HUNTER COLLECTED BLOOD SAMPLES FOR COMPARING THE PHYSICAL CONDITION OF TWO QUÉBEC MOOSE POPULATIONS

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Résumé: Des échantillons de sang d'orignaux (*Alces alces*) tués dans une réserve faunique où la densité était relativement élevée et le rapport de sexes proche de 1:1 furent comparés à des échantillons récoltés dans des zones de chasse adjacentes où la densité était plus basse et le rapport des sexes variable entre 1:3 et 1:2. Ces échantillons furent récoltés entre la mi-septembre et la mi-octobre; leur hématocrite, et leur teneur en azote urée, en phosphore inorganique, en phosphatase alkaline et en albumine furent déterminés. Il fallut en moyenne trois jours aux échantillons pour parvenir à la station biologique. Environ 76% des échantillons subirent de l'hémolyse, ce qui affecta les concentrations d'azote urée, de phosphore et d'albumine. Le taux d'azote urée varia en fonction du temps, réflétant une baisse de la prise de protéine au cours de la période d'étude. La comparaison des deux populations ne révéla des différences significatives que pour l'albumine. La pauvre qualité des échantillons de sang récoltés par les chasseurs a probablement diminué la possibilité de trouver plus de différences en comparant les deux populations.

Abstract: Blood samples from moose (*Alces alces*) harvested in a game reserve where density was relatively high and sex ratio close to 1:1 were compared with samples collected in adjacent hunting zones where density was lower and adult bull:cow ratios varied between 1:3 and 1:2. Samples were collected between mid-September and mid-October, and analysed for hematocrit, blood urea nitrogen (BUN), inorganic phosphorus, alkaline phosphatase and albumin. There was an average of 3-days delay between animal death and delivery of samples to us. Some 76% of the samples were hemolysed which in turn had a significant effect on BUN, phosphorus, and albumin concentration. BUN levels varied with date, this reflecting a decrease in protein intake during the period studied. Comparing blood values between the populations showed a significant difference only in albumin. The poor quality of blood samples collected by hunters probably lowered the possibility of finding more differences when comparing the 2 populations.

Crête et al. (1981) found that moose (*Alces alces*) hunting differed markedly between game reserves and surrounding hunting zones in southwestern Québec. Hunting pressure (hunter-days/km^2) and harvests were respectively 25-100 and 2-7 times lower in the reserves than outside because of controlled hunts (Bouchard and Moisan 1974), resulting in winter densities at least twice higher in the reserves. Since the moose season in Québec has coincided with the rut for many years and since adult males are more vulnerable than females and calves at this time (Fraser 1976; Crête et al. 1981) because of their greater mobility (Roussel et al. 1975) and lower wariness, lower

winter bull:cow ratios have been related to hunting pressure (Crête et al. 1981). Contrarily to what was observed in Alaska (Bishop and Rausch 1974), unbalanced sex ratios have been associated with decreasing productivity in Québec (Crête et al. 1981).

Differences in density and in sex ratios should affect rutting behavior in moose. In hunting zones one should expect the rut to last longer, the males to serve more females, to wander on longer distances and to spend less time feeding. Moreover growth of young bulls could be depressed by their early participation into the rut as suggested by Bubenik (1972). More oestrus cycles and associated fasting (Crête and Jordan 1981) should occur in hunting zones. On the other hand in the reserves, animals of both sexes should face more social interactions during the rut. We tested the hypothesis of lower forage intake and poorer physical condition for moose in the hunting zones than in the reserves. We examined blood because it offers a good index of health and forage intake (Franzmann and LeResche 1978; Seal et al. 1978b).

STUDY AREA, METHOD AND MATERIALS

Blood samples were collected in La Vérendrye reserve and in the surrounding zone F_3, H_2 and J_1 during fall of 1978 and 1979 (fig. 1); this area of southwestern Québec (approx. 47°N, 77°W) was selected because it was possible to stop hunters en route to their hunting sites to solicit assistance in collecting blood. The region is vegetated with forests of mixed northern hardwoods and

