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Prognostication of hypocalcemia in dairy goats

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Metabolic diseases significantly affect the health, productivity and reproductive performance of goats. Therefore, accurate prediction and early diagnosis are extremely important for their effective treatment and prevention. The aim of this study was to investigate the use of linear regression and ROC analyses for prognostication hypocalcemia in dairy goats. The study included clinically healthy (n = 321) and subclinically hypocalcemic (n = 216) pregnant and lactating animals. The concentration of total calcium and ionised calcium, the activity of total alkaline phosphatase and its bone and intestinal isoenzymes were determined in blood serum using standardised methods. According to linear regression analysis and ROC analysis, the determination of ionised calcium content in the blood serum of goats has high diagnostic value for prognostication hypocalcemia, and it is advisable to measure total calcium concentration for monitoring studies of its metabolism. Based on the results of regression analysis, we did not find a statistically significant relationship between total calcium and its ionised fraction with the activity of total alkaline phosphatase and its bone and intestinal isoenzymes in the serum of goats, as the coefficients of determination in linear regression analysis were low. However, based on ROC analysis between these values, we found high diagnostic significance of total alkaline phosphatase and its isoenzymes for prognostication of hypocalcemia in goats.

Keywords: total calcium; ionised calcium; alkaline phosphatase; isoenzymes; linear regression analysis; ROC analysis; Youden index.

Introduction

Modern livestock breeding faces a number of problems related to metabolic disorders, which significantly affect the health, productivity and reproductive performance of animals. Metabolic diseases lead to economic losses due to the costs of treatment and culling of sick animals. In this regard, early diagnosis of metabolic diseases, in particular hypocalcemia, is of particular importance, as it allows for the timely detection of disorders, prevents the development of clinical forms of pathologies and minimises negative consequences for animals (Wu, 2020). According to Levchenko et al. (2001), the general direction of research is to penetrate the secrets of compensatory and adaptive reactions, searching among them for specific markers for early diagnosis and targeted prevention of a particular pathology.

Hypocalcemia is one of the most common metabolic diseases, affecting primarily dairy goats and having a subclinical and clinically pronounced course (Bayoumi et al., 2021; Serrenho et al., 2021).

The prevalence of clinical hypocalcemia in goats. Simões et al. (2024) found it in less than 5.0% of the studied population. Sakhaee et al. (2023) diagnosed subclinical disease in 24.2% of dairy animals, with the prevalence of the disease found in 6.8% of goats with one foetus and in 39.4% of goats that gave birth to two or more kids. According to Munn et al. (2024), the development of subclinical hypocalcemia in goats increases the mortality of young animals, especially in the first weeks after birth.

According to the results of studies by Cajueiro et al. (2021), a decrease in total calcium concentration in blood serum was found in pregnant goats starting from the 80th day of pregnancy, which is associated, in particular, with the peculiarities of the physiological state of goats and foetuses (Ismail et al., 2008; Hotsuliak & Sakhniuk, 2024). According to Barłowska et al. (2021), in lactating animals, calcium metabolism disorders are caused by its increased excretion from the body, especially in the first weeks after kidding due to its secretion into colostrum and milk.

Determination of total calcium concentration in blood serum is commonly used to diagnose subclinical hypocalcemia in animals (Oetzel, 1988; Goff, 2008). Only a few studies have evaluated the level of ionised calcium, as it is the biologically available form of this macroelement (Martinez et al., 2014; Neves et al., 2017). According to the results of studies by Karapinar et al. (2024), a discrepancy was found between the concentration of total calcium and its ionised fraction in clinically healthy cows and cows with subclinical hypocalcemia. The authors believe that the level of total calcium in blood serum is not an objective indicator of animal nutrition. Therefore, one of the important stages of early diagnosis of hypocalcemia is the determination of the content of ionised calcium and its share in the total calcium structure.

Currently, there is a search for informative biomarkers to predict hypocalcemia before the onset of clinical signs of the disease, but even now, animals at risk are often assessed based on total calcium concentration, as well as age, productivity, medical history, and feed analysis. In this context, in addition to ionised calcium, enzymes, in particular alkaline phosphatase and its isoenzymes, are attracting research interest as potential indicators of early metabolic disorders in the development of hypocalcemia (Minisola et al., 2025).

Calcium exists in three different fractions in blood plasma: approximately 50.0% is in ionised (free) form, 40.0% is bound to proteins, and 10.0% forms complexes with certain anions (lactate, citrate, inorganic phosphate, and bicarbonate). According to Melendez et al. (2022), the concentration of ionised calcium (iCa) in animal blood serum is one of the main clinical markers of circulating physiologically active total calcium (tCa). It is the ionised fraction of calcium that is its biologically active form, and changes in its content occur under the action of hormones, cell receptors and enzymes involved in the homeostasis of this vital macroelement in animals. According to the authors, the concentration of free calcium itself is a reasonably informative indicator for determining calcium homeostasis in high-yielding dairy cows.

Determining the concentration of this element and its ionised fraction in goat blood serum is a key aspect in diagnosing hypocalcemia, which mostly develops during pregnancy and in the first third of lactation. Early diagnosis of calcium metabolism disorders allows the detection of subclinical forms of the disease, prevents the development of complications, and ensures the high effectiveness of therapeutic and preventive measures (Goff, 2014; Silva et al., 2022; Cao et al., 2025).

According to Schini et al. (2022) and Wu et al. (2024), alkaline phosphatase and its isoenzymes are important biochemical markers that reflect the metabolic status, liver function, bone tissue, and immune system of animals. Their activity can vary in both pregnant and lactating goats, especially during the so-called ‘transitional period’,

and serve as indicators of subclinical hypocalcemia in dairy goats. According to the authors, despite their diagnostic value, these indicators have not been sufficiently studied, especially in pregnant goats and during the first third of lactation.

In order to predict the development of postpartum hypocalcemia, Arnold et al. (2024) evaluated the informative value of total calcium, ionised calcium and alkaline phosphatase activity in high-yielding cows 2 weeks before expected calving and on the day of calving. Based on the results of their research, they established a direct correlation between enzyme activity in late gestation and total calcium levels after calving (r = + 0.64). According to the authors, in addition to the above indicators for predicting the disease in cows in the early stages of pathology development, there is a high probability of the presence of unidentified diagnostic markers that directly affect calcium metabolism. According to Eckermann (2007), alkaline phosphatase is closely related to bone tissue metabolism and can serve as a biomarker for predicting early diagnosis of hypocalcemia in cattle during calving. Thus, in the clinical form of the disease, the author diagnosed a significant decrease in enzyme activity in the blood serum of cows and an increase in fractional calcium excretion compared to healthy animals, especially during the so-called 'transitional period.' However, no analysis of the diagnostic value of this enzyme was performed.

The aim of this study was to investigate the use of linear regression and ROC analyses to predict hypocalcemia in dairy goats based on a set of biochemical parameters, in particular, total calcium, ionised calcium, alkaline phosphatase and its isoenzymes.

Material and methods

The research protocol was approved by the Ethics Committee of the Bila Tserkva National Agrarian University (Protocol No. 25-2 dated 03.07.2025). The keeping, feeding, care and all procedures involving animals were carried out in accordance with the international requirements of the Law of Ukraine 'On the Protection of Animals from Cruel Treatment' (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, 18 March 1986, ETS No. 123) and the 'General Ethical Principles for Animal Experiments' adopted by the First National Congress on Animal Bioethics (Kyiv, 2001). The research was conducted in compliance with all principles of humanity, as provided for by the European Community Directive. All procedures on goats were performed in accordance with ARRIVE recommendations, without violating the provisions of Council Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes. The microclimate in the room during the study complied with zoohygienic standards (DSTU 7823:2015 Livestock farms. Requirements for microclimate parameters in livestock buildings).

