Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 57 - 67
Received 16 February 2021; Accepted 08 March 2021.

The Histological and Histochemical Structure of the Rectum in the Cockatiel (*Nymphicus hollandicus*)

Ameer. M. Taha[1] and Ali. A. Abed[1]

[1]Department of Biology, College of Education for Pure Science, University of Mosul, Iraq.

amhamdany@uomosul.edu.iq

https://orcid.org/0000-0003-0306-7545

Abstract:

Birds are among the most important vertebrate animals on the surface of the Earth, so they are widely studied in terms of environment, nutrition, reproduction, migration as well as their internal structure. This study aimed to recognize the histological and histochemical structure of the Rectum in Cockatiel *Nymphicus hollandicus*, which is one of the beautiful ornamental birds that are raised in homes, by using compound optical microscopy and histological and histochemical techniques. The histological results showed that the rectal wall was consisted of four tunica: the mucosa; submucosa; muscularis; and finally serosa. The mucosa formed villi of various shapes extending towards the rectal lumen. These villi is covered with a simple columnar epithelial tissue with a large number of secretory goblet cells, but their spread differs among the villi. Themucosa's lamina propria is composed of loose connective tissue that is rich in blood vessels and also contains intestinal glands or Lieberkuhkn's crypts that appeared and spread below the villi. While the lymph nodes not observed in this bird, and it is one of the striking results. The submucosa is composed of loose connective tissue. In contrast, the muscularis consisting of smooth muscles of two arrangements, theouter longitudinal andinner circular the. As for histochemical results, epithelial tissue cells of both types and secretory cells of the intestinal glands have shown positive responses to histochemical techniques that used, but to varying degrees. The stronger responses have emerged to detect mucous and carbohydrates detection techniques, indicating a widespread of these substances in this organ. The study concluded that the rectal composition in this bird is proportional to its function and the nature of the food it eats and that the materials secreted from its tissues contribute to the completion of this function.

Keywords: Cockatiel; *Nymphicus hollandicus*; rectum; Lieberkuhkn's crypts.

Introduction

The cockatiel (*Nymphicushollandicus*) is an Australian bird belonging to the order Psittaciformes; family Psittacidae; subfamily Cacatuinae (BirdLife International 2012). It was originally endemic to Australia, but now these birds are most popular pets with globalspread (Alcarazet al.,2016). In Iraq, this bird became one of the ornamental birds that spread widely in homes as well as other types of parrots, which feed entirely on the grains provided by the breeder.

The bird's class contains more than 9000 living species distributed in 199 families, and birds have a wide range of phenotypic, physiological, and functional modifications that make them live and adapt anywhere (Clark et al., 2009). The

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 57 - 67
Received 16 February 2021; Accepted 08 March 2021.

ecological diversification of birds' habitats and their subsequent feeding methods, as well as the types of foods they feed on, are known to be a source of large diversity in the composition of the gastrointestinalsystem (Dziala-Szczepanczyk and Wesolowska, 2008).

In birds, the large intestine composed of the rectum and pair of caeca, the primary function of which is to absorb water and ions, and to keep the minimum undigested litter to keep the bird lightweight, because excessive waste in the bird's body impedes its speed. The rectum in birds contains many relatively flat villi and a few goblet cells, in contrast to the colon in mammals, which includes many goblet cells and free from villi. Also, the rectum in the bird contains a relatively small number of crypts and is shorter than it is in the colon (Whittow, 1998).

The histological structure of the digestive system and the large intestine in birds is affected by the diet. Some studies have shown that the total size of the large intestine and its surface area in some birds varies with the type of food (Ricklefs, 1996; Dehkordi and Ghahremani, 2016).In between and within species, the large intestine has significant morphological and functional contrast, For example, manyspecies of familiar bird, such asducks and chickens, have individual large intestine, which aids in digestion as well as water balance.

Between all the birds' species, the absorption capacity and water preservation are different. In birds,water retention and excretion performed by the production of the concentrated urine than plasma (Sjaastad et al. 2004). Studies have shown that the rectum has a significant role in re-absorbing as much water as possible before excreta disposal, especially in birds that have a high metabolic rate relative to their mass and activity (Al-Hamdany, 2012).

Therefore, these study came toidentify the histological components and the histochemical structure of the rectum in Cockatiel (*Nymphicushollandicus*).

Materials and methods

1- Birds collection

Ten adult birds of Cockatiel *Nymphicushollandicus* (five males and five females) were obtained from a local pet store in Mosul / Iraq. The weights of these birds ranged between 101-120g, and their lengths ranged between 25-30 cm, and they were all about the same age (18 months ±10 day). The birds were transferred to the histology and anatomy laboratory at the Education College for Pure Sciences / University of Mosul, where they were placed in an iron cage with dimensions of 60X60X60 and left for a week to ensure their health and safety from diseases and provided with food and water daily.

