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# HISTOLOGICAL STUDY OF DERMIS OF CATTLE BREEDS OF MAHARASHTRA IN DIFFERENT CLIMATIC CONDITION

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**Abstract:** The present study was conducted on skin samples of 4 – 6 years of age healthy Deoni, Red kandhari, Dangi and Gaolao breeds of cattle managed under hygienic conditions on farm in different regions of Maharashtra. The skin samples, 10 of each breed were obtained surgically from loin region during winter and summer seasons separately.
The dermis was consisted of connective tissue fibers, cells, hair follicles, sweat glands, sebaceous glands, arrector Pili muscles and blood vessels. Dermis showed two distinct layers namely outer, thin, cellular papillary layer and inner, thick, fibrous reticular layer.
**Keywords:** Histology, Dermis, cattle Breeds, Climatic condition.

## Material and Methods

The present study was conducted in the Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Parbhani (M.S.). The experiment was carried out on 40 female cattle of 4 – 6 years of age belonging to different breeds located in different regions of Maharashtra state during winter (November - February) and summer (March – June) seasons, separately. The skin samples, 10 of each were obtained from loin region of healthy Deoni, Red kandhari, Gaolao and Dangi breeds of cattle, managed under hygienic conditions on the farm in different regions of Maharashtra state. Tissue pieces of 5 mm size were cut to preserve in following fixatives for the histomorphological study.

1. 10% Neutral buffered formalin
2. 10% formalin
3. Bouin's fluid

After fixation, tissues were washed in running tap water for overnight. These were then processed for routine paraffin technique. The tissues were first passed through ascending grades of alcohol, cleared in xylene, infiltrated in three changes of paraffin (melting point 580-600C) and then embedded in paraffin by employing manual tissue processing schedule suggested by Drury and Wallington (1980).

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The longitudinal and transverse tissue sections of 4 to 5 µ thickness were obtained on manually operated rotary microtome. The sections were mounted on glass slides and dried at room temperature for 24 hours and were preserved carefully for staining. The following staining methods were used for histomorphological studies.

a) Harri's Haematoxylin and Eosin stain for normal histoarchitectural study (Mukharjee, 1992).

b) Van Gieson's stain for collagen fibers (Singh and Sulochana, 1996).

c) Masson's trichrome method for collagen fibers (Luna, 1968).

d) Silver impregnation stain for Reticular fibers (Mukherjee, 1992).

e) Wilder's method for reticulin (Luna, 1968).

f) Verhoeff's stain for elastic fibers (Mukharjee, 1992).

g) Crossman's modification of Mallory's triple stain for collagen and elastic fibers (Singh and Sulochana, 1996).

h) Periodic acid Schiff's (PAS) stain for carbohydrate like glycogen, mucin and reticulin (Mukharjee, 1992).

The micrometrical recordings were taken under simple microscope by micrometer scale after caliberation at 10X (1µ = 15.38 graduations) and 40 X power (1µ = 3.30 graduation) magnifications.

The data collected was subjected to the statistical analysis as per the standard procedure suggested by Panse and Sukhatme (1967).

**Results and Discussion**

Dermis was thick fibrous and vascular layer of skin, attached to the epidermis by basement membrane. The dermis in all breeds of cattle and seasons under present study revealed irregular network of collagen, elastic and reticular fibers with cells of connective tissue, vessels, nerves, hair follicles, sweat glands, sebaceous glands and arrector pili muscles. Microscopically, dermis showed two layers i.e. thin, superficial papillary layer and thick, deeper reticular layer (Plate 1).

It was observed that, connective tissues of papillary layer blends with the connective tissues of the reticular layer. However, the maximum number of sweat glands was found in between these two layers, where the connective tissue of dermis was sparse. This area of sweat gland population indicated the line of demarcation between the papillary and reticular layer of dermis in all breeds of cattle and seasons under present study (Plate 2).

Similar reports were made by Akers and Denbow (2008), Monteiro-Riviere (2007), Samuelson (2007) in domestic animals, Aslan *et al.* (2004) in Zavot breed of cattle and Mugale *et al.* (2001) in Deoni cattle. They reported that dermis was composed of superficial papillary and deeper reticular layer, consisted of irregular network of connective tissue fibers, sweat glands, sebaceous glands, hair follicles, arrector pili muscles, connective tissue cells, vessels and nerves. In contrast to the present observation, Nagaraju *et al.* (2012) in cattle and Monteiro-Riviere (2007) stated that stratum reticularis of dermis (reticular layer) was not so distinct and not clearly demarcated from stratum papillaris (papillary layer).

