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Histomorphological and histochemical structure of the midgut and hindgut of the Caucasian honey bee (*Apis mellifera caucasia*)

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Abstract: The Caucasian honey bee (*Apis mellifera caucasia* subspecies of *Apis mellifera* L.) is one of the most common and prominent honey bee breeds in Turkey. The morphometry of endemic honey bee breeds has been extensively studied, but little attention has been given to the microscopic morphology of these bees. The aim of the current study was to describe for the first time the histomorphology of the midgut and hindgut of the Caucasian honey bee in Turkey. A total of 20 local Caucasian adult honey bee workers were sampled for histomorphological and histochemical analysis. The midgut epithelium consisted of epithelial cells with different morphologies. Acidic, neutral, and mixed mucosubstances were found in the luminal surfaces of the cells and peritrophic membranes. The ileum mostly consisted of an epithelium containing columnar cells that usually had basal nuclei. The rectum of adult workers had a single-layered epithelium externally, involving a layer of inner circular and outer longitudinal muscles. There were 6 long hollow rectal pads in the median-anterior area of the rectum. These results contribute in detail to our understanding of the histomorphology of the Caucasian (*A. m. caucasia*) honey bee.

Keywords: Caucasian (*A. m. caucasia*) honey bee, hindgut, histochemistry, histomorphology, honey bee, midgut

1. Introduction

In addition to being the primary insect species in terms of pollination of plants within the agricultural ecosystem, the honey bee (*Apis mellifera L.*) adds billions of dollars to the economy of livestock breeding [1–5]. In accordance with the conditions of the environment it has adapted to, the morphology, behavior, biology, and physiology of the honey bee varies; this results in high diversity among bee species around the world [6,7]. Previous studies have indicated that this variation results in differences in the structure of the digestive tract of the honey bee [8,9].

The digestive system of insects comprises 3 parts: the foregut, midgut, and hindgut. In honey bees, the foregut consists of the pharynx and the esophagus, the midgut consists of the ventriculus/real stomach, and the hindgut consists of the ileum and the rectum [9]. The ventriculus is recognized as the primary organ of pollen digestion and absorption, while the ileum and rectum are considered to be mostly responsible for osmotic control via the absorption of water and ions [10,11].

Honey bees are constantly exposed to several different stress factors in their ecosystem, such as pesticides [12], pathogens [13], and poor nutrition and/or malnutrition [14]. The midgut and intestines are the main organs affected by these negative stimulants. Midgut and intestinal mucosa act as a protective shield and induce the immune response against many honey bee pathogens [14]. For this reason, these organs are often the first areas that are attacked and infected by honey bee pathogens and other vectors [15]. In order to examine these interactions at either a cellular or tissue level in a comprehensive manner, a good level of familiarity with the normal morphology of the midgut and the intestines, as well as the honey bee species, is required.

This study was conducted to reveal for the first time the histomorphological features of the midgut and intestinal mucosa in the physiological conditions of the Caucasian (*A. m. caucasia*) honey bee, which is one of the endemic honey bee species in Turkey.

2. Materials and methods

All experimental procedures were approved by the Animal Ethics Committee of Ankara University (2016-8-89). A total of 20 Caucasian (*A. m. caucasia*) adult honey bee workers were sampled for histomorphological and histochemical analysis. Workers were selected from F1 colonies that had already been assessed in terms of health status, external

