



## Color Stability of Fallow Deer (*Dama dama*) *Infraspinatus*, *Longissimus Thoracis et Lumborum*, and *Biceps Femoris* Muscles During Refrigerated Storage

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**Abstract:** Fallow deer (*Dama dama*) meat comprises a relatively small proportion of the game meat market in South Africa, despite having huge potential. To exploit its market potential, the quality attributes of fresh meat from fallow deer need to be characterized. Limited studies have been undertaken on the color stability of economically important muscles in game species. Therefore, the objective of the present study was to examine the color stability of 3 major muscles, i.e., *infraspinatus* (IS), *longissimus thoracis et lumborum* (LTL), and *biceps femoris* (BF), from fallow deer. The IS, LTL, and BF muscles were removed from both sides of 12 (6 male and 6 female) fallow deer carcasses. The muscles were fabricated into 2.5-cm steaks. The steaks were aerobically over-wrapped and stored at 2°C for 8 d. Meat pH, instrumental color, surface myoglobin redox forms, and metmyoglobin reducing activity were evaluated at specific intervals. Data were analyzed using mixed model repeated measures ANOVA, with gender, muscle, and time as fixed effects. The IS muscle exhibited greater ( $P < 0.05$ ) pH, surface redness, color stability, oxymyoglobin content, and metmyoglobin reducing activity than the LTL and BF counterparts. In addition, surface metmyoglobin and total iron contents were lower in IS than in LTL and BF. While the IS demonstrated stable redness throughout the storage, the LTL and BF remained color stable only for 1 to 2 d. These findings suggested that fallow deer IS muscle is more color stable than the LTL and BF during refrigerated storage.

**Keywords:** color stability, fallow deer, game meat, muscles, myoglobin

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## Introduction

Fallow deer (*Dama dama*) are not indigenous to South Africa, and while it is uncertain when they

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were first introduced to South Africa from Europe, the first record of their occurrence is at Newlands House, Cape Town in 1869 (Mills and Hes, 1997). The fallow deer adapted well to the South African climate, and consequently their population grew rapidly (Curry et al., 2012). They have been translocated to various areas within South Africa, and they can be found on many game farms as well as free roaming throughout South Africa. Despite the drastic increase in their population, fallow deer meat still represents a relatively small proportion of the game meat market in South Africa (Hoffman and Wiklund, 2006).

Fallow deer are one of the most commonly farmed cervid species in Europe, Canada, and the US, and the popularity of deer farming as a whole is increasing globally (Hoffman and Wiklund, 2006; Chakanya et

al., 2016). This popularity may stem from the relatively low-input production system required to rear fallow deer and the minimal impact their husbandry has on the environment (Volpelli et al., 2003). Thus, market potential exists for the increased production of fallow deer for export and local consumption within South Africa. While venison commonly refers to meat obtained from domesticated/farmed cervids in countries outside South Africa, African game meat is still obtained from wild and free-roaming cervids (Hoffman and Wiklund, 2006).

To create a sustainable and reliable fallow deer meat market, products of consistent and high quality need to be delivered to the consumers (Hutchison et al., 2010). To achieve this, the factors affecting the quality of fallow deer meat need to be investigated. Although some research has been conducted on the quality of venison harvested from farmed fallow deer, limited research has been conducted on game meat from fallow deer in South Africa. One important quality attribute of fallow deer meat that needs to be investigated is its color stability. The color of fresh red meat largely dictates the willingness of a consumer to purchase (Faustman and Cassens, 1990; Risvik, 1994; Mancini and Hunt, 2005; Suman et al., 2014). Thus, desirable and consistent meat color, and prolonged color stability will result in increased sales and revenue for the game meat industry (Neethling et al., 2017).

The objective of the present study was to determine the color stability of 3 economically important muscles in fallow deer carcasses, i.e., *infraspinatus* (IS), *longissimus thoracis et lumborum* (LTL), and *biceps femoris* (BF), during aerobic refrigerated storage.

## Materials and Methods

### Animal harvesting and muscle collection

Twelve (6 male and 6 female) mature fallow deer were harvested (average bled weight  $51.8 \pm 12.16$  kg) on the farm at Brakkekuil ( $34^{\circ}18'24.0''S$  and  $20^{\circ}49'3.9''E$ ), Witsand, Western Cape Province, South Africa. The animals were harvested according to standard operating procedure (SU-ACUM14-001SOP issued by the Stellenbosch University Animal Care and Use Committee). The animals were harvested at night to minimize stress and were shot in the head or the high neck area with a .308 caliber rifle. Once shot, the animals were exsanguinated in the field (2 to 3 min after being shot). As far as possible, all steps were taken to prevent or minimize any unnecessary ante-mortem stress experienced by the animals. The exsanguinated,

undressed carcasses were transported within 15 min to abattoir facilities at Brakkekuil where the legs, head and skin were removed and the animals were eviscerated.

After dressing, the carcasses were hung by both hind legs in a cold room ( $2^{\circ}C$ ) for 24 h. Subsequently, the entire *infraspinatus* (IS), *longissimus thoracis et lumborum* (LTL), and *biceps femoris* (BF) muscles were removed from the left and right sides of each carcass. All visible intermuscular and subcutaneous fat were removed, and the muscles were individually vacuum packaged in a composite plastic bag (70  $\mu$ m polyethylene and nylon; moisture vapor transfer rate of  $2.2 \text{ g}^{-2} 24 \text{ h}^{-1} 1 \text{ atm}^{-1}$ ,  $O_2$  permeability of  $30 \text{ cm}^{-3} \text{ m}^{-2} 24 \text{ h}^{-1} 1 \text{ atm}^{-1}$ , and a  $CO_2$  permeability of  $105 \text{ cm}^{-3} \text{ m}^{-2} 24 \text{ h}^{-1} 1 \text{ atm}^{-1}$ ; Freddy Hirsch, Cape Town, South Africa) with a residual pressure of 5 mbar (Multivac, Model C200; Sepp Haggemuller, Wolfertschwenden, Germany). The vacuum-packaged muscles were transported under refrigeration to the Department of Animal Sciences at Stellenbosch University and were placed in a cold room ( $2^{\circ}C$ ) on arrival. To minimize any temperature variations, the samples were laid out on racks in a single layer inside the cold room.

### Muscle fabrication

The muscles were stored for 48 h at  $2^{\circ}C$  prior to the color stability study. Since the variations between the muscles from left and right sides were considered negligible, the muscles from the left and right sides of each carcass were considered an experimental unit. The muscles were removed from the vacuum packaging and blotted dry with paper towel to remove any surface moisture. The anterior and posterior ends of the muscles were removed and were not used. Each muscle was then cut at the center and perpendicular to the muscle fiber direction into 2 halves, and three 2.5-cm steaks were cut perpendicular to the muscle fiber direction from each half. This approach provided 6 steaks per muscle, and 12 steaks per experimental unit. Steaks from each muscle (IS, LTL, and BF) and carcass were packed in a single layer in polystyrene trays (16 cm  $\times$  21 cm; Freddy Hirsch, Cape Town, South Africa) and overwrapped with low-density polyethylene (LDPE) film (moisture vapor transfer rate of  $585 \text{ g}^{-2} 24 \text{ h}^{-1} 1 \text{ atm}^{-1}$ ,  $O_2$  permeability of  $25,000 \text{ cm}^{-3} \text{ m}^{-2} 24 \text{ h}^{-1} 1 \text{ atm}^{-1}$ , and a  $CO_2$  permeability of  $180,000 \text{ cm}^{-3} \text{ m}^{-2} 24 \text{ h}^{-1} 1 \text{ atm}^{-1}$ ; Freddy Hirsch).

### Refrigerated storage

The color stability study was conducted during 8 d refrigerated storage. The overwrapped trays were

placed in a single layer in the cold room at 2°C to minimize variations between samples in terms of light exposure, temperature, and gas permeation through the LDPE film. The packaged steaks were stored under fluorescent lights (OSRAM L58W/640, Energy saver, Cool White, 4600 Lumen), and the samples were taken at d 0, 1, 2, 4, 6, and 8.

