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J Vet Res 61, 59-67, 2017
DOI:10.1515/jvetres-2017-0008

REVIEW ARTICLE

Beef tenderness improvement by dietary vitamin D_3 supplementation in the last stage of fattening of cattle

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Received: January 19, 2016 *Accepted: March 10, 2017*

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 D_3 to the feed for a short period before slaughter (7–10 days) is one of the natural ways to enhance the tenderness. Vitamin D_3 is responsible for Ca^{2+} 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 D_3 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 D_3 to improve beef tenderness.

Keywords: beef, texture, feed system, vitamin D_3.

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 1ºC. 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 D_3, 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 antioxidant 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 sarcomere, 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. *longissiumus dorsi*) obtained from steers, which were fed feed supplemented with vitamin D_3.

Table 1 Effect of vitamin D_3 supplementation in the last phase of fattening on tenderness (WBSF value) of beef (own study)

Authors	Animals	Vitamin dose D_3/d/animal	Muscle	WBSF (kG)				Observation
				Day 3	Day 7	Day 14	Day 21	
Lipińska *et al.* (21)	Crossbreed Holstein-Friesian x Limousin: bulls 18–19 months of age	0 x 10^6 IU D_3 (placebo)	Centre roast	---	3.43	3.62	---	Addition of 7 x 10^6 IU D_3 and 10 x 10^6 IU D_3 of vitamin D_3, improved tenderness of each of the valuated muscles. The addition of a higher dose of vitamin D_3 (10 x 10^6 IU) resulted in greater tenderness enhancement, which was underlined after 14 days of ageing, especially in the case of knuckle and top side.
			Top butt	---	3.63	3.19	---	
			Inside round	---	3.69	3.82	---	
		3.5 x 10^6 IU D_3 start supplementation 10 days before slaughter, application for 6 days (10th day – slaughter)	Centre roast	---	3.09	3.25	---	
			Top butt	---	3.46	3.94	---	
			Inside round	---	3.88	4.18	---	
		7 x 10^6 IU D_3 start supplementation 10 days before slaughter, application for 3 days (10th day – slaughter)	Centre roast	---	3.32	3.10	---	
			Top butt	---	4.32	**3.19**	---	
			Inside round	---	3.69	3.60	---	
		10 x 10^6 IU D_3 start supplementation 10 days before slaughter, application for 3 days (10th day – slaughter)	Centre roast	---	3.53	**3.08**	---	
			Top butt	---	3.48	3.31	---	
			Inside round	---	4.18	**3.53**	---	
Rafalska, (33)	Crossbreed Holstein-Friesian x Simmental: bulls 18 months of age	0 x 10^6 IU D_3 (placebo)	Strip loin	---	3.55	3.29	**2.28**	The addition of vitamin D_3 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 10^6 IU dose of vitamin D_3 there was no significant decrease in WBSF values between the samples aged 14 and 21 days.
			Eye of round		3.86	3.69	**3.41**	
			Top round		3.57	3.15	**2.31**	
		3.5 x 10^6 IU D_3 start supplementation 1 day before slaughter	Strip loin	---	2.54	1.93	**1.90**	
			Eye of round		3.45	3.41	**3.28**	
			Top round		2.65	**2.09**	2.36	
		7 x 10^6 IU D_3 start supplementation 1 day before slaughter	Strip loin	---	2.05	**1.74**	1.8	
			Eye of round		3.45	**2.76**	2.56	
			Top round		2.44	**2.03**	1.88	
		10 x 10^6 IU D_3 start supplementation 1 day before slaughter	Strip loin	---	1.76	1.74	1.69	
			Eye of round		3.24	**2.47**	2.58	
			Top round		2.11	2.03	2.1	
Lobo-Jr *et al.* (22)	Nellore-type steers over 30 months of age	0 x 10^6 IU D_3 (placebo) and no shade	Strip loin	10.0	8.7	---	**7.0**	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.
		0 x 10^6 IU D_3 (placebo) and with shade (50% UV filtration ratio)	Strip loin	10.9	9.0	---	**7.6**	
		2 x 10^6 IU of vitamin D_3 for 2 days before slaughter and no shade	Strip loin	11.6	10.6	---	**7.6**	
		2 x 10^6 IU of vitamin D_3 for 2 days before slaughter with	Strip loin	10.09	9.3	---	**7.3**	