2- Birds Dissection

The birds were dissected using the animal guide of ethical Committee / University of Mosul. The birds were sacrificed by anaesthetizing them By placing them immediately in place at> 70% CO_2. Then the feathers were removed from the ventral region. After fixing the bird on a dissection dish, a longitudinal incision was made in the ventral area. The rectum was removed and transferred to a Petri dish containing 75% physiological solution. Then the rectum was cut into pieces suitable for the histological process.

3- Histological preparation

The histological preparation steps were performed according to the method of (Al-Hajj 2010). Rectal samples were fixed with neutral formalin for 24 h. The samples were then washed with running water to remove traces of the fixative. The dehydration process was carried out using increasing concentrations of ethyl alcohol, using the concentration

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 57 - 67
Received 16 February 2021; Accepted 08 March 2021.

of 60%, 70%, 80%, 90%, 95% for half an hour for each concentration and then placed in absolute alcohol for two hours and twice.The clearing process was done with xylene for half an hour and then covered with paraffin wax to prepare the wax blocks.The wax blocks were sectioned using a Vic Science rotating microtome with a thickness of 5-7 μm. The tissue sections were carried to glass slides for staining.

4- Staning Process

The staining process included histological stains histochemical techniques that were:

4-1- Heamatoxylin and Eosin Stain (H&E): prepared and dyed according to (Al-Tarwa et al., 2009). After removing the wax from the slides using xylene, pass down in a series of ethyl alcohol. The samples were placed with Heamatoxylin stain for (6 minutes), then transferred to acidified alcohol (15-30 seconds), stained with eosin for (30 seconds). Then moved to ascend concentrations of Ethyl alcohol, then placed in xylene. The slides were covered with slides covers byDibutylphthalate Polystyrene Xylene(D.P.X).

4-2- Azan stain (AZ): that prepared and stained, as in (Suvarna et al., 2019). First of all, remove the wax, by xylene and ethyl alcohol. They transferredfor 5 minutes in distilled water (D.W). Stain with Azoczrmine solution in the oven (56-60 ° C) for (75 minutes). Then wash it with distilled water and distinguish it with a blue aniline solution (15 minutes). Dipped in acid alcohol, then stir in the Phosphonic acid solution for (75 minutes). They then placed in a blue aniline solution and orange G stain for (70 minutes). Wash with running water, then transfer to ascending alcohol concentrations, clearing with xylene and cover slides with covers using D.P.X.

4-3-Mallory's Trichrome stain (TS): was prepared and dyed, according to (Al-Attar et al., 1982). The wax was removing and delivering the tissue to its natural position as in the previous stains. It is placed in an acid solution of fuchsin for (25 minutes), then washedfor (3 minutes)with running water. Transfer to Phosopho-Molybdic acid solution for (2 minutes). Then it is washed quickly with running water and transferred to a solution consisting of aniline blue, orange G, and oxalic acid for (45 seconds) and washed with distilled water. The samples are dehydrated with ethyl alcohol, clearing with xylene, and the slides covered with their covers.

4-4- Bromophenol blue (BP) technique: which used to detect proteins, and prepared and stainedas the stapes of (El-Banhawy et al., 1996). The wax is removed from the sections by transferred them to xylene, then to absolute ethyl alcohol. They dye with Bromophenol blue solution for (15 minutes). Wash with an aqueous acetic acid solution for (20 minutes) to remove traces of excess dye. Then put it in distilled water for (3 minutes). The sections dehydrated with ethyl alcohol, clearing with xylene.

4-5- Sudan black B (SB) technique: which used to detect lipids, which was prepared and dyed according to (El-Banhawy et al., 1996). After removing the wax, transfer the samples to xylene, then to 70% ethyl alcohol. The sections stained with Sudan Black B solution for (30 minutes). Wash with 70% ethyl alcohol for (5 minutes) to remove traces of excess dye. Then wash it with running water and dye it with a neutral red solution (1%) for (one minute). Transfer to absolute alcohol, then to xylene, then cover with slide covers.

4-6- AlcianBlue (AB) pH 1 and pH 2.5 techniques used to detect carbohydrates prepared and dyed as in (Suvarna et al., 2019). The wax removed, and the sections were transferred to xylene, then to ethyl alcohol concentrations, thenfor (5 minutes)in D.W. Stain with Alician blue solution for (35 minutes). Then with running water, wash for (10 minutes). Transfer to dyeing with eosin solution for a period of (30 seconds) and

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 57 - 67
Received 16 February 2021; Accepted 08 March 2021.

then wash with running water for (3 minutes). Transfer to absolute alcohol, then to xylene, then cover with slide covers.

