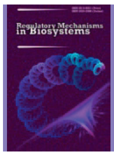

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Metabolism of calcidiol and calcium in pregnant and lactating goats

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The aim of our study was to investigate the dynamics of the metabolism of one of the active metabolites of vitamin D – $25OHD_3$ and its effect on the metabolism of total and ionized calcium in pregnant and lactating goats. The subject of the study was Zaanen and Laman goats: pregnant (75–90 and 120–140 days of pregnancy) and lactating animals (0–2 and 15–25 days after kidding). The content of $25OHD_3$ in the blood serum of goats was in the range of 9.8–54.2 ng/mL, including 10.4–32.4 ng/mL in the pregnant animals (75–140 days) and 9.8–54.2 ng/mL in the lactating animals (0–25 days). The dynamics of calcidiol in the blood serum of the pregnant goats was characterised by a 1.35-fold increase in its concentration on the 120–140th days compared to the animals 75–90 days of kidding, with maximum values on the 0–2nd day safter kidding and a decrease in its content on the 15–25th days of lactation. In clinically healthy goats with optimal serum calcium content, the total concentration of $25OHD_3$ ranged from 10.4 to 54.2 ng/mL, including 10.4–32.4 ng/mL in the pregnant animals and 12.1–54.2 ng/mL in the lactating animals. In the subclinical course of hypocalcaemia, the serum calcidiol content in the blood of goats was in the range of 9.8–29.8 ng/mL, including in pregnant animals – from 11.0 to 21.0 ng/mL, during the first 15–25th days of lactation – 9.8–29.8 ng/mL. At the optimal content of ionised calcium in the blood serum of goats, the concentration of calcidiol was in the range of 10.4–54.2 ng/mL, against 9.8–29.8 ng/mL in animals with hypocalcaemia. There is a positive correlation between the values of 25OHD3 and total calcium in the blood serum of goats with subclinical hypocalcaemia.

Keywords: $25OHD_3$; total calcium; ionised calcium; hypocalcaemia; statistics.

Introduction

Goat breeding is a promising sector in the structure of the global livestock industry. Today, the number of goats in the world is one billion, fewer than cattle and sheep. However, between 2006 and 2016, the number of goats increased by 19.3%, while cattle and sheep increased by only 6.7% and 6.8%, respectively (Bayoumi et al., 2021; Nair et al., 2021; Kirgiafini et al., 2024). Goats are hardy and do not require significant maintenance costs. Thanks to their breeding ability, these animals adapt to various climate changes (Martin et al., 2017; Paim et al., 2019; Massender et al., 2022).

Proper nutrition of animals involves providing the body with all the nutrients necessary for vital activity: proteins, carbohydrates, lipids, vitamins and minerals. The calculation of livestock rations takes into account the concentration of nutrients and biologically active substances necessary for maintaining health, as well as the body's need to ensure high animal productivity under modern intensive animal husbandry technologies. This applies to all components of the diet, including fat-soluble vitamins (A, D, E, K) (Vlizlo, 2015; Combs & McClung, 2016). One of the most vital fat-soluble vitamins for animals is vitamin D. It is represented by two main forms: D_2 (ergocalciferol) and D_3 (cholecalciferol). It is also called the 'sunshine vitamin' because 7-dehydrocholesterol (7-DHC) is isomerised to vitamin D_3 in the epidermis under the influence of UV radiation within 3 days (Holick & Slominski, 2024). According to Bikle (2010), the main epidermal cells, keratinocytes, also contain enzymes that can form one of the active metabolites of vitamin D, $1,25(OH)_2D_3$ (calcitriol) (Kohler et al., 2013; Hribar et al., 2023). Ergocalciferol is ingested by animals with feed or feed additives. The largest source of vitamin D_2 is sun-dried vegetable feeds, as they contain ergosterol, which undergoes photoisomerisation and is converted to ergocalciferol when exposed to sunlight (Houghton & Vieth, 2006).

Vitamins D_2 and D_3 function as prohormones, are biologically inactive and the only difference between them is the structure of their side chains. A two-stage enzymatic hydroxylation process is required to convert ergo- and cholecalcifolate vitamins into active compounds. In the first stage of hydroxylation in the liver, vitamins D_2 and D_3 are converted to 25-hydroxyvitamin D ($25OHD_3$) by 25-hydroxylases. The kidneys are the second site of hydroxylation, where 1α-hydroxylase converts $25OHD_3$ to 1,25-dihydroxyvitamin D_2 or D_3 (calcitriol). However, the rate of conversion is under homeostatic control due to its dependence on the concentration of circulating parathyroid hormone. 1α-Hydroxylation in the kidneys depends on the level of ionised calcium in the plasma (Holick, 2002; Jones, 2008). Dittmer & Thompson (2010) note that at low plasma ionised calcium concentrations, active renal 1α-hydroxylation of $25OHD_3$ occurs to form calcitriol, and at optimal levels, calcidiol undergoes 24-hydroxylation to an inactive metabolite. In contrast to hepatic 25-hydroxylases, renal 1α-hydroxylase activity is strictly controlled by parathyroid hormone (PTH), $1,25(OH)_2D_3$ itself, calcitonin (CT), and fibroblast growth factor 23 (FGF_{23}) (Norman, 2008; Kumar et al., 2012).

