African Journal of Biotechnology Vol. 9(35), pp. 5775-5778, 30 August, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.1748
ISSN 1684–5315 ©2010 Academic Journals

Full Length Research Paper

Increase of hepatic nitric oxide levels in a nutritional model of fatty liver in broiler breeder hens

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Accepted 2 July, 2010

Fatty liver hemorrhagic syndrome (FLHS) is a metabolic condition of laying hens characterized by the accumulation of excess fat in the liver and liver hemorrhage. It suggested that oxidative damage to the cellular and organelle membranes of the liver increases the susceptibility of the liver to hemorrhage. However, a local chemical component that may affect the incidence of hemorrhage in liver, by affecting the blood pressure of liver, is nitric oxide. To clarify the effects of high (20 and 40% more than normal) and low (20% less than normal) food intake on plasma and liver oxidative products, malondialdehyde (MDA) and protein carbonyl (PC), and also nitric oxide (NO) in over fed broiler breeder hens, 198 hens (30 weeks old) were fed for 30 days (two 14-day period). Feed intake, body weight (BW), egg production (EP), plasma NO, MDA and CP were measured at the end of each 14-day periods. Food intake did not reduce during 4 weeks of experiment in hens fed with 20 and 40% above their estimated requirement. Increased food intake resulted in increased body weight gain significantly ($P < 0.05$). Egg production significantly declined in hens provided with C+40% diet ($P < 0.05$). Liver MDA, NO and PC concentrations were increased in C+40% hens in second period of experiment ($P < 0.05$). Liver hemorrhage score of hens fed C+40% diet significantly was higher in the second period of the experiment. It was concluded that an increase in liver hemorrhage in over fed broiler breeder can be associated with the oxidative stress components (MDA, PC) and liver NO concentration.

Key words: Fatty liver, nitric oxide, malondialdehyde, protein carbonyl, broiler breeder.

INTRODUCTION

In broiler breeder hens, the capacity for rapid early growth coupled with free access to feed leads to enhanced adult fatness (Havenstein et al., 2003). These undesirable outcomes appear to arise from increases in food intake that occurred concomitantly with genetic selection for rapid early growth (Barbato, 1994; Richards 2003). In adulthood, persistence of the trait of increased, voluntary food intake results in actual food intakes in excess of the requirement for optimal adult health and performance as indicated by reduced livability and increased incidence of metabolic disease such as ascites, fatty liver, and kidney syndrome (Griffin and Goddard, 1994; Robinson et al., 1993; Julian, 1998). Chen et al. (2006) reported nearly 2-fold increase in liver hemorrhage score (LHS) of hens fed 290 g of feed/hen per day (feed satiated hens) in comparison to hens fed 145 g of feed/hen per day, which reflects liver steatosis that was associated with increased liver hemorrhage.

It suggested that oxidative damage to the cellular and organelle membranes of the liver increases the susceptibility of the liver to hemorrhage (Squires and Leeson, 1988; Spurlock and Savage, 1993). Oren et al. (2009) reported that triglyceride (TG) accumulation in primary

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Abbreviations: FLHS, Fatty liver hemorrhagic syndrome; **MDA,** malondialdehyde; **PC,** protein carbonyl; **NO,** nitric oxide; **BW,** body weight; **EP,** egg production; **TBARS,** thiobarbituric acid-reactive substances; **LHS,** liver hemorrhage score; **TG,** triglyceride; **ME,** metabolisable energy; **SEM,** standard error of mean.

hepatocytes leads to oxidative stress. This in turn causes disturbances in the normal physiological functions of the body by way of increase in free radical generation. Oxygen radicals have been shown to catalyze the oxidative modification of lipids resulting in lipid peroxidation. Lipid peroxides are formed by auto-oxidation of polyunsaturated fatty acids primarily in cell membranes resulting in membrane damage (Kawamura et al., 1992). Malondialdehyde (MDA) is a by-product of lipid per-oxidation used as an index of the rate of tissue reaction chain. In addition, MDA is used as an indicator of oxidative stress in cells and tissues (Bouchard et al., 1999; Madebo et al., 2003). Another product of oxidative stress in higher animals, which was used as an index of protein oxidation, is protein carbonyl (PC).

Furthermore, feed-satiated hens had significantly greater plasma leptin and insulin concentrations (Chen et al., 2006). Leptin and insulin share some common signaling mechanisms, including activation of phosphatidylinositol 3-kinase (Kim et al., 2000), which is involved in insulin-stimulated NO synthesis (Zeng et al., 2000). As far as we know., there is no previous research that studied MDA, PC and NO levels relevant to fatty liver hemorrhagic syndrome in hens. In this study, we aimed to investigate the changes of MDA, PC, and NO levels in plasma and liver of broiler breeder hens fed with different level of feed intake.

