A brief Analysis of the Health Status of Dairy Cows by Determining Serum Biochemical Parameters

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Abstract
The research was carried out to follow the state of health of dairy cattle by determining the biochemical parameters in the serum. Animals of the BNR breed from a farm in the Moldova area were studied and blood samples were collected, later the following serum parameters were analyzed: albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, gamma-GT, total proteins, triglycerides, and urea. At the same time, the obtained milk production was correlated with the biochemical parameters of bovine serum, thus there were distinctly significant positive correlations between milk production and aspartate aminotransferase (AST), respectively urea, and an insignificant correlation was between milk production and albumin. Following the biochemical analyses, the liver parameters were very slightly above the limit provided by the specialized literature.

Keywords: biochemical parameters, bovine serum, health status.

1. Introduction

Serum biochemical indicators play an essential role in monitoring cow health. These indicators provide essential information about the physiological and metabolic state of the animal, allowing veterinarians and farmers to make informed decisions [1]. Regular monitoring of these markers can help early detection, ensuring timely intervention and disease health outcomes [2]. By analyzing serum biochemical indicators, potential health problems can be identified before they manifest clinically, enabling proactive health management strategies [3]. Dairy cattle can be affected by production diseases, related to nutrition or poor management. Metabolic profile testing is essential for detecting metabolic disorders in dairy cattle [4]. Blood and milk biochemical parameters are checked during lactation to monitor animal health and milk production, especially in those at increased risk of metabolic diseases. Major health problems occur especially during childbirth and breastfeeding, with negative energy balance states leading to excessive fat accumulation in the liver [5]. Clinical and laboratory monitoring during transition and early lactation has a crucial role in detecting subclinical nutritional and metabolic diseases in dairy cattle [6]. Lipid metabolites are relevant in the pathogenesis of some metabolic diseases and adaptation to stress, with NEFA accumulating as triglycerides in the liver and affecting various blood parameters in cases of hepatic lipidosis [7]. As the excretory function of the liver cells declines, the levels of metabolites such as TBil, ammonia, and bile acids increase in the blood. Fatty liver and hepatocyte damage lead to the release of enzymes into the blood such as ALT, AST, ALP, LDH, and GGT. These enzymes can be used as indicators of postpartum liver function [8]. Also, the evaluation of enzyme activity in milk can be useful for the

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early diagnosis of subclinical diseases in cattle [9]. Modern management practices involve frequent health monitoring of high-yielding dairy cows to control milk production and quality [10]. Regulated testing of serum biochemical indicators is beneficial for disease prevention, improving animal welfare, and increasing farm profitability [11].
Establishing reference values for cows helps to quickly identify deviations, facilitating the early detection of health problems and reducing the costs of intensive treatments.

2. Materials and methods

Just like humans, animals also need to be tested periodically. Cattle are a source of meat and milk for humans, and the quality indicators of these two products depend on whether the animal is healthy. In order to identify as early as possible any deviation in the activity of the animal's body, it is necessary to carry out a biochemical blood test.
Accurate biochemical analysis begins with the proper collection of the blood sample. The analysis is problems performed to determine metabolic or lack of nutrients in an animal.The 15 blood samples were collected 4 to 6 h after milking and feeding from the tail vein into 9 ml clot activator vacutainer tubes.
After collection, blood samples were centrifuged for 5 minutes at 3000 rpm in a Hettich Zentrifugen Rotofix 32A Centrifuge and the resulting supernatant is called serum (Figure 1).

Figure 1. Centrifugation of blood samples and the serum obtained after centrifugation

