DIETARY OXIDATION AFFECTS SERCA ACTIVITY AND DRIP LOSS IN BREAST MUSCLE OF BROILER CHICKEN

Wangang Zhang[1,2], Shan Xiao[1,3], Eun Joo Lee[1,2] and Dong U. Ahn[1,2*]

[1]Department of Animal Science, Iowa State University, Ames, IA 50011, USA

[2]Department of Agricultural Biotechnology, Major in Biomodulation, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-921, Korea, [3]College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China. [*]Dong U. Ahn (phone: +1-515-294-6595; fax: +1-515-294-9143; e-mail: duahn@iastate.edu)

***Abstract*—**This study was designed to test the hypothesis that oxidation conditions in diet can lead to oxidative stress regulating sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity and eventually influence meat quality. A total of 120 of 28 d-old broiler chickens were randomly assigned to one of three dietary treatments: control, oxidized diet (5% oxidized oil) and antioxidants-added diet (500 IU vitamin E and 200 ppm butylated hydroxyanisole) and fed for 2 weeks. At 42 d, breast muscles were separated from carcasses immediately after slaughter, frozen in liquid nitrogen, and then stored at -80 ^{o}C. Lipid and protein oxidation were measured by fluorometric thiobarbituric acid-reactive substances and carbonyl content respectively. The activity of SERCA was determined by nicotinamide adenine dinucleotide (NADH) degradation. Addition of 5% oxidized oil in diet significantly increased lipid oxidation in breast muscle compared to control group ($P < 0.05$). The carbonyl content (protein oxidation) in breast meat from chickens fed oxidized diet was higher than that of control and antioxidant groups ($P < 0.05$). The lipid oxidation of muscle from chickens fed antioxidants-supplemented diet was lower than that from oxidized and control diets ($P < 0.05$). Oxidized diet group showed higher drip loss at day 1 and 3 compared to control and antioxidant-supplemented diet groups ($P < 0.05$). No significant difference was found about drip loss between control and antioxidant supplemented groups ($P > 0.05$). Significant differences were detected between control and oxidized diet group for SERCA activity measured at 0.01 and 0.02 mM of calcium ($P < 0.05$). Different dietary treatments did not show significant effects on body weight gain, feed consumption, feed efficiency, cooking loss and color measurement ($P > 0.05$). This suggested that oxidation of diet can induce oxidative stress in animal, which can be an important factor for the variation in meat quality.

***Index Terms*— Chicken, dietary oxidation, drip loss, protein oxidation, SERCA activity**

I. INTRODUCTION

Lipid oxidation is known to cause quality problems by forming off-odor and off-flavor compounds and decreasing nutritive values in meat. Dietary addition of unsaturated fatty acids may be related to increased levels of lipid oxidation (Warnants, VanOeckel & Boucque, 1996). However, limited research has been reported about the effects of dietary addition of oxidized oil on protein oxidation and meat quality. Protein oxidation can cause fragmentation and conformational changes of protein secondary and tertiary structures to modify their functions. Oxidation-induced intermolecular bonds including disulfide, dityrosine and other intermolecular bridges can lead to protein aggregation and polymerization to change protein proteolytic properties (Morzel, Gatellier, Sayd, Renerre & Laville, 2006). These changes can influence physical and chemical properties of proteins including solubility, hydrophobicity, water holding capacity, meat tenderness, gelation functions and even nutritional values (Liu & Xiong, 2000; Rowe, Maddock, Lonergan & Huff-Lonergan, 2004). Cytoplasmic calcium concentrations can be mainly mediated by two enzymes including SERCA and calcium release channel (RyR) in skeletal muscle (Stokes & Wagenknecht, 2000). SERCA is responsible for calcium uptake from cytoplasm into sarcoplasmic reticulum, and thus inactivation of SERCA is related to increased levels of calcium in sarcoplasm of skeletal muscle cells (Jorgensen & Anderson, 1988). Increased release of calcium from sarcoplasmic reticulum can cause muscle contraction and increase the production of lactate, which further increase the rate and extent of pH decline during the conversion of muscle to meat postmortem (Huff-Lonergan & Lonergan, 2005; Lundstrom, Essen-Gustavsson, Rundgren, Edforslilja & Malmfors, 1989). Therefore, inactivation of SERCA could cause high drip loss and even lead to pale, soft and exudative meat (PSE). SERCA contains 24-29 cysteines per molecule depending on different isoforms, and thus, SERCA is highly sensitive to oxidative stress and nitrosylation (Zhao, Lytton & Burchiel, 1996). In current study, we hypothesized that the addition of oxidized oil in diet may cause oxidation including lipid and protein and thus regulate meat quality in chicken breast.