The study was conducted on 1–4-year-old goats of the Saanen, Alpine and Laman dairy breeds. The study subjects were clinically healthy (n = 321) and subclinically hypocalcemic (n = 216) pregnant and lactating animals.

The material for the study was blood samples collected in disposable Vacumed (Italy) tubes with a blood coagulation activator and gel using the ante-mortem jugular vein puncture method. Blood sampling was performed from 8:00 to 10:00 a.m. before feeding the animals.

Biochemical analysis of goat blood serum using standardised methods determined the concentration of total calcium (reaction with calcium arsenate III), ionised calcium (ion exchange absorption method), the activity of total alkaline phosphatase and its bone and intestinal isoenzymes (reaction with 4-nitrophenyl phosphate; Sigma-Aldrich, Switzerland) (Levchenko et al., 2010; Vlizlo et al., 2012). The measurements were carried out in the research laboratory of the Department of Propaedeutics and Medicine of Internal Diseases of Animals and Poultry named after V. I. Levchenko using a Stat Fax 4500+ biochemical analyser.

The results of the biochemical studies are presented in accordance with the International System of Units (SI), which is recommended for use in clinical laboratory practice (Levchenko et al., 2010). Data analysis was performed using the Statistica 12.0 software package (StatSoft Inc., USA, 2014) and MedCalc version 22.017 (MedCalc Software Ltd., Belgium, 2024). The arithmetic mean (x) and standard deviation (SD) were determined. The normality of the distribution of indicators was assessed using the Shapiro-Wilk test, and the equality of variance was assessed using the Levene criterion. To compare the differences between the mean indicators, the Tukey criterion was used, where differences were considered statistically significant at $P < 0.05$ for all data (Petrovska et al., 2022).

Linear regression equations were used to model the relationship between variable 'y' (total calcium and ionised calcium concentration) and vector variable 'x' (alkaline phosphatase activity and its isoenzymes). Regression analysis allows one to establish the type of relationship between factors and makes it possible to predict the value of one dependent variable based on the value of another independent variable. The nature of the relationship between the indicators was checked using scatterplots. The quality of the calculated regression was assessed using the coefficient of determination (R^2): the stronger the relationship, the closer the coefficient of determination is to unity. Conversely, in the absence of a relationship, this coefficient tends towards zero (Montgomery et al., 2021; Park, 2025).

To evaluate the diagnostic effectiveness of the indicators, ROC analysis was used to determine the area under the ROC curve (AUC), which characterises the test's ability to distinguish between the presence or absence of disease. An AUC value of up to 0.5 indicates low discriminatory ability (randomness), while an increase to 1.0 indicates high accuracy in distinguishing between healthy and diseased animals. This test reflects the dependence of the number of correctly and incorrectly classified cases with a 95% confidence interval (95% CI) (Hoo et al., 2017).

Using the optimal cut-off value, we calculated the sensitivity, specificity, diagnostic efficiency of the biomarker, and the Youden index (J) – a statistical indicator for assessing the effectiveness of a diagnostic test. This index ranges from 0 to 1 (or from 0 to 100%), with values closer to 1 indicating relatively high effectiveness of the biomarker under study, and a decrease in the index indicating low test informativeness (Hughes, 2015).

Results

The study was conducted on clinically healthy and subclinically hypocalcemic lactating dairy goats (n = 537) in the summer-autumn and winter-spring periods during 2022–2025. Animals with optimal clinical and biochemical status, including a minimum physiological concentration of total calcium in blood serum of 2.20 mmol/L and ionised calcium of 0.47 mmol/L (Hotsuliak & Sakhniuk, 2024), were considered healthy.

Taking into account the diversity of the research results obtained and in order to establish their informative value for the early diagnosis of hypocalcemia, we conducted a comprehensive analysis of individual indicators and their combinations in different variations for total calcium metabolism, its ionised fraction, as well as the activity of total alkaline phosphatase and its isoenzymes in the blood serum of clinically healthy goats and during the subclinical course of hypocalcemia during pregnancy and during the first two months of the lactation period. Statistical methods were used – linear regression and ROC analyses in biological studies. The physiological limits of serum concentrations of total calcium, ionised calcium, total alkaline phosphatase activity and its intestinal and bone isoenzymes in goats were taken as 2.20–2.90 mmol/L; 0.47–1.20 mmol/L; 12.6–412.2 U/L; 5.5–70.5 U/L and 7.3–401.3 U/L (Hotsuliak & Sakhniuk, 2024b; Sakhniuk et al., 2025).

We found that the concentration of total calcium in the blood serum of all studied goats was within the range of 1.28–2.87 mmol/L (2.237 ± 0.068 mmol/L), including in clinically healthy animals – 2.20–2.87 mmol/L ($P = 5.0 \times 10^{-10}$; Table 1), and in subclinical hypocalcemia – 1.28–2.15 mmol/L. At the same time, the level of ionised calcium fraction in all studied animals varied in the range of 0.25–1.30 mmol/L (0.762 ± 0.204 mmol/L), in particular, in clinically healthy animals – 0.47–1.30 mmol/L (0.852 ± 0.175 mmol/L; $P = 5.0 \times 10^{-10}$; Table 1), which is 1.35 times higher than in sick animals.

Table 1
Metabolism of total and ionized calcium (mmol/L) in goat blood serum

Biochemical parameters	Clinical condition of goats			
	clinically healthy (n = 321)		patients with hypocalcemia (n = 216)	
	x ± SD	Lim	x ± SD	Lim
Total calcium	2.412 ± 0.144	2.20–2.87	1.970 ± 0.155***	1.28–2.15
Ionized calcium	0.852 ± 0.175	0.47–1.30	0.630 ± 0.017***	0.25–1.05

Notes: * – P < 0.05; ** – P < 0.01; *** – P < 0.001 – clinically healthy goats versus patients with subclinical hypocalcemia.

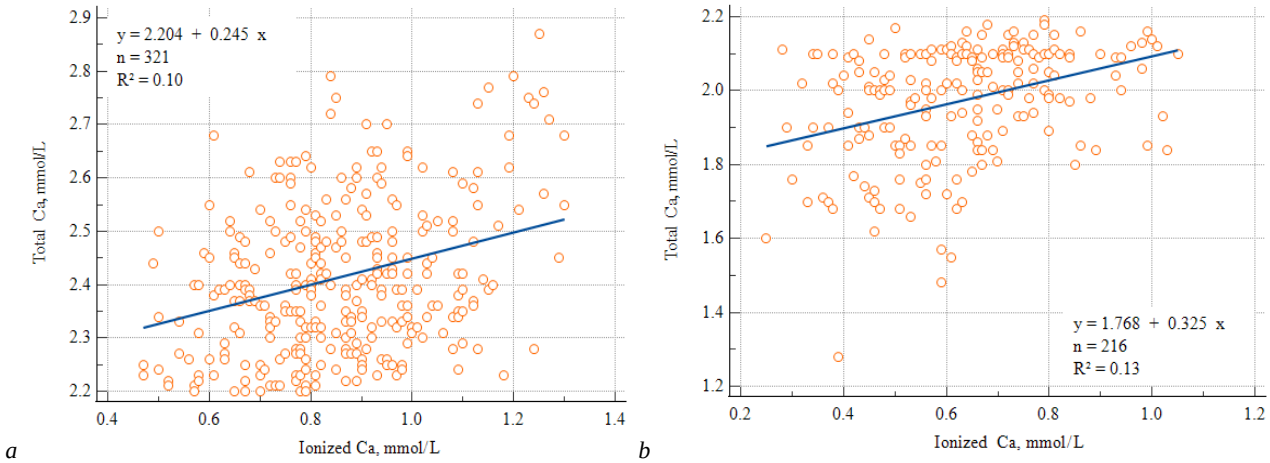
Based on the analysis of individual indicators of total calcium and ionised calcium, optimal values for both parameters were diagnosed in 59.8% of the 537 goats examined. At the same time, in 32.4% of animals with reduced total calcium content, the concentration of its ionised fraction was within physiological limits (0.47–1.05 mmol/L). In another 7.8% of goats, a simultaneous decrease in both indicators was diagnosed.

