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VITAMIN D-STATUS OF CALVES AT THE FIRST MONTH OF LIFE AFTERDIFFERENTROUTES OF CHOLECALCIFEROLINPUT TO COWS

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Vitamin D-status of early postnatal calves obtained from high-performance cows were treated with cholecalciferol orally or intramuscularly in winter-stall period was investigated.Vitamin D was added to the diet in daily dose of 30 IU per 1 kg of body weight for a month starting 7–10 days before the predicted date of calving, and then in 5–7 day after calving. Intramuscularly vitamin D was administered: the first time — at 7–10 days before the predicted date of calving and three more times starting from 5–7th day after calving every 7 days at a dose of 210 IU per 1 kg of body weight for each input.For studies in calves for took blood 5–7- and 28–30-th day after birth. In the blood examined the contents of the 25-hydroxyvitamin D, total calcium and its fractions, inorganic phosphorus, magnesium and alkaline phosphatase activity.

It was established that calves derived from cows that in the last days of gestation and after calving were treated with cholecalciferol orally or intramuscularly had higher blood levels of 25-OHD_3, total calcium, bounded with protein calcium and ultrafiltrated calcium, inorganic phosphorus, magnesium and lower activity of alkaline phosphatase compared to calves from cows of the control group.

Blood serum of calves born by cow sthat were intramuscular injected with vitamin D_3 characterized by significantly higher content of 25-OH D_3, calcium and inorganic phosphorus at 5–7th and 28–30th day after birth, compared to calves of control group. Supplementation of cow diet with cholecalciferol showed the effectt on these calf blood parameters onlyon 28–30thday after birth.

Thus, the introduction of cholecalciferol cows in late pregnancy and early lactation is essential to ensure their calves this vitamin in the early postnatal period.

Keywords: COWS, CALVES, VITAMIN D, METABOLISM, BLOOD, 25-HYDROXYCHOLECALCIFEROL, CALCIUM, PHOSPHORUS, MAGNESIUM, ALKALINE PHOSPHATASE

D-ВІТАМІННИЙ СТАТУС ТЕЛЯТ У ПЕРШИЙ МІСЯЦЬ ЖИТТЯ ЗА РІЗНИХ СПОСОБІВ ВВЕДЕННЯ ХОЛЕКАЛЬЦИФЕРОЛУ КОРОВАМ

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У зимово-стійловий період досліджували D-вітамінний статус телят раннього постнатального періоду розвитку, які були отримані від високопродуктивних корів, котрим перорально і парентерально вводили холекальциферол. Перорально холекальциферол вводили коровам до корму щоденно у добовій дозі 30 МО на 1 кг маси тіла впродовж місяця, починаючи за 7−10 днів до прогнозованої дати отелення, та з 5−7-го дня після отелення. Внутрішньом'язово вітамін D вводили: перший раз — за 7−10 днів до прогнозованої дати отелення і ще тричі починаючи з 5−7-го дня після отелення через кожні 7 днів у дозі 210 МО на 1 кг маси тіла за одне введення. Для досліджень у телят брали кров на 5–7 і 28–30-й день після народження. У крові досліджували

вміст 25-гідроксивітаміну D, кальцію загального і його фракцій, фосфору неорганічного, магнію та активність лужної фосфатази.

Встановлено, що телята, отримані від корів, яким в останні дні тільності та після отелення вводили холекальциферол перорально і внутрішньом'язово, характеризувалися більшим вмістом у крові 25-OHD$_3$, кальцію загального, протеїнзв'язаного і ультрафільтрованого, неорганічного фосфору, магнію та нижчою активністю лужної фосфатази, ніж телята, отримані від корів контрольної групи. Телята, отримані від корів, яким вітамін D вводили внутрішньом'язово характеризувалися вірогідно вищим вмістом 25-OHD$_3$, кальцію і неорганічного фосфору на 5–7-й і 28–30-й день після народження, порівняно із контрольними. Додавання коровам холекальциферолу до корму проявляло свій вплив на досліджувані показники у крові телят лише на 28–30-й день після народження.

Отже, введення холекальциферолу коровам в кінці тільності і на початку лактації є важливим для забезпечення їхніх телят цим вітаміном у ранній постнатальний період.

