FULL PAPER *Internal Medicine*

Effects of Oral Administration of "Rumen-Bypass" Vitamin D_3 on Vitamin D and Calcium Metabolism in Periparturient Cows

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ABSTRACT. Eleven late-pregnant Jersey cows were assigned to two groups; a group (PO-RBVD group) consisting of five cows treated with an oral administration of 10 million I.U. of an encapsulated form of vitamin D_3 ("rumen-bypass" VD_3; $RBVD_3$) and another group (IMVD group) consisting of the other six treated with an intramuscular injection of 10 million I.U. of vitamin D_3 (VD_3). The cows received the $RBVD_3$ or VD_3 administration at 7 days before the expected parturition. The changes in the plasma concentrations of vitamin D metabolites, ionized Ca (Ca^{++}) and inorganic phosphorus (iP) were evaluated. Of the vitamin D metabolites, the plasma 25-hydroxyvitamin D concentrations in PO-RBVD group increased significantly after the $RBVD_3$ administration and remained in high levels that were significantly higher than those in IMVD group. This suggested that $RBVD_3$ was absorbed rapidly and excellently from the post-ruminal digestive tract without the degradation by ruminal microorganisms. The plasma Ca^{++} and iP concentrations in PO-RBVD group tended to be higher after the administration and around parturition than those in IMVD group. From these observations, it was suggested the oral $RBVD_3$ administration had more potent ability to prevent parturient paresis compared with the VD_3 injection used widely in Japan.—KEY WORDS: calcium, cattle, parturition, "rumen-bypass", vitamin D_3.

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The use of vitamin D (VD; general name of vitamin D_2 and D_3) has been popular in attempt to prevent parturient paresis in cows. These treatments include a single intramuscular injection of 10 million I.U. of vitamin D_3 (VD_3) before 1 week of the expected parturition [4, 11, 13, 17, 24] and continuous feeding of 20 to 30 million I.U. of VD for five consecutive days before calving [3, 5, 6]. Although parental administration of VD_3 has been widely used in Japan, large oral doses of VD has not been used because of instability of the prophylactic effect and inconvenience due to continuous feeding and necessity of extremely massive doses of VD. Once VD is orally administered to cows, VD metabolism actually begins in the rumen [10]. VD is converted to some unidentified metabolites having antivitamin D activities by rumen microorganisms, and as much as 80% of the VD disappears from the incubation media of the rumen fluid over 24 hrs [10]. The ruminal degradation of VD is the reason why continuous feeding of extremely massive doses of VD is needed for maximum protection against milk fever [10]. Several studies have reported that dietary proteins, amino acids and vitamin AD_3E premix encapsulated by hydrogenated oil or polymer can bypass the rumen with minimization of the ruminal degradation in cows [1, 15, 16, 23]. Therefore, we suspect that VD in a form that prevents the degradation by ruminal microorganisms will bypass the rumen and provide the prophylactic effect against parturient paresis. In the present study, cows received a single oral administration of an encapsulated form of VD_3 ("rumen-bypass" VD_3; $RBVD_3$) before calving. The objective of this study was to determine the effects of $RBVD_3$ on VD and Ca metabolism after the oral administration and around parturition. These effects were compared with a single intramuscular injection of VD_3 that was one of the popular prophylaxis in Japan.

MATERIALS AND METHODS

Animals: Eleven late-pregnant Jersey cows (aged 3 to 8 years) were assigned to two groups; a group (PO-RBVD group) consisting of five cows treated with an oral administration of $RBVD_3$ and another group (IMVD group) consisting of the other six treated with an intramuscular injection of commercial VD_3 preparation. The cows stayed in an outside paddock during dry period until 8 days before the expected parturition. They were housed in an individual pen until 3 days after calving. They thereafter stayed in another outside paddock during 9 a.m. to 3 p.m., and were

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housed in a stanchion during 3 p.m. to 9 a.m. The cows were fed a good quality hey, corn silage, and commercial concentrate during dry period and after parturition. Commercial Ca and P supplements were added in the diet after calving. It was calculated that the cows were consuming 42 to 45 g of Ca and 27 to 31 g of phosphorus (P) per day during at least 2 weeks before the expected parturition, and 223 to 232 g of Ca and 73 to 79 g of P per day after calving.

