Effects of Different Types of Vitamin D3 on Milk Production Performance, Blood Parameters, and Calcium-Phosphorus Metabolism in Dairy Cows: Postprint

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Abstract

This study was conducted to investigate the effects of different types of vitamin D3 (vitamin D3 and 25-hydroxy D3) on milk production performance, blood parameters, and calcium and phosphorus metabolism in Holstein lactating dairy cows. Forty-five healthy Holstein lactating dairy cows with similar milk yield, parity, milk composition, and days in milk were selected and randomly allocated into 3 groups, with 15 replicates per group and 1 cow per replicate. The control group was fed a basal diet, experimental group I was supplemented with 25,000 IU/(head · d) of vitamin D3 in the basal diet, and experimental group II was supplemented with 60 mg/(head · d) of 25-hydroxy D3. The experiment consisted of a 10-day preliminary period followed by a 60-day formal experimental period. The results showed: 1) Milk protein, milk calcium, and milk zinc contents in experimental groups I and II were significantly higher than those in the control group ($P<0.05$), while milk somatic cell count was significantly lower ($P<0.05$); there were no significant differences in dry matter intake, milk yield, or other milk composition parameters among all groups ($P>0.05$). 2) Blood osteocalcin content in experimental groups I and II was significantly higher than that in the control group ($P<0.05$), whereas blood bone resorption marker content was significantly lower ($P<0.05$); no significant differences were observed among groups in blood parathyroid hormone, calcium, phosphorus, or magnesium contents, or alkaline phosphatase activity ($P>0.05$). 3) Milk calcium output in experimental groups I and II was significantly higher than that in the control group ($P<0.05$), while fecal calcium output was significantly lower ($P<0.05$); calcium and phosphorus retention and apparent digestibility in experimental groups I and II were significantly higher than those in the control group ($P<0.05$). In conclusion, dietary supplementation with vitamin D3 and 25-hydroxy D3 can significantly increase calcium and phosphorus apparent di-

gestibility and retention, significantly elevate milk protein, milk calcium, and milk zinc contents, significantly reduce milk somatic cell count, improve milk quality, and thereby enhance production performance in dairy cows.

Full Text

Effects of Different Types of Vitamin D3 on Milk Production Performance, Blood Indices, and Calcium-Phosphorus Metabolism in Dairy Cows

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Abstract

This experiment was conducted to investigate the effects of different types of vitamin D3 (vitamin D3 and 25-hydroxyvitamin D3) on milk production performance, blood indices, and calcium-phosphorus metabolism in lactating Holstein dairy cows. Forty-five healthy lactating Holstein cows with similar milk yield, parity, milk composition, and days in milk were randomly assigned to three groups, with 15 replicates per group and one cow per replicate. The control group was fed a basal diet, trial group I received the basal diet supplemented with 25,000 IU/(head · d) of vitamin D3, and trial group II received the basal diet supplemented with 60 mg/(head · d) of 25-hydroxyvitamin D3. The pre-experimental period lasted 10 days, followed by a 60-day formal experimental period. The results showed that: (1) Milk protein, calcium, and zinc contents in trial groups I and II were significantly higher than those in the control group ($P<0.05$), while milk somatic cell count (SCC) was significantly lower ($P<0.05$). No significant differences were observed among groups in dry matter intake (DMI), milk yield, or other milk composition parameters ($P>0.05$). (2) Blood osteocalcin content in trial groups I and II was significantly higher than in the control group ($P<0.05$), whereas blood bone resorption marker content was significantly lower ($P<0.05$). No significant differences were found among groups in blood parathyroid hormone, calcium, phosphorus, or magnesium contents, or in alkaline phosphatase activity ($P>0.05$). (3) Milk calcium output in trial groups I and II was significantly higher than in the control group ($P<0.05$), while fecal calcium output was significantly lower ($P<0.05$). Calcium and phosphorus deposition amounts and apparent digestibility in trial groups I and II were significantly higher than in the control group ($P<0.05$). In conclusion,

dietary supplementation with vitamin D3 and 25-hydroxyvitamin D3 can significantly improve calcium and phosphorus apparent digestibility and deposition, significantly increase milk protein, calcium, and zinc contents, significantly reduce SCC, and improve milk quality, thereby enhancing dairy cow production performance.

