Journal of Wildlife Diseases, 46(1), 2010, pp. 1–12

SITE AND AGE CLASS VARIATION OF HEMATOLOGIC PARAMETERS FOR FEMALE GREATER SAGE GROUSE (*CENTROCERCUS UROPHASIANUS*) OF NORTHERN NEVADA

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ABSTRACT: Decreases in Greater Sage Grouse (*Centrocercus urophasianus*) numbers throughout the western United States have been attributed to declining habitat quantity and quality. Improving our understanding of how interannual ecologic site variability affects nutritional status and fitness of different bird age classes will lead to improved land management and conservation strategies. Greater Sage Grouse were sampled from two Population Management Units located in northern Nevada, United States: Tuscarora (TU) and Lone Willow (LW) during 15 March–11 April 2004 and 14–20 March 2005. Twenty (16 yearlings, four adults) and 17 (7 yearlings, 10 adults) female Sage Grouse were captured and bled during 2004, and 12 (four yearlings, eight adults) and 14 (10 yearlings, four adults) were sampled during 2005 in TU and LW, respectively. Samples were evaluated to examine the effect of site, age, and year on specific hematologic and serum chemistry parameters. Several differences between age classes, sites, and years were detected for a number of fitness indicators; however, actual values fell within normal ranges of variation for Sage Grouse or other avian species. Differences were also detected for several parameters more closely related to reproductive fitness, including total plasma and serum proteins, and serum calcium and phosphorus. Yearlings had lower plasma protein ($P<0.0001$) and lower serum protein than did adults ($P=0.0003$). In 2004, TU yearlings had lower serum calcium levels than the adults, and in 2005, LW yearlings had lower levels than adults ($P=0.008$). Females on the TU site had lower serum phosphorus than the LW females ($P<0.0001$). Overall, adult females weighed more than yearlings ($P=0.0004$). Lower values found in yearlings, and on the TU management unit, indicate a lower production potential, particularly in unfavorable years. A lower intrinsic ability of yearlings to reproduce, combined with lower nutrition potentials and associated annual variations on certain types of habitat combinations, indicate that conservation measures must be flexible and based on local prescriptions. Fitness parameters of Sage Grouse should be used to assess effects of land management practices and conservation on Sage Grouse populations in order to provide more certainty of the outcome, whether positive, neutral, or deleterious.

Key words: *Centrocercus urophasianus*, hematologic parameters, Nevada, Sage Grouse.

INTRODUCTION

The US Fish and Wildlife Service, on 12 January 2005, issued a decision that the Greater Sage Grouse (*Centrocercus urophasianus*) did not warrant listing as threatened or endangered (Federal Register 50 CFR part 17; the finding is now under a new review). The decision was based heavily on recommendations contained in the various state conservation plans. These plans identify continued research efforts as a critical and vital part of Sage Grouse conservation, given that Greater Sage Grouse population levels have experienced a relative decline throughout much of their range (Girard, 1937; Connelly and Braun, 1997). Declines in Greater Sage Grouse abundance in the Great Basin have been attributed to lower Sage Grouse productivity associated with declining habitat quantity and quality (Crawford and Lutz, 1985; Klebenow, 1985). Because both the amount and quality of available forage affects the nutritional status of an animal, it is important to understand how ecologic site variability and potential, in turn, affect the annual variation of nutritional status in different bird age classes. Habitat analysis can only define, on average, the nutritional potential of a given area. Because animals select diets that have a higher nutritional quality than the average quality available to them, an assessment of the nutritional status of individual animals using a partic-

ular habitat is a better indicator of habitat quality (Emlen, 1966; MacArthur and Pianka, 1966).

Research has shown that some blood parameters are indicative of the nutritional status of prelaying Greater Sage Grouse hens and that these parameters could also provide insight into the nutritional quality of prelaying-nesting habitat (Dunbar et al., 2005). Dunbar et al. (2005) were also the first to report blood parameter ranges for Greater Sage Grouse (both hens and yearlings); however, samples were pooled from similar ecologic site locations in Nevada and Oregon. Understanding the effect that ecologic site potential can have on hematologic values of Sage Grouse populations that use different sites with different potentials can assist in determining what types of ecologic sites may limit Sage Grouse productivity. Although the two habitat sites (Tuscarora and Lone Willow) included in this study are considered to be ecologically similar and managed under the same land-use scenario, the Lone Willow site is considered to be the most productive Sage Grouse habitat in North America (Pope and Goldie, 2004). The objective of this study was to determine and compare annual variation in hematologic values for adult and yearling Greater Sage Grouse females from two distinct, but generally similar, Population Management Units (PMU) of northern Nevada.

