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## Vitamin D Provision in High-Yield Dairy Cows During Winter Housing Period

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**Aim.** To investigate the vitamin D status in highly productive cows during winter housing period and effect of cholecalciferol by various ways of vitamin D<sub>3</sub> injection to cows in last days of gestation and after calving.

**Methods.** Enzyme-linked immunoassay, spectrophotometry. **Results.** It has been stated that intramuscular injection of cholecalciferol into cows caused increase of the vitamin D<sub>3</sub> active metabolite – 25-OHD<sub>3</sub>, calcium, phosphorus and magnesium levels together with decrease of alkaline phosphatase level in pre- and post-natal periods. Oral supplementation makes little influence on the studied blood parameters of cows. **Conclusions.** Extrabuccal administration and oral supplementation of cholecalciferol in winter housing period to high-yield cows in the last days of gestation and after calving is accompanied by increased levels of its metabolites and their effect on mineral metabolism in the postnatal period. The nature of these changes depends on the mode of vitamin D administration and the physiological state of the cows.

**Key words:** Vitamin D, 25-hydroxycholecalciferol, metabolism, cattle.

### INTRODUCTION

The influence of fat-soluble vitamins (vitamin D, in particular) upon some metabolism stages in the cattle organism attracts attention of both Ukrainian and foreign researchers.

For a long period of time vitamin D<sub>3</sub> was supposed to act as a hormone regulating calcium and phosphorus homeostasis only. However, the recent explorations provide strong evidences that the vitamin takes part in the plenty of various biochemical processes [1–5]. Particularly, cholecalciferol regulates important physiological functions of the organism, and its deficiency results in negative consequences, such as pathologies and diseases [5–7].

The metabolic processes are intensified in the organism of a cow in pre- and post-natal periods due to changes in endocrine profile, inter-organs rearrangement of both constructive and energetic substrates, vitamins and minerals. All that is dedicated to fetal growth, placenta and milk gland's functions [1, 2]. That is why, the decrease of calcium, mineral phosphorus and vitamin D<sub>3</sub> active metabolite – 25-OHD<sub>3</sub> levels is observed in the cows' blood during pregnancy and after calving [1–3, 5, 7]. The descent of both calcium and phosphorus concentration, which play an important part in the whole range of principal metabolic processes, causes

the disturbance in cows' metabolism. Given the mass calving happens in winter and spring, when the vitamin D rate decreases in forage, the deficiency of this very vitamin is fixed in this very period.

Despite the topicality of the issue, the problem of cholecalciferol standard values in blood for various age groups and also with a glance to physiological state has been still unsolved together with the question of vitamin D dosage. That is why, the explorations dedicated to the development of efficient administration of vitamin D<sub>3</sub> to incalvers for provision of its optimal rate for the lactation period and prevention of post-natal pathologies continue to be relevant.

### MATERIALS AND METHODS

The current research has surveyed the cows of the Ukrainian Black-and-White dairy breed. All animals belong to one and the same age group, are of almost equal body weight, report the same lactation performance (more than 5.5 thousand kg of the previous total lactation yield) and physiological state. The experiment was performed during the winter housing period. The animals were divided into three groups, five cows in each (one – control and two – experimental). The cows in all groups were kept in the same conditions and got with balanced feeding. The grouping was based on the breeding date and veterinary examination results.

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The blood of all cows was tested for vitamin D state of the body at the very beginning of the experiment. After that, the animals got vitamin D in various modes of administration. The 1<sup>st</sup> group of cows (the control one) never got additional cholecalciferol. The animals in the 2<sup>nd</sup> (experimental) group received the daily dose of vitamin D<sub>3</sub> (30 IU per each kg of body weight) every day during a month per oral, starting from 7–10 day, up to the expected calving date, and later – since 5–7 day after calving. The cows from the 3<sup>rd</sup> (experimental) group were injected with vitamin D<sub>3</sub> intramuscular: the first injection – 7–10 days before calving and later – three more times since 5–7 day after calving (each seven days, total dose – 210 IU per each kg of body weight for one injection).

