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HEMATOLOGIC AND BLOOD CHEMICAL CHARACTERISTICS OF FERAL HORSES FROM THREE MANAGEMENT AREAS

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ABSTRACT: Blood was collected from 486 feral horses of mixed sex and age classes captured from three wild horse management areas in Nevada and Oregon from December 1985 to February 1986. Males were significantly outnumbered by females in the Flanigan area, but both sexes were represented in approximately equal numbers in the Wassuk and Beaty's Butte areas. Hematology and chemistry values averaged 16.4 ± 0.11, 46.3 ± 0.28, 9.9 ± 0.07, 6.9 ± 0.10, 47.1 ± 0.24, 16.6 ± 0.09, 35.2 ± 0.09, 10.4 ± 0.14 and 23.4 ± 0.25 for hemoglobin (HGB), hematocrit (HCT), red blood cells (RBC), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), cortisol (F) and serum urea nitrogen (SUN), respectively. Statistically significant differences in HGB, HCT, RBC, WBC, MCV and MCH levels occurred with respect to age ($P \leq 0.001$). Serum F levels were lower in immature animals than in either subadult or adults in all areas. Flanigan horses appeared in the poorest condition and had the lowest HGB, HCT and RBC counts while the values for Wassuk horses were significantly higher ($P \leq 0.001$). Serum F levels were lowest in the Flanigan horses. A significantly lower ($P \leq 0.001$) proportion of adult mares had progesterone levels consistent with pregnancy in the Flanigan horses versus those from the other two areas. These data are consistent with a subjective evaluation of the condition of the horses.

Key words: Feral horses, *Equus caballus*, hematology, serum chemistry, trapping and handling stress, field survey.

INTRODUCTION

Enactment of the Wild Free-Roaming Horse and Burro Act in 1971 (PL 92-195) by the Congress of the United States has resulted in increased growth of many populations of feral horses (*Equus caballus*) in the western United States. The Bureau of Land Management (BLM) estimated the population of feral horses and burros in western North America to exceed 50,000 animals and has proposed appropriate management levels at 25,000 to 30,000 animals (Boyles, 1986). The implied overstocking may result in depletion of forage in certain herd management areas. Proper management of feral horse populations in western North America is dependent upon knowledge of population sizes, rates of increase and mortality, and other factors affecting herd health. Reproduction and mortality are at least partially dependent on the physical condition of the animals.

Blood data from wild-caught animals may contribute to the pool of scientific information on its own merits but data on blood characteristics and composition from wildlife populations could be helpful in comparing health and disease status among populations of the same species (Seal et al., 1978). This study was undertaken to compare the condition of three feral horse herds by hematologic and metabolic measurements and to establish a reference range of values in undrugged feral horses. Data are presented that relate blood hematological parameters, serum urea nitrogen levels and progesterone and cortisol levels to subjective estimation of body condition in populations of feral horses from three different management areas.

MATERIALS AND METHODS

One hundred eighty feral horses were captured from the Flanigan management unit (40°0′ to 40°15′N, 119°40′ to 120°0′W) in northwestern Nevada during December 1985, 143 horses from the Wassuk Mountains (38°35′ to 38°55′N, 118°50′ to 118°55′W) of west central Nevada during January 1986 and 163 horses from the Beaty's Butte management area (42°0′ to 42°25′N, 119°05′ to 119°25′W) in southern Oregon during February 1986. The captured horses represent-