Thus, the combination of optimal values of total calcium and its ionised fraction was established in 59.8% of the total number of goats studied. The physiological values of the free fraction of calcium in one third of animals (32.4%) with hypocalcemia based on total calcium content confirm the need for simultaneous monitoring of its ionised fraction. In 7.8% of goats, a simultaneous decrease in both indicators was found, in which the ionised calcium content did not exceed 0.46 mmol/L, and its share was 21.0%.

Based on the ambiguity of the results obtained, we conducted a detailed analysis of these values in clinically healthy goats. It was found that in 55.8% of animals, the concentration of free calcium was quite high – from 0.77 to 1.30 mmol/L (1.04 ± 0.127 mmol/L). In another 44.2% of animals, with optimal values of total calcium and ionised calcium, the limits of the latter did not exceed the minimum physiological limit (0.47–0.91 mmol/L; 0.702 ± 0.096 mmol/L).

Thus, based on the analysis of the research results, it was established that in 100.0% of clinically healthy goats, the concentration of total calcium and its ionised fraction was within physiological values.

The data presented in Figure 1 indicate a correlation between total calcium and ionised calcium in the blood serum of clinically healthy goats and goats with subclinical hypocalcemia, which is confirmed by the coefficient of determination ($R^2 = 0.10$; $R^2 = 0.13$) and a high degree of reliability (P < 0.001; P < 0.001; see Fig. 1a and 1b).



a *b*

Fig. 1. The results of linear regression analysis between total calcium and ionized calcium in the blood serum of goats: *a* – clinically healthy (n = 321); *b* – sick with subclinical hypocalcemia (n = 216)

The calculated optimal threshold value for the concentration of the ionised fraction of calcium (≥ 0.75 mmol/L) under the ROC curve (AUC = 0.816; P < 0.001; 95% confidence interval: 0.781–0.848; J = 49.2%; Fig. 2) indicates a high degree of reliability of the proposed prognostic model. Thus, according to the data of linear regression analysis and ROC analysis, the determination of ionised calcium content in the blood serum of goats has high diagnostic value for predicting hypocalcemia, and it is advisable to measure the concentration of total calcium for monitoring studies of its metabolism.

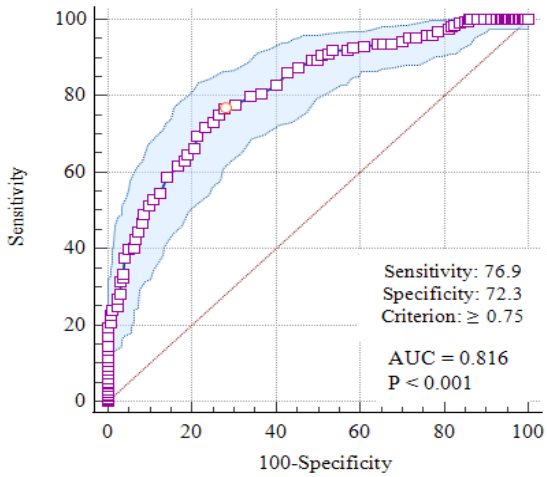


Fig. 2. ROC curve of the diagnostic value of the ionized calcium fraction in the blood serum of goats (Ca_{total} > 2.2 mmol/L, n = 537)

The next stage of the work was to study the informative value of alkaline phosphatase and its isoenzymes for predicting calcium metabolism disorders in goats using a similar statistical analysis of the specified values.

When studying the metabolism of the enzyme, it was found that in all the goats studied, its activity in blood serum was within the range of 26.0–1087.0 U/L, including in clinically healthy goats – 26.0–922.8 U/L (Table 2), and its maximum values were 1.18 times lower than in the subclinical course of hypocalcemia. In 82.1% of the animals studied, enzyme activity was within physiological limits. Hyperfermentemia was diagnosed in another 17.9% of goats, including 19.7% of pregnant goats and 16.5% of lactating goats.

Table 2
Metabolism of total alkaline phosphatase and its isoenzymes (U/L) in goats blood serum

Biochemical parameters	Clinical condition of goats			
	clinically healthy (n = 321)		patients with hypocalcemia (n = 216)	
	x ± SD	Lim	x ± SD	Lim
Total alkaline phosphatase	212.4 ± 199.7	26.0–922.8	266.9 ± 221.7*	27.7–1087.0
Intestinal alkaline phosphatase	204.3 ± 197.0	24.5–905.3	257.0 ± 215.7*	25.2–988.0
Bone alkaline phosphatase	38.0 ± 32.5	5.5–187.0	56.3 ± 61.2***	5.5–485.6

Notes: see Table 1.

Based on the analysis of individual indicators of total calcium concentration and total alkaline phosphatase activity, normal values

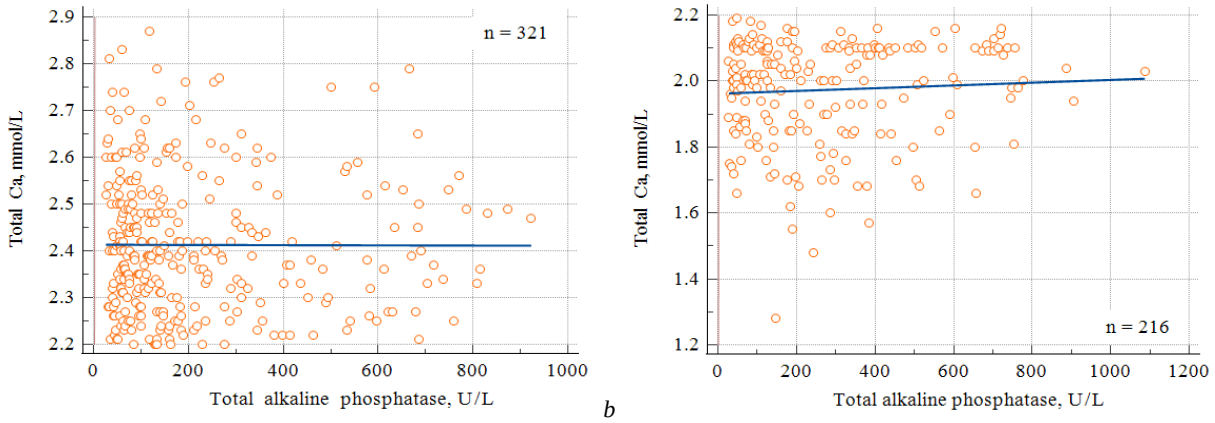
for both parameters were diagnosed in 50.7% of all examined goats. In 9.0% of animals, an increase in enzyme activity was observed at optimal macroelement concentration. In 31.5% of goats with reduced total calcium levels, enzyme activity was within the reference values, and in another 8.8% of the studied population with hypocalcemia, hyperfermentemia of total alkaline phosphatase was diagnosed.

An analysis of total calcium metabolism and total alkaline phosphatase activity in clinically healthy animals showed that in 84.7% of them the values were within physiological limits. In another 15.3% of goats with optimal macroelement values, increased enzyme activity was diagnosed, which indicates its elimination beyond the destroyed cells.