The main function of vitamin D is to increase the concentration of calcium in the blood plasma to a level that supports optimal bone mineralisation and other vital body functions. One of the active forms of vitamin D, $1,25(OH)_2D_3$, is a 'secosteroid hormone' that is involved in the homeostasis of minerals in the body (Komisarenko et al., 2017). When serum calcium levels decrease due to insufficient calcium in the diet or increased need due to growth, pregnancy or lactation, the synthesis of hormonal $1,25(OH)_2D_3$ increases and, due to its genomic action, leads to intensive absorption of this essential macronutrient in the intestine. When optimal serum calcium metabolism decreases due to impaired intestinal absorption, $1,25(OH)_2D_3$ together with parathyroid hormone mobilise bone calcium and increase its reabsorption in the distal renal tubules (Fleet & Schoch, 2010; Christakos et al., 2011). In addition, the anterior pituitary hormone prolactin has a direct effect on 1α-hydroxylase (CYP27B1), which activates the increase in blood concentrations of 1,25-dihydroxycholecalciferol, which in its turn increases the absorption of this vital macronutrient in the intestine, in particular during lactation (Ajibade et al., 2010).

Lack of vitamin D in animals leads to the development of osteomalacia, which is characterised by impaired bone mineralisation. In the early stages of bone pathology, the level of $25OHD_3$ and $1,25(OH)_2D_3$ decreases (Eder & Grundmann, 2022; Schild et al., 2023). In particular, vitamin D deficiency in adult goats leads to the development of nutritional and fibrous osteodystrophy, secondary osteodystrophy, rickets, other metabolic diseases and reduced animal productivity. With the deepening of osteomalacia, the concentration of calcium and phosphorus in the serum of animals decreases and the activity of alkaline phosphatase increases (Goyal et al., 2016; Handel et al., 2016; Holick & Slominski, 2024). According to the results of research by Mona et al. (2012) and Sharma et al. (2017), kids and lambs were diagnosed with rickets with pronounced clinical signs: anorexia, growth retardation, scoliosis, lameness, joint enlargement and curvature of the thoracic limbs. Among the biochemical changes in the blood serum were an increase in alkaline phosphatase activity, hypocalcaemia and hypophosphatemia.

Despite the multidirectional positive functions of vitamin D, its excess is toxic to animals, causing mineralisation of soft tissues due to the development of persistent hypercalcaemia and hyperphosphatemia. The pathogenesis of D-hypervitaminosis is based on increased bone resorption due to the Ca-mobilising effect of $1,25(OH)_2D_3$, which leads to calcium deposition in the lungs, kidneys, and heart. Vitamin D toxicosis in animals is associated with severe gastrointestinal disorders, hypertension, heart rhythm disturbances, and neurological symptoms (seizures) (Safadi et al., 1999; Dittmer & Thompson, 2010; Hodnik et al., 2020).

The aim of the study was to investigate the metabolism of $25OHD_3$ in the blood serum of clinically healthy and hypocalcemic pregnant and lactating goats and its relationship with the metabolism of total calcium and its ionized fraction.

Material and methods

The maintenance, feeding, care and all procedures with animals were carried out in accordance with the international requirements of the Law of Ukraine "On Protection of Animals from Cruelty" (Kyiv, 2006, No. 1164-IV) and in accordance with the basic principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, France, March 18, 1986, ETS No. 123) and the "General Ethical Principles for Experiments on Animals" adopted by the First National Congress on Animal Bioethics (Kyiv, 2001). The research was performed in compliance with all principles of humanity, as stipulated by the European Community Directive.

The research was conducted at the Department of Propedeutics and Medicine of Internal Diseases of Animals and Poultry named after V. I. Levchenko, the Interfaculty Research Laboratory of Molecular Genetic and Immunological Research of Bila Tserkva National Agrarian University, and Ukrainian farms specialising in dairy goats.

The microclimate in the premises during the research complied with zoohygienic standards (DSTU 7823:2015 Livestock farms. Requirements for microclimate parameters of livestock premises). The animals were clinically examined, their diets were analysed, and blood was taken for biochemical analysis.

The material for the study was blood samples, which were collected in disposable vacuum tubes from Vacumed (FL Medical, Torrella, Italy) with a blood clotting activator and gel by intravital jugular vein puncture. Blood sampling was performed from 8:00 to 10:00 a.m. before feeding of the animals, taking into account all the rules of veterinary septic and antiseptic.