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drivers, and colony outputs. The bees were cryoanesthetized and dissected in a 100 mM NaCl solution. To prevent damage to the samples, the abdomen was gently cut and the organs were harvested using forceps, scalpel, and scissors. Initially, the revealed digestive tract was examined with Ringer's solution to check for the presence of any pathogen elements. The samples that were determined to be healthy were fixed in 10% neutral buffered formalin solution for 18 h, dehydrated in graded ethanol solutions, cleared with xylol, and then embedded in paraffin. After paraffin embedding, the specimens were cut into 5-µm sections. Crossmon's modified Mallory's trichrome stain was applied to the sections in order to determine the general morphological structure [16]. The following staining procedures were used for the general histochemistry of mucosubstances: periodic acid–Schiff (PAS) reaction to detect neutral mucosubstances, alcian blue (AB) staining at pH 2.5 to assess the nature of the acidic mucosubstances and to detect selective characterization of sulfated mucosubstances, and combined stainings with AB at pH 2.5 and PAS. To prove the presence of acidic mucosubstances, toluidine blue (TB) staining was also performed [17]. Images were captured with a Leica DFC 450 digital camera integrated to a Leica DM2500 light microscope.

3. Results

The macroscopic examination revealed that the midgut was located in the anterior and dorsal portion of the abdomen and had a U-shaped structure. It was noted that the midgut exhibited a color ranging from pale yellowish brown to dark brown depending on the content. There were tangled whitish or yellowish Malpighian tubes about the midgut that emptied into the tract at the ventriculus and ileum junction (Figure 1).

At the microscopic level, we found that the midgut wall was surrounded by a double layer of muscles showing striated muscle morphology, oriented in various directions (Figure 2A). The epithelium of the midgut consisted of epithelial cells with different morphologies. Among these cells, the most notable ones were epithelial cells with columnar or elongate structure. At the bottom, just above the basal membrane, there was also a group of relatively short-length cells. We also observed another type of cell group that has secretory epithelial cell characteristics (Figure 2B). One of the most striking results of midgut epithelium examination was that the cell nucleus size, shape, and location differed throughout the epithelium.

The epithelium's structure was not regular, but it appeared to be studded with projections, possibly villi, in