Thus, according to the results of biochemical studies, a combination of optimal values of total calcium and total alkaline phosphatase was established in 50.7% of pregnant and lactating animals. However, according to the results of the analysis, the assessment of the herd for the content of these values in clinically healthy goats is more informative, as indicated, in particular, by the optimal values of these indicators in 84.7% of the animals studied, as well as an increase in the activity of total alkaline phosphatase in 15.3% of them at the optimal concentration of total calcium.

Based on the results of regression analysis, we did not find a strong correlation between total calcium and total alkaline phosphatase in the blood serum of clinically healthy goats. However, among sick animals with subclinical hypocalcemia, the coefficient of determination was higher than in clinically healthy animals.

As a result of ROC analysis, the optimal threshold value for total alkaline phosphatase activity in goat milk was ≤ 187.9 U/L (sensitivity – 65.1%, specificity – 51.9%, area under the curve (AUC) – 0.575 (95% confidence interval: 0.532–0.618; J = 17.0%; P < 0.003; Fig. 4). The area under the ROC curve (AUC) was 0.575, indicating a low but statistically significant diagnostic value of this indicator (P < 0.003). At the same time, the Youden index, which summarises sensitivity and specificity, was 17.0%, which also indicates the informative value of total alkaline phosphatase as a biomarker for the early diagnosis of subclinical hypocalcemia in goats.



a *b*

Fig. 3. Results of linear regression analysis between total calcium and total alkaline phosphatase in goats blood serum: *a* – clinically healthy (n = 321); *b* – sick with subclinical hypocalcemia (n = 216)

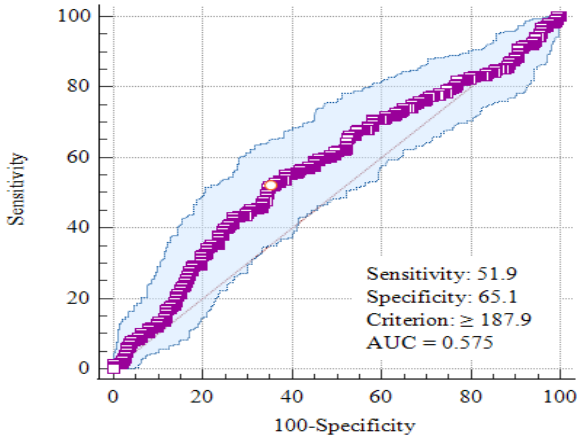


Fig. 4. ROC curve of diagnostic significance of total alkaline phosphatase in the blood serum of goats (Ca_{total} > 2.2 mmol/L; n = 537)

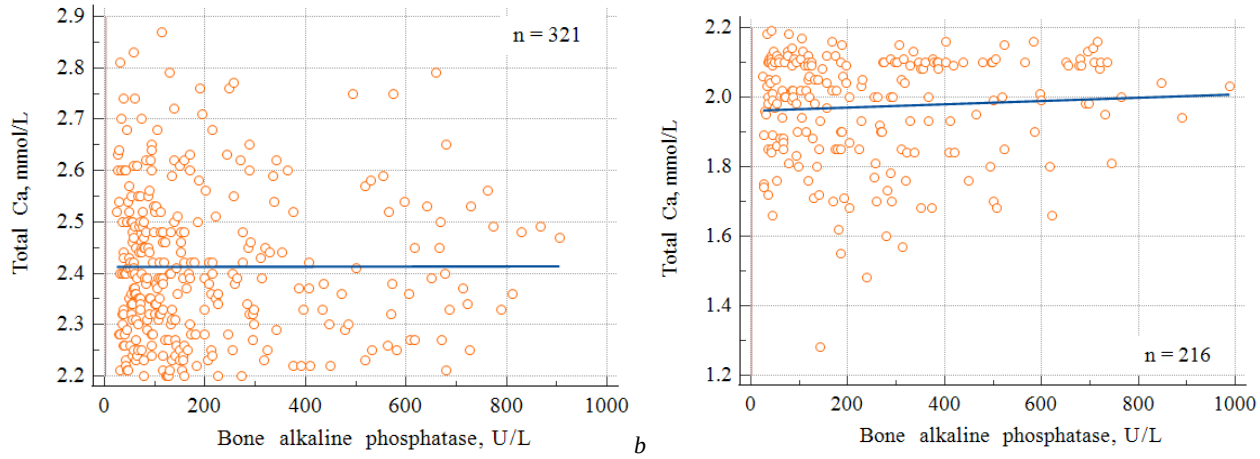
The next stage of the work was the application of regression and ROC analyses to evaluate total calcium and bone isoenzyme alkaline phosphatase as prognostic indicators in goats. It was found that the activity of the isoenzyme in the blood serum of pregnant and lactating goats ranged from 24.5 to 988.0 U/L, including 24.5 to 905.3 U/L in clinically healthy animals, and the difference from animals with hypocalcemia was significant (Table 2; P = 4.2 x 10^{-3}).

When analysing the individual indicators of these values, their optimal values were established in 50.7% of the total number of goats studied. In 9.0% of animals, ostase activity was elevated at physiological concentrations of the macroelement. In 31.5% of goats with reduced total calcium levels, the activity of the bone isoenzyme of alkaline phosphatase was within the normal range, and in another 8.8% of animals with hypocalcemia, hyperfermentemia of the isoenzyme was detected.

A similar comparison of total calcium concentration and ostase activity in clinically healthy animals indicates that these values were within physiological limits in 84.7% of goats. In another 15.3% of goats with optimal macroelement values, bone isoenzyme hyperfermentemia was diagnosed, which is evidence of metabolic disorders in bone tissue, in particular, the development of dystrophic processes in it due to D-hypovitaminosis. Thus, according to the results of biochemical studies, in 84.7% of clinically healthy animals, the concentration of total calcium and the activity of bone isoenzyme were within optimal limits. In another 15.3% of goats, with physiological values of this macroelement, ostase hyperfermentemia was diagnosed, indicating a high probability of developing pathology in bone tissue at a subclinical level.

Based on the results of regression analysis of these values, we also found no strong correlation in the blood serum of clinically healthy goats, as between total calcium and total alkaline phosphatase, but among sick animals, the coefficient of determination was higher than in clinically healthy ones. According to the results of studies and ROC analysis, the optimal threshold value of bone isoenzyme activity in goat milk was ≤ 172.7 U/L (sensitivity – 53.7%, specificity – 64.5%, area under the curve (AUC) – 0.575 (95% confidence interval: 0.532–0.618; P < 0.003; Fig. 6). The results of the ROC analysis confirm the statistical significance of bone alkaline phosphatase isoenzyme according to the Youden index – 18.2%.

Based on the results of linear regression and ROC analysis of bone alkaline phosphatase isoenzyme activity, it can be concluded that it has high prognostic value according to ROC analysis for predicting subclinical hypocalcemia in goats, since the coefficients of determination were low. At the next stage of the study, our attention was focused on studying the informativeness of intestinal alkaline phosphatase isoenzyme. It was found that its activity in the blood serum of all studied goats was within a wide range – from 5.5 to 485.6 U/L, including in clinically healthy animals – 5.5–187.0 U/L, which is 1.48 times less than in the subclinical course of hypocalcemia.



a *b*

Fig. 5. The results of linear regression analysis between total calcium and bone isoenzyme of alkaline phosphatase in the blood serum of goats: *a* – clinically healthy (n = 321); *b* – sick with subclinical hypocalcemia (n = 216)

