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Accelerated tissue aging and increased oxidative stress in broiler chickens fed allopurinol☆

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Abstract

Uric acid has been hypothesized as being one of the more important antioxidants in limiting the accumulation of glycosylated endproducts in birds. Study 1 was designed to quantitatively manipulate the plasma concentrations of uric acid using hemin and allopurinol while study 2 determined their effects on skin pentosidine, the shear force value of *Pectoralis major* muscle, plasma glucose, body weight and chemiluminescence monitored oxidative stress in broiler chickens. Hemin was hypothesized to raise uric acid concentrations thereby lowering oxidative stress whereas allopurinol was hypothesized to lower uric acid concentrations and raise measures of oxidative stress. In study 1 feeding allopurinol (10 mg/kg body weight) to 8-week-old broiler chicks ($n = 50$) for 10 days decreased plasma uric acid by 57%. However, hemin (10 mg/kg body weight) increased uric acid concentrations 20%. In study 2, 12-week-old broiler chicks ($n = 90$) were randomly assigned to either an ad libitum (AL) diet or a diet restricted (DR) group. Each group was further divided into three treatments (control, allopurinol or hemin fed). Unexpectedly, hemin did not significantly effect uric acid concentrations but increased ($P < 0.05$) measures of chemiluminescence dependent oxidative stress in both the DR and AL birds probably due to the ability of iron to generate oxygen radicals. Allopurinol lowered concentrations of uric acid and increased ($P < 0.05$) the oxidative stress in the AL birds at week 22, reduced ($P < 0.05$) body weight in both the AL and DR fed birds at 16 and 22 weeks of age, and markedly increased ($P < 0.001$) shear force values of the *pectoralis major* muscle. Skin pentosidine levels increased ($P < 0.05$) in AL birds fed allopurinol or hemin fed birds, but not in the diet restricted birds at 22 weeks. The significance of these studies is that concentrations of plasma uric acid can be related to measures of oxidative stress, which can be linked to tissue aging.

Keywords: Uric acid; Allopurinol; Skin pentosidine; Oxidative stress; Broiler chickens

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1. Introduction

Most avian species live longer than mammals of comparable body size (Lindstedt and Calde, 1976). The maximum longevity of birds is known to range from 4 years for blue jays (*Cyanocitta cristata*) to 64 years for Macaws (*Ara macao*). The longevity of birds is somewhat surprising, since they exhibit many traits that should render them susceptible to the degenerative processes of aging. These traits have been reviewed (Holmes and Austad, 1995) and include: metabolic rates as much as two to two and a half times greater than those of mammals of similar body size, (Lindstedt and Calde, 1976; Beuchat and Chong, 1997) blood sugar levels typically two to six times higher and body temperatures approximately 3°C higher than mammals (Ku and Sohal, 1993). Each of these factors should increase the rate of oxygen free radical production and accelerate the formation of Maillard products. Without unique protective mechanisms against the potential for oxidative damage, birds would be short lived and age more rapidly than mammals. However, avian species have higher levels of circulating antioxidants (tocopherols, carotenoids and uric acids) compared to comparably sized mammals (Schweiger et al., 1991; Ku and Sohal, 1993; Shapiro et al., 1997). Apparently birds have evolved mechanisms to limit the damage caused by the production of increased amounts of oxygen free radicals.

Uric acid is a circulating antioxidant that is ubiquitous and demonstrates a positive correlation with maximum life span across species (Ames et al., 1981; Cutler, 1984a,b). It is a potent scavenger of free radicals in humans and many animals (Parmley et al., 1992; Hellsten et al., 1997). Humans, the longest living primates, have comparatively high levels of uric acid because they lack uricase, the terminal degradative enzyme present in monkeys and other mammals (Ames et al., 1981; Schreiber et al., 1986). The lower levels of uric acid in macaques correlate with their shorter life span as compared with humans (Short et al., 1997).

It has been demonstrated in vitro (Becker, 1993; Hellsten et al., 1997) that urate has the ability to scavenge peroxides, hydroxyl radical species and hypochlorus acid. Following the reperfusion injury that occurs after a myocardial infarction, uric acid concentrations increase in plasma. Whether this represents a compensatory response remains unclear (Parmley et al., 1992). It has been proposed that lower tissue concentrations of the glycoxidation product, pentosidine, in birds as compared with mammals, are due to a more efficacious avian antioxidant system (Iqbal et al., 1999a,b) which includes a plasma concentration of uric acid approximately twofold greater than that in humans (Schweiger et al., 1991; Bishop et al., 1992). Skin pentosidine (Ps) is a tissue crosslink that forms within the matrix of a protein (Sell et al., 1992) and is linked to an increased rate of aging in birds (Iqbal et al., 1999a,b).