Ключові слова: КОРОВИ, ТЕЛЯТА,ВІТАМІНD, МЕТАБОЛІЗМ, КРОВ 25-ГІДРОКСИХОЛЕКАЛЬЦИФЕРОЛ, КАЛЬЦІЙ, ФОСФОР, МАГНІЙ, ЛУЖНА ФОСФАТАЗА

D-ВИТАМИННЫЙ СТАТУС ТЕЛЯТ В ПЕРВЫЙ МЕСЯЦ ЖИЗНИ ПРИ РАЗНЫХ СПОСОБАХ ВВЕДЕНИЯ ХОЛЕКАЛЬЦИФЕРОЛА КОРОВАМ

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В зимне-стойловый период исследовали D-витаминный статус телят раннего постнатального периода развития, которые были получены от высокопродуктивных коров, которым перорально и парентерально вводили холекальциферол. Перорально холекальциферол вводили коровам в корм ежедневно в дозе 30 МЕ на 1 кг массы тела в течение месяца, начиная за 7–10 дней до прогнозируемой даты отела, и с 5–7-го дня после отела. Внутримышечно витамин D вводили: первый раз — за 7–10 дней до предполагаемой даты отела и еще трижды начиная с 5–7-го дня после отела через каждые 7 дней в дозе 210 МЕ на 1 кг массы тела за одно введение. Для исследований в телят брали кровь на 5–7 и 28–30-й день послерождения. В крови исследовали содержание 25-гидроксивитамина D, кальция общего и его фракций, фосфора неорганического, магния и активность щелочной фосфатазы

Установлено, что телята, полученные от коров, которым в последние дни стельности и после отела вводили холекальциферол перорально и внутримышечно, характеризировались высшим содержанием в крови 25-OH D3, кальция общего, протеинсвязанного и ультрафильтруючегося, неорганического фосфора, магния и более низкой активностью щелочной фосфатазы, чем телята полученные от коров контрольнойг руппы. Телята, полученные от коров, которым витамин D вводили внутримышечно; характеризировались достоверно высшим содержанием 25-OH D3, кальция и неорганического фосфора на 5–7-й и 28–30-й день после рождения, по сравнению с контрольными. Добавление коровам холекальциферола в корм проявляло своевлияние на исследуемые показатели в крови телят только на 28–30-й день после рождения.

Следовательно, введение холекальциферола коровам в конце стельности и в начале лактации является важным для обеспечения телят этим витамином в ранний постнатальный период.

Ключевые слова:КОРОВЫ, ТЕЛЯТА, ВИТАМИН D, МЕТАБОЛИЗМ, КРОВЬ, 25-ГИДРОКСИХОЛЕКАЛЬЦИФЕРОЛ, КАЛЬЦИЙ, ФОСФОР, МАГНИЙ, ЩЕЛОЧНАЯ ФОСФАТАЗА

Feeding and housing conditions of pregnant cows have a significant impact on the viability of newborn calves and their physiological maturity, growth, development and implementation of the genetic productivity potential. The vitamin D plays important role in ensuring vital functions of calves in early postnatal periods. In young cattle 1–12 months old, mild D-deficient condition is diagnosed in more than 40 %. Calves born in the autumn and winter are sick more often [1–4].

It is known that newborn calves have a low level of vitamin D in the blood plasma and liver [5]. The supplement of their mothers by vitamin D and its levels in colostrum and milk consumed the offspring has a direct impact on D-vitamin status of the body in the early postnatal period [3, 6–11]. Research by B. W. Hollis et al. (1983) found that there is a relationship between level of 25-hydroxyvitamin D in blood of cows and its level in milk [12].

Vitamins D_2 and D_3 are biologically inert compounds. They pass a series of consistent transformations for the manifestation of theirs biological actions. The first stage of this transformation occurs in the liver under the influence hydroxylized enzymes to form 25-OH D. Further it's transformation to active metabolites depends on the level of calcium and phosphorus in the blood. During hypocalcemia and hypophosphatemia, 25 OH D is converted to 1,25-$(OH)_2$ D in kidney, but for norm- and hypercalcemia hydroxylation occurs on 24- or 26-th position of carbon to form 24,25-$(OH)_2$ D and 26,25-$(OH)_2$ D, respectively. Partially the transport form of 25-OH D enters into the fat and muscle tissues, where it can create tissue depot of indefinite existence [13].

