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ANALYSIS OF BLOOD BIOCHEMICAL PROFILE OF ENDANGERED ROMANIAN GREY COWS ACCORDING TO AGE

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Abstract

This study was conducted to analyze the blood biochemical parameters with aim to monitor the health status of 32 Romanian Grey Steppe cattle being in a national genetic conservation program due to their biological characteristics such as rusticity, adaptability to severe environmental conditions, increased resistance to disease, and longevity. A number of 8 biochemical parameters were statistically analyzed, respectively: GLI (glucose), CHO (cholesterol), TP (total protein), Ca (calcium), ALAT (alanine aminotransferase), AST (aspartate aminotransferase), P (phosphorus), and ALB (albumin). The cattle were between 2-24 years old. The results were analyzed by age category, respectively 2-10 years and 11-24 years. Mean values of GLI, CHO, ALB, and TP were higher in the older cattle group, while mean values of ASAT, ALAT, Ca, and P were higher in younger cattle. Monitoring the health status of endangered cattle breeds is an important aspect of genetic conservation program. In this research, the results of the biochemical profile fell within the range of the reference values for both age categories, an aspect that demonstrates the resistance, longevity, and genetic characteristics of this breed.

Key words*: biochemical profile, blood, cattle, Grey Steppe.*

INTRODUCTION

The importance of studying the biochemical profile of the Romanian Grey Steppe cattle breed derives from their special biological characteristics that have distinguished this native breed over time, such as adaptability and increased resistance to diseases and severe climate and habitat conditions, rusticity and longevity.

Currently, this breed is in danger of extinction and any research on it brings valuable results for genetic conservation efforts (Davidescu et al., 2022; Ciobanu et al., 2023).

The bovine species is the most exposed to climate changes that are increasingly accentuated in the present, therefore the Romanian Grey Steppe could be an effective alternative to improved breeds, due to its special qualities of resistance and adaptability to different stress factors (Matei et al., 2020; Davidescu et al., 2023).

The nutritional state of animals is a fundamental indicator of their productivity and general health (Usturoi et al., 2022). In order to detect nutritional issues, Payne et al. (1970) first tested metabolic profiles by examining biochemical parameters in animal blood.

Monitoring blood parameters in cows, such as glucose, cholesterol, total protein, calcium, phosphorus, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and albumin, serves several important purposes in managing animal health and ensuring optimal farm performance (Roland et al., 2014).

The levels of glucose, cholesterol, total proteins, and albumin can provide insights into nutritional intake and animal metabolism. These parameters indicate whether the cow is receiving sufficient essential nutrients to support health and productivity (Mădescu et al., 2021).

ALT and AST levels are associated with liver function. Increased levels of these enzymes may indicate liver stress or injury and may provide information about liver health (Coroian et al., 2017).

Calcium and phosphorus are essential for bone and nervous system health. Proper monitoring can help to prevent issues related to deficiencies in these minerals (Biswal et al., 2017).

Total protein and phosphorus levels can influence reproductive health. Regular

monitoring can help ensure optimal conditions for reproduction and maintain fertility.

Continuous monitoring of biochemical parameters allows for early identification of health problems or nutritional deficiencies. This provides an opportunity for prompt intervention and treatment to prevent the development of more severe conditions.

Knowing the health status of animals and their biochemical parameters can contribute to optimizing production performance, including milk production and reproduction rates (Marin et al., 2020; Vidu et al., 2015).

Understanding glucose levels and other parameters can help to evaluate diet quality and adjust feeding to meet the nutritional needs of animals.

Age can influence the levels of biochemical parameters in the blood, and this influence may vary depending on the species, breed, and environmental conditions. In general, for cows, certain trends were observed in relation to age and blood parameters. It is important to note that these trends are generalizations and there is significant individual variability (Könyves et al. (2017).

