Beef tenderness improvement by dietary vitamin D₃ supplementation in the last stage of fattening of cattle

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Abstract

Tenderness is the most important characteristic of meat, determining consumer approval. There are numerous methods of its improvement, although of diverse effectiveness. Addition of vitamin D₃ to the feed for a short period before slaughter (7–10 days) is one of the natural ways to enhance the tenderness. Vitamin D₃ is responsible for Ca²⁺ mobilisation in serum and increase in activity of proteolytic enzymes belonging to calpains, which results in significant improvement of beef tenderness and reduction of ageing time. The use of vitamin D₃ is an application tool determining tenderness improvement of beef with substantial reduction in processing costs. Moreover, shorter post mortem ageing process will exceed the retail display time, which will consequently reduce losses due to unsold meat being returned from shops to the manufacturers. Based on the results of studies conducted over the last 15 years, this paper presents the possibility and the effects of the use of vitamin D₃ to improve beef tenderness.

Keywords: beef, texture, feed system, vitamin D₃.

Introduction

The quality of beef is not easy to determine since both sensory (tastiness, juiciness) and textural (tenderness) components must be considered in terms of consumer expectations (14). Consumers perceive meat quality through a variety of attributes: tenderness, colour, nutritional value, tastiness, water-holding capacity, fat content, and also safety (32, 37). Accordingly, cattle production should focus on high quality of obtained meat, especially when consumption of beef in Poland is constantly decreasing – 3.9 kg in 2005, 2.4 kg in 2010, and 1.08 kg/per/year in 2015 (36). By contrast, beef consumption is much higher in countries like Argentina (55.7 kg/per/year), Brasil (39.8 kg/per/year), USA (38.2 kg/per/year), or Australia (35.3 kg/per/year) (1 4). These countries have the highest consumption values of beef per capita. In order to improve or equalise the quality, ageing process is applied. Unfortunately, in Poland and other Eastern European countries there are only few facilities able to perform proper meat ageing. The reason for this limitation is higher demand for refrigerated storage capacity with stabilised temperature of 1°C. In those countries, carrying out ageing process for 21 days is not feasible due to technical and economic reasons. Therefore, it is necessary to deliver alternative methods for quality improvement, adequately designed for current technical situation of meat sector.

Numerous factors contribute to overall beef quality, both genetic (breed, gender) and environmental (feeding system, breeding conditions) (24, 29), through which production process can be controlled (40). Animal feeding is one of the major factors determining quality of meat (23). Feeding type, or rather its intensity and modifications applied (feed composition, addition of biologically active substances) (8), are responsible for daily weight gain and better carcass conformation in a shorter time, e.g. through using metabolically active compounds – metabolic modifiers. These compounds can be divided into six main groups: anabolic steroids, somatotropin, β-blockers (ractopamine, zipaterol), vitamins administered together with feed at very high doses (vitamin D₃, vitamin E, vitamin A), dedicated lipids (conjugated dienes of linoleic acid), and other metabolically active compounds (chromium,
magnesium, manganese) (10). Substances belonging to the first three groups significantly modify animal body functions to increase production efficiency. Moreover, since 1996 the use of growth stimulants, such as ractopamine hydrochloride, has been forbidden in EU countries, hence the number of invasive solutions is reduced. It is worth mentioning that only some of them are used in order to improve meat quality, especially in terms of tenderness.

The majority of the substances are used to enhance mostly the visual aspects of meat, such as colour, marbling, and texture, as well as properties determining technological utility, basically pH and water-holding capacity. Most commonly used solution is modification of feed composition and addition of substances which increase feed conversion ratio, daily gain weight, and dressing percentage (10), without influencing physical properties (colour, tenderness) or tissue composition of carcasses, which determine the nutritional value of meat (11).

Nowadays, feed additives contributing to a specific nutritional value are more frequently used for instance through change in fatty acids profile (1, 6), increasing antioxidative status (5, 8) or improving oxidative stability of lipids (vitamin E) in beef (17). In terms of beef tenderness, one of the tools is enrichment of feed with vitamin D$_3$.

This vitamin occurs naturally in both animal organisms and in animal feed before slaughter. Increasing vitamin D$_3$ content in the feed shortly before slaughter (8–10 days) results in higher absorption of calcium from the gastrointestinal system (12, 27). It is assumed that improvement of meat tenderness happens due to Ca$^{2+}$ ions activation, which has an important role during muscle tissue contraction. Additionally, these ions are responsible for activation of enzymes from calpain group, which determine proteolysis process during post mortem ageing period, thus accelerating its duration (25, 26, 27). The aim of this paper was to evaluate the possibilities of meat tenderness improvement and shortening of the ageing process due to high doses of vitamin D$_3$ addition to feed in the last phase of cattle fattening period.

**Beef tenderness and the use of Ca$^{2+}$ activating compounds**

Juiciness, tastiness, and tenderness belong to the most important textural properties and sensory attributes, which determine both perception and consumer approval of beef (40, 41). However, tenderness is considered the most changeable determinant of customer satisfaction (20), which is also indicated by market demand. Moreover, numerous studies have shown that consumers are willing to pay more for meat of guaranteed, repeatable tenderness (4, 13).

