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Differences in serum iron concentrations between the summer and winter in Noma horses

Keiichi HISAEDA[1], Tetsushi ONO[1,2], Takako SHIMOKAWA-MIYAMA[1], Akihisa HATA[1], Eri IWATA[1], Yasuharu HIASA[3], Emi OHZAWA[4], Teruaki TOZAKI[5], Harutaka MURASE[6], Masaki TAKASU[7,8], Naohito NISHII[9] and Hitoshi KITAGAWA[1,9]*

[1]Faculty of Veterinary Medicine, Okayama University of Science, 1-3 Ikoino-oka, Imabari, Ehime 794-8555, Japan
[2]Joint Faculty of Veterinary Medicine, Yamaguchi University,1677-1 Yoshida, Yamaguchi 753-8515, Japan
[3]Hiasa Animal Clinic, 2-6-8 Koushouji, Imabari, Ehime 794-0805, Japan
[4]Noma Horse Preservation Society, 8 Nomakou, Imabari, Ehime 794-0082, Japan
[5]Laboratory of Racing Chemistry, 1731-2 Tsuruta, Utsunomiya, Tochigi 320-0851, Japan
[6]Equine Department, Japan Racing Association, 1-1-1 Nishi-Shimbashi, Minato-ku, Tokyo 105-0003, Japan
[7]Gifu University Institute for Advanced Study, Gifu University, Gifu 501-1193, Japan
[8]Center for One Medicine Innovative Translational Research (COMIT), Gifu University, Gifu 501-1193, Japan
[9]Joint Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, Gifu 501-1193, Japan

We examined the differences in serum iron (Fe) concentrations and related variables between summer and winter in Noma horses. Blood samples were collected from 37 clinically normal horses seven consecutive times: September 2018, February 2019, October 2019, February 2020, September 2020, February 2021, and February 2022. Serum Fe concentrations ranged from 74 µg/dl to 316 µg/dl with a median of 176 µg/dl. The concentrations were lower in stallions compared with mares and geldings, tended to be low at 10–14 years of age, and then increased with age. Serum Fe concentrations were repeatedly low in summer and high in winter. Total iron-binding capacity (TIBC), Fe-saturation rate, hemoglobin (Hb), hematocrit (Ht), MCV, MCH, albumin, cholesterol, sodium (Na), potassium (K), chloride (Cl), and calcium (Ca) were lower in summer than in winter. However, creatinine, total protein, inorganic phosphorus, and Mg were higher in summer. The unsaturated iron-binding capacity, RBC count, blood urea nitrogen (BUN), glucose, and AST levels were not significantly different. Serum Fe concentrations were positively correlated with Hb, TIBC, Fe saturation rate, Ht, MCV, MCH, creatinine, albumin, glucose, cholesterol, AST, Na, Cl, and Ca, but negatively correlated with BUN and K. In Noma horses, serum Fe concentrations might be higher than the reference values for horses and consistently decrease in summer in parallel with Hb and MCV. The lowering of the serum Fe concentrations in summer may be due to a combination of the effects of Fe loss from sweating, dermatitis, insect bites, dietary composition, and/or unknown factors.

Keywords: *lowering in summer, Noma horse, seasonal change, serum iron concentration*

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The Noma horse is native to Japan. It has been used for working in lower mountainous areas and terraced agricultural fields due to its light gait, ability to withstand coarse foods, and capacity to carry a load of 70 kg over steep slopes. Since 1978, Noma horses have been kept as exhibition animals at a public ranch for conservation purposes. The number of Noma horses increased to 85 in 2008, but has been decreasing since then. We initiated periodic medical checkups for Noma horses in 2018, conducting them twice-

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*Corresponding author. e-mail: kitagawa.hitoshi.f1@a.gifu-u.ac.jp

yearly until 2020 and annually from 2021 onwards. Based on the results of these medical examinations, we have previously reported the reference values for Noma horses [27], seasonal and sex differences [26], and age differences [19]. However, we did not report the serum iron (Fe) concentrations. The concentration can be measured to assess Fe metabolism through its transport. The concentration of Fe increases in animals with hemolytic anemia and dyserythropoiesis and in those with Fe overload, and is generally low in animals with Fe deficiency, inflammation, and excess storage pools and following the administration of glucocorticoids. [14]. Excess and deficiency of Fe, as well as other trace elements, is also correlated with immune function [2, 10, 36]. Although these abnormalities are not observed in Noma horses, confirming Fe status may be important for health management.

