Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Metabolism of alkaline phosphatase and acid phosphatase in goats

V. Sakhniuk, M. Hotsuliak, V. Marchuk, A. Kharchenko, M. Savcheniuk

Bila Tserkva National Agrarian University, Bila Tserkva, Ukraine

Article info

Received 10.06.2025
Received in revised form 02.07.2025
Accepted 05.08.2025

Bila Tserkva National Agrarian University, Soborna st., 8/1, Bila Tserkva, 09117, Ukraine. Tel.: +38-068-603-21-89. E-mail: mhotsuliak@btsau.edu.ua

Sakhniuk, V., Hotsuliak, M., Marchuk, V., Kharchenko, A., & Savcheniuk, M. (2025). Metabolism of alkaline phosphatase and acid phosphatase in goats. Regulatory Mechanisms in Biosystems, 16(3), e25143. doi:10.15421/0225143

Goat breeding is a branch of world livestock production that has a rich history, modern achievements and gradual development in different countries of the world due to the widespread introduction of innovative technologies. The purpose of this study was to determine the physiological limits of alkaline and acid phosphatase activity in blood serum, as well as to study the dynamics of these parameters in clinically healthy and subclinical hypocalcaemic dairy goats. The subjects of the study were pregnant and lactating Zaanen, Alpine and LaMancha goats. The activity of total alkaline phosphatase (ALP) and its bone and intestinal isoenzymes, acid phosphatase (ACP), concentration of total calcium, and ionised calcium were determined in goat serum by standardised methods. The measurements were performed on a Stat Fax 4500+ biochemical analyzer. The physiological limits of the activity of total alkaline phosphatase (12.6–412.2 U/L), its intestinal (5.5–70.5 U/L) and bone isoenzymes (7.3–401.3 U/L), and acid phosphatase (0.92–11.56 U/L) in the blood serum of clinically healthy goats were established. In the subclinical course of hypocalcemia, the activity of ALP in the serum of goats was 266.9 ± 15.1 U/L, which is 1.30 times higher than in clinically healthy animals (212.4 ± 11.2 U/L). In particular, the activity of intestinal and bone isoenzymes of ALP in diseased animals was 1.48 and 1.26 times higher compared to clinically healthy goats. The activity of acid phosphatase in the subclinical course of hypocalcemia averaged 9.02 ± 0.48 U/L (0.63–58.80 U/L), which was 1.45 times higher than in clinically healthy animals (6.24 ± 0.26 U/L; 1.00–27.60 U/L) and is evidence of increased resorption in goat bone tissue.

Keywords: isozymes; intestinal alkaline phosphatase; bone alkaline phosphatase; acid phosphatase; total calcium; ionized calcium; hypocalcemia.

Introduction

The modern introduction of technological methods in production systems and genetic improvement of herd productivity has led to the emergence of various metabolic diseases in ruminants (Brozos et al., 2010; Constable & Radostis, 2017; Hernandez et al., 2020). Metabolic disorders negatively affect the health of the herd and lead to significant economic losses. In recent decades, the main focus has been on the study of calcium metabolism in various metabolic disorders in small ruminants, in particular, postpartum hypocalcemia.

The periods of late pregnancy and early lactation are critically important for goats. At this time, the female body undergoes significant biochemical changes and adaptations to a new physiological state. During this period, animals are especially susceptible to many metabolic diseases, including due to the lack of vitamins and essential macro- and microelements in the diet. Therefore, early diagnosis of diseases is extremely important, as it primarily determines the treatment and prevention of pathologies. In addition, timely diagnosis is a key aspect for saving animal life and prevents inefficient use of veterinary drugs (Yatoo et al., 2023).

Alkaline phosphatase (ALP) belongs to the group of hydrolase enzymes that catalyzes the hydrolysis of a wide range of phosphate mono-, di-, and triesters, inorganic pyrophosphates, phosphoramidates, phosphonate, sulfate, and sulfonate monoesters in alkaline environments, as well as at physiological pH (Le-Vinh et al., 2022). This membrane-bound glycosylated zinc-containing metalloenzyme is one of the most abundant enzymes in nature, as it has been found in humans and animals, as well as in plants, bacteria, and algae, indicating its involvement in fundamental biochemical processes (Millan, 2006; Van Loo et al., 2010). The activity of alkaline phosphatase in animals increases in the presence of magnesium and chlorine ions, and its physiological activity requires an optimal ratio of these minerals in the body. Despite the fact that alkaline phosphatase is one of the most studied enzymes, its physiological function is not yet fully understood (Fernandez & Kidney, 2007).

The determination of alkaline phosphatase activity in animal serum is of great prognostic value in the diagnosis of various diseases (Sharma & Prasad, 2013). In particular, an increase in enzyme activity is observed in bone and liver pathologies, endocrine diseases (hyperadrenocorticism, diabetes mellitus, hypothyroidism and hyperparathyroidism) and genetic diseases, as well as in vitamin D deficiency in the diet of animals. High levels of ALP may indicate active bone resorption, as this enzyme is a byproduct of osteoblast activity (Sato et al., 2002; Fernandez & Kidney, 2007).

In animals, alkaline phosphatase is encoded by two gene loci and, accordingly, forms two isoenzymes: tissue-independent and intestinal (Brun et al., 2014). Liver, bone, kidney, and placental ALP is encoded by a tissue-independent locus with subsequent modification, which leads to the formation of its different isoforms. Some types of this enzyme are formed by its post-translational modification (gene product). Thus, animals have many isoforms of ALP, but only 2 isoenzymes, while humans have 4 (Fernandez & Kidney, 2007).

The bone isoenzyme alkaline phosphatase is a glycoprotein localized in osteoblast membranes that reflects their activity in the process of bone formation and accounts for a significant proportion of the total enzyme activity. Therefore, it is obvious that determining the activity of this isoenzyme serves as a marker of bone remodeling (Van Hoof & De Broe, 1994; Grote-Koska et al., 2016; Minisola et al., 2025).

