



## Inosine ameliorates the effects of hemin-induced oxidative stress in broilers

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### ABSTRACT

The objective of these studies was to determine whether inosine, a precursor of the antioxidant uric acid, can ameliorate hemin-induced oxidative stress. Dietary inclusion of inosine was begun either before or after hemin-induced oxidative stress. Broilers (4 weeks) were divided into four treatment groups (Control, Hemin, Inosine, Hemin/Inosine). Throughout the study control birds ( $n=10$ ) were injected daily with a buffer solution, while hemin birds ( $n=10$ ) were injected daily (i.p.) with a 20 mg/kg body weight hemin buffer solution. Leukocyte oxidative activity (LOA) and concentrations of plasma uric acid (PUA) were measured. Results from the first study showed that hemin birds had increased levels of LOA ( $P=0.0333$ ) and lower PUA ( $P=0.1174$ ). On day 10, control and hemin birds were subdivided into inosine birds ( $n=5$ ) and hemin/inosine birds ( $n=5$ ). These birds were given 0.6 M/kg of feed/day of dry inosine. Plasma concentrations of uric acid and LOA were then measured on day 15. Results showed that inosine raised concentrations of PUA ( $P=0.0001$ ) and lowered LOA ( $P=0.0044$ ) as induced by hemin. In the second study pretreatment of broilers with hemin prevented the increase in LOA induced by hemin ( $P=0.0001$ ). These results show that modulating the concentrations of uric acid can markedly affect oxidative stress.

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### 1. Introduction

Although oxygen is important to life on earth, it acts as a two edged sword providing numerous advantages on one side, but on the other hand, has the potential to create multiple forms of toxicity (Fridovich, 1983). It is the consumption of oxygen and the direct incorporation of it into biomolecules that lead to the production of reactive oxygen species (ROS). Metabolic processes that naturally occur during aerobic energy catabolism are responsible for creating ROS, which will ultimately lead to the oxidative damage of proteins, lipids and DNA (Benzie, 2000). Reactive oxygen species are the fundamental cause of numerous negative biochemical changes that occur throughout an organism's life and are indicative of oxidative stress. Once reactive oxidative species are created, the body's own natural defense systems come into play in order to minimize their generation and help counteract the damages that are brought on by these highly reactive and unstable species. Over time, constant exposure to these deleterious species leads to cellular damage and life threatening diseases.

Evolution's answer to this oxygen paradox relies on a wide range of antioxidant defense mechanisms that are extensive, diverse, coordi-

nated and effective on many different levels (Benzie, 2000). Protection from ROS is a highly complex system that takes into consideration which reactive species is generated, how it is generated, where it is generated and what target is being damaged (Halliwell, 1998). Therefore, it is safe to say that no one antioxidant is 100% effective against all types of ROS (Papežíková et al., 2005). Each antioxidant plays a significant role in protecting every species from oxidative onslaughts. Overcoming these oxidative attacks is crucial to the survival and longevity of each species.

Exogenous antioxidants that can only be taken in through the diet are becoming an ever more popular movement. The increase in trying to identify these possible antioxidants rises from finding a treatment, or prevention, for diseases caused by ROS. Recent studies have shown that fortifying diets with substances like fruits and vegetables can help aid individuals in fighting cancer, heart disease, and many other types of illnesses (Leonard et al., 2003). One such substance that has come into play is resveratrol, a polyphenol found in grapes, red wine, and other foods (Leonard et al., 2003; Borra et al., 2005). It has the ability to scavenge  $\cdot\text{OH}$  and  $\text{O}_2\cdot$  while inhibiting lipid peroxidation and DNA damage brought on by Fenton reactions (Leonard et al., 2003). Resveratrol may also aid in a type of redox regulation in platelets due to the fact that ROS are present in blood platelets and act as second messengers (Olas et al., 2004). While recent studies have shown that resveratrol is a scavenger of ROS, they have also shown that it has many useful properties including anti-inflammatory, antibacterial

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activities, as well as prevention of cancer and coronary heart disease (Chen et al., 2005; Imamura et al., 2002; Leonard et al., 2003). Despite the fact that resveratrol has protective properties, its mechanism of action is not totally understood and requires further studies (Imamura et al., 2002).

