Tr. J. of Veterinary and Animal Sciences
23 (1999) 483–487

Collagen Content and Electrophoretic Analysis of Type I Collagen in Breast Skin of Heterozygous Naked Neck and Normally Feathered Commercial Broilers

Güldehen BILGEN
Ege University, Faculty of Agriculture, Department of Animal Science, İzmir-TURKEY

Gülgün OKTAY, Zeynep TOKGÖZ, Gül GUNER
Dokuz Eylül University, Faculty of Medicine, Department of Biochemistry, İzmir-TURKEY

Servet YALÇIN
Ege University, Faculty of Agriculture, Department of Animal Science, İzmir-TURKEY

Received: 18.02.1999

Abstract: This study was conducted to evaluate the breast skin collagen content and electrophoretic analyses of type I collagen in heterozygous naked neck and normally feathered commercial chicks. A total of 72 birds from each genotype were randomly selected at 7 weeks and slaughtered. Breast skin was separated from each carcass and was analysed for collagen content and gel electrophopresis of type I collagen was performed. Males had significantly higher level of skin collagen content than females in both genotypes. In the naked neck chickens α_1, and α_2 bands were highly intensive than the commercial ones indicating that the collagen has become cross-linked by non-reducible covalent bands. On the other hand, normally feathered chickens had higher intensity of β and γ bands than the naked neck chickens.

Key Words: naked neck gene, skin tears, type I collagen.

Heterozigot Çıplak Boyunlu ve Normal Tüylü Ticari Etlik Piliçlerin Göğüs Derisinde Tip I Kollageninin Elektroforetik Analizi ve Deri Kollagen İçeriği

Özet: Çalışma, heterozigot çıplak boyunlu ve normal tüylü ticari piliçlerde göğüs derisinde kollagen içeriğinin ve tip I kollageninin elektroforetik analizini değerlendirme amacı ile yürütülmüştür. Her bir genotipten 72 piliç 7. haftada rastgele seçilerek kesilmiştir. Göğüs derileri karkastan ayrıldıktan sonra kollagen içeriği saptanmış ve tip I kollageninin jel elektroforezi gerçekleştirilmiştir. Her iki genotipte deri kollagen düzeyi erkek piliçlerde dişilerden önemli düzeyde yüksek bulunmuştur. Çıplak boyunlu piliçlerde α_1 ve α_2 bantlarının normal ticari genotipe göre daha yoğun olması, kollagenin indirgen olmayan kovalent bağlarla çapraz bağlı hale geldiğini göstermektedir. Öte yandan, normal ticari genotipte β ve γ bantlarının daha yoğun olduğu gözlenmiştir.

Anahtar Sözcükler: Çıplak boyunluluk geni, deri yırtıkları, tip I kollagen

Introduction

Collagen is one of the principle structural proteins of the skin. Collagens are a large family of structural proteins in the extracellular matrix of eukaryotes (1). Being the most abundant animal protein in mammals, collagen accounts for about 30% of all proteins. The collagen molecules, after being secreted by the cells, assemble to form the characteristic fibers responsible for the functional integrity of tissues such as bone cartilage, skin, and tendon (2). They contribute as a structural framework to other tissues such as blood vessels and most organs. Cross-links between adjacent molecules are a prerequisite for the collagen fibers to withstand the physical stress to which they are exposed.

Different collagen types, which differ in chemical and physical properties, such as electrophoretic mobility and molecular weight, have been identified (3, 4). Before 1969, type I collagen was the only mammalian collagen known. The basic molecule composed of three polypeptide chains was called "tropocollagen" or "collagen molecule". The latter term has survived (3,5).

The collagen I molecule consists of two identical chains, called α_1.(I), hydrogen bonded to each other and to a third chain with a different aminoacid sequence termed α_2. This collagen was therefore given the molecular formula [α_1 (I)]$_2$ α_2. Type I collagen that was evaluated in this study is the most abundant form of collagen. It comprises between 80% and 99% of the total collagen in skin, tendon, bone (6). The proportion of type I collagen in the particular tissue can vary at different sites during development, with age and with pathology (3).

The molecular genetics of the chicken has so far been concentrated on genes whose main interest lies in evolutionary comparison with mammals, for example, the globins and the collagens (1).