MATERIALS AND METHODS

Birds management, laying performance and sampling

One hundred and ninety-two (192) commercially reared arbor acres plus fast feathering broiler breeder hens (30 weeks old) were used for this study. Hens were given the same regime in accordance with Arbor Acres Plus nutritional recommendations. Hens were fed by soybean and corn based breeder layer mash that provided 11.5 MJ of metabolisable energy (ME)/kg and 155 g/kg crude protein of diet. Diet composition was calculated from the published values for feed ingredients (NRC, 1994). Feed was placed between 0700 to 0730 h within a 15 L: 9 D photoperiod in which lights were turned on at 0600 h. Hens had free access to water throughout the experiment. Egg production and food intake were recorded daily. Hens were allocated to four different levels of food intake: Control (163 g/day), T (-20%) (20% less than control food intake, that is, 130.4 g/day), T (+20%) (20% more than control food intake, that is, 195.6 g/day) and T (+40%) (40% more than control food intake, that is, 228.2 g/day) with 6 replicates and 8 hens per each for 30 days. Eggs were collected from each group at 24 h intervals. Whole eggs were weighed on an electronic balance.

At the end of 15 and 30 days of experiment (period 1 and 2, respectively), 24 birds from each group were anesthetized and then killed and weight of liver was measured and liver steatosis and hemorrhage were judged using a 5-point scale (Walzem et al., 1993). Immediately, after LHS determination, liver samples were frozen in liquid nitrogen and stored at −80°C for subsequent analysis.

However, blood samples were collected into the anticoagulant tubes from each bird and then were centrifuged at 3000 ×g for 10 min and plasma were collected. Blood samples were kept on ice and protected from light until they are processed to prevent any artifactual oxidation during the experiment. Plasma samples were stored at −20°C until assayed for plasma MDA, PC and NO.

Determination of total protein

The total protein in the liver tissue was determined by a method modified from that of Lowry and colleagues (Peterson, 1979).

MDA, PC and NO analysis

Lipid peroxidation was determined as thiobarbituric acid-reactive substances (TBARS) in plasma and liver samples by method of Placer et al. (1966). The values of TBARS material were expressed in terms of malondialdehyde (MDA, nmol/ml plasma or nmol/g tissue). Protein Carbonyl ELISA Kit (STA-310; Cell Biolabs, Inc., San Diego) analyzed the PC of the plasma samples. The carbonyl content of the liver of control and treated animals was assayed by the method of Levine et al. (1990) with some modifications (cited by Parihar et al., 2003). Plasma NO concentration was measured by the colorimetric assay kit (Nitric Oxide Assay Kit Shinjuku, Tokyo, Japan plasma). Liver NO was measured by the method described by Satish et al. (2006).

Statistical analysis

All results are represented as mean ± SEM. Comparisons were made between control and treatment (T) groups using one-way ANOVA (Duncan's multiple range tests) (SAS package, Version 8.2, SAS Institute, Cary, NC), with $P < 0.05$ accepted as significant.

RESULTS

Egg production traits

Food intake did not reduce during 4 weeks of experiment in hens have fed with 20 and 40% above their estimated requirement. Average body weight of hens is shown the in Table 1. Increased food intake resulted in increased body weight gain significantly ($P < 0.05$). Hens fed with 20% lower food intake of their requirement have shown decrease in body weight. Egg production was significantly ($P < 0.05$) affected by the level of feed intake, but the most reduction belong to hens fed C+40% diet. One week after the hens were provided with C+40% of feed, egg production declined and reached 77.2% for the first period of experiment and ultimately reached 70.1% for the second period ($P < 0.05$).

Plasma and liver NO, MDA and PC concentrations

In the second period of experiment, for malondialdehyde (MDA), a lipid peroxidation product, the experiment shows that hens fed either C+20% or C+40% diets had significantly ($P < 0.05$) higher levels of plasma MDA and liver MDA and there was no significant difference in plasma MDA between groups in the first period of the study (Table 3).

During the first period of experiment, plasma and live

Table 1. Egg production and LHS in broiler breeder hens

Parameters	Period 1				Period 2			
	Control	C-20%	C+20%	C+40%	Control	C-20%	C+20%	C+40%
EP (%)	83.1^{a} ±0.52	81.6^{a} ±0.84	83.0^{a} ±1.00	77.2^{b} ±1.20	81.6^{a} ±1.05	78.0^{b} ±0.96	76.3^{b} ±0.87	70.1^{c} ±1.95
BW (gr)	3416^{c} ±44.0	3181^{d} ±45.5	3553^{b} ±50.2	3730^{a} ±48.0	3514^{c} ±47.5	3107^{d} ±49.1	3692^{b} ±46.1	4071^{a} ±51.0
LHS	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00	0.43 ±0.28

Mean values bearing different superscripts (a, b, c) within a row in each period differ significantly (P < 0.05).

