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Effect of Allopurinol and Inosine Administration on Xanthine Oxidoreductase Gene Expression in Selected Tissues of Broiler Chickens

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Abstract: Uric acid is considered the most significant factor in amelioration of oxidative burden in birds. Uric acid is formed in the terminal reactions of purine degradation by the enzyme xanthine oxidoreductase (XOR). In this study, inosine, a purine precursor, was fed to 3 groups of 5 birds: Group 1 was fed 0 (control), Group 2, 0.6 mols inosine/kg feed (INO) and Group 3, INO treatment plus 50 mg allopurinol/kg BM (INOAL). Allopurinol is a known inhibitor of XOR and thereby reduces uric acid (UA). INOAL birds showed lower total liver XOR activity ($p = 0.005$) but kidney XOR activity was not affected. Both INO and INOAL treated birds had higher plasma and kidney UA concentrations than controls. Liver uric acid (LUA) was significantly reduced in INOAL birds when compared to other treatments. XOR gene expression was increased ($p = 0.007$) in the liver tissue of INOAL birds when compared to CON and INO birds. However, there were no significant changes in XOR gene expression in the kidney tissue. To our knowledge, this is the first report of XOR gene expression measured under these conditions. These results suggest that regulation of UA production is tissue dependent. The results also indicate a compensatory effect of allopurinol on XOR gene expression which can be linked to a decrease in antioxidant protection from UA.

Key words: Uric acid, xanthine oxidoreductase gene expression, oxidative stress

INTRODUCTION

Xanthine oxidoreductase (XOR) catalyzes the terminal reactions in purine degradation to uric acid (UA), a potent antioxidant for birds and humans. Specifically, XOR catalyzes the formation of UA from hypoxanthine and xanthine. XOR exists in two interconvertible forms: xanthine oxidase (XO) and xanthine dehydrogenase (XD). In mammals, XOR is predominantly found in the XD form, which can be converted to XO either irreversibly via proteolysis or reversibly by oxidation of the sulfhydryl residues (Hille and Massey, 1981). In the avian, a low, but detectable activity of XO has been measured in the liver and kidney (Remy and Westerfeld, 1951; Nishino *et al*., 1989; Harrison, 2002; Carro *et al*., 2009a). Despite this discovery, the majority of previous research in birds has focused on activity of XD.

XOR reaction products include UA and reactive oxygen species (ROS), specifically the generation of peroxide and superoxide radicals. Both radical types are implicated in exacerbating oxidative damage to various tissues (Galbusera *et al*., 2006). In contrast UA is a potent antioxidant in birds (Simoyi *et al*., 2002; Stinefelt *et al*., 2005). In most mammals, uricase converts UA to allantoin before excretion. In birds, humans, reptiles and some higher primates, uricase is absent due to the evolutionary loss of the UOX gene encoding for uricase and therefore UA concentrations in the plasma remain elevated (Oda *et al*., 2002). Increased UA concentrations have been shown to reduce oxidative stress (Simoyi *et al*., 2002), while a reduction in UA dramatically increases oxidative stress (Klandorf *et al*., 2001; Carro *et al*., 2009b). The gene structure of XOR has been determined in several species including humans, mice, chickens and insects. However, it should be noted that to our knowledge the gene sequences for either XD or XO specifically have not been determined for chickens and only xanthine oxidoreductase has been sequenced.

Previous studies have investigated the effects of diet on chicken XD activity (Scholz and Featherston, 1967; Itoh *et al*., 1978), developmental patterns of XD in the embryo and the post-hatching period (Lee and Fisher, 1972) and tissue XOR distribution in broilers (Carro *et al*., 2009a; Settle *et al*., 2012), but to our knowledge little is known about the regulation of XOR gene expression in birds.

The gene loci that code for human and mouse XOR have been identified and the associated genes have been assigned to chromosomes 2p 22 and 17 (Harrison, 2002; Cazzaniga *et al*., 1994). *Drosophila* genes tend to be more compact with only four or five exons (Terao *et al*., 1997). In mammals the exon-intron structure is highly conserved and contains >60 kb and approximately 36 exons (Xu *et al*., 1996). Mammalian and avian cDNA sequences have also been reported. Avians, specifically chickens, have a sequence that corresponds to 1358 amino acids whereas mammalian enzymes have a range from 1330-1335 with approximately 90% homology between themselves (Harrison, 2002). The Mo-Co binding site is the most

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conserved of the amino acid sequences and has a 94% homology between human, rat and mouse XOR (Xu *et al*., 1995). Mutations in the XOR gene in humans, more specifically concerning the XD form, have been linked to inheritable xanthinuria.