The concentration of $25OHD_3$ was determined in the blood serum of animals by enzyme-linked immunosorbent assay using a Stat Fax 2100 analyser (Avareness Technology Inc, USA). The study was performed using the 25-OH Vitamin D Total (Vit D-Direct) test system (Monobind Inc, USA) (Zerwekh, 2008; Holick, 2009).

During the biochemical study of blood in goats, total calcium (reaction with calcium arsenase III) and ionised calcium (by ion-exchange absorption) were determined by standardised methods. The measurements were performed on a Stat Fax 4500+ biochemical analyser.

The results of biochemical studies are presented according to the international system of units recommended for use in clinical laboratory practice. Statistical calculations were performed using the standard Statistica 12.0 software package (StatSoft Inc, USA, 2014). The arithmetic mean (x), standard deviation (SD), and Pearson's correlation coefficient (r) were determined. The Shapiro-Wilk test was used to determine the normality of the distribution, and the equality of variance was determined by the Levene test. To compare differences between means, we used the Tukey test, where differences were considered statistically significant at $P < 0.05$ for all data (Gastwirth et al., 2009; Sedgwick, 2012; Petrovska et al., 2022).

Results

The study was carried out on the livestock of pregnant (75–90 and 120–140 days of pregnancy) and lactating goats (0–2 and 15–25 days after kidding) of Zaanen and Laman breeds in summer-autumn and winter-spring periods.

When studying the metabolism of $25OHD_3$ in pregnant goats, we found the values of this fat-soluble vitamin in the blood serum ranging from 10.4 to 32.4 (18.0 ± 5.3) ng/mL. When analysing the concentration of 25-hydroxycholecalciferol in the blood serum of goats, depending on the physiological state and lactation period, we found that on the 75–90th days of pregnancy its content ranged from 10.4 to 20.8 (15.3 ± 3.6) ng/mL (Fig. 1). In 50.0% of the animals of this group, the concentration of calcidiol was in the range of 10.4–14.7 (12.5 ± 1.83) ng/mL, and in the same number of studied animals, its level was in the range of 15.9–20.8 (18.0 ± 1.94) ng/mL.

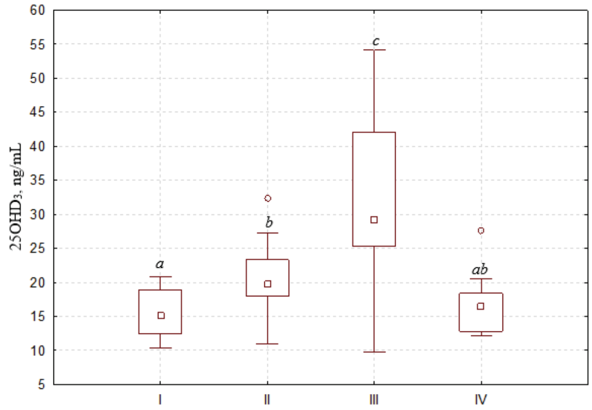

Fig. 1. Concentration of $25OHD_3$ in the blood serum of pregnant and lactating goats; abscissa axis – animal groups: I – 75–90 days of pregnancy; II – 120–140 days of pregnancy; III – 0–2 days after kidding; IV – 15–25 days of lactation; along the ordinate axis – units of measurement (ng/mL); different letters in the same coordinate system indicate samples that differ significantly from each other according to the results of the Tukey test ($P < 0.05$); small square – median; upper and lower boundaries of the rectangle – 25% and 75% quartiles; vertical line – minimum and maximum values, circles and asterisks – outliers; n = 12

On the 120–140th days of pregnancy, the concentration of $25OHD_3$ in the blood serum of goats was in the range of 11.0–32.4 (20.7 ± 5.48) ng/mL, which is 35.3% higher than in the animals of the previous period of the study and is statistically significant ($P = 0.01$; Fig. 1). At the same time, in 58.3% of animals, the level of 25-hydroxycholecalciferol was in the range of 11.0–19.9 ng/mL, and in 41.7% of goats its content was higher than the average value in the group and reached 32.4 ng/mL.

Thus, with the approach to kidding, we found a pronounced increase in the concentration of $25OHD_3$ in the blood serum of goats, which is confirmed by the presence of a positive correlation ($r = +0.12$) between the groups of animals of the first and second studies during pregnancy.

We established that the concentration of 25-hydroxyvitamin D in the blood serum of lactating goats was in a wide range of values – from 9.8 to 54.2 (24.6 ± 12.2) ng/mL, which is 1.37 times higher compared to pregnant animals ($P = 0.045$; Fig. 1). In this context, we analysed the results of studies of a group of animals in the first days after kidding and in the third to fourth weeks of lactation. Thus, the level of the active metabolite of vitamin D in the blood serum of goats on the 0–2nd days after kidding averaged 32.6 ± 12.5 ng/mL and was 2.13 and 1.57 times higher compared to goats on the 75–90th and 120–140th days of pregnancy, respectively (Fig. 1). A positive correlation of medium (r_1 = + 0.40) and weak (r_2 = + 0.21) degrees was established between the content of $25OHD_3$ in the blood serum of goats on days 0–2 after kidding and the first and second studies during the period of pregnancy, which, in our opinion, is an objective evidence of the dynamics of calcidiol metabolism in the so-called 'transition period' when the physiological state of goats changes.