For at least 50 years, factors affecting tenderness of beef and its variability were the subject of research. Tenderness is primarily determined by two muscle components, namely the connective tissue content and the mechanism of muscle contraction (19). After death, ATP reserve drops to zero. Calcium pump ceases its function and high concentration of Ca$^{2+}$ ions is sustained in the sarcoplasm, which leads to post mortem shrinkage. Only after degradation of contractile proteins does muscle relaxation occurs and the post mortem shrinkage disappears.

Moreover, various factors differently affect formation of tenderness during the ageing period. These factors can be divided into two groups according to their nature: intravital (age, gender, breed, muscle, fat content, and diet), and ante mortem, meaning animal handling for 48 h prior to slaughter (transportation conditions, conditions in the cattle warehouse: access to water and feed). Additionally, post mortem factors (mainly correct process of cooling the carcasses – the rate of pH and temperature change in time) also have an important role (40). Synergistic effect of these factors results in beef tenderness being determined by pH decrease, as a function of carcass temperature, muscle calpain activity, sarcomeres length (15, 31), amount and ratio of individual fractions of collagen, and type and size of muscle fibres (33).

One of the most effective methods of enhancing meat tenderness is submission to ageing process. Myofibrillar protein proteolysis, which relies on Ca$^{2+}$ dependent enzymes, μ-calpain and m-calpain, determines the rate and intensity of changes occurring during ageing, which are responsible for meat tenderness (15).

Over the past twenty years, the majority of studies on tenderness factors were focused on identifying changes which occur in meat due to endogenous proteolytic system during the ageing process. It is associated with partial protein, glycogen, and other components of muscle tissue degradation, which leads to the development of technological, culinary, and nutritional properties, such as tenderness and reduction of its variability (23), tastiness, juiciness, colour, and water-holding capacity (19).

For a long time it was believed that ageing process depends largely on the activity of proteolytic enzymes from the group of lysosomal cathepsins. Nowadays, greater role in meat tenderisation during storage is associated with proteolytic calpain system. This system consists of μ-calpain and m-calpain activated by Ca$^{2+}$ ions and endogenous calpain inhibitor - calpastatin. This inhibitor has a major role in the regulation of calpain system in muscle tissue after slaughter (15). This relationship is strongly influenced by pH value of the environment and also partially reversible, due to the fact that proteolytic enzymes require certain pH to their activation and reactions. Both μ-calpain and m-calpain are located in myofibrils, where the μ-calpain is located in 66% around boundary Z-line of the sarcomere, with its remaining parts in I-band (20%) and also in A-band (14%). Meanwhile, m-calpain is located on the Z-line of the sarcomere in 52% and in I- and A-bands in 27% and 21%, respectively (17, 18).
Myofibrillar proteins such as titin, nebulin, filamin, desmin, troponin T and I, and tropomyosin are substrates for the enzymes from calpain family. The main function of these proteins is to secure proper structure of muscular fibres, and their degradation is responsible for the post mortem textural changes, particularly relating to tenderness. Therefore, they are very significant for the ageing process (17, 30). This process also leads to structural changes in muscle fibres. Boundary Z-lines of the sarcomeres disintegrate, followed by structure relaxation and myofibrils fragmentation. Proteins, such as desmin and troponin-T, are being degraded, and cross connections between myofibrils become weak, resulting in increased length of sarcomeres. Moreover, cytoskeletal proteins – titin and nebulin, that stabilise spatial distribution of thick and thin myofilaments in the sarcromere, become degraded. The breakdown of proteins responsible for sustaining costamere structure - talin and vinculin, leads to formation of new polypeptides with molecular weights of 95 kDa and 28-32 kDa, due to degradation of structural proteins (15).

Proteolysis during ageing process is initiated by μ-calpain, which is activated at low concentration of Ca\(^{2+}\) ions released from sarcoplasmic reticulum of mitochondria when rigor mortis occurs (15). Usually this happens about 6 hours after slaughter at pH of 6.3. As the concentration of Ca\(^{2+}\) increases, m-calpain is being activated (after about 16 h after slaughter), which is responsible for further tenderisation process (19). Interestingly, calpains achieve optimum activity at pH 6.0-7.0 (meat with such pH values is not accepted by consumer nor industry), however, their stability is low. Half-life periods of their full activity are different - for μ-calpain about 1 day, while for m-calpain about 10 days (17).

The mechanism of tenderisation of meat during ageing involves five steps. An increase in Ca\(^{2+}\) ion concentration activates the calpains, which initiates tenderisation process. Calpains are inactivated by binding with calpastatin when pH increases. This step depends on the pH changes. The proteolysis of myofibrillar proteins occurs due to calpains activity (tenderisation process). The active calpains are inactivated because of autolysis (17).

Post mortem ageing, performed under standard conditions, is usually a long-term and expensive process, which does not always bring the desired effects. Research has been conducted on the shortening of this process and increasing its effectiveness, particularly in terms of tenderness enhancement. This can be achieved by using different technological treatments, applied individually or in various combinations, in order to accelerate proteolysis process: lower rate of cooling the carcasses after slaughter, strict temperature control during refrigerated storage, slinging carcasses by hip bone, electrostimulation of carcasses immediately after slaughter, and use of compounds based on Ca\(^{2+}\) (calcium chloride and calcium propionate) (26, 34). The aim of each of these modifications is to increase meat tenderness by limiting sarcomere shrinkage, proteolytic enzymes activity, and reducing tension of certain muscles (e.g., leg muscles). Degradation of myofibrillar proteins results in the release of exogenous Ca\(^{2+}\), that is the substrates for the enzymes belonging to calcium-dependent proteases (calpain). Accelerated proteolytic changes during ageing occur due to increased activity of m-calpain, which, under standard conditions after slaughter, is activated with a delay, because it requires a higher concentration of Ca\(^{2+}\) (19).