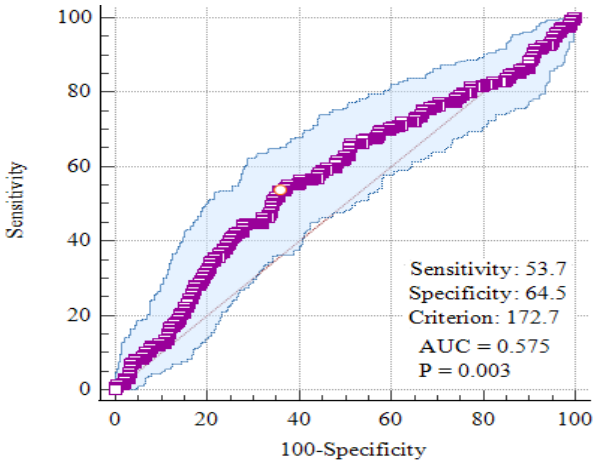


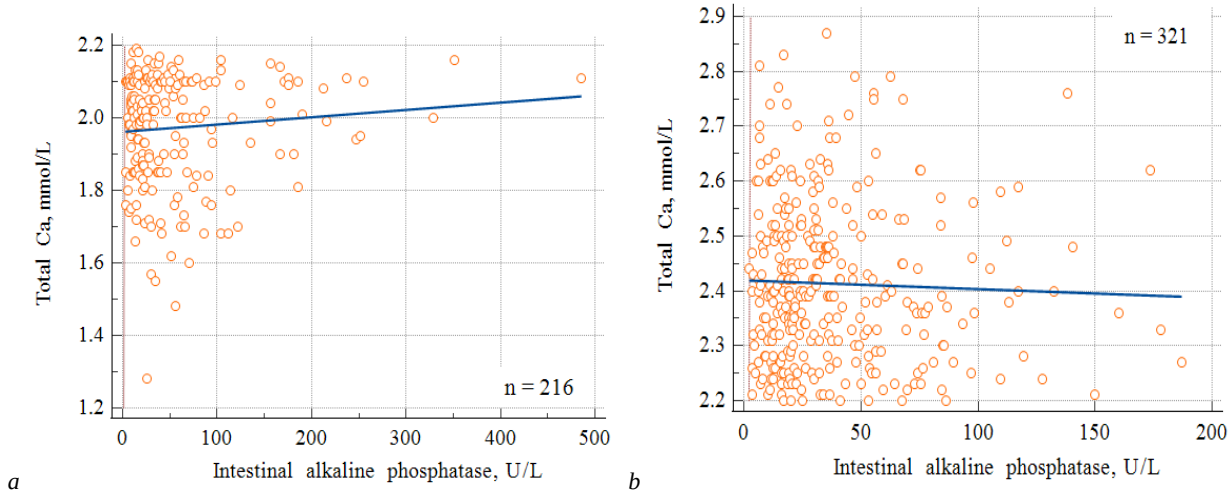
Fig. 6. ROC curve of the diagnostic value of bone isoenzyme alkaline phosphatase in the blood serum of goats (Ca_{total} > 2.2 mmol/L; n = 537)

Optimal values of this isoenzyme activity were found in 82.3% of goats from the entire studied population, in particular in 78.9% of pregnant goats and in 84.8% of lactating animals, while increased activity was diagnosed in 17.7%, including 21.1% of pregnant goats and 15.2% of lactating goats.

Based on the analysis of individual indicators of total calcium concentration and intestinal alkaline phosphatase isoenzyme activity, optimal values of both parameters were diagnosed in 51.4% of the 537 examined goats. In another 8.4% of animals, increased isoenzyme activity was found at physiological concentrations of total calcium, which, in our opinion, may indicate the development of inflammatory processes in the intestine. In 9.3% of goats with hypocalcemia, hyperfermentemia of this isoenzyme was detected.

At the same time, analysis of the metabolism of these indicators in clinically healthy animals shows that in 86.0% of them they were within physiological limits. In another 14.0% of goats, hyperfermentemia of the isoenzyme was diagnosed at optimal total calcium values.

According to the results of linear regression analysis, it was established that the correlation between total calcium and intestinal isoenzyme of alkaline phosphatase was low (Fig. 7a and 7b).



a *b*

Fig. 7. Regression analysis between total calcium and intestinal isoenzyme of alkaline phosphatase in the blood serum of goats: *a* – clinically healthy (n = 321); *b* – sick with subclinical hypocalcemia (n = 216)

According to the results of our studies and ROC analysis, the optimal threshold value of intestinal alkaline phosphatase isoenzyme activity in pregnant and lactating goats was ≤ 58.5 U/L, with an area under the curve (AUC) of 0.556. Analysis of this test indicates the high diagnostic value of intestinal alkaline phosphatase isoenzyme for the early diagnosis of hypocalcemia in goats, since the specificity of the test was quite high (80.9%), allowing reliable identification of healthy animals (confidence interval: 0.512–0.598; J – 12.4%; P < 0.032; Fig.8).

Based on the results of linear regression and ROC analysis of total alkaline phosphatase activity and its bone and intestinal isoenzymes, it can be concluded that they have high prognostic value for the early diagnosis of subclinical hypocalcemia according to ROC analysis, while the coefficients of determination of linear regression were low.

Given the importance of ionised calcium in the metabolism of total calcium in goats, further analysis of the research results was aimed at studying the relationship between its concentration and the activity of total alkaline phosphatase and its isoenzymes using similar methods of mathematical analysis of biological objects.

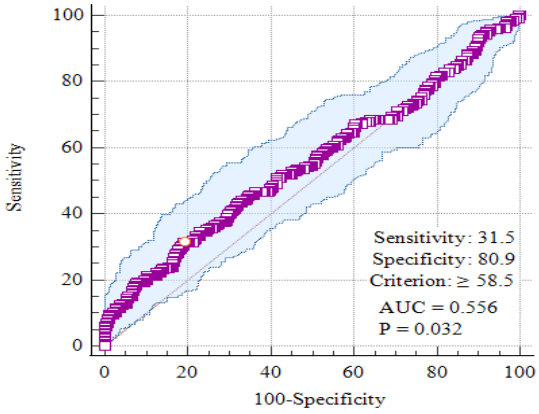
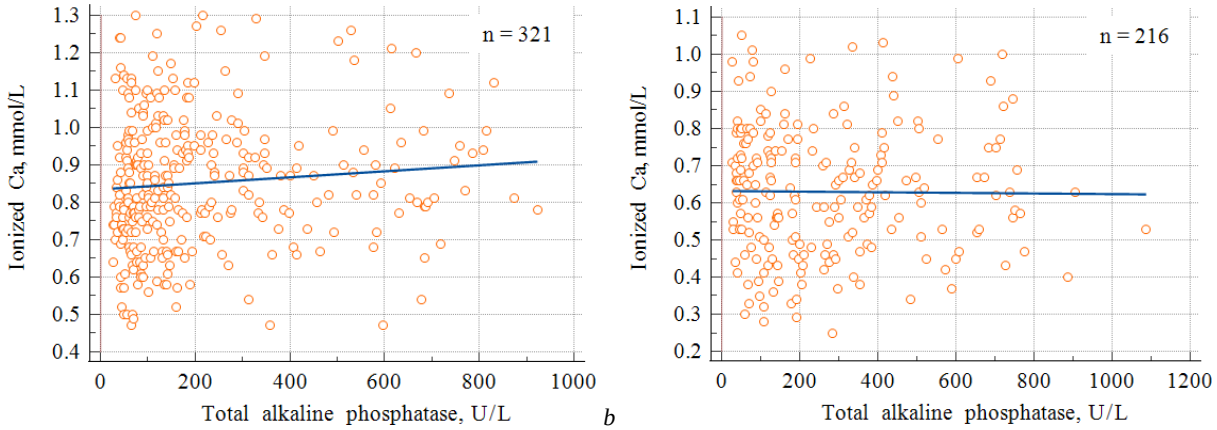


Fig. 8. ROC curve for the diagnostic value of intestinal isoenzyme alkaline phosphatase in the blood serum of goats (Ca_{total} > 2.2 mmol/L; n = 537)

Analysis of the research results shows a combination of optimal values of ionised calcium and total alkaline phosphatase in 75.6% of the total goat population. In 16.6% of animals with optimal free calcium content, an increase in the activity of total alkaline phosphatase to 1087.0 U/L was diagnosed, compared to 9.0% in animals with similar total calcium analysis.

