

Age-dependent haemogram and sex-dependent serum biochemistry values in semi-feral Konik horses

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Altersabhängiges Blutbild und geschlechterabhängige Serumwerte in halbwilden Konik-Pferden

In europäischen Naturschutzprojekten dienen halbwilde, freilaufende Konikpferde zunehmend dem Erhalt offener Landschaften. Deren Gesundheitsstatus kann jedoch gegenwärtig nur begrenzt eingeschätzt werden, da keine Normalwerte zu wichtigen Blutparametern etabliert sind. Unsere Studie zielte daher auf die geschlechts- und altersabhängige Erfassung von Blutbild und definierten biochemischen Serumwerten in gesunden, halbwilden Koniks.

Die Blutentnahme erfolgte bei 53 weiblichen und 18 männlichen (8 unkastriert, 10 kastriert) Konik-Pferden zweier mitteldeutscher Naturreservate. Diese waren unterschiedlich alt (9–266 Monate) und ohne Krankheitsanzeichen. Die Analyse der Blutproben erfolgte in einem akkreditierten Labor.

Die Auswertungen ergaben alters-, aber keine geschlechtsabhängigen Unterschiede in der Leukozytenzahl. Unter den Leukozyten kam es mit zunehmendem Alter besonders zur Abnahme an Lymphozyten, weshalb das Granulozyten-Lymphozyten-Verhältnis altersabhängig anstieg. Die Serumwerte ausgewählter biochemischer Parameter waren dagegen unabhängig vom Alter, zeigten aber oft geschlechtsspezifische Unterschiede. Diesbezüglich hatten die männlichen Koniks höhere Werte für Gesamtprotein, Triglyzeride und die Enzymaktivitäten von alkalischer Phosphatase, Aspartat-Aminotransaminase und γ -Glutamyltransferase als die Stuten. Die Geschlechterdifferenzen in den jeweiligen Enzymaktivitäten beschränkte sich aber auf die unkastrierten Tiere. Die Hengste zeigten daneben auch höhere Serumwerte für Kalzium und Selen als die Wallache oder Stuten. Soweit die jeweilige Gruppengrößen dies zuließen, wurden dann abhängig von Alter und Geschlecht Referenzwerte für die gemessenen Blutparameter ermittelt. Diese erleichtern nun die Einschätzung des Gesundheitsstatus von

Abstract

Semi-feral, free-roaming Konik polski horses are used in some European countries for preserving semi-open pasture landscapes. The estimation of their health status is still limited by insufficient data on various blood parameters. Therefore, our study aimed at the sex- and age-dependent analysis of haemogram and selected biochemistry parameters in healthy, semi-feral Koniks. In order to reach this aim, we took blood samples from 53 female and 18 male (8 uncastrated, 10 castrated) Koniks living in two Middle German nature reserves. They were of different age (9–266 months) and without signs of illness. Blood samples were analysed by an accredited laboratory.

We identified age- but not sex-dependent changes in the white blood cell count (WBC). Higher age mainly caused a decrease in lymphocytes. Therefore, WBC correlated negatively and granulocyte-to-lymphocyte ratio positively with increasing age. Serum values of selected biochemical parameters did not depend on age but showed some sex-related differences. In this regard, serum total protein, triglyceride and the enzymatic activities of alkaline phosphatase, aspartate transaminase and γ -glutamyltransferase were higher in males than females. However, the sex dependency of these enzymatic activities was restricted to uncastrated males. They also showed higher serum values for calcium and selenium than castrated males or all females. As far as the respective group sizes permitted, we then calculated age- or sex-dependent reference interval values for all parameters analysed. These values improve now the estimation of the health status of semi-feral, free-roaming Konik horses and provide a stable basis for future studies.

Keywords: blood, breed, castrated, free-roaming, reference value

halbwilden Konikpferden und bilden zudem eine stabile Basis für Nachfolgestudien.

Schlüsselwörter: Blut, freilaufend, kastriert, Rasse, Referenzwerte

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Introduction

Breeds of horse (*Equus caballus*) are developed for various uses. They are loosely divided into cold-blooded, warm-blooded and thoroughbred (“hot-blooded”) horses according to their character, as well as into large and small horses according to their body size. Important subgroup of the small horses is the pony type. The term pony is commonly used as synonym for the entire group of small horses. Ponies and other small horses serve as working or leisure animals. In case of their use in competitions, ponies should not exceed more than 147 cm body high at the withers.¹

Nowadays, at least 150 pony breeds are known. One of them is the Konik polski horse (*Equus caballus gmelini Antonius*) which belongs to the warm-blooded horses.² In contrast to many other breeds of ponies, Koniks are not only used as working and leisure animals but also for restoring and preserving landscapes as semi-open pasture habitats.³ According to their use, they are kept in either traditional stables as domestic animals or naturalistic conditions as semi-feral, free-roaming animals.² Konik populations can be found in Poland, the country of their origin, and in some other European countries.³

Animal welfare-friendly husbandry of domestic or semi-feral Koniks includes health monitoring and, if necessary, medical treatment of the individual horse. In this regard, blood samples are commonly collected and analysed in a laboratory to identify possible diseases by pathological alterations of haemogram values and/or specific biochemistry parameters. The correct interpretation of analysis values requires reference interval (RI) data for particular laboratory measurements. RIs correspond to the values within the 2,5th and 97,5th percentiles of a data set determined for a healthy population.^{4,5} However, they are influenced by biological, environmental and technical factors as well as the number of healthy individuals included in the study.^{5,6} Highly critical is also the definition of “healthy individuals” as it requires various clinical investigations of the animals before and after sample collection. In real settings, comprehensive investigations of animals are not always feasible. Moreover, an animal number of at least 40 (better 120) animals as suggested by the guideline⁵ is not always possible to reach. This is particularly the case for wildlife or half-wildlife, minor species and rare breeds as well as certain genders or age groups.

