

ELECTROPHORETIC PATTERN OF SERUM PROTEINS IN CLINICALLY HEALTHY DAIRY COWS

Carmen IONITA, Roxana Mariana IGNATESCU, Valerica DANACU, Lucian IONITA

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: roxana.ignatescu@yahoo.com

Abstract

For the diagnosis of diseases in cattle, apart from the clinical examination of the animal, the veterinarian also needs some practical, fast, cheap and, above all, available tools. The importance of this study lies in reducing the number of known paraclinical examinations that are often difficult to manage during a rural or farm field trip and are also expensive. Because clinical signs are often nonspecific, the general clinical examination of clinically healthy cows can only lead to a suspicion of disease without a necessary tool for confirmation or discovery of an insidious pathological process. For this reason, serum protein electrophoresis (SPE) is an important component of laboratory diagnostic evaluations for the measurement of serum proteins. In our study we used 8 clinically healthy cows of which 4 lactating cows and 4 weaned cows. Although there have been fluctuations in the value of various protein fractions and low specificities have been achieved in the diagnosis of certain diseases, the determination of the serum protein profile in ruminants and the correct interpretation of their results are very useful for clinicians in the clinical diagnosis of healthy and diseased animals and can serve as basis for other specific laboratory examinations, going up to protein fractions.

Key words: albumins, biochemistry, blood serum, protein fractions, proteins.

INTRODUCTION

Veterinary medicine has advanced a lot in recent years, but no matter how advanced the techniques available for diagnosis, in the evaluation of internal diseases of dairy cows we can never depart from the basic methods, starting from the complete clinical examination and exhaustive anamnesis, followed by the investigations common paraclinical tests such as hematological examination and microscopic evaluation of blood smear, serum biochemistry, urine examination, etc. In all animal species, gestation and lactation are characterized by intense metabolic changes and changes in many haematological and to chemical components, including variables related to protein metabolism (Bossuyt, 2006).

In clinical medicine, electrophoresis is mainly used to quantitatively separate and classify (electrophoretic pattern or pattern) the proteins in a biological sample (serum, urine, cerebrospinal fluid, peritoneal or pericardial fluid, etc.) and immunoelectrophoresis allows the qualitative detection of components to be named (Keren, 2003).

Electrophoresis is an analytical technique based on the migration of charged particles in a solution under the influence of an electric field (Thrall et al., 2012).

Protein fractions migrated by electrophoresis were identified in dairy cows, from cathode to anode, as albumin, alpha 1 - and alpha 2 - globulins, beta 1 - and beta 2 -globulins, and gamma globulins (Jackson et al., 2015; Ionita, 2024).

Factors regulating immunoglobulin production by normal and disease-associated plasma cells. Blood electrophoresis varies according to age, physiological state, body condition score, etc. The availability and speed of this investigation, its non-invasive nature, low cost and important diagnostic information justify the efforts made to obtain optimal results and a quality interpretation.

MATERIALS AND METHODS

Serum protein electrophoresis is a technique that consists in the separate migration of serum protein fractions on a semisolid gel, in the presence of an electric current.

The proteins are arranged on the gel in bands, according to their size and electrical charge, then the protein bands are colored with a special staining solution, so that they can be visualized. After the gel has dried, a densitometer measures the bands: the densitometer provides a visual pattern or graph of the protein bands, as well as a calculation of the relative percentage of each protein band in the total protein.

The only individual protein that is distinctly separated by electrophoresis is albumin, which has a clinical connotation only when it has low values compared to normal.

The biological material was represented by 8 collected blood samples, from which the serum was extracted and used for electrophoresis. The electrophoresis was carried out using the EP Line 1.0 electrophoresis system (Figure 1), consisting of an EP SA200 voltage source, an EP MB2 x 40 migration bath and a dedicated EP Line analysis, calculation and results interpretation system 1.0



Figure 1. The EP Line 1.0 electrophoresis system (original)

This system is adapted to a computer that allows data analysis and interpretation.