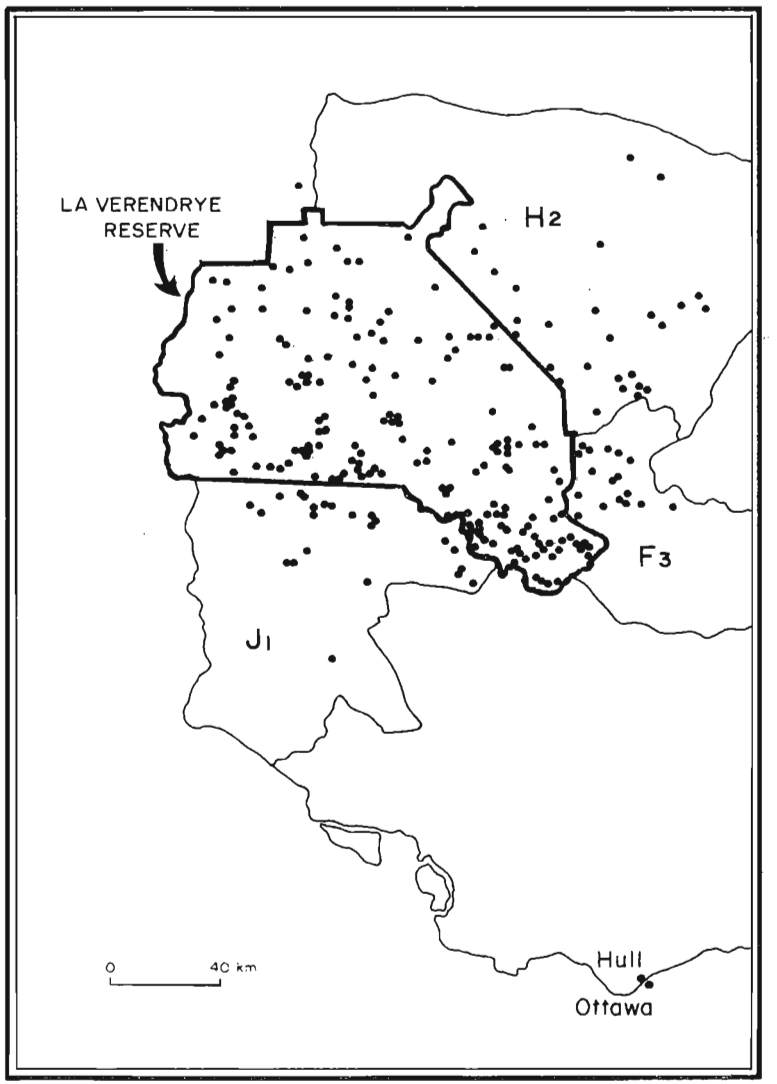

Figure 1. Kill locations of moose from which blood samples were drawn, southwestern Québec, September-October 1978 and 1979. F_3, H_2 and J_1 constitute the name of the hunting zones

boreal conifers and includes numerous shallow lakes and ponds (Crête et al. 1981). Productivity and composition of both summer and winter forage species do not differ notably between the zones (Crête and Jordan 1982). Preliminary data on body and organ weight (Verme and Ozoga 1980) have indicated comparable forage quality for the two areas (M. Crête, unpubl. data). Winter aerial counts by helicopter (Crête and St-Hilaire 1979) yielded density estimates of 0.3 and 0.1 moose km^{-2} respectively in the reserve and in the 3 hunting zones; winter sex ratio (excluding calves) was 39% in the reserve and 25-36% outside (Crête et al. 1981; unpubl. data).

During the 2 seasons, approximately 500 pairs of 7-ml disposable culture tubes containing 50 IU heparin were distributed to hunters in La Vérendrye reserve and 1,500 to hunters in the heavily hunted zones. Hunters were asked to fill both tubes with blood from the heart as soon as possible and to gently shake them; they were also asked to keep blood samples stored at a cool temperature. When samples were received at the checking station, hematocrit was determined within 12 hours; the whole sample was then centrifuged and the plasma deep frozen. Coagulated or totally hemolyzed samples were rejected. At the end of each hunting season, plasma samples were shipped frozen to our laboratory where they were processed within 1 month. The age of adult moose from which a blood sample had been collected was determined by sectioning the 1st incisor (Sergeant and Pimlott 1959); calves were aged by their size and many yearlings by tooth wear. Information was grouped by calves, yearlings, and older animals. Blood samples from unaged animals were discarded. Samples were classified according to 3 periods of collection: September 15-25,

September 26 - October 5, October 6-20.

