INVESTIGATION OF EQUINE HEMATOLOGICAL CONSTITUENTS IN CENTRAL TAIWAN. I. DISTRIBUTION OF THE BLOOD CELL PARAMETERS AND THE BIOCHEMICAL COMPOSITIONS OF SERUM

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Summary

A total of 109 heads of horses and ponies from eight horseback riding clubs nearby Central Taiwan were investigated to evaluate the blood parameters and the biochemical compositions of scrum for the documentation of clinical pathological diagnosis and exercise physiology. Blood samples were collected from the jugular vein of resting horses. The sex difference in the blood traits were compared both in horses and in ponies. Results shows that total plasma proteins (PP) and hematocrit (PCV) were found higher in male horses than in females (p < 0.05). The sexual effect also exertes a significant influence on the leucocyte (WBC) count, but not on the erythrocyte (RBC) concentration. According to the differential counts of leucocytes, the number of monocytes and lymphocytes was higher in the male pony than that of in male horse. A close relationship between the erythrocyte sedimention rate (ESR) and the other blood parameters were found especially in PCV, RBC concentration, and plasma protein level. The average ESR observed at 60 minutes were 21.80 ± 21.87 mm, 39.50 ± 18.90 mm and 43.73 ± 17.89 mm in stallions, geldings, and mares, respectively. Most of the biochemical components of horse serum detected were distributed in normal ranges, although some of the items show a great variation in such a large sample size.

(Key Words: Horse, Pony, Leucocyte Differential Count, Blood Cell Parameters, ESR, PCV, Serum, Biochemical Compositions)

Introduction

There were no more than eight hundreds heads of horses until 1990 in Taiwan (COA investigation, 1990). Most of the horses in this Island were come from United States, Japan, Australia, New Zealand, Germany and even Mainland China for the dressage competitions and show jumping except the race track running. Recently, the number of horses has been increased to one thousand heads or more within the past two years owing to increasing importation. The Council of Agriculture has also shown interesting in promotion of raising horses for leisure-riding or as a pet animals for Taiwan people. Unfor-

This investigation was firstly focused on the blood components and the serum compositions of horses and ponies to evaluate the normal ranges of these physiological parameters of equine in Taiwan for the documentation of clinical veterinary and exercise physiology.

Materials and Methods

Blood samples of 109 horses from 8 farms or horseback riding clubs in Central Taiwan, including 46 mares, 15 stallions, and 48 geldings were taken from the jugular vein with commercial EDTA-rinsed syringe. All the horses allowed to

tunately, it is still quite difficult to refer any imformations related to horse management, nutrition, physiology, reproduction, and veterinary medicine due to inadequate documentation under such a particular environment of subtropical Taiwan. Therefore, it is necessary to perform series of evaluations as a standard reference in equine science in Taiwan.

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be withdrawn blood were under resting condition. The age of horses was determined following the principles described by Ensminger (1977) when the identification records of the horses were unavailable.

Fresh blood samples were used to examine pH value immediately and then the complete blood counts (CBC) was performed. The concentration of erythrocytes (RBC), leucocytes (WBC), and thrombocytes were estimated with the Neubauer chamber of the hematocytometer (Bailey et al., 1984). Total plasma protein (PP) was measured by a refractometer (TS meter). The fibrinogen, hemoglobin (Hb), erythrocyte sedimentation rate (ESR), the hematocrits or packed cell volume (PCV) and the Wintrobe erythrocyte indexes, i.e., the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), were based on the Wintrobe's methods described by Schalm et al. (1982a); whereas the ESR was test with the Wintrobe's tube and recorded at six consecutive 10-minute intervals for one hour. The blood films for differential leucocyte counts were stained with the modified Wright's stain for 4-6 minutes (Schalm et al., 1982a).

The serum was separated from blood cells by centrifugation (3,500 rpm, 1,500G, for 15 minutes) and was stored at -40°C before being analysed. The biochemical parameters and the electrolytes of the serum, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (T-BIL), direct bilirubin (D-BIL), total scrum protein (TP), albumin (ALB), globulin (GLO), blood urea nitrogen (BUN), creatinine (CRE), uric acid, triglyceride, total cholesterol (CHO), blood glucose (GLU), creatine phosphokinase (CPK), lactate dehydrogenase (LDH), magnesium, calcium, sodium, iron, potassium and inorganic phosphorus were determined by the Automatic analyzer 7050 (Hitachi) or atomic absorption analyzer.

