CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Vitamin D-biofortified beef: a comparison of cholecalciferol with synthetic versus UVB-mushroom-derived ergosterol as feed source
Authors	Duffy, Sarah K.;O'Doherty, John V.;Rajauria, Gaurav;Clarke, Louise C.;Hayes, Aoife;Dowling, Kirsten G.;O'Grady, Michael N.;Kerry, Joseph P.;Jakobsen, Jette;Cashman, Kevin D.;Kelly, Alan K.
Publication date	2018-02-21
Original Citation	Duffy, S. K., O'Doherty, J. V., Rajauria, G., Clarke, L. C., Hayes, A., Dowling, K. G., O'Grady, M. N., Kerry, J. P., Jakobsen, J., Cashman, K. D. and Kelly, A. K. (2018) 'Vitamin D-biofortified beef: A comparison of cholecalciferol with synthetic versus UVB-mushroom-derived ergosterol as feed source', Food Chemistry, 256, pp. 18-24. doi: 10.1016/j.foodchem.2018.02.099
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://www.sciencedirect.com/science/article/pii/S0308814618303364 - 10.1016/j.foodchem.2018.02.099
Rights	© 2018 Elsevier Ltd. All rights reserved. This manuscript version is made available under the CC-BY-NC-ND 4.0 license - http://creativecommons.org/licenses/by-nc-nd/4.0/
Download date	2026-09-08 03:46:21
Item downloaded from	https://hdl.handle.net/10468/7768

UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Accepted Manuscript

Vitamin D-biofortified beef: a comparison of cholecalciferol with synthetic versus UVB-mushroom-derived ergosterol as feed source

Sarah K. Duffy, John.V. O'Doherty, Gaurav Rajauria, Louise C. Clarke, Aoife Hayes, Kirsten G. Dowling, Michael N. O'Grady, Joseph P. Kerry, Jette Jakobsen, Kevin D. Cashman, Alan K. Kelly

PII: S0308-8146(18)30336-4
DOI: https://doi.org/10.1016/j.foodchem.2018.02.099
Reference: FOCH 22483

To appear in: *Food Chemistry*

Received Date: 18 September 2017
Revised Date: 26 January 2018
Accepted Date: 19 February 2018

Please cite this article as: Duffy, S.K., O'Doherty, John.V., Rajauria, G., Clarke, L.C., Hayes, A., Dowling, K.G., O'Grady, M.N., Kerry, J.P., Jakobsen, J., Cashman, K.D., Kelly, A.K., Vitamin D-biofortified beef: a comparison of cholecalciferol with synthetic versus UVB-mushroom-derived ergosterol as feed source, *Food Chemistry* (2018), doi: https://doi.org/10.1016/j.foodchem.2018.02.099

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Vitamin D-biofortified beef: a comparison of cholecalciferol with synthetic versus UVB-mushroom-derived ergosterol as feed source

Sarah K. Duffy[a], John. V. O'Doherty*[a], Gaurav Rajauria[a], Louise C. Clarke[a], Aoife Hayes[b], Kirsten G. Dowling[b], Michael N. O'Grady[c], Joseph P. Kerry[c], Jette Jakobsen[d], Kevin D. Cashman[b], Alan K. Kelly[a]

[a]School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland.

[b]Cork Centre for Vitamin D and Nutritional Research, School of Food and Nutritional Sciences, University College Cork, Cork, Ireland.

[c]Food Packaging Group, School of Food and Nutritional Sciences, University College Cork, Cork, Ireland.

[d]National Food Institute, Technical University of Denmark, Søborg, Denmark.

***Corresponding author**

Email: john.vodoherty@ucd.ie (Prof. J. V. O'Doherty)

Phone: +353-1-716 7128

Fax: +353-1-716 1103

ACCEPTED MANUSCRIPT

Abstract

This study investigates dietary fortification of heifer feeds with cholecalciferol and ergocalciferol sources and effects on beef total vitamin D activity, vitamer, respective 25-hydroxymetabolite contents, and meat quality. Thirty heifers were allocated to one of three dietary treatments [(1) basal diet+4000 IU of vitamin D_3 (Vit D_3); (2) basal diet+4000 IU of vitamin D_2 (Vit D_2); and (3) basal diet+4000 IU of vitamin D_2-enriched mushrooms (Mushroom D_2)] for a 30 day pre-slaughter period. Supplementation of heifer diets with Vit D_3 yielded higher ($p < 0.001$) *Longissimus thoracis* (LT) total vitamin D activity (by 38-56%; $p < 0.05$) and serum 25-OH-D concentration (by 20-36%; $p<0.05$), compared to that from Vit D_2 and Mushroom D_2 supplemented animals. Irrespective of vitamin D source, carcass characteristics, sensory and meat quality parameter were unaffected ($p>0.05$) by the dietary treatments. In conclusion, vitamin D_3 biofortification of cattle diets is the most efficacious way to enhance total beef vitamin D activity.

Keywords: cholecalciferol; ergocalciferol; vitamin D_2-enriched mushrooms; heifers; *Longissimus thoracis.*

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

1. Introduction

Vitamin D deficiency and associated health risks are very much to the forefront of public health policy, particularly in Europe and northern latitudes where a high prevalence of vitamin D deficiency has been observed (Cashman et al., 2016). Recent estimates from national surveys in Europe indicate that as much as 55 and 100% of adults (19–64 years) and older adults (> 64 years) have inadequate vitamin D intakes when compared to the Estimated Average Requirement (EAR) (Roman Viñas et al., 2011). Consequently, there is a definite need for innovatively designed natural food-based vitamin D-enhancement strategies which cover a range of stable food sources reflective of diversity in dietary patterns and fortification policies (Black, Seamans, Cashman and Kiely, 2012; Guo, Kliem, Lovergrove and Givens, 2017; Verkaik-Kloosterman, Seves and Ocké, 2017; Wilson, Tripkovic, Hart and Lanham-New, 2017).

The fortification of animal feeds to naturally enhance the vitamin D content of a wide range of food types is an enhancement strategy with high consumer appeal and offers the potential to increase vitamin D intakes at a population level (Duffy et al., 2017; Milešević, Samaniego, Kiely, Glibetić and Roe, 2018). Beef, is one such food that is a likely target for vitamin D biofortification. Additionally, beef is one of the few dietary staple foods which contain vitamin D and more importantly the 25-OH-D metabolite which is more biologically active at increasing total vitamin D content (Roseland, Philips, Patterson, Pehrsson and Taylor, 2018; Uusitalo et al., 2011). Indeed, previous work has successfully demonstrated the ability to enhance the content of vitamin D and/or 25-OH-D through short term dietary supplementation of vitamin D_3, potentially allowing for a 'High in vitamin D' claim on the beef product, along with a modest improvement in beef tenderness (Duffy et al., 2017).

