TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2016) 40: 514-520

doi:10.3906/vet-1506-28

Influence of dietary vitamin D_3 supplementation on the sarcomere length, Warner–Bratzler shear force, shortening of ageing time, and sensory acceptance of culinary beef muscles

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Received: 08.06.2015 • **Accepted/Published Online:** 16.02.2016 • **Final Version:** 27.06.2016

Abstract: The aim of this study was to determine the effects of dietary vitamin D_3 supplementation on ageing time, tenderness, and consumer acceptance of 3 muscles: longissimus lumborum, semitendinosus, and semimembranosus from crossbreed bulls. Bulls (n = 48) were divided into 4 experimental groups (n = 12 each) that were administered ground oats supplemented with various amounts of vitamin D_3 (0.0, 3.5, 7.0, or 10.0×10^6 IU) at 1 day before slaughter. To determine the effect of vitamin D_3 addition, changes in tenderness were measured by sarcomere length and Warner–Bratzler shear force (WBSF). In addition, all of the muscles were examined organoleptically by a team of 20 panelists. The results show that the dose of vitamin D_3 significantly ($P < 0.01$) increased the sarcomere length and reduced the WBSF-value during ageing, as compared to meat samples from the control group (without vitamin D_3). The 7.0×10^6 IU dose was found to be the most effective ($P < 0.01$). Consumer research data confirmed the findings of the instrumental analyses.

Key words: Beef meat, tenderness, vitamin D_3, sarcomere length, Warner–Bratzler shear force (WBSF)

1. Introduction

Tenderness is an important quality feature of culinary beef and influences consumer acceptance (1–4). In consumer evaluations, tenderness is the result of the impression received both as an ease of meat shredding in the initial phase of biting, ease of shredding for the smaller parts during chewing, and as a feeling left by some of the meat pieces present after chewing. Tenderness is determined by many factors, which can be divided into intravital (breed, sex, age, diet) and postmortem (time and temperature of carcass conditioning) (5).

Meat texture is formed as a result of the ageing process, which constitutes endogenous changes in the meat microstructure after slaughter. The literature identifies 2 main trends of meat tenderization theory: enzymatic and nonenzymatic. Takahashi's calcium theory is an example of a nonenzymatic theory of tenderizing, associated with improving tenderness through increasing bivalent calcium ion concentrations, which cause weakening of myofibril structure (6). However, enzymatic theory is currently the most popular approach for creating tender meat. According to the enzymatic theory, the changes that occur during ageing are the results of the intracellular action of enzymes, called calpains. There are 2 main types of calpains (μ- and m-calpain), which are located in the region of the Z-line and in the I and A band of muscles. Calpains require Ca^{2+} ions to achieve their biological activity (7).

Vitamin D_3 has a direct effect on the body's regulation of the calcium balance. Vitamin D_3 is present in animals as a cholecalciferol (cholesterol derivative) and, after the hydroxylation processes, in the liver and kidneys in its biologically active forms: 25-hydroxyvitamin D_3 and 1α-25-dihydroxyvitamin D_3. Vitamin D_3 allows the transportation of calcium ions through the plasma membrane by allowing production of a double-hydrolyzed form of vitamin D, which affects the activation of enzymes responsible for the hydrolysis of inositol lipids. As a result of this process, the IP3 (1, 4, 5-triphosphate) and DAG (diacyloglicerol) compounds are formed, which bind to proteins present in the membrane of calcium channels, thus initiating their opening (3,8).

Previous studies on animals fed with vitamin D_3 have shown that increasing calcium ion concentrations in the blood plasma improve meat tenderness. Furthermore, the addition of vitamin D_3 to animals' feed reduced ageing time from 21 to 14 days, and in some cases to 7 days (1,2,4–6,8–12).

