Research in Veterinary Science 189 (2025) 105630

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Modeling climate change effects on some biochemical parameters in horse

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ARTICLE INFO

Keywords:
Biochemical profile
Blood biomarkers
Climate change
Global warming
Horse
Seasons

ABSTRACT

Changes in the global climate pose a severe threat to human and animal welfare and productivity.

Total proteins (TP), globulins (GLOB), albumins (ALB), alkaline phosphatase (ALP), creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), blood urea nitrogen (BUN), γ-glutamyl transferase (GGT), and creatinine (CREA) were evaluated during a three-year monitoring period (2021−23) on 16 Thoroughbred retired mares from the regional Golkoy Breeding Farm in Kastamonu- Turkey. The following thermal and hygrometric parameters were gathered: ambient temperature (AT), relative humidity (RH) and ventilation (VT), and the Temperature-Humidity Index (THI) was then calculated. Blood samples were collected on the first of each month from January 2021 to December 2023 and the obtained serum was used for the analysis, variations in environmental parameters were correlated to changes in biochemical profile. Two-way for repeated measure ANOVA showed a significant effect of month for AT (<0.0001), RH (<0.0001), and THI (<0.0001), and on TP ($p < 0.001$), GLOB ($p < 0.001$), ALB ($p < 0.0001$), ALP ($p < 0.01$), CK ($p < 0.01$), LDH ($p < 0.001$), AST ($p < 0.0001$), BUN ($p < 0.0001$), GGT ($p < 0.0001$), and CREA ($p < 0.0001$). ALP, CK, LDH, AST, and BUN values increased during the hottest periods, while GGT showed decreasing values during the summer. CREA showed positive correlation with AT, and LDH and CREA exhibited negative correlation with RH. These results may be useful for the monitoring of horses' physiological conditions as a result of climate change.

1. Introduction

Several examples of changes in meteorological characteristics brought on by climate change have shown themselves in the past century as temperature variability and extreme weather. (Luna-Cerón et al., 2024). Compared to 1850–1900, the Earth's surface temperature rose by approximately 1.1 °C (Tollefson, 2021). Accompanying this, extreme heat events now occur 4.8 times more frequently than during the period 1850–1900 (Ebi, 2022). Meanwhile, other extreme climates, such as cold waves, droughts, floods, etc., are also becoming increasingly frequent. Periods of extreme cold occur in some northern mid-latitudes in winter, possibly due to disruption of the stratospheric polar vortex regulated by biological rhythms (Cohen et al., 2021; Aragona et al., 2024a). Extreme temperatures associated with weather change can induce denaturation of proteins affecting metabolism and membrane characteristics, alteration of core body temperature, organ damage and increase the susceptibility to the development of cardiovascular and renal diseases as well as the increasing risk of heatstroke, myocardial infarction and mortality in humans (Zhang et al., 2023; Hashim, 2010; Brownlow and Mizzi, 2023; Lacetera, 2019). Climate change is expected to exert an overwhelming negative effect on livestock health and welfare. Effects of climate change on animals may be due primarily to increased temperatures and frequency and intensity of heat waves (Zakari et al., 2015). It was previously shown that severe weather events could negatively affect the hematological profile, reproduction, athletic performance, physiological adaptability to exercise (Satué et al., 2014; Koubkova et al., 2002; Zakari et al., 2015; Deniz et al., 2024a) and physiological adaptability to transport and stressful events in horses (Aragona et al., 2024b, 2024c; Deniz et al., 2024b; Piccione et al., 2001; Kim et al., 2023; Medica et al., 2017). The health status of organs and proper functioning of the organism are carried out by monitoring hematological and biochemical indicators over time, providing useful information regarding performance and fitness in horses (Hinchcliff et al., 2005; Bhat et al., 2023; Andriichuk and Tkachenko, 2015; Piccione

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https://doi.org/10.1016/j.rvsc.2025.105630
Received 13 December 2024; Received in revised form 24 March 2025; Accepted 25 March 2025
Available online 26 March 2025

Table 1
Seasonal three-year monitoring data for ambient temperature (°C), relative humidity (%), ventilation (m/s), and THI. Superscript letters denote significance of Bonferoni post hoc tests: ^{a} vs January, ^{b} vs February, ^{c} vs March, ^{d} vs April, ^{f} vs June, ^{g} vs July, ^{h} vs August, ^{i} vs September, ^{m} vs October, ^{n} vs November.

