Study the variation of serum protein electrophoresis in dairy cows affected by metritis and mastitis

KHELAF SAIDANI[1], METREF AHMED KHIREDINE[2*], AOUANE NEDJMA[3]

[1] Veterinary science institute, Saad Dahleb university, Blida 1, Laboratory of Local Animal Resources, Higher National Veterinary School of Algiers Algeria
[2*] Veterinary science institute, Saad Dahleb university, Blida 1, Algeria
[3] Higher National Veterinary School of Algiers, SPA laboratory

SUMMARY

The objective of this study was to evaluate the electrophoretic pattern of serum proteins in dairy cows suffering from mastitis and metritis, and to explore the influence of these inflammatory diseases on the concentrations of different serum protein fractions. 148 dairy cows were enrolled in the study, including 23 cows with clinical signs of various inflammatory diseases metritis (n=13), mastitis (n=10). The cows were of local breed and their crossbreeds at the different ages. 125 clinically healthy dairy cows were taken as the control group. The cows were blood sampled for the determination of total serum proteins and serum protein fractions. The separation of plasma proteins on an agarose gel using an alkaline buffer (pH 9.1) by electrophoresis in a semi-automatic system (HYDRASYS). The protein fractions were divided into albumin, α_1-, α_2-, β, and γ-globulins. Statistical analyses were expressed as mean ± standard deviation (SD). The Shapiro-Wilk test was used to check normality according to Kappes before performing parametric tests such as Student T test and Analysis of variance. One-way nonparametric analysis of variance was applied to compare groups (Kruskal-Wallis test), when the assumptions of one-way ANOVA were not met. In cows with metritis and mastitis significant changes for albumin ($P < 0.01$) and, ($P < 0.05$) for α_1, α_2, TP (Total Proteins). Significant changes were found in the level of α_1-globulins zone, similar changes were observed also in the level of α_2-globulins zone, with BCS (p value < 0,01). The low significant are found about β globulins with others factors (F value <0,5), in opposite with γ globulins, her level increased significantly with all factors (P value <0,1), especially with age (P value <0,001). The albumin to globulin ration being inversely proportional to globulin concentrations, this resulted in a lower ration in sick cattle (P value <0,01), can serve as indicator to manage the transition period. Our results suggest marked influence of mastitis and metritis diseases on the concentrations of serum protein fractions in dairy cows along with age and BCS (body condition score). The results could be useful for veterinary practitioners in the early diagnosis in reproductive disorders such as metritis and mastitis.

KEY WORDS

Dairy Cows; SPE; Metritis; Mastitis.

INTRODUCTION

As in other African countries, the Algerian livestock productivity is low due to various constraints, such us infectious and parasitic diseases, various reproductive disorders, quantitatively and qualitatively poor nutrition, low genetic potential of native breeds, and traditional husbandry systems (1). Fertility related problems, both types of mastitis, and lameness are the three most relevant production diseases in dairy cows (2). Uterine inflammatory condition in bovine farms increase health coasts, reduce food consumption, along with reducing milk production and it is an important cause of early culling (3). It is the most important postpartum disorder in dairy cattle (4). Likewise, bovine mastitis is the most prevalent and relevant in Algerian dairy cows, generating significant low profitability (5). What makes the situation worse is that these reproductive disorders are generally associated with each other (6). On other hand, serum protein electrophoresis is currently regarded as the standard technique for fractionation of serum proteins in clinical biochemistry, Its interpretation in combination with the data collected for each individual has made it possible to confirm clinically apparent cases and to discover other asymptomatic cases. Serum protein electrophoresis as a practical tool for diagnosis in rural medicine: the ease of its realization, the simplicity of its method, the rapidity of its response (less than one hour), the quality of its information. The interpretation of the biochemical parameters measured can only be meaningful for an individual, (a diagnostic choice, remains exclusively the domain of the clinician). The study of the tracings allowed us to discover that electrophoresis alone can provide an answer to a large number of clinical diagnostic problems. Its interpretation in combination with all the data collected for each individual allowed us to confirm clinically apparent cases and to discover other asymptomatic cases (8), It can provide important information for the diagnosis, prognosis, monitoring of various diseases, Serum protein electrophoresis has been studied in animals medicine, in particular for the clinical diagnosis of diseases characterized by dys-