Recent advances in recombinant DNA technology have opened the way for the study of the structure and regulation of the collagen gene. Recently, chicken collagen genes have been cloned by recombinant DNA techniques. Analysis of these genes has contributed important general concepts to the understanding of gene structure and function in higher organisms.

The α_2 type I collagen gene of chicken was cloned from a genomic DNA library and found to be at least 30-40kb long, containing more than 50 exons (7). The α_1 type I collagen gene has also been cloned at the cDNA sequence (8).

Chicken genes that have been cloned are α1 type I, α_2 type I, α_1 type II, α_1 type III, α_1 type IX, and α_2 type IX (1, 9).

Skin tears occur during defeathering in poultry slaughter houses, are associated with downgrading and results in economic losses to the broiler industry. Studies on this issue (10, 11) have shown that skin tearing is affected by strain, sex, skin strength, dietary factors and skin collagen content of the skin. In recent years, the naked neck gene has received much attention because of its association with heat tolerance (12). It has been shown that under high constant or natural ambient temperatures, heterozygous naked neck broilers are superior to their normally feathered counterparts (13,14). Cahaner et al. (13) found higher levels of collagen in homozygous naked neck (Na/Na) birds than in heterozygous (Na/na) and normally feathered (na/na) birds. However, electrophoretic analyses of type 1 collagen in the skin of naked neck birds have not previously been carried out. Therefore, this study was conducted to evaluate the skin collagen content and electrophoretic analyses of type I collagen in heterozygous naked neck and normally feathered commercial chicks.

Materials and Methods

A total of 360 chicks from each genotype –the heterozygous naked neck genotype and a normally feathered commercial genotype–were used. At 7 weeks, 72 birds were randomly selected and slaughtered. The breast was separated from each carcass and the skin was removed.

Collagen assay procedure:

Hydroxyproline was used to determine the collagen content of chicken skin. The amino acid hydroxyproline was determined as described by Reddy and Enwemeka (15). The presence of the amino acid hydroxyproline in collagen (about 13%) is a unique feature because this amino acid occurs in only a few proteins, such as elastin (about 1%). Therefore, hydroxyproline has been used for many years as a means of determining amount of collagen present in a tissue.

For the assay, 0-ring screw-capped high temperature glass tubes were used. Aliquates of standard Hyp and test samples were hydrolyzed in alkali (20 mg tissue /50µl 2 N NaOH). The hydrolyzed samples were then mixed with a buffered chloramine- T reagent and oxidation was allowed to proceed for 25 min. at room temperature. The chromophore was then developed with the addition of Erlich's reagent, and the absorbance of reddish purple complex was measured at 550 nm with a JACOBS 550 spectrophotometer. Absorbance values were plotted against the concentration of standard Hyp, and the presence of Hyp in unknown tissue extracts was determined from the standard curve. The collagen values were calculated with the assumption that 12.5% of the collagen is hydroxyproline (16). Data were subjected to analysis of variance by SAS (17).

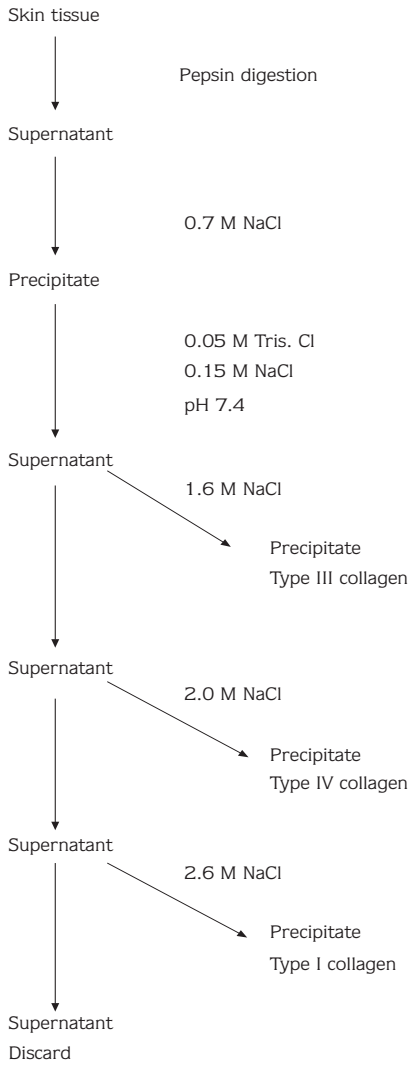
Solubilization of collagen:

Chicken skin tissues were homogenized in 10-20 volume of 0.5 M acetic acid with a polytron homogenizer. Since most of the collagenous components of connective tissues are relatively insoluble, partial proteolytic digestion is required for their extraction. For this reason, pepsin was added at a pepsin: wet tissue ratio of 1:100 v/w and incubated at 4˚C for 16h. This mixture was centrifuged at 10 000g for 30 min. to remove insoluble residue. The insoluble residue was re-extracted as above to increase the yield.