In the current study, altered uric acid levels are linked with measures of oxidative injury. The specific objectives were to determine the effects of allopurinol and hemin on uric acid levels in broiler chickens and on the accumulation of Ps and shear values (SV) of *pectoralis major* muscle. Allopurinol is a structural analogue of the natural purine base, hypoxanthine and it, and its metabolite oxypurinol, are potent inhibitors of xanthine oxidase, the enzyme involved in the conversion of xanthine to uric acid and thereby lower plasma uric acid levels. Allopurinol is thus hypothesized to enhance measures of oxidative injury whereas hemin, which increases the plasma concentrations of uric acid (Miller et al., 1993) is hypothesized to lower measures of oxidative injury.

2. Materials and methods

2.1. Birds and management

The purpose of the first experiment was to define the dose of allopurinol and hemin required to manipulate concentrations of plasma uric acid in broiler chicks. Broiler chicks ($n = 60$; Ross × Ross, mixed sex) approximately 6 weeks of age were obtained from Ross Breeders and maintained under standard husbandry practices. These included recommended brooders and temperatures, bell drinkers and pan feeders. Specifications for space, temperature, light and husbandry was adhered to (Ross Breeders, 1996). At eight weeks of age, 50 broilers were divided into five groups: control, allopurinol fed (5 mg/kg and 10 mg/kg body wt.) and hemin fed (5 mg/kg and 10 mg/kg body wt.). Blood was sampled for plasma uric acid measurements at day 3 and 10 of the experimental diet.

Table 1
The amount of feed (g/day) provided on a daily individual basis for the diet restricted (DR) groups[a]

Age (weeks)	Ad libitum (AL)	Diet restricted (DR)
12	203	130
13	232	150
14	220	160
15	208	165
16	211	165
17	208	165
18	201	165
19	208	165
20	202	165
21	201	165
22	206	165

[a]The average daily food intake of the ad libitum fed birds is also presented.

The principle experiment involved birds of 12 weeks of age. The remaining birds were divided into two main treatment groups, a diet restricted (DR) group and a group with free access to food (AL) with three sub-treatments within each group (control, allopurinol fed and hemin fed; 2×3 factorial) (Table 1). The DR birds were fed a limited allowance diet (Ross Breeders, 1996). Allopurinol (10 mg/kg) and hemin (10 mg/kg) supplemented feeds were prepared every week based on the weight of the bird the previous week. Mortality figures for each group were determined at the end of the study. Birds were killed after 4 and 10 weeks of consuming their respective diets.

2.2. Electron spin resonance (ESR) measurement

ESR spin trapping was used in vitro to determine the possible contribution of allopurinol and oxypurinol to antioxidant activity in the treated animals by measuring short-lived free radical intermediates (Shi et al., 1997). This technique involves the interaction of a short lived oxygen free radical with a diamagnetic compound (spin trap) to form a relatively long-lived free radical product, which can be detected by ESR. The intensity of the adduct signals corresponds to the amount of short-lived oxygen free radicals that can form an adduct with the spin trap. Reactants are mixed in a test tube with a total final volume of 0.50 ml and are then transferred to a flat cell for the ESR measurements. All ESR measurements are recorded using a Varian E4 spectrometer and a flat cell assembly.

Hyperfine splitting was measured to 0.1 G directly from magnetic field separations using potassium tetraperoxochromate (K_3CrO_8) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) as the standards. The relative radical concentration was estimated by multiplying half of the peak height by (ΔH_{pp}) (Shi et al., 1997). ΔH_{pp} represents peak to peak width.

2.3. Pentosidine (Ps) determination

Birds ($n = 5$) from the second study were randomly selected from each dietary group at 16 and 22 weeks of age and killed by electrical stunning. Approximately 1 g of skin was removed from the abdominal area, washed with normal saline and stored at -80°C for analysis. A collagen digest was prepared as previously described (Monnier et al., 1986; Monnier, 1990). Briefly, this technique involves skin preparation (removal of the epidermis and adipose layers and very fine mincing), delipidation in a chloroform-methanol mixture (2:1), and rehydration in 50% methanol followed by hydrolysis in 6 mol/l HCl at 110°C for 18 h under nitrogen. The samples were placed in a Speed Vac Centrifuge and the vacuum desiccated samples were reconstituted with 250 μl dd H_2O and filtered using a Costar® Spin-X® centrifuge tube filter. Collagen content is estimated by the modified Stagmen and Stalder method using a hydroxyproline standard. This method assumes that hydroxyproline makes up 14% of the total collagen (Maekawa et al., 1970). The measurement of Ps was accomplished by reverse phase HPLC (Iqbal et al., 1997). One milligram of skin collagen digest in 100 μl water/0.01 mol/l heptaflurobutyric acid (HFBA) is injected into a 0.46 × 25 cm Vydac 218TP104 (10 μm) C-8 column connected to a Waters HPLC. The apparatus consisted of two pumps (Waters™ 600 Controller), an auto sampler (Waters™ 717), and a scanning florescence detector (Waters™ 474). A linear gradient of 12–42% acetonitrile from 0 to 25 min in water and 0.01 M HFBA at flow rate of 1 ml/min achieve separations. An on line scanning fluorescence detector at an excitation wavelength of 325 nm/emission wavelength 370 nm detects the Ps peak. Quantification of Ps was made by comparison of peak areas with a Ps standard injected under identical conditions. A software package (Millennium 2.1) was used for peak integration.