Corresponding Author:
Metref Ahmed Khiredine (metref_ahmed@univ-blida.dz)
(makvetdz16211@gmail.com)

proteinemia (leishmaniasis, ehrlichiosis, feline infectious peritonitis) or to identify the presence of inflammation. Many proteins of the acute phase of inflammation, considered diagnostically important, migrate in this fraction. Alpha1-antitrypsin, α1-acid glycoprotein, α1-antichymotrypsin, α1-ketoprotein, serum amyloid A, and α1 -lipoprotein have been identified in the α1-globulin area, whereas haptoglobin, α2-microglobulin, α2-macroglobulin, ceruloplasmin, α2-antiplasmin and α2-lipoprotein in the α2 globulin fraction (9). The aim of this study was to determine the utility of electrophoresis in describing and determining changes in protein fractions in dairy cows affected by inflammatory diseases, including mastitis and metritis, compared to clinically healthy animals, to improve health monitoring on a dairy farm, opposite than others methods, which most used in the world like the somatic cell count (SCC) and somatic cell score (SCS), (10). Our study also provides information on the electrophoretic serum protein profile of local breed cows and crossbreeds with European cattle breeds including Holstein and Montbeliard, in the context of Algerian farming.

Table 1 - Concentrations of total serum proteins, serum protein fractions, respectively, in healthy cattle, cattle suffering mastitis and metritis (mean ± SD).

Variables	Healthy cattle (n=125)		Sick cattle (n=23)		p value
	Mean	SD	Mean	SD	
TP(gr/l)	76.13710	18.50350	71.04167	11.96909	0.321
Alb(gr/l)	31.03040	7.897562	28.00667	7.435625	0.09068.
α1G(gr/l)	2.937339	1.859106	2.583750	1.608209	0.4283
α2G(gr/l)	8.121613	2.203964	7.605417	1.865268	0.2804
βG(gr/l)	6.404677	1.819585	6.359583	1.821546	0.9492
γG(gr/l)	27.55089	11.386268	26.48500	7.638249	0.7811
A to G Ratio	0.7474821	0.2451507	0.6836461	0.2215226	0.2514

Table 2 - Effects of the factors on Total serum protein, by Multivariable parametric ANOVA.

Levels	Sum of squares	Degree of freedom	F value	Pr(>F)
Age	18.288	6	3.2509	**0.005512** **
BCS	1.341	3	0.4768	0.699044
Status	5.370	1	5.7274	**0.018350** *
Age. * BCS	11.056	13	0.9071	0.548024
Age * Status	5.980	5	1.2756	0.279386
BCS * status	0.804	2	0.4287	0.652386
age * BCS * status	9.054	4	2.4143	0.052970 .
Residuals	105.946	113		

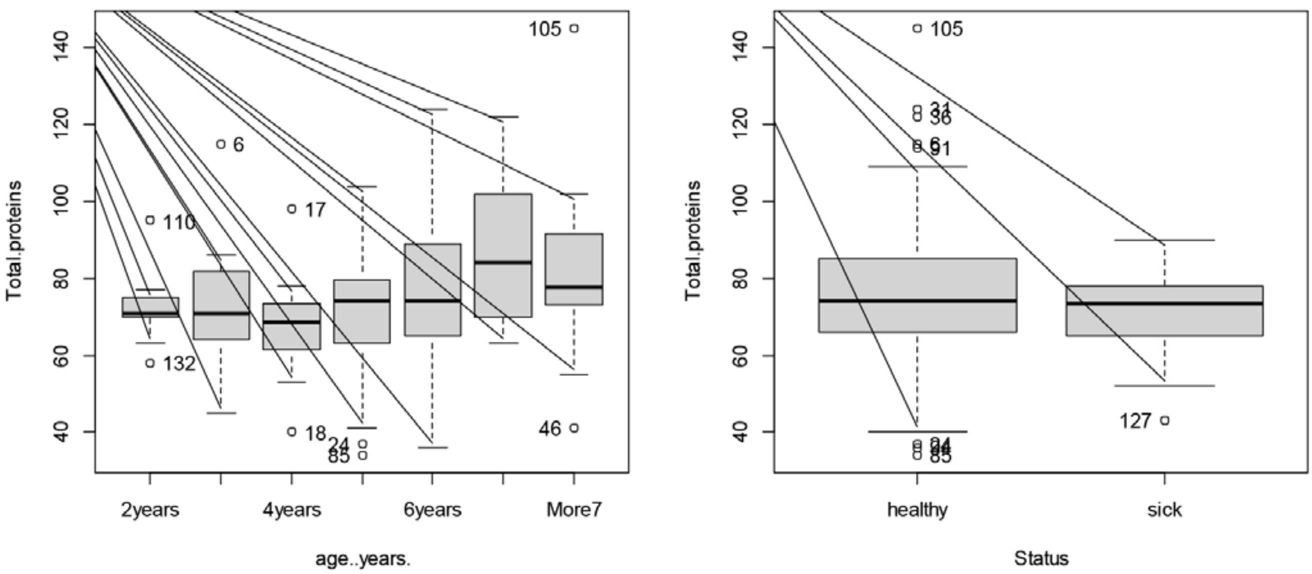

Figure 1 - Boxplots showing effects of age and reproductive disorders on total proteins.