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Studies conducted previously, by other scientists, have found that the long-term supplementation of high doses of vitamin D_3 (for example more than 7.5×10^6 IU) has an adverse impact on the physical condition of animals. Therefore, the main object of the present report was to investigate whether using a reduced dose of vitamin D_3 has a positive effect on meat texture properties. Moreover, unusually for this kind of research, it was decided to see the influence of vitamin D_3 supplementation on consumer opinion.

2. Materials and methods

2.1. Animals, diet, and feeding

Bulls (n = 48) used in this study were crosses of Holstein–Friesian × Simmental deriving from commercial crossbreeding. The paternity breed bull was Eugen for all animals in the experiment. All bulls were 18 months old and weighed about 700.0 ± 21.0 kg. The experiment, divided into a local and a laboratory part, was performed in January 2014. The local part lasted 10 days on a homestead located in the Warmia and Mazury district. It included the isolation of the experimental bulls from the rest of herd and their feeding until slaughter. During the experiment, isolated bulls were divided into 4 groups of 12 animals each. For 10 days all groups were fed with fodder consisting of rye haylage, crushed oats, beet decoction, and a premix of ground grain and fodder chalk as a source of calcium ions, which was administered at a rate of 0.22 kg × day^{-1} × $animal^{-1}$. On the day before slaughter, once, before the morning feeding, the suitable groups were feed with vitamin D_3 in amounts of 0.0 (placebo dose), 3.5, 7.0, or 10.0×10^6 IU. The vitamin D used in the experiment was in the form of a dry powder stabilized with DL-α-tocopherol (Merck Millipore). Before administering, it was dissolved in a small amount of water and mixed with crushed oats. The next day, the bulls were transported to a slaughterhouse located in the Mazovia district for slaughter and the butchering of carcasses into individual culinary elements.

2.2. Sample collection

After slaughter the carcasses were classified as confirmation class R +, according to the EUROP grading, and the level of fat deposition was 2. The experimental material used in this study was muscle: semitendinosus (EYE075), semimembranosus (TOP073), and longissimus lumborum (STR045). Separation elements for muscles and then for the specific research samples were conducted at the Division of Engineering in Nutrition, Department of Human Nutrition on Consumer Sciences, Warsaw University of Life Sciences in Warsaw. The samples were vacuum-packed using a vacuum packaging machine (EDESA, model VAC-10DT, Spain) in polyethylene bags and aged in a refrigerator for 7, 14, and 21 days.

2.3. Measurement of Warner–Bratzler shear force

After ageing, all samples were cooked to an endpoint internal temperature of 71 °C using a waterbath (Memmert, model WNE14, Germany). From each prepared sample, 6 2.54 cm long cylinders were excised along the muscle fibers. The analysis was made using a Universal Testing Machine (Instron, model 5965, USA) with a measuring head speed of 20.0 mm × min^{-1} and a capacity of 500.0 N. Measurement of shear force was done using a cutting test by cutting each cylinder across the line of muscle fibers, using a Warner–Bratzler device (V-blade) setting. The Warner–Bratzler shear force (WBSF) [N] is the maximum value of the force achieved during the cutting of the sample.

2.4. Measurement of sarcomere length

The measurement of sarcomere length was made by laser diffraction, according to the modification of methodology described by Cross et al. (13) and Koolmarees et al. (14).

A helium-neon laser (Optel, model HNL-03, Poland) with constant laser wavelength value amounting to 632.8 nm was used in this analysis. Sarcomere length was calculated from the following formula:

$$L = \left(\lambda . \sqrt{1 + \frac{D^2}{T^2}} \right) / 100 ,$$

where L is the length of the sarcomeres [μm], λ is the laser wavelength [nm], D is the distance from the sample holder to the screen [mm] (constant value in the experiment and amounting to 100.0 mm), and T is the average of 25 readings of the distance between zero and the first diffraction band [mm].

2.5. Sensory analysis

After a suitable period of ageing, the examined muscles were sensory analyzed, which was conducted in accordance with the methodology of Meilgaard et al. (15) and the Polish Standards (16–18).