On the other hand, uric acid may be a partial answer to oxidative stress, and the diseases that attack each organism as a result of it. Uric acid is arguably one of the most important non-enzymatic extracellular antioxidants (Ames et al., 1993). It is considered a physiologically useful antioxidant and radical scavenger because it has the ability to interact with biological oxidants and radicals, the end products of these interactions are physiologically less harmful than the original components, and it is also found in high enough concentrations within the tissues to insure significant reactions (Becker, 1993). However, uric acid is also viewed as a preventative antioxidant because it not only intercepts strong oxidants like hydroxyl radicals, peroxyxynitrite, singlet oxygen, lipid hydroperoxides, and hypochlorous acid, but has the ability to form redox inactive complexes with transition metal ions (Papežiková et al., 2005; Stinefelt et al., 2005). Such defensive antioxidants have the ability to diminish the activity of metal ions by decreasing their ability to participate in Fenton chemistry, which leads to a decrease in oxidative damage.

Humans, birds, reptiles and higher primates have increased levels of uric acid as compared to other mammals because they lack the primary terminal purine oxidative enzyme uricase, which is responsible for degrading uric acid into allantoin (Benzie, 2000; Hediger et al., 2005; Iqbal et al., 1999). However, in the presence of oxidative stress urate can be converted to allantoin and other by-products of oxidation (Hediger et al., 2005). In fact, allantoin concentrations have been measured in the plasma of birds, which suggests that non-enzymatic pathways are being exemplified by the reaction of uric acid and reactive oxygen species in order to generate this compound (Simoyi et al., 2003).

Uric acid has been shown to have a positive association with maximum life span across species (Hediger et al., 2005; Iqbal et al., 1999; Klandorf et al., 2002). Therefore, higher plasma uric acid concentrations produce a greater life span of the individual species. Evidence of this occurs in the avian species, since they have greater longevity for life compared to animals of comparable body size. Birds, when compared to other species of similar size, have increased metabolic rates, higher body temperatures and high glucose levels which should make them more susceptible to oxidative stress and radical onslaughts, however the opposite occurs (Hediger et al., 2005; Simoyi et al., 2002). It has been hypothesized that uric acid is the antioxidant responsible for the increased life span of numerous species including primates, reptiles and birds.

In the current study, altered uric acid levels are linked inversely to measurements of oxidative stress. The specific objective was to determine if oral inosine treatment, which increases uric acid production, might serve as a potential therapy for oxidative stress in broiler chickens. Inosine is a naturally occurring purine that can be derived from the oxidative deamination of adenosine (Mabley et al., 2005), and is directly involved in the formation of uric acid via hypoxanthine and xanthine production via xanthine oxidase, which increases plasma uric acid levels when supplemented in the diet. Inosine is thus hypothesized to decrease oxidative injury whether it is supplemented before or after the onset of oxidative stress.

## 2. Materials and methods

### 2.1. Birds and management

One-day-old broiler chicks (*Gallus gallus*) ( $n=40$ ; Ross  $\times$  Cobb; mixed sex) were obtained from Pilgrim Farms in Moorefield, WV (USA) and maintained under standard husbandry practices. Broiler chicks were floor reared and given a starter diet *ad libitum* in pan feeders and

drinking water, and temperatures were maintained at recommended levels for 6 weeks. Provisions for space, temperature, light, and husbandry were rigidly followed (Ross Breeders, 1996). Blood samples were obtained from the wing vein and placed into heparinized tubes for measurement of plasma uric acid (PUA) and leukocyte oxidative activity (LOA). Preliminary studies on hemin dose response indicated that hemin, fed to broilers over a seven week period, significantly decreased body weight, as well as decreased feed intake when compared to control groups. Therefore, body weights (BW) were taken biweekly until termination of the experiment. Feed intake was observed throughout the experiment. Broilers were killed at 6 weeks of age.

### 2.2. Uric acid determination

Blood samples were collected and plasma uric acid ( $n=5$  per treatment group) was determined using a commercially available Uric Acid Reagent kit (Sigma Chemicals, St. Louis, MO, USA).