Inhibition of the XOR enzyme results from feeding allopurinol or oxypurinol, as well as from feeding molybdenum-deficient diets that are high in tungsten (Harrison, 2002). Allopurinol is used to treat gout and hyperuricemia in humans. In birds, allopurinol decreases plasma uric acid (PUA) levels (Klandorf *et al*., 2001; Simoyi *et al*., 2002; Carro *et al*., 2009b), but information on its effects on XOR activity is scarce and the effects may be tissue dependent (Woodward *et al*., 1972; Carro *et al*., 2009b). Previous studies in our lab have demonstrated inosine fed with allopurinol can lead to a residual toxic effect in the liver tissue of broilers due to reduction of UA concentrations within the tissue (Settle *et al*., 2012). In order to better understand factors that regulate XOR activity, UA levels were increased by exogenous administration of inosine to broilers. While there was no effect on activity of XOR, it was hypothesized that allopurinol would have a down-regulating effect on UA production; however this could potentially have a compensatory effect such that XOR gene expression would increase in response to the reduced antioxidant defense provided by UA.

MATERIALS AND METHODS

All experimental protocols were approved by the West Virginia University Animal Care and Use Committee.

Animals and experimental diets: Twenty Cobb x Cobb mixed sex chicks at one day of age were donated from Pilgrim's Pride Hatchery (Moorefield, WV) and fed a commercial starter diet until three weeks of age before being offered a commercial grower diet. At 5 weeks of age, 15 birds were selected and divided into 3 homogeneous groups according to their body mass (BM) and PUA concentrations. Each group was randomly assigned to one of 3 treatments CON (control), INO (inosine) and INOAL (inosine with allopurinol). Birds in the control group were fed a commercial broiler diet free of antibiotic additives. Birds in the INO group were provided the control diet supplemented with inosine at a rate of 0.6 mols inosine per kg of feed. Birds in the INOAL group received the control diet containing inosine and allopurinol at 50 mg per kg BM over a short period of time to assess the effect of decreased uric acid on XOR gene expression and activity. This dose of inosine had been previously shown to raise PUA levels in broilers (Simoyi *et al*., 2002). Body weight and feed intake were monitored over the experimental period. At the end of the experimental period, birds in each group were euthanized by cervical fracture and tissues were collected. The right kidney was removed as well as the liver. Tissues were snap frozen in liquid nitrogen and stored in -80°C until analysis.

cDNA synthesis: Total mRNA from 1 mg of either kidney or liver tissue was extracted using RNA-Bee isolation medium (Tel-Test, TX) and quantified using a Genequant spectrophotometer. Quality was assessed using a denaturing formaldehyde-agarose gel. First strand cDNA was synthesized by using Life Technologies' Superscript II Indirect cDNA Labeling System Life, Carlsbad, CA) according to the manufacturer's protocol. Each reaction mixture contained 2.5 µL of 2.5 mM dNTP mixture, 5.0 µL of 5x reverse transcriptase buffer, 2.0 µL of 0.1M Dithiothreitol (DTT), 0.5 µL RNasin (Promega) and 2.0 µL random hexamer primers (Roche Scientific). The final cDNA concentration was 1.8 µg/µL. Each sample was heated to 70°C for five minutes and then 4°C for five minutes in a MJ Research PTC-200 DNA Engine (MJ Research Inc. Watertown, MA, USA). At this time, 1 µL reverse transcriptase (Super Script II, Life Technologies, USA) was added to each sample and cycled at 37°C for sixty minutes, 90°C for five min and 4°C for five min. Samples were stored at -80°C until analysis with real-time RT-PCR less than 48 h after synthesis.