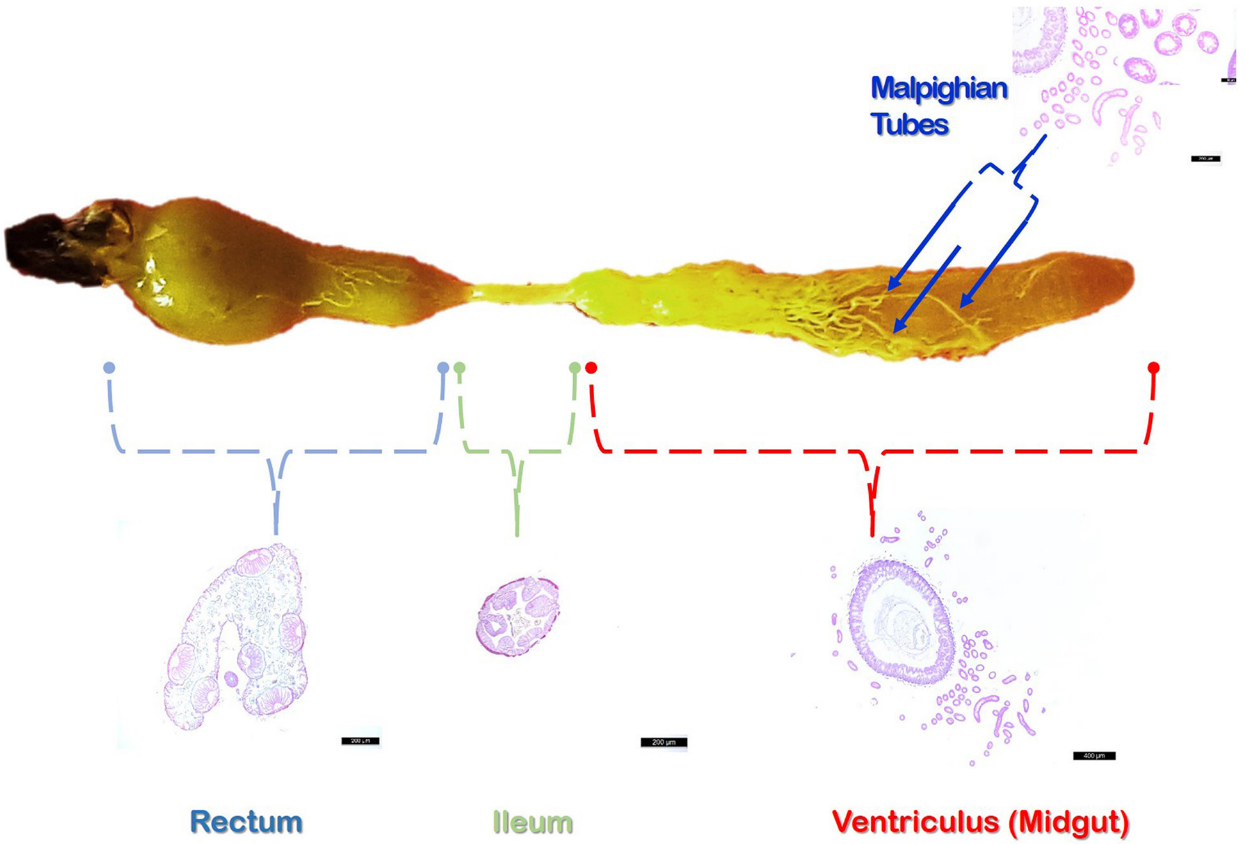

Figure 1. General macroscopic and microscopic appearance of the midgut and hindgut of the Caucasian honey bee (*A. m. caucasia*).