Among all horse breeds, the Konik polski horse also belongs to the minor breeds. Still, pioneer work for defining Konik-specific blood values had been done by Polish colleagues.

Niedzwiedz *et al.*⁷ and Kedzierski & Pluta⁸ reported values for various serum biochemistry parameters analysed in populations of 74 and 12 healthy Koniks, respectively. Witkowska-Pilaszewicz *et al.* published values for common haematological parameters and also serum biochemistry parameters from 41 Koniks.⁹ These studies included animals kept under controlled conditions in private stabling and/or semi-controlled conditions in a research station.^{7–9} Niedzwiedz *et al.* did not perform husbandry-dependent evaluations in their scientific work.⁷

The husbandry of Koniks under semi-controlled conditions is used in environmental projects in Europa including Germany.³ They are often kept on pasture together with other semi-feral animals like Heck cattle (*Bos taurus*). Both species are robust and highly adaptable to different habitats and, therefore, well suited to restore and stabilise half-open landscapes in selected areas. The number of independent studies contributing to the definition of valid Konik-related RI values for common and more specific blood parameters is still limited, especially for the semi-feral, free-roaming animals. As this would be helpful for estimating their health status, our study aimed at the quantification of various haematological and biochemical parameters including electrolytes and minerals in semi-feral Konik horses, their comparison with known values from Koniks living in more controlled conditions, and the calculation of RI values. In order to reach this aim, we analysed these parameters in blood samples from an age-heterogenous population of male and female Koniks being kept under semi-controlled conditions in two Middle German nature reserves.

Material and Methods

Animals

The study population comprised 71 Konik polski horses of both sexes kept full-time in two different nature reserves of the German state of Saxony-Anhalt (Table 1). These reserves were Wulfener Bruch (142 ha split into 5 parcels of 23 to 38 ha) in the middle part and Oranienbaumer Heide (800 ha) in the eastern part of Saxony-Anhalt, which experiences the subcontinental type of climate. Wulfener Bruch is a typical lowland landscape with wet meadow areas, whereas Oranienbaumer Heide mainly comprises heathland areas. Koniks were co-settled with Heck cattle in both reserves with the aim of keeping the landscape as semi-open pasture area. Although 39 horses were born elsewhere, none of them

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arrived from other places within the last three years. They always had *ad libitum* access to water and mineralized salt blocks (Ralinger, Mineralstein, Ralinger Salz Handels-GmbH, Föhren, Germany). Additional grass hay was only provided in case of insufficient pasture conditions.

All Konik horses were checked visually every day for health conditions including body condition score, hoof status, injuries or ectoparasites. To get also an insight into the endoparasite status pooled faecal samples were examined for endoparasites twice a year. Deworming had only been performed on demand. Horses in this study did not receive any immunization through vaccination. Ten males had been castrated (Table 1). As blood samples had been taken in February, all mares were in anoestrus phase. None of the mares was pregnant or lactating. Of the originally 74 horses, we excluded three mares from the statistical analysis because of Laminitis disease at a later time. Therefore, our analysis included 71 horses without clinical signs of diseases around the time of blood sample collection.

Blood analysis

Each reserve we took blood from all horses at one day between 10 am to 4 pm. In order to reach this, Koniks were pre-collected in well-known paddocks one day before. Here they had free access to grass hay and water. At the day of study, they were consecutively kept under forced confinement conditions for visual health check and blood sampling. Detailed health checks (*i.e.*, measuring of the body temperature, auscultation of the body) were not performed as this had only been possible under immobilization. Blood was taken by puncture of the jugular vein (20 G needle) and vacuum method into S-Monovette® tubes (Sarstedt, Nümbrecht, Germany). We obtained blood samples in potassium EDTA and in serum tubes (7,5 mL volume each). Serum tubes were gently mixed, incubated for 60 min at ambient temperature and then centrifuged at 2000 g (20 min). After aspiration and transfer of the serum into new tubes, all samples were kept cool (4–8 °C) in cooling boxes until analysis. They were transferred overnight to an accredited laboratory (LABOKLIN GmbH & Co. KG, Bad Kissingen, Germany) that performed all analyses at the next morning.

Our analyses included measurements of haemogram and various serum biochemical parameters (protein, metabolites, enzyme activities, electrolytes and minerals). All haemogram parameters were determined by flow cytometry in a Sysmex XT 2000i Automated Hematology Analyzer (Sysmex, Norderstedt, Germany). While most biochemical parameters were determined by photometry-based methods in the Cobas® 8000 Modular Analyzer series (Roche, Ludwigsbuurg, Germany), selenium was quantified by atomic absorption spectroscopy using the ZEE nit 650P instrument (Analytik Jena, Jena, Germany).

Ethics approval

Blood collection from Konik horses was approved by the local animal welfare commissions (file number 203.m-42502-3-879 MLU_G).

Statistics

Data presentations and statistical analyses were performed by use of the software OriginPro 2019 (OriginLab Corporation; Northampton, MA). If not indicated otherwise, we presented our data consistently as median with total range because some data sets were not normally distributed (Shapiro-Wilk test) and/or too small for the calculation of percentiles. Differences between two evaluation groups were tested for significance by Student's t-test (parametric) or Mann-Whitney test (non-parametric). Differences between three evaluation groups were tested for significance by ANOVA statistics with Bonferroni post-hoc procedure (parametric) or by Kruskal-Wallis-ANOVA statistics with Dunn's procedure (non-parametric). Age dependencies were tested by regression analysis with ANOVA statistics and Pearson correlation. P values below 0,05 indicate significant changes.