MATERIALS AND METHODS

Greater Sage Grouse were sampled from two ecologically similar PMUs located in northern Nevada, USA (Fig. 1). The Tuscarora PMU (TU) is located approximately 72 km northeast of Winnemucca, Nevada and is comprised of the Tuscarora and Snowstorm Mountains and the Sheep Creek Range, an area of about 650,000 ha. Elevations range from about 1,600 m to over 2,300 m. The Lone Willow PMU (LW) is located approximately 100 km northwest of Winnemucca and is comprised of the Montana, Trout Creek, Bilk Creek, and Double-H Mountains, an area of about 192,000 ha. Elevations range from 1,200 to over 2,500 m.

Sage grouse capture was conducted during the breeding season, 15 March–11 April 2004 (March 2004) and 14–20 March 2005 (March 2005). All captures were conducted with the use of spotlight trapping techniques (Geisen et al., 1982; Wakkinen et al., 1992). A portable generator served as the power source for a 2-million candle-power spotlight. The generator was affixed to an aluminum frame backpack. During capture, one individual carried the generator and directed the spotlight to identify grouse eye-shine and occupy the bird's attention. Additional members of the capture team carried long-handled fishing nets to affect capture.

At the initial capture, each hen was weighed and then aged as a yearling or adult. (Eng, 1955; Crunden, 1963). Hens were placed in a narrow fabric bag hung from a spring scale. Scales were precalibrated with a 1,000 g standard weight. Female Sage Grouse captured in 2004 were banded with a serial-numbered aluminum size 14 leg band (Petersen, 1980) in order to exclude repeated captures of the same bird in 2005. Bird age was determined by wing-molt and plumage characteristics. Juveniles have feathers that are more pointed, mottled, and narrow than are adult feathers. In yearling grouse, the two outermost primaries (P9 and P10) are retained from juvenile plumage, while the other eight primaries (P1–P8) are molted, taking on adult primary characteristics. The two remaining juvenile primaries are pointed, unlike the other eight primaries, and this trait allows yearling grouse to be differentiated from adult grouse.

Approximately 1.5 ml of blood was collected by venipuncture of the cutaneous ulnar vein of each female grouse (Campbell, 1995). Heparin was run through the needle prior to venal insertion in order to minimize blood coagulation when warm blood contacted the cold needle. The blood sample was then used to fill two microtainers, each with 0.75 ml of blood. One microtainer contained ethylenediaminetetraacetic acid (EDTA) and the other had no anticoagulant. The microtainer with EDTA was then gently mixed, once blood was added, and then refrigerated. This microtainer of whole blood was used to conduct a differential complete blood count that included percent packed cell volume (PCV), white blood cell count (WBCC) including differential and absolute cell volumes, and total plasma protein concentration.

The blood placed in the microtainer with no anticoagulant was centrifuged within 6 hr of collection at 3,200 RPM for 15 min and then separated. The serum was then refrigerated

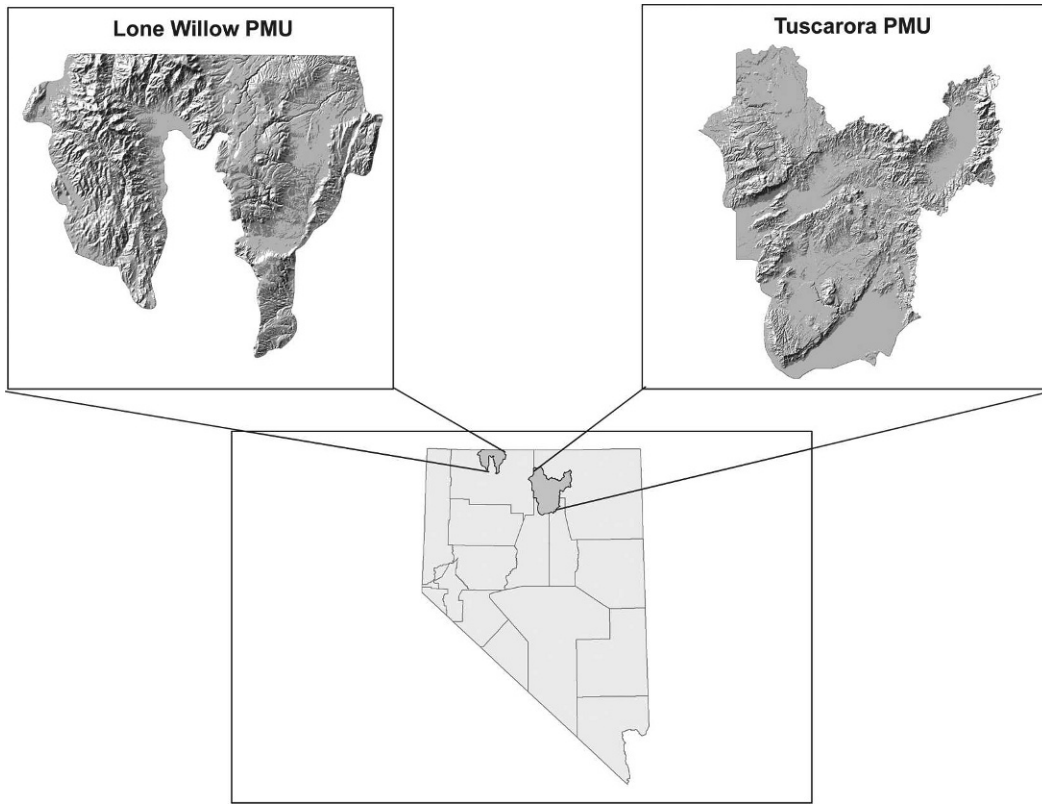

FIGURE 1. Location map of Lone Willow and Tuscarora Population Management Units, northern Nevada, United States.