ed the majority of horses in each management area. Free-ranging bands were herded into corral traps on a band by band basis by a professional roundup service utilizing a Bell B1 helicopter (Bell Helicopter Textron, Inc., P.O. Box 482, Fort Worth, Texas 76101, USA). As many as seven bands could be held at one time without mixing. Often one or two bands would be held overnight for processing the next morning. These animals were provided water and hay ad libitum. All animals were visually and subjectively graded according to condition. Animals were considered in excellent condition if they appeared fat and the backbone and ribs were not visible. They were considered in good condition if they had prominent withers but ribs and backbone were not visible. Animals in fair condition had visible backbone and ribs that were barely visible. Animals in poor condition had visible ribs and animals in very poor condition exhibited prominent ribs and hipbones. Band condition was graded based on condition of the majority of animals in that band. In almost every case all animals within a band were considered to be in similar condition. Although condition of individual bands within an area ranged from excellent to poor, each area was assigned an overall condition based on the average number of bands fitting in that rating. The animals were placed in a squeeze chute where they were aged and bled. Ages were estimated according to tooth eruption and wear (Ensminger, 1969) and a blood sample was collected from the jugular vein utilizing a 35-ml syringe and 15-ga needle. A portion of the sample was transferred to a tube containing sodium EDTA and mixed thoroughly. The remainder was transferred to plain glass tubes and allowed to clot for 2–12 hr. Blood was kept in styrofoam containers until processed. EDTA tubes for hematology were shipped airmail in styrofoam containers to the senior author's laboratory every other day. Clotted blood was centrifuged in a tabletop centrifuge every evening. The serum was removed and stored in capped plastic tubes at −20 C until assayed.

Complete blood counts were performed immediately after arrival at the laboratory as described by Seal et al. (1977). Parameters measured included hemoglobin (HGB), hematocrit (HCT), red blood cell count (RBC) and white blood cell count (WBC). Mean corpuscular hemoglobin concentration (MCHC), mean cell volume (MCV) and mean corpuscular hematocrit (MCH) were calculated. Serum urea nitrogen (SUN) was analyzed colorimetrically by the method of Fawcett and Scott (1960) as modified by Chaney and Marback (1962) utilizing reagents and procedure included in the Sigma Urea Nitrogen kit (Sigma Chemical Company, St. Louis, Missouri 63160, USA). Serum progesterone (P4) and cortisol (F) levels were estimated by radioimmunoassay and competitive protein binding assay, respectively, as previously described (Plotka et al., 1980; Seal et al., 1983). Corpus luteum (CL) function was considered active when the serum P4 level was 1.5 ng/ml or greater (Plotka et al., 1975).

Statistics in the text are means (± standard errors). Blood and chemistry comparisons were made using ANOVA, correlations were made using Least Squares regression analysis and sex, age and P4 ratios were compared using Chi-square analysis. All analyses were performed utilizing the Number Cruncher Statistical System version 4.21 for the IBM computer (J. L. Hintze, Kaysville, Utah 84037, USA). This program follows the procedures outlined by Snedecor and Cochran (1967) and Ostle (1969). For two- and three-way ANOVA, animals were divided into three age groups: immature, <2 yr old; subadults, 2–3 yr old; adult, >3 yr old. Actual ages for the animals were used in the correlation analyses.

RESULTS

Population characteristics

Horses captured ranged in estimated age from 0.5 to ≥20 yr (6.0 ± 0.3). The median age for horses from the Flanigan and Beaty's Butte areas was 4 yr and from the Wassuk area was 6 yr. Overall males and females were present in approximately equal numbers in the Wassuk (1:1.03) and Beaty's Butte (1:1.06) areas. However, males were outnumbered by females (1:1.27) in the Flanigan area. Females outnumbered males in the 0.5 and 1.5 age class by 2.1:1 in Flanigan and 1.7:1 in Beaty's Butte areas. There were only seven subadult females and 20 subadult males in Beaty's Butte.

Overall, the horses from the Flanigan area were in the poorest condition and horses from the Wassuk were in the best condition. Generally, all animals within a specific band were in similar condition.

Hematology

Mean HGB, HCT, RBC and WBC levels differed significantly ($P < 0.001$) among areas. For all age groups, Flanigan horses

TABLE 1. Hematology and chemistry of immature (<2 yr old) feral horses from three management areas.