	Experimental conditions		AMBIENT TEMPERATURE (°C)	RELATIVE HUMIDITY (%)	VENTILATION (m/s)	Temperature-humidity index (THI)
2021	WINTER	January	1	80	6.55	34.6
		February	2.25	79	6.51	38.60
		March	7	75	6.98	46.45
	SPRING	April	13.75 ^{abc}	70 ^{abc}	6.8	56.94 ^{abc}
		May	14.75 ^{abcd}	70 ^{abc}	5.63	58.55 ^{abcd}
		June	19.5 ^{abcd}	67 ^{abc}	7.11	67.1 ^{abcd}
	SUMMER	July	19.75 ^{abcde}	62 ^{abcde}	6.12	65.51 ^{abcde}
		August	14.5 ^{abcde}	59 ^{abcde}	6.37	58.06 ^{abcde}
		September	9.75 ^{abcde}	62 ^{abcde}	4.71	51.32 ^{abcde}
	AUTUMN	October	6.5 ^{abcefghi}	68 ^{abcegi}	4.59	46.23 ^{abcefghi}
		November	2 ^{abcfghi}	72 ^{abcegi}	5.84	39.07 ^{abcfghi}
		December	1 ^{cefghimn}	82 bc^{cefghimn}	6.31	36.21 ^{cefghimn}
	Experimental conditions		AMBIENT TEMPERATURE (°C)	RELATIVE HUMIDITY (%)	VENTILATION (m/s)	Temperature-humidity index (THI)
2022	WINTER	January	−1.5	80	6.45	32.48
		February	0.75	79	5.42	36.22
		March	0	75	7.01	46.4
	SPRING	April	9.25 ^{abc}	70.51 ^{abc}	7.51	50.17 ^{abc}
		May	12.25 ^{abcd}	70.46 ^{abc}	6.1	54.68 ^{abcd}
		June	16.75 ^{abcd}	68.69 ^{abc}	6.57	62.15 ^{abcd}
	SUMMER	July	18.75 ^{abcde}	62 ^{abcde}	6.76	65.75 ^{abcde}
		August	22.5 ^{abcde}	59 ^{abcde}	5.15	69.09 ^{abcde}
		September	16.5 ^{abcde}	62 ^{abcde}	5.84	60.90 ^{abcde}
	AUTUMN	October	11 ^{abcefghi}	68.1 ^{abcegi}	5.43	52.88 ^{abcfghi}
		November	7.25 ^{abcfghi}	75 ^{abcegi}	4.94	47.14 ^{abcfghi}
		December	4 ^{cefghimn}	82 ^{bccefghimn}	4.29	41.07 ^{cefghimn}
	Experimental conditions		AMBIENT TEMPERATURE (°C)	RELATIVE HUMIDITY (%)	VENTILATION (m/s)	Temperature-humidity index (THI)
2023	WINTER	January	1.75	81	4.03	37.55
		February	0.75	79	7.89	36.22
		March	4.5	75	6.23	42.57
	SPRING	April	8.5 ^{abc}	71 ^{abc}	6.61	49.1 ^{abc}
		May	11.5 ^{abcd}	70.36 ^{abc}	4.5	53.57 ^{abcd}
		June	16.5 ^{abcd}	68.73 ^{abc}	4.88	61.05 ^{abcd}
	SUMMER	July	19.75 ^{abcde}	63 ^{abcde}	6.39	65.57 ^{abcde}
		August	22.25 ^{abcde}	59 ^{abcde}	6.86	68.83 ^{abcde}
		September	18.75 ^{abcde}	62 ^{abcde}	6.53	64.09 ^{abcde}
	AUTUMN	October	12 ^{abcefghi}	68 ^{abcegi}	5.62	54.37 ^{abcfghi}
		November	6.75 ^{abcfghi}	76 ^{abcegi}	9.3	45.99 ^{abcfghi}
		December	3.5 ^{cefghimn}	82.13 ^{bccefghimn}	3.92	40.26 ^{cefghimn}

et al., 2025). Some biochemical indices concerning to muscular and metabolic function include plasma proteins and specialized enzymes catalyzing specific reactions (Alberghina et al., 2016; Satué et al., 2014; Fazio et al., 2023; Aragona et al., 2024d). These parameters, including CREA and BUN values, naturally undergo external influence due to seasonality and climatic changes, leading to decline in vital organs function and welfare as observed in humans (Aimo et al., 2022). Increasing knowledge of specific metabolic changes during seasons, over time, in vital organs offers the possibility to improve performance and welfare in horses. This may provide insight into how alterations in environmental exposures related to climate change and seasonal variations affect critical physiological markers that govern health status, diagnosis, and performance. This objective of the present study was achieved by monitoring a 3-year period and the possible correlation among the investigated parameters (Total proteins- TP, globulins- GLOB, albumins- ALB, alkaline phosphatase -ALP, creatine kinase- CK, lactate dehydrogenase- LDH, aspartate aminotransferase- AST, blood urea nitrogen- BUN, γ-glutamyl transferase- GGT and creatinine CREA) in order to observe the seasonal variation of these parameters over time and the possible effects of increased global warming on the horse's physiological status. The assessment and examination of seasonal variations in physiological markers enhance comprehension of the impact of heat fluctuations on physiology and related acclimatization/adaptation in mammals. Since it is already known that the parameters of the biochemical profile can be affected by seasonal changes and environmental variations in humans, then it would be interesting to observe the response of these same parameters in the horse as that may represent a predictive index related to the proper functioning of the organism.

2. Materials and methods

The present experimental protocol was approved by Kastamonu University Animal Experiments Local Ethics Committee (Decision no: 2024/52).