The blood for tests was collected from the jugular vein before morning feeding in the following dates: before vitamin administration, in the 5<sup>th</sup>–7<sup>th</sup> day since calving (after the first intramuscular injection) and in the 30<sup>th</sup> day since calving (after five days after the final injection).

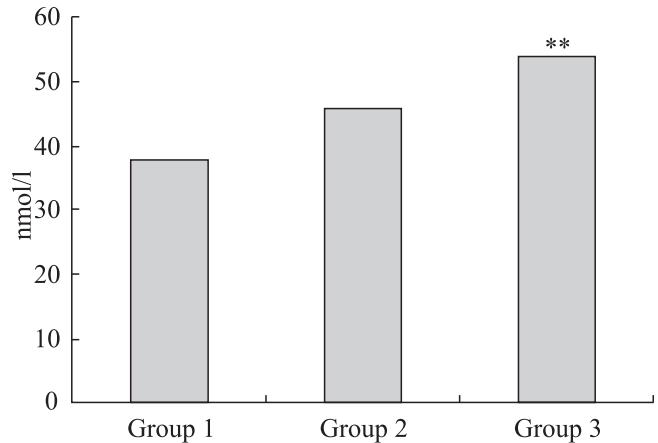
The concentration of 25-OHD<sub>3</sub> in the blood of the examined animals was detected by means of the enzyme-linked immunoassay using the Immunodiagnostik test system (Germany). The calcium, mineral phosphorus and magnesium content and alkaline phosphatase (AP) potency were detected using the Pliva Lachema biological test kits (the Czech Republic) applying the techniques described in the mentioned paper [8]. The AP isoenzymes potency was detected using inhibitors [9]. The obtained data were processed statistically by the Statistica software.

### RESULTS AND DISCUSSION

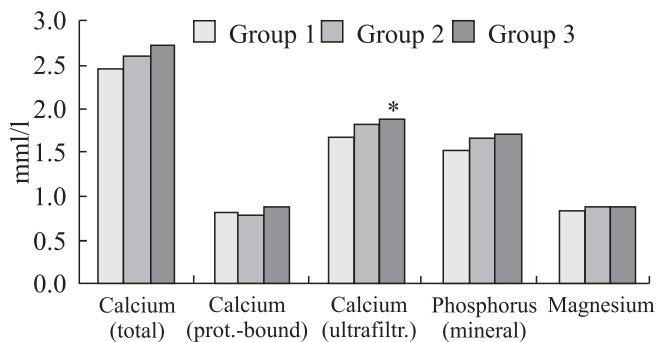
Vitamin D provision rate of an animal organism is detected by 25-OHD<sub>3</sub> concentration in blood [1, 4, 11]. 25-OHD<sub>3</sub> content in blood serum of the cows depends upon age, breed, housing conditions and clinical state [5, 10–12].

The performed research reported that the content of 25-OHD<sub>3</sub> in the blood serum of the cows during winter housing period in 7–10 days before calving (before the first administration of the agent) and in the 5<sup>th</sup>–7<sup>th</sup> day after the calving was almost equal: 38 nmol/l. The content of 25-OHD<sub>3</sub> in the cows' blood after the administration of cholecalciferol performed in various modes in the 5<sup>th</sup>–7<sup>th</sup> day after calving differed from this parameter of the cows from the control group (Fig. 1) In particular, 25-OHD<sub>3</sub> concentration in the blood of the cows from the 3<sup>rd</sup> group appeared to be higher for 42 per cent compared to the control group ( $p < 0.01$ ). The supplementing of cholecalciferol to the cows' forage (daily dose – 30 IU per each kg of body weight) made no essential impact on the concentration of 25-hydroxycholecalciferol in the blood in the 5<sup>th</sup>–7<sup>th</sup> day after calving compared to the control group.