Table 3 - Effects of the factors on Albumin fraction by Multivariable parametric ANOVA.

Levels	Sum of squares	Degree of freedom	F value	Pr(>F)
Age	2.986	6	1.0827	0.377083
BCS	1.297	3	0.9404	0.423681
Status	3.611	1	7.8558	**0.005963** **
Age. * BCS	9.333	13	1.5617	0.106805
Age * Status	9.728	5	4.2320	**0.001454** **
BCS * status	0.789	2	0.8577	0.426861
Age * BCS* status	1.952	4	1.0618	0.378867
Residuals	51.948	113		

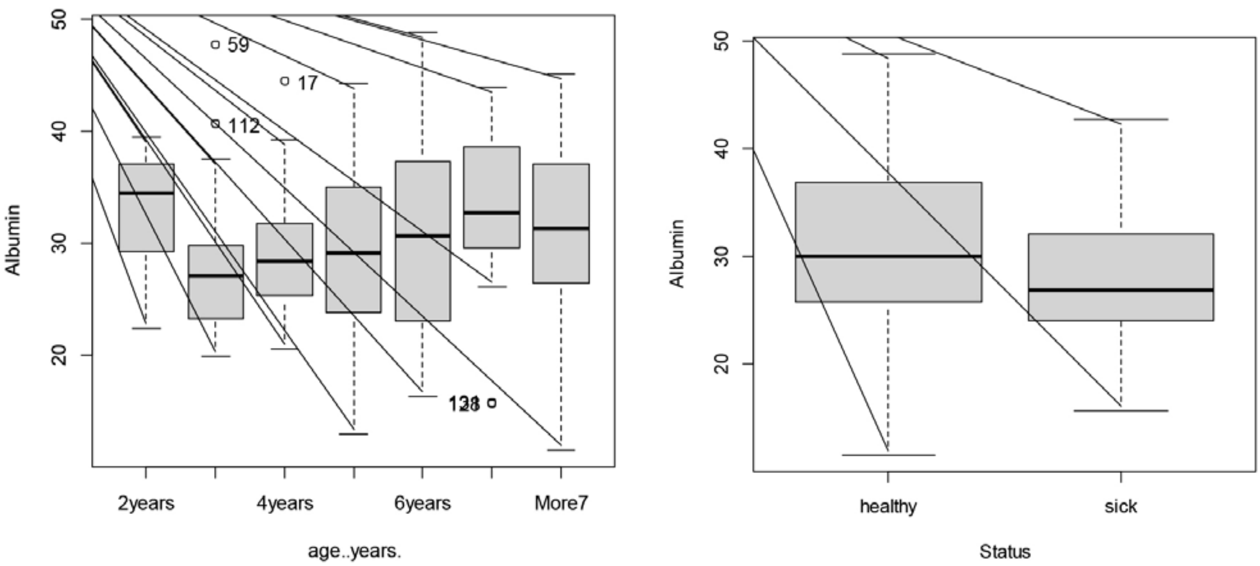

Figure 2 - Boxplots showing effects of age and reproductive disorders on serum albumin fraction concentration.

Table 4 - Effects of the factors on α1 Globulin fraction by Multivariable parametric ANOVA.

Levels	Sum of squares	Degree of freedom	F value	Pr(>F)
Age	3.278	6	1.7405	0.1179729
BCS	6.050	3	6.4257	**0.0004665** ***
Status	0.530	1	1.6887	0.1964111
Age. * BCS	6.677	13	1.6365	0.0852748
Age * Status	0.514	5	0.3273	0.8956002
BCS * status	0.331	2	0.5277	0.5914230
Age * BCS* status	2.831	4	2.2548	0.0675742
Residuals	35.466	113		

MATERIAL AND METHODS

Animals: 148 multiparous dairy cows are included for this study, belonging to twenty-three breeding farms, were included. The cows, of local breed and their crossbreeds, were from different age categories and in different stages of lactation. The twenty-three enrolled dairy farms represented different feeding and husbandry systems, in order to get random sampling.