4-7- Periodic acid & Schiff Reagent (PAS) technique : also used to detect carbohydrates, which was prepared and stained according to (Suvarna et al., 2019). After removing the wax and transferring the sections to xylene, then to ethyl alcohol, pull them into distilled water (2 minutes). They moved to a periodic acid solution for (10 minutes). Wash it by water for (10 minutes). Wash it with distilled water for (2 minutes). The Schiff Reagent solution is transferred for (15 minutes) and then washed with running water for (5 minutes). Stain with Heamatoxylin stain solution for (6 minutes). Then wash them with running water for (5 minutes). Transfer to absolute alcohol, then to xylene, then cover with slide covers.

Results andDiscussion

Histological results

The results showed that the rectal wall is composed of the four layers of the digestive tract wall, which are the mucosa, submucosa, muscularis, and serosa also appeared in the rest of the birds (Líman et al., 2002; Zaher et al., 2012; khaleal and Salman,2016; Pandit et al., 2018).The mucous layer appeared in the rectum of this bird in the form of villi with multiple shapes, including conical, pyramidal, filamentous, and other structures. Some of this villi appeared length from the other, and the average length of this villi was (113.067±4.432µm), while the average thickness (41.733±1.491µm). In some areas, these villi are so compact that the distances between the villi are almost non-existent, while in other areas, they are far apart from each other, and the gaps between them are clear and wide (Figures1,2,3,4).The villi increase the surface area of the rectum cavity and thus increase the absorption area. These villi appeared in other birds, which are short in *Elanus caeruleus* (Hamdi et al., 2013), Pin-tailed sandgrouse (Al-Jeraisy,2017), and zebra finch (Al-Duleemy, 2019). These villi in *Coturnix coturnix* were numerous and leaf-like (Zaher et al., 2012), whereas in common kestrel and white-eared bulbul that have short finger-like, tongue-shaped, and leaf-like shape with a flat apical surface (khaleal and Salman,2016).

These villi covered with a simple columnar epithelial tissue based on a straight, basal membrane, its average thickness (2.44±0.142µm), these cells are compact, and their side borders do not appear clearly in most areas. The average length of these cells (14.613±2.812µm) and their width (5.84±1.231µm) and the nuclei of these cells are basal and spheres with an average diameter (4.756±0.823µm). Between these cells, there are secretory goblet cells that are characterized by large spherical secretory vesicles. The distribution of these cells between the epithelial cells is uneven, as in some villi are dominant and in other villi are smaller in number. Still, they are many in general in particular at the top of villi (Figures 4,5,6,7,8).The columnar epithelial tissue that covered the villi is evident in all studied birds (Abo-shaeir,2001; Samte, 2008; AL-Aredhi,2013; Taki-El-Deen, 2017). The epithelial tissue distinguished by its many goblet cells, and these cells appeared in other birds as *Melopsittacus undulates* and the Zagh (Hamad,2008). It has been observed that the goblet cells gradually increase in number from the duodenum to the rectum (Zaher et al., 2012). This increase in these cells in the rectum can be attributed to the need for their secretions to facilitate the movement of excreta and put it out of the bird's body (Eroschenko, 2005; Hamad,2008).

The lamina porpria is formed of loose connective tissue rich in blood vessels, collagen and muscle fibers, lymphocytes, and other cells. The lamina porpria that

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 57 - 67
Received 16 February 2021; Accepted 08 March 2021.

inserted inside the villi, to be a lamina porpria cone, characterized by containing muscle and collagen fibers that support these villi as well as large blood vessels in some villi (Figures 8,9,10,11,12), which also appeared in the other of the birds (Majeed et al.,2009; Nasrin et al.,2012).The lamina porpria also contains intestinal glands or Lieberkuhkn's crypts, which appeared in the form of compound tubular glands. Its secretory units are in the form of a single row below the villi, and in some areas, they are in the form of two rows. Most of the secretory units of these glands are circular and have an average diameter (63.653±4.254µm). The secretory cells inside the secretory units are columnar and characterized by their secretory activities, as most of these cells occupied by secretory vesicles as well as the cavity of these units, which is very small (Figures 1,2,3,8,9,10,11,12), these glands also appeared in the rest of birds (Al-Sheshani, 2006). A network of muscle fibers surrounds these units to support them and seek to secrete their contents into the spaces between villi. These muscle fibers return to the muscularis mucosa layer (Figures 5,7,10,11).