**Improvement of beef tenderness using vitamin D\(_3\) supplementation**

One of the alternative methods to improve meat tenderness is the use of vitamin D\(_3\), which is responsible for mobilisation of Ca\(^{2+}\). Supplementation with high doses of vitamin D\(_3\) in the last phase of fattening results in increased concentrations of Ca\(^{2+}\) in serum and muscle tissue, causing significant improvement of tenderness in meat obtained from cattle (3, 27, 35). Wheeler et al. (39) found that higher level of calcium in the muscles is responsible for larger enzymatic activity of calpains, which promotes the process of proteolysis. Swanek et al. (35) as one of the first teams working on improving beef tenderness proved that vitamin D\(_3\) supplementation of feed for cattle resulted in a significant improvement in tenderness of longissimus dorsi muscle.

The greatest tenderness improvement was demonstrated after 7 days of ageing. Therefore, on the basis of conducted studies it was concluded that vitamin D\(_3\) supplementation of feed may have a similar effect on meat tenderness as other systems based on activation of Ca\(^{2+}\).

Increase in meat tenderness through application of vitamin D\(_3\) to animals is explained by increase in the level of Ca\(^{2+}\) within the muscle tissue, which affects activation of calpain enzymes (3). formulation of molecules of about 30-kDa, which are products of troponin-T degradation due to calpain activity is indication of post mortem proteolysis and tenderisation process (27). Vitamin D\(_3\) supplementation greatly increases the concentration of Ca\(^{2+}\) through the additional effect of 1,25 - dihydroxy vitamin D\(_3\) (3). Skeletal muscles are an important target destination for vitamin D\(_3\).

It has been proved that vitamin D\(_3\) supplementation of feed causes an increase in binding of Ca\(^{2+}\) within the Z-line of sarcomeres and an increase in Ca\(^{2+}\) concentration in the cytosol of skeletal muscle cells. Higher concentration of Ca\(^{2+}\) in muscle tissue can increase the ability of calcium-dependent proteolytic enzymes to break troponin-T into smaller polypeptides of about 30 kDa for 14 days after slaughter, thereby enhancing meat tenderness (25, 26). Enhancement in beef tenderness may result from increased intensity of the proteolysis process during post mortem ageing due to a higher concentration of Ca\(^{2+}\) inside muscle cells (26). This was confirmed by Swanek et al. (35), who showed higher concentrations of Ca\(^{2+}\) in plasma and the longest muscle (m. longissimus dorsi) obtained from steers, which were fed feed supplemented with vitamin D\(_3\).
Table 1 Effect of vitamin D₃ supplementation in the last phase of fattening on tenderness (WBSF value) of beef (own study)

| Authors            | Animals                                      | Vitamin dose D₃/d/animal | Muscle         | WBSF (kG) | Observation                                |
|--------------------|----------------------------------------------|--------------------------|----------------|-----------|--------------------------------------------|
|                    |                                              |                          | Day 3 | Day 7 | Day 14 | Day 21 |                          |                                                   |
| Lipińska et al.    | Crossbreed, Holstein-Friesian x Limousin:    | 0 x 10⁶ IU D₃ (placebo)  | Centre roast   | ---    | 3.43   | 3.62 | --- | Addition of 7 x 10⁶ IU D₃ and 10 x 10⁶ IU D₃ of vitamin D₃ improved tenderness of each of the evaluated muscles. The addition of a higher dose of vitamin D₃ (10 x 10⁶ IU) resulted in greater tenderness enhancement, which was underlined after 14 days of ageing, especially in the case of knuckle and top side. |
|                    | bulls 18–19 months of age                    | 3.5 x 10⁶ IU D₃ start   | Top but        | ---    | 3.63   | 3.19 | --- |                            |
|                    | supplementation 10 days before slaughter,     | Inside round             | ---          | 3.69   | 3.82   | --- |                            |
|                    | application for 6 days (10⁶ day – slaughter)  |                          |               |        |        |    |                            |
|                    | 7 x 10⁶ IU D₃ start                         | Centre roast             | ---          | 3.32   | 3.10   | --- |                            |
|                    | supplementation 10 days before slaughter,    | Top but                  | ---          | 4.32   | 3.19   | --- |                            |
|                    | application for 3 days (10⁶ day – slaughter)  | Inside round             | ---          | 3.69   | 3.60   | --- |                            |
|                    | 10 x 10⁶ IU D₃ start                        | Centre roast             | ---          | 3.53   | 3.08   | --- |                            |
|                    | supplementation 10 days before slaughter,    | Top but                  | ---          | 3.48   | 3.31   | --- |                            |
|                    | application for 3 days (10⁶ day – slaughter)  | Inside round             | ---          | 4.18   | 3.53   | --- |                            |
| Rafalska, (33)     | Crossbreed, Holstein-Friesian x Simmental:   | 0 x 10⁶ IU D₃ (placebo)  | Strip loin    | ---    | 3.55   | 3.29 | 2.28 | The addition of vitamin D₃ caused a decrease in WBSF values during ageing in all analysed muscles. The greatest decreases in WBSF values were recorded for strip loin and top round. However, in the case of 7.0 x 10⁶ IU dose of vitamin D₃ there was no significant decrease in WBSF values between the samples aged 14 and 21 days. |
|                    | bulls 18 months of age                       | 3.5 x 10⁶ IU D₃ start   | Eye of round  | ---    | 3.86   | 3.69 | 3.41 |                            |
|                    | supplementation 1 day before slaughter       | Top round                | ---          | 3.57   | 3.15   | 2.31 |                            |
|                    | 7 x 10⁶ IU D₃ start                         | Strip loin               | ---          | 2.54   | 1.93   | 1.90 |                            |
|                    | supplementation 1 day before slaughter       | Eye of round             | ---          | 3.45   | 3.41   | 3.28 |                            |
|                    | 10 x 10⁶ IU D₃ start                        | Strip loin               | ---          | 2.65   | 2.09   | 2.36 |                            |
|                    | supplementation 1 day before slaughter       | Eye of round             | ---          | 2.44   | 2.03   | 1.88 |                            |
| Lobo-Jr et al.     | Nellore-type steers over 30 months of age     | 0 x 10⁶ IU D₃ (placebo)  | Strip loin    | ---    | 1.76   | 1.74 | 1.69 | No effect associated with the dose of vitamin D₃ addition and/or sunlight exposure was showed on WBSF values during ageing. This fact may be related to animal age (over 30 months) and maturity stage which are also important factors of determination the WBSF values due to a decrease in collagen properties (especially solubility) that occurs with age. |
|                    | and with shade (50% UV filtration ratio)     | Strip loin               | 10.0         | 8.7    | ---    | 7.0  |                            |
|                    | 2 x 10⁶ IU of vitamin D₃ for 2 days before   | Strip loin               | 10.9         | 9.0    | ---    | 7.6  |                            |
|                    | slaughter and no shade                       |                          | 11.6         | 10.6   | ---    | 7.6  |                            |
|                    | 2 x 10⁶ IU of vitamin D₃ for 2 days before   | Strip loin               | 10.09        | 9.3    | ---    | 7.3  |                            |
|                    | slaughter with 2 x 10⁶ IU of vitamin D₃       |                          |              |        |        |    |                            |