Collecting data: Information concerning each dairy cow, including age in years, Body condition score (BCS) and clinical examination, was carefully recorded in an Excel sheet, pending descriptive and then inferential analyses. this study, the statistical unit was the dairy cow. For each enrolled animals, serum protein fraction, albumin and globulin were collected along with the clinical data. The BCS was assessed according to Roche (11), and the cows were clinically examined according to standard examination procedures. Clinical examinations included the assessment of the overall health

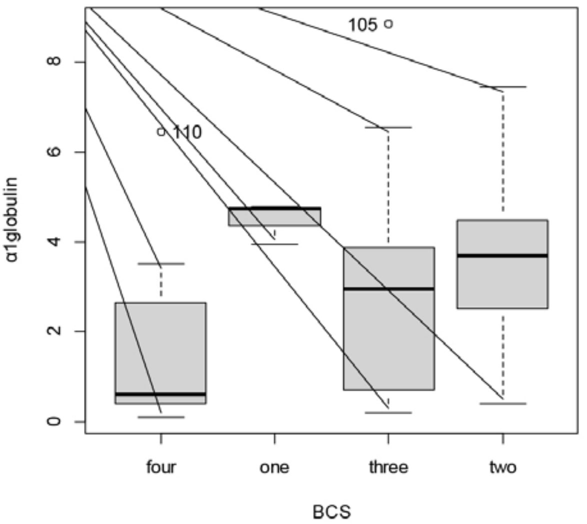

Figure 3 - Boxplot showing effect of BCS on α1 Globulin fraction concentration.

status of the animals (food intake, behavior), inspection and recording of body temperature, respiratory and pulse rates, and a detailed evaluation of the organ systems (12). According to the clinical findings, the cows were divided into the following groups:
cows with clinical metritis (n=10) with abnormal enlarged uterus, obvious red-brownish watery or viscous off-white purulent uterine discharge, accompanied by foul odor; they showed no signs of systemic illness within 21 days after parturition (12).
cows with clinical mastitis (n=13); the cows suffering from mastitis showed clinical signs such as redness, hardness, swelling and pain in the udder, or changes in milk color, and presence of clots in the milk (12). In three cases out of 13, the clinical bovine mastitis was associated either with hypo-calcaemia, retained placenta or repeat breeding.
And then, 125 clinically healthy dairy cows without any signs of diseases and in good general condition were taken as a control group.

Sample and sampling: After an initial clinical examination and

Table 5 - Effects of the factors on α2Globulin fraction by Multivariable parametric ANOVA.

Levels	Sum of squares	Degree of freedom	F value	Pr(>F)
Age	1.5779	6	1.8349	0.09848 .
BCS	0.0016	3	0.0038	0.99967
Status	0.3464	1	2.4170	0.12282
Age. * BCS	1.5719	13	0.8436	0.61356
Age * Status	1.0461	5	1.4598	0.20858
BCS * status	0.2744	2	0.9571	0.38709
Age * BCS* status	1.0973	4	1.9140	0.11292
Residuals	16.1956	113		

Table 6 - Effects of the factors on β Globulin fraction by Multivariable parametric ANOVA.

Levels	Sum of squares	Degree of freedom	F value	Pr(>F)
Age	0.8833	6	1.2080	0.3074
BCS	0.0072	3	0.0197	0.9962
Status	0.1049	1	0.8610	0.3554
Age. * BCS	2.0632	13	1.3023	0.2219
Age * Status	0.9782	5	1.6054	0.1643
BCS * status	0.3817	2	1.5662	0.2133
Age * BCS* status	0.9704	4	1.9907	0.1007
Residuals	13.7708	113		

Table 7 - Effects of the factors on γ Globulin fraction by Multivariable parametric ANOVA.