Vitamin D exists in two prominent forms, (i) cholecalciferol (vitamin D_3) produced by the human body through ultraviolet B (UVB) rich sun light exposure and from animal-based food

ACCEPTED MANUSCRIPT

products, or (ii) ergocalciferol (vitamin D_2) derived from exposing ergosterol, a common steroid found in plants, fungi and yeasts (Holick et al., 2008). Cashman et al. (2014), in their investigation of the contribution of food-derived vitamin D_2 to overall vitamin D nutritional status in the human population, suggested that vitamin D_2, as well as possibly 25-OH-D_2 naturally present in beef, may be of potential importance. In terms of enhancing the natural level of vitamin D in meat via biofortification, the choice of vitamer (e.g. vitamin D_2 or D_3) for fortification purposes has been highlighted as an important consideration (Cashman, 2012). In Europe, Article 9t (b) of Council Directive 70/524/EEC allows a maximum content of 4000 IU of vitamin D/kg of complete feeding stuff or of the daily ration of cattle, but as either vitamin D_2 or D_3. This assumes equivalence between both vitamers, but there has been a growing body of evidence from human nutrition studies to suggest that vitamin D_2 may be less effective in raising total serum 25-OH-D compared to an equivalent amount of vitamin D_3 (Tripkovic et al., 2012). This may also be the case for cattle, with implications for uptake into muscle.

It is of note that many species of mushrooms have a high ergosterol content which offers the potential to form vitamin D_2, if they are exposed to UVB radiation (Kalaras, Beelman and Elias, 2012). Indeed, mushroom-derived vitamin D_2 is an under-investigated potential novel food-based source for application in the production of vitamin D-biofortified beef and other red meats, and could potentially be a more efficacious, cost-effective and renewable source compared to synthetic vitamin D_3 and vitamin D_2 alternatives (Itkonen et al., 2016). Therefore, the objective of this study was to assess the effects of addition of synthetic vitamin D_3 and vitamin D_2, as well as UVB-exposed mushroom-derived vitamin D_2, to the diets fed to beef heifers, at the EU allowable level. The study also includes total vitamin D activity, as well as individual vitamers and their 25-hydroxyvitamin metabolites and subsequent effects on meat quality.

ACCEPTED MANUSCRIPT

2. Material and methods

2.1. General

All experimental procedures described in this work were approved by the University College Dublin Animal Research Ethics Committee (AREC-14-05-Kelly) and conducted under experimental license from the Department of Health in accordance with the cruelty to animal act 1876 and the European Communities (amendments of cruelty to animal act, 1876) Regulations (1994).

2.2. Experimental design and dietary treatments

Thirty continental heifers were blocked on the basis of live weight and age and randomly allocated to one of three dietary treatments: (1) basal diet + 4000 IU of vitamin D_3/kg of feed (Vit D_3); (2) basal diet + 4000 IU of vitamin D_2/kg of feed (Vit D_2) and (3) basal diet + 4000 IU of vitamin D_2-enriched mushrooms (Mushroom D_2). Dietary treatments were offered for the final 30 d period pre-slaughter. The basal diet consisted of a standard *ad-libitum* finishing regime of concentrates and forage (straw) offered at a ratio of 90:10. Diets were formulated to meet nutrient requirements of finishing beef heifers and the basic diet contained 110 g/kg of crude protein and 11.4 MJ/kg of metabolizable energy. Detailed ingredient composition and chemical analysis of the diets are presented in Tables 1 and 2. The vitamin D_3 levels in the experimental diets were chosen to comply with EU regulations. The 4000 IU of vitamin D_3/kg/feed in bovine diets is the maximum inclusion rate permitted in the EU (EFSA, 2012). The vitamin D_3 was sourced from DSM, Nutritional Products Limited, UK. The vitamin D_2 was sourced from A & Z Food Additives Co., Limited, Zhejiang, China. The dried vitamin D_2-enriched mushrooms were sourced from Monaghan Mushrooms, Ireland. The mushroom vitamin D_2 content was naturally enhanced, following exposure to synthetic UVB at a dose strength of 1.5 J/cm^2 for 3 s, as previously described by Stepien et al. (2013). The dried

ACCEPTED MANUSCRIPT

mushroom powder was included at 1.82 g/kg of feed, this inclusion level was added to obtain 4000 IU of vitamin D_2/kg of feed. The vitamin D_2-enriched mushroom powder was analysed by high-performance liquid chromatography, for vitamin D concentration prior to diet manufacture.

2.3. Feed management and live weight

Heifers were housed in a slatted floor building; dietary treatments were equally represented across 5 pens of 6 heifers per pen with a 2.5 m^2 animal space allowance. Heifers were individually fed using a Calan Broadbent controlled feeding system (American Calan, Northwood, New Hampshire 03261, USA). Each animal was fitted with a unique key hung from a neck cord. The animal's sensor key recognises the electronic circuit board on each feeder and unlocks the feed door. Feed was weighed in and refusals weighed back on a daily basis to monitor dry matter intake; refusals were discarded daily. Feed samples were taken at diet manufacture and weekly throughout the experimental period for chemical analysis and stored at -20 °C pending laboratory analysis. Heifers were weighed weekly throughout the experiment, using a 'Weigh Crate' (O'Donovan's Engineering, Cork, Ireland) and the 'Winweigh' software package (Tru-test Ltd., Auckland, New Zealand). Average daily gains during the experimental period were calculated, using linear regression of live weight against recording date for each heifer and using the REG procedure in SAS (SAS , 2006).

2.4. Carcass analysis

Post-slaughter carcass weight was determined for each heifer (hot carcass weight × 0.98). The video imaging analysis carcass classification system (VBS 2000, E+V, Germany) mechanically assigned each carcass side a carcass conformation and fat score on a 15 point scale, using the EU Beef Carcass Classifications Scheme (Hickey, Keane, Kenny, Cromie and Veerkamp, 2007). The *Longissimus thoracis* (LT) (the cube roll, commercial cut that

ACCEPTED MANUSCRIPT

begins between the 5th and 6th rib and ends between the 10th and 11th rib) was excised after 14 d of wet ageing at 4 °C, as described by Moran et al. (2017). Thereafter, the LT was cut into 2.5 cm thick steaks for vitamin D analysis, chemical composition and tenderness analysis. All steaks were vacuum-packed and frozen at –20 °C prior to analysis.

2.5. Chemical analysis of feed

Feed samples were analysed for dry matter (DM), ash, nitrogen (N), gross energy (GE), ether extract (EE) and neutral detergent fibre (NDF). All samples were dried at 55 °C for 72 hours (h), milled and passed through a 1 mm screen (Christy and Norris, Chelmsford, England) prior to analysis. Feed DM was determined after drying overnight (16 h minimum) at 105 °C. The crude ash content of the diets was determined at 550 °C for 6 h, after ignition of weighed samples in a muffle furnace (Nabertherm, Bremen, Germany).The GE of feed was measured, using an adiabatic bomb calorimeter (Parr Instruments, IL, USA). The N content of the diets was determined as N × 6.25, using a LECO FP 528 instrument (LECO Instruments, USA). Ether extract concentration (g/kg DM) of feed was determined, using light petroleum ether and Soxtec instrumentation (Tecator, Sweden). The NDF content of the feed was determined with a Fibretec extraction unit. All samples were measured in duplicate.

