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Safety evaluation of dietary levels of 25-hydroxyvitamin D_3 in growing calves

Pietro Celi[a,b,*], Scot Williams[a], Mark Engstrom[a], Joseph McGrath[a,c], James La Marta[a]

[a] DSM Nutritional Products, Animal Nutrition and Health, Wurmisweg 576, 4303 Kaiseraugst, Switzerland
[b] Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC 3010, Australia
[c] School of Environment and Rural Science, The University of New England, Armidale, NSW 2350, Australia

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ABSTRACT

The objective of this study was to evaluate the safety of 25-OH-D_3 addition to the diet of bovine calves. The investigation was conducted as a feeding experiment for 90 days. 40 calves were allotted to four groups: T1 - control group which received 30 IU vitamin D_3/kg feed, and three experimental groups, which received 25-OH-D_3 in increasing dosages: T2 - 25-OH-D_3 at 1.7 µg/kg, T3 - 25-OH-D_3 at 5.1 µg/kg and T4 - 25-OH-D_3 at 8.5 µg/kg. All calves in the four groups gained weight continually: no growth depression was observed. No adverse effects of 25- OH-D_3 were observed for any of the hematology and serum chemistry parameters measured or during the routine clinical examinations. Plasma 25-OH-D_3 concentration was higher ($p < 0.05$) in groups T2, T3 and T4 compared to that observed in group T1. Tissues content of 25-OH-D_3 was significantly higher in in groups T2, T3 and T4 than in group T1. In the post-mortem evaluation, no adverse effects of the different 25-OH-D_3 doses were observed, neither during the gross pathology nor in the histological examination. The results of this study show explicitly that there were no adverse effects of 25-OH-D_3 compared to the control group, supplemented with vitamin D_3.

1. Introduction

Vitamin nutrition is a challenging and dynamic field for ruminant nutritionists. While the National Research Council (NRC) publications for dairy cattle (NRC, 2001), beef Cattle (NRC, 2000), sheep (NRC, 1985) and other small ruminants (NRC, 2007), define vitamin requirements for vitamins A, D and E it is important to highlight that the current NRC requirements for these vitamins are based on data that are at least 30 years old and, in the case of vitamin D, greater than 50 years old (Weiss, 1998). Moreover, the approach used to calculate these requirements was based on prevention of deficiencies rather than feeding vitamins in amounts and ratios appropriate to their life stage and growing conditions. The current recommended cholecalciferol (Vit. D_3) requirement for calves is 30 IU/kg/day (NRC, 2001). Justification for the current NRC recommendation is limited.

Vitamin D is an essential component of physiological processes in relation to calcium and phosphorus homeostasis and development and maintenance of skeletal health. Bone health is increasingly attracting interest in the international scientific community because of its links with ruminant production and health (Lean et al., 2014). In the current ruminant husbandry systems, confinement of production livestock is common. Considering that a significant proportion of cattle and calves are housed under conditions with little or no exposure to sunlight, in the absence of sufficient sun exposure for dermal conversion, vitamin D becomes an essential nutrient. The vitamin D status of such calves and heifers, in particular, may not be adequate depending on some management practices (Nelson et al., 2016a). Young, growing animals have a greater requirement for Vitamin D than mature animals, in light of their rapid growth of both structural (skeleton) and soft tissues. While mature cattle might receive adequate vitamin D from exposure to direct sunlight or from consumption of forages, this might not be the case for calves and heifers. Newborn calves get vitamin D in the colostrum from their mothers. Later, the neonatal calf receives vitamin D in commercial milk replacers, however levels of vitamin D in these supplements may need to be re-evaluated, given recent evidence suggesting vitamin D status of calves fed milk replacers might not be ideal (Nelson et al., 2016a).

25-hydroxyvitamin D_3 (25-OH-D_3) became commercially available for use in animal feed in the late 1990's and is sold under the brand name Hy-D by DSM Nutritional Products. 25-OH-D_3 is the first metabolite in the sequential transformation of vitamin D3 to the physiologically active hormone 1,25-dihydroxycholecalciferol [1,25-$(OH)_2$-D_3].

* Corresponding author. DSM Nutritional Products, Animal Nutrition and Health, Wurmisweg 576, 4303 Kaiseraugst, Switzerland.
E-mail address: pietro.celi@dsm.com (P. Celi).

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In birds, dietary supplementation with 25-OH-D_3 is more efficiently absorbed and is more metabolically active than vitamin D (Bar et al., 1980; Fritts and Waldroup, 2003). However, data regarding cut-offs of plasma 25-OH-D_3 that define the lower limit of adequacy or sufficiency in the calf are sparse. Therefore, the aim of this study was to assess the safety of dietary 25-OH-D_3 in growing calves.

2. Material and methods

This study was conducted at the Amatsigroup Inc. facilities in Terre Haute, IN, United States. The study protocol was assessed and approved by the Amatsigroup, Inc. Institutional Animal Care and Use Committee prior to the beginning of the study.

2.1. Animals and treatments

Forty weaned Holstein calves (20 males and 20 females) were used in a 90-day feeding trial to assess the effects of feeding diets supplemented with Vitamin D_3 or with increasing dietary supplementation levels of 25-OH-D_3. All calves were acclimated for 14 days prior the beginning of the 90 days treatment and were 3.5 months old and had an average body weight (BW ± SD) of 106 ± 14 kg. Calves were identified with colored, individually numbered, ear tags (one in each ear). Calves were chosen as they are the most sensitive class of animals within the target species for the intended use of the test item.