Keywords: vitamin D3; 25-hydroxyvitamin D3; lactating dairy cows; calcium-phosphorus metabolism

Introduction

Vitamin D3 is a vitamin closely associated with calcium-phosphorus metabolism in the body and plays a crucial role in maintaining blood calcium-phosphorus deposition and bone mineralization. However, vitamin D3 has low biological activity and must first be converted to 25-hydroxyvitamin D3, then to its final active form, 1,25-dihydroxyvitamin D3 [1,25-(OH) D], in the kidneys. Horst et al. [1] and Gast et al. [2] reported that direct supplementation of 1,25-(OH) D in dairy cow diets can effectively increase plasma calcium content and calcium absorption in the small intestine. Due to the short half-life of 1,25-(OH) D , both vitamin D3 and its intermediate metabolite 25-hydroxyvitamin D3, which has a longer half-life, have become research hotspots [3]. Liao et al. [4] found that dietary supplementation with 25-hydroxyvitamin D3 significantly improved disease resistance in piglets. Research has shown that oral administration of 25-hydroxyvitamin D3 to peripartum Jersey cows significantly increased serum 25-hydroxyvitamin D3 concentration from 77.50 ng/mL to 119.00 ng/mL compared to vitamin D3, alleviating postpartum paresis [5]. Currently, 25-hydroxyvitamin D3 has been commercially produced and applied in broiler chickens [6], breeder ducks [7], pigs, and peripartum dairy cows, demonstrating superior advantages in absorption and transport compared to conventional vitamin D3. However, research on 25-hydroxyvitamin D3 in lactating dairy cows remains limited, and few domestic studies have investigated whether dietary supplementation with 25-hydroxyvitamin D3 can increase calcium and phosphorus contents in raw milk when dietary calcium requirements and appropriate calcium-phosphorus ratios are met. Therefore, this experiment was conducted to study the effects of dietary vitamin D3 and 25-hydroxyvitamin D3 supplementation on milk production performance, blood indices, and calcium-phosphorus metabolism in dairy cows, aiming to provide experimental data for producing high-calcium milk sources and calcium-enriched dairy products from the source of production.

1.1 Experimental Materials

The vitamin D3 and 25-hydroxyvitamin D3 used in this experiment were provided by DSM (China) Limited, with contents of 500,000 IU/g and 1.25%, respectively.

1.2 Experimental Design

The trial selected 45 healthy Holstein dairy cows with similar milk yield, parity, milk composition, and days in milk (150–220 days), which were randomly divided into three groups with 15 replicates per group and one cow per replicate. The basal diet was formulated according to NRC (2001) . The control group was fed the basal diet without any vitamin D3 supplementation, trial group I received the basal diet supplemented with 25,000 IU/(head · d) vitamin D3, and trial group II received the basal diet supplemented with 60 mg/(head · d) 25-hydroxyvitamin D3. The vitamin D3 supplementation level was based on NRC (2001) and previous studies by Li Xin [8] and Gao Daoping et al. [9], while the 25-hydroxyvitamin D3 level followed DSM' s recommended dosage.

1.3 Management

The experiment was conducted at Shanghai Zhenhua Dairy Farm. Cows were fed total mixed ration (TMR) with tie-stall feeding management. Feeding and milking were performed daily at 03:30, 11:00, and 19:30. Cows had free access to water throughout the trial period. The experimental period lasted 70 days, including a 10-day pre-experimental period and a 60-day formal experimental period.

1.4 Sample Collection

1.4.1 Diet and Orts During the experimental period, diet and orts samples were collected every 15 days to calculate dry matter intake (DMI). Collected diet samples were dried in a 65°C oven for 48 hours, equilibrated for 48 hours, then ground into air-dried samples and stored for analysis.

1.4.2 Fecal and Urine Samples The total collection method for feces and urine was employed [10]. After the final milk and blood sampling in the barn, total feces and urine were collected continuously for three days. Daily fecal collections were mixed, and exactly 4% of the total fecal weight was weighed and mixed with 10% tartaric acid (1/4 of fecal weight) to prepare air-dried fecal samples for analysis. Urine was collected 24 hours using urine bags, with 200 mL of 10% dilute sulfuric acid pre-added to collection buckets. Ten percent of the total daily urine volume was collected and stored at -20°C for analysis.

