Changes values of Serum proteins electrophoresis by body condition score, physiological stage and age in healthy cattle

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SUMMARY

The objective of the present study was to determine the influence of age, BCS (Body Condition Score) and physiological status to the electrophoresis of serum proteins in clinically healthy cattle. we studied 161 clinically healthy cattle. Blood samples were taken into jugular vein and analyzed for the values of serum protein fractions using agarose gel electrophoresis, in a semi-automatic system (HYDRASYS). an automated biochemical analyzer Cobas 6000 (ROCHE) are using to determine Total protein value. the standard value (Mean ± SD) has reported. The study obtained the same concentrations for albumin and globulins fractions with other works. the results marked shift in the values of some protein fractions, consequence of the studying parameters: The values of γ-, α_1-globulins and Albumin in cattle were high significant with all categories of age ($P< 0.001$, $P<0.01$, $P< 0.05$ respectively). Also, the albumin/globulin ratio is of special interest for clinicians because the A/G ratio, is higher significant influence of age ($P<0.0001$). A high significant influence was found in the concentrations of α1-globulins with different stage of physiological status ($P < 0.0001$). Significant changes in relation to the stage of pre- or post -partum period was observed also in the concentrations of β-, γ-globulins, ($P < .05$, $P<0.01$ respectively). Then, a high significant relation between the A/G ratios and the physiological status ($P < .0001$). finely, only α1 globulins zone have a high significant with different grade of BCS ($P < .001$). In fact, of our results, we can better understand how age, BCS and physiological status interact to serum protein profiles in healthy cattle, pay particular attention to age when interpreting serum protein electrophoretic profiles. This knowledge is essential for optimizing nutritional strategies and health management practices on the farm and should be taken into consideration when interpreting the serum protein profile.

KEY WORDS

Cattle, Serum Electrophoresis, Body score, Age, physiological stage.

INTRODUCTION

The electrophoresis of serum protein in evaluating dysproteinemias has great potential for the clinical diagnostic setting (1,2). The laboratory evaluation of serum protein electrophoretic profiles is an important diagnostic tool for clinicians. It is benefits to identify and quantify protein fractions as well as to determine the physiologic serum protein electrophoretic fractions, in order to identify animals with altered serum protein values due to changes in homeostasis or disease (3,4). Animal age and nutrition have an important impact on the concentrations of biochemical variables that are commonly analyzed (5,6). A high-yielding dairy cow undergoes the most difficult and crucial periods during the late gestation and early lactation, which are associated with the change from gestational non-lactating to non-gestational lactating states (7). The body condition varies significantly according to the physiological stage; hence, the interest in the conduct of the dry period, which is a strategic and determining period for the nutritional future of the animal and the herd, his impact on SPE is significant by nutrition condition. The body condition of the animals is one of the indicators of the efficiency and safety of a ration. Some authors have reported that the variations in the serum protein profile and shift in albumin and globulin concentrations may occur not only under pathological, but also under physiological conditions (8). Agarose gel electrophoresis of serum proteins in bovine samples identified five fractions comprising albumin, α1 and α2, β, and γ-globulins. There are very few reports published about more detailed serum protein profiles in cattle (9,2). It would therefore be important to determine if the serum protein electrophoretic pattern of investigated animals is also influenced by the animals' age, body score condition, or physiological status. For that, the aim of the present study is to quantify the serum protein electrophoresis on agarose gel in healthy cattle through the influence of ages, BCS (body score condition), physiological stage. and it is essential to determine the standard concentrations of each major protein fraction also in cattle, especially when we contribute to standardize the methods of protein electrophoresis and the quantification of serum protein fractions in cattle.

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MATERIAL AND METHODS

Ethical approval: The research was approved by the Research Ethics Committee of our scientist university committee. In accordance with local laws.