and shipped to the diagnostic laboratory for use in a serum chemistry panel to provide information about uric acid, calcium, phosphorus, creatine kinase, aspartate aminotransferase (AST), glucose, albumin, and total serum protein concentration in circulatory blood.

While collecting blood, several drops of fresh blood were used to prepare two smears for each grouse using the slide-and-coverslip method (Campbell, 1995); these were used to determine white blood cell (WBC) differential and absolute counts. Whole blood and serum samples were kept refrigerated along with blood smears and shipped within 48 hr of collection by overnight delivery to the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Oregon State University, Corvallis, Oregon, USA for all blood chemistry and cell count determinations.

Serum was used in a serum chemistry panel to provide information about uric acid, calcium, phosphorus, calcium:phosphorus ratio, creatine kinase, AST, glucose, albumin, and total serum protein concentration in circulatory blood. Serum biochemistries were performed on an automated chemistry analyzer (Hitachi 717 Biochemical Analyzer, Roche/Boehringer Manheim, Indianapolis, Indiana, USA) using standard reagents and methods. Packed cell volumes were measured by the standard capillary tube method after centrifugation of a microhematocrit tube. Plasma proteins were determined by refractometer (Dunbar et al., 2005).

Total WBCCs were performed by estimation from a blood smear using 40× magnification and described in more detail in Dunbar et al. (2005). The slide-and-coverslip method has been the standard method used for all published Sage Grouse research (Dunbar et al., 2005) because of the small amount of blood that is available from birds of this size. This analysis employed the same laboratory and laboratory personnel as all previous Sage Grouse research. Although it may not be the most preferable method, our results are comparable to all other Sage Grouse blood value information.

A 2×2×2 factorial design was used to

TABLE 1. Pooled spring 2004–2005 means (±SE) and ranges (n=63) for weight and blood parameters in female Sage Grouse from TU and LW Management Units, northern Nevada.

Measure	Mean	Range (min–max)
Hen weight (g)	1,334.8 (±12.5)	1,115–1,550
Hematology		
Total WBC (μl)	4,671.4 (±49.8)	4,000–5,500
Absolute heterophils (μl)	914.6 (±33.9)	452–1,632
Absolute lymphocytes (μl)	3,690.1 (±52.6)	3,024–5,207
Absolute monocytes (μl)	40.2 (±3.9)	0–106
Absolute eosinophils (μl)	66.1 (±9.8)	0–418
Absolute basophils (μl)	11.9 (±2.8)	0–84
Packed cell volume (%)	58.6 (±0.8)	36–72
Total plasma protein (g/dl)	6.2 (±0.1)	4.1–9.2
Serum chemistry		
Glucose (mg/dl)	335.5 (±4.3)	230–406
Total serum protein (g/dl)	4.0 (±0.06)	2.7–5.0
Albumin (g/dl)	1.9 (±0.03)	1.2–2.4
Creatine kinase (CK; u/l)	2,403.7 (±155.0)	909–7,809
Aspartate aminotransferase (AST; u/l)	423.4 (±10.8)	246–654
Serum calcium (mg/dl)	20 (±1.0)	10.1–44.1
Serum phosphorus (mg/dl)	6.5 (±0.3)	3.4–16.5
Calcium:phosphorus (ratio)	3.2 (±0.08)	0.9–4.34
Uric acid (mg/dl)	7.2 (±0.3)	2.4–12.6

analyze body weight and blood parameter relationships. The design included three main effects; two sites (Tuscarora PMU and Lone Willow PMU), two capture times (2004 and 2005), and two age groups (yearling and adult). Data were analyzed using the SAS program FXQLQLQL (Fernandez, 2005). Interactions of age×site, age×year, site×year, and age×site×year were also examined. Differences were determined at the 95% confidence interval ($P<0.05$) for all comparisons. In addition to the aforementioned analyses, all samples collected were pooled across area, age group, and year to create a reference range of Sage Grouse blood values.

RESULTS

Twenty and 17 female Sage Grouse were captured in TU and LW, respectively, during 2004 (15 March–11 April 2004). In TU, the female age ratio was 16 yearlings and four adults. In LW, the ratio was seven yearlings and 10 adults. A separate group of birds were captured in 2005. Twelve and 14 Sage Grouse hens were captured in TU and LW, respectively, during 2005 (14–20 March 2005). In TU, the female age ratio was four yearlings and eight adults; in LW, the female age ratio was 10 yearlings and four adults.

