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RESEARCH PAPER

Comparative Study of Ultrastructural Observations on Blood Cells of Local Omani and Cobb 500 Broiler Chickens

Shadha K. Al-Aufi[1], Haytham Ali[1], Hani M. El-Zaiat[1,2], Kaadhia Al-Kharousi[1], Salma R. Al-Adwani[1], Yasmin El Tahir[1], Abdulrahman Al Nabhani, Mohamed A. Al Kindi and Waleed Al-Marzooqi[1,*]

دراسة مقارنة للملاحظات فوق الهيكلية على خلايا الدم للدجاج المحلي العماني واللاحم كوب ٥٠٠

شذى خ. العوفي[١]، هيثم علي[١]، هاني م. الزيات[١،٢]، كاذية الخروصي[١]، سلمى ر. العدواني[١]، ياسمين الطاهر[١]، عبدالرحمن النبهاني[٣]، محمد أ الكندي، وليد المرزوقي[١،*٣]

Abstract. The local Omani chicken represents a long-established indigenous chicken breed in the Sultanate of Oman. Many farmers in Oman raise local chickens for meat and egg production. Therefore, to ensure an enhanced production of local breed chickens, it is important to assess their health performance. The present study was conducted to describe the comparative ultrastructural details of the blood cells of Omani and Cobb 500 chickens. Twenty apparently healthy 35-old birds (10 per strain) of either sex reared at the Poultry Research Unit at the Agricultural Experiment Station, Sultan Qaboos University. The blood was collected from wing vein into tubes containing EDTA as anticoagulant. The blood was further processed for transmission electron microscopic study. The results obtained showed that all blood cells of both Cobb 500 and Omani chicken were similar. The heterophil nucleus had a multi-lobed nucleus. The number of nuclei lobes ranges from 2 to 3. The Eosinophil had a distinctive appearance and mostly had a lobulated nucleus. Basophil was round in appearance and had four types of granules (dense granules, mottled granules, web or net granules and myelin granules) which can be seen clearly. The lymphocytes were the smallest leukocytes and it was characterized as small round cell with few cytoplasmic process. The monocytes were round in shape with kidney-shaped or indented nucleus. The nucleus had more heterochromatin patches compared to euchromatin. The thrombocytes were distinguished from other cells by their dense nucleus and the large vacuoles found in the cytoplasm. In conclusion, this study shows great similarities in the ultrastructure of all blood cells composition between local Omani and Cobb 500 broiler breeds.

Keywords: Blood cells, Cobb 500, Omani local chicken, Ultrastructure.

الملخص: يمثل الدجاج العماني المحلي سلالة دجاج محلية راسخة في سلطنة عمان. يقوم العديد من المزارعين في عمان بتربية الدجاج المحلي لإنتاج اللحوم والبيض. لذلك ، لضمان تعزيز إنتاج الدجاج المحلي ، من المهم تقييم أدائهم الصحي. أجريت هذه الدراسة لوصف التفاصيل للمقارنة فوق الهيكلية لخلايا الدم في الدجاج العماني والدجاج اللاحم كوب 500. عشرون طائرا يبدو بصحة جيدة يبلغ عمرها 35 عاما (10 طيور لكل سلالة) من كلا الجنسين تربى في وحدة بحوث الدواجن في محطة التجارب الزراعية بجامعة السلطان قابوس. تم جمع الدم من الوريد المجنح إلى أنابيب تحتوي على EDTA كمضاد للتخثر. تمت معالجة الدم بشكل أكبر للدراسة المجهرية الإلكترونية للارسال. أظهرت النتائج التي تم الحصول عليها أن جميع خلايا الدم لكل من Cobb 500 والدجاج العماني كانت متشابهة. كان للنواة غير المتجانسة نواة متعددة الفصوص. يتراوح عدد فصوص النوى من 2 إلى 3. كان للحمضات مظهر مميز وكان لها في الغالب نواة مفصصة. كان البازوفيل مستديرا في المظهر وكان لديه أربعة أنواع من الحبيبات (حبيبات كثيفة ، حبيبات مرقشة ، حبيبات شبكية أو صافية وحبيبات المايلين) والتي يمكن رؤيتها بوضوح. كانت الخلايا الليمفاوية أصغر الكريات البيض وتميزت بأنها خلية مستديرة صغيرة مع عدد قليل من العمليات السيتوبلازمية. كانت الخلايا الوحيدة مستديرة الشكل مع نواة على شكل الكلى أو المسافة البادئة. كان لدى النواة بقع غير متجانسة أكثر مقارنة باليوكروماتين. تم تمييز الصفيحات الدموية عن الخلايا الأخرى من خلال نواتها الكثيفة والفجوات الكبيرة الموجودة في السيتوبلازم. في الختام ، تظهر الدراسة أوجه تشابه كبيرة في البنية الفائقة لجميع تكوين خلايا الدم بين سلالات العمانية المحلية و الدجاج اللاحم Cobb 500.