RI values were calculated by use of the MedCalc statistical software (MedCalc Software Ltd., Ostend, Belgium) according to the guidelines.^{5,10} In detail, we performed Box-Cox transformation of the data sets, removed possible outliers (Tukey test) and calculated the lower and upper reference level (2,5th and 97,5th percentile, respectively) with 90% confidence interval (CI). CI values were calculated by

Table 1: Konik population each reserve.

	male			female
	all	uncastrated	castrated	all
Animals (n)	18	8	10	53
Wulfener Bruch	12	8	4	20
Oranienbaumer Heide	6	–	6	33
Median age (months)	79	87 (9–205)	74 (35–148)	95 (11–266) ^a

^aP < 0,05 vs. all males for the median data with total range (two group statistics).

bootstrapping statistics as recommended for small sample size. Then, CI values were checked if they do not exceed 1/5 of the width of the RI value.

Results

We assessed haemograms and selected serum factors in blood samples of 71 Konik horses depending on age and sex. Female Koniks (mares) were higher in number and age than the male Koniks (Table 1). 97 months was the mean age and 90 months the median age of the total population. Male Koniks were either uncastrated (stallions) or castrated (geldings) (Table 1). Therefore, we tested for the general effect of sex and the additional effect of castration of the male animals. Moreover, we compared our data with data sets reported for Konik populations in Poland.^{7–9}

Haemogram analyses did not reveal different counts for red or white blood cells between male and female Koniks (Table 2). Only the number of thrombocytes tended to be lower in the mares (Table 2). MCH calculations also revealed slightly lower values for the erythrocyte haemoglobin content in stallions (Table 2). Comparisons of haemogram values of this study with published values indicated differences in red blood cell-related parameters and thrombocyte counts (Table 2).

Although the haemogram did not show clear sex-dependent differences, we identified a significant influence of age (Figure 1). The influence of age is shown by correlation statistics and two group statistics (Figure 1). For the two group statistics we split our data at the median age (90 months) into

Koniks younger or older than this cut-off value. Another splitting was not performed as it had result in too small groups for later RI calculations. In detail, the total number of white blood cells decreased with age (Figure 1A). This decrease mainly based on the age-dependent reduction of lymphocytes, whereas the absolute counts of neutrophil and eosinophil granulocytes remained stable with age (data not shown). Therefore, increasing age correlated negatively with the percentage of lymphocytes and positively with the percentages of neutrophils and eosinophiles (Figure 1B–D). As basophile granulocytes are negligible (Table 2), the granulocytes-to-lymphocytes ratio (GLR) strongly increased with age (Figure 1E). In contrast to the white blood cells, most red blood cell-related parameters did not change with age (data not shown). Only the erythrocyte haemoglobin content (MCH) depended on age (Figure 1F).

In contrast to haemogram, increasing age did not influence any factor measured in the serum samples of the Konik population used in our study (Table 3). Although many factors did also not differ between male and female animals, we identified sex-related differences in the serum levels of proteins (total, albumin, globulin), metabolites (triglycerides), minerals (calcium, selenium) and enzymatic activities (alkaline phosphatase, aspartate transaminase, γ -glutamyltransferase). The serum levels of these factors were always higher in males than females (Table 3). Sub-analysis of the castration status of the male Koniks revealed that the sex dependency of most of them primarily based on differences between stallions and mares (Figure 2). Only the serum protein level (Figure 2A) and the serum level of albumin or globulin alone (data not shown) were independent of the castration. Sex-dependent differences in the serum

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Table 2: Haematological parameters of semi-feral Konik horses.

Parameter	units	male			female	reported
		all	uncastrated	castrated	all	all ^a
Red blood cells	T/L	10,0	11 (8,5–12,1)	9,5 (8,5–10,5)	9,3 (6,2–11,3)	7,7 ^c
Haematocrit	L/L	0,42	0,43 (0,35–0,46)	0,42 (0,33–0,46)	0,40 (0,33–0,49)	0,35
Haemoglobin	g/L	154	154 (142–182)	156 (127–178)	152 (107–177)	113
MCH	pg	16	15 (13–17)	16 (15–17) ^a	16 (13–18) ^a	15 ^c
White blood cells	G/L	8,8	10,8 (7,0–12,0)	8,7 (6,6–15,3)	8,2 (5,1–12,1)	7,5 ^c
Neutrophils	%	49	45 (37–56)	50 (36–57)	50 (27–78)	–
Eosinophils	%	7	7 (5–14)	7 (3–12)	6 (0–13)	–
Basophils	%	1	1 (0–1)	1 (1–7)	1 (0–5)	–
Lymphocytes	%	39	42 (26–52)	38 (25–51)	39 (14–68)	48
Monocytes	%	4	4 (3–6)	4 (3–5)	4 (2–8)	12
GLR	–	1,50	1,26 (0,83–2,73)	1,54 (0,86–2,76)	1,44 (0,41–5,80)	–
Thrombocytes	G/L	203	255 (161–363)	202 (112–242)	176 (49–412) ^b	403

Median data with total range compared with mean data reported in another study. ^aP < 0,05 vs. uncastrated males (three group statistics). ^bP < 0,10 vs. all males (two group statistics). ^cEffect of age for all ponies including Koniks (Pearson correlation statistics).⁹

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triglyceride level had only been determined for the total population of male Koniks (Table 3).

Subsequent comparisons of our values with published values on Konik serum factors indicated clear differences in the activities of aspartate transaminase, creatine kinase and γ -glutamyltransferase as well as in the levels of glucose and total bilirubin (Table 3). While the serum activities of these enzymes were lower in the Konik population of our study, the values for glucose and total bilirubin were higher (Table

3). Only three of 71 horses had glucose concentrations below 4 mmol/L, the median value of the Niedzwiedz study.⁷ Other serum parameters had been determined in a comparable range, or they could not be compared due to missing data sets on Koniks in the literature (Table 3).