Animals and clinical examination: The study was carried out on 161 cows. Various ages, all clinically healthy and sourced from a standard dairy farm, were included in this study. We consider the cattle are healthy if the clinical examination is normal without sick signs, according to Smith et al, (2021) (10). The BCS factor (Body Condition Score), from 1 to 5, was also considered, it was assessed according to Roche et al (2009) (11). The age factor was divided into five groups as they grow and production: 0-6 months: calves; 6 months - 1 years: heifers and young bull; 1-2 years: breeding period; 2-8 years: in production; Over 8 years: reform and culling period. For the physiological status, we deviated our study groups into five groups: Dry period: it includes the subject in the dry period; Lactating cow: the subject in production; Youngs cattle: the subjects before starting production; Pre-partum: early week before calving; post-partum: early week after calving.

Sample and sampling: The blood sampling is performed on the jugular vein. For each animal, three blood tubes are taken from tubes of the Vacutainer® type, for a volume of 4 ml (the first on the dry tube, the second citrate and the last on the heparin tube). 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).

Laboratory analysis: 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 Amido-schwarz and the excess of dye

Table 1 - Values of serum protein fractions and A/G ratios in clinically healthy cattle according to the age categories.

	The age groups (mean ± SD)					P-value
Proteins	0-6M	6M-1Y	1Y-2Y	2Y-8Y	Over 8Y	<0.0001
TP (gr/l)	71.56 ±11.9	68.53±5.89	73.35± 7.64	69.71 ± .22	72.37 ± .38	0.62
Alb(gr/l)	35.54 ±3.81	32.81±8.47	30.79 ±7.59	35.14±12.23	29.22±7.60	0.04
α1G(gr/l)	3.66 ±0.90	3.14 ±1.32	2.72 ±1.72	3.36 ±0.66	.68 ±2.07	0.00
α2G(gr/l)	7.45 ±1.67	7.28 ±1.82	7.76 ±2.17	7.19 ±1.63	8.16 ±2.27	0.61
βG(gr/l)	5.49 ±1.44	5.88 ±1.38	6.26 ±1.82	5.68 ±1.22	6.13 ±1.77	0.29
γG(gr/l)	19.39 ±7.29	19.40±8.89	25.80±10.47	18.33 ±5.58	27.15±7.96	0.00
Alb/G	.047 ±0.25	0.96 ±0.25	0.77 ±0.24	1.02 ±0.28	.714 ±0.22	0.00

Table 2 - Values of serum protein fractions and A/G ratios in clinically healthy cattle according to the physiological status.

	The physiological status (mean ± SD)					P-value
Proteins	Dry period	PRP (pre-partum)	PSP (post-partum)	Dairy cows	Young cattle	<0.0001
TP(gr/l)	7.46 ±13.54	71.78 ±9.75	70 ±0.00	73.42±18.51	67.98±14.86	0.20
Alb(gr/l)	34.66±10.32	32.47 ±5.64	29.55 0.63	30.89 ±8.15	33.0±7.09	0.35
α1G(gr/l)	1.66 ±1.76	2.10±1.41	.35±0.21	3.01 ±1.72	3.49 ±0.90	0.00
α2G(gr/l)	8.80±1.79	7.26 ±1.84	9.00 ±0.70	7.81 ±2.19	7.05 ±1.67	0.01
βG(gr/l)	6.93±1.37	5.99 ±1.57	6.30 ±0.56	6.22 ±1.85	5.53 ±1.34	0.02
γG(gr/l)	25.40 ±5.95	23.92 ±6.79	24.85±2.05	25.47±11.06	18.81±8.59	0.00
Alb/G	0.83 ±0.27	0.87± 0.25	0.72± 0.02	0.78± 0.25	1.00± 0.25	0.00

Table 3 - Values of serum protein fractions and A/G ratios in clinically healthy cattle according to the BCS.