### 2.3. L-012-based chemiluminescence as a measurement of oxidative stress

Chemiluminescence techniques are functional assays to quantify the release of oxidants from cells or tissues (Van Dyke, 1987). Chemiluminescence measures oxidative mechanisms which emanate from white cells—a combination of neutrophils and macrophages. In addition it can be linked to the antioxidant level in the blood since the higher the luminescence, the less natural antioxidants exist in the blood. The combination of total antioxidant activity and oxidant expression provides a sensitive measurement of oxidant stress. L-012-based chemiluminescence (L-BCL) was used to determine the amount of LOA as described by Iqbal et al. (1999). Two mL of blood from 4- and 5-week-old birds ( $n=20$ ) was carefully layered on 2 mL of monopolyresolving medium in a  $13 \times 100$  mm #10 Falcon tube and leukocytes were isolated by centrifugation at 2000 g for 20 min. To a 3 mL luminometer tube was then added 100  $\mu$ L of leukocytes, 100  $\mu$ L L-012 solution, 200  $\mu$ L PBS (pH=7.4) and 100  $\mu$ L phorbol myristate acetate (PMA). The reaction tube was then placed into the luminometer (Berthold model LB 9505C) with the temperature set at 37 °C. Oxidative stress was determined by measuring the integrated luminescence generated over 20 min using KINB software. Results were reported as counts per minute (CPM). Measured luminescence was corrected based on the number of leukocytes present in each reaction tube.

### 2.4. Buffer mixture

Throughout both studies a buffer solution was used in order to solubilize hemin. The hemin-phosphate buffer solution (pH=7.4) contained 1 mL of dimethylsulfoxide (DMSO) and an amount of hemin (20 mg/kg BW) adjusted for each bird, according to weight.

### 2.5. Preliminary study for effect of inosine on kidney morphology

Ten four-week-old broilers, were divided into two groups consisting of inosine-treated ( $n=5$ ) and control birds ( $n=5$ ). On day 1 inosine was included in the grower diet of the inosine birds, at 0.6 M/kg of feed/day while the control birds were fed a grower diet. Birds in both control and inosine treatments were given feed and water *ad libitum*. After 7 days the birds were killed by cervical fracture, and the abdominal cavity was opened ventrally to reveal the kidneys. The dorsal aorta just cranial to the kidneys was cannulated, and 20 mL of 0.1 M phosphate buffer (pH 7.2) was injected to clear the kidneys of blood. Immediately following this procedure, 20 mL of 10% neutral buffered formalin (NBF) was injected to fix the kidneys. The kidneys were carefully dissected out of the synsacrum and immersed and stored in 10% NBF.

**Table 1**  
Effects of inosine on the kidney and nephron components when fed inosine diets for 1 week

Structure	Control	Inosine
Cortex	83.3±4.9	88.6±0.7
Medulla	8.6±2.9	6.0±1.3
Blood vessels	8.1±2.3	5.4±0.9
Glomeruli	11.1±2.9	10.2±2.4
Proximal tubules	63.9±2.6	63.5±3.2
Distal tubules	7.8±2.5	7.7±3.0
Cortical collecting tubules	2.9±1.4	4.4±3.3
Cortical capillaries	14.3±2.1	14.2±3.0
Thick limbs of Henle	42.7±0.4	42.3±3.1
Thin limbs of Henle	4.1±1.5	4.1±1.4
Collecting ducts	38.6±3.7	33.3±3.4
Medullary capillaries	14.6±2.9	20.3±4.1

Data are means±S.D. Kidney components include the cortex, medulla and major blood vessels. Renal cortical components include glomeruli, proximal tubules, distal tubules, cortical collecting tubules and capillaries. Renal medullary components include thick limbs of Henle, thin limbs of Henle, collecting ducts and capillaries.

The tissue was then processed routinely for light microscopy through a series of graded alcohols, toluene then into paraffin wax. The tissue was embedded in paraffin wax and cut at 5 µm in an unbiased manner at 10 equally-spaced intervals along its length (Mayhew, 1991). The resulting sections were stained with hematoxylin and eosin.

Volumes of the kidney components (cortex, medulla, major blood vessels) and nephron components (renal corpuscle, proximal tubule, loops of Henle, distal tubule, collecting ducts) were estimated by point counting using the Cavalieri Principle (Gundersen et al., 1988).