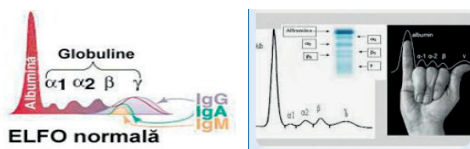


Figure 2. Normal electrophoresis (Bossuyt, 2006)

After the gel has dried, a densitometer measures the bands: the densitometer provides a visual pattern or graph of the protein bands, as well as a calculation of the relative percentage of each protein band in the total protein. The only individual protein that is distinctly separated by electrophoresis is albumin, which has clinical connotations only when it is significantly lower than normal (in fact albumin in pregnant cows is slightly lower). The electrophoretogram divides the protein fraction of serum or plasma into its constituent components, which include albumin

and globulins. There are three globulin fractions: alpha, beta and gamma (Figures 3 and 4).

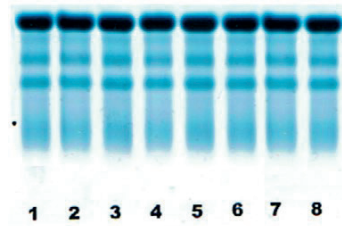


Figure 3. Samples with migrated and stained protein fractions (original)

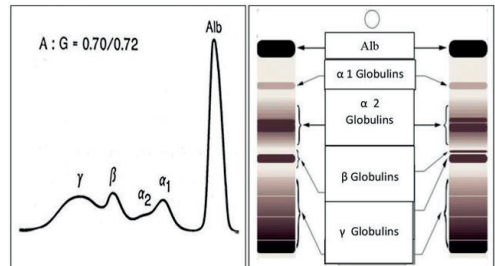


Figure 4. Example of healthy bovine serum protein electrophoresis (after Ionita L., 2024)

RESULTS AND DISCUSSIONS

The study was carried out in a farm of 50 cows from which we chose 8 dairy cows, of which 4 were lactating and 4 were weaned. The age of the monitored cows was between 3.5 and 6 years and they were in their second to fourth gestation. The cows were in good nutritional condition with an average body weight of about 550 kg. The health status of the animals during the evaluated period was observed daily. All animals were clinically healthy.

A. Dairy cows

Table 1. Blood biochemical examination of proteins

Lactating cows	Total protein g/dl	Albumin g/dl	Globulin g/dl	Report A/G	
1	5.50	1.81	3.69	0.49	
2		6.90	3.82	3.08	1.24
3		6.30	4.32	1.98	2.19
4		6.40	4.39	2.01	2.19
Mediate		6.28	3.59	2.69	1.53
Physiological values		5.8-8.5	2.5-3.7	3.3-4.8	0.45-1.31

From the analysis of Table 1, in dairy cows normal values of total serum proteins, but regarding albumin, 3 cows had increased albumi-

nemia values (probably slightly dehydrated) and 1 sample was found below normal values (low during pregnancy).

Regarding the globulins, it is found that in samples 3 and 4 they are found to be low compared to normal. It should be noted that in samples 3 and 4 albumin was significantly increased, globulins significantly decreased, and the A/G ratio is above the physiological limit.

Regarding the average of the sample, it is found that the total proteins and albumin are within physiological limits, the globulins are low and the A/G ratio is increased above the physiological limit. This requires performing electrophoresis to see what happens to the protein fractions of these 4 samples.

Sample 1 - Dairy cow

Table 2. The results of the blood biochemical examination

Sample	Total protein g/dl	Albumin g/dl	Globulin g/dl	Report A/G
1.	5.50	1.81	3.69	0.49
Physiological values	5.8-8.5	2.5-3.7	3.3-4.8	0.45-1.31

In the blood biochemical examination (Table 2), total proteins and albumin are below normal values and globulins and the A/G ratio are within physiological values

Table 3. Electrophoresis of protein fractions

Parameter	Result g/dl	Result (%)	Reference (%)
Alb	1.81	32.94	27-38
(H) Alpha 1	0.53	9.7	4-8
(H) Alpha 2	0.73	13.32	4-8
(H) Beta	1.18	21.37	6-10
(H) Gama	1.25	22.67	12-22

Analysis result:

Total protein = 5.50 g/dl, Report A/G = 0.49

From the Table 3 and Figure 5 of the electrophoretic examination in this animal, it is found that all globulin fractions are above normal values, albumin having normal values.