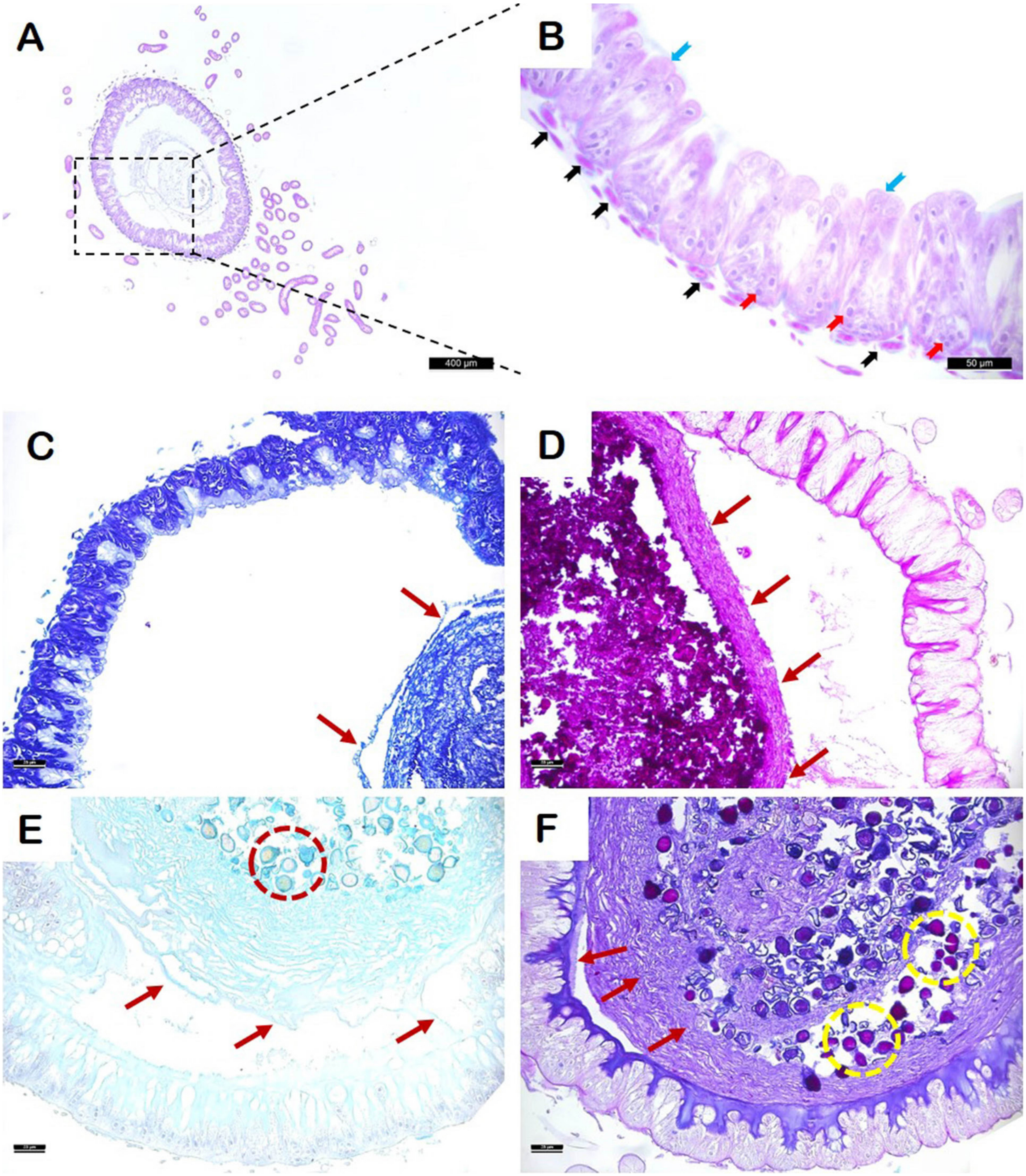

Figure 2. Light microscopic view of the midgut. A) Transverse sections of midgut and Malpighian tubes by Mallory's trichrome staining. B) General view of midgut epithelium. Columnar epithelial cells (blue arrow), basal cells (red arrow), muscle layer (black arrow). C) General view of TB staining. Peritrophic membrane (red arrow). D) General view of PAS staining. Strong reaction in the peritrophic membrane (red arrow) and apical surface of epithelial cells. E) General appearance of AB staining. Peritrophic membrane (red arrow), content surrounded by peritrophic membrane in the lumen (red circle). F) General appearance of PAS/AB staining. Peritrophic membrane (red arrow), content surrounded by peritrophic membrane in the lumen (yellow circle).

sections, between which there were pits and crypts. The presence of highly concentrated and intensely stained peritrophic membranes covering the lumen just above the epithelial cell layer was noted. It was also observed that the peritrophic membrane was connected to the epithelium and surrounded the midgut contents in the lumen (Figures 2C–2F).

Specific localization of acidic (Figures 2C and 2E), neutral (Figure 2D), and mixed mucosubstances (Figure 2F) was found in the luminal surfaces of the cells and peritrophic membranes. The reaction was strong in the luminal peritrophic membranes, especially those containing food.

Macroscopically, the ileum had a long tubular structure between the ventriculus and the rectum. In transverse sections, we observed that the mucosa formed protrusions facing the lumen that had 4 to 6 folds. This structure was surrounded by several muscle layers, which also had striated muscle morphology. Single-row epithelium layers had different cell types varying from cubic to columnar, with a nucleus structure in a basal location. Besides that, squamous epithelial cells were also present in the junction with the rectum. Histochemical reactions of mucosubstances were weak on the surface of the epithelium, as well as peritrophic membranes in the lumen (Figure 3).

We observed that the rectum had a wider, pouch-like structure compared to the ileum. We also noticed that the structure of the rectum had a flexible nature that could expand due to increased content. We found that the rectum was surrounded by circular muscles. There was a simple squamous epithelium lining the mucosal folds of the rectum. Although the lining was folded, there were no villi or microvilli-like structures. There were 6 long hollow rectal pads in the median-anterior area of the rectum. Each rectal pad had specialized epithelial structures that consisted of 3 different cell types, which were principal, basal, and junctional cells. The principal cells had long, columnar structures that reached the lumen, and the nucleus location was close to basal. Basal cells were cubic cells with 2 rows and a round nucleus. A lumen separated the basal cells from the principal cells. Junctional cells had a thin flattened structure and formed a passage between the rectum, epithelium, and principal cells. The rectum's histochemical staining patterns were similar to those of the ileum (Figure 4).