الكلمات المفتاحية: خلايا الدم، كوب 500، دجاج عماني محلي، بنية فائقة.

Introduction

The local chicken is considered an essential part of human livelihood, and it contributes to the food security of low-income families as a significant source of meat and eggs in more than 80% of poor households in the developing regions around the world (Shaat and Al-Habsi, 2016). Cobb 500 is superlatively suitable to the Middle East region, adapting well to warmer climates with integrators benefiting from the breed's efficiency right through the production chain. One of the main characteristic of Cobb 500 broiler breed is the great feed conversion and there for that make it the best choice in broilers breed selection (Hult, 2015). Many farmers in Oman raise local chickens for meat and egg production. To ensure an enhanced production of local breed chickens, it is important to assess their health performance first (Shaat and Al-Habsi, 2016).

The immune system is built up by many lymphoid tissues, molecules and leukocytes, which defend the body from infectious agents, such as viruses, bacteria, parasites and fungi. Besides protecting the body from pathogens, the immune system plays a role in maintaining body hemostasis by restoring and removing damaged endogenous tissues and cells. Leukocytes are produced predominantly in the bone marrow. After differentiation and maturation, leukocytes migrate to the blood, lym-

Waleed Al-Marzooqi[1,*] (✉) walmar@squ.edu.om, [1]Department of Animal and Veterinary Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, Muscat, Sultanate of Oman, [2]Department of Animal Production, Faculty of Agriculture, University of Alexandria, Alexandria, Egypt, Department of Pathology College of Medicine and Health Sciences, Sultan Qaboos University, Oman.

phatic system and peripheral tissues to perform their different immunological functions (Hult, 2015). Avian leukocytes include mononuclear cells (monocytes and lymphocytes), granulocytes (basophils, eosinophils and heterophils) and thrombocytes (Jones, 2015).

In recent years, transmission electron microscopy has become necessary to study biological and molecular ultrastructures. However, very little electron microscopy in chicken blood cells have been reported, and no survey on local Omani chicken has been done so far. The main aim of this study was to compare the ultrastructural observations of the blood cells of local Omani and Cobb 500 broiler chickens raised under intensive management.

Material and Methods

Ethical Approval

This study was approved in 2019 by the Animal Research Ethics Board at Sultan Qaboos University.

Resource Population

This study was conducted on both Cobb 500 broilers (n=10) and local Omani chickens (n=10) that were apparently healthy of age 35 days old of either sex reared at the Poultry Research Unit at the Agricultural Experiment Station, Sultan Qaboos University. Feed and water were provided ad libitum.

Sample Processing

Blood samples were obtained using disposable syringes needles (23 gauges) from the wing vein. Samples were collected into tubes containing ethylenediamine teraacetic acid (EDTA). The samples were kept on ice and transported to the Electron Microscopy lab in the College of Medicine, immediately after collection to prevent any cells ultrastructure alteration. The identification and classification of blood cells were achieved using the transmission electron microscopy (TEM). Samples for electron microscopy were prepared as previously described (Johnson et al., 2020). Briefly, to obtain a buffy coat layer, samples were centrifuged at 400 g for 20 minutes within 90 minutes of collection. The plasma was removed, and the buffy coat was coated with Karnovsky's fixative (2 percent glutaraldehyde in 2.5 % paraformaldehyde in 0.2 M phosphate buffer, pH 7.38) on top. The fixative was withdrawn and the buffy coat layers were peeled off and dissected into 2 × 1 × 1 mm blocks and placed in a Petri dish containing fresh Karnovsky fixative for an additional 90 minutes after overnight refrigeration. A tissue processor from Leica Electron Microscopy was used to process the blocks. Spurr's epoxy resin embedding media was used to embed tissues, which were polymerized overnight. Light microscopy was used to examine sections stained with 1 % toluidine blue in 1% borax. Ultra-sectioning with an ideal thickness of 60–90 nm and staining with uranyl acetate and Reynold's lead citrate were used to identify locations with the highest concentration of lymphocytes. A Jeol JEM-1230 transmission electron microscope with an 80 kV volt-age was used to examine sections on grids. MSC SI0031 Gatan CCD camera and Digital Micrograph (TM) 1.85.1535 software were used for digital imaging and acquisition. Prior to screening samples, images were automatically gain-corrected using gain and dark standards and calibrated using a carbon grating replica (Robinson, Geraci, Sonnenblick, & Factor, 1988).