One of the noticeable results of this bird is the absence of lymph nodules in this organ, but lymphocytes widely spread among the contents of the other lamina porpria (Figures 3,5,10,11). The submucosa is composed of loose connective tissue, and its components overlap with lamina porpria components in most parts (Figures 1,2,3,11), and this result differs from what found in many birds as in starling, zebra finch, Pin-tailed sandgrouse, and other birds (Khaleel and Atiea, 2017; Al-Jeraisy,2017; Al-Duleemy, 2019). Perhaps the reason for this is due to the nature of feeding this bird on one type of food, and therefore it is less vulnerable to pathogens through food.

The muscularis layer is composed of two secondary layers of smooth muscles, the first layer has a circular arrangement with an average thickness (54.828±4.493µm) and is in the form of large muscle bundles, and the second layer has a longitudinal arrangement. It's an average thickness (18.927±3.269µm) and somewhat disassembled, so some lost in histological preparation (Figures 1,2,3,4,8,11,12). The serosa layer composed form of a simple squamous epithelial tissue, under whichlocated a loose connective tissue. Between the muscularis layer and the serosa layer, there are blood vessels and Auerbach's plexus (Figures 1,2,3,4).The submucosa, muscularis, and serosa layers appeared similar to the rest of the studied birds (Hamdi et al., 2013; Khaleel and Atiea, 2017; Al-Jeraisy,2017; Al-Duleemy, 2019).

Histochemical results

Table (1) indicates the histochemical response for the epithelial cells and intestinal glands of histochemical techniques used in this study. The table shows a weakly positive reaction to both the epithelial tissue and the intestinal glands of the BP technique, which indicates the presence of a few secretions of protein substances in the secretions of epithelial tissue and secretory cells of the intestinal glands (Figures 7,12). These structures also showed a weakly positive response to SB technique, as indicated in Table (1), this suggests the presence of a few secretions of fatty substances in the secretions of epithelial cells and gastrointestinal glands (Figures 8). On the other hand, Table (1) shows a medium positive response in the epithelial tissue and goblet cells and a weak positive response in the secretory units of the intestinal glands to PAS technique and this indicates a medium to the low presence of neutral mucous substances and polysaccharides in the secretions of these tissues, respectively (Figures 2,6,11).

While Table (1) indicates a very positive response to the AB pH 1 technique in both epithelial cells and secretory cells of the intestinal glands, as a result of the presence of large quantities of sulfurous mucus substances with severe acidity, that shown very clearly in the apical surfaces of epithelial cells, thegoblet cells' vesicles as well as the lumen of secretory units and the secretory cells of the intestinal glands (Figures 1,9). Also, the epithelial tissue response was medium positive for AB pH 2.5 technique and very positive in the secretory units to the same technique as indicated in that table (1). That shows a medium secretion in the epithelial tissue and high secretion of intestinal glands of the sulfurous mucus substances with weak acidity (Figure 4).

The epithelial tissue, goblet cells, as well as intestinal glands showed a weakly positive response to BP and SB techniques, and the same result appeared in *Coturnix coturnix* (Zaher et al., 2012), also these results are in agreement with (El-Banhawy et al.,1993) and (El-Sayyad,1995). While for carbohydrates and mucus substances, the epithelial cells showed a medium positive response to PAS technique and weak positive response in the glands, strong responses in both tissues to AB pH 1 and pH 2.5 techniques. The similar response appeared in *Coturnix coturnix*, starling, zebra finch, Pin-tailed sandgrouse, common kestrel, and white-eared bulbul and others birds (El-Banhawy et al., 1993; Indu et al., 2011; Hu et al., 2011; Zaher et al., 2012; khaleal and Salman,2016; Khaleel and Atiea, 2017; Al-Jeraisy,2017; Al-Duleemy, 2019). This large amount of mucus substances may imply the need to increase the protection of mucosa and lubrication of fecal expulsion (Wang and Peng, 2008).

Conclusions

It concluded from the current study that the structure of the gastrointestinal tract in birds, especially the rectum, has an important role in maintaining the specific weight of the bird, and thus helps the bird to fly with the least possible effort by eliminating waste and absorbing nutrients and water with high efficiency. Also, the materials secreted from the tissues of rectum contribute to the protection of the mucosa layer and facilitate the excretion of waste.

Acknowledgments

The authors thank and appreciate the Presidency of Mosul University, represented by Professor Dr. Qusay Kamal Al-Din Al-Ahmadi and the Deanship of the College of Education for Pure Sciences for facilitating the provision of chemicals and using laboratory instrumentsin the laboratories of Mosul University / Iraq.