Administration of $RBVD_3$ or VD_3: The cows of PO-RBVD group treated with an oral administration of 10 million I.U. of $RBVD_3$ mixed with a small amount of the concentrate at 3 p.m. of 7 days before the expected parturition. The $RBVD_3$ was in the form of VD_3 resin encapsulated by hydrogenated oil that was supplied by Nippon Soda Co., Ltd., Japan (Pro Vit D_3-1000). The cows of IMVD group treated with an intramuscular injection of 10 million I.U. of VD_3 dissolved in an liposoluble medium (Osvitan-1000, Denka Pharmacy Co., Ltd., Japan) at 3 p.m. of 7 days before the expected parturition. If cow did not calve within 1 week, the cows received an additional administration of $RBVD_3$ or VD_3 at 8 days after the previous administration.

Plasma biochemistry: Blood was taken with two heparinized syringes from the external jugular vein at 3 p.m. of every day before calving, at time soon after calving (0 days), and at 0.25, 0.5, 1, 2, 3 and 5 days (i.e., 6, 12, 24, 48, 72 and 120 hrs) after the time of calving. The blood from one syringe was immediately used to determine the plasma concentration of ionized Ca (Ca^{++}) by 288 Blood Gas System (Ciba Corning Diagnostics Corp., U.S.A.). The blood from another syringe was immediately centrifuged to separate plasma, which was frozen at −20°C until analyzed. The plasma concentration of 25-hydroxyvitamin D (25-OHD) was measured by a modified method of a competitive protein binding assay described by Proszynska *et al.* [18]. The plasma concentration of 1,25-dihydroxyvitamin D ($1,25\text{-}(OH)_2D$) was measured by a radioreceptor assay described by Reinhardt *et al.* [21]. In the present study, total concentrations of $25\text{-}OHD_2$ and $25\text{-}OHD_3$ (or $1,25\text{-}(OH)_2D_2$ and $1,25\text{-}(OH)_2D_3$) were expressed as the concentrations of 25-OHD (or $1,25\text{-}(OH)_2D$), because $25\text{-}OHD_3$ (or $1,25\text{-}(OH)_2D_3$) cannot be separated from $25\text{-}OHD_2$ (or $1,25\text{-}(OH)_2D_2$). The plasma inorganic P (iP) concentration was determined by a molybdenum method [2].

Ca treatment for parturient paresis: If cow developed parturient paresis after calving, the cow received Ca treatment (an intravenous infusion of 500 m*l* of 25 % Ca borogluconate solution) [19, 25]. The criteria to start this treatment were that the cow was in recumbency and was unable to stand up itself.

Data analysis: The data in each group were expressed as means ± standard deviation. Firstly, the data during 0 to 3 days after the administration were evaluated. If cow received the repeated administration, the data after the second administration were excluded. Student's *t*-test was done to compare each timed value between PO-RBVD and IMVD groups. Analysis of variance was used to observe the effects in each group. If the effects were significant, the timed values were compared with the value at 0 day by Bonferroni multiple comparison test. In the next, the data around parturition (during -2 to 5 days after parturition) were also evaluated. If cow received Ca treatment after calving, the data after the treatment were excluded. Student's *t*-test was done to compare each timed value between PO-RBVD and IMVD groups. In all of the analysis, significance was set at $P<0.05$.

RESULTS

Duration between the administration and calving: The cows of PO-RBVD group calved at 3.2 ± 1.9 days after the oral RBVD3 administration. The cows of IMVD group calved at 6.0 ± 3.1 days after the VD_3 injection. One cow of IMVD group received repeated VD_3 injections before calving.

Development of parturient paresis: Two cows of PO-RBVD group and two cows of IMVD group developed parturient paresis within 1 day (during 6 to 24 hr) after calving. They stood within 12 hr after Ca treatment.

