

Peroxide Status in Tissues of Heat-Stressed Broilers

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ABSTRACT : The peroxidation status of tissues was estimated in broilers under acute or chronic heat stress (32°C, 24 h, 5×24 h) in the present study. The results showed that the lipid peroxide (LPO) concentrations in plasma and liver were elevated ($p<0.05$) by acute heat stress, and were not influenced in kidney ($p>0.05$). At the same time, no significant change of superoxide dismutase (SOD) activity in the liver, kidney or plasma was observed. Under chronic heat exposure, the SOD activity in liver was increased ($p<0.05$) and the LPO concentrations in the liver and plasma were restored to the normal levels. The LPO level in kidney was not affected by chronic heat stress ($p>0.05$), but SOD activity was significantly decreased ($p<0.01$). The results suggested that the peroxidation was induced by acute heat stress and disappeared along with the time of heat exposure, and the peroxidation reactions were different among tissues. (*Asian-Aus. J. Anim. Sci.* 2000. Vol. 13, No. 10 : 1373-1376)

Key Words : Broiler, Heat Stress, Peroxidation, Superoxide Dismutase, Lipid Peroxide

INTRODUCTION

Free radicals are chemicals that have one or more unpaired electrons in their outer orbit. The free radical is produced in normal metabolism processes of the body. There are different kinds of free radicals that comprise oxygen radicals and cellular sources of free radicals. The oxygen radicals are the most important and consist of superoxide, hydroxyl, hydrogen peroxide, and nitric oxide radicals. It has been estimated that as much as 2% of the oxygen consumed by mitochondria is used to form superoxide radicals (Chance et al., 1979).

In normal conditions, the generated free radicals are extinguished by the antioxidant protective system, which includes protective compounds and enzymatic systems. The antioxidant protective compounds include vitamin E, β -carotene, ascorbic acid, glutathione, uric acid, etc. The protective enzymatic systems comprise superoxide dismutase (SOD), catalase (CAT), and GSH recycling enzymes (GSH peroxidase and GSH reductase) (Duthie et al., 1989).

If the amount of free radicals exceeds the reduction capacity of the antioxidant protective system, the free radicals can attack the polyunsaturated fatty acids (PUFA) of membrane and results in lipid peroxidation. Lipid peroxidation has numerous detrimental effects on cell function, such as the crosslinking of proteins resulting from the peroxidation which can inactivate membrane bound enzymes, and the physical changes within the membrane can alter tertiary structure of membrane proteins. So the free

radical can cause damage to cell structure and function. Cong and Fang (1992) reported that the free radical damage occurred in rats that suffered from γ -ray. Song and Gao (1995) reported that the lipid peroxide content in plasma and liver was elevated significantly in rats exposed to a cold environment. Mocanu et al. (1993) reported that the CAT activity rose in heat-stressed rats. Though many studies have been conducted to investigate the harmful effect of heat stress on growth performance (Deyhim and Teeter, 1991; Perket and Qureshi, 1992), nutrients metabolism (Al-Batshan and Hussein, 1999; Hussein and Al-Batshan, 1999), and the biochemical indices (Lin et al., 2000) of birds, little information about peroxide status of the animal body is known for birds suffering heat stress.

The present experiment was conducted to evaluate the effect of heat stress on the peroxide status of broilers.

MATERIALS AND METHODS

Experimental animal

A single factor experiment was conducted in this study. Forty-eight broilers six-weeks of age were used and divided into three groups according to body weight. All the experimental birds were raised at 18-20°C and 50-60% relative humidity (RH). The light regime was 23L:1D. The birds had free access to feed and water during the experimental period. The experimental diet was formulated on the basis of recommendations by the NRC (1994) (table 1).

Experimental methods

Tissue: As the experiment began, the birds of control group were maintained at 20°C, and the birds of the two experimental groups were exposed to an environment of 32°C with 60% RH. After 24 h heat

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Received March 16, 2000; Accepted May 10, 2000

exposure, 8 birds of each group were chosen to provide a blood sample via wing vein and then killed. The liver and kidney were taken and washed with ice cold 0.9% NaCl (the liver was perfused via portal vein). After washing with physiological water, the tissues were homogenized in a ratio of 1 g of wet tissue to 9 ml of 1.15% KCl by using a glass homogenizer. After 5 d heat exposure, the above procedure was repeated and the blood and tissues were taken from another 8 birds.

