Normal Hematologic and Serum Biochemical Reference Intervals for Juvenile Wild Turkeys

Authors: Bounous, Denise I., Wyatt, Roger D., Gibbs, Penelope S., Kilburn, J.V., and Quist, Charlotte F.

Source: Journal of Wildlife Diseases, 36(2) : 393-396

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-36.2.393

Journal of Wildlife Diseases, 36(2), 2000, pp. 393–396

Normal Hematologic and Serum Biochemical Reference Intervals for Juvenile Wild Turkeys

Denise I. Bounous,[1,5] **Roger D. Wyatt,**[2] **Penelope S. Gibbs,**[3] **J.V. Kilburn,**[2] **and Charlotte F. Quist**[4] [1] Department of Pathology, [2] Department of Poultry Science, [3] Medical Microbiology and Parasitology, and [4] Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, Georgia, USA, 30602; [5] Corresponding author (e-mail: dbounous@calc.vet.uga.edu).

ABSTRACT: Blood samples taken from 48 4-mo-old wild turkeys (*Meleagris gallopova silvestris*) were used to establish reference intervals for hematology and serum chemistry values. The study was conducted during September and October 1996. Packed cell volume, total and differential white cell counts, total protein, albumin, glucose, calcium, uric acid, triglyceride concentrations, as well as aspartate transaminase (AST) and lactate dehydrogenase (LDH) activities were assayed. Reference intervals from wild turkeys are similar to those reported for domestic turkeys.

Key words: Hematology, *Meleagris gallopova silvestris*, reference intervals, serum chemistry, wild turkey.

Reference intervals for hematology and serum chemistry have been established and reported in most domestic mammalian species (Jain, 1986). However, limited information is available for domestic avian species, and even less has been established for wild avian species (Kaneko et al., 1997; Zinkl, 1986). Limited blood parameters for small numbers of wild turkeys ($n = 4$ penreared poults; number of poults not given for wild caught or killed poults) have been reported, but the assays performed were limited to hemoglobin, packed cell volume, cholesterol, glucose, and total protein, and the methodologies were not current (Lisano and Kennamer, 1977; Johnson and Lange, 1939). Publications for parameters in domestic turkeys are limited to packed cell volume, cholesterol, glucose, calcium, and total protein (Rhian et al., 1944; Bell et al., 1957; Bell and Sturkie, 1965). Reference intervals for white blood cell counts and differentials, serum albumin, uric acid, and triglyceride concentrations as well as aspartate transaminase (AST) and lactate dehydrogenase (LDH) activities have not been previously reported for domestic or wild turkeys. The normal reference intervals presented for hematology and chemistry values should add significantly to the interpretation of clinical findings in populations of wild turkeys.

Reference intervals are especially important when clinical evaluation is based on interpretation of multiple analytes that are simultaneously measured. This work was conducted to establish reference values for hematology and serum chemistry parameters for clinically normal juvenile wild turkeys as a prelude to a toxicology trial with low levels of aflatoxins (Quist et al., 2000). The following analytes are chosen because they are part of the routine laboratory testing performed in our laboratory for evaluation of avian health: packed cell volume, total and differential white cell counts, total protein, albumin, glucose, calcium, uric acid, cholesterol, and triglyceride concentrations, as well as AST and LDH activities.

Forty-eight wild turkeys were used in the study, which was conducted between September and October 1996. Unresolvable logistic difficulties prohibited collection of wild turkey eggs from the field. First, despite cooperation of turkey biologists from several states, collection of sufficient numbers of eggs to allow a study of this magnitude was not possible. Secondly, this method of collection would not result in chicks of a uniform age necessary to allow comparisons between groups. Subsequently, a number of game breeders from several states were screened to determine appropriate histories on source of the birds. With consultation of turkey biologists from several state fish and wildlife

TABLE 1. Normal reference intervals for hematology values for 4-mo-old wild turkey poults (n = 47).