## 2.6. Experimental design study 1

At 4 weeks of age, 20 broilers were weighed, individually banded, and randomly divided into four groups: control, hemin injected (20 mg/kg body mass (BW) w/ buffer), inosine fed (0.6 M/kg of feed/day), and hemin/inosine. The broilers received a grower diet and drinking water *ad libitum*. On day 1, each bird ( $n=20$ ) was injected daily with 2 mL of buffer solution or hemin buffer solution. Throughout the study control birds ( $n=10$ ) were injected daily with a buffer solution, while hemin birds ( $n=10$ ) were injected daily intraperitoneally with a 20 mg/kg BW hemin buffer solution. Blood was drawn from the wing vein of each bird on day 8 and measured for levels of leukocyte oxidative activity (LOA) and plasma uric acid (PUA). On day 10, control and hemin birds were subdivided into inosine fed birds ( $n=5$ ) and hemin/inosine fed birds ( $n=5$ ). These birds were provided 0.6 M/kg of feed/day of inosine mixed in with the feed. Blood was then taken on day 15 and measured for levels of LOA and PUA. On day 16 the birds were euthanized.

## 2.7. Experimental design study 2

Four-week-old broilers ( $n=20$ ) were weighed, individually banded, and divided into four groups: control, hemin injected (20 mg/kg BW w/ buffer), inosine fed (0.6 M/kg of feed/day), and hemin/inosine. The broilers received a grower diet and drinking water *ad libitum*. Beginning on day 1, inosine/hemin birds ( $n=5$ ) were pretreated with 0.6 M/kg of feed/day inosine mixed in with their feed while control birds ( $n=15$ ) were given a standard grower diet with no supplementation. On day 3, blood samples were drawn and measured for LOA and PUA levels. After the bleeding, the control birds were further divided into control, hemin and inosine groups. Hemin buffer was then injected into the hemin and hemin/inosine groups and the standard buffer was injected into the control and inosine groups. At the same time, inosine was added to the diet of the inosine group. On day 5, blood samples were obtained and once again measured for levels of LOA and PUA. On day 6, the birds were euthanized.

## 2.8. Statistical analysis

One-way ANOVAs were used to compare the volumes of the kidney and nephron components. Significance was set at the 95% confidence interval for the preliminary study done on inosine dosage.

Data were analyzed by analysis of variance (ANOVA) and performed with PC SAS software (1995). A general linear models procedure was used to determine significant differences among treatment means. Fisher's least significant difference test was used to compare group means. Differences were considered significant at  $P\leq 0.05$ .

## 3. Results

### 3.1. Preliminary study on dosage of inosine on kidney morphology

One-way ANOVAs revealed that there was no difference in percent of cortex, medulla or major blood vessels between treatment groups. Further, our results indicated no differences between treatment groups in the components of the nephrons (renal corpuscle, proximal and distal tubule, loop of Henle, cortical collecting tubule, collecting ducts and capillaries) (Table 1).

### 3.2. Study 1

#### 3.2.1. Growth response

In contrast to the preliminary studies done on inosine dose response in which hemin was added to the diet, differences in body weight (BW) were significant between individual treatment groups over a 16 day period (Table 2). On day 1, birds were weighed for the first time revealing no significant differences in BW between treatments. On day 6, the birds were weighed revealing significant differences in BW between treatments ( $P<0.0237$ ). Body weights in the inosine group were significantly elevated as compared to both hemin-treated groups. Similarly, BW in the control group was also elevated as compared to the inosine/hemin group. No significant differences were found between the hemin-treated groups or between the control and inosine-treated groups. Again, on day 10, birds were weighed and a significant difference in BW was measured ( $P<0.014$ ). BW in the hemin/inosine-treated group was reduced as compared to the control and inosine-treated groups. No significant differences were found between the control and inosine groups or between the control and hemin-treated groups. On day 16 there was a significant difference in BW between treatments ( $P<0.004$ ). Control birds were heavier than both hemin-treated groups but were not significantly different from the inosine-treated group. The inosine-treated group differed from the hemin/inosine-treated group, but did not differ from the hemin group. For the duration of the study, control and inosine-treated groups never significantly differed in BW and hemin and hemin/inosine groups never differed in BW.

**Table 2**

Study 1: Growth performance of broilers administered hemin, followed by the inclusion of inosine in the diet

Body mass (kg)±S.D.				
Treatment	Day 1	Day 6	Day 10	Day 16
Control	1.44±0.11	1.65±0.09 <sup>ab</sup>	2.12±0.06 <sup>a</sup>	2.64±0.15 <sup>a</sup>
Hemin	1.36±0.12	1.49±0.11 <sup>bc</sup>	1.78±0.26 <sup>b</sup>	2.24±0.24 <sup>bc</sup>
Hemin/inosine	1.32±0.09	1.44±0.14 <sup>c</sup>	1.77±0.18 <sup>b</sup>	2.06±0.21 <sup>c</sup>
Inosine	1.39±0.12	1.68±0.17 <sup>a</sup>	2.09±0.23 <sup>a</sup>	2.37±0.24 <sup>ab</sup>
ANOVA <i>P</i> -value	0.434	0.024	0.014	0.004
Fischer's LSD value	–	0.177	0.264	0.286

Values are means±S.D. Means with different superscripts within each column differ significantly ( $P<0.05$ ).