Chemical analysis: The lipid peroxide (LPO) content in liver and kidney was determined by the method of Ohkawa et al. (1979) as following: 0.2 ml of plasma anticoagulated with heparin was mixed with 4.0 ml of 1/12 N H₂SO₄ and 0.5 ml of 10% phosphotungstic acid. After 5 min, the mixture was centrifuged for 10 min at 3000 rpm and the upper liquid was removed. The deposit was purified with the mixture solution containing 2.0 ml of 1/12 N H₂SO₄ and 0.5 ml of 10% phosphotungstic acid, and centrifuged for 10 min at 3000 rpm. The purified deposit was mixed with 2.0 ml distilled water and 0.5 ml of 0.67% thiobarbituric acid (Sigma Comp.), and the mixture was heated at 100°C for 60 min. After cooling with tap water, 2.0 ml of the mixture of n-butanol and pyridine (15:1, v/v) was added to the mixture and shaken for 1 min. The absorbance of the organic layer was measured at 532 nm with UVIKON 810 ultravioletsemester after centrifugation at 4000 rpm for 10 min. In the standard tube, 0.2 ml of 1,1,3,3-tetramethoxypropane (Sigma product) was used to replace the deposit of test-tube.

The SOD activity of tissue and plasma was determined by a modified method of pyrogallol acid

autoxidation (Deng et al., 1991). The autoxidation rate was determined by the following procedure. At 25°C, 10 ml of 50 μl/L pyrogallol acid was mixed with 4.5 ml of 50 mmol/L, pH 8.30 K₂HPO₄-KH₂PO₄, the absorbency was measured at 325 nm every 30 s, and the autoxidation rate was required to be controlled at 0.070 OD per min. The SOD activity was measured when the pyrogallol acid was added to the mixture containing 10 μl sample (V2) and 4.5 ml of K₂HPO₄-KH₂PO₄ buffer, and the absorbency (A) of the mixture at 325 nm was recorded every 30 second. The activity of SOD was calculated by the formula:

$$\text{SOD (unit/ml): } [(0.070-A/\text{min})/0.070$$

$$\times 100\%]/50\% V1/V2$$

V1: volume of final mixture, ml;

V2: volume of sample, ml.

Statistical analysis

Data obtained were analyzed by using variance procedure of SAS package (1989), and treatment means were compared using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The results showed that there were no significant changes in activity of SOD of plasma, liver and kidney ($p>0.05$) after 24-hour heat exposure. The LPO concentration was elevated in the liver ($p<0.05$), and plasma ($p<0.05$), but unaffected in the kidney ($p>0.05$). For a longer time of heat exposure (5×24 h), SOD activity was increased significantly in the liver ($p<0.01$), but decreased significantly in kidney ($p<0.01$), and was not changed significantly in plasma ($p>0.05$). The LPO levels in the liver, kidney and plasma had no significant difference in 5-day heat exposure group ($p>0.05$), compared to the control group (table 2, 3).

When the animal was suffering from heat stress, the secretion of catecholamine (including epinephrine and norepinephrine) by chromaffin tissue of adrenal gland would be increased (Edens and Siegel, 1975). As the chromaffin part was innervated by the sympathetic nervous system, so the secretion of

Table 1. Composition of experimental diet

Ingredients	%	Calculated analysis
Corn	64.88	ME (kJ/kg) 13.41
Soybean meal (44% CP)	24.53	CP (%) 18.0
Corn oil	4.44	Ca (%) 0.83
Fish meal	3.00	EP (%) 0.36
Bone meal	1.00	Met (%) 0.33
Limestone	1.00	Lys (%) 0.90
DL-Methionine	0.03	
Salt	0.12	
Mineral and vitamin premix ¹	1.00	

¹ Mineral and vitamin premix provided per kg of diet: manganese, 100 mg; zinc, 75 mg; iron, 80 mg; iodine, 0.65 mg; copper, 80 mg; selenium, 0.35 mg; retinyl acetate, 0.008 MIU; cholecalciferol, 0.002 MIU; vitamin E, 0.009 MIU; vitamin K, 0.6 mg; thiamin, 1.0 mg; riboflavin, 4.5 mg; niacin, 56 mg; pantothenic acid, 8.2 mg; pyridoxine, 1.9 mg; biotin, 0.05 mg; folic acid, 0.6 mg; vitamin B₁₂, 0.009 mg; and choline chloride, 400 mg.

