Age-Related Changes in Meat Tenderness and Tissue Pentosidine: Effect of Diet Restriction and Aminoguanidine in Broiler Breeder Hens

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ABSTRACT

The nonenzymatic glycosylation of tissue protein contributes to the formation of crosslinks that leads to structural and functional deterioration in the long-lived tissue protein, collagen. The accumulation of these crosslinks thus contributes to the objectionable toughness of meat from aged animals, decreases its economic value, and limits its use in whole muscle foods. The objectives of this study were to determine the effectiveness of diet restriction and the crosslinking inhibitor, aminoguanidine (AG), on reducing the accumulation of crosslinks, thereby improving meat tenderness in broiler breeder hens. The glycoxidation product, pentosidine, was also measured in skin (Ps) to determine whether changes in its concentrations correlated with the changes in shear value (SV). Chicks (n = 450) were randomly assigned to four treatment groups from 8 to 125 wk after hatch: ad libitum (AL), diet restricted (DR), AL and DR groups supplemented with 400 ppm AG each (AL+AG and DR+AG, respectively). Shear value was measured with an Instron Universal Mechanical Machine. Skin pentosidine was isolated by reverse phase HPLC. There was an age-related, linear increase in SV (P < 0.0001, r = 0.96), which correlated (r = 0.86) with the age-related increase in Ps in AL hens. Diet restriction retarded SV (P < 0.0001) over the sampling period. In general, SV values for AL+AG were similar to those measured in DR, whereas no additive effect was observed for AG in DR birds. It was concluded that there was a linear increase in meat toughness (SV) with age that correlates with the accumulation of Ps, and that the decline in meat tenderness can be retarded by DR or AG. Secondly, the effect of DR on accumulation of Ps was so pronounced that AG supplementation did not further enhance this effect.

(Key words: diet restriction, aminoguanidine, meat tenderness, pentosidine, aging)

1999 Poultry Science 78:1328–1333

INTRODUCTION

Meat of spent fowls becomes objectionably tough with increasing age and this toughness limits its use in whole muscle foods, resulting in considerable economic losses to the poultry industry (Shimokomaki et al., 1972; Nakamura et al., 1975). For this reason, spent fowl meat has been traditionally used in less profitable, comminuted or retorted products in which small particle size or thermal processing is used to reduce toughness (Nurmahmudi and Sams, 1997). This problem is of particular importance to the poultry industry, which produces spent fowl as a by-product of table and hatching egg production. Approximately 130,000,000 spent fowl are disposed of in the U.S. annually (Vandepopuliere and Lyons, 1996); thus, the potential economic benefit to be gained by tenderizing this meat is tremendous.

The age-related decline in meat tenderness is due to many factors. Recent studies have suggested that glucose-derived, nonenzymatic collagen crosslinking plays a role in the normal aging process as well as the accelerated tissue aging associated with the hyperglycemic state (Brownlee et al., 1988). The structural, long-lived tissue protein collagen is the major component of connective tissue and functions as the basic supporting structure of tissues and organs. Nonenzymatic collagen crosslinking in muscle may contribute to the decline in meat tenderness of aged animals, although its importance in skeletal muscle has not been established (Bailey, 1989; Klandorf et al., 1996). The glycosylation theory of aging suggests that modification of proteins by glucose and associated browning or Maillard reactions lead to the gradual crosslinking, polymerization, development of brown color, and fluorescence characteristic of age-associated changes in tissue protein collagen (Cerami, 1985; Monnier, 1989). Accumulation of these crosslinks are postulated to lead...
to structural and functional deterioration of tissue protein and contribute to the pathophysiology of diabetic complications (Monnier, 1989; Bucala and Cerami, 1992; Vlassara et al., 1994; Cefalu et al., 1995). One such crosslink, pentosidine, has been isolated and characterized in numerous animal species (Sell and Monnier, 1989; Iqbal et al., 1997), and is an imidazole [4, 5b] pyridinium molecule containing a lysine and an arginine residue crosslinked by a pentose.