The analysis of individual parameters indicates that the level of $25OHD_3$ in the blood serum of 16.7% of the studied goats in the first days after kidding was low and ranged from 9.8–16.8 ng/mL, in 50.0% of animals – in the range of 20.5–37.2 ng/mL, in another 33.3% the concentration of this active metabolite of vitamin D was higher than 40.0 ng/mL (40.8–54.2 ng/mL). Thus, in 83.3% of the studied goats, the concentration of 25-hydroxyvitamin D in the first two days after kidding was quite significant and ranged from 21.3 to 54.2 ng/mL.

Starting from the third week of lactation (days 15–25), we found a significant decrease in the concentration of $25OHD_3$ in the blood serum of goats to 16.8 ± 4.4 ng/mL (12.1–27.6 ng/mL), which is almost twice less compared to animals 0–2 days after kidding with a high degree of confidence ($P = 1.49 \times 10^{-4}$; Fig. 1) and a direct positive correlation (r = + 0.40). In the vast majority of lactating goats studied (83.3%), its content was in the range of 12.1–18.9 ng/mL (15.0 ± 2.47 ng/mL), however, in no case did it decrease to 9.8 ng/mL (the minimum value in goats in the first days after kidding). At the same time, in 16.7% of goats, the level of calcidiol was quite high and ranged from 24.0 to 27.6 ng/mL (25.8 ± 2.55 ng/mL). Thus, even with increasing lactation, in more than 25% of the studied goats, the concentration of $25OHD_3$ in the blood serum was higher than 18–20 ng/mL and reached values of 25–27.6 ng/mL.

Thus, based on the results of studying the metabolism of 25-hydroxyvitamin D in the blood serum of pregnant and lactating Zaanen and Laman goats, we found its minimum (9.8–11.0 ng/mL) and maximum (47.8–54.2 ng/mL) values, as well as a probable increase in the concentration of this active metabolite of vitamin D with the approach to kidding and in the first two days of the postpartum period. In particular, at 75–90 days of pregnancy in 50% of the studied animals, the concentration of calcidiol was in the range of 15.9–20.8 ng/mL, at 120–140 days – in 41.7% of goats – in the range of 20.1–32.4 ng/mL, in 83.3% of lactating goats on the 0–2nd days after kidding – 20.5–54.2 ng/mL, and, starting from the third-fourth week of lactation, 83.3% of them showed a decrease in its content to 12.1–18.9 ng/mL.

In the context of the obtained research results, we conducted a comprehensive analysis of the metabolism of calcidiol, total calcium and its ionised fraction in the blood serum of goats at different kidding periods and during the first three weeks of lactation. Animals with a minimum physiological concentration of total calcium in the blood serum of 2.20 mmol/L and an ionised calcium fraction of 0.47 (min) and 1.2 (max) mmol/L (19.0–55.0% of total Ca) were considered clinically healthy (Hotsuliak & Sakhniuk, 2024).

We found that in clinically healthy goats with optimal total calcium content, the concentration of $25OHD_3$ in the blood serum ranged from 10.4 to 54.2 ng/mL, including 10.4–32.4 ng/mL in pregnant and 12.1–54.2 ng/mL in lactating goats (Fig. 2a). In the subclinical course of hypocalcaemia, the calcidiol content in the serum of goats was in the range of 9.8–29.8 ng/mL (17.5 ± 5.86 ng/mL), including in pregnant animals – from 11.0 to 21.0 ng/mL (15.5 ± 3.42 ng/mL), during the first 15–25 days of lactation – 9.8–29.8 ng/mL (19.5 ± 7.96 ng/mL). In the analysis of individual parameters in goats 75–90 days of pregnancy with optimal total calcium content, the average concentration of 25-hydroxycholecalciferol was 17.0 ± 4.13 ng/mL (10.4–20.8 ng/mL) against 14.0 ± 2.22 ng/mL (11.0–16.3 ng/mL) in animals with hypocalcaemia (Fig. 2a).