Real-time RT-PCR: Primers for XOR (Forward: 5'CTGCAGGATGCCTGCCGCTT3'; Reverse: 5'GCATGG GCTTGGGTGCTGGT3') and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were designed using Primer 3 software (Howard Hughes Institute). Each sample was assayed in triplicate for the XOR and GAPDH primers on a 96-well plate. GAPDH (forward: 5'GACGTGCAGGAACACTA3'; reverse: 5'CTTGGACTT TGCCAGAGAGG3') was selected as a normalizer for this experiment as there were no differences in expression patterns due to treatment. A pooled sample, that contained cDNA samples from all treatments, was used for both analyses of both primer efficiencies. Primers were diluted to 5 µM/µL concentration in nuclease free water. A serial dilution was used to obtain a standard curve for each primer and efficiency was calculated. Primer efficiencies were determined from the slope of the regression line calculating the log of the cDNA concentrations versus the Ct value using the equation: $E = 10^{(-1/Slope)}$. The efficiencies were used to calculate the relative mRNA abundance using the "efficiency corrected relative expression" equation (Equation 3; Pfaffl, 2001). Real time RT-PCR was performed using IQ Sybr Green Supermix (Bio Rad Inc., USA) on a Bio Rad CFX 96 Real-Time System (Bio Rad Inc., USA). Each PCR reaction mixture contained 10 µIQ Sybr Green Supermix, 3 µL diluted cDNA (1:10), 1 µL each of the forward and reverse primer (diluted to 5 µ/µL) and 5 µL of nuclease-free water. Each sample was run in triplicate on a 96

well plate. The PCR reaction cycle was as follows: 95°C for 3 min, 95°C for 15 sec, 62°C for 30 sec, 70°C for 30 sec (repeated for 40 cycles). Then intervals of 95°C and 60°C for 5 min. Relative expression was determined using the efficiency corrected relative expression method (Pfaffl, 2001).

Xanthine oxidase and xanthine dehydrogenase activities: Previously, tissue preparation and enzyme activity determination have been described in detail by Settle *et al.* (2012). Briefly, frozen tissue samples (0.5 g) were homogenized in 4 mL of ice-cold 0.1 M TRIS buffer (pH = 7.8) using a Polytron PT 2100 (Kinematika AG, Littau, Switzerland) for 20 s at 19,000 rpm. The homogenate was centrifuged (14000 x g, 4°C, 30 min) and 1 mL of the supernatant fraction was immediately chromatographed on Sephadex G-25 (PD-10 desalting colums; GE Healthcare, Piscataway, NJ, USA) and equilibrated with 1.5 mL of 0.1 M TRIS buffer (4°C). The eluates were stored on ice and assayed for XO and XD activities within 1-2 h after homogenizing the tissue.

XO and XD activities were assayed by measuring the formation of UA when xanthine was incubated with the eluates. Each eluate was incubated with 100 μM xanthine in the presence of ambient oxygen (XO activity) or with 100 μM xanthine and 0.67 mM NAD^+ (XO+XD activity). The reaction mixture contained 200 μL of eluate and 2.8 mL of 0.1 M TRIS buffer (pH = 7.8) with 100 μM xanthine. The tubes containing the reaction mixtures were incubated at 41°C for 30 min and absorbance was measured at 294 nm in a Beckman Spectrophotometer DU 640 (Beckman Instruments, Fullerton, CA). The amount of UA produced was determined from the difference between the absorbance values at 30 and 0 min, as compared to external standards of known UA concentration. Blanks containing xanthine and xanthine plus NAD^+ were incubated along with the sample tubes to correct for nonenzymatic oxidation of xanthine. Each assay was performed in duplicate.

One unit of activity was defined as the production of 1 nanomol of UA per min at 41°C and pH 7.8 using 100 μM xanthine as substrate in the presence (XO+XD activity) or absence of 0.67 mM NAD^+ (XO activity). XD activity was calculated as the difference between total (XO+XD) and XO activity. XO and XD activities are expressed as units per mg of protein, but XO and XD activity in total liver was calculated as units per g of liver multiplied by liver weight. Specific activities were expressed in terms of units per mg of protein in the eluate. The protein content in the eluate was assessed by Bio Rad Protein Assay (Bio-Rad Chemical Division, USA) on a spectrophotometer according to the method of Bradford (1976) using serum albumin as standard.