In the statistical models, the effects of sexes were analysed on both of the horse and the pony with the General Linear Model (GLM) of Statistic Analysis System (SAS). Some of the items described by Schalm et al. (1982b) and Sato et al. (1978) were analysed with the ages as a covariate. Means of the different criteria were compared by the Least Square Means of t-test.

Results

The distribution of blood parameters

The comparisons of blood parameters between horse and pony in different sexual categories are listed in table I. Data show that most of the blood parameters, including the pH value, erythrocyte number, thrombocyte (or platelets) concentration, hemoglobin concentration, fibrinogen content, plasma protein fibrinogen ratios (PP:F) and the Wintrobe's indexes, were not significantly influenced by sexes, regardless of horses of in ponies. However, as it is shown in table 1, the sexes and groups (horse and pony) exert a significant effect (p < 0.05) on leucocyte count, total plasma protein, and the hematocrit.

Results of the leucocyte differential counts are presented in table 2, which reveals significant effect among categories only in the percentages of lymphocytes and monocytes. Correlation coefficients (r) between PCV and ESR detected in 10-minute interval were ranged from -0.35 to -0.91 (data not shown). The relationships between sedimentation rate of crythrocytes (ESR) and various packed cell volume (PCV) are shown in figure 1. This results indicated that the higher the PCV value, the slower the blood cells settling down.

Serum compositions

The distribution and comparison of the serum biochemical parameters and mineral or electrolyte concentration are list in table 3 and table 5 from which reveal a wide range of variation for such a large sample size.

Data show no significant difference between horse and pony in statistical analysis are AST, ALT, BUN, LIDH, and blood glucose concentration of each group, whereas the alkaline phosphotase of gelding was higher than that of stallion as the same trend as it presented in total cholesterol. The lists in table 4 are those of the biochemical items of scrum demonstrate significant different (p < 0.05) between horse and pony in different sexual criteria, which are T-bilirubin, D-bilirubin, albumin, creatinine, uric acid, triglyceride, and creatine phosphokinase. Serum protein is highest in gelding of pony and lowest in mare of horse. Globulin concentration also possess the similar tendency, whereas the average A:G ratio is apparently higher in horse than that of in pony.

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TABLE 1. COMPARISON OF BLOOD PARAMETERS BETWEEN HORSES AND PONIES IN DIFFERENT SEXUAL CATEGORIES*

		Horse			Pony	_=
Criteria	Stallion	Gelding	Mare	Stallion	Gelding	Mare
	n = 8	n = 40	n = 40	n = 7	n = 8	<u>n</u> = 6
Age, year	7.63±1.36 ^{bcd}	9.28±0.62°	$10.95 \pm 0.62^{\rm ac}$	4.57 ± 1.46^{bd}	6.25 ± 1.36 ^d	7.83 ± 1.57abcc
pH value	7.47 ± 0.03	7.50 ± 0.02	7.50 ± 0.02	7.49 ± 0.04	7.48 ± 0.03	7.50 ± 0.05
RBC, 10 ⁶ /μl	10.82±0.75	10.25±0.35	9.48 ± 0.36	10.66±0.80	10.74上0.71	9.97±0.81
WBC, 10³/μl	6.67 ± 0.66 ^b	7.59±0.32 ^{ab}	7.23+0.31 ^{8b}	6.45±0.76 ^b	8.24±0.83ab	8.93±0.83ª
Platelets, 104/µl	8.47±2.89	13.14±1.33	12.14±1.39	6.89±3.07	12.68±2.75	10.38±3.11
Hb, g/dl	10.52 ± 0.65	9.92 ± 0.31	10.05 ± 0.31	10.19 ± 0.74	10.49 ± 0.81	10.67 ± 0.81
PCV, %	46.42 ± 2.13^{b}	41.62±1.00°c	39.77 ± 1.00^{n}	$43.29 \!\pm\! 2.42^{\rm ab}$	44.88 ± 2.17bc	43.10 ± 2.45^{ab}
PP, g/dl	7.28±0.17 [∞]	6.95 ± 0.08^{ab}	6.75 ± 0.08^{a}	7.27 + 0.20 bc	7.39 ± 0.18^c	7.24 ± 0.20 bc
Fibrinogen, g/dl	0.25 ± 0.05	0.27 ± 0.02	0.25 ± 0.02	0.32 ± 0.05	0.30±0.05	0.31±0.06
MCV, fl	44.09 ± 3.04	41.79±1.40	43.78 ± 1.45	41.63 ± 3.23	42.94 ± 2.89	46.08 ± 3.27
MCH, pg	10.11 ± 1.21	10.27 ± 0.58	11.11 ± 0.59	9.91 ± 1.31	10.09 ± 1.44	12.76 ± 1.43
MCHC, g/dl	22.92 + 1.48	24.34 + 0.72	25.67 ± 0.70	23.77±1.71	24.28 ± 1.87	25.74 ± 1.86
ESR, mm/hr	17.38±6.44 ^b	43.16±2.96ac	$44.91 \pm 2.92^{\rm a}$	26.86 ± 6.89^{t}	22.13±6.44bcd	36.08±7.44°b
PP : F**	33.31 ± 8.43	35.87±3.82	35.38 ± 3.77	28.58±9.01	24.61 ± 8.43	26.85±9.73