	BCS (mean ± SD)				P-value
Proteins	1	2	3	4	<0.0001
TP(gr/l)	70.62±10.17	80.66±22.61	72.62 ±17.44	69.73 ±15.21	0.37
Alb(gr/l)	30.32±4.63	35.27 ±8.06	32.74 ±8.45	31.81 ±7.63	0.50
α1G(gr/l)	1.45 ±1.43	3.96±0.75	3.05 ±1.63	3.13 ±1.32	0.00
α2G(gr/l)	7.82 ±2.12	8.06 ±1.57	7.62 ±2.11	7.42±1.86	0.79
βG(gr/l)	6.41 ±1.42	6.15 ±2.19	6.08 ±1.70	5.81 ±1.56	0.54
γG(gr/l)	24.59 ±6.45	27.21±18.54	23.11 ±10.23	21.54 ±8.98	0.40
Alb/G	0.78 ±0.19	0.91 ±0.41	0.88 ±0.27	0.90 ±0.26	0.46

is removed in acidic medium. Electrophoretic profiles are visually analyzed to detect abnormalities. Densitometry gives a precise relative quantification of each individualized zone. 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, α1- and α2- 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 et al (2020) (12), before performing parametric tests such as Student 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 γ-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 BCS, age and physiological status. 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. Data were analyzed using the R (R Core Team, 2023) (13) statistical software (Version 4.3.1).

RESULTS AND DISCUSSION

Overall, the study obtained the same concentrations for albumin and globulins fractions with other works (9,6), we have reported, all the standard value (Mean ± SD) in the: Table 1, Table 2 and Table 3.

The value of γ-globulins in cattle was high significant with all categories of ages ($P< 0.001$). Significant influence of age was detected also in the values of α_1-globulins and Albumin ($P<0.01$, $P< 0.05$ respectively). These findings are similarly reported by another author (14,15), serious inflammatory conditions are associated with higher concentrations of α-globulins, caused by the fact that the most of acute phase proteins (haptoglobin, ceruloplasmin, α1-acid glycoprotein, α1-antitrypsin) occur in this fraction. This is explained by the consequence of transition from fetal to neonatal the life and then from newborn to young animal necessitates major physiological adjustments (16). Higher values of α1-globulins in cattle obtained in our study may be related to the exposure of animals to changing nutritional and rearing factors, or it associated with the normal process of growth due to physiological factors (17). The higher concentrations of α1-globulins in cattle recorded in our study may reflect the accumulation of α1-fetoprotein in the blood serum of young animals. this protein belongs to the electrophoretic migrating α1 zone and is synthesized by the developing fetus and then by the parenchymal cells of the liver (10). The β-globulin, α_2-globulins and TP (total proteins) fraction did not differ significantly between the age groups of animals. However, the albumin/globulin ratio is of special interest for clinicians because the A/G ratio, is higher significant influence of age ($P<0.0001$). Presumably caused by changing globulin patterns during development (8,15). Therefore, it allows systematic classification of the electrophoretic profile and identification of dysproteinemias, and should be interpreted with attention paid to which part of the ratio has changed. Our study shown, the age is an important factor that may affect the concentrations of different fraction of serum proteins separated by electrophoresis (Table 1, Figure 1), according to Fayos et al (2005) (18). In other hand, many authors stated that the most of biochemical parameters in young animals differ from the reference intervals used in adults (19,20). Moreover, a high significant influence was found in the concentrations of α1-globulins with different stage of physiological status ($P < 0.0001$). we observe a significant drop in her value after calving (postpartum period) and they increased till the lactating period and dry period. Opposite changes were observed in the concentrations of α2-globulins. Their lowest mean concentration was recorded before parturition, which slight increased after parturition ($P < 0.01$) with further slight decrease till the of lactation (Table 2, Figure 2). However, we note an augmentation of their concentration at the dry period. Our results are similar for α2-globulins, but differ with α1-globulins compared to those found by other authors in dairy cows and sheep (4,9). These later, reported a trend towards increased values of α-globulins in the postpartum period. The increases of the alpha fractions are predominantly caused by increases in the concentrations of acute phase proteins of inflammation like: Alpha1-antitrypsin, α1-acid glycoprotein and α1-lipoprotein have been identified in the α1-globulin fraction, while haptoglobin, α2-microglobulin, α2- macroglobulin, ceruloplasmin and α2-lipoprotein in the α2-globulin fraction (10). It is the consequence of the inflammatory processes occurring in the urogenital system and mammary gland in the period after calving. Intense changes in the concentrations of many acute phase proteins were reported in dairy cows with post-partum diseases (21). The physiological processes taking place around the time of parturition, especially increase in myometrial activity, involution of the uterus, as well as degeneration and regeneration of the endometrium, may also be responsible for higher concentrations of acute phase proteins in blood serum of cows after calving (22). However, the changes in the concentrations of some serum proteins during the periparturient period should not be considered as a result of pathological processes (23). Significant changes in relation to the stage of pre- or post -partum period was observed also in the concentrations of β- globulins ($P < 0.05$). Their values decreased before calving, with a subsequent increase postpartum. The slight lower concentrations of β-globulins in cows before calving observed in our study might be caused by the transfer of transferrin, which is one of the main proteins from this fraction, into the mammary gland for the synthesis of colostrum and milk (24, 15). Furthermore, the β-globulin fraction includes some other proteins, including complement, which are involved in the inflammatory and stress responses and, thus, may be responsible for increasing concentrations of β-globulins in cows' post-partum (25, 9). Similar trend of significantly increasing values was observed in the concentrations of γ-globulins ($P < 0.01$). The lower concentrations of γ-globulins in pre-partum may be associated with the transport of immunoglobulins from the blood stream across the mammary barrier and into the lacteal secretion during the synthetases of colostrum (26). In the overhand, the blood-derived immunoglobulins are essential for the synthesis of

