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VITAMIN A STATUS OF WILD MALLARDS (*ANAS PLATYRHYNCHOS*) WINTERING IN SASKATCHEWAN

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ABSTRACT: Vitamin A status of wild male mallards (Anas platyrhynchos) overwintering in Saskatchewan, Canada was determined. Vitamin A levels $<0.2 \ \mu$ g hepatic retinyl palmitate/g liver, occurred in 6% and 25% of male mallards sampled in 1991 to 1992 and 1992 to 1993, respectively. There was no temporal trend in vitamin A levels over either winter. Squamous metaplastic lesions, commonly associated with vitamin A deficiency in domestic animals, were not observed in any bird; hence, they were not a good indicator of vitamin A status in wild mallards. Serum retinol was not a good indicator of vitamin A status in wild mallards. Many mallards in good body condition had low vitamin A levels; thus, we propose that good body condition and ample fat stores are not indicative of overall health of the bird.

Key words: Anas platyrhynchos, mallard, vitamin A deficiency, body condition, retinol, retinyl palmitate, winter.

INTRODUCTION

There is a trend for mallards (Anas platyrhynchos) and other waterfowl to winter north of their traditional wintering grounds in areas where water remains open vear round (Bellrose, 1976). These birds feed almost exclusively on waste grain that contains only trace amounts of vitamin A, through the 3 to 5 mo of winter (Jorde et al., 1983; Clark and Sugden, 1990). During the winter of 1990 to 1991, vitamin A deficiency was diagnosed in mallards found dead near Gardiner Dam, Saskatchewan, Canada (51°15'N, 106°50'W) (Wobeser and Kost, 1992). These birds were from a population which remained near the dam throughout the winter. Vitamin A deficiency also was reported in wintering wild black ducks (Anas rubripes) in Massachusetts (USA) (Hagar, 1950).

Vitamin A is a collective term for compounds with similar biological activity to retinol, including retinyl esters and beta carotenes. Clinically deficient birds may have grossly visible white plaques in the esophagus. These keratin-dilated, mucous glands lined with stratified squamous epithelium are considered typical of vitamin A deficiency. Squamous metaplasia also has been reported in the trachea, nasal cavity, and ureter of domestic birds (Jungherr, 1943). Loss of muco-epithelial surfaces in vitamin A deficiency may provide a portal of entry for disease-causing agents (Jungherr, 1943). Vitamin A plays an integral role in the cellular immune function of many species (Sijtsma et al., 1990; Friedman et al., 1991). Vitamin A deficient chickens have exhausted lymphoid cell stores and are unable to mount an effective immune response when challenged with infectious agents (Bang et al., 1975; Nauss, 1984).

Our objectives were to determine: a) vitamin A levels in mallards wintering at Gardiner Dam during 1991 to 1992 (Winter 1) and 1992 to 1993 (Winter 2), b) the relationship between body size or condition and levels of vitamin A in tissues, c) the presence of lesions of vitamin A deficiency in wild mallards and the relationship between lesions and tissue levels of vitamin A, and d) vitamin A values in mallards shot by hunters during fall migration.

METHODS AND MATERIALS

During Winter 1, approximately 3,000 mallards wintered below Gardiner Dam in an area free of ice, downstream from the electricity generating turbines. The birds preferred to stage, protected from the wind, along a small creek with a maximum width of 6 m which flowed from the infrastructure of the dam for about 150 m to the river. The level of the creek and river varied daily by >1 m depending on electrical needs. Between December 1991 and February 1992, 34 male mallards were collected at the creek by shooting. Blood sampling was attempted with limited success immediately upon retrieval of the carcasses. The carcasses were held at ambient temperature (-10 to -37 C) for up to 3 hr before postmortem examination.

