

Effects of Calcium Formate and Calcium Lactate on Production Performance, Milk Composition, Blood Biochemical Indices, and Calcium and Phosphorus Metabolism in Lactating Dairy Cows (Postprint)

Authors: Shu Zhang, Lu Na, Wang Yajing, Shao Wei, Li Shengli, Muzhishen

Date: 2018-12-25T00:00:00+00:00

Abstract

This experiment was conducted to investigate the effects of calcium formate and calcium lactate on production performance, milk composition, blood biochemical parameters, and calcium and phosphorus metabolism in 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 to 3 groups, with 15 replicates per group and one cow per replicate. The control group was fed a basal diet, while experimental group I and experimental group II replaced 50% of the limestone in the basal diet with calcium formate and calcium formate + calcium lactate, respectively, and the calcium levels were consistent across the three diets. The preliminary period lasted 10 days, and the formal experimental period lasted 60 days. The results showed: 1) Compared with the control group, the milk protein percentage in experimental groups I and II was significantly increased ($P < 0.05$); milk calcium content and milk fat percentage both increased, but the differences were not significant ($P > 0.05$). There were no significant differences in dry matter intake, milk yield, lactose percentage, or milk phosphorus content among all groups ($P > 0.05$). 2) The plasma osteocalcin (OC) content in experimental groups I and II was significantly higher than that in the control group ($P < 0.05$). There were no significant differences in serum calcium, phosphorus, parathyroid hormone (PTH) content, alkaline phosphatase (ALP) activity, or plasma bone resorption marker (CTX) content among all groups ($P > 0.05$). 3) The apparent digestibility of calcium and phosphorus in experimental groups I and II was significantly higher than that in the control group ($P < 0.05$), calcium and phosphorus balance was significantly higher than that in the control group ($P < 0.05$), and fecal calcium and phosphorus contents were significantly lower than those in the control group ($P < 0.05$). In conclusion,

replacing partial limestone with calcium formate and calcium lactate in dairy cow diets significantly increased the milk protein percentage in lactating dairy cows, also improved milk fat percentage and milk calcium content, significantly increased the apparent digestibility of calcium and phosphorus, and reduced calcium and phosphorus excretion.

Full Text

Effects of Calcium Formate and Calcium Lactate on Performance, Milk Composition, Blood Biochemical Indices, and Calcium and Phosphorus Metabolism in Lactating Dairy Cows

**ZHANG Shu^{1,2}, LU Na^{1,2}, WANG Yajing², SHAO Wei¹, LI Shengli², MU Zhishen³

¹College of Animal Science and Technology, Xinjiang Agricultural University, Urumqi 830052, China

²State Key Laboratory of Animal Nutrition, Beijing Engineering Technology Research Center of Raw Milk Quality and Safety Control, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China

³Inner Mongolia Mengniu Dairy (Group) Co., Ltd., Hohhot 011500, China

Abstract

This experiment investigated the effects of calcium formate and calcium lactate on production performance, milk composition, blood biochemical indices, and calcium and phosphorus metabolism in lactating dairy cows. Forty-five healthy Holstein cows with similar milk yield, parity, milk composition, and days in milk were randomly assigned to three groups (n=15 per group). The control group received a basal diet, while trial groups I and II received experimental diets in which 50% of the limestone in the basal diet was replaced by calcium formate (group I) or a combination of calcium formate and calcium lactate (group II), with calcium levels standardized across all diets at 1.23%. The study consisted of a 10-day preliminary period followed by a 60-day experimental period. The results showed that: (1) Compared with the control group, milk protein percentage was significantly higher in both trial groups ($P < 0.05$), while milk fat percentage and calcium content showed increasing trends without statistical significance ($P > 0.05$). No significant differences were observed among groups in dry matter intake, milk yield, lactose percentage, or milk phosphorus content ($P > 0.05$). (2) Plasma osteocalcin (OC) concentrations were significantly elevated in both trial groups relative to the control ($P < 0.05$). Serum calcium, phosphorus, and parathyroid hormone (PTH) concentrations, serum alkaline phosphatase (ALP) activity, and plasma bone resorption marker (CTX) levels did not differ significantly among groups ($P > 0.05$). (3) Calcium and phosphorus apparent digestibility and retention were significantly higher in both trial

groups compared with the control ($P < 0.05$), while fecal calcium and phosphorus excretion were significantly lower ($P < 0.05$). In conclusion, partially replacing limestone with calcium formate and calcium lactate in dairy cow diets significantly improved milk protein percentage, enhanced milk fat percentage and calcium content, increased calcium and phosphorus apparent digestibility, and reduced calcium and phosphorus emissions.