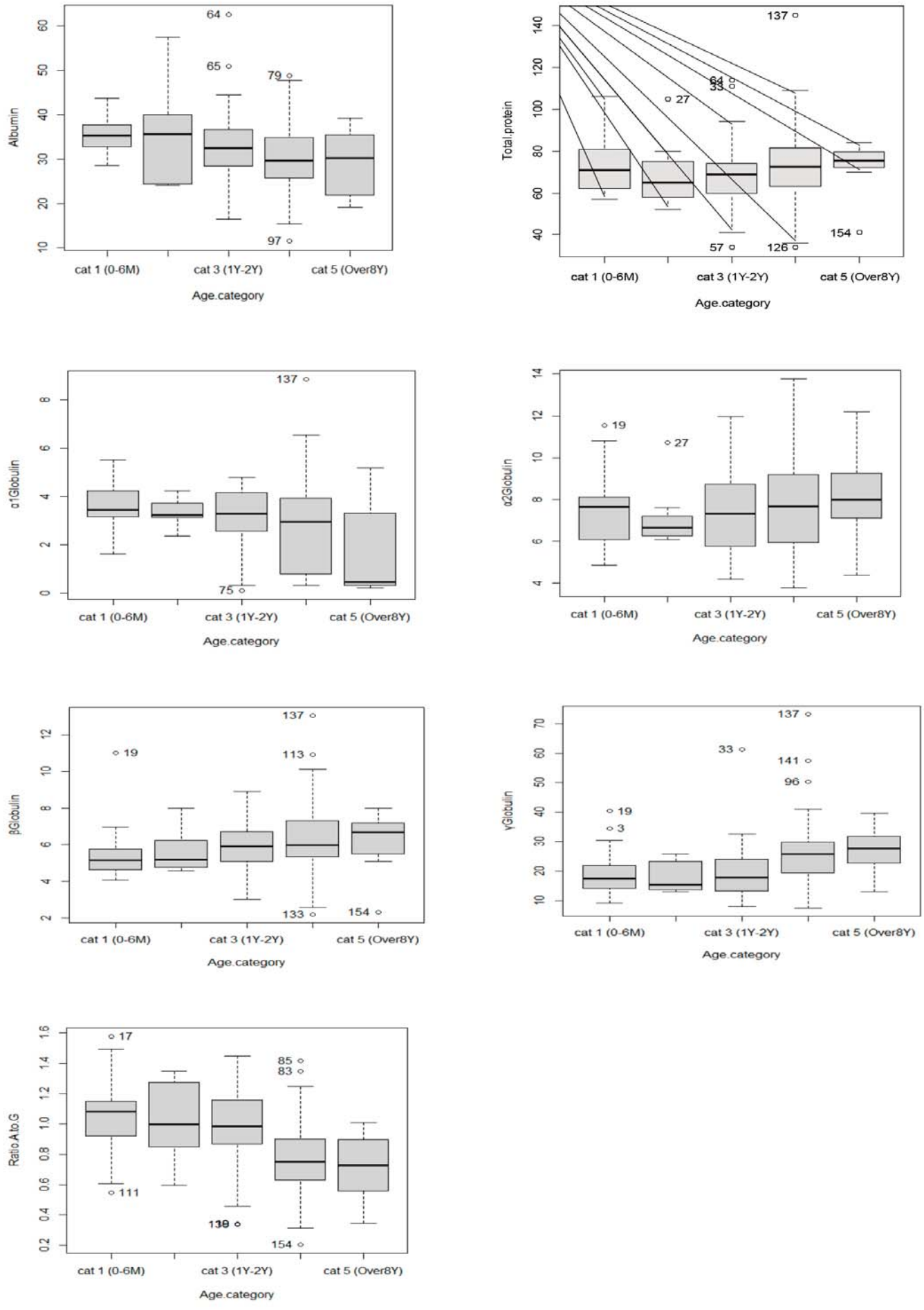

Figura 1 - Changes of serum protein fraction according to the age categories.