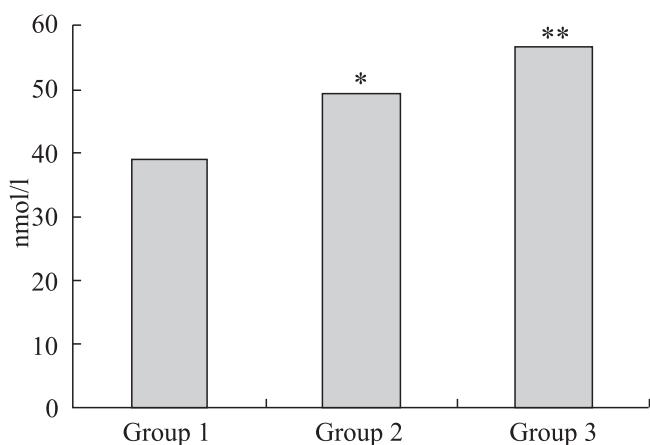
Vitamin D administration to the cows by means of various modes comes amid the changes in mineral turnover. It is known that calcium homeostasis is regu-



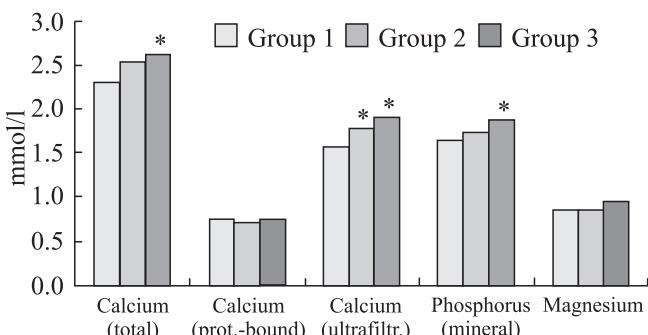
**Fig. 1.** 25-OHD<sub>3</sub> content (nmol/l) in the blood serum of the cows in the 5<sup>th</sup>–7<sup>th</sup> day after calving conditional upon various modes of vitamin D<sub>3</sub> administration ( $M \pm m$ ,  $n = 5$ ); \*\* $p < 0.01$  compared to the control group



**Fig. 2.** Mineral elements content (mmol/l) in the blood serum of the cows in the 5<sup>th</sup>–7<sup>th</sup> day after calving conditional upon various modes of vitamin D<sub>3</sub> administration ( $M \pm m$ ,  $n = 5$ ); \* $p < 0.05$  compared to the control group



**Fig. 3.** 25-OHD<sub>3</sub> content (nmol/l) in the blood serum of the cows in the 30<sup>th</sup> day after calving conditional upon various modes of vitamin D<sub>3</sub> administration ( $M \pm m$ ,  $n = 5$ ); \* $p < 0.05$ ; \*\* $p < 0.01$  compared to the control group



**Fig. 4.** Mineral elements content (mmol/l) in the blood serum of the cows in the 30<sup>th</sup> day after calving conditional upon various modes of vitamin D<sub>3</sub> administration ( $M \pm m$ ,  $n = 5$ ); \* $p < 0.05$  compared to the control group

lated by means of impact on the intestinal absorption of calcium, its further reabsorption in kidneys and skeletal mobilization by calcium-regulating hormones (parat-hormone, calcitonin), and also by the concentration of other hormones and phosphorus via their influence upon vitamin D<sub>3</sub> metabolism [3, 4, 7]. In the blood serum calcium is both protein-bound (albumins, globulins) and ultrafiltrated fraction, able to work its way through colloid membranes. The ultrafiltrated fraction consists of both calcium ionized and combined with citric, phosphoric and carbonic acids.

The total calcium content demonstrated the growth trend in the blood serum of the cows from the 2<sup>nd</sup> and 3<sup>rd</sup> groups in the 5<sup>th</sup>–7<sup>th</sup> day after calving (Fig. 2). However, only the difference between the ultrafiltrated calcium content in the blood serum of the cows from the 3<sup>rd</sup> and control groups was obvious ( $p < 0.05$ ). The min-

eral phosphorus and magnesium concentration in the blood serum of the cows belonging to the experimental groups made no essential difference from the parameters of the control group after the first administration of cholecalciferol.