The intestinal isoenzyme of alkaline phosphatase (intestinal ALP) is localized in the brush border of the enterocytes (ectoenzyme). The active enzyme is a dimeric protein of two identical monomers, and its stabilization depends on the presence of magnesium. This brush border isoenzyme is involved in fatty acid absorption and plays an important role in maintaining gut microbial homeostasis and intestinal barrier function due to its ability to dephosphorylate lipopolysaccharide (LPS) (Estaki, 2014). According to the literature (Brun et al., 2006; Brun et al., 2011; Lallès, 2013), calcium has a stimulating effect on the activity of the intestinal isoenzyme alkaline phosphatase in animals.

Strom et al. (1991) and Centeno et al. (2004) found that the expression of the intestinal ALP isoenzyme is controlled by $1,25(OH)_2$ D3, and the interaction between the isoenzyme and calcium leads to changes in its activity (Strom et al., 1991). Experimental administration of the intestinal isoenzyme inhibitor L-phenylalanine to animals

increases the percentage of calcium absorption. Thus, the activity of this enzyme may play a role in the regulation of the TRPV5/6 channel through changes in pH depending on the calcium concentration in the intestinal lumen (Brun et al., 2009; Brun et al., 2011).

In diseases of the bone system, considerable attention is paid to determining the activity of acid phosphatase, as it is also an iron-containing enzyme expressed in large quantities by osteoclasts that resorb bone, macrophages, and dendritic cells (Schini et al., 2022). ACP is directly involved in bone remodeling and in the primary immune response (Hayman, 2008; Ek-Rylander & Andersson, 2009; Anand & Srivastava, 2012), and an increase in enzyme activity indicates an increase in the intensity of bone resorption (Bull, 2002).

The aim of the study is to determine the physiological limits of alkaline and acid phosphatase activity in blood serum, as well as to study the dynamics of these parameters in clinically healthy and subclinical hypocalcaemic dairy goats.

Material and methods

The research protocol was approved by the Ethics Committee of Bila Tserkva National Agrarian University (Protocol No. 25-2 of 03.07.25).The animals were housed, fed, cared for and all procedures performed 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, 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 stipulated by the European Community Directive.

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. Blood samples were taken from 8:00 to 10:00 am before feeding, taking into account all the rules of veterinary septic and antiseptic.

The work was carried out on the livestock of Zaanen, Alpine and LaMancha dairy goats. The subjects of the study were clinically healthy pregnant goats (n = 146) and lactating goats (n = 175), as well as pregnant goats (n = 82) and lactating animals (n = 134) of 1–4 years of age suffering from subclinical hypocalcemia.

Table 1
Number of goats studied in the study of monitoring of serum ALP and ACP in clinically healthy and hypocalcaemic goats (n = 537)

Technology group	Date				Total
	2022	2023	2024	2025	
75–90 days of pregnancy	–	23 (20/3)	98 (61/37)	–	121
120–140 days of pregnancy	0/5	–	45 (24/21)	57 (41/16)	107
0–2 days after kidding	–	–	40 (14/26)	60 (24/36)	100
15–25 days of lactation	5 (1/4)	–	39 (33/6)	32 (18/14)	76
50–60 days of lactation	5/0	14 (10/4)	95 (64/31)	19 (6/13)	133

The activity of total alkaline phosphatase (ALP) and its bone and intestinal isoenzymes (by the method of Wagner, Putilin and Harabuga) was determined in goat serum by standardised methods; acid phosphatase (ACP) (by reaction with 4-nitrophenylphosphate, Sigma-Aldrich, Switzerland); total calcium concentration (reaction with calcium arsenase III) and ionised calcium (by ion-exchange absorption; Levchenko et al., 2010). The measurements were performed in the research laboratory of internal and metabolic diseases of animals and poultry of the Department of Propedeutics and Medicine of Internal Diseases of Animals and Poultry named after V. I. Levchenko using a Stat Fax 4500+ biochemical analyzer.

The results of biochemical studies are presented according to the International System of Units (SI), recommended for use in clinical laboratory diagnostics.

Statistical calculations were performed using the standard software package Statistica-12 (StatSoft Inc., USA, 2014). The data are presented in the tables as x ± SE (mean ± standard error). The Shapiro-Wilk test was used to determine the normality of the distribution, and the equality of variance was determined by the Levene test. One-way analysis of variance (ANOVA) and Tukey's post hoc test were used to compare groups on data with a normal distribution, while the Kruskal-Wallis test was used for groups whose distributions were not normal. In addition, the Mann-Whitney U-test or t-test for independent samples was used to assess differences between two different groups. A statistically significant P-value was defined as less than 0.05 (Gastwirth et al., 2009; Kim, 2015; 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, 15–25, 50–60 days after kidding) of Zaanen, Alpine and LaMancha breeds of dairy breeds in the summer-autumn and winter-spring periods during 2022–2025. Animals with optimal clinical and biochemical status indicators, including a minimum physiological concentration of total calcium in the blood serum of 2.20 mmol/L and a proportion of ionized calcium of at least 0.47 mmol/L, were considered healthy (Hotsuliak & Sakhniuk, 2024).

One of the most pressing issues of laboratory diagnostics in veterinary medicine is the lack of clearly defined reference values for the activity of total alkaline phosphatase, its isoenzymes and acid phosphatase in blood serum. Today, there are significant discrepancies in their values in the available literature. In this regard, there was a need to establish physiological limits for the activity of ALP and its isoenzymes in the blood serum of purebred goats kept on Ukrainian farms. For this purpose, 321 head of clinically healthy pregnant and lactating animals with optimal clinical study results and blood serum biochemical parameters were selected.

The physiological limits of total alkaline phosphatase activity in the blood serum of clinically healthy goats (212.4 ± 199.8 U/L) are as follows: min – 12.6; max – 412.2 U/L. In 84.7% of goats, the metabolism of total alkaline phosphatase was within the specified limits (Table 2).

