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# Influence of dietary vitamin $D_3$ supplementation on the color changes in *longissimus lumborum, vastus lateralis, semitendinosus,* and *semimembranosus* beef muscles

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**Abstract:** The aim of this study was to determine the effect of dietary vitamin $D_3$ supplementation on changes in color and heme pigment (myoglobin and metmyoglobin) in 4 beef muscles. The muscles came from 48 crossbred bulls that were subjected to supplementation with vitamin $D_3$ at 4 different doses: 0.0 (control group), 3.5, 7.0, and $10.0 \times 10^6$ IU. The $3.5 \times 10^6$ IU dose was administered daily for 6 days, before the morning feeding. Four days before slaughter, the supplementation was withdrawn. The dose of 7.0 and $10.0 \times 10^6$ IU was administered for 3 days and withdrawn 7 days before slaughter. The data show that supplementation with vitamin $D_3$ had a negative effect on beef meat color ($P < 0.05$). All analyzed muscles with vitamin $D_3$ had lightness ($L^*$) and yellowness ($b^*$) values higher than those of the control group ($P < 0.05$). Furthermore, redness ($a^*$) decreased and metmyoglobin increased as a function of vitamin $D_3$ dose. These negative effects on meat color occurred with 7.0 and $10.0 \times 10^6$ IU doses. The muscle microstructure was imaged and it showed the effects of higher $Ca^{2+}$ concentrations on the deterioration of meat color.

**Key words:** Beef, color, vitamin $D_3$, transmission electron microscope, color components, heme pigments content

## 1. Introduction

The color of fresh meat is one of the major quality traits affecting beef acceptability at the point of purchase (1–3). In the case of red meat such as beef, a bright red or cherry color is associated with freshness and naturalness. Therefore, even small negative changes in color can cause financial losses in the meat industry. In the United States, annual losses due to meat discoloration reach over 1 billion dollars (4,5).

The color of meat depends on the concentration and chemical form of the base heme pigment (the hemoglobin molecule without protein portion), which is a myoglobin. In fresh meat, there are 3 redox types of myoglobin: deoxymyoglobin (DMb), oxymyoglobin (OMb), and metmyoglobin (MetMb). The form of the pigment depends on the presence of a ligand that is connected to the heme iron atom and its valence electrons. DMb results in a purple-red color. In the presence of oxygen, it becomes OMb (a light pink-red pigment). Another conversion is transformation to the MetMb form, which is characterized by a brown color. This form of the heme pigment is the most unacceptable form in raw meat (6).

Factors influencing meat color are aging time, bloom development, kind of analyzed muscle, and even the breed and sex of the animal. Furthermore, packaging influences beef, including modified atmosphere packaging. This promotes blooming and improves the surface redness. Vacuum packaging is the worst at preserving red color. Preharvest strategies can also be used. This approach uses a feeding system (grass/forage, pasture, and feedlot/grain finishing), feed composition, and vitamins and mineral supplementation to modulate color. The main vitamins used to improve fresh beef color are vitamin E (an antioxidant used to prevent lipid oxidation), vitamin A (restricted to finishing diets), vitamin C, and vitamin $D_3$ (5,7–9).

Vitamin $D_3$ is widely used to improved beef tenderness, but there is no information about its effect on color. Montgomery et al. (10,11) showed that feeding with very high concentrations of vitamin $D_3$ ($7.5 \times 10^6$ IU) daily for 9 days before slaughter resulted in an increase in calcium ion concentration in the plasma and muscles of 18-month-old cattle. Wiegand et al. (12) claimed that increases in calcium ions due to vitamin $D_3$ supplementation positively influenced the values of $L^*$ and $a^*$. In turn, Wilborn et al. (13) showed that vitamin $D_3$ added to pig feed reduced the $L^*$ value, but subjective color scores increased. Furthermore, Hansen et al. (14) and Lahucky et al. (15)

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claimed that improving the color of meat via vitamin $D_3$ is associated with increasing the value of bivalent calcium ions. Thus, $Ca^{2+}$ may prevent lipid oxidation through interaction with superoxide radicals.

To date, conducted studies have shown that vitamin $D_3$ has an effect on the color of meat from pigs, but there is no information about the influence of this vitamin on beef meat color. There are many articles about the effect of vitamin $D_3$ on beef meat texture, especially tenderness. Therefore, the aim of this study was to investigate the effect of dietary vitamin $D_3$ supplementation on color changes in beef muscles.

## 2. Materials and methods

### 2.1. Animals, diet, and feeding

The experimental material used in this study consisted of muscles from 48 bull carcasses (Holstein-Friesian × Limousine) from commercial crossbreeding. The paternity breed bull of all experimental bulls was Benzene. The carcasses were classified according to EUROP grading, as confirmation class R, and the level of fat deposition as two. All animals were 18 months old and weighed about 610.0 ± 24.5 kg.