Results

The ultrastructural observations of Omani local chicken and Cobb 500 brioler chickens were examined. In general, we found that the ultrastructural features of blood cells of both strains were similar and are therefore described together.

Heterophils

The heterophil nucleus was surrounded by a nuclear membrane. In most cells, the nucleus has a higher quantity of heterochromatin than euchromatin. Mature polymorphonuclear cells have a multi-lobed nucleus. The number of nuclei lobes ranges from 2 to 3 (Figures 1a and 1b). Different organelles were detected in the cytoplasm such as mitochondria, vesicles, centrioles and central body. In addition, pseudopodia were seen frequently in the heterophil peripheral cytoplasm. In addition, two types of intracellular granules were seen; dense granules and less dense granules. Dense granules can be either oval or spherical. They are cytoplasmic membrane-bounded electron dense granules. The size of these granules ranges from 120-575 nm. Multiple numbers were demonstrated. Less dense granules (specific granules) are lighter than dense granules and smaller in size. They are membranous granules. Their size ranges from less than 200-270 nm.

Eosinophil

Eosinophils had a distinctive appearance and showed small pseudopodia (Figures 2a and 2b). Mostly they had a lobulated nucleus. Heterochromatin and euchromatin were in equal quantities. The nucleus often was located in the center of the cell. Major structures were detected in both broiler Cobb 500 and Omani chicken were Golgi apparatus. Invaginations resembling vacuoles in the granules are seen approximately a quarter the size of the granule and different sizes of dense granules surrounding with a membrane were present in this cell.

Basophil

Basophils are round in appearance. Small pseudopodia were detected (Figures 3 and 4). The single nucleus showed indentation. Heterochromatin and euchromatin were distributed equally in Cobb 500 breed. In the Omani breed, the euchromatin distribution was more

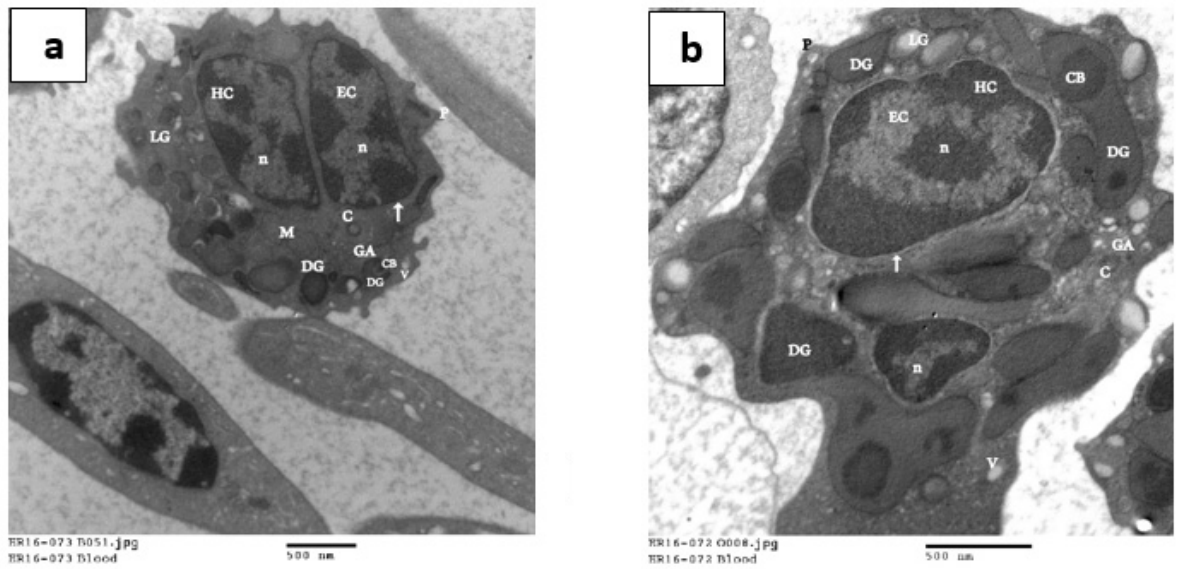

Figure 1. Transmission electron micrograph of (a) Cobb 500 broiler and (b) Omani local chicken. In both panel (a and b), a heterophil showing bi-lobed nucleus (n), heterochromatin (HC), Euchromatin (EC) and nuclear membrane (arrow) were observed. The cytoplasm includes dense granules (DG), less dense granules (LG), centriole (C), Golgi apparatus (GA) mitochondria (M), dissected central body (CB), pseudopodia (P) and Vesicles (V)

