Open Access

Int. J. Trop. Vet. Biomed. Res.
Vol. 9 (1): 47-55; May 2024

www.jurnal.usk.ac.id/IJTVBR
E-ISSN : 2503-4715

Histology and histomorphometry of the skin in the ventral abdominal region of domestic fowl (*Gallus gallus domesticus*), duck (*Anas platyrhynchos*), and doves (*Columba livia*)

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Abstract

This study aims to determine the histological and histomorphometric characteristics of the skin of domestic fowl (*Gallus gallus domesticus*), ducks (*Anas platyrhynchos*), and doves (*Columba livia*) in the ventral abdominal region. Each study sample consisted of six individuals. The samples were processed into histological preparations with a thickness of 5 µm and then stained using Haematoxylin-Eosin (HE). The results showed that the histological structure of the skin of domestic fowl, ducks, and doves is relatively thin compared to the structure of mammalian skin and consists of the epidermis, dermis, and hypodermis (subcutis). The histomorphometric data analysis showed that the thickness of the epidermal layer of the skin in the ventral abdominal region of domestic fowl, ducks, and doves was not significantly different ($P>0.05$), but there were significant differences ($P<0.05$) in the thickness of the dermis and hypodermis. It can be concluded that a similarity exists in the histological structure of the skin of domestic fowl, ducks, and doves, but differences are observed in the histomorphometric properties of the skin in the ventral abdominal region of these birds.

Keywords: Aves, histology, histomorphometry, skin

Citation: Zainuddin, Z., Rahmi, E., Akmal, M., Masyitha, D., Ismail, I., Hamny, H., Pratiwi, S., & Zulkifli, B. (2024). Histology and histomorphometry of the skin in the ventral abdominal region of domestic fowl (Gallus gallus domesticus), duck (Anas platyrhynchos), and doves (Columba livia). *The International Journal of Tropical Veterinary and Biomedical Research*, 9(1), 47-55.

Introduction

Chickens, ducks, and pigeons belong to the class Aves. Aves have wings formed by the modification of the front extremities and legs that are adapted to their habitats, such as walking, swimming, or perching. Aves include a class with many orders that live in diverse habitats, including the order Anseriformes (ducks, geese, swans), which have broad beaks covered with many sensory organs, short legs, and short tails. The order Galliformes (chickens, peacocks, turkeys) have short beaks and a pair of legs used for scratching for seeds. The order Columbiformes (pigeons and doves) have slender and short beaks with soft skin ceres at the base of the beak, as well as toes that are longer than the tarsus (Brotowidjoyo, 1994).

The most commonly kept Aves by humans are chickens (Galliformes) and ducks (Anseriformes). Pigeons were originally wild Aves that were later domesticated (Kurniawan dan Arifianto, 2017). The characteristics of Aves include having skin covered with feathers, wings for movement or flight, a pair of legs, a beak, being homoiothermic, having lungs and air sacs, and inhabiting land, water, and air (Astuti, 2007). Additionally, feathers are the most distinctive characteristic of Aves, complementing their complex skin epidermis. Based on the presence of feathers, the skin of Aves is classified into *apteria* (featherless areas) and *pterylae*

(feathered areas) (Zhang et al., 2017). Research conducted by Joseph et al. (2018) also explains that the skin of chickens without feathers is thinner compared to the areas with feathers.

In general, the skin of Aves consists of three layers: the epidermis, dermis, and hypodermis (Rakhmawati and Sulistyoningsih, 2019). The hypodermis of chickens contains more fat compared to the muscles or meat. It has been reported that the skin of village chickens has the lowest fat content compared to broiler chickens and red chickens (Rakhmawati and Sulistyoningsih, 2019). Duck skin, which ranges from white to pink, is covered by feathers. The thickness of duck skin varies across different parts of the body, with the ventral surface being thicker than the dorsal surface. The sternum and abdomen's skin is relatively denser than other body parts. Duck skin's subcutaneous layer (hypodermis) contains fat deposits that cushion the feather follicles (Joseph et al., 2018). According to Yadav et al. (2020), the skin of Aves tends to be thin, which helps reduce body weight as an adaptation for flight. It is known that the skin of the Red-headed bunting (*Emberiza bruniceps*) bird is pink or pale red, elastic, loose, and attached to the underlying muscles, facilitating movement during flight. Polygonal-shaped cells are arranged to form gaps, making the bird's skin more elastic for flight adaptation.