The criterion for evaluation of the need for vitamin D, which suggested R. L. Horst et al. (1994) is the concentration of 25-hydroxycholecalciferol in blood. The level of 25-hydroxycholecalciferol is considered as a total reflection of the endogenous formation of cholecalciferol in the skin and its receipts from feed or vitamin preparations. In healthy dairy cows concentration of 25-OH D_3 in plasma is in the range of 20 to 50 ng/ml. Values less than 5 ng/ml is considered as a sign of deficiency of vitamin D; if the concentration exceeds 200 to 300 ng/ml, it is evidence of the development of hypervitaminosis, and causes intoxication by vitamin D [14].

In addition, today the issue of normal values of concentration of 25-OH D_3 in serum is debatable. There is no doubt in fact that «normal» can be considered such level of 25-OH D_3, which ensures the implementation of cholecalciferol effects in all organs that contain specific receptors for its hormonally active form — 1,25 $(OH)_2$ D_3 [15]. Research has established that in the early postnatal period animals, can adapt to low levels of vitamin D in their body variously, which depends on the species [16].

Despite the large number of studies that being conducted by different research groups in order to establish normal levels of vitamin D in the blood of humans and animals, remains important questions regarding the optimal level of vitamin D in the body of calves in early postnatal period because of various factors and ways of correction. This issue is topical not only in Ukraine, but also in other European countries, because of very little information about the actual content of vitamin D in the feed in various regions, the loss of it's prolonged storage ability and genetic ability of cows to accumulate vitamin D in the liver and adipose tissue by the action sunlight during the grazing period. Moreover, no data about the levels of 25-OH D_3 in plasma, which define the lower limit of adequacy or sufficiency of the body and its effects on metabolism in calves during the first month of life.

In this context, the aim of this study was to investigate the contents of the active metabolite of vitamin D_3 — 25-OH D_3, the concentration of calcium and its fractions, inorganic phosphorus, magnesium and alkaline phosphatase activity in the blood of calves recieved from high-yielding cows which were parenteral injection and oral supplementation of vitamin D_3 in the last days of gestation and after calving.

Materials and methods

Studies were conducted in the three groups of dairy calves in pilot farm «Pasichna» of Institute of forage and agricultural Podillya NAAS of Ukraine, located in the natural geographical areas of Podillya. The experiment was performed during the winter housing period. The calves of all groups were obtained from high-yielding cows of the Ukrainian Black-and-White dairy breed that were kept in and the same conditions and got with balanced feeding. Calves born from these cows were divided into three groups. The 1st group of calves (the control one) were obtained from cows that never got additional cholecalciferol. The calves of 2nd (experimental) group derived from cows that received the daily dose of vitamin D_3 (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 calves of 3rd (experimental) group derived from cows that injected with vitamin D_3 intramuscular: the first injection — 7−10 days before calving and later — three more times since 5−7 day after calving (each seven days, dose — 210 IU per each kg of body weight for one injection).

The blood for research was collected from the jugular vein of calves in the following dates: at 5th–7th days old (after the first intramuscular injection) and at 28–30th days old (after five days after the final injection).

The concentration of 25-OH D_3 in the blood of the examined animals was detected by means of the enzymelinked immunoassay using the test system developed by the Immunodiagnostik (Germany). The method is based on the competitive binding of 25-OH D_3 serum and 25OH D_3-biotin with vitamin D_3-binding protein (VDBP), that immobilized on 96-well immunological plates.

The content of calcium (total, bounded with protein and ultrafiltrated), inorganic phosphorus, magnesium and alkaline phosphatase (ALP) activity were detected using the biological test kits produced by the Pliva Lachema firm (the Czech Republic) applying the techniques described in the mentioned paper [17]. The AP isoenzymes activities were detected using techniques described in the mentioned paper [18] and expressed as U/l — the number of micromoles of 4-nitrophenol released by the enzyme contained in 1 liter of serum for 1 min under these conditions. The obtained data were processed statistically by the Statistica software. The results of the mean values was considered statistically significant at: $p<0.05$ — *, $p<0.01$ — ** and $p<0.001$ — ***, compared to a control group of calves.

Results and discussion

Vitamin D provision rate of an animal organism is detected by 25-OH D_3 concentration in blood, which reflects the total number of vitamin D of endogenous and exogenous origin [1, 5, 13, 15]. 25-OH D_3 content in blood serum of the cattle ows depends upon age, breed, housing conditions and clinical state [1, 5–9, 15].