2.6. Blood sampling, serum and muscle calcium determination

Prior to slaughter serum samples were taken via the jugular vein, using lithium/heparin vacationers (BD- Plymouth, UK). The blood was stored overnight at 4 °C and centrifuged at 4720 *g* for 20 minutes (min) at 4 °C (40R centrifuge, Thermo Fisher Scientific, Ireland), after which the serum layer was subsequently removed from the blood cell layer and stored in 1.5 ml tubes at -20 °C until required for further vitamin D and calcium (Ca) analysis. Serum and LT Ca was determined with an atomic absorption spectrophotometer (Varian 50, Varian, Santa Clara, CA, USA), using the method of Foote, Horst, Huff-Lonergan, Trenkle, Parrish and Beitz. (2004), with minor modifications. Briefly, serum samples were prepared and

ACCEPTED MANUSCRIPT

measured in duplicate, 100 µl of plasma were diluted with 5 ml of 0.1% lanthanum oxide solution. A standard curve was prepared by using 0, 5, 10 and 15 mg/dl of $CaCl_2$. *Longissimus thoracis* samples were measured similarly in duplicate; approximately 5 g of wet tissue were excised from each beef steak, homogenised using a blender (Waring commercial blender), and dried overnight at 105 °C. Meat samples were then ashed at 550 °C in a muffle furnace (Nabertherm, Bremen, Germany) for 6 h. Ashed samples were suspended in 25 ml of 3 N hydrochloric acid. Samples were then analysed by diluting 1 ml of hydrochloric acid preparation with 4 ml of 0.1% lanthanum oxide solution.

2.7. Longissimus thoracis chemical composition

Longissimus thoracis samples were analysed for moisture, ash, N and inter-muscular fat in duplicate. Moisture and ash were determined according to the method of AOAC 2005 (950.46B and 920.153). First, approximately 50 g of LT tissue were excised from each beef steak, trimmed of external fat and connective tissue and homogenised using a blender (Waring commercial blender). *Longissimus thoracis* moisture, ash, N and inter-muscular fat content were determined as previously described for chemical analysis of feed. An internal standard ERM-BB501 (LGC standards, Middlesex, UK) was used for the calibration of LT chemical analysis.

2.8. Vitamin D analysis of LT, serum and feed

The 'total vitamin D activity' of LT steaks and vitamin D_3, 25-OH-D_3, vitamin D_2 and 25-OH-D_2 metabolite contents of experimental diets were analysed using modifications of a sensitive liquid chromatography-tandem mass spectrometry (LC/MS/MS) method, as described elsewhere by Roseland et al. (2016). Total vitamin D activity of LT was defined as [vitamin D_3 + (25-OH-D_3 × 5) + vitamin D_2 + (25-OH-D_2) × 5]. The conversion factor of 5 is applied to the 25-OH-D_3 and 25-OH-D_2 content on the basis of efficacy data from a

ACCEPTED MANUSCRIPT

randomized controlled trial with oral vitamin D_3 and 25-OH-D_3 in healthy adults (Cashman et al., 2012), and is a factor commonly used in several food-composition tables (Finglas et al., 2015). It should be noted, however, that equivalent data do not exist for vitamin D_2 and 25-OH-D_2, so we assumed a conversion factor of 5 in our calculation of total vitamin D activity of LT. Serum 25-OH-D_3 and 25-OH-D_2 were analysed by LC-MS/MS, as described by Cashman et al. (2013). Serum total 25-OH-D concentration was calculated as [25-OH-D_3 + 25-OH-D_2].

2.9. Warner-Bratzler shear force (WBSF) determination of LT

Warner Bratzler shear force analysis was carried out according to Wheeler, Shackelford and Koohmaraie (1996), with some modifications. Briefly, LT samples were de-frosted overnight at 4 °C, trimmed of external fat, weighed and cooked in open vacuum bags in a circulating water bath (model no. Y38, Grant Instruments Ltd., Barrington, Cambridge CB2 IBR, UK) set at 72 °C, until an internal temperature of 70 °C was achieved. Internal temperature was monitored by placing a thermocouple in the geometric centre of each steak; four steaks were cooked per water bath to ensure water circulation was consistent around all samples. Steaks were cooled sufficiently at room temperature and were placed in storage bags (to prevent dehydration) and were stored at 4 °C overnight. Coring was carried out on chilled samples after 24 h, eight (1.25 cm diameter) cores, parallel to longitudinal orientation of fibres, were collected from each sample and sheared, using an Instron universal testing machine (Model no. 5543, Instron Europe, High Wycombe, Bucks, UK) instrument equipped with a Warner Bratzler shearing device. Cores were sheared at a crosshead speed of 200 - 250 mm/min. Cores that were not uniform in diameter or containing obvious connective tissue defects were discarded. Calibration was carried out before each analysis; load cell was 500 Newtons (NT) and the instrument was allowed to warm for 30 mins before use. Before each run, 3 blank runs below 1 NT were recorded; blanks were also recorded after every 10 samples. For

ACCEPTED MANUSCRIPT

analysis of the data, Instron Series IX Automated Materials Testing System software for Windows (Instron Corporation, Bucks, UK) was employed.

2.10. Sensory evaluation

Sensory analysis of cooked beef steaks (n = 30) was performed in duplicate (10 mins/session) by a total of 40 naïve assessors (untrained) (70% males, 30% females ranging in age from 21-63 years) over two analysis days, as described by O'Sullivan, Byrne, and Martens, (2003). On each analysis day, beef steaks (n = 5) from each dietary treatment were covered with aluminium foil and cooked in an oven (Zanussi Professional, Model 10 GN1/1, Conegliano, Italy) at 200 ºC until an internal meat temperature of 72 ºC was reached. Following cooking, steaks were cooled, cut into 2 cm × 2 cm cubes and pooled for each treatment group, from which cubes were randomly selected, placed on plates and identified with random three-digit codes. Sample presentation order (3 samples/plate × 2 plates = 6 samples/panellist) was randomised to prevent any flavour carryover effects. Prior to serving to panellists, beef samples were re-heated in a microwave oven for 20 seconds to release the meat odour and flavour. Sensory analysis was undertaken in University College Cork's sensory laboratory panel booths in accordance with the ISO (2007) international standard regulations. Assessors were provided with water to cleanse their pallets between samples. Hedonic sensory analysis descriptors were appearance, odour, liking of texture, liking of flavour and overall acceptability. Off-flavour was selected as an intensity sensory analysis descriptor. Assessors were asked to rate samples for each attribute by marking a point on a 10 cm line scale ranging from 0 (extremely dislike/none) to 10 (extremely like/extreme) with anchor points on each end.