Table 2
Physiological limits of the activity of ALP and its izoenzymes and acid phosphatase in the blood serum of clinically healthy goats

Parameter, unit of measurement	x ± SE	Physiological limits	SD	within the normal range	
				n = 321	%
Total alkaline phosphatase, U/L	212.4 ± 11.2	12.6–412.2	199.8	272	84.7
Intestinal alkaline phosphatase, U/L	38.0 ± 1.8	5.5–70.5	32.5	266	82.9
Bone alkaline phosphatase, U/L	204.3 ± 11.0	7.3–401.3	197.0	272	84.7
Acid phosphatase, U/L	6.24 ± 0.26	0.92–11.56	5.32	287	89.4

Based on the results of the study, we conducted a comparative analysis of the metabolism of ALP activity and its isoenzymes and total calcium and its ionized fraction in the blood serum of goats at different gestation periods and during the first two months of lactation. It was found that in 321 clinically healthy goats with optimal serum total calcium content (2.41 ± 0.01 mmol/L; 2.23–2.87 mmol/L), the

values of ALP activity ranged from 26.0 to 923.0 U/L (212.4 ± 11.2 U/L). In particular, in 146 pregnant goats with a concentration of total calcium in the blood serum in the range of 2.20–2.79 mmol/L (2.40 ± 0.01 mmol/L), the activity of ALP was in the range of 27.0–873.8 U/L (229.4 ± 18.5 U/L; Table 3), incl, in 79.5% of the studied goats its activity was in the range from 27.0 to 401.8 U/L (126.4 ± 7.9 U/L), in another 20.5% – in the range of 414.4–873.8 U/L (628.1 ± 21.6 U/L).

In 175 clinically healthy lactating goats (54.5% of the total number of clinically healthy animals) with optimal serum total calcium (2.42 ± 0.01 mmol/L; 2.23–2.87 mmol/L), the enzyme activity ranged from 26.0 to 923.0 U/L (198.1 ± 13.4 U/L; Table 3) and was somewhat lower compared to clinically healthy pregnant animals (Table 3). In 89.1% of the studied lactating goats, the activity of ALP was in the range from 26.0 to 407.4 U/L (148.0 ± 7.8 U/L), and in 10.9% – in the range of 414.9–922.8 U/L (610.1 ± 32.8 U/L).

Table 3

Dynamics of alkaline phosphatase activity in the blood serum of clinically healthy and hypocalcemic pregnant and lactating goats (x ± SE, n = 537)

Technology group	Clinical condition of goats			
	clinically healthy	Lim	goats with hypocalcemia	Lim
75–140 days of pregnancy	229.4 ± 18.5^{b}	27.0–873.8	255.5 ± 21.4^{bc}	39.8–776.4
75–90 days of pregnancy	235.3 ± 25.2^{bc}	27.0–809.5	253.0 ± 26.0^{bc}	45.0–702.8
120–140 days of pregnancy	222.2 ± 27.3^{ab}	32.2–873.8	258.0 ± 33.9^{bc}	39.8–776.4
0–60 days of lactation	198.1 ± 13.5^{ab}	26.0–923.0	273.8 ± 20.5^{bc}	27.7–1087.0
0–2 days after kidding	156.1 ± 20.9^{a}	29.6–535.4	249.9 ± 29.0^{bc}	27.7–1087.0
15–25 days of lactation	212.6 ± 29.9^{ab}	35.0–923.0	344.7 ± 60.1^{c}	30.1–905.0
50–60 days of lactation	208.1 ± 18.3^{ab}	26.0–831.4	269.3 ± 31.1^{bc}	31.0–760.0

Note: different letters indicate that data sets are significantly (P < 0.05) different from each other within the entire table according to the Tukey test.

An increase in the activity of ALP was detected in 15.3% of the total number of clinically healthy animals, mainly among goats on the 75–90th and 120–140th days of pregnancy, which is, respectively, 5.6% and 3.7% of the total number of studied livestock. Among lactating goats, an increase in the activity of ALP was detected in 6.0% of animals, mainly on the 50–60th day of lactation (3.1%).

In the subclinical course of hypocalcemia (1.98 ± 0.01 mmol/L; 1.28–2.19 mmol/L), the activity of ALP was in a wide range – from 27.7 to 1087.0 U/L (266.9 ± 15.1 U/L), which is 1.3 times more than in clinically healthy animals and is statistically significant (P = 8.4 x 10^{-3}; see Table 3). In 21.8% of diseased goats, the enzyme values were higher than the maximum physiological limit (618.3 ± 21.5 U/L; 413.9–1087.0 U/L). In particular, in pregnant goats, the activity of ALP in the blood serum was 11.4% higher compared to clinically healthy animals (255.5 ± 21.4 U/L; 39.8–776.4 U/L).

In the blood serum of lactating goats with subclinical hypocalcemia, the activity of ALP was 38.3% higher compared to clinically healthy animals with a high degree of confidence (273.8 ± 20.5 U/L; 27.7–1087.0 U/L; P = 4.1 x 10^{-3}; see Table 3), and in 23.9% of the studied goats of this technological group, the activity of this enzyme ranged from 417.4 to 1087.0 U/L (629.8 ± 28.1 U/L), which is significantly higher than the maximum physiological value.

In this context, we analyzed the results of studies of clinically healthy and sick lactating goats, in particular, in the first days after giving birth, the third-fourth weeks and the second month of lactation. Thus, the activity of ALP in the blood serum of clinically healthy goats on the 0–2nd day after kidding was 156.1 ± 20.9 U/L (29.6–535.4 U/L) and was 1.51 times lower than in goats on the 75–90th days of pregnancy (P = 2.95 x 10^{-2}; Table 3). In animals with subclinical hypocalcemia, the enzyme activity during this period was 249.9 ± 29.0 U/L and was in a wide range (27.7–1087.0 U/L), which is 1.60 times (60.2%) higher compared to clinically healthy animals with a high degree of confidence (P = 5.0 x 10^{-2}; Table 3).