### **Instrumental color evaluation**

The surface color of the steaks was measured instrumentally at each time point (American Meat Science Association, 2012) using a color-guide 45°/0° colorimeter (Model 6801, BYK-Gardner, Geretsried, Germany) equipped with 11-mm aperture, illuminant D65, and 10° observer angle. Calibration of the colorimeter was done using the standards provided by the manufacturer. The overwrap was removed prior to color measurement, and the color was measured directly on the surface of the steaks. After the color measurement, the specific sample was removed for further analyses. On d 0, the steaks, overwrapped to prevent surface desiccation, were bloomed for 60 min at 2°C prior to color measurement.

Measurements were taken at 5 different locations on each steak, and the average of the 5 measurements for each attribute was calculated and used for statistical analysis. The instrumental color parameters measured were  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness) values. In addition, the hue angle and chroma were calculated from the  $a^*$  and  $b^*$  values (American Meat Science Association, 2012).

Concurrent to the surface color parameters, the reflectance was measured from 400 to 700 nm at 10-nm increments. The reflectance data were used to calculate the ratio of reflectance at 630 nm and 580 nm, i.e.,  $R$  (630/580), as an indirect estimate of surface color stability (American Meat Science Association, 2012). The consumer acceptability cut-off value of 3 was employed for  $R$  (630/580) as reported previously (Purchas et al., 2010).

### **Surface myoglobin redox forms**

The reflectance data from meat surface can be used to calculate myoglobin (Mb) redox forms (American Meat Science Association, 2012). The reflectance was measured at the isobestic wavelengths of Mb (473, 525, 572, and 730 nm). If the colorimeter does not measure up to 730 nm, a wavelength of 700 nm can be used, as was the case in this study. Wavelengths which were not specifically measured, but required for the calculations (473, 525, and 572 nm), were calculated using integration (American Meat Science Association, 2012). All

required reflectance values were first converted to their corresponding reflex attenuation, which is the logarithm of the reciprocal of reflectance. The reflex attenuation values were subsequently used to calculate the percentages of the various Mb redox forms, present at the surface of fresh meat, using the equations according to American Meat Science Association (2012).

### **Meat pH**

The pH values of the meat samples were determined at each time point using the iodoacetate method (Jeacocke, 1977). The meat sample (0.5 g) was homogenized in 5 mL of a 5 mM sodium iodoacetate and 150 mM KCl (adjusted to pH 7 with KOH) solution. The pH of the homogenate was measured in duplicate with a calibrated Jenway 3510 bench top pH meter (IJEN351201, Lasec SA, Cape Town, South Africa).

### **Oxygen consumption rate (OCR)**

The OCR was measured at 3 time points (d 0, 4, and 8). A 3 cm × 3 cm × 2 cm size meat sample was removed from the center of each steak. If the steak surface was not freshly cut (i.e., on d 4 and 8), a thin section of the surface layer was removed to expose a fresh surface. The samples were allowed to bloom for 2 h at 2°C to ensure uniform oxygenation and covered in LDPE (moisture vapor transfer rate of 585 g<sup>-2</sup> 24 h<sup>-1</sup> 1 atm<sup>-1</sup>, O<sub>2</sub> permeability of 25,000 cm<sup>-3</sup> m<sup>-2</sup> 24 h<sup>-1</sup> 1 atm<sup>-1</sup>, and a CO<sub>2</sub> permeability of 180,000 cm<sup>-3</sup> m<sup>-2</sup> 24 h<sup>-1</sup> 1 atm<sup>-1</sup>; Freddy Hirsch) to prevent desiccation. The samples were vacuum packaged in a composite plastic bag (70 μm polyethylene and nylon; moisture vapor transfer rate of 2.2 gm<sup>-2</sup> 24 h<sup>-1</sup> 1 atm<sup>-1</sup>, O<sub>2</sub> permeability of 30 cm<sup>-3</sup> m<sup>-2</sup> 24 h<sup>-1</sup> 1 atm<sup>-1</sup> and a CO<sub>2</sub> permeability of 105 cm<sup>-3</sup> m<sup>-2</sup> 24 h<sup>-1</sup> 1 atm<sup>-1</sup>; Freddy Hirsch) with a residual pressure of 5 mbar (Multivac, Model C200; Sepp Haggenmuller). The bloomed surface reflectance was scanned (700 to 400 nm) immediately after packaging. These measurements were used to calculate the initial % oxymyoglobin (%OMb), using the relevant spectral values at 473, 525, 572, and 700 nm based on the equations (American Meat Science Association, 2012). The scanned samples were subsequently placed in a water bath and incubated at 25°C for 20 min. After incubation, the surface reflectance was scanned again, and these measurements were used to calculate the final %OMb. The OCR was calculated using the equation below (American Meat Science Association, 2012).

$$\text{OCR} = \frac{[(\text{Initial \%OMb} - \text{Final \%OMb}) / \text{Initial \%OMb}] \times 100}{\text{Time}}$$

### Metmyoglobin reducing activity (MRA)

The MRA was measured at 3 time points (d 0, 4, and 8). A 3 cm × 3 cm × 2 cm sample was removed from the center of each steak. The samples were submerged in 0.3% NaNO<sub>2</sub> (w/w) for 20 min to induce metmyoglobin (MMb) formation. After 20 min the samples were removed, blotted dry and vacuum packaged in a composite plastic (70 μm polyethylene and nylon; moisture vapor transfer rate of 2.2 g<sup>-2</sup> 24 h<sup>-1</sup> 1 atm<sup>-1</sup>, O<sub>2</sub> permeability of 30 cm<sup>-3</sup> m<sup>-2</sup> 24 h<sup>-1</sup> 1 atm<sup>-1</sup>, and a CO<sub>2</sub> permeability of 105 cm<sup>-3</sup> m<sup>-2</sup> 24 h<sup>-1</sup> 1 atm<sup>-1</sup>) with a residual pressure of 5 mbar (Multivac, Model C200; Sepp Haggemuller). The surface reflectance was measured (400 to 700 nm) immediately after packaging. The samples were incubated in a water bath at 30°C for 2 h after which the surface reflectance was scanned once more. The %MMb (pre-incubation as well as post-incubation) on the surface was calculated based on the K/S ratios and according to established formulas using the relevant spectral values at 525, 572, and 700 nm (American Meat Science Association, 2012). The MRA was calculated using the following equation.

$$\text{MRA} = 100 \times [(\% \text{ pre-incubation surface MMb} - \% \text{ post-incubation surface MMb}) / \% \text{ pre-incubation surface MMb}]$$

### Iron content

Total iron content was determined on d 0 using HNO<sub>3</sub>-digested samples analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Briefly, a 0.5-g sample was pre-digested (allowed to digest at room temperature) in concentrated HNO<sub>3</sub> for 20 min. The samples were then digested fully in a MARS microwave digester (MARS 240/50; CEM Corporation, Matthews, NC) at 1600 W, 800 psi and 200°C for 10 min (ramp time of 25 min and cool down time of 25 min). The samples were diluted to 50 mL (1:100 dilution) with deionized water. The diluted, digested samples were then analyzed using a Thermo ICap 6200 ICP-AES (Thermo Electron Corporation, Milan, Italy). The ICP-AES was calibrated using NIST traceable standards and verified with a control standard. The results were corrected for the dilution factor resulting from the digestion procedure.

### Myoglobin concentration

Myoglobin concentration was measured in the samples taken on d 0. Myoglobin was extracted according to the method of Tang et al. (2004). Briefly,

a 10-g sample was homogenized (P-8; Kinematica, Littau, Switzerland) in 100 mL cold 40 mM potassium buffer (adjusted to pH 6.8) and allowed to extract for 60 min at 4°C. The extract was then centrifuged (Sigma 2-16 K, Wirsam Scientific, Cape Town, South Africa) for 30 min at 4,000 rpm at 4°C. A small quantity of sodium dithionite (3 to 5 μg) was added to the supernatant to convert all the Mb to deoxymyoglobin (DMb). The absorbance of the supernatant was measured at 433 nm (A<sub>433</sub>) spectrometrically (Spectrostar Nano, BMG Labtech, Ortenberg, Germany). The Mb concentration was calculated as follows (American Meat Science Association, 2012).

$$\text{Mb (mg/g meat)} = A_{433} \times (1 \text{ M Mb} / 114,000) \times [(1 \text{ mol/L}) / \text{M}] \times (17,000 \text{ g Mb/mol Mb}) \times (1000 \text{ mg/g}) \times \text{dilution factor of } 0.10 \text{ L/10 g meat}$$

### Statistical analyses

Mixed model repeated measures ANOVA was used to investigate the differences between muscles, taking into account gender and storage time effects. Thus gender, muscle, and storage time were treated as fixed effects, and animal nested in gender as random effect. For post hoc testing, the Fisher least significant difference (LSD) test was used. Correlation analyses were done using Pearson correlation. A 5% significance level ( $P < 0.05$ ) was used as guideline for significant effects and correlations.