Uric acid analysis: As described by Settle *et al.* (2012), the UA concentration in plasma was measured in the supernatant fraction by using a commercially available cholorometric diagnostic kit (Sigma Diagnostic kit procedure 685; Sigma Diagnostics, St. Louis, MO). This method employs uricase to generate H_2O_2 which reacts with 4-amino antipyrine (4-AAP) and TBHB in the presence of peroxidise to form a quinoneimine dye measured on a spectrophotometer. The resulting change in absorbance at 520 nm is proportional to UA concentration in the sample.

Concentration of UA in liver and kidney samples was measured using the technique described for PUA analysis using 40 μL of the supernatant fraction from the homogenization of tissues for XOR analysis. Briefly, tissues were homogenized in 2 mL of ice-cold 0.1 M TRIS buffer (pH = 7.8) and centrifuged at 14000 x g at 4°C for 30 min. Concentrations of UA were analyzed in 40 μL of the supernatant fraction by the method described previously.

Statistical analyses: Tukey's multiple comparison test was used to assess differences among means. All analyses were conducted using the PROC GLM of SAS (SAS institute, 2002) for both studies. Correlations between UA concentration in liver and kidney were determined by Pearson correlation analysis using the PROC CORR of SAS software. Significance was as p = 0.05. Statistical significance for gene expression was assessed using the analysis of Variance (ANOVA) procedure of the Ri 386 software. When significant differences occur ($p<0.05$), means were separated by the least squares mean procedure and then adjusted for multiple comparisons using the Tukey's multiple comparison test.

RESULTS

Body weights, PUA, tissue uric acid and XOR activity: Results for body mass, PUA, tissue uric acid and XOR activity have been reported by Settle *et al.* (2012). Briefly, there were no differences (p = 0.853) in initial BM between the experimental groups (Table 1), after 1 week of treatment BM of the control birds was higher ($p<0.001$) than that of the INO and INOAL birds. No differences ($p>0.05$) were detected between INO and INOAL groups As shown in Table 1, PUA concentrations were similar (p = 0.847) in all groups at the onset of the study. After 3 days of treatment, INO birds had higher ($p<0.001$) PUA levels than controls and INOAL birds. The INOAL birds had significantly higher PUA levels than control birds. After 6 days of treatment, PUA concentrations remained elevated ($p<0.001$) in INO and INOAL birds as compared to control birds, but there were no differences ($p>0.05$) between INO and INOAL groups.

The effects of experimental treatments on XO and XD activities and UA concentrations in the liver and kidney are shown in Table 2 and 3, respectively. After 6 days of treatment, INOAL birds had lower (p = 0.001) liver

Table 1: Effects of experimental treatments (CON: 0; INO: 161 g of inosine/kg feed; INOAL: 161 g of inosine plus 0.5 g of allopurinol/kg feed) on plasma uric acid concentrations and body mass (BM) and in broilers (n = 5)

			---------- Plasma uric acid (mg/dL) ----------		
Treatment	Initial BM (kg)	Final BM (kg)	Day 0	Day 3	Day 6
CON	2.26	2.87[b]	5.12	4.41[a]	4.46[a]
INO	2.19	2.65[b]	4.93	18.6[c]	14.3[b]
INOAL	2.23	2.07[a]	4.96	13.9[b]	12.0[b]
SEM	0.080	0.110	0.530	1.070	1.133
p =	0.853	<0.001	0.847	<0.001	<0.001

[a,b,c]For each variable, means within a column lacking a common superscript differ (p<0.05). SEM: Standard error of the mean

Table 2: Effects of experimental treatments (CON: 0; INO: 161 g of inosine/kg feed; INOAL: 161 g of inosine plus 0.5 g of allopurinol/kg feed) on liver weight, activities of xanthine oxidase (XO) and xanthine dehydrogenase (XD) and uric acid concentration in the liver of broilers (n = 5)

		--------- Activity (units/mg protein) ---------				---------- Uric acid ----------	
Treatment	Liver weight (g)	XO	XD	XO+XD	Total XO+XD activity (units/liver)	mg/g wet tissue	Total mg in the liver
CON	60.8[b]	7.92	37.9	45.9	24.7[b]	0.262[b]	15.5[b]
INO	53.6[b]	7.97	37.2	44.6	20.9[ab]	0.266[b]	14.3[b]
INOAL	41.2[a]	7.33	35.7	43.7	16.2[a]	0.093[a]	3.76[a]
SEM	2.82	0.559	2.50	3.00	1.49	0.0333	1.582
p =	0.001	0.673	0.812	0.875	0.005	0.005	<0.001