The experiment was performed in February of 2014 and was divided into two parts: local and laboratory. The first (local) part lasted 10 days and included isolation of the experimental bulls from the rest of herd and modulation of their feeding until slaughter (feed and water were always available for all bulls throughout the experiment). The isolated bulls were divided into 4 groups. These varied in terms of feed with appropriate doses of vitamin $D_3$ (Table 1).

The composition of the feed is presented in Table 2. Vitamin $D_3$ was in the form of dry powder stabilized via DL-α-tocopherol (Merck Millipore). Previously weighed doses of vitamin $D_3$ were dissolved in a small amount of water and mixed with 0.5 kg of ground oats. Each animal was fed this mixture daily for the defined time (Table 1) before morning feeding. Animals were slaughtered after 10 days of finishing in a local slaughterhouse in Mazovia.

**Table 2.** Composition of finishing diets in present study.

| Ingredient | Dry matter basis [%] |
|---|---|
| Ground oats | 70.0 |
| Rye silage | 20.0 |
| Beet decoction | 7.0 |
| Fodder chalk | 2.5 |
| Primasan® | 0.5 |
| Vitamin $D_3$ | 0.00035–0.001 |

### 2.2. Sample collection

The *longissimus lumborum*, *semitendinosus*, *semimembranosus*, and *vastus lateralis* muscles were collected from each animal. All samples were vacuum-packed with a vacuum-packaging machine (EDESA, model VAC-10DT, Spain) in polyethylene bags with refrigerator aging for 10 days.

### 2.3. Determination of pH value

The pH value was measured by inserting a pH glass electrode into each sample, compensated with a temperature measurement probe. The temperature during measurements was 10 °C. All measurements were repeated three times. The final score was the arithmetic average of the 3 measurements. The measurement was performed using a pH meter (Testo, model Testo 205, Germany). The pH meter was calibrated using pH 4 and pH 7 buffers. The

**Table 1.** Supplementation dosing.

| Dosage parameters | Experimental group | | | |
|---|---|---|---|---|
| | I* | II* | III* | IV* |
| HF × LM[a] (n = 48) | | | | |
| Dose of vitamin $D_3$[b] | 0.0 | 3.5 | 7.0 | 10.0 |
| Time of supplementation[c] | 0 | 6 | 3 | 3 |
| Time of feeding without supplementation[c] | 10 | 4 | 7 | 7 |
| Total time of finishing[c] | 10 | 10 | 10 | 10 |

*The number of experimental groups where the first (I) is the control group.
[a]HF × LM: Holstein-Friesian × Limousine crossbreed bulls.
[b]Expressed in $10^6$ IU $day^{-1}$ $animal^{-1}$.
[c]Expressed in days.

pH measurement was the main criterion for distinguishing normal from DFD (dark, firm, and dry meat) beef meat.

**2.4. CIE L*a*b* determination**

Measurements of the color components L* (lightness), a* (redness), and b* (yellowness) were performed on the surface of each meat sample after 30 min of conditioning (blooming) at 8 °C. Measurements were made using a Minolta CR400 Chroma Meter (Minolta, Japan) with the following settings: light source $D_{65}$; observer 10°; aperture 8 mm. Before the measurement, the device was calibrated using a calibration plate. For each sample, the measurement was performed 10 times in various steak locations. These were then averaged. A Konica Minolta camera coupled with the software Spectra Magic was used to determine the values of a* and b*. It calculated the chroma (saturation index, C*) and hue angle (h°). The data were used to calculate the absolute color difference (ΔE) between meat with and without vitamin $D_3$. The ΔE value was calculated according to the following formula:

$$\triangle E = \sqrt{(L_1^* - L_1^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2} \ ,$$

where $\Delta E$ is the absolute color difference; $L_1^*$, $a_1^*$, $b_1^*$ are the means of the color components determined for meat without vitamin $D_3$; and $L_2^*$, $a_2^*$, $b_2^*$ are the means of color components determined for meat with vitamin $D_3$.

The obtained ΔE values were analyzed using the CIE standards. According to these criteria, ΔE values between 0 and 2 are indiscernible and those between 2 and 3.5 are discernible by an experienced observer. Values over 3.5 are very noticeable.

**2.5. Determination of heme pigments**

The heme pigments (myoglobin and metmyoglobin content) were measured according to the method described by Krzywicki (16). Absorbance was evaluated using a Shimadzu UV-1800 spectrophotometer (Shimadzu Co., Japan).

**2.6. Computer image analysis**

Computer image analysis was used to take a picture of beef muscle surfaces after 30 min of blooming at 8 °C. Beef steaks were placed on matte black background. Pictures were taken using a digital camera (QImaging MicroPublisher 5.0 RTV, QImaging, Canada) with a lens (Computar, Japan) and with resolution of 2560 × 1920. The digital camera was equipped with a polarizing filter. All pictures were taken under daily fluorescent light emitted by two OSRAM DULUX L lamps (36 W) with a color temperature of 5400 K and a color rendering index of $R_a \geq 90$ (Osram GmbH, Germany). The digital camera and both lamps were placed on a Kaiser tripod (Kaiser Fototechnik GmbH & Co. KG, Germany). The distance between the lens and the steak was 300 mm. Each image was taken and saved in TIFF format.