The plasma assays run were (1) blood-urea nitrogen (BUN) for estimating protein intake (Kirkpatrick et al. 1975, Bjargov et al. 1976, Carver et al. 1978, Seal et al. 1978b, Bahnak et al. 1979); (2) inorganic phosphorus for estimating energy intake (Bjarghov et al. 1976, Seal et al. 1978b) (this assay was performed in 1978 only); (3) alkaline phosphatase for estimating growth in young animals (Hyvärinen et al. 1977, Seal et al. 1978a, Barrett and Chalmers 1979, Franzmann et al. 1980); (4) albumin for estimating long-term nutritional status and, indirectly, levels of infections (Seal 1978). Hematocrit served as an indicator of overall physical condition (Bjarghov et al. 1976, Franzmann and LeResche 1978).

Hematocrit was determined with a micro-hematocrit centrifuge at 5500G for 10 min. BUN was measured with diacetyl-monoxime without deproteinization of plasma (Sigma Chemical Company: reagent #535). Inorganic phosphorus (Wortlington Diagnostics, Freeholel, N.J.) and alkaline phosphatase (Boerhinger Mannhein Canada) assays were performed on an auto-analyzer (Trace TM III Clinical Chemistry System, Beckman). Total serum protein was measured using the biuret reaction, and the percentage of albumin, alpha and beta globulin were estimated after separation by micro-electrophoresis (ACI, Analytical Chemist Inc., Palo Alto, Cal.) on agar film (Universal Electrophoresis Film Agarose, Corning ACI) using a densitometer (Clifford, Model 445).

Since hemolysis was common, we separated blood samples in 3 quality categories; the degree of hemolysis was estimated by summing the concentration of alpha-2 and beta globulin, since hemoglobin is

included with those 2 proteins during the electrophoresis (Schultze and Heremans 1966). The 3 classes were: (1) no hemolysis, alpha 2+ beta globulin ≤ 1.30 g/dl, (2) light hemolysis = alpha 2 + beta globulin 1.31 - 2.0 g/dl, (3) high hemolysis = alpha 2 + beta globulin ≥2.01 g/dl.

Analysis of variance with unequal cell frequencies was used to evaluate the influence of serum quality, hunting regime, sex and age, and date of collection on blood constituents. A SPSS statistical package (Nie et al. 1975) was used, and only 2-way interactions are considered for discussion. Chi-square tests were performed for comparing 2 frequency distributions and t-tests for comparing 2 means. Means are presented with their standard errors.

RESULTS AND DISCUSSION

Between September 15 and October 20 of both years, collections from La Vérendrye reserve yielded 185 usable samples, and from adjacent hunting zones there were 42. Because of relatively small samples from the hunting zones and because the package for the analysis of variance was restricted to 5 factors, results from the 2 years had to be pooled. We estimate, based on overall hunter success in the 2 zones, that return of blood samples was approximately 70% in the Reserve and 25% outside. Greater personal contact between hunters and project personnel and the privilege of hunting in a game reserve appear responsible for better cooperation there. Time elapsed between kill and processing of blood at the checking station averaged 2.7 ± 0.13 days (n = 136) for the Reserve and 3.4 ± 0.31 days

Table 1. Mean and coefficient of variation for 5 blood constituents from moose collected by hunters in southwestern Québec during the breeding seasons of 1978 and 1979

	Mean	Coefficient of variation
Hematocrit (vol. %)	34.0 (208)[a]	49
BUN (mg/dl)	16.4 (225)	38
Phosphorus (mg/dl)	12.3 (95)	53
Alkaline phosphatase (IU/liter)	246.7 (171)	169
Albumin (g/dl)	3.8 (224)	19

[a] Sample size.

(n = 20) for hunting zones: this difference is not significant ($p > 0.05$). Among acceptable blood samples (n = 227), 24% had no hemolysis, 36% fell in quality class 2, and 40% in class 3; the distribution of samples among the quality classes was not significantly different for both regions ($p > 0.10$).

Table 1 shows that variation of blood-constituent level among samples was generally high, with an extreme value for alkaline phosphatase. Compared to other data for free-ranging moose (LeResche et al. 1974, Franzmann and LeResche 1978) and for pronghorns (*Antilocapra americana*: Barrett and Chalmers 1977), our coefficients of variation were similar for albumin, 1.5-2.0 times higher for BUN, inorganic phosphorus and alkaline phosphatase, and about 4 times higher for hematocrit. High variability in hematocrit in animals taken by shooting was documented by Wesson III et al. (1979a).