2.4. Cooking time and shear value evaluation

Birds were electrically stunned and bled using a modified Kosher technique (Kenney et al., 1996; Iqbal et al., 1999b). The *pectoralis major* muscle was isolated, refrigerated at 4°C for 4 h, vacuum packed, and stored at −20°C until further processed. The breast muscle was weighed and then cooked to an internal temperature of 70°C on a Farberware Smokeless Indoor Grill. The endpoint internal temperature was monitored with an industrial data logger, equipped with a copper-constant thermocouple. Cooked muscle was cooled to room temperature and refrigerated overnight at 4°C. Slices of approximately 1.2–1.3 cm were cut perpendicular to the fiber orientation of the muscle. From each sample, four to five cores were removed from the thickest portion of the cooked muscle. Shear values (SV) were determined by using an Instron Universal Mechanical Machine. A Warner-Bratzler Apparatus was attached to a 50-kg load cell and tests were performed at a cross head speed of 127 mm/min. Output from a LVDT conditioner was acquired by a computer equipped with a DT 2805 data acquisition board. Signals were processed with the HP-VEE software package.

2.5. Uric acid and glucose determination

Plasma uric acid ($n = 5$ per treatment group) was determined using a commercially available uric acid reagent (Sigma Chemicals). Plasma glucose was measured using an YSI 2700 Select Biochemistry analyzer.

2.6. Luminol-based chemiluminescence (LBCL) as a measurement of oxidative stress

Chemiluminescence techniques are functional assays to quantify the release of oxidants from cells or tissues (Van Dyke, 1987; Radi et al., 1993). Luminol-based chemiluminescence (LBCL) oxidative stress was used to define the amount of oxidation stress as described by Iqbal et al. (1999a). One milliliter of blood from 16-and 22-week-old birds ($n = 5$) was carefully layered on 5 ml in mono-polyresolving medium in a 13 × 100 mm # 10 Falcon tube (2027) (ICN 16-980-49) and leukocytes were isolated by centrifugation at 300 × g for 5 min.

The total number of leukocytes was counted using a routine hemocytometric technique. To a 3-ml luminometer tube was then added 100 μl of leukocytes (10^9/l), 100 μl luminol (10^{-5} M) solution, 200 μl phosphate buffered saline (pH 7.4) and 100 μl phorbol myristate acetate (PMA). Luminol reacts with hypochlorite with the production of photons. The reaction tube is placed in a luminometer with the temperature control set at 37°C. Oxidative stress is determined by measuring the integrated luminescence generated over 20 min using KINB software — Berthold luminometer. Results are reported as Bq (Becquerels). Measured luminescence is corrected for each group based on the number of leukocytes present in each reaction vessel.

2.7. Statistical analysis

The experimental treatments were arranged as a two by three factorial. The main effects were feeding method (ad libitum or restricted) and feed additive (none, or hemin or allopurinol. Data were analyzed using the general liner models procedure of SAS (version 6.04) (SAS, 1990). In the event of a significant F-value ($P < 0.05$), multiple comparisons were made using the Student–Neuman–Keuhls procedure.

3. Results

3.1. Study 1

There was a dose dependent reduction in concentrations of uric acid in allopurinol treated chicks. Compared to controls, plasma uric acid was lowered 57% ($P < 0.05$) in allopurinol (10 mg/kg body wt.) treated chickens (data not shown). In contrast, the uric acid concentration was 20% greater ($P < 0.05$) at d 10 in the hemin fed chickens (10 mg/kg body wt.) but was not significant for birds fed the lower dose. No significant effects of either treatment on uric acid levels were measured at day 3. Fig. 1a shows the effect of H_2O_2 on the $^{\cdot}OH$ (hydroxyl free radical) generation from a reaction mixture recorded 3 min after reaction initiation from a pH of 7.4 phosphate buffer containing 1 mM DMPO, 0.1 mM H_2O_2 and 0.02 mM Fe (II). The intensity of