## Results

The interactions between the main effects for the parameters are presented in Table 1. Only the results for significant interactions ( $P < 0.05$ ) and individual main effects ( $P < 0.05$ ) are presented and discussed.

### Instrumental color

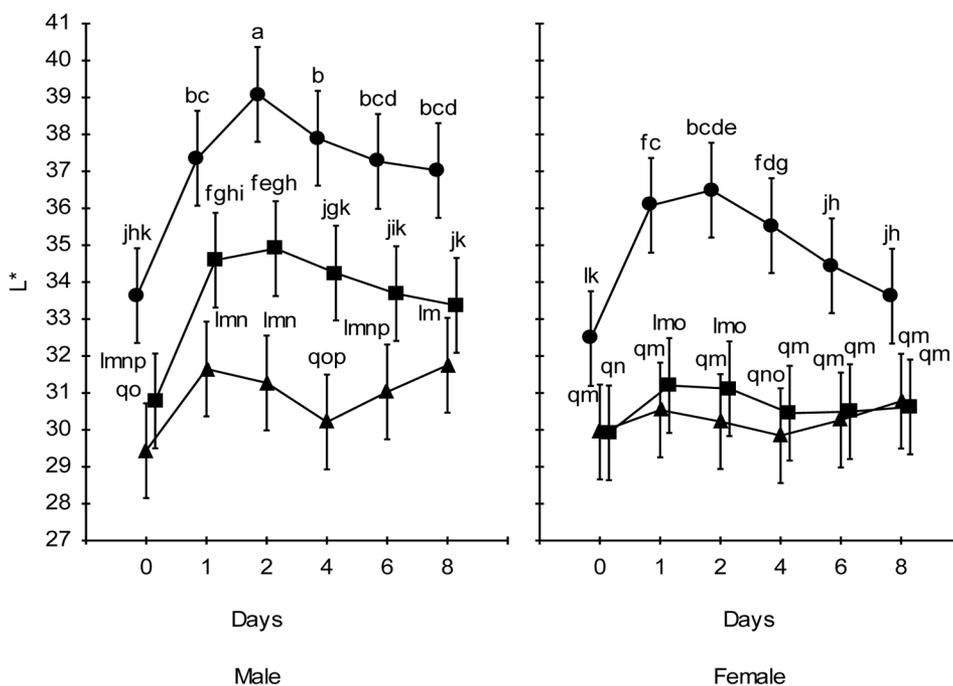
A gender, muscle, and storage time interaction ( $P < 0.05$ ) was observed for the  $L^*$  values (Table 1). In both males and females, the IS exhibited the greatest ( $P < 0.05$ )  $L^*$  values throughout the storage (Fig. 1). In the males, BF demonstrated greater ( $P < 0.05$ )  $L^*$  values than LTL on all storage days, except on d 0. In contrast, the  $L^*$  values of LTL and BF from female fallow deer were similar ( $P > 0.05$ ) throughout the storage.

The data on  $a^*$  values also demonstrated a gender, muscle, and storage time interaction ( $P < 0.05$ ; Table

**Table 1.** The *P*-values<sup>1</sup> indicating the impact of gender (G), muscle (M), and storage time (T) on the various color stability attributes measured for fallow deer meat

Attributes	G × M × T	M × T	G × T	G × M	Gender	Muscle	Storage time
<i>L*</i> value	<b>0.039</b> <sup>1</sup>	<b>0.000</b>	<b>0.005</b>	<b>0.009</b>	<b>0.016</b>	<b>0.000</b>	<b>0.000</b>
<i>a*</i> value	<b>0.004</b>	<b>0.000</b>	<b>0.000</b>	0.568	0.140	<b>0.000</b>	<b>0.000</b>
<i>b*</i> value	<b>0.003</b>	<b>0.000</b>	<b>0.003</b>	0.208	<b>0.014</b>	<b>0.000</b>	<b>0.000</b>
Chroma	<b>0.002</b>	<b>0.000</b>	<b>0.001</b>	0.502	<b>0.040</b>	<b>0.000</b>	<b>0.000</b>
Hue	<b>0.017</b>	<b>0.000</b>	<b>0.000</b>	<b>0.024</b>	0.579	<b>0.000</b>	<b>0.000</b>
R(630/580)	0.399	<b>0.000</b>	0.137	0.892	0.053	<b>0.000</b>	<b>0.000</b>
%DMb	0.141	<b>0.000</b>	<b>0.010</b>	<b>0.002</b>	<b>0.002</b>	<b>0.000</b>	<b>0.000</b>
%OMb	0.384	<b>0.000</b>	<b>0.032</b>	0.279	<b>0.004</b>	<b>0.000</b>	<b>0.000</b>
%MMb	0.918	<b>0.000</b>	<b>0.036</b>	0.452	<b>0.035</b>	<b>0.000</b>	<b>0.000</b>
pH	0.927	<b>0.000</b>	0.824	0.845	0.132	<b>0.000</b>	<b>0.000</b>
MRA	0.145	0.052	<b>0.046</b>	<b>0.005</b>	<b>0.043</b>	<b>0.000</b>	<b>0.001</b>
OCR	0.218	<b>0.014</b>	0.577	0.140	0.727	<b>0.001</b>	<b>0.000</b>
Total iron	–	–	–	0.536	0.594	<b>0.000</b>	–
Non-heme iron	–	–	–	0.700	0.382	<b>0.000</b>	–
Total Mb	–	–	–	0.341	0.530	<b>0.001</b>	–

<sup>1</sup>*P*-values in bold indicate a significant interaction/difference at *P* < 0.05.

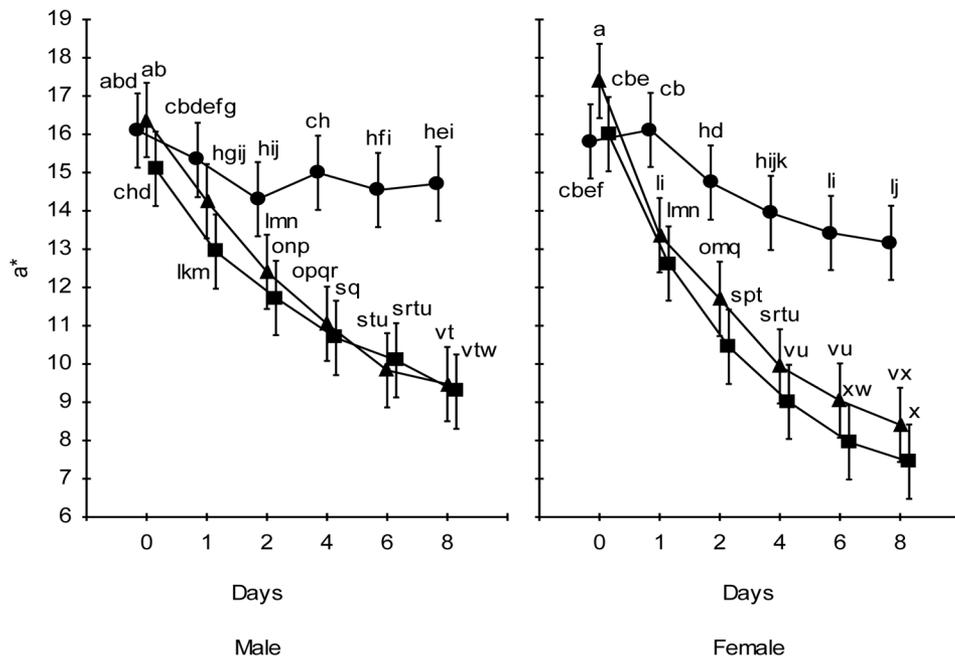


**Figure 1.** The changes in *L\** values (lightness) of 3 fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum*, and (■) *biceps femoris*, from male and female carcasses, stored at 2°C. <sup>a-q</sup>Means without common letters are different (*P* < 0.05).