In the present study, it was observed that papillary layer of dermis composed of loose connective tissue with variety of connective tissue cells. The dermal papillae were the upward projections of superficial papillary layer between epidermal pegs. They were highly vascular than the remainder part of papillary layer in all breeds of cattle and seasons under present study (Plate 3). In agreement with the present findings Samuelson (2007) in domestic animals reported that papillary layer of dermis was composed of loose web of extracellular matrix. He stated that nourishment of epidermis was assisted by the capillary bed within each dermal papilla. Similar observations were also made by Akers and Denbow (2008) in domestic animals, who reported that papillary layer formed finger like projections called dermal papillae that contained capillaries.

In all breeds of cattle and seasons under present study, it was noticed that the collagen fibers were predominant over the other fiber types in papillary layer. These fibers were more compact and parallel to the skin surface below the epidermis. Whereas, cross and longitudinal section of skin revealed, longitudinal as well as concentric arrangement of collagen fibers around hair follicles, sebaceous glands and formed sheath surrounding to the sweat glands. In remainder of papillary layer collagen fibers were loosely interwoven and interposed between myofibers of arrector pili muscles (Plate 4).

The presence of compact arrangement of collagen fibers below the epidermis could be attributed for providing strong attachment between the epidermis and dermis. Similarly, presence of concentric and longitudinal arrangement of collagen fibers around hair follicles and sebaceous glands might be ascribed to provide firmness to these structures.

This observation of present study corroborates with the findings made by Calhoun and Stinson (1981) in domestic animals. They reported circularly and longitudinally arranged network of collagen fibers formed connective sheath around hair follicles.

It was noticed that, in all breeds of cattle and seasons under present study, elastic fibers in papillary layer were very few than the other fiber type, distributed predominantly in middle to lower part of papillary layer. They were sparse in superficial part of papillary layer. Elastic fibers were fine branched and were arranged parallel to the skin surface forming loose network. These fibers were found intermixed with the concentrically arranged collagen fibers around the root of hair follicles and arranged as a single fiber layer surrounding to sweat and sebaceous glands, blood vessels and finely interposed between the myofibers of arrector pili muscle. However, they were more at the ends of arrector pili muscle.

In line with the present observations, Bhayani and Vyas (1991) in Gir cattle reported presence of network of elastic fibers around hair follicles. Similar observation was made by Monteiro-Riviere (2007) in domestic animals. They stated that arrector pili muscle was anchored by elastic fibers. Although, Mugale *et al.* (2001) in Deoni cattle reported similar findings on morphology, amount, distribution of elastic fibers, but the observations of present study regarding the orientation of elastic fibers is in contrast where in they reported that elastic fibers were arranged perpendicular to the skin surface in papillary layer.

The presence of elastic fibers around root of hair follicles and wall of sweat and sebaceous glands observed during present study might be correlated to provide elasticity for the enlargement of hair follicle during its growth and for distension of glands caused by accumulation of their secretory contents. The presence of these fibers at ends of arrector pili muscle may aid in relaxation of this muscle after its contraction. During present study, sections stained for reticular fibers showed distinct thin reticular fiber layer below and along the epidermis and around the sweat glands. Reticular fibers were fine and loosely interwoven in the papillary layer. However, it formed dense lattice below the epidermis, around the hair follicles and sebaceous glands. These fibers were observed around the wall of blood vessels, interposed in the tunica media of large blood vessels and in between the myofibers of arrector pili muscle.

These observations of the present study regarding the distribution of reticular fibers confirm the observations recorded by Mugale *et al.* (2001) in Deoni breed of cattle. They reported abundant distribution of reticular fibers at dermo-epidermal junction and around sweat glands, sebaceous glands and blood vessel wall. In agreement with the present findings, Calhoun and Stinson (1981) in domestic animals stated that epidermal part of the hair follicle was separated from the dermis by basal lamina associated with reticular fiber.

The distinct thin reticular fiber layer below and along the epidermis and around sweat gland confirms the presence of reticular lamina which is an integral part of basement membrane of epidermis and sweat gland epithelium. The dense lattice of reticular fibers below the epidermis, around hair follicles and sebaceous glands may serve as a scaffold for support of these skin components.