Based on the results obtained, we analysed these values in clinically healthy goats. It was found that in 84.7% of animals, the concentration of free calcium and the activity of total alkaline phosphatase were within physiological limits. In another 15.3% of goats in this group, with optimal free calcium values, enzyme hyperfermentemia was diagnosed.

Based on the results of regression analysis, we did not find a strong correlation between the ionised fraction of calcium and total alkaline phosphatase in the blood serum of clinically healthy goats. However, among animals with subclinical hypocalcemia, the coefficient of determination was higher than in clinically healthy animals (Fig. 9).



a *b*

Fig. 9. Results of linear regression analysis between ionised calcium and total alkaline phosphatase in goats' blood serum: *a* – clinically healthy (n = 321); *b* – sick with subclinical hypocalcemia (n = 216)

It has been established that total alkaline phosphatase activity ≥ 167.1 U/L indicates a risk of subclinical hypocalcemia in animals (sensitivity – 64.0%; specificity – 57.9%).

Thus, the optimal threshold value of total alkaline phosphatase activity (≥ 167.1 U/L) calculated by ROC analysis (AUC = 0.619; P < 0.001; 95% confidence interval: 0.576–0.660; J = 21.9%; Fig. 10) for ionised calcium and linear regression analysis indicators indicates the statistically significant diagnostic value of the enzyme for the early diagnosis of hypocalcemia in goats.

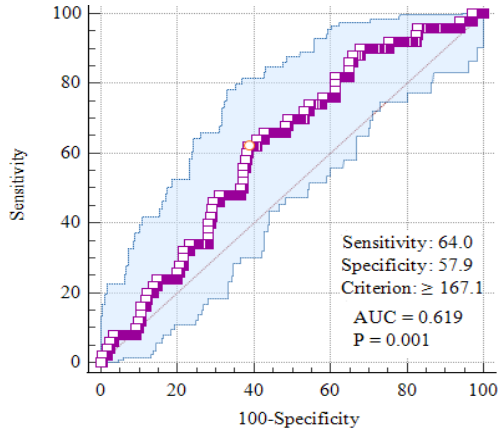


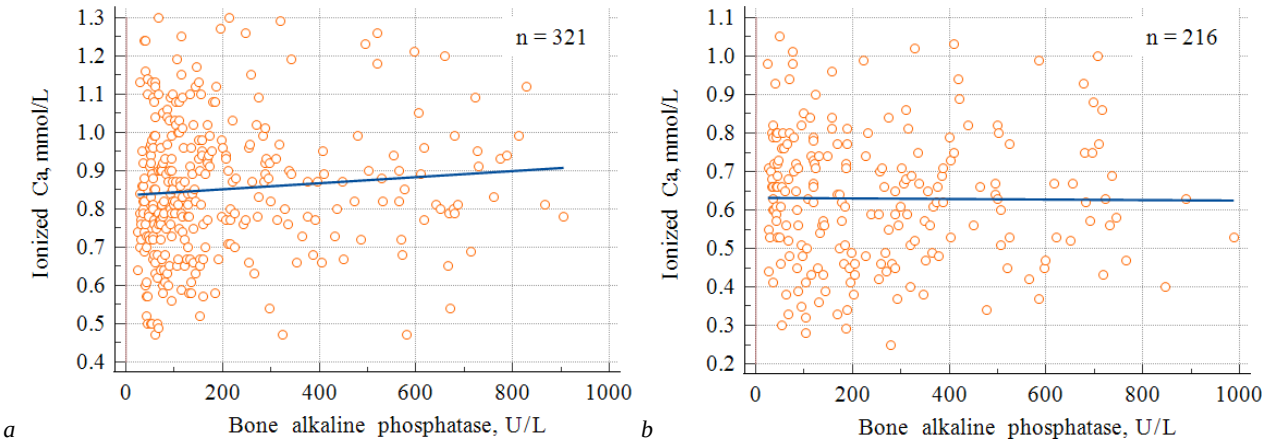
Fig. 10. ROC curve of diagnostic significance of total alkaline phosphatase in the blood serum of goats ($Ca_{ionised}$ > 0.47 mmol/L; n = 537)

The results obtained indicate that the high prognostic value of total alkaline phosphatase activity is confirmed by ROC analysis, while according to linear regression analysis, the correlation with the ionised calcium fraction was weak (Fig. 9). When studying the metabolism of bone isoenzyme alkaline phosphatase in the blood serum of all goats examined, its activity was found to be within the range of 24.5–988.0 U/L, in particular, in clinically healthy animals – in the range from 24.5 to 905.3 U/L. Analysis of the results of studies of the concentration of the ionised fraction of calcium and the activity of the bone isoenzyme of alkaline phosphatase indicates a combination of optimal values for both indicators in 75.6% of animals from the total studied population, in particular, in 72.8% of pregnant goats and in 77.7% of lactating goats. In 16.6% of goats, with optimal free calcium content, ostase activity was elevated above 401.3 U/L (normal).

Analysis of these indicators in clinically healthy animals shows that in 84.7% of them these values were within physiological limits. In another 15.3% of goats, hyperfermentemia of this isoenzyme was diagnosed at optimal free calcium values.

Thus, according to the results of biochemical studies, in 84.7% of clinically healthy animals, the concentration of ionised calcium and the activity of the bone isoenzyme were within optimal limits. In 15.3% of goats, ostase hyperfermentemia was diagnosed at physiological free calcium values, indicating latent metabolic disorders in bone tissue without changes in the concentration of the ionised fraction of calcium in the blood serum of goats, and may be an objective marker in the early prediction system for this pathology in goats.

When performing linear regression analysis, we found a slightly higher level of correlation between ionised calcium and bone isoenzyme of alkaline phosphatase in the blood serum of clinically healthy animals than in animals with subclinical hypocalcemia, however, the coefficient of determination was low (Fig. 11). The optimal threshold value of ostase activity (≥ 173.4 U/L) calculated by ROC analysis (AUC = 0.614; P < 0.001; 95% confidence interval: 0.571–0.655; J = 23.8%; Fig. 12) for ionised calcium indicates the statistically significant diagnostic value of the isoenzyme for the early diagnosis of hypocalcemia in goats. Thus, bone alkaline phosphatase isoenzyme activity ≥ 173.4 U/L indicates a high probability of subclinical hypocalcemia in animals (sensitivity – 62.0%; specificity – 61.8%).



a *b*

Fig. 11. Regression analysis between ionised calcium and bone isoenzyme of alkaline phosphatase in goats blood serum: *a* – clinically healthy (n = 321); *b* – sick with subclinical hypocalcemia (n = 216)

