procedure described by Strange et al. (1977). Five grams of samples were mixed with 30 mL of distilled deionized water at 25°C, and the pH was measured using an Accumet AR25 pH meter (Fisher Scientific, Pittsburg, PA, USA).

3.2.4. Instrumental color

The CIE (1976) *L** (lightness), *a** (redness), and *b** (yellowness) values were recorded at three random locations on the oxygen-exposed surface of each loin chop (King et al., 2023), using a HunterLab LabScan XE colorimeter (Hunter Associations Laboratory, Reston, VA), equipped with a 1.27-cm-diameter aperture, illuminant A, and a 10° standard observer. Prior to measurement, the colorimeter was calibrated using standard black and white plates. On day 0 of storage, the oxygen permeable film was

removed from the packages, and the chops were allowed to bloom for 2 hours at 2°C before assessing their color attributes. The reflectance was measured across the spectrum from 400 to 700 nm. The ratio of reflectance 630/580nm was determined as an indirect measurement of surface color stability (King et al., 2023). Additionally, the absolute differences in color coordinates (Deltas; Δ) were determined between the days 0 and 6. Deltas for lightness (Δ*L**), redness (Δ*a**), and yellowness (Δ*b**) were expressed as negative (–) or positive (+) results, whereas the total color difference or Delta E (ΔE) was only positive. The Deltas were expressed as Δ*L** = difference between lighter and darker (+ = lighter; – = darker); Δ*a** = differences between red and green (+ = redder; – = greener); Δ*b** = differences between yellow and blue (+ = yellower, – = bluer). The total color change or Delta E (ΔE), which represents the color change over a period, was calculated using the average of initial color readings (day 0) and the final readings (day 6), according to King et al. (2023):

$$\Delta E = [(\Delta L^*)^2 + (\Delta^{a^*})^2 + (\Delta b^*)^2]^{0.5}$$

3.2.5. Lipid oxidation (TBARS)

Lipid oxidation was assessed using the thiobarbituric acid assay (Yin et al., 1993). Duplicate 5-gram samples were homogenized with 22.5 mL of an 11% trichloroacetic acid (TCA) solution and then filtered through Whatman No. 1 filter paper (GE Healthcare, Little Chalfont, UK). Subsequently, 1.5 milliliters of the resulting aqueous filtrate were added with an equal volume of 20 mM thiobarbituric acid (TBA) solution and incubated at 25°C for 20 hours. A blank of 20 mM TBA and 11% TCA was simultaneously incubated with the

other samples. The absorbance at 532 nm was measured using a UV-2401PC spectrophotometer (Shimadzu Inc., Columbia, MD), and the findings were reported as thiobarbituric acid reactive substances (TBARS).

3.2.6. Metmyoglobin reducing activity (MRA)

Metmyoglobin reducing activity (MRA) was assessed following the method proposed by Sammel et al. (2002). The 2.54-cm thick lamb loin chops were immersed in a 0.3% sodium nitrite solution (Sigma-Aldrich Co., St. Louis, MO) for 30 minutes, at room temperature, to facilitate metmyoglobin formation. After this period, the chops were removed from the solution, blotted dry, and vacuum-sealed (99% vacuum; Sipromac Model 600A; Drummondville, Quebec, Canada) in Prime Source vacuum pouches (3 mil, Bunzl Koch Supplies Inc., Kansas City, MO). The reflectance spectra were recorded from 400 to 700 nm on the surface light exposed using a colorimeter (HunterLab LabScan XE) to determine pre-incubation surface metmyoglobin levels (King et al., 2023). Subsequently, the samples were incubated for 2 hours at 30°C to allow metmyoglobin reduction. After incubation, the surface reflectance was rescanned to determine post-incubation metmyoglobin levels. MRA was calculated using the following formula:

MRA = 100 × [(% pre-incubation surface metmyoglobin - % post-incubation surface metmyoglobin)/% pre-incubation surface metmyoglobin]

3.2.7. Statistical analysis

The experimental design was a split-plot, with the red clover diet as the whole plot and storage days as the sub-plot. The LL muscles from Twenty-four (n = 24) lamb carcasses were utilized in this study. The experimental units were the LL muscles obtained from each lamb carcass. Subsequently, chops fabricated from these carcasses and assigned for 0, 3, or 6 days were designated as sub-plot experimental units. The analysis of variance was assessed using the PROC GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). The Least Square means were separated using the PDIFF option with a Tukey-Kramer adjustment, and the differences among means were considered statistically different at a 5% level.

3.3. Results and discussion

3.3.1. Carcass characteristics

There was no effect of diet ($P > 0.05$) on live weight, carcass conformation, quality grade (flank fat streaking), overall grade, ribeye area, fat thickness, body wall thickness, neck, foreshank, right shoulder, rack, loin, left leg, right leg, and dressing percentage (Table 3.2). However, there was an effect of diet ($P < 0.05$) on hot carcass weight, cold carcass weight, and left shoulder cut. The hot carcass weight, cold carcass weight, and the left shoulder cut were greater ($P < 0.05$) in lambs supplemented with 2.5% and 7.5% of red clover compared with control and 5% red clover hay supplemented counterparts. The observed differences could be attributed to the feed efficiency and increased growth rate of lambs fed with red clover (Weinert-Nelson et al., 2023). Red clover (*Trifolium pratense*)

is rich in soluble phenolics, such as formononetin and biochanin A, which act enhancing the efficiency of nutrient digestibility, most notably protein utilization (Kagan et al., 2015; Flythe and Kagan, 2010, Harlow et al., 2020) in the gastrointestinal tract (Lee et al., 2004; Cassida et al., 2000). Therefore, supplementation of red clover may have increased the feed efficiency and average daily gain, leading to the increase of growth performance and live weight of animals (Weinert-Nelson, 2023).

In partial agreement, Stenberg et al. (2020) evaluated the effects of different feeding systems such as the silage (composed by 18% red clover, 76% timothy (*Phleum pratense* L.), and 6% white clover (*Trifolium repens* L.)); cultivated pasture (composed by 10% red clover, 50% timothy (*Phleum pratense* L.), 20% meadow fescue (*Festuca pratensis* Huds.), 15% perennial ryegrass (*Lolium perenne* L.), and 5% white clover (*Trifolium repens* L.)); and semi-natural pasture (composed by trees and shrubs without red clover) on the carcass traits and meat quality of lambs and reported similar conformation, fatness, and dressing percentage between the carcass from lamb fed with diets containing 18% of red clover silage and 10% of red clover pasture. However, no differences in the carcass weights were observed between the red clover containing diets. Kok et al. (2019) evaluated the carcass characteristics and meat color of lambs fed either a mix of plantain (*Plantago lanceolata*) and red clover (*Trifolium pratense*), or a perennial ryegrass (*Lolium perenne*) and white clover pasture (*Trifolium repens*) mix and reported greater carcass weight of lambs grazed on the plantain-red clover. Turner et al. (2015) evaluated the carcass characteristics and meat quality in meat-goat kids finished on red clover (*Trifolium pratense* L.), birdsfoot trefoil (*Lotus corniculatus* L.), and chicory (*Cichorium intybus* L.)

pastures and documented greater cold carcass weight in red clover supplemented goats than their chicory fed counterparts. The authors reported that red clover supplemented goats exhibited the tendency of greater live body weight, ribeye area, back fat thickness and body wall thickness compared to their chicory fed counterparts. No differences were observed in dress out percentage. In contrary, Girard et al. (2015) evaluated the effect of various silages prepared from red clover (*Trifolium pratense*), alfalfa (*Medicago sativa*), sainfoin (*Onobrychis viciifolia*), and birdsfoot trefoil (*Lotus corniculatus*) forage legumes and reported lower slaughter weight, hot carcass weight, and dressing percentage of lambs supplemented with red clover than their birdsfoot trefoil and sainfoin counterparts. Fraser et al. (2004) evaluated the carcass characteristics and meat quality of lambs finished on red clover (*Trifolium pratense*), lucerne (*Medicago sativa*), or perennial ryegrass (*Lolium perenne*) swards and documented similar cold carcass weight in all the treatment groups.