	Flanigan		Wassuk		Beaty's Butte		Significance	
	Male ($\bar{x}$ ± SE[a])	Female ($\bar{x}$ ± SE)	Male ($\bar{x}$ ± SE)	Female ($\bar{x}$ ± SE)	Male ($\bar{x}$ ± SE)	Female ($\bar{x}$ ± SE)	Sex (P)	Area (P)
n	14	30	8	12	19	27		
Hgb, g/dl	13.3 ± 0.4	13.9 ± 0.2	16.6 ± 0.5	18.1 ± 0.3	14.6 ± 0.4	15.4 ± 0.8	≤0.001	≤0.001
Hct, % vol	39.6 ± 1.0	41.5 ± 0.6	46.5 ± 1.2	49.1 ± 1.0	41.3 ± 0.8	43.5 ± 0.7	≤0.003	≤0.001
Red cells × 10^6	9.1 ± 0.2	9.6 ± 0.2	11.6 ± 0.3	12.5 ± 0.3	9.8 ± 0.2	10.3 ± 0.2	≤0.002	≤0.001
MCV, fl	43.4 ± 0.9	43.7 ± 0.6	40.2 ± 1.2	39.2 ± 1.0	42.4 ± 0.8	42.3 ± 0.7	NS[b]	≤0.001
MCHC, g/dl	33.4 ± 0.4	33.6 ± 0.3	35.7 ± 0.4	36.9 ± 0.6	35.3 ± 0.6	35.3 ± 0.4	NS	≤0.001
MCH, pg	14.5 ± 0.3	14.6 ± 0.2	14.3 ± 0.4	14.4 ± 0.2	14.9 ± 0.2	14.9 ± 0.2	NS	NS
White cells × 10^3	8.2 ± 0.6	8.2 ± 0.4	6.8 ± 0.7	8.3 ± 0.6	9.6 ± 0.5	9.2 ± 0.4	NS	≤0.004
Urea N, mg/dl	25.8 ± 1.4	24.3 ± 0.9	20.8 ± 1.8	22.2 ± 1.5	20.0 ± 1.2	24.0 ± 1.0	NS	≤0.04
Progesterone, ng/ml	NA[c]	0.4 ± 0.4	NA	1.0 ± 0.7	NA	1.8 ± 0.5	NA	NS
Cortisol, μg/dl	6.7 ± 1.0	7.3 ± 0.5	11.0 ± 0.7	10.4 ± 0.6	9.5 ± 0.4	10.3 ± 0.4	NS	≤0.001

[a] Mean ± standard error.
[b] Not significant.
[c] Not analyzed.

TABLE 2. Hematology and chemistry of subadult (2 to 3 yr old) feral horses from three management areas.

	Flanigan		Wassuk		Beaty's Butte		Significance	
	Male ($\bar{x}$ ± SE[a])	Female ($\bar{x}$ ± SE)	Male ($\bar{x}$ ± SE)	Female ($\bar{x}$ ± SE)	Male ($\bar{x}$ ± SE)	Female ($\bar{x}$ ± SE)	Sex (P)	Area (P)
n	18	15	15	16	20	7		
Hgb, g/dl	13.6 ± 0.2	14.7 ± 0.3	18.6 ± 0.3	18.5 ± 0.2	15.7 ± 0.3	15.3 ± 0.3	NS[b]	≤0.001
Hct, % vol	39.6 ± 0.8	42.7 ± 1.0	51.5 ± 0.8	51.5 ± 0.6	44.8 ± 0.8	43.8 ± 0.9	NS	≤0.001
Red cells × 10^6	8.4 ± 0.2	9.2 ± 0.2	11.7 ± 0.1	11.9 ± 0.2	9.6 ± 0.2	9.2 ± 0.3	NS	≤0.001
MCV, fl	47.4 ± 1.1	46.4 ± 0.6	43.8 ± 0.5	43.3 ± 0.7	47.0 ± 0.8	47.8 ± 1.4	NS	≤0.001
MCHC, g/dl	34.4 ± 0.6	34.6 ± 0.4	36.1 ± 0.2	36.0 ± 0.3	35.0 ± 0.3	34.8 ± 0.5	NS	≤0.03
MCH, pg	16.3 ± 0.3	16.0 ± 0.2	15.8 ± 0.2	15.6 ± 0.2	16.4 ± 0.3	16.6 ± 0.4	NS	≤0.02
White cells × 10^3	7.0 ± 0.4	6.4 ± 0.4	5.0 ± 0.3	5.3 ± 0.4	8.0 ± 0.4	7.8 ± 0.6	NS	≤0.001
Urea N, mg/dl	23.4 ± 1.2	24.1 ± 1.3	22.7 ± 1.3	22.2 ± 1.3	23.8 ± 1.1	26.8 ± 1.9	NS	NS
Progesterone, ng/ml	NA[c]	0.9[d] ± 0.8	NA	2.6[d] ± 0.9	NA	5.8[d] ± 1.3	NA	≤0.01
Cortisol, μg/dl	8.2 ± 0.6	8.7 ± 0.7	12.4 ± 0.6	10.1 ± 1.1	11.7 ± 0.4	11.3 ± 0.7	NS	≤0.001