16 healthy, non-pregnant retired Thoroughbred mares aged between 10 and 20 years old and with a mean body weight of 560 ± 20 kg were enrolled in the study as a convenience sample, from the regional Golkoy Breeding Farm in Kastamonu- Turkey (Latitude: 41.371; Longitude: 33.7756; Altitude: 800.0 m). All mares were housed into individual boxes (6 and 12 m^2) and turned out to adjacent paddocks for a number of hours depending on seasons and weather conditions and were managed by experienced stable staff. Prior to the start of the study, horses underwent physical and clinical examination to establish health status (body temperature, heart rate, respiratory rate) to be included in the study. Horses with diseases or altered clinical examination, pregnant or exercising horses were excluded. Each horse was regularly dewormed every three month and the annual vaccinations against influence and Tetano were delivered two weeks apart from the blood sampling to avoid any confounding effects on the blood parameters caused by immune activation. Mares were fed with good quality hay and concentrates: 44 % oatmeal (2.64 kg), 24 % crushed barley (1.44 kg), 12 % maize (0.72 kg), 15 % soybean meal (0.9 kg), 4 % bran (0.24 kg), salt, concentrated pellet feed (6 kg), dry grass (5 kg), alfalfa (4 kg) twice a day at 06:00 and 18:00 and water was provided ad libitum. Ambient temperature- AT °C; relative humidity- RT %; and ventilation- VT m/s were obtained by the Kastamonu Meterology Directorate to monitor microclimatic conditions during the experimental protocol. The

Table 2
Mean and Standard Deviation (SD) of ALP, CK, LDH, AST and CREA investigated each month during the seasonal three-years monitoring period. Superscript letters denote significance of Bonferoni post hoc tests: ^{a} vs January, ^{b} vs February, ^{c} vs March, ^{d} vs April, ^{f} vs June, ^{g} vs July, ^{h} vs August, ^{i} vs September, ^{m} vs October, ^{n} vs November.

	Experimental conditions		ALP		CK		LDH		AST		CREA	
2021	WINTER	January	116.3	25.43	214.4	36.9	265.9	49.38	280.1	25.25	1.215	0.12
		February	128.4	22.06	224.6	40.3	280.2	36.94	301.4	27.24	1.358	0.12
		March	137.6	25.62	194.9 ^{b}	27.48	264.1	39.46	310.3	38.72	1.196 ^{b}	0.11
	SPRING	April	140.3 ^{a}	18.75	190.5 ^{b}	35.17	290.9	31.26	305.5	25	1.187 ^{b}	0.11
		May	121.7 ^{d}	15.11	230.6	55.52	252.9 ^{d}	34.46	304.8	32.61	1.236 ^{b}	0.09
		June	129.7	21.57	232.4	56.7	284.8	53.51	311.6	31.26	1.222 ^{b}	0.14
	SUMMER	July	125.6	25.96	215.9	50.87	307.3 ^{e}	63.06	306.2	31.65	1.241	0.10
		August	138.1	24.78	180.8	34.18	289.6	59.49	280.4	29.14	1.301	0.13
		September	123.7 ^{h}	24.5	184.6 ^{f}	43.21	267.9	58.32	271.8 ^{f}	24.64	1.278	0.14
	AUTUMN	October	122.6	25.74	211.8	42.81	287.1	59.34	277.4	25.9	1.254	0.14
		November	122.5	19.38	208.4 ^{h}	32.27	270.6 ^{m}	53.76	276	24.61	1.268	0.13
		December	122.3	21.84	194	34.9	252.5 ^{d}	42.43	286.6	38.57	1.108 ^{bh}	0.11
	Experimental conditions		ALP		CK		LDH		AST		CREA	
2022	WINTER	January	115.4	16.31	207.6	43.69	255.5	43.87	283.6	41.56	1.213	0.11
		February	137.6	20.82	214.8	41.56	282.3	44.76	304	31.01	1.266	0.13
		March	135.9	22.73	227.4	54.18	295.9	46.87	321.9	31.17	1.164 ^{b}	0.08
	SPRING	April	131.1	21.88	213.8	53.09	287.1	48.78	312.5	28.03	1.236	0.09
		May	126	20.65	206.8	34.93	253 ^{d}	55.55	315.1	30.15	1.161 ^{b}	0.12
		June	122.3	23.2	179.9 ^{be}	31.99	238.5 ^{bcd}	44.29	303.1	24.84	1.329 ^{acde}	0.12
	SUMMER	July	122.8	26.24	201.1	38	258.2	46.67	294.4	34.8	1.399 ^{bcde}	0.10
		August	126.9	24.86	216.4	50.46	338.9 ^{abdefg}	46.56	299.4	37.78	1.376 ^{ace}	0.12
		September	120.1	18.46	210.1	38.68	264.1 ^{h}	41.96	283.6	28.15	1.377 ^{ce}	0.16
	AUTUMN	October	128.2	26.96	211.2	41.38	271.3 ^{h}	45.93	277.9 ^{cde}	22.9	1.188 ^{gi}	0.13
		November	119.9	24.47	208.1	36.5	281.3	50.02	295.1	40.7	1.178 ^{fghi}	0.09
		December	115.5	22.05	202	36.87	244.4 ^{hn}	41.83	288.8	35.23	1.223 ^{gi}	0.10
	Experimental conditions		ALP		CK		LDH		AST		CREA	
2023	WINTER	January	112.8	23.95	223.2	53.5	260.9	41.52	277.8	21.41	1.181	0.11
		February	133.6	30.25	222.5	48.9	268.9	44.24	294.3	35.49	1.215	0.11
		March	130.1	22.62	208.7	30.47	242.1	40.01	299.1	36.01	1.248	0.10
	SPRING	April	129.6	29.78	181 c	31.28	252.5	39.9	286.7	29.81	1.278	0.11
		May	123.3	19.13	211.5	48.29	276.4 ^{c}	49.58	310.8 ^{d}	34.35	1.284	0.12
		June	124.7	19.03	177.6 c	39.63	271.9 ^{c}	49.52	297.4	31.69	1.289	0.11
	SUMMER	July	128.6	25.26	191.8	41.43	292.5	51.24	295.9	37.92	1.331	0.10
		August	137.5	28.5	209.6	35.23	288.8	60.8	300.1	35.68	1.186 ^{g}	0.08
		September	132.9	30.91	183.2 h	27.32	253.4 ^{h}	43.99	276.4	36.62	1.154 ^{g}	0.09
	AUTUMN	October	123.4	25.36	209.1 ^{i}	47.75	275.6 ^{i}	55.78	269.3	37.87	1.226 ^{i}	0.10
		November	120.3 ^{h}	27.29	181.2	42.74	273.8	52.63	268.3	41.68	1.139 ^{g}	0.12
		December	116.4	19.37	201.5	46.58	272.3	47.49	274.2 ^{e}	29.71	1.06 ^{bcdefghimn}	0.09