The assessment team consisted of 20 panelists who declared that they consumed beef at a minimum once per week. Analyzed descriptors were tenderness, juiciness, flavor, and overall acceptability. Evaluation of intensity of specified traits was performed using a method of scaling with a structured, unipolar, 10-point scale with assayed boundary. The heat treatment of samples was performed using a contact grill with cast iron plates (Silex, model S-Tronic 161 GR OV, Germany). The temperature of the upper and lower plate during the treatment was constant at between about 190 and 210 °C. The treatment was carried out for 5 min to obtain a temperature of 71 °C in the geometric center of the sample. All panelists received grilled and specially coded samples of about 30 g weight with a temperature, during serving, of about 60 °C in the geometric center for independent analysis. Samples from each combination of ageing period and vitamin D_3 dose

were assessed 6 times. The total number of examined samples was 120.

2.6. Statistical evaluation

Data were subjected to statistical analysis (Statistica 10.0) using analysis of variance (ANOVA) for a factorial ($P < 0.05$ and $P < 0.01$) and post-hoc analyses (NIR-Fisher test) to determine the interaction between the dose of vitamin D_3 and changes in sarcomere length, WBSF values, shortening of ageing time, and consumer acceptance of 4 different sensory descriptors. All results are summarized in Tables 1 and 2 in the form of mean values ($\bar{x}$) and standard deviations (± SD), indicating the significance of the interactions. The results of statistical analyses of consumer tests are presented in Figures 1 to 3, indicating the homogeneous groups.

3. Results and discussion

3.1. The effect of dietary vitamin D_3 supplementation on sarcomere length

By multifactor ANOVA ($P < 0.01$), it was found that the dose of vitamin D_3 and ageing time both affected sarcomere length (Table 1). After 7 days of ageing, it was observed that increasing doses of vitamin D_3 resulted in extended sarcomeres in all analyzed muscles. Compared with the control group, the smallest addition of vitamins (3.5×10^6 IU) resulted in sarcomere extension by approximately 0.2 μm in all muscles.

Increasing the addition of vitamin D_3 to 7.0×10^6 IU resulted in elongation of the sarcomeres by about 0.6 μm in STR045 and by approximately 0.1 μm in EYE075 and TOP073, as compared with the 3.5×10^6 IU dose ($P < 0.01$). Further increasing the dose of vitamin D_3 to 10.0×10^6 IU resulted in a slight increase in sarcomere length compared to the 3.5×10^6 IU dose, although this did not reach statistical significance. After 7 days of ageing, the longest sarcomeres were found in the striploin (STR045) and the shortest from eye round (EYE075) meats. A similar trend was obtained after 14 and 21 ageing days. However, there are no statistically significant differences ($P < 0.01$) between the sarcomere lengths obtained after 14 and 21 days of ageing. This finding suggests that extension of the ageing period to 21 days does not provide substantial improvement in tenderness compared to 14 days ageing.

For the 3 analyzed muscles, the best dose of vitamin D_3 for improving tenderness was 7.0×10^6 IU, which simultaneously induced the shortening of ageing time from 21 to 14 days. Unfortunately, there are as yet no available data on the influence of vitamin D_3 on sarcomere length. Most researchers associate tenderness improvement with activation of proteolytic enzymes by increasing the concentration of calcium ions. Myofibrillar protein breakdown, which determines the tenderness of meat by increasing the sarcomere length can be detectable by SDS-PAGE electrophoresis. Using this method, Swanek et al.

Table 1. The effect of dietary vitamin D_3 supplementation on sarcomere length [μm] in beef muscles during ageing (n = 48).