**2.7. Meat microstructure**

The digital images of the four selected muscle samples were taken by the Department of Botany, Faculty of Agriculture and Biology of Warsaw University of Life Sciences using a transmission electron microscope (TEM, Philips-Morgagni). Samples were previously prepared with a Leica UCT ultramicrotome (sections of 90 nm in thickness). The preparations were observed with an FEI M268D Morgagni TEM with an SIS Morada digital camera at 14,000× magnification and SISiTEM software.

**2.8. Statistical analysis**

Statistical analysis was conducted using Statistica 10.0 (StatSoft, USA). To verify the normality of the distribution, a Shapiro–Wilk test was conducted. One-way analysis of variance (ANOVA) and a post hoc test (NIR-Fisher test) were performed to determine the effect of vitamin $D_3$ on changes in color, pH value, and heme pigment in the muscles ($P < 0.05$). Analysis of correlation coefficients was performed using Pearson's correlation for $P \leq 0.05$.

**3. Results**

The effects of vitamin $D_3$ supplementation on color parameters are presented in the form of correlation coefficients in Table 3. Based on these results, vitamin $D_3$ affected metmyoglobin content in the analyzed muscles and their discoloration. This was shown by the high correlation coefficients ranging from 0.7135 for *longissimus lumborum* muscle (P = 0.009) to 0.9522 (P = 0.000) for the *semitendinosus* muscle. A similar relationship was obtained in the case of ΔE: increasing the dose of vitamin $D_3$ increased the degree of discoloration. The correlation coefficients in this case ranged from 0.5975 (P = 0.040) for the *semimembranosus* muscle to 0.8332 (P = 0.001) for the *vastus lateralis* muscle.

The color component values that changed under dietary vitamin $D_3$ supplementation are depicted in Table 4. The results presented in Table 4 show that increasing the vitamin $D_3$ dose increased L* (lightness). Versus the control group, a dose of $3.5 \times 10^6$ IU resulted in a slight increase in lightness (L*) for all analyzed muscles. However, the highest brightness characterized muscle *semitendinosus* at $7.0 \times 10^6$ IU (45.92). The values of lightness (L*) obtained for the muscles *longissimus lumborum* and *vastus lateralis* at $7.0 \times 10^6$ IU were 38.79 and 38.72, respectively. Those results were the lowest lightness values of all analyzed muscles. The values of parameter L* obtained for *longissimus lumborum*, *vastus lateralis*, and *semitendinosus* at 7.0 and $10.0 \times 10^6$ IU were different ($P < 0.05$). Moreover, for all L* values, a dose of $10.0 \times 10^6$ IU gave lower values than those achieved at $7.0 \times 10^6$ IU. The differences in L* values between the mentioned doses of vitamin $D_3$ ranged from 0.55 to 2.60.

**Table 3.** Analysis of correlation between dose of vitamin $D_3$ (cumulative analysis for all doses) and color traits of four selected beef muscles.

| Color traits | Correlation between dose of vitamin $D_3$ and color characteristics of beef | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *longissimus lumborum* | | *semitendinosus* | | *semimembranosus* | | *vastus lateralis* | |
| | P-value | R coefficient | P-value | R coefficient | P-value | R coefficient | P-value | R coefficient |
| C* | 0.192 | –0.4047 | 0.306 | –0.3227 | 0.393 | –0.2715 | 0.254 | –0.3575 |
| h° | 0.510 | 0.2109 | 0.603 | –0.1675 | 0.096 | 0.5029 | 0.273 | –0.3443 |
| Mb | 0.139 | –0.4529 | 0.130 | –0.4627 | 0.000* | 0.8559 | 0.000* | –0.8582 |
| MetMb | 0.009* | 0.7135 | 0.000* | 0.9522 | 0.010* | –0.7053 | 0.001* | 0.8073 |
| pH | 0.145 | 0.4467 | 0.534 | 0.1995 | 0.573 | –0.1813 | 0.724 | –0.1141 |
| L* | 0.978 | –0.0089 | 0.169 | 0.4244 | 0.973 | 0.0257 | 0.032* | –0.6182 |
| a* | 0.225 | –0.3782 | 0.267 | –0.3487 | 0.211 | –0.3890 | 0.116 | –0.4779 |
| b* | 0.204 | –0.3947 | 0.869 | 0.0535 | 0.425 | –0.2544 | 0.036* | –0.6089 |
| ΔE | 0.023* | 0.6465 | 0.003* | 0.7736 | 0.040* | 0.5975 | 0.001* | 0.8332 |

*P ≤ 0.05 for Pearson's correlation; C* - saturation index (chroma); h° - hue angle; Mb - myoglobin; MetMb - metmyoglobin; L* - lightness; a* - redness; b* - yellowness; ΔE - absolute color difference.