Finally, we calculated RI values with 90 % CI of the upper and lower limit for each parameter studied. Data of our initial age- and sex-dependent analyses indicated if this was possible for all Konik horses or for subgroups only. Sub-

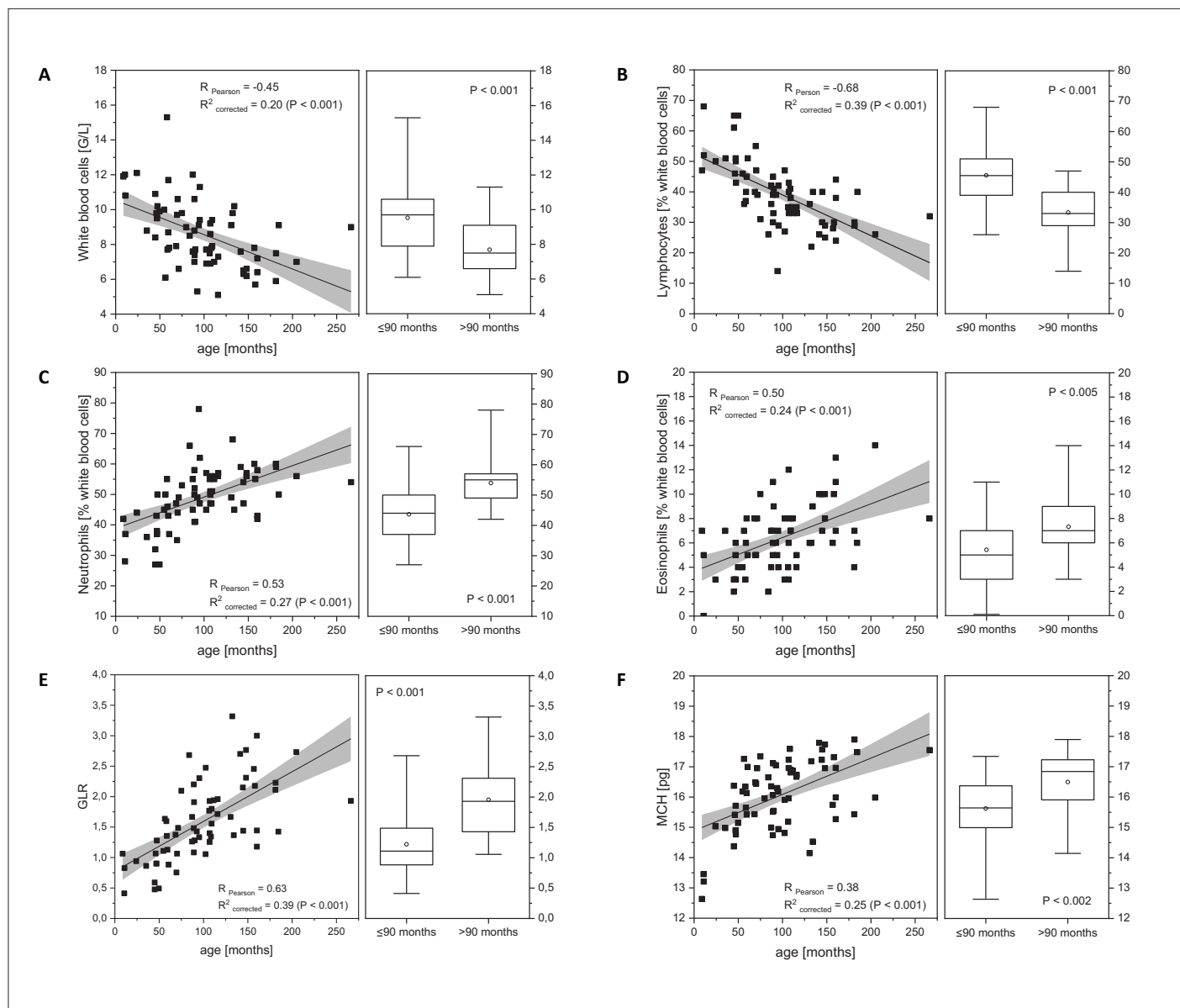


Figure 1: Effect of increasing age on the white blood cell count (A), percentage and relation of the white blood cell subpopulations (B–E) and the red blood cell-related MCH values (F). On the left, data are given as dot plot graph with correlation factors and regression line with 95% CI of the linear fit statistics. On the right, data are given as box plot graph according to cut-off value (median age) of 90 months. Box plot (interquartile range) graphs are presented with median line, mean (O), whiskers (total range) and P values (two group statistics; n=36 horses ≤90 months vs. n=35 horses >90 months).

groups not reaching the critical number of at least 20 individuals per group^{5, 10} (*i.e.*, stallions, geldings) were omitted from the RI calculations. As some biochemical parameters did not show significant differences between geldings and mares (Table 2, Figure 2), we calculated the data for the total population of geldings and mares. Table 4 summarizes all RI values determined for the haematological and biochemical parameters. In none case the CI value exceeds 1/5 of the width of the RI value (Table 4).

Discussion

This study reports blood values of common haematological and selected biochemical parameters in semi-feral, free-roaming Konik horses and their partial dependency on age or sex. In particular, we identified an age-dependent decrease in lymphocytes but not granulocytes and, therefore,

significant increase in the granulocyte-to-lymphocyte ratio with higher age. Although the number of erythrocytes did not depend on age, increasing age changed their haemoglobin content. Sex-dependent changes we mainly found for some serum biochemical parameters. This includes the levels of proteins (total, albumin, globulin), triglycerides, minerals (calcium, selenium) and activities of marker enzymes (alkaline phosphatase, aspartate transaminase, γ -glutamyltransferase). Castration of the male Koniks often abolished these sex-dependent differences. Based on these findings, this study then reports RI values each parameter and describes some differences to blood values reported for Koniks living in more controlled conditions.