3.3.2. Meat pH

There was no diet x storage interaction (P = 0.3194) for pH (Table 3.3). There was no effect of diet (P = 5989) but an effect of storage (P = 0.001) for the muscle pH (Table 3.3). All samples exhibited a decrease (P < 0.05) in pH from day 0 to 6 of storage.

The observed results could be attributed to the similar plasma glucose concentrations (mmol L^{-1}) in both red clover fed and control lambs (Fraser et al., 2004; Speijers et al., 2004). Fraser et al. (2004) evaluated the production performance and meat quality of grazing lambs finished on red clover (*Trifolium pratense*), lucerne (*Medicago*

sativa), and perennial ryegrass (*Lolium perenne*) and observed similar glucose content in all types of forages. Additionally, the glucose concentrations observed were within the normal range for sheep, indicating that the lambs were not restricted in energy requirements in their diets. The similar energy intake of control and red clover-fed animals is important to guarantee a similar plasma glucose and muscle glycogen content (Immonen et al., 2000), which will be converted into lactic acid, under postmortem anaerobic conditions, reducing the muscle pH (Apaoblaza et al., 2017; Immonen et al, 2000).

In support of our results, Kok et al. (2019) examined the impacts of diet composed of mix of plantain (*Plantago lanceolata*) and red clover (*Trifolium pratense*); and a mix of perennial ryegrass (*Lolium perenne*) and white clover pasture (*Trifolium repens*) and observed similar ultimate pH in semimembranosus muscle from lambs raised in both feeding systems. Girard et al. (2015) evaluated the effect of diet prepared from red clover (*Trifolium pratense*), alfalfa (*Medicago sativa*), sainfoin (*Onobrychis viciifolia*), and birdsfoot trefoil (*Lotus corniculatus*) and observed no differences in pH in lamb longissimus thoracis et lumborum muscles of animals in any feeding system. Stenberg et al. (2020) examined the impacts of different feeding systems such as silage (18% red clover, 76% timothy, and 6% white clover); cultivated pasture (10% red clover, 50% timothy, 20% meadow fescue, 15% perennial ryegrass, and 5% white clover); and semi-natural pasture (composed by trees and shrubs without red clover) on the carcass characteristics and meat quality of lambs, and documented no differences in pH values in the longissimus lumborum muscles from lambs in all the feeding systems. In contrast, Kim

et al. (2013) evaluated the effect of different forage-feeding regimes with ryegrass (*Lolium perenne*), lucerne (*Medicago sativa*), chicory (*Cichorium intubus*), plantain (*Plantago lanceolata*) and red clover (*Trifolium pratense*) and reported lower pH in lamb longissimus dorsi muscles from red clover and ryegrass supplemented lambs. Campbell et al. (2011) determined the effects of forages such as the red clover (*Trifolium pratense*), brassica 'Goliath' (*Brassica napus, cv* Goliath), brassica 'Winfred' (*Brassica napus*, cv Winfred), turnip (*Brassica rapa*), radish (*Raphanus sativa*), pasture (*Lolium perenne*), or plantain (*Plantago lanceolata*) on lamb carcass characteristics and meat color and reported greater pH in short loins from red clover finished lambs.

In support of our results regarding decrease of pH during storage, Girard et al. (2015) examined the effect of diet prepared from red clover (*Trifolium pratense*), alfalfa (*Medicago sativa*), sainfoin (*Onobrychis viciifolia*), and birdsfoot trefoil (*Lotus corniculatus*) forage legumes and documented a decrease in postmortem pH in all lamb longissimus thoracis et lumborum muscle samples over 24 hours of chilled storage.

3.3.3. Lightness (L values)*

There was no diet x storage interaction (P = 0.2343) for lightness (Table 3.3). Also, there was no effect of diet (P = 0.7878) and storage (P = 0.1922) for *L** values. The observed similarities in lightness values could be attributed to the muscle fiber type (Sirin et al., 2017), and muscle pH (Abril et al., 2001). The longissimus lumborum muscle is mainly composed of muscle fiber type IIB, which uses glycogen as an energy source resulting in a postmortem pH drop, and an increase in light reflectance, affecting *L** values

(Abril et al., 2001). Supporting our results, Stenberg et al. (2020) reported no differences in lightness in the longissimus lumborum muscles from lambs fed with silage (18% red clover, 76% timothy, and 6% white clover); cultivated pasture (10% red clover, 50% timothy, 20% meadow fescue, 15% perennial ryegrass, and 5% white clover); and semi-natural pasture (composed by trees and shrubs without red clover). Campbell et al. (2011) evaluated the impact of forages on carcass attributes and lamb color and reported similar lightness in short loins from lambs finished either on red clover (*Trifolium pratense*), brassica 'Goliath' (*Brassica napus, cv* Goliath), brassica 'Winfred' (*Brassica napus*, cv Winfred), turnip (*Brassica rapa*), radish (*Raphanus sativa*), pasture (*Lolium perenne*), or plantain (*Plantago lanceolata*) forages. Luciano et al. (2019) examined the effect of diet with timothy grass (*Phleum pretense*); binary mixture of timothy and sainfoin (*Onobrychis viciifolia*; 50:50); binary mixture of timothy and red clover (*Trifolium pratense*; 50:50); ternary mixture of timothy, sainfoin and red clover (50:25:25) respectively and reported no differences in lightness in lamb longissimus thoracis et lumborum muscles regardless the forage type. Kok et al. (2019) evaluated the influence of diet (mix of plantain (*Plantago lanceolata*) and red clover (*Trifolium pratense*) and a mix of perennial ryegrass (*Lolium perenne*) and white clover pasture (*Trifolium repens*)) and documented similar lightness in in semimembranosus muscle from lambs raised in both feeding systems. Girard et al. (2015) examined the impact diet prepared from red clover (*Trifolium pratense*), alfalfa (*Medicago sativa*), sainfoin (*Onobrychis viciifolia*), and birdsfoot trefoil (*Lotus corniculatus*) forage legumes and reported no differences in lightness in lamb longissimus thoracis et lumborum *muscles* of animals from any feeding system. In contrast, Kim et al.

(2013) documented greater lightness in longissimus dorsi muscles from lambs fed red clover (*Trifolium pratense*), compared to their counterpartes fed with ryegrass (*Lolium perenne*), lucerne (*Medicago sativa*), chicory (*Cichorium intubus*), plantain (*Plantago lanceolata*), on day 1 of storage.

Regarding Delta Δ*L**, the samples exhibited overall positive values (Table 3.3), except by 2.5-RC, indicating an increase pattern in lightness values during storage. CON (Δ*L** = 2.45) exhibited the greatest increase in lightness, compared to 5-RC (Δ*L** = 0.64) and 7.5-RC (Δ*L** = 0.71) samples. 2.5-RC (Δ*L** = -0.58) was the only the samples that exhibited negative values, meaning a decrease pattern in lightness during storage.

3.3.4. Redness (a values)*

There was a diet x storage interaction (*P* = 0.0206) for redness. There was no effect of diet (*P* = 0.7786) but an effect of storage (*P* = 0.0079) for *a** values (Figure 3.1). All samples exhibited a decrease in redness (*P* < 0.05) from day 0 to day 6 of storage. The observed decrease in redness could be attributed to lipid oxidation and metmyoglobin accumulation in the loin chops (Faustman et al., 2010). The increase of lipid oxidation (as observed in our results, Figure 3.5) generates free radicals and reactive oxygen species capable to enhance the myoglobin oxidation and metmyoglobin accumulation on the meat surface (Faustman et al., 2010; Abraham et al., 2017). This is turn, may have contributed to the decrease of surface redness (*a** values) during storage.