When comparing our data to two other studies of moose blood conducted during the same season (LeResche et al. 1974, Franzmann and LeResche 1978), hematocrit was generally lower in our samples. The low values were attributed to our animals being shot which generally results in lower hematocrit readings than when animals are restrained in captivity or chemically immobilized (Jacobson et al. 1978; Wesson IIIet al. 1979a, b). BUN levels were slightly lower in our study than in one other study (Franzmann and Leresche 1978), but this is probably not related to the shooting. Phosphorus levels were approximately double in our animals; blood phosphorus is increased by drug immobilization (LeResche et al. 1974), while the influence of shooting is unknown. Continued metabolism in red blood cells between collection and the processing, which frees inorganic phosphorus, is probably responsible for most of this apparent increase (Simesen 1970). Our alkaline phosphatase levels seemed comparable to those reported elsewhere but shooting impact on this parameter is also unknown. Albumin concentration was slightly lower than or similar to levels observed elsewhere. Shooting does not affect albumin concentration in cottontail rabbits (*Sylvilagus floridanus*: Jacobson et al. 1978). BUN and alkaline phosphatase levels decrease by 5-7% after prolonged frozen storage (> 100 days) (Hunter and Madin 1978). Total protein and inorganic phosphorus are not affected by storage.

The degree of hemolysis (Table 2) had a significant effect ($p < 0.05$) on levels of BUN, inorganic phosphorus, and albumin. The higher BUN concentration in class 3 was caused by the interference of free hemoglobin with the colorimeter reading: deproteinization of the plasma samples would have alleviated this interference and is recommended by us. Phosphorus readings increased with hemolysis because organic phosphorus from lysed cells was being added (Simesen 1970). Furthermore, there was a significant interaction ($p < 0.05$) between sample quality and sex for phosphorus and alkaline phosphatase concentration (fig. 2). Higher phosphorus levels for females in quality class 3 are difficult to explain; variations for alkaline phosphatase are still more intricate.

Date of collection had a significant influence only on BUN level. Mean concentration was 18.6 ± 0.70 (n = 68), 15.9 ± 0.66 (n = 102) and 14.6 ± 0.67 (n = 55) mg/dl respectively for the first, the second and the third period of collection. The decrease in BUN concentration during the breeding season was associated with the

Table 2. Mean values of 5 blood constituents according to the degree of hemolysis of the samples as estimated by pooled concentration of alpha 2 and beta globulin. Samples were collected by moose hunters in south-western Québec between Septembre 15 and October 20, in 1978 and 1979.

	Sample quality class[a]			Significance
	1	2	3	of F
Hematocrit (vol. %)	30.0 ± 2.0[b]	34.0 ± 1.8	36.0 ± 2.0	0.19
	(49)[c]	(74)	(85)	
BUN (mg/dl)	15.8 ± 0.70	14.6 ± 0.62	18.4 ± 0.72	< 0.01
	(55)	(81)	(89)	
Phosphorus (mg/dl)	10.6 ± 0.54	12.7 ± 0.38	14.2 ± 1.94	0.04
	(35)	(35)	(25)	
Alkaline phosphatase	203 ± 40.6	188 ± 31.5	340 ± 383.5	0.09
(IU/liter)	(50)	(60)	(61)	
Albumin (g/dl)	3.9 ± 0.09	3.7 ± 0.08	4.0 ± 0.09	0.03
	(55)	(82)	(87)	

[a] Class 1 = alpha 2 + beta globulin ≤ 1.30 g/dl; class 2 = alpha 2 + beta globulin 1.31 - 2.00 g/dl: class 3 = alpha 2 + beta globulin ≥ 2.01 g/dl.

[b] $s_{\bar{x}}$.

[c] Sample size.