1.4.3 Milk Samples Milk yield was recorded every 15 days during the formal experimental period. Starting from day 1 of the formal period, milk samples were collected every 15 days and mixed in a 4:3:3 ratio from morning, midday, and evening milkings, totaling 100 mL. Fifty milliliters were refrigerated at 4°C and transported to Mengniu Ma' anshan Division Laboratory for routine index testing, while the remaining 50 mL was stored at -20°C for determination of calcium, phosphorus, magnesium, and zinc contents in milk.

1.4.4 Blood Samples Blood samples were collected every 15 days starting from day 1 of the formal period. Five cows were randomly selected from each group, and 5 mL of blood was collected from the tail vein before morning feeding using both regular vacuum tubes and lithium heparin anticoagulant tubes. Blood samples were centrifuged at 4,000 r/min for 10 minutes. Serum was aliquoted into 1.5 mL tubes and stored at -20°C, while plasma was aliquoted into 1.5 mL tubes and stored at -80°C for analysis.

1.5 Laboratory Analyses

1.5.1 Nutritional Components of Diet, Orts, and Feces Determinations of dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, calcium, and phosphorus in diet, orts, and feces samples followed methods described by Zhang Liying [11].

1.5.2 Milk Analysis Milk samples were analyzed for protein, fat, lactose percentages, and somatic cell count (SCC) using a milk composition analyzer (Foss FT120) and somatic cell counter (Foss 5000). Milk calcium, magnesium, and zinc contents were determined by Hitachi ZA3000 atomic absorption spectrophotometer, while milk phosphorus content was measured according to methods described by Zhang Liying [11].

1.5.3 Blood Indices Serum was used to determine alkaline phosphatase (ALP) activity and contents of calcium, phosphorus, magnesium, and parathyroid hormone (PTH). Plasma was used to determine 25-hydroxyvitamin D3, bone resorption marker (CTX), and osteocalcin (OC) contents. All blood samples were sent to Beijing Labtech Technology Development Company for analysis.

1.6 Statistical Analysis

Data were analyzed using SPSS 19.0 software for one-way ANOVA. Duncan' s multiple comparison test was used for significance testing, with $P<0.05$ considered significant and $0.05 P<0.10$ considered a significant trend. Results are expressed as mean ± standard deviation.

Results

2.1 Effects of Different Vitamin D3 Types on DMI, Milk Yield, and Milk Composition

As shown in Table 2 , milk protein, calcium, and zinc contents in trial groups I and II were significantly higher than in the control group ($P<0.05$), with no differences between the two trial groups ($P>0.05$). Somatic cell count (SCC) in trial groups I and II was significantly lower than in the control group ($P<0.05$),

with trial group II significantly lower than trial group I ($P<0.05$). No significant differences were observed among groups in DMI, milk yield, or other milk composition parameters ($P>0.05$).

2.2 Effects of Different Vitamin D3 Types on Blood Indices

As shown in Table 3 , blood osteocalcin (OC) content in trial groups I and II was significantly higher than in the control group ($P<0.05$), while blood CTX content was significantly lower ($P<0.05$), with no significant differences between the two trial groups ($P>0.05$). Blood 25-hydroxyvitamin D3 content in trial groups I and II showed an increasing trend compared to the control group ($P=0.09$). No significant differences were found among groups in blood PTH, calcium, phosphorus, or magnesium contents, or in ALP activity ($P>0.05$).

2.3 Effects of Different Vitamin D3 Types on Calcium-Phosphorus Metabolism

As shown in Table 4 , milk calcium output in trial groups I and II was significantly higher than in the control group ($P<0.05$), while fecal calcium output was significantly lower ($P<0.05$), with no differences between the two trial groups ($P>0.05$). Calcium and phosphorus deposition amounts in trial groups I and II were significantly higher than in the control group ($P<0.05$), with calcium deposition in trial group II significantly higher than in trial group I ($P<0.05$). Calcium and phosphorus apparent digestibility in trial groups I and II was significantly higher than in the control group ($P<0.05$).