Starting from the third week of lactation (days 15–25), there is a tendency to increase the activity of ALP in the serum of clinically healthy goats to 212.6 ± 29.9 U/L (35.0–923.0 U/L), which is 1.36 times higher compared to animals 0–2 days after kidding (Table 3). In animals with subclinical hypocalcemia, the enzyme activity was 1.62 times higher compared to clinically healthy animals at the same lactation period with a statistically significant difference (P = 3.1 x 10^{-2}; Table 3).

On the 50–60th days of lactation, the activity of ALP in the blood serum of clinically healthy animals was slightly lower compared to the previous period of the study and 22.7% lower compared to sick goats at an identical period of lactation (Table 3). Thus, according to the results of the study, it was found that the activity of total alkaline phosphatase in the blood serum of goats with subclinical hypocalcemia was 1.30 times higher compared to clinically healthy animals (P = 8.4 x 10^{-3}; Table 3). In particular, a tendency to increase the activity of the enzyme was noted in pregnant goats, and a significant increase (by 38.3%) in lactating goats, indicating serious disturbances in its metabolism in the blood serum of animals with hypocalcemia (P = 4.1 x 10^{-3}; Table 3).

Given the multidirectional functions of total alkaline phosphatase, an important aspect of the analysis is the study of the activity of its intestinal and bone isoenzymes. The analysis of the metabolism of these isoenzymes is impossible without establishing their physiological limits.

Based on the calculation of the standard deviation x ± SD (204.3 ± 197.0 U/L), we established physiological limits for the activity of the bone isoenzyme alkaline phosphatase in the blood serum of clinically healthy goats: min – 7.3; max – 401.3 U/L. In 84.7% of goats, the activity of the isoenzyme was within the defined limits, and its share in the structure of total alkaline phosphatase was 96.2% (Table 2).

We found that in 321 clinically healthy goats the activity of bone isoenzyme ALP (ostease) in the blood serum ranged from 24.5 to 95.3 U/L (204.3 ± 11.0 U/L).

An increase in the activity of the bone isoenzyme ALP was detected in 15.3% of clinically healthy animals, mainly among goats on the 75–90th and 120–140th days of pregnancy, which is, respectively, 5.6% and 3.8% of the studied livestock, and its share in the structure of total alkaline phosphatase was 98.3%. In particular, among lactating goats, an increase in this isoenzyme was detected in 5.9% of animals, mostly in the 50–60th days of lactation (3.1%), which, in our opinion, has low diagnostic information content.

It was found that in the blood serum of clinically healthy pregnant goats the activity of bone isoenzyme ALP was in a wide range from 25.8 to 868.0 U/L (223.2 ± 18.3 U/L), including in 79.0% of animals. In 79.5% of animals – in the range of 25.8–396.6 U/L (121.3 ± 7.7 U/L), in another 20.5% – 406.4–868.0 U/L (617.2 ± 21.6 U/L).

In clinically healthy lactating goats, the activity of bone isoenzyme ALP tended to decrease (by 15.5%) compared to pregnant animals (Table 4), including in 89.1% of the studied animals, the activity of bone isoenzyme ALP was in the range from 24.5 to 387.0 U/L, in another 10.9% – in the range of 409.7–905.3 U/L, however, such cases were not widespread and did not indicate the development of pathology in these animals.

In goats with subclinical hypocalcemia, the activity of bone isoenzyme ALP in the blood serum was 1.26 times higher compared to clinically healthy animals (257.0 ± 14.7 U/L; P = 9.9 x 10^{-3}; Table 4). In particular, in pregnant animals, the value of the isozyme tended to increase, in lactating animals it was significantly higher (38.7%) compared to clinically healthy goats with a statistically significant difference (P = 4.7 x 10^{-3}; Table 4). This indicates bone resorption in diseased animals, because the proportion of bone isozyme in the structure of total alkaline phosphatase was 97.3%.

Table 4
Dynamics of bone isoenzyme ALP activity in the blood serum of clinically healthy and hypocalcemic pregnant and lactating goats (x ± SE, n = 537)

Technology group	Clinical condition of goats			
	clinically healthy	Lim	goats with hypocalcemia	Lim
75–140 days of pregnancy	223.2 ± 18.3^{b}	25.8–868.0	249.5 ± 21.2^{bc}	36.4–765.8
75–90 days of pregnancy	228.3 ± 24.8^{bc}	25.8–789.4	245.8 ± 25.7^{bc}	42.5–681.4
120–140 days of pregnancy	216.8 ± 27.2^{ab}	29.9–868.0	253.1 ± 33.6^{bc}	36.4–765.8
0–60 days of lactation	188.6 ± 13.1^{ab}	24.5–905.3	261.6 ± 19.8^{bc}	25.2–988.0
0–2 days after kidding	147.9 ± 20.1^{a}	25.8–520.0	235.0 ± 27.6^{abc}	25.2–988.0
15–25 days of lactation	203.5 ± 29.3^{ab}	30.7–905.3	335.3 ± 58.6^{c}	26.1–890.4
50–60 days of lactation	197.7 ± 18.1^{ab}	24.5– 829.1	259.1 ± 30.3^{bc}	28.5–737.1

Note: see Table 3.

Our results indicate that in the subclinical course of hypocalcemia, especially during lactation, there is a significant increase in the activity of bone isoenzyme ALP in the serum of animals, which is a biomarker for the early diagnosis of calcium metabolism disorders. In particular, the proportion of bone isoenzyme in the structure of total alkaline phosphatase in diseased goats was 96.6% compared to 96.2% in clinically healthy animals.

Based on the calculation of the standard deviation x ± SD (38.0 ± 32.5 U/L; n = 321), the physiological limits of the activity of the intestinal isoenzyme alkaline phosphatase in the blood serum of clinically healthy goats are as follows: min – 5.5; max – 70.5 U/L. In 82.9% of goats, the activity of the isoenzyme was within the defined limits, and its share in the structure of total alkaline phosphatase was 17.9% (Table 2). We have found that in 321 clinically healthy goats, the activity of the intestinal isoenzyme ALP in the blood serum ranged from 3.4 to 187.0 U/L (38.0 ± 1.8 U/L). The analysis of individual indicators shows that in the vast majority of the studied pregnant goats (84.2%) the activity of the isoenzyme was in the range from 3.4 to 69.9 U/L (29.5 ± 1.6 U/L), in 15.8% – in the range of 72.9–187.0 U/L (98.0 ± 5.8 U/L). In clinically healthy lactating goats, the activity of the intestinal isoenzyme ALP was somewhat lower (by 10.2%; Table 5), with the largest proportion of this isoenzyme (87.4%) being within physiological limits, in another 12.6% – in the range of 72.5–178.2 U/L, and in some cases reached 105.2–178.2 U/L (Table 5).