It was established that the content of 25-OHD_3 in the blood of calves aged 5–7 days was lower, and the concentration of calcium and inorganic phosphorus — higher than in the blood of their mothers after parturition [19]. We found a lower concentration of 25-OH D_3 in the blood of newborn calves compared to the content of their mothers for 5–7 days after calving apparently confirmed by studies of several authors, that the activity of 25-hydroxylase in the liver of newborns was very low [20].

Fig. 1. The content of 25-OH D_3 (nmol/l) in the blood serum of the calves conditional upon various modes of vitamin D_3 administration to cows (M ± m, n = 5)

The content of 25-hydroxycholecalciferol in serum of calves in the control group at 5–7-days age old was 31.4±2.56 nmol/l and slightly increased for the 28–30-days (Fig. 1, 2). The injection of cholecalciferol to cows by different ways led to an increase of 25-OH D_3 concentration in blood up their calves at 5–7th and 28–30th days old. Thus, at 28–30th days after birth,concentration of 25-OH D_3 in the calves blood in 2nd and 3rd groups were 25 % and 29 % higher ($p<0.05$) in comparison to calves of the control group ($p<0.05$). In 5–7th days age the 25-hydroxycholecalciferol content was significantly higher only in calves derived from cows that vitamin D was administered intramuscularly ($p<0.05$).

Thus, the 25-hydroxycholecalciferol concentration in the blood of calves during first days after birth depends on the content of this metabolite in the blood of their mothers and in the consumed colostrum and milk. Our data are consistent with studies on other animal species and human, the level of vitamin D in the blood of pregnant women is shown in its level in the blood of their offspring [6–8; 10, 12].

The content of total calcium in the blood of all groups calves in the 5–7-th day after birth was higher in comparisant to its rates in 30-day age (Fig. 2, 3). The high concentration of total calcium in the blood of calves during the first days after birth is also likely to be the result of a high concentration of 1,25-$(OH)_2$ D in maternal blood and its effect on placental calcium transport, as confirmed by studies on sheep [21].

The injection of cholecalciferol to cows before and after calving was accompanied by an increase in total calcium and its fractions in the blood of their calves. Thus, at the 5–7 days after birth the total calcium content in the serum of calves from the 3rd group was higher by 11 % ($p<0.05$), protein-bound — 15 % ($p<0.01$) and ultrafiltrated — 9 % ($p<0.05$) compared to that of the control group calves. In the 2nd group of calves at this age, significant difference in the contents protein-bound calcium was only observed.