II. MATERIALS AND METHODS

2.1 Animals and feed

One hundred and twenty one-day-old boilers were fed with corn-soybean meal basal diet during the first 4 weeks. After 4 weeks, the birds were randomly distributed into 3 dietary treatments with 4 replications each. The birds were housed in groups of 10 in 12 pens under standard conditions of temperature, humidity and ventilation.

2.2 Sample preparation

At the end of feeding trial (d 42), the birds were slaughtered following the standard USDA procedure. Immediately after slaughter, 2 birds from each pen were randomly selected and one side of breast muscle from each selected bird was sampled. The samples were frozen in liquid nitrogen and stored at -80 ^{o}C until analyzed for SERCA activity, and lipid and protein oxidation. The chickens were chilled for 1 d and the other side of breast was sampled and used to determine subsequent drip and cooking loss, and color measurement.

2.2 Lipid oxidation

Lipid oxidation was determined using fluorometric thiobarbituric acid reactive substance (TBARS) as described by Jo and Ahn (1998). The fluorescence was measured by a fluorometer (Model 450 digital Fluorometer, Turner Corporation, Dubuque, IA USA) with 520 nm excitation and 550 nm emission at gain 5.

2.3 Protein oxidation

Protein carbonyl content was determined after derivatization with 2,4-dinitrophenylhydrazine (DNPH) as described by Lund, Hviid, Claudi-Magnussen and Skibsted (2008) with minor modifications. The carbonyl content was calculated as nmol/mg protein using an absorption coefficient of 22,000 $M^{-1}cm^{-1}$ as described by Levine, Williams, Stadtman and Shacter (1994).

2.4 Color measurement

Color of breast muscles were measured using a HunterLab MiniScan XE colorimeter (Hunter Laboratory Inc., Reston, VA) with D65 illuminant and 10° standard observer. The instrument was calibrated against blank and white references prior to use. Four random readings per sample were taken and averaged for CIE L* (lightness), a* (redness), and b* (yellowness) values.

2.5 Drip loss and cooking loss

To determine drip loss, whole breast samples were placed in individual plastic bags under atmospheric conditions at 4 °C. Immediately prior to being placed in bags, breast samples were dried by towel and the initial weights were recorded. After 1 d of storage, samples were removed from their individual bags and towel dried and weighed again. The breasts were then placed in new bags and stored for an additional 2 d. Following 3 d of storage, breast samples were again towel dried and weighed. Drip loss after 1 or 3 d of storage was calculated as a difference between final and initial weight expressed as a percentage of the initial weight: [(initial weight – final weight)/initial weight] x 100%.

For cooking loss, breast samples were weighed and placed in a polyethylene bag before cooking in waterbath. Internal temperature of samples was maintained at 74 °C for 20 min with the use of a thermometer probe inserted into the center of the monitored sample. After cooking, samples were towel surface dried and weighed. Cooking loss was determined as the percentage of weight loss between raw and cooked breast samples.

2.6 Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity

Sarcoplasmic reticulum (SR) was isolated using the method described by Dremina, Sharov, Kumar, Zaid, Michaelis and Schoneich (2004) with minor modifications. Ca^{2+}-dependent and basal ATPase activities of SERCA were measured by NADH degradation in the presence or absence of calcium ionophore thapsigargin at pH 7 (Chu, Dixon, Saito, Seiler & Fleischer, 1988).

2.7 Data analysis

All data were analyzed using SAS version 9.1 (NC, US) and significance was reported at the $P<0.05$ level. General Linear Procedure and analysis of variance (ANOVA) were used to determine the significance of the effects of diet.

III. RESULTS AND DISCUSSION

3.1 Feed intake and growth performance

The weights of broiler chickens were similar among three groups when the treatments were started (Table 1). After 2 weeks of feeding with three different diets, dietary treatments did not show any significant effects on weight gain between 4 and 6 weeks or on final body weight at week 6 ($P > 0.05$). The feed consumption between 4 to 6 weeks was not significantly different among three treatments ($P > 0.05$). No significant difference was found for feed efficiency (weigh gain/feed intake) during the experiment period ($P > 0.05$). Previous studies have shown that feeding oxidized fat

or oil significantly lowered weight gain and feed efficiency in broiler chickens (Chae, Lee & Lee, 2002). No significant differences on growth performance and feed consumption in current study may be partly due to the short period of feeding trial with oxidized oil compared to other studies.

3.2 Drip loss and color

After 1 d of storage under atmospheric conditions at 4 °C, the breast meat from chickens fed with diet containing oxidized oil showed significantly high drip loss ($P < 0.05$). The drip loss of meat at d 1 from oxidized oil group was 63% and 44% higher than control and antioxidant-supplemented group, respectively. This tendency was also detected after 3 d of storage. The control and antioxidant-supplemented group had significantly lower drip loss compared to oxidized oil group ($P < 0.05$). This is consistent with other studies which showed that oxidative stress could increase drip loss (Feng, Zhang, Zheng, Xie & Li, 2006). However, no significant differences in drip loss were found between control and antioxidant-supplemented diet group after 1 and 3 d of storage ($P > 0.05$). Cooking loss was not influenced by the three diet treatments ($P > 0.05$).