According to research conducted by Kurniawati et al. (2020), the thickness of the skin layers varies depending on breed, species, health status, age, body location, and environmental or habitat conditions. Based on Mumford et al. (2007), cited by Andriani et al. (2007), stated that the thickness of the epidermal layer varies according to body part, reproductive cycle, sex, and habitat or environmental conditions. Renaudeau et al. (2006), cited by Apriani et al. (2019), noted that the thickness of the hypodermis is influenced by diet, genetics, hormones, and the animal's activity.

Currently, research on the histology and histomorphometry of the skin in village chickens (*Gallus gallus domesticus*), ducks (*Anas platyrhynchos*), and pigeons (*Columba livia*) in the ventral abdominal region is very limited. Kiladze (2008) reported on the morphometry and collagen stromal structure of village chicken skin. Rakhmawati and Sulistyoningsih (2019) reported on the comparison of fat thickness in the skin layers of various consumption chicken types. Fabian et al. (2021) reported the presence of smooth muscle in the fascicles of pigeon skin dermis, forming attachments with myotendinous feather follicles, which play a role in flight activity and thermoregulation.

Previous research has reported that, histologically and electrophysiologically, the wing skin of pigeons contains vibration-sensitive receptors (*Herbst corpuscles*) that function to detect changes in vibration frequency in the surrounding environment. This enables pigeons to respond quickly to stimuli and enhances their ability to adapt to various situations (Horster, 1990). Hashim et al. (2020) reported on the histology of the head skin of Cairina moschata (*Muscovy duck*) during the post-egg period. However, comprehensive reports on the histology and histomorphometry of the skin in village chickens (*Gallus gallus domesticus*), ducks (*Anas platyrhynchos*), and pigeons (*Columba livia*) in the ventral abdominal region are still rare. Based on this background, there is a need for research on the histology and histomorphometry of the skin in village chickens, ducks, and pigeons in the ventral abdominal region.

Materials and Method

Sample Preparation

This study uses skin samples from the ventral abdominal region of three types of Aves: six village chickens (*Gallus gallus domesticus*), six ducks (*Anas platyrhynchos*), and six pigeons (*Columba livia*), all male and aged 4-6 months. The samples were prepared for histological examination using hematoxylin and eosin (HE) staining, and their histological structures were observed. Histomorphometry measurements included the thickness of the epidermal to hypodermal layers, using averages from

three orientation points measured at two edge points and one central point.

Materials

The equipment used includes a measuring cylinder, organ storage containers, a minor surgery set, tweezers, an aluminum tray, petri-disc, tissue paper, staining jars, dropping pipettes, an oven, an embedding processor, a basket, a microtome, a microtome knife, object glass, cover glass, labeling paper, a slide warmer at 37 ºC, a water bath, a light microscope (Olympus CX31), and Toupview software.

The materials used are village chicken skin, duck skin, pigeon skin, 0.9% NaCl physiological solution, neutral buffered formalin (NBF) as a fixative, distilled water, Xylol, alcohol with graded concentrations (70%, 80%, 90%, 95%, and absolute), 96% alcohol, paraffin, hematoxylin and eosin, and Entellan®.

Results and Discussion

Histological Structure of Aves Skin

Based on the observations, the histological structure of the skin in village chickens, ducks, and pigeons is very thin. This is consistent with Spearman's (1996) report that Aves skin is very thin and elastic. The histological structure of Aves skin consists of three layers: the epidermis, dermis, and hypodermis. This aligns with Rakhmawati and Sulistyoningsih's (2019) statement that the histological structure of Aves skin comprises three layers: the epidermis, dermis (corium), and hypodermis (*subcutis*).

The observations indicate that the epidermal layer consists of the *stratum basale*, *stratum intermedium*, and *stratum superficial*. The *stratum granulosum* in Aves is not distinctly visible. The *stratum lucidum* is not observed in Aves skin due to the very short duration of its formation from the *stratum granulosum*, generally forming only a very thin layer. Cells in each epidermal layer undergo mitosis towards the skin surface, forming a cornified layer (*stratum corneum*). The presence of this cornified layer signifies that the skin functions as a protective barrier against mechanical abrasion and temperature balance, as well as allowing the exchange of substances (excretion and respiration) (Mulyani, 2003). Kurniawati et al. (2020) explain that the skin is the outermost and largest organ covering the body surface and plays a role in body homeostasis.