Keywords: calcium formate; calcium lactate; lactating dairy cows; calcium and phosphorus metabolism

Introduction

Calcium is the most abundant and one of the most essential mineral elements in animal bodies, playing a critical role in maintaining normal function of nervous and muscular tissues [1-2]. Late gestation and lactation represent the physiological stages with the greatest calcium demand in dairy cows, making the selection of appropriate calcium supplements an effective measure for improving nutritional status. Additionally, whether organic calcium supplementation can increase milk calcium content to produce calcium-enriched dairy products has become a research focus. Current calcium additives on the market are primarily classified as inorganic calcium (e.g., calcium hydrogen phosphate, limestone) or organic calcium (e.g., calcium formate, calcium lactate). Calcium formate is a novel additive product containing approximately 31% calcium and 69% formic acid. Calcium lactate serves as a high-quality calcium source with high water solubility and generally superior absorption compared to other calcium-based additives. In the stomach, it dissociates into calcium ions and lactate, providing both calcium supplementation and energy. Both formic and lactic acids are propionate precursors that undergo fermentation in the rumen via the tricarboxylic acid cycle to generate propionate [3]. For ruminants, propionate is the primary source of glucose, with 90% being absorbed by the liver and converted to glucose. Wang et al. [4] demonstrated that adding calcium propionate to early-lactation cow diets at 200 g/d significantly improved energy balance and body condition while meeting calcium requirements. Zuo et al. [5] reported that supplementing 0.5% calcium lactate in Holstein cows significantly increased milk yield and fat percentage. These studies investigated organic calcium supplementation as an addition to basal diets. Therefore, this experiment systematically evaluated differences between organic calcium sources and limestone by replacing a portion of limestone with calcium lactate and calcium formate to investigate their effects on production performance, milk composition, blood biochemical indices, and calcium and phosphorus metabolism.

1. Materials and Methods

1.1 Experimental Materials Calcium formate and calcium lactate were provided by Hangzhou Guogu Biological Technology Co., Ltd., with calcium

contents of 30% and 13%, respectively. Limestone was provided by Shanghai Zhenhua Dairy Farm with a calcium content of 34%.

1.2 Experimental Design and Animal Management Forty-five healthy Holstein cows with similar milk yield, parity, milk composition, and days in milk (150–220 days) were randomly allocated to three groups (n=15 per group). The control group received a basal diet, while trial groups I and II received diets in which 50% of the limestone was replaced by calcium formate (group I) or a combination of calcium formate and calcium lactate (group II). All diets were formulated to contain identical calcium levels (1.23%) according to NRC (2001) standards. Diet composition and nutrient levels are presented in Table 1 .

The feeding trial was conducted at Shanghai Zhenhua Dairy Farm from May to July 2017. Cows were housed in tie-stall barns and fed total mixed rations three times daily at 03:30, 11:00, and 19:30, coinciding with milking times. Fresh water was available ad libitum. The experimental period lasted 70 days, including a 10-day preliminary period and a 60-day data collection period.

1.3 Sample Collection **1.3.1 Feed and Orts** Feed and Orts were collected every 15 days throughout the trial to calculate dry matter intake. Samples were dried at 65°C for 48 hours, equilibrated for 48 hours, and ground for subsequent analysis.

1.3.2 Fecal and Urine Samples A total collection method was employed [6]. Following the final milk and blood sampling, feces and urine were collected continuously for three days. Daily fecal collections were mixed, and 4% of the total weight was subsampled and mixed with 10% tartaric acid (one-quarter of the fecal weight) before drying. Urine was collected using urine bags over 24 hours into containers pre-treated with 200 mL of 10% sulfuric acid; 10% of the daily urine volume was sampled and stored at -20°C.

1.3.3 Milk Samples Milk yield was recorded every 15 days during the experimental period. Milk samples were collected at 15-day intervals and pooled in a 4:3:3 ratio (morning:afternoon:evening) to obtain 100 mL total volume. Fifty milliliters were refrigerated at 4°C for routine analysis at the Mengniu Ma' anshan Laboratory, while the remaining 50 mL was stored at -20°C for calcium and phosphorus determination.

1.3.4 Blood Samples Blood samples were collected at 15-day intervals from five randomly selected cows per group before morning feeding. Five milliliters of blood were drawn from the coccygeal vein into both plain and lithium heparin vacuum tubes. Serum was separated by centrifugation at 4,000 rpm for 10 minutes and stored at -20°C, while plasma was stored at -80°C.

1.4 Analytical Methods **1.4.1 Nutrient Analysis of Feed, Orts, and Feces** Dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, calcium, and phosphorus

contents were determined according to standard methods [7].

