

ESTIMATION OF HYDROXYPROLINE AND COLLAGEN IN THE SKIN OF DEER, SHEEP AND GOAT

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Received: 25-10-2019 Accepted 31-10-2019

ABSTRACT

The present study was conducted using skin samples of spotted deer, sheep and crossbred goat to estimate the quantity of hydroxyproline and collagen in the specimens. The specimens were collected from dorsal lumbar regions of the deer brought for post-mortem at the Pathology department of the College from Thrissur zoo and from Forest Department and of sheep and goat, which were freshly slaughtered at the Meat Technology Unit of the College. The samples were analysed for hydroxyproline as a measure of collagen content at the Meat Technology Unit of the College. Skin predominantly consisted of Type I collagen. Variation in the collagen content has been observed among the species under study. The dermal collagen content was

more in the wild species.

Key words: Collagen, Deer, Goat, Hydroxyproline, Skin

INTRODUCTION

Organs and tissues of body contain a complex extracellular matrix, which maintains structure and plays important roles in its function. Collagen is the most abundant of extracellular matrix protein and its content in a tissue depends on both synthetic and degradative processes. Any change in deposition of collagen depends on an alteration in balance of these processes. In growth, development, wound healing, these processes are tightly regulated to maintain or restore tissue architecture and function. In pathologies with fibrosis, there is a net increase in deposition

of extracellular matrix proteins, including collagens. Fibrotic diseases affecting organs and tissues exhibit a major pathogenic feature as the excessive and disorganized disposition of collagens, severely limiting the tissue function due to changes in both synthetic and degradative pathways. Tissues show relative abundance of hydroxyproline in collagen, compared with other proteins and hence facilitate the use of former as a relatively specific marker of collagen content and metabolism.

$C_5H_9O_3N$, is a common non-proteinogenic amino acid, abbreviated as HYP, in Protein Data Bank. Hydroxyproline is a major component of collagen and has a chemical structure of 4-hydroxypyrrolidine-2-carboxylic acid. It plays key role for collagen stability, and permits sharp twisting of collagen helix. It is also found in few proteins other than collagen and hence used as indicator to determine the collagen content of tissues indirectly. Since skin predominantly contains Type I collagen and species variation in collagen content is observed, this study was undertaken to estimate collagen content in skin of spotted deer, sheep and cross-bred goat.

MATERIALS AND METHODS

Specimens were collected from dorsal lumbar region of spotted deer brought for post mortem at College of Veterinary and Animal

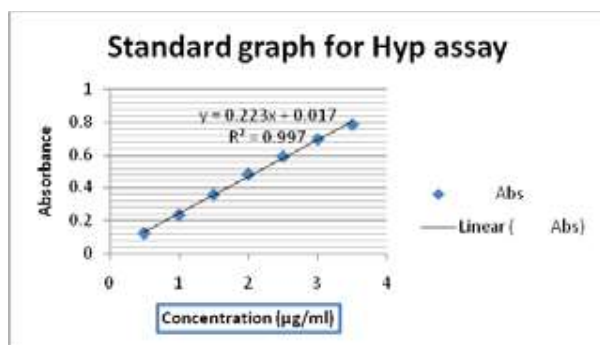
Sciences, Mannuthy from Thrissur zoo/ Forest department and of sheep and goat, freshly slaughtered at Meat Technology Unit, Mannuthy. Samples were analysed for hydroxyproline as a measure of Collagen content at Meat Technology Unit of the College using Chloramin T method for hydroxyproline estimation. Fat and hair on skin samples were removed, moisture removed using a hot air oven and fat extracted and discarded using Socs Plus Solvent Extraction System (Pelican Equipments, India).

Specimen was hydrolysed in a solution of hydrochloric acid. Resultant hydroxyproline was oxidised with chloramin-T and reacted with 4-dimethyl amino benzaldehyde (DMAB). The red colour developed was measured photometrically using Chloramin T method (Stegemann, and Stadler, 1967; AOAC, 1990).

Read absorbance at 560 nm immediately in a spectrophotometer. Concentration calculated from regression equation computed from reference values.

- $Y = ax + b$
- $Y = \text{slope} \times \text{concentration} + \text{intercept}$
- $\text{Absorbance} = 0.223 \times \text{concentration of Hyp} + 0.017$
- $\text{Concentration of sample } (\mu\text{g/ml}) =$

Absorbance of sample + 0.017/0.223



Serial dilution of standard hydroxyproline from 0.5 to 3.5 concentration was prepared and absorbance was measured at 560 nm. Standard graph was plotted and was used to estimate the quantity of hydroxyproline in the test samples.

RESULTS AND DISCUSSION

The mean values of hydroxyproline and collagen content of the skin samples are in shown in Table 1. The study that the dermal collagen content was more in the deer. Further, histologically there was a direct relationship between skin collagen and dermal thickness.

Woessner (2007) also presented a method for the quantitative determination of hydroxyproline in biological materials containing as little as one part of hydroxyproline in 4000 parts of amino acids. The method was applied to study the hydroxyproline distribution in cell particulates, tissue fluids and purified plant and animal proteins and found significant amounts of hydroxyproline in crystalline preparations of pepsin, elastase and ficin.

As per the reports of Muralidharan and Ramesh (2005), percentage of collagen content of skin in cattle and buffalo were 73.90 ± 0.64 and 77.5 ± 0.40 respectively.

Hydroxyproline is a constant amino acid, specific to collagen and hence, present study also confirmed that estimation of hydroxyproline gives an indirect indication of collagen content in the skin. In the present study, the hydroxyproline and collagen estimation of the skin samples indicated that the dermal collagen content was more in the wild species.

Table 1. Mean collagen content and percent of skin in deer, sheep and goat

No	Species	Hyp (µg/ml)	Hydroxyproline (mg/100mg)	Collagen (µg/ml)	Collagen (%)
1.	Deer	3.830±0.235	10.214±0.680	28.569±1.754	76.201±5.076
2.	Sheep	3.706±0.081	9.595±0.231	27.647±0.607	73.400±0.882
3.	Goat	2.822±0.599	7.634±0.133	21.055±0.447	56.948±0.992

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