During December 1992, approximately 4,000 mallards were in the same area. Eighty-nine male mallards were sampled during four collections from December 1992 to March 1993. During December, January and February, birds were trapped in the small creek using $1.2 \text{ m} \times$ $1.2 \text{ m} \times 0.6 \text{ m}$ weld wire funnel traps baited with grain. Traps were baited only on the day of sampling. Ten male mallards were shot in January for comparison with birds captured in bait traps in the same area on the same day. The sample of ducks in March 1993 was collected by shooting because a drop in water level made trapping impossible. Shot birds were handled the same as birds collected in Winter 1. Live-trapped birds were transported for 1 hr to the Western College of Veterinary Medicine, Saskatoon, Saskatchewan, where they were killed by carbon dioxide asphyxiation. Blood, collected by intracardiac puncture from birds that had ceased respiration, was allowed to clot protected from light at 37 C for several hours and then centrifuged at 3,500 rpm at 10 C for 15 min while protected from light. Sera were frozen in liquid nitrogen (-170 C) and then stored at -80C until analyzed for vitamin A. Handling of the blood and sera was as consistent as possible. Minor variations in tissue handling had no effect on vitamin A levels (Honour, 1994). Postmortem procedures were as described by Honour et al. (1995). At the beginning of each postmortem examination, a sample taken from the same location of each liver was wrapped in non-porous plastic and foil, frozen in liquid nitrogen (-170)C) and then stored at -80 C until shipped for vitamin A analysis. Vitamin A analyses of livers and sera were performed by the Biomarker Laboratory, Environment Canada, National Wildlife Research Centre, Hull, Quebec, Canada using a method described by Honour et al. (1995). Samples of esophagus, trachea and kidney were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μ m and stained with hematoxylin and eosin. Head-beak (maximum linear distance from beak tip to occipital crest of skull) and keel lengths (maximum linear length of skinned keel) were recorded for all birds collected during Winter 2. The content of the crop, proventriculus and ventriculus was examined and identified; the weight of the contents was subtracted from the body weight of all birds. Birds were categorized as juvenile or adult on the basis of wing feather characteristics (Carney, 1992). Observed body condition (OBC) was scored on a scale of 1 to 5: 1, no observable fat; 2, fat overlying the abdominal viscera; 3, pericardial fat as well as subcutaneous fat; 4, large amounts of fat in the peri-esophageal and tracheal regions; 5, fat in all the aforementioned locations, together with subcutaneous fat of >5mm depth and fat dorsal to the kidneys. To evaluate body condition based on mass and skeletal size, a standardized body score (SBS) was calculated for mallards collected in Winter 2 by regressing body weight on the sum of head-beak and keel lengths. A mean of the SBS for all the wild drakes was determined and the difference between each bird's SBS and this mean is reported as a residual.

While the study concentrated on males, 10 females were shot inadvertently in Winter 1 and three females were shot in Winter 2. Hepatic vitamin A content was determined for these females.

Tissue samples were collected from 22 mallards shot by hunters at Last Mountain Lake (51°05'N, 105°10'W), Saskatchewan Landing (50°39'N, 107°59'W) and Gardiner Dam, Saskatchewan, between 15 September and 15 October 1991 (hereafter referred to as "migratory" mallards). Serum was collected from only two of these birds. Birds were frozen, either intact or after removal of the pectoral muscles, within 2 hr of death. Liver samples from migratory birds were frozen at -20 C for up to 2 wk before being frozen in liquid nitrogen but this method of handling the livers did not appear to affect the vitamin A concentrations (Honour, 1994). Histological examination was not performed on these ducks.

Mean monthly temperatures for the Gardiner Dam area were supplied by Environment Canada, Saskatoon, Saskatchewan, Canada.

All statistical analyses were done using Statistix 4.0 (Analytical Software, St. Paul, Minnesota, USA). Continuous data were tested for normality using the Wilk-Shapiro/Rankit test at a 50% confidence level (Shapiro and Wilk, 1965). Hepatic retinyl palmitate (HRPT) and hepatic retinol (HROL) data always failed to meet criteria for normality and subsequently were analyzed using non-parametric tests. The SBS and serum retinol (SROL) appeared to be normally distributed and were analyzed using parametric tests. To compare the normally distributed statistics between months and years, one-way analysis of variance (OAV) was performed. If a significant difference was detected using OAV, a Scheffe's pairwise comparison of the means was used to determine which samples were significantly different. The Kruskal-Wallis OAV was used to compare the HROL and HRPT levels between months within years and between years (Daniel, 1978). To test whether age of the birds varied between months a chi-squared contingency table analysis was performed. To test for independence of OBC and month sampled, a log likelihood ratio was used. Linear regression was performed to compare the HROL to HRPT concentrations. Transformation of SROL and HRPT by taking the natural log of each parameter improved the linear regression between SROL and HRPT; a constant of 0.5 was added to HRPT values prior to the transformation to avoid error due to zero values (Zar, 1984). To determine if there were differences between birds that were shot and those that were trapped, comparisons of vitamin A levels and body measurements were made using the Student's t-test (SBS, SROL) and the Mann-Whitney U-test (HROL, HRPT).