Ageing time [days]	Dose of vitamin D_3 [10^6 IU]			
	0	3.5	7	10
STR045 (m. longissimus lumborum)				
7	1.72 ± 0.03 aA	1.91 ± 0.04 aB	2.48 ± 0.11 aC*	2.55 ± 0.05 a*C*
14	1.78 ± 0.01 aA	2.50 ± 0.05 bB*	2.69 ± 0.02 bC*	2.71 ± 0.01 a*C
21	2.27 ± 0.03 bA	2.55 ± 0.005 bB*	2.70 ± 0.005 bB*	2.83 ± 0.03 a*B*
EYE075 (m. semitendinosus)				
7	1.44 ± 0.06 aA	1.63 ± 0.01 aB	1.74 ± 0.05 aC	1.79 ± 0.06 aC
14	1.57 ± 0.02 bA*	1.66 ± 0.03 aA*	1.85 ± 0.002 bB	1.89 ± 0.04 aB
21	1.60 ± 0.01 bA	1.71 ± 0.06 abAB	1.94 ± 0.04 aB*	1.87 ± 0.07 bB*
TOP073 (m. semimembranosus)				
7	1.66 ± 0.04 a*A	1.86 ± 0.02 aB	1.95 ± 0.005 aC	1.98 ± 0.05 aC
14	1.78 ± 0.01 ab*A	1.95 ± 0.01 ab*A	2.18 ± 0.02 bB	2.49 ± 0.02 cC
21	1.94 ± 0.002 bA	2.08 ± 0.05 bB	2.51 ± 0.01 cC	2.30 ± 0.02 bB

($\overline{X}$ ± SD); a…c are mean values, analyzed vertically, denoted by different superscripts differ statistically at $P < 0.01$/* at $P < 0.05$; A…C are mean values, analyzed horizontally, denoted by different superscripts differ significantly at $P < 0.01$/* at $P < 0.05$.

Table 2. The effect of dietary vitamin D_3 supplementation on Warner–Bratzler shear force (WBSF) [N] of beef muscles during ageing (n = 48).

Ageing time [days]	Dose of vitamin D_3 [10^6 IU]			
	0	3.5	7	10
STR045 (m. longissimus lumborum)				
7	35.51 ± 2.86 bC	25.42 ± 3.18 bB	20.55 ± 6.52 bA	17.63 ± 8.36 aA
14	32.92 ± 2.72 bB	19.35 ± 2.95 aB	17.43 ± 8.67 aA	17.42 ± 2.89 aA
21	20.28 ± 3.26 aB*	19.05 ± 1.95 aAB	18.03 ± 3.67 aAB	16.94 ± 3.88 aA*
EYE075 (m. semitendinosus)				
7	38.60 ± 2.53 b*B	34.58 ± 1.39 aA	34.59 ± 1.38 b*A	32.43 ± 3.06 aA
14	36.98 ± 6.04 abC	34.14 ± 8.90 aBC	27.68 ± 2.87 abAB*	24.79 ± 3.81 aA
21	34.19 ± 4.93 a*B*	32.83 ± 3.57 aB	25.64 ± 3.82 a*A*	25.88 ± 1.87aA*
TOP073 (m. semimembranosus)				
7	35.78 ± 9.55 b*C	26.55 ± 1.64 bB	24.38 ± 3.60 b*A	21.15 ± 2.87 aA
14	31.55 ± 3.39 abC	20.98 ± 3.21 aB	20.33 ± 5.21 aA	20.32 ± 3.45 aA
21	23.15 ± 8.70 a*B*	23.63 ± 2.34 aB	18.83 ± 3.59 a*A*	21.07 ± 4.31 aA*

($\overline{X}$ ± SD); a…c are mean values, analyzed vertically, denoted by different superscripts differ statistically at $P < 0.01$/* at $P < 0.05$; A…C are mean values, analyzed horizontally, denoted by different superscripts differ significantly at $P < 0.01$, * at $P < 0.05$.

(19) observed an increase in the amount of myofibrillar protein degradation products with a weight of 30 kDa after 14 days of aging for a 7.5×10^6 IU dose of vitamin D_3. Furthermore, the Montgomery group have reported an increase in the amount of myofibrillar protein degradation products after 14 days of ageing at doses of 5.0 and 7.5×10^6 IU (5,10,12).