^{*} Different superscripts (a,b,c,d) in the same row stand for significant difference between each two categories (p < 0.05).

TABLE 2. COMPARISON OF LEUCOCYTE DIFFERENTIAL COUNT BETWEEN HORSES AND PONIFS IN CENTRAL TAIWAN*

Citania	Horse			ропу		
Criteria (%)	Stallion n = 8	Gelding n = 40	Маге <u>п</u> = 40	Stallion n = 7	Gelding n = 8	Mare n = 6
Buffy coat	0.54 ± 0.04	0.47 ± 0.02	0.47 ± 0.02	0.42 ± 0.04	0.53 ± 0.04	0.50 ± 0.04
Banded Neutrophils	7.73±2.52	11.57±1.34	9.37 ± 1.21	9. 29 ± 2 .92	7.42±2.96	13.85±2.90
Mature Neutrophils	43.36±4.44	34.24±2.35	34.25+2.12	38.23 ± 5.14	28.71 ± 5.22	31.86±5.11
Lymphocytes	s 34.53±5.79°	40.11 ± 3.07^{ab}	41.10±2.77 ^{ab}	42.20±6.69ªb	53.41±6.80 ^b	43.12±6.66ªb
Monocytes	4.73±1.51 ^{ab}	$4.76 \pm 0.80^{\rm a}$	5.56+0.7288	7.13±1.74ab	8.83±1.77 ^b	4.83±1.73ab
Eosinophils	7.23 ± 3.21	8.58 ± 1.70	7.70±1.54	1. 4 9±3.72	3.19 ± 3.78	5.23 ± 3.70
Basophils	0.55 ± 0.41	1.08 ± 0.22	1.01 ± 0.20	0.24 ± 0.47	0.58 ± 0.48	0.23 ± 0.47
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[•] Different superscripts (a,b) in the same row stand for significant different between each two categories (p < 0.05).

^{**} PP : F indicates Plasma protein : Fibrinogen ratio.

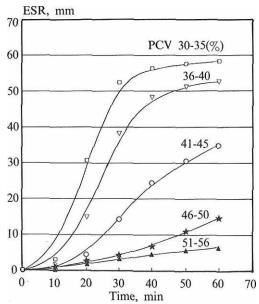


Figure 1. The relationship between erythrocyte sedimentation rate (ESR) and packed cell volume (PCV) of equine.

Discussion

In this investigation, the distribution of blood characteristes detected from the horses and ponies had been pooled together (data not shown). It was shown that the pH values maintained at the physiological condition. The PCV was ranged from 30-56% with the average of $41.62 \pm 6.46\%$, which was similar to the figure (39.4 \pm 7.1%) revealed by Garcia and Beech (19.86). The average hemoglobin concentration and MCH were 10.10 g/dl and 10.69 pg, respectively, which were a little lower than the normal value (13.03 g/dl and 13.99 pg) when compared to that of the report from Kniil et al. (1969). However, the rests of the traits were all similar to the results released by the same authors.