In support of our results, Kok et al. (2019) reported similar redness in semimembranosus muscles from lambs raised in mix of plantain (*Plantago lanceolata*)

and red clover (*Trifolium pratense*); and a mix of perennial ryegrass (*Lolium perenne*) and white clover pasture (*Trifolium repens*). Luciano et al. (2019) examined the effect of silage diet with red clover (Trifolium pratense cv. Mervious), sainfoin (Onobrychis viciifolia cv. Perly), and timothy grass (Phleum pretense cv. Liglory) and reported greater redness in longissimus thoracis et lumborum muscles from lambs supplemented with timothy grass and the ternary mixture of timothy, sainfoin, and red clover. Girard et al. (2015) examined the effect of diet prepared from red clover (*Trifolium pratense*), alfalfa (*Medicago sativa*), sainfoin (*Onobrychis viciifolia*), and birdsfoot trefoil (*Lotus corniculatus*), and documented no differences in redness in lamb longissimus thoracis et lumborum muscles from animals of any feeding system. Stenberg et al. (2020) reported no differences in redness values in the longissimus lumborum muscles from lambs fed with silage (18% red clover, 76% timothy, and 6% white clover); cultivated pasture (10% red clover, 50% timothy, 20% meadow fescue, 15% perennial ryegrass, and 5% white clover); and semi-natural pasture (composed by trees and shrubs without red clover). In contrary, Kim et al. (2013) documented lower redness in red clover supplemented lamb longissimus dorsi on day 1 of storage when compared to ryegrass (*Lolium perenne*), lucerne (*Medicago sativa*), chicory (*Cichorium intubus*), plantain (*Plantago lanceolata*), and red clover (*Trifolium pratense*) feeding. The decrease of redness during storage was reported by Luciano et al. (2019) in longissimus thoracis et lumborum muscles from lambs fed with timothy grass (*Phleum pretense*); binary mixture of timothy and sainfoin (*Onobrychis viciifolia*; 50:50); binary mixture of timothy and red clover (*Trifolium pratense*; 50:50); ternary mixture of timothy, sainfoin and red clover (50:25:25) during 7 days of storage at 4°C. Additionally,

Campbell et al. (2011) also documented a decreased in redness in loin muscles from lambs finished with red clover (*Trifolium pratense*), brassica 'Goliath' (*Brassica napus,* cv Goliath), brassica 'Winfred' (*Brassica napus*, cv Winfred), turnip (*Brassica rapa*), radish (*Raphanus sativa*), pasture (*Lolium perenne*), or plantain (*Plantago lanceolata*) forages during 7 days of refrigerated storage at 4°C.

Regarding Δ*a**, all samples exhibited negative values (Figure 3.1), indicating a decrease pattern in *a** values during storage. However, CON samples exhibited greater decrease in *a** values (Δ*a** = -1.55) compared to their red clover counterparts (2.5-RC Δ*a** = -0.17; 5-RC Δ*a** = -0.80; 7.5-RC Δ*a** = -0.44).

3.3.5. Yellowness (b values)*

There was no diet x storage interaction (P = 0.0763) for yellowness (Table 3.3). There was no effect of diet (P = 0.5098) but an effect of storage (P < 0.0001) for the *b** values (Table 3.3). All samples exhibited an increase in yellowness (P < 0.05) from day 0 to 6.

In support of our results, Luciano et al. (2019) documented similar yellowness in longissimus thoracis et lumborum muscles from lambs fed with timothy grass (*Phleum pretense*); binary timothy and sainfoin (*Onobrychis viciifolia*; 50:50); timothy and red clover (*Trifolium pratense*; 50:50); or timothy, sainfoin and red clover, respectively. Kok et al. (2019) reported similar yellowness in semimembranosus muscle from lambs raised in a mix of plantain (*Plantago lanceolata*) and red clover (*Trifolium pratense*), and a mix of perennial ryegrass (*Lolium perenne*) and white clover pasture (*Trifolium repens*).

Stenberg et al. (2020) documented similar yellowness in the longissimus lumborum muscles from lambs finished in silage (composed by 18% red clover, 76% timothy (*Phleum pratense* L.), and 6% white clover (*Trifolium repens* L.)); cultivated pasture (composed by 10% red clover, 50% timothy (*Phleum pratense* L.), 20% meadow fescue (*Festuca pratensis* Huds.), 15% perennial ryegrass (*Lolium perenne* L.), and 5% white clover (*Trifolium repens* L.)); and semi-natural pasture (composed by trees and shrubs without red clover). In contrast, Girard et al. (2015) reported lower yellowness in lamb longissimus thoracis et lumborum muscles from lamb animals supplemented with red clover (*Trifolium pratense*), then their counterparts fed with alfalfa (*Medicago sativa*), sainfoin (*Onobrychis viciifolia*), and birdsfoot trefoil (*Lotus corniculatus*).

Regarding storage, Luciano et al. (2019) observed an increase in yellowness in the longissimus thoracis et lumborum muscles from lambs fed with red clover (*Trifolium pratense* cv. Mervious), sainfoin (*Onobrychis viciifolia* cv. Perly), and timothy grass (*Phleum pretense* cv. Liglory) during 7 days of storage.

Regarding Delta Δ*b**, all samples exhibited positive values (Table 3.3), indicating an increase pattern in *b** values during storage. CON (Δ*b** = 0.82) and 5-RC (Δ*b** = 0.86) exhibited lower increase in yellowness compared to the 2.5-RC (Δ*b** = 1.53) and 7.5-RC (Δ*b** = 1.40) samples.

3.3.6. Color stability (R630/580)

There was no effect of diet ($P = 0.8249$) for color stability (Figure 3.2). However, there was a diet x storage interaction ($P = 0.005$) and an effect of storage ($P < 0.0001$) for

R630/580 (Figure 3.2). All samples exhibited a decrease ($P < 0.05$) in color stability from day 0 to day 6 of storage period. The observed decline in color stability could be attributed to the myoglobin oxidation and the accumulation of metmyoglobin into meat surface (Faustman et al., 2010). The ratio R630/580 indicates surface discoloration, with greater ratio means great redness and color stability (King et al., 2023). The increase of lipid oxidation (as observed in our results; Figure 3.5) during storage may have contributed to myoglobin oxidation, and metmyoglobin accumulation, leading to the decline in R630/580 (Faustman et al., 2010).

In support, Luciano et al. (2019) reported an increase of metmyoglobin formation and consequently decrease in color stability in the longissimus thoracis et lumborum muscles of animals fed with red clover (*Trifolium pratense* cv. Mervious), sainfoin (*Onobrychis viciifolia* cv. Perly), timothy grass (*Phleum pretense* cv. Liglory) during 7 days of storage. In contrast, Kim et al. (2013) examined the effect of ryegrass (*Lolium perenne*), lucerne (*Medicago sativa*), chicory (*Cichorium intubus*), plantain (*Plantago lanceolata*) and red clover (*Trifolium pratense*) and reported that the meat from lambs fed red clover had inferior color stability compared to their ryegrass and plantain fed counterparts on day 7 of storage. Campbell et al. (2011) lower color deterioration in lamb loin from red clover-fed (*Trifolium pratense*) animals than their counterparts, fed with brassica 'Goliath' (*Brassica napus, cv* Goliath), brassica 'Winfred' (*Brassica napus*, cv Winfred), turnip (*Brassica rapa*), radish (*Raphanus sativa*), pasture (*Lolium perenne*), and plantain (*Plantago lanceolata*) during 7 days of storage.

3.3.7. Hue angle

There was no effect of diet ($P = 0.6615$) for hue angle (Figure 3.3). However, there was a diet x storage interaction ($P = 0.0174$) and an effect of storage ($P < 0.0001$) for hue angle (Figure 3.3). All samples exhibited an increase ($P < 0.05$) in hue angle from day 0 to day 6 of storage. Hue angle value indicates the color changes over time towards discoloration. High values indicate less red, more metmyoglobin, and a brown color (King et al., 2023). The observed increase in hue angle could be attributed to the increase of metmyoglobin on the meat surface during storage (King et al., 2023; Luciano et al., 2011, Luciano et al., 2009a). The rate of metmyoglobin oxidation can be enhanced by the increase of lipid oxidation (as observed in our findings; Figure 3.5) through the production of secondary products (Faustman et al., 2010; Suman et al., 2007), thereby contributing to the increase of hue angle.