[a] Mean ± standard error.
[b] Not significant.
[c] Not analyzed.
[d] Means with similar superscripts were not significantly different with respect to progesterone level.

TABLE 3. Hematology and chemistry of adult (≥4 yr old) feral horses from three management areas.

	Flanigan		Wassuk		Beaty's Butte		Significance	
	Male ($\bar{x}$ ± SE[a])	Female ($\bar{x}$ ± SE)	Male ($\bar{x}$ ± SE)	Female ($\bar{x}$ ± SE)	Male ($\bar{x}$ ± SE)	Female ($\bar{x}$ ± SE)	Sex (*P*)	Area (*P*)
n	45	53	42	38	34	41		
Hgb, g/dl	15.5 ± 0.2	15.0 ± 0.2	20.5 ± 0.2	18.7 ± 0.2	17.7 ± 0.3	15.9 ± 0.1	≤0.001	≤0.001
Hct, % vol	45.0 ± 0.8	43.6 ± 0.6	55.2 ± 0.5	51.2 ± 0.5	50.2 ± 0.9	45.5 ± 0.4	≤0.001	≤0.001
Red cells × 10^6	9.0 ± 0.2	8.6 ± 0.2	11.4 ± 0.2	10.6 ± 0.2	10.1 ± 0.2	9.2 ± 0.1	≤0.001	≤0.001
MCV, fl	50.1 ± 0.7	51.3 ± 0.6	48.6 ± 0.6	48.3 ± 0.6	49.8 ± 0.7	49.6 ± 0.6	NS[b]	≤0.004
MCHC, g/dl	34.6 ± 0.3	34.4 ± 0.3	37.1 ± 0.2	36.5 ± 0.2	35.3 ± 0.3	35.0 ± 0.2	NS	≤0.001
MCH, pg	17.3 ± 0.3	17.6 ± 0.3	18.0 ± 0.2	17.6 ± 0.2	17.4 ± 0.2	17.3 ± 0.2	NS	NS
White cells × 10^3	7.1 ± 0.3	6.8 ± 0.2	4.7 ± 0.2	4.8 ± 0.2	6.8 ± 0.3	7.1 ± 0.3	NS	≤0.001
Urea N, mg/dl	22.8 ± 0.8	22.6 ± 0.7	22.0 ± 0.8	22.3 ± 0.8	27.7 ± 0.9	24.2 ± 0.8	NS	≤0.003
Progesterone, ng/ml	NA[c]	3.9[d] ± 0.5	NA	4.0[d] ± 0.3	NA	5.6[d] ± 0.5	NA	≤0.01
Cortisol, μg/dl	8.8 ± 0.4	10.2 ± 0.4	11.4 ± 0.4	11.2 ± 0.4	12.3 ± 0.5	12.7 ± 0.3	NS	≤0.001

[a] Mean ± standard error.
[b] Not significant.
[c] Not analyzed.
[d] Means with similar superscripts were not significantly different with respect to progesterone level.

had the lowest HGB, HCT and RBC levels and Wassuk horses had the highest (Tables 1–3). Statistically significant differences in HGB, HCT, RBC, WBC, MCV and MCH occurred with respect to age ($P < 0.001$). Sex by age interactions were significant for HGB, HCT and RBC ($P \leq 0.001$) but not for WBC or erythrocyte parameters. A significant area by sex interaction occurred for HGB only ($P < 0.05$) and a significant area by age interaction was apparent for RBC and WBC ($P \leq 0.02$ and $P \leq 0.03$, respectively). Hemoglobin and HCT levels had low but significant positive correlations with age ($r = 0.32$ and 0.31, respectively, $P < 0.001$).