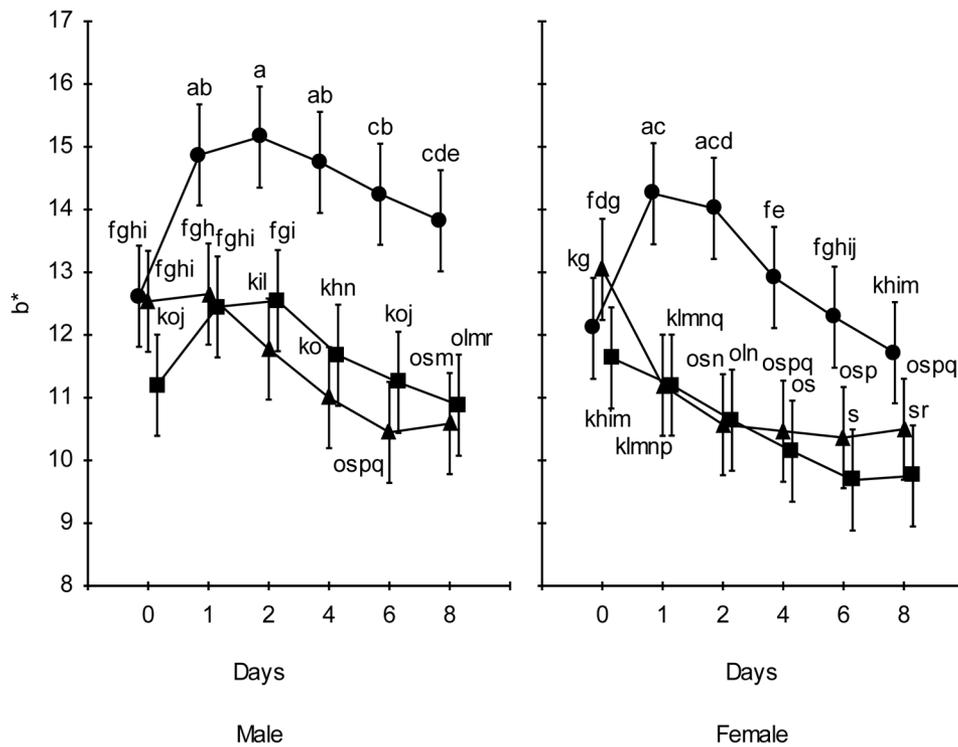
1). In males, on d 0, *a\** values of the IS muscles did not differ (*P* > 0.05) from those of the LTL and BF (Fig. 2). In contrast, in the females, LTL differed from the IS and BF on d 0 (*P* < 0.05), with the LTL having the highest (*P* < 0.05) *a\** values. A decline (*P* < 0.05) in *a\** values was observed for all the muscles over the storage, with the least decrease observed for the IS

of both genders. Overall, the IS muscle demonstrated greatest *a\** values in both genders.

The results for *b\** values demonstrated a gender, muscle, and storage time interaction (*P* < 0.05; Table 1). Overall, IS muscles from males and females exhibited greatest (*P* < 0.05) *b\** values after d 1 to 2 of the storage compared to their LTL and BF counterparts (Fig. 3).



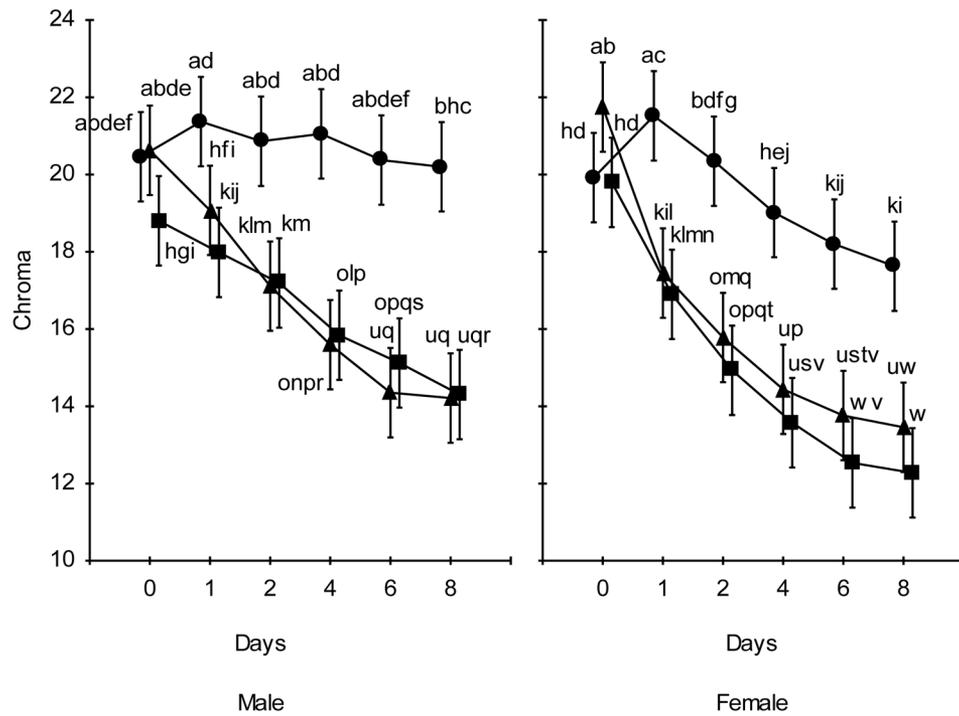
**Figure 2.** The changes in  $a^*$  values (redness) of 3 fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum*, and (■) *biceps femoris*, from male and female carcasses, stored at 2°C. <sup>a-x</sup>Means without common letters are different ( $P < 0.05$ ).



**Figure 3.** The changes in  $b^*$  values (yellowness) of 3 fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum*, and (■) *biceps femoris*, from male and female carcasses, stored at 2°C. <sup>a-s</sup>Means without common letters are different ( $P < 0.05$ ).

Gender, muscle, and storage time interactions were observed for the chroma values ( $P < 0.05$ ; Table 1). A decrease in chroma ( $P < 0.05$ ) was observed for all muscles from male and female fallow deer, except the IS from

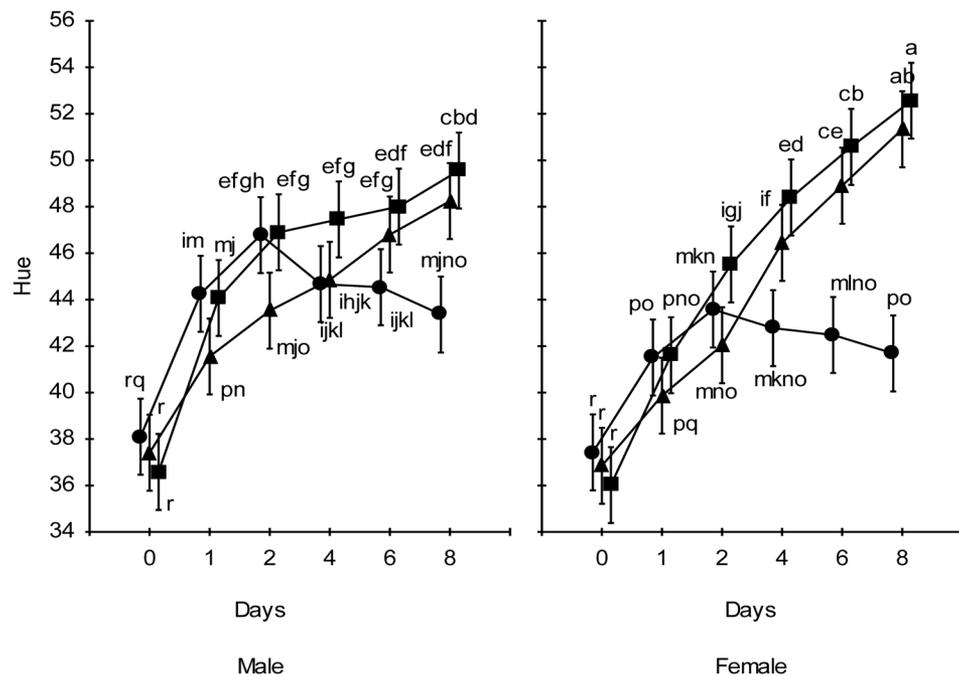
males, which exhibited no overall change ( $P > 0.05$ ; Fig. 4). Data for hue angle exhibited a gender, muscle, and storage time interaction ( $P < 0.05$ ; Table 1). An increase in hue values during storage was observed in all the mus-