References

1. Abo-shaeir,W.A.M.(2001).*Macroscopic and microscopic anatomy on the digestive system of some vertebrate animals*. Ph.D. Thesis. Faculty of sciences. AL- Azhar university. Egypt.
2. Al- Hamdany, A.M. (2012).*Comparative Anatomical, Histological with Developmental Study at light and Electron microscopic level of eye and alimentary canal for three species of birds which differ in nutrient nature*. Ph.D. Thesis.Education College, Mosul University.Iraq.
3. Al- Sheshani, A.S.Y. (2006). *Anatomical and histological comparative study of alimentary tract in two types of birds' grainivorous bird, (Columba Livia*

Gmelin, 1789) and carnivorous birds, (Accipiter nisus Linnaeus, 1758). M.Sc.Thesis. University of Tikrit. Iraq.

4. AL-Aredhi, J.A.M. (2013). *Comparative anatomical studies of gastrointestinal tract for three wild Iraqi birds' black- shouldered kite Elanus caeruleus, Green-winged teal Anas crecca and the common quail Coturnix coturnix*. M.Sc.Thesis.University of Kufa.Irqa.
5. Al-Attar, A.A., Al-Allaf, S.M.,&Al-Mukhtar, K.A. (1982). *Microscopic preparations*.1st ed. Ministry of Higher Education and Scientific Research. Iraq.
6. Alcaraz, L. D., Hernández, A. M., & Peimbert, M. (2016). Exploring the cockatiel (*Nymphicus hollandicus*) fecal microbiome, bacterial inhabitants of a worldwide pet. *PeerJ*, 4, e2837. doi:10.7717/peerj.2837
7. Al-Duleemy, A.S. (2019). *Comparative Anatomical and histological study of the Digestive canal and histochemistry of the mucin in two types of birds*.M.Sc.Thesis.College of Education for Pure Sciences, University of Mosul, Iraq.
8. Al-Hajj, H.A. (2010). *Optical Microscopy, Theory and Practice*. 2nd ed. Al-Masirah House for Publishing, Distribution, and Printing, Amman,Jordan.
9. Al-Jeraisy, F.N. (2017). *Anatomical histological study and histochemical comparison of the digestive system in three types of vertebrates belonging to three different classes.*M.Sc.Thesis..College of Education for Pure Sciences, University of Mosul, Iraq,.
10. Al-Tarwa, M.M., Othman, J.M., Abu Dayyeh, M.,&Al-Ratrout,O.K.(2009).*The basics of histology*. 1st ed. Oman House of Culture for Publishing and Distribution. Jordon.
11. Birdlife International. *Nymphicus hollandicus*. In: IUCN red list of threatened species. Version 2013.1. Available in: <Available in: http://www.iucnredlist.org .
12. Clark, P., Boardman, W.S.,&Raidal, S.R.(2009).*Atlas of Clinical Avian Hematology*. A John Wiley & Sons, Ltd., Publication. USA.
13. Dehkordi, R.A.,&Ghahremani, P. (2016). Developmental study of rectum in broiler chicken: A stereological and morphometrical study.*Veternary Research Forum*,7(1):41–45.
14. Dziala-Szczepanczyk, E.,& Wesolowska, I. (2008). Morphometric characteristics of esophagus and intestine in tufted ducks (*Aythya fuligula*) wintering on the baltic coastal areas in north-western Poland. *Electronic Journal of Polish Agricultural Universities*,11(4):1-35.
15. El-Banhawy, M.A., Khattab, F.I.,&El-Ganzoury, M.A. (1990).*The foundations of theoretical and practical histochemistry*.1 st ed. Academic Library. Cairo. Egypt.
16. El-Banhawy, M., Mohallal, M.E., Rahmy, T.R.,& Moawad, T.I. (1993). A comparative histochemical study on the proventriculus and ileum of two birds with different feeding habits. *Journal of the Egyptian German Society Zoology*,11(C):155-174.
17. El-Sayyad, H.I. (1995). Structural analysis of the alimentary canal of-hatching Youngs of the owl *Tytoalbaalba*. *Journal of the Egyptian German Society Zoology*,16(C): 185-202.
18. Eroschenko, V.P. (2005). *diFiore's Atlas of Histology with Functional Correlations*.10th Ed .Cambridge University Press.USA.

