Research Notes

Comparison of Plasma Uric Acid Levels in Five Varieties of the Domestic Turkey, *Meleagris gallopavo*


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**ABSTRACT** Plasma uric acid (PUA) is a consensus physiological biomarker for many phenotypes in vertebrates because it is a reliable indicator for processes such as oxidative stress and tubular function. In birds, it is considered a major antioxidant and is also the primary endproduct of nitrogen metabolism. Despite this importance, knowledge of baseline levels of PUA in physiologically normal birds, including the turkey, *Meleagris gallopavo*, is limited. Here, we compared PUA levels in a total of 106 apparently normal male and female birds at 8 and 32 wk of age from 5 strains of the domestic turkey, including Bourbon Red, Narragansett, Blue Slate, Royal Palm, and Spanish Black. Though differences in PUA were not significant at 8 and 32 wk of age, BW, variety, and sex effects were highly significant. When adjusted for BW, female birds had, on average, a higher PUA per kilogram of BW than male birds. When adjusted for both sex and BW, Royal Palm birds had the lowest average PUA, and Blue Slate had the highest PUA. Results of these investigations represent the first comparative analysis of PUA in physiologically normal turkey varieties. They suggest that differences in basal plasma levels of uric acid in physiologically normal turkeys are influenced by sex, weight, and genetic background but may be independent of age.

**Key words**: plasma uric acid, turkey, body weight, age, sex

2006 Poultry Science 85:1791–1794

**INTRODUCTION**

The *American Standard of Perfection* (American Poultry Association, 2001) describes the 8 known varieties of the domestic turkey, *Meleagris gallopavo*, as a single breed. Knowledge of the physiological and biochemical differences and similarities of these varieties, however, remains very limited. This paucity in our understanding of these turkey varieties, including Royal Palm (RP), Bourbon Red (BR), Blue Slate (BS), Spanish Black (SB), Narragansett (NG), Broad-Breasted Bronze, and the White Holland, is probably due to the research emphasis by both academic and industry scientists on commercial turkeys, which are a hybrid of one or more birds.

Many genetic, morphological, and physiological studies have been conducted on commercial turkeys, making them one of the most understood poultry species. However, noncommercial or heritage turkeys, though their appeal as a meat source continues to increase (Shriver, 2003), have been studied little. Recent efforts initiated by the American Livestock Breeds Conservancy seek to increase our understanding of the relatedness among noncommercial domesticated turkeys. For example, Smith et al. (2005) used molecular marker systems to show that the RP strain was genetically much less related to the BR, NG, BS, and SP strains. These genetic studies provide a foundation for further analysis of morphological and physiological differences, including plasma uric acid (PUA), among these varieties.

Uric acid, in addition to being a waste product, is considered a biomarker for many physiological characteristics in vertebrates, including birds, because it cannot be broken down in vivo. It is considered an important antioxidant (Hare and Johnson, 2003), as PUA has been shown to scavenge free radicals (Whiteman and Halliwell, 1996), chelate transition metals (Rowley and Halliwell, 1985), and block peroxynitrite, a toxic product of free radical reaction with nitric oxide (Whiteman and Halliwell, 1996). Recently, for example, uric acid was shown to protect mice against secondary damage following spinal cord injury by blocking the toxic effects on primary spinal neurons of peroxynitrite (Scott et al., 2005). Because PUA is considered an antioxidant, 1 physiological trait which it has been implicated to affect is oxidative stress (Cutler, 1984). This implication is based on studies showing that, in vitro, PUA protects against oxygen-mediated damage (Ames et al., 1981), inhibits lipid peroxidation (Smith and Lawing, 1983), affects nitrite oxidation of hemoglobin (Smith and Nunn, 1984), and reduces hyaluronic acid degradation (Liu et al., 1984). The role of PUA as an antioxidant is further supported by reports that it can bind some metallic ions.
to form complexes that inhibit oxidations (Rowley and Halliwell, 1985).

Previous investigations involved the chicken and turkey evaluated PUA levels, thus, antioxidant status, in response to dietary supplements in commercial birds (Simoyi et al., 2003). Lacking, however, are strain comparisons for PUA of turkeys on a standard diet. This need for the establishment of reference PUA levels in noncommercial domesticated turkeys is further supported by a recent observation (Gyenai, 2006) that 5 noncommercial turkey strains respond differently to toxin-induced dilated cardiomyopathy, an abnormality believed to be influenced by oxidative stress (Yucel et al., 1998). In the present work, we compared PUA levels in the following varieties of noncommercial domestic turkeys: BR, BS, NG, RP, and SB. Additional objectives included the evaluation of the effect of age and sex on PUA concentration in the 5 turkey varieties.