All blood values collected in 2004 and 2005 were pooled and means, standard error (SE), and reference ranges were calculated for bird weight and each blood constituent (Table 1). No two- or three-way interactions were detected for absolute heterophils, eosinophils, or basophils, PCV, total plasma protein, glucose, albumin, creatine kinase, serum phosphorus, and uric acid.

A three-way interaction was detected for serum calcium levels (P=0.044; Table 2). During 2004, TU yearlings had lower serum calcium levels (12.9±1.1 mg/dl) than did adults (19.3±2.3 mg/dl; P=0.016), but no differences were indicated for 2005. Lone Willow females exhibited an opposite trend from TU females; no difference was detected between yearlings and adults during 2004, while yearlings had lower serum calcium values (14.5±1.5 mg/dl) than did adults in 2005 (24.2±2.3 mg/dl; P=0.0008). During 2004, TU yearlings had lower values

TABLE 2. Means (±SE) for serum calcium by age, site, and year in northern Nevada, spring 2004–2005.

	TU[b]		LW[c]	
	Yearlings	Adults	Yearlings	Adults
Year[a]	Serum Calcium (mg/dl)			
2004	12.9 (±1.1)[f,h]	19.3 (±2.3)[e]	28.2 (±1.7)[d,g]	28.9 (±1.5)[d]
2005	17.8 (±2.3)[d,g]	22.4 (±1.6)[d]	14.5 (±1.5)[e,h]	24.2 (±2.3)[d]

[a] 2004 = (15 March–11 April 2004), 2005 = (14–20 March 2005).

[b] n = 16 yearlings 2004, four yearlings 2005, four adults 2004, eight adults 2005.

[c] n = seven yearlings 2004, 10 yearlings 2005, 10 adults 2004, four adults 2005.

[d,e,f] Row means with different superscript differ at $P<0.05$.

[g,h] Column means with different superscript differ at $P<0.05$.

(12.9±1.1 mg/dl) than did LW yearlings (28.2±1.7 mg/dl; $P<0.0001$), and TU adults had lower values than did LW adults ($P<0.0001$). Site had no effect on serum calcium levels in 2005 for adult birds; however, TU yearlings had greater ($P<0.0001$) values than LW yearlings (17.8±2.3 and 14.5±1.5 mg/dl, respectively). Year had no effect on serum calcium levels of TU and LW adults; however, a year effect was detected for TU and LW yearlings. Serum calcium levels were greater ($P<0.0001$) in 2005 for TU yearlings compared to 2004 (17.8±2.3 and 12.9±1.1 mg/dl, respectively), whereas values were lower ($P<0.0001$) in 2005 for LW yearlings compared to 2004 (14.5±1.5 and 28.2±1.7 mg/dl, respectively).

An age×site interaction was detected for WBCC ($P=0.025$), absolute lymphocytes ($P=0.023$), and absolute monocytes ($P=0.010$; Table 3); a year×age interaction was detected for AST ($P=0.039$); and a site×year interaction detected for bird weight ($P=0.003$), WBCC ($P=0.013$), calcium:phosphorus ratio ($P=0.01$), and serum protein ($P=0.040$; Table 4).

Within sites, WBCC did not differ between bird age classes. Within age classes, TU yearlings had greater values (4,841±104 µl) than LW yearlings (4,588±114 µl; $P=0.023$), but adults did not differ between sites. There were no site differences in 2004, but TU females had a higher WBCC (4,962±114 µl) in 2005 than did LW females (4,615±114 µl; $P=0.033$). Tuscarora females experienced lower WBCC in 2004 than in 2005 (4,465±104 and 4,962±114 µl, respectively; $P=0.002$), while LW females were not different between years. Although an overall site×age interaction was detected for absolute lymphocytes, there were no detectable differences between age classes within sites or between yearlings or adults of TU and LW, indicating no biologic significance.

Tuscarora yearlings had lower monocyte (29±8 µl) values than the adults (55±9 µl; $P=0.035$), and there was no difference between LW age classes (Table 3). There was no site effect on yearlings; however, TU adults had greater monocyte values (55±9 µl) than did LW adults (26±8 µl; $P=0.023$) across years.

There was no difference between adult (398±22 u/l) and yearling AST levels (394±17 u/l) in 2004, although yearlings had higher values (489±22 u/l) than did adults in 2005 (407±23 u/l; $P=0.010$). Yearling AST levels were higher in 2004 than in 2005 ($P=0.0009$), and there was no year effect on adults.

Both a site×year interaction ($P=0.003$) and an age effect ($P=0.0004$) were present for Sage Grouse weights (Table 4). During 2004, there were no differences between female weights in TU and LW; however, TU females had heavier weights than the LW females in 2005 ($P=0.012$). TU females did not have weight fluctuations

TABLE 3. Means (±SE) for total WBC and absolute lymphocyte and monocytes, with regard to age and site, northern Nevada.

