15

BARD

FINAL REPORT
PROJECT NO. IS-1604-89

**Collagen Metabolism *In Vivo* and in Cell Culture as
Related to Skin Quality in Broilers**

M. Pines, M. Yamauchi, I. Plavnik, S. Hurwitz

הספרייה המרכזית
למדעי החקלאות
בית דגן

1993

630.72

BAR/PIN

2nd copy

BARD
P.O. Box 6
Bet Dagan, Israel

Date: December 1993

BARD Project No I-1604-89

Title:

**Collagen Metabolism *In Vivo* and in Cell Culture as Related to
Skin Quality in Broilers**

Investigator's names

M. Pines
M. Yamauchi
S. Hurwitz
I. Plavnik

Investigators institution

ARO, The Volcani Center, Bet Dagan, Israel
University of North Carolina, Chapel Hill, NC, USA
ARO, The Volcani Center, Bet Dagan, Israel
ARO, The Volcani Center, Bet Dagan, Israel

Project Starting Date: August 1990

Type of report: Final

Principal Investigator

Institution Authorizing Official

Mark Pines

b. Table of Contents

c.	Abstract.....	3
d.	Objectives.....	4
e.	Research reports.....	5
Collagen as a major contributor to skin integrity		
	Skin tearing in broilers in relation to skin collagen: Effect of sex, strain, and diet.....	5
	Increased skin tearing in broilers and reduced collagen synthesis in skin <i>in vivo</i> and <i>in vitro</i> in response to the coccidiostat halofuginone.....	13
Environmental factors and skin collagen in broilers		
	The use of dietary ascorbic acid in the prevention of skin tearing in broiler chickens: Effect on collagen synthesis.....	18
	The use of dietary ascorbic acid in the prevention of skin tearing in broiler chickens: Interaction with environmental temperature and humidity.....	29
Collagen metabolism in skin fibroblasts		
	Growth hormone and insulin growth factor I regulate collagen gene expression and extracellular collagen in cultures of avian skin fibroblasts.....	36
	Effect of N-terminal modified analogs of growth hormone on collagen synthesis in avian skin fibroblasts.....	45
	Halofuginone: an inhibitor of collagen type I synthesis.....	51
Postranslational modification of collagen - cross linking		
	Developmental changes in skin collagen biosynthesis pathway and cross-linking in post-hatch male and female chickens.....	57
	Strain and sex dependent collagen cross-linking in chickens skin.....	72
Genetic selection for skin collagen in broilers		
	Genetic and phenotypic association between skin tearing and skin collagen, protein and fat in broilers.....	77
f.	Cooperation.....	86
g.	Evaluation of research achievements.....	87
h.	List of publications.....	88

Abstract

Skin tearing in broilers was found to be inversely related to the collagen concentration when strain, sex, and dietary protein and energy were varied. Halofuginone commonly used as a coccidiostat and previously identified as an important causative factor in skin tearing, reduced broiler skin collagen content. In cultured skin fibroblasts, halofuginone inhibited collagen synthesis and collagen type I gene expression. These results suggest that a) collagen is a major determinant of skin strength. b) The increase in skin tearing during processing, induced by halofuginone, is caused by direct suppression of skin collagen synthesis.

Dietary vitamin C, a cofactor in synthesis of collagen, appeared not to alter skin collagen, even under the most stressful conditions of temperature and relative humidity.

The developmental changes in the skin collagen biosynthesis pathway in male and female chickens were evaluated. Skin collagen content increased with age and was higher in males than in females. This is probably a consequence of higher expression of collagen type I genes [$\alpha 1(I)$ or $\alpha 2(I)$] in male than in female skin. At all ages examined, the levels of the collagen cross-link dihydroxylysinoxonorleucine (DHLNL) were higher in male compared with female skin. These results provide additional evidence which shows that the higher tensile strength of male skin compared with female is due to the elevated skin collagen content, resulted from increased expression in collagen type I genes on the one hand, and the higher amount of DHLNL collagen cross-links on the other.

The endocrine control of collagen type I synthesis by skin fibroblasts was investigated. Growth hormone (GH) and insulin-like growth factor I (IGF-I) inhibited type I procollagen gene expression. Collagen degradation was enhanced by IGF-I, but not modified by GH, suggesting that GH and IGF-I cooperate in regulating collagen biosynthesis, but collagen degradation is affected only by IGF-I. Collagen synthesis by skin fibroblasts was also used to evaluate the structure-function relationships of GH.

A genetic selection program was initiated to improve skin quality. Offspring from parents with high collagen content had higher levels of collagen compared with offspring of parents with low skin collagen content. The higher skin collagen resulted in decrease in skin tearing during processing without affecting growth and feed efficiency. It is concluded that heavy losses to the poultry industry caused by damage to broiler skin during processing could be reduced by selection for skin collagen. This selection program is now examined in detail, and if found applicable, will be included in the Israeli Selection Program.