In the present study, the dermal reticular layer was composed of dense irregular connective tissue, chiefly of smooth muscular tissue and collagen fibers. The smooth muscle fiber bundles were irregularly arranged. The size and number of smooth muscle fiber bundles were more in the middle and deeper part than that of superficial part of reticular layer in all breeds of cattle and seasons under present study.

The collagen fibers in the dermal reticular layer were arranged as irregularly distributed bundles. These were more in the superficial part of reticular layer. These fibers were found interposed in the muscle fibers of smooth muscle bundles in all breeds of cattle and season under present study. In all breeds of cattle and season in the present investigation, the elastic fibers in the dermal reticular layer were very few than the other fiber type. These fibers were interposed in the fibers of smooth muscular tissue. These were found surrounding to the blood vessels and formed of internal elastic lamina of large blood vessels.

In the present study, the reticular fibers in dermal reticular layer were course than that of the papillary layer. These were found around the blood vessels and interposed in the fibers of smooth muscle bundles.

The average values of thickness of papillary layer are presented in table 1. The average thickness of papillary layer during summer season was recorded as 682.40 ± 16.13 μm, 527.38 ± 8.72 μm, 581.36 ± 2.56 μm and 540.91 ± 3.68 μm. Whereas, during winter season it was recorded as 750.08 ± 3.36 μm, 645.34 ± 3.36 μm, 690.56 ± 8.46 μm and 680.87± 1.00 μm in Deoni, Red Kandhari, Dangi and Gaolao breeds of cattle, respectively. There was a significant increase in the thickness of papillary layer in winter season in all breeds of cattle under present study. The present observation can be justified by the reports of Nay and Hayman (1963) who stated that papillary layer was significantly greater in thickness in winter than summer season in different European dairy cattle breeds.

The average thickness of papillary layer showed significant statistical variation between different breeds of animals in both season under present work. However, non-significant difference was observed between Red kandhari and Gaolao cattle during summer season and between Dangi and Gaolao cattle during winter season.

The average thickness of papillary layer in all Breeds of cattle and season during present work is found to be lower than the reports made by Walkar (1960) in different indigenous African cattle breeds, Nay and Hamen (1963) in different European dairy cattle breeds during summer and winter season, Patel *et al.* (1988) in Jersey X Kankrej and Holstein X Kankrej cross breed cows, Bhayani *et al.* (1989) in adult Kankrej cow, Aslan *et al.* (2004) in Zavot breed of cattle.

This variation in thickness of papillary layer may be due to breed or climatic differences. In support to these variations in thickness of papillary layer among breeds, Walker (1960) reported breed wise significant difference in the papillary layer in different indigenous African cattle breeds.

The average values of reticular layer thickness are presented in table 2. The average thickness of reticular layer during summer season was 3740.67 ± 53.13 µm, 3055.69 ± 4.12 µm, 3592.46 ±9.68 µm and 3661.82 ± 34.74 µm. Whereas in winter season, it was 3870.99 ± 9.10 µm, 3240.10 ± 4.91 µm, 3750.87 ± 3.49 µm and 3810.70 ± 2.91 µm in Deoni, Red Kandhari, Dangi and Gaolao cattle, respectively. The significant increase in the thickness of reticular layer was observed during winter season in all breeds of cattle under present investigation.

The significant difference in terms of reticular layer thickness was observed between Deoni, Red Kandhari, Dangi and Gaolao cattle in both seasons.

The average thickness of reticular layer recorded in the present study in all breeds of cattle and season is found to be less than the thickness of reticular layer reported by Walker (1960) in skin over second last rib area in different African indigenous cattle breeds, Bhayani *et al.* (1989) in skin of mid side region at 12th intercostal space in Kankrej cow and reports of Aslan *et al.* (2004) who reported average thickness of reticular layer from different body region in Zavot cattle breed.

Patel *et al.* (1988) reported less thickness of reticular layer in skin of mid side region between 12th – 13th intercostal space in two cross breed cows up to age of 42 months, Bhayani and Vyas (1990) in skin of mid side region in adult Gir cows. Similarly, as compared to present recordings, Muralidharan and Ramesh (2005) noted less thickness of reticular layer in Sindhi cattle and more thickness of it in Jersey and Jersey X Sindhi cows.

However, the average thickness of reticular layer in Dangi cattle during summer season in the present study is within the range of reports made by Bhayani and Vyas (1990) in adult Gir cow. These variations between thickness of reticular layer of present study and the reports made by other workers may be attributed to age, breed, body region and seasonal differences.