Levels	Sum of squares	Degree of freedom	F value	Pr(>F)
Age	17.284	6	3.1429	**0.006908** **
BCS	1.772	3	0.6445	0.587969
Status	1.035	1	1.1288	0.290290
Age. * BCS	10.327	13	0.8667	0.589608
Age * Status	3.478	5	0.7590	0.581255
BCS * status	1.348	2	0.7353	0.481621
Age * BCS*status	10.872	4	2.9655	**0.022624** *
Residuals	103.572	113		

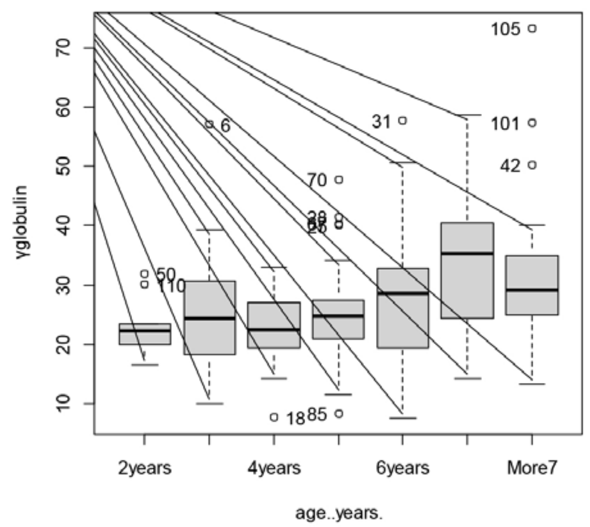

Figure 4 - Boxplot showing effect of age on γ Globulin serum protein fraction.

diagnosis, blood samples from the cows being evaluated are taken from the jugular vein and collected from Vacutainer® tubes, in a volume of 4 ml, for determination of total serum protein concentrations and separation of serum protein fractions. The samples are then placed in a cooler then centrifuged at 3000 rpm, for 10 minutes a ROTOFIX 32 A centrifuge (Hettich). The serum/plasma was then frozen at - 4 ° C. The concentrations of total proteins (TP, g/l) were determined according to the biuret method on an automated biochemical analyzer Cobas 6000 (ROCHE) Designed by Hitachi High-technologies corporation. The chemical reactions to obtain the dosage are adapted and modified according to the requirements of the designer; as well as all the pre-dosing steps (calibration and balancing). Zone electrophoresis on agarose gel was used to separate serum protein fractions using an automated electrophoresis system Hydrasys allowing the separation of plasma proteins on an agarose gel using an alkaline buffer (pH 9.1) by electrophoresis in a semi-automatic system (HYDRASYS). Normal serum proteins are separated into five major fractions. The HYDRASYS system makes it possible to carry out all the sequences until the gel is ready for qualitative or quantitative analysis. The separated proteins are stained with a solution of Amidoschwarz and the excess of dye is removed in acidic medium. Electrophoretic profiles are visually analyzed to detect abnormalities. Densitometry gives a precise relative quantification of each individualized zone (see appendix). The reading of the gel by densitometry makes it possible to define the relative concentrations (percentages) of each fraction. The protein fractions were divided into the following bands: albumin, α_1- and α_2- globulins, β globulins, and γ-globulins. Each fraction was expressed according to the optical density in absolute concentrations (g/l) calculated from the concentrations of total serum proteins. The ratios of albumin to globulins (A/G) were calculated also.

***Statistical analyses*:** All obtained results were expressed as mean ± standard deviation (SD). The Shapiro-Wilk test was used to check normality according to Kappes (13) before performing parametric tests such as Student T test and Analysis of variance. One-way nonparametric analysis of variance was applied to compare groups (Kruskal-Wallis test), when the assumptions of one-way ANOVA were not met.
The five serum protein fraction concentrations (albumin, α1 and α2, β and g-globulins) identified and measured by electrophoretic separation, along with total protein, were served as quantitative response variables for testing hypothesis. The main factor considered is especially the presence/absence of genital tract disorder (binary variable) and mammary inflammation, such metritis, mastitis, repeat breeding or retained placenta. The Tukey multiple-comparison test was applied for post hoc comparison.
To be able to perform multifactorial analyses of variance, the non-Gaussian distributed variables were transformed by the square root function, rather than using Napierian logarithm transformation, which would give negative values.
The age factor was divided into seven categories: two years, three years, four years, five years, six years, seven and more than 7 years. The BCS factor (Body condition score), from 1 to 4, was also taken into account.
Data were analysed using the R (14) statistical software (Version 4.3.1).

RESULT AND DISCUSSION

The data referring to the relative and absolute concentrations of serum protein fractions in cows during the evaluated period expressed as average values and standard deviations, including the significance of differences in the results between the sample collections are presented in Tables 1 to 8. Univariate statistical analysis showed slight alterations in the serum protein profile of dairy cows affected by mastitis and metritis (Table 1). However, multivariable analyzes (Tables 2 to 8) showed large, statistically sig-

Table 8 - Effects of the factors on Albumin to Globulin ratio by Multivariable parametric ANOVA.