prominent than heterochromatin. Nucleoli were present in some cells. In both breeds, the mitochondria could be seen. Four types of granules (Dense granules, Mottled granules, Web or net granules and Myelin granules) could be seen (Figures 5 and 6). Dense granules were darker granules, and some of them had a little internal structure which can be seen. They range from 100-280 nm in Cobb 500 chicken and 100-250 nm in Omani chicken. Mottled granules, they are less dense than the first granules. In Cobb 500 chicken they range from 150-200 nm and from 200-250 nm in local chicken. Web or net granules, looser than mottled granules and had ability to break down. They range from 80-100 nm in both breeds. Myelin granule, these granules size was 250 nm in Cobb 500 and 120 nm in Omani chicken.

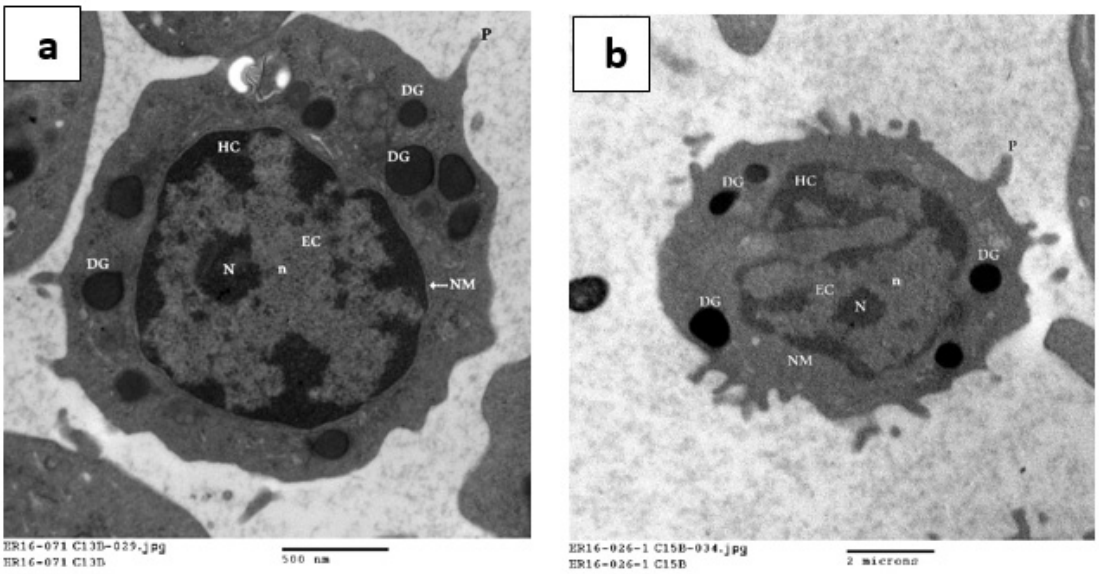

Figure 2. Transmission electron micrograph presenting eosinophil from (a) Cobb 500 broilers and (b) Omani local chicken. Nucleus (n), nucleolus (N), heterochromatin (HC), euchromatin (EC) and nuclear membrane (NM). This cell also shows centric nucleus and various sized of dense granules (DG). Pseudopodium (P) is also detected