Other parameters analyzed here include a* (redness) and b* (yellowness). Table 4 shows that vitamin $D_3$ in animal feed contributed to a decline in a* values. This was especially true at 7.0 and 10.0 × $10^6$ IU of vitamin $D_3$. This gave the smallest redness values. Moreover, low and statistically significant a* values were obtained for *longissimus lumborum* muscle at 7.0 × $10^6$ IU of vitamin $D_3$ (15.43). Statistically ($P < 0.05$), the highest a* values were obtained for *vastus lateralis* muscle at 10.0 × $10^6$ IU of vitamin $D_3$ (24.78). In *semitendinosus* and *semimembranosus* muscles, the differences between 7.0 and 10.0 × $10^6$ IU were minimal, despite the increased vitamin $D_3$ ($P < 0.05$). Only in *vastus lateralis* muscle were significant differences in the a* value between 0.0 and 3.5 × $10^6$ IU of vitamin $D_3$ obtained ($P < 0.05$). In *longissimus lumborum*, *semimembranosus*, and *semitendinosus* increasing the dose of vitamin $D_3$ increased the value of b*. For *vastus lateralis*, increasing the vitamin $D_3$ dose did not increase the b* value. The highest values of b* were obtained at 7.0 or 10.0 × $10^6$ IU of vitamin $D_3$. However, only *vastus lateralis* and *longissimus lumborum* showed differences between those two doses that were significant ($P < 0.05$). The highest b* value was in *semimembranosus* at 7.0 × $10^6$ IU of vitamin $D_3$ (13.52). The lowest was in *vastus lateralis* at 10.0 × $10^6$ IU of vitamin $D_3$ (10.12).

The highest values of hue angle (h°) and declining chroma (C*) values were obtained for all muscles at 7.0 × $10^6$ IU of vitamin $D_3$. In addition, the absolute discoloration (ΔE) of all analyzed muscles was higher than 3.5. This means that the changes in color were very noticeable (Table 4; Figure 1).

The effects of vitamin $D_3$ doses on pH, myoglobin, and metmyoglobin values are given in Table 5. It was concluded that the addition of vitamin $D_3$ decreased the amount of myoglobin and simultaneously increased the amount of metmyoglobin. The lowest content of myoglobin was obtained from 7.0 × $10^6$ IU of vitamin $D_3$ (from 1.14% in the *semitendinosus* muscle to 2.16% for the *vastus lateralis* muscle). For most of the analyzed muscles, a dose of 7.0 × $10^6$ IU of vitamin D caused a statistically significant ($P < 0.05$) increase in the amount of metmyoglobin (from 32.17% for *vastus lateralis* to 38.23% for *longissimus lumborum*). High doses of vitamin $D_3$ significantly decreased the metmyoglobin content. In the case of *semitendinosus* muscle, increasing the dose of vitamin $D_3$ to 10.0 × $10^6$ IU progressively increased the metmyoglobin and the myoglobin contents.

It is clear that the muscles in the control group were characterized by poorly visible and small Z lines and I bands. Furthermore, the extension of Z lines and I bands proves that advanced progression of muscle proteolysis is due to increasing vitamin $D_3$ doses. For a dose of vitamin $D_3$ at 7.0 × $10^6$ IU, the Z line and I band were the largest for *longissimus lumborum* muscle (A) and *semimembranosus* (C). We observed far-reaching changes in proteolysis as seen by a displacement in the muscle fibers.

**Table 4.** The effect of dietary vitamin $D_3$ supplementation on the changes in color values for the four selected beef muscles.