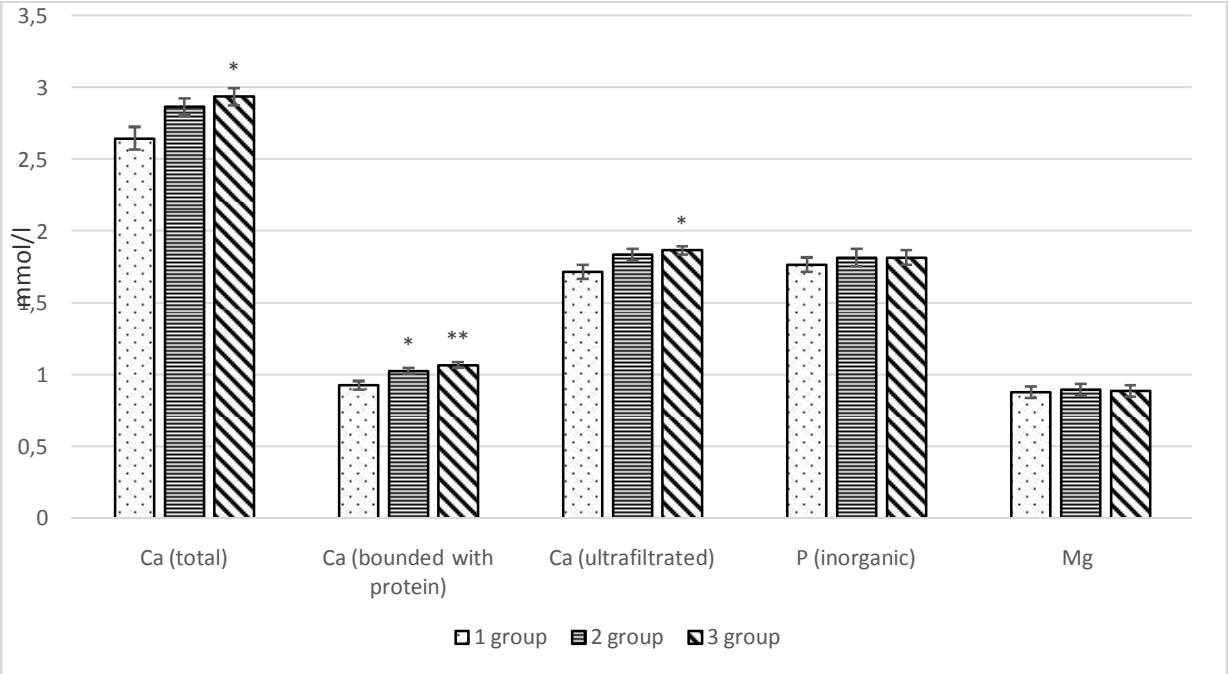

Fig. 2. The content of mineral elements (mmol/l) in the blood serum of the calves in the 5th–7th day age old (M ± m, n = 5)

In the 28–30-day after birth the total calcium content in the blood serum of calves from the 2nd group was higher by 11 % ($p<0.05$) and 3rd group — by 14 % ($p<0.01$) in comparison to parameters of the control group (Fig. 3). Thus, the content of protein-bound calcium in serum of calves from the 2nd group was higher by 22 % ($p<0.001$) and 3rd — 26 % ($p<0.001$). Content ultrafiltrated calcium in this age was significantly higher only in calves from the 3rd experimental group.

In the control and experimental groups of calves aged 5–7-days, there was insignificant difference between values of concentration of inorganic phosphorus (1.77–1.82 mmol/l) in the blood (Fig. 2). The content of phosphorus in the blood was increased in control as well as in experimental groups of calves aged 28–30 days and amounted in accordance: 1.83±0.05; 1.91±0.06 and 2.02±0.05 mmol/l (Fig. 3). The injection of cholecalciferol to cows before and after calving of vitamin D by different ways was leading to a significant increase in the concentration of inorganic phosphorus in the blood of calves only the 3rd group on the 28–30th day after birth.

Also, there were found no significant differences on the concentration of magnesium in the blood of calves of both research groups in the 5–7-day as well as in the 28–30-day age, in comparisant to the performance of calves in the control group.