Figure 3. Transverse section of the ileum. A) PAS staining. Muscle layer (black arrow). B) Mallory's trichrome staining. Muscle layer (black arrow).

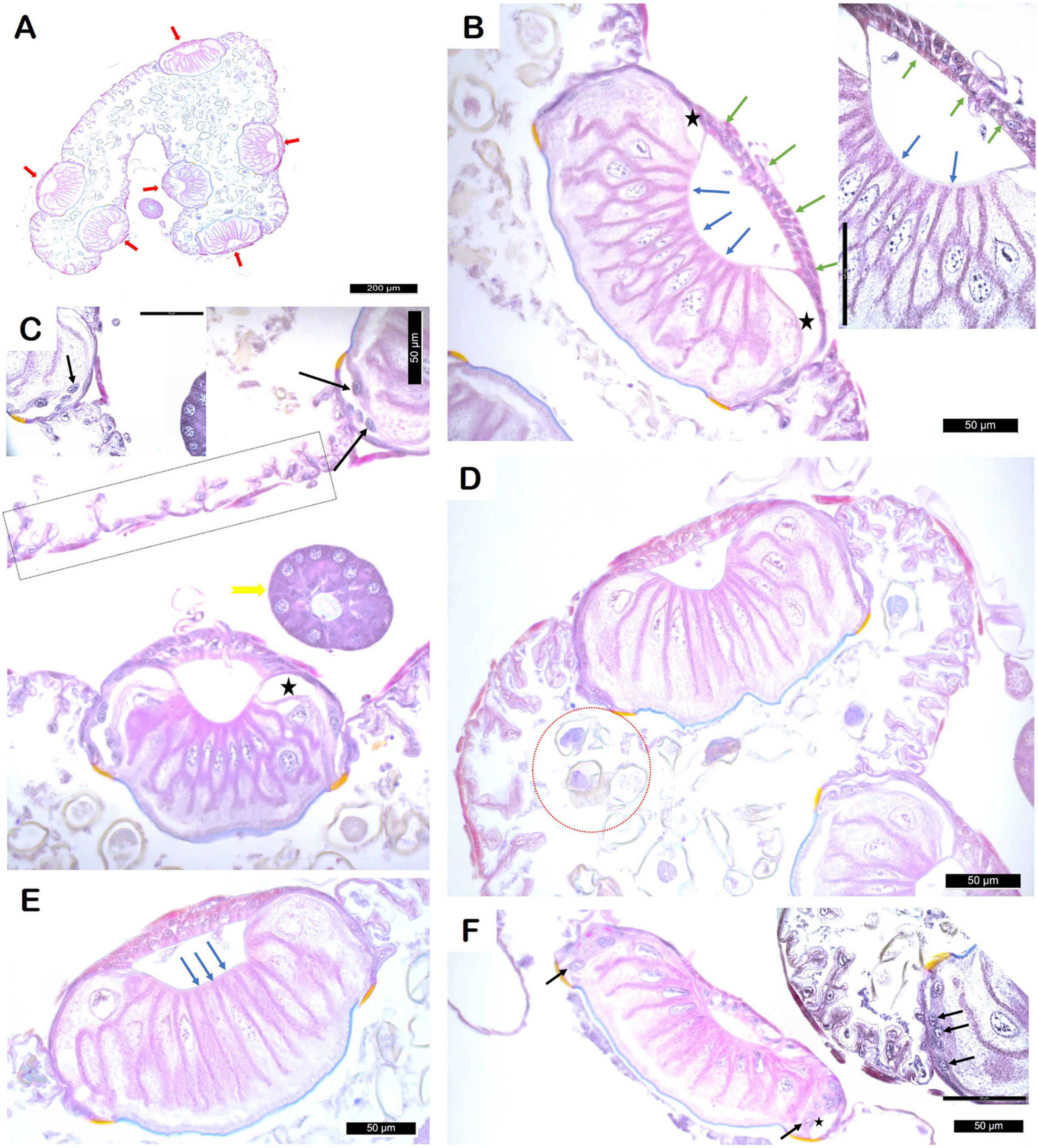

Figure 4. General light microscopic view of the rectum, Mallory's trichrome staining. A) Rectal pads (red arrow). B) Basal cells (green arrow). The lumen (black star) separating the basal and the principal cells (blue arrows) from each other. C) Simple squamous epithelium of rectum (black rectangle). Junction cells (black arrow). Malpighian tube (yellow arrow), lumen (black star). D) The content of rectum surrounded with peritrophic membrane (red circle). E) Principal cells reaching the lumen (blue arrow). F) Junction cells (black arrow) and the lumen (black star).

4. Discussion

It is known that the morphological diversity of insect digestive tracts is directly related to variables such as regional topography, nutritional sources, environmental factors, and sociability [18]. In addition to these variables, it has previously been suggested that phylogenetic relationships may have an effect on gut morphology among bees [9]. In Turkey, studies of the honey bee are mostly related to morphometric features of domestic honey bee species [7,19–22]. Although these studies are potentially influential in determining the morphological characteristics of domestic honey bees, there is little information about the histomorphology of the gut. Since morphometric studies do not achieve sufficient detail in gut morphology, we examined the gut structure of the Caucasian honey bee (*A. m. caucasia*) at light microscope level.

Several researchers have proposed that the peritrophic membrane is a glycosaminoglycan-, glycoprotein-, and protein-rich secretion of the midgut epithelium [11,15,23]. These researchers also observed that the staining intensity of the peritrophic membrane is different among bees. Although histochemical staining results of this study confirmed the chemical composition of the peritrophic membrane of the Caucasian honey bee, differences in intensity of reactions might be associated with the endemic flora and ecosystem. These results may support the findings of Gajger et al. [10], who observed that bees fed with different nutrients had different histochemical staining patterns in the peritrophic membranes.