Table 2. Effect of feeding level on plasma and liver NO concentrations

Parameters	Period 1				Period 2			
	Control	C-20%	C+20%	C+40%	Control	C-20%	C+20%	C+40%
Plasma NO (µM/L)	35.7 ±3.8	36.8 ±4.6	38.4 ±5.0	40.1 ±5.0	32.8^{c} ±2.3	44.3^{b} ±3.8	39.9^{bc} ±3.4	59.1^{a} ±3.2
Liver NO (nmol/mg protein)	3.5 ±0.21	3.4 ±0.19	3.6 ±0.23	3.9 ±0.24	3.1^{b} ±0.20	3.2^{b} ±0.21	3.4^{b} ±0.27	4.4^{a} ±0.28

Mean values bearing different superscripts (a, b, c) within a row in each period differ significantly (P < 0.05).

Table 3. Effect of feeding level on plasma and liver PC and MDA concentrations

Parameters	Period 1				Period 2			
	Control	C-20%	C+20%	C+40%	Control	C-20%	C+20%	C+40%
Plasma PC (nmol/ml)	10.42 ±1.6	12.45 ±1.9	11.28 ±2.2	14.62 ±2.0	11.79 ±1.4	10.67 ±1.5	13.91 ±1.8	18.31 ±2.6
Liver PC (nmol/mg protein)	20.13 ±2.4	25.27 ±1.8	21.65 ±2.3	26.52 ±2.8	19.71^{b} ±1.6	20.14^{b} ±2.5	25.91^{b} ±2.6	40.84^{a} ±3.5
Plasma MDA (nmol/ml)	2.68 ±0.25	2.57 ±0.26	2.82 ±0.27	3.01 ±0.28	2.51^{bc} ±0.23	2.06^{c} ±0.21	3.06^{ab} ±0.27	3.43^{a} ±0.29
Liver MDA (nmol/mg protein)	3.31 ±0.32	3.51 ±0.31	3.76 ±0.31	3.93 ±0.35	3.55^{b} ±0.33	2.86^{c} ±0.29	4.16^{ab} ±0.30	4.57^{a} ±0.37

Mean values bearing different superscripts (a, b, c) within a row in each period differ significantly (P < 0.05).

NO concentration did not shown significant difference between hens with different level of feed intake, but in C-20% and C+40% groups, the plasma and liver NO concentration increased at second period of experiment significantly (P < 0.05; Table 2). Plasma PC concentration did not change between treatments in both period of experiment significantly (P < 0.05), however, in the second period of experiment, the liver PC concentration of hens fed C+40% diet was significantly higher (P < 0.05; Table 3). Plasma MDA, NO and PC concentrations were increased in C+40% hens in the second period (P < 0.05; Table 3). The hens with higher plasma and liver MDA, PC and NO concentrations (C+40%) displayed significant increase in liver hemorrhage (P < 0.05; Table 1).

DISCUSSION

Increases of liver hemorrhage and impaired egg-production in hens fed C+40% diet indicate typical experience of fatty liver syndrome. In addition, significant increase of plasma and liver MDA levels in these hens showed the presence of the oxidative stress. The increased level of MDA implies increase in lipid per-oxidation. It has been reported that overproduction of free oxygen radicals, which is related to the rate of antioxidant consumption, causes oxidative stress (Czuczejko et al., 2003). The increased free radical and malondialdehyde (MDA) produced due to lipid peroxidation react with biological structures such as proteins, lipids, carbohydrates

and DNA and cause damage (McCord, 2000). One of the major products of lipid peroxidation, MDA, has been reported to promote cross-linking bonds in the cell membrane and leads to unfavorable effects such as changes in ion permeability and enzyme activity. Lipid peroxidation is also responsible for tissue damage (cited by Cevat Nisbet et al., 2007).

NO is a regulator of vascular tone (Orozco et al., 2003) which is produced by constitutive endothelial or neural NO synthesis, or in higher concentrations, by inducible NO synthase (NOS_2). In rats, locally produced NO is important for the maintenance and increase of rat ovary blood flow during the preovulatory period (Kenrokuro et al., 2002). Hence, modulating blood perfusion in the liver may be one of the significant mechanisms by which NO can affect incidence liver hemorrhage in overfed broiler breeder hens in this study. However, it has been demonstrated that nitric oxide (NO) is an important mediator of hepatotoxicity, and the changes in its generation or actions may contribute to pathologic states. It has been proposed that the high production of NO causes injury, perhaps through the generation of potent radicals (Hafize et al., 2005).

In broiler breeder hens, it has been observed (Chen et al., 2006) that plasma leptin concentration was highly correlated with the degree of adiposity. In addition, elevated leptin correlated with liver hemorrhage. In over fed hens, significant increase in plasma and liver NO levels may be associated with increased plasma leptin (Oren et al., 2009). In addition, leptin increased plasma concentrations of nitric oxide metabolites (nitrates-nitrites) in rat (Jerzy et al., 2002).

The results of our study strongly suggest that one of the main reasons for liver hemorrhage in over fed broiler breeder is high level of liver NO. It was concluded that liver injury in fatty liver syndrome can be associated with increased level of liver NO.

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