	Yearlings[a]	Adults[b]
WBC (µl)		
TU	4,841 (±104)[c]	4,588 (±114)
LW	4,522 (±92)[d]	4,755 (±110)
Absolute lymphocytes (µl)		
TU	3,827 (±114)	3,509 (±125)
LW	3,534 (±101)	3,755 (±121)
Absolute monocytes (µl)		
TU	29 (±8)[e]	55 (±9)[c,f]
LW	44 (±7)	26 (±8)[d]

[a] $n = 20$ in TU, 17 in LW.

[b] $n = 12$ in TU, 14 in LW.

[c,d] Column means with different superscript differ at $P<0.05$.

[e,f] Row means with different superscript differ at $P<0.05$.

TABLE 4. Means (±SE) for weight, total WBC, and total serum protein, with regard to site and time, northern Nevada, spring 2004–2005.

Year[a]	TU[b]	LW[c]
Weight (g)		
2004	1,352 (±21)	1,402 (±19)[d]
2005	1,354 (±23)[f]	1,271 (±22)[e,g]
Total WBC (µl)		
2004	4,465 (±104)[e,g]	4,662 (±92)[f]
2005	4,962 (±114)[d,f]	4,615 (±110)[g]
Total serum protein (g/dl)		
2004	4.1 (±0.1)[f]	3.8 (±0.1)[e,g]
2005	4.0 (±0.1)	4.2 (±0.1)[d]
Calcium:phosphorus (ratio)		
2004	3.3 (±0.2)	3.4 (±0.1)[d]
2005	3.5 (±0.2)[f]	4.2 (±0.2)[e,g]

[a] 2004 = (15 March–11 April 2004), 2005 = (14–20 March 2005).

[b] $n = 20$ in 2004, 12 in 2005.

[c] $n = 17$ in 2004, 14 in 2005.

[d,e] Column means with different superscript differ at $P<0.05$.

[f,g] Row means with different superscript differ at $P<0.05$.

between years; however, lower weights were observed for LW females between 2004 and 2005 ($P<0.0001$). Overall, adults had heavier weights (1,318.5±21.9 g) than did juveniles (1,206.9±13.1 g; $P=0.0004$).

A site×year interaction ($P=0.01$) was observed for the serum calcium:phosphorus ratio (Table 4). No difference was detected between TU and LW females in 2004. In 2005, the calcium:phosphorus ratio was greater ($P=0.001$) in TU than in LW females (3.5±0.2 and 2.7±0.2, respectively). Tuscarora females were not affected by year, while a decrease in calcium:phosphorus ratio was seen in LW females ($P=0.026$) for 2005 compared to 2004 (3.4±0.2 and 2.7±0.2, respectively). Age had no effect ($P=0.07$) on the calcium:phosphorus ratio (Table 5).

Both a site×year interaction ($P=0.040$) and an age effect ($P=0.0003$) were detected for total serum protein levels. In 2004, serum protein was greater ($P=0.001$) in TU than in LW females (4.1±0.1 and 3.8±0.1 g/dl, respectively; Table 4). No difference, however, was detected between TU and LW females in 2005. Tuscarora females were not affected by year, while an increase in serum protein was seen in LW females ($P=0.022$) for 2005 compared to 2004 (4.2±0.1 and 3.8±0.1 g/dl, respectively). Overall, adult females (4.2±0.09 g/dl) had higher serum protein levels than did yearlings (3.8±0.08 g/dl; $P=0.0003$). There was no site effect for total plasma protein; however, plasma protein was greater ($P<0.0001$) in adults (6.9±0.2 g/dl) than in yearling birds (5.8±0.2 g/dl). A year effect ($P=0.004$) was also detected with lower plasma protein levels reported for 2004 (6.0±0.2 g/dl) as compared to 2005 (6.7±0.2 g/dl).

There were no age, site, or year effects for absolute lymphocytes, eosinophils, and basophils (Tables 5–7). There was an age effect on total plasma protein (5.8±0.2 and 6.9±0.2 g/dl for yearlings and adults; $P<0.0001$), albumin (1.8±0.4 and 2.0±0.5 g/dl for yearlings and adults; $P=0.006$), and creatine kinase (2,696±223 and 1,866±255 u/l for yearlings and adults; $P=0.017$; Table 5). There was a site effect on PCV (61±1.0 and 57±1.3% for TU and LW females; $P=0.011$), serum phosphorus (5.3±0.4 and 8.0±0.4 mg/dl

TABLE 5. Means (±SE) for hematology and serum chemistry, by age, in northern Nevada, spring 2004–2005.