Regarding the blood glucose level, this biochemical parameter can vary with age, as metabolism itself can be influenced by the stage of development and hormonal activity. In general, glucose levels are higher in younger animals (Cozzi et al., 2011).

However, cholesterol concentrations may increase with age, often reflecting changes in the metabolism and energy needs of the animal.

Total protein levels, including albumin, can vary with age, reflecting the health status of animals, growth stage, or reproductive status.

Calcium and phosphorus are two critical electrolytes for bone health and the nervous system, respectively. Their levels vary depending on the specific nutritional requirements of each developmental stage.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are enzymes that reflect liver function. Their levels can vary depending on various conditions including liver disorders and injuries (Obućinski et al., 2019).

It is important to consider that variations in these parameters can be influenced by several factors, including diet, management, overall health, and genetic factors. Specific studies are advisable to evaluate these influences within the analyzed animal population, considering all relevant factors, to identify significant differences between groups and understand how parameter variation is linked to age (Popa et al., 2022).

The aim of monitoring these parameters is to maintain animal health, prevent health issues, and ensure efficient and sustainable farm production.

MATERIALS AND METHODS

Thirty-two Romanian Grey Steppe cows, divided into two groups according to age category (2–10 years old, respectively 11–24 years old farmed in north-east of Moldova, were analysed in this study (Figure 1).

Figure 1. Romanian Grey Steppe cattle (original photo)

All animals were clinically healthy. Their health status was evaluated based on a thorough clinical exam. The cows were kept in stables with temporary access to pastures. During the experiment, cows were fed with hay (95% dry matter, 9.5% crude protein and 1.5% crude fat, respectively) and water was available ad libitum.

At the time of blood sample collection, all cows were considered clinically healthy. A total of 64 blood samples were collected, including 2 blood samples from each cow. Blood was obtained from the jugular vein of each selected cow in the morning before feeding. Blood was collected in test tubes with and without EDTA as an anticoagulant and refrigerated at a temperature of 4°C, and immediately delivered to the laboratory for further analysis. Biochemical blood parameters were analysed using an automated biochemistry analyser-Cormay ACCENT S120 (Figure 2).

Figure 2. Automated chemistry analyzer - Accent S120 (medicalexpo.com/prod/cormay-diagnostics/product)

The obtained results were interpreted statistically to verify the degree of homogeneity as well as the accuracy of the applied methods.

RESULTS AND DISCUSSIONS

In young animals, higher levels of glucose may exist because of increased energy requirements during the period of rapid growth and development. In older animals, glucose levels may be more stable or decrease depending on overall health and sugar metabolism management.

Cholesterol concentrations in young animals may be lower because they have not yet reached metabolic maturity, whereas in older animals, cholesterol levels may increase, reflecting changes in metabolism and energy needs.

The total protein levels in young animals may be higher because they require proteins for growth and development. In older animals, protein levels can be influenced by overall health and nutritional requirements related to reproductive function or maintenance of body condition.

Minerals, such as calcium and phosphorus, have higher concentrations in young animals because these substances are essential for bone development. In older animals, levels may remain high but can also vary based on specific nutritional requirements for reproduction or maintenance of bone health.

Liver enzymes ALT and AST may be elevated in young animals due to injuries or physiological stress associated with the growth period. In contrast, in older animals, levels may vary depending on overall liver health, potentially increasing in the presence of liver disorders or lesions. The concentrations of biochemical blood parameters of Podolian Grey Steppe cows analysed in this study are shown in Table 1 and Figure 11.