[a,b]For each variable, means within a column lacking a common superscript differ (p<0.05). SEM: Standard error of the mean

Table 3: Effects of experimental treatments (CON: 0; INO: 161 g of inosine/kg feed; INOAL: 161 g of inosine plus 0.5 g of allopurinol/kg feed) on activities of xanthine oxidase (XO) and xanthine dehydrogenase (XD) and uric acid concentration in the kidney of broilers (n = 5)

	------ Activity (units/mg protein) ------			
Treatment	XO	XD	XO+XD	Uric acid (mg/g wet tissue)
CON	5.80	34.2	40.0	0.084[a]
INO	5.49	31.5	37.0	0.596[b]
INOAL	5.22	31.0	36.2	0.139[a]
SEM	0.341	2.28	2.56	0.1193
p =	0.510	0.573	0.554	0.006

[a,b]For each variable, means within a column lacking a common superscript differ (p<0.05). SEM: Standard error of the mean

weights than birds in CON and INO groups, whereas no differences (p>0.05) between CON and INO groups were detected. In addition, there were no differences (p = 0.673 to 0.875) between treatments in XO, XD, or XO+XD activity at the end of the study. However, control birds had higher (p<0.05) total enzyme activity in the liver (units/liver) than INOAL birds, with no differences (p>0.05) measured between control and INO groups. Concentrations of UA in the liver of INOAL birds, expressed both in mg per g of tissue and as total mg in the liver, were lower (p<0.001) than those in the liver of control and INO birds, while there were no differences (p>0.05) between INO and CON birds.

No differences (p = 0.510 to 0.573) in UA existed between any of the groups in enzyme activity of the kidney were detected (Table 3).

Kidney UA concentrations in CON birds were lower (p<0.05) than those found in the INO treatment, but no difference (p>0.05) was detected between CON and INOAL groups.

XOR gene expression: There was an upregulation of XOR gene expression in liver tissue (Fig. 1) of the INOAL group when compared to both the CON (p = 0.007) and INO (p = 0.008) groups. There was no difference (p = 0.996) in XOR gene expression in the liver between CON and INO groups. There was no significant difference in kidney XOR (Fig. 2) gene expression between CON, INO and INOAL groups (p = 0.462, p = 0.906, p = 0.264, respectively).

DISCUSSION

The liver and kidney have been documented as sources of XOR activity in avian species (Harrison, 2002; Strittmatter, 1965; Carro *et al*., 2009b). The most studied form of XOR is XD, which is the more readily available form in the tissues. Little attention has been paid to XO in birds, although it has been identified in several tissues including the liver, kidney, intestine and pancreas of broilers (Remy and Westerfield, 1951; Strittmatter, 1965; Carro *et al*., 2009a).

In this study, inosine, a precursor known to increase UA concentrations in birds (Simoyi *et al*., 2001), was administered separately and in combination with allopurinol (Settle *et al*., 2012). To our knowledge, this is the first study to investigate the effect of combining allopurinol and inosine on XOR activity, UA concentrations and XOR gene expression. As previously reported, the administration of inosine did not affect birds BM, but the INOAL birds showed a decreased final BM at the end of the study as compared with control and INO birds. This is in accordance with previous observations in birds fed the same allopurinol dose (Carro *et al*., 2009b).

As in previous studies (Della Corte and Stirpe, 1967; Simoyi *et al*., 2002) birds in the INO group had

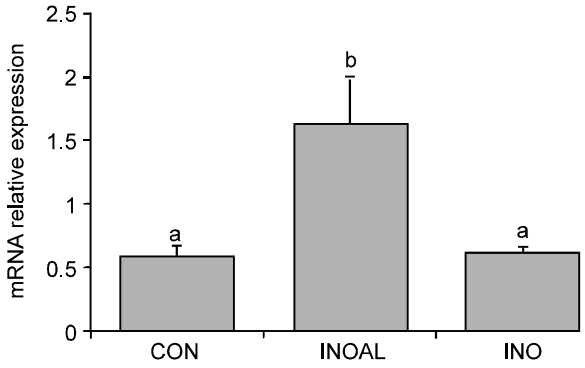

Fig. 1: Effects of experimental treatments (CON: 0; INO: 161 g of inosine/kg feed; INOAL: 161 g of inosine plus 0.5 g of allopurinol/kg feed) XOR gene expression in the liver tissue of broilers (n = 5). [a,b]For each variable, means a bar lacking a common superscript differ ($p<0.05$). Error bars represent SEM for each treatment