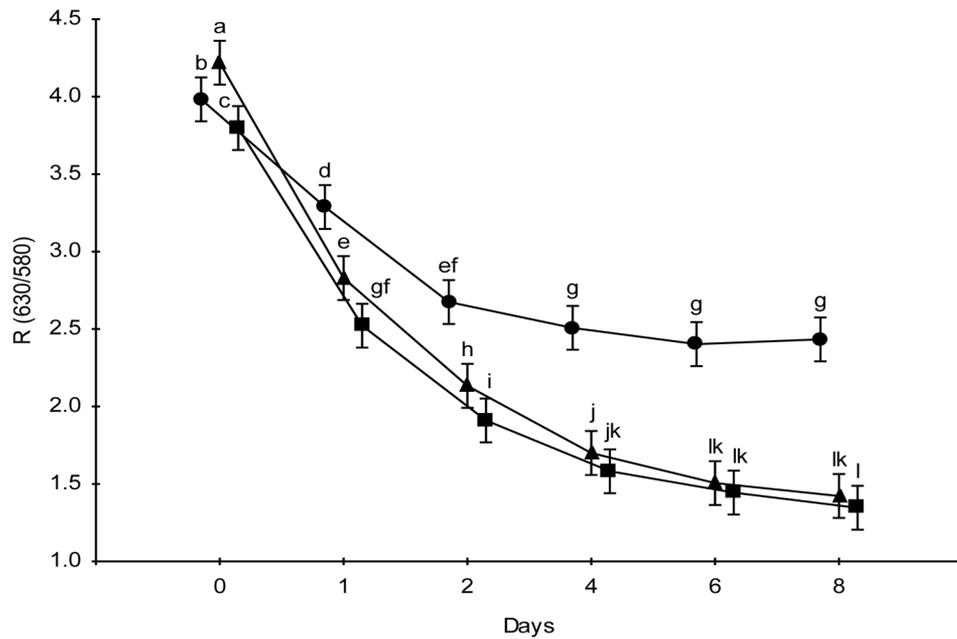
**Figure 4.** The changes in chroma of 3 fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum*, and (■) *biceps femoris*, from male and female carcasses, stored at 2°C. <sup>a-w</sup>Means without common letters are different ( $P < 0.05$ ).

cles from both male and female fallow deer (Fig. 5). A muscle and time interaction ( $P < 0.05$ ) was observed for the R (630/580) values, while gender had no effect ( $P >$

0.05; Table 1). The R (630/580) values decreased ( $P < 0.05$ ) for all the muscles during the storage, with a rapid decline in BF and LTL compared to IS (Fig. 6).



**Figure 5.** The changes in hue of 3 fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum*, and (■) *biceps femoris*, from male and female carcasses, stored at 2°C. <sup>a-r</sup>Means without common letters are different ( $P < 0.05$ ).

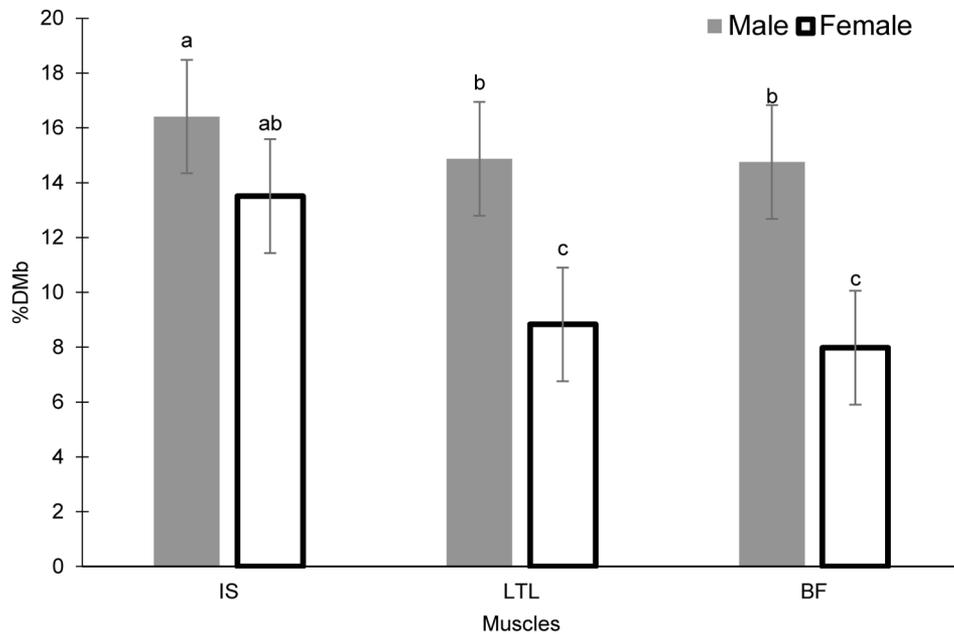


**Figure 6.** The changes in R (630/580) of 3 fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum*, and (■) *biceps femoris*, stored at 2°C. <sup>a-l</sup>Means without common letters are different ( $P < 0.05$ ).

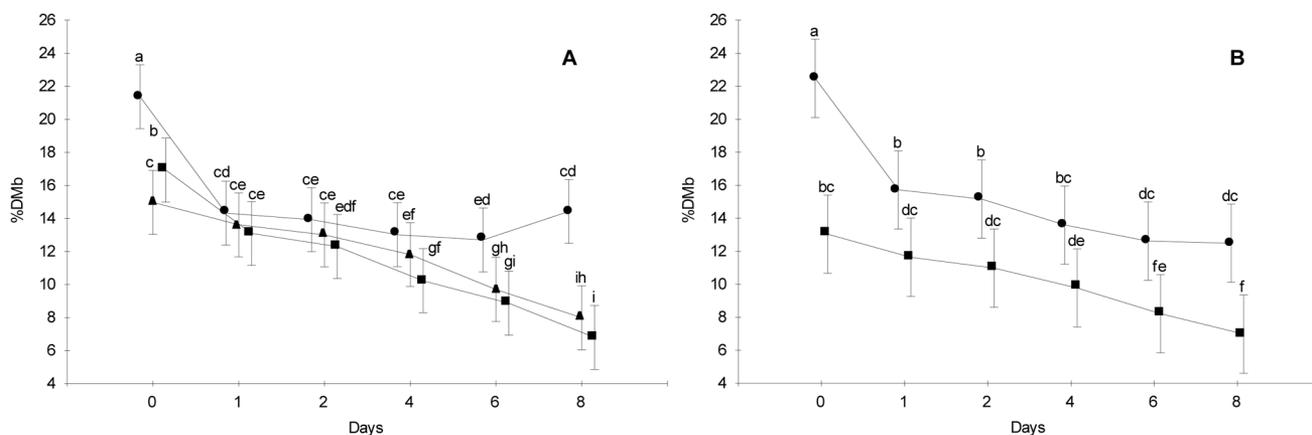
**Surface Mb redox forms**

For the %DMb, muscle × storage time, gender × time, and gender × muscle interactions were observed (Table 1). The gender × muscle interactions indicated that LTL and BF from the males demonstrated greater ( $P < 0.05$ ) %DMb than their counterparts from females,

whereas the IS from male and female fallow deer exhibited no differences ( $P > 0.05$ ; Fig. 7). In addition, IS from both males and females exhibited greater ( $P < 0.05$ ) %DMb than their LTL and BF counterparts (Fig. 7). The %DMb decreased ( $P < 0.05$ ) in all the muscles during storage (Fig. 8A). A decrease ( $P < 0.05$ ) in %DMb was observed for muscles from both genders



**Figure 7.** The %DMb of 3 fallow deer muscles, *infraspinatus* (IS), *longissimus thoracis et lumborum* (LTL), and *biceps femoris* (BF), from male and female carcasses, stored at 2°C. <sup>a-c</sup>Means without common letters are different ( $P < 0.05$ ).



**Figure 8.** (A) The changes in %DMb of 3 fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum*, and (■) *biceps femoris*, stored at 2°C; (B) changes in %DMb of muscles from (●) male and (■) female fallow deer, stored at 2°C. <sup>a-i</sup>Means without common letters are different ( $P < 0.05$ ).

(Fig. 8B). Nonetheless, the muscles from male fallow deer exhibited greater ( $P < 0.05$ ) %DMb than their counterparts from female throughout the storage.