Measure	Yearlings[a]	Adults[b]
Hematology		
Absolute heterophils (μl)	1,102 (±157)	922 (±180)
Absolute eosinophils (μl)	66 (±15)	62 (±17.2)
Absolute basophils (μl)	11 (±4)	15 (±4.8)
Packed cell volume (%)	59 (±1)	59 (±1.3)
Total plasma protein (g/dl)	5.8 (±0.2)[d]	6.9 (±0.2)[c]
Serum chemistry		
Glucose (mg/dl)	337 (±6)	342 (±6.4)
Albumin (g/dl)	1.8 (±0.4)[d]	2.0 (±0.5)[c]
Creatine kinase (U/L)	2,696 (±223)[c]	1,866 (±255)[d]
Phosphorus (mg/dl)	6.1 (±0.4)	7.2 (±0.4)
Calcium:phosphorus (ratio)	3.1 (±0.1)	3.3 (±0.1)
Uric acid (mg/dl)	7.0 (±0.3)	7.7 (±0.4)

[a] $n = 37$.

[b] $n = 26$.

[c,d] Row means with different subscripts differ at $P<0.05$.

for TU and LW females; $P<0.0001$), and uric acid (6.7±0.4 and 8.0±0.3 mg/dl for TU and LW females; $P=0.012$; Table 6). Sampling year affected total plasma protein (6.0±0.2 and 6.7±0.2 g/dl for 2004 and 2005; $P=0.004$), glucose (326±6 and 352±6 mg/dl for 2004 and 2005; $P=0.003$), albumin (2.0±0.4 and 1.8±0.05 g/dl for 2004 and 2005; $P=0.013$), and uric acid (8.2±0.3 and 6.5±0.4 mg/dl for 2004 and 2005; $P=0.001$; Table 7).

DISCUSSION

Published information on hematologic parameters of the Greater Sage Grouse is limited (Davis, 2003; Dunbar et al., 2005), particularly with respect to ecologic site potentials and associated interannual variations resulting from weather fluctuations. This study compared adult and yearling Sage Grouse hematologic parameters across two years and two sites (two different Population Management Units) of northern Nevada.

White blood cell count, AST, monocytes, lymphocytes, heterophils, eosinophils, basophils, creatine kinase, glucose, and uric acid levels all serve as indicators of individual bird fitness (Seal, 1978; Campbell, 1995; Dunbar et al. 2005). Even though differences were detected between age classes, sites, and years, actual values fell within the range of variation delineated by Davis (2003), Pope and Goldie (2004) and Dunbar et al. (2005). It is, therefore, doubtful that they were biologically significant, given the particular year and associated ecologic site potentials. However, these differences can serve to indicate which parameters may indicate habitat limitations, predator stress, or any number of additional factors that may limit Sage Grouse chick production when, in any given year, the habitat potential on a particular combination of ecologic sites is well below optimum.

There was a site effect on spun PCV. Tuscarora females had higher values than did LW females, although both exceeded PCV of other avian species. Packed cell volume is expressed as a percent, measuring red blood cell mass in relation to volume of whole blood (Amand, 1986). Although not yet established for Sage Grouse, the PCV range expected for most caged birds is 35–55% (Campbell, 1995) or 37–53% (Amand, 1986). Higher values

TABLE 6. Means (±SE) for hematology and serum chemistry, by capture site, northern Nevada, spring 2004–2005.

Measure	TU[a]	LW[b]
Hematology		
Absolute heterophils (µl)	1,141 (±175)	883 (±163)
Absolute eosinophils (µl)	62 (±17)	67 (±16)
Absolute basophils (µl)	16 (±5)	10 (±4)
Packed cell volume (%)	61 (±1)[c]	57 (±1.3)[d]
Total plasma protein (g/dl)	6.3 (±0.2)	6.4 (±0.2)
Serum chemistry		
Glucose (mg/dl)	333 (±6)	346 (±6.2)
Albumin (g/dl)	2 (±0.04)	2 (±0.04)
Creatine kinase (u/l)	2,107 (±248)	2,455 (±230)
Serum phosphorus (mg/dl)	5.3 (±0.4)[d]	8.0 (±0.4)[c]
Uric acid (mg/dl)	6.7 (±0.4)[d]	8.0 (±0.3)[c]

[a] $n = 32$ in TU.

[b] $n = 31$ in LW.

[c,d] Row means with different subscripts differ at $P<0.05$.

can be caused by many factors including photoperiod, age, sex, elevation, and dehydration, while lower values (anemia) can be caused by hemorrhage, hemoparasites, egg-laying, or mineral deficiencies (Amand, 1986). Blood parasites can also induce anemia; however, Dunbar et al. (2003) found no differences in PCV of birds infected with *Leukocytozoon* spp. (51% PCV during breeding season and 47% PCV during brood rearing) and uninfected birds (53% PCV during breeding season and 45% PCV during brood rearing). A PCV mean of 55.78% has been previously recorded for Sage Grouse hens during spring (Davis, 2003), showing that Sage Grouse may be expected to fall towards the higher limit of the general range for avian PCV.