3.2. The effect of dietary vitamin D_3 supplementation on the Warner–Bratzler shear force

Vitamin D_3 significantly impacted shear force values during ageing ($P < 0.05$). Regardless of whether the analyzed meat contained vitamin D_3, ageing time reduced shear force (WBSF) (Table 2). The addition of vitamin D_3 caused a decrease in shear force during ageing in all analyzed muscle. This finding confirms the effects of vitamin D on regulation of calcium ion concentrations, which are actively involved in the activation of proteolytic enzymes.

The largest decrease in WBSF values were recorded for STR045 and TOP073 muscles. Furthermore, similar to sarcomere length, shear force values decreased at the 7.0 to 10.0×10^6 IU supplementation levels ($P < 0.01$) for all analyzed muscles, as compared to unsupplemented muscles. However, when considering the samples from each dose of vitamin separately, there was no statistically significant decrease in shear force between the 14 and 21 day-aged samples that had received a dose of 7.0×10^6 IU, for all muscles types. Therefore, extending the ageing time to 21 days does not improve the shear force values compared to 14 days ageing.

Swanek et al., analyzing the longissimus dorsi et thoracis muscle, have found that the optimum reduction of shear force value was achieved after 14 days of ageing. Although the lowest shear force-value was reached after 21 days of ageing, the difference in shear force between the 2 periods of ageing was not significant. In studies by Montgomery et al. for the same muscle, there was a reduction in tenderness obtained with a dose 7.5×10^6 IU after 14 days of ageing. Analogous results for the longissimus have been reported by Karges et al. (7). In addition, they proved that prolonged administration of vitamin D_3 (from 4 to 6 days before slaughter) caused a greater decrease in the shear force after 14 days of ageing for a dose of vitamin D_3 equal to 6.0×10^6 IU. In addition, Montgomery et al. (5) and Foote et al. (2) report similar results for the longissimus dorsi and semimembranosus muscles. In the former cited publication, 4 different doses of vitamin D_3 (0, 0.5, 1.0, and 5.0×10^6 IU × day^{-1} × $animal^{-1}$) were tested and it was demonstrated that the greatest decline in the value of shear force was reached after 14 days of ageing at a dose of 5.0