The epidermis is composed of stratified squamous epithelium with a cornified layer on its superficial part. Cells in the basal layer gradually move towards the superficial layer, differentiating until they eventually die and slough off. The dermis of Aves skin consists of two layers: the *stratum compactum*, located beneath the basal layer of the epidermis, and the *stratum laxum*. However, the boundary between these layers is not clear due to their intermingling. The dermal layer consists of connective tissue, blood vessels, feather follicles, and musculus erector pili surrounding the feather follicles (Kalangi, 2013).

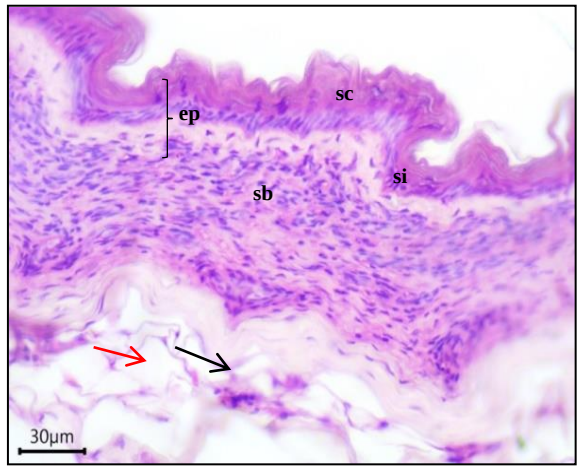

Figure 1. Histological image of the epidermis in the ventral abdominal region of village chicken (*Gallus gallus domesticus*) showing the epidermis (ep), *stratum basale* (sb), *stratum intermedium* (si), *stratum corneum* (sc), *stratum compactum* of the dermis (black arrow), and *stratum laxum* of the dermis (red arrow). HE staining with magnification 400x, scale bar 30 µm.

The hypodermis is the layer beneath the *stratum laxum* of the dermis and is made up of loose connective tissue with fine collagen fibers, fat cells, and blood vessels. The fat cells in the hypodermis are referred

to as panniculus adiposus (Kalangi, 2013). These fat cells are round with empty cytoplasm due to lysis during dehydration, causing the nucleus to be pulled to the edge of the cell membrane. Yadav et al. (2020) describe that the skin in Aves class is covered with feathers, except for the skin on the head and feet in some species. According to Weir and Lunam (2004), the fat layer in the skin supports relatively large feathers extending into the dermis. Kurniawati et al. (2020) state that feather follicles are formed by germinal cells at the base of the follicles scattered among the dermis. The histological depiction of the Aves skin epidermis is shown in Figure 1.