In support, Kim et al. (2013) reported similar hue angle values in lamb loins from animals fed with ryegrass (*Lolium perenne*), lucerne (*Medicago sativa*), chicory (*Cichorium intubus*), plantain (*Plantago lanceolata*) and red clover (*Trifolium pratense*) on day 1 of storage. Fraser et al. (2004) evaluated the carcass characteristics and meat quality of lambs finished on red clover (*Trifolium pratense*), lucerne (*Medicago sativa*), and perennial ryegrass (*Lolium perenne*) and reported an increase of hue angle values in lamb *longissimus dorsi* during 13 days of storage. Luciano et al. (2019) documented an increase in hue angle in lamb longissimus thoracis et lumborum muscles from lamb fed with timothy grass (*Phleum pretense*); mixture of timothy and sainfoin (*Onobrychis viciifolia*;

50:50); mixture of timothy and red clover (*Trifolium pratense*; 50:50); and mixture of timothy, sainfoin and red clover (50:25:25) respectively during 7 days of storage.

3.3.8. Chroma

There was a diet x storage interaction (P = 0.0427) for chroma. Additionally, there was no effect of diet (P = 0.6454) but an effect of storage (P = 0.0006) for chroma (Figure 3.4). Chroma increased from day 0 to day 3, followed by a decrease on day 6 of storage. Chroma or saturation index indicates the intensity of meat color where high values denote more intense or vivid color. Chroma is calculated from redness (a^* values) and yellowness (b^* values) according to the equation $(a^{*2} + b^{*2})^{½}$ (King et al., 2023). Both redness (Figure 3.1) and yellowness (Table 3.3) exhibited a decrease pattern from day 3 to 6, which probably contributed to observed results in chroma. In agreement, Luciano et al. (2019) documented no effect of diet with timothy grass (*Phleum pretense*); mixture of timothy and sainfoin (*Onobrychis viciifolia*; 50:50); mixture of timothy and red clover (*Trifolium pratense*; 50:50); mixture of timothy, sainfoin and red clover (50:25:25) on chroma of lamb longissimus thoracis et lumborum. However, there was an effect of storage, where chroma decreased during 7 days of storage. In contrast, Kim et al. (2013) documented greater chroma in longissimus dorsi muscles from lambs fed with red clover (*Trifolium pratense*) than their counterparts fed with ryegrass (*Lolium perenne*), lucerne (*Medicago sativa*), chicory (*Cichorium intubus*), and plantain (*Plantago lanceolata*) on day 1 of storage. Additionally, the authors reported a decrease in chroma during 7 days of storage.

Regarding storage, Fraser et al. (2004) examined the carcass traits and meat quality of lambs finished on red clover (*Trifolium pratense*), lucerne (*Medicago sativa*), and perennial ryegrass (*Lolium perenne*) swards and reported a decrease of chroma values in lamb longissimus dorsi muscles during 13 days of display.

3.3.9. Lipid oxidation (TBARS)

There was no diet x storage interaction (P = 0.543) and no effect of diet (P = 0.5254) for TBARS. However, there was an effect of storage (P < 0.013) for lipid oxidation (Figure 3.5). All samples exhibited an increase (P < 0.05) in lipid oxidation from day 0 to day 6 of storage (Figure 3.5). The increase in lipid oxidation during storage could be attributed to the decline in the redox capacity of the meat and the production of free radicals, which act triggering lipid oxidation reactions (Min and Ahn, 2005). Additionally, myoglobin is a heme protein containing a central iron atom, which can undergo oxidation and generate reactive intermediates capable to enhance both myoglobin and lipid oxidation (Faustman et al., 2010).

Supporting our results, Luciano et al. (2019) examined the impact of timothy grass (*Phleum pretense*); mixture of timothy and sainfoin (*Onobrychis viciifolia*; 50:50); mixture of timothy and red clover (*Trifolium pratense*; 50:50); and mixture of timothy, sainfoin and red clover (50:25:25) silage diet and reported an increase of the lipid oxidation (TBARS values) in lamb longissimus thoracis et lumborum muscle over 4 days of storage from all feeding systems. Kim et al. (2013) documented an increase of lipid oxidation in *longissimus dorsi* from lambs fed with ryegrass (*Lolium perenne*), lucerne

(*Medicago sativa*), chicory (*Cichorium intubus*), plantain (*Plantago lanceolata*) and red clover (*Trifolium pratense*) during 7 days of storage.

3.3.10. Metmyoglobin reducing activity (MRA)

There was no diet x storage interaction (P = 0.9955) for MRA (Figure 3.6). Additionally, there was no effect of diet (P = 0.4284), but an effect of storage (P < 0.0001) for MRA. The MRA values decreased from day 0 to day 3, followed by an increase on day 6. MRA is the ability of the postmortem muscle to donate an electron to MMb (Fe^{3+}) to form DMb (Fe^{2+}), and delay meat discoloration (Ramanathan et al., 2019b, Ramanathan et al., 2014). The observed increase in MRA from day 3 can be attributed to the mitochondrial damage (Nair et al., 2018). Mitochondrial degradation towards the end of storage might lead to the release of reducing enzymes from the mitochondria, which might be responsible for the observed increase in MRA at 6^{th} day of storage.

In contrast, Kim et al. (2013) examined the impact of different forage-feeding regimes with ryegrass (*Lolium perenne*), lucerne (*Medicago sativa*), chicory (*Cichorium intubus*), plantain (*Plantago lanceolata*) and red clover (*Trifolium pratense*) and reported that all lamb longissimus dorsi muscle samples exhibited decreased in color reversing ability (CRA) over 7 days of retail display. Additionally, the authors also documented that the red clover supplemented lamb samples had numerically greater CRA than their chicory, lucerne, plantain, and ryegrass fed counterparts at day 1 and day 7 of refrigerated retail storage.

3.3.11. Delta E

Delta E expresses the total color change and was calculated from the beginning (day 0) to the end (day 6) of storage. Delta E values are positive and high values reflect greater changes in overall color. CON exhibited greater color discoloration (ΔE = 3.01) than their red clover 2.5-RC (ΔE = 1.65), 5-RC (ΔE = 1.34), and 7.5-RC (ΔE = 1.63) counterparts, during 6 days of storage. The observed difference in lamb discoloration can be attributed to the presence of bioactive compounds in red clover, such as isoflavones (biochanin A, formononetin) (Luciano et al., 2019), which have strong antioxidant capacity and are related to enhance the antioxidant capacity in different animal products (Luciano et al., 2019; Singh et al., 2021). This in turn, may have contributed to the lower discoloration in the LL muscles from animals fed with different concentrations of red clover.

3.4. Conclusions

The findings of the present study indicate that supplementation of red clover (2.5%, 5%, or 7.5%) did not influence overall lamb carcass characteristics, instrumental color (*L**, *a**, *b**, R630/580, hue angle, chroma), and oxidative stability (MRA and lipid oxidation). Hot carcass weight, cold carcass weight, and left shoulder cuts were greater in red clover (2.5% and 7.5%) supplemented lambs. Additionally, there was an effect of storage increasing yellowness, hue angle, MRA, and lipid oxidation; and decreasing pH, redness, R630/580, and chroma. Lamb chops from animals fed with red clover (2.5%, 5%, or 7.5%) exhibited lower delta color change (ΔE) than control samples, indicating lower

color deterioration over the storage. These findings suggest that the red clover diet can be a promise feeding in lamb production without compromising fresh meat color.

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Table 3.1: Dietary composition of 0% (CON), 2.5%, 5%, and 7.5% red clover diets containing 85:15 concentrate:forage.