Regarding seasonal alterations in serum Fe concentrations in horses, Hobo [15] compared the clinical test results of retired horses in spring (April to July) and autumn (October to January of the following year) and reported no major differences in serum Fe concentrations between these periods. Gromadzka-Ostrowska *et al.* [13] compared serum Fe concentrations in Shetland ponies across January, March, June, and October and reported that the concentrations decreased with age and tended to be higher in March. Noma horses are kept as exhibition animals and are not subjected to heavy exercise loads like racehorses. They do not show obvious symptoms of anemia, but laboratory test results indicate lower red blood cell (RBC) count, hemoglobin (Hb) concentrations, hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) values in the summer [26]. Therefore, we considered that climates exceeding the upper critical temperature for horses [7] lead to energy loss and a decline in nutritional condition. In humans, minerals such as Fe and magnesium are lost through excessive sweating in high temperatures or labor-intensive environments, and athletes, especially women who lose Hb-Fe through menstruation [6, 8], may experience changes in serum Fe concentrations due to environmental changes. In this study, we examined the differences in serum Fe concentrations and related variables between summer and winter in Noma horses.

Materials and Methods

Animals

Blood samples from 37 clinically normal Noma horses were collected seven consecutive times: September 2018, February 2019, October 2019, February 2020, September 2020, February 2021, and February 2022. They were all raised at Nomauma Highland, a public ranch for Noma horses (Imabari, Ehime, Japan). The experimental horses included sixteen mares, eleven stallions, and ten geldings, ranging in age from 1 to 26 years as of September 2018, and 4 to 29 years as of February 2022.

Breeding environment

Nomauma Highland comprises three stables. Each stable has a sandy grazing paddock without grass and a roofed feeding and resting facility. The Noma horses are housed at night but are allowed to roam freely during the day, as shown in Fig. 1.

Foods and water

Each adult horse was fed 500 g of Sudan hay, 250 g of hay cubes, 150 g of wheat bran, 25 g of pressed barley, and 25 g of pressed soybeans twice a day (around 8:00 and 16:00). All food was purchased from JA West Japan Kumiai Feed Co., Ltd., Kobe, Hyogo, Japan. Horses consume little fresh grass. No Fe-containing supplements were administered. The feed quantity was almost the same throughout the year but was slightly higher during the winter season. Water was freely available. The mineral components of drinking water were analyzed by a specialized testing company (La Belle Vie Laboratory Inc. Tokyo, Japan) and were within the standard values: Fe, <0.001 mg/*l*; sodium (Na), 23.0 or 23.4 mg/*l* ; potassium (K), 1.1 mg/*l*; magnesium (Mg), 6.0 mg/*l*; calcium (Ca), 39.8 or 40.7 mg/*l*; copper, <0.002 or 0.006 mg/*l*; and manganese, 0.001 or <0.00002 mg/*l*.

Climate

According to the weather observation data for Imabari City [20], the maximum temperatures in September 2018, October 2019, and September 2020 exceeded 30°C. Therefore, we analyzed the data for these summer periods. On

Fig. 1. State of the first pasture field. Horses are kept in a facility with a covered open stable.

the other hand, the minimum winter temperatures (February 2019, 2020, 2021, and 2022) ranged from –0.9 to –2.8. The maximum temperatures during this period were 14.1 to 21.7°C, indicating that Imabari City's climate is not extremely cold.