Objectives

1. To identify nutritional and environmental factors deleterious or favorable to skin strength and to evaluate their effect on skin collagen concentration, collagen types and crosslinking, and on skin cell proliferation.
2. To identify and evaluate endocrine, paracrine and autocrine factors which control skin cell proliferation and collagen synthesis and degradation, including collagen genes expression.
3. Initiation of practical implementation of results through field tests and computer models used for economical optimization.

Cooperation

Experimental protocols and distribution of research efforts were coordinated, and results were discussed during exchange visits of principal investigators. Skin samples from various experiments performed in Israel were send for collagen cross-linking determination at Chapel Hill.

Evaluation of research achievements

Of practical importance is the finding that skin tearing in broilers was inversely related to the collagen concentration in skin when strain, sex, halofuginone and dietary protein and energy were varied, suggesting that collagen was a major determinant of skin strength. Nutritional factors such as dietary protein or ascorbic acid, and environmental factors were not found to be of major practical consequence. On the other hand, a strong genetic component of skin collagen concentration, in association with skin strength was discovered. On the basis of these results, a genetic selection program to increase broiler skin collagen in order to improve skin quality and reduce skin tears was initiated. This selection program is now examined in detail, and if found applicable, will be included in the Israeli Selection Program and will reduce the estimated annual loss of \$ 5-10 million in Israel alone.

The mechanism of the involvement of the coccidiostat halofuginone in inducing skin tearing was identified as the inhibition of collagen synthesis by skin fibroblasts. This was further narrowed down as a specific inhibition of collagen type I gene expression at the transcription level. The finding of a drug which specifically affects transcription at a very low concentrations (10^{-10} M), is of major scientific importance.

The findings concerning the involvement of IGF-I and growth hormone in regulation of collagen synthesis is of importance in adding more detail to the understanding of the control of synthesis of this important protein on the one hand, and elucidation of some of the function of growth hormone in avian species, on the other. With regard to the practical aspects, these results can also contribute to the understanding of the possible association between growth and skin integrity.

List of publications

- Granot, I., Bartov, I., Plavnik, I., Wax, E., Hurwitz, S. and Pines, M. (1991) Increased skin tearing in broilers and reduced collagen synthesis in skin *In vivo* and *in vitro* in response to the coccidiostat halofuginone. Poult. Sci. 70: 1559-1563.
- Granot, I., Pines, M., Plavnik, I., Wax, E., Hurwitz, S. and Bartov, I. (1991) Skin tearing in broilers in relation to skin collagen: Effect of sex, strain, and diet. Poult. Sci. 70: 1928-1935.
- Granot, I., Halevy, O., Hurwitz, S. and Pines, M. (1991) Growth hormone and insulin growth factor I regulate collagen gene expression and extracellular collagen in cultures of avian skin fibroblasts. Mol. Cell. Endocrinol. 80: 1-9.
- Granot, I., Hurwitz, S., Halevy, O. and Pines, M. (1993) Halofuginone: an inhibitor of collagen type I synthesis. Biochim. Biophys Acta 1156:107-112.
- Granot, I., Halevy, O., Tchelet, A., Sakal, E., Gertler, A., Vogel, T., Hurwitz, S. and Pines, M. (1993) Effect of N-terminal modified analogs of growth hormone on collagen synthesis in avian skin fibroblasts. Mol. Cell. Endocrinol. 92:241-246.
- Cahaner, A., Gutman, M. and Pines, M. (1993) Genetic and phenotypic association between skin tearing and skin collagen, protein and fat in broilers. Poult. Sci. 72: 1832-1840.
- Pines, M., Hurwitz, S., Schickler, M. and Yamauchi, M. (1994) Developmental changes in skin collagen biosynthesis pathway and cross-linking in post-hatch male and female chickens. Submitted.

Articles and lectures published in Proceeding of Symposia.

- Pines, M., Granot, I. and Hurwitz, S. (1988) Relationship between skin collagen content and strength in broilers. The XXVI Annual Convention of World's Poultry Science Association, Zichron Yaacov.
- Cahaner, A., Granot, I. and Pines, M. (1989) The genetics of skin collagen content in broilers and its potential application in breeding against skin tears. The XXVI Annual Convention of World's Poultry Science Association, Zichron Yaacov.
- Goussac, D., Meyuhas, S., Navon, S., Rav-Hon, s., Rolf, Y., Granot, I and Pines, M (1989) Effect of nutritional treatments on skin tearing of female broilers. The XXVI Annual Convention of World's Poultry Science Association, Zichron Yaacov.
- Pines, M., Granot, I., Halevy, O. and Shmuel Hurwitz (1990) Halofuginone: An specific inhibitor of collagen type I synthesis The XXV Annual Convention of World's Poultry Science Association, Zichron Yaacov.

משרד המבחן
משרד המבחן
משרד המבחן