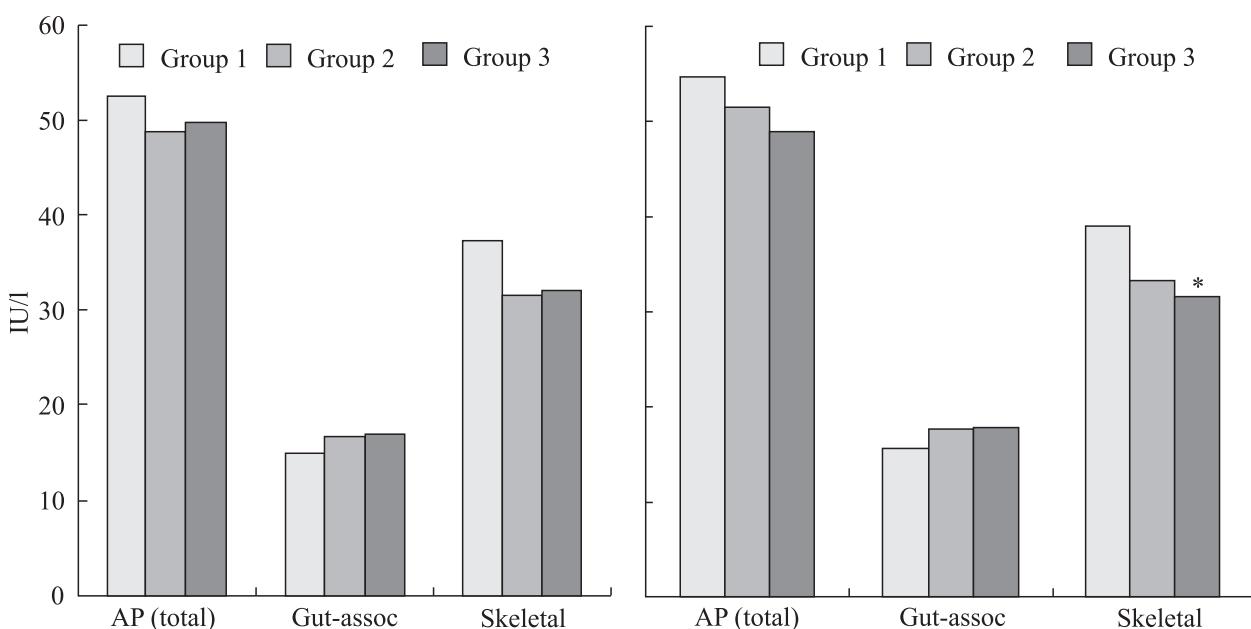
The data from Fig. 3 state that supplementation of cholecalciferol during one month manifested itself in effect of its active metabolites. So, at the 30<sup>th</sup> day after calving 25-OHD<sub>3</sub> content in the blood was obviously higher conditional upon both extrabuccal administration and oral supplementation of vitamin D compared to the control group ( $p < 0.05$ ;  $p < 0.01$ ).

Biochemical data analysis of the blood reports that intramuscular injection of cholecalciferol causes difference in calcium content – both total and ultrafiltrated, protein-bound – in the blood serum of the cows between the 3<sup>rd</sup> and control groups (Fig. 4).

The total calcium content in the blood serum of the cows from the 3<sup>rd</sup> group was 14 per cent higher compared to the control group ( $p < 0.05$ ). The ultrafiltrated calcium rate was reliably higher both at extrabuccal administration and oral supplementation of vitamin D. Such changes are caused by influence of vitamin D<sub>3</sub> upon calcium intestinal absorption in the cows and connected to increased physiological requirements in ionized calcium due to beginning of lactation.

The mineral phosphorus content conditional upon long-term administration of cholecalciferol raises up, though the reliable differences compared to the control group ( $p < 0.05$ ) were reported in the cows of the 3<sup>rd</sup> group only.