**MATERIALS AND METHODS**

**Birds and Sample Collection**

A total of 106 day-old birds from BR, NG, RP, SB, and BS turkey varieties were obtained from Privett Hatcheries (Las Cruces, NM). The phylogenetic relatedness of these varieties has recently been described (Smith et al., 2005). Briefly, using 3 molecular marker systems, including microsatellites and single nucleotide polymorphisms, the RP strain was shown to be less closely related to the BR, BS, NG, and SB varieties. The birds in the present work were raised together from hatch using standard methods and according to the guidelines of Virginia Tech’s Institutional Animal Care and Use Committee. Birds were fed ad libitum standard marsh diets with protein and carbohydrate contents adjusted according to age. Water was also provided ad libitum to all birds.

Blood was collected from each bird at 8 and 32 wk of age (WOA) by brachial venipuncture into tubes containing EDTA as anticoagulant. Birds at 8 WOA were fed a standard diet containing 22% CP. At 32 WOA, the birds were sexually mature and were on a standard diet containing 14% CP. At the time of blood collection, each bird was weighed using a standard scale.

**PUA Assay**

Blood samples were either processed immediately for use in the PUA assay or after overnight storage at 4°C. Plasma was obtained by centrifuging the blood samples at approximately 2,000 x g for 15 min at 4°C, followed by storage at −20°C until ready for use. Duplicate plasma samples were assayed for PUA using an optimized commercial enzymatic assay (Diazyme Laboratories, San Diego, CA), which was based on the following coupled reactions

\[
\text{uricase} \\
\text{uric acid + O}_2 + 2\text{H}_2\text{O} \rightarrow \text{allantoin + CO}_2 + \text{H}_2\text{O}_2
\]

where ESPAS = N-ethyl-N-sulfopropyl-m-anisidine.

\[
2\text{H}_2\text{O}_2 + \text{ESPAS + 4-aminopyrene} \rightarrow \text{quinine dye + 4H}_2\text{O}
\]

The quinine dye was monitored by a spectrophotometer at 600 nm. Following optimization, the manufacturer’s recommended protocol was modified by doubling the reagents in all the PUA assays in the present work. The modification reduced the SE from 25 to 14% of repeated measurements on the same birds. The decrease in SE was not justified by the additional cost for reagents when the volumes were increased above twice that recommended by the manufacturer.

**Statistical Analysis**

The data were analyzed using the GLM procedure of SAS (SAS Institute, 2002). Variety, sex, and age were included in the analyses as fixed factors. Interactions were also included in the analyses. Both the Student’s t-test and Duncan’s multiple range tests were used to evaluate sex differences and strain differences. Additionally, the regression option was used to adjust data following the ANOVA test of significance within each strain. Data adjusted for BW were also compared between sexes and among varieties. Comparisons were considered significant at \(P < 0.05\).

**RESULTS AND DISCUSSION**

The unadjusted PUA concentrations at 8 and 32 WOA are presented in Table 1. Though significant only for SB females, the concentration of PUA in birds at 32 WOA was higher in both males and females and in all the 5 strains. Except for NG birds, PUA levels in males were higher at both ages. Additionally, strain differences were significant at both ages, with significantly lower \(P < 0.05\) PUA concentration in the RP birds. Differences among the strains for PUA concentration persisted even after adjusting the data for BW and sex using regression coefficients. The RP strain had the lowest and the SB strain had the highest PUA concentration at 32 WOA (Figure 1). The differences among BR, BS, and NG birds were not significant and were at least 10% lower and higher than the SB and RP birds, respectively. When adjusted for BW, females had a higher concentration of PUA in all the strains, though the difference between males and females was significant only in NG, RP, and SB strains (Figure 2).

Differences between male and female birds observed here appear to be inconsistent with a previous report by Culleton et al. (1999) that found that, in humans, males had a 33% higher concentration of PUA than females. Because our unadjusted data are consistent with this report, it is probable that the differences observed in humans are a result of differences in BW between age-matched males and females.

Among the surprises in the current work was the lack of a consistent significant difference between birds at 8 and 32 WOA in PUA concentration. Earlier work in wild birds (Okumura and Tasaki, 1969; Lumeij and Bruijne,
Table 1. Summary of the average plasma uric acid (PUA) level found using different individual birds for each breed and gender