Mean life expectancy of Konik horses is nearly 20 years.³ As the mean age of Koniks in our study was about 8 years and the range between 9 months and 22 years, we studied a horse population with many animals in the early or mid-

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Table 3: Selected biochemical parameters in serum samples of semi-feral Konik horses.

Parameter	units	male	female	reported		
				all ⁷	all ⁹	male ⁸
Protein, total	g/L	77,7 (55,5–94,2)	67,7 (55,3–81,9) ^b	71,2	66,1	68,4
Albumin	g/L	30,2 (27,0–35,8)	28,6 (23,0–34,8) ^b	38,0	36,1	–
Globulin	g/L	46,3 (25,3–59,2)	37,8 (26,5–49,8) ^b	33,2	30,0	–
Triglycerides	mmol/L	0,63 (0,14–1,81)	0,37 (0,12–1,57) ^b	0,10	0,33 ^a	0,31
Cholesterol	mmol/L	2,4 (1,6–3,3)	2,4 (1,9–3,8)	2,7	–	–
Glucose	mmol/L	5,3 (4,7–8,7)	5,5 (2,8–8,2)	4,0	3,8	6,2
Urea	mmol/L	6,1 (3,8–8,1)	6,1 (1,6–9,7)	4,7	8,2 ^b	–
Creatinine	μ mol/L	100 (75–130)	96 (53–265)	136	78,0 ^b	–
Bilirubin, total	μ mol/L	20,9 (12,2–45,0)	23,8 (11,6–50,7)	12,0	12,3 ^b	–
Alkaline phosphatase	U/L	171 (94–302)	148 (86–264) ^b	216	211	–
Aspartate transaminase	U/L	125 (117–271)	146 (79–196) ^b	433	395	–
Creatine kinase	U/L	229 (140–637)	226 (95–996)	328	599 ^b	–
μ -Glutamyltransferase	U/L	13,2 (3,7–62,4)	6,7 (3,6–65,0) ^b	–	55,0 ^{a, b}	–
Glutamate dehydrogenase	U/L	4,4 (1,3–54)	2,6 (0,9–17,4)	–	–	–
Lactate dehydrogenase	U/L	598 (386–937)	625 (338–1226)	–	–	604
Electrolytes & minerals						
Calcium	–	3,0 (2,8–3,2)	2,8 (2,5–3,3) ^b	3,0	–	–
Copper	–	14,9 (13,7–21,1)	14,3 (10,7–47,6)	–	–	–
Iron	–	23,2 (11,9–27,4)	22,5 (8,4–34,5)	–	–	–
Magnesium	–	0,8 (0,6–1,1)	0,8 (0,6–1,0)	0,9	–	–
Phosphorus, inorganic	–	1,0 (0,7–2,4)	1,1 (0,5–2,1)	1,2	–	–
Potassium	mmol/L	4,5 (2,7–5,4)	4,6 (3,9–8,0)	3,7	–	–
Sodium	mmol/L	136 (130–140)	134 (127–142)	141	–	–
Selenium	μ g/L	78,3 (53,9–115)	72,6 (42,0–97,6) ^b	–	–	–
Zinc	μ mol/L	11,6 (7,4–22,6)	11,4 (8,0–85,4)	–	–	–

Median data with total range compared with data reported in other studies. As available, we compared with data for median⁷ or mean⁹ of the entire group (male and female) or with mean data of the stallion group.⁸ P < 0,05 for the effect of age (Pearson correlation statistics) and ^bsex (two group statistics).