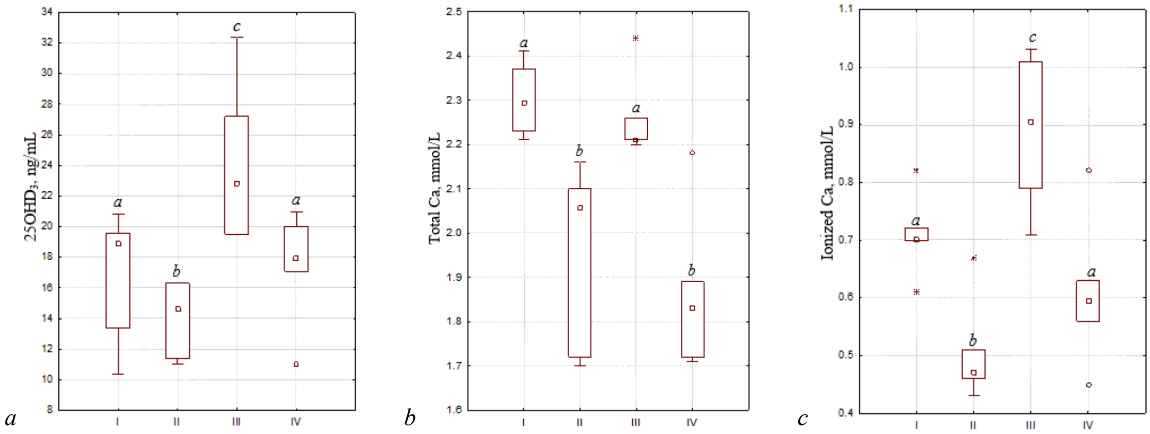

Fig. 2. Metabolism of $25OHD_3$, total and ionised calcium in the blood serum of clinically healthy and hypocalcaemic goats: *a* – concentration of $25OHD_3$ (ng/mL) in the blood serum; *b* – total calcium (mmol/L) in the blood serum; *c* – ionised calcium (mmol/L) in the blood serum; abscissa axis – animal groups: I – clinically healthy goats 75–90 days of pregnancy; II – animals with subclinical hypocalcaemia 75–90 days of pregnancy; III – clinically healthy goats 120–140 days of pregnancy; IV – animals with subclinical hypocalcaemia 120–140 days of pregnancy; other designations are described in detail in Figure 1; n = 6

Between the average values of $25OHD_3$ and total calcium in the blood serum of clinically healthy goats on days 75–90 of pregnancy, we found an average positive correlation (r = +0.57). The concentration of the ionised calcium fraction in the blood serum of clinically healthy animals of this group was found to be in the range of 0.61–0.82 (0.70 ± 0.07) mmol/L (30.7% of Ca total) against 0.43–0.67 (0.50 ± 0.09) mmol/L ($P = 1.36 \times 10^{-3}$; 25.0% of Ca total) – in the subclinical course of hypocalcaemia (see Fig. 2c). At the optimal content of ionised calcium in the blood serum of goats, calcidiol was in the range from 10.4 to 20.8 (17.0 ± 4.13) ng/mL, against 14.0 ± 2.22 (11.0–16.3) ng/mL in animals with hypocalcaemia (Fig. 2a). A positive correlation of medium (r = +0.52) and weak (r = +0.29) degrees was found between the values of 25-hydroxycholecalciferol and ionised calcium in the blood serum of clinically healthy goats of this group in animals with subclinical hypocalcaemia.

On the 120–140th days of pregnancy, the concentration of calcidiol in clinically healthy animals was in the range of 19.5–32.4 (24.00 ± 5.27) ng/mL, which was 41.2% higher than in the animals of the previous period of the study ($P = 4.9 \times 10^{-2}$; Fig. 2a). At the same time, the values of total calcium in the blood serum of these goats were in the range of 2.21–2.44 (2.30 ± 0.09) mmol/L (Fig. 2b).

Thus, according to the results of the study, an increase in the concentration of $25OHD_3$ in the blood serum of animals from 21.0 to 32.4 ng/mL was found with the approach to kidding.

In the subclinical course of hypocalcaemia, the values of $25OHD_3$ were quite variable and ranged from 11.0 to 21.0 ng/mL, and the mean value of this active metabolite (17.5 ± 3.5 ng/mL) was 27.1% lower compared with clinically healthy animals of this group (P = 0.05; Fig. 2a). The dynamics of a probable increase in the concentration of ionised calcium in clinically healthy animals of 120–

140 days of age to 0.90 ± 0.14 (0.71–1.03) mmol/L were established, compared with the previous study (P = 0.026; Fig. 2c), as well as in comparison with the results of studies in female goats with hypocalcaemia 0.60 ± 0.18 (0.45–0.82) mmol/L; P = 0.0057; Fig. 2c). At the optimal content of ionised calcium in the blood serum of goats, the level of calcidiol was in the range from 19.5 to 32.4 (24.0 ± 5.3) ng/mL against 17.5 ± 3.5 (11.0–21.0) ng/mL in the subclinical course of the pathology (P = 0.05) with a positive correlation between the values in diseased animals (r = + 0.54).