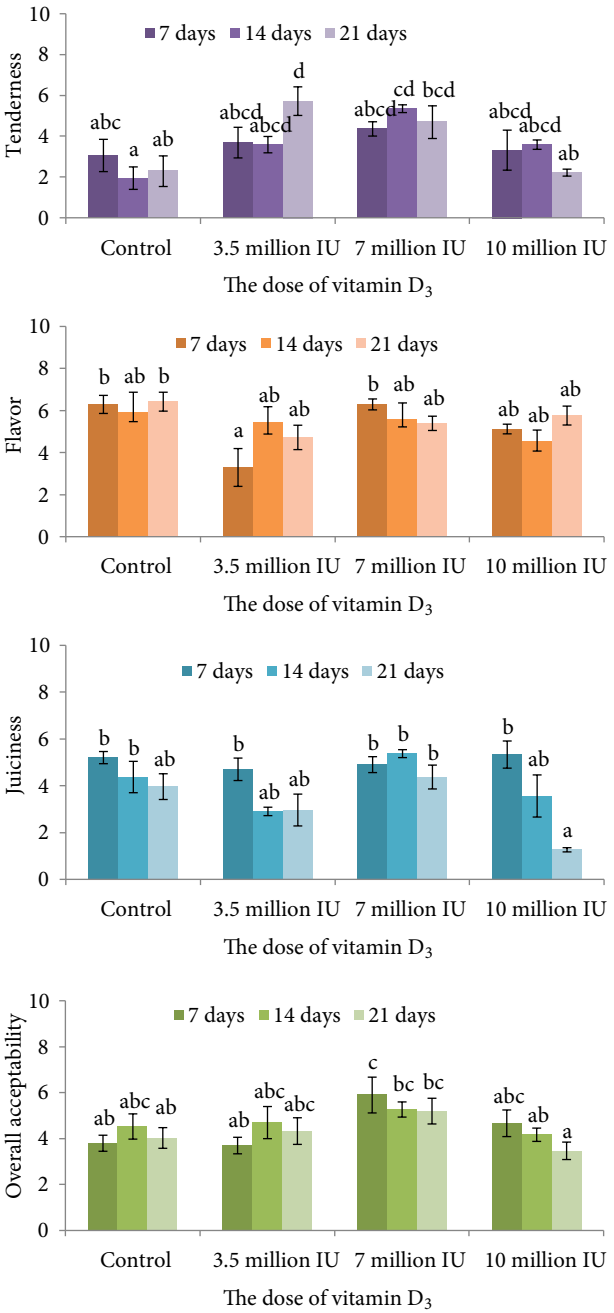

Figure 1. The effect of dietary vitamin D_3 supplementation on the sensory analysis of semitendinosus (EYE075) muscle during ageing (a…d bars denoted by different superscripts differ significantly at $P < 0.01$).

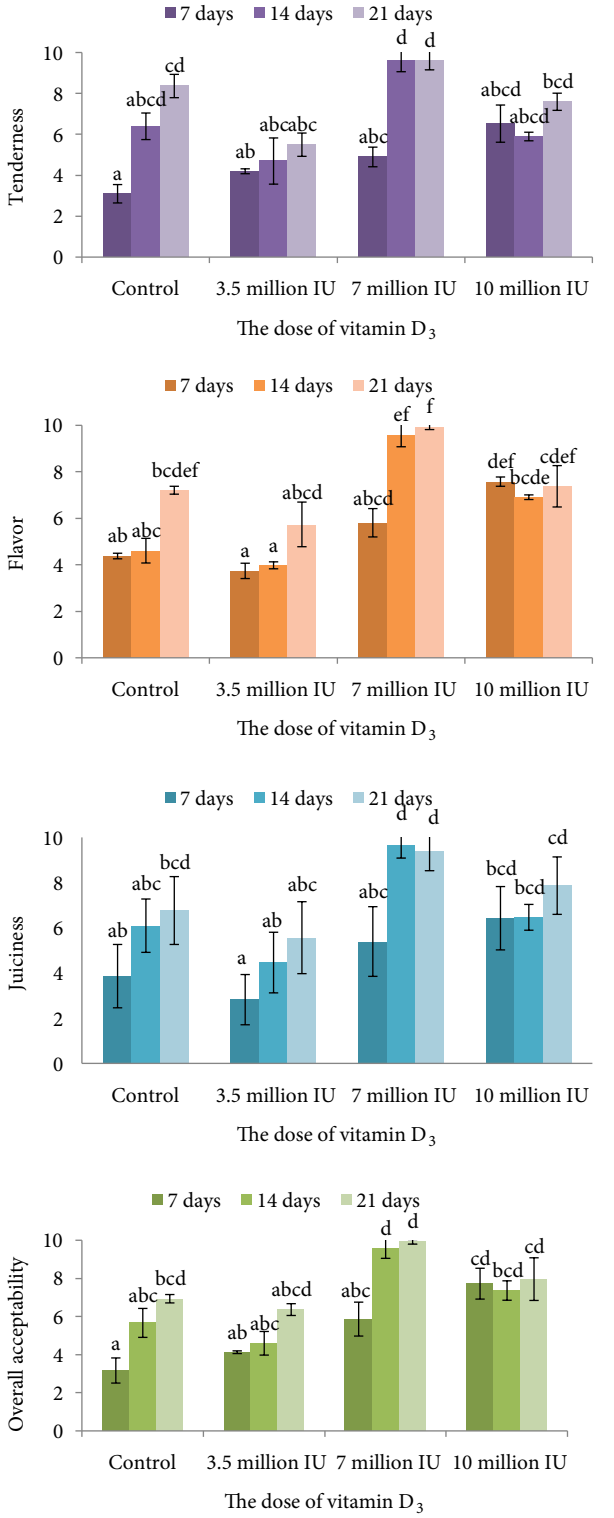

Figure 2. The effect of dietary vitamin D_3 supplementation on the sensory analysis of longissimus lumborum (STR045) muscle during ageing (a…d bars denoted by different superscripts differ significantly at $P < 0.01$).