Ingredient, %	Treatment			
	0.0%-RC (CON)	2.5%-RC	5.0%-RC	7.5%-RC
Ground Orchardgrass Hay	15.0	12.5	10.0	7.5
Ground Red Clover Hay	-	2.5	5.0	7.5
Ground Corn	50.6	51.1	51.5	52.2
DDGS	28.0	27.6	27.2	26.5
Liquid Molasses	3.0	3.0	3.0	3.0
Ground Limestone	1.1	1.1	1.0	1.0
Salt	0.63	0.63	0.63	0.63
Dicalcium Phosphate	0.63	0.63	0.63	0.63
Ammonium Chloride	0.5	0.5	0.5	0.5
Sheep Premix[2]	0.3	0.3	0.3	0.3
Vit ADE (IU/lb)	0.1	0.1	0.1	0.1
E-20 (IU/lb)	0.1	0.1	0.1	0.1

[1] Ingredients are expressed as a percentage of the total diet.

[2] Provimi PMX.

Table 3.2: Carcass characteristics of Polypay ram lambs finished either on control, 2.5%, 5%, or 7.5% red clover hay[1].

Carcass Characteristics	Treatment				*P*-values
	CON	2.5-RC	5-RC	7.5-RC	Diet
Live Weight (kg)	57.43 ± 0.19	59.17 ± 0.68	59.40 ± 0.79	60.08 ± 1.29	0.18
Hot Carcass Weight (kg)	26.11 ± 0.19^{b}	27.66 ± 0.27^{a}	26.60 ± 0.52^{ab}	27.69 ± 0.39^{a}	0.01
Cold Carcass Weight (kg)	25.84 ± 0.21^{b}	27.39 ± 0.25^{a}	26.33 ± 0.51^{ab}	27.58 ± 0.33^{a}	0.01
Ribeye Area (cm^2)	17.42 ± 0.30	17.31 ± 0.78	17.07 ± 0.66	17.69 ± 0.85	0.93
Fat Thickness (cm)	0.31 ± 0.03	0.35 ± 0.04	0.35 ± 0.06	0.83 ± 0.35	0.16
Body Wall Thickness (cm)	1.78 ± 0.05	1.86 ± 0.16	1.78 ± 0.16	1.95 ± 0.11	0.75
Neck (kg)	1.04 ± 0.04	1.07 ± 0.05	0.99 ± 0.05	1.13 ± 0.06	0.25
Foreshank; IMPS # 210 (kg)	1.26 ± 0.05	1.29 ± 0.02	1.31 ± 0.06	1.31 ± 0.03	0.89
Left Shoulder; IMPS # 207 (kg)	2.50 ± 0.06^{b}	2.76 ± 0.05^{a}	2.47 ± 0.07^{b}	2.76 ± 0.06^{a}	< 0.01
Right Shoulder; IMPS # 207 (kg)	2.65 ± 0.05	2.83 ± 0.12	2.83 ± 0.06	2.81 ± 0.05	0.27
Rack; IMPS # 204 (kg)	2.60 ± 0.05	2.78 ± 0.06	2.71 ± 0.18	3.05 ± 0.15	0.09
Loin; IMPS # 232 (kg)	2.18 ± 0.06	2.54 ± 0.14	2.20 ± 0.10	2.38 ± 0.11	0.08
Left leg; IMPS # 233A (kg)	4.09 ± 0.10	4.17 ± 0.10	4.41 ± 0.14	4.38 ± 0.09	0.14
Right leg; IMPS # 233A (kg)	4.41 ± 0.10	4.51 ± 0.08	4.40 ± 0.12	4.46 ± 0.14	0.89
Dressing Percentage (%)	45.46 ± 0.45	46.77 ± 0.74	44.80 ± 0.88	46.59 ± 0.60	0.17
Conformation[2]	(C^0) 583.33 ± 30.73	(C^+) 600.00 ± 25.82	(C^0) 583.33 ± 16.67	(C^0) 583.33 ± 30.73	0.96

Quality Grade (FFS)[3]	(Sm) 320.00 ± 8.16	(Mt) 406.67 ± 22.16	(Sm) 376.67 ± 26.79	(Sm) 328.33 ± 50.95	0.19
Overall Grade[2]	(C^+) 616.67 ± 16.67	(C^+) 600.00 ± 25.82	(C^+) 633.33 ± 21.08	(C^+) 600.00 ± 51.64	0.86

CON = control, 2.5-RC = 2.5% red clover, 5-RC = 5% red clover, 7.5-RC = 7.5% red clover.

[a-b] Means without common superscripts are different ($P < 0.05$).

[1] Results are expressed as mean ± standard error of the mean (SEM).

[2] 400 = low choice (C^-), 500 = average choice (C^0), 600 = high choice (C^+), 700 = low prime (P^-), 800 = average prime (P^0), 900 = high prime (P^+)

[3] FFS = flank fat streaking. 100 = traces (Tr), 200 = slight (Sl), 300 = small (Sm), 400 = modest (Mt), 500 = moderate (Md), 600 = slightly abundant (Sa), 700 = moderately abundant (Ma), 800 = abundant (Ab)

Table 3.3: Meat pH, lightness (*L**) and yellowness (*b**) values of longissimus lumborum chops from Polypay ram lambs finished either on control, 2.5%, 5%, or 7.5% red clover hay during refrigerated storage (2°C) for 6 days[1] under aerobic packaging.

Parameter	Treatment	Days of storage			Delta
		0	3	6	
*L** value	CON	35.71 ± 0.53	37.54 ± 0.64	38.16 ± 0.65	Δ *L** = 2.45
	2.5-RC	38.57 ± 0.68	37.39 ± 0.82	37.99 ± 1.26	Δ *L** = -0.58
	5-RC	36.94 ± 1.00	37.67 ± 1.05	37.58 ± 1.02	Δ *L** = 0.64
	7.5-RC	36.79 ± 0.51	37.39 ± 0.51	37.50 ± 0.68	Δ *L** = 0.71
*b** value	CON	14.05 ± 0.29^{c}	15.61 ± 0.39^{a}	14.87 ± 0.26^{b}	Δ *b** = 0.82
	2.5-RC	13.46 ± 0.19^{c}	16.30 ± 0.38^{a}	14.99 ± 0.37^{b}	Δ *b** = 1.53
	5-RC	14.46 ± 0.35^{c}	15.15 ± 0.42^{a}	15.32 ± 0.29^{b}	Δ *b** = 0.86
	7.5-RC	13.92 ± 0.34^{c}	16.55 ± 0.50^{a}	15.32 ± 0.38^{b}	Δ *b** = 1.40
Meat pH	CON	5.75 ± 0.04^{a}	5.70 ± 0.04^{b}	5.66 ± 0.03^{b}	-
	2.5-RC	5.75 ± 0.04^{a}	5.62 ± 0.03^{b}	5.70 ± 0.02^{b}	-
	5-RC	5.69 ± 0.02^{a}	5.67 ± 0.04^{b}	5.67 ± 0.02^{b}	-
	7.5-RC	5.73 ± 0.03^{a}	5.62 ± 0.03^{b}	5.66 ± 0.02^{b}	-

CON = control, 2.5-RC = 2.5% red clover, 5-RC = 5% red clover, 7.5-RC = 7.5% red clover.
[a-b] Means without common superscripts are different ($P < 0.05$).
[1] Results are expressed as mean ± standard error of the mean (SEM).

Figure 3.1: Redness (*a** values) of longissimus lumborum chops from Polypay ram lambs finished either on control (CON), 2.5% red clover hay (2.5-RC), 5% red clover hay (5-RC), or 7.5% red clover hay (7.5-RC) during 6 days of refrigerated storage. Standard error bars are indicated. [a-b] Means within a treatment or day of storage with different letters are different ($P < 0.05$).