**Table 1: Mean and SE of papillary layer thickness of the skin during summer and winter seasons in Deoni, Red Kandhari, Dangi and Gaolao cattle**

Thickness of papillary layer (µm)

| Season | Deoni | Red Kandhari | Dangi | Gaolao |
|---|---|---|---|---|
| **Summer** | 682.40 A2 ± 16.13 | 527.38 C2 ± 8.72 | 581.36 B2 ± 2.56 | 540.91 C2 ± 3.68 |
| **Winter** | 750.08 A1 ± 3.36 | 645.34 C1 ± 3.36 | 690.56 B1 ± 8.46 | 680.87 B1 ± 1.00 |

**Table 2: Mean and SE of reticular layer thickness of the skin during summer and winter seasons in Deoni, Red Kandhari, Dangi and Gaolao cattle**

Thickness of reticular layer (µm)

| Season | Deoni | Red Kandhari | Dangi | Gaolao |
|---|---|---|---|---|
| **Summer** | 3740.67 A2 ± 35.13 | 3055.69 D2 ± 4.12 | 3592.46 C2 ± 9.68 | 3661.82 B2 ± 34.74 |
| **Winter** | 3870.99 A1 ± 9.10 | 3240.10 D1 ± 4.91 | 3750.87 C1 ± 3.49 | 3810.70 B1 ± 2.91 |