The animals were further classified into 2 groups, i.e. the horse and the pony. In each of the group includes different sexes (stallion, mare and gelding) of the animals. The least square means were adjusted with the ages as a covariate

TABLE 3. DISTRIBUTION OF BIOCHEMICAL PARAMETERS AND MINERAL OR ELECTROLYTE CONTENT OF HORSE SERUM

Biochemical parameters (Abbreviation)	N	Minimum	Maximum	Mean \pm S.D.
Aspartate aminotransferase (AST), $\mu \parallel$	78	92.00	636.00	213.17 ± 73.77
Alanine aminotransferase (ALT), μ / l	78	2.00	18.00	6.49 ± 3.14
Alkaline phosphatase (ALP), µ/l	78	68.00	456.00	224.15 ± 78.09
Total bilirubin (T-B1L), mg/dl	78	0.36	3.47	1.21 ± 0.50
Direct bilirubin (D-BIL), mg/l	78	0.24	1.44	0.55 ± 0.21
Total serum protein (TP), g/dl	99	4.80	8.40	6.99 ± 0.54
Albumin (ALB), g/dl	99	1.90	3.70	2.69 ± 0.32
Globulin (GLO), mg/dl	99	2.90	5.50	4.30 ± 0.49
Albumin/Globulin ratio, A/G	99	0.40	0.90	0.64 ± 0.12
Blood urea nitrogen (BUN), ang/dl	98	9.60	29.90	16.36 ± 4.19
Creatinine (CRE), mg/dl	76	0.50	1.80	1.27 ± 0.26
Uric acid (UA), mg/dl	75	0.10	0.60	0.35 + 0.11
Triglyceride (TG), mg/dl	98	7.00	94.00	25.92 ± 14.15
Total cholesterol (CHO), mg/dl	98	49.00	122.00	85.15 ± 14.37
Glucose (GLU), mg/dl	98	42.00	235.00	90.91 + 21.37
Creatine phosphokinase (CPK), µ/1	98	48.00	643.00	133.43 ± 82.98
Lactate dehydrogenase (LDH), MI	35	88-00	795.00	418.14 ± 164.30
Potassium (K), mg/l	104	106.70	343.50	161.19 ± 36.33
Sodium (Na), mg/l	106	3942.00	7542.00	5746.00 ± 715.89
Iron (Fe), mg/I	99	1.01	4.26	2.41 ± 0.66
Magnesium (Mg), mg/dl	42	1.70	9.40	3.06 ± 1.61
Calcium (Ca), mg/dl	95	8.79	15.27	11.35 ± 1.03
Phosphorus (P), mg/dl	97	1.70	8.90	3.07 ± 1.07
Calcium/Phosphorus ratio (Ca : P)	95	1.19	6.69	3.98 ± 0.99

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TABLE 4. COMPARISON OF BIOCHEMICAL PARAMETERS BETWEEN HORSE SERUM AND PONY SERUM OF DIFFERENT SEXUAL CRITERIA

	Horse			Pony		
ltems	Mare	Stallion	Gelding	Mare	Stallion	Gelding
Serum protein g/dl	n, 6.79±0.09ª	7.33±0.20°	7.00 ± 0.08^{ab}	6.92±0.22 ^{ab}	7.18±0.18 ^{ab}	8.07±0.28°
T-bilirubin, mg/dl	1.24±0.09 ⁿ	1.87±0.18°	1.23±0.08ª	0.48±0.20 ^b	1.00 ± 0.16^{B}	1.31±0.31 ^{BC}
D-bilirubin, mg/l	0.55 ± 0.04^{a}	0.84 ± 0.08^{c}	0.55±0.04 ⁸⁴	0.30 ± 0.09^{h}	0.43 ± 0.07 abd	0.62±0.13 ^{ac}
Albumin, g/dl	2.61 ± 0.05^{a}	3.01±0.12°	2.77±0.05 ^{cd}	2.27±0.14 ^b	2.62±0.[] ^{ad}	2.69±0.18abc
Globulin, mg/dl	4.18 ± 0.08^{a}	4.32±0.19 ⁸⁶	4.23±0.08ab	4.65±0.21 ^b	4.56±0.17 ^b	5.37±0.27°
A : G ratio	0.64 ± 0.02^{acd}	0.71 ± 0.05^{a}	$0.66 \pm 0.02^{\mathrm{ac}}$	0.50 ± 0.05^{b}	0.59±0.04 ^{bcd}	0.51±0.07 ^{bd}
Creatinine, mg/dl	1.29 ± 0.06^{ab}	1.43±0.11 ^a	1.30 ± 0.05 ^{8b}	1.19±0.12ªb	1.17±0.10 ^{ab}	0.96±0.19 ⁶
Uric acid, mg/dl	0.35 ± 0.02^a	0.32 ± 0.08^a	0.33±0.02°	0.34 ± 0.06^{a}	0.35 ± 0.05^{a}	0.58±0.08 ^b
Triglyceride, mg/dl	28.45±2.40°c	22.62±5.51 ^{ad}	20.28 ± 2.28 ^d	40.35±6.16 ^{bc}	37.38±4.95 ^{bc}	27.59±7.92 ^{cd}
Creatine phospokina	136.22±14.85° ase, μ/I	7 9. 2 9±34.17 ^a	128.96±14.1 2 ª	155.69 ± 38.21 ac	127.62 ± 30.71ª	247.36±49.16 ^{bc}