Table 5
Dynamics of intestinal isoenzyme ALP activity in the blood serum of healthy and hypocalcemic pregnant and lactating goats (x ± SE, n = 537)

Technology group	Clinical condition of goats			
	clinically healthy	Lim	goats with hypocalcemia	Lim
75–140 days of pregnancy	40.3 ± 2.6^{ab}	3.4–187.0	64.5 ± 6.6^{b}	11.5–351.1
75–90 days of pregnancy	37.8 ± 2.8^{a}	6.4–112.4	60.8 ± 6.2^{b}	12.1–185.3
120–140 days of pregnancy	43.5 ± 4.8^{ab}	3.4–187.0	68.1 ± 11.6^{bc}	11.5–351.1
0–60 days of lactation	36.2 ± 2.5^{a}	3.7–178.2	51.2 ± 6.0^{ab}	2.90–485.6
0–2 days after kidding	30.5 ± 6.3^{a}	5.4–178.2	38.0 ± 6.1^{a}	2.90–252.0
15–25 days of lactation	40.4 ± 4.4^{ab}	3.7–173.6	95.5 ± 23.6^{c}	5.60–485.6
50–60 days of lactation	36.1 ± 3.3^{a}	4.1–160.0	46.2 ± 7.8^{ab}	3.20–255.2

Note: see Table 3.

An increase in the activity of intestinal isoenzyme ALP was detected in 14.0% of the total number of clinically healthy animals, including among pregnant goats (7.1%) and 6.9% among lactating goats (mainly on days 15–25 and 50–60 of lactation).

In the blood serum of goats with subclinical hypocalcemia, the activity of the intestinal isoenzyme ALP was 48.2 % (56.3 ± 4.5 U/L; 2.9–485.6 U/L) higher compared to clinically healthy animals with a high degree of probability ($P = 6.6 \times 10^{-5}$; Table 5), in particular, in pregnant and lactating animals this index was 60.0% and 41.4% higher than in healthy animals, and in 18.7% of them (25 head) the values were significantly higher than the upper physiological limit ($P = 2.5 \times 10^{-4}$; $P = 3.2 \times 10^{-2}$; Table 5).

Thus, the increase found by us in the activity of intestinal isoenzyme ALP in pregnant and lactating goats with subclinical hypocalcemia once again confirms its role as an important biomarker for the early diagnosis of calcium homeostasis disorders in animals.

A group of phosphatase enzymes plays a major role in the regulation of phosphorus and calcium metabolism.The analysis of the available literature shows a limited number of studies on the metabolism of acid phosphatase in the serum of ruminants, in particular, in goats. In view of this, there is a need to establish physiological limits for the activity of this enzyme for further use in diagnostic practice and research. Based on the calculation of the standard deviation x ± SD (6.24 ± 5.32 U/L; n = 321), the physiological limits of acid phosphatase activity in the blood serum of clinically healthy goats are as follows min – 0.92; max – 11.56 U/L. In 89.4% of animals, the activity of acid phosphatase was within the defined limits.

When studying the metabolism of acid phosphatase in the blood serum of clinically healthy goats, it was found that its activity ranged from 1.0 to 27.6 U/L (6.24 ± 0.26 U/L). In particular, in the group of pregnant goats, the activity of the enzyme varied in a wide range – from 1.0 to 27.6 U/L (7.5 ± 0.5 U/L), and in the vast majority of them (81.5%) it was in the range from 1.0 to 11.8 U/L (4.9 ± 0.2 U/L), and in another 18.5% – in the range of 13.0–27.6 U/L (19.1 ± 0.7 U/L). In clinically healthy lactating goats, the activity of acid phosphatase was 30.7% lower compared to pregnant animals (5.2 ± 0.2 U/L; 1.2–15.8 U/L; $P = 2.0 \times 10^{-5}$; Table 6), and 96.6% of the enzyme values were within the range of physiological limits.

Table 6
Dynamics of acid phosphatase activity in the blood serum of clinically healthy and hypocalcemic pregnant and lactating goats (x ± SE, n = 537)

Technology group	Clinical condition of goats			
	clinically healthy	Lim	goats with hypocalcemia	Lim
75–140 days of pregnancy	7.5 ± 0.5^{ab}	1.0–27.6	9.6 ± 0.7^{bc}	0.63–41.3
75–90 days of pregnancy	6.5 ± 0.7^{ab}	1.0–25.8	9.5 ± 1.1^{bc}	0.63–41.3
120–140 days of pregnancy	8.8 ± 0.7^{b}	1.7–27.6	9.8 ± 0.8^{bc}	2.7–29.4
0–60 days of lactation	5.2 ± 0.2^{a}	1.2–15.8	8.7 ± 0.7^{b}	1.3–58.8
0–2 days after kidding	4.9 ± 0.3^{a}	2.2–10.7	12.0 ± 1.3^{c}	1.9–58.8
15–25 days of lactation	4.5 ± 0.3^{a}	1.5–12.2	5.5 ± 0.4^{ab}	2.1–9.8
50–60 days of lactation	5.7 ± 0.3^{a}	1.3–15.8	5.9 ± 0.3^{a}	1.3–14.3

Note: see Table 2.

Thus, the activity of acid phosphatase in the blood serum of clinically healthy goats is significantly higher than in lactating goats, which may indicate the peculiarities of its metabolism during this physiological period and requires further study.