RESULTS

Levels of HRPT in the birds collected during the two winters were highly variable, with a range of over 1,200-fold (Table 1). Birds with levels of HRPT below the detection limit of 0.4 μ g/g were found in December 1991 (n = 2), and December 1992, and January and February 1993 (n = 5), but no temporal trend was evident. There was no difference in HRPT levels among sampling months within Winter 1 (P = 0.42) but HRPT levels among months within Winter 2 approached a significant difference (P = 0.083). The overall median HRPT level in Winter 1 was higher (P =0.004) than that found in Winter 2.

Hepatic retinol levels did not differ among months within Winter 1 (P = 0.90) nor did they differ between years (P =0.14) (Table 1). Monthly variation in HROL levels approached a significant difference in Winter 2 (P = 0.098). Birds with levels of HROL below detection limits were found in all months of Winter 1; these birds had up to 148.5 μ g HRPT/g liver. Detectable HROL was present in all birds examined in Winter 2.

Only ten serum samples were collected

585-1,060 356 - 1,169 234-1,012 755-1,030 342-1,025 358-988 TABLE 1. Hepatic retinol (HROL), hepatic retinyl palmitate (HRPT) and serum retinol (SROL) of wild male mallards collected at Gardiner Saskatchewan, Canada during the winters of 1991 to 1992 to 1993. Range SROL (µg/l) $791 \pm 266 \\ 848 \pm 150 \\ 693 \pm 191 \\ 668 \pm 219 \\ 627 \pm 201 \\ 911 \pm 91$ Mean ± SD **Å** Number sampled g 21 21 21 3.2-1,690.5 11.1-1,045.1 0-597.3 0-504.3 0-676.4 5 - 620.50-1,323Range HRPT• (µg/g liver) 61.2 [28.1 [75.8 78.7 15.4 15.4 72.3 Median Number sampled 0.1-11.65 0-6.33 0.6-37.45 0-11.470.1-4.85 0-6.490.1-35.1 Range HROL• (µg/g liver) Median 1.68 1.15 2.42 8. Number sampled 2 21 22 14 December 1992 February 1992 February 1993 December 199 anuary 1992 anuary 1993 March 1993

HROL and HRPT levels were not normally distributed. ND = not done.

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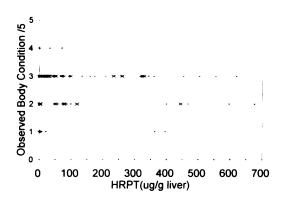


FIGURE 1. Observed body condition (OBC) and hepatic retinyl palmitate (HRPT) levels of wild male mallards sampled at Gardiner Dam, Saskatchewan during the winter of 1992 to 1993.

in Winter 1 and none of these was from December. Serum retinol levels did not differ (P = 0.71) between samples from January and February, 1992 (Table 1). Levels of SROL differed among months of Winter 2. The SROL levels in the March 1993 sample were higher than in the other three samples from Winter 2 (P > 0.05). The SROL level also was higher (P =0.0076) in Winter 1 than in Winter 2. There was no temporal trend in the SROL levels.

Hepatic retinol had good linear correlation with HRPT (r = 0.748, P < 0.005) but SROL correlated poorly with HRPT (linear, r = 0.472, 0.025 < P < 0.05). The natural log of (HRPT + 0.5) correlated well with natural log of SROL (r = 0.754, P < 0.005). When SROL levels were compared to HRPT levels, only one bird had <300 µg SROL/l and <2 µg HRPT/g liver; 17 birds had ≥300 µg SROL/l and <2 µg HRPT/g liver, while 68 birds had ≥300 µg SROL/l and ≥2 µg HRPT/g liver.

No histological lesions characteristic of vitamin A deficiency were found in the esophagus, trachea, kidney, or nasal cavity of any of the wild birds. Lesions associated with parasitism, and inflammatory cells infiltrates in the esophagus were noted in some individuals.