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Table 1 (continued)

		shade (50% UV filtration ratio)						
		2 x 10^6 IU of vitamin D_3 for 8 days before slaughter and no shade	Strip loin	9.7	**7.6**	---	**6.0**	
		2 x 10^6 IU of vitamin D_3 for 8 days before slaughter with shade (50% UV filtration ratio)	Strip loin	10.06	9.2	---	**7.0**	
Hansen *et a*l. (13)	Bonsmara: steers, 9 months of age	0 x 10^6 IU D_3 (placebo)	Strip loin	---	---	3.5	---	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.
		1 x 10^6 IU for 9 days prior slaughter	Strip loin	---	---	4.6	---	
		7 x 10^6 IU for 3 days prior slaughter	Strip loin	---	---	4.3	---	
		7 x 10^6 IU for 6 days prior slaughter	Strip loin	---	---	4.7	---	
		7 x 10^6 IU start supplementation 13 days before slaughter, application for 6 days	Strip loin	---	---	4.6	---	
Carnagey *et al*. (4)	Crossbreed heifers	**Control** (no 25-OH D_3, no vitamin E)	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.
		25-OH D_3 – 500 mg 25-OH D_3 administered at once 7 days before slaughter	Strip loin	3.85	3.61	**3.36**	---	
		Vitamin E- 1000 IU vitamin E administered daily with feed for 104 days before slaughter	Strip loin	**3.64**	3.05	**3.35**	---	
		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	3.76	3.80	3.95	---	
Foote *et al.* (12)	Crossbreed steers	0 x 10^6 IU D_3 (placebo) – control group	Strip loin Top round Chuck steak	--- --- ---	3.24 3.23 ---	3.45 3.23 2.33	3.03 4.44 2.64	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
		5 x 10^6 IU D_3 (administered daily for 9 days, slaughter after 2 days)	Strip loin Top round Chuck steak	--- --- ---	3.15 3.02 ---	2.84 3.33 2.13	2.82 4.43 2.61	
		125 mg 25-OH D_3 once only	Strip loin Top round	--- ---	3.03 2.80	3.14 3.45	2.57 3.80	

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Table 1 (continued)

		(slaughter after 4 days)	Chuck steak	---	---	2.27	2.68	prior to slaughter and the dose must be upgraded.
		500 µg $1,25(OH)2D_3$ once only (slaughter after 3 days)	Strip loin Top round Chuck steak	--- --- ---	3.62 3.29 -	3.17 3.65 2.51	2.84 4.71 2.85	
Montgomery *et al.* (26)	Crossbreed steers: *Bos Taurus* x *English*, *Bos Taurus* x *Continental* and *Bos indicus*	0×10^6 IU D_3 (placebo) – control group	Strip loin Top round Top butt Mock tender	5.52 5.56 5.06 4.90	5.49 5.38 4.24 4.14	5.02 4.86 4.38 4.41	5.02 5.21 3.69 4.40	Significant interaction was proved between vitamin D_3 dose x muscle x ageing period and WBSF. Vitamin D_3 supplementation causes an increase in Ca^{2+} 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_3 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_3 is used.
		0.5×10^6 IU D_3	Strip loin Top round Top butt Mock tender	5.15 4.16 4.64 4.36	4.48 4.80 4.29 4.08	4.04 3.92 4.05 4.31	4.27 4.59 3.58 4.31	
		1.0×10^6 IU D_3	Strip loin Top round Top butt Mock tender	4.76 4.11 5.06 4.94	4.75 4.37 4.22 4.17	3.99 4.32 4.00 4.39	4.19 4.68 3.31 4.65	
		5.0×10^6 IU D_3 For 8 days before slaughter	Strip loin Top round Top butt Mock tender	4.76 4.46 5.03 5.06	4.54 4.42 4.25 4.44	4.21 4.07 3.87 5.02	4.17 4.87 3.52 4.76	
Rider Sell *et al*. (34)	Crossbreed Angus cull cows (78 months of age)	$0 \times$ IU D_3 (placebo)	Strip loin	4.72	5.30	3.68	3.89	Addition of vitamin D_3 improved tenderness of beef meat (strip loin) obtained from beef cull cows. However, this kind of meat requires at least 14 days of ageing.
		5.0×10^6 IU D_3	Strip loin	**4.15**	6.69	5.11	4.53	
		7.5×10^6 IU D_3 7 days before slaughter	Strip loin	4.67	**3.66**	4.19	3.76	
Montgomery *et al.* (25)	Crossbreed steers	0 IU – Control (placebo)	Strip loin Top round	--- ---	2.80 4.50	2.92 4.01	2.45 4.16	Supplementation with vitamin D_3 of 0.5×10^6 IU/day/animal, improved tenderness of tested muscles (with no negative impact on intravital traits).
		0.5×10^6 IU D_3	Strip loin Top round	--- ---	2.22 3.67	2.79 4.40	2.56 3.59	
		1×10^6 IU D_3	Strip loin Top round	--- ---	2.53 3.52	2.81 3.95	2.48 3.80	
		2.5×10^6 IU D_3	Strip loin Top round	--- ---	2.58 4.29	3.01 4.27	2.67 4.14	
		5×10^6 IU D_3	Strip loin Top round	--- ---	2.47 3.63	2.84 4.25	2.52 4.04	
		7.5×10^6 IU D_3 9 days before slaughter	Strip loin Top round	--- ---	2.31 4.38	2.97 4.14	2.57 3.89	
Montgomery *et al.* (27)	Crossbreed Continental x British: steers 23 months of age	$0 \times$ IU D_3 – placebo	Strip loin Top round	3.58 ---	3.32 3.97	3.25 3.91	3.38 3.74	Addition of vitamin D_3 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 5×10^6 IU of vitamin D_3. Increase in tenderness can be explained by higher intracellular concentration of Ca^{2+} available during proteolysis in ageing process.
		5×10^6 IU D_3	Strip loin Top round	3.11 ---	**3.20** 3.56	2.80 3.37	2.90 3.32	
		7.5×10^6 IU D_3 9 days before slaughter (10th day – slaughter)	Strip loin Top round	3.17 ---	**2.78** 3.32	2.89 3.37	3.02 3.56	