2.11. Statistical analysis

Data were checked for normality and homogeneity of variance by histograms, qq plots, and formal statistical tests as part of the UNIVARIATE procedure of SAS (SAS, 2006). Data that

ACCEPTED MANUSCRIPT

were not normally distributed were transformed by raising the variable to the power of lambda. The appropriate lambda value was obtained by conducting a Box-Cox transformation analysis, using the TRANSREG procedure of SAS. The transformed data were used to calculate P values. The corresponding least squares means (LSM) and standard errors of the non-transformed data are presented in the results for clarity. Animal performance, vitamin D meat content, serum 25-OH-D and shear force data were analysed, using a randomised mixed model ANOVA with the MIXED procedure of SAS. Fixed effects in the statistical model include dietary treatment and pen. Block was included as a random effect. Differences between treatments were determined by F-test, using Type III sums of squares. The PDIFF command incorporating the Tukey test was applied to evaluate pairwise comparisons between treatment means. Mean values were considered to be statistically significantly different when $p < 0.05$ and considered a tendency when $p < 0.10$ but > 0.05. Least square means are reported with pooled standard errors (SEMs).

3. Results

3.1. Animal performance and carcass data

The effects of dietary treatment on heifer performance and carcass characteristics are presented in Table 3. Dietary treatment had no effect ($p > 0.05$), on any of the animal performance parameters examined, including DM intake, slaughter weight and average daily gain. Similarly, carcass weight, kill out %, carcass conformation and fat score were not affected ($p > 0.05$) by dietary treatment**.**

3.2. Vitamin D activity in LT steaks and serum 25-OH-D

The effects of dietary treatment on serum 25-OH-D_3 and 25-OH-D_2, separately and combined as serum total 25-OH-D, are presented in Table 4. Results showed that heifers offered the Vit D_3 exhibited the highest ($p < 0.001$) serum 25-OH-D_3 content (compared to

ACCEPTED MANUSCRIPT

other dietary treatments) and serum 25-OH-D_3 concentrations did not differ between heifers offered the Vit D_2 or Mushroom D_2 in this 30 day feeding period. As expected, heifers offered the Vit D_2 and Mushroom D_2 had a greater ($p < 0.001$) serum 25-OH-D_2 content compared to the Vit D_3 treatment group. Serum total 25-OH-D content was highest ($p < 0.001$) for heifers offered the Vit D_3, compared to either, Vit D_2 or Mushroom D_2, with no significant difference ($p > 0.05$) between these latter two groups. The effects of dietary treatments on LT vitamin D compounds (vitamin D_3, 25-OH-D_3, vitamin D_2 and 25-OH-D_2) and LT total vitamin D activity (i.e. vitamin D_3 + 25-OH-D_3 × 5 + vitamin D_2 + 25-OH-D_2 × 5) are presented in Table 4. As expected, heifers offered the Vit D_3 had higher LT vitamin D_3 ($p < 0.001$) and LT 25-OH-D_3 metabolites, compared to either Vit D_2 or Mushroom D_2, and showed no significant difference ($p > 0.05$) between these latter groups. Also, as expected, heifers offered the Vit D_2 and the Mushroom D_2 exhibited the highest ($p < 0.001$) LT vitamin D_2 and LT 25-OH-D_2 metabolites compared to the Vit D_3 treatment.

For total vitamin D activity, heifers offered the Vit D_3 had the highest ($p < 0.05$) compared to either of the two vitamin D_2 supplementation sources. Additionally, LT total vitamin D activity did not differ ($p > 0.05$) between the Vit D_2 and the Mushroom D_2 treatment groups.

3.3. Calcium activity in serum and LT steaks and beef WBSF values, sensory analysis and Longissimus thoracis chemical composition

The effects of dietary treatment on serum and LT Ca activity are presented in Fig. 1 (a-b) and WBSF values are presented in Table 5. Calcium concentrations in serum and beef were not different ($p > 0.05$) across the vitamin D supplementation treatment groups. Dietary treatment did not alter ($p > 0.05$) WBSF values. Vitamin D source had no significant impact ($p > 0.05$) on any of the sensory parameters of the LT evaluated, including appearance, odour, texture, flavour, overall acceptance and off-flavour. For LT muscle, the chemical composition

ACCEPTED MANUSCRIPT

(including moisture, protein, ash or inter muscular fat components) was not affected with average values across treatment of 69.6%, 1.0%, 25.9% and 3.4% for moisture, ash, protein and inter-muscular fat, respectively, as presented in Table 5.

4. Discussion

As a stable dietary food for many populations, beef is now recognised as an important natural source of vitamin D, and the biofortification of livestock feeds is a strategy shown to successfully boost vitamin D beef levels even further (Duffy et al., 2017). This approach has strong consumer appeal and when combined with other food-wide strategies could go some way in bridging the gap between current population vitamin D intakes and recommended requirements (Cashman et al., 2016).

The choice of vitamin D compound to be added to the livestock feeds is an important consideration in terms of the overall biofortification approach (Cashman, 2012). Accordingly, the present study assessed the effects of vitamin D_3 and vitamin D_2 (synthetic and naturally-derived from UVB-exposed mushrooms) fortification of livestock feeds on beef total vitamin D activity, as well as on its vitamer and associated 25-hydroxy metabolite content, and on key aspects of meat quality. Of importance, results showed that supplementation of livestock feeds with Vit D_3 led to significantly greater (38-56%) total vitamin D activity of the resulting beef steak than that from either Vit D_2 or Mushroom D_2 treatment sources (all provided at the EU allowable level for vitamin D). These values are comparable to those in a previous study by Duffy et al. (2017), where similar animals and diets were fed the Vit D_2 and Mushroom D_2 and had an (100 and 60%) increase in total LT activity compared to the negative control (0 IU).

It is important to stress that while there were no differences in animal performance and carcass characteristics, which in this sense supports the European Food Safety Authority's

ACCEPTED MANUSCRIPT

Panel on Additives and Products or Substances used in Animal Feed assumption of equivalence of vitamin D_2 and D_3, from a human nutrition perspective there are differences. The meat value from the vitamin D_2-supplemented animals was less, such that typical UK average serving sizes of steak (144 g) arising from the LT of Vit D_3- Vit D_2- and Mushroom D_2–supplemented groups would have 2.0, 1.5 and 1.3 µg of total vitamin D activity, respectively. Consumption of a typical serving size of vitamin D_3-biofortified meat would contribute ~20% to the current EAR of 10 µg/d (Institute of Medicine, 2011). Despite modestly less overall vitamin activity compared to that arising from vitamin D_3-biofortified beef, there was no difference in total vitamin D activity in LT of animals supplemented with Vit D_2 and Mushroom D_2, and consumption of typical serving sizes of steaks from these would contribute ~15 and ~13% to the EAR, respectively. This livestock study, to the author's knowledge, is the first to use and test dried UVB-exposed mushroom as a natural novel feed ingredient. The analytical data for the vitamin D-biofortified beef, irrespective of which form of vitamin D was used, are also important as it is deficient in many food compositional databases, particularly the contribution attributed to 25-OH-D_3 and 25-OH-D_2. Fundamentally, in order to make a nutrition claim on the vitamin D status of beef, certified vitamin D and 25-OH-D contents will be a necessity and in time will likely be a labelling prerequisite (Taylor et al., 2014).