1.4.2 Milk Analysis Milk protein, fat, and lactose percentages were analyzed using a Foss FT120 milk composition analyzer. Milk calcium content was determined by atomic absorption spectrophotometry (Hitachi ZA3000), and milk phosphorus content was measured according to Zhang [7].

1.4.3 Blood Biochemical Indices Serum PTH concentration was measured by ELISA (Langdun Biological Technology, Shanghai). Serum ALP activity and calcium and phosphorus concentrations were determined using assay kits (Nanjing Jiancheng Bioengineering Institute). Plasma OC and CTX concentrations were measured using specific ELISA kits for osteocalcin and type I collagen.

1.5 Statistical Analysis Data were analyzed using one-way ANOVA in SPSS 19.0. Duncan's multiple range test was used for post-hoc comparisons. Significance was declared at $P < 0.05$, and results are expressed as means \pm standard deviation.

2. Results

2.1 Effects on Dry Matter Intake, Milk Yield, and Milk Composition As shown in Table 2, milk protein percentage was significantly higher in both trial groups compared with the control ($P < 0.05$). Milk fat percentage and calcium content exhibited increasing trends without statistical significance ($P > 0.05$). No significant differences were observed among groups in dry matter intake, milk yield, lactose percentage, or milk phosphorus content ($P > 0.05$).

2.2 Effects on Blood Biochemical Indices Table 3 shows that plasma OC concentrations were significantly elevated in both trial groups relative to the control ($P < 0.05$). Serum calcium, phosphorus, and PTH concentrations, serum ALP activity, and plasma CTX content did not differ significantly among groups ($P > 0.05$).

2.3 Effects on Calcium and Phosphorus Metabolism As presented in Table 4, both trial groups exhibited significantly higher calcium and phosphorus apparent digestibility and retention compared with the control ($P < 0.05$), while fecal calcium and phosphorus excretion were significantly lower ($P < 0.05$).

3. Discussion

3.1 Effects on Dry Matter Intake, Milk Yield, and Milk Composition Previous research by Huang et al. [8] demonstrated that 0.5% calcium formate supplementation in piglet diets did not significantly affect feed intake or growth performance, whereas 1.0% supplementation improved feed intake, daily gain, and feed conversion ratio. Our findings align with these results, showing that calcium formate and calcium lactate had no significant effects on dry matter intake or milk yield in lactating dairy cows.

Calcium formate and calcium lactate dissociate in the stomach into calcium ions and organic acid anions, providing both calcium supplementation and glucogenic precursors (formic and lactic acids) that ferment in the rumen to produce propionate via the tricarboxylic acid cycle [3]. For ruminants, propionate serves as the primary glucose precursor, with 90% being absorbed by the liver and converted to glucose. Wang et al. [4] reported that calcium propionate supplementation at 200 g/d in early-lactation cows improved energy balance and body condition. In our study, the lack of significant change in milk fat percentage may be attributed to the relatively low inclusion rates of organic calcium sources. In trial group I, calcium formate inclusion was 0.34% of diet DM, equivalent to 83.1 g/d based on a dry matter intake of 22.27 kg/d. In trial group II, calcium formate and calcium lactate inclusion rates were 0.17% and 0.39%, respectively, providing 41.6 g/d of formate and 95.3 g/d of lactate based on 22.25 kg/d dry matter intake.

The significantly higher milk protein percentage observed in both trial groups likely resulted from supplementation with glucogenic precursors (formic and lactic acids), which promote gluconeogenesis and increase plasma glucose and insulin concentrations. Patton et al. [9] confirmed that feeding glucogenic precursors significantly elevates plasma glucose and insulin levels, with insulin secretion enhancing amino acid uptake by mammary tissue and thereby increasing milk protein synthesis. Milk calcium and protein metabolism are closely correlated through casein micelle interactions [10-12]. Approximately 80% of milk protein consists of casein, which contains 72% of milk calcium, with the remainder present as free ions [13]. Consequently, milk calcium content in both trial groups increased in parallel with milk protein percentage, showing an upward trend compared with the control group, though the difference was not statistically significant.

3.2 Effects on Blood Biochemical Indices Serum calcium and phosphorus concentrations reflect mineral metabolism balance in dairy cows. Blood mineral concentrations typically remain stable due to dual regulation by PTH and calcitonin, along with other factors that prevent short-term dietary effects [14]. PTH is a critical peptide hormone regulating bone turnover and calcium-phosphorus metabolism. Zhu et al. [15] reported that PTH promotes calcium absorption and mobilizes calcium release from bone tissue, while also regulating phosphorus balance through non-transcriptional pathways and facilitating phosphorus release from bone [16-17]. The absence of significant differences in serum calcium, phosphorus, and PTH concentrations among groups in our study indicates that limestone, calcium formate, or mixed calcium sources all provided adequate calcium and phosphorus without differentially stimulating bone calcium mobilization.