Continued on next page
shade (50% UV filtration ratio)

| Study          | Treatment Details                                                                 | Meat Type 1 | Meat Type 2 | Meat Type 3 | Meat Type 4 |
|---------------|-----------------------------------------------------------------------------------|-------------|-------------|-------------|-------------|
| Hansen et al. (13) Bonsmara: steers, 9 months of age | 2 x 10^6 IU of vitamin D_3 for 8 days before slaughter and no shade  
7 x 10^6 IU for 3 days prior slaughter  
7 x 10^6 IU for 6 days prior slaughter  
7 x 10^6 IU start supplementation 13 days before slaughter, application for 6 days | Strip loin 9.7 | --- | 7.6 | --- | 6.0 | None of the vitamin D_3 supplementation levels were found to decrease significantly WBSF values. However, application of vitamin D_3 for the first 6 days resulted in increased calcium ion content in the meat tissue. This treatment also improved beef colour. |
| Carnagey et al. (4) Crossbreed heifers | Control (no 25-OH D_3, no vitamin E)  
25-OH D_3 – 500 mg 25-OH D_3 administered at once 7 days before slaughter  
25-OH D_3 – 500 mg 25-OH D_3 administered at once 7 days before slaughter and 1000 IU vitamin E daily with feed for 104 days before slaughter | Strip loin 3.79 | 3.04 | 3.60 | --- | --- | Single application of 500 mg 25-OH D_3 for 7 days before slaughter may have a role in tenderness improvement of roast beef obtained from heifers. This dose was similarly effective as 0.5 to 7.5 x 10^6 IU D_3 in previous studies, at the same time not causing high vitamin D_3 and its metabolites concentration in blood. Vitamin E supplementation may also influence changes in tenderness during ageing. Surprisingly, combination of 25-OH D_3 and vitamin E supplementation did not show higher impact on meat quality. |
| Foote et al. (12) Crossbreed steers | 0 x 10^7 IU D_3, vitamin E (placebo)  
5 x 10^7 IU D_3, vitamin E (administered daily for 9 days, slaughter after 2 days)  
125 mg 25-OH D_3 once only | Strip loin 3.24 | --- | 3.45 | 3.03 | --- | It was proved that a dose of 125 µg 1,25(OH)_2D_3 administered once 4 days prior to slaughter was ineffective in terms of WBSF value change. However, the differences in individual muscles response to the experiment may be due to different muscle fibres types. In order to maximize the assumed effects, supplementation period... |
Table I (continued)