**Table 3**

Study 1: Feed intake of broilers administered hemin, followed by the inclusion of inosine in the diet

Feed intake over 16 days	
Treatment	Feed intake (kg)
Control	9.34
Hemin	6.98
Hemin/inosine	7.33
Inosine	9.30

Descriptive data on the overall feed intake for each treatment group over a 16 day period.

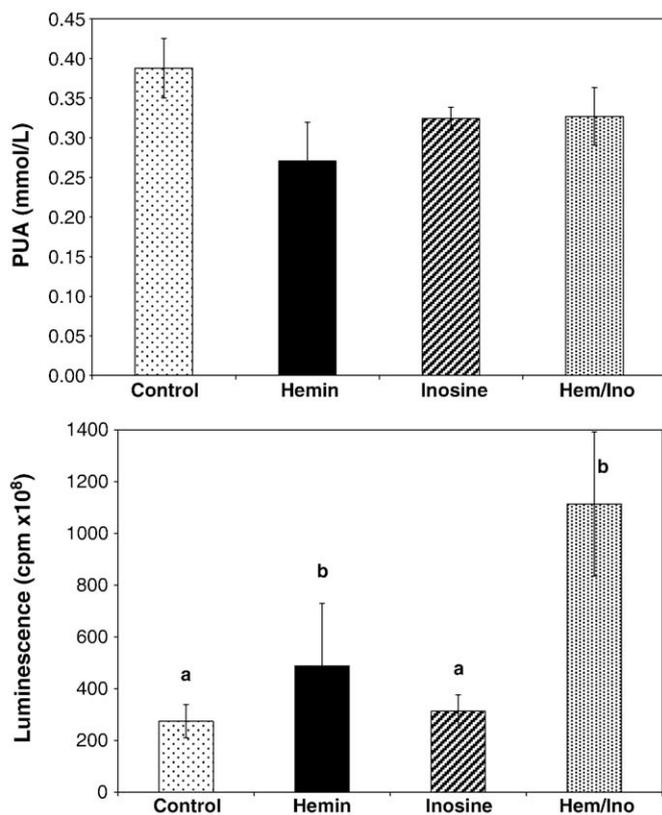
**3.2.2. Feed intake**

Feed intake differed between treatments (Table 3). The control group, as well as the inosine group consumed more feed (kg) than did the hemin or hemin/inosine groups over a period of 16 days.

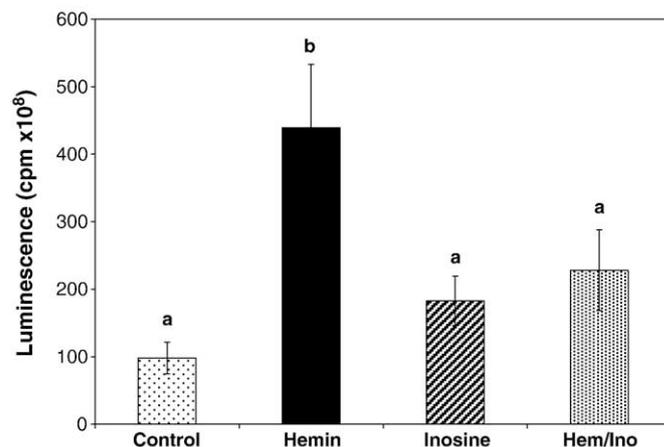
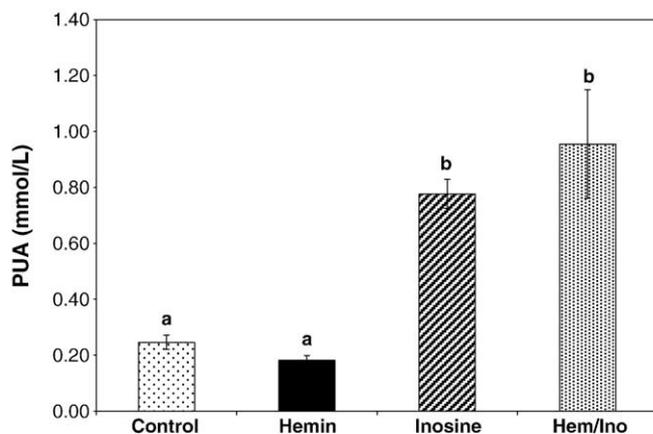
**3.2.3. PUA and LOA**