The modestly lower effectiveness of vitamin D_2 supplementation, be it as synthetic or via the more natural UVB-exposed mushroom source, in terms of resulting total vitamin D activity in the beef, is likely to be associated with the significantly lower (by 20-36%) serum total 25-OH-D in these two treatment groups compared to that of the vitamin D_3-supplemented group. This would have consequences for uptake into the muscle tissue. While this is, to our knowledge, one of the first such comparisons in livestock, the findings agree with an increasing body of evidence from intervention studies with vitamin D_2 versus D_3 in human

ACCEPTED MANUSCRIPT

subjects which likewise suggest a lower effectiveness of supplement vitamin D_2 in raising serum total 25-OH-D (Tripkoic et al., 2012). Various mechanisms have been proposed to explain these findings. For example, vitamin D_3 sources may increase systematic 25-OH-D to a greater extent due to a lower affinity of circulating vitamin D-binding protein for vitamin D_2 compared to vitamin D_3, leading to a more rapid metabolism and clearance of vitamin D_2 from blood (Armas, Hollis and Heaney, 2004) or a higher affinity of the hepatic-based 25-hydroxylase enzyme for vitamin D_3 compared to vitamin D_2 (Horst and Littledike, 1982). There has also been recent evidence to suggest that increasing the vitamin D_2 intake (by supplementation or even by UVB-exposed mushrooms) in healthy humans and increasing serum 25-OH-D_2 concentrations, leads to a concomitant decrease in serum 25-OH-D_3 concentrations, thus limiting the overall response of serum total 25-OH-D (Cashman, Kiely, Seamans and Urbain, 2016).

Skeletal muscle cells are a target organ for vitamin D metabolites, as the vitamin D receptor is expressed in large quantities in muscle cells (Hamilton, 2010) and thus may benefit from vitamin D enrichments. While structurally, vitamin D_2 differs from vitamin D_3 by the addition of a double bond, metabolism of both vitamers occurs through the same pathway in cattle (Horst and Littledike, 1982). Although both forms contribute to the overall signalling events of vitamin D, vitamin D_3 is considered the predominant form in cattle (Horst and Littledike, 1982). Vitamin D_3-enriched diets supplemented prior to slaughter have also been reported to improve post-mortem proteolysis and reduce shear force values, thereby improving beef tenderness; however in the majority of these cattle studies, supplementation was at ultra-high levels, posing potential toxicity concerns (Montgomery, Parrish, Beitz, Horst, Huff-Lonergan, and Trenkle, 2000). Our group has shown (Duffy et al., 2017) that raising vitamin D_3 (at allowable EU supplementation) levels in the diet modestly improved beef tenderness (indicative by a decrease in shear force values of LT muscle), which was

ACCEPTED MANUSCRIPT

most likely due to the interactive roles that vitamin D and Ca play in the muscle proteolysis and tenderisation process (Swanek et al., 1999). However, the role that vitamin D_2 plays in meat tenderisation is not at all elucidated. In the present study, instrumental shear force values were similar for all dietary treatment, regardless of vitamin D dietary source. This may be due to the fact that, even though serum 25-OH-D levels differed modestly between the vitamin D_3- and D_2-supplemented groups, the Ca levels were unaffected and free Ca and Ca-dependent proteases are both centrally involved in the *post mortem* tenderization process of beef (Swanek et al., 1999). The differences in serum 25-OH-D between the vitamin D_3- and D_2-supplemented groups may not have been of a magnitude to induce changes in serum Ca, which was seen in our previous study where serum 25-OH-D changes in heifers were considerable due to the vitamin D_3 dose-related (0, 2000 and 4000 IU/kg) design of the trial (Duffy et al., 2017). The lack of effect in terms of instrumental shear force values of LT muscle between groups may explain the lack of effect on any of the sensory parameters evaluated, including appearance, odour, texture, flavour, overall acceptance and off flavour in the present study. The *post mortem* ageing process can be identified by enhancement of beef sensory quality. However the present findings indicate that vitamin D source supplementation causes no negative alteration on sensory evaluation in aged beef; this is in line with our previous study (Duffy et al., 2017) which reported no difference in sensory properties compared to the control (0 IU of vitamin D_3/ kg of feed) when heifer diets were enriched with vitamin D_3 (2000 IU – 4000 IU of vitamin D_3/ kg of feed). Furthermore, these findings are also in line with those of Montgomery et al. (2000) who reported no differences in any of the key sensory traits for aged steaks of beef cattle supplemented with super-nutritional levels (0 – 7.5×10^6) of vitamin D_3.

ACCEPTED MANUSCRIPT

4. Conclusion

Vitamin D_3 supplementation of cattle diets is more effective for increasing total beef vitamin D content than are natural or synthetic vitamin D_2 sources for a 30 day feeding period. The biofortification of heifer diets with 4000 IU of vitamin D_3 will contribute 20% per 100 g of beef to the EAR for vitamin D. Irrespective of vitamin D source, no negative alterations to any sensory or meat quality parameters is an important finding from a consumer acceptance viewpoint.

Conflict of interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication.

Acknowledgments

This research is funded by the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 613977 (ODIN) and the Irish Department of Agriculture, Food and The Marine under its Food Institutional Research Measures.

References

AOAC.950.46B. (2005). Moisture in Meat and Meat Products. Official methods of analysis, 18th edition. AOAC International, Gaithersburg, MD, USA.

AOAC.920.153. (2005). Ash in Meat and Meat Products. Official methods of analysis, 18th edition. AOAC International, Gaithersburg, MD, USA.

Armas, L. A., Hollis, B. W., & Heaney, R. P. (2004). Vitamin D_2 is much less effective than vitamin D_3 in humans. *The Journal of Clinical Endocrinology & Metabolism, 89*(11), 5387-5391.

ACCEPTED MANUSCRIPT

Black, L. J., Seamans, K. M., Cashman, K. D. & Kiely, M. (2012). An updated systematic review and meta-analysis of the efficacy of vitamin D food fortification. *The Journal of Nutrition*, *142*, 1102-1108.

Burild, A., Frandsen, H. L., Poulsen, M. & Jakobsen, J. (2014). Quantification of physiological levels of vitamin D_3 and 25-hydroxyvitamin D_3 in porcine fat and liver in subgram sample sizes. *Journal of Separation Science, 37,* 2659-2663.

Cashman, K. D. (2012). The role of vitamers and dietary-based metabolites of vitamin D in prevention of vitamin D deficiency. *Food and Nutrition Research*, *56*(1), 5383.

Cashman, K. D., Dowling, K. G., Skrabakova, Z., Gonzalez-Gross, M., Valtuena, J., De Henauw, S., Moreno, L., Damsgaard, C. T., Michaelsen, K. F., Molgaard, C., Jorde, R., Grimnes, G., Moschonis, G., Mavrogianni, C., Manios, Y., Thamm, M., Mensink, G. B., Rabenberg, M., Busch, M. A., Cox, L., Meadows, S., Goldberg, G., Prentice, A., Dekker, J. M., Nijpels, G., Pilz, S., Swart, K. M., Van Schoor, N. M., Lips, P., Eiriksdottir, G., Gudnason, V., Cotch, M. F., Koskinen, S., Lamberg-Allardt, C., Durazo-Arvizu, R. A., Sempos, C. T. & Kiely, M. (2016). Vitamin D deficiency in Europe: pandemic? *The American Journal of Clinical Nutrition*, *103*, 1033-44.