Effects after $RBVD_3$ or VD_3 administration: The plasma 25-OHD concentrations in PO-RBVD group increased significantly from 55.4 ± 27.5 *n*g/m*l* at 0 day to about 125 to 145 *n*g/m*l* during 1 to 3 days after the RBVD3 administration ($P<0.05$ or 0.01) (Fig. 1). The 25-OHD levels during 1 to 3 days were significantly higher in PO-RBVD than in IMVD group ($P<0.05$ or 0.01). The plasma 25-OHD concentrations in IMVD group did not change significantly (around 54 to 75 *n*g/m*l*). There were no significant changes in the plasma $1,25\text{-}(OH)_2D$ concentrations (around 30 and 62 *p*g/m*l*) in both PO-RBVD and IMVD groups (Fig. 1). The plasma Ca^{++} and iP concentrations in PO-RBVD group (about 1.31 to 1.38 mmol/*l* and 6.0 to 7.6 mg/d*l*, respectively) tended to be higher than those in IMVD group (about 1.22 to 1.27 mmol/*l* and 5.0 to 6.1 mg/d*l*, respectively) during 1 to 3 days, although there were no significant differences between the groups (Fig. 2).

Effects around parturition: Although the plasma 25-OHD concentrations did not show marked changes in each group (Fig. 3), the levels in PO-RBVD group (about 134 to 185 *n*g/m*l*) were significantly higher than those in IMVD group (about 70 to 90 *n*g/m*l*) during -2 to 2 days after parturition ($P<0.05$ or 0.01). The cows of PO-RBVD group showed the small increase in the plasma $1,25\text{-}(OH)_2D$ levels after parturition (80 to 94 pg/m*l*), whereas the cows of IMVD group revealed the marked rise after parturition (more than 100 *p*g/m*l*). There were no significant differences in the each timed values between PO-RBVD and IMVD groups. The plasma Ca^{++} concentrations in PO-RBVD group seemed to be higher than those in IMVD group around parturition except for 0 to 1 day (Fig. 4). At -2 and 3 days after parturition, the plasma Ca^{++} levels in PO-RBVD group (1.43 ± 0.08 and 1.21 ± 0.04 mmol/*l*, respectively) were

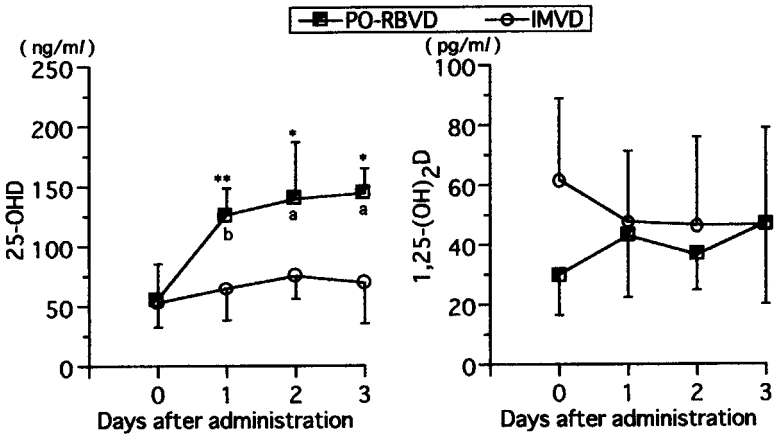

Fig. 1. Changes in the plasma 25-OHD and 1,25-$(OH)_2D$ concentrations after the $RBVD_3$ or VD_3 administration in PO-RBVD and IMVD groups. *P<0.05 and **P<0.01: compared with the value of IMVD group. [a] P<0.05 and [b] P<0.01: compared with the value at 0 day in PO-RBVD group.