Differences were detected for several parameters more closely related to reproductive fitness (Seal, 1978; Campbell, 1995; Dunbar et al., 2005); these include total plasma and serum protein, calcium, and phosphorus. Yearlings had lower total plasma protein levels than did adults, and all females had lower total plasma protein values in 2004 than in 2005. Although birds at both capture sites had values on the high end, or in excess of the established range for other avian species (Amand, 1986), they corresponded well within the range of 5.6–7.2 g/dl previously recorded during the spring for Sage Grouse females in northern Nevada (Davis, 2003; Pope and Goldie, 2004; Dunbar et al., 2005).

Values for avian serum protein ordinarily fall within 3.5–5.5 g/dl, with determination of both serum protein and serum albumin concentrations providing a reliable indication of the protein content of avian blood (Campbell, 1995). Even with the variations between Sage Grouse age, site, and year, all values fell within the expected ranges for other avian species (Olsen and Orosz, 2000). Total serum protein levels have been found to average 4.3±0.5 g/dl for Sage Grouse females in other studies in northern Nevada (Pope and Goldie, 2004), indicating that all the values in this study were within expected ranges, except for the LW 2004 values. The serum protein values found in LW during 2004 are lower than all other reported values, and we speculate that some subset of late-winter weather conditions may be responsible.

Serum calcium values of 25.1, 31.6, and 21.4 mg/dl were recorded in spring for

TABLE 7. Means (±SE) for hematology and serum chemistry, by time, northern Nevada, spring 2004–2005.

Measure	2004[a]	2005[b]
Hematology		
Absolute heterophils (μl)	974 (±157)	1,050 (±180)
Absolute eosinophils (μl)	68 (±15)	60 (±17.2)
Absolute basophils (μl)	16 (±4)	10 (±4.8)
Packed cell volume (%)	57 (±1)	61 (±1.3)
Total plasma protein (g/dl)	6.0 (±0.2)[d]	6.7 (±0.2)[c]
Serum chemistry		
Glucose (mg/dl)	326 (±6)[d]	353 (±6)[c]
Albumin (g/dl)	2.0 (±0.04)[c]	1.8 (±0.05)[d]
Creatine kinase (u/l)	2,405 (±223)	2,157 (±255)
Serum phosphorus (mg/dl)	6.7 (±0.4)	6.6 (±0.4)
Uric acid (mg/dl)	8.2 (±0.3)[c]	6.5 (±0.4)[d]

[a] $n = 37$ in 2004.

[b] $n = 26$ in 2005.

[c,d] Row means with different superscript differ at $P<0.05$.

Sage Grouse females in other studies in northern Nevada by Davis (2003), Pope and Goldie (2004), and Dunbar et al. (2005), respectively. Our TU bird values were consistently below these in both 2004 and 2005, and our LW birds were below those levels in 2005 as compared to Davis (2003) and Pope and Goldie (2004).

Females in TU had lower serum phosphorus levels than those in LW. Values of both sites were lower than others recorded (8.03, 11.6, and 7.3 mg/dl) for spring samples in northern Nevada (Davis, 2003; Pope and Goldie, 2004; Dunbar et al., 2005). Woodard et al. (1979) found that high calcium and low phosphorus ratios in poultry diets were responsible for effects such as poor growth, severe paralysis, and high mortality, especially in chicks. Although phosphorus values found in this study are lower than previously recorded for Sage Grouse, it may be just as important to compare calcium:phosphorus ratios as it is to calculate values of each individual mineral. Ratios of 3.3:1–3.5:1 were most common in this study; however, LW females across age groups had lower values (2.7:1) than the TU and LW females in 2004, or the TU females in 2005. Additionally, Dunbar et al. (2005) reported an odds ratio analysis for calcium:phosphorus ratio that demonstrated a 70% decline in the predicted odds of at least one chick surviving until 1 August, if the value levels increased 1 unit (3:1 to 4:1). These authors suggested ratios approaching 4:1 may not be conducive to chick production and likely represented phosphorus to be inadequate in the diet. Our ratio values were within the recorded range, and ratio differences between sites within years reflect changes in calcium rather than in phosphorus.

