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HEMATOLOGICAL AND BLOOD CHEMICAL VALUES OF MALLARD, *Anas p. platyrhynchos,* DRAKES BEFORE, DURING AND AFTER REMIGE MOULT

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Abstract: Hematological and blood chemical values were determined for wild and captive mallards, *Anas p. platyrhynchos,* for the late spring period prior to remige moult through early fall migration. Hemoglobin, packed cell volume, erythrocyte and total leukocyte counts, as well as the number of heterophils and lymphocytes, declined significantly during and after remige moult compared to values recorded prior to remige moult. During fall migration mallard drakes had elevated levels of glucose and uric acid. No significant changes were observed in total protein, alkaline phosphatase, glutamic oxaloacetic transaminase or creatine phosphokinase in pre- or post-moult periods. The hematological and biochemical values should prove useful in providing background information on mallard drakes, and documenting baseline data for intra and interspecies comparisons with diseased birds.

INTRODUCTION

A variety of environmental stressors, both external (temperature, light) and internal (bacterial organisms, parasites),[17] influence the homeostatic state of birds. This is even more evident when these factors are compounded by man-made stressors such as heavy metals[4] and breakdown compounds of insecticides.[8,9] Indices which can be used to monitor the physiological status of birds and their ability to cope with these stressors are useful tools for waterfowl managers requiring information on factors influencing the density of discrete populations.

Both hematological and biochemical blood values serve as indicators of the physiological state of birds. Changes in certain hematological and biochemical features may indicate either localized or systemic disease and/or the influence of toxic pollutants. To detect such influences, baseline data on healthy birds must be established for the annual cycle of each sex.

Hematological data exists for several species of waterfowl[1,3,5,10,11] but data are limited on seasonal patterns.[6,16,30] There is a paucity of seasonal biochemical data. Kocan[5] presented values on plasma protein fractions of fall diving ducks while Kocan and Pitts[6] provided information on plasma proteins, glucose and cholesterol for canvasback *(Aythya valisineria).*

This paper reports on the hematological and biochemical changes in captive and wild drake mallards, *Anas p. platyrhynchos,* observed from the nesting and post-nesting period to the start of fall migration.

MATERIALS AND METHODS

Adult male mallards, hatched in captivity from eggs collected in the wild, and maintained at the Delta Waterfowl Station, Delta, Manitoba, were the major sample component in this study. These birds were maintained on a high protein diet, a combination of turkey starter and wheat.[29] Twelve drakes were sampled for blood on at least three occasions between mid-June and late August, the usual period of moult for this species. Five wild drakes were collected by shooting in early June near Hanley, Saskatchewan,

and another 10 were sampled in mid-August at a bait trap site on the Quill Lakes, Saskatchewan.

Blood samples (2.5 ml) were taken from the cutaneous vein of the elbow with a heparinized syringe and injected into an EDTA treated glass tube.[1] Blood was extracted from the heart of each mallard collected near Hanley.

All samples, regardless of collection location, were drawn between 0600 and 0800 solar time to reduce physiological variability. To stabilize various enzyme systems the cellular portion of the blood was removed by centrifugation immediately upon collecting the sample.[27] All the plasma samples were clear, therefore chylomicremia was discounted as a factor in various analytical results.[27] The blood tubes were chilled in a freezer chest, containing three 500 ml freezettes for transport from the field to the laboratory.

Hematology

Erythrocytes (RBC) were counted manually in an improved Neubauer hemocytometer after the sample was diluted in Gower's solution. The leukocyte counts (WBC), in which the sample was treated with Turk's solution, included all cells within four 1 mm squares of the hemocytometer. Duplicate counts were made on most drakes sampled.

The packed cell volume (PCV) was determined after centrifuging blood in capillary tubes for 5 min in a micro-hematocrit centrifuge.

Calculation for the determination of mean cell size, hemoglobin content and concentration in erythrocytes followed Swenson (p. 32ff).[20] The morphometric determination of average RBC length and width were measured from selected blood smears throughout the sample period.

Differential counts were determined following methods described for avian blood.[7,31] The blood smears were prepared immediately upon collection, air dried, and later stained with Wright's solution, then buffered at pH 6.8 with Sorensen's solution.