Levels	Sum of squares	Degree of freedom	F value	Pr(>F)
Age	0.12589	6	1.0999	0.36687
BCS	0.06962	3	1.2166	0.30704
Status	0.02945	1	1.5437	0.21664
Age. * BCS	0.35045	13	1.4133	0.16397
Age * Status	0.22119	5	2.3191	**0.04787 ***
BCS * status	0.04560	2	1.1953	0.30642
Age *BCS* status	0.16743	4	2.1944	0.07406
Residuals	2.15545	113		

nificant differences between the protein profile of diseased versus healthy dairy cows. This is why several factors must be taken into account simultaneously, such as age, BCS, presence of diseases, physiological status, etc., to avoid biased interpretation of the results. In the healthy cow, previously studies showed that there are dynamic changes in the concentrations of serum total proteins and in their electro- phoretic fractions in dairy cows during the last week of pregnancy and early stages of lactation (15). Indeed, the total serum protein is made up of numerous protein fractions (16), and diagnostic information could be obtained by detecting changes in the electrophoretic serum protein profile (17). The relative concentrations of all the separated protein fractions during the inflammatory diseases like metritis and mastitis in cows (table 1), showed significant changes primarily for albumin for α_1 globulin, α_2 globulin, as well as Total Proteins when data were analyzed by multivariate statistical methods (P < 0.01, P <0 .05 respectively). The level of albumin decreased significantly for sick subjects, described in table 2 and it have significant changes according to age and BCS (F value < 0,01). We can explain this, because albumin is a negative marker for nutritional status, when the animal is sick the consequence is the appetite decrease, and then there is a direct impact for liver function, may be caused by its decreased synthesis in the liver and increased utilization in the udder for lactation (18). Albumin is considered as the best marker and fundamental element of nutrition, it is the most abundant plasma protein and the major component of fetal bovine serum, it is also the best indicator of malnutrition (19). hypoalbuminemia can result from an inflammatory process, liver or kidney failure, lower hemoglobin levels, may therefore be caused by a lack of synthesis, which may be an indication of severe hepatic disorders. However, hypoalbuminemia may result from renal leakage caused by glomerulopathy. It may also be the result of severe inflammation of the intestine leading to protein loss. Hypo-albuminemia is therefore not specific to liver disease. It is also not very sensitive since it only appears at the end of the evolution of a hepatic disease (20). Significant changes were found in the level of α_1-globulins zone, similar changes were observed also in the level of α_2-globulins zone, with BCS (p value < 0,01). Similarly, others authors found in dairy cows and ewes a trend of increasing values of α-globulins during post- partum (21). The alpha fraction includes many diagnostically important acute phase proteins. Alpha$_1$-antitrypsin, α_1-acid glycoprotein and α_1-lipoprotein have been identified in the α_1-globulin fraction, while haptoglobin, α_2-microglobulin, α_2- macroglobulin, ceruloplasmin and α_2-lipoprotein in the α_2-globulins fraction (22), The increases of the alpha fractions are predominantly caused by increases in the concentrations of these proteins, which may be related to inflammatory processes occurring in the urogenital system and mammary gland (23). However, significantly lower levels of β globulins were recorded in diseased cattle specially when studied with others factors (P value <0,5) while γ globulin levels significantly increased, acting simultaneously with age and BCS (P value <0.01), especially with age (F value <0,001). This condition is may be associated with the transport of immunoglobulins from the blood stream (24). Both mastitis and metritis are characterized by increased neutrophilic count and reveals that the viability of the neutrophils showed prolonged maintenance during mastitis and metritis (25), it's a response of the innate immune system. It is well known that the increases of the alpha fractions are predominantly caused by increases in the concentrations of these proteins, which may be related to inflammatory processes occurring in the urogenital system and mammary gland in the period after calving. However, we must distinguish the variation of these concentration between healthy and sick cows, the changes in the concentrations of some serum proteins during the periparturient period should not be considered as a result of pathological processes (26). The albumin to globulin ration being inversely proportional to globulin concentrations, this resulted in a lower ration in sick cattle (P value <0,01), interacting especially with age, it was observed an increase in mastitis and metritis occurrence as cows getting older (27). Furthermore, albumin to globulin ratio can serve as indicator to manage the transition period, which is crucial to the profitability of dairy farming. Indeed, the fertility of high-producing cows is compromised by drastic changes, either metabolic, hormonal and physiological, during the transition period; with less consumption of dry matter and increase in requirements to sustain production; what engenders weight loss and body condition, in addition to an increase in the presence of anestrus, days per first heat, number of days open and the number of services per conception (28), along with various metabolic and infectious pathologies (29). The dry cow management and nutrition have been the subject of much research over the last 30 years, implementing many health and nutritional strategies (30).