Prelaying nutrition has been shown to affect the breeding success of Sage Grouse (Barnett and Crawford, 1994) as well as other galliforms, with more nutritious diets resulting in larger clutches, larger eggs, greater hatching success, and greater chick survival (Moss et al., 1975; Beckerton and Middleton, 1982, 1983). Dietary protein is important for grouse reproduction, with higher prelaying amounts positively correlated with clutch size, hatching success, and chick survival in Red Grouse (*Lagopus lagopus scoticus*) (Beckerton and Middleton, 1982). Consumption of forbs (herbs other than grasses) contributes a large amount of protein to the diet of a prelaying Sage Grouse (Barnett and Crawford, 1994), and it was noted through our vegetation monitoring that TU had

less key forbs than did LW during 2005, perhaps providing a less-nutritious forage base to the grouse population in that area. Although no site differences were seen in plasma and serum protein levels, yearlings had consistently lower plasma and serum protein values than did adults. A 1.0 g/dl difference in serum protein level has been shown to impact productivity in Sage Grouse, with females having the higher values experiencing a greater ability to renest (Dunbar et al., 2005); this perhaps indicates a lowered intrinsic ability of yearlings to renest, resulting in lower reproductive success potential. As ground-nesting birds, nest predation is quite prevalent, and renesting can contribute heavily to overall yearly reproductive success and chick recruitment.

Likewise, this study found that age had an influence on serum calcium levels. Other studies reported no age effects on serum calcium levels (Davis, 2003; Pope and Goldie, 2004), suggesting the hypothesis that this parameter may differ by age, under stressful conditions, when no differences are found in unstressed populations. Phosphorus was not found to differ by age, but TU had consistently lower phosphorus levels than did LW. Intestinal absorption is the most common source of both serum calcium and phosphorus, indicating a lower amount of both in TU. In 2004, key forbs, known to have high concentrations of calcium and phosphorus (Barnett and Crawford, 1994; Davis, 2003), had higher levels of vegetative cover in LW. Age differences were seen in LW during 2005 that were not seen in 2004; however, winter 2004–2005 was milder, having less snowfall and comparable temperatures, than was winter 2003–2004 (Desert Research Institute weather report, unpubl. data). Although calcium and phosphorus were lower in certain age classes and in certain site years, no statistical differences in calcium:phosphorus ratios were detected during the prenesting period. This demonstrates functioning homeostatic mechanisms which likely assist populations living in sites with lower nutritional forage bases to compensate for inadequacies through maintenance of required ratios. This may allow for production of healthy chicks (Woodard et al., 1979) while having the least deleterious effects possible on the bone calcium reserves of the female grouse. Deciphering the calcium and phosphorus fluctuations is beyond the scope of this study, due to the variety of factors that can impact these parameters.

Lower phosphorus values detected in this study, as compared to others, may also be due, in part, to different sampling methods. Hemolysis produces a false increase in phosphorus concentrations due to the leakage of intercellular phosphorus (Thrall et al., 2004). Serum used in this study was separated directly after centrifugation, whereas serum samples used in other studies (Davis, 2003; Pope and Goldie, 2004) may have had prolonged contact with the separated cells.

Yearlings consistently weighed less than adults, corresponding to findings of previous studies (Beck and Braun, 1978; Remington and Braun, 1988); however, this study indicates that weight disparities by age will become more marked under stressful conditions. If yearling weight gain is inhibited during extreme winter conditions, causing their entrance into the breeding season with lower energy reserves, breeding success the following year may be impeded regardless of the availability of nutritious spring forbs.

Physiologic parameters can provide information to complement habitat studies. Some variation from year to year is expected in wild populations, and long-term data collection in any given area will be required to establish expected values for that area. It is also probable that reproductive success and population health may be impacted by physiologic parameters not investigated in this model (e.g., reproductive hormones). The information from this study includes hematologic data from two habitats and may

provide a practical guide for assessing the physiologic condition of Sage Grouse hens in populations under similar conditions.

Wildlife studies customarily measure habitat parameters, and the physiologic health of the animal is overlooked, although it may prove quite important in understanding limiting factors for a population. The nutritional content in a species of forb or shrub is site and year dependent (Davis, 2003), indicating that Sage Grouse populations could inhabit different areas, having similar habitat parameters, and receive different physiologic contributions from the forage base. Analyzing long-term vegetation trend data in conjunction with physiologic indices will provide important information about habitat, climactic, geographic, or other differences affecting Sage Grouse populations.

Physiologic parameters should also be used to assess the effects of certain land management practices on Sage Grouse populations in small geographic areas. Controversial practices, such as prescribed fire, could be conducted in conjunction with physiologic data collection, allowing nutritional health of the population to be assessed pre- and postburn. This would provide for more certainty as to the effects, either positive or deleterious, of land management practices. More information is required for blood parameters under a variety of physiologic, environmental, and geographic conditions in order to develop predictable responses regarding annual variation and long-term trends in vegetative compositions of various habitats used by Sage Grouse populations.

ACKNOWLEDGMENTS

Our gratitude is extended to K. Kulinsky (University of Nevada-Reno technician) and all the undergraduate students that collected data for this project. We appreciate the Nevada Bureau of Land Management for providing the study areas and the Nevada Division of Wildlife for providing bird capture permits for the project. The project was funded by the Nevada Arid Rangeland Initiative and the Nevada Agriculture Experiment Station. Procedures involving Sage Grouse handling were approved by the University of Nevada Institutional Animal Care and Use Committee (A03/04-07).

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Received for publication 15 September 2008.