Tibbetts [24] showed that the carbohydrate composition of glycoprotein secretions might change to increased viscosity as a protection mechanism. Gajger et al. [10] also compared healthy and *Nosema*-infected honey bee intestinal mucous substance secretion profiles and they found that neutral/acidic mucous substance secretion was lost in the infected group. This concurs with our results that Caucasian honey bees have healthy mucous substance secretion in spite of the fact that a different honey bee species was used.

It has been reported that the ileum has no distinctive features for the differentiation of bee breeds at macroscopic level [25]. In agreement with that, we observed similar morphology in all Caucasian honey bees. In a study [9] examining the mucosal layers of the ileum in a more detailed manner, it was reported that the epithelial cells show both columnar and cubic morphology, but mostly cubic. In comparison to these findings, in our study we observed a higher number of columnar cells in the epithelium. We believe that this phenomenon is likely due to the extent of epithelial remodeling related to feeding habits and rectal content.

Snodgrass [26] reported that rectal glands of honey bees and stingless bees have long, pad-like structures and consist of principal and basal cells. Studies in later years [27,28] showed that the structure of rectal glands can vary in shape (fusiform, oval, spherical, and hollow) among bee species. While the results obtained in our study concur with the results of Snodgrass [26], morphological differences described by other researchers [27,28] may be associated with the possible differences in water absorption mechanisms among bee species.

Midgut and hindgut mucosa of honey bees withstand several stress factors caused by various serious pathogens throughout their life cycle. The most important criterion for evaluation of these pathogenic alterations, and for the selection of treatment and/or preventative methods, is having good knowledge of healthy midgut and hindgut morphology. It was concluded that results of this study of the Caucasian honey bee will provide information for researchers in their studies of all bee species. The histomorphology and physiological mechanisms of the midgut and hindgut mucosal cells certainly need further investigation.

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