The OBC did not appear to correlate well with HRPT levels (Fig. 1). Based on the chi-squared probability of all OBC of

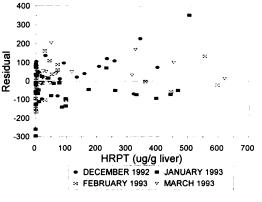


FIGURE 2. Relationship between body size of wild male mallards wintering at Gardiner Dam, Saskatchewan, 1992 to 1993, and hepatic retinyl palmitate levels (HRPT). Body size is represented as the residual of body weight (g) regressed on the sum of headbeak and keel lengths (mm).

both years (P = 0.001), the OBC varied among months sampled. The OBC of birds sampled in Winter 1 varied among months (P = 0.006). Birds sampled in December 1991 had lower OBC than birds sampled in January and February 1992. The OBC of birds sampled in Winter 2 varied among months (P = 0.053). The trend was for birds sampled in January 1992 to have lower OBC than those sampled in February and March 1993. There was no difference in the OBC between years when months were paired. The SBS of birds sampled in January 1993 were lower than those of birds sampled in December 1992 and March 1993 but did not differ from birds sampled during February 1993. There was no correlation between residuals of SBS and HRPT levels (Fig. 2).

Age of birds varied between months of Winter 1 and the same months of Winter 2 (P = 0.003). There was a variation of age among months of Winter 1 (P = 0.002); also, an apparent variation in age among months of Winter 2 may be significant (P= 0.059). The mean age of birds in the December 1991 sample was lower than that of the February 1992 sample. There was a trend to sample younger birds in December 1992 than in March 1993. Based

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on an OAV (SROL, SBS) or Kruskal-Wallis analysis of variance (HROL, HRPT, OBC) between juveniles and adults over both vears, there was no difference in the SROL or SBS (P = 0.28, P = 0.24, respectively), borderline significantly lower HROL in juveniles (P = 0.067) and lower OBC and HRPT in juveniles (P = 0.016, P = 0.043, respectively).

No differences (P > 0.05) were found in HRPT, HROL, OBC (Mann-Whitney U-test), SROL, SBS (two-tailed t-test) or age (chi-square test) between the samples of birds collected by shooting and those collected by trapping in January, 1993.

Male mallards collected during fall migration by hunters had vitamin A levels ranging from 0.47 to 21.97 μ g HROL/g liver and 9.0 to 645.6 μ g HRPT/g liver (Table 2). These levels were not different from vitamin A levels of males sampled in December 1991 (HROL, P = 0.072; HRPT, P = 0.106). Females collected by hunters had vitamin A levels ranging from 0.36 to 12.8 μ g HROL/g liver and 27.5 to 724.7 μ g HRPT/g liver; these did not differ from levels in males sampled in December 1991 (HROL, P = 0.43; HRPT, P = 0.19).

Females shot during winter sampling at Gardiner Dam had levels of hepatic vitamin A within the range of levels for males from the same sample date (Table 3). The maximum values of HRPT in an individual female in both Winters 1 and 2 was much lower than the maximum level found in males in the respective years.

Mean monthly temperatures at Gardiner Dam, Saskatchewan ranged from -3.5C to -16.5 C in the winter preceding the study (1990 to 1991), 1.2 C to -9.6 C in Winter 1 and -0.8 C to -15.9 C in Winter 2.

DISCUSSION

Vitamin A deficiency occurs in mammals when hepatic levels drop below 20 μ g retinol/g liver (Goodman, 1988). Diagnosis of vitamin A deficiency in birds has depended on histological evidence of squamous metaplasia of mucous secreting

		HROL• (µg/g	(µg/g liver)		HRPT• (µg/g liver)	ç liver)		SROL (µg/l)	
Sex	Number sampled Median	Median	Range	Number sampled	Median	Range	Number sampled	Mean ± SD	Range
Males	13	4.22	0.47-21.97	13	411.70	9.02-645.60	ND∲	ND	ND⊳
Females	6	2.80	0.36-12.8	6	112.14	27.46-724.70	61	925 ± 85	840-1,010

Hepatic retinol (HROL), hepatic retinyl palmitate (HRPT) and serum retinol (SROL) of wild mallards shot by hunters at several locations

TABLE 2.