The mineral metabolism disorders in the cows' blood comes amid the changes in alkaline phosphatase po-



**Fig. 5.** Alkaline phosphatase potency (IU/l) and its isoenzymes in the 5<sup>th</sup>–7<sup>th</sup> and 30<sup>th</sup> days after calving ( $M \pm m$ ,  $n = 5$ ); \* $p < 0.05$  compared to the control group

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tency, especially its isoenzymes (Fig. 5). In particular, four intramuscular injections of cholecalciferol result in negligible raise of the AP gut-associated isoenzyme, while the skeletal one decreases its potency for 23 per cent ( $p < 0.05$ ). The decline in skeletal AP potency gives evidences on decrease in calcium reabsorption from osseous tissue due to vitamin D effect.

However, after the first intramuscular injection and every day oral supplementation of vitamin during 7 days before calving (daily dose – 30 IU/kg of body weight) the potency of total AP slightly decreased due to raise of AP gut-associated isoenzyme activity, together with decline in skeletal isoenzyme potency. Though, the differences in the parameters of both experimental and control groups were negligible.

In general, the obtained results give evidences on the fact that the supply of down-calving cows with vitamin D during winter-spring housing period is of great importance for physiological metabolism of cholecalciferol, calcium, phosphorus and magnesium in the cattle organism both during post-natal period and intensity peak of the lactation.

### CONCLUSIONS

The cholecalciferol administration in the high-yield dairy cows during winter housing period in the last days of pregnancy and after calving comes amid increasing of its active metabolites' content, while the latter depends upon both the mode of administration and physiological state of the animals.

Both extrabuccal administration and oral supplementation of vitamin D<sub>3</sub> in the last days of pregnancy and during a month after calving result in the raise of calcium in total and also its fractions, mineral phosphorus and magnesium content together with decline of alkaline phosphatase potency in general in the organisms of high-yield dairy cows in the post-natal period, especially at the end of experiment. The nature of these changes depends upon the vitamin D mode of administration.

### Забезпеченість вітаміном D

### високопродуктивних молочних корів у зимово-стійловий період утримання

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**Мета.** Дослідити D-вітамінний статус високопродуктивних корів у зимово-стійловий період, а також дію холекальциферолу за різних способів введення вітаміну D<sub>3</sub> коровам в останні дні тільності та після отелення.

**Методи.** Імуноферментний аналіз, спектрофотометрія  
**Результати.** Встановлено, що внутрішньом'язове введення холекальциферолу коровам спричиняє підвищення рівня активного метаболіту вітаміну D<sub>3</sub> – 25-OHD<sub>3</sub>,

кальцію, фосфору і магнію та зниження активності лужної фосфатази в до- та післяродовий періоди. Пероральне введення чинить незначний вплив на досліджувані показники крові корів. **Висновки.** Парентеральне і пероральне введення холекальциферолу у зимово-стійловий період високопродуктивним коровам в останні дні тільності і після отелення супроводжується зростанням рівня його активних метаболітів та їхнім впливом на мінеральний обмін у післяотельний період. Характер цих змін залежить від способу введення вітаміну D та фізіологічного стану корів.

**Ключові слова:** вітамін D, 25-гідроксихолекальциферол, метаболізм, велика рогата худоба.

### Обеспеченность витамином D высокопродуктивных молочных коров в зимне-стойловый период содержания

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**Цель.** Исследовать D-витаминный статус высокопродуктивных коров в зимне-стойловый период, а также действие холекальциферола при различных способах введения витамина D<sub>3</sub> коровам в последние дни стельности и после отела. **Методы.** Имуноферментный анализ, спектрофотометрия. **Результаты.** Установлено,

что внутримышечное введение холекальциферола коровам вызывает повышение уровня активного метаболита витамина D<sub>3</sub> – 25-OHD<sub>3</sub>, кальция, фосфора и магния, а также снижение активности щелочной фосфатазы в до- и послеродовый периоды. Пероральное введение оказывает незначительное влияние на исследуемые показатели крови коров. **Выводы.** Парентеральное и пероральное введение холекальциферола в зимне-стойловый период высокопродуктивным коровам в последние дни стельности и после отела сопровождается повышением уровня его активных метаболитов и их влиянием на минеральный обмен в период после родов. Характер этих изменений зависит от способа введения витамина D и физиологического состояния коров.

**Ключевые слова:** витамин D, 25-гидроксихолекальциферол, метаболизм, крупный рогатый скот.

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