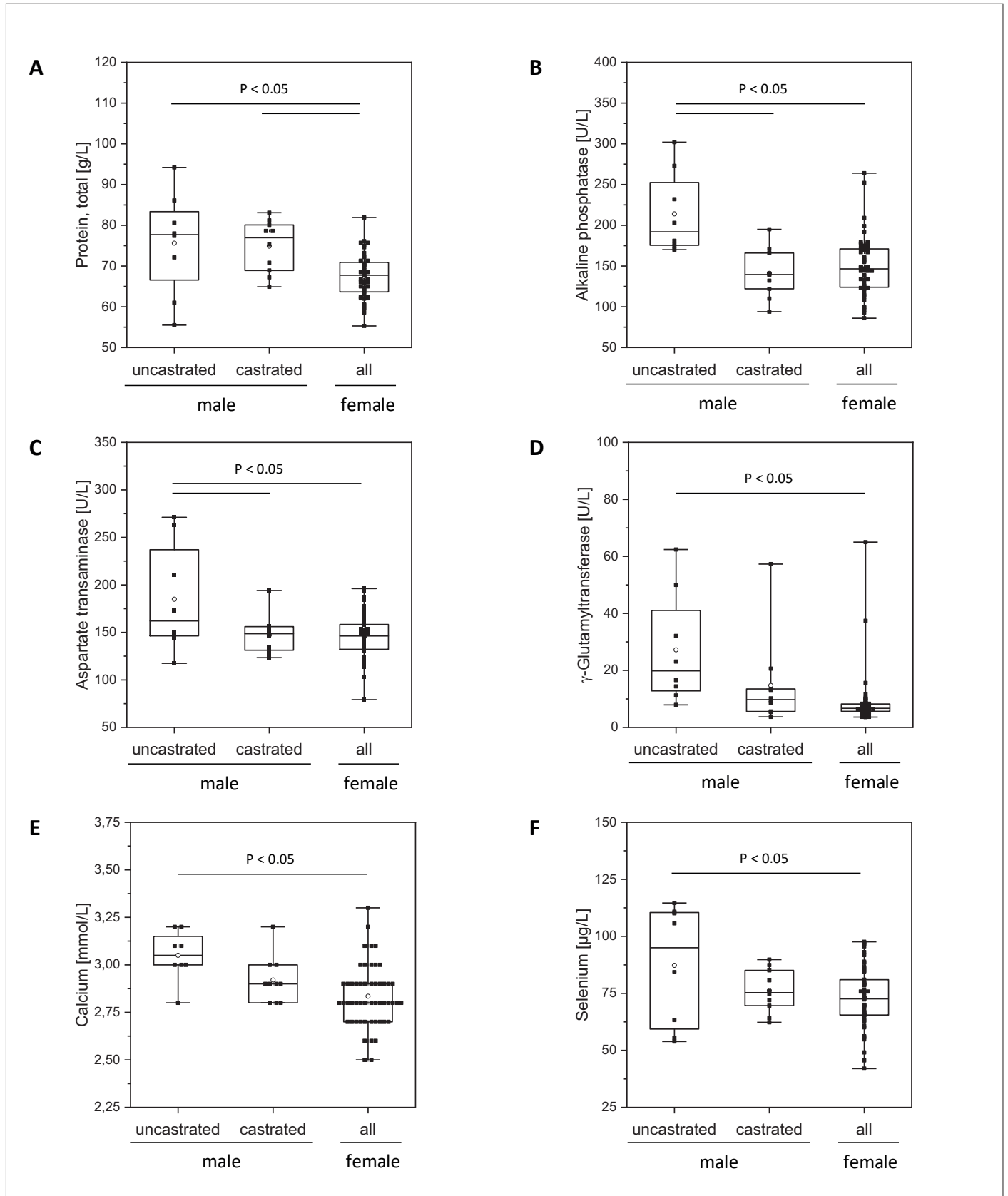


Figure 2: Serum levels of biochemical parameters (A–D) and minerals (E–F) in male Koniks depending on the castration status and female Koniks. Data are given as box plot (interquartile range) graph with median line, mean (O), individual data (■) and whiskers (total range). P < 0,05 as indicated (ANOVA statistics with post-hoc analysis).

dle adulthood and few animals in the pre- or late adulthood. Polish studies only included adult Koniks which were aged 3–15 years⁷, 2–16 years⁹, or 7–13 years⁸ but the mean age was comparable with that in our study. They analysed more female than male Koniks^{7,9} or only stallions.⁸ Our study also included more female than male horses, a fact that limits sex-dependent evaluations. Additional differences in some blood values between stallions and geldings within

the group of male Koniks enhances this limitation. Castration-mediated changes we identified for the activities of some serum enzymes indicating the influence of androgens on their metabolism in distinct tissues, such as liver, muscle and bone, and/or release into the blood. In this regard, serum activities of alkaline phosphatase, aspartate transaminase and γ -glutamyltransferase differ between male and female horses only in case of stallions. Analogue differenc-

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Table 4: Blood RI values calculated for all semi-feral Konik horses or subgroups as appropriate.

	Parameter	units	all	subgroups	
				≥90 months	≥90 months
Haemogram	Red blood cells	T/L	7,6 (7,3–7,9) – 11 (11–12)	n.n.	n.n.
	Haematocrit	L/L	0,33 (0,32–0,35) – 0,48 (0,47–0,51)	n.n.	n.n.
	Haemoglobin	g/L	126 (122–131) – 178 (174–183)	n.n.	n.n.
	MCH	pg	n.a.	12 (10–13) – 17 (17–18)	14 (13–15) – 18,5 (18–19)
	White blood cells	G/L	n.a.	6,2 (5,6–6,9) – 14 (13–16)	5,1 (4,6–5,6) – 11 (10–12)
	Neutrophils	%	n.a.	23 (21–30) – 64 (58–69)	42 (40–45) – 73 (67–80)
	Eosinophils	%	n.a.	1 (0–2) – 11 (10–13)	3 (2–4) – (12–16)
	Basophils	%	0 – 6 (5–7)	n.n.	n.n.
	Lymphocytes	%	n.a.	27 (23–31) – 70 (62–77)	17 (12–21) – 44–50)
	Monocytes	%	3 – 6	n.n.	n.n.
	GLR		n.a.	0,4 (0,3–0,5) – 2,6 (2,1–3,0)	1,1 (0,9–1,2) – 4,7 (3,5–6,7)
	Thrombocytes	G/L	79 (55–102) – 280 (266–293)	n.n.	n.n.
Biochemistry				geldings and mares	mares
	Protein, total	g/L	n.a.	n.a.	57 (55–59) – 79 (76–81)
	Albumin	g/L	n.a.	n.a.	23 (22–25) – 34 (33–35)
	Globulin	g/L	n.a.	n.a.	28 (27–31) – 49 (47–51)
	Triglycerides	mmol/L	n.a.	n.a.	0,1 (0,1–0,2) – 1,3 (1,0–1,7)
	Cholesterol	mmol/L	1,7 (1,6–1,8) – 3,3 (3,1–3,5)	n.n.	n.n.
	Glucose	mmol/L	4,4 (4,2–4,6) – 6,8 (6,5–7,1)	n.n.	n.n.
	Urea	mmol/L	4,1 (3,8–4,4) – 8,3 (8,0–8,7)	n.n.	n.n.
	Creatinine	μmol/L	68 (65–73) – 129 (124–135)	n.n.	n.n.
	Bilirubin, total	μmol/L	11 (10–12) – 53 (47–60)	n.n.	n.n.
	Alkaline phosphatase	U/L	n.a.	91 (84–101) – 235 (214–258)	n.n.
	Aspartate transaminase	U/L	n.a.	110 (105–116) – 191 (181–200)	n.n.
	Creatine kinase	U/L	124 (114–135) – 605 (479–777)	n.n.	n.n.
	μ -Glutamyltransferase	U/L	n.a.	3,7 (3,3–4,1) – 16 (13–19)	n.n.
	Glutamate dehydrogenase	U/L	1,1 (0,9–1,2) – 24 (14–45)	n.n.	n.n.
Lactate dehydrogenase	U/L	392 (367–420) – 1110 (994–1227)	n.n.	n.n.	
Minerals & electrolytes	Calcium	mmol/L	n.a.	2,5 (2,5–2,6) – 3,2 (3,1–3,3)	n.n.
	Copper	μmol/L	11 (11–12) – 24 (21–28)	n.n.	n.n.
	Iron	μmol/L	13 (12–15) – 33 (31–35)	n.n.	n.n.
	Magnesium	mmol/L	0,59 (0,55–0,62) – 1,05 (1,0–1,1)	n.n.	n.n.
	Phosphorus, inorganic	mmol/L	0,53 (0,47–0,60) – 1,8 (1,7–2,0)	n.n.	n.n.
	Potassium	mmol/L	3,4 (3,2–3,7) – 5,8 (5,5–6,2)	n.n.	n.n.
	Sodium	mmol/L	129 (127–130) – 140 (139–141)	n.n.	n.n.
	Selenium	μg/L	n.a.	47 (43–52) – 98 (94–103)	n.n.
Zinc	μmol/L	7,7 (7,4–8,1) – 29 (21–49)	n.n.	n.n.	