19. Taki-El-Deen, F. M. A. (2017). Histological and Histochemical Studies on the Alimentary Canal of Spur-Winged Lapwing *Vanellus Spinosus*. *The Egyptian Journal of Hospital Medicine*, 67(1), 314–321. doi:10.12816/0036642
20. Hamad, R.S. (2008). *Histological and histological comparison study of the gut of the lovebird (Melopsittacus undulates Gould, 1840) and the Zagh (Corvus f. Frugilegus Linnaeus, 1758).*M.Sc.Thesis..College of Science, Tikrit University, Iraq.
21. Hamdi, H., El-Ghareeb, A., Zaher, M., &AbuAmod, F. (2013). Anatomical, histological and histochemical adaptations of the avian alimentary canal to their food habits: II- *Elanus caeruleus*. *IJSER*,4:1355-1364.
22. Hu, X., Guo, Y., Li, J., Yan, G., Bun, S., & Huang, B. (2011). Effects of an early lipopolysaccharide challenge on growth and small intestinal structure and function of broiler chickens. *Canadian Journal of Animal Science*, 91(3), 379–384. doi:10.4141/cjas2011-008
23. Indu, V.R., Lucy, K.M., Sreeranjini, A.R., Ashok, N.,&Chungath, J.J. (2011). A comparative study on the histomorphology of ileum and colorectum in peafowl (*Pavo cristatus*). *J. Int. Vet. Am.*,9(2):35-38.
24. Khaleal, I.M.,& Salman, R.J. (2016). A comparative histological study of ceca and rectum in common kestrel (*Falco tinnunculus*) and white-eared bulbul (*Pycnonotus leucotis*) according to their food type. *The Iraqi Journal of Veterinary Medicine*, 40(2):48-56.
25. Khaleel, I.M.,&Atiea, G.D. (2017). Morphological and Histochemical Study of Small Intestine in Indigenous Ducks (*Anas platyrhynchos*). *Agric. and Veter. Sci. J. V.* 10 (7): 19-27.
26. Líman, N., Aslan, S.,&Gülmez, N.(2002). The histological observations on the large intestine of the goose (*Anser anser*) during the pre- and post-hatching periods. Journal of Veterinary Medical Science,64(8), 705–709.doi.org/10.1292/jvms.64.705 .
27. Majeed, M.F., Al- Asadi, F.S., Al. Nassir, F.S.,&Rahi, E,H. (2009). The morphological and histological study of the caecum in broiler chicken. *Bas J Vet Res.*,8:19-25.
28. Nasrin, M., Siddiqi, M. N. H., Masum, M. A., & Wares, M. A. (2012). Gross and histological studies of digestive tract of broilers during postnatal growth and development. *Journal of the Bangladesh Agricultural University*, 10(1), 69–77. doi:10.3329/jbau.v10i1.12096
29. Pandit, K., Dhote, B. S., Mahanta, D., Sathapathy, S., Tamilselvan, S., Mrigesh, M., & Mishra, S. (2018). Histological, Histomorphometrical and Histochemical Studies on the Large Intestine of Uttara Fowl. *International Journal of Current Microbiology and Applied Sciences*, 7(03), 1477–1491. doi:10.20546/ijcmas.2018.703.176
30. Ricklefs, R. E. (1996). Morphometry of the Digestive Tracts of Some Passerine Birds. *The Condor*, 98(2), 279–292. doi:10.2307/1369146
31. Samte, L. (2008). *Gross morphometric, Light and electron microscopic studies on the large intestine of Kadaknath fowl*. M.Sc.Thesis.G.B.P.U.A. & T. Pantnagar.
32. Sjaastad, O.V., Hove, K.,&Sand, O. (2004). *Physiology of domestic animals*. Oslo: Scandinavian Veterinary Press.USA.
33. Suvarna, K., Layton, C.,&Bancroft, J. (2019). *Bancroft's Theory and Practice of Histological Techniques*. 8th ed .Elsevier Limited.USA.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 57 - 67
Received 16 February 2021; Accepted 08 March 2021.

34. Wang, J. X., & Peng, K. M. (2008). Developmental Morphology of the Small Intestine of African Ostrich Chicks. *Poultry Science*, 87(12), 2629–2635. doi:10.3382/ps.2008-00163
35. Whittow, G.C. (1998). *Sturkie's Avian Physiology*. 5th ed. Academic Press.USA.
36. Zaher, M., El-Ghareeb, A., Hamdi, H.,&AbuAmod F. (2012). Anatomical, histological and histochemical adaptations of the avian alimentary canal to their food habits:I-*Coturnix coturnix*. *Life Science Journal*,9(3): 253-275.

Table (1) the response of the of Rectum tissues to the histochemical techniques

Tissue / Technique	Epithelial tissue	Intestinal glands
AB pH 1	+++	+++
AB pH 2.5	++	+++
PAS	++	+
BP	+	+
SB	+	+

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 57 - 67
Received 16 February 2021; Accepted 08 March 2021.