Presently, there is no economically feasible way to increase meat tenderness by decreasing collagen crosslinking in spent fowl meat (Kondaiah and Panda, 1992; Woods et al., 1997). Numerous studies have demonstrated that diet restriction (Masoro et al., 1989; Cefalu et al., 1995) and the crosslinking inhibitor, aminoguanidine (AG) (Brownlee et al., 1986; Khatan et al., 1988; Oxlund and Andreassen, 1992; Wu, 1995; Klandorf et al., 1996), retard the accumulation of crosslinks over an animal’s life time. Whether diet restriction, AG or both, can improve meat tenderness (SV) as compared to that of birds that consumed feed ad libitum has not been determined. In addition, the glycoxidation product, pentosidine, was measured in skin (Ps) to determine whether changes in its concentration correlated with internal changes in SV of the Pectoralis major muscle.

MATERIALS AND METHODS

Birds and Management

Day-old broiler breeder (Cobb × Cobb) female (n = 450) chicks were placed in electrically heated battery brooders and provided ad libitum access to feed until 4 wk of age. At this time, chicks were randomly assigned to four treatment groups: ad libitum (AL group); diet restricted (DR group); AL with 400 ppm aminoguanidine (AG); and DR with 400 ppm AG in feed (AL+AG and DR+AG groups, respectively). Birds were fed accordingly throughout the study. The DR chicks were fed daily with a limited allowance diet according to the Cobb Management Guide. At the selected dose of AG, a significant reduction in the accumulation of fluorescent end products occurred in the P. major muscle was then isolated, vacuum packed, and stored at −20 C until thermal processing.

Breast Muscle Collection. Tissue samples were obtained at 12-wk intervals from 8 to 92 wk and then again at 125 wk of age. Hens (n = 5) from each group were randomly selected for slaughter. Birds were electrically stunned and bled using a modified kosher technique. The carcass was eviscerated and stored for 4 h at 4 C. The P. major muscle was then isolated, vacuum packed, and stored at −20 C until thermal processing.

Measurement of Shear Value. The frozen P. major muscles were thawed overnight at 4 C and then brought to room temperature before cooking. The P. major muscle from each bird was cooked to an internal temperature of 70 C on a Farberware Smokeless Indoor Grill. Endpoint internal temperature (CT) was monitored with an Industrial Datalogger equipped with a copper-constant thermocouple. Cooked muscle was cooled to room temperature and refrigerated overnight at 4 C. Slices of 1.27 cm were cut perpendicular to the fiber orientation of the muscle. Four to six 1.27-cm diameter cores were removed from these slices parallel to the fiber orientation through the thickest portion of the cooked muscle. A Warner-Bratzler shear value (SV) was determined by using an Instron Universal Mechanical Machine. A Warner-Bratzler apparatus was attached to a 50-kg load cell and tests were performed at a cross head speed of 127 mm/min. Output from a LVDT conditioner was acquired by a computer equipped with a DT 2805 data acquisition board. Signals were processed with the HP-VEE software package.

Breast Weight and Cook Yield. Raw and cooked breast weights (BRW) were recorded. Cook yield (CY) was calculated by expressing cook weight (CW) as a percentage of uncooked weight.

Pentosidine Determination

Preparation of Collagen Digest. Hens (n = 5) from the AL group at all time points were randomly selected and euthanatized as described earlier. Approximately 1 g of skin was removed from the breast area, washed with normal saline, and stored at −80 C until assayed. The collagen digest used for Ps determination was prepared according to the techniques described by Monnier et al. (1986) and Sell et al. (1992). Briefly, this involved the removal of epidermal and adipose layers from skin samples, freezing in liquid nitrogen, and mincing. The minced samples were delipidated overnight in a chloroform:methanol (2:1) solution. Samples were rehydrated in a 50% methanol solution and hydrolyzed in 6 N HCl at 110 C for 18 h. All tubes were flushed with N2 prior to capping for...
heating. Subsequent to the hydrolysis, the samples were placed into a Speed Vac centrifuge vacuum drier until the HCl was evaporated. Samples were reconstituted in 250 μL H2O and filtered using a Costar® Spin-X® centrifuge tube filter. A modified Stegman and Stadler spectrophotometric method was used for collagen estimation, using a hydroxyproline standard (Maekawa et al., 1970).