CONCLUSION

Significant alterations were found regarding electrophoretic serum protein fraction levels, which reflect the response of the organism to metritis and mastitis occurrence, and these variations interrelate with age and BCS. Our results could be useful for veterinary practitioners in the early diagnosis, prevention and to find out the best monitoring solutions primarily in bovine reproductive disorders such as metritis and mastitis. Furthermore, the serum albumin level is a reliable parameter for evaluating the nutritional status of dairy cows, mainly during transition period.

Ethical approval

The research was approved by the research ethics committee of our university (N° 52/ D.G.E.F/2024)

Acknowlegment

The authors are grateful and would like to thank the breeders for their helpful, to the laboratory staff and to the scientific committee of Saad Dahleb Blida 1 University

Funding

The presented data are not a result of a funded project or grant

Conflict of interest

The authors declare that they have no competing interests

References

1. Lobago F., Bekana M., Gustafsson H., Kindahl H (2006). Reproductive performances of dairy cows in smallholder production system in Selalle, Central Ethiopia. Tropical Animal Health and Production. 2006;38(4):333-342. doi: 10.1007/s11250-006-4328-1.
2. Bennett, R. M., K. Christiansen, and R. S. Clifton-Hadley (1999). Estimating the costs associated with endemic diseases of dairy cattle. J. Dairy Res. 66:455-459.