<table>
<thead>
<tr>
<th>Breed</th>
<th>Sex/n1</th>
<th>8 WOA</th>
<th>32 WOA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bourbon Red</td>
<td>Male/16</td>
<td>4.63 ± 0.85&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;,c&lt;/sup&gt;</td>
<td>4.69 ± 0.57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Female/16</td>
<td>3.35 ± 1.20&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;,b&lt;/sup&gt;</td>
<td>4.17 ± 0.60&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Narragansett</td>
<td>Male/6</td>
<td>3.87 ± 1.44&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;,AB&lt;/sup&gt;</td>
<td>3.94 ± 0.64&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;,AB&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Female/16</td>
<td>4.23 ± 1.67&lt;sup&gt;c&lt;/sup&gt;&lt;sup&gt;,C&lt;/sup&gt;</td>
<td>4.63 ± 0.57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Royal Palm</td>
<td>Male/8</td>
<td>3.49 ± 1.05&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;,AB&lt;/sup&gt;</td>
<td>3.89 ± 0.47&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;,AB&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Female/13</td>
<td>3.18 ± 1.61&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;,A&lt;/sup&gt;</td>
<td>3.34 ± 0.74&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;,A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spanish Black</td>
<td>Male/10</td>
<td>5.01 ± 1.37&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;,D&lt;/sup&gt;</td>
<td>5.41 ± 0.47&lt;sup&gt;c&lt;/sup&gt;&lt;sup&gt;,C&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Female/10</td>
<td>3.88 ± 1.01&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;,B&lt;/sup&gt;</td>
<td>5.21 ± 0.54&lt;sup&gt;c&lt;/sup&gt;&lt;sup&gt;,C&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blue Slate</td>
<td>Male/8</td>
<td>5.02 ± 0.68&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;,D&lt;/sup&gt;</td>
<td>5.32 ± 0.58&lt;sup&gt;c&lt;/sup&gt;&lt;sup&gt;,C&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Female/8</td>
<td>2.73 ± 1.40&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;,A&lt;/sup&gt;</td>
<td>3.13 ± 0.54&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;,A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means with the same superscript within a row are not significantly different (P > 0.05).

<sup>A–D</sup>Means with the same superscript within a column are not significantly different (P > 0.05).

<sup>1</sup>Where n = the number of birds analyzed from each sex.

<sup>2</sup>Values are means ± SEM of PUA. WOA = weeks of age.

1985) indicated that a change in diet from younger to older birds, primarily in CP content, as was done here, results in a significant change in uric acid. Because birds are uricotelic, the lack of change in PUA in older birds may be due to negligible changes in structural proteins, which is normally observed when diets of animals are changed from high-protein and low-energy feed to low-protein and high-energy feed (Singer, 2003).

Knowledge of the base concentration of PUA in physiologically normal turkeys provides a foundation for establishing its association, if any, with abnormal or disease conditions. In humans, for example, PUA or serum uric acid level has been reported to have a positive relationship with cardiovascular diseases and mortality (Fang and Alderman, 2000). Though not a consensus, there is evidence to support a strong positive association between uric acid level and increased risk for cardiovascular disease. As a model for human dilated cardiomyopathy (Genao et al., 1996), the reference values described here for different turkey varieties may be useful in further defining the etiology of cardiovascular diseases, which are influenced significantly by oxidative stress.

Another phenotype for which PUA could be an important biomarker in birds is longevity or, more specifically, the relationship between longevity and oxidative stress, the imbalance between free radicals and antioxidants. In birds, the lack of uricase makes PUA of particular interest, as it is believed to function directly as an antioxidant (Machin et al., 2004). It is known that birds generally have several-fold higher levels of PUA than mammals, including humans (De Boeck and Stockx, 1978). This high level increases the likelihood that PUA will act as an antioxidant in vivo to reduce the effects of the products of oxidation, including free radicals. This capability will likely also lead to beneficial consequences, including increased longevity, as has been observed in some birds (Austad, 1997). The relatively long life span of some birds, despite a higher metabolic rate, when compared with mammals of proportionate size, has led to an interest in using birds as models for aging (Holmes and Ottinger, 2003). One hypothesis as to the reason for this longevity is their ability to deal with reactive oxygen species, molecules in the body that cause oxidative stress. Birds may lessen the threat of reactive oxygen species by having a higher level of antioxidants present. Therefore, levels of uric acid may show a correlation with life span.

In birds, Simoyi et al. (2003) previously suggested that allantoin, the product of PUA oxidation, is a useful mea-
ure of oxidative stress status. This appears to be consistent with earlier investigations that showed allantoin to be a valuable endogenous marker for the status of oxidative stress (Hicks et al., 1993). In the Simoyi et al. (2003) studies, 4-wk-old commercial turkeys on inosine-supplemented diets showed marginal changes in PUA relative to the levels in control birds and chickens on the similar diet. The higher PUA status, in chickens of age and weight similar to commercial turkeys, was attributed to the higher requirement for antioxidants for the faster-growing chickens. This may explain the lack of differences between turkeys at the 2 ages evaluated in the present work. Consistent with the present work, Simoyi and Klandorf (2003) reported a small increase in PUA concentration in 10-wk-old commercial turkeys above the average level observed in 8-wk-old birds on a standard nonfructose diet.

In the present work, we have described comparative values for a physiologically important biomarker in varieties of turkeys that remain little studied. Our understanding of these varieties provides an opportunity to improve their performance as well as to use these varieties for improvement of the commercial turkey. Knowledge of the physiologically normal values of PUA in these varieties could be useful in evaluating the relationship between oxidative stress and cardiovascular diseases and longevity, links that continue to be tenuous.

ACKNOWLEDGMENTS

We thank the staff of the Virginia Tech Turkey Farm for help with the birds. Help from H. Wang, Department of Food Science and Technology, Virginia Tech, with the PUA assay is also gratefully acknowledged. Partial funding for this project was provided by the Virginia Agriculture Council. The work was in partial fulfillment of the Virginia Tech Honors in Biochemistry program by Stefanie Hartman.

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