Determination methods

Peripheral blood was collected from the jugular vein before noon. After sampling, the blood samples were kept on ice and transported to the university, and the sera were separated. Laboratory tests were performed on the day of blood collection at the Biomedical Research and Service Center of Okayama University of Science. Blood and biochemical tests were performed with a blood cell counter (Celltac α, Nihon Kohden Corporation, Tokyo, Japan) and an autoanalyzer (3100, Hitachi High-Tech Science Corp., Tokyo, Japan), respectively. The determined variables were Fe, unsaturated iron-binding capacity (UIBC), Hb, RBC, Ht, MCV, MCH, blood urea nitrogen (BUN), creatinine (CRE), total protein (TP), albumin, glucose, cholesterol, asparate transaminase (AST), Na, K, chloride (Cl), Ca, inorganic phosphorus (IP) and Mg. The total iron-binding capacity (TIBC= Fe [μg/d*l*]+ UIBC [μg/d*l*] and Fe saturation rate (Fe [μg/d*l*]/TIBC μg/d*l*) * 100) were calculated, respectively [35]. Hb, UIBC, TIBC, and saturation rate were analyzed as Fe-related variables. This study used data from regular health checkups. The experimental protocols were approved by the Animal Care and Use Committee of Okayama University of Science, Japan (approval number: 2018-37).

Statistics

Data are expressed as median, minimum, and maximum values. Differences in serum Fe concentrations by age and by sex (mares, stallions, and geldings), and by age (1 to 4 years, 5 to 9 years, 10 to 14 years, 15 to 20 years, and over 20 years) were examined using the Kruskal-Wallis test and Scheffe's pairwise comparison method. Differences in the data between the seven blood sampling times were tested using Friedman's test and Scheffe's paired comparison method. Differences between summer and winter data were tested using the Mann-Whitney U test. Correlations between serum Fe concentration and other variables were analyzed using Spearman's rank correlation coefficients. Statistical analyses were performed using commercially available software (Bell Curve in Excel; Social Survey Research Information Co., Ltd., Tokyo, Japan). A P-value of <0.05 was considered significant.

Results

As shown in Table 1, the serum Fe concentrations in the Noma horses ranged from 74 to 316 μg/d*l*, with a median value being 176 μg/d*l*. The concentrations in stallions were significantly lower than those in mares and geldings (P<0.01). There were no significant differences in serum Fe concentrations between the ages of 1–4, 5–9, and 10–14 years. The concentrations tended to be low at 10–14 years of age and increased with age.

Figure 2 shows the serum Fe concentrations at each sampling time. Serum Fe concentrations were repeatedly low in summer and high in winter. Significant differences were observed between September 2018 and February 2019, 2020, and 2022; between February 2019 and September 2020; between October 2019 and February 2020 or 2022; between February 2020 and September 2020; and between September 2020 and February 2021 or 2022. Serum Fe concentrations were significantly lower in summer than in winter (P<0.001).

Table 2 shows the changes in the Fe-related variables. The Hb concentrations tended to show similar changes as

Table 1. Serum Fe concentrations in Noma horses

Classification	No. of samples	Serum Fe concentration (μg/d*l*)			Kruskal-Wallis test	Multiple comparison
		Median	Minimum	Maximum	*P*-value	*P*-value
All samples	259	176	74	316		
Gender					<0.001	
Mares	112	189	108	316		<0.001 for stallions
Stallions	77	134	74	290		
Geldings	70	193	113	286		<0.001 for stallions
Age					<0.001	
1 to 4	39	178	121	269		
5 to 9	38	160	104	282		
10 to 14	65	145	74	260		
15 to 19	55	182	81	290		=0.016 for age 10 to 14
>20	62	204	108	316		=0.005 for age 5 to 9 and <0.001 for age 10 to 14