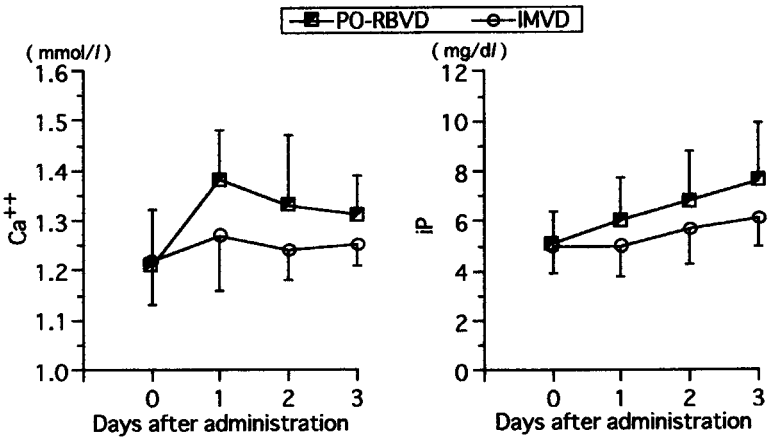

Fig. 2. Changes in the plasma Ca^{++} and iP concentrations after the $RBVD_3$ or VD_3 administration in PO-RBVD and IMVD groups.

significantly higher than those in IMVD group (1.28 ± 0.06 and 1.11 ± 0.04 mmol/*l*, respectively; P<0.05). The plasma Ca^{++} concentrations decreased to about 0.9 mmol/*l* in PO-RBVD group and to about 0.87 mmol/*l* in IMVD groups during 0 to 1 day after parturition. The plasma iP concentrations in PO-RBVD group seemed to be higher than those in IMVD group around parturition. At 3 days after parturition, the plasma iP level in PO-RBVD group (4.1 ± 0.7 mg/d*l*) was significantly higher than that in IMVD group (2.9 ± 0.9 mg/d*l*; P<0.05). The cows of PO-RBVD group revealed the decrease in the plasma iP levels (about 3.5 mg/d*l*) at 0 and 1 days after parturition, and those of IMVD group showed the low iP concentrations (about 2.6 to 3.2 mg/d*l*) during 0 to 3 days.

DISCUSSION

Once VD enters the blood, it accumulates to the liver [8–10, 20]. The increase in the plasma 25-OHD after the VD_3 administration is thought to be caused by the acceleration of the production of 25-OHD in the liver, because VD is converted into 25-OHD by the hepatic microsomal and mitochondrial 25-hydroxylase enzymes [8, 9, 10, 20]. The mitochondrial 25-hydroxylase enzyme, especially, can hydroxylate VD to 25-OHD under excess concentration of VD over the physiological range, because this enzyme is immense in quantity [12]. The 25-OHD is present in the plasma at concentrations of 20 to 50 ng/m*l* in the normal cows and at 200 to 300 ng/m*l* in VD toxicosis [9]. In the present study, the plasma 25-OHD concentrations in PO-RBVD group after the $RBVD_3$ administration increased significantly to approximately 2 to 3 times of the values in the normal cows. The plasma 25-OHD levels around

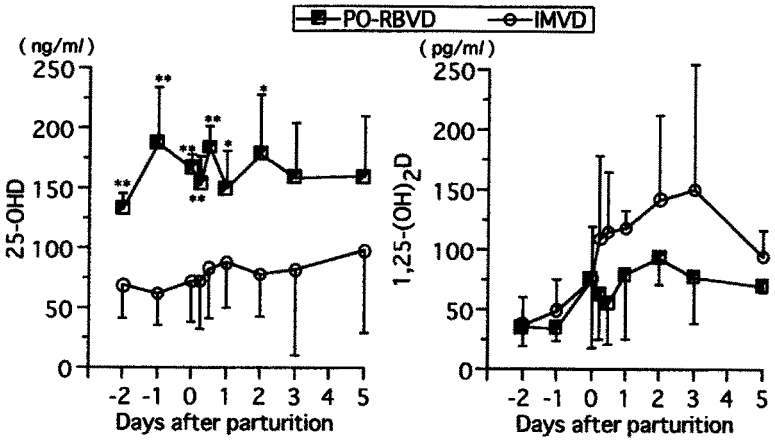

Fig. 3. Changes in the plasma 25-OHD and 1,25-$(OH)_2$D concentrations around parturition in PO-RBVD and IMVD groups. *P<0.05 and **P<0.01: compared with the value of IMVD group.