Table 1. The concentrations of biochemical blood parameters of Podolian Grey Steppe cows

No.	Parameter	UM	Age category					
			2-10 years			11-25 years		
			$\bar{X}$	±*Sx*	*Variation limits*	$\bar{X}$	±*Sx*	*Variation limits*
1.	GLI (glucose)	mg/dL	**58.0**	1.60	45.0-71.0	**50.3**	1.42	43.1-57.4
2.	CHO (cholesterol)	mg/dL	**172**	5.40	149-245	**182.3**	6.02	167-198
3.	TP (total protein)	g/dL	**9.14**	0.50	8.2-10.1	**8.22**	0.46	7.3-8.92
4.	Ca (calcium)	mg/dL	**11.4**	2.10	10.5-12.3	**9.55**	1.98	8.88-10.2
5.	P (phosphorus)	mg/dL	**7.92**	3.30	7.20-8.20	**6.73**	2.87	6.34-7.12
6.	ALAT (alanine aminotransferase)	U/L	**26.5**	2.50	11.2-64.5	**40.5**	2.76	15.3-66.1
7.	AST (aspartate aminotransferase)	U/L	**35.3**	3.10	30.0-45.0	**40.4**	3.42	32.4-48.2
8.	ALB (albumin)	%	**40.0**	1.50	38.0-42.0	**46.5**	1.73	42.0-51.0

The table provides data regarding the average concentrations of eight biochemical parameters in the blood of 32 cows from Romanian Grey Steppe cows divided into two age categories: 2-10 years and 11-25 years. A higher average glucose level was observed in younger cows (58.0 mg/dL) than that in older ones (50.3 mg/dL) (Figure 3).

Cholesterol, triglycerides, and different density lipoproteins are important constituents of the lipid fraction of the body. Cholesterol is unsaturated alcohol of the steroid family of compounds, and it is essential for the normal function of all animal cells and is a fundamental element of their cell membranes (Puvača et al., 2016; Puvača et al., 2015).

For cholesterol, the average appears to increase with age, with values of 172 mg/dL for younger cows and 182.3 mg/dL for older ones (Figure 4).

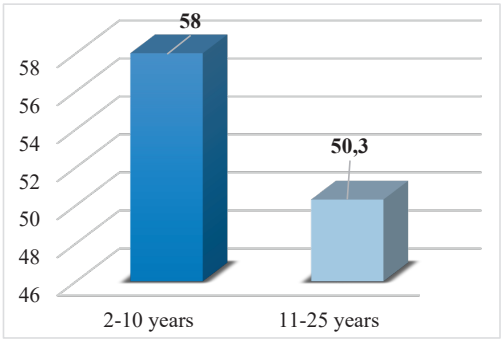

Figure 3. Glucose concentration mg/dL

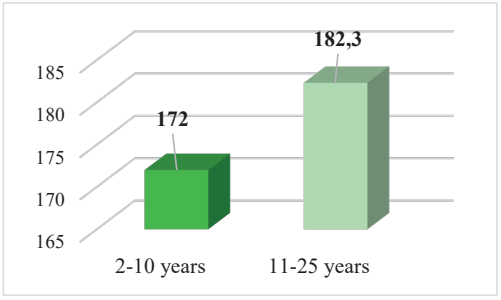

Figure 4. Cholesterol concentration mg/dL

Contrary to expectations, the average concentration of total proteins decreased in older cows (8.22 g/dL) compared to younger ones (9.14 g/dL) (Figure 5). Similarly, calcium levels in the blood were higher in younger cows (11.4 mg/dL) than in older ones (9.55 mg/dL) (Figure 6). A similar trend was observed for phosphorus, with average concentrations decreasing with age (Figure 7).