After centrifugation, the liquid component, immediately transferred to an Eppendorf tube, with a lid, must use a pipette. If not analyzed immediately, it should be stored and transported at -20°C or lower.
An analysis of blood sera was performed to determine the following biochemical parameters: ALB (albumin), ALKP (alkaline phosphatase), ALT/GPT (alanine aminotransferase), AST/GOT (aspartate aminotransferase), CHOL (cholesterol), gamma- GT, total protein, TRIG (triglycerides) and BUN (urea). The BioSystems BA200 analyzer, which has a dynamic baseline equipped with SMART LED technology, was used to determine the assays.
The principle of the method is based on passing the known wavelength through a sample and measuring the amount of light energy that is transmitted. This is done by placing a photocell on the opposite side of the respective sample. A beam of light is made up of photons; when a photon encounters an analyte molecule there is a chance of the photon being absorbed by the analyte. This absorption reduces the number of photons in the light beam, thus reducing the light intensity. All molecules absorb radiant energy at certain wavelengths. Those that absorb energy in the visible spectrum are known as pigments. Proteins and nucleic acids absorb light in the ultraviolet range. The intensity of color in a sample depends on the amount of solution present in that sample. Light transmission is not a linear function, but predominantly exponential. This analyzer also has the most flexibility, highly accurate dosing, and a small system that requires little maintenance. It has the ability to load both samples and reagents. Proper storage and handling of samples is essential. Ensuring the reliability and accuracy of sample collection procedures is an essential step in biochemical analysis.

3. Results and discussion

Biochemical parameters change their values, which is characteristic of certain pathological conditions, therefore a biochemical profile is recommended whenever it is considered necessary. The cows that were the object of the research were from the G_1 group, which implies an average milk production of 31 liters. Regarding the level of lactation, the animals of the G_1 group 70% are primiparous, 20% secondariparous and 10% multiparous.
The serum samples were analyzed from a biochemical point of view, and the determined parameters were in number of 9, so in Table 1 the results of the parameters of the biochemical profile are highlighted.

Table 1. The results of the biochemical profile of the serum samples from the G_1 group

Nr. Crt.	Milk production	Albumin (g/L)	ALP-DEA (U/L)	ALT-GPT (U/L)	AST-GOT (U/L)	Cholesterol (mg/dL)	Gamma-GT (U/L)	Total protein (g/L)	Triglycerides (mg/dL)	Urea (mg/dL)
1	33.79	38	38	105	30	8.81	0.66	65	73	11
2	28.83	37	37	107	27	7.94	0.72	28	69	0.1
3	32.68	37	37	71	20	9.07	0.88	25	79	7
4	26.33	33	33	66	25	8.72	0.67	43	71	7
5	27.78	36	36	79	19	8.73	0.9	37	62	6
6	32.38	26	26	99	16	7.4	0.57	24	60	5
7	29.77	36	36	73	23	8.55	0.69	17	68	11
8	32.64	34	34	80	19	8.53	0.85	27	69	4
9	29.72	34	34	111	28	8.98	0.73	44	81	6
10	33.40	32	32	88	25	7.98	0.64	18	82	4
11	28.06	34	34	84	22	8.45	0.83	13	70	12
12	35.50	36	36	109	30	8.51	0.68	83	80	3
13	27.10	40	40	107	37	9.68	0.65	32	76	10
14	34.25	36	36	62	30	7.88	0.66	27	70	9
15	33.09	34	34	69	21	7.61	0.51	30	65	9
Min.	**26.33**	**26**	**62**	**16**	**58**	**99**	**13**	**60**	**0.1**	**21**
Max.	**35.50**	**40**	**111**	**37**	**190**	**292**	**83**	**82**	**12**	**48**
Average	**31.02**	**34.87**	**87.33**	**24.80**	**102.27**	**201.73**	**34.20**	**71.67**	**6.94**	**35.80**
Dev.s.	**2.93**	**3.20**	**17.51**	**5.60**	**35.20**	**55.77**	**18.66**	**6.79**	**3.40**	**6.61**
CV %	**0.09**	**0.09**	**0.20**	**0.23**	**0.34**	**0.28**	**0.55**	**0.09**	**0.49**	**0.18**
Reference interval		**29-39**	**27-127**	**5-18**	**60-125**	**163-397**	**6-17.4**	**59-81**	**10-19**	**10-25**

Table 2 highlights the statistical estimators resulting from the blood biochemical examination. The animals taken in the study are from the G_1 group, whose milk production means an amount of 31 liters on average.