3. Van Dorp, R.T.E., S.W. Martin, M.M. Shoukri, J.P.T.M. Noordhuizen and J.C.M. Dekkers. (1999). An epidemiologic study of disease in 32 registered Holstein dairy herds in British Columbia. Can. J. Vet. Res., 63: 185-192
4. Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO (2006). Defining postpartum uterine disease in cattle. Theriogenology 65: 1516-1530. doi: 10.1016/j.theriogenology.2005.08.021
5. Saidani K, López Sc, Ziam H, Hamiroune M, Righi S, Díez Bp, Panadero Fr, Fernández Rg. (2018). La mastitis bovina clínica en el norte de Argelia: factores de riesgo y plan de control. Livestock Research for Rural Development. 30(8).
6. Haile A., Tsegaye Y., Tesfaye N (2014). Assessment of major reproductive disorders of dairy cattle in urban and per urban area of hosanna, southern Ethiopia. Animal and Veterinary Sciences. 2014;2(5):135-141. doi: 10.11648/j.avs.20140205.11
7. Compton CWR, Heuer C, Thomsen PT (2017). Invited review: A systematic literature review and meta-analysis of mortality and culling in dairy cattle. J Dairy Sci. 2017,Jan [cited 2019 Feb 6];100(1):1-16. 10.3168/jds.2016-11302
8. Metref A, Melizi M (2017). Importance of serum protein electrophoresis in bovine medicine (easy tool for diagnosis). Global Veterinaria. 2017;19(2): 509-516
9. Bossuyt, X. (2006). Book: Advances in serum protein electrophoresis. Advances in Clinical Chemistry. Vol 42. 2006.
10. Gil, A. K. R., Aguirre-Salado, C. A., Fonseca-Rodríguez, O., Ballesteros-Rodea, G., Del Socorro Hernandez-Arteaga, L. E., & Álvarez-Fuentes, G. (2022). What do we have learned about mastitis spatial analysis during the last 30 years?. *Large Animal Review*, *28*(3), 153-160.
11. Roche J R, Friggens N.C., Kay J.K , Fisher M.W., Stafford K.J., Berry D.P (2009). Invited review: Body condition score and its association with dairy cow productivity, health, and welfare, Journal of Dairy Science,Volume 92, Issue 12.
12. Jackson P.G.G., Cockcroft P.D.(2002) Clinical Examination of Farm Animals. Blackwell Science Ltd., Blackwell Publishing Company; Oxford, UK: 2002. p. 320.
13. Kappes, R.; Knob, D. A.; Thaler Neto, A.; Alessio, D. R. M.; Rodrigues, W. B.; Scholz, A. M. and Bonotto, R. (2020) Cow's functional traits and physiological status and their relation with milk yield and milk quality in a compost bedded pack barn system. Revista Brasileira de Zootecnia 49:e20190213. https://doi.org/10.37496/rbz4920190213
14. R Core Team R. (2023). A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/
15. Tóthová CS, Mudro P and Nagy O (2017). The electrophoretic pattern of serum proteinin dairy cows with inflammatory diseases. Acta Veterinaria Beograd 67: 178-190. https://doi.org/10.1515/acve-2017-0016
16. Metref AK, Nedjma A, Djamila K and Mohamed M (2021). Abnormalities of serum protein electrophoresis in cattle and comparison between assays for markers of inflammation and markers of liver alteration. International Journal of Veterinary Science 10(3): 208-213. https://doi.org/10.47278/journal.ijvs/2021.038
17. Eckersall PD (2008). Proteins, proteomics, and the dysproteinemias. In: Kaneko JJ, Harvey JW, Bruss ML, editors. Clinical biochemistry of domestic animals. 6th ed. London: Elsevier Academic Press; p. 117-155.
18. Celeska I, Janevski A, Dzadzovski I, Ulchar I, Kirovski D (2015). The dynamics of biochemical parameters in blood of clinically healthy Holstein cows from day 5 before to day 60 after calving. Maced. Vet. Rev. 2015;38(2):189-193.
19. Mario P. Smuts, Sonya de Bruyn, Peter N. Thompson, Dietmar E. Holm (2019). Serum albumin concentration of donor cows as an indicator of developmental competence of oocytes, Theriogenology, Volume 125, 2019, Pages 184-192
20. Warren OJ, Smith AJ, Alexious C (2019). The inflammatory response to cardiopulmonary bypass: part 1-mechanisms of pathogenesis. J Cardiothorac Vasc Anesth. 2009;23:223-31.
21. Tóthová, C., Nagy, O., Ková , G., & Nagyová, V. (2016). Changes in the concentrations of serum proteins in calves during the first month of life. Journal of Applied Animal Research, 44(1), 338-346.
22. Smith B, van Metre D, Pusterla N. Book (2021) Large Animal Internal Medicine. 6th ed. Elsevier Inc.; 2021. pp. 435-442
23. Shaodong Fu, Bo Yang, Yabin Gao, Yawei Qiu, Naiyan Sun, Zhi Li, Shiyuan Feng, uanyuan Xu, Jinqiu Zhang, Zhenhua Luo, Xiangan Han, Jinfeng Miao (2024). A critical role for host-derived cystathionine-β-synthase in Staphylococcus aureus-induced udder infection, Free Radical Biology and Medicine, Volume 210, 2024, Pages 13-24
24. Piccione G, Messina V, Alberghina D, Giannetto C, Caselle S and Assenza A (2012). Seasonal variations in serum protein fractions of dairy cows during different physiological phases. Comparative Clinical Pathology 21: 1439-1443. https://doi 10.1007/s00580-011-1311-7
25. Rambault M, Gilbert FB, Roussel P, Tessier A, David V, Germon P, Winter N, Remot A (2023). Neutrophils expressing major histocompatibility complex class II molecules circulate in blood and milk during mastitis and show high microbicidal activity. J Dairy Sci. 2023 Jun;106(6):4245-4256. doi: 10.3168/jds.2022-22728. Epub 2023 Apr 18. PMID: 37080786.
26. Kurpi ska A, Jarosz A, Skrzypczak WF (2014). Proteomic studies in pregnant and lactating cows - a review. J Anim Feed Sci. 23:203-211.
27. Navid Ghavi Hossein-Zadeh, Mehrnaz Ardalan (2011). Cow-specific risk factors for retained placenta, metritis and clinical mastitis in Holstein cows. Vet Res Commun. 2011 Aug;35(6):345-54. doi: 10.1007/s11259-011-9479-5. Epub 2011 May 17. PMID: 21584779.
28. Cardoso F. (2017) The transition period in dairy cattle, physiology, and nutritional consideration, an overview. Journal of Animal Science. Vol. 95 (2), pp. 27 - 39.
29. Esposito G, Irons P, Webb E, Chapwanya A (2014). Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Animal Reproduction Science. Vol. 144 (3), pp. 60 - 71.
30. Hoffman M, Reed S, Pillai S, Jones A, McFadden K, Zinn S. Govoni K (2017). Physiology and Endocrinology Symposium: The effects of poor maternal nutrition during gestation on offspring postnatal growth and metabolism. Journal of Animal Science. Vol. 95 (5), pp. 2222 - 2232.