| Treatment | Crossbreed steers: Bos Taurus x English, Bos indicus | Montgomery et al. (26) | Montgomery et al. (25) | Montgomery et al. (27) |
|-----------|------------------------------------------------------|------------------------|------------------------|------------------------|
|           | (slaughter after 4 days)                             | Chuck steak            | Strip loin              | Strip loin              | Strip loin              |
|           |                                                      | ---                    | 3.62                   | 3.17                   | 2.84                   |
|           |                                                      | Top round              | 3.29                   | 3.65                   | 4.71                   |
|           |                                                      | Chuck steak            | 2.51                   | 2.85                   |                        |
|           | 500 µg                                               | 5.22                   | 5.49                   | 5.02                   | 5.02                   |
|           | 1.25(OH)2D3                                         | 5.56                   | 5.38                   | 4.86                   | 5.21                   |
|           | once only (slaughter after 3 days)                   | 5.06                   | 4.24                   | 4.38                   | 3.69                   |
|           |                                                      | 4.90                   | 4.14                   | 4.41                   | 4.40                   |
|           |                                                      | Strip loin             | 5.15                   | 4.48                   | 4.04                   |
|           |                                                      | Top round              | 4.16                   | 4.80                   | 3.92                   |
|           |                                                      | Top butt               | 4.64                   | 4.29                   | 4.05                   |
|           | 0.5 x 10³ IU D3                                     | Mock tender            | 4.36                   | 4.08                   | 4.31                   |
|           |                                                      | Strip loin             | 4.76                   | 4.75                   | 3.99                   |
|           |                                                      | Top round              | 4.11                   | 4.37                   | 4.32                   |
|           |                                                      | Top butt               | 5.06                   | 4.22                   | 4.00                   |
|           |                                                      | Mock tender            | 4.94                   | 4.17                   | 4.39                   |
|           | Montgomery et al. (26)                               | Strip loin             | 4.76                   | 4.54                   | 4.21                   |
|           |                                                      | Top round              | 4.46                   | 4.42                   | 4.07                   |
|           |                                                      | Top butt               | 5.03                   | 4.25                   | 3.87                   |
|           |                                                      | Mock tender            | 5.06                   | 4.44                   | 5.02                   |
|           | Crossbreed steers: Angus cull cows (78 months of age) | Strip loin             | 4.72                   | 5.30                   | 3.68                   |
|           | Strip loin <sup>6</sup>                              | 5.15                   | 6.69                   | 5.11                   |
|           | Strip loin                                          | 4.15                   | 6.69                   | 5.11                   | 4.53                   |
|           | Strip loin                                          | 4.67                   | 3.66                   | 4.19                   | 3.76                   |
|           | 0 x IU D3 (placebo)                                 | Strip loin             | 2.80                   | 2.92                   | 2.45                   |
|           |                                                      | Top round              | 4.50                   | 4.01                   | 4.16                   |
|           | 0.5 x 10³ IU D3                                     | Strip loin             | 2.22                   | 2.79                   | 2.56                   |
|           |                                                      | Top round              | 3.67                   | 4.40                   | 3.59                   |
|           | 1 x 10³ IU D3                                       | Strip loin             | 2.53                   | 2.81                   | 2.48                   |
|           |                                                      | Top round              | 3.52                   | 3.95                   | 3.80                   |
|           | 2.5 x 10³ IU D3                                     | Strip loin             | 2.58                   | 3.01                   | 2.67                   |
|           |                                                      | Top round              | 4.29                   | 4.27                   | 4.14                   |
|           | 5 x 10³ IU D3                                       | Strip loin             | 2.47                   | 2.84                   | 2.52                   |
|           |                                                      | Top round              | 3.63                   | 4.25                   | 4.04                   |
|           | 7.5 x 10³ IU D3                                     | Strip loin             | 2.31                   | 2.97                   | 2.57                   |
|           |                                                      | Top round              | 4.38                   | 4.14                   | 3.89                   |
|           | 0 x IU D3 (placebo)                                 | Strip loin             | 3.58                   | 3.32                   | 3.25                   |
|           |                                                      | Top round              | 3.97                   | 3.91                   | 3.74                   |
|           | Crossbreed steers: Continental x British; steers 23 months of age | Strip loin             | 3.11                   | 3.20                   | 2.80                   |
|           | Strip loin                                          | 3.56                   | 3.37                   | 3.32                   |
|           | Strip loin                                          | 3.17                   | 2.78                   | 2.89                   |
|           | Strip loin                                          | 3.32                   | 3.37                   | 3.56                   |

Significant interaction was proved between vitamin D<sub>3</sub> dose x muscle x ageing period and WBSF. Vitamin D<sub>3</sub> supplementation causes an increase in Ca<sup>2+</sup> concentration in the muscles, activates the calpain system, accelerates myofibrillar proteins degradation, including troponin T, and enhances tenderness of various bovine muscles. Improvement of beef tenderness through vitamin D<sub>3</sub> supplementation was shown. Improvement of WBSF value was observed in LM, SM, and GM already after 3 days of ageing; therefore long-term ageing process is not required when vitamin D<sub>3</sub> is used.

Addition of vitamin D<sub>3</sub> improved tenderness of beef meat (strip loin) obtained from beef cull cows. However, this kind of meat requires at least 14 days of ageing.

Supplementation with vitamin D<sub>3</sub> of 0.5 x 10³ IU/day/animal, improved tenderness of tested muscles (with no negative impact on intravital traits).

Addition of vitamin D<sub>3</sub> caused a decrease in shear force value by 0.5 kG in both tested muscles in comparison with the control group. The greatest improvement was observed with ageing of 14 days (P<0.5), although it was concluded that the most effective dose was 5 x 10³ IU of vitamin D<sub>3</sub>. Increase in tenderness can be explained by higher intracellular concentration of Ca<sup>2+</sup> available during proteolysis in ageing process.
Selected results of the addition of vitamin D₃ on physical properties of meat, in particular its tenderness, are presented in Table 1.