Data for %OMb demonstrated muscle  $\times$  storage time and gender  $\times$  time interactions (Table 1). The %OMb decreased ( $P < 0.05$ ) during the storage in all 3 muscles (Fig. 9A) from male and female fallow deer (Fig. 9B). The %OMb for all the muscles plateaued ( $P > 0.05$ ) after d 4. The gender  $\times$  storage time interaction showed that the muscles from female animals had greater %OMb ( $P < 0.05$ ) than their counterparts from males on d 0 and 1, after which gender had no impact ( $P > 0.05$ ; Fig. 9B).

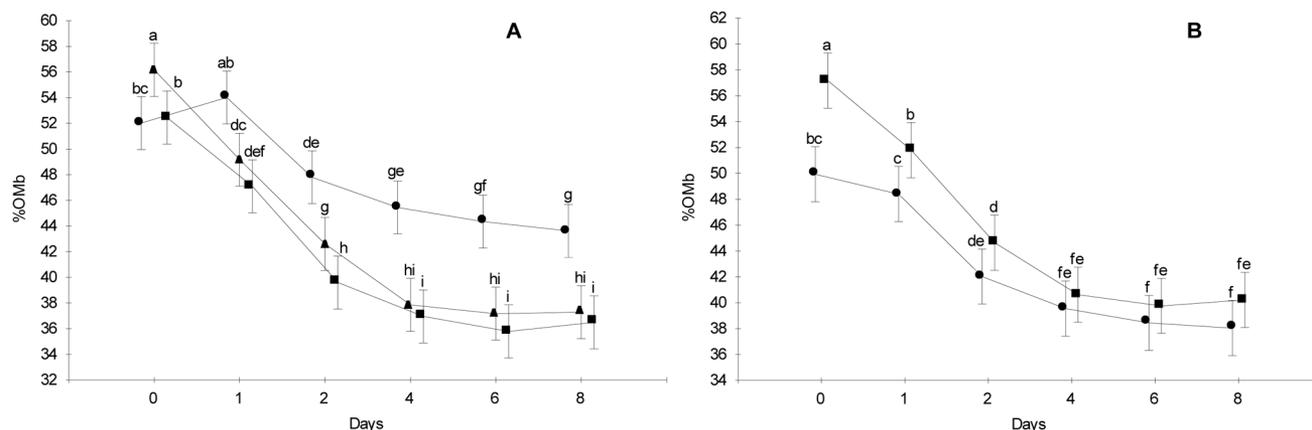
Muscle  $\times$  storage time as well as gender  $\times$  time interactions were observed for %MMb (Table 1). An increase ( $P < 0.05$ ) in %MMb was observed for the muscles (Fig. 10A) in both males and females (Fig. 10B). The increase ( $P < 0.05$ ) in %MMb was slower for the IS in comparison to the LTL and BF (Fig. 10A), and the IS exhibited the lowest ( $P < 0.05$ ) %MMb throughout the storage. The

muscles from female fallow deer demonstrated greater ( $P < 0.05$ ) %MMb than their counterparts from males on all the days, except d 1 and 2 (Fig. 10B).

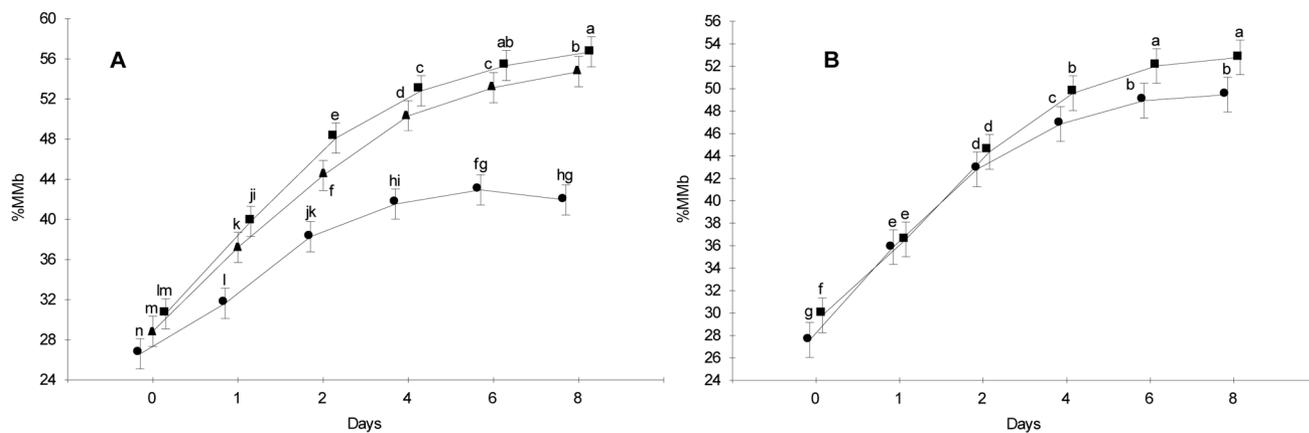
### Biochemical attributes

A muscle  $\times$  storage time interaction ( $P < 0.05$ ) was observed for the pH, while gender had no effect ( $P > 0.05$ ; Table 1). The IS had the highest ( $P < 0.05$ ) pH values and the LTL the lowest ( $P < 0.05$ ) values during the storage (Fig. 11).

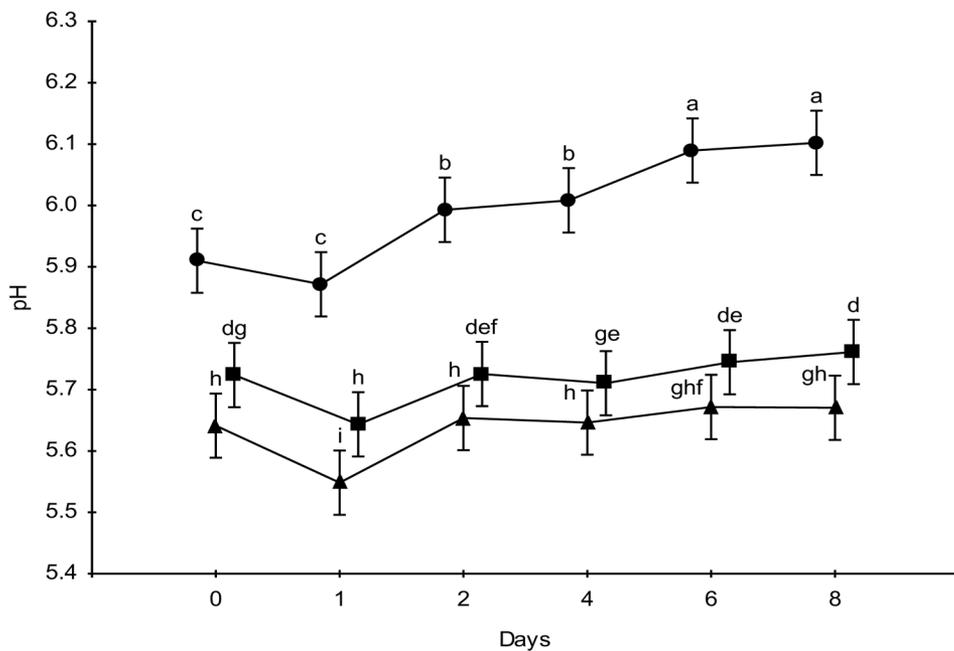
Data for MRA exhibited gender  $\times$  time and gender  $\times$  muscle interactions ( $P < 0.05$ ; Table 1). The LTL and BF from male fallow deer demonstrated greater ( $P < 0.05$ ) MRA than their counterparts from females, whereas IS from males and females did not differ ( $P > 0.05$ ) in MRA (Fig. 12). In addition, IS from both males and females exhibited greater ( $P < 0.05$ ) MRA than their LTL and BF counterparts. There was an initial increase ( $P < 0.05$ ) in MRA from d 0 to 4 for all the



**Figure 9.** (A) The changes in %OMb of 3 fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum*, and (■) *biceps femoris*, stored at 2°C; (B) changes in %OMb of muscles from (●) male and (■) female fallow deer, stored at 2°C. <sup>a-i</sup>Means without common letters are different ( $P < 0.05$ ).



**Figure 10.** (A) The changes in %MMb of 3 fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum*, and (■) *biceps femoris*, stored at 2°C; (B) changes in %MMb of muscles from (●) male and (■) female fallow deer, stored at 2°C. <sup>a-n</sup>Means without common letters are different ( $P < 0.05$ ).