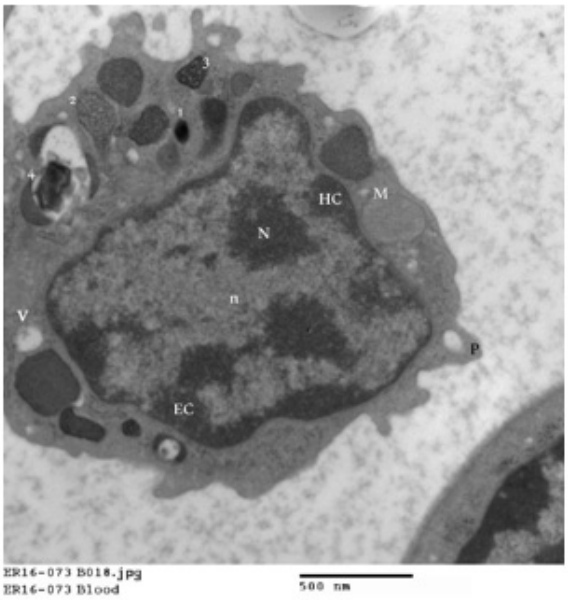

Figure 3. Transmission electron micrograph of Cobb 500 breed basophil showing a single central nucleus (n), nucleolus (N), heterochromatin (HC), euchromatin (EC), vessel (V) and mitochondria (M) Four different types of granules: (1) dense (2) mottled (3) web or net form (4) myelin figure also can be seen

Lymphocyte

In both chicken breeds' peripheral blood, lymphocytes were the smallest leukocytes. lymphocyte was round in shape (Figure 7a and 7b). The nucleus was located in the center of the cell and showed a higher nucleus to cytoplasm ratio. The nucleus was round in shape, and it contained an equal quantity of euchromatin and heterochromatin. Different structures were observed in the cytoplasm, such as different sizes and number of mitochondria. In some cells, there were up to six mitochondria. In addition, multi-vascular body, few short endoplasmic reticulum strands, beta-glycogen granules and less than 0.1 μm measures small clear vesicles were detected.

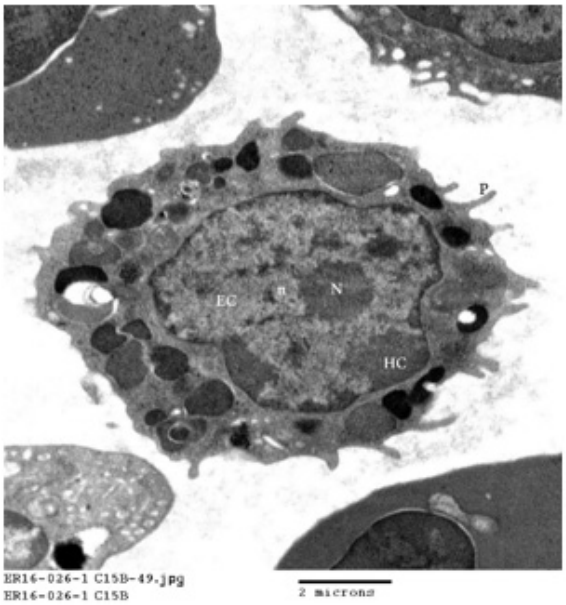

Figure 5. Transmission electron micrograph of Omani breed basophil showing single central nucleus (n), nucleolus (N), heterochromatin (HC), euchromatin (EC) and pseudopodia like structures (P)

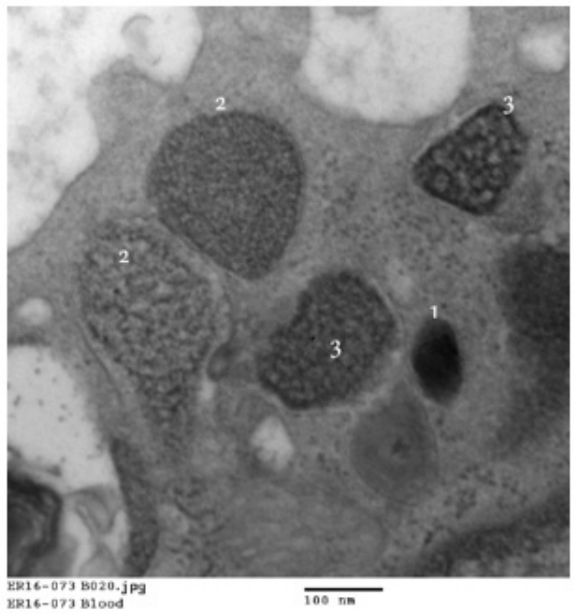

Figure 4. Higher magnification of figure-3 showing dense (1), mottled (2), web or net form (3) granules

Monocyte

Monocyte can be easily identified due to their kidney-shaped or indented nucleus (Figures 8 and 9). Monocyte contained less heterochromatin compared to euchromatin even though monocyte heterochromatin were not as dense as lymphocyte. Heterochromatin was always found towards the edge of the nucleus. The identification of the nuclear membrane was better in monocyte compared to other leucocytes. Both Cobb 500 and Omani breeds had a similar shape and structure. In the majority of monocyte cells, a single layer of rough endoplasmic reticulum surrounded the other nuclear membrane. There was no smooth endoplasmic reticulum detected. Many mitochondria of different sizes were frequently observed in each cell. Some of the mitochondria were small and round with a dense matrix, and others were larger with more prominent cristae and lighter matrix. Golgi apparatus was detected more often in this cell than in other leukocytes. Many cells contain lysosomes with different densities. Large empty vesicles were seen in the cytoplasm. Pinocytotic vesicles were also detected. Pseudopodium was seen in the majority of the cell.