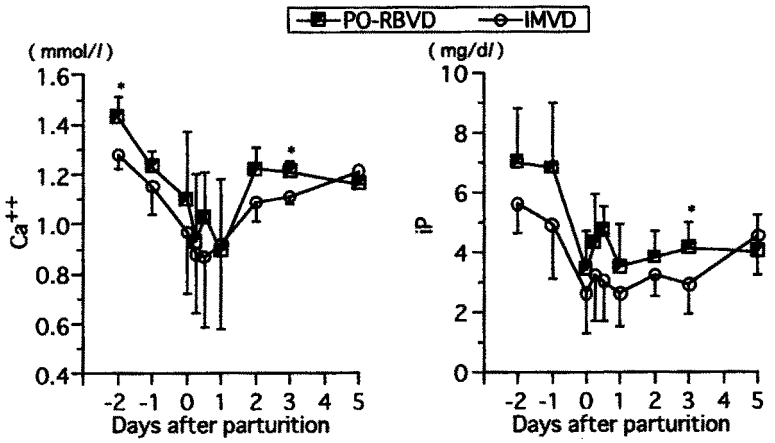

Fig. 4. Changes in the plasma Ca^{++} and iP concentrations around parturition in IMVD and PO-RBVD groups. * P<0.05: compared with the value of IMVD group.

parturition were significantly higher in PO-RBVD group than in IMVD group, although the levels in IMVD group were higher than the values in the normal cows. These results suggest that the most of $RBVD_3$ administered orally bypassed the rumen to be absorbed from the post-ruminal digestive tract. In addition, the results suggest that the absorption of 10 million I.U. of $RBVD_3$ from the digestive tract was more rapid and excellent compared with that of the same dose of VD_3 from an injection site of the muscles.

Hollis *et al.* [7] reported that the plasma 25-OHD concentrations did not change until 7 days after an intramuscular injection of 15 million I.U. of VD_3 to pregnant cows and that the values increased significantly at 28 days. Their results [7] seemed to be similar to the data of the present study, because the plasma 25-OHD concentrations in IMVD group did not show significant increase during at least 3 days after the VD_3 injection. On the other hand, Naito *et al.* [14] and Takaki *et al.* [22] reported that the plasma 25-OHD concentrations increased immediately after an intramuscular injection of 10 million I.U. of VD_3 to lactating cows and pregnant cows. This discrepancy may be due to different solvents of VD_3. The VD_3 used by Hollis *et al.* [7] and in the present study was dissolved in an liposoluble medium, whereas Naito *et al.* [14] and Takaki *et al.* [22] used commercial VD_3 that had been reported as a form of VD_3 dissolved in an aqueous medium [4]. We supposed that the absorption of VD_3 in the liposoluble medium from an injection site of the muscle might be slower than that of VD_3 in the aqueous medium, and we suspect that the VD_3 in liposoluble medium may distribute easily to the fat tissues before the accumulation into the liver.

Once 25-OHD is formed, it can be converted directly into at least four metabolites. Of these metabolites, 1,25-$(OH)_2$D, the most active form of VD, is converted from 25-OHD by 1α-hydroxylase in the kidney [20]. The control of 1α-hydroxylation process is tightly regulated by directly

parathyroid hormone (PTH) and P and indirectly by Ca [9, 10, 20]. For example, the decline in the plasma Ca concentration stimulates to secrete PTH from the parathyroid gland, and PTH stimulates the activation of 25-OHD by up-regulating 1α-hydroxylase enzyme to form 1,25-$(OH)_2$D [9]. When the plasma Ca is high, PTH secretion is depressed, and 1,25-$(OH)_2$D synthesis is depressed [9]. In the present study, there was no significant elevation of the plasma 1,25-$(OH)_2$D concentrations in PO-RBVD group, although the plasma 25-OHD concentrations increased significantly. We suggested that the cows described here maintained the tight control of kidney 1α-hydroxylase enzyme during the high plasma 25-OHD concentrations after the oral $RBVD_3$ administration.

