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Source: Journal of Wildlife Diseases, 26(1) : 78-82

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-26.1.78

Journal of Wildlife Diseases, 26(1), 1990, pp. 78–82

DIFFERENTIAL WHITE BLOOD CELL VALUES OF THE MALLARD (*ANAS PLATYRHYNCHOS*) ACROSS DIFFERENT AGES AND REPRODUCTIVE STATES

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ABSTRACT: Differential white blood cell counts were recorded for adult mallards (*Anas platyrhynchos*) of both sexes during several stages of reproduction: pre-egg laying, egg laying, incubating, molting, and postreproductive. Similar counts were made for young birds from 5 to 60 days of age. No significant ($P \leq 0.05$) differences amongst the cell ratios due to sex or reproductive state of the adult birds were noted. Nonlaying and laying birds had similar numbers of thrombocytes which were significantly greater than thrombocyte numbers of incubating, molting or postreproductive birds. Young birds had a decrease in the percent lymphocytes from 50 to >60 days of age and a concomitant, compensating increase in percent heterophils. Thrombocyte numbers increased from 5 days of age to a peak at 18 days of age, after which they did not vary significantly.

Key words: Mallard, *Anas platyrhynchos*, hematology, leukocytes, white blood cells, differential, reproduction, experimental study.

INTRODUCTION

Hematological values are useful for detecting the effects of environmental, infectious, parasitic, or toxicologic stresses on animals. White blood cell counts in particular provide evidence of an immune response to an infectious or parasitic disease or an indication of toxicant-induced immunosuppression or stimulation. To utilize this information to its full extent in either controlled laboratory experimentation or in evaluating the health of wild-caught animals, normal values must be available for comparison.

Data on hematological values of the mallard (*Anas platyrhynchos*) are available to a limited extent in the literature. A review of duck hematology is presented by Hemm and Carlton (1967). Shave and Howard (1976) published hematologic values of a variety of captive waterfowl, including mallards, but did not investigate any possible differences due to season or reproductive state. Driver (1981) examined the influence of the remige molt on red blood cell counts and total and differential white blood cell counts in mallard drakes. The present study examined influences of reproductive state of mallards of both sexes and age of young birds on the ratios of white blood cell types.

MATERIALS AND METHODS

Blood was collected from 50 captive breeding pairs of mallards as previously described (Fairbrother et al., 1990). Two blood samples were drawn from each bird within each reproductive period. Reproductive condition was designated as pre-egg laying (if the birds were in breeding plumage but had not yet begun laying eggs), egg laying, incubating, molting, and postreproductive (when the molt was complete). Reproductive condition of the male was considered the same as that of the female with whom he was paired until he began the postreproductive molt.

Thin film blood smears were made and stained with Wright-Giemsa stain (Dein, 1984). Cells were observed under 100× magnification (oil-emersion) and classified as heterophil, lymphocyte, eosinophil, basophil, monocyte or thrombocyte according to criteria specified by Dein (1984). A total of 100 white blood cells were counted per slide to determine the percentage of the first five cell types. Thrombocytes were enumerated as the number per 10 high-power fields of view.

Due to small sample sizes, young birds between the ages of 50 and 59 days were grouped into "50 days" age class, and birds of 60 to 69 days were grouped as age "60 days." Prior to statistical analysis, data from the two samples collected from each bird within the same reproductive period were averaged to give one value per bird per reproductive period. Effect of sex and reproductive state on the relative ratios of heterophils, lymphocytes, eosinophils, basophils and monocytes of adult mallards was examined using the chi-squared (χ^2) statistic.

TABLE 1. Leukocyte percentages in adult mallards (*Anas platyrhynchos*) of different reproductive states ($\bar{x}$ ± SE).

Reproductive state*	*n*	Lymphocytes	Heterophils	Basophils	Monocytes	Eosinophils
Females						
PE	42	60 ± 1.4	35 ± 1.5	2.2 ± 0.2	2.2 ± 0.3	0.7 ± 0.15
EL	21	58 ± 3.0	37 ± 3.0	3.2 ± 0.5	1.8 ± 0.2	0.8 ± 0.30
INC	18	62 ± 1.5	33 ± 1.7	3.0 ± 0.4	1.9 ± 0.2	0.2 ± 0.07
MOLT	13	68 ± 2.1	28 ± 2.4	2.1 ± 0.5	1.8 ± 0.5	0.1 ± 0.08
PR	44	57 ± 1.6	37 ± 1.4	3.2 ± 0.3	3.2 ± 0.3	0.2 ± 0.06
Males						
PE	41	58 ± 1.8	36 ± 1.9	3.4 ± 0.4	1.9 ± 0.2	0.9 ± 0.18
EL	20	59 ± 3.0	36 ± 3.1	2.6 ± 0.4	1.9 ± 0.2	0.6 ± 0.18
INC	16	66 ± 1.4	29 ± 1.4	2.2 ± 0.3	2.5 ± 0.3	0.2 ± 0.17
MOLT	23	67 ± 1.9	27 ± 2.0	2.9 ± 0.4	2.9 ± 0.4	0.3 ± 0.10
PR	50	54 ± 1.6	38 ± 1.5	3.6 ± 0.3	3.6 ± 0.3	0.4 ± 0.10

* PE, Pre-egg laying; EL, Laying; INC, Incubating; MOLT, Molting; PR, Postreproductive. Males were classified in the same reproductive state as the female with whom they were paired until they began the postreproductive molt.