temperature- humidity index (THI) was calculated according to the formula reported by Thom (1959):

$$THI = [0.8 \times AT + (RH/100) \times (AT–14.4) + 46.4]$$

(Hartmann et al., 2015; Mader et al., 2006)

2.1. Blood sampling.

Blood samples were collected via jugular venipuncture into 4 ml vacutainer tubes (Terumo Corporation, Japan) with clot activator before the morning feeding (05:30) (hh.mm) every first day of each month from January 2021 to December 2023. Serum was obtained by centrifugation at 3000 r.p.m. for 10 min and placed in separate eppendorf test tubes at −20 °C until analysis, performed when each sample at the end of experimental period was collected. From the obtained sera TP, GLOB, ALB, ALP, CK, LDH, AST, BUN, GGT and CREA concentration were determined. The obtained sera were analyzed to assess the concentration of TP using a specific kit (Respons 910–12,311 99 10,920) working on photometric test according to biuret method Proteins form a violet blue color complex with copper ions in alkaline solution. The absorbance of the color is directly proportional to the concentration. GLOB and ALB parameters were analyzed by means of a specific kit (Respons 910–10,220 99 10,923) using a photometric test with bromocresol green. ALP and AST were analyzed by using a specific kit (Respons 910–10,441 99 10,920; 126,019,910,920) using a kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). CK and LDH were analyzed by a specific kit (Respons 910–11,641 99 10,921; 14,251 99 10,920) working on the otimized UV test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) for CK and LDH. BUN was analyzed by a specific kit (Respons 910–13,101 99 10,920) using enzymatic photometric test in which, in the first step, the substrate urea is hydrolyzed by urease to ammonium and bicarbonate ions. In the presence of 2-Oxoglutarate and NADH, the ammonium ions are catalyzed by glutamate dehydrogenase (GLDH). The amount of reduced NADH, measured by the change of absorption at 340 nm, is proportional to the amount of urea present in the sample. GGT was analyzed by a specific kit (Respons 910–12,801 99 10,920) using kinetic photometric test according to IFCC (International Federation of Clinical Chemistry). CREA was analyzed using a specific kit (Response 910–11,711 99 10,920) using kinetic test without deproteinization. Serum biochemical parameters were evaluated using a DiaSys respons- 910 Vet Analyzer (DiaSys Diagnostic System, USA, Wixom, MI).

2.2. Statistical analysis

Normal distribution of the data was tested using Kolmogorov-Smirnov tests. Two-way repeated measures analysis of variance (ANOVA) followed by Bonferroni post-hoc multiple comparison tests was performed to investigate the effect of month (season) and year on the analyzed parameters.

Regression lines between the monthly values of each biochemical parameter and, 95 % confidence interval for monthly mean AT and RH for every year were determined and Pearson correlation coefficient (r)

Table 3
Mean and Standard Deviation (SD) of TP, GLOB, ALB, BUN and GGT investigated each month during the seasonal three-years monitoring period. Superscript letters denote significance of Bonferroni post hoc tests: ^{a} vs January, ^{b} vs February, ^{c} vs March, ^{d} vs April, ^{f} vs June, ^{g} vs July, ^{h} vs August, ^{i} vs September, ^{m} vs October, ^{n} vs November.