In the first two days after kidding, the concentration of 25-hydroxycholecalciferol in the blood serum of goats was in a wide range of values – from 9.8 to 54.2 ng/mL with a sufficiently high mean value (32.6 ± 12.5 ng/mL), which is 2.13 and 1.57 times higher than in the groups of animals at 75–90 and 120–140 days of pregnancy ($P = 3.36 \times 10^{-5}$; P = 0.034; Fig. 1). An individual analysis of calcidiol and total calcium in the blood serum of goats of this group showed that with the content of the active metabolite from 9.8 to 16.8 ng/mL, the concentration of total calcium averaged 1.60 ± 0.42 (1.28–1.87) mmol/L, with 2.10 ± 0.22 (1.71–2.31) mmol/L. In 33.0% of goats with $25OHD_3$ levels over 40 ng/mL, the content of the essential macronutrient averaged 2.30 ± 0.16 (2.22–2.54) mmol/L. The concentration of calcidiol in the blood serum of clinically healthy animals 0–2 days after kidding was 40.7 ± 11.2 (21.3–54.2) ng/mL and had a pronounced tendency to increase compared to the previous period of the study (P = 0.014; Fig. 3a), as well as in relation to the same group of goats (0–2 days after kidding) with hypocalcaemia – 24.2 ± 7.32 (9.8–29.8) ng/mL; P = 0.022; Fig. 3a).

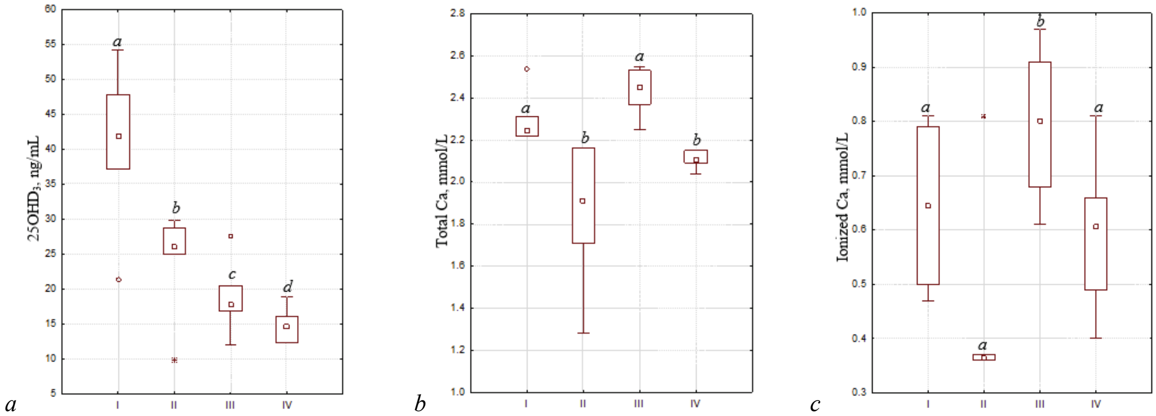

Fig. 3. Metabolism of $25OHD_3$, total and ionised calcium in the blood serum of clinically healthy and lactating goats with hypocalcaemia: *a* – concentration of $25OHD_3$ (ng/mL) in the blood serum; *b* – total calcium (mmol/L) in the blood serum; *c* – ionised calcium (mmol/L) in the blood serum; abscissa axis – animal groups: I – clinically healthy goats on the 0–2nd days after kidding; II – sick animals with subclinical hypocalcaemia on the 0–2nd days after kidding; III – clinically healthy goats on the 15–25th days of lactation; IV – sick animals with subclinical hypocalcaemia on the 15–25th day sof lactation; other designations are described in detail in Figure 1; n = 6

A positive correlation of medium degree (r = +0.53) was established between the concentration of calcidiol and total calcium in the blood serum of clinically healthy goats of this group, in animals with subclinical hypocalcaemia the correlation was significantly higher (r = +0.70). Thus, there is a direct correlation between the provision of animals with calcidiol and the concentration of total calcium in the blood serum of goats in the first days after kidding.

The dynamics of ionised calcium metabolism in goats in the first two days after kidding was characterised by a significant decrease in its concentration to 0.60 ± 0.16 (0.36–0.81) mmol/L, which is 24.0% less than in animals 120–140 days of kidding (P = 0.03; Figs. 2c and 3c), including in clinically healthy animals – 0.60 ± 0.14 (0.47–0.81) mmol/L, which is 1.50 times higher than the value in the subclinical course of hypocalcaemia (P = 0.01; Fig. 3c). At the optimal level of the ionised calcium fraction in the blood serum of goats of this group, the concentration of $25OHD_3$ was significantly higher compared to animals with hypocalcaemia (P = 0.022; Fig. 3a). A positive correlation (r = +0.10) was established between the concentration of calcidiol and ionised calcium in the blood serum of clinically healthy goats of this group, and in animals with subclinical hypocalcaemia the correlation was almost three times higher (r = +0.25).