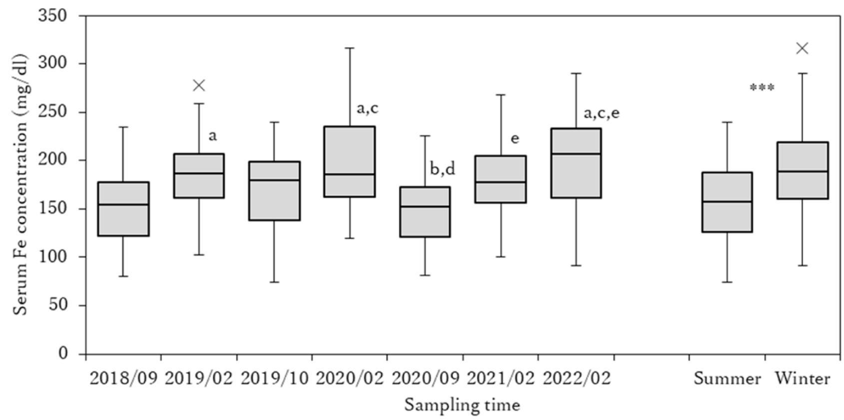

Fig. 2. Serum Fe concentrations at each sampling time.
a: Significantly different from September 2018 (P<0.05), b: Significantly different from February 2019 (P<0.05), c: Significantly different from October 2019 (P<0.05), d: Significantly different from February 2020 (P<0.05), e: Significantly different from September 2020 (P<0.05), ***: Significantly different between summer and winter (P<0.001).

Table 2. Changes in serum Fe-related variables

Variable		Date of sample collection							Friedman test	Season		Difference between summer and winter
		18-Sep	19-Feb	19-Oct	20-Feb	20-Sep	21-Feb	22-Feb		Summer	Winter	
		(n=37)	(n=37)	(n=37)	(n=37)	(n=37)	(n=37)	(n=37)	P-value	(n=111)	(n=148)	P-value
Hemoglobin	Median	12.8	13.6	13.1	13.7[a]	13.2	13.3	13.5	<0.001	13.1	13.5	0.0034
(g/dl)	Minimum	10.7	11.6	10.8	12.1	11.2	11.3	11.5		10.7	11.3	
	Maximum	15.3	17	15.7	15.6	16.1	16	16.7		16.1	17	
Unsaturated iron	Median	274	266	277	270	283	290	274	0.0906	274	273	0.6869
binding capacity	Minimum	174	33	123	43	60	55	99		60	33	
(μg/dl)	Maximum	359	350	366	370	347	348	409		366	409	
Total iron	Median	419	449	434	460[a,b]	437	452	467[a,b]	<0.001	433	461	<0.001
binding capacity	Minimum	266	289	262	314	285	267	324		262	267	
(μg/dl)	Maximum	524	593	563	595	525	551	632		563	632	
Saturation rate	Median	35.8	39.8	36.4	40.8	34.8	38.7	42.4	0.277	35.8	39.8	<0.001
(%)	Min	22.6	28.4	26	28.6	24.4	25.5	21.9		22.6	28.4	
	Max	54.5	88.7	61.8	86.3	79	82.8	71.7		54.5	88.7	

Results of Scheffe's paired comparison for differences between the data on the sampling days. [a]: Significantly different from 2018/09 (P<0.01), [b]: Significantly different from 2020/09 (P<0.01).

the serum Fe concentrations, being low in summer and high in winter, although they were within the reference ranges. There were no significant differences in UIBC between summer and winter. TIBC and the Fe saturation rates were also lower in summer.

Table 3 shows the differences in other laboratory test results between summer and winter. Although the differences were small, the Ht, MCV, and MCH values and concentrations of albumin, cholesterol, Na, K, Cl, and Ca were lower in summer than in winter, similar to the serum Fe concentrations. In contrast to the Fe concentrations, the creatinine, TP, IP, and Mg concentrations were higher in the summer. There were no significant differences in the RBC count, concentrations of BUN, glucose, and AST activity between summer and winter.