On day 8 the PUA concentration of hemin and hemin/inosine treatments was not different when compared to control and inosine treatments ( $P < 0.12$ ) (Fig. 1). On day 15 after the administration of inosine into the feed, PUA concentrations of inosine and hemin/inosine treatments were significantly higher ( $P < 0.0001$ ) compared to control and hemin treatments as shown in Fig. 2. Hemin treatment was contrasted against all other treatment groups and found to have lower PUA concentrations ( $P < 0.0007$ ).

The LOA on day 8 for hemin and hemin/inosine treatments was increased compared to control and inosine treatments ( $P < 0.0333$ ). In a one-way ANOVA for least significant differences, the hemin groups were significantly higher in measurements of LOA when compared to control and inosine groups ( $P \leq 0.05$ ) (Fig. 1). Once inosine had been added to the diet, results on day 15 indicated that the hemin/inosine



**Fig. 1.** Study 1: Effects of hemin on plasma uric acid concentrations (PUA) and measurements of leukocyte oxidative activity (LOA) (Day 8). All broilers fed a grower diet and injected (i.p.) with a control or hemin injection for 8 days. Treatments with different letters differ significantly ( $P \leq 0.05$ ).



**Fig. 2.** Study 1: Effects of hemin and inosine on PUA concentrations and measurements of LOA (Day 15). All broilers fed a grower diet and injected (i.p.) with a control or hemin injection for 15 days. All inosine treatments had inosine supplemented in the feed for 1 week. Treatments with different letters differ significantly ( $P \leq 0.05$ ).

treatment reduced LOA to levels measured in inosine fed birds on day 8 (Figs. 1 and 2). Control and inosine treatments continued to have low levels of LOA ( $P \leq 0.0044$ ). Fig. 2 also revealed that the hemin group maintained higher levels of LOA when compared to all other treatments ( $P \leq 0.001$ ).

**3.3. Study 2**

**3.3.1. Growth response**

Within the short duration of the study there were no significant differences in BW measured between any of the four groups (Table 4).

**3.3.2. PUA and LOA**

On day 3, the PUA concentration in the pretreated hemin/inosine group was significantly increased ( $P \leq 0.05$ ) when compared to the control birds (Fig. 3). On day 5, after the hemin injections and inosine

**Table 4**

Study 2: Effect of diet on growth performance with pretreatment of inosine

Pretreatment of inosine bird nasses (kg)	
Treatment	Average body mass (kg)
Control	1.93
Hemin	1.86
Hemin/inosine	1.84
Inosine	1.73

Descriptive data on average body weight of broilers pretreated with inosine for 1 week.