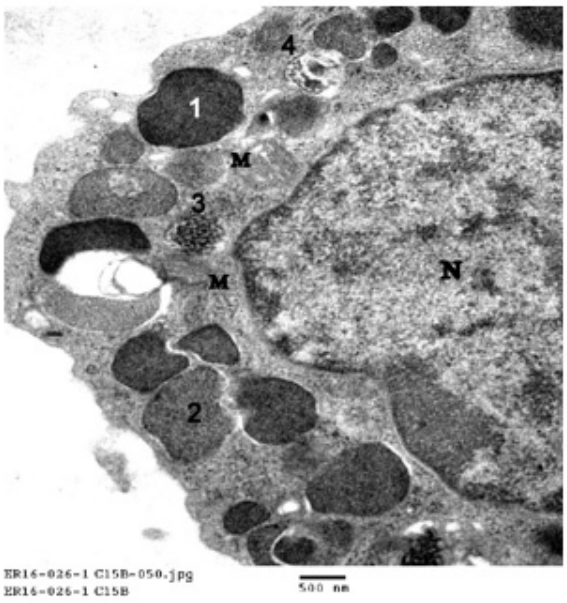

Figure 6. Higher magnification of figure-8 showing the nucleus (N) of the basophil with 4 different types of granules: (1) dense (2) mottled (3) web or net form (4) myelin figure. Mitochondria (M) also can be seen

Thrombocyte

Thrombocyte was distinguished from other cells by their dense nucleus and the large vacuoles found in the cytoplasm. Heterochromatin was predominating over euchromatin, and that made the nuclei detection easier. In many cells, the nucleolus was irregular, large and dense (Figures 10 and 11). The primary cell structure detected in in the cytoplasm of the thrombocyte cells in both breed was vacuoles. There were two types of vacuoles; large vacuoles were around 1 µm. The majority of large vacuoles were empty except from one or two dense granules measuring 50-100 nm. Small vacuoles can be detected all around the cytoplasm, measuring less than 250 nm. Pinocytotic vesicles, mitochondria and myelin figure was also detected.

Erythrocyte

Erythrocytes in both breeds were similar under transmission electron microscopy. The cytoplasm is moderately dense because of hemoglobin content, without notable organelles. The erythrocytes have a narrower, more elongated, elliptical shape. Artifactual separation of the nucleus from the cytoplasm is evident (Figures 12a and 12b).

Discussion

Chicken heterophil is a multilobulated oval or round cell with rod-like eosinophilic granules. Cobb 500 and Omani chickens had similar heterophil traits. Euchromatin was detected in the nucleus center region, while heterochromatin was found in patches around the perimeter, similar to the findings of Mohd et al. (2016). Single beta granules were always recognized as glycogen. There was evidence of the Golgi apparatus, and mitochondria were frequently spherical and tiny. Other bird species with similar traits include ducks, geese, turkeys, pigeons, quail, and guinea-fowl (Maxwell, 1973). The goose and duck heterophils, on the other hand, lacked the small, spherical, dense, membrane bound granules seen in chickens.

Heterophil possesses two types of granules: dense cytoplasmic granules (primary granules) and short round or oval less dense granules (secondary granules)