	Experimental conditions		TP		GLOB		ALB		BUN		GGT	
2021	WINTER	January	6.706 ^{b}	0.309	2.738	0.303	3.969	0.162	13.81	1.559	23.38	3.757
		February	6.631	0.433	2.444	0.416	4.188	0.255	14	1.862	24.19	3.351
		March	6.806	0.537	2.444	0.416	4.188	0.255	15.31	2.056	26.81 ^{b}	3.229
	SPRING	April	7.038	0.501	3.044 ^{bc}	0.466	3.994	0.165	16.13 ^{ab}	2.363	28.5 ^{b}	3.615
		May	6.556 ^{cd}	0.483	2.444 ^{d}	0.412	4.113	0.203	13.63 ^{cd}	1.408	26.38	3.828
		June	7.125 ^{bce}	0.437	3.056 ^{bce}	0.487	4.069	0.196	18.38 ^{abcde}	1.962	24.63 ^{de}	3.757
	SUMMER	July	6.9 ^{bef}	0.446	3.031 ^{bce}	0.487	3.869 ^{bcdef}	0.178	19.13 ^{abcde}	1.586	22.5 ^{cdef}	3.367
		August	6.7	0.426	2.419 ^{d}	0.504	4.281 ^{adg}	0.187	16.31 ^{aeg}	1.815	21.06 ^{cde}	2.816
		Sptember	6.975 ^{h}	0.482	2.713 ^{h}	0.487	4.263 ^{ag}	0.2	18.56 ^{abce}	2.555	23.13 ^{cde}	2.217
	AUTUMN	October	6.913 ^{h}	0.44	2.638 ^{h}	0.449	4.275 ^{adg}	0.202	17.19 ^{abei}	2.639	22 ^{cde}	2.129
		November	6.6 ^{fim}	0.371	2.506	0.414	4.094 ^{him}	0.173	15.81 ^{gim}	2.509	24.25 ^{dh}	2.543
		December	6.363 ^{cdfgim}	0.437	2.419 ^{dfg}	0.432	3.944 ^{him}	0.109	15.88 ^{afg}	1.821	23.38 ^{cd}	2.446
	Experimental conditions		TP		GLOB		ALB		BUN		GGT	
2022	WINTER	January	6.594	0.37	2.425	0.427	4.169	0.185	16.25	1.844	24.31	3.554
		February	6.956	0.479	2.381	0.497	4.581 ^{a}	0.323	15.75	1.528	26.75	4.123
		March	7.056	0.514	2.525	0.584	4.531 ^{a}	0.258	14.56	1.315	26.38	2.729
	SPRING	April	6.888	0.48	2.463	0.561	4.425 ^{a}	0.291	15	1.966	25.69	2.626
		May	7.12 ^{a}	0.312	2.513	0.535	4.519 ^{a}	0.254	15.63 ^{c}	1.455	24.44	3.521
		June	6.756 ^{c}	0.568	2.388	0.599	4.369 ^{abc}	0.241	15.69 ^{c}	1.448	23.19 ^{d}	2.198
	SUMMER	July	6.494 ^{e}	0.43	2.588	0.479	3.906 ^{abcdef}	0.205	16.81 ^{c}	1.424	21.5 ^{bcd}	3.327
		August	6.625	0.553	2.675	0.521	3.95 ^{bcdef}	0.231	17.25 ^{c}	1.183	22.94 ^{bc}	4.219
		September	7.05	0.518	3.119 ^{abdf}	0.544	3.931 ^{abcdef}	0.224	17 ^{c}	1.897	21.5 ^{bcd}	2.28
	AUTUMN	October	6.975	0.503	3.056 ^{abf}	0.532	3.919 ^{abcdef}	0.211	16.31 ^{c}	1.778	20.63 ^{bcd}	3.052
		November	6.563 ^{eim}	0.411	2.613 ^{im}	0.44	3.95 ^{abcdef}	0.193	17.88 ^{cm}	1.746	24.56 ^{im}	1.825
		December	6.663 ^{im}	0.423	2.6 ^{im}	0.438	4.063 ^{bcdeim}	0.216	17.5 ^{cm}	2.309	23.56 ^{m}	2.25
	Experimental conditions		TP		GLOB		ALB		BUN		GGT	
2023	WINTER	January	6.919	0.442	3.044	0.424	3.875	0.224	15.75 ^{a}	1.571	24.13	2.872
		February	6.763	0.622	3.019	0.512	3.819	0.172	18	1.414	25.13	3.594
		March	6.8	0.622	2.994	0.584	3.806	0.244	19.13 ^{a}	1.668	24.44	2.988
	SPRING	April	6.175 ^{abc}	0.463	2.55 ^{c}	0.52	3.625 ^{ac}	0.195	16.31 ^{c}	1.852	24.19	4.135
		May	6.875 ^{d}	0.587	3.094 ^{d}	0.63	3.781 ^{d}	0.214	19.63 ^{ad}	2.094	25.75	3.697
		June	6.488 ^{de}	0.573	2.775 ^{de}	0.581	3.713	0.189	17.56 ^{ce}	1.75	24.38	3.757
	SUMMER	July	6.325 ^{a}	0.515	2.75	0.574	3.575 ^{ab}	0.161	19.25 ^{ad}	1.949	23.25	3.661
		August	6.575 ^{d}	0.373	2.663	0.528	3.913 ^{g}	0.26	17.19 ^{a}	1.515	22.81	3.92
		September	6.613 ^{d}	0.497	2.731	0.553	3.881 ^{dg}	0.183	17.56 ^{a}	1.548	19.56 ^{abcdefh}	2.421
	AUTUMN	October	6.863 ^{dhi}	0.469	2.931 ^{hi}	0.521	3.931 ^{dg}	0.178	19.31 ^{adhi}	1.58	20.94 ^{abcef}	2.407
		November	6.794 ^{d}	0.455	3.069 ^{dhin}	0.49	3.725 ^{im}	0.257	19.38 ^{adhi}	1.821	20 ^{abcefh}	3.055
		December	6.475 ^{mn}	0.477	2.638 ^{m}	0.388	3.838	0.303	19.13 ^{ahi}	1.408	23.94 ^{aimn}	2.462