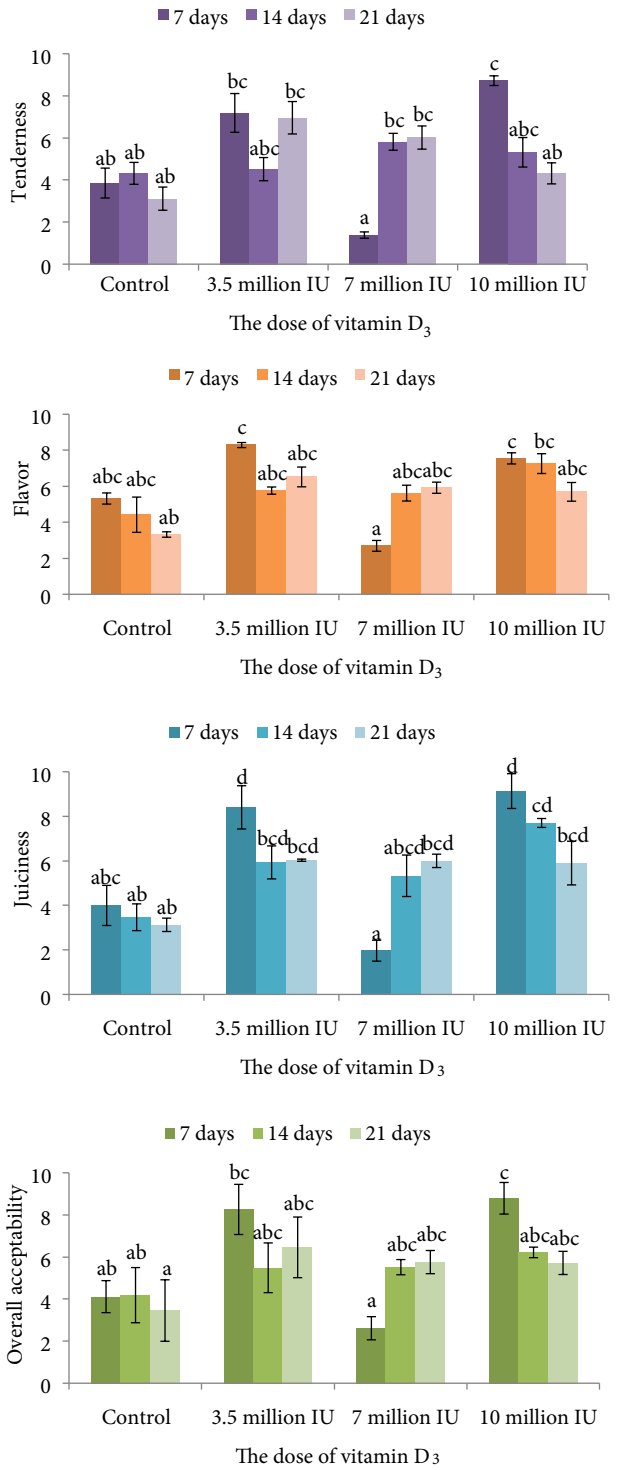

Figure 3. The effect of dietary vitamin D_3 supplementation on the sensory analysis of semimembranosus (TOP073) muscle during ageing (a…d bars denoted by different superscripts differ significantly at $P < 0.01$).