Serum ALP plays an important role in calcium digestion, absorption, secretion, and bone mineralization [18]. CTX, present in mature bone collagen, is released during osteoclast-mediated bone resorption, with elevated levels indicating en-

hanced bone resorption. Osteocalcin (OC) is a bone-specific non-collagenous protein that serves as a bone turnover marker, with plasma OC concentrations reflecting bone calcium flux rates. Our results showed no significant differences in serum ALP activity or plasma CTX concentrations among groups, indicating comparable bone resorption levels. However, plasma OC concentrations were significantly higher in both trial groups, suggesting enhanced calcium and phosphorus deposition in bone compared with the control. This indicates that while all calcium sources met physiological requirements, calcium formate and calcium lactate may accelerate bone calcium deposition, though the specific mechanisms require further investigation.

3.3 Effects on Calcium and Phosphorus Metabolism Calcium and phosphorus are essential mineral elements for bone growth and metabolism [18]. Research indicates that calcium lactate is a highly bioavailable calcium source with excellent absorption characteristics [19-20]. Calcium ion absorption requires an acidic environment, and factors affecting gastrointestinal pH consequently influence calcium absorption [21]. Organic acids such as calcium formate and calcium lactate can enhance rumen microbial fermentation and synthesis, promoting calcium absorption [3]. Ma et al. [22] demonstrated that rats fed calcium lactate exhibited significantly higher bone calcium content and bone mineral density compared with those fed calcium carbonate (the primary component of limestone) at equivalent intake levels. Although we did not measure gastrointestinal pH in this study, the enhanced calcium absorption and bone deposition observed with organic calcium sources may be related to reduced gastrointestinal pH. Furthermore, since calcium and phosphorus are regulated by interconnected biological and physicochemical mechanisms in the gastrointestinal tract, cellular fluid, and bone-blood system [23-24], the increased bone calcium deposition in both trial groups was accompanied by significantly enhanced phosphorus deposition.

Environmental concerns regarding livestock waste have become increasingly prominent, with livestock manure production in China exceeding industrial solid waste in 2009, primarily from cattle (approximately 46.97% of total manure). Bai et al. [25] reported that average phosphorus utilization efficiency in Chinese dairy farms ranges from only 10-22%, substantially lower than levels in the United States, Australia, and New Zealand (19-35%). Gourley et al. [26] documented phosphorus use efficiencies of 6-158% across 41 Australian dairy farms, with such variability impacting feed resource utilization, animal health, and environmental quality. While most research has focused on dietary calcium-to-phosphorus ratios, few studies have examined the effects of calcium formate and calcium lactate on calcium and phosphorus metabolism in ruminants. Our findings demonstrate that these organic calcium sources not only reduce calcium excretion but also decrease phosphorus emissions, thereby mitigating environmental pollution and improving farm ecological efficiency.

4. Conclusion

Partial replacement of limestone with calcium formate and calcium lactate in dairy cow diets significantly increased milk protein percentage, improved milk fat percentage and calcium content, enhanced calcium and phosphorus apparent digestibility, and reduced calcium and phosphorus excretion.