evaluated. Data were analyzed using statistical software Graph Pad Prism v. 9.5.1 (Graphpad Software Ldt., USA). Data were reported as means ± standard deviation (SD) and a p-value less than 0.05 was considered statistically significant.

3. Results

The analyzed data were normally distributed ($p > 0.05$). The values for all investigated parameters during the monitoring period were within the reference ranges for horses (Kaneko et al., 2008; Hinchcliff et al., 2005). The month variable, showed a significant effect on AT (<0.0001), RH (<0.0001), and THI (<0.0001). Table 1 presented the results of a Bonferroni post-hoc multiple comparison of environmental characteristics. The two-way for repeated measures ANOVA showed a significant effect of month on TP ($p < 0.001$), GLOB ($p < 0.001$), ALB ($p < 0.0001$), ALP ($p < 0.01$), CK ($p < 0.01$), LDH ($p < 0.001$), AST ($p < 0.0001$), BUN ($p < 0.0001$), GGT ($p < 0.0001$) and CREA ($p < 0.0001$). Bonferroni post-hoc multiple comparison of biochemical parameters was shown in Table 2–3. A significant effect of year was observed for GLOB ($p < 0.01$), ALB ($p < 0.0001$), and BUN ($p < 0.0001$) as shown in Fig. 1. No significance was observed for VT. During the experimental period, a positive correlation between CREA ($p < 0.01$; $r = 0.34$) and AT has been observed and a negative correlation has been reported between GGT ($p < 0.01$; $r = -0.44$) and AT. The present results showed a positive correlation of RH with GGT ($p < 0.001$; $r = 0.46$), and a negative correlation of RH with LDH ($p < 0.01$; $r = -0.40$) and CREA ($p < 0.001$; $r = -0.48$) as shown in Fig. 2.

4. Discussion

Blood parameters are important tool that helps daily management to monitor health and metabolic status of horses. The biochemical profile in clinically normal horses is influenced by many factors such as animal's physiological status, physical activity, management system and seasons (Satué et al., 2014). The present study, examined seasonal fluctuations during a three-year period in the biochemical profile of horses as an indicator for possible climate change effect. A significant variation of monthly values was observed for all the investigated parameters during the three-years protocol. Seasonal changes affected TP, GLOB ALB concentrations in 2021, 2022, and 2023, showing a significant periodic monthly trend as observed in Table 3. In mammals, periodic oscillations in biological parameters are influenced by the presence of a biological clock, regulated by specific external synchronizers such as light, temperature, and climate changes (Coskun et al., 2023; Giannetto et al., 2022; Aragona et al., 2023; Refinetti, 2006). Biological rhythms of chemical constituents commonly measured in serum may indicate seasonal variations in metabolic functions or may have physiologic and pathophysiologic importance in humans. In accordance with previous studies, our results showed an increase in total proteins and globulins during the summer months over the three years of monitoring. The increase in total proteins could be a physiological attempt to maintain an expanded plasma volume. Variations in serum protein concentration have been observed in lactating cows and buffaloes during the spring and summer seasons. This increase during the summer period may be due to the rise in osmotic pressure exerted by globulins. High levels of