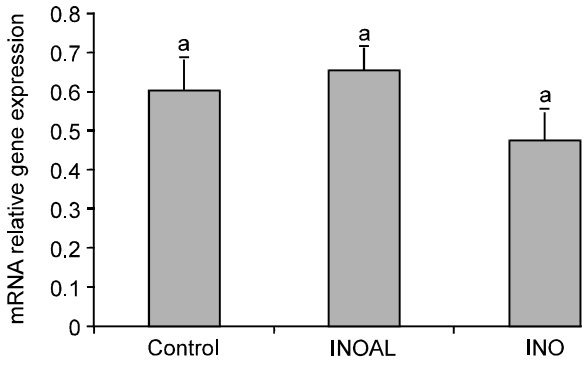

Fig. 2: Effects of experimental treatments (CON: 0; INO: 161 g of inosine/kg feed; INOAL: 161 g of inosine plus 0.5 g of allopurinol/kg feed) XOR gene expression in the kidney tissue of broilers (n = 5). [a,b]For each variable, means a bar lacking a common superscript differ ($p<0.05$). Error bars represent SEM for each treatment

significantly higher PUA than controls after 3 and 6 days of treatment, with INOAL birds having intermediate PUA concentrations but higher than in controls. This suggests that allopurinol was unable to completely inhibit XOR activity. Unexpectedly, liver UA concentrations were not affected by the administration of inosine, despite the higher UA concentrations observed in plasma and kidney of INO birds compared with the controls (3.2 and 7.1 times higher, respectively). These results suggest a different regulating mechanism of UA concentrations in liver and kidney.

Although it was reported that there were no differences in the XOR activity in the liver between groups, when total XO+XD activity in the liver was calculated, the INOAL birds showed significantly lower total enzyme activity, indicating that allopurinol was inhibiting enzyme activity as compared to control birds, which confirms the results observed in Settle *et al.* (2012) when birds fed diets containing allopurinol (25 mg/kg BW) were supplemented with uric acid exhibited no change in XO or XD activity, but demonstrated a reduction in total enzyme activity. In accordance with that observation, the birds in the INOAL group exhibited significantly lower UA concentrations in the liver as compared with control and INO birds. This observation suggests a pronounced residual effect of allopurinol or oxypurinol, regardless of the presence of inosine which is converted into UA. The lack of differences in liver UA concentrations and XOR activity between control and INO birds indicates that inosine had no effect on UA concentrations or enzyme activity in the liver.

To our knowledge, this is the first report of XOR gene expression in birds. The chicken XD gene was first cloned by Sato *et al.* (1995) and was found to contain 1358 nucleotides. In this study, there was a significant induction of XOR gene expression in liver tissue of INOAL birds. Given that the liver UA content was significantly decreased it can be hypothesized that the liver may be vulnerable to oxidative damage due to a reduction in antioxidant defense. Simoyi *et al.* (2002) showed that a decrease in uric acid of the plasma can be linked to an increase in leukocyte oxidative activity, which suggests that the increase in XOR gene expression represents a compensatory mechanism to reduce oxidative damage to this tissue. A study by Dupont *et al.* (1992) reported that there was an increase in XD/XO gene expression at the transcriptional level in rat endothelial cells under treatment with IFN-γ, linking an increase in this gene with an increase in pro-inflammatory cytokines. Furthermore, there is evidence that uric acid, a scavenger of peroxynitrite, exhibits protective properties in the inhibition of CNS inflammation as well as the blood-CNS barrier that has been compromised by peroxynitrite damage (Hooper *et al.*, 2000). It is suggested that there is an increase in oxidative damage and inflammation in INOAL birds which may be directly related to the reduction in uric acid in the liver thereby causing a concomitant increase in the XOR gene expression.