| Color traits | Dose of vitamin $D_3$* | | | |
|---|---|---|---|---|
| | 0.0 | 3.5 | 7.0 | 10.0 |
| *m. longissimus lumborum* | | | | |
| L* | $35.20^{a} \pm 1.14$ | $37.30^{b} \pm 0.98$ | $38.79^{c} \pm 0.79$ | $35.07^{a} \pm 2.10$ |
| a* | $23.61^{b} \pm 1.83$ | $23.74^{b} \pm 2.42$ | $15.43^{a} \pm 1.89$ | $21.24^{b} \pm 1.83$ |
| b* | $12.64^{a} \pm 0.96$ | $12.79^{a} \pm 0.86$ | $13.18^{b} \pm 0.33$ | $12.44^{a} \pm 0.78$ |
| h° | $24.54^{a} \pm 1.46$ | $26.26^{ab} \pm 0.74$ | $29.49^{b} \pm 1.70$ | $24.94^{a} \pm 0.73$ |
| C* | $23.47^{a} \pm 2.47$ | $21.19^{a} \pm 2.59$ | $19.72^{a} \pm 1.23$ | $21.36^{a} \pm 1.93$ |
| ΔE | - | $4.16^{a} \pm 0.57$ | $6.48^{a} \pm 2.48$ | $4.19^{a} \pm 1.14$ |
| *m. semitendinosus* | | | | |
| L* | $41.96^{a} \pm 1.02$ | $42.07^{a} \pm 1.38$ | $45.92^{c} \pm 0.84$ | $43.32^{b} \pm 1.25$ |
| a* | $24.74^{ab} \pm 2.26$ | $25.15^{b} \pm 1.25$ | $23.46^{a} \pm 1.45$ | $24.60^{ab} \pm 1.91$ |
| b* | $9.84^{a} \pm 0.61$ | $10.49^{ab} \pm 1.09$ | $11.10^{b} \pm 1.11$ | $9.75^{a} \pm 0.93$ |
| h° | $28.19^{ab} \pm 0.94$ | $25.93^{a} \pm 1.29$ | $29.42^{b} \pm 3.03$ | $25.65^{a} \pm 0.37$ |
| C* | $28.25^{a} \pm 2.09$ | $27.17^{a} \pm 2.62$ | $26.19^{a} \pm 1.25$ | $26.92^{a} \pm 1.49$ |
| ΔE | - | $3.82^{a} \pm 0.69$ | $4.16^{a} \pm 0.82$ | $4.13^{a} \pm 0.24$ |
| *m. semimembranosus* | | | | |
| L* | $36.02^{a} \pm 0.69$ | $37.46^{b} \pm 1.28$ | $39.16^{c} \pm 1.12$ | $36.81^{ab} \pm 0.97$ |
| a* | $27.10^{c} \pm 0.86$ | $23.02^{a} \pm 1.47$ | $18.44^{a} \pm 2.16$ | $24.65^{b} \pm 1.22$ |
| b* | $12.35^{a} \pm 0.93$ | $11.86^{a} \pm 0.75$ | $13.52^{b} \pm 0.96$ | $11.85^{a} \pm 0.62$ |
| h° | $24.99^{a} \pm 0.89$ | $25.58^{a} \pm 0.79$ | $27.38^{b} \pm 1.36$ | $26.05^{ab} \pm 0.37$ |
| C* | $27.76^{a} \pm 2.26$ | $25.61^{a} \pm 1.79$ | $23.90^{a} \pm 2.54$ | $26.50^{a} \pm 0.85$ |
| ΔE | - | $4.41^{a} \pm 1.49$ | $5.82^{a} \pm 1.32$ | $3.65^{a} \pm 0.76$ |
| *m. vastus lateralis* | | | | |
| L* | $36.74^{b} \pm 1.87$ | $36.81^{b} \pm 0.45$ | $38.72^{c} \pm 0.78$ | $34.62^{a} \pm 1.23$ |
| a* | $28.05^{c} \pm 1.03$ | $24.78^{b} \pm 1.09$ | $21.98^{a} \pm 2.23$ | $25.12^{b} \pm 1.45$ |
| b* | $11.91^{b} \pm 1.02$ | $11.34^{b} \pm 0.95$ | $10.80^{a} \pm 1.07$ | $10.12^{a} \pm 0.48$ |
| h° | $25.59^{bc} \pm 0.73$ | $25.01^{ab} \pm 0.91$ | $26.65^{c} \pm 0.71$ | $23.57^{a} \pm 0.92$ |
| C* | $27.97^{a} \pm 2.09$ | $27.27^{a} \pm 0.74$ | $25.16^{a} \pm 1.31$ | $26.53^{a} \pm 0.98$ |
| ΔE | - | $3.69^{a} \pm 0.45$ | $3.95^{a} \pm 0.83$ | $4.56^{a} \pm 0.38$ |

Mean ± SD; $^{a...d}$ - mean values differ in rows on the basis of post hoc NIR-Fisher test criteria for $P < 0.05$; *doses of vitamin $D_3$ are expressed in $10^6$ IU $day^{-1}$ $animal^{-1}$; C* - saturation index (chroma); h° - hue angle; Mb - myoglobin; MetMb - metmyoglobin; L* - lightness; a* - redness; b* - yellowness; ΔE - absolute color difference.