**Skin Collagen Pentosidine.** Age-related changes in Ps from 8 to 92 wk of age were determined in a separate study (Iqbal et al., 1999). For purposes of establishing a relationship with SV in this study, Ps is reported in AL-fed birds only from 8 to 125 wk of age. A replicate study investigating the accumulation of Ps in the skin of broiler breeder hens until 68 wk of age was previously published (Iqbal et al., 1997). Estimation of Ps was done by a modified reversed phase HPLC method (Iqbal et al., 1997). One milligram of acid-hydrolyzed collagen digest in 100 μL of water/0.01 M heptafluorobutyric acid (HFBA) was injected into a 0.46 × 25 cm Vydac 218TP104 (10 μm) C-18 column connected to a Shimadzu HPLC. The apparatus consisted of two model LC-600 pumps, a model SIL-6B auto injector, a model RF 551 fluorescence monitor (excitation 325 nm, emission 370 nm). Separations were achieved by application of a linear gradient of 12 to 42% acetonitrile from 0 to 20 min in water and 0.01 M HFBA. Quantification of pentosidine was made by comparison of peak areas with a pentosidine standard injected under identical conditions. A software package (Shimadzu CLASS-VP 4.2) was used to analyze the data.

**Statistical Analyses**

Data were analyzed by the General Linear Models procedure using a 2 × 2 factorial design for each age group (SAS Institute, 1990). Regression analysis was carried out to determine the correlation between SV and Ps. Covariate analyses were carried out with age, CT, and BRW as covariates of SV in order to eliminate their potential effects on treatments. The Student-Newman-Keuls Range method was used to test the significance of difference between means.

**RESULTS AND DISCUSSION**

In the present study, the potential contribution of non-enzymatic crosslinks to meat tenderness has been addressed in broiler breeder hens over a 125-wk period. In skin and muscle, Type I and III collagen are the two predominant genetic types of collagen (Lawrie, 1991) and therefore a specific tissue crosslink, Ps, was used as an indicator of glycative and oxidative tissue damage in these tissues (Baynes, 1991). In mammals, pentosidine has been found to increase over the life span of the individual (Grandhee and Monnier, 1991; Dyer et al., 1991b; Cefalu et al., 1995). In agreement with these studies, there was an age-related, linear increase in Ps (P < 0.001, r = 0.95) that correlated (r = 0.86) with the linear increase in SV of P. major muscle in AL hens (Figure 1). Nakamura et al. (1975) reported that SV of P. major muscle increased with age (r = 0.955) in different strains of White Leghorn chicken up to 520 d of age. Similar observations were also reported by Awonorin and Ayoade (1992) in broilers; Robertson et al. (1984) in buffalo and Brahman-Shorthorn steers, and Batchet et al. (1962) on lamb cuts from different ages.

Because crosslinks play an important role in the normal aging process and diabetes, the utility of DR and a pharmacological agent (AG) to attenuate the formation of these crosslinks was investigated. Various methods have been used to degrade the intrinsic collagen network to improve the texture of meat from older animals. These approaches include: acid marinades (Arganosa and Marriott, 1989), injection of plant proteinases such as papain (Brooks et al., 1985; Cronlund and Woychik, 1987), Ca2+ ion marinades and postmortem injections (Koohmaraie et al., 1988, 1989, 1990; Papa et al., 1989; Young and Lyon, 1989; Young et al., 1989), postmortem injection of bacterial collagenases into muscle (Foegeding and Larick, 1986; Cronlund and Woychik, 1987), and activation of postmortem collagen proteolysis by the action of neutral proteases (Koohmaraie et al., 1987; Morgan et al., 1991; Birkhold et al., 1992; Nurmahmudi and Sams, 1997). However, most of these studies did not address changes in collagen and meat quality in older animals. Diet restriction is one of the most extensively used antiaging measures (Weindruch and Walford, 1988; Synder, 1989) and has been reported to extend the life span of rodents (Berg and Simms, 1960; Yu et al., 1982), reduce glycosylation (Masoro et al., 1989; Cefalu et al., 1995; Beuchat and Chong, 1997), and delay the onset of a number of major age-associated diseases (Yu, 1994). In our study, DR decreased SV of P. major muscle; the effect became particularly pro-