The use of high doses of vitamin D₃ for a short period (3–6 days) does not have a negative effect on the feedlot performances of beef cattle (2, 3). Furthermore, the addition of vitamin D₃ in the last stage of fattening of cattle is associated with an increase in total calcium concentration in the muscle tissue, regardless of muscle type (3), which may have a significant impact on the ageing process. Montgomery et al. (26) and Caragney et al. (3, 4) have demonstrated an increase in the extent of protein degradation, especially troponin-T and other proteins responsible for the sarcomere integrity due to enhanced action of calcium-dependent proteases m- and μ-calpain. However, the vitamin D₃ addition can affect negatively colour parameters, mainly reduce lightness (21). However, this relationship is not confirmed by Hansen et al. (13) and Lobo-Jr et al. (22). In earlier studies (26, 34), no correlation between the addition of vitamin D₃ and sensory traits of beef such as juiciness, flavour, and overall palatability has been shown, while Rafalska (33) indicated a positive effect of vitamin D₃ addition (7.5 and 10 x 10⁶ IU) on analysed sensory traits (tenderness, juiciness, and flavour). In addition, the highest marks for tenderness, juiciness, and flavour were awarded for strip loin and top round aged for 14 days with 10 x 10⁶ IU of vitamin D₃. This fact confirms the possibility of shortening the ageing time from 21 to 14 days. In the formation of meat flavour upon heating small molecular weight water-soluble compounds and lipids are contributed. The study indicates that the beef ageing carried out for more than 21 days may decrease the flavour. Beef ageing performed for 35 days may also increase the metallic off-flavour (7).

Breeding cattle for slaughter based on the use of innovative components determining feed quality and management methods will increase the efficiency of livestock production, as well as improve the quality of produced beef. Increasing the amount of selected feed ingredients can have a positive impact not only on carcass properties, but also on the quality of the meat. One of these components is vitamin D₃, which administered to animals for a short period before slaughter causes mobilisation of Ca²⁺ in the plasma, resulting in a significant improvement in tenderness of beef. It has been confirmed in many studies conducted in the 20th and 21st century.

Increased Ca²⁺ concentration in the muscle due to administration of vitamin D₃ is responsible for higher activity of proteolytic enzymes from calpain group. Use of vitamin D₃ in high doses (0.5 x 10⁶ to 7.5 x 10⁶ IU/hd/d) for a short time (4 – 10 days) prior to slaughter improves beef tenderness (m. longissimus thoracis) after 7 days of ageing (38). Significant increase in tenderness of beef was observed by Montgomery et al. (25, 27), who reported that addition of vitamin D₃ (7.5 x 10⁶ IU D₃ for 9 days before slaughter) contributed to a decline in shear force value of about 0.5 kG (m. longissimus lumborum and semimembranosus) compared to the control group. In the experiment performed by Vergas et al. (38) it was demonstrated that addition of vitamin D₃ (6 x 10⁵ IU/hd/d) and combination of vitamin D₃ and vitamin E (6 x 10⁵ IU/hd/d and 1000 IU/hd/d, respectively) resulted in shorter ageing period compared to the control group, as well as lower WBSF value for the tested muscles (m. longissimus lumborum), which was less than 3.86 kG, allowing to classify them as “very tender” according to Destefanis et al. (9).

Furthermore, studies conducted worldwide and discussed in this paper demonstrated no impact of vitamin D₃ administered for a short period prior to slaughter (10 days) on the production rates, such as feed intake, daily weight gain, and feed conversion ratio. Animal diet supplemented with high doses of vitamin D₃ can potentially lead to improved tenderness and larger consumer approval of beef. Addition of substances

| Table 1 (continued) | Steers: crossbreed | Crossbreed Continental x British: steers 23 months of age | Strip loin | Strip loin | Strip loin | Strip loin | Strip loin |
|----------------------|-------------------|----------------------------------------------------------|------------|------------|------------|------------|------------|
| Swane et al. (35)    | Angus x Hereford and crossbreed Salers x Charolaise | 0 x 10⁶ IU D₃ (placebo), 5 x 10⁶ IU D₃ 7 days before slaughter (7th day – slaughter) | 0 x 10⁶ IU D₃ (placebo), 5 x 10⁶ IU D₃ 10 days before slaughter (10th day – slaughter) | 3.58 | 3.97 | 3.11 | 3.56 |
| Montgomery et al. (28) | 0 x 10⁶ IU D₃ (placebo), 5 x 10⁶ IU D₃ | Strip loin | Top round | 3.58 | 3.97 | 3.20 | 3.47 |
|                      | 7.5 x 10⁶ IU D₃ 10 days before slaughter (10th day – slaughter) | Strip loin | Top round | 3.17 | 2.89 | 3.28 | 3.56 |

Strip loin – m. longissimus lumborum, top round – m. semimembranosus, eye of round – m. semitendinosus, mock tender – supraspinatus, top blade – infraspinatus, top butt - m. gluteus medius, center roast – m. rectus femoris, inside round – m. adductor femoris
increasing the intensity of proteolysis process by causing higher concentration of Ca^{2+}, such as vitamin D₃, leads to a shorter ageing process, e.g. 21 vs 14 days, depending on the type of muscle. Thus, it can be an alternative solution to other methods of tenderness enhancement. Summarising, improved beef tenderness, which largely translates into quality improvement and reduction of ageing time due to vitamin D₃ feed supplementation, positively affects the economic indicators. Ageing process requires financial expenses due to the cost of storage of large quantities of meat, costs of large refrigerated warehouses and their operation. Reducing the ageing time will also significantly prolong shelf-life (retail display). The use of vitamin D₃ feed supplementation will reduce the amount of unsold meat being returned from the retailers to meat processing plants.