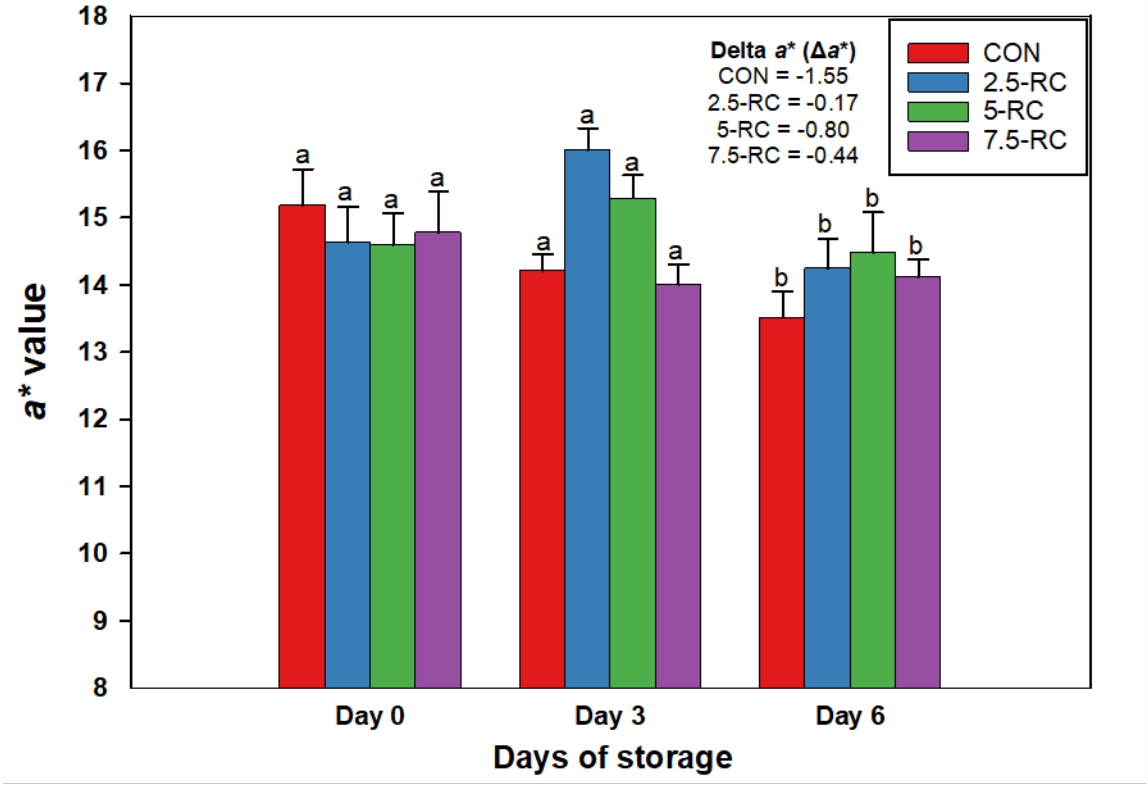


Figure 3.1: Color stability (R630/580) of longissimus lumborum chops from Polypay ram lambs finished either on control (CON), 2.5% red clover hay (2.5-RC), 5% red clover hay (5-RC), or 7.5% red clover hay (7.5-RC) during 6 days of refrigerated storage. Standard error bars are indicated. [a-b] Means within a treatment or day of storage with different letters are different ($P < 0.05$).

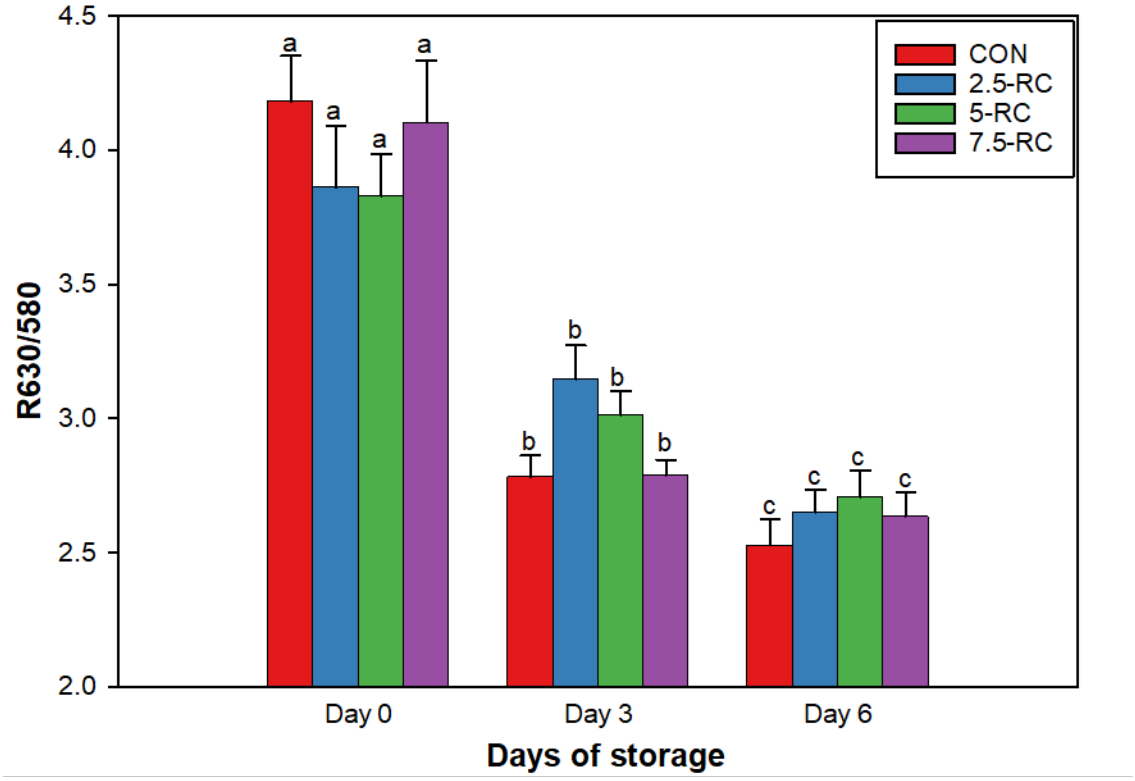


Figure 3.3: Hue angle of longissimus lumborum chops from Polypay ram lambs finished either on control (CON), 2.5% red clover hay (2.5-RC), 5% red clover hay (5-RC), or 7.5% red clover hay (7.5-RC) during 6 days of refrigerated storage. Standard error bars are indicated. [a-b] Means within a treatment or day of storage with different letters are different ($P < 0.05$).