Cashman, K. D., Kiely, M., Seamans, K. M. & Urbain, P. (2016). Effect of Ultraviolet Light–Exposed Mushrooms on Vitamin D Status: Liquid Chromatography–Tandem Mass Spectrometry Reanalysis of Biobanked Sera from a Randomized Controlled Trial and a Systematic Review plus Meta-Analysis. *The Journal of Nutrition*, *146*(3), 565-575.

Cashman, K. D., Kinsella, M., McNulty, B. A., Walton, J., Gibney, M. J., Flynn, A. & Kiely, M. (2014). Dietary vitamin D_2–a potentially underestimated contributor to vitamin D nutritional status of adults? *British Journal of Nutrition*, *112*, 193-202.

Cashman, K. D., Seamans, K. M., Lucey, A. J., Stocklin, E., Weber, P., Kiely, M., & Hill, T. R. (2012). Relative effectiveness of oral 25-hydroxyvitamin D_3 and vitamin D_3 in raising

ACCEPTED MANUSCRIPT

wintertime serum 25-hydroxyvitamin D in older adults. *The American Journal of Clinical Nutrition*, *95*(6), 1350-1356.

Cashman, K.D., Kiely, M., Kinsella, M., Durazo-Arvizu, R. A., Tian, L., Zhang, Y., Lucey, A., Flynn, A., Gibney, M. J., Vesper, H. W., Phinney, K. W., Coates, P. M., Picciano, M. F. & Sempos, C. T. (2013). Evaluation of Vitamin D Standardization Program protocols for standardizing serum 25-hydroxyvitamin D data: a case study of the program's potential for national nutrition and health surveys. *The American Journal of Clinical Nutrition*, *97*, 1235-1342.

Duffy, S. K., O'Doherty, J. V., Rajauria, G., Clarke, L. C., Cashman, K. D., Hayes, A., O'Grady, M. N., Kerry, J. P & Kelly, A. K. (2017) Cholecalciferol supplementation of heifer diets increases beef vitamin D concentration and improves beef tenderness. *Meat Science, 134,* 103-110.

European food safety authority (EFSA). (2012) Scientific opinion on the safety and efficy of vitamin D_3 (cholecalciferol) as a feed additive for chickens for fattening, turkeys, other poultry, pigs, piglets (suckling), calves for rearing, calves fro fattening, bovines, pvines, equines, fish amd other animal species or categories, based on a dossier submitted by DSM. *EFSA Journal, 10*(12), 2968-2993.

Finglas, P. M., Roe, M. A., Pinchem, H. M., Berry, R., Church, S. M., Dodhia, S. K., Farron-Wilson, M., & Swan, G. (2015). McCance. & Widdowson's The composititon of foods: seventh sumary edition. Cambridge: Royal Society of Chemistry. pp. 644.

Foote, M. R., Horst, R. L., Huff-Lonergan, E. J., Trenkle, A. H., Parrish, F. C., JR. & Beitz, D. C. (2004). The use of vitamin D_3 and its metabolites to improve beef tenderness. *Journal of Animal Science*, *82,* 242-9.

ACCEPTED MANUSCRIPT

Guo, J., Kilem, K. E., Lovergrove, J. A., Givens, D. I. (2017) Effect of production system, supermarket and purchase date on the vitamin D content of eggs at reatil. *Food Chemistry,* 221, 1021-1025.

Hamilton, B. 2010. Vitamin D and Human Skeletal Muscle. *Scandinavian Journal of Medicine & Science in Sports*, 20, 182-190.

Hickey, J. M., Keane, M. G., Kenny, D. A., Cromie, A. R., & Veerkamp, R. F. (2007). Genetic parameters for EUROP carcass traits within different groups of cattle in Ireland. *Journal of Animal Science, 85*(2), 314-321.

Holick, M. F., Biancuzzo, R. M., Chen, T. C., Klein, E. K., Young, A., Bibuld, D., Reitz, R., Salameh, W., Ameri, A. & Tannenbaum, A. D. (2008). Vitamin D_2 is as effective as vitamin D_3 in maintaining circulating concentrations of 25-hydroxyvitamin D. *The Journal of Clinical Endocrinology & Metabolism, 93*(3), 677-681.

Horst, R. L., & Littledike, E. T. (1982). Comparison of plasma concentrations of vitamin D and its metabolites in young and aged domestic animals. *Comparative Biochemistry and Physiology, Part B, 73*(3), 485-489.

Institute of Medicine (2011). Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: The National Academies Press.

International Organisation for standarization (2007). ISO 8589, Sensory analysis- General guidance for the design of test rooms, 1-16.

Itkonen, S. T., Skaffari, E., Saaristo, P., Saarnio, E. M., Erkkola, M., Jakobsen, J., Cashman, K. D., & Lamberg-Allardt, C. (2016). Effects of vitamin D_2-fortified bread v. supplementation with vitamin D_2 or D_3 on serum 25-hydroxyvitamin D metabolites: an 8-week randomised-controlled trial in young adult Finnish women. *British Journal of Nutrition, 115*(07), 1232-1239.

ACCEPTED MANUSCRIPT

Kalaras, M. D., Beelman, R. B., & Elias, R. J. (2012). Effects of postharvest pulsed UV light treatment of white button mushrooms (Agaricus bisporus) on vitamin D_2 content and quality attributes. *Journal of Agricultural and Food Chemistry, 60*(1), 220-225.

Milešević, J., Samaniego, L., Kiely, M., Glibetić, M.,& Roe, M. (2018). Specialized food composition dataset for vitamin D content in foods based on European standards: Application to dietary intake assessment. *Food Chemistry, 240,* 544-549.

Montgomery, J. L., Parrish, F. C., JR., Beitz, D. C., Horst, R. L., Huff-lonergan, E. J. & Trenkle, A. H. (2000). The use of vitamin D_3 to improve beef tenderness. *Journal of Animal Sciece, 78*, 2615-21.

Moran, L., O'Sullivan, M. G., Kerry, J. P., Picard, B., McGee, M., O'Riordan, E. G., & Moloney, A. P. (2017). Effect of a grazing period prior to finishing on a high concentrate diet on meat quality from bulls and steers. *Meat Science, 125*, 76-83.

O'Sullivan, M., Byrne, D. & MartenS, M. (2003). Evaluation of pork colour: sensory colour assessment using trained and untrained sensory panellists. *Meat Science*, *63*, 119-129.

Verkaik-Kloosterman, J., Seves, S. M., & Ocké, M. C. Vitamin D concentrations in fortified foods and dietary supplements intended for infants: Implications for vitamin D intake. *Food Chemistry*, 221, 629-635.

Roman Viñas, B., Ribas Barba, L., Ngo, J., Gurinovic, M., Novakovic, R., Cavelaars, A., De Groot, L. C. P. G. M., Van't Veer, P., Matthys, C. & Serra Majem, L. (2011). Projected Prevalence of Inadequate Nutrient Intakes in Europe. *Annals of Nutrition and Metabolism, 59,* 84-95.