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References

1. Alfaia C.P.M., Alves S.P., Martins S.I.V., Costa A.S.H., Fontes C.M.G.A., Lemos J.P.C., Bessa R.J.B., Prates J.A.M.: Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. Food Chem 2009, 114, 939–946.
2. Aragon S.N., Ribeiro F.R.B., Hosford A.D., Thompson A.J., Hergenreder J.E., Jennings M.A., Corley J.R., Johnson B.J.: Influence of yeast cell wall supplementation during the finishing phase on feedlot steer performance, carcass characteristics, and postmortem tenderness. Prof Anim Sci 2016, 32, 192–200.
3. Carnagey K.M., Huff-Lonergan E.J., Lonergan S.M., Trenkle A., Horst R.L., Beitz D.C.: Use of 25-hydroxyvitamin D₃ and dietary calcium to improve tenderness of beef from the round of beef cows. J Anim Sci 2008, 86, 1637–1648.
4. Carnagey K.M., Huff-Lonergan E.J., Trenkle A., Wertz-Lutz A.E., Horst R.L., Beitz D.C.: Use of 25-hydroxyvitamin D₃ and vitamin E to improve tenderness of beef from the longissimus dorsi of heifers. J Anim Sci 2008, 86, 1649–1657.
5. Castillo C., Pereira V., Abuelo A., Hernández J.: Effect of supplementation with antioxidants on the quality of bovine milk and meat production. Sci World J 2013, 2013, 1–8.
6. Daley C.A., Abbott A., Doyle P.S., Nader G.A., Larson S.: A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutr J 2010, 9, 10.
7. Dashdorj S., Amna T., Hwang I.: Influence of specific taste-active components on meat flavor as affected by intrinsic and extrinsic factors: an overview. Eur Food Res Technol 2015, 241, 157–171.
8. Descalzo A.M., Sancho A.M.: A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentina. Meat Sci 2008, 79, 423–436.
9. Desteфанi G., Brugiapaglia A., Barge M.T., Dal Molin E.: Relationship between beef consumer tenderness perception and Warner–Bratzler shear force. Meat Sci 2008, 78, 153–156.
10. Dikeman M.E.: Effects of metabolic modifiers on carcass traits and meat quality. Meat Sci 2007, 77, 121–135.
11. Dunshea F.R., D’Souza D.N., Petrick D.W., Harper G.S., Warner R.D.: Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. Meat Sci 2005, 71, 8–38.
12. Foote M.R., Horst R.L., Huff-Lonergan E.J., Trenkle A.H., Parrish F.C., Jr, Beitz D.C.: The use of vitamin D₃ and its metabolites to improve beef tenderness. J Anim Sci 2004, 82, 242–249.
13. Hansen S., Frylinck L., Strydom P.E.: The effect of vitamin D₃ supplementation on texture and oxidative stability of beef loin from steers treated with zilpaterol hydrochloride. Meat Sci 2012, 90, 145–151.
14. Hocquette J.-F., Botreau R., Picard B., Jacquet A., Beitz D.W., Scollan N.D.: Opportunities for predicting and manipulating beef quality. Meat Sci 2012, 92, 197–209.
15. Huff-Lonergan E., Zhang W., Lonergan S.M.: Biochemistry of postmortem muscle - lessons on mechanisms of meat tenderization. Meat Sci 2010, 86, 184–195.
16. Juárez M., Dugan M.E.R., Al Viñal N., Basarab J.A., Baron V.O.S., McAllister T.A., Aalhus J.L.: Beef quality attributes as affected by increasing the intramuscular levels of vitamin E and omega-3 fatty acids. Meat Sci 2012, 90, 764–769.
17. Juszczak-Kubiak E., Rosochacki S.J.: Znaczenie kalpain i katesypin w procesie degradacji białek mięśniowych w stanie post mortem podczas kruszenia i dojrzewania mięsa. Roczn Inst Przem Mizę 2002, 39, 77–89.
18. Juszczak-Kubiak E., Rosochacki S.J.: Geny warunkujące jakość mięsa u bydła – proteoliza w mięśniach a kruchość wołowiny. Przegląd Hod 2007, 75, 4–6.
19. Kemp C.M., Sensky P.L., Badrige R.G., Butter P.J., Parr T.: Tenderness – an enzymatic view. Meat Sci 2010, 84, 248–256.
20. Li, T., Wang L., Liu Y.: A new insight into the role of calpains in post-mortem meat tenderization in domestic animals: a review. Asian-Aust J Anim Sci 2013, 26, 443–454.
21. Lipinska A., Polorak A., Wierzbicka A., Wyszisz J.: The effect of packaging method and dietary vitamin D₃ supplementation on the quality of beef in rectus femoris, gluteus medius, and adductor femoris beef muscles. Turk J Vet Anim Sci 2016, 40, 505–513.
22. Lobo-Jr. A.R., Delgado E.F., GB. Mourão G.B., Pedereca A.C.M.S., Berndt A., Demarchi J.J.A.A.: Interaction of dietary vitamin D₃ and sunlight exposure on B. indicus cattle: Animal performance, carcass traits, and meat quality. Livestock Sci 2012, 145, 196–204.
23. Melucci L.M., Panarace M., Feula P., Villarreal E.L., Grigioni G., Carduza F., Soria L.A., Mezzadra C.A., Arco E.M., Mazzucco J.P., Corva P.M., Irueta M., Rogberg-Muñoz A., Miquel M.C.: Genetic and management factors affecting beef quality in grazing Hereford steers. Meat Sci 2012, 92, 768–774.
24. Moczewska M., Potorak A., Wierzbicka A.: Impact of the ageing process on the intensity of post mortem proteolysis and tenderness of beef from crossbreeds. Bull Vet Inst Pulawy 2015, 59, 361–367.
25. Montgomery J.L., Carr M.A., Kerth C.R., Hilton G.G., Price B.P., Gayleyn M.L., Horst R.L., Miller M.F.: Effect of vitamin D₃ supplementation level on the postmortem tenderization of beef from steers. J Anim Sci 2002, 80, 971–981.
26. Montgomery J.L., King M.B., Gentry J.C., Barham D.C., Horst R.L., Hilton G.G., Blanton J.R., Jr, Horst R.L., Gayleyn M.L., Morrow K.L., Wester D.B., Miller M.F.: Suplemental vitamin D concentration and biological type of steers: II. Tenderness, quality, and residues of beef. J Anim Sci 2004, 82, 2092–2104.
27. Montgomery J.L., Parrish F.C., Jr, Bietz D.C., Horst R.L., Huff-Lonergan E.J., Trenkle A.H.: The use of vitamin D₃ to improve beef tenderness. Anim Sci 2000, 78, 2615–2621.
28. Montgomery J.L., Parrish F.C., Jr., Bietz D.C., Horst R.L., Huff-Lonergan E.J., Trenkle A.H.: Feeding supplemental dietary vitamin D₃ to improve beef tenderness. Beef Research Report -
Iowa State University, 1998, A.S. Leaflet R1549. http://www.extension.iastate.edu/Pages/ansci/beefreports/asl-1549.pdf.