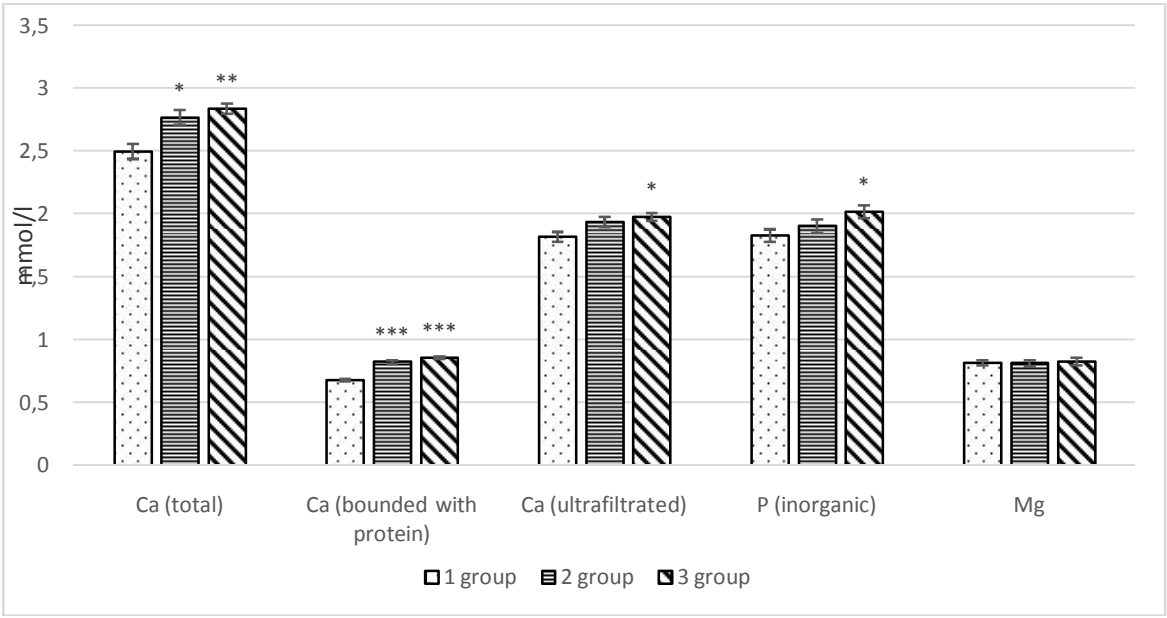

Fig. 3. The content of mineral elements (mmol/l) in the blood serum of the calves in the 28th–30th day age old (M ± m, n = 5)

The activity of alkaline phosphatase in the blood serum of calves in 5–7th after birth was 160–166 IU/L (Fig. 4). By the 28–30 days age the enzyme activity in the blood of calves somewhat increased especially due to increasing bone isoenzyme (Fig. 5). The administration of cholecalciferol to cows before and after calving by different ways were accompanied by a decrease in total alkaline phosphatase activity and bone isoenzyme activity and increased intestinal isoenzyme in the blood of calves experimental groups. Significant difference was only observed in the intestinal isoenzyme activity in the blood of calves at the 28–30th-day of age that were obtained from cows injected intramuscularly vitamin D.

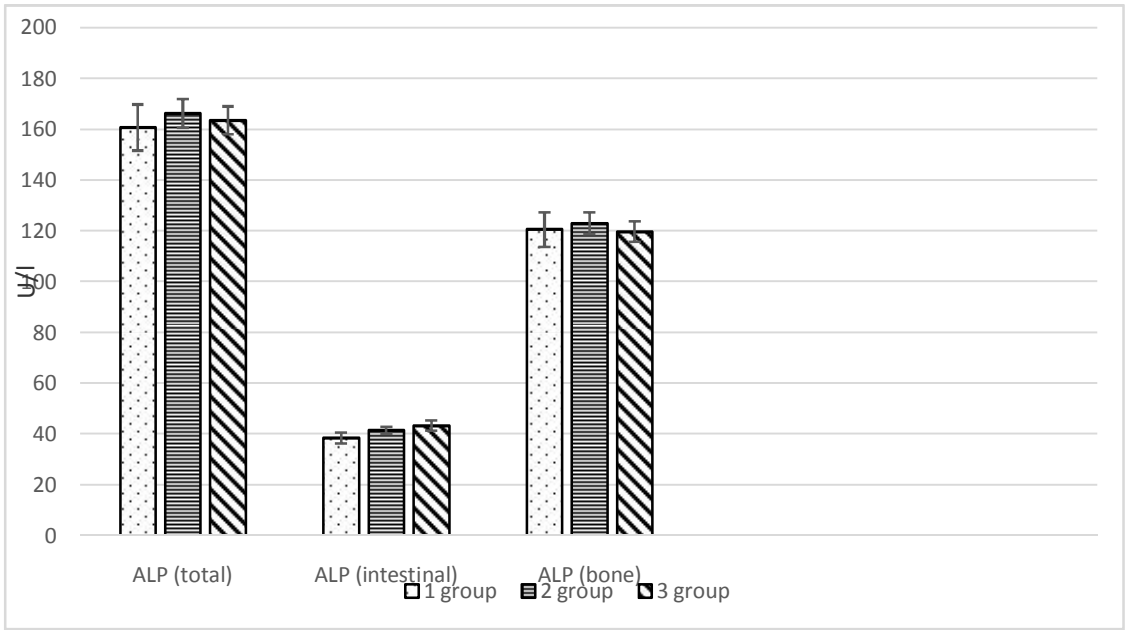

Fig. 4. The activity of alkaline phosphatase (IU/l) and its isoenzymes in the 5th–7th days age old (M ± m, n = 5)