RI values are given with 90% (Bootstrap) CI for the 2,5th and 97,5th percentiles, respectively. Values ≥ 10 have been rounded to zero decimal places. Number of blood data can be less than the number of horses (Table 1) due to removal of outliers (0–3 depending on the parameter). n.a.: not applicable due to dependencies on age, sex or castration status (Tables 2–3, Figures 1–2) n.n.: not necessary to calculate.

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es between mares and stallions but not geldings have also been identified for the serum concentrations of calcium and selenium. Surprisingly, studies on Koniks^{7,9} or other horse breeds¹⁰ did either not analyse or identify such castration-associated effects. Witkowska-Pilaszewicz *et al.* also described moderate differences in the serum activity of the γ -glutamyltransferase between male and female Koniks⁹ but, in contrast to us, they⁹ and Niedzwiedz *et al.*⁷ did not describe a general sex dependency of the serum protein levels.

Aspartate transaminase in serum samples mainly originates from liver, heart and skeletal muscle.¹¹ Therefore, changes in the aspartate transaminase activity can only be interpreted together with the serum activity of the muscle-related creatine kinase.¹¹ The activities of both enzymes, and also of the γ -glutamyltransferase, were clearly higher in the studies of the Polish colleagues.^{7,9} This discrepancy eventually derives from differences in husbandry conditions. While we included only semi-feral, free-roaming Koniks, they studied Koniks living under more controlled conditions and used for other purposes.^{7,9} Moreover, technical differences are well-conceivable as they used other types of automated analysers.

Electrolytes and minerals including trace elements are essential for a well-balanced metabolism and, therefore, health in horses. In some cases, Niedzwiedz *et al.* analysed the same elements in Koniks as we did.⁷ This included calcium, magnesium, potassium and inorganic phosphorus, of which the serum concentrations had been comparable. Moreover, we analysed the serum concentrations of copper, iron, magnesium, selenium and zinc. Compared with RI data for horses in general and/or ponies in particular^{10, 12, 13} most elements were concentrated within the respective RI. Only the mean serum level of selenium was close to the lower reference limit of 70 $\mu\text{g/L}$.¹² As selenium levels depend on the chemical composition of the soil in areas used for pasture, both nature reserves used for keeping the Koniks of our study seem to offer marginal amounts of selenium only. Still, they did not show classical signs of selenium deficiency such as degradation of the musculature, tendency to infections or brittle coat and hair loss.¹⁴

Differences in distinct serum enzyme activities are not the only discrepancy between the data of our study and the Polish studies.^{7,9} Another discrepancy can be observed for the serum glucose concentrations which are commonly higher in the Konik population of our study. Changes in the serum glucose level usually depend on food and/or stress. In our case, higher glucose concentrations might be caused by the less compliance of the semi-feral Koniks to human handling and, therefore, higher stress level compared with the horse populations used in the Polish studies.^{7,9}

In contrast to some biochemical parameters showing sex-dependent changes, haemogram values often changed with increasing age. Age-dependent changes were mainly observed

for the number of lymphocytes and its relation to the number of granulocytes, the so-called GLR value. In detail, increasing age causes higher GLR values due to an age-dependent decrease of lymphocytes but not granulocytes. This is a well-known phenomenon also observed in horses including ponies.^{9, 10} It is mainly explained by the higher susceptibility of lymphocytes than granulocytes to telomere shortening-induced cell death.¹⁵ In many cases, GLR is only calculated for the number of neutrophils per lymphocytes.¹⁰ We decided for the GLR calculation by including all types of granulocytes because the age-dependent decrease in lymphocytes also affected the percentage of eosinophils. Although GLR values are popular markers for biological age of humans,¹⁶ they are still less applied to horses or other animal species.

In addition to the leukocyte parameters, red blood cell-related values can depend on age. This has been shown by Witkowska-Pilaszewicz *et al.* for the total number of erythrocytes and MCH values in Koniks and other ponies.⁹ While these authors found a negative correlation between red blood cell count and age but positive correlation between MCH values and age, we only identified an age dependency of the MCH values. Larger erythrocyte (mean corpuscular) volumes, enable higher amounts of haemoglobin per cell.^{9, 10} We did not observe an effect of age on the red blood cell count because the number for erythrocytes in the Konik population of this study was clearly higher than in the population of Witkowska-Pilaszewicz *et al.*,⁹ whereas the number of thrombocytes was only half as high. Although these authors did not analyse serum iron,⁹ it indirectly indicates less iron availability in their horses. Differences in the erythrocyte counts also explain the different serum bilirubin values between our both studies.⁹ Also, Niedzwiedz *et al.* detected relatively low serum bilirubin concentrations in their Konik population suggesting lower erythrocyte counts as well.⁷