An increase in acid phosphatase activity was detected in 10.6% (34 head) of the total number of clinically healthy animals, which is 8.7% of the studied livestock. An increase in enzyme activity was diagnosed in 1.9% of lactating animals, mostly on the 50–60th day of lactation.

In the subclinical course of hypocalcemia, the activity of acid phosphatase in the serum of goats was 1.45 times higher compared to clinically healthy animals with a high degree of probability ($P = 2.3 \times 10^{-7}$; Table 6). A tendency to increase in the activity of the enzyme ($P = 3.8 \times 10^{-2}$) was noted in pregnant goats compared to healthy animals. In lactating sick goats, the values of acid phosphatase were 67.3% higher compared to clinically healthy animals ($P = 3.8 \times 10^{-8}$; Table 6).

Thus, the activity of acid phosphatase in the blood serum of goats with subclinical hypocalcemia is significantly increased, especially in lactating animals, indicating its enhanced metabolic activity and the significant importance of this enzyme in pathological processes associated with impaired calcium metabolism in animals.

Discussion

Metabolic disorders have a significant impact on the physiological state and productivity of small dairy ruminants. One of the most common metabolic disorders in goats is hypocalcemia. A decrease in serum calcium levels in animals usually occurs a few weeks before and after giving birth. The mechanism of development of hypocalcemia is based on a sharp or gradual decrease in the concentration of the essential macronutrient in the blood serum of animals to 2.2 mmol/L or less. The decrease in calcium content also occurs due to insufficient supply of vitamin D, calcium and phosphorus in the diet, as well as due to an imbalance between these vital macronutrients (Goff, 2008; Hotsuliak & Sakhniuk, 2024; Simões & Margatho, 2024).

The determination of the activity of alkaline phosphatase and its isozymes in clinically healthy and hypocalcemic animals is of great diagnostic value, as this enzyme plays an important role in the functioning of cell membranes, ensuring optimal metabolism in organs and body systems, and is one of the main markers of bone metabolism. The main sources of this enzyme in the body are the liver, bone osteoblasts, intestinal mucosa, placenta, kidneys, and segmented leukocytes (McComb et al., 2013; Povorozniuk & Balatska, 2013; Chornii & Shmanko, 2017). According to the results of Kaneko (1997), the physiological limits of total alkaline phosphatase activity in clinically healthy goats are in the range of 93.0–387.0 U/L, according to Djuricic et al. (2011) – in the range of 48.1–125.5 U/L. The study by Al-Rukibat et al. (2020), indicates that the activity of ALP in the serum of clinically healthy goats was in the range of 8.0–960.0 U/L (213.0 ± 39.0 U/L).

The analysis of the study results revealed that in the subclinical course of hypocalcemia, the activity of total alkaline phosphatase in the serum of goats was 1.30 times higher compared to clinically healthy animals ($P = 8.4 \times 10^{-3}$), including 11.4% in pregnant goats and 38.3% in lactating goats ($P = 4.1 \times 10^{-3}$). The reason for the increase in enzyme activity may be the development of dystrophic processes in bone tissue or excessive calcium loss during pregnancy and lactation (Varshney et al., 2018). Bandarra et al. (2011) and Sharma et al. (2017) found a significant increase in serum ALP activity in animals with a decrease in total calcium, including in bone tissue damage (osteoporosis, osteomalacia, and osteodystrophy). In goat bone diseases, the increase in enzyme activity is mainly due to its bone isoenzyme (Töyräs et al., 2002).

We have found that in the subclinical course of hypocalcemia, the activity of intestinal and bone isoenzymes of ALP in the serum of goats was 1.48 and 1.26 times higher compared to clinically healthy animals ($P = 6.6 \times 10^{-5}$; $P = 9.9 \times 10^{-3}$), which indicates their high informativeness in the early diagnosis of calcium metabolism disorders.

The researchers give a special place to acid phosphatase, which is involved in the catalysis of phosphorus esters in blood plasma and tissues. An increase in the activity of ACP indicates an increase in bone destruction and is also observed in various diseases accompanied by increased bone resorption, including osteoporosis, hyperparathyroidism, and renal osteodystrophy (Khopta et al., 2025).

The values of acid phosphatase activity in the blood serum of goats with subclinical hypocalcemia were 1.45 times higher compared to clinically healthy animals. The activity of this enzyme is a more specific indicator for assessing bone health, as this enzyme is found in osteoclasts and its increase indicates an increase in the intensity of bone resorption, which increases with osteodystrophy (Ferreira & Drüeke, 2000; Maslak & Sobakar, 2015; Jørgensen et al., 2021). According to the literature (Fukumoto, 2014), the activity of acid phosphatase in the blood serum of clinically healthy Zaanen goats averages 2.80 ± 0.37 U/L, while in diseased animals it is 3.80 ± 0.28 U/L, which is 35.7% more and is a characteristic sign of impaired D-vitamin and calcium-phosphorus metabolism in goats.

Conclusions

The physiological limits of total ALP, its intestinal and bone isoenzymes, and acid phosphatase were established. In clinically healthy goats, the activity of ALP ranged from 26.0–923.0 U/l, in particular, in pregnant goats – 27.0–873.8 U/L, in lactating goats – 26.0–923.0 U/L. In 21.8% of the diseased animals, the enzyme values were higher than the maximum physiological limit, including 18.3% of the pregnant and 23.9% of the lactating animals. In clinically healthy goats, the activity of the bone isoenzyme ALP was in the range of 24.5–95.3 U/L, in diseased goats, the activity of ostease was 1.26 times higher. The activity of the intestinal isoenzyme ALP in clinically healthy goats was 38.0 ± 1.8 U/L, in particular, in the group of pregnant animals – 40.3 ± 2.6 U/L, in lactating animals – 36.2 ± 2.5 U/L. In hypocalcaemia, 23.1% of goats had isoenzyme values higher than the maximum physiological limit. The activity of ACP in clinically healthy goats ranged from 1.0–27.6 U/L. In 21.8% of sick goats, the enzyme values were higher than the maximum physiological limit.