The forty selected animals were randomized into four treatment groups, each group containing ten animals, five males and five females. The animals were ranked by weight within each sex to form, for each sex, four blocks of five animals. In each weight/sex block, one animal was randomized to each of the four groups. The eight weight/sex blocks of animals were randomized to the two sides of the building (East and West) so that one side of the building contained two male weight blocks and three female weight blocks and the other side of the building contained three male weight blocks and two female weight blocks. Randomization was made using a computer random number generator with Excel®. The treatments were as follows:

- T1: (Control) basal diet supplemented with 0.75 µg (30 IU) Vit. D_3/kg BW;
- T2: Basal diet supplemented with 1.7 µg 25-OH-D_3/kg BW/day (1X);
- T3: Basal diet supplemented with 5.1 µg 25-OH-D_3/kg BW/day (3X);
- T4: Basal diet supplemented with 8.5 µg 25-OH-D_3/kg BW/day (5X).

Vit. D_3 was supplied as 4 mg ROVIMIX® D-500 (DSM Nutritional Products Ltd) per kg feed and the 25-OH-D_3 by 40 mg ROVIMIX® Hy·D® 1.25% (DSM Nutritional Products Ltd) per kg feed.

2.2. Diets and feeding

All animals were fed a base pelleted diet (Purina Animal Nutrition, MN, USA) formulated to comply with NRC recommendations (NRC, 2001). Three feed rations were provided for use with this study: Ration A, Ration B, and Ration C, as detailed below. Ration A: Control feed containing Vitamin-D_3 (for use in Group T1; this ration had no added 25-OH-D_3); Ration B: Feed containing 25-OH-D_3 (for use in Groups T2, T3 and T4) and Ration C: feed containing no added Vitamin-D_3 or 25-OH-D_3 (for use in Groups T1, T2, T3 and T4). During the acclimation period, all animals received Ration A at a rate to achieve 30 IU Vitamin-D_3 per kg BW/day. This is the same dose rate that calves in the T1 group received while on study; this was achieved by weighing feed out into bags labelled for each individual calf. Calves requiring additional feed were offered 1.5 kg (from Day −14 through Day −9) or 2.0 kg (from Day −9 through Day −1) of Ration C after they consumed their entire allocation of Ration A (T1) or B (T2, T3 and T4). From Day 1 calves were received the experimental diets as described below. During the treatment period, target doses of Vitamin-D_3 or 25-OH-D_3 were calculated for each calf based upon the most recent body weight. The appropriate ration (Ration A for T1 or Ration B for T2, T3 and T4) was then weighed out into a bag labelled as the first portion for each specific calf. A second portion of feed, diluent Ration C, was offered at a rate to achieve a total feed intake of 2.75–3.00% of the body weight for each animal, to maintain acceptable daily body weight gains. This second portion was calculated based upon the most recent body weights and was weighed out into bags labelled as the second portion for each specific animal. Calves were offered their first portion each morning; once their first portion was consumed, they were offered their second portion as well as alfalfa cubes, beet pulp moistened with water or Timothy Balance Cubes. The amount of feed remaining each morning was recorded along with the amount of feed wasted. During the treatment phase of the study, the targeted total amount of feed offered to each animal ranged from 2.75% (Day 1 through Day 8) to 3.00% (Day 9 through Day 90) of their individual body weight. Beginning on Day 44, animals were not given any feed in excess of 4.5 kg; instead, additional Timothy Balance Cubes were provided.

Calves were weighed on a weekly interval starting from Day −14 (beginning of acclimation period), until the end of the study and doses of Ration A and B were adjusted accordingly.

2.3. Housing and management

The calves were individually housed in two rooms (West and East) of twenty pens each of approximately 6 m^2. The animals were housed over slatted floors which were cleaned daily. Pens were identified with a label containing the study number, the animal identification and pen number. Housing room temperatures and humidity were recorded daily and ranged from 10.2 to 27.1 C° and 33–91%, respectively, in the West room and from 10.8 to 25.7 C° and 33–91%, respectively, in the East room. Calves were fed once daily in the morning throughout the study. Calves were fed individually, and individual feed intake was recorded daily throughout the study. Animals also received alfalfa cubes, beet pulp moistened with water and/or timothy hay cubes during the study. Calves were fed alfalfa cubes from Day −14 till day 38, then beginning on Day 39 calves were offered beet pulp moistened with water and alfalfa cubes were no longer offered. Beginning on Day 41, calves were given timothy hay cubes, a cubed mixture of timothy hay and beet pulp, along with beet pulp moistened with water. Beet pulp moistened with water was no longer offered after Day 60. These changes in dietary fiber were made due to an apparent sensitivity to the high quality of the alfalfa cubes which lead to occurrence of bloating which was observed in all treatment groups. As an additional precautional measure in addition to the increase in the portion of dietary fiber offered, all calves were vaccinated against *Clostridium Perfringens* Type A on Day 40. Two calves from Group T3 were severely affected by bloating and were euthanized. Fresh potable water was offered *ad libitum*.

Each animal was observed daily for general health and behavior from the beginning of acclimation to the end of the study by qualified personnel. A detailed physical examination was conducted by a veterinarian on Day −14, Day 1, Day 29, Day 60 and Day 90. The following parameters were evaluated: ocular system, nervous system, musculoskeletal system, integumentary system, cardiovascular system (heart rate: HR), respiratory system (respiratory rate: RR), reproductive system, urinary system, lymphatic system, gastrointestinal system, behavior, rectal temperature (RT), feces (consistency, color and mucus, blood), signs of illness.

2.4. Sampling

2.4.1. Feed sampling and composition

Feed samples (300 g) from each ration and of alfalfa and timothy Hay cubes were collected on Days 1, 29, 60 and 90. The samples were analyzed for dry matter (AOAC 930.15), crude protein (AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93), crude fat (AOAC 920.39), starch (AOAC