Hemoglobin concentration in g/100 ml was determined by adding 20 μl of well-mixed whole blood to 5 ml of Drabkin's reagent. The stoppered cuvette was inverted until the sample was well mixed and then allowed to stand for 10 min to ensure completion of the reaction. After colour development the samples were centrifuged at 1800 rpm for 5 min. The supernate decanted and the optical density of the supernatant measured in a spectrophotometer set at 540 μm. The readings were compared with those on a standard curve prepared from hemoglobin standards.[27]

Plasma Biochemistry

Plasma was analysed for total protein,[25] glucose,[26] alkaline phosphatase,[24] serum glutamic oxaloacetic transaminase (SGOT),[22] creatine phosphokinase (CPK),[21] and uric acid[22] by the Clinical Chemistry Laboratory, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, using an Abbott ABA 100 system.[2]

Plasma protein fractions were electrophoretically separated on Beckman Electrophoresis membranes in Beckman B-2 barbitol buffer (pH 8.8, ionic strength 0.05) at 250 VDC for 30 min. The membranes were stained with Ponceau S, rinsed in 5% glacial acetic acid, soaked in 95% ethanol for 1 min and dried at 100 C for 15 min. The acetate strip was then read in an integrating Beckman densitometer.[3]

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[1] Vacutainer: Becton, Dickinson and Comp., 2464 South Sheridan Way, Mississauga, Ontario, L5J 2M8, Canada.

[2] Abbott Laboratories, Diagnostics Division, 820 Mission Street, South Pasadena, California 91103, USA.

[3] Beckman Instruments, 2500 Habour Blvd., Fullerton, California 92634, USA.

Analyses for calcium, inorganic phosphate, urea and lactic acid dehydrogenase[27] were conducted only on samples drawn from Hanley mallards.

Pattern of moult

Hematological and biochemical results were sorted according to five categories which were established for this study of moult in mallards. The stage within remige moult as well as pre- and post-remige moult was based on descriptions of plumage and moult outlined in Oring[12] and Palmer[13] in addition to field observations and measurements. The groupings used were: A. Definitive Alternate plumage (breeding plumage); B. Pre Basic moult; C. Remige moult (loss of primary and secondary flight feathers); D. Remige development (primary remiges less than 120 mm); and E. Definitive Basic plumage (birds capable of sustained flight).

Statistical analyses, analysis of variance and Student-Newman-Keutz[18] tests were conducted on the sorted data of wild trapped and captive drakes. Then the wild shot drakes were compared to the combined group. The tabular results are presented to provide information both as to collection site and to illustrate changes, if any, during moult.

RESULTS

Hematology. Significant differences were measured for Hb, PCV, total WBC count and WBC differentials during the period of remige moult (C), remige development (D) and the attainment of Definitive Basic plumage (E) (Table 1).

Hemoglobin and PCV were high prior to the remige moult, decline during this period and gradually increased. The values measured after the remige moult were lower ($P<0.05$) than those prior to moult. The total counts for cellular elements, erythrocytes and leukocytes, suggested a marked decline which begin during pre-basic moult for WBC and during the remige moult for RBC; both differed from the proceeding values ($P<0.001$). The pattern with the white blood cell differentials for heterophils, eosinophils and monocytes was essentially the same as the total WBC counts; whereas the lymphocyte numbers paralleled the total RBC counts, all elements differed ($P<0.001$) from the pre-moult values.

The morphology of erythrocytes did not vary significantly ($P>0.05$) either as to the stage of moult or to collection site (Table 2).

Plasma Biochemistry. Values obtained for glucose, uric acid, three enzymes, and protein fractions are summarized in Table 3. Blood from mallards collected at Hanley also was analysed for calcium (Ca), inorganic phosphorous (P), and lactic acid dehydrogenase (LDH); the mean values (± 1 standard error): Ca 4.6±0.4 mg/100 ml, P 1.6±0.2 mg/100 ml, urea 2.8±0.4 mg/100 ml, and LDH 537±68 μl.

Differences were found for glucose ($F = 3.66$, $P<0.025$), uric acid ($F = 3.47$, $P<0.05$), and alkaline phosphatase ($F = 7.44$, $P<0.001$). The uric acid and alkaline phosphatase values during and subsequent to remige moult were different from those measured prior to remige moult ($P<0.05$). Glucose values measured during the Definitive Basic plumage period (E) were greater than measurements proceeding this period ($P<0.05$). Within the plasma protein fractions, levels of albumin during the Definitive Basic plumage were 10-20% higher than the values recorded in the previous periods ($P<0.05$), whereas post-remige moult values of α-2 globulin were significantly lower than those recorded earlier ($P<0.05$).

No pre-albumin peak was detected in plasma from drakes in breeding plumage (A) or from samples drawn from fall migrants at Quill Lakes (Q).

Wild-bait trapped drakes were combined with data from captive mallard drakes by plumage class and stage of

TABLE 1. Hematological values of mallard drakes prior to, during and following remige moult. Means and standard errors are given for the three collection areas: Hanley (H, wild and shot), Delta (A-E captive) and Quill Lakes (Q, wild, bait capture).