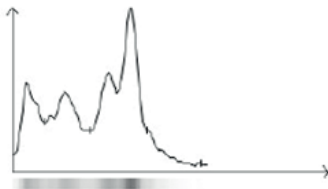


Figure 5. The graphic aspect of the migration of protein fractions

Sample 2 - Dairy cow

Table 4. The results of the blood biochemical examination

Sample	Total protein g/dl	Albumin g/dl	Globulin g/dl	Report A/G
2	6.90	3.82	3.08	1.24
Physiological values	5.8-8.5	2.5-3.7	3.3-4.8	0.45-1.31

From Table 4 in the blood bio-chemical examination total proteins and the A/G ratio were within physiological limits, albumin increased, and globulins decreased

Table 5. Electrophoresis of protein fractions

Parameter	Result (g/dl)	Result (%)	Reference (%)
Alb	3.82	56.34	27-38
(H) Alpha 1	0.38	5.44	4-8
(H) Alpha 2	0.57	8.32	4-8
(H) Beta	1.00	14.43	6-10
(H) Gamma	1.14	16.47	12-22

Analysis result:

Total protein = 6.90 g/dl, Report A/G = 1.24

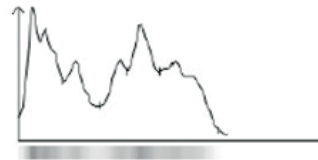


Figure 6. The graphic aspect of the migration of protein fractions

From the Table 5 and Figure 6 it follows that albumins and fractions Alpha 2 and beta were increased and fractions alpha 1 and gamma globulins are within normal limits.

Sample 3 - Dairy cow

Table 6. The results of the blood biochemical examination

Sample	Total protein g/dl	Albumin g/dl	Globulin g/dl	Report A/G
3	6.30	4.32	1.98	2.19
Physiological values	5.8-8.5	2.5-3.7	3.3-4.8	0.45-1.31

Table 6 shows that total proproteins were within physiological limits, albumin was significantly increased, globulins were low, and the A/G ratio was very high.

Table 7. Electrophoresis of protein fractions

Parameter	Result (g/dl)	Result (%)	Reference (%)
Alb	4.32	68.60	27-38
(H) Alpha 1	9.31	4.98	4-8
(H) Alpha 2	0.54	8.58	4-8
(H) Beta	0.92	14.57	6-10
(H) Gamma	0.20	3.15	12-22

Analysis result:

Total protein = 6.30 g/dl, Report A/G = 2.19

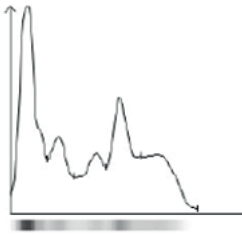


Figure 7. The graphic aspect of the migration of protein fractions

Table 6 and Figure 7 show that the $\alpha 1$ fraction was normal, the other globulins were increased, and the gamma fraction was decreased.

Sample 4 - Dairy cow

Table 8. The results of the blood biochemical examination

Sample	Total protein g/dl	Albumin g/dl	Globulin g/dl	Report A/G
4	6.40	4.39	2.01	2.19
Physiological values	5.8-8.5	2.5-3.7	3.3-4.8	0.45-1.31

Table 8 shows that total proteins were within physiological limits and albumins and the A/G ratio were very high and globulins very low.