Roseland, J. M., Patterson, K. Y., Andrews, K. W., Phillips, K. M., Phillips, M. M., Pehrsson, P. R., Dufresne, G. L., Jakobsen, J., Gusev, P. A. & Savarala, S. (2016). Interlaboratory Trial for Measurement of Vitamin D and 25-Hydroxyvitamin D [25(OH) D] in Foods

ACCEPTED MANUSCRIPT

and a Dietary Supplement Using Liquid Chromatography–Mass Spectrometry. *Journal of Agricultural and Food Chemistry, 64*, 3167-3175.

Roseland, J. M., Philips, K. M., Patterson, K. Y., Pehrsson., Taylor, C. L. (2018) Vitamin D in Foods: An Evolution of Knowledge. Vitamin D (fourth edition. Vol. 2: Health, Disease and Therapeutics). Academic Press, 41-77.

SAS Institute Inc., (2006). Base SAS® 9.13 Procedures Guide (Second edition. Vols. 1, 2, 3 and 4. Cary, NC;SAS Institute Inc.

Stepien, M., O'Mahony, L., O'Sullivan, A., Collier, J., Fraser, W. D., Gibney, M. J., Nugent, A. P. & Brennan, L. (2013). Effect of supplementation with vitamin $D_{(2)}$-enhanced mushrooms on vitamin D status in healthy adults. *Journal of Nutritional Science*, *2*, e29, 1-9.

Swanek, S. S., Morgan, J. B., Owens, F. N., Gill, D. R., Strasia, C. A., Dolezal, H. G., & Ray, F. K. (1999). Vitamin D_3 supplementation of beef steers increases longissimus tenderness. *Journal of Animal Science, 77*(4), 874-881.

Taylor, C. L., Patterson, K. Y., Roseland, J. M., Wise, S. A., Merkel, J. M., Pehrsson, P. R. & Yetley, E. A. (2014). Including Food 25-Hydroxyvitamin D in Intake Estimates May Reduce the Discrepancy between Dietary and Serum Measures of Vitamin D Status. *The Journal of Nutrition*, *144*, 654-659.

Tripkovic, L., Lambert, H., Hart, K., Smith, C. P., Bucca, G., Penson, S., Chope, G., Hyppönen, E., Berry, J., vieth, R. & Lanham-new, S. 2012. Comparison of vitamin D_2 and vitamin D_3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. *American Journal of Clinical Nutrition, 95(6),* 1357-1364.

Uusitalo, U., Kronberg-Kippila, C., Aronsson, C. A., Schakel, S., Schoen, S., Mattisson, I., Reinivuo, H., Silvis, K., Sichert-Hellert, W., Stevens, M., Norris, J. M., Virtanen, S. M.,

ACCEPTED MANUSCRIPT

& the, TEDDY study group. (2011). Food composition database harmonization for between-country comparisons of nutrient data in the TEDDY Study. *Journal of Food Composition and Analysis : an official publication of the United Nations University, International Network of Food Data Systems, 24*(4-5), 494-505.

Wheeler, T., Shackelford, S., & Koohmaraie, M. (1996). Sampling, Cooking, and Coring Effects on Warner-Bratzler Shear Force Values in Beef. *Journal of Animal Science, 74*, 1553-1562.

Wilson, L. R., Tripkovic, L., Hart, K. H., Lanham-New, S. A. (2017). Vitamin D deficiency as a public health issue: using vitamin D_2 or vitamin D_3 in future fortification strategies. *Proceedings of the Nutrition Society, 76,* 392-399.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

List of Figures

Fig. 1. Effect of dietary treatment on (a) serum calcium (Ca) concentration and (b) LT Ca content.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Highlights

UVB-exposed vitamin D_2-rich mushroom used as a natural novel feed ingredient

Dietary biofortification can successfully enhance beef vitamin D content

Vitamin D_3 is efficient supplementation source to boost beef vitamin D activity

Consumption of vitamin D_3-biofortified diet can contribute ~20% to the current EAR

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

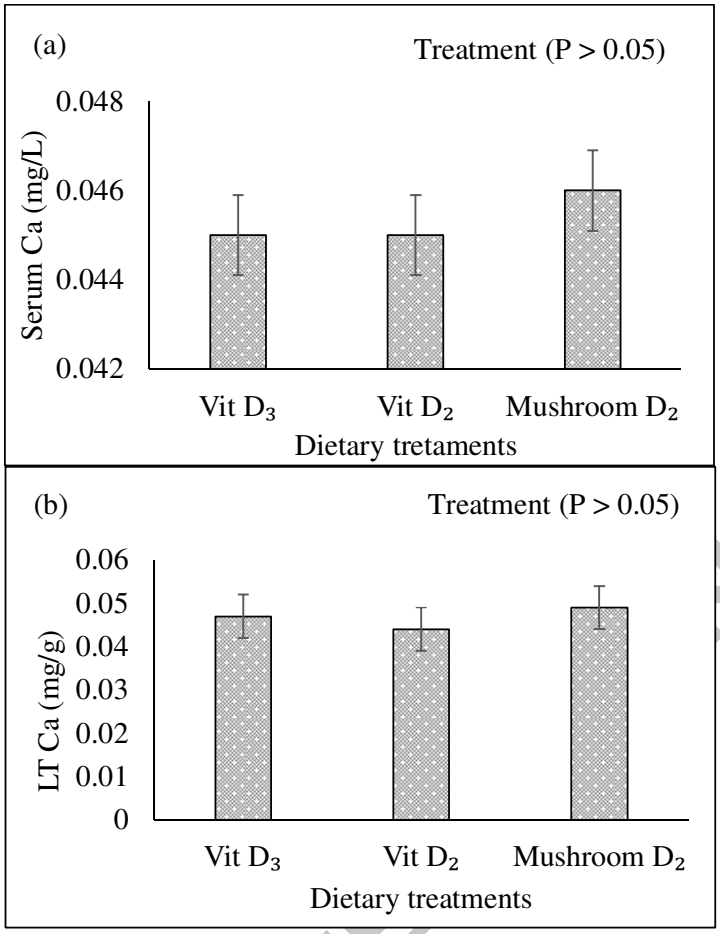

Fig. 1.

ACCEPTED MANUSCRIPT

Table 1

Ingredients' composition of the basal diet offered to heifers over the experimental period. Ingredients (g/kg), unless otherwise indicated.