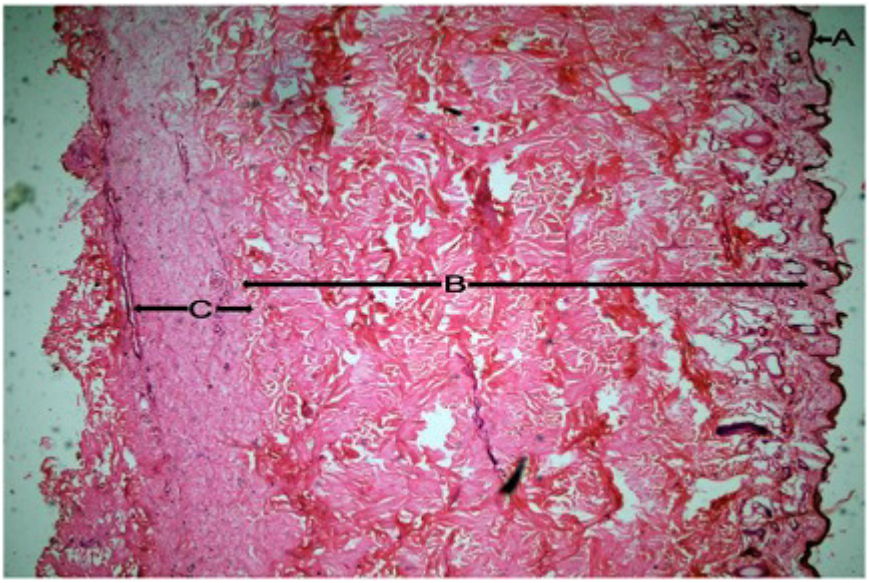


**PLATE 1: Photomicrograph of skin of Deoni cattle in summer season showing**
B. Dermis (Haematoxylin and Eosin, X 40)

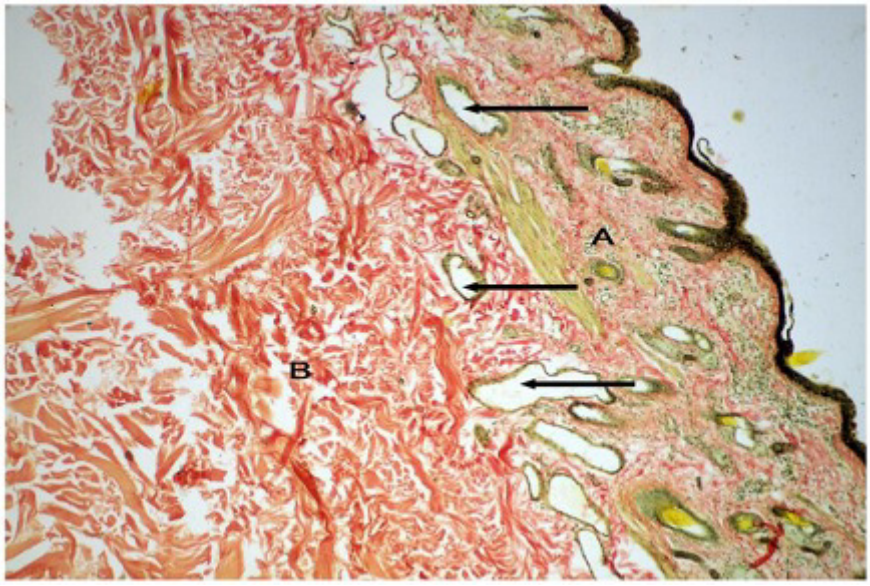


**PLATE 2: Photomicrograph of skin of Deoni cattle during summer season**
A. Papillary layer
B. Reticular layer
(Van Gieson's, X 100)

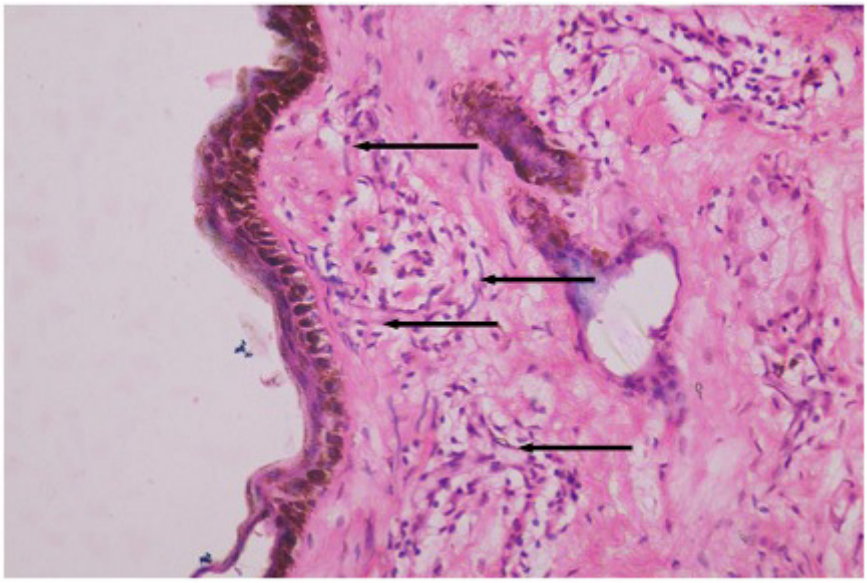

**PLATE 3: Photomicrograph of skin of Deoni cattle during summer season**
**Arrow showing capillary network in the dermal papilla**
(Haematoxylin and Eosin, X 400)

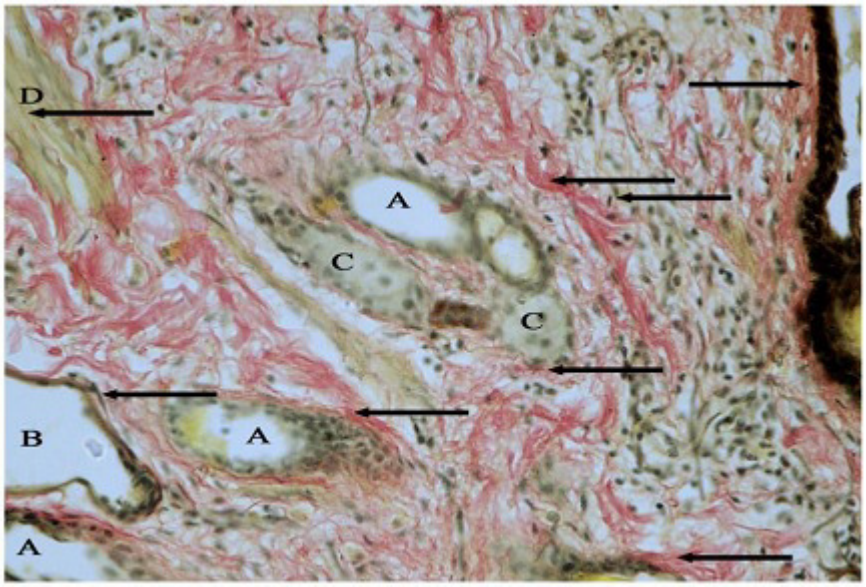


**PLATE 4: Photomicrograph of skin of Deoni cattle during summer season Arrow showing distribution of collagen fibers in papillary layer of dermis**
D. Arrector Pili muscle (Van Gieson's, X 400)

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