Since significant sex by age interactions were present, the data were analyzed by two-way ANOVA within age groups. With this technique, significant sex differences occurred for HGB, HCT, and RBC levels among immature and adult horses, but not in subadults. In immature animals, females had higher values than males ($P \leq 0.003$, Table 2). However, in adult animals, males had higher values than females ($P = 0.001$, Table 3).

Significant sex differences were not noted in MCV, MCHC or MCH. However, in immature and adult animals MCV and MCHC were significantly different among areas ($P \leq 0.004$) and in subadults MCV, MCHC and MCH were different among areas ($P \leq 0.02$). White blood cell counts were significantly lower in Wassuk horses than in either Flanigan or Beaty's Butte horses in all age groups ($P \leq 0.004$).

Serum chemistry

Serum urea nitrogen differed significantly among areas in adult ($P \leq 0.003$) and immature ($P \leq 0.04$) horses but not in subadults (Tables 1–3). Serum urea nitrogen levels were slightly but significantly ($P < 0.04$) higher in immature animals from the Flanigan area than in the same aged animals from either the Wassuks or Beaty's Butte. However, in adults SUN levels were significantly higher in the Beaty's Butte animals ($P < 0.003$).

Serum cortisol levels ranged from 2 to 18 μg/dl. Significant differences occurred among areas and among age groups within area ($P < 0.001$). The area by age interaction was significant also ($P < 0.03$). However, there were no differences between cortisol levels in males and females. Cortisol levels were lower in immature animals than in either subadults or adults in all areas. Horses in all age groups in the Flanigan area had lower cortisol values than horses in the other areas (Tables 1–3). However, the age relationship was consistent with the other areas.

Corpus luteum function

A significantly higher ($P < 0.001$) proportion of adult mares ≥4 yr old had P4 levels <1.5 ng/ml in the Flanigan area (20/52) than in the Wassuk area (4/38) and Beaty's Butte area (7/41). In addition, only one of 15 subadults (2–3-yr-old) had an active CL in the Flanigan area as compared with half or more in the Wassuk (8/16) and Beaty's Butte (4/7) areas. Five of 27 yearlings showed CL activity in Beaty's Butte as compared with one of 30 in Flanigan and one of 12 in the Wassuk range. These results are reflected in the average P4 levels (Tables 1–3).

DISCUSSION

Trapping and handling stress

All the blood samples for this study were collected from undrugged horses handled in a squeeze chute. We attempted to standardize procedures as much as possible; however, the animals differed in terms of distance herded, length of time in the trap, ambient temperatures, and observed behavior. Most of these factors should be randomized both within and between areas because the roundups in each area took several days and herds within each area were gathered near the trap site as well as from several kilometers away. The major consequence would be to increase the variance within an area and thus reduce the significance of differences among areas.

However, interpretation of the results still must consider the probable effects of collection and handling procedures upon measured blood parameters (Franzmann, 1972; Seal et al., 1972a; Schalm et al., 1975). The results affected may include HGB, HCT, RBC and serum cortisol. Archer and Clabby (1965) observed that packed cell volume (PCV) of the horse increased promptly upon exertion, twitching and handling. An increase in circulating red cell mass as a result of splenic contraction may occur with handling; thus, resting horses from any of the populations might have lower values than those reported here. Total erythrocyte count is increased also by stress and exercise in horses (Hawkey, 1977). Although seasonal changes in blood parameters have been reported, they should not be a factor in this study because all collections were conducted during the winter.

Hematology

The hematologic values reported here are within the range for "hot-blooded" breeds (Thoroughbred, quarterhorse, Appaloosa, Standardbred, and Arabian; Jain, 1986) and are similar to values reported by Seal et al. (1985) for feral horses captured in the Pah Rah and Pine Nut Herd management areas of Nevada. These values are slightly higher than values published for "cold-blooded" horses (Jain, 1986). Packed cell volume, RBC and HGB can increase with excitement (Searcy, 1969; Swenson, 1970). Wild horses are likely to be more excited than domestic animals during blood sampling and this could account for the higher hematological levels observed in our study.