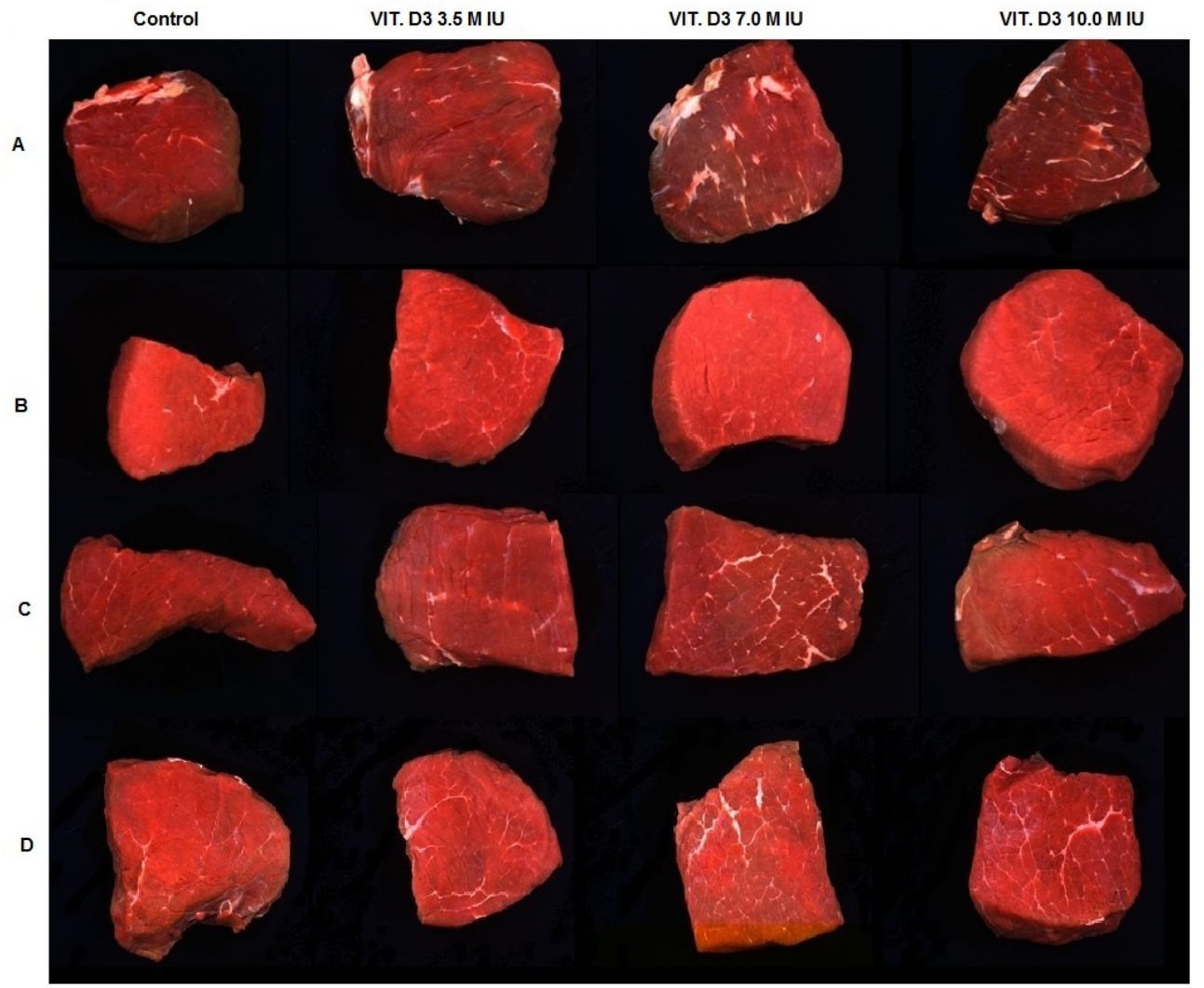


**Figure 1.** The effect of dietary vitamin $D_3$ supplementation with different doses (control ($0.0 \times 10^6$ IU), $3.5 \times 10^6$ IU, $7.0 \times 10^6$ IU, and $10.0 \times 10^6$ IU) on the changes in general appearance of four selected beef muscles (A – *longissimus lumborum*; B – *semitendinosus*; C – *semimembranosus*; D – *vastus lateralis*).

## 4. Discussion

McKenna et al. (17) showed that higher lightness was achieved in the *semitendinosus* muscle and lower L* values (lightness) in *longissimus* muscle. Moreover, the parameter L* played a large role in maintaining the color stability of beef muscles. Montgomery et al. (10,11) found no effect of vitamin $D_3$ supplementation on lightness. Enright et al. (18) observed no effect of vitamin $D_3$ on general improvement of beef meat color. On the other hand, when 500,000 IU $day^{-1}$ of vitamin $D_3$ was added to pig feed, there was a small decline in the L* value for *longissimus* muscle (12). These results were confirmed by Wilborn et al. (13) and demonstrated that adding vitamin $D_3$ to the pig feed in an amount from 40,000 to 80,000 IU $kg^{-1}$ $feed^{-1}$ reduced the overall improvement of meat lightness. In this experiment, increased lightness for the *longissimus lumborum* muscle agrees with the results obtained by Hope-Jones et al. (2), who compared the effects of supplementation with vitamin $D_3$ and zilpaterol on changes in color components. In their experiment, supplementation of vitamin $D_3$ at $7.0 \times 10^6$ IU for 6 days before slaughter improved the lightness in a statistically significant way ($P < 0.05$) for meat samples aged for 14 days under vacuum.

Results for a* (redness) and b* (yellowness) from animals untreated with vitamin $D_3$ correspond to those obtained by other authors for beef meat quality without defects (normal meat) (19,20). Values of a* achieved by Hope-Jones et al. (2) were considerably lower for a dose of $7.0 \times 10^6$ IU of vitamin $D_3$ administered 3 days before slaughter than our data collected under the same supplementation parameters

(vitamin $D_3$ dose and supplementation time). Obtained changes are probably due to shortening of the aging time and using a vacuum. In this study aging was done for 10 days, while Hope-Jones et al. (2) used 14 days of aging. Less red beef and main changes ($P < 0.05$) in the values of b* were also reported in other articles (2,5,14). Small changes in the values of parameter b* can be explained by very short blooming times (30 min), as in the case of beef full stabilization of this parameter requires about 80 min (2). The addition of vitamin $D_3$ to swine feed increases the a* parameter, which is responsible for improving the attractiveness of the meat color. Parameter b* remained unchanged despite the vitamin $D_3$, vitamin E, and bivalent ions ($Mg^{2+}$) (12,13,15,21,22).