Discussion

3.1 Effects of Different Vitamin D3 Types on DMI, Milk Yield, and Milk Composition

Martinez et al. [12] found that supplementing vitamin D3 and calcium to diets with different dietary cation-anion differences (DCAD) had no significant effects on postpartum DMI or milk yield in dairy cows. The basal diet in this experiment was formulated according to NRC (2001) recommendations for sodium (0.19%), potassium (1.02%), and chloride (0.25%), resulting in a DCAD of 270 mEq/kg. Our results are consistent with the above findings, showing that dietary supplementation with exogenous vitamin D3 in a positive DCAD diet had no significant effect on DMI or milk yield.

In this experiment, milk calcium, zinc, and protein contents in trial groups I and II were significantly higher than in the control group, while no significant differences were observed in milk fat, lactose, phosphorus, or magnesium contents. This outcome primarily resulted from the coordinated secretion of milk calcium, protein, and zinc. Dietary vitamin D3 supplementation promotes the formation of calcium-binding proteins in intestinal mucosal cells, thereby enhancing active calcium absorption and increasing calcium deposition in the body [2,13],

which concurrently elevates milk calcium content. Additionally, two-thirds of milk calcium is bound to protein, and milk calcium content is significantly positively correlated with milk protein content (correlation coefficient = 0.725) [14]. Rodríguez et al. [15] reported a significant positive correlation between milk calcium and zinc contents.

Somatic cell count (SCC) represents the number of cells per milliliter of milk, primarily comprising leukocytes (neutrophils, lymphocytes) and macrophages [16]. Mathieu et al. [17] noted that vitamin D3, particularly its active form 1,25-(OH) D , plays an important role in regulating immune responses in animals. Vitamin D primarily mediates the differentiation and maturation of T lymphocytes, B lymphocytes, and macrophages, as well as cytokine and immunoglobulin secretion [18-20]. Liao et al. [4] reported that dietary 25-hydroxyvitamin D3 supplementation in weaned piglets could inhibit inflammatory responses and reduce leukocyte counts. In this experiment, both types of exogenous vitamin D3 significantly reduced SCC, with 25-hydroxyvitamin D3 supplementation showing a more pronounced effect. This may be attributed to vitamin D3' s involvement in immune responses, which enhances mammary gland defense function and reduces leukocyte counts, thereby decreasing SCC.

3.2 Effects of Different Vitamin D3 Types on Blood Indices

Blood calcium, phosphorus, and magnesium levels reflect the metabolic balance of these ions in dairy cows. Hypocalcemia caused by low blood calcium can lead to mastitis and milk fever [21-22]. Yang Yuju [23] reported that both injection and oral administration of vitamin D could increase blood calcium content in dairy cows, though not significantly. Our results are consistent with these findings, showing that exogenous vitamin D3 and 25-hydroxyvitamin D3 slightly increased serum calcium content without significant differences. Goff [24] demonstrated that low blood magnesium is also an important factor causing milk fever, and hypomagnesemia can affect calcium homeostasis and induce hypocalcemia. Li Dagang [25] found that adding anionic salts and vitamin D3 to peripartum cow diets significantly increased blood magnesium content at calving. In this experiment, no significant differences in serum magnesium content were observed among groups, possibly due to differences in the physiological stage of the experimental animals. Yang Yuju [23] also noted that combined use of vitamin D3 and high-phosphorus diets significantly increased blood phosphorus content on day 60 of the experiment. In contrast, our experimental diets maintained constant phosphorus content with only exogenous vitamin D3 supplementation, resulting in non-significantly increased serum phosphorus in trial groups. These results indicate that dietary vitamin D3 supplementation effectively maintains ion homeostasis in dairy cows.

Calcium homeostasis in dairy cows is regulated by multiple mechanisms involving various hormones and enzymes. Blood PTH and 25-hydroxyvitamin D3 work together to regulate blood calcium stability [26]. PTH is one of the most important peptide hormones regulating bone turnover and skeletal