The prospect of further research is to study the D-vitamin and endocrine status of goats in clinically healthy and hypocalcaemic goats of different physiological and technological groups.

The authors declare that they have no conflicts of interest.

References

Al-Rukibat, R., Ismail, Z., Al-Zghoul, M. B., & Hananeh, W. (2020). Establishment of reference intervals of selected blood biochemical parameters in Shami goats. Veterinary Clinical Pathology, 49(4), 665–668.

Anand, A., & Srivastava, P. K. (2012). A molecular description of acid phosphatase. Applied Biochemistry and Biotechnology, 167(8), 2174–2197.

Araujo, C. A., Nikolaus, J. P., Morgado, A. A., Monteiro, B. M., Rodrigues, F. A., Vechiato, T. A., & Sucupira, M. C. (2014). Energy and hormonal profile of Santa Ines ewes from the middle third of gestation to postpartum. Pesquisa Veterinária Brasileira, 34(12), 1251–1257.

Bandarra, P. M., Pavarini, S. P., Santos, A. S., Antoniassi, N. A. B., Cruz, C. E., & Driemeier, D. (2011). Nutritional fibrous osteodystrophy in goats. Pesquisa Veterinária Brasileira, 31(10), 875–878.

Brozos, C., Mavrogianni, V. S., & Fthenakis, G. C. (2010). Treatment and control of Peri-Parturient metabolic diseases: Pregnancy toxemia, hypocalcemia, hypomagnesemia. Veterinary Clinics of North America Food Animal Practice, 27(1), 105–113.

Brun, L. R. M., Brance, M. L., Rigalli, A., & Puche, R. C. (2006). Effect of calcium on rat intestinal alkaline phosphatase activity and molecular aggregation. Journal of Enzyme Inhibition and Medicinal Chemistry, 21(6), 757–763.

Brun, L. R., Brance, M. L., & Rigalli, A. (2011). Luminal calcium concentration controls intestinal calcium absorption by modification of intestinal alkaline phosphatase activity. British Journal of Nutrition, 108(2), 229–233.

Brun, L. R., Brance, M. L., Lombarte, M., Lupo, M., Di Loreto, V. E., & Rigalli, A. (2014). Regulation of intestinal calcium absorption by luminal calcium content: Role of intestinal alkaline phosphatase. Molecular Nutrition and Food Research, 58(7), 1546–1551.

Brun, L. R., Traverso, A., & Rigalli, A. (2008). Aggregation and inhibition of rat intestinal alkaline phosphatase by high concentrations of calcium. Reversibility of the processes. Journal of Enzyme Inhibition and Medicinal Chemistry, 24(3), 691–696.

Bull, H. (2002). Acid phosphatases. Molecular Pathology, 55(2), 65–72.

Centeno, V. A., Díaz de Barboza, G. E., Marchionatti, A. M., Alisio, A. E., Dallorso, M. E., Nasif, R., & Tolosa de Talamoni, N. G. (2004). Dietary calcium deficiency increases Ca^{2+} uptake and Ca^{2+} extrusion mechanisms in chick enterocytes. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 139(2), 133–141.

Chornii, A., & Shmanko, V. (2017). Riven' markeriv remodeliuvannia kistkovoyi tkanyny u osib iz pervynnym hipotyreozom [Level markers of bone remodeling in patients with primary hypothyroidism]. Journal of Education, Health and Sport, 7(4), 269–278 (in Ukrainian).

Constable, P. D., & Radostis, O. M. (2017). Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs, and goats. 11th ed. Saunders Company, Philadelphia.

Djuricic, D., Dobranic, T., Grizelj, J., Gracner, D., Harapin, I., Stanin, D., Samardzija, M. (2010). Concentrations of total proteins and albumins, and AST, AP, CK and GGT activities in the blood serum boer and saanen goats during puerperium. Reproduction in Domestic Animals, 46(4), 674–677.

Ek-Rylander, B., & Andersson, G. (2009). Osteoclast migration on phosphorylated osteopontin is regulated by endogenous tartrate-resistant acid phosphatase. Experimental Cell Research, 316(3), 443–451.

Estaki, M. (2014). Interplay between intestinal alkaline phosphatase, diet, gut microbes and immunity. World Journal of Gastroenterology, 20(42), 15650.

Fernandez, N. J., & Kidney, B. A. (2007). Alkaline phosphatase: beyond the liver. Veterinary Clinical Pathology, 36(3), 223–233.

Ferreira, A., & Drüeke, T. B. (2000). Biological markers in the diagnosis of the different forms of renal osteodystrophy. The American Journal of the Medical Sciences, 320(2), 85–89.

Fukumoto, S. (2014). Phosphate metabolism and vitamin D. BoneKEy Reports, 3, 497.

Gastwirth, J. L., Gel, Y. R., & Miao, W. (2009). The impact of Levene's test of equality of variances on statistical theory and practice. Statistical Science, 24(3), 343–360.

Goff, J. P. (2008). The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. The Veterinary Journal, 176(1), 50–57.

Grote-Koska, D., Klauke, R., Brand, K., & Schumann, G. (2016). Alkaline phosphatase activity – pH impact on the measurement result. Clinical Chemistry and Laboratory Medicine, 55(7), e146–e149.

Hayman, A. R. (2008). Tartrate-resistant acid phosphatase (TRAP) and the osteoclast/immune cell dichotomy. Autoimmunity, 41(3), 218–223.

Hernandez, J., Benedito, J. L., & Castillo, C. (2020). Relevance of the study of metabolic profiles in sheep and goat flock. Present and future: A review. Spanish Journal of Agricultural Research, 18(3), e06R01.

Hotsuliak, M. M., & Sakhniuk, V. V. (2024). Hipokaltsiiemiia kitnykh i laktuiuchykh kiz (poshyrennia, etiolohiia, metody diahnostyky) [Hypocalcaemia in pregnant and lactating goats (distribution, etiology, diagnostic methods)]. Scientific Messenger of LNU of Veterinary Medicine and Biotechnology, 26(115), 101–111 (in Ukrainian).