3.3 Protein and lipid oxidation

Dietay addition of oxidized rapseed oil and soybean oil was reported to lower lipid stability in both raw and precooked broiler meat during storage (Jensen, Engberg, Jakobsen, Skibsted & Bertelsen, 1997). In current study, feeding broiler chicken with 5% oxidized oil significantly increased lipid oxidation in breast muscle than control group ($P < 0.05$). Oxidized diet showed higher carbonyl content compared to control and antioxidant-supplemented groups ($P < 0.05$). Antioxidants addition lowered the levels of TBARS in muscle ($P<0.05$), but no significant effects were detected on carbonyl content compared with control group ($P > 0.05$).

3.4 SERCA activity

SERCA and RyR are the major enzymes to regulate the levels of cytoplasmic calcium in skeletal muscle (Stokes & Wagenknecht, 2000). Klebl, Ayoub and Pette (1998) reported that SERCA activity was inactivated by protein oxidation and tyrosine nitration in rabbit muscle. In current study, addition of oxidized oil in diet decreased the specific SERCA activity measured in the calcium level of 0.01 and 0.02 mM at pH 7 than control group ($P < 0.05$). Decreased SERCA activity may cause faster rate of pH decline and lower ultimate pH, which increase drip loss during chiling and storage.

IV. CONCLUSION

Incidence of PSE conditions has been a major problem for meat industry. However, the causes and the consequences for PSE meat in poultry have not been clearly explained. Current study showed that dietary oxidation could contribute to the variation of drip loss in broiler breast meat. Increased drip loss in meats from oxidized diet might be related to increased oxidative stress, which led the increased protein oxidation and the decreased SERCA activity.

ACKNOWLEDGEMENT

This study was supported jointly by Iowa State University, and WCU (World Class University) program (R31-10056) through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology.

REFERENCES

Chae, B. J., Lee, K. H., & Lee, S. K. (2002). Effects of feeding rancid rice bran on growth performance and chicken meat quality in broiler chicks. *Ansian-Australian Journal of Animal Science, 15,* 266-273.

Chu, A., Dixon, M. C., Saito, A., Seiler, S., & Fleischer, S. (1988). Isolation of sarcoplasmic reticulum fraction referable to longitudinal tubules and junctional terminal cisternae from rabbit skeletal muscle. *Methods in Enzymology, 157*, 36-46.

Dremina, E. S., Sharov, V. S., Kumar, K., Zaid, A., Michaelis, E. K., & Schoneich, C. (2004). Anti-apoptotic protein Bcl-2 interacts with and destabilizes the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA). *Biochemical Journal, 383,* 361-370.

Feng, J. H., Zhang, M. H., Zheng, S. S., Xie, P., & Li, J. Q. (2006). The effect of cyclic high temperature on mitochondrial ROS production, Ca^{2+}-ATPase activity and breast meat quality of broilers. *Chinese Journal of Veterinary Science, 37,* 1304–1311.

Gupta, S., McArthur, C., Grady, C., & Ruderman, N. B. (1994). Stimulation of vascular Na^{+}-K^{+}-ATPase activity by nitric oxide: a cGMP-independent effect. *The American Physiological Society, 266,* H2146-H2151.

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water holding capacity in meat: The role of postmortem biochemical and structural changes. *Meat Science, 71,* 194-204.

Jensen, C., Engberg, R., Jakobsen, K., Skibsted, L. H., & Bertelsen, G. (1997). Influence of the oxidative quality of dietary oil on broiler meat storage stability. *Meat Science, 47,* 211-222.

Jo, C., & Ahn, D. U. (1998). Fluorometric analysis of 2-thiobarbituric acid reactive substances in turkey. *Poultry Science, 77,* 475-480.

Jorgensen, P. L., & Andersen, J. P. (1988). Structural basis for E1-E2 conformational transitions in Na, K-pump and Ca-pump proteins. *Journal of Membrane Biology, 103,* 97-120.

Klebl, B. M., Ayoub, A. T., & Pette, D. (1998). Protein oxidation, tyrosine nitration, and inactivation of sarcoplasmic reticulum Ca^{2+}-ATPase in low-frequency stimulated rabbit muscle. *FEBS letter, 422,* 381-384.

Levine, R. L., Williams, J. A., Stadtman, E. R., & Shacter, E. (1994). Carbonyl assays for determination of oxidatively modified proteins. *Methods in Enzymology, 233*, 346-357.