Table 2. The effect of heat stress on the activity of SOD in plasma, liver and kidney (Unit/ml, Unit/g, wet weight)

	Control group	HS group, 24 h	HS group, 5 × 24 h
Plasma	259.5 ± 27.2	258.3 ± 39.1	274.8 ± 40.4
Liver	585.1 ± 89.5 ^B	617.6 ± 75.9 ^B	701.5 ± 65.3 ^A
Kidney	672.1 ± 112.8 ^A	630 ± 74.8 ^A	388.1 ± 53.0 ^B

^{A,B} Means within the same row with different superscript differ significantly ($p<0.01$).

catecholamine in response to stress was immediate (Hillman et al., 1987). Haggendal et al. (1987) reported that experimental stress (restraint stress) could result in the reaction of sympathetic nervous system and adrenal system, and resulted in the release of catecholamine which could increase free radicals and induce injury of heart cell. Singal et al. (1982) reported that catecholamine-induced changes involve free radicals, by promoting lipid peroxidation which increases membrane permeability. When the production of free radicals increased, lipid peroxidation in cells occurred and the content of lipid peroxide (LPO) rose (Duthie et al., 1989). So the concentration of LPO was an effective index to reflect the peroxidation-state of tissue. Cong and Fang (1992) reported that the free radical damage occurred in rats suffered γ -ray and observed the increase in LPO content. Song and Gao (1995) reported that the lipid peroxide content of plasma and liver was elevated significantly in rats exposed to a cold environment. In the present experiment, the LPO concentrations in plasma and liver were increased in the birds exposed to high temperature for 24 h, which indicated the acute heat stress induced peroxidation of the tissues. The increase in LPO concentration might be caused by two possible reasons. One might be an increase in production of free radicals that resulted in the elevation of LPO. Another reason might be a decrease of activities of protective enzymes. The protective enzymatic systems, which comprised superoxide dismutase (SOD), catalase (CAT), and GSH recycling enzymes, could reduce the amount of free radicals and repair the free radical damage of tissue. Among these enzymes, SOD is most important and it catalyzes the dismutation of O_2 to H_2O_2 . So the changes in the enzyme activity under heat stress might have an important effect on the free radicals damage. Our results showed that the activities of SOD in different tissues were not affected by acute heat exposure, compared with control group. That indicated the increase of LPO concentration was induced by elevated production of free radicals, and was not the result of a reduction in SOD activity.

The results of our experiment showed that the LPO content restored to the normal level at the fifth

day of heat exposure. That means the free radicals damage disappeared. This change coincided with the change of SOD activity in the experiment, which showed the SOD activity in the liver was increased significantly by a long period of heat exposure ($p < 0.01$). The results suggested the elevated activity of SOD resulted in the reduction of LPO concentration in liver. As the activity of SOD was not increased at the first 24 h of heat exposure, this result supported the conclusion of Song and Gao (1995) who indicated that the elevated activity of SOD was the compensation to elevated LPO concentration. Mocanu et al. (1993) reported that heat stress could result in a rise of CAT, and the increase of CAT was thought to be implicated in a rise of antioxidation ability of tissue. The activity of SOD in the plasma had no significant change, though it had a trend to be increased along with the extension of heat exposure time.

However, unlike the changes in liver and plasma in the experiment, no significant change of LPO content was observed in the kidney ($p > 0.05$) at different heat exposure times, and the SOD activity was not affected by acute heat stress, but was decreased by long term heat exposure. The results suggested that the lipid peroxidation process in different tissues might respond differently to heat stress. The reasons need to be investigated further.

IMPLICATIONS

Acute heat stress (32°C, 24 h) resulted in a significant increase in LPO levels in plasma and liver of broilers ($p < 0.05$), and had no significant effect on SOD activity in liver and kidney. When the exposure time was prolonged, the SOD activity of liver was elevated significantly, and meanwhile the LPO concentration of liver and plasma restored to normal level. The SOD activity of kidney was decreased by long term heat exposure, but the LPO level was not affected. The results suggested that the peroxidation only takes place in some organs of heat-stressed broilers, and the peroxidation disappears along with arousing of antioxidation ability.