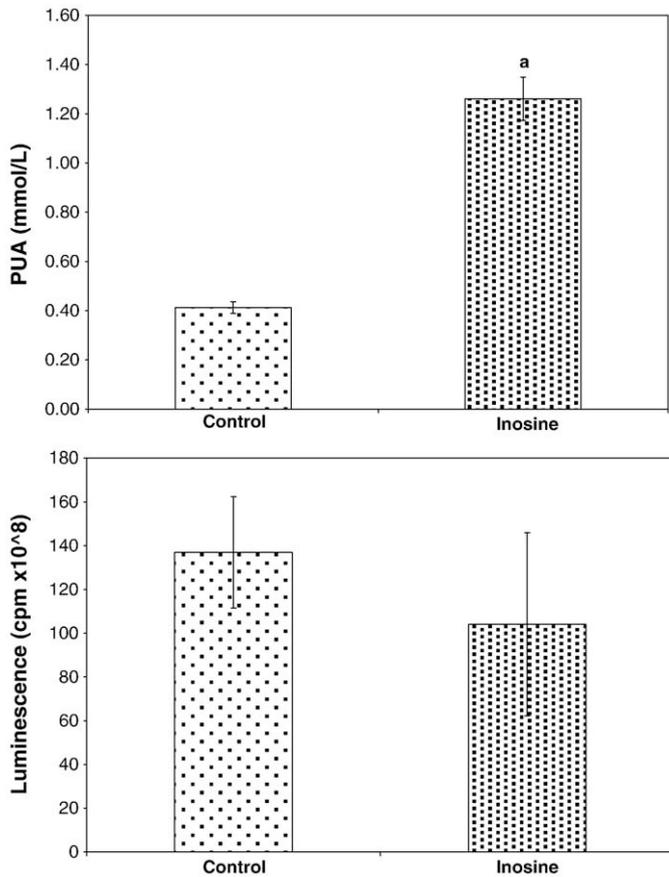


Fig. 3. Study 2: Effects of inosine on PUA concentrations and measurements of LOA (Day 3). All broilers fed a grower diet while inosine treatment was pretreated with purine supplemented feed. Treatments with a letter differ significantly ( $P \leq 0.05$ ).

supplementation, the PUA concentrations in inosine and hemin/inosine supplemented groups remained elevated as compared to control and hemin groups ( $P < 0.001$ ) (Fig. 4).

Comparison of LOA on day 3 for the control and hemin/inosine groups revealed no significant differences (Fig. 3). On day 5 measurements of LOA showed that all treatment groups had reduced levels of LOA as compared to the hemin group ( $P < 0.0033$ ). Inosine, as well as hemin/inosine groups had levels of LOA that were not significantly different from that of the control group (Fig. 4).

#### 4. Discussion

It has been established that birds have remarkable longevity for their body size (Holmes and Austad, 1995) despite the fact that they also have much higher metabolic rates, body temperatures and blood glucose concentrations when compared to mammals (Ogburn et al., 1998). Theoretically, birds should sustain more damage from processes that lead to ROS. However, the exact opposite occurs which would suggest that birds have a highly developed mechanism for dealing with oxidative stress. Studies have shown that uric acid plays a significant role in limiting oxidative stress and thus increasing uric acid concentrations via inosine supplementation reduces oxidative stress in chickens (Simoyi et al., 2002).

In previous studies, the inclusion of inosine (0.6 mol/kg) in the diet of broilers for 3 weeks increased plasma concentrations of uric acid and decreased oxidative stress (Simoyi et al., 2002). In agreement with this study the inclusion of inosine in the diets of broilers at a dose of 0.6 mol/kg for 7 days resulted in an increase in concentrations of plasma uric acid concomitant with a decrease in oxidative stress. However, Simoyi et al. showed that inosine, given over a three week

period of time, increased kidney weight threefold compared to controls. Our results indicated that the administration of inosine for a seven day period did not affect kidney weight or ultrastructure (Table 1). In the avian kidney, uric acid is bound to a protein in the proximal tubule, and chronic increased uric acid levels may lead to a change in the morphology of the kidney (Simoyi et al., 2002). This suggests that short bouts of inosine included in the diets of broilers may be advantageous in treating oxidative damage.

In a previous study, Klandorf et al. (2001) showed that hemin was associated with an enhanced elevation of oxidative stress in broilers. Hemin is a rich source of iron, which generates ROS via Fenton chemistry. A simple mixture of an iron salt and  $H_2O_2$  can set into motion, a chain of events that will lead to the generation of free radicals and/or oxidants (Halliwell and Gutteridge, 1990). From this, we concluded that by supplementing broiler diets with hemin we could increase oxidative stress and therefore could potentially develop a model for testing the efficacy of certain compounds to determine whether or not they could serve as a useful antioxidant or treatment against oxidative damage.

In the preliminary studies, the graded administration of hemin to the diets of the broiler chicks resulted in characteristic dose dependent increases in oxidative stress as well as a significant decrease in concentrations of uric acid. However, after 3 to 7 days the levels of oxidative stress decreased and uric acid returned to control levels. The reduction in oxidative stress was associated with a reduction in food intake, which resulted in a decline in body weight. A possible explanation for this was that the palatability of the diet was reduced, which led to the decline in food intake. Once they stopped