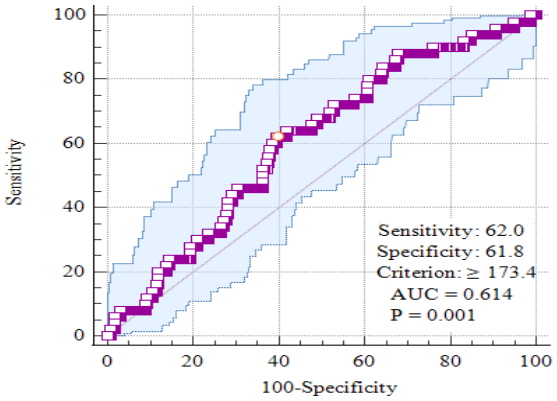


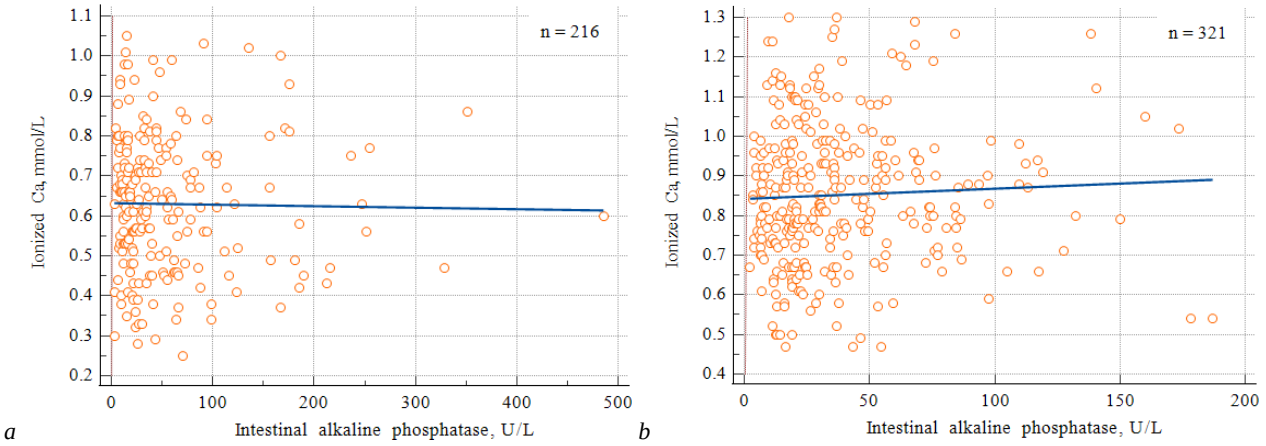
Fig. 12. ROC curve of diagnostic significance of bone isoenzyme of alkaline phosphatase in the blood serum of goats ($Ca_{ionised}$ > 0.47 mmol/L; n = 537)

Based on the results of linear regression and ROC analysis of bone alkaline phosphatase isoenzyme activity, it can be concluded that it has high prognostic value according to ROC analysis for predicting subclinical hypocalcemia in goats. According to the results of linear regression analysis, the dependence of these variables in clinically healthy and sick animals was low.

The activity of intestinal isoenzyme of alkaline phosphatase in the blood serum of all studied goats was within the range of 5.5–485.6 U/L, in particular, in clinically healthy goats – 5.5–187.0 U/L. Analysis of the results of studies of the concentration of the ionised fraction of calcium and the activity of the intestinal isoenzyme of alkaline phosphatase indicates a combination of optimal values for both indicators in 76.2% of the total livestock, in particular, in 72.8% of pregnant goats and in 78.6% of lactating goats. In 16.0% of goats with optimal free calcium content, the activity of the intestinal isoenzyme of alkaline phosphatase was elevated.

In the vast majority of clinically healthy goats (86.0%), the concentration of ionised calcium and the activity of intestinal isoenzyme alkaline phosphatase were within physiological limits. In another 14.0% of animals in this group, with optimal ionised calcium values, an increase in the activity of this enzyme above the upper physiological limit was diagnosed.

It was established that, according to the results of linear regression analysis, the relationship between the ionised calcium fraction and the intestinal isoenzyme of alkaline phosphatase in the blood serum of clinically healthy and sick goats with subclinical hypocalcemia was insignificant (Figs. 13a and 13b).



a *b*

Fig. 13. Regression analysis between ionised calcium and intestinal isoenzyme of alkaline phosphatase in goats blood serum: *a* – clinically healthy (n = 321); *b* – sick with subclinical hypocalcemia (n = 216)

The optimal threshold value of intestinal isoenzyme alkaline phosphatase activity (≥ 58.5 U/L) calculated by ROC analysis (P < 0.005; 95% confidence interval: 0.533–0.619; J = 14.7%; Fig. 14) for ionised calcium indicates the high diagnostic significance (AUC = 0.577) of the intestinal isoenzyme for the early diagnosis of hypocalcemia in goats (sensitivity – 36.0%; specificity – 78.5%) compared to ROC analysis for total calcium.

Based on the results of linear regression and ROC analyses, it can be concluded that intestinal isoenzyme of alkaline phosphatase has high diagnostic value for predicting subclinical hypocalcemia in goats only according to ROC analysis, since the relationship between ionised calcium and isoenzyme was insignificant, as confirmed by the low level of determination.

Thus, the results of studying the metabolism of total calcium and ionised calcium in the blood serum of clinically healthy goats indicate a simultaneous combination of their optimal values with the activity of total alkaline phosphatase and its bone and intestinal isoenzymes in the vast majority of animals in this group (84.7–86.0%). An increase

in the activity of total alkaline phosphatase and its isoenzymes with physiological indicators of the essential macroelement and its ionised fraction was found in 14.0–15.3% of goats. Thus, analysis of the metabolism of these indicators in clinically healthy goats is more informative for the early diagnosis of this pathology than monitoring all studied animals, including those with subclinical hypocalcemia.

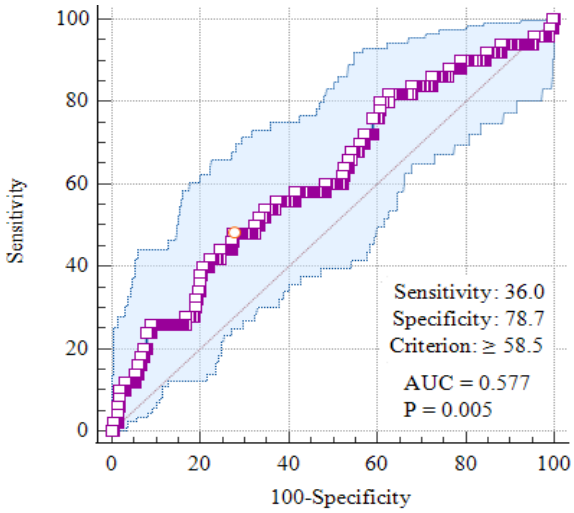


Fig. 14. ROC curve of diagnostic significance of intestinal isoenzyme of alkaline phosphatase in the blood serum of goats ($Ca_{ionised}$ > 0.47 mmol/L; n = 537)

The results of ROC analysis between total calcium, ionised calcium and total alkaline phosphatase activity, its bone and intestinal isoenzymes indicate their high diagnostic value for predicting subclinical hypocalcemia. However, according to the results of linear regression analysis, the dependence of these variables in clinically healthy and sick animals was low, indicating the low diagnostic significance of this test.

Discussion

The main objective of our work was to apply linear regression and ROC analyses to predict hypocalcemia in dairy goats based on a set of biochemical parameters, including total calcium, ionised calcium, alkaline phosphatase, and its bone and intestinal isoenzymes.

According to Arnold et al. (2024) and Yatoo et al. (2023), the threshold values for determining subclinical and clinically significant hypocalcemia are 2.0 and 1.1 mmol/L of total calcium in goat blood serum, respectively. The results of our studies are consistent with the data of these authors (Hotsuliak & Sakhniuk, 2024b).

Ionised calcium (iCa) is a biologically active fraction of calcium, the concentration of which is regulated by parathyroid hormone, calcitonin and 25(OH) D^3 and directly regulates vital physiological processes. Unlike total calcium (tCa), the level of ionised calcium is a more sensitive indicator of its homeostasis, as it changes under the influence of blood pH and protein content, making it more informative for assessing calcium metabolism in animals. The ratio of ionised calcium (iCa) to total calcium (tCa) in animal blood serum depends on various factors, including age, genetics, body condition and physiological status (pregnancy, lactation) (Silva et al., 2022). According to Cao et al. (2025), measuring only the concentration of total calcium can lead to misinterpretation of the results. According to the results of studies by Jose-Cunilleras et al. (2025), a wide range of percentages of ionised calcium in the structure of total calcium was observed in the blood serum of clinically healthy cows and cows with subclinical hypocalcemia.