^{*} Different superscripts (a,b,c,d) in the same row stands for significant difference between each two categories (p < 0.05).

TABLE 5. COMPARISON OF MINERALS OR ELECTROLYTES LEVELS OF HORSE SERUM IN DIFFERENT SEXUAL CRITER.A

Items	Mare	Stallion	Gelding	
Iron, mg/l	2.62 ± 0.10^{n}	2.32± 0.19 ^{ab}	2.23 ± 0.09^{h}	
Sodium, mg/l	5768.83 ± 111.49	5734.47 + 186.56	5729.04 ± 103.22	
Potassium, mg/l	168.88± 5.5▮	155.69 ± 9.65	155.80 ± 5.27	
Magnesium, mg/dl	3.26 ± 0.37	2.44 ± 0.61	3.08 ± 0.41	
Calcium, mg/dl	10.99 ± 0.16^{a}	11.84 ± 0.26^{b}	$11.51 \pm 0.15^{\circ}$	
Phosphorus, mg/dl	3.12 ± 0.17	3.31 ± 0.28	2.95 ± 0.17	
Ca : P ratio	3.94 ± 0.16	3.71 ± 0.26	4.11 ± 0.15	

^{*} Different superscripts (a,b) in the same row stands for significant difference between each two categories (p < 0.05).

because there were significantly defferent in age among all the categories (table 1), which might affect some of the blood characteristics (Schalm et al., 1982b). The PCV value and total plasma protein shown in table I were significantly higher in stallion than in mare, which gives totally the same trend as the RBC concentration, although the RBC concentration only slightly higher, but not significant both in male horse and male pony. This result quite coincided with the report

of Schalm et al. (1982b) in Thoroughbred and Arabian horse.

It is well known that the concentration of blood cells in bloodstream is affected by several factors, including age (Sato et al., 1978), feedstuffs (Kerr and Snow, 1982), and anesthesia (Steffey et al., 1980). Especially when animals are under stress, in exciting condition and during or right after taking exercises, the blood cells will be released from the splenic contraction, which results in an immediate elevation of erythrocyte and/or leucocyte levels in the peripheral circular system of horse (Garcia and Beech, 1986; Rose, 1982; Schalm et al., 1982b; Snow et al., 1982). The variation of erythrocyte number in table 1 and the leucocyte differential counts didn't show obvious difference among horses except for lymphocyte and monocyte number (table 2). The exact reasons were not clear. It is probably due to the variation in different physiological excitement or training programs of the animals while being sampled. Also, it might be comfound by the effect of different breeds from which we cannot extract the error sum of square in the statistical model, because the original identifications of most horses were unavailable in this field investigation. As to the buffy coat, which primarily composed of thrombocytes (or platelets) and lencocytes, is only a rough estimation of leucocyte level in blood samples. Accordingly, it is hardly to find any consistency with the leucocyte concentration among different categories of the animals.