	Pre-remige moult			Remige moult		Post-remige moult	
Parameter	H	A	B	C	D	E	Q
Number	2	7	5	1	5	7	5
Hemoglobin	17.5	16.7	15.1	16.6	12.0	13.4	13.8
(g/100 ml)	± 0.20	± 0.09	± 0.29		± 0.14	± 0.12	± 0.30
Packed cell volume	39.8	38.5	41.2	48.0	33.9	39.2	40.1
(%)	± 0.38	± 0.31	± 0.79		± 0.66	± 0.39	± 0.76
Erythrocytes	3.18	3.02	2.63	2.50	2.48	2.45	1.92
(x 10^6/mm^3)	± 0.03	± 0.02	± 0.05		± 0.07	± 0.03	± 0.03
MCV (μm^3)	156.6	127.5	156.7	192.0	136.7	160.0	208.9
	± 1.29	± 1.03	± 3.00		± 2.66	± 1.59	± 3.96
MCH (pg)	55.0	55.3	57.4	66.4	48.4	54.7	71.9
	± 0.6	± 0.3	± 1.1		± 0.6	± 0.5	± 1.6
MCHC (%)	35.1	43.4	36.7	34.6	35.4	34.2	34.4
	± 0.4	± 0.2	± 0.7		± 0.4	± 0.3	± 0.7
Leukocytes	—	26.47	8.03	8.28	8.42	7.19	5.33
(x 10^3/mm^3)		± 0.49	± 0.46		± 0.35	± 0.39	± 0.44
Heterophils	—	13.33	3.01	3.81	3.91	2.61	3.02
(x 10^3/mm^3)		± 0.49	± 0.17		± 0.16	± 0.22	± 0.39
Eosinophils	—	1.44	0.32	0.49	0.34	0.34	0.25
(x 10^3/mm^3)		± 0.66	± 0.05		± 0.02	± 0.04	± 0.02
Lymphocytes	—	10.42	4.36	3.64	3.69	4.02	1.97
(x 10^3/mm^3)		± 0.57	± 0.33		± 0.21	± 0.18	± 0.20
Monocytes	—	0.57	0.15	0.17	0.18	0.11	0.04
(x 10^3/mm^3)		± 0.04	± 0.02		± 0.03	± 0.01	± 0.01
Basophils	—	0.71	0.21	0.17	0.13	0.13	0.06
(x 10^3/mm^3)		± 0.08	± 0.04		± 0.02	± 0.02	± 0.01

TABLE 2. Morphometry of erythrocytes of mallards for Delta and Quill Lakes ($\bar{x} \pm$ standard error and range).

	Pre-remige moult		Remige moult	Post-remige moult	
Parameter	A	B	D	E	Q
Cell length	12.5± 0.20	12.9± 0.21	12.4± 0.20	13.2± 0.20	12.9± 0.14
(μ)	(11.2-13.6)	(12.0-13.6)	(12.0-13.6)	(12.0-14.4)	(12.0-13.6)
Cell width	6.8±0.17	7.1± 0.14	6.6± 0.12	6.2± 0.10	6.8± 0.13
(μ)	(6.4- 8.0)	(6.4- 8.0)	(6.4- 7.2)	(6.4± 7.2)	(6.4- 7.2)
Nuclear length	6.0± 0.13	6.1± 0.12	5.2± 0.13	5.6± 0.18	5.2± 0.13
(μ)	(5.6- 6.4)	(5.6- 6.4)	4.8- 5.6)	(4.8- 6.4)	(4.8- 5.6)
Nuclear width	2.7± 0.12	2.7± 0.12	2.6± 0.11	2.6± 0.11	2.5± 0.08
(μ)	(2.4- 3.2)	(2.4- 3.2)	(2.4- 3.2)	(2.4- 3.2)	(2.4±- 3.2)

moult; in comparisons to the wild-shot drakes the only difference from captive and trapped drakes occurred in the uric acid content ($P<0.05$). For bait-trapped and captive drakes assigned to the same plumage class, differences were detected for the following parameters: lymphocyte number, total protein, albumin, uric acid and alkaline phosphatase ($P<0.05$). These differences are attributed to a more phenologically advanced state for the Quill Lake sample, an aspect not fully accounted for within the classification. The differences were not considered sufficient to merit separation of captive from wild stocks.

DISCUSSION

Under ideal conditions, internal physiological changes associated with growth and reproduction, and behaviour mechanisms (e.g., territoriality) operate in concert thereby giving any internal component (e.g., packed cell volume) its normal range. If a component is considered at any point within the annual cycle, the net influence of these stressors on the internal component result in a discrete value within the normal range as either one of many peaks or troughs. Thus the variation measured in both hematological and plasma biochemical components for the mallard drake in the period from post-breeding through moult to early fall migration mirror the operation and/or effect of these mechanisms.