Hotsuliak, M., & Sakhniuk, V. (2024). Metabolizm kaltsiiu ta yoho fraktsiinoho skladu u klinichno zdorovykh kiz [Calcium metabolism and its fractional composition in clinically healthy goats]. Scientific Bulletin of Veterinary Medicine, 192, 28–42 (in Ukrainian).

Jørgensen, H. S., Behets, G., Viaene, L., Bammens, B., Claes, K., Meijers, B., Evenepoel, P. (2021). Diagnostic accuracy of noninvasive bone turnover markers in renal osteodystrophy. American Journal of Kidney Diseases, 79(5), 667–676.

Kaneko, J. J. (1997). Serum proteins and the dysproteinemias. Clinical biochemistry of domestic animals. 5th ed. Academic Press. Pp. 117–138.

Khopta, N. S., Romaniuk, A. L., Netchitajlo, L. J., & Ersteniuk, A. M. (2025). Metabolic processes in the body and bones of experimental animals under conditions of exposure by cadmium and nitrite ions. The Animal Biology, 27(1), 15–20.

Kim, T. K. (2015). T test as a parametric statistic. Korean Journal of Anesthesiology, 68(6), 540–546.

Lallès, J. (2013). Intestinal alkaline phosphatase: Novel functions and protective effects. Nutrition Reviews, 72(2), 82–94.

Levchenko, V. I., Holovakha, V. I., & Kondrakhin, I. P. (2010). Metody laboratornoyi klinichnoyi diahnostyky khvorob tvaryn [Methods of laboratory clinical diagnosis of animal diseases]. Agrarian Education, Kyiv (in Ukrainian).

Le-Vinh, B., Akkuş-Dağdeviren, Z. B., Le, N. N., Nazir, I., & Bernkop-Schnürch, A. (2022). Alkaline phosphatase: A reliable endogenous partner for drug delivery and diagnostics. Advanced Therapeutics, 5(2), 2100219.

Maslak, Y., & Sobakar, A. (2015). Etiopatohenez osteodystrofiyi u kiz zaanenskoyi porody [Etiopathogenesis of osteodystrophy in Zaanen goats]. Bulletin of Poltava State Agrarian Academy, 1–2, 119–123 (in Ukrainian).

McComb, R. B., Bowers Jr., G. N., & Posen, S. (2013). Alkaline phosphatase. Springer Science & Business Media, Berlin, Heidelberg.

Millán, J. L. (2006). Alkaline phosphatases: Structure, substrate specificity and functional relatedness to other members of a large superfamily of enzymes: Structure, substrate specificity and functional relatedness to other members of a large superfamily of enzymes. Purinergic Signal, 2(2), 335–341.

Minisola, S., Cipriani, C., Colangelo, L., Labbadia, G., Pepe, J., & Magnusson, P. (2025). Diagnostic approach to abnormal alkaline phosphatase value. Mayo Clinic Proceedings, 100(4), 712–728.

Petrovska, I. R., Salyha, Y. T., & Vudmaska, I. V. (2022). Statystychni metody v biolohichnykh doslidzhenniakh [Statistical methods in biological research]. Agrarian Science, Kyiv (in Ukrainian).

Povorozniuk, V., & Balatska, H. (2013). Rol' markeriv remodeliuvannia kistkovoyi tkanyny u diahnostytsi systemnoho osteoporozu [The role of bone remodelling markers in the diagnosis of systemic osteoporosis]. Art of Treatment, 2–3, 12–14 (in Ukrainian).

Sato, J., Okada, K., Fukuda, S., Sato, R., Yasuda, J., & Naito, Y. (2002). Serum activities of Tartrate-resistant acid phosphatase and bone specific alkaline phosphatase as indices of bone metabolism in the cow. Journal of Veterinary Medical Science, 64(7), 653–655.

Schini, M., Vilaca, T., Gossiel, F., Salam, S., & Eastell, R. (2022). Bone turnover markers: Basic biology to clinical applications. Endocrine Reviews, 44(3), 417–473.

Sharma, D. K., Sonawane, G. G., & Swarnkar, C. P. (2017). Clinico-hemato-biochemical study of two commercial feed supplements for amelioration of rickets in growing male lambs. Comparative Clinical Pathology, 27(1), 231–238.

Sharma, U., Pal, D., & Prasad, R. (2013). Alkaline phosphatase: An overview. Indian Journal of Clinical Biochemistry, 29(3), 269–278.

Simões, J., & Margatho, G. (2024). Metabolic periparturient diseases in small ruminants: An update. Applied Sciences, 14(21), 10073.

Strom, M., Krisinger, J., & DeLuca, H. F. (1991). Isolation of a mRNA that encodes a putative intestinal alkaline phosphatase regulated by 1,25-dihydroxyvitamin D-3. Biochimica et Biophysica Acta – Gene Structure and Expression, 1090(3), 299–304.

Töyräs, J., Nieminen, M., Kröger, H., & Jurvelin, J. (2002). Bone mineral density, ultrasound velocity, and broadband attenuation predict mechanical properties of trabecular bone differently. Bone, 31(4), 503–507.

Van Hoof, V. O., & De Broe, M. E. (1994). Interpretation and clinical significance of alkaline phosphatase isoenzyme patterns. Critical Reviews in Clinical Laboratory Sciences, 31(3), 197–293.

Van Loo, B., Jonas, S., Babtie, A. C., Benjdia, A., Berteau, O., Hyvönen, M., & Hollfelder, F. (2010). An efficient, multiply promiscuous hydrolase in the alkaline phosphatase superfamily. Proceedings of the National Academy of Sciences, 107(7), 2740–2745.

Varshney, J., Kumar, G., & Singh, S. K. (2018). Evaluation of promising biochemical markers of nutritional osteodystrophy in goats. Small Ruminant Research, 169, 86–89.

Yatoo, M. I., Parray, O. R., Ul Haq, R. I., & Mushtaq, M. (2023). Diagnostic techniques in goats. Principles of Goat Disease and Prevention. Wiley, Hoboken, New Jersey. Pp. 237–249.