There was not a significant difference in the kidney tissue with respect to XOR gene expression, indicating that there are tissue-specific differences in regulation of UA. A study by Suzuki *et al.* (1984) using a rat model, suggested that liver and kidney tissue may show differences in sensitivity to allopurinol doses such that a range of 10-100 mg/kg BW/day can be toxic resulting in hepatic necrosis and renal damage in some cases. In the case of the kidney, it was found that relative kidney weight, creatinine and BUN increased at doses above 10 mg/kg BW, indicative of renal damage and it was

concluded that the kidney may be more sensitive to allopurinol, but the mechanism was not determined (Suzuki *et al*., 1984). In birds uric acid is packaged in protein vesicles in the kidney before secretion (Braun and Dantzler, 1997) and the insoluble uric acid crystals are excreted along with fecal material from the cloaca (Skinner *et al*., 2001). Uric acid is actively transported by MRP4 from the proximal tube epithelium (Bataille *et al*., 2008). The mechanism of transport from the avian liver is, to our knowledge, unknown. These results also indicate that the regulation of uric acid differs between tissues.

Conclusion: In conclusion, these studies show that allopurinol had a residual toxic effect in the liver and kidney, as indicated by chronically depressed UA concentrations in both tissues. The low UA tissue concentrations reported by Settle *et al*. (2012) are an indication of increased susceptibility to oxidative stress, due to the inverse relationship that UA has with oxidative stress. There was a significant increase in XOR gene expression in liver tissue, indicative that a reduction in concentrations of uric acid may initiate a compensatory up-regulation of the XOR gene to restore antioxidant protection to the tissue. However, this was not demonstrated in kidney tissue, which indicates that the mechanisms regulating UA production in liver and kidney may differ. Future work with the allopurinol model of inflammation will further increase our understanding of the role that XOR has within the avian and the importance in the regulation of uric acid in the antioxidant defense system.

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REFERENCES

Bradford, M.M., 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. Anal. Biochem., 72: 248-254.

Bataille, A.M., J. Goldmeyer and J.L. Renfro, 2008. Avian renal proximal tubule epithelium urate secretion is mediated by Mrp4. Am. J. Physiol. Integr. Comp. Physiol., 295: 2024-2033.

Braun, E. and W. Dantzler, 1997. Vertebrate renal system. In: Handbook of Physiology.Comparative Physiology. Bethesda, MD: Am. Physiological Society, Sect., 8: 481-576.

Carro, M.D., E. Falkenstein, K.P. Blemings and H. Klandorf, 2009a. Determination of xanthine oxidoreductase activity in broilers: effect of pH and temperature of the assay and distribution in tissues. Poult. Sci., 88: 2406-2414.

Carro, M.D., E. Falkenstein, W.J. Radke and H. Klandorf, 2009b. Effects of allopurinol on uric acid concentrations, xanthine oxidoreductase activity and oxidative stress in broiler chickens. Comp. Biochem. Physiol. C., 151: 12-17.

Cazzaniga, G., M. Terao, Lo P. Schiavo, F. Galbiati, F. Segalla, M.F. Seldin and E. Garattino, 1994. Chromosomal mapping, isolation and characterization of the mouse xanthine dehydrogenase gene. Genomics, 23: 390-402.

Della Corte, E. and F. Stirpe, 1967. Regulation of xanthine dehydrogenase in chick liver: further experiments on the effects of inosine, actinomycin D and other factors. Biochem. J., 102: 520-524.

Dupont, G.P., T.P. Hueksteadt, B.C. Marshall, U.S. Ryan, J.R. Michael and J.R. Hoidal, 1992. Regulation of xanthine dehydrogenase and xanthine oxidase activity and gene expression in cultured rat pulmonary endothelial cells. J. Clin. Invest., 89: 197-202.

Galbusera, C., P. Orth, D. Fedida and T. Spector, 2006. Superoxide radical production by allopurinol and xanthine oxidase. Biochem. Pharmacol., 71: 1747-1752.

Harrison, R., 2002. Structure and function of xanthine oxidoreductase: where are we now? Free Radic Biol. Med., 33: 774-797.

Hooper, D.C., G.S. Scott, A. Zborek, T. Miksheeva, R.B. Kean, H. Koprowski and S.V. Spitsin, 2000. Uric acid, a peroxynitrite scavenger, inhibits CNS inflammation, blood-CNS barrier permeability changes and tissue damage in a mouse model of multiple sclerosis. FASEB J., 14: 691-698.