The present study showed the cows of IMVD group had the decrease in the plasma Ca^{++} levels during 0 to 1 day after parturition and the decline in the plasma iP concentrations during 0 to 3 days. The plasma 1,25-$(OH)_2$D concentrations in this group increased markedly after parturition. This increase in the 1,25-$(OH)_2$D levels seemed to be a response to the decline in the plasma Ca^{++} and iP concentrations. The cows of PO-RBVD group showed only a tendency of mild increase in the plasma 1,25-$(OH)_2$D concentrations after parturition (Fig. 3), although the cows had the decrease in the plasma Ca^{++} and iP concentrations during 0 to 1 day after calving. These different degree of the rise in the plasma 1,25-$(OH)_2$D levels was thought to be due to the different degree of the elevation in the plasma Ca^{++} and iP concentrations after the oral $RBVD_3$ administration and VD_3 injection. The plasma Ca^{++} and iP concentrations in PO-RBVD group tended to be higher after the oral $RBVD_3$ administration and were significantly higher at -2 and/or 3 days after parturition than those in IMVD group.

Conrad *et al.* [3] reported that a massive oral doses (20 to 30 million I.U.) of VD increased Ca and P absorption from the digestive tract and induced an increase in the blood Ca and iP levels. Hollis *et al.* [7] reported that bone resorption was activated immediately by an increase in the plasma 25-OHD concentrations after the injection of 15 million I.U. of VD_3. We suggested that the higher values of the plasma Ca^{++} and iP concentrations in PO-RBVD group resulted from increased intestinal absorption and bone resorption of Ca and P due to higher concentrations of the plasma 25-OHD.

In the present study, the single oral administration of 10 million I.U. of $RBVD_3$ at 7 days before the expected calving showed the evidence of rapid and excellent absorption of $RBVD_3$ from the digestive tract and the tendency to maintain the higher plasma levels of Ca^{++} and iP. These results suggested that the $RBVD_3$ administration provided more potent ability to prevent parturient paresis compared with the VD_3 injection used widely in Japan. However, the occurrence of parturient paresis in the several cows of both PO-RBVD and IMVD groups suggested that the prophylaxis with VD_3 was not always complete, as shown in the previous investigations [4, 11].