The "expected" values for the χ^2 analysis were generated using a maximum likelihood estimate because of the nonindependence of the cell percentages. A similar statistic was used to determine if age of the young bird affected the cell ratios. A $\log_{10}(N + 1)$ transformation was performed on thrombocyte numbers of adult birds to normalize the data prior to conducting a split plot analysis of variance (ANOVA) to determine if sex or reproductive state affected the number of cells. A one-way ANOVA was conducted on a square root transformation of number of thrombocytes in young birds to look for differences due to age. Significant ANOVA tests were followed by a Tukey's pair-wise comparison test to determine which means differed. Differences were considered significant at $P \leq 0.05$. All statistical analyses were conducted using the SAS statistics package for VAX mainframe computers (SAS, 1985). Values were reported as $\bar{x}$ ± one SE.

RESULTS

There were no significant differences in the relative ratios of heterophils, lymphocytes, eosinophils, basophils and monocytes due to sex or reproductive state (Table 1). Thrombocyte numbers were similar for both sexes but varied with reproductive state. Pre-egg laying and laying birds had similar numbers of thrombocytes which were significantly greater than thrombocyte numbers of incubating, molting or postreproductive birds (Fig. 1).

There was a significant interaction of age and cell type for heterophils, lymphocytes, eosinophils, basophils and monocytes. Birds aged 5 days to 42 days had the same relative ratios of cells with percent lymphocytes (73 ± 2%) higher than in adults (59 ± <1%) and percent heterophils (24 ± 3%) lower than in adults (34 ± <1%). Beginning between 42 and 50 days, the percent lymphocytes decreased to 40 ± 4% and percent heterophils increased to 50 ± 3% while the other cell types remained constant (Fig. 2). Therefore, between age 60 days and adult, percent lymphocytes increased again and heterophils decreased. Thrombocyte numbers were low in 5-day-old birds and increased until day 18 when they reached a plateau at a mean of 20 ± <1 cells/10 high-powered fields of view (Fig. 3). The small number of thrombocytes (9 ± 4) in birds 42 days of age may reflect the small sample size ($n = 2$); thrombocyte numbers among birds 18 to 60 days of age were not statistically different.

DISCUSSION

The ratio of leukocyte cell types observed in the adult mallards of the present study is similar to previous studies with ducks (Hemm and Carlton, 1967; Lucus

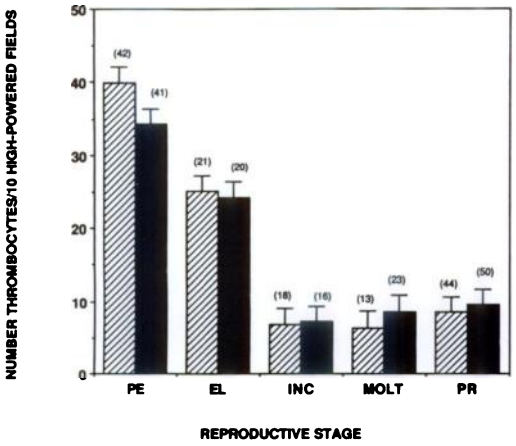

FIGURE 1. Mean (±SE) number of thrombocytes per 10 high-powered (100×) microscope fields from adult male (■) and female (▨) mallards of different reproductive states. PE, Pre-egg laying; EL, Laying; INC, Incubating; MOLT, Molting; PR, Postreproductive. Males were classified in the same reproductive state as the female with whom they were paired until they began the postreproductive molt. Numbers above the bars indicate sample sizes.

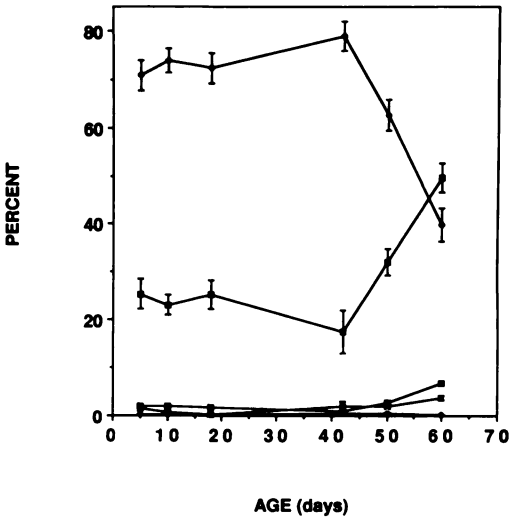

FIGURE 2. Leukocyte differential of young mallards. -⊡- basophils; -◆- eosinophils; -■- heterophils; -◇- lymphocytes; -x- monocytes.