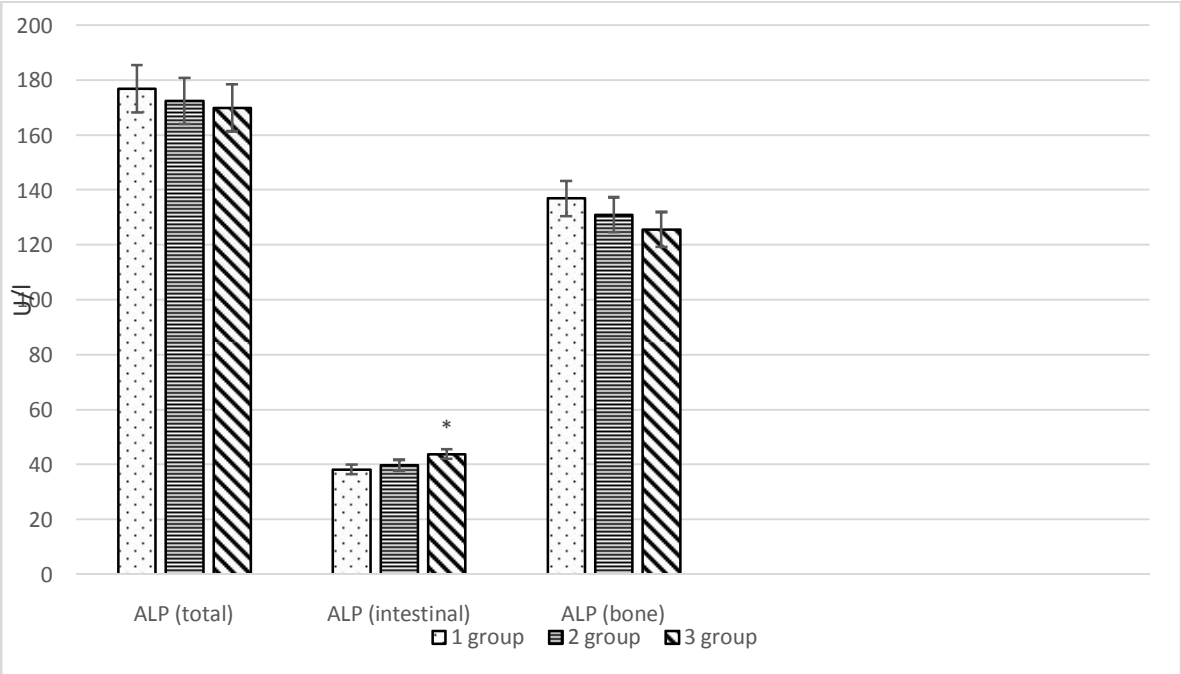

Fig. 5. The activity of alkaline phosphatase (IU/l) and its isoenzymes in the 28th–30th days age old (M ± m, n = 5)

The obtained results indicate that the provision of vitamin D and calcium and phosphorus metabolism in calves aged up to month depended on the content of cholecalciferol in the body of cows in late pregnancy and early lactation periods. Also it indicates about more active absorption of this vitamin from maternal colostrum and milk due to the beneficial effects of active metabolites cholecalciferol on the functional state of organs (intestine, liver, and kidneys) participating at its absorption and metabolism.

Conclusions

The intramuscular injection of cholecalciferol to cows: the first time — for 7–10 days before the predicted calving date and three more times every 7 days starting from 5–7 day after calving at a dose of 210 IU per kg of body weight for one injection, and by daily adding of cholecalciferol to the feed every day at a daily dose of 30 IU per kg of body weight for a month, starting 7–10 days before the predicted date of calving, and 5–7 day after calving in winter-stall period, is accompanied by increase in D-vitamin status of their calves from birth to 30 days of age. Calves derived from cows that were introduction cholecalciferol orally and intramuscularly were characterized with higher levels of 25-OH D_3 in blood, total calcium and its fractions, inorganic phosphorus, magnesium and lower activity of alkaline phosphatase than calves from cows of the control group. The level of these changes depends on way of introduction, the quantity of injected vitamin and age of animals.

To prospect of further researches. The prospect for further research is the study the biological action of vitamin D in the body of cattle in different geographical areas of Ukraine in different age periods of development and different physiological periods in health and disease.

1. Levchenko V. I., Vlizlo V. V., Kondrahin I. P. et al. ; za red. Levchenka V. I. The clinical diagnosis of internal diseases. Bila Cerkva, 2004. 608 p. (In Ukrainian).

2. Levchenko V. I., Tihonjuk L. A., Apuhovska L. I. Diagnosis of early forms of D-hypovitaminosis in calves over the content of phosphorus and 2.3 dyfosfohlitseratu in erythrocytes. *Bulletin of Agricultural Science,* 1981, № 9, pp. 73–76 (in Ukrainian).