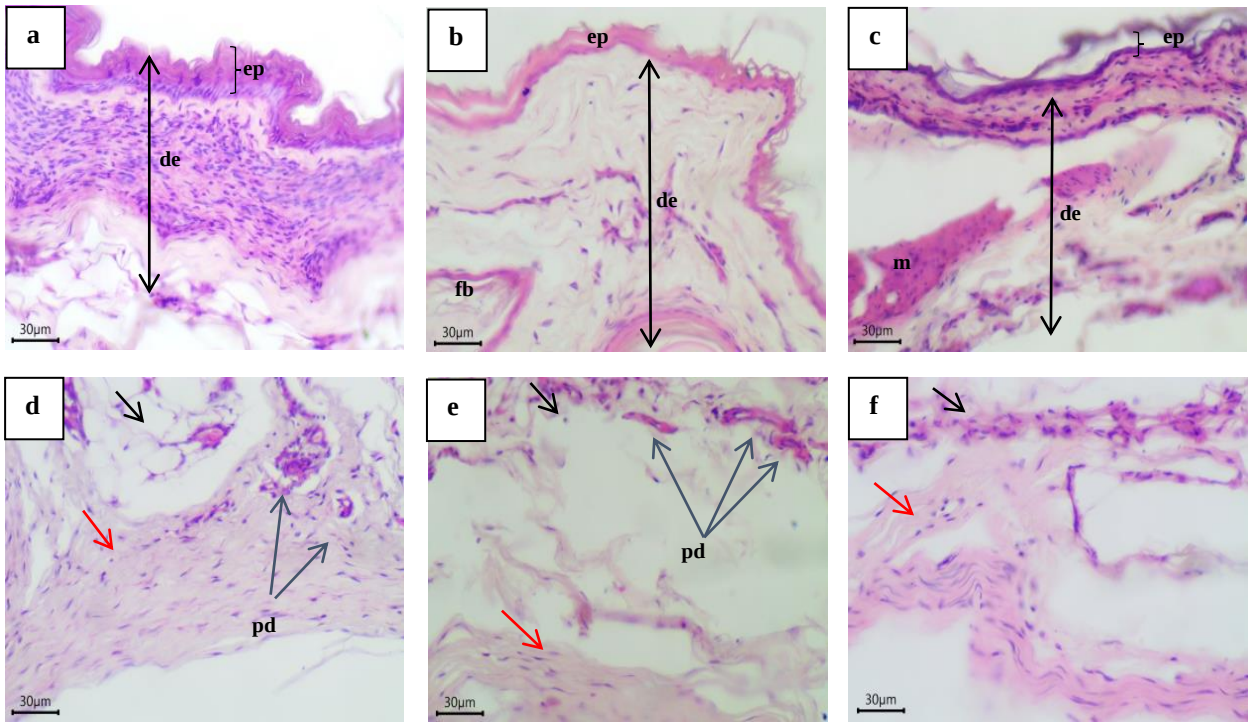

Figure 2. Histological image of the ventral abdominal skin: a) village chicken, b) duck, and c) pigeon, showing the presence of epidermis (ep); dermis (de); arrector pili muscle (m); feather follicle (fb). HE staining with 400x magnification, scale bar 30 µm. Histological image of the hypodermis of the ventral abdominal skin: d) village chicken, e) duck, and f) pigeon, showing the presence of fat cells (black arrow), collagen connective tissue (red arrow), and blood vessels (pd). HE staining with 400x magnification, scale bar 30 µm.

Skin in the Abdominal Region of Village Chicken, Duck, and Pigeon

Observation results on the ventral abdominal skin of village chicken, duck, and pigeon show the *stratum laxum*. The *stratum compactum* is located just below the epidermis and appears as denser connective tissue compared to the *stratum laxum*. The dermal layer contains connective tissue, blood vessels (arteries and veins), feather follicles, and muscles around the feather follicles. The connective tissue commonly found in this layer is collagen connective tissue. Collagen connective tissue appears as a fine pink staining with HE staining. This is consistent with Mulyani's (2003) statement that the dermal layer is rich in collagen connective tissue. Mansoori et al. (2013) also reported on the dermal structure of the ostrich post-biopsy, showing the presence of collagen and elastic connective tissue in the skin dermis. Chu (2012) also explained that collagen fibers are the dominant component in the dermal layer.

According to Marks et al. (2000), collagen is a protein that is the main component of connective tissue, commonly found in tendons, bones, blood vessels, and skin. Collagen fibers run in three directions: some bundles run parallel to the epidermis, perpendicular, and alternating (Weir and Lunam, 2004). The histological image of the

ventral abdominal skin of village chicken, duck, and pigeon is presented in the Figure 2 a, b, c. While, Figure 2 d, e, f shows the histological image of the hypodermis of the ventral abdominal skin.

Collagen plays a role in maintaining the structure, stability, and strength of the dermal layer (skin) (Widiyastuti and Megantara, 2021). In collagen connective tissue, many fibroblast cells are found. Fibroblast cells appear as flat, purplish cells over collagen fibers. This is consistent with the description by Wangko and Karundeng (2014) that fibroblasts are flattened cells with oval-shaped nuclei.

In the dermal layer, feather follicles of varying sizes can be found. The feather follicles are surrounded by epithelial cells known as the root sheath. Kalangi (2013) explains that feathers are keratin structures with histological similarities, including the presence of epidermis, dermis, and hypodermis layers. The epidermis layer consists of *stratum basale*, *stratum intermedium*, and *stratum superficial*, with the surface undergoing keratinization. This keratinized layer is formed from the *cytomorphosis* process of cells in the basal layer, which then differentiate and die, eventually peeling off. This aligns with Kalangi's (2013) report that during the mitosis process, skin cells differentiate, enlarge, and form keratin filaments. Upon reaching the skin surface, these cells die and gradually peel off.