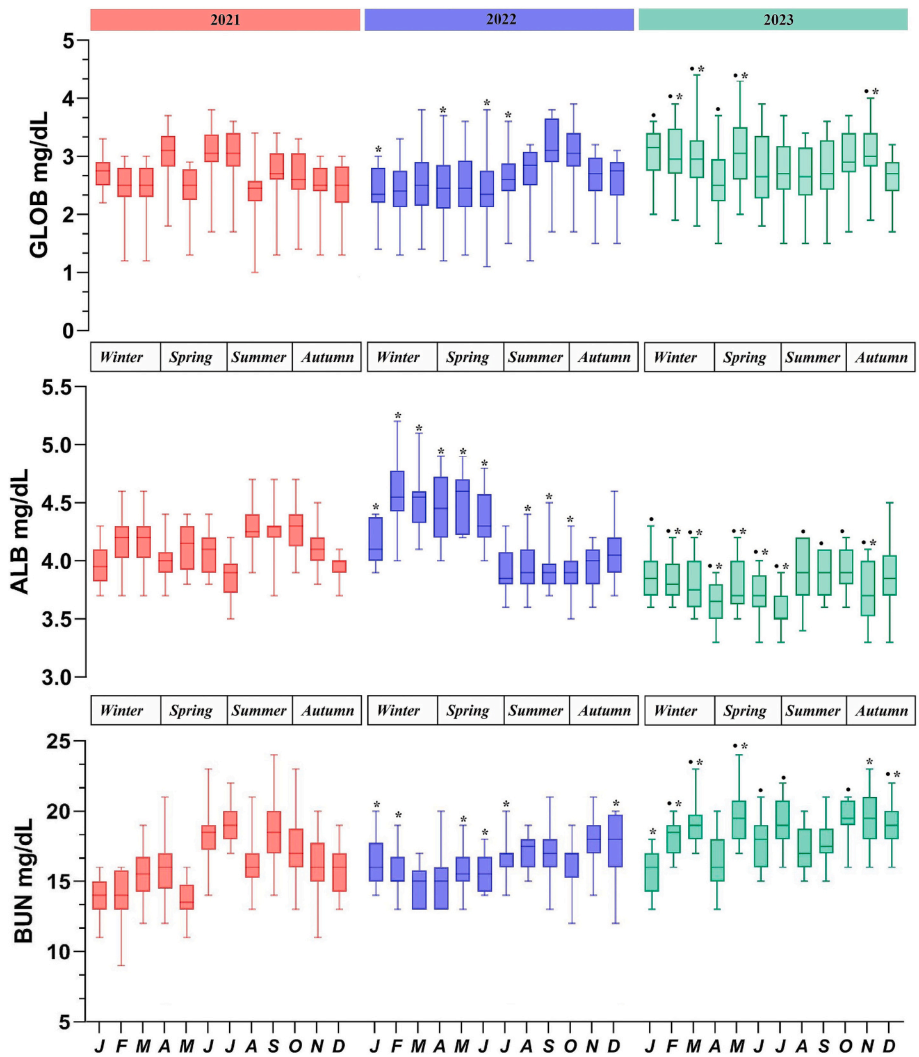

Fig. 1. Min and Max values ± standard deviation (± SD) of GLOB, ALB, and BUN obtained by a monthly monitoring during a three-years (2021–2023) period. Significances among years ($p < 0.05$): * vs 2021 • vs 2022.

globulins are almost always caused by dehydration. Dehydration leads to hemoconcentration through the reduction of fluid volume and the consequent hyperproteinemia. The increase in globulin and creatinine levels in the presence of high environmental temperatures suggests that this adaptation could be linked to a metabolic response as observed in cows (Mazzullo, 2014).

ALP, CK, LDH, AST and BUN showed increasing values in spring and summer, compared to winter and autumn, during the three- years monitoring periods (Gidlow et al., 1986).

The present results are in agreement with what has been reported in horses of different breeds that associate increases in these parameters in the summer months with muscle activity and balanced feed intake during seasonal variations (Vranković et al., 2015). This was in contrast to what obtained in humans that observed increasing CK values associated with low temperatures and consequently increased myocardial damage (Zhang et al., 2023; Fazio et al., 2023).

The changes in the mentioned parameters, which are considered indicators of liver function, could vary according to food intake during different seasons, in fact when food intake decreases in excessively hot periods, energy requirements could be met through hepatic gluconeogenesis and thus the increase in these parameters (Duda et al., 2020; Giannetto et al., 2025; Muthuramalingam et al., 2020). Moreover, AST have key roles in gluconeogenesis and formation of urea, explaining the consequent elevated BUN concentration (Oduye, 1976).

CREA showed higher values in February during 2021 consistently with previous findings in children and goats (Okere, 2022) and an opposite trend with increasing values was observed during summer 2022 and 2023. Recent studies have shown a seasonal variation with an increase in the summer months of CREA over 20 years in humans possibly due to the environmental temperature increasing (Aimo et al., 2022). Therefore, it would be appropriate to expand this study to a longer period of monitoring in the horse. During the three years period the highest GGT concentration was observed in winter. GGT enzyme catalyzes the transfer of glutamyl groups between peptides and is involved in glutathione reactions (Hinchcliff et al., 2005; Colakoglu et al., 2017; Abd Ellah et al., 2016). A decrease in blood glutathione peroxidase enzymes has been observed during the hottest seasons than during winter in cows and goats as well in humans (Alila-Johansson et al., 2003; Colakoglu et al., 2017). One explanation could be the haemoconcentration and haemodilution of intravascular substances caused by seasonal changes in vascular tone. The vascular tone in the summer is reduced due to high temperature. Conversely, the vascular tone increases in the winter due to low temperature (Miyake et al., 2009).