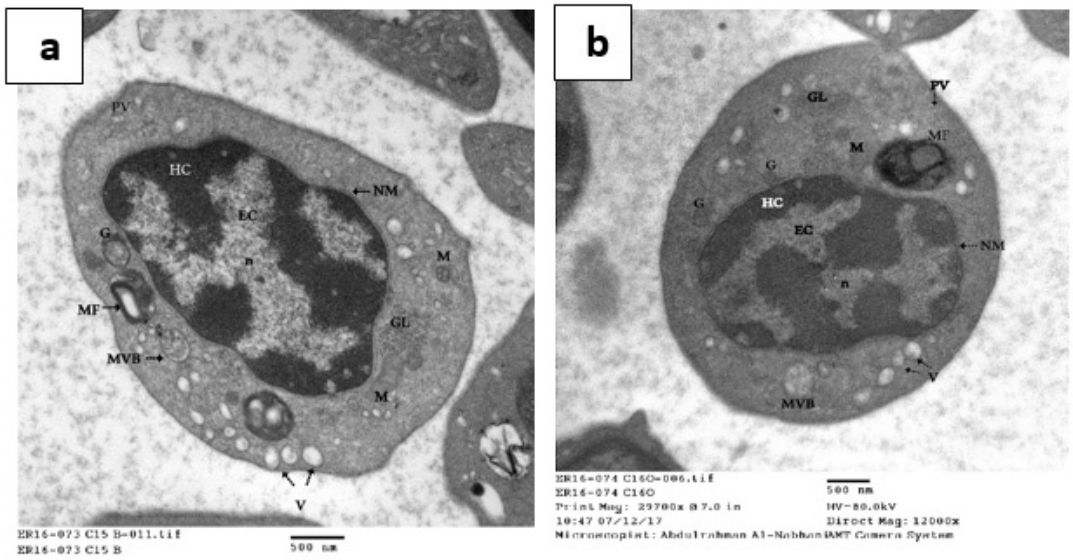

Figure 7. Transmission electron micrograph presenting lymphocyte from Cobb 500 (a) and Omani Local chicken (b). Nucleus (n), heterochromatin (HC), euchromatin (EC), mitochondria (M), pinocytotic vesicle (PV), Multi-vascular body (MVB), dense electron granules (G), small empty vesicles (V), beta-glycogen granules (GL) and myelin figure (MF)

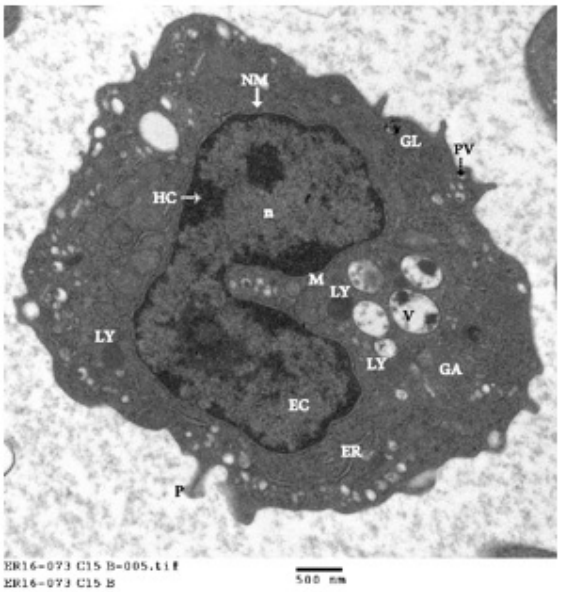

Figure 8. Transmission electron micrograph of Cobb 500 breed monocyte with a kidney shaped nucleus (n). This cell shows a distinct double nuclear membrane (NM), heterochromatin (HC) and euchromatin (EC). Mitochondria (M), endoplasmic reticulum (ER), Golgi apparatus (GA), dense lysosome (LY), beta-glycogen granules (GL), pinocytotic vesicles (PV) and empty vesicles (V) can also be seen. Pseudopodia (P) can also be detected

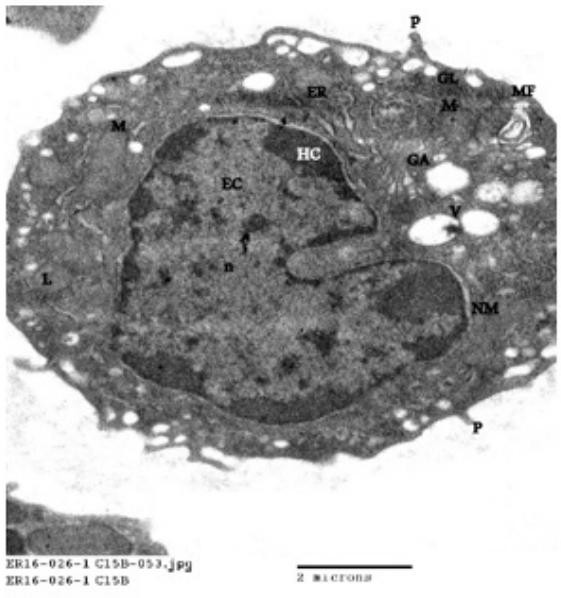

Figure 9. Transmission electron micrograph of Omani breed monocyte showing kidney shape nucleus (n), This cell shows a distinct double nuclear membrane (NM), heterochromatin (HC) and euchromatin (EC). Mitochondria (M), endoplasmic reticulum (ER), Golgi apparatus (GA), dense lysosome (L), beta-glycogen granules (GL), myelin figure (MF) and empty vesicles (V) can also be seen. Pseudopodia (P) can also be detected