Cells in the *stratum basale* are low cylindrical or cuboid with slightly oval nuclei near the basal membrane. Kalangi (2013) states that the basal layer of the epidermis consists of cells aligned above the basal membrane, with cylindrical or cuboid shapes. Cells in the *stratum intermedium* are polygonal and arranged in 3-4 layers. The *stratum corneum* in the epidermis consists of keratinized cells appearing as dead, peeling skin cells. Mansoori et al. (2013) report that the outermost layer of the ostrich epidermis consists of keratinized cells that tend to detach from the skin surface as flakes, giving the skin a dry appearance. Cells in the *stratum corneum* are flattened without nuclei, forming keratin lamellae. Menon et al. (1991) report that the pigeon *stratum corneum* is loose and more permeable than mammalian skin, contributing to the bird's elasticity and flexibility during flight.

The dermal layer of avian skin consists of two layers: the *stratum compactum* and the hard layer produced by the epithelium forming the feather follicles. The presence of feather follicles contributes to the skin's thickness. This aligns with Lucas and Stettenheim (1972), who state that dermal thickness increases in areas with more than one feather follicle. Around the feather follicles, musculus erector pili is found. This smooth muscle appears circular in cross-section and plays a role in feather contraction. According to Barathi et al. (2019), smooth muscle fibers in the dermis attach to feather follicles, and elastic tendons are visible at the attachment points. Blood vessels and nerves supporting feather presence are also found around the feather follicles. Judoamidjoyo (1981) states that avian skin layers are generally loose, with abundant fatty tissue and vasculature.

Blood vessels in the skin consist of arteries and veins. Arteries have thicker walls compared to veins. The presence of blood vessels in the dermis plays a role in maintaining body homeostasis and delivering nutrients to the epidermis. Ardha et al. (2014) explain that in the dermis, the connective tissue matrix houses randomly distributed connective tissue cells, loose collagen fibers, and capillaries that nourish the avascular epidermal cells and regulate body temperature. Peltonen et al. (1998) also explains that vascularization in the skin, including the abdomen, increases blood flow to provide fluid for skin evaporation.

The dermal layer has numerous fat cells. This aligns with Homberger and de Silva's (2000) explanation that fat deposits in the avian dermis act as hydraulic tissues aiding feather movement within the dermis. No secretory glands, such as sweat glands, are found in the dermis. This is a distinctive histological feature of avian skin compared to mammalian skin. According to Ulupi and Ihwantoro (2014), avian skin lacks sweat

glands but is covered by feathers that help regulate body temperature.

The hypodermis layer is bordered by the dermis and separated by a relatively thin elastic lamina. The hypodermis consists of collagen connective tissue, elastic fibers, fat cells, and blood vessels. This aligns with Kalangi (2013), who states that the hypodermis layer has looser connective tissue with fine collagen fibers. Skin fat in chickens' forms from the breakdown of carbohydrates into fat reserves. Feed factors can affect the presence of skin fat in chickens. Mulyani (2003) explains that high energy content in chicken feed results in high body fat content and deposition in the skin. Village chickens are reported to have lower subcutaneous fat deposition compared to other consumption chickens. This can be explained by genetic factors and feed factors affecting slow growth and minimal body fat accumulation (Rakhmawati and Sulistyoningsih, 2019).

Table 1. Mean and standard deviation of histomorphometry of avian skin

Thickness parameter (µm)	Species		
	A1	A2	A3
General Thickness	1201.74±223.59[a]	654.61±152.27[b]	675.18±243,.61[b]
Thickness of the epidermis layer	14.76±3.05[a]	12.55±3.54[a]	15.65±3.57[a]
Thickness of the dermis layer	996.52±133.56[a]	399.47±114.36[b]	350.08±57.57[b]
Thickness of the hypodermis layer	274.61±39.99[a]	251.54±43.71[a]	88.82±10.68[b]

[a,b] Differences in notation within the same row indicate significant differences (P<0.05). Ayam kampung (A1), duck (A2), and pigeon (A3).

According to Homberger and de Silva (2000), the subcutaneous layer of duck skin contains fat deposits acting as hydrostatic pads during feather movement. Ardha et al. (2014) explain that the subcutaneous tissue (hypodermis) is located below the dermis layer. Fat cells form a layer of varying thickness depending on body location, gender, and nutritional status. This fat tissue connects the skin to the underlying fascia. Vascular tissue is also present in the hypodermis layer.