Hille, R. and V. Massey, 1981. Studies on the oxidative half-reaction of xanthine oxidase. J. Biol. Chem., 256: 9090-9095.

Itoh, R., T. Nishino, C. Usami and K. Tsushima, 1978. An immunochemical study of the changes in chicken liver xanthine dehydrogenase activity during dietary adaptation. J. Biochem., 84: 19-26.

Klandorf, H., D.S. Rathore, M. Iqbal, X. Shi and K. Van Dyke, 2001. Accelerated tissue aging and increased oxidative stress in broiler chickens fed allopurinol. Comp. Biochem. Physiol. C., 129: 93-104.

Lee, P.C. and J.R. Fisher, 1972. Effect of allopurinol on the accumulation of xanthine dehydrogenase in liver and pancreas of chicks after hatching. Arch. Biochem. Biophys., 148: 277-281.

Nishino, T., T. Nishino, L. Schopfer and V. Massey, 1989. The reactivity of chicken liver xanthine dehydrogenase with molecular oxygen. J. Biol. Chem., 264: 2518-2527.

Oda, M., Y. Satta, O. Takenaka and N. Takahata, 2002. Loss of urate oxidase activity in hominoids and its evolutionary implications. Mol. Biol. Evol., 19: 640-653.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res., 29: 45-45.

Remy, C. and W.W. Westerfeld, 1951. The effect of diet on xanthine dehydrogenase in chicken tissues. J. Biol. Chem., 193: 659-667.

Sato, A., T. Nishino and K. Noda, 1995. The structure of chicken liver xanthine dehydrogenase: cDNA cloning and the domain structure. J. Biolog. Chem., 270: 2818-2826.

SAS Institute Inc., 2002. SAS user's guide: Statistics. SAS Institute Inc., Cary, NC.

Scholz, R.W. and W.R. Featherston, 1967. Effect of alterations in protein intake on liver xanthine dehydrogenase in the chick. J. Nutr., 95: 271-277.

Settle, T., M.D. Carro, E. Falkenstein, W. Radke and H. Klandorf, 2012. The effects of allopurinol, uric acid and inosine administration on xanthine oxidoreductase activity and uric acid concentrations in broilers. Poult. Sci., 91: 2895-2903.

Simoyi, M., K. Van Dyke and H. Klandorf, 2002. Manipulation of plasma uric acid in broiler chicks and its effect on leukocyte oxidative activity. Am. J. Physiol. Regulatory Integrative Comp. Phsyiol., 282: 791-796.

Skinner, K.A., S. Tan and D.A. Parks, 2001. Uric Acid Metabolism. Encyclopedia of Life Sciences. Nature Publishing Group/www.els.net., pp: 1-8.

Stinefelt, B., S.S. Leonard, K.P. Blemings, X. Shi and H. Klandorf, 2005. Free Radical Scavenging, DNA protection and inhibition of lipid peroxidation mediated by uric acid. Ann. Clin. Lab. Sci., 35: 37-45.

Strittmatter, C.F., 1965. Studies on avian xanthine dehydrogenases: properties and patterns of appearance during development. J. Biol. Chem., 240: 2557-2564.

Suzuki, Y., J. Sudo and T. TanAbe, 1984. Allopurinol toxicity: its toxic organ specificity between the liver and kidney in the rat. J. Toxicol. Sci., 9: 343-351.

Terao, M., M. Kurosaki, S. Zanotta and E. Garattini, 1997. The xanthine oxidoreductase gene: structure and regulation. Biochem. Soci. Transact., 25: 14170-14175.

Xu, P., X.L. Zhu, T.P. Huecksteadt, A.R. Brothman and J.R. Hoidal, 1995. Assignment of human xanthine dehydrogenase to chromosome 2p 22. Genomics, 23: 289-291.

Xu, P., T.P. Hueksteadt and J.R. Hoidal, 1996. Molecular cloning and characterization of the human xanthine dehydrogenase gene. Genomics, 34: 173-180.

Woodward, W.D., P.C. Lee, N.W. DeLapp and J.R. Fisher, 1972. Induction of chick liver xanthine dehydrogenase by purines. Arch. Biochem. Biophys., 153: 537-542.