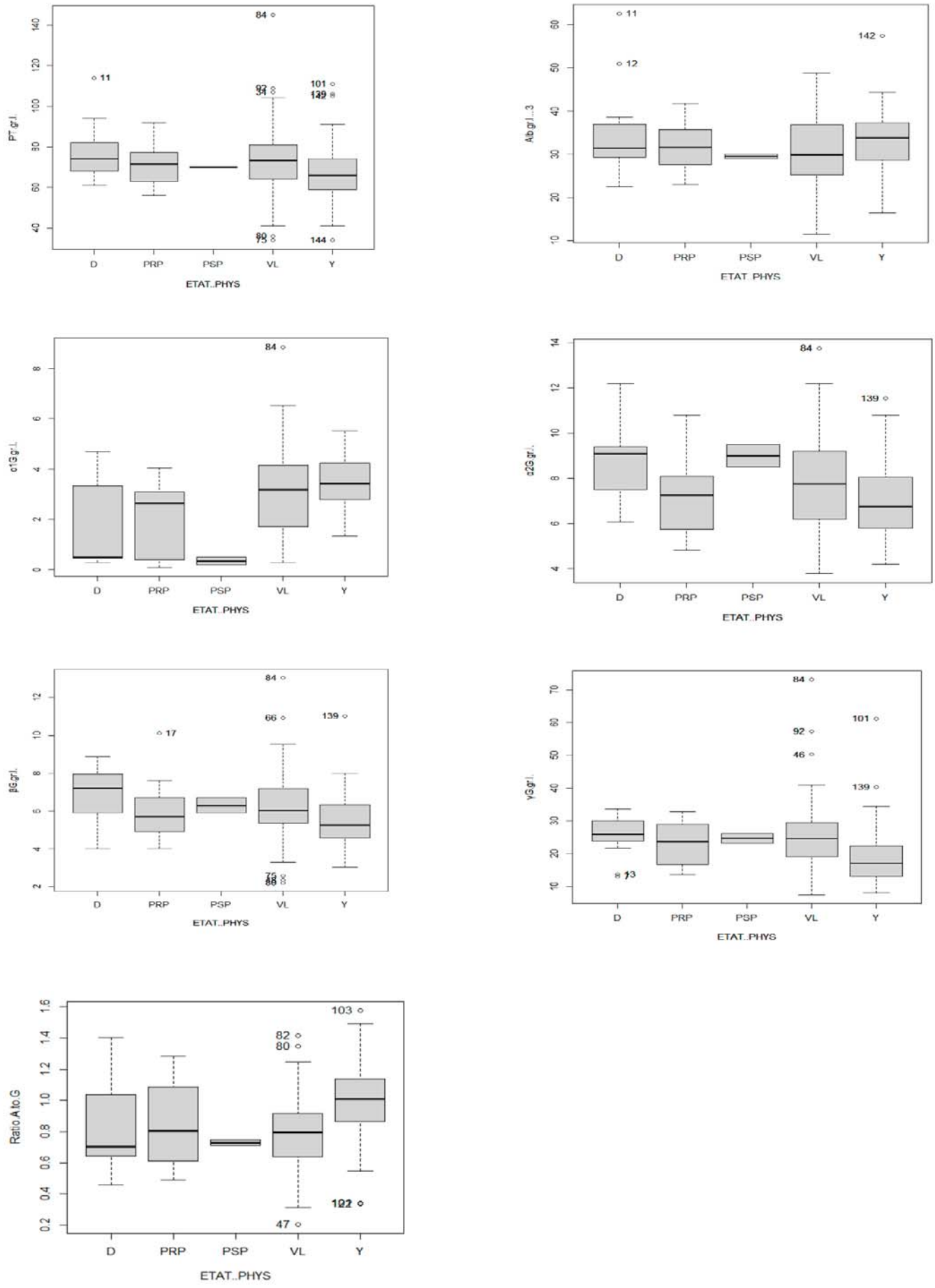

Figura 2 - Changes of serum protein fraction according to the physiological status.