Histomorphometry of the Skin

Histomorphometry is defined as a method of measuring cells or tissues (Wahyuni et al., 2012). Histomorphometry is performed to measure the thickness of the skin from the epidermis to the hypodermis, as well as the thickness of each of its layers. The results of the histomorphometry observations of the abdominal skin of native chickens, ducks, and pigeons are presented in Table 1.

Based on measurements of the epidermal layer thickness of the ventral abdominal skin in free-range chickens, ducks, and pigeons, there was no significant difference (P>0.05). Mumford et al. (2007) cited by Andriani et al. (2017) stated that the thickness of the epidermal layer varies depending on the body part, reproductive cycle, gender, and habitat or environmental conditions. This indicates that the thickness of the skin epidermis is not influenced by species differences but is influenced by body regions. Histologically, the epidermis of the ventral abdominal skin in free-range chickens, ducks, and pigeons has the same structure, which plays a role in protection against mechanical activities and acts as a barrier limiting the entry and exit of substances. Menon and Menon (2000) reported that avian epidermis contains unique *sebokeratinocytes* that secrete sebum-like lipids during keratinization. This secretion forms a double lipid layer that provides a permeability barrier to the skin.

Statistical tests showed that the dermal thickness of the ventral abdominal skin of free-range chickens differed significantly (P<0.05) from that of pigeons. The thickness of the skin layer in free-range chickens is also related to their agonistic habitus, thus the skin plays a role in body protection. This is in line with Wardi et al. (2019), who stated that roosters have a habit called agonistic behavior, characterized by

vigilance and attacking by pecking or using sharp spurs. The thickness of the dermal layer is related to the presence of feather follicles, erector pili muscles, vascularization, connective tissue, and dermal fat. Lucas and Stettenheim (1972) cited by Joseph et al. (2018) reported that the skin of birds that are rarely or not feathered is thinner than feathered skin, so the presence of feather follicles contributes to the thickness of the dermal layer.

Statistical tests showed that the thickness of the hypodermis of the ventral abdominal skin of free-range chickens differed significantly ($P<0.05$) from that of pigeons. Renaudeau et al. (2006) cited by Apriani et al. (2019) explained that the thickness of the hypodermis layer is influenced by diet, genetics, hormones, and animal activity. Based on statistical tests, the thicker hypodermal layer was found in the abdominal skin of free-range chickens. This is consistent with Wahyu (1997), who stated that the fat content in chickens is mostly found under the skin and less under the muscles (meat). The formation of skin fat comes from carbohydrates and fat reserves. Rakhmawati and Sulistyoningsih (2019) reported that, compared to other types of consumption chickens such as broilers and red chickens, the skin fat content of free-range chickens is actually lower.

Research by Damayanti (2006) stated that ducks are waterfowl used as a source of protein. Chavez (2000) reported that in Pekin laying ducks, the skin fat content is higher than in Muscovy ducks because nutrients in the feed are mostly converted for reproductive organs and maintaining body temperature. The ducks used in this study were meat ducks kept in cages, where the feed composition was dominated by protein, most of which was converted into meat, resulting in minimal skin fat deposits, but habitat conditions were not a variable used in this study. The hypodermal thickness of pigeon abdominal skin was also relatively thin compared to free-range chickens. The relatively thinner skin layer of pigeons compared to free-range chickens and ducks is related to their habitat and activities dominated by flying behavior. Thus, thin and elastic skin will facilitate the movement of pigeons during flight. This is as reported by Kadri et al. (2016), where the percentage of pigeon flying behavior was 69.93% and walking behavior was 30.07%. Samudera et al. (2016) reported that pigeon fat content tends to be low in the chest and generally high in the thighs.

Conclusion

Based on the research findings, it can be concluded that the histological structure of the ventral abdominal skin in free-range chickens, ducks, and pigeons comprises the epidermis, dermis, and hypodermis layers, mirroring the typical skin structure of vertebrate animals. Histomorphometric analysis reveals no significant differences in the thickness of the epidermal layer. However, there are notable differences in the thickness of the dermis and hypodermis layers. Specifically, the dermis and hypodermis layers in free-range chickens' ventral abdominal skin are significantly thicker than those in ducks and pigeons.

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