Parameter	Reference interval
Packed cell volume (%)	31–42
Leukocytes (cells/μl)	10,362–46,487
Heterophils	4,057–27,607
Lymphocytes	4,221–34,267
Monocytes	0–3,952
Eosinophils	0–438
Basophils	0–2,207

TABLE 2. Normal reference intervals for serum biochemistry analytes for 4-mo-old wild turkey poults (n = 47).

Analyte	Reference interval
Total protein (gm/dl)	3.6–5.5
Albumin (gm/dl)	1.1–2.1
Glucose (mg/dl)	215–500
Calcium (mg/dl)	11.4–14.6
Uric acid (mg/dl)	3–17
Cholesterol (mg/dl)	60–220
Triglycerides (mg/dl)	159–290
Aspartate aminotransferase (IU/L)	255–499
Lactic dehydrogenase (IU/L)	420–1,338

agencies and the Chief Biologist of the National Wild Turkey Federation (Edgefield, South Carolina, USA) a breeder was selected. The poults were obtained as one-day-old chicks from a certified commercial game breeder (Toubl Game Bird Farms, Beloit, Wisconsin, USA). These birds were supplemented with a single clutch of age matched wild turkey eggs obtained from the Georgia Department of Natural Resources (Social Circle, Georgia, USA). All poults were raised in battery brooders until 14-days-old, and then moved to floor pens with cedar shavings at the University of Georgia Poultry Diagnostic and Research Center (Clarke County, Georgia, USA; 33°5′N, 83°22′W). They were fed standard poultry starter rations (23% protein, UGA Poultry Science Feed Mill, Athens, Georgia, USA) and water ad libitum for 4 wk, followed by standard poultry grower ration (21% protein, UGA Poultry Science Feed Mill) until four mo of age. At this time blood was collected by capturing and hooding the poults and bleeding from the ulnar vein. Packed cell volume was determined by the microhematocrit method. Leukocyte counts were performed using a manual hemocytometer method. Blood was diluted into an eosinophil Unopette system (Becton Dickinson and Co., Franklin Lakes, New Jersey, USA) using phloxine dye for counting granulocytes (Campbell, 1995). Peripheral blood smears were stained by routine methods with Wright's stain. Differential white cell counts and erythrocyte morphology observations were performed by one individual (DIB) on all the blood smears. Serum chemistries, including total protein, albumin, glucose, calcium, uric acid, cholesterol, and triglyceride concentrations, as well as AST and LDH activities were performed on an Abbott Spectrum Series II analyzer (Abbott Laboratories, Abbott Park, Illinois, USA). Serum was separated within 1 hr of collection and assays were conducted on the day of collection.

Reference intervals were determined using recommendations of the International Federation of Clinical Chemists (IFCC) (Lumsden, 1998). Reference intervals for hematology are presented in Table 1, and intervals for serum chemistry are presented in Table 2. All population and analyte measurements were tested for Gaussian distribution with the method of Kolmogorov-Smirnov, and the following data were found to have normal distributions: calcium, LDH, cholesterol, triglyceride, PCV, TWBC, and basophils. The reference intervals for these data were calculated parametrically without any transformation and reported in Tables 1 and 2. For data analyzed parametrically, the 2.5 and 97.5 percentiles were estimated by taking the mean and subtracting or adding 1.96 standard deviations, respectively (Solberg, 1996). Monocyte counts were transformed to a Gaussian distribution using a square root transformation, and the reference interval reported in Table 2 is the

square of the result obtained from the transformed data; the analysis was parametric. Lymphocytes, heterophils, and eosinophils were analyzed nonparametrically and reported in Table 2. Total protein, albumin, glucose, and AST, and uric acid data were calculated using a nonparametric method and are reported in Table 1. Data analyzed with the nonparametric method were ranked for analysis; the 2.5 and 97.5 percentiles were obtained by adding one to the number of samples taken and multiplying by 0.025 or 0.975, respectively (Solberg, 1996; Koepke, 1998). The rank number for each parameter or analyte was reconverted to original measurements before reporting.