References

- [1] VILLEREAL M L, PALFREY H C. Intracellular calcium and cell function[J]. *Annual Review of Nutrition*, 1989, 9(1): 347-376.
- [2] BRONNER F, PANSU D. Nutritional aspects of calcium absorption[J]. *The Journal of Nutrition*, 1999, 129(1): 9-12.
- [3] YANG Chengjian. Regulation of ruminal methane production by disodium fumarate and analysis of related rumen microbial flora in goats[D]. PhD Thesis. Nanjing: Nanjing Agricultural University, 2011.
- [4] WANG Cong, LIU Qiang, HUANG Yingxiang, et al. Effects of calcium propionate on body condition and energy balance of dairy cows in early lactation[J]. *China Feed*, 2008(17): 18-20.
- [5] ZUO Yuping, QU Chunhong, FENG Chengli, et al. Feeding trial of Tiduo-zhuang calcium lactate feed additive in dairy cows[J]. *Feed Industry*, 1993, 14(2): 40-41.
- [6] CAO Z J, MA M, YAN X Y, et al. A simple urine-collecting apparatus and method for cows and heifers[J]. *Journal of Dairy Science*, 2009, 92(10): 5224-5228.
- [7] ZHANG Liying. Feed analysis and feed quality detection technology[M]. 4th ed. Beijing: China Agricultural University Press, 2016: 370.
- [8] HUANG Jianhua, ZHANG Shuiyin, YANG Fengmei. Effects of calcium formate on performance of piglets[J]. *Journal of Nanchang College*, 2006, 12(2): 101-102.
- [9] PATTON R S, SORENSON C E, HIPPEN A R. Effects of dietary glucogenic precursors and fat on intake carbohydrate status transition dairy cows[J]. *Journal of Dairy Science*, 2004, 87(7): 2122-2129.
- [10] BIJL E, VAN VALENBERG H J F, HUPPERTZ T, et al. Protein, casein, and micellar salts in milk: current content and historical perspectives[J]. *Journal of Dairy Science*, 2013, 96(9): 5455-5464.
- [11] POULSEN N A, RYBICKA I, POULSEN H D, et al. Seasonal variation in content of riboflavin and major minerals in bulk milk from three Danish dairies[J]. *International Dairy Journal*, 2015, 42: 6-11.
- [12] GAUCHERON F. The minerals milk[J]. *Reproduction Nutrition Development*, 2005, 45(4): 473-483.
- [13] FAN Jinbo, WANG Pengjie, ZHOU Suzhen, et al. Research progress on structure and physicochemical properties of casein micelles[J]. *Science and Technology of Food Industry*, 2014, 35(3): 396-400.
- [14] YAO Meirong. Effects of diets with different concentrate-to-forage ratios on digestion and metabolism of calcium, phosphorus, magnesium, zinc and lacta-

- tion performance in Holstein dairy cows[D]. Master's Thesis. Urumqi: Xinjiang Agricultural University, 2004.
- [15] ZHU Xing, HOU Jiafa, ZHANG Yongmei, et al. Changes of PTH, CT and E2 in caged layers with osteoporotic due to calcium deficiency[J]. Chinese Journal of Veterinary Science, 2001, 21(1): 72-74.
- [16] BACIC D, LEHIR M, BIBER J, et al. The renal Na⁺/phosphate cotransporter NaPi-IIa is internalized receptor-mediated endocytic route in response to parathyroid hormone[J]. Kidney International, 2006, 69(3): 495-503.
- [17] YANG Jing, CHEN Lin. Intestinal phosphorus absorption and its regulatory mechanisms[J]. Chinese Journal of Osteoporosis and Bone Mineral Research, 2014, 7(1): 77-81.
- [18] WANG Jian, WANG Dong, HE Jianping, et al. Effects of oral calcium supplementation on calcium and phosphorus metabolism in Gansu zokor[J]. Chinese Journal of Zoology, 2010, 45(4): 46-51.
- [19] KRUGER M C, GALLAHER B W, SCHOLLUM L M. Bioavailability of calcium is equivalent from milk fortified with either calcium carbonate or milk calcium in growing male rats[J]. Nutrition Research, 2003, 23(9): 1229-1237.
- [20] LEE W T K, JIANG J, LAI X J, et al. Calcium absorption from calcium fortified soymilk and cow's postmenopausal Chinese women with suboptimal serum 25-hydroxyvitamin D status[J]. Bone, 2008, 43(Suppl.1): S31-S32.
- [21] CHU Hanping. Study on appropriate supply of calcium and phosphorus in dairy cows[D]. Master's Thesis. Tai'an: Shandong Agricultural University, 2005.
- [22] MA Dandan, ZHU Wenli, LONG Zhu, et al. Comparison of effects of different calcium sources on bone mineral density in rats[J]. Food and Nutrition in China, 2010(9): 64-68.
- [23] GU Jianhong, LIU Jundong, ZHAO Ruiying, et al. Effects of different calcium-phosphorus ratios on osteoclastogenesis and bone resorption function of cultured Muscovy duck osteoclasts in vitro[J]. Acta Veterinaria et Zootechnica Sinica, 2007, 38(12): 1357-1361.
- [24] FENG Yanglian. Ruminant nutrition[M]. Beijing: Science Press, 2004: 13, 595.
- [25] BAI Z H, MA L, OENEMA O, et al. Nitrogen and phosphorus use efficiencies in dairy production in china[J]. Journal of Environmental Quality, 2013, 42(4): 990-1001.
- [26] GOURLEY P, DOUGHERTY W J, WEAVER D M, et al. Farm-scale nitrogen, phosphorus, potassium and sulfur balances and use efficiencies on Australian dairy farms[J]. Animal Production Science, 2012, 52(10): 929-944.

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

Source: ChinaXiv – Machine translation. Verify with original.