The sedimentation rate in this experiment was observed in every 10-minute's interval for 1 hour. The highest rate of sedimentation was found at 20-30 minutes after observation (circa 1.0-1.4 mm/min) and tended to be slow down afterwards (figure 1). It was almost completely settled down within 1-2 hours due to the character of horse blood cells much readily tends to rouleaux formation. This phenomenon is quite different from the other domestic species, especially the ruminants, which need 24 hours to fall only 2.2-4.0 mm in a normal condition (Schalm et al., 1982 b).

Our data further demonstrated that the ESR measured at the 60th (ESR 60) minute was highly correlated to the distance of settling of the blood cells at the interval between the 20th and the 30th minute (r = 0.79, p < 0.0001). It probably

revealed that the time required to determine the ESR of horse blood could be shorten to 30 minutes if more time was not allowed to take a clinical measurement.

The AST (or GOT, glutamic oxalacetic transaminase) and ALT (or GPT, glutamic pyruvic transaminase) are two enzymes widely used as an indicator for clinical diagnosis of hepatitis in human. When the liver function was damaged, the concentration of the enzymes will tend to elevate in serum. However, the determination of ALT is only useful in diagnosis of hepatic function of small animals and primates since livers of larger species like horse, cattle, and sheep contain only insignificant amounts of ALT (Cornelius, 1989). The ALT level also used for detection of neuromuscular disorders of domestic animals and physical activity in horses (Cardinet III, 1989). As shown in table 4, the average AST value is $213.17 \pm 73.77 \ \mu/l$, which is only slightly lower than that of a healthy horse described by Steffey et al. (1980) and Kaneko (1989) (226-366 µ/l), and so is the GTP value (Kaneko, 1989; Sato et al., 1978). In this investigation, regardless the methodology used in testing, the AST and ALT levels were examined with the serum collected from the animals in different breeds and different training program or physiological conditions of each horse. It can be realized that the levels of these two emzymes were slightly varied from the normal value but still within an acceptable range. The ALP, T-BIL, D-BIL, TP, ALB, BUN, CRE, LDH, total cholesterol and blood glocose were in a normal range for a resting horse (Steffey ct al., 1980; Snow et al., 1982). However, the average concentration of uric acid was slightly higher than the resultants of Snow et al. (1982; 0.35 ± 0.11 vs. 0.23 mg/dl).

Potassium is the major intracelluar cation of body tissue in which contains 105 mmol/1 (4106 mg/l) in human blood cells. This value is approximately 23 times higher than that of in extracellular fluids, namely 137-207 mg/l (Tietz, 1976). As it was shown in table 3 and table 5, the concentration of potassium in serum was similar to both the normal value of human serum and horse serum described by Kerr and Snow (1982). In the concentration of calcium and phosphorus, the ranges in this investigation coincided with the baseline revealed by Kerr and Snow (1982) and Steffey et al. (1980). The cal-

cium/phosphorus ratio of serum was in an average of 3.98 ± 0.99 (table 3). There are no significant difference in calcium/phosphorus ratio between different sexes (table 5). It is still to be explained that the sodium and the magnesium concentrations evaluated in this paper were far above the normal value reported by Snow et al. (1982), i.e. 5746 mg/l vs. 3220 mg/l in sodium and 3.06 mg/dl vs. 1.7 mg/dl in magnesium, respectively.

We noticed a normal range of serum protein list in table 3. Although changes in the levels of serum proteins might be attributed to individual differences, hemoconcentration, and hepatic disease, it can be considered as a convenient parameter of water balance and other organ disorders of animal body.

The determination of BUN is used for evalnation of kidney function. This test is usually requested along with the serum creatinine test for differential diagnosis of prerenal, renal and postrenal hyperuremia. Because of less sensitivity of blood creatinine concentration in response to the renal impaired, it is not considered as a moderate or severe kidney damage until the creatinine level is above 2.4 mg/dl in serum, of which the normal values are 0.5-1.2 mg/dl in human, 1.0-2.7 mg/dl in swine, and 1.2-1.9 mg/dl in horse and sheep (Kaneko, 1989; Tietz, 197 6). Our data in table 3 demonstrates a normal value of 1.27 ± 0.26 mg/dl and in table 5 shows the level of the stallion of horse is higher than that of a castrated pony (p < 0.05). The different between horse and pony in those items list in table 4 might be attributable to the breed effect and different state of excitement of the animals during sampling. The pony usually performed much more sensitive than the horse did when being withdrawn blood by jugular venipuncture.

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