calcium-phosphorus metabolism. Zheng Jiasan et al. [26] observed that rumen-protected vitamin D3 supplementation in peripartum cows increased blood PTH and 25-hydroxyvitamin D3 content gradually prepartum, peaking at calving, then decreasing postpartum. Goff et al. [27] and Weiss et al. [28] demonstrated that dietary 25-hydroxyvitamin D3 supplementation could affect blood PTH content in peripartum cows. McDermott et al. [29] reported that dietary vitamin D3 supplementation at 250,000 IU/d significantly increased blood 25-hydroxyvitamin D3 content in peripartum cows. Horst et al. [1] found that oral 25-hydroxyvitamin D3 linearly increased blood 25-hydroxyvitamin D3 content within two days post-calving. Weiss et al. [28] showed that feeding vitamin D3 and 25-hydroxyvitamin D3 from 15 days prepartum to 7 days postpartum increased plasma 25-hydroxyvitamin D3 content more than threefold in the 25-hydroxyvitamin D3 group compared to the vitamin D3 group. In this experiment, dietary supplementation with different vitamin D3 types slightly increased blood PTH content without significant differences and increased blood 25-hydroxyvitamin D3 content without significant differences. The inconsistency with other studies is mainly because most other research focused on peripartum cows, during which massive calcium loss for milk secretion stimulates bone calcium mobilization and intestinal calcium absorption, causing disordered calcium and hormone secretion that triggers large PTH and 25-hydroxyvitamin D3 production to meet calcium demands.

Blood alkaline phosphatase (ALP) plays an important role in calcium digestion, absorption, secretion, and ossification [30]. C-terminal telopeptide of type I collagen (CTX) exists in mature bone collagen; when osteoclast activity increases, bone collagen dissolves and releases type I collagen, and CTX content rises when bone resorption is enhanced. Osteocalcin (OC) is a bone turnover marker and the most important specific non-collagen protein in bone matrix. Blood OC content reflects bone calcium turnover rate in dairy cows. Li Dagang [25] reported that dietary vitamin D3 supplementation had no significant effect on blood ALP activity. Zhao Zhen et al. [31] monitored blood indices in 246 elderly individuals and found that blood OC content decreased with decreasing blood 25-hydroxyvitamin D3 content, with the vitamin D-deficient group having significantly lower blood OC content than the vitamin D3 group. In this experiment, dietary vitamin D3 and 25-hydroxyvitamin D3 supplementation had no significant effect on blood ALP activity but significantly increased blood OC content and decreased blood CTX content, indicating that exogenous vitamin D3 supplementation is beneficial for improving bone turnover efficiency, enhancing bone formation, and alleviating bone metabolism disorders.

3.3 Effects of Different Vitamin D3 Types on Calcium-Phosphorus Metabolism

Calcium and phosphorus play crucial roles in bone growth and metabolism and are essential mineral elements for animal skeletal development and health maintenance [32]. Vitamin D3 plays an important role in calcium-phosphorus

metabolism in dairy cows by promoting calcium salt deposition and mineralization in bone [23,33]. Research indicates that 25-hydroxyvitamin D3 is more effectively absorbed and has stronger biological activity than vitamin D3 [34]. Current studies show that direct dietary supplementation with 25-hydroxyvitamin D3 not only shortens the metabolic process of vitamin D3 in the body but also avoids absorption and utilization issues caused by intestinal damage and liver or kidney dysfunction [35].

Ye Hui et al. [6] reported that dietary 25-hydroxyvitamin D3 supplementation in yellow-feathered broilers could effectively replace vitamin D3, and supplementation at 70–90 g/kg significantly improved calcium apparent and true digestibility. Due to rapid growth in broilers, high requirements for calcium and phosphorus deposition in bone exist, whereas few reports address vitamin D3 and 25-hydroxyvitamin D3 effects on bone calcium deposition in lactating dairy cows. In this experiment, supplementation with different vitamin D3 types in dairy cow basal diets resulted in significantly higher calcium and phosphorus apparent digestibility and deposition in trial groups compared to the control group, indicating that vitamin D3 supplementation effectively improves calcium and phosphorus utilization and increases their deposition in bone to meet calcium demands during lactation.

Conclusion

1. Dietary supplementation with vitamin D3 and 25-hydroxyvitamin D3 significantly increased milk calcium, zinc, and protein contents, significantly reduced SCC, and had no significant effects on DMI or milk yield.
2. Dietary supplementation with vitamin D3 and 25-hydroxyvitamin D3 significantly increased blood OC content and significantly decreased blood CTX content; blood calcium, phosphorus, and hydroxyvitamin D3 contents increased but without significant differences.
3. Dietary supplementation with vitamin D3 and 25-hydroxyvitamin D3 significantly increased calcium and phosphorus deposition amounts and apparent digestibility, with 25-hydroxyvitamin D3 supplementation showing superior effects.

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Note: Figure translations are in progress. See original paper for figures.