Table 2. Statistical estimators regarding the biochemical examination of the analyzed bovine serum

Biochemical Parameters	Reference interval *(The Merck Veterinary manual)*	Statistical estimators				
		$\bar{x}$	s	CV %	Min.	Max.
Albumin (g/L)	29-39	34.87	3.20	0.09	26	40
ALP-DEA (U/L)	27-127	87.33	17.51	0.20	62	111
ALT-GPT (U/L)	5-18	24.80	5.60	0.23	16	37
AST-GOT (U/L)	60-125	102.27	35.20	0.34	58	190
Cholesterol (mg/dL)	163-397	201.73	55.77	0.28	99	292
Gamma-GT (U/L)	6-17.4	34.20	18.66	0.55	13	83
Total protein (g/L)	59-81	71.67	6.79	0.09	60	82
Triglycerides (mg/dL)	10-19	6.94	3.40	0.49	0.1	12
Urea (mg/dL)	10-25	35.80	6.61	0.18	21	48

The albumin content of bovine serum recorded an average value of 34.87 g/L, the minimum being 26 g/L, and the maximum value being 40 g/L. The minimum and maximum limits were not within the reference range, but the average was within the standards. Regarding the studied character, it showed a very good homogeneity, with the value of the coefficient of variation being 0.09% (table 2).

Alkaline phosphatase (ALP-DEA) is a very important parameter that can indicate liver disease, Cushing's syndrome, or active bone growth in young animals. The average values obtained in the present case fell within the limits mentioned in the reference standard, thus the average value obtained was 87.33 u/L, with a minimum of 62 g/L and a maximum of 111 g/L. The coefficient of variation obtained in the case of alkaline phosphatase was 0.20%, which indicates a very good homogeneity within the analyzed batch.

Alanine aminotransferase (ALT-GPT) is an indicator of liver disease. For this parameter, the reference standard imposes values between 5 and 18 U/L, the average we obtained being 6.8 U/L higher than the maximum allowed by the standard [12]. The value of the coefficient of variation was less than 10%, expressing a very homogeneous population.

Regarding aspartate aminotransferase AST-GOT (a parameter that, if it is rising, indicates liver and muscle diseases), the average value obtained was 102.27 U/L, the minimum being 58 U/L and the maximum 190 U/L IT. The coefficient of variation that was obtained this time too was a very small one, of 0.34%, which indicates a very good homogeneity within the analyzed batch.

In the case of the following parameters, the average values obtained fell within the limits provided by the reference standard, as follows: cholesterol, the average obtained was 201.73 mg/dL, the average value being by the reference range 163-397 mg/dL and total proteins, the average obtained was 71.67 g/L, falling within the limits of 59-81 g /L (Figure 2).

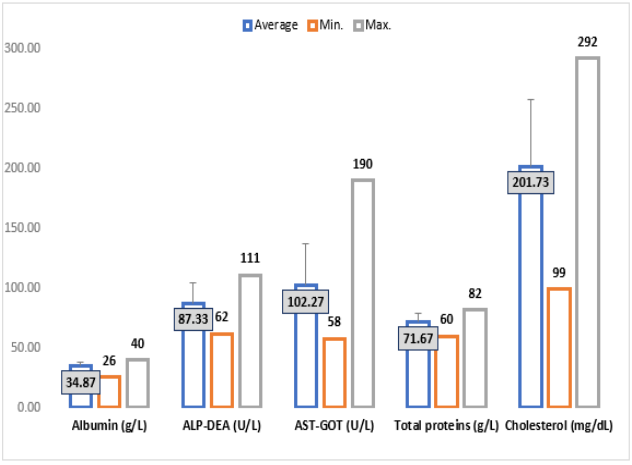

Figure 2. The dynamics of the values of the blood parameters according to the standard

The average values of the blood biochemical parameters analyzed that did not fall within the limits provided by the specialized literature and that showed a significant increase were: alanine aminotransferase (ALT/ALT) - 24.80 U/l, 6.8 percent more than the maximum allowed, glutamyl transpeptidase (gamma-GT) - 34.20 U/l, 16.8 percent more than the maximum allowed and urea - 35.80 mg/dL, 10.8 percent more (Figure 3). The only parameter that did not fall within the limits provided by the specialized literature and that showed a significant decrease was represented by triglycerides. The reference standard requires a minimum value of 10 mg/dL and a maximum of 19 mg/dL. The average obtained by us following the determinations (6.94 mg/dL) was lower by 3.94 mg/dL than the minimum allowed by the reference interval standard (Figure 3).