$\times 10^6$ IU $\times$ day^{-1} $\times$ animal^{-1}. In the latter publication, the authors tested the different forms of vitamin D (vitamin D_2, 25-hydroxyvitamin D_3, and 1,25-dihydroxyvitamin D_3) and found that the greatest decrease in WBSF occurred with 25-hydroxyvitamin D_3 after 8 days of ageing (10,19).

3.3. The effect of dietary vitamin D_3 supplementation on the sensory descriptors

All analyzed muscles (semitendinosus, semimembranosus, and longissimus lumborum) were further assessed by consumer analysis, the results of which are presented in Figures 1 to 3.

Based on the consumer analysis, semitendinosus (EYE075) (Figure 1) aged for 7 days and supplemented with 7.0 and 10.0 $\times 10^6$ IU vitamin D_3 scored highest in terms of tenderness, juiciness, and flavor. The highest overall acceptability rate was reported for the 7.0 $\times 10^6$ IU vitamin D_3 samples. Extending the ageing period to 14 days resulted in a significant increase in the assessment of tenderness for the samples that had received a dose of 10.0 $\times 10^6$ IU but a decrease in tenderness for the 7.0 $\times 10^6$ IU samples.

Prolongation of the ageing time to 21 days resulted in an increase in the tenderness of samples that had received 10.0 $\times 10^6$ IU of vitamin D_3 and a slight increase in tenderness at a dose of 7.0 $\times 10^6$ IU. The Warner–Bratzler cutting data showed no statistically significant differences in the tenderness of the EYE075 meat between doses of 7.0 and 10.0 $\times 10^6$ IU of vitamin D_3. Consumer analysis revealed a difference between those 2 doses in terms of tenderness. According to the panelist, the samples that had received a dose of 10.0 $\times 10^6$ IU of vitamin D_3 and aged for 14 days were the most tender.

Figure 2 shows that extending the ageing time for the samples that had received 7.0 $\times 10^6$ IU of vitamin D_3 resulted in an improvement in all analyzed descriptors (tenderness, juiciness, flavor, and overall acceptability). For a 10.0 $\times 10^6$ IU dose, the samples aged for 14 days were considered the most tender, juicy, and flavorsome. Interestingly, those results are not congruent with those obtained by the instrumental analyses.

As in the case of the longissimus muscle, the semimembranosus muscle (TOP073) samples with highest marks for tenderness, juiciness, and flavor were aged for 14 days and with a dose 10.0 $\times 10^6$ IU. For the 7.0 $\times 10^6$ IU dose, an extension of the ageing time resulted in a decrease of tenderness and overall acceptability (Figure 3). Comparing the results of instrumental and sensory analysis, it can be seen that the most effective dose of vitamin D_3 that significantly improved the tenderness of semimembranosus muscle was 10.0 $\times 10^6$ IU. Moreover, this dose allowed a shortening of the ageing time from 21 to 14 days.

Unfortunately, our data are unable to support a shortening of the ageing time to less than 14 days. The results confirm that, for the analyzed muscles, a dose of 7.0×10^6 IU vitamin D_3 is able to decrease the shear force to an optimum level within 14 days of ageing. Based on our analyses, the lowest tenderness was reported for the semitendinosus muscle, while the highest tenderness was found in the longissimus muscle. Moreover, our tenderness analysis allowed a clear separation between various doses of vitamin D_3 and ageing times. According to the panelists, improvement of meat tenderness was achieved after 14 days of ageing for the 10.0 and 7.0×10^6 IU doses, thus confirming the data obtained for the measurement of changes in sarcomeres length and shear force values. In addition, we found that that the samples with a high score of tenderness were also characterized by high juiciness and much higher notes for overall acceptability than the samples evaluated as less tender. A similar relationship was obtained for the flavor. Samples with greater juiciness were characterized by higher values of taste and odor.

Acknowledgments

This research was carried out within the Project no WND-POIG.01.03.01-00-204/09 Optimizing of Beef Production in Poland According to the "from Fork to Farm" Strategy, cofinanced by the European Regional Development Fund under the Innovative Economy Operational Programme 2007–2013.

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