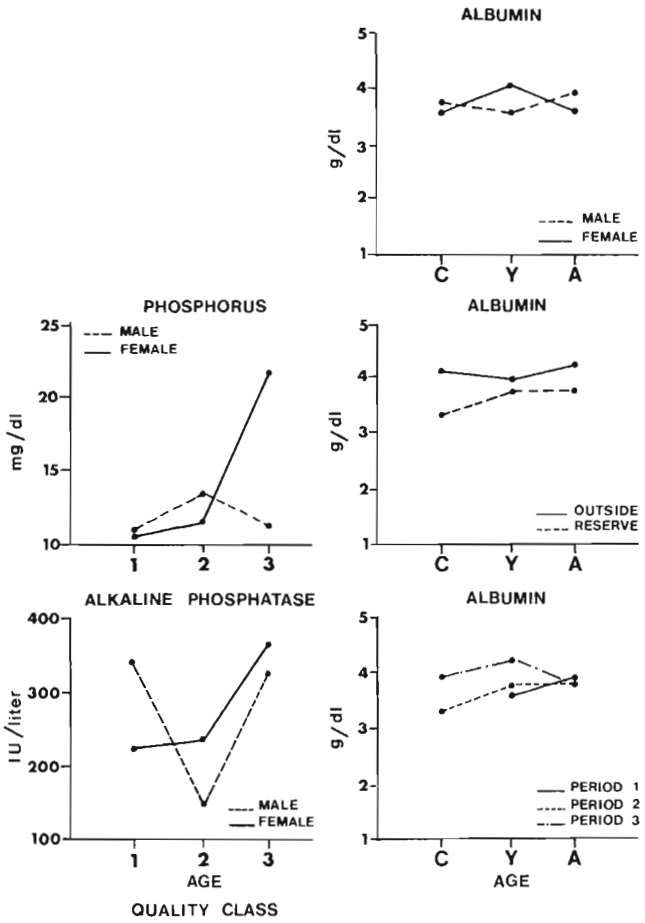

Figure 2. Mean values observed for albumin, phosphorus and alkaline phosphatase when signficant ($P < 0.05$) 2-way interactions were found.

switching of diets from leaves to twigs (Crête and Jordan 1981), where protein levels in twigs were less than half those in leaves (Crête and Jordan 1982). LeResche et al. (1974) also reported such a decrease in BUN level in fall for moose. Lower BUN levels in late fall also reflect weight lost associated with the rut (Franzmann et al. 1978).

None of the 5 blood constituents showed significant difference by age or by sex. It is surprising not to have found an age related difference for alkaline phosphatase since higher levels of this enzyme in growing animals have been well documented in moose and other ungulates (Barrett and Chalmers 1977, Hyvärinen et al. 1977, Seal et al. 1978a, Franzmann et al. 1980). Time lag between collection and processing of blood samples is probably responsible for not finding such a pattern. Seal et al. (1978a) found no age difference during winter in hematocrit, BUN, P and albumin for white-tailed deer (*Odocoileus virginianus*). On the other hand Franzmann and LeResche (1978) found no sex difference in September for hematocrit, phosphorus and albumin, but differences were significant in October. In Minnesota, there was no sex difference for hematocrit in October, but in December females were higher (LeResche et al. 1974). Seal et al. (1978b) found no sex difference among white-tailed deer during winter for the same 5 blood constituents.

Only albumin level differed between our 2 zones, being lower in La Vérendrye reserve than in adjacent hunting zones (respectively 3.8 $\pm$ 0.05 (n = 185) and 4.1 $\pm$ 0.13 (n = 39) g/dl). However it exhibited significant ($P < 0.05$) age x sex, age x region and age x collection period interactions (fig 2). The age x sex and the age x period interactions were probably caused by bias in sampling. In the first case, 39% (n = 35) of yearling females sampled originated

from the hunting zones where albumin level was higher than in the reserve as compared to 18% (n = 33) for yearling males. For the age x period interaction, yearling samples from hunting zones represented 32% (n = 37) of the total during the second period, and 47% (n = 15) for the third one, while 18% (n = 37) and 14% (n = 36) of the adult samples came from the hunting zones for the corresponding periods. When comparing with other age categories, it is difficult to explain the greater difference in albumin concentration for calves, which seems responsible for age x region interaction, because of the small sample size (n = 4 for the reserve and 5 outside). Franzmann et al. (1980) found low albumin concentration for moose neonates as it is well documented for other cervids (Chapman 1977); increase of albumin to adult level could have been slower in the reserve than outside for unknown reasons, although this increase should happen early after birth (Chapman 1977).

Albumin concentration appeared to be really higher outside than in the reserve since the tendency was the same for the 3 age categories (fig. 2). Lower albumin concentration in the reserve could be associated with greater incidence of parasitism there (mainly *Echinococcus granulosus*, *Cysticercus tenuicollis* and *Cysticercus tarandi*), since Seal (1978) found low albumin level for acute infection in white-tailed deer. However the effect of parasite loads on albumin is unknown.

It is difficult to reject or not the hypothesis of poor physical condition in the hunting zones. Since blood samples from both areas were submitted to the same treatment, one could argue that the lack of important difference indicates that the 2 populations

were comparable. However time lag between blood collection and delivery to us influenced all samples, even those without hemolysis, and this could have masked true differences. Hunter collected blood samples are probably not suitable for finding fine differences between 2 cervid populations.

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