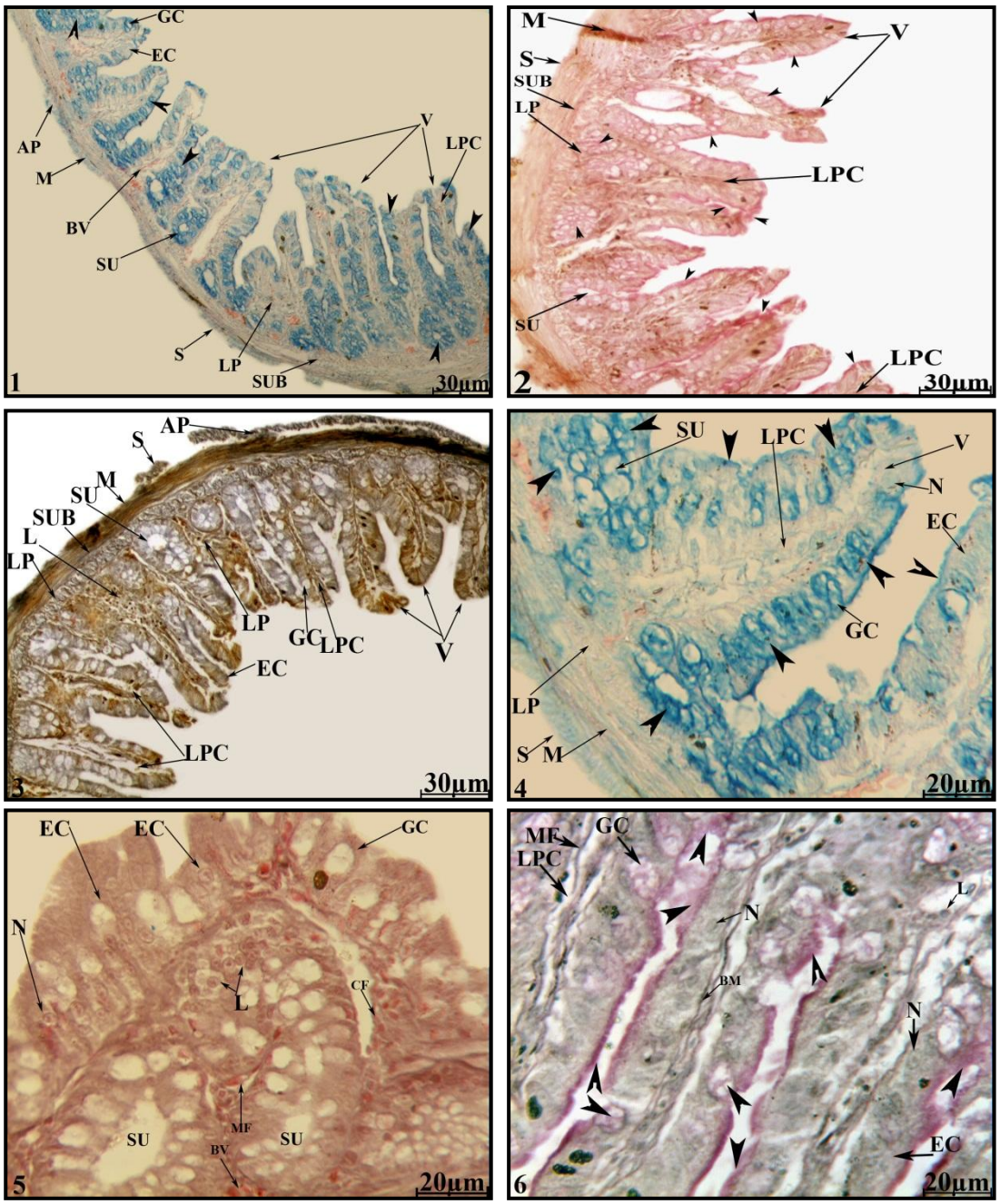

Figure (1) the Rectum in Cockatiel. AB pH 1 technique. Figure (2) the Rectum in Cockatiel. PAS technique. Figure (3) the Rectum in Cockatiel. TS stain. Figure (4) the Rectum in Cockatiel. AB pH 2.5 technique. Figure (5) the Lamina porpria of Rectum in Cockatiel. AZ stain. Figure (6) the Rectum in Cockatiel. PAS technique.

Abbreviations (V) villi; (GC) Goblet cells; (EC) Epithelial cells; (LP) Lamina propria; (LPC) Lamina porpria cone; (SUB) Submucosa; (BV) Blood vessels; (SU) Secretory units; (S) Serosa; (M) Muscularis; (AP) Auerbach's plexus; (N) Nucleus; (BM) Basement membrane; (MF) Muscles fibers; (L) Lymphocytes;

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 57 - 67
Received 16 February 2021; Accepted 08 March 2021.