Liu, G., & Xiong, Y. L. (2000). Electrophoretic pattern, thermal denaturation, and in vitro digestibility of oxidized myosin. *Journal of Agricultural and Food Chemistry, 48,* 624-630.

Lund, M. N., Hviid, M. S., Claudi-Magnussen, C., & Skibsted, L. H. (2008). Effects of dietary soybean oil on lipid and protein oxidation in pork patties during chill storage. *Meat Science, 79,* 727-733.

Lundstrom, K., Essen-Gustavsson, B., Rundgren, M., Edforslilja, I., & Malmfors, G. (1989). Effect of halothane genotype on muscle metabolism at slaughter and its relationship with meat quality – a within-litter comparison. *Meat Science, 25,* 251–263.

Morzel, M., Gatellier, P. H., Sayd, T., Renerre, M., & Laville, E. (2006). Chemical oxidation decreases proteolytic susceptibility of skeletal muscle proteins. *Meat Science, 73,* 536-543.

Rowe, L. J., Maddock, K. R., Lonergan, S. M., & Huff-Lonergan, E. (2004). Oxidation environments decrease tenderization of beef steaks through inactivation of μ-calpain. *Journal of Animal Science, 82,* 3254-3266.

Sotkes, D. L., & Wagenknecht, T. (2000). Calcium transport across the sarcoplasmic reticulum: Structure and function of Ca^{2+}-ATPase and the ryanodine receptor. *European Journal of Biochemisty, 267,* 5274-5279.

Warnants, N., VanOeckel, M. J., & Boucque, C. V. (1996). Incorporation of dietary polyunsaturated fatty acids in pork tissues and its implications for the quality of the end products. *Meat Science, 44,* 125-144.

Zhao, M., Lytton, J., & Burchiel, S. W. (1996). Inhibition of sarco-endoplasmic reticulum calcium ATPase (SERCA) by polycyclic aromatic hydrocarbons: lack of evidence for direct effects on cloned rat enzymes. *International Society for Immunopharmacology, 18,* 589-598.

Table 1: Effects of dietary treatments on growth and feed intake in broiler chickens

	Control	Oxidized oil	Antioxidant
4 week weight (kg)	1.371±0.030	1.415±0.028	1.339±0.010
6 week weight (kg)	2.743±0.068	2.778±0.065	2.669±0.039
Weight gain (kg)	1.372±0.048	1.362±0.039	1.331±0.029
4-6 week feed intake (kg)	2.387±0.049	2.419±0.075	2.320±0.038
Gain/feed (kg/kg)	0.574±0.011	0.563±0.003	0.573±0.003

*: Means within the same row with different superscripts are significantly different (P<0.05).

Table 2: Effects of dietary treatment on meat color of chicken breast

	Control	Oxidized oil	Antioxidant
L value	63.6±2.3	63.0±3.1	63.7±3.1
a value	9.3±1.1	9.6±1.9	9.5±1.3
b value	12.6±2.3	12.2±1.9	12.2±2.1

*: Means within the same row with different superscripts are significantly different (P<0.05).

Table 3: Effects of dietary treatments on drip and cooking loss in broiler chickens

	Control	Oxidized oil	Antioxidant
Day 1 drip loss (%)	$0.422±0.053^{a}$	$0.687±0.067^{b}$	$0.477±0.046^{a}$
Day 3 drip loss (%)	$0.794±0.075^{a}$	$1.372±0.144^{b}$	$0.862±0.092^{a}$
Cooking loss (%)	21.20±0.81	21.50±0.97	20.23±1.18

*: Means within the same row with different superscripts are significantly different (P<0.05).

Table 4: Effects of dietary treatments on protein and lipid oxidation in broiler chickens

	Control	Oxidized oil	Antioxidant
Protein oxidation (nmol/mg protein)	$0.55±0.05^{a}$	$0.70±0.05^{b}$	$0.55±0.04^{a}$
Lipid oxidation (fluorometric reading)	$19.60±3.08^{b}$	$27.40±3.40^{c}$	$13.00±0.62^{a}$

*: Means within the same row with different superscripts are significantly different (P<0.05).

Table 5: Effects of dietary treatments on non-specific and specific SERCA activity (μmole Pi/mg protein/min)

	Control	Oxidized oil	Antioxidant
No-specific activity	298.03±60.62	222.20±25.68	313.75±56.49
Activity measured at 0.01 mM calcium	$315.59±38.90^{b}$	$229.59±26.87^{a}$	$342.58±19.94^{b}$
Activity measured at 0.02 mM calcium	$553.22±54.97^{b}$	$378.63±33.62^{a}$	$479.42±49.80^{ab}$

*: Means within the same row with different superscripts are significantly different (P<0.05).