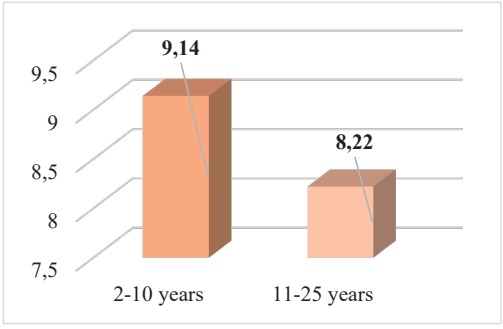

Figure 5. Total protein concentration g/d

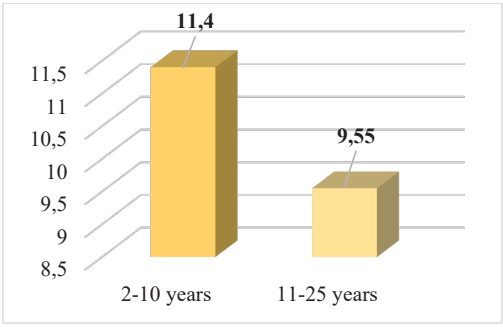

Figure 6. Calcium concentration mg/d

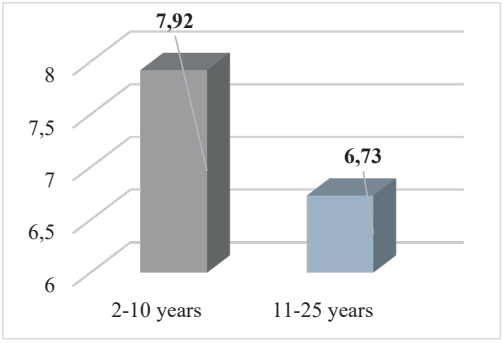

Figure 7. Phosphorus concentration mg/dL

The liver enzymes ALAT and AST show higher values in older cows, suggesting a possible association with overall liver health (Figures 8 and 9). Finally, the average albumin levels increased with age, indicating potential changes in the metabolic health of older cows (Figure 10).

Ruminant liver cells do not show high ALT activity, and the increased activity of that enzyme in the serum during liver damage, even in necrosis, is insignificant (Stojević et al., 2005).