On days 15–25 after kidding, there was a gradual increase in total calcium to 2.30 ± 0.19 mmol/L with a simultaneous decrease in $25OHD_3$ in the serum of lactating goats compared to animals on days 0–2 of lactation (2.1 ± 0.33 mmol/L; Fig. 3b). The individual analysis of 25-hydroxycholecalciferol and total calcium in the blood serum of these animals revealed that in 83.3% of the studied lactating goats the concentration of $25OHD_3$ was 15.00 ± 2.47 (12.1–18.9) ng/mL and the level of total calcium in them ranged from 2.04–2.55 (2.22 ± 0.17) mmol/L against 2.51 ± 0.05 mmol/L in goats with an average value of calcidiol 25.8 ± 2.55 (24.0–27.6) ng/mL.

Thus, the dynamics of $25OHD_3$ metabolism in the blood serum of clinically healthy lactating goats from two to three weeks after kidding were characterised by a significant decrease in its concentration to 18.8 ± 5.11 (12.1–27.6) ng/mL compared to animals 0–2 days after kidding (P = 0.002; Fig. 3a). The content of the active metabolite of the vitamin in sick animals with subclinical hypocalcaemia was 14.80 ± 2.63 (12.3–18.9) ng/mL and had a pronounced tendency to decrease (by 21.3% less) compared to clinically healthy goats of this group (Fig. 3a). On the 15–25th days of lactation, against the background of a probable decrease in the concentration of 25-hydroxycholecalciferol to 12.1 ng/mL and a tendency to increase total calcium, there was a gradual slight increase in the content of ionised calcium to 0.70 ± 0.16 mmol/L, and its ratio to total calcium was 0.32:1, against 0.26:1 on the 0–2nd day after kidding. In clinically healthy lactating goats, the level of ionised calcium in the blood serum was 0.80 ± 0.15 (0.61–0.97) mmol/L, which was 1.33 times higher than in sick animals (0.60 ± 0.14 mmol/L; P = 0.0038; Fig. 3a). The ratio of ionised calcium to total calcium in healthy goats was 0.33:1, while in sick animals with hypocalcaemia it was 0.28:1. At the optimal content of total and ionised calcium in the blood serum of lactating goats of this group, the concentration of calcidiol was at the level of 18.8 ± 5.1 (12.1–27.6) ng/mL against 14.8 ± 2.6 (12.3–18.9) ng/mL in animals with subclinical pathology (Fig. 3a).

According to the results of laboratory tests of blood serum of pregnant and lactating Zaanen and Laman goats of dairy breeds, it was found that the concentration of calcidiol in 50.0% of clinically healthy goats of different physiological groups varied in a fairly wide range – from 10.4 to 54.2 ng/mL, averaging 25.2 ± 11.6 ng/mL. Instead, in goats with hypocalcaemia, this figure was lower and averaged 17.5 ± 5.86 ng/mL (within the range of 9.8–29.8 ng/mL; P = 0.0016), which is 31.0% less than in clinically healthy animals.

Discussion

Due to the intensive development of goat breeding and the growth of products entering the world market, the issue of increasing productivity and monitoring the health of these animals is gaining special attention. To ensure high competitiveness, farms should focus on achieving high product quality, as well as on creating favourable conditions for their maintenance and providing goats with all the necessary nutrients, in particular, vitamin D (Lopes et al., 2012; Ebrahimi et al., 2014; Renna et al., 2025).

Vitamin D is involved in intestinal calcium absorption with regulation of calcium-phosphorus haemostasis, in bone formation and remodelling, and has anti-inflammatory and immunomodulatory effects. Insufficient solar UV exposure or vitamin D deficiency in the diet can cause D-hypovitaminosis in animals, which leads to osteomalacia, osteodystrophy and rickets in young animals. In particular, in ruminants, with optimal calcium supply in the diet, vitamin D stimulates bone formation and mineralisation, and in hypocalcaemia, it increases calcium mobilisation from bone tissue and its absorption in the intestine (Monna et al., 2012; Alabada & Saleh, 2020; Hurst et al., 2020). In particular, it affects joint health through a number of mechanisms, including maintaining calcium homeostasis, enhancing bone metabolism by stimulating the absorption of this essential macronutrient, and regulating chondrocyte function (Zhang et al., 2025). Vitamin D is required for the absorption of minerals from feed and plays a key role in osteogenesis, osteosynthesis, embryonic development and other vital functions (Nelson et al., 2016; Hakeem et al., 2025).

According to the literature (Kohler et al., 2013; Golder et al., 2021; Zillinger et al., 2024), the level of vitamin D supply in animals is determined primarily by the concentration of one of the active metabolites, 25-hydroxyvitamin D, in the blood serum, which depends, in particular, on the age, breed, housing conditions and clinical condition of the animals.

Studies by Norman (2011), Weber et al. (2014) and Nemeth et al. (2017) state that the optimal level of $25OHD_3$ in the serum of cows, goats and sheep is in the range of 30 to 60 ng/mL (75–150 nmol/L), and values below 10 ng/mL indicate vitamin D deficiency in animals, which leads to impaired bone metabolism and calcium homeostasis.