Increasing the dose of vitamin $D_3$ reduced C* (chroma) and resulted in a small increase in h° (hue angle) (14). Decreases in the C* value resulted in a brown color (23). These negative changes in meat color (appearance of a brown color and darkening of meat) were confirmed both in the results collected in Tables 4 and 5 and Figure 1 and in the literature. Many authors have shown the existence of a linear relationship between the meat pH value and it lightness (L*) (3,6,24,25). However, the pH values obtained in this study do not indicate any meat quality defects (Table 5). The dark color of the analyzed muscles can indicate the presence of DFD defects. The obtained pH values were from 5.4 to 5.8, which are generally recognized as suitable for the pH of normal meat (23,26).

Factors affecting growth in meat MetMb content are storage temperature, pH, MetMb reductase activity, the partial pressure of oxygen, and the oxidation of lipids (27). The decline in the a* value correlates with an increase in meat MetMb content (23).

The pH values in these muscles do not suggest that they have a negative impact on the meat color, and therefore the cause of this phenomenon must be due to another feature. The features that affect the color changes are affected by pH and heme pigment as well as the structure of the analyzed muscles (28). Therefore, to explain the changes in meat color obtained from animals supplemented with vitamin $D_3$, images of microstructure were obtained with a TEM (Figure 2).

The microstructure changes were probably associated with increases in calcium ion concentration, which was a

**Table 5.** The effect of dietary vitamin $D_3$ supplementation on the changes in myoglobin (Mb) and metmyoglobin (MetMb) concentration as well as pH values in four beef muscles.

| Trait | Dose of vitamin $D_3$* | | | |
|---|---|---|---|---|
| | 0.0 | 3.5 | 7.0 | 10.0 |
| *m. longissimus lumborum* | | | | |
| Mb** | $7.56^{d} \pm 0.37$ | $2.77^{b} \pm 0.61$ | $1.58^{a} \pm 0.11$ | $4.87^{c} \pm 0.37$ |
| MetMb** | $13.15^{a} \pm 2.73$ | $22.85^{b} \pm 0.77$ | $38.23^{d} \pm 0.45$ | $26.71^{c} \pm 1.00$ |
| pH | $5.59^{ab} \pm 0.04$ | $5.56^{a} \pm 0.05$ | $5.59^{b} \pm 0.01$ | $5.60^{ab} \pm 0.02$ |
| *m. semitendinosus* | | | | |
| Mb** | $4.57^{d} \pm 0.14$ | $2.41^{b} \pm 0.21$ | $1.14^{a} \pm 0.19$ | $2.61^{c} \pm 0.20$ |
| MetMb** | $20.16^{a} \pm 1.41$ | $30.06^{b} \pm 0.86$ | $33.92^{b} \pm 1.91$ | $47.10^{c} \pm 1.02$ |
| pH | $5.64^{b} \pm 0.04$ | $5.44^{a} \pm 0.01$ | $5.43^{a} \pm 0.05$ | $5.66^{b} \pm 0.02$ |
| *m. semimembranosus* | | | | |
| Mb** | $1.23^{a} \pm 0.02$ | $2.03^{b} \pm 0.05$ | $1.91^{b} \pm 0.01$ | $4.27^{c} \pm 0.17$ |
| MetMb** | $24.93^{a} \pm 0.87$ | $34.46^{b} \pm 2.08$ | $37.50^{b} \pm 1.93$ | $24.89^{a} \pm 2.19$ |
| pH | $5.61^{b} \pm 0.03$ | $5.65^{a} \pm 0.03$ | $5.61^{a} \pm 0.02$ | $5.65^{a} \pm 0.04$ |
| *m. vastus lateralis* | | | | |
| Mb** | $5.25^{c} \pm 0.21$ | $2.34^{b} \pm 0.24$ | $2.16^{a} \pm 0.25$ | $2.55^{b} \pm 0.20$ |
| MetMb** | $17.05^{a} \pm 1.88$ | $21.88^{a} \pm 2.66$ | $32.17^{b} \pm 1.99$ | $28.72^{b} \pm 2.06$ |
| pH | $5.51^{ab} \pm 0.03$ | $5.57^{a} \pm 0.04$ | $5.59^{a} \pm 0.01$ | $5.54^{a} \pm 0.05$ |

Mean ± SD; $^{a...d}$ - mean values differ in rows on the basis of post hoc NIR-Fisher test criteria for $P < 0.05$; *doses of vitamin $D_3$ are expressed in $10^6$ IU $day^{-1}$ $animal^{-1}$; **myoglobin (Mb) and metmyoglobin (MetMb) contents are expressed in %.