Tables 4 and 5 and Fig. 3 show the correlations between the serum Fe concentrations and other laboratory test results. Significant correlations with the serum Fe concentrations were observed for Hb (Fig. 3A), TIBC, and Fe saturation rates but not with UIBC. Significant positive correlations with serum Fe concentrations were observed for Ht (Fig. 3B), MCV, MCH, creatinine, albumin (Fig. 3C), glucose, cholesterol, AST, Na (Fig. 3D), Cl, and Ca (Fig. 3E). BUN (Fig. 3F) and K (Fig. 3G) concentrations were negatively and significantly correlated with the Fe concentration (Table 4). The serum Fe concentrations did not correlate

Table 3. Differences in laboratory tests between summer and winter

Variable	Summer (n=111)			Winter (n=148)			*P*-value
	Median	Minimum	Maximum	Median	Minimum	Maximum	
Red blood cell ($10^4/\mu l$)	743	599	1016	764	600	1046	0.2227
Hematocrit (%)	37	29.6	45.3	38.8	31.6	50.4	<0.001
MCV (fl)	49.6	37.4	55.2	51	39.7	57.8	<0.001
MCH (pg)	17.6	13.3	19.6	17.9	13.9	20.1	0.023
BUN (mg/d*l*)	16	8.9	25.7	17.1	7.5	26.1	0.0564
Creatinine (mg/d*l*)	1.04	0.55	1.5	0.94	0.52	1.37	<0.001
Total protein (g/d*l*)	6.81	5.34	8.77	6.61	5.21	7.74	0.0026
Albumin (g/d*l*)	3.5	2.51	4.13	3.82	2.86	4.87	<0.001
Glucose (mg/d*l*)	103	69	149	104	74	161	0.576
Cholesterol (mg/d*l*)	79	53	114	95	60	126	<0.001
AST (IU/*l*)	386	231	867	365	239	672	0.1043
Sodium (mEq/*l*)	140	132	145	144	136	151	<0.001
Potassium (mEq/*l*)	4.26	1.67	6.21	4.47	1.42	6.27	0.0187
Chlorine (mEq/*l*)	102	94	108	106	99	114	<0.001
Calcium (mg/d*l*)	12.4	10.6	14.2	12.9	11.3	14.4	<0.001
IP (mg/d*l*)	2.8	1.6	5.1	2.5	1.5	4.1	0.0043
Magnesium (mg/d*l*)	2.1	1.7	2.6	2.05	1.6	2.7	<0.001

MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; BUN, blood urea nitrogen; AST, aspartate transaminase; IP, inorganic phosphorus.

Table 4. Correlations between serum Fe concentration and Fe-related variables

Fe-related variable	Correlation with serum Fe (n=259)	
	Correlation coefficient	*P*-value
Hemoglobin (g/d*l*)	0.1434	0.021
Unsaturated iron binding capacity (μg/d*l*)	0.0113	0.8563
Tortal iron binding capacity (μg/d*l*)	0.5888	<0.001
Saturation rate (%)	0.8012	<0.001

significantly with the RBC count, TP, IP, or Mg (Fig. 3H). To summarize these results, TIBC, the saturation rate, Hb, Ht, albumin, cholesterol, Na, Cl, and Ca showed the same behavior as the serum Fe concentration: they were lower in summer and showed positive and significant correlation with the serum Fe concentration. In contrast, UIBC, BUN, creatinine, glucose, TP, AST, K, IP, and Mg behaved differently from the serum Fe concentrations: they were not low in summer and did not show significant positive correlation with the serum Fe concentration.