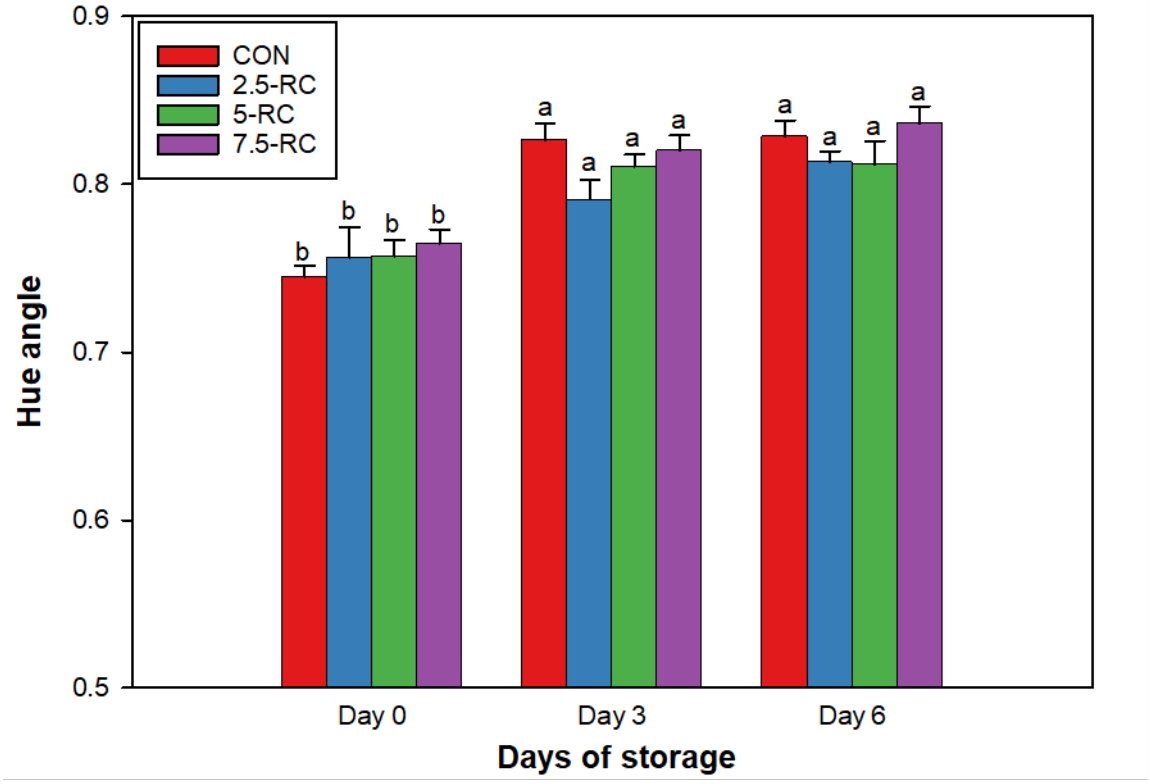


Figure 3.4: Chroma of longissimus lumborum chops from Polypay ram lambs finished either on control (CON), 2.5% red clover hay (2.5-RC), 5% red clover hay (5-RC), or 7.5% red clover hay (7.5-RC) during 6 days of refrigerated storage. Standard error bars are indicated. [a-b] Means within a treatment or day of storage with different letters are different ($P < 0.05$).

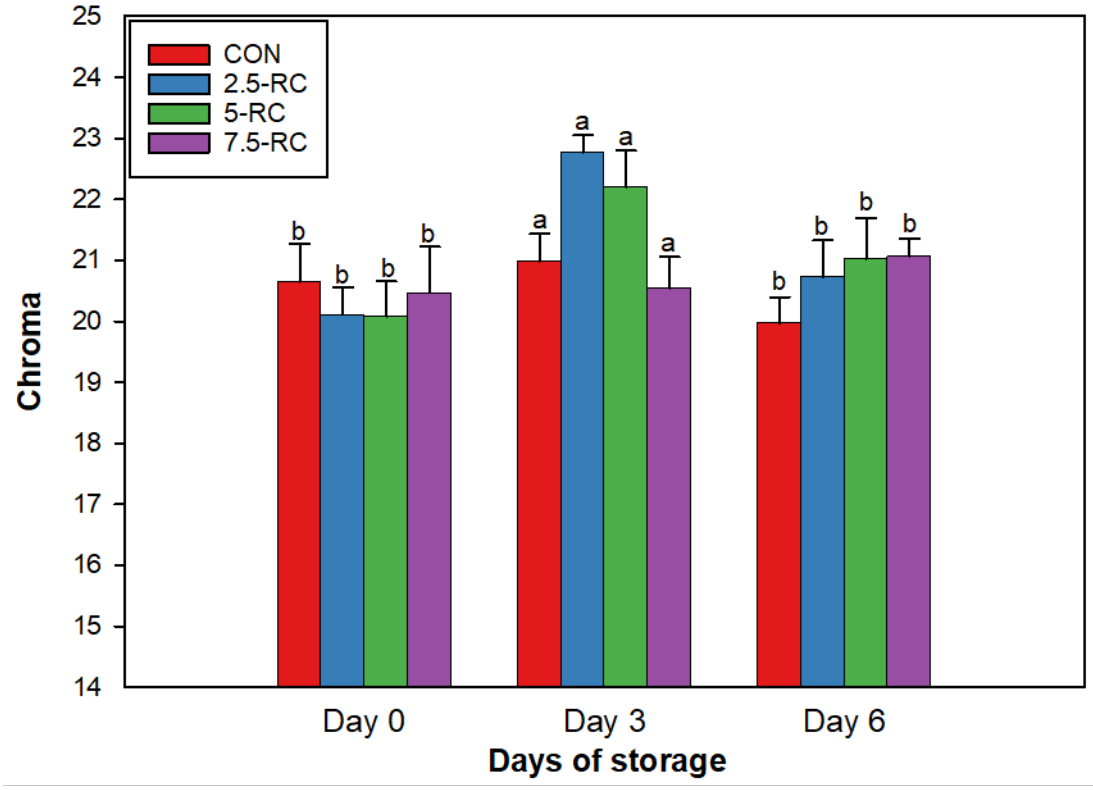


Figure 3.5: Lipid oxidation (TBARS) of longissimus lumborum chops from Polypay ram lambs finished either on control (CON), 2.5% red clover hay (2.5-RC), 5% red clover hay (5-RC), or 7.5% red clover hay (7.5-RC) during 6 days of refrigerated storage. Standard error bars are indicated. [a-b] Means within a treatment or day of storage with different letters are different ($P < 0.05$).

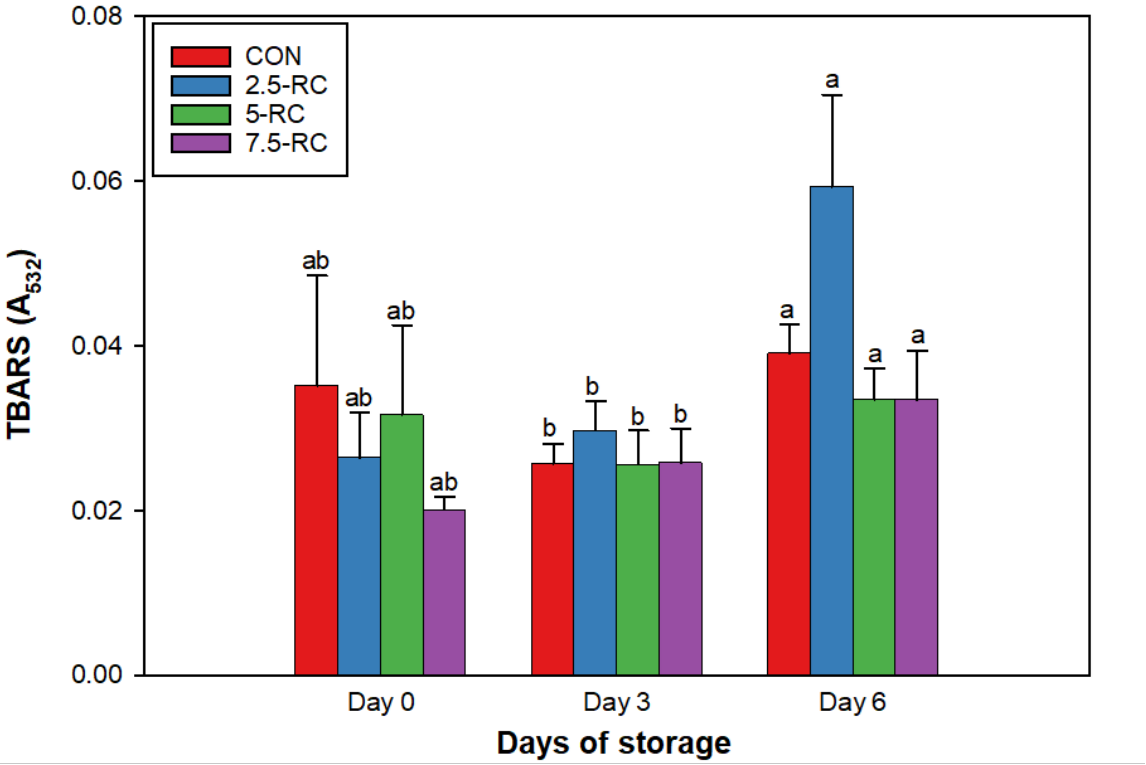
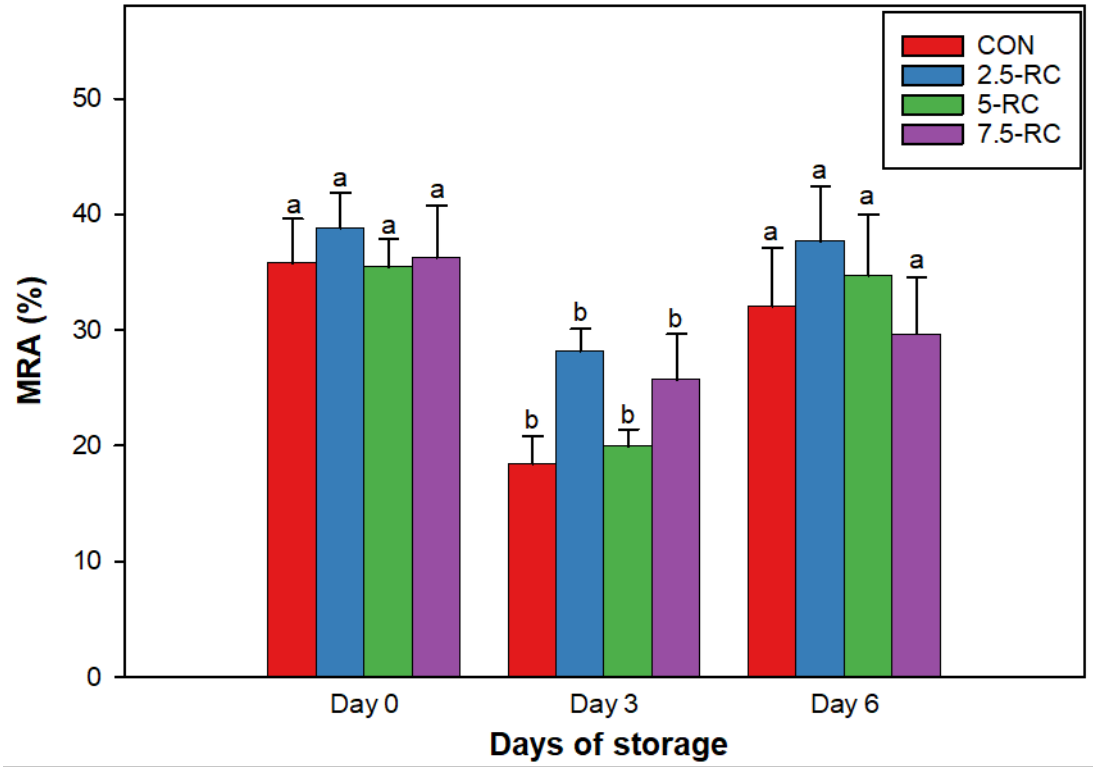