· HROL and HRPT levels were not normally distributed not done ii

g

		HROL· (µg/g liver)	liver)		HRPT• (µg/g liver)	; liver)		SROL (µg/l)	
	Number sampled	Median	Range	Number sampled	Median	Range	Number sampled	x ± SD	Range
December 1992	4	1.02	0-135	4	112.0	64.5-205.6	0	ND	ND
January 1992	6	0.3	0-0.6	67	191.5	10.4-273.0	1	ND	868
February 1992	4	0.36	0-1.58	4	36.9	25.2-91.5	e	935 ± 209	625-1,174
January 1993	I	ND	4.2	I	ND	118.9	0	ND	ND
March 1993	6	0.7	0.5-0.8	61	12.5	4.8-20.2	0	ND	QN

ND = not done

Hepatic retinol (HROL), hepatic retinyl palmitate (HRPT) and serum retinol (SROL) concentrations in female mallards collected during the

epithelial surfaces (Jungherr, 1943). This occurred in 10 of 14 captive mallards with $<2 \,\mu g \, HRPT/g \, liver (Honour et al., 1995).$ If vitamin A deficiency is defined as a state in which mallards have $<2 \ \mu g \ HRPT/g$ of liver, two (5.9%) of 34 wild mallards in Winter 1 and 23 (25%) of 92 wild mallards in Winter 2 were vitamin A deficient. The absence of histological lesions in the wild ducks with $<2 \mu g$ HRPT/g liver supports the conclusion that histological evaluation is a very conservative diagnostic indicator of vitamin A deficiency (Honour et al., 1995). The lack of squamous metaplasia in any of the wild mallards with levels of $<2 \mu g$ HRPT/g of liver could result from a difference in vitamin A requirements between wild and captive mallards. The captive mallards that became deficient were second generation wild strain birds and thus should not be physiologically different from the wild mallards. Detrimental effects on reproduction and the immune system may occur prior to clinical evidence of squamous metaplasia (Nauss, 1984); thus, some wild mallards with low levels of HRPT/g liver may have had undetected immune and reproductive dysfunction associated with suboptimal levels of vitamin A.

Vitamin A levels in the diet prior to consuming a deficient diet can markedly affect the vitamin A status of ducks over time because vitamin A is stored in the liver. It is difficult to define the minimum amount of stored vitamin A required for mallards to survive the winter on an all grain diet. Captive mallards appeared to remain vitamin A-replete throughout the winter if they began with a group median of approximately 600 µg HRPT/g liver (Honour et al., 1995). If this median value was applied to the wild ducks, only two of 17 ducks collected in December 1991 and one of 21 ducks collected in December 1992 had vitamin A stores sufficient to last through the winter. Experimental mallards that had a median of 37 μ g HRPT/g liver prior to consuming an all grain diet, became vitamin A deficient within 2 mo

TABLE 3.

(Honour et al., 1995). If this is similar to wild mallards feeding on grain during the winter, three of 17 birds in December 1991 and nine of 21 birds in December 1992 could be expected to become deficient if feeding solely on grain.

Because there were individual wild ducks with as much as 1,600 μ g HRPT/g liver during the winter and there was a lack of a temporal trend in the HRPT levels in these ducks, a dietary source of vitamin A may have been available to at least some of the ducks during winter. Food materials found in the proventriculus of shot mallards, included wheat (Triticum aestivum), barley (Hordeum vulgare), wild mustard seed (Brassica kaber), wild oats (Avena fatua), faba beans (Vicia faba), and unidentified algae. Of these, only algae are reported to be a source of vitamin A (Goodwin, 1984). Variation in HRPT between the two winters may have been related to weather conditions. Winter 1 was less severe than Winter 2 so that much more open water was available for use by ducks and more ducks may have had access to algae in Winter 1 than Winter 2. The winter of 1990 to 1991, when ducks with vitamin A deficiency were found dead at Gardiner Dam (Wobeser and Kost, 1992), had average temperatures similar to Winter 2.

Captive mallards with no detectable HROL also had no detectable HRPT (Honour et al., 1995). Some wild birds from Winter 1 had no detectable HROL but had up to 148 μ g HRPT/g liver. The reason for this difference between captive and wild birds is not known. Internal standards were used routinely during detection of the vitamin A forms, which makes measurement error unlikely. Chronic exposure to 3,3',4,4',5,5' hexabromobiphenyl caused a decrease in liver retinol and retinyl esters with serum retinol being affected when there was inadequate dietary vitamin A (Jensen et al., 1987). Polychlorinated biphenyls (PCB's) decreased vitamin A stores in liver of exposed adult doves and decreased vitamin A in yolk of eggs from

exposed birds (Spear et al., 1989). Dioxins decreased vitamin A stores in liver of rats (Thunberg et al., 1980). Levels of these toxins were not measured in the mallards so we can not rule out the possibility of toxins affecting vitamin A levels in these birds.