Table 5. Correlations between the serum Fe concentration and laboratory test results

Variable	Correlation with serum Fe (n=259)	
	Correlation coefficient	*P*-value
Red blood cell ($10^4/\mu l$)	–0.0667	0.2851
Hematocrit (%)	0.1648	0.0079
Mean corpuscular volume (f*l*)	0.3952	<0.001
Mean corpuscular hemoglobin (pg)	0.363	<0.001
Blood urea nitrogen (mg/d*l*)	–0.3035	<0.001
Creatinine (mg/d*l*)	0.151	0.015
Total protein (g/d*l*)	0.0409	0.5123
Albumin (g/d*l*)	0.4511	<0.001
Glucose (mg/d*l*)	0.1478	0.0173
Cholesterol (mg/d*l*)	0.5527	<0.001
Asparate transaminase (IU/*l*)	0.1287	0.0385
Sodium (mEq/*l*)	0.2946	<0.001
Potassium (mEq/*l*)	–0.2137	<0.001
Chlorine (mEq/*l*)	0.3498	<0.001
Calcium (mg/d*l*)	0.16	0.0099
Inorganic phosphorus (mg/d*l*)	–0.0731	0.2414
Magnesium (mg/d*l*)	0.0084	0.8926

Discussion

According to textbooks, the serum Fe concentration in horses is 91–199 μg/d*l* [14, 29, 32]. Osbaldiston *et al.* [28] reported that the serum Fe concentration in Thoroughbreds was 109 ± 12 μg/d*l*, and that the concentrations in American Quarter horses were higher, at 154 ± 34 μg/d*l*. Another report indicated mean serum Fe concentrations of 253 ± 66 μg/d*l* in Warm-blooded horses, 202 ± 87 μg/d*l* in half-breds, 166 ± 67 μg/d*l* in Haflinger horses, 183 μg/d*l* in heavy horses, and 366 μg/d*l* in Arabian Haflingers [30]. Summarizing all the data for Noma horses, the serum Fe concentrations ranged