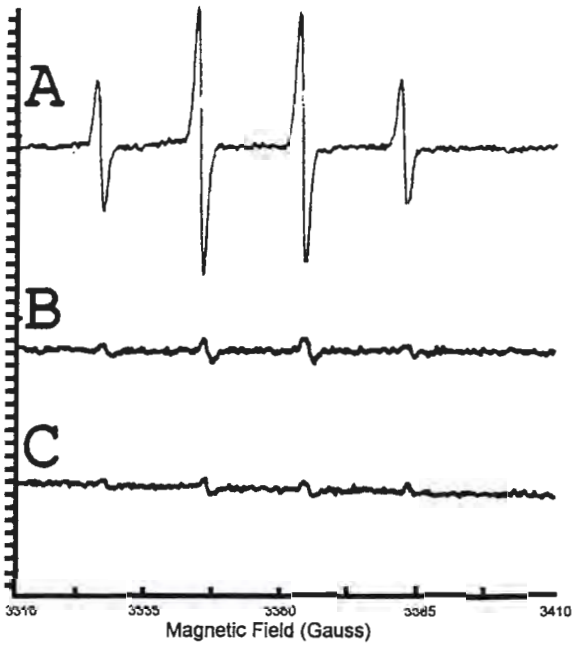

Fig. 1. The effect of H_2O_2 on the hydroxyl radical generation. (a) Electron spin resonance (ESR) spectrum recorded 3 min after reaction initiation from a pH of 7.4 phosphate buffer containing 1 mM DMPO, 0.1 mM H_2O_2 and 0.02 mM Fe (II). (b) Same as (a), but with saturated allopurinol. (c) Same as (a), but with saturated oxypurinol.

the ·H generation was decreased ninefold when a saturated solution of allopurinol (Fig. 1b) and oxypurinol (Fig. 1c) was added. A 40% and 30% reduction, respectively, in intensity of the ·O (superoxide free radical) radical production was documented with addition of allopurinol and oxypurinol addition (Fig. 2).

3.2. Study 2

3.2.1. Plasma uric acid and glucose levels

Allopurinol ($P < 0.05$) reduced the plasma concentrations of uric acid, both in AL and DR birds at week 16 although the reduction was not significant at week 22 in AL birds (Fig. 3). Uric acid concentrations were marginally higher ($P = 0.05$) in the AL birds as compared with the DR controls at week 16. There was no significant effect of hemin on uric acid concentrations in either the DR or AL groups. No significant differences in plasma glucose were found between the treatment groups (Table 2). Concentrations of plasma glucose ranged from 224 ± 3 to 235 ± 4 mg/dl at 16 weeks and from 226 ± 7 to 239 ± 8 at 22 weeks.

3.2.2. Skin pentosidine

At 16 weeks, AL birds provided the Control diet exhibited higher skin concentrations of Ps (an index of glycosylation) compared with values in the DR group (Fig. 4). Neither allopurinol nor hemin altered skin Ps levels in DR of AL groups at 16 weeks of age. There were no differences in skin Ps levels in Control-fed AL or DR groups at 22 weeks of age. There were no differences in skin Ps in the DR group due to allopurinol or hemin treatments. However, in the AL group, allopurinol and hemin increased Ps levels sixfold above levels in the AL birds fed the Control diet (Fig. 4).

3.2.3. Chemiluminescence measurement of oxidative stress

Hemin increased the oxidative stress assessed by the chemiluminescence method utilized ($P < 0.05$) in the AL birds at 16 weeks and at 22 weeks in both the DR and AL groups. Allopurinol increased ($P < 0.05$) the oxidative stress in the AL group at week 22 (Fig. 5). Both hemin and al-

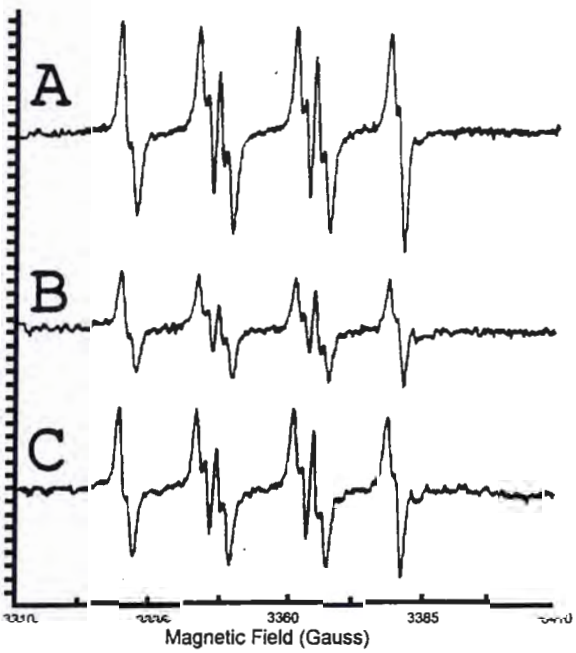

Fig. 2. The intensity of superoxide radical generation with xanthine oxidase. (a) ESR spectrum recorded 3 min after reaction initiation from a pH of 7.4 phosphate buffer containing 50 mM DMPO, 0.35 mM xanthine, 0.05 unit xanthine oxidase and 0.3 mM Datapac. (b) Same as (a), but with saturated allopurinol. (c) Same as (a), but with saturated oxypurinol.