We found no differences between birds collected by shooting and trapping. Sampling ducks by shooting and bait trapping has been reported to result in a bias towards younger birds in poorer condition (Reinecke and Shaiffer, 1988; Heitmeyer et al., 1993), although it is disputed that the observed bias is due to location (Dufour et al., 1993).

Observed body condition was not a good indicator of hepatic vitamin A levels and we conclude that vitamin A deficiency can be present in birds that have good fat stores. This result is in agreement with those of experimental trials (Honour et al., 1995). Some of the mallards found dead at Gardiner Dam in 1991 by Wobeser and Kost (1992) were in moderate body condition but had squamous metaplasia of esophageal glands suggestive of vitamin A deficiency. Observed body condition is a qualitative measurement with poor reproducibility between investigators (Owen and Cook, 1977) and body weight is not necessarily a good indicator of body condition (Owen and Cook, 1977; Johnson et al., 1985). Total lipid extraction and other methods of estimating fat content have been used as indicators of survivability, health status, and condition of waterfowl (Ringelman, 1988). Our observations provide evidence that it may be unwise to assume that mallards in good body condition are in a good state of health if micronutrient supplies are inadequate. Other methods of determining body condition use a combination of a linear skeletal measurements and body weight to correct for skeletal size (Johnson et al., 1985). The SBS provided a measure of body weight relative to skeletal length. We did not find a correlation between SBS and vitamin A levels.

Serum retinol levels in other species have been reported to be conserved in the presence of decreasing HRPT until a critical value in the liver is reached, followed by a rapid decrease in serum vitamin A (Goodman, 1988). In wild mallards collected during the two winters, SROL had a moderate, natural log curvilinear relationship with the natural log of HRPT, and was evidence for a conservation of SROL as levels of hepatic vitamin A decreased. In captive mallards, levels $<300 \,\mu g \, SROL/l$ were a diagnostic indicator of $<2 \mu g$ HRPT/g (Honour et al., 1995). In wild mallards, the sensitivity of 300 μ g SROL/l to predict <2 μ g HRPT/g liver was only 5.5%, while the specificity and positive predictive value was 100% and both the accuracy and the negative predictive value was 80%. Serum retinol appears to be of limited use for determining the vitamin A status of depleted wild birds, since the poor sensitivity makes it a poor screening test (Smith, 1991). The reason for this poor predictability using SROL in wild birds is unknown; however, factors such as the length of time on a deficient diet and trace levels of vitamin A in a deficient diet may confound serum levels.

Because the method of aging used is dependant on the timing of pre-basic molt, we are uncertain if the age differences observed, such as a higher proportion of adults in the February and March samples than in earlier months, reflected a true difference in age or a molt phenomenon (Honour, 1994). Because of uncertainty related to the method of aging during winter, we chose not to make any conclusions regarding vitamin A status related to age of the ducks.

The range of vitamin A levels in liver and serum of migrating mallards sampled during autumn was similar to that found in drakes overwintering at Gardiner Dam. Mallards with low levels of vitamin A in the fall have probably selected feeds that do not contain high levels of vitamin A or vitamin A precursors such as beta-carotene. Females collected by hunters during fall migration had lower median HRPT levels than did the migrating males and were similar to the winter levels in males. In contrast, the median level of HRPT in females collected in winter was higher than that of males. However, no general conclusions can be drawn because of the small sample size.

The great variation in vitamin A levels among individual mallards may reflect foraging success. Cold weather and hierarchical status alter feeding sites of ducks (Jorde et al., 1983). At Gardiner Dam, the thermally favorable site was the creek that remained ice-free; the limited algae available at this site might improve the vitamin A status of individuals. However, this area is very small and algae production would be minimal. We did not see any obvious correlation between body size and vitamin A levels, so we cannot draw any conclusions regarding the effect foraging success may have on vitamin A levels. However, birds that selected foods other than carotene-poor grains should have the highest tissue levels of vitamin A.

Mallards wintering at northern locations may face several problems, including inadequate energy for spring migration and reproduction, and inadequate protein for molt (Pawlina et al., 1993). If the findings of this study apply to birds wintering in other northern locations, subclinical vitamin A deficiency may be occurring and its effect on immune function in wild populations should be further studied.

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