Global warming impact natural seasonal changes that reflect the physiological changes that horses undergo during the year. A significant effect of the year was observed for the studied parameters. GLOB showed a decrease from 2021 to 2022 during January, February, April,

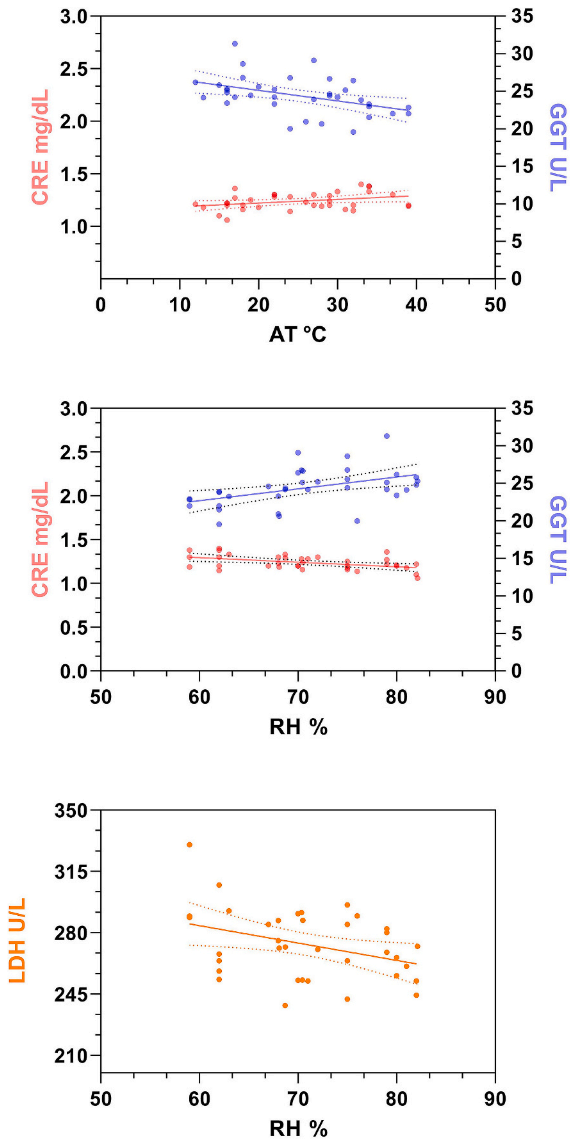

Fig. 2. Regression lines and Pearson correlation coefficient (*r*) between the significant monthly values of GGT, CREA and LDH and 95 % confidence interval for AT and RH for all years.

June and July and an increase from 2022 to 2023 during January and a decrease from 2022 to 2023 during April. Increasing values of GLOB were observed in 2023 compared to 2021 and 2022 during February, March, May and November. ALB significantly increased from 2021 to 2022 during winter and spring (January, February, March, April, May, June) and decreased during summer 2022 (August, September, October), while a general decreasing was observed during 2023 compared to 2021 and 2022. BUN showed an increase from 2021 to 2022 during January, February, March, April, May and December and an additional increasing was observed in 2023 during January, February, March, May, June, July, October, November and December.

The above mentioned parameters did not show a correlation with changes in environmental parameters, so the changes observed over the three years monitoring period could possibly be attributable more to periods of dehydration in spring and summer or simply to a dietary aspect of nutrition that inevitably can undergo changes over the years, also depending on the quality of the food itself, which as observed for the physiological aspects of the animals, is going to be negatively affected by climate changes. According to the obtained results, a positive correlation was found between increased environmental temperature and CREA as opposite to RH as well as LDH.

Similarly, GGT was observed to have an inversely proportional relationship with the increase in AT and proportional with the increase in RH. Environmental variations could regulate and influence body temperature, that influences mammals' physiological functions. Typically, an increase in body temperature correlates with heightened enzyme activity. Proteins in the body, such as enzymes, start to denature and lose functionality at elevated temperatures (Cao and Wang, 2016). Given that climate models forecast an increase in both the frequency and intensity of heat waves and cold spells in the forthcoming years, it is essential to comprehend and incorporate the potential molecular pathways responsible for organismal harm at elevated and reduced temperatures.

5. Conclusion

Biochemical parameters are used as biological markers to assess the health status of domestic animals. The investigated parameters in horses showed seasonal variation that can consequently influence hepatic and renal function. The three years of monitoring did not demonstrate a negative influence on the physiological response of the horse, considering the biochemical profile, but are a springboard for future studies to evaluate the impact of these parameters on the organism in the long term, in order to ensure the welfare of the horse linked to an unavoidable condition of climate change and global warming.

CRediT authorship contribution statement

Ömer Deniz: Investigation, Conceptualization. **Francesca Aragona:** Writing – original draft, Formal analysis, Data curation. **Gaetana Pezzino:** Visualization, Software, Conceptualization. **Enrico Cancellieri:** Visualization. **Serkan Bozaci:** Visualization. **Kenan Çağrı Tümer:** Visualization, Investigation, Conceptualization. **Francesco Fazio:** Writing – review & editing, Supervision, Methodology.

Declaration of generative AI and AI-assisted technologies in the writing process

The use of AI and AI-assisted technologies was not included during the writing process.

Funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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