Table 9. Electrophoresis of protein fractions

Parameter	Result (g/dl)	Result (%)	Reference (%)
Alb	4.39	68.63	27-38
(H) Alpha 1	0.34	6.30	4-8
(H) Alpha 2	0.36	5.64	4-8
(H) Beta	1.19	18.57	6-10
(H) Gamma	0.12	1.86	12-22

Analysis result:

Total protein = 6.40 g/dl, Report A/G = 2.19

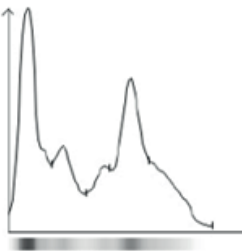


Figure 8. The graphic aspect of the migration of protein fractions

From the data obtained by electrophoresis, this sample shows an increase in albumin and β fractions and a very significant decrease in γ fractions (Table 9 and Figure 8).

B. The following results were observed in **weaned cows** in the period of mammary rest

Table 10. Blood biochemical examination of proteins

Lactating cows	Total protein g/dl	Albumin g/dl	Globulin g/dl	Report A/G
1	5.50	1.81	3.69	0.49
2	6.90	3.82	3.08	1.24
3	6.30	4.32	1.98	2.19
4	6.40	4.39	2.01	2.19
Mediate	6.28	3.59	2.69	1.53
Physiological values	5.8-8.5	2.5-3.7	3.3-4.8	0.45-1.31

From the Table 10 it follows that the average of the sample is within physiological limits in 3 samples, being only small fluctuations in a single sample (3) where the significant increase in albumin caused a significant decrease in globulins and the A/G ratio was well above the limit physiological.

Sample 1 - Weaned cow

Table 11. The results of the blood biochemical examination

Sample	Total protein g/dl	Albumin g/dl	Globulin g/dl	Report A/G
1	7.10	2.77	4.33	0.64
Physiological values	5.8-8.5	2.5-3.7	3.3-4.8	0.45-1.31

From Table 11 all the parameters of the analyzed protein profile are within normal limits.

Table 12. Electrophoresis of protein fractions

Parameter	Result (g/dl)	Result (%)	Reference (%)
Alb	2.77	38.96	27-38
(H) Alpha 1	0.41	5.79	4-8
(H) Alpha 2	0.60	8.48	4-8
(H) Beta	0.99	13.88	6-10
(H) Gamma	2.33	32.88	12-22

Analysis result:

Total protein = 7.10 g/dl, Report A/G = 0.64

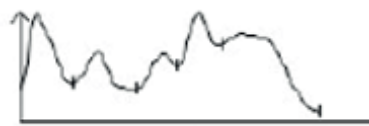


Figure 9. The graphic aspect of the migration of protein fractions

The electrophoresis results show that the $\alpha 1$ fraction was normal, the other globulins being slightly increased.

Sample 2 - Weaned cow

Table 13. The results of the blood biochemical examination

Sample	Total protein g/dl	Albumin g/dl	Globulin g/dl	Report A/G
2	6.40	3.03	3.37	0.90
Physiological values	5.8-8.5	2.5-3.7	3.3-4.8	0.45-1.31

From Table 13 all the parameters of the analyzed protein profile are within normal limits.

Table 14. Electrophoresis of protein fractions

Parameter	Result (g/dl)	Result (%)	Reference (%)
Alb	3.03	47.28	27-38
(H) Alpha 1	0.43	6.75	4-8
(H) Alpha 2	1.03	16.04	4-8
(H) Beta	1.17	18.35	6-10
(H) Gamma	0.74	11.57	12-22

Analysis result:

Total protein = 6.40 g/dl, Report A/G = 0.90

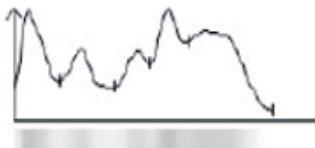


Figure 10. The graphic aspect of the migration of protein fractions

From the Table 14 and the Figure 10, we can see normal values of Alpha 1 globulins, albumins and β globulins are increased and gamma globulins are very slightly decreased compared to normal.