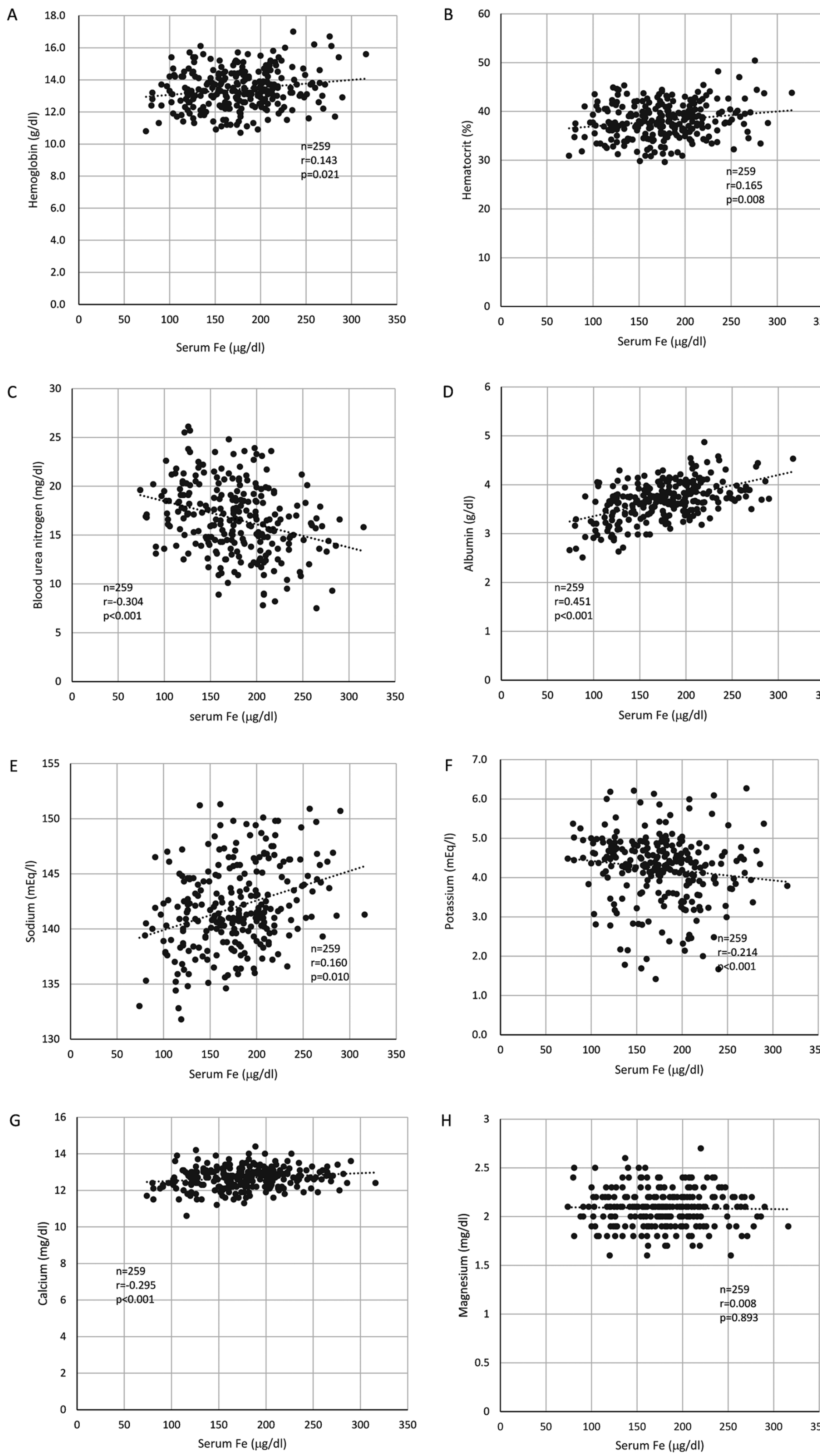

Fig. 3. Correlations between serum iron concentrations and major laboratory test results.

from 74 to 316 μg/d*l*, with a median of 176 μg/d*l*. They tended to be higher than the reference values described in textbooks and those in Thoroughbreds and American quarter horses, although they were lower than those in Schorr's horses [30]. No age or sex differences were found in the serum Fe concentrations in horses reported previously [28, 30, 31]. A significant positive correlation has been observed between age and the blood (serum) Fe concentration [3]. Although there were some differences compared with other reports, possibly because of the varying living or feeding conditions, the serum Fe concentrations of Noma horses might be within the physiological ranges for horses, as no symptoms associated with Fe deficiency or excess were observed. In the Noma horses, the serum Fe concentrations were lower in stallions, tended to be low at 10–14 years of age, and increased with age. However, the reasons for the observed sex and age differences among the Noma horses remain unclear.

In Noma horses, the serum Fe concentrations were repeatedly low in summer and high in winter. Lower serum Fe concentrations in summer have not been observed in other horse breeds [13, 15]. It has been questioned whether the reference serum Fe concentration for Noma horses should be based on summer or winter values. In this study, we hypothesized that the winter serum Fe concentration should be the reference value, as Noma horses naturally live in cold regions and the hot climate of Imabari City is unsuitable for them. Low serum Fe concentrations are caused by increase demand, loss of Fe, and/or suppression of mobilization, and this includeds a reduced intake of Fe due to iron-deficient diets or malabsorption from the intestine, blood loss, mineral loss through sweat, over hydration from excessive drinking, and suppression of mobilization, possibly as a result of infections, inflammation, or malignancies.

Generally, feeding a Fe-deficient diet or long-term malabsorption decreases the serum Fe concentration [11]. Food containing Fe-chelators, such as tannic acid, may also induce mineral malabsorption [22]. However, Fe deficiency is rare in adult herbivores, as their Fe requirements are low and they obtain sufficient Fe by eating Fe-rich roughage or directly from soil [17, 34]. In Noma horses, the calculated Fe content in their feed is greater than the required amounts [25]. Although the volume was slightly higher in winter, the Noma horses were fed almost the same amount of food with almost the same composition, so it is unlikely that the diet directly influenced the decline in serum Fe concentrations in summer.