Ingredient	(g/kg)*
Rolled barley	250
Ground maize	250
Beet pulp nuts	140
Soy hulls	140
Rape meal	70
Wheat distillers dried grain	70
Cane molasses	50
Minerals and vitamins[¥]	30
Concentrate : forage ratio	90:10

*Vitamin D_3 was added to the basal diet in order to obtain 3 levels of dietary treatments: (1) basal diet + 4000 IU of vitamin D_3/kg of feed (Vit D_3); (2) basal diet + 4000 IU of vitamin D_2/kg of feed (Vit D_2); (3) basal diet + 4000 IU of vitamin D_2-enriched mushrooms/kg of feed (Mushroom D_2).
Vitamin D_2-enriched diets was added to the at an inclusion level of 1.82 mushroom D_2 g/kg of feed to obtain the inclusion level of 4000 IU/kg of feed.
[¥]The premix provided vitamins and minerals (per kg diet) as follows: 5000 IU vitamin A, 1000 IU vitamin E, 25 mg cupric sulphate pentahydrate, 1000 mg ferrous sulphate monohydrate, 67 mg calcium iodate anhydrous, 1000 mg manganous oxide, 1678 mg zinc oxide, 8 mg sodium selenite

ACCEPTED MANUSCRIPT

Table 2

The analysed chemical profile of the experimental diets during the experimental period.

Item (g/kg)	Dietary treatments*		
	Vit D_3	Vit D_2	Mushroom D_2
Concentrate			
Dry matter	827	826	828
Ash	52.8	55.0	52.9
Crude protein (N × 6.25)	102	103	103
Ether extract	16.9	16.1	16.4
Neutral detergent fibre	198	198	199
Vitamin D (IU/kg)[¥]	4320	4290	4276
Straw			
Dry matter	880	881	880
Ash	80.8	80.4	80.8
Crude protein (N × 6.25)	38.8	38.6	38.8
Ether extract	12.5	12.2	12.4
Neutral detergent fibre	844	844	843

*Vitamin D_3 was added to the basal diet in order to obtain 3 levels of dietary treatments: (1) basal diet + 4000 IU of vitamin D_3/kg of feed (Vit D_3); (2) basal diet + 4000 IU of vitamin D_2/kg of feed (Vit D_2); (3) basal diet + 4000 IU of vitamin D_2-enriched mushrooms/kg of feed (Mushroom D_2).

[¥]Cholecalciferol concentration of experimental diets measured using sensitive liquid chromatography-tandem mass spectrometry as described by (Burild et al., 2014), while the content of Vitamin D_2 in the other two groups were calculated

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 3

Effect of cholecalciferol inclusion on animal performance and carcass characteristics (LSM ± SEM[¥]).

Variable	Dietary treatments[*]			SEM[¥]	*p*-value
	Vit D_3	Vit D_2	Mushroom D_2		
DM intake (kg)	9.37	9.41	9.39	0.217	0.747
Vitamin D intake (IU/day)	40892	41269	40994	173.039	0.304
End weight (kg)	624.80	626.30	628.00	9.749	0.973
Average daily gain (kg)	1.01	0.98	0.99	11.953	0.741
Carcass weight (kg)	342.75	344.00	345.37	4.715	0.925
Kill out %	54.89	54.98	55.03	0.600	0.968
Carcass conformation (1-15)	8.90	9.60	9.45	0.300	0.498
Fat score	10.30	10.15	9.89	0.618	0.657

*Vitamin D_3 was added to the basal diet in order to obtain 3 levels of dietary treatments: (1) basal diet + 4000 IU of vitamin D_3/kg of feed (Vit D_3); (2) basal diet + 4000 IU of vitamin D_2/kg of feed (Vit D_2); (3) basal diet + 4000 IU of vitamin D_2-enriched mushrooms/kg of feed (Mushroom D_2).

[¥] LSM = Least square mean; SEM = Standard error of the mean.

Table 4

Effect of treatments on serum 25-OH-D_3, serum 25-OH-D_2, serum total 25-OH-D and LT vitamin D_3, LT 25-OH-D_3, LT vitamin D_2, LT 25-OH-D_2 and total LT vitamin D concentration. (Least-square means ± SEM).

Variable	Dietary treatments[*]			SEM[¥]	*p*-value
	Vit D_3	Vit D_2	Mushroom D_2		
Serum (nmol/l)[†]					
25-OH-D_3	145[a]	82.2[b]	70.2[b]	9.323	0.001

ACCEPTED MANUSCRIPT

25-OH-D_2	20.0[a]	56.1[b]	51.2[b]	3.717	0.001
Total 25-OH-D	166[a]	138[b]	121[b]	11.279	0.025
LT (µg/100g)[†]					
Vitamin D_3	0.09[a]	0.02[b]	0.02[b]	0.009	0.001
25-OH-D_3	0.23[a]	0.12[b]	0.09[b]	0.012	0.001
Vitamin D_2	< 0.01[a]	0.03[b]	0.01[a]	0.003	0.002
25-OH-D_2	0.03[a]	0.09[b]	0.07[b]	0.006	0.001
Total vitamin D activity[§]	1.38[a]	1.10[b]	0.88[b]	0.082	0.004

*Vitamin D_3 was added to the basal diet in order to obtain 3 levels of dietary treatments: (1) basal diet + 4000 IU of vitamin D_3/kg of feed (Vit D_3); (2) basal diet + 4000 IU of vitamin D_2/kg of feed (Vit D_2); (3) basal diet + 4000 IU of vitamin D_2-enriched mushrooms/kg of feed (Mushroom D_2).

[¥]LSM = Least square mean; SEM = Standard error of the mean

[a,b,c] Means within a row with different superscripts differ ($p < 0.01$)

[†]n = 10 per dietary treatment group for serum and n = 7 per dietary treatment group for LT

[§]Total vitamin D activity is calculated as vitamin D_3 + 25-OH-D_3 (× 5) + vitamin D_2 + 25-OH-D_2 (× 5) (Cashman et al., 2012)

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 5

Effect of experimental diets on mean sensory scores, chemical composition and Warner Bratzler shear force values of *Longissimus thoracis* (LT; 14 d-aged) samples from vitamin D-supplemented heifers (LSM ± SEM[¥]).

Variable	Dietary treatments[*]			SEM[¥]	*p*-value
	Vit D_3	Vit D_2	Mushroom D_2		
Sensory evaluation (0-10)					
Overall acceptance	6.39	6.23	6.11	0.202	0.612
Appearance	6.52	6.65	6.50	0.211	0.873
Odour	6.29	6.30	6.42	0.197	0.868
Texture	6.00	5.94	5.83	0.256	0.887
Flavour	6.35	6.27	6.26	0.218	0.945
Off-flavour	1.26	1.58	1.42	0.229	0.604
Chemical composition (g/kg)					
Moisture	69.50	69.72	69.54	0.295	0.112
Ash	1.05	1.03	1.052	0.192	0.769
Protein (N × 6.25)	25.9	25.7	26.0	0.399	0.899
Inter muscular fat	3.63	3.39	3.21	0.579	0.878
Shear force values (NT)					
WBSF	36.15	37.85	38.40	2.455	0.782

*Vitamin D_3 was added to the basal diet in order to obtain 3 levels of dietary treatments: (1) basal diet + 4000 IU of vitamin D_3/kg of feed (Vit D_3); (2) basal diet + 4000 IU of vitamin D_2/kg of feed (Vit D_2); (3) basal diet + 4000 of vitamin D_2-enriched mushrooms/kg of feed (Mushroom D_2).

[¥]LSM = Least square mean; SEM = Standard error of the mean.

WBSF = Warner-Bratzler shear force.