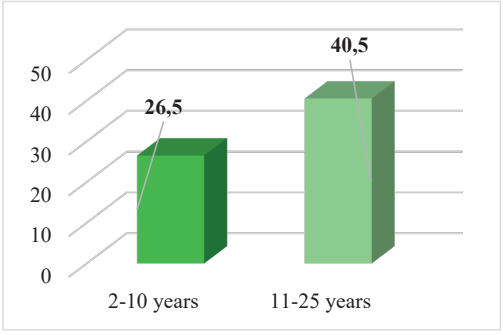

Figure 8. Alanine aminotransferase concentration U/L

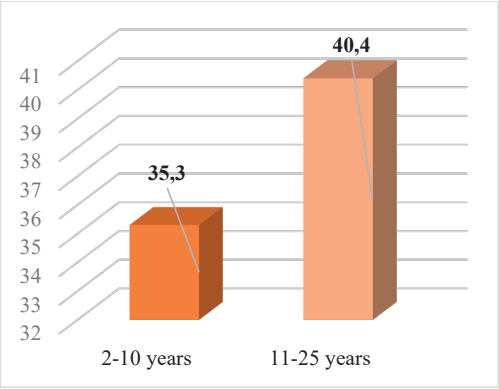

Figure 9. Aspartate amino transferase concentration U/L

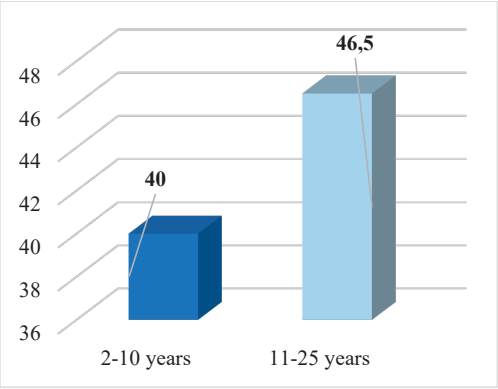

Figure 10. Albumin concentration %

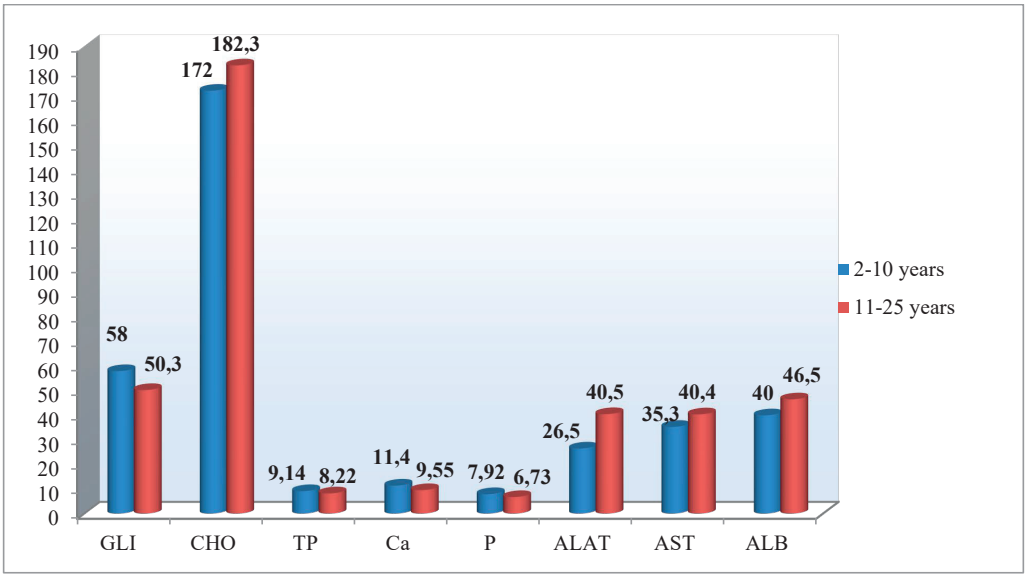

Figure 11. Average of biochemical parameters concentration in blood for Romanian Grey Steppe cows analysed in two age categories (2-10 years; 11-25 years)

Overall, these results contribute valuable insights into age-related physiological variations in Romanian Grey Steppe cows and provide a foundation for future research in understanding the factors influencing these changes.

Asimilar investigation has been conducted in other research (Bedenicki et al., 2014) on the Istrian cattle breed, which pointed constant genetic similarity to Podolian grey steppe cattle breed.

Much research has shown different values of biochemical factors that are understandable because of the many differences of genetic factors between different cattle breeds and many existing paragenetic factors (Keros et al., 2013).

RECOMMENDATIONS REGARDING THE MONITORING OF BIOCHEMICAL PARAMETERS IN ENDANGERED ROMANIAN GREY CATTLE

Monitoring biochemical parameters in endangered Romanian cattle is crucial for ensuring their health and welfare. The authors made the following recommendations. Permanent monitoring of biochemical parameters in the blood by people with knowledge in the field, such as veterinarians and researchers in the field. This will provide a reference point for evaluating deviations from normal values.

Scheduling regular veterinary check-ups for cattle to assess overall health status. During these check-ups, biochemical parameters such as blood glucose levels, liver enzymes, kidney function tests, and electrolyte levels should be evaluated.

The development of customized monitoring plans based on the age, sex, reproductive status, and specific health concerns of individual animals. Therefore, young calves, pregnant cows, and aging cattle may require different monitoring protocols.

The use of portable diagnostic equipment to test biochemical parameters. This enables rapid and convenient monitoring without the need to transport cattle to veterinary clinics.

Implementation of a schedule for regular blood sampling to monitor key biochemical parameters. This could be done quarterly, semi-annually, or annually, depending on the specific needs of the cattle population and availability of resources.

Assurance that cattle are receiving a balanced diet and meets their nutritional requirements. Poor nutrition can affect biochemical parameters such as serum protein, mineral, and vitamin levels.

Environmental monitoring considers environmental factors that may influence biochemical parameters such as temperature, humidity, and exposure to toxins or pollutants. In addition, it is important to minimize stressors in the cattle environment to maintain optimal health.

The development of comprehensive monitoring protocols and the effective interpretation of biochemical data.