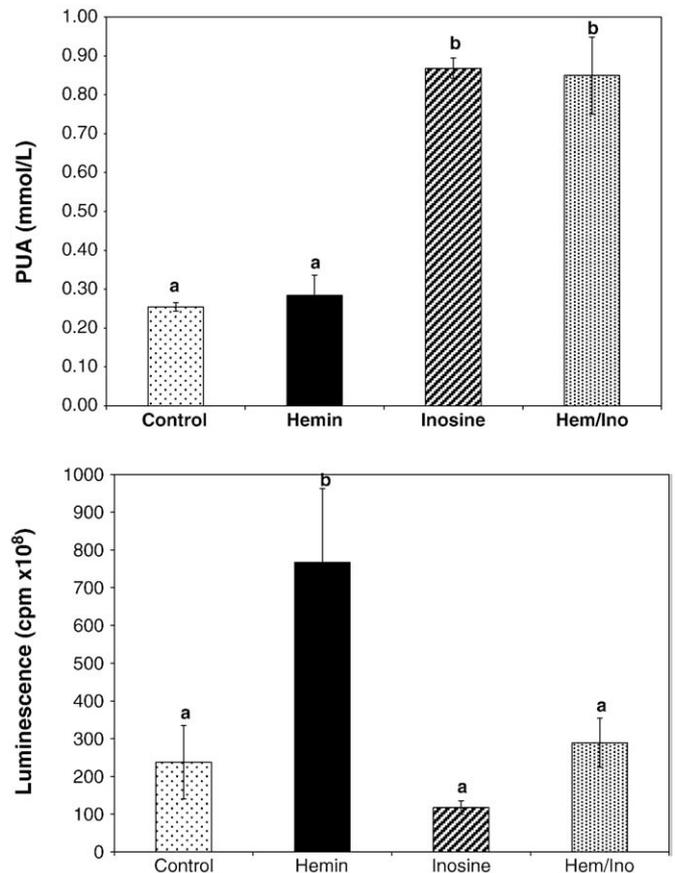


Fig. 4. Study 2: Effects of inosine and hemin on PUA concentrations and measurements of LOA (Day 5). All broilers fed a grower diet and injected (i.p.) with a control or hemin injection for 2 days. Inosine treatment was pretreated with purine supplemented feed for 5 days. Treatments with different letters differ significantly ( $P \leq 0.05$ ).

consuming the diet, they reduced the consumption of hemin, which lead to a decrease in oxidative stress. For each of these preliminary studies we found inconsistent increases in oxidative stress in hemin-treated birds, which led to the conclusion that the intake of hemin was variable among the birds. For this reason we needed to ensure a more reliable way to administer the correct dose of hemin in order to consistently increase oxidative stress. Thus in the two principal studies we injected the birds with hemin rather than by administering it in the feed. Birds were injected daily (i.p.) to insure that they each received the correct dose of hemin according to body weight. Once we had established a steady state level of oxidative stress, where measurements of LOA remained elevated, then we could supplement inosine into the diet.

In our first study we demonstrated that dietary supplementation of inosine effectively reduced hemin-induced oxidative stress in vivo. During the first phase of the study, hemin significantly increased the onset of oxidative stress and decreased uric acid levels in broilers injected with hemin before inosine was supplemented into the diets (Fig. 1). Birds injected with hemin had significantly reduced body weight (Table 2) when compared to birds that were being injected with a placebo. Once inosine was supplemented into the diet, concentrations of uric acid were increased (Fig. 2) while oxidative stress declined almost to control levels (Fig. 2).

Our second study demonstrated that pretreatment with inosine limited the development of oxidative stress in hemin injected broilers. Birds were supplemented with inosine for 3 days, which resulted in a significant increase in uric acid (Fig. 3), which was not attenuated by hemin administration (Fig. 4). However, pretreatment with inosine significantly attenuated the increase in oxidative stress in hemin injected broilers (Fig. 4).

In conclusion, the present investigation specifically demonstrates that inosine inclusion increases uric acid, which can ameliorate the effects of hemin-induced oxidative stress. Further, the administration of inosine, a precursor to uric acid, has the ability to either limit the increase in oxidative stress associated with a pro-oxidant or reduce the magnitude of a stress in an animal already oxidatively stressed. Reductions in uric acid concentrations have previously been observed in humans with multiple sclerosis (Koprowski et al., 2001) and Parkinson's disease (Schwarzschild et al., 2008) and treatment with inosine of patients with multiple sclerosis has been demonstrated to ameliorate the symptoms of the disease. Inosine may thus represent a treatment modality for disorders associated with inflammation and oxidative stress.

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