Purification of type I collagen

Due to its triple-helical structure, a large proportion of the collagen molecule is resistant to pepsin digestion up to 4˚C, whereas most other tissue contituents are substantially degraded. By salt fractionation of the pepsin extracts, a solution containing essentially pure collagen was obtained. Collagens were fractionated initially from the 0.5 M acetic acid extract by addition of solid NaCl to yield 0.7M. The flow diagram in Figure 1 illustrates this procedure and the salt concentration (18).

Figure 1. Purification procedure for type I collagen



Gel electrophoresis of type I collagen

The purity of this collagen preparation was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) by the Laemmli (19) system with an 10% resolving gel. The gel was allowed to set for 45 min. Distilled water was pipetted over the gel to prevent oxygen inhibition of the polymerisation reaction. The 4% stacking gel was then prepared on top of the resolving gel. The comb was placed carefully to prevent formation of air bubbles. This gel was allowed to set for 30 min. Samples were diluted prior to electrophoresis using sample buffer to give a final protein concentration of 1 µg/ml. Samples were boiled at 100˚C for 5 min. Gels were run at 25 mA per gel for 45 min. On completion of electrophoresis, gels were fixed and stained with Coomassie Blue R-250 for 1 h. The gel was then transferred to destained solution and subsequently photographed. Type I collagen purified from rat tail tendon was used as a standard.

Results

Mean collagen contents of naked neck and normally feathered chickens were found as 2.44 and 2.35%, respectively (Table 1). Significant differences between sexes were obtained for collagen content, which was significantly higher in males than in females in both genotypes.

Five protein bands were found from origin point to migration side ($\gamma 1_2 2$; $\beta 1_2$; $\beta 1,2$; α_1; α_2) by SDS-PAGE (Figure 2).

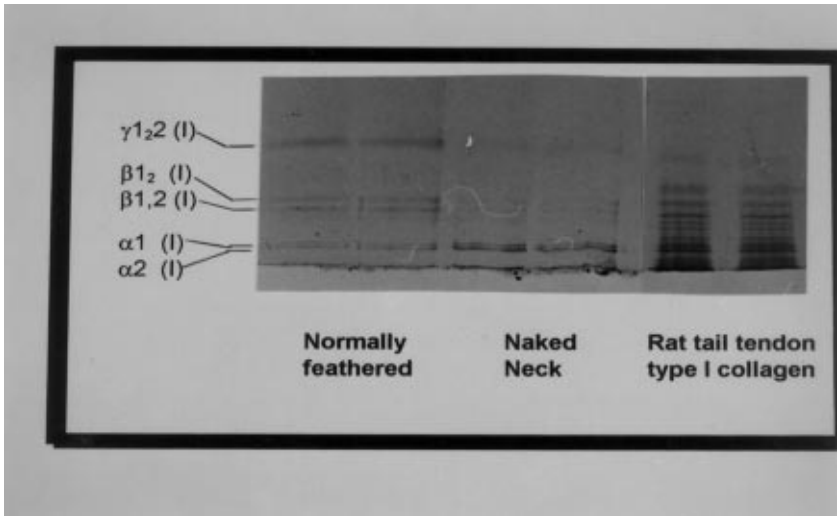


Figure 2. SDS-PAGE of Type I collagen from naked neck and normally feathered commercial broilers.