3. Kurtjak B. M., Janovich V. G. *Fat-soluble vitamins in veterinary medicine and animal husbandry.* Lviv, Triada Pljus, 2004. 426 p. (In Ukrainian).

4. Maslova T. V., Egorova G. G. Etiological factors of development of D defytsyt status of calves. *Advances modern natural science*, 2005, № 10, p. 68 (in Russian).

5. Horst R. L., Reinhardt T. A. Vitamin D metabolism in ruminants and its relevance to the periparturient cow. *J. Dairy Sci,* 1983, Vol. 66, pp. 661–678.

6. Goff J. P., Horst R. L., Littledike E. T. Effect of maternal vitamin D status at parturition on the vitamin D status of the neonatal calf. *J. Nutr.,* 1982, 112, pp. 1387–1393.

7. Barlet J. P., Argemi B., Davicco M. & Lefaivre J. Plasma concentrations of 25-hydroxyvitamin D in pregnant and lactating ewes and foetal and newborn lambs. *J. Endocrinol.*, 1978, 79, pp. 149–150.

8. Van Saun R J. Vitamin D-responsive rickets in neonatal lambs. *Can Vet J.*, 2004, vol. 45, pp. 841–844.

9. Reinhardt T. A., Horst R. L., Goff J. P. Calcium, phosphorous, and magnesium homeostasis in ruminants. *Vet. Clin. North Am. Food Anim. Pract.,* 1988, 4, pp. 331–350.

10. Luk'janova E. M., Antipkin Ju. G., Omel'chenko L. I., Apuhovskaja L. I. *Vitamin D & its role in the Provision of health of children and pregnant women.* K., Ekspert B, 2005. 230 p. (In Ukrainian).

11. Dittmer K. E., Thompson K. G. Vitamin D Metabolism and Rickets in Domestic Animals : A Review. *Vet. Pathol,* 2011, vol. 48, pp. 389–407.

12. Hollis B. W., Lambert P. W., Horst R. L. *Factors affecting the antirachitic content of native milk.* Page 157 in Perinatal calcium and phosphorus metabolism. M. F. Holick, T. K. Ray, and C. S. Anaste, ed. Elsevier Sci. Publ., B. V., 1983.The Netherlands.

13. DeLuca H. F. The vitamin D system in the regulation of calcium and phosphorus metabolism. *Nutr. Rev.*, 1979, 37, pp. 161–193.

14. Horst R. L., Goff J. P., Reinhardt T. A. Calcium and vitamin D metabolism in the dairy cow. *J. Dairy Sci.,* 1994, 77, pp. 1936–1951.

15. Norman A. W. From vitamin D to hormone D: Fundamentals of the vitamin D endocrine system essential for good health. *Am. J. Clin. Nutr.,* 2008, 88, pp. 491–499.

16. Goff J. P., Horst R. L., Littledike E. T. Effect of sow vitamin D status at parturition on the vitamin D status of the neonatal piglets. *J. Nutr.,* 1984, pp. 114–163.

17. Vlizlo V. V., Fedoruk R. S., Ratych I. B. et al. ; za red. Vlizlo V. V. Laboratory methods of research in biology, veterinary medicine: a guide. Lviv: SPOLOM, 2012. 764 p. (In Ukrainian).

18. Vagner V. K., Putilin V. M., Harabuga G. G. *Questions of medical chemistry*, 1981, 27, № 6, pp. 752–754 (in Russian).

19. Yuskiv L. L., Vlizlo V. V. Vitamin D Provision in High-Yield Dairy Cows During Winter Housing Period. *Agricultural Science and Practice*, 2014, vol. 1 (1), pp. 42–46.

20. Gascon-Barre M., Plourdo V., Haddad P., Martial J. *Fetal and neonatal uptake and microsomal C-25 hydroxylation of vitamin D by the rat liver*. Page 654 in Vitamin D. Chemical, biochemical and clinical update. A. W. Norman, K. Schaefer, H.-G. Grigolt, and D. V. Herra, h,ed. de Gruyter, 1985, Berlin.

21. Durand, D., G. D. Braithwaite, and J.-P. Baler. The effect of la-hydroxycholecalciferol on the placental transfer of calcium and phosphate in sheep. *Br. J. Nutr.,* 1983, 49, pp. 475–483.