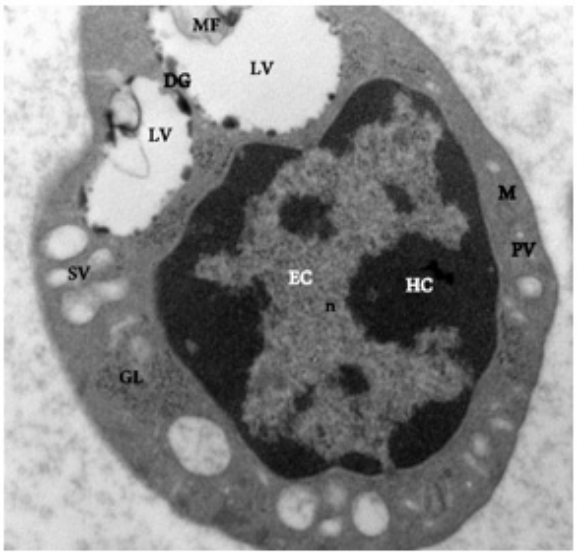

Figure 10. Transmission electron micrograph of Cobb 500 breed thrombocyte showing a single nucleus (n), heterochromatin (HC) and euchromatin (EC). Small dens granules (DG) attached to large vacuoles (LV), small vacuoles (SV), beta glycogen granules (GL), pinocytotic vesicle (PV) and mitochondria (M) can be detected

in chicken. The dense granules contain a central body that play an important role during phagocytosis (Mohd et al., 2016). Heterophil central body was also reported in duck, goose, turkeys and pigeons (Maxwell, 1973). Dense granules of both Cobb 500 and Omani breed were measured and there were no significant differences in dense granules of both breed. Cobb 500 chicken dense granules measurements were within the range reported previously (Mohd et al., 2016), which were 400-1600 nm in length. Similar observations have been observed in duck and goose, with lengths ranging from 260 to 300 nm in turkey and pigeon (Maxwell, 1973).

No obvious ultrastructure differences were observed in the lymphocyte cells between Cobb 500 breed and the Omani breed examined. Except for the cytoplasm to nucleus ratio, which was lower than monocytes, they had a similar diameter to monocytes. This was in line with Maxwell's (1973) and Mohd et al.'s results (2016). The nucleus of a medium-sized lymphocyte featured one to two nucleoli, which were encircled by a chromatin rim. Despite the fact that it shares some characteristics with both tiny lymphocytes and monocytes, medium sized lymphocytes were previously overlooked as a unique cell type in avian peripheral blood. However, granules detected in the medium-sized lymphocytes of the two chicken strains studied were comparable to those seen in six bird species including domestic fowl (Maxwell, 1973; Mohd et al., 2016).

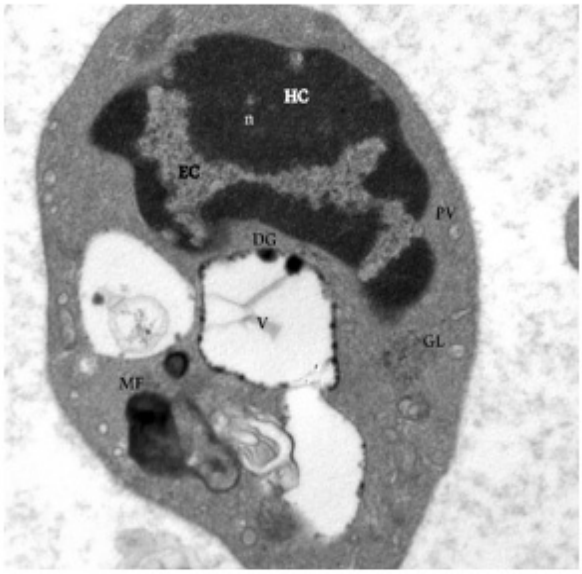

Figure 