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REFERENCES

1. Chalupa, W. 1974. Rumen bypass and protection of proteins and amino acids. *J. Dairy Sci.* 58: 1198–1218.
2. Chen, P. S., Jr., Toribara, T. Y. and Warner, H. W. 1956. Microdetermination of phosphorus. *Anal. Chem.* 28: 1756–1766.
3. Conrad, H. R., Hansard, S. L. and Hibbs, J. W. 1956. Studies on milk fever in dairy cows. V. The effect of massive oral doses of vitamin D on absorption, excretion, retention and blood levels of calcium and phosphorus. *J. Dairy Sci.* 39: 1697–1705.
4. Gregorovic, V., Skusek, F., Kesnar, F. and Beks, L. 1967. Crystalline vitamin D3 for the prevention of milk fever in cattle. *Vet. Rec.* 81: 161–162.
5. Hibbs, J. W. and Conrad, H. R. 1960. Studies on milk fever in dairy cows. VI. Effect of prepartal dosage levels of vitamin D on milk fever incidence. *J. Dairy Sci.* 43: 1124–1129.
6. Hibbs, J. W. and Pounden, W. D. 1955. Studies on milk fever in dairy cows. IV. Prevention by short time prepartum feeding of massive doses of vitamin D. *J. Dairy Sci.* 38: 65–72.
7. Hollis, B. B., Conrad, H. R. and Hibbs, J. W. 1977. Changes in plasma 25-hydroxycholecalciferol and selected blood parameters after injection of massive doses of cholecalciferol or 25-hydroxychorecalciferol in nonlactating dairy cows. *J. Nutr.* 107: 606–613.
8. Horst, R. L. 1986. Regulation of calcium and phosphorus homeostasis in the dairy cows. *J. Dairy Sci.* 69: 604–616.
9. Horst, R. L., Goff, J. P. and Reinhardt, T. A. 1994. Calcium and vitamin metabolism in the dairy cows. *J. Dairy Sci.* 77: 1936–1951.
10. Horst, R. L. and Reinhardt, T. A. 1983. Vitamin D metabolism in ruminants and its relevance to the periparturient cows. *J. Dairy Sci.* 66: 661–678.
11. Julien, W. E., Conrad, H. R., Hibbs, J. W. and Crist, W. L. 1977. Milk fever in dairy cows. VIII. Effect of injected vitamin D_3 and calcium and phosphorus intake on incidence. *J. Dairy Sci.* 60: 431–436.
12. Littledike, E. T. and Horst, R. L. 1982. Vitamin D3 toxicity in dairy cows. *J. Dairy Sci.* 65: 749–759.
13. Naito, Y., Saito, M., Watanabe, T. and Murakami, D. 1984. Effects of administered vitamin D_3 on calcium metabolism in cows before and after parturition. *J. Jpn. Vet. Med. Assoc.* 37: 297–302 (in Japanese).
14. Naito, Y., Watanabe, E., Sato, R. and Murakami, D. 1987. Effects of large amounts of vitamin D_3 injection on plasma vitamin D_3 metabolites in lactating cows. *Jpn. J. Vet. Sci.* 49: 121–127.
15. Papas, A. M., Vivini, J. L., Clark, J. H. and Peirce-Sandner, S. 1984. Effects of rumen-protected methionine on plasma free amino acids and production by dairy cows. *J. Nutr.* 114: 2221–2227.
16. Papas, A. M., Sniffen, C. J. and Muscato, T. V. 1984. Effects of rumen-protected methionine for delivering methionine postruminally in dairy cows. *J. Dairy Sci.* 67: 545–552.
17. Payne, J. M. and Manston, R. 1967. The safety of massive doses of vitamin D_3 in the prevention of milk fever. *Vet. Rec.*

81: 214–216.
18. Proszynska, K., Lukaszkiewicz, J., Jarocewicz, N. and Lorec, R. S. 1985. Rapid method for measuring physiological concentration of 25-hydroxyvitamin D levels in blood serum. *Clin. Chem. Act*a 153: 85–92.
19. Radostits, O. M., Blood, D. C. and Gay, C. C. (eds.) 1994. Metabolic diseases: parturient paresis (milk fever). pp. 1314–1328. *In*: Veterinary Medicine, 8th ed. Bailliere Tindall, London.
20. Reinhardt, T. A., Horst, R. L. and Goff, J. P. 1988. Calcium, phosphorus, and magnesium homeostasis in ruminants. *Vet. Clin. North Am.: Food Anim. Pract.* 4: 331–350.
21. Reinhardt, T. A., Horst, R. L., Orf, J. W. and Hollis, B. W. 1984. A microassay for 1,25-dihydroxyvitamin D not requiring high performance liquid chromatography: application to clinical studies. *J. Clin. Endocrinol. Metab.* 58: 91–98.
22. Takaki, H., Naito, Y., Sato, R. and Murakami, D. 1992. Effects of injection of vitamin D_3 on plasma concentrations of vitamin D metabolites. *J. Jpn. Vet. Med. Assoc.* 45: 480–483 (in Japanese).
23. Takemura, K., Osame, S., Ichijo, S. and Sarashina, T. 1990. Application of vitamin AD_3E premix of liquid and rumen bypass types in dairy cows. *J. Jpn. Vet. Med. Assoc.* 43: 864–868 (in Japanese).
24. Tutt, J. B. 1963. Report on clinical trial of Duphafral D.3-1000 for the prevention on hypocalcaemia in dairy cows. *Vet. Rec.* 75: 469–470.
25. Yates, D. J. and Hunt, E. 1990. Disorder of calcium metabolism. pp. 1315–1322. *In*: Large Animal Internal Medicine (Smith, B. P. ed.), C. V. Mosby Company, St. Louis.