According to our data, the physiological limits of ionised calcium in goats' blood serum are 0.47–1.20 mmol/L (Hotsuliak & Sakhniuk, 2024b). Based on the analysis of individual indicators of total calcium and ionised calcium, optimal values for both parameters were diagnosed in 59.8% of the 537 goats studied. It should be noted that in 32.4% of animals with a reduced content of essential macroelements, the concentration of free calcium was within physiological limits (0.47–1.05 mmol/L), which was also observed by Ott et al. (2021) and Leno et al. (2017). In another 7.8% of animals, a decrease in both indicators was diagnosed, with the maximum content of the latter not exceeding 0.46 mmol/L. Thus, analysis of the metabolism of these indicators in clinically healthy goats is more informative for early diagnosis of this disease than monitoring all studied animals, including those with subclinical hypocalcemia.

According to the results of ROC analysis (AUC = 0.816), a concentration of ionised calcium ≥ 0.75 mmol/L indicates a high risk of subclinical hypocalcemia in goats (sensitivity – 76.9%; specificity – 72.3%; P < 0.001). According to linear regression analysis, the relationship between total calcium and ionised calcium in the blood serum of clinically healthy goats and goats with subclinical hypocalcemia indicates a correlation between them (R^2 = 0.10; R^2 = 0.13).

Alkaline phosphatase is considered to be a marker of bone metabolism, and an increase in its activity disrupts the balance between bone formation and resorption and increases the likelihood of developing hypocalcemia (Leno et al., 2017).

When analysing individual indicators of total calcium concentration and total alkaline phosphatase activity, optimal values for both parameters were diagnosed in 50.7% of the goats studied. In 9.0% of animals with optimal concentrations of this essential macroelement, increased enzyme activity was observed, which was also observed in the blood serum of clinically healthy lactating cows (Sato et al., 2005). In 31.5% of the goats studied, with a reduced level of total calcium, the enzyme activity was within the normal range, which does not exclude initial disturbances in calcium metabolism in animals (Arnold et al., 2024).

In particular, the analysis of total calcium metabolism and total alkaline phosphatase activity in clinically healthy animals showed that in 84.7% of them the values were within physiological limits. In another 15.3% of goats with optimal macroelement values, enzyme hyperfermentemia was diagnosed, which indicates the informative value of this indicator for predicting the subclinical course of the pathology.

Analysis of the results of studies of the concentration of the ionised fraction of calcium and the activity of total alkaline phosphatase showed a combination of optimal values for both parameters in 75.6% of the animals studied, in particular, in clinically healthy goats these values were diagnosed in 84.7%, which indicates the higher informative value of this indicator among clinically healthy goats than when monitoring all studied animals.

Bone isoenzyme of alkaline phosphatase (ostase) is one of the most sensitive markers that reflects osteoblast activity and bone remodelling processes. It is synthesized and expressed at high levels by osteoblasts simultaneously with osteoid secretion (Vimalraj, 2020). This isoenzyme activates the hydrolysis of phosphate esters on the surface of osteoblast cells, while ensuring a high extracellular concentration of inorganic phosphate for osteoid mineralisation, and also promotes the breakdown of pyrophosphates, which are inhibitors of ionised calcium and phosphorus deposition in osteoid (Millán, 2006).

Unlike total alkaline phosphatase, whose activity can vary under the influence of various physiological and pathological conditions (hepatic, intestinal and placental isoenzymes), it is the bone isoenzyme of alkaline phosphatase that allows a more accurate assessment of bone metabolism in animals. This is especially important during pregnancy and early lactation, when calcium requirements increase and the risk of hypocalcemia rises. Therefore, determining the activity of bone isoenzyme alkaline phosphatase in the blood serum of animals makes it possible to detect latent disturbances in calcium homeostasis even before the appearance of clinical symptoms, which makes it a valuable information marker for the early diagnosis of hypocalcemia in goats (Liesegang et al., 2006; Camassa et al., 2017; Makris et al., 2022).

When analysing individual indicators of total calcium concentration and bone alkaline phosphatase isoenzyme activity, optimal values for both parameters were diagnosed in 50.7% of the goats studied, while in another 9.0% of animals, increased ostase activity was found at physiological concentrations of the essential macroelement.

In 31.5% of animals with reduced total calcium levels, bone isoenzyme activity was within normal limits, while in another 8.8% of

goats, hyperfermentemia of total alkaline phosphatase was detected. According to the results of studies by Staric & Zadnik (2010), it was found that in the subclinical course of hypocalcemia in cows, ostase activity was higher compared to clinically healthy animals, but was within the reference values.

According to the literature (Brun et al., 2006; Brun et al., 2012; Santos et al., 2022), calcium has a stimulating effect on the activity of intestinal alkaline phosphatase isoenzyme in animals. It has been established that the concentration of calcium in the intestinal lumen directly correlates with the activity of the intestinal isoenzyme of alkaline phosphatase. In particular, this isoenzyme activates its enzymatic action when the concentration of the macroelement increases with a noticeable linear dependence ($r = + 0.946$; $P < 0.01$) and is inactivated when the latter decreases. According to Fawley & Gourlay (2016), impaired expression or function of the intestinal isoenzyme alkaline phosphatase is observed in intestinal inflammation, dysbiosis, and bacterial localisation.

The analysis of total calcium metabolism and intestinal alkaline phosphatase isoenzyme activity in clinically healthy animals showed that in 86.0% of them the values were within physiological limits. In another 16.0% of goats, with optimal macroelement values, hyperfermentemia of this isoenzyme was diagnosed, which indicates that this indicator is more informative for the early diagnosis of subclinical hypocalcemia among clinically healthy goats than when comparing these values among all studied animals.

When analysing the results of studies of the concentration of the ionised fraction of calcium and the activity of the intestinal isoenzyme of alkaline phosphatase, a combination of optimal values of both indicators was found in 76.2% of the animals studied, compared to 86.0% among clinically healthy goats.

Thus, the results of ROC analysis between total calcium, ionised calcium with the activity of total alkaline phosphatase and its bone and intestinal isoenzyme showed high diagnostic value for predicting the subclinical course of hypocalcemia. However, linear regression analysis did not reveal a significant correlation between these variables.

Conclusions

According to linear regression analysis and ROC analysis (AUC = 0.816), determining the content of ionised calcium in the blood serum of goats has high diagnostic value for prognostication of hypocalcemia, while measuring total calcium concentration is advisable for monitoring its metabolism. Based on the results of regression analysis, we did not find a statistically significant relationship between total calcium, its ionised fraction, and the activity of total alkaline phosphatase and its bone and intestinal isoenzymes in the blood serum of goats. However, when performing ROC analysis between these values, we established the high diagnostic significance of total alkaline phosphatase and its isoenzymes for the early diagnosis of hypocalcemia in goats, in particular, the area under the ROC curve (AUC) of the enzyme was 0.575, bone – 0.575 and intestinal isoenzyme of alkaline phosphatase- 0.556, for total calcium not less than 2.2 mmol/L, respectively. In a similar ROC analysis for ionised calcium in the blood serum of goats, not less than 0.47 mmol/L, the area under the ROC curve was higher, in particular, total alkaline phosphatase – 0.619, ostase – 0.614 and intestinal isoenzyme of alkaline phosphatase – 0.577.

Further research should focus on studying the vitamin D and endocrine status in clinically healthy goats and goats with subclinical hypocalcemia from different physiological and technological groups.

The authors declare that there is no conflict of interest.

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