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Table 1 (continued)

Swanek *et al.* (35)	Steers: crossbreed Angus x Hereford and crossbreed Salers x Charolaise	0 x10⁶ IU – Control (placebo)	Strip loin	---	4.70	4.03	4.58	Use of vitamin D_3 for 7 days prior to slaughter caused a decrease in WBSF after 7 days of ageing versus control group. Moreover, it was concluded that addition of a higher dose of vitamin D_3 (7.5 x 10^6 IU) resulted in greater tenderness enhancement, which was mostly visible after 14 days of ageing.
		5 x10^6 IU D_3 7 days before slaughter (7th day – slaughter	Strip loin	---	4.12	3.87	3.60	
Montgomery *et al.* (28)	Crossbreed Continental x British: steers 23 months of age	0 x 10^6 IU D_3 (placebo),	Strip loin	3.58	3.32	3.25	3.38	Supplementation with vitamin D_3, improves tenderness of evaluated muscles, regardless the ageing period; however, the highest tenderness was observed after 14 days. Dose of 5 x 10^6 IU D_3 was without doubt more effective than 7.5 x 10^6 IU D_3.
			Top round	3.97	3.91	3.74	3.87	
		5 x 10^6 IU D_3	Strip loin	3.11	3.20	**2.80**	2.90	
			Top round	3.56	3.37	3.32	3.42	
		7.5 x 10^6 IU D_3 10 days before slaughter (10th day – slaughter)	Strip loin	3.17	2.89	**2.78**	3.02	
			Top round	3.32	3.37	3.56	3.42	

Strip loin – *m. longissimus lumborum*, top round – *m. semimembranosus*, eye of round – *m. semintendinosus*, mock tender – *supraspinatus*, top blade – *infraspinatus*, top butt - *m. gluteus medius*, center roast – *m. rectus femoris*, inside round – *m. adductor femoris*

Selected results of the addition of vitamin D_3 on physical properties of meat, in particular its tenderness, are presented in Table 1.

The use of high doses of vitamin D_3 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 D_3 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 D_3 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 D_3 and sensory traits of beef such as juiciness, flavour, and overall palatability has been shown, while Rafalska (33) indicated a positive effect of vitamin D_3 addition (7.5 and 10 x 10^6 IU) on analysed sensory traits (tenderness, juiciness, and flavour). In addition, the highest marks for tenderness, juiciness, and flavour were showed for strip loin and top round aged for 14 days with 10 x 10^6 IU of vitamin D_3. 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 metalic 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 D_3, which administered to animals for a short period before slaughter causes mobilisation of Ca^{2+} 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 Ca^{2+} concentration in the muscle due to administration of vitamin D_3 is responsible for higher activity of proteolytic enzymes from calpain group. Use of vitamin D_3 in high doses (0.5 x 10^6 to 7.5 x 10^6 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 D_3 (7.5 x 10^6 IU D_3 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 D_3 (6 x 10^6 IU/hd/d) and combination of vitamin D_3 and vitamin E (6 x 10^6 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 D_3 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 D_3 can potentially lead to improved tenderness and larger consumer approval of beef. Addition of substances increasing the intensity of proteolysis process by causing higher concentration of Ca^{2+}, such as vitamin D_3, 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 D_3 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 D_3 feed supplementation will reduce the amount of unsold meat being returned from the retailers to meat processing plants.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The research was performed within the Project no POIG.01.03.01-00-204/09 Optimising of Beef Production in Poland. According to "From Farm to Fork" Strategy co-financed by the European Regional Development Fund under the Innovative Economy Operational Programme 2007–2013.

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