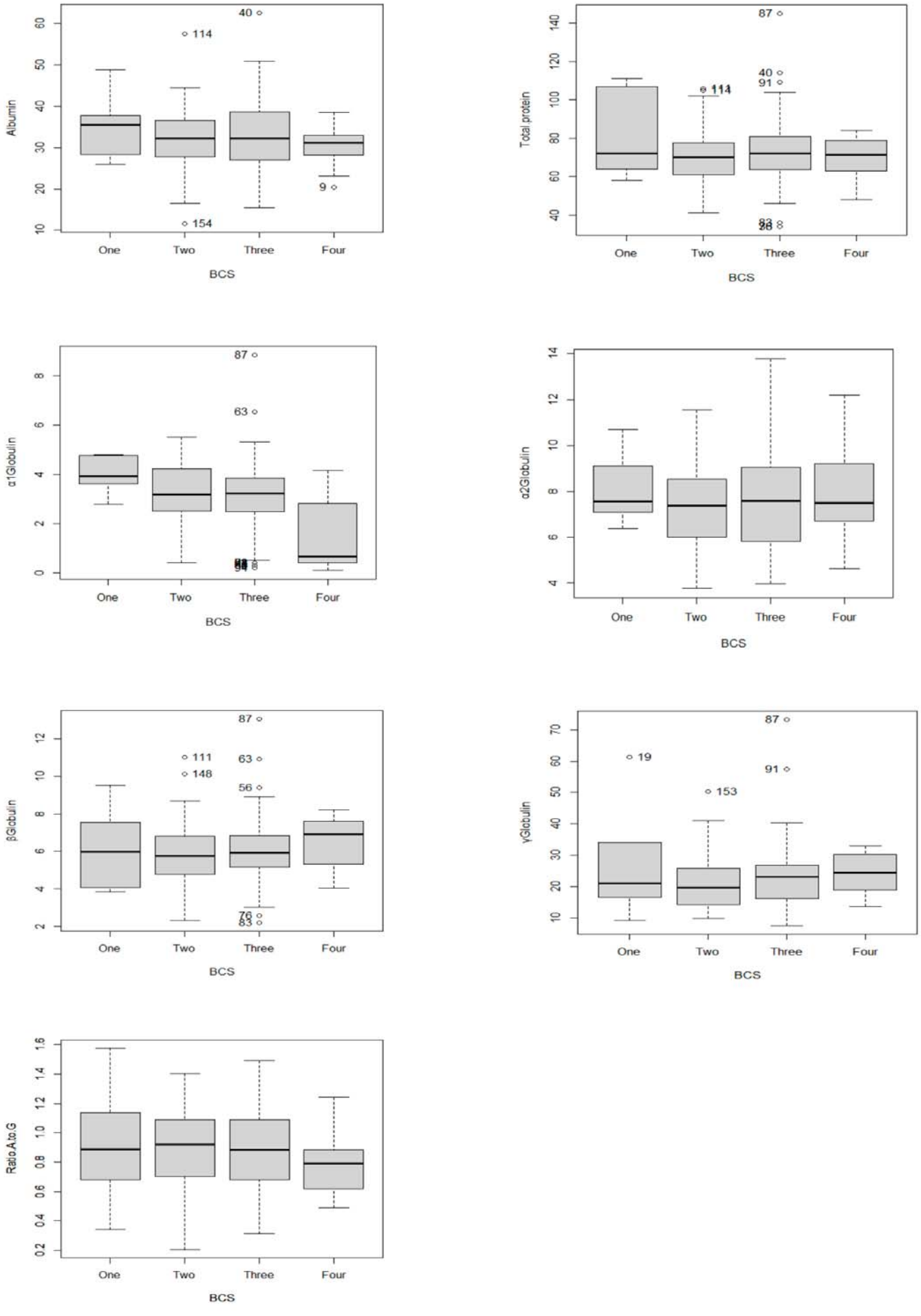

Figura 3 - Changes of serum protein fraction according to the (BCS).

colostrum and to provide the newborn animal with passive immunity (27,28). On the other hand, we note a high significant relation between the A/G ratios and the physiological status ($P < 0.0001$). The ratio differences observed in cows around the time of parturition may reflect the transport of immunoglobulins and some proteins to the mammary gland. The determination of A/G ratio in this period may be of great importance allowing the detection of changes in serum proteins and may serve as an indicator of hepatic functions (10). We observed no marked fluctuation in the concentrations of Albumin in dairy cows in the period from before to after calving. But some authors, similarly to our results, found a general decrease of albumin concentrations (29, 1). The Albumin and TP (total proteins) did not differ significantly between the physiological status stage of cattle. The higher concentrations of albumin in cows before parturition are difficult to interpret because of the decreased plasma volume in this period (9). Overall, our results are similar according with were previously found in cows during peripartum period (9). The lower concentrations of TP after calving may reflect the transfer of immunoglobulins from the bloodstream to the mammary gland for the synthesis of colostrum and milk (6). Also, our result reported in (Table 3, Figure 3) shown only α1 globulins zone have a high significant effect with different grade of BCS ($P < 0.001$). We did not find an influence about other parameters. Furthermore, some authors show a strong relationship between the decrease of the body condition score and the increase of acute phase proteins in dairy cows around parturition which indicates the process of a certain form of non-specific inflammation at loss of body fat (11). This could suppose the involvement of the different components of this fraction in changes occur in the energy balance status, since dairy cows need to mobilize body reserves to meet the nutritional demands of milk synthesis or lost body weight in scarcity season, causing negative energy balance until nutrient intake covers the demands (11).