Reference interval determination can be difficult in wild species because the stress and physiologic effects of handling can affect the results of laboratory tests. The turkeys in our group were acclimated over a 4 mo period; however, they were extremely excitable and restraint added to their agitation. Although excitability may have influenced our results, this would be the case in any situation of blood collection from wild turkeys, and therefore, may actually represent a norm for experimental studies.

The mean (±SD) packed cell volume (36.7%) for our poults was slightly less than those previously reported for 11-mo-old pen-reared (45.5 ± 3.1) or wild-caught (41.8 ± 4.0) eastern wild turkeys (Lisano and Kennamer, 1977). It is possible that the younger age of our poults was a factor in this difference or that the techniques for packed cell volume determination were different. One poult was eliminated from the data set because a septic joint was confirmed at necropsy and by culture. The total leukocyte counts are higher than expected. Although we examined each poult at necropsy, it could be possible, although unlikely, that an obscure underlying inflammatory disease could be a contributor to some of the higher heterophil counts. Chickens and domestic turkeys have a white blood cell distribution with lymphocytes as the most numerous leukocytes (Johnson and Lange, 1939; Zinkl, 1986). This also would be expected in wild turkeys and is confirmed by our data. Excitement can cause an epinephrine release that can result in lymphocytosis and sometimes neutrophilia in mammals (Duncan et al., 1994). This also has been observed in avian species (Campbell, 1995). Lymphocytosis attributable to stress-induced has been reported to occur in fowl (Shapiro and Schechtman, 1949). The number of basophils in our wild turkey samples was greater than anticipated. Early inflammation, hypersensitivity, and stress or nonspecific injury has been associated with a basophilia in poults that is not associated with corticosteroids (Campbell, 1995). Our findings emphasize the variability that presents with the experimental study of wild species and underscores the importance of complete analysis for evaluation of health status.

We established a reference interval for total protein as 3.6 to 5.5 gm/dl. Mean (±SD) total protein for 11-mo-old eastern wild turkeys has been reported as 4.7 ± 0.5 gm/dl for males and 4.6 ± 0.5 gm/dl for females (Lisano and Kennamer, 1977) and as an interval of 4.85 to 6.01 gm/dl (Martin et al., 1981). Our reference interval of 215 to 500 gm/dl is comparable to blood glucose values for 11-mo-old penned juvenile wild turkeys, which was reported as 375.5 ± 33.1 gm/dl in the winter months and 350.8 ± 30.6 gm/dl in the spring months (Lisano and Kennamer, 1977). The reference interval for cholesterol for our 4-mo-old wild turkeys is 60 to 220 mg/dl. Mean serum cholesterol concentration of 16-wk-old domestic turkeys has been reported as 249 mg/dl (Speckman and Ringer, 1962). The reference interval established for AST and LDH includes some values that would be interpreted as increased in other species. Increased concentrations of AST and LDH in our turkeys could occur with mild exertional myopathy or muscle damage; however, these turkeys had received no

previous intramuscular injections or venipuncture prior to blood collection that would contribute to activity of these enzymes (Lumeij, 1997). Some diversity could be attributable to diet or to the innate differences in domestic and wild poults. Variations in measurements of analytes also can occur as a result of different laboratory methodologies used for the assays.

We have used accepted statistical methods to establish reference intervals using current laboratory techniques and a large number of wild turkeys. The reference intervals for wild turkeys established in this study indicate that they are comparable to domestic turkeys.

This study was funded by a grant from National Wild Turkey Federation. We are also grateful to R. L. Brooks, Jr., J. Brown, M. Teglas, T. Cornish, J. Wlodkowski, C. Sewell, and B. Marchinton for their assistance. Financial assistance also was received via sponsorship from the fish and wildlife agencies of Alabama, Arkansas, Florida, Georgia, Kansas, Kentucky, Louisiana, Maryland, Mississippi, Missouri, North Carolina, Puerto Rico, South Carolina, Tennessee, Virginia, and West Virginia, [Federal Aid to Wildlife Restoration Act (50 Stat. 917)], and through Grant Agreement 1445-GT09-96-0002, U.S. Geological Survey, U.S. Department of the Interior.