Loss of Fe due to sweating during strenuous sports or work in hot environments may contribute to decreased serum Fe concentrations [8], although the main causes of Fe deficiency in humans are malnutrition and blood loss [4, 11, 12] Horses are the only species other than humans that depend on sweating and evaporative cooling as their primary thermoregulation mechanism [7, 9, 23, 24]. Body temperature, respiration rate, and sweating are higher in horses with unshaded pens compared with those with shaded pens in hot and sunny summers [16]. Serum Fe concentrations are lowered in conjunction with exercise stress, and endurance exercise might also affect Fe metabolism by increasing iron excretion through sweat in horses [18]. Imabari city, Ehime prefecture, where Noma horses are bred, has a hot environment with temperatures exceeding 30°C in summer. The body surface temperatures of Noma horses measured in September 2020 sometimes exceeded 50°C under sunlight [37]. In such situations, Noma horses likely sweat to avoid the elevation of their body temperatures, and Fe loss through sweat may contribute to decreased serum Fe concentrations. However, the Fe and mineral loss may be mild because they do not sweat profusely and do not undergo strenuous exercise. Due to excessive sweating, the serum concentrations of sweat components such as Na, K, Ca, Cl, and Mg should also decrease [1]. In Noma horses, the serum concentrations of Ca, Na, Cl, and K were low in summer, and there were positive correlations between the Fe concentrations and parameters other than the K concentrations, as hypothesized; however, the Mg concentrations were high, unlike those of Fe, and were not correlated with the Fe concentrations. The excretion of Fe through sweat may be not the only cause of the decrease in serum Fe concentration, but we consider that this mechanism is significantly involved in the decline in serum Fe levels during summer.

Massive hemorrhage may cause a decrease in serum Fe concentrations. However, the Noma horses did not show any obvious bleeding symptoms; therefore, bleeding could be the cause of the low serum Fe concentrations. Aside from bleeding, Noma horses experience dermatitis in summer that is associated with allergens from blood-sucking insects [37]. Insect bites are unlikely to be severe enough to reduce blood Hb and serum Fe concentrations, but they may be partially responsible. The inflammation they cause reduces the serum Fe concentrations by decreasing Fe mobilization [5, 21, 33]. In Noma horses, skin inflammation may play a role in lowering serum Fe concentrations; however, the relationship remains unclear.

Excessive water intake to cope with high body temperatures can lead to temporary overhydration, inducing temporary decreases in serum Fe concentrations. However, in the Noma horses, the mineral components of the drinking water, including Fe, were low and within the standard values. Large amounts of water intake were not confirmed by animal keepers during the summer season. Moreover, the creatinine, total protein, IP and Mg levels were high in summer, and the Fe concentrations were not significantly correlated with the total protein and Mg concentrations. On the contrary, the BUN concentrations were negatively correlated with the Fe concentrations. Overdrinking and overhydration are not direct causes of the decrease in serum Fe concentrations in summer.

It has been revealed that the serum Fe concentrations were higher in Noma horses compared with the so-called reference values for horses. We also found that the serum Fe concentrations repeatedly decreased in summer and increased in winter, paralleling the blood Hb concentrations, MCV, and MCH, which were within the reference values, while RBCs became microcytic in summer. Although identifying the cause of the decrease in serum Fe concentration in summer is difficult, fluctuations may be due to Fe loss from sweating, blood dilution from drinking excessive water, loss of hemoglobin-Fe caused by blood-sucking insects, a decrease in Fe mobilization associated with dermatitis, the amount and composition of foods, and/or unknown factors. It is unclear whether the fluctuation in the serum Fe concentration is a characteristic of Noma horses. Conducting similar studies on the seven other Japanese native horse breeds may help elucidate the cause of seasonal fluctuations, because their genetic lineage may differ from that of racehorses. In Noma horses, supplementation with Fe is not necessary because the serum Fe concentrations are within the reference ranges. However, heat countermeasures may be necessary during the hot season, considering the abnormal climate in recent years. In addition, it is necessary to set reference values for summer and winter based on laboratory test results including the serum Fe concentrations, in Noma horses.

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