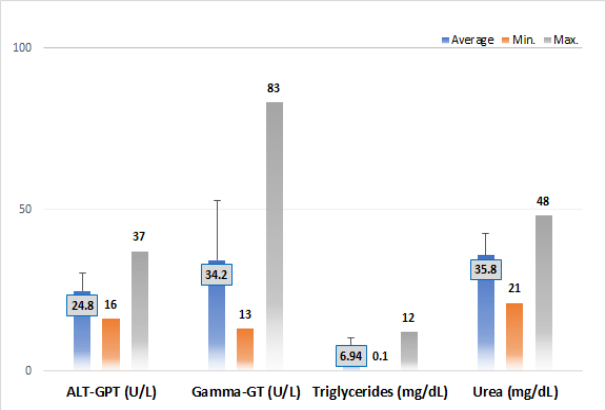

Figure 3. Dynamics of non-standard blood parameter values

Elevated alanine aminotransferase is an indicator of liver disease. Nonhepatic conditions such as inflammatory gastrointestinal disease, heart failure, and hemolytic anemia can cause mild to moderate ALT elevations that can be up to four to five times normal. Approximately 24 hours are required for the half-life of ALT in plasma. Animals receiving glucocorticoids, anticonvulsants, or biliary stasis may have moderately elevated ALT levels [13].

Elevated gamma glutamyltransferase may indicate disease located in the liver. This enzyme called GGT is present throughout the body but is especially concentrated in the liver. GGT can leak into the blood when the liver is damaged. Damage to the bile ducts or liver disease can indicate high levels of GGT in the blood. Blood GGT levels can be used to identify chronic, subacute, or acute diseases. An increase in both liver enzymes can be

related to fatty liver syndrome in ruminants [14]. A low triglyceride content showed that the animals studied were not in a positive energy balance [8]. The increase in urea content indicates an alteration of liver function, but also other changes, such as those related to dehydration, heart disease, shock, and urethral obstructions. Serum urea levels can be increased to a greater extent by the excessive intake of protein in feed, which is not differentiated in the rumen and thus passes into the blood [9].

At the same time, the obtained milk production was correlated with the biochemical parameters of bovine serum, thus there were distinctly significant positive correlations between milk production and aspartate aminotransferase (AST), respectively urea (Table 3).

Table 3. Correlation between milk production and analyzed biochemical parameters of bovine serum

		Milk production	Albumin	ALP-DEA (U/L)	ALT-GPT (U/L)	AST-GOT (U/L)	Cholesterol (mg/dL)	Gamma-GT (U/L)	Total Protein (g/L)	Triglycerides (g/L)	Urea (mg/dL)
Milk production (L)	Correlation Pearson	1	.002	-.419**	-.178	.290**	.194	.214*	-.214*	-.665**	.740**
	Sig. (2-tailed)		.985	.000	.094	.006	.067	.043	.043	.000	.000
	N	15	15	15	15	15	15	15	15	15	15

Distinctly significant negative correlations were between milk production and ALP (alkaline phosphatase), and triglycerides, and an insignificant correlation was between milk production and albumin, respectively cholesterol.

4. Conclusions

In conclusion, it is observed that the average values of the analyzed blood biochemical parameters that did not fall within limits provided by the specialized literature and that recorded a higher content were represented by alanine aminotransferase (ALT/ALT) - 24.80 U / l, 6.8 percent more than the maximum allowed, glutamyl transpeptidase (gamma-GT) - 34.20 U/l, 16.8 percent more than the maximum permitted and urea - 35.80 mg/dL, 10.8 percent more. The only parameter that showed a significant decrease was represented by triglycerides, this parameter did not fall within the limits provided by the specialized literature. All dairy cattle involved in the study were healthy at the time of sample collection, but the results of biochemical analyses may indicate some mild liver disorders. The reduced triglyceride content was probably because the studied animals were in a negative energy balance at that time.

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