CONCLUSION

Our findings represent a meaningful contribution to the studies of electrophoretic separation of serum proteins in cattle, because a larger animal numb are used for quantifications of serum protein fractions in order to yield satisfactory results. Presented results showed a marked influence of age on the concentrations of several serum protein fractions. The most marked age-related differences between different categories were observed in the concentrations of α1- , γ-globulins, Albumin and the ratio A/G. This shift in the concentrations of albumin and globulin fractions suggests that the age of evaluated animals should be taken into consideration when interpreting serum protein electrophoretic profiles. For the physiological status significant changes were found in all evaluated variables during the study stages. Except for Albumin and total proteins. They reflect the physiological period of evaluated animals should be taken into consideration when interpreting serum protein profile. And the consequence of the organism response to changes occurring in this critical period and may be related to during the pre-partum, post-partum, lactation period and dry-period.

Except, the concentrations of α_1-globulins were higher significant in BCS and different physiological status than others. The results confirm the need for age and physiological status a specific reference values also for these variables when considering precise interpretation of laboratory results with respect to dysproteinemias. The obtained data would be useful for clinicians in the comparison between healthy and ill cattle for dysproteinemias and various physiological conditions, providing a basis for further specific laboratory investigations.

Conflict of interest

The authors declare that they have no competing interests.

Authors contribution

NA conceived and designed the project. AKM executed the experiment and analyzed the blood samples. KS analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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