Sample 3 - Weaned cow

Table 15. The results of the blood biochemical examination

Sample	Total protein g/dl	Albumin g/dl	Globulin g/dl	Report A/G
3	6.90	4.31	2.59	1.68
Physiological values	5.8-8.5	2.5-3.7	3.3-4.8	0.45-1.31

From the Table 15 the total proteins are in physiological values, the albumin and the A/G ratio are significantly increased, and the globulins are at low values.

Table 16. Electrophoresis of protein fractions

Parameter	Result (g/dl)	Result (%)	Reference (%)
Alb	3.82	56.34	27-38
(H) Alpha 1	0.38	5.44	4-8
(H) Alpha 2	0.67	8.32	4-8
(H) Beta	1.00	14.43	6-10
(H) Gamma	1.14	16.47	12-22

Analysis result:

Total protein = 6.90 g/dl, Report A/G = 1.68

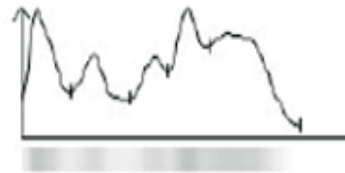


Figure 11. The graphic aspect of the migration of protein fractions

From Table 16 and Figure 11, alpha 1 albumins and gamma globulins are in physiological values and albumin and alpha 2 and beta fractions are increased.

Sample 4 - Weaned cow

Table 17. The results of the blood biochemical examination

Sample	Total protein g/dl	Albumin g/dl	Globulin g/dl	Report A/G
3	6.70	3.42	3.28	1.04
Physiological values	5.8-8.5	2.5-3.7	3.3-4.8	0.45-1.31

From Table 17 all the parameters of the analyzed protein profile are within normal limits.

Table 18. Electrophoresis of protein fractions

Parameter	Result (g/dl)	Result (%)	Reference (%)
Alb	3.42	51.01	27-38
(H) Alpha 1	0.37	5.54	4-8
(H) Alpha 2	0.55	8.18	4-8
(H) Beta	1.22	18.20	6-10
(H) Gamma	1.14	17.06	12-22

Analysis result:

Total protein = 6.70 g/dl, Report A/G = 1.04

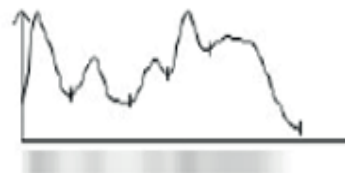


Figure 12. The graphic aspect of the migration of protein fractions

From Table 18 and Figure 12, alpha 1 albumins and gamma globulins are in physiological values and albumin and alpha 2 and beta fractions are increased.

CONCLUSIONS

1. Electrophoresis is a practical and economical alternative in the clinical exploration of ruminant medicine.
2. Electrophoresis is a non-invasive test that complements the quantitative characterization of proteinemia with information on fraction composition that can also be followed in evolution.
3. Even if by biochemical determinations the total proteins and albumins are in physiological values, by electrophoresis significant fluctuations of the protein fractions may appear which may betray an asymptomatic or chronic pathology in clinically healthy dairy cows.
4. Despite this low specificity in the diagnosis of certain diseases, the determination of the serum

protein profile in ruminants and the correct interpretation of their results are very useful for clinicians in the diagnosis of healthy and diseased animals and can serve as a basis for other specific laboratory examinations.

REFERENCES

- Bossuyt, X. (2006). Advances in serum protein electrophoresis. *Adv. Clin. Chem.*, 42, 43-80.
- Ionita, L. (coord.) (2024). *Pathology and veterinary medical clinic*. Craiova, RO: Printera Publishing House.
- Jackson, D., & Elsawa, S. (2015). Factors regulating immunoglobulin production by normal and disease-associated plasma cells. *Biomolecules*, 5, 20-40.
- Keren, D.F. (2003). *Protein Electrophoresis in Clinical Diagnostic*. London, UK: Hodder Arnold Publishing House.
- Thrall, M.A., Weiser, G., Allison, R.W., & Campbell, T.W. (2012). *Veterinary Haematology and Clinical Chemistry*. Iowa, USA: John Wiley & Sons Publishing House.