**Figure 11.** The changes in pH of 3 fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum*, and (■) *biceps femoris*, stored at 2°C. <sup>a-i</sup>Means without common letters are different ( $P < 0.05$ ).

muscles from both genders (Fig. 13). Subsequently, a decrease ( $P < 0.05$ ) was observed for the muscles from females, but not in the ones from the male animals.

While gender did not influence ( $P > 0.05$ ) OC (oxygen consumption), a muscle  $\times$  storage time interaction ( $P < 0.05$ ) was observed (Table 1). A decrease ( $P < 0.05$ ) in OC was observed in all 3 muscles (Fig. 14). While the LTL exhibited greatest ( $P < 0.05$ ) OC on d 4, the IS demonstrated the lowest ( $P < 0.05$ ) OC on d 8.

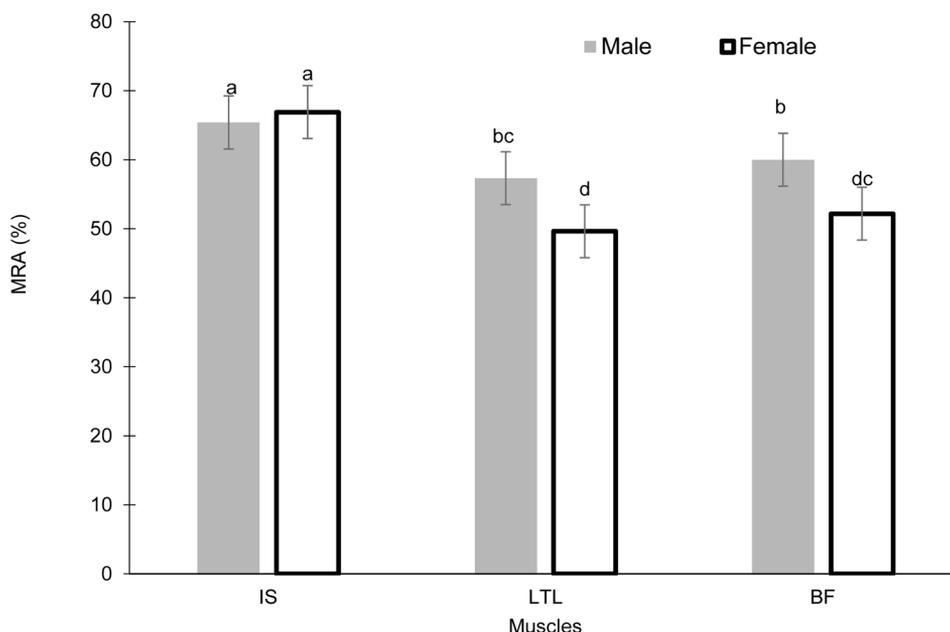
Muscle source influenced total iron and Mb contents, while gender had no effect on these attributes (Table 2). The LTL and BF had the greatest ( $P < 0.05$ ) and the IS the

lowest ( $P < 0.05$ ) total iron contents (Table 2). The IS and LTL had lower ( $P < 0.05$ ) Mb content than the BF.

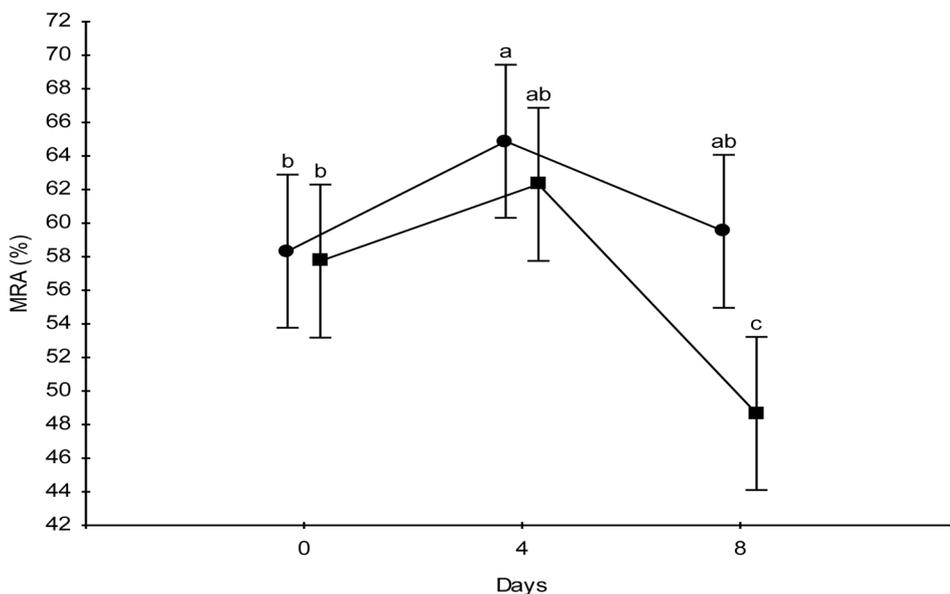
## Discussion

### Surface color stability

The results for  $L^*$  in the present study indicate that the IS muscle is lighter in appearance compared to the LTL and BF counterparts (Fig. 1). The BF muscles from males were lighter in comparison to their LTL counter-



**Figure 12.** The metmyoglobin reducing activity (MRA) of 3 fallow deer muscles, *infraspinatus* (IS), *longissimus thoracis et lumborum* (LTL), and *biceps femoris* (BF), from male and female carcasses, stored at 2°C. <sup>a-d</sup>Means without common letters are different ( $P < 0.05$ ).

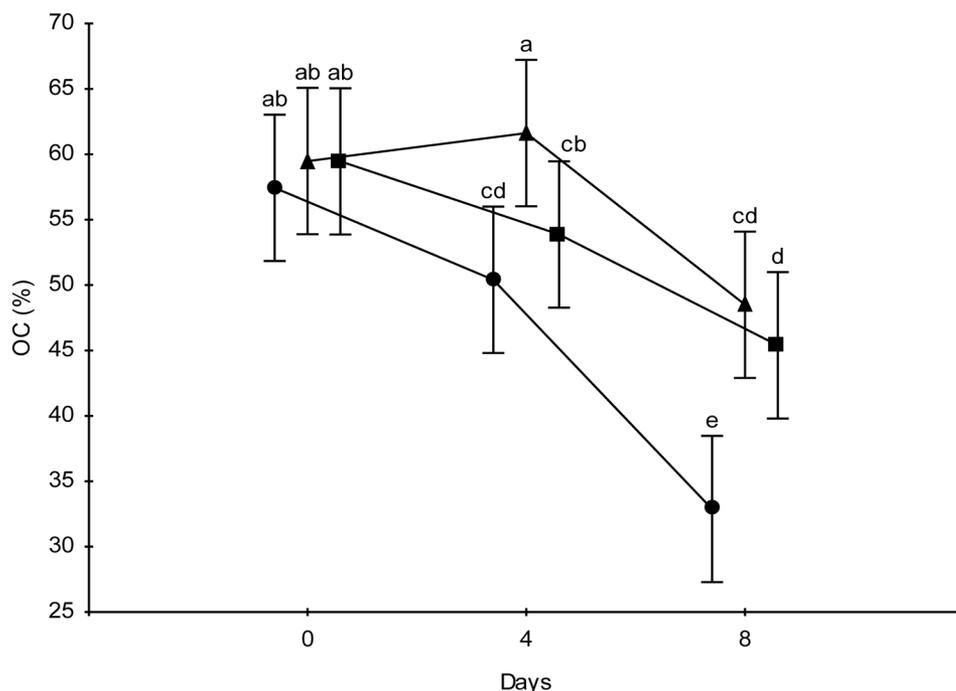


**Figure 13.** The changes in metmyoglobin reducing activity (MRA) of muscles from (●) male and (■) female fallow deer. <sup>a-c</sup>Means without common letters are different ( $P < 0.05$ ).

parts, whereas the LTL and BF from females had similar values. Interestingly, the IS and LTL did not differ in total Mb concentration, whereas the BF had the greatest Mb concentration (Table 2). In addition, no gender differences were observed for total Mb concentration in the present study (Table 1). Therefore, the theorized relationships between Mb concentration and  $L^*$  values (Vestergaard et al., 2000; Díaz et al., 2002; Kritzing et al., 2003; Daszkiewicz et al., 2011) as well as the propensity to dis-

color rapidly (Farouk et al., 2007; Purchas et al., 2010) were not readily observable in fallow deer muscles.