REFERENCES

- Al-Batshan, H. A. and E. O. S. Hussein. 1999. Performance and carcass composition of broilers under heat stress: I. The effects of dietary energy and protein. *Asian-Aus. J. Anim. Sci.* 12:914-922.
- Hussein, E. O. S. and H. A. Al-Batshan. 1999. Performance and carcass composition of broilers under heat stress: II. The effects of dietary lysine. *Asian-Aus. J. Anim. Sci.* 12:923-931.
- Chance, B., H. Sies and A. Boveris. 1979. Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* 59:527-605.

Table 3. The effect of heat stress on the level of LPO in plasma, liver and kidney (nmol/ml, nmol/g, wet weight)

	Control group	HS group, 24 h	HS group, 5×24 h
Plasma	13.2 ± 5.2 ^b	22.3 ± 7.5 ^a	14.2 ± 6.0 ^b
Liver	178.0 ± 35.3 ^b	212.3 ± 23.7 ^a	170.2 ± 27.7 ^b
Kidney	317.2 ± 64.4	272.2 ± 66.2	316.2 ± 41.1

^{a,b} Means within the same row with different superscript differ significantly ($p < 0.05$).

- Cong, J. and Y. Z. Fang. 1993. Stress-induced oxidative injuries. *Advances in Free Radical Life Sciences*. 1:28-34.
- Deng, B. Y., Q. S. Yuan and W. J. Li. 1991. Modified method of pyrogallol acid autooxidation to measure SOD activity. *Progress in Biochemistry & Biophysics*. 18:163.
- Deyhim, E. and R. G. Teeter. 1991. Sodium and potassium chloride drinking water supplementation effects on acid-base balance and plasma corticosterone in broilers reared in thermoneutral and heat-distressed environment. *Poult. Sci.* 60:1583-1586.
- Duncan, D. B. 1955. Multiple range and multiple F test. *Biometrics*. 11:1-42.
- Duthie, G. G., K. W. J. Wahle and W. P. T. James. 1989. Oxidants, antioxidants and cardiovascular disease. *Nutr. Res. Rev.* 2:52-62.
- Edens, F. W. and H. S. Siegel. 1975. Adrenal responses in high and low ACTH response lines of chickens during acute heat stress. *Gen. Comp. Endocrinol.* 25:64.
- Haggendal, J., L. Jonsson, G. Johansson, S. Bjurström, J. Carlsten and K. Thoren-Tolling. 1987. Catecholamine induces free radicals in myocardial cell necrosis on experimental stress in pigs. *Acta Physiol. Scand.* 131:447-452.
- Hillman, P. E., N. R. Scott and A. van Tienhoven. 1987. Physiological responses and adaptations to hot and cold environments. In: *Stress Physiology in Livestock*, V. III. Poultry, M. K. Yousef, CRC Press Inc. pp. 43-54.
- Lin, H., R. Du, X. H. Gu, F. C. Li and Z. Y. Zhang. 2000. Study on the Plasma Biochemical Indices of Heat-stressed Broilers. *Asian-Aus. J. Anim. Sci.* (In press).
- Mocanu, M. M., S. E. Steare, M. C. W. Evans, J. H. Nugent and D. M. Tellon. 1993. Heat stress attenuates free radical release in the isolated perfused rat heart. *Free Rad. Biol. Med.* 15:459-463.
- National Research Council. 1994. *Nutrient Requirement of Poultry*, 9th Ed. National Academy Press, Washington, DC.
- Ohkawa, H., N. Ohishi and K. Yagi. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochem.* 95:351-358.
- Perket, P. R. and M. A. Qureshi. 1992. Performance and immunity of heat-stressed broilers fed vitamin- and electrolyte-supplemented drinking water. *Poult. Sci.* 71:88-97.
- SAS. 1989. *SAS User's Guide: Statistics*, SAS Institute Inc., Cary, NC, U. S. American.
- Singal, P. K., N. Kapur, K. S. Dhillon, R. E. Beamish and N. S. Dhalla. 1982. Role of free radicals in catecholamine-induced cardiomyopathy. *Can. J. Physiol. Pharmacol.* 60:1390-1397.
- Song, Z. H. and Y. Gao. 1995. Effect of sea buckthorn oil and vitamin C on lipid peroxidation of rat after cold exposure. *Acta Nutrimenta Sinica.* 17:27-31.