According to the results of our studies, the concentration of calcidiol in the blood serum of goats increased with the approach to kidding: on the 120–140th days of pregnancy, its content was 35.3% higher compared to animals 75–90 days of pregnancy with maximum values in the period 0–2 days after kidding. On the 15–25th days of lactation, the concentration of vitamin D metabolite was 1.94 times lower compared to animals in the first two days after kidding (Fig. 1). Our results are in line with the data of Liesegang et al. (2007), who established the dynamics of increasing the concentration of $25OHD_3$ and 1,25-dihydrocholecalciferol in the blood serum of goats before kidding with maximum values on the 2–3rd days after kidding and a gradual decrease in its content by 4–5 weeks of lactation.

According to Goyal et al. (2016), increased vitamin D levels in pregnant ewes are associated with a disruption of the negative feedback mechanism involving VDR-mediated regulation of CYP24A1, and increased maternal $25OHD_3$ levels correlated with increased levels of renal 25-hydroxylases CYP2J and CYP2R1. It is believed that before kidding and during the kidding, there is an increase in 25OHD metabolism and a decrease in vitamin D binding protein concentration, which are possible explanations for the decrease in serum $25OHD_3$ in early lactation animals (Nelson et al., 2016). Our research results are in line with the data of foreign authors (Cleal et al., 2017), who found the level of $25OHD_3$ in the serum of ewes at late lactation in the range of 24 to 28 ng/mL with low total calcium levels, since during pregnancy the intensity of mineral metabolism significantly increases for fetal development and preparation of the body for lactation (Babazadeh et al., 2022). According to the results of research by Ryan (Ryan & Kovacs, 2020), during pregnancy, the level of $1,25(OH)_2D_3$ in females increases significantly. The increase in calcitriol concentration during this period is due to the increased expression and functionnal activity of CYP27B1 in the mother's kidneys and placenta (Kirby et al., 2013).

On the 15th–25th days of lactation, the concentration of calcidiol in the blood serum of clinically healthy goats showed a pronounced decrease compared to the 0–2nd days after kidding (P = 0.00015; Fig. 3a). Such dynamics of $25OHD_3$ metabolism are consistent with the results of studies by Muscher et al. (2011), according to which the level of vitamin D metabolite in the blood serum of clinically healthy lactating Zaanen goats ranged from 12 to 46 ng/mL. According to the literature (Nelson et al., 2016; Holcombe et al., 2018; Mora-Gutierrez et al., 2024), cows in early lactation also showed a decrease in serum calcidiol, while in late lactation and dry periods it was significantly higher. A number of scientists (Chun et al., 2014; Olsen et al., 2016) have linked the decrease in the active metabolite of vitamin D (calcidiol) in the serum of animals to the effect of the onset of lactation on vitamin D-binding protein (DBP), which is synthesised in the liver and transports $25OHD_3$ to tissues and maintains its concentration in the circulatory system. The decrease in serum $25OHD_3$ levels in lactating goats may also be due to its excretion in milk (Rai et al., 2022).

The metabolite $25OHD_3$ is a precursor of the active hormone 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$), which activates the expression of genes related to calcium transport and bone remodelling. The concentration of calcitriol in the circulation is strictly controlled by calcidiol, even at low concentrations, in response to the endocrine system's response, which controls calcium-phosphorus homeostasis. A minimum concentration of $25OHD_3$ in animal serum is necessary for optimal functioning of the immune and cardiovascular systems and the gastrointestinal tract (Babazadeh et al., 2022).

The prospect of further research is to study the relationship between the metabolism of calcidiol, $1,25(OH)_2D_3$, parathyroid hormone and calcitonin and their effect on the metabolism of total calcium and its fractions in clinically healthy and hypocalcaemic pregnant and lactating goats.

Conclusions

The metabolism of $25OHD_3$ in the blood serum of Zaanen and Laman goats was characterised by values from 9.8 to 54.2 ng/mL, including in pregnant animals (days 75–140) – 10.4–32.4 ng/mL, in lactating animals (days 0–25) – 9.8–54.2 ng/mL. The dynamics of calcidiol in the blood serum of pregnant goats was characterised by an increase in its concentration on the 120–140th days compared to animals at 75–90 days of gestation (r = +0.12), with maximum values in the first 0–2 days after kidding and a probable decrease in its content on the 15–25th days of the lactation period. In clinically healthy goats with optimal serum calcium content, the total concentration of $25OHD_3$ ranged from 10.4 to 54.2 ng/mL against 9.8–29.8 ng/mL in subclinical hypocalcaemia. At the optimal content of ionised calcium in the blood serum of goats, the concentration of calcidiol was in the range of 10.4–54.2 ng/mL against 9.8–29.8 ng/mL in animals with hypocalcaemia.

The authors declare that there is no conflict of interest with respect to their contributions and the results of the study.

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