LITERATURE CITED

BELL, D. J., W. M. MCINDOE, AND D. GROSS. 1957. Tissue components of the domestic fowl. 3. The non-protein nitrogen of plasma and erythrocytes. Biochemical Journal 71: 355–364.

———, AND P. D. STURKIE. 1965. Chemical constituents of blood. *In* Avian physiology, 2nd Edition. P. D. Sturkie (ed.). Comstock Publishing Associates, Ithaca, New York, pp. 32–84.

CAMPBELL, T. W. 1995, Avian hematology. *In* Avian hematology and cytology, T. W. Campbell (ed.). Iowa State University Press, Ames, Iowa, pp. 3–19.

DUNCAN, J. R., K. W. PRASSE, AND E. A. MAHAFFEY. 1994. Veterinary laboratory medicine, 3rd Edition. Iowa State Press, Ames, Iowa, 300 pp.

JAIN, N. C. 1986. Schalm's veterinary hematology, 4th Edition. Lea and Febiger, Philadelphia, Pennsylvania, 1221 pp.

JOHNSON, E. P., AND C. J. LANGE. 1939. Blood alterations in typhlohepatitis of turkeys, with notes on the disease. The Journal of Parasitology 25: 157–167.

KANEKO, J. J., J. W. HARVEY, AND M. L. BRUSS. 1997. Clinical biochemistry of domestic animals, Academic Press, San Diego, California, 932 pp.

KOEPKE, J. A. 1998. Introduction to quality control and quality assurance in hematology. *In* Clinical hematology, 2nd Edition. E. A. Stein-Martin, C. A. Lotspeich-Steinger, J. A. Koepke, (eds.). Lippincott-Raven Publishers, Philadelphia, Pennsylvania, pp. 563–578.

LISANO, M. E., AND J. E. KENNAMER. 1977. Values of several blood parameters in eastern wild turkeys. Poultry Science 56: 157–166.

LUMEIJ, J. T. 1997, Avian clinical biochemistry. *In* Clinical biochemistry of domestic animals, 5th Edition. J. J. Kaneko, J. W. Harvey, and M. L. Bruss (eds.). Academic Press, San Diego, California, pp. 857–879.

LUMSDEN, J. H. 1998. "Normal" or reference values: Questions and comments. Veterinary Clinical Pathology 27: 102–106.

MARTIN, R. M., M. E. LISANO, AND J. E. KINNEMAR. 1981. Plasma estrogens, total protein, and cholesterol in the female eastern wild turkey. The Journal of Wildlife Management 45: 798–802.

QUIST, C. F., D. I. BOUNOUS, J. V. KILBURN, V. F. NETTLES, AND R. D. WYATT. 2000. The effect of dietary aflatoxin on wild turkey poults. Journal of Wildlife Disease 36: In press.

RHIAN, M., W. O. WILSON, AND A. L. MOXON. 1944. Composition of blood of normal turkey. Poultry Science 23: 224–229.

SPECKMAN, E. W., AND R. K. RINGER. 1962. The influence of reserpine on plasma cholesterol, hemodynamics and arteriosclerotic lesions in the broad breasted bronze turkey. Poultry Science 41: 40–45.

SHAPIRO, A. B., AND A. M. SCHECHTMAN. 1949. Effect of adrenal corticol extract on the blood picture and serum proteins of fowl. Proceeding of the Society of Experimental Biology and Medicine 70: 440–445.

SOLBERG, H. E. 1996, Establishment and use of reference values. *In* Tietz fundamentals of clinical chemistry, 4th Edition. C. A. Burtis and E. R. Ashwood (eds.). W. B. Saunders Co., Philadelphia, Pennsylvania, pp. 182–191.

ZINKL, J. G. 1986. Avian hematology. *In* Schalm's veterinary hematology, 4th Edition. N. C. Jain (ed.). Lea and Febiger, Philadelphia, Pennsylvania, pp. 256–273.

Received for publication 19 February 1999.