Early intervention for any abnormal findings by consulting veterinarians and implementing appropriate interventions. The early detection of health issues can significantly improve treatment outcomes and prevent further complications.

By implementing these recommendations, we can effectively monitor the biochemical parameters of endangered Romanian cattle and contribute to their genetic conservation.

Modern methods for monitoring biochemical parameters from blood in cattle utilize advanced technology and instrumentation to provide accurate and efficient results.

In addition, the authors recommend the application of modern methods with high accuracy for monitoring biochemical parameters in cattle blood.

Automated Chemistry Analyzers: These analyzers automate the process of biochemical analysis from blood samples. They can measure a wide range of parameters including electrolytes, enzymes, metabolites, and proteins. Automated chemistry analyzers offer high throughput and accuracy, making them suitable for large-scale monitoring programs.

Point-of-Care Testing (POCT) Devices: POCT devices allow for rapid on-site analysis of blood samples without the need for laboratory equipment. These handheld devices can measure specific biochemical parameters such as glucose and electrolytes within minutes, making them ideal for immediate diagnostic purposes in field settings.

Enzyme-Linked Immunosorbent Assay (ELISA): ELISA is a sensitive technique used to quantify specific proteins or hormones in blood samples. It is commonly employed to measure concentrations of hormones such as cortisol, insulin, and progesterone in cattle. ELISA kits are available commercially and offer high specificity and sensitivity.

High-Performance Liquid Chromatography (HPLC): HPLC is a chromatographic technique used to separate, identify, and quantify components in a mixture. It is often utilized for the analysis of small molecules such as amino acids, organic acids, and vitamins in blood samples from cattle. HPLC provides excellent resolution and accuracy for quantitative analysis.

Mass Spectrometry (MS): MS is a powerful analytical technique that can identify and quantify molecules based on their mass-to-charge ratio. It is commonly used for targeted or untargeted metabolomic analysis of blood samples to assess metabolic profiles in cattle. MS offers high sensitivity and specificity, allowing for the detection of a wide range of metabolites.

Biosensors: Biosensors are devices that combine a biological sensing element with a transducer to detect and quantify specific analytes in biological samples. Biosensors can be designed to measure various biochemical parameters such as glucose and cholesterol in

cattle blood. They offer real-time monitoring capabilities and can be integrated into wearable or implantable devices for continuous monitoring.

Next-Generation Sequencing (NGS): NGS technologies enable comprehensive analysis of the genetic and transcriptomic profiles of cattle. By sequencing the RNA molecules present in blood samples, NGS can provide insights into gene expression patterns and metabolic pathways related to various biochemical parameters. NGS can be particularly useful for investigating complex diseases and traits in cattle populations.

These modern methods offer diverse approaches for monitoring biochemical parameters in cattle, allowing for accurate diagnosis, research, and management of health conditions. The choice of method depends on factors such as the specific parameters of interest, sample size and available resources.

CONCLUSIONS

It is in general consensus that a complete biochemical parameters profile is an important and powerful tools to monitor response to therapy, and the severity of illness of cows.

The results of our research have shown the influence of different age of cows on biochemical parameters values. The obtained results have mainly demonstrated a significant influence of breed on the parameters mentioned above.

It has been shown that the values of biochemical parameters were generally situated within the reference intervals. Due to the shortage of studies on the clinical biochemistry of the Romanian Podolian Grey Steppe, the obtained results represent a novelty and contribution to achieving a better understanding of the metabolic profile for estimating the physiological status, and for future diagnostic purposes, but more investigation in this field is certainly more than necessary.

It is also concluded that traditional methods for assessment of cow's nutritional status should be combined with their blood biochemical profiles. Routine laboratory analyses of blood biochemical profile can help reduce financial losses significantly by facilitating early diagnosis of diseases.

Moreover, these results are indispensable for an adequate genetic conservation program of the breed.

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