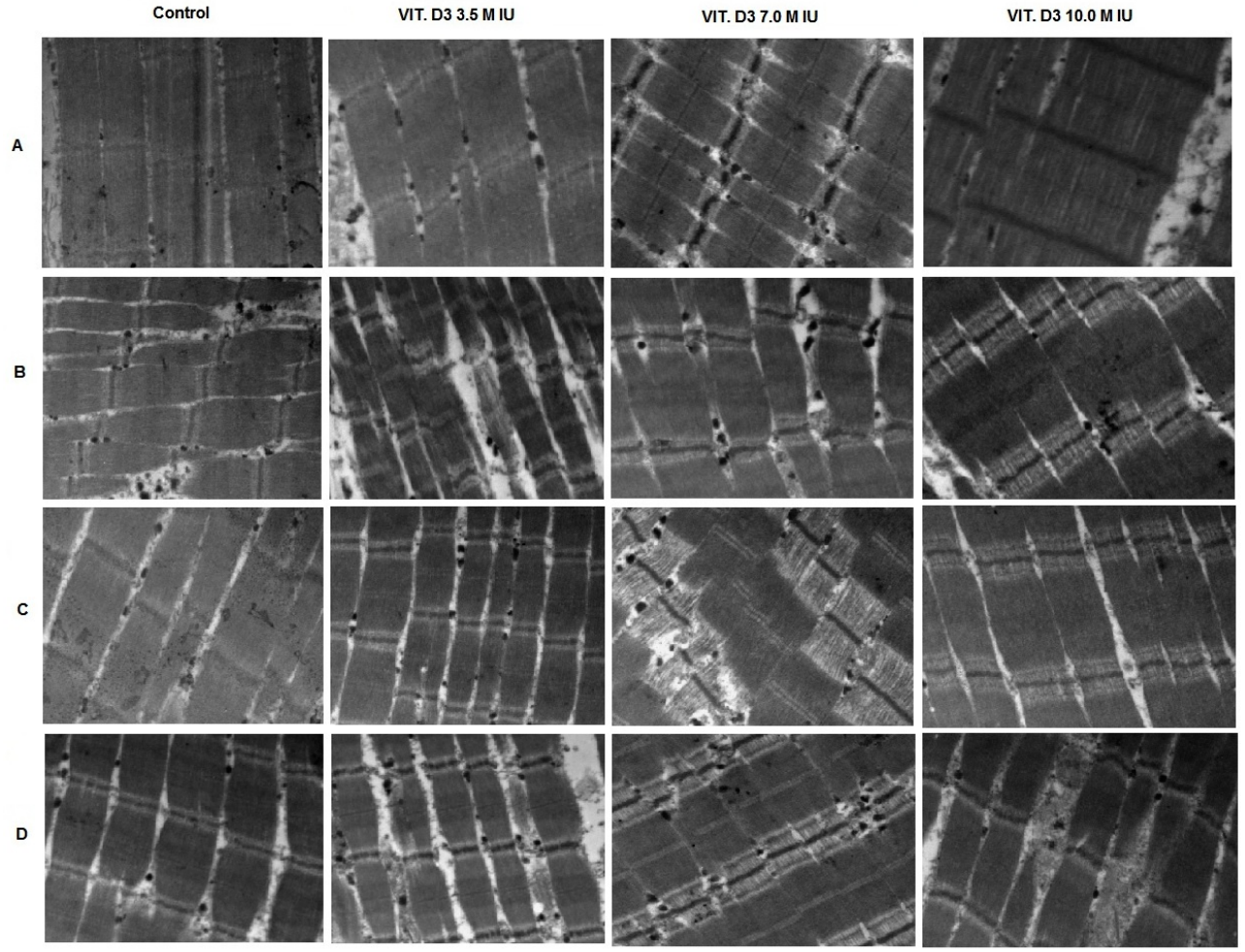


**Figure 2.** The effect of dietary vitamin $D_3$ supplementation with different doses (control ($0.0 \times 10^6$ IU), $3.5 \times 10^6$ IU, $7.0 \times 10^6$ IU, and $10.0 \times 10^6$ IU) on the microstructure changes in four selected beef muscles (A – *longissimus lumborum*; B – *semitendinosus*; C – *semimembranosus*; D – *vastus lateralis*); pictures were collected using a TEM at 14,000× magnification.

direct effect of vitamin $D_3$ action. Many researchers have described the effect of vitamin $D_3$ on the tenderness of beef meat and the effect of vitamin D on $Ca^{2+}$ concentration in muscles (10,11,29). According to Hansen et al. (14), the effect of vitamin $D_3$ at high concentrations of $Ca^{2+}$ can prevent oxidation of lipids through interactions with peroxide radicals.

Moreover, studies carried out on beef *longissimus* muscle marinated in a calcium salt solution showed that the brine increased $Ca^{2+}$ in the muscle and simultaneously darkened the meat. In addition, increases in meat calcium contributed to its more rapid discoloration (30).

In summary, the obtained results allow us to conclude that vitamin $D_3$ supplementation significantly affected the color of the selected muscles. This was confirmed not only by the obtained values of color parameters and the amount of heme pigments but also by captured images of the muscle microstructure. The results of this article show the effect of vitamin $D_3$ on calcium ion activation, in consequence enhancing the darker color of muscle.

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