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13 Savant Instruments, Farmingdale, NY 11735.
14 Corning Costar Corp., Cambridge, MA 02140.
15 Vydac, Hesperia, CA 92345.
16 Shimadzu, Inc., Columbia, MD 21040.
17 Vincent M. Monnier, Cleveland, OH 44120.
The effect of age, diet restriction (DR) and aminoguanidine (AG) on shear value (SV). Each bar represents mean (n = 5) ± SEM. No common letters differ (P < 0.001) at 68 and 80 wk and (P < 0.0001) at 92 and 125 wk between ad libitum (AL) and DR groups at those time points only. pf = peak force in kilograms.

Supplementation with AG reduced SV at 68 wk and thereafter. However, no additional decrease in SV was recorded (P > 0.05) for the DR+AG group (Figure 2). This finding is consistent with a previous study in broiler breeder hens in which SV of B. femoris muscle were not affected in DR birds supplemented with AG (Klandorf et al., 1996). It appears that AG is relatively ineffective in DR animals. This could be due to the fact that glycosylation is finite and is limited in amounts that range from ~0.4 to 20% by weight of covalently attached carbohydrates, depending on the collagen’s tissue of origin (Eyre, 1980; Lawrie, 1991). Furthermore, pentosidine accounts for <1% of the crosslinks formed during in vitro browning reaction of protein with glucose (Dyer et al., 1991). Diet restriction probably left little or no role for AG to play supplementation in reducing the glycosylation process and subsequently reduction in the meat toughness. Although the mode of action of AG in lowering SV values in the AL group is not clearly understood, AG has been found to lower oxidative stress (Iqbal et al., 1999) in broiler breeder hens. Whether its mode of action is comparable to that of DR has yet to be established.

There was also a linear, age-related increase in BRW (P < 0.0001, r = 0.64) until 44 wk and CT until 20 wk. These observations are consistent with those of Awonorin and Ayoade (1992) in broilers and turkeys, and Toumy and Lechnir (1964) in beef. Diet restriction significantly reduced BRW and CT (P < 0.0001). Covariate analyses did not eliminate the treatment effects when BRW, CT, and age were kept as covariates of SV. In general, there was no consistent effect of AG supplementation on BRW and CT; however, a marked decrease in CT was observed at 80 wk in the AL+AG group (Figures 3 and 4). No consistent effect of DR and AG was observed on CY (data not reported).

In summary, increased meat toughness (SV) with age correlates with accumulation of Ps in AL animals. The decline in meat tenderness can be retarded by DR or supplementation with AG. Furthermore, the effect of DR on crosslink accumulation is so pronounced that AG supplementation cannot further enhance this effect. The results of this study have potential implications, particularly for the use of skin as an indicator of age-related changes in muscle. Using this approach, a skin sample can be used for pentosidine estimation and, hence, as a predictor of meat tenderness. Secondly, the data support the use of AG in the diets of AL animals as to...
improve meat tenderness. Future studies could address pentosidine accumulation in specific muscles and relate these changes to SV.

ACKNOWLEDGMENTS

The authors wish to thank Nabil H. Al-Humadi, NIH/NIH, Morgantown WV 26505, for his assistance in the HPLC analysis; Edwin C. Townsend, Agriculture and Forestry Experimental station, West Virginia University, Morgantown, WV 26506, for his help in statistical analyses; and Hakan Kocamis and Lorree L. Probert, Division of Animal and Veterinary Sciences, West Virginia University, Morgantown, WV 26506, for technical assistance.

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