In general, values for immature animals were lower than in subadults or adults. This is consistent for published data on MCV in Equidae (Hawkey, 1977) but contrasts with data on erythrocyte counts. Hawkey (1977) reported that in horses, the total erythrocyte count is influenced by age and in wild Equidae the erythrocyte count is higher and MCV lower in juveniles than in adults. The opposite results seen in RBC in the present study may be confounded by the effects of stress. Immature animals did not appear as frightened as mature animals in response to handling. The lower cortisol values in immature animals supported this conjecture.

The lower hematological values seen in the Flanigan area as compared with values in the Wassuk and Beaty's Butte areas are consistent with the concept that hematological values are an indicator of condition of these animals. The Flanigan horses appeared in much poorer condition than either the Wassuk or Beaty's Butte horses.

Serum chemistry

The lack of a direct correlation of SUN with condition of the horses in the three areas was not surprising. Serum urea nitrogen is affected by a number of nutritional and physiological factors among which are catabolism of body protein (Guada et al., 1976; Bahnak et al., 1979) and quality of nutrition, such as the amount of dietary protein and/or energy (Preston et al., 1961, 1965; Kirkpatrick et al., 1975). Further information about the diet the animals were on and other environmental factors is needed before a complete interpretation of the SUN levels can be made.

Serum F levels varied with age and area. The lower levels of F in the Flanigan horses are of interest since it might be suspected that F levels would be increased during periods of nutritional stress (Seal et al., 1972a). Possibly the ability of the horse pituitary-adrenal axis to respond to the stress of handling is diminished when animals are already nutritionally stressed. Seal et al. (1972b) observed a similar effect in deer placed on different nutritional diets.

Since the horse is seasonally polyestrous with anestrus occurring from November through March (Kirkpatrick and Turner, 1983), P4 levels >1.5 ng/ml during the winter should indicate that an animal is pregnant. The majority of studies indicate that feral horses are sharply seasonal with

respect to breeding and foaling (Kirkpatrick and Turner, 1986). Baseline P4 levels during anestrus or estrus prior to ovulation during a cycle have been reported to be <0.8 ng/ml in domestic horses (Plotka et al., 1975; Ginther, 1979). Seal and Plotka (1983) used a P4 level of 2.4 ng/ml in conjunction with an estradiol level of 70 pg/ml in feral horses from the Challis, Idaho herd to determine that 88 of 99 (88%) adult mares were pregnant. Regardless of the P4 level for discrimination, it was apparent from the number of animals having elevated P4 levels and the average P4 levels within age class (Tables 1–3) that there were fewer mares with CL function in the Flanigan herd than in either the Wassuk herd or the Beaty's Butte herd.

Several factors may influence the fecundity of feral horse herds including genetics, ecology of the ranges, population density, age structure and sex ratios. The higher proportion of nonpregnant mares in the Flanigan herd could be a consequence of any or all of these factors. Because the Flanigan horses appeared to be in the poorest condition, it is possible that inadequate nutrition may have been a major contributing factor in the lower number of animals with elevated P4 levels in this herd. It is unlikely that the age structure could account for the differences in luteal function because the average age of adult mares in the Flanigan area was 8.3 ± 1.1 yr versus 6.7 ± 0.5 yr for the adult mares in the Beaty's Butte area. The mares in the Wassuk area were slightly older than the Beaty's Butte mares (10.2 ± 0.8 yr versus 6.7 ± 0.5 yr) but the percentage of mares with luteal function in these two herds was similar (90% versus 83%, respectively).

Another contributing factor for the lower reproduction in the Flanigan mares could be the less stable band structure. Berger (1983) reported that the foaling rate of stable bands (bands in which there was no change of the band stallion) was 82% while the foaling rate of unstable bands (bands in which stallions had been replaced by new males) was 38%. The Flanigan unit had been gathered by the BLM in September 1985, 3 mo before we collected our samples. A total of 350 horses were removed from the area including 165 adult mares and 129 stallions. This collection could have disrupted the structure of many bands and caused the loss of several dominant stallions and the exchange of dominant stallions.

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