Figure 3.6: Metmyoglobin reducing activity (MRA) of longissimus lumborum chops from Polypay ram lambs finished either on control (CON), 2.5% red clover hay (2.5-RC), 5% red clover hay (5-RC), or 7.5% red clover hay (7.5-RC) during 6 days of refrigerated storage. Standard error bars are indicated. [a-b] Means within a treatment or day of storage with different letters are different ($P < 0.05$).



SUMMARY

The per capita consumption of lamb is increasing worldwide, and meat color is the most important attribute determining consumers' purchase decisions. Fresh lamb has a light red color and any deviation to brown color can result in consumers' rejection. Lamb color can be influenced by dietary strategies. The use of milk replacer during the pre-weaning phase and supplementation of red clover forage during the finishing phase can significantly impact the color stability of fresh lamb. Artificial raising is commonly utilized in lamb production to raise orphan, triplet, or surplus lambs and help them to gain weight, growth, and survival. Supplementation with red clover is an alternative feeding strategy for the lamb industry due to its high nutritive value and positive effects in lamb performance.

The first experiment evaluated the influence of artificial raising with milk replacer on carcass characteristics and color stability of longissimus lumborum (LL) chops from ram lambs during refrigerated storage. Polypay ram lambs were raised conventionally with ewes (CR) or artificially on milk replacer (AR). After 60 days, the lambs were fed *ad libitum* and finished on a high-forage (50:50 forage:concentrate), or on a high-concentrate diet (85:15 concentrate:forage) in confinement. The results indicated that the milk replacer and diet had no influence ($P > 0.05$) on the overall carcass characteristics, surface redness, yellowness, hue, chroma, color stability (R630/580), pH, and MRA of LL chops. Storage resulted in a decrease ($P < 0.05$) of color stability (R630/580) and MRA, and an increase ($P < 0.05$) of lipid oxidation, yellowness, and hue value in CR and AR chops. The use of milk

replacer as a pre-weaning strategy can be successfully utilized in lamb production without compromising fresh meat color.

The second experiment investigated the impact of red clover (*Trifolium pratense* L.) supplementation on the carcass characteristics, color stability, and lipid oxidation of LL chops from ram lambs during refrigerated storage. Polypay ram lambs were raised either on control, consisted of 85:15 concentrate: roughage with orchardgrass (*Dactylis glomerata* L.) without red clover (*Trifolium pratense* L.); 2.5% red clover with 12.5% orchardgrass; 5% red clover with 10% orchardgrass; or 7.5% red clover with 7.5% orchardgrass. The results demonstrated that the hot carcass weight, cold carcass weight, and lamb shoulder weight increased ($P < 0.05$) with supplementation of red clover (2.5% and 7.5%). Red clover supplementation had no effect ($P > 0.05$) on the surface redness, yellowness, hue, chroma, color stability (R630/580), pH, and MRA of LL chops. Storage resulted in an increase ($P < 0.05$) of yellowness, lipid oxidation, and hue angle, and a decrease ($P < 0.05$) of meat pH, color stability (R630/580), redness, and chroma in all samples. Additionally, lower color change (ΔE) ($P < 0.05$) was observed in chops from red clover supplemented lambs during the storage compared to their control counterparts. The supplementation with red clover can be utilized in lamb production without compromise of the fresh lamb color.

In summary, artificial raising and red clover supplementation did not exert negative effect on fresh lamb color stability. Dietary supplementation with red clover might offer antioxidant protection by decreasing the color change of lamb LL.

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PROFESSIONAL POSITIONS

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Veterinarian (12/2021 – 05/2022), Birds and Pet Animal Clinic, Rajshahi, Bangladesh

Veterinarian (01/2022 – 03/2022), Zilla Veterinary Hospital, Rajshahi, Bangladesh

AWARDS AND HONORS

2024 Graduate Student Congress Conference Award, University of Kentucky

2024 Graduate Student Congress Professional Development Award, University of Kentucky

PUBLICATIONS

1. **Mondal, K.**, Ragsdale, M. E., Rentfrow, G., Ely, D. G., Davis, B. E., Weinert-Nelson, J., Flythe, M. D., Wang, Y., Salim, A. P., Suman, S. P. Red clover supplementation does not influence the color stability of lamb longissimus lumborum muscles. American Meat Science Association Annual Reciprocal Meat Conference, Jun 16-19, 2024, Oklahoma City, OK, Abstract # 68.

2. **Mondal, K.**, Purvis, K. G., Rentfrow, G., Ely, D. G., Davis, B. E., Weinert-Nelson, J., Flythe, M. D., Wang, Y., Suman, S. P. Color and oxidative stability of longissimus lumborum muscles from artificially raised ram lambs finished on a high-concentrate diet. American Society of Animal Science Southern Section Annual Meeting, Jan 27-30, 2024, Louisville, KY, Abstract # 1660750.

3. **Mondal, K.**, Rentfrow, G., Ely, D. G., Parsley, K. G., Davis, B. E., Weinert-Nelson, J., Flythe, M. D., Wang, Y., Suman, S. P. Color stability and lipid oxidation in longissimus lumborum muscles from ram lambs artificially raised on milk replacer as a pre-weaning strategy. American Meat Science Association Annual Reciprocal Meat Conference, Jun 25-28, 2023, St. Paul, MN, Abstract # 102.

Koushik Mondal