The hematological results of the mallard drakes are similar to other waterfowl,[3,16,30] and to the red-winged blackbird *(Agelaius phoeniceus)*.[15] Hemoglobin values parallel those of other dabbling and diving ducks;[1,3,10] the differences relate to season, species and/or technique. Seasonal similarities in RBC counts exist between Canada geese and mallard studied in North Dakota[16] and the mallards of this study. Although differential counts taken as a grand average are similar to earlier studies,[7,10] no comparable seasonal patterns have been reported.

TABLE 3. Plasma biochemical values for mallard drakes prior to, during and post moult (mean ± standard error), for three collection areas: Hanley (H), Delta (A-E) and Quill Lakes (Q).

	Pre-remige moult			Remige moult		Post-remige moult	
Parameter	H	A	B	C	D	E	Q
Number	4	8	9	2	8	7	10
Glucose (mg/100 ml)	196. ± 5.7	273 ± 3.9	208 ± 3.1	215 ± 7.8	230 ± 4.5	235 ± 2.8	304 ± 8.0
Alkaline phosphotase (μ/l)	59 ± 1.8	71 ± 3.7	71 ± 2.3	145 ± 7.0	85 ± 3.1	105 ± 9.5	181 ± 6.0
Glutamic oxaloacetic transaminase (μ/l)	208 ± 2.6	44 ± 2.7	49 ± 3.1	35 ± 5.7	28 ± 2.2	48 ± 7.8	84 ± 2.0
Creatine phosphokinase (μ/l)	—	675 ± 81	980 ± 96	557 ±156	372 ± 56	2212 ±316	1486 ± 91
Uric acid (mg/100 ml)	10.2 ± .5	6.9 ± 0.2	7.8 ± 0.2	6.3 ± 0.2	4.5 ± 0.2	5.5 ± 0.2	7.8 ± 0.2
Total protein (gm/100 ml)	4.20 ± 0.02	4.49 ± 0.09	4.61 ± 0.09	3.61 ± 0.07	4.16 ± 0.5	4.12 ± 0.07	3.56 ± 0.04
Pre Albumin (gm/100 ml)	—	0.063 ± 0.005	0.053 ± 0.002	0.063 ± 0.014	0.054 ± 0.003	0.037 ± 0.002	0.037 ± 0.002
Albumin (gm/100 ml)	—	2.135 ± 0.04	2.119 ± 0.04	1.882 ± 0.03	1.914 ± 0.03	2.076 ± 0.06	1.528 ± 0.04
α-1 Globulin (gm/100 ml)	—	0.368 ± 0.01	0.485 ± 0.01	0.350 ± 0.02	0.521 ± 0.02	0.451 ± 0.02	0.360 ± 0.04
α-2 Globulin (gm/100 ml)	—	0.188 ± 0.01	0.254 ± 0.04	0.191 ± 0.01	0.163 ± 0.03	0.123 ± 0.03	0.104 ± 0.01
β globulin & fibrinogen (gm/100 ml)	—	1.531 ± 0.17	1.407 ± 0.10	0.935 ± 0.07	1.289 ± 0.09	1.312 ± 0.12	1.329 ± 0.08
γ globulin (gm/100 ml)	—	0.261 ±0.04	0.291 ± 0.05	0.189 ± 0.04	0.223 ± 0.03	0.158 ± 0.01	0.206 ± 0.01

Ronald *et al.*[15] suggest that increased numbers of monocytes and lymphocytes signify increased fat synthesis coincident with fall migration. However, no substantial increase in these cellular components was detected in the mallards sampled (Table 1). Plasma glucose levels increased in both wild and captive mallards as fall migration approached. This is consistent with the result reported for migrating European robins *(Erithacus rubecula).*[2] The glucose circadian rhythm[2,14,28] was not examined in this study.

The decline in total protein and RBC's during remige moult, followed by the gradual increase after moult, suggests that some protein utilization was shifted to feather production. The changes in protein fractions appeared to be proportional to the change in total protein during this period.

Changes in plasma enzymes, especially SGOT, LDH, and CPK, relate to one of two causes. In the case of Hanley mallards the elevated levels of SGOT and LDH reflect a drastic physiological change which occurred when the birds were shot.[5] The high levels of CPK recorded for individual drakes both at Delta and the Quill Lakes relate to muscle damage as a result of capture and handling. Comparable levels of CPK have been found in cannon-netted geese that had severe myopathy similar to capture myopathy in mammals (G. Wobeser, 1980, pers. comm.).

Although the sample size in this study was limited, these data provide baseline information on the physiologic seasonal variation in chemical and hematological values as related to the moult period. Additional study is required to monitor the influences of harsh environment, varying food supply, man-made stressors (insecticides, drainage, etc.) and disease upon the biochemical and cellular components of blood in waterfowl.

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