Genotype	Sex		Mean	Significance (P values)
	Male	Female		
Naked neck	2.72	2.15	2.44	<.001
Normally feathered commercial	2.58	2.11	2.35	<.001

Table 1. Skin collagen content (%) of naked neck and normally feathered commercial broilers

Discussion

The arrangement of collagen fibres plays an important role in determining the proporties of a tissue. For instance, the collagen fibres in skin are randomly arranged as a criss-cross lattice, which confers the necessary degree of flexibility and extensibility to skin (3). This study was carried out to elucidate any possible change in proportions of the different collagen bands in naked neck and normal broilers. In the present study, the α_1, and α_2 bands of the naked neck chickens were higher intensity than those of commercial chickens, indicating that the collagen has become cross-linked by non-reducible covalent bands. In contrast, normally feathered chickens had higher β and γ bands than the naked neck chickens. The cause of the increase in the γ collagen components in the normally feathered chickens is the formation of endogenous intermolecular cross-links, which are presumed to contribute significantly to dermal stability, and the retarding of the migration of these cross-link. However, it is not possible to conclude that normally feathered birds have stronger skin because the skin strength of birds did not determine in this study. It has been planning to continue the near future to evaluate from the point of view skin tearing.

References

1. Bulfield, G.: Molecular genetics. In Poultry Breeding and Genetics, 1990, pp.543-584. (Ed. Crowford, R. D). The Netherlands.
2. Freeman, W. H.: Biochemistry. 1988, pp. 261-281. 3th (ed.Stryer, L.), New York.
3. Burgeson, R. E. and Marcel, E. N.: Collagen types: Molecular structure and tissue distribution. Clinical Orthopaedics and Related Research, 1992, 282:251-272.
4. Djavani, M., Yenice, S., Kırkalı, G., Güner, G., Sessiz, H. T.: Alterations of collagen content in kidney of diabetic rabbits. Biochem. Soc. Trans. 1993, 21: 275-.
5. Metzler, D. E.: Biochemistry, the chemical reactions of living cells. International edition, Academic press. 1977, pp.64-76.
6. Miller, E. J. and Gay, S.: The collagens an overview and update. Methods Enzymol. 1987, 144:3-41.
7. Ohkubo, H., Vogeli, G., Mudryj, M., Auvedimento, V. E., Sullivan, M., Paston, I. and de Crombrugghe, B.: Isolation and characterization of overlapping genomic clones covering the chicken α_2 type I collagen gene. Proc. Nat. Acad. Sci. 1980, 77:7059-7063.
8. Fuller, F. and Boedtler, H. : Sequence determination and analysis of the 3' region of the chicken pro-α_1 (I) and pro- α_2 (I) collagen messenger ribonucleic acids including the carboxy-terminal propeptide sequence. Biochemistry, 1981, 20:996-1006.
9. Walchl, C., Koller, E., Trueb, J., and Trueb, B.: Structural comparison of the chicken genes for α_1 (VI) and α_2 (VI) collagen. European Journal of Biochemistry, 1992, 205:583-589.
10. Bilgili, S. F., Eckman, M. K. and Bushong, R. D.: Broiler skin strength: influence of age, sex and feathering rate. J. Applied Poultry Res., 1993, 2:135-141.
11. Granot, I., Pines, M., Plavnik, I., Wax, E., Hurwits, S., Bartov, I.: Skin tearing in broiler in relation to skin collagen: effects of sex, strain and diet. Poultry Science, 1991, 70:1928-1935.
12. Merat, P.: Potential usefulness of the (Na) naked neck gene. J. World's Poultry Sciences, 1986, 42: 124-142.
13. Cahaner, A., Deeb, N., Gutman, M.: Effects of plumage-reducing naked neck (Na) gene on the performance of fast growing broilers at normal and high ambient temperature. Poultry Science, 1993, 72:767-775.
14. Yalçın, S., Testik, A., Özkan, S., Settar, P., Çelen, F. & Cahaner, A.: Performance of naked neck and normal broilers in hot, warm and temperate climates. Poultry Science, 1997, 76: 930-937.
15. Reddy, G. K. and Enwemeka, C. S.: A simplified method for the analysis of hydroxyproline in biological tissues. Clinical Biochemistry, 1996, 29:225-229.
16. Edward, C. A., O'Brien, W. D.J. R.: Modified assay for determination of hydroxyproline in a tissue hydrolyzate. Clin. Chim. Acta, 1980, 104:161-167.

17. SAS, Institute: SAS User's Guide (Cary, NC, SAS Institute), 1986.

18. Duance, U. C.: Purification of collagen types from various tissues. In: Protein purification applications: a practical approach. E. I. V. Horrs and S. Angal, pp. 137-141 , 1990.

19. Laemmli, U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 1983, 227:680-685.