Contrary to the general suggestion that the meat from male animals is darker than that of females, whenever a difference was observed in  $L^*$  values, the muscles from male fallow deer were lighter than their counterparts from female animals. In contrast, no gender effect was reported for the  $L^*$  values of the blesbok muscles (Neethling et al., 2016).



**Figure 14.** The changes in oxygen consumption (OC) of 3 fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum*, and (■) *biceps femoris*, stored at 2°C. a-e Means without common letters are different ( $P < 0.05$ ).

**Table 2.** Total iron and myoglobin contents of *infraspinatus* (IS), *longissimus thoracis et lumborum* (LTL), and *biceps femoris* (BF) muscles from fallow deer on d 0 of refrigerated storage

Attribute	IS	LTL	BF	SEM
Total iron ( $\mu\text{g/g}$ )	35.60 <sup>b</sup>	41.40 <sup>a</sup>	41.59 <sup>a</sup>	1.427
Myoglobin ( $\text{mg/g}$ )	8.79 <sup>b</sup>	9.16 <sup>b</sup>	9.70 <sup>a</sup>	0.269

<sup>a,b</sup>Means in a row without common superscripts are different ( $P < 0.05$ ).

As observed in blesbok muscles (Neethling et al., 2016), strong correlations were noted in the fallow deer muscles between %OMb and  $a^*$  ( $r = 0.82$ ;  $P < 0.05$ ), chroma ( $r = 0.75$ ;  $P < 0.05$ ), and R (630/580) ( $r = 0.85$ ;  $P < 0.05$ ) values. In addition, strong correlations existed between  $a^*$  and chroma ( $r = 0.97$ ;  $P < 0.05$ ), and R (630/580) ( $r = 0.92$ ;  $P < 0.05$ ), as well as chroma and R (630/580) ( $r = 0.83$ ;  $P < 0.05$ ). These strong correlations supported the relationships between the aforementioned attributes and their robustness in measuring surface redness/color stability. Similarly, strong correlations were documented between %MMb and hue ( $r = 0.83$ ;  $P < 0.05$ ) substantiating the relationship between these attributes and their usefulness in measuring surface discoloration (browning) of meat. Similarly, strong correlations between %MMb and hue were also reported in the blesbok muscles (Neethling et al., 2016).

The results for the surface color attributes and surface Mb redox forms indicated that the IS was the most color

stable among the 3 fallow deer muscles. It maintained the greatest  $a^*$ , chroma, R (630/580), and %OMb values, and had the lowest hue and %MMb values. Similarly, Neethling et al. (2016) reported that the IS was also the most color stable among the 3 muscles (LTL, IS, and BF) from blesbok. Overall, the results of the present study also suggested that the LTL and BF have similar color stabilities. In contrast, distinct differences in color stabilities exist between these 3 muscles (LTL, IS, and BF) from beef; the beef IS, BF and LTL were categorized as muscles with “very low”, “low”, and “high” color stabilities, respectively (McKenna et al., 2005). This difference between beef and game may be attributed to the species-specific variations in fresh meat color (Mancini and Hunt, 2005; Neethling et al., 2017).

Overall, the chroma followed a trend similar to that of the  $a^*$  values (Fig. 4) and indicated that regardless of gender, the IS was the most color stable fallow deer muscle, whereas the LTL and BF had similar color stabilities. The data on hue value further supported the results of  $a^*$  and chroma, with the IS being the most color stable, while the LTL and BF have similar color stabilities (Fig. 5). The hue values for fallow deer IS exhibited a trend similar to that was previously reported in the IS from blesbok (Neethling et al., 2016).

The R (630/580) results also indicate that the IS muscle is the most color stable and that the LTL and BF are the least color stable (Fig. 6). Neethling et al. (2016) reported similar trend for the R (630/580) in the bles-

bok muscles. Based on the R (630/580) cut-off value 3 (Purchas et al., 2010), none of the fallow deer muscles remained acceptable after d 1. Similarly, the cut-off value of 3 for R (630/580) did not reflect the color stability of the blesbok muscles (Neethling et al., 2016), wherein a cut-off of 2 better reflected the color stabilities. This appears to be the case for the fallow deer muscles in the present study, in which a cut-off of 2 indicated that the IS remained acceptable in color throughout the storage, whereas the LTL and BF were unacceptable after d 2.

The %OMb results indicated that the IS muscle was the most color stable (Fig. 9A). The %MMb results also indicated that the IS muscle is the most color stable and that the BF is less color stable than the LTL (Fig. 10A). Overall, the %OMb and %MMb results reflected the trend for the  $a^*$  value and hue angle.

### **Biochemical attributes influencing surface color stability**

The Mb oxidation is delayed at high pH, whereas it is accelerated at low pH (Gotoh and Shikama, 1974; Ledward, 1985; Gutzke and Trout, 2002). Thus, muscles with high pH will be more color stable than those with low pH. In the present study, the greater color stability of the IS than the LTL and BF is explained partially by its greater pH. However, the LTL, which had a color stability similar to that of the BF, had a lower pH than the BF. This indicates that factors other than pH also play a role in the color stability of fallow deer muscles. Contrary to these results, Neethling et al. (2016) reported a strong correlation between pH and %OMb in the blesbok muscles, with the more color stable muscles also having correspondingly higher pH and vice versa (Neethling et al., 2016). This inconsistency indicates that the effect of pH on the color stability of muscles in game animals is species-specific. While some researchers have noted that higher pH values decrease the oxidation of Mb (Brown and Mebine, 1969; O'Grady et al., 2001), others have noted no link between pH and rate of discoloration (Hood, 1980; McKenna et al., 2005).

The MRA refers to the enzymatic and non-enzymatic systems in the post-mortem muscles, which possess the ability to reduce MMb to DMb (Bekhit and Faustman, 2005). The DMb can subsequently be converted to Omb. Thus, greater MRA often leads to an increased color stability. The results (Fig. 12) suggested that MRA did play a role in the color stability of the fallow deer muscles. Furthermore, the color stability differences noted for the muscles may also be explained by the greater overall MRA in the IS compared to the LTL and BF (Fig. 12).

The OC of meat refers to the residual mitochondrial activity post-mortem. The mitochondria compete with Mb for oxygen, thus leading to a decrease in the amount of oxygen available to bind to the Mb. Consequently, less Omb is formed and conditions ideal for MMb formation are created, ultimately resulting in a decline in the color stability (Bendall and Taylor, 1972; O'Keefe and Hood, 1982; Ledward, 1985). The results of the present study do not wholly support the notion that high OC leads to a decline in color stability (Fig. 14). Additionally, Neethling et al. (2016) did not find a definitive relationship between OC and color stability in blesbok muscles. Conversely, McKenna et al. (2005) observed that beef BF had higher OC in comparison to LTL and IS, with the LTL having the lowest OC.

Endogenous prooxidants in the muscle, such as iron, can also decrease the color stability of the muscle. The iron serves as a catalyst for lipid oxidation, which consequently destabilizes color (Faustman et al., 2010). Since both heme and non-heme iron have been associated with accelerating lipid oxidation (Igene et al., 1979; Chen et al., 1984), the total iron concentration of meat should also be indicative of muscle color stability. While IS, the most color stable fallow deer muscle, exhibited the lowest total iron content, LTL and BF had similar total iron contents and demonstrated similar color stability.

Greater Mb concentrations are often associated with the accelerated discoloration in beef (Jeong et al., 2009; King et al., 2011). However, the results from the present study did not agree with this association observed in beef; while fallow deer IS and LTL demonstrated lower Mb content than BF, IS was more color stable than LTL and BF. In agreement, the Mb concentration was not found to be indicative of color stability in blesbok muscles as well (Neethling et al., 2016). Similarly, other studies have also reported that the relationship between Mb concentration and color stability in beef muscles is inconclusive (Sammel et al., 2002; McKenna et al., 2005; Canto et al., 2015).

### **Conclusions**

The findings on  $a^*$  value, chroma, R (630/580), surface Mb redox forms, MRA, and pH, indicated that IS muscle from fallow deer is the most color stable among the 3 muscles (IS, LTL, and BF) investigated in this study. In addition, LTL and BF from fallow deer exhibited similar color stability and discolored more rapidly than the IS. The game industry may employ muscle-specific strategies to retail and improve marketability of fresh meat from fallow deer.

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