29. Onopiuk A., Półtorak A., Wierzbicka A.: Influence of postmortem muscle glycogen content on the quality of beef during aging. J Vet Res 2016, 60, 301–307.

30. Paredi G., Raboni S., Bendixen E., de Almeida A.M., Mozzarelli A.: “Muscle to meat” molecular events and technological transformations: the proteomics insight. J Proteomics 2012, 75, 4275–4289.

31. Polati R., Menini M., Robotti E., Millioni R., Marengo E., Novelli E., Balzan S., Cecconi D.: Proteomic changes involved in tenderization of bovine Longissimus dorsi muscle during prolonged ageing. Food Chem 2012, 135, 2052–2069.

32. Półtorak A., Wyrwisz J., Moczkowska M., Mareinkowska-Lesiak M., Stelmasiak A., Rafalska U., Wierzbicka A., Sun D.-W.: Microwave vs. convection heating of bovine Gluteus medius muscle: impact on selected physical properties of final product and cooking yield. Int J Food Sci Technol 2014, 50, 958–965.

33. Rafalska U.K.: Influence of dietary vitamin D₃ supplementation on the sarcomere length, Warner-Bratzler shear force, shortening of ageing time, and sensory acceptance of culinary beef muscles. Turk J Vet Anim Sci 2016, 40, 514–520.

34. Rider Sell N.R., Mikel W.B., Xiong Y.L., Behrends J.M.: Vitamin D₃ supplementation of cull cows: Effects on longissimus and semitendinosus muscle tenderness. J Anim Sci 2004, 82, 225–230.

35. Swanek S.S., Morgan J.B., Owens F.N., Gill D.R., Strasie C.A., Dolezal H.G., Ray F.K.: Vitamin D₃ supplementation of beef steers increases longissimus tenderness. J Anim Sci 1999, 77, 874–881.

36. Świtlik K.: Ceny detaliczne i spożycie mięsa. Rynek Mięsa, Stan i Perspektywy, 2016, 51, 55–64.

37. Van Wezemael L., DeSmet S., Ueland Ø., Verbeke W.: Relationships between sensory evaluations of beef tenderness, shear force measurements and consumer characteristics. Meat Sci 2014, 97, 310–315.

38. Vargas D.N., Down A.E., Webb D.S., Han H., Morgan J.B., Dolezal H.G.: Effects of dietary supplementation of feedlot steers with vitamins E and D₃ on live performance, carcass traits, shelf-life attributes and longissimus muscle tenderness. Anim Sci Res Rep 1999, 59–66. http://www.ansi.okstate.edu/research/research-reports-1/1999/1999-1%20Vargas%20Research%20Report.pdf.

39. Wheeler T.L., Koehler M., Shackelford S.D.: Effect of postmortem injection time and post-injection aging time on the calcium-activated tenderization process in beef. J Anim Sci 1997, 75, 2652–2660.

40. Wyrwisz J., Moczkowska M., Kurek M., Stelmasiak A., Półtorak A., Wierzbicka A.: Influence of 21 days of vacuum-aging on color, bloom development, and WBSF of beef semimembranosus. Anim Sci Pap Rep 2012, 30, 339–351.