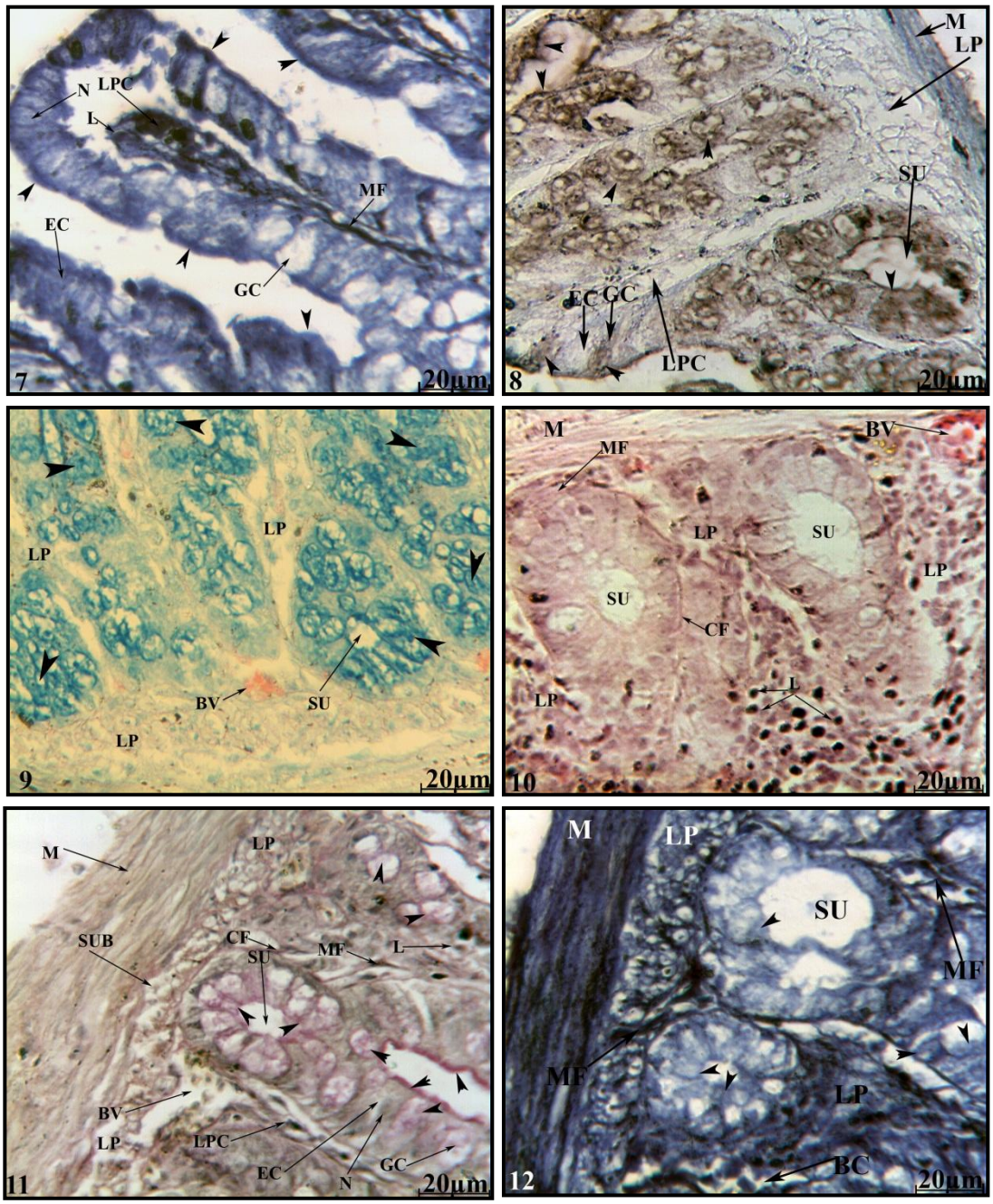

Figure (7) the Villi of Rectum in Cockatiel. BP technique. Figure (8) the Rectum in Cockatiel. SB technique. Figure (9) the Lamina porpria of Rectum in Cockatiel. AB pH 1 technique. Figure (10) the Lamina porpria of Rectum in Cockatiel. H&E stain. Figure (11) the Rectum in Cockatiel. PAS technique. Figure (12) the Rectum in Cockatiel. BP technique.

Abbreviations (V) villi; (GC) Goblet cells; (EC) Epithelial cells; (LP) Lamina propria; (LPC) Lamina porpria cone; (SUB) Submucosa; (BV) Blood vessels; (SU) Secretory units; (S) Serosa; (M) Muscularis; (AP) Auerbach's plexus; (N) Nucleus; (BM) Basement membrane; (MF) Muscles fibers; (L) Lymphocytes; (CF) Collagen fibers; (arrowhead) positive response.