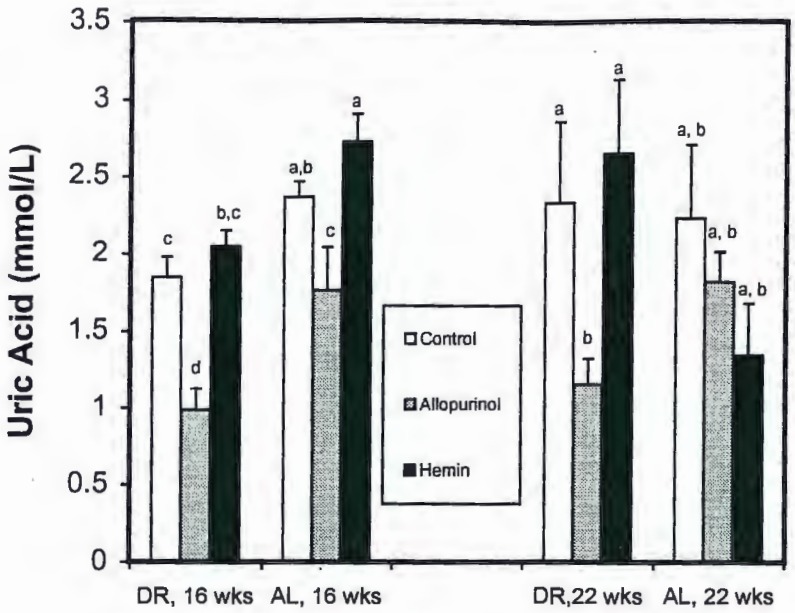

Fig. 3. Effect of allopurinol and hemin on plasma uric acid concentrations at 16 and 22 weeks of age. Means with no common letters differ significantly ($P < 0.05$).

lopurinol ($P < 0.05$) increased the total leukocyte counts at 16 and 22 weeks (Table 2). This effect was much more pronounced in hemin fed birds. A 1.7- and threefold increase in the total leukocyte count at week 16 and 22, respectively, was measured in the hemin fed birds (Table 2).

3.2.4. Mortality, body and breast weight, and shear force

The mortality of the allopurinol fed birds was 40%, compared to 7 and 13% in the control and hemin fed birds. Allopurinol reduced body weight in both treatment (DR and AL) groups at week

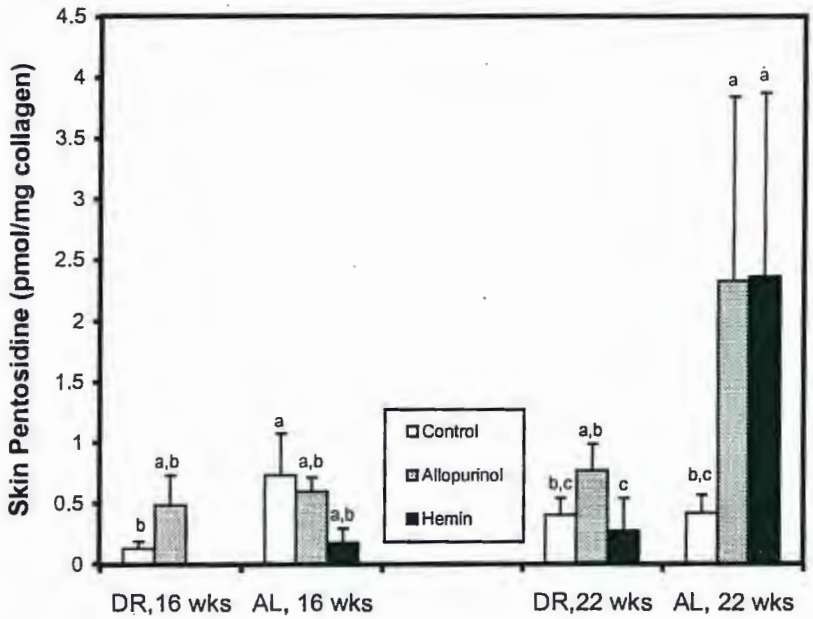

Fig. 4. Effect of allopurinol and hemin on skin pentosidine (Ps) at 16 and 22 weeks of age. Means with no common letters differ significantly ($P < 0.05$).