and Jamroz, 1961; Magath and Higgins, 1934; Mulley, 1980), geese (Williams and Trainer, 1971), red-winged blackbirds (*Agelaius phoeniceus*) (Ronald et al., 1968), chickens (Olson, 1937; Sturkie, 1986), and Japanese quail (*Coturnix coturnix japonica*) (Nirmalan and Robinson, 1971). In general, adult birds have a preponderance of lymphocytes, averaging about 60 to 70% of the total white blood cell (WBC) count. Heterophils are the next most common, comprising about 35% of the total number of cells. In some birds this ratio is reversed with heterophils being the most prominent cell type. This reversal has been seen in Demoiselle cranes (*Anthropoides virgo*) (Conetta et al., 1974), ring-necked pheasants (*Phasianus colchicus*), and ostriches (*Struthio* sp.) (Sturkie, 1986). The Demoiselle crane was reported to have 57% heterophils, 39% lymphocytes and no eosinophils. Historically, differential leukocyte counts have been hampered by a lack of agreement on how to differentiate heterophils and eosinophils. Conetta et al. (1974) may have included eosinophils in their "heterophil" count. Some mammals (e.g., dog and cat) have a greater percentage of neutrophils (functional equivalent of heterophils) than lymphocytes while others (e.g., cow, sheep, goat and pig) are similar to the mallard and have a greater percentage of lymphocytes (Schalm et al., 1975). The significance of the differences in lymphocyte to heterophil ratio among various avian species is unknown.

Although the present study did not find any significant effects of reproductive state on the WBC ratios, Williams and Trainer (1971) observed a seasonal variation in cell types of blue geese (*Chen caerulescens*) that may have been related to breeding physiology. Nirmalan and Robinson (1971), in a study of reproductive Japanese quail, found that only eosinophils were affected by reproductive state, increasing two-fold during egg laying. Driver (1981) observed a decrease in percent heterophils during and immediately after the remige molt of mallards. Significant changes in cell ratios due to age similar to those observed in the present experiment were reported by Lucus and Jamroz (1961) for the mallard. Olson (1937) found no age differences in chickens while Nirmalan and Robinson (1971) reported a higher percent of het-

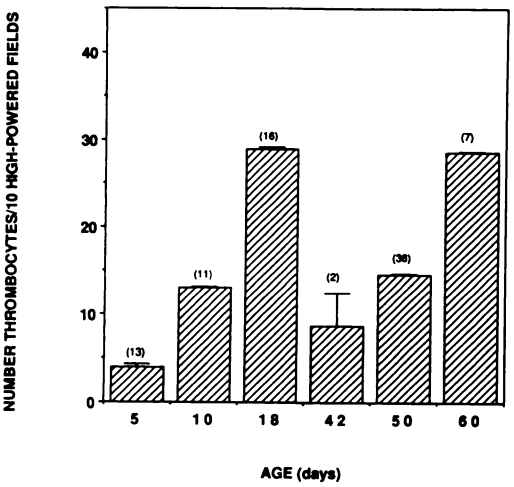

FIGURE 3. Mean (±SE) number of thrombocytes per 10 high-powered (100×) microscope fields from young mallards. Numbers above the bars indicate sample sizes.

erophils and lower percent of lymphocytes in young Japanese quail as compared to adults.

This study examined only the relative ratios of the different leukocyte cell types and did not measure the actual numbers of the cells. Subsequent studies in our laboratory have shown adult male mallards housed under similar conditions have an average total WBC count of 14,350 cells/mm^3. This is lower than the average of 23,400 cells/mm^3 from a literature survey conducted by Hemm and Carlton (1967) but within the reported range of 11,500 to 51,200 cells/mm^3. We achieved similar results from replicate counts done by the same observer using the direct method and the indirect phloxine-B method (Campbell and Coles, 1986; Dein, 1984). Other authors (Hemm and Carlton, 1967; Nirmalan and Robinson, 1971; Ronald et al., 1968) have reported an increase in total numbers of leukocytes with increasing age of birds. In the present study, thrombocyte numbers increased in young birds from 5 to 18 days of age and reached a plateau slightly higher than nonreproductive adult values. Probably the remaining leukocytes followed a similar pattern.

Total and differential leukocyte counts can provide valuable information in laboratory and field settings about the state of the immune system and whether birds have been exposed to infectious or immunotoxic agents (Campbell and Coles, 1986). The data from this study are based on a larger sample size than used in previously reported studies. These data provide baseline information from normal, healthy birds kept under ideal conditions to which other measurements can be compared when determining the effects of natural or anthropogenic biotic and abiotic stressors on waterfowl health.

ACKNOWLEDGMENTS

We thank M. Fix for technical assistance, D. Zirkle for statistical consultation, and S. Dominguez for his helpful review of the manuscript. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. Environmental Protection Agency.

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Received for publication 28 April 1989.