Important aim of the present study had been the comparison of common and specific blood values on semi-feral, free-roaming Konik horses with blood values reported by others for Koniks living in more controlled conditions. Another important aim had been the calculation of RI values each blood parameter. Ideally, RI calculations comprise animal populations larger than 120 healthy individuals.^{4, 5} As Koniks are still a minor breed of horses it took already some effort to find a location with a few dozen animals for our study. Because of the relatively low number of animals and the need for age- and/or sex-related subgrouping, the calculation of Konik-related RI values was only possible with some limitations. Further limitations had been natural restrictions in monitoring their health as well as some technical features (*i.e.*, long period of the day for taking blood, time until blood analysis in the laboratory). Moreover, our RI values refer to blood analysis performed by certain types of automated analysers. However, these technical limitations are common limitations in daily veterinary practice and should, therefore, not impair the quality of our RI values.

In conclusion, our study contributes to the health monitoring of Konik horses by presenting age- and sex-dependent blood values. Although many blood values presented are suited as RI values, further studies are needed to clarify the RI values for certain subgroups, especially the stallions, and to clarify distinct differences between data identified by us or other studies.

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Hémogramme en fonction de l'âge et valeurs biochimiques sériques en fonction du sexe chez les chevaux Konik semi-sauvages

Les chevaux Konik Polski semi-sauvages en liberté sont utilisés dans certains pays européens pour préserver les paysages de pâturages semi-ouverts. L'estimation de leur état de santé est encore limitée par le manque de données sur divers paramètres sanguins. C'est pourquoi notre étude visait à analyser, en fonction du sexe et de l'âge, l'hémogramme et certains paramètres biochimiques chez des Konik semi-sauvages en bonne santé. Pour ce faire, nous avons prélevé des échantillons de sang sur 53 femelles et 18 mâles (8 non castrés, 10 castrés) vivant dans deux réserves naturelles d'Allemagne centrale. Ils étaient d'âges différents (9-266 mois) et ne présentaient aucun signe de maladie. Les échantillons de sang ont été analysés par un laboratoire accrédité.

Nous avons identifié des changements dans la numération des globules blancs (WBC) qui dépendent de l'âge mais non du sexe. L'augmentation de l'âge a principalement entraîné une diminution des lymphocytes. Par conséquent, le nombre de globules blancs est corrélé négativement et le rapport granulocytes/lymphocytes positivement avec l'augmentation de l'âge. Les valeurs sériques de certains paramètres biochimiques ne dépendaient pas de l'âge, mais présentaient certaines différences liées au sexe. À cet égard, les protéines totales sériques, les triglycérides et les activités enzymatiques de la phosphatase alcaline, de la transaminase aspartate et de la γ -glutamyltransférase étaient plus élevés chez les mâles que chez les femelles. Cependant, la dépendance de ces activités enzymatiques par rapport au sexe était limitée aux mâles non castrés. Ces derniers présentaient également des valeurs sériques de calcium et de sélénium plus élevées que les mâles castrés ou que toutes les femelles. Dans la mesure où la taille des groupes respectifs le permettait, nous avons ensuite calculé des valeurs d'intervalle de référence dépendant de l'âge ou du sexe pour tous les paramètres analysés. Ces valeurs améliorent désormais l'estimation de l'état de santé des chevaux Konik semi-sauvages en liberté et fournissent une base stable pour les études futures.

Mots clés: sang, race, castré, libre, valeur de référence

Valori dell'emogramma e della biochimica sierica dipendenti dall'età e dal sesso nei cavalli Konik semi-selvatici

I cavalli Konik polski semi-selvatici, liberi di vagare, sono utilizzati in alcuni paesi europei per preservare paesaggi di pascoli semi-aperti. La stima del loro stato di salute è ancora limitata da dati insufficienti su vari parametri ematici. Pertanto, il nostro studio aveva l'obiettivo di analizzare i parametri dell'emogramma e della biochimica sierica predefiniti in relazione al sesso e all'età nei Konik sani e semi-selvatici. Per raggiungere questo obiettivo, abbiamo prelevato campioni di sangue da 53 femmine e 18 maschi (8 non castrati, 10 castrati) di cavalli Konik che vivevano in due riserve naturali della Germania centrale. Erano di diverse età (9-266 mesi) e senza segni di malattia. I campioni di sangue sono stati analizzati da un laboratorio accreditato.

Abbiamo identificato cambiamenti dipendenti dall'età, ma non dal sesso, nel conteggio dei globuli bianchi (WBC). L'età più avanzata ha principalmente causato una diminuzione dei linfociti. Pertanto, il WBC è risultato correlato negativamente e il rapporto granulociti-linfociti positivamente con l'età crescente. I valori sierici dei parametri biochimici selezionati non dipendevano dall'età, ma mostrano alcune differenze legate al sesso. In questo senso, le proteine totali sieriche, i trigliceridi e le attività enzimatiche della fosfatasi alcalina, dell'ast e della γ -glutamyltransferasi erano più alte nei maschi rispetto alle femmine. Tuttavia, la dipendenza dal sesso di queste attività enzimatiche era limitata ai maschi non castrati. Essi mostrano anche valori sierici più elevati per il calcio e il selenio rispetto ai maschi castrati o a tutte le femmine. Per quanto le dimensioni dei gruppi lo hanno permesso, abbiamo quindi calcolato valori di intervallo di riferimento dipendenti dall'età o dal sesso per tutti i parametri analizzati. Questi valori migliorano ora la stima dello stato di salute dei cavalli Konik semi-selvatici e liberi di vagare e forniscono una base stabile per studi futuri.

Parole chiave: *Coxiella burnetii*, aborto, nati morti, capra, zoonosi

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