Table 2
Effect of allopurinol and hemin on plasma glucose and total leukocyte count ($n = 5$ per treatment group)[a]

Group (Week)	Plasma glucose (mg/dl)		TLC × 10^5	
	16	22	16	22
DR-C	231 ± 4	234 ± 5	15.1 ± 0.0*	24.6 ± 7.4
DR-A	229 ± 7	239 ± 8	32.2 ± 2.4*	66.3 ± 14.2*
DR-H	224 ± 3	231 ± 7	42.3 ± 2.3*	97.8 ± 35*
AL-C	233 ± 3	239 ± 8	22.8 ± 2.0	25.3 ± 5.0
AL-A	224 ± 10	226 ± 7	37.08 ± 2.2*	73.6 ± 21.0*
AL-H	235 ± 4	233 ± 5	43.0 ± 1.7*	130.7 ± 29*

[a] *Abbreviations:* DR, diet restriction; AL, Ad libitum; C, control; A, allopurinol; H, hemin. Values are means ± S.E. *$P < 0.05$ compared within a column.

22 (Fig. 6). Overall, there was a 49% and 35% reduction in breast weight in the DR and AL birds fed allopurinol, respectively. The allopurinol fed birds had shear force values that were increased 88% and 58% ($P < 0.05$) in the DR and AL groups respectively at week 22 as compared with controls (Fig. 7). There was no significant effect of allopurinol on the shear force values at 16 weeks. Hemin had no effect on shear values.

4. Discussion

Previous studies have established that birds have higher concentrations of antioxidants in their bodies and appear more efficient in dealing with oxidative stress as compared to mammals (see Introduction). It has been hypothesized that uric acid plays an important role in limiting oxidative stress and the subsequent accumulation of glycosylation endproducts such as Ps in birds (Iqbal et al., 1999a; Klandorf et al., 1999). In previous studies, doses of allopurinol from 2 to 50 mg/kg were used to lower uric acid levels in laboratory rats (Klein et al., 1975), 6.5 mg/kg in dogs, and in varying doses, from 30 to 50 mg/kg, in ethanol fed turkey poults (Czarnecki et al., 1987). In the present study, administration of allopurinol to broiler chickens at a dose of 10 mg/kg decreased plasma uric acid concentrations. The uric acid lowering effect of allopurinol is a consequence of its inhibition of xanthine oxidase, the enzyme involved in the conversion of hypoxanthine to

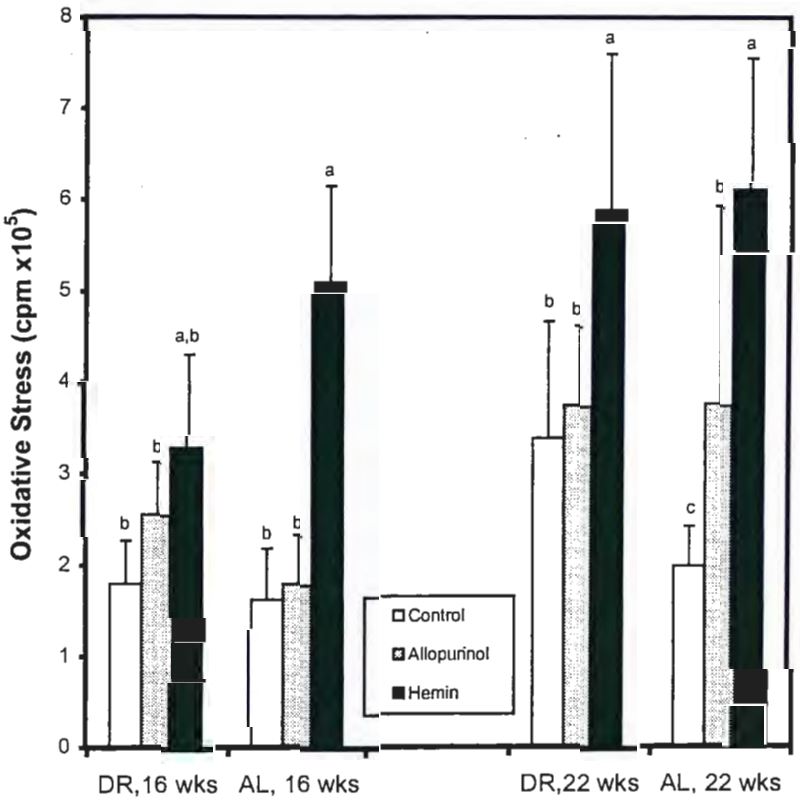

Fig. 5. Effect of allopurinol and hemin luminol induced chemiluminescence detected oxidative stress at 16 and 22 weeks of age. Means with no common letters differ significantly ($P < 0.05$).

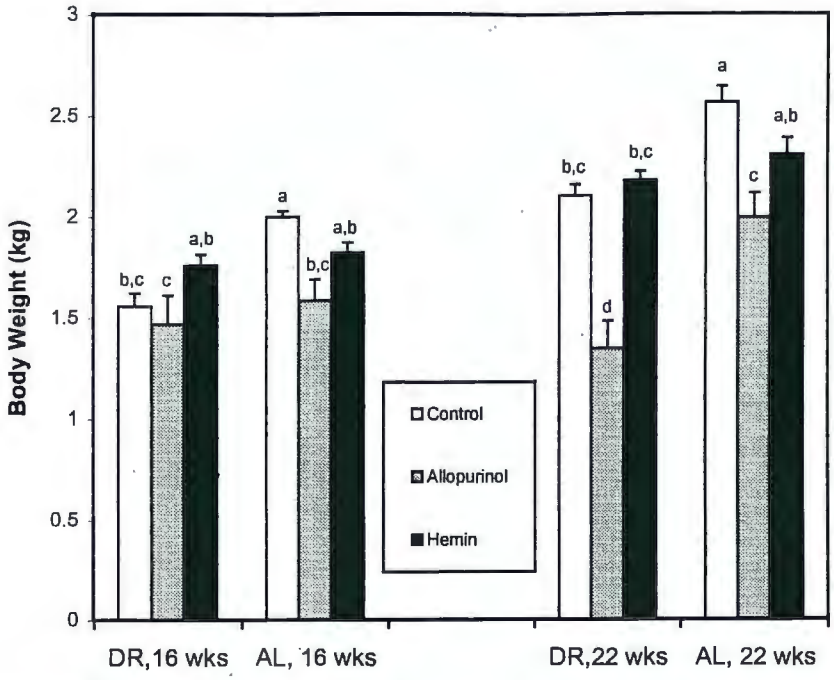

Fig. 6. Effect of allopurinol and hemin on body weight at 16 and 22 weeks of age. Means with no common letters differ significantly ($P < 0.05$).

xanthine and xanthine to uric acid. A decrease in the uric acid concentration of allopurinol fed birds was accompanied by a decrease in the body and breast muscle weight and an increase in

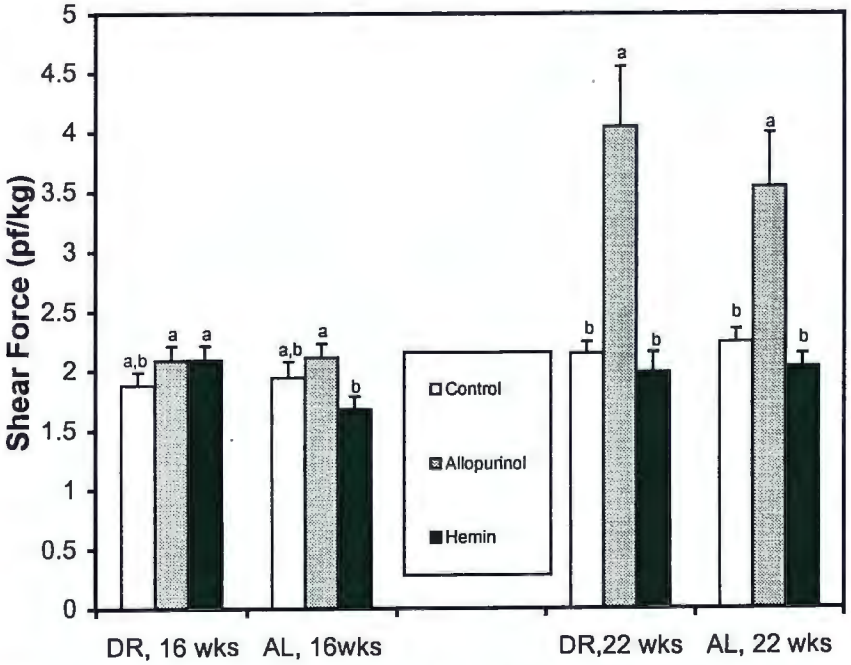

Fig. 7. Effect of allopurinol and hemin on shear values (SV) at 16 and 22 weeks of age. Means with no common letters differ significantly ($P < 0.05$).

tissue aging as evidenced by an increase in shear force values (88% and 58% increase) in the DR and AL group, respectively.

Faure et al. (1990) have reported that allopurinol inhibited radiation-induced lipid peroxidation in rat erythrocytes and suggested that it has a direct antioxidant action. In their experiments, the allopurinol concentration was reported to be 20 mM, nearly 200 times the usual level achieved in serum with pharmacological doses of allopurinol. The daily administration of allopurinol to cats (50 mg/kg per day) produced serum concentrations of only 12.0 fM. In the current study, the antioxidant effects of allopurinol (10 mg/kg body wt.) are unlikely to be present as the concentrations that would have likely been achieved were much too low. However, an increased (40%) mortality in allopurinol treated birds was evident at week 22, although the results from Study 1 did not suggest that the dose of allopurinol selected was toxic. This result supports the view that uric acid is critical for dealing with the elevated oxidative stress observed in avians (see Introduction). There are several reports in the literature documenting toxicity of allopurinol in humans. As a result of hypertonicity as has been reported in turkey poults (Czarnecki et al., 1987) and humans (Hande et al., 1984), plasma oxypurinol concentrations become much greater than that required to prevent uric acid formation. Other investigators have suggested that a reduced uric acid excretion during long-term therapy with allopurinol alters the tubular reabsorption of oxypurinol as uric acid and oxypurinol share the same transport mechanism (Berlinger et al., 1985). Allopurinol is readily absorbed and is rapidly converted to oxypurinol, which is excreted unchanged in the urine (Appelbaum et al., 1982). In humans, a decrease in the intake of protein alters the tubular transport of uric acid (Mehta, 1983). While it minimally affects the renal clearance of allopurinol, it has a marked effect on the renal clearance of oxypurinol. Other reported side effects of allopurinol include gastrointestinal intolerance and vasculitis (Mehta, 1983; Fox and Kelley, 1985). Additional studies are required to determine whether any of these side effects are responsible for the increased mortality observed in the chickens used in the study and has been reported for turkey poults.

Hemin (10 mg/kg body wt.) was without effect on concentrations of both uric acid and shear force in both the DR and AL treated birds. Interestingly, hemin was associated with an enhanced measurement of oxidative stress. As hemin is a blood product and a source of iron, the increase in oxidative stress observed in this study may have been due to the well-known ability of iron to convert hydrogen peroxide to hydroxyl radical in the Haber–Weiss reaction (Tappel, 1985). The relationship between metal ions, oxygen radicals, and tissue damage has been reviewed (Aust et al., 1985; Ryan and Aust, 1992). In our studies, hemin administration resulted in oxidative stress assessed by chemiluminescence ($P < 0.001$) and increased the total leukocyte count in the AL (at 16 and 22 weeks) and DR (at 16 weeks) birds. These results were found to be in agreement with previous studies (Aust et al., 1985; Ryan and Aust, 1992; Grinberg et al., 1999) that ascribe an increased oxidative stress capacity to hemin treatment.

The present studies did not suggest any effects of allopurinol or hemin treatment on plasma glucose concentrations. However, changes in plasma uric acid concentrations were associated ($P < 0.001$) with changes in the accumulation of Ps in skin tissue with duration of treatment. In agreement with previous studies in birds (Iqbal et al., 1999a), concentrations of Ps were lower in DR birds compared to AL fed birds at 16 weeks although not at 22 weeks. Further, concentrations of Ps and possibly other glycosylated proteins were not associated with elevated concentrations of plasma glucose. These results differ with studies in mammals where variable effects of diet restriction on glucose levels have been reported. For example, a 10% decrease in blood glucose concentrations in DR rats was associated with a decrease in the glycosylation of proteins, which might be caused by a consequence of a reduced oxidative stress in the DR chickens (Ross, 1972; Masoro et al., 1989; Sell et al., 1996). While several studies have firmly established that collagen glycosylation is increased with age, others have failed to demonstrate anything but a cursory relationship between protein cross-linking and glycosylation, either in vivo or in vitro (Guitton et al., 1981; Le Pape et al., 1984; Monnier, 1990; Lyons et al., 1991). As an example, the amount of

glycosylated hemoglobin in hummingbirds, which have plasma glucose concentrations in excess of 650 mmol/l, is only 2–5%, which is much less than measured in mammals, which have levels ranging from 6 to 8% (Beuchat and Chong, 1997).

The reduction in uric acid concentrations, in both the DR and AL birds with allopurinol feeding was associated with an increase in the shear force value of the *pectoralis major* muscle. This observation supports the hypothesis that uric acid is an important antioxidant in birds. The reason for the increase in SF values has not been established although the increase Ps may be a result of an increased level of oxidative stress and resultant glycosylation. A reduction in the concentration of antioxidants accelerated the formation of glycoxidation products. This finding is supported by the work of others (Yu et al., 1982; Youngman et al., 1992; Yu, 1993).

In conclusion, the present investigation has documented that a reduction in uric acid concentration in chickens is associated with increase in oxidative stress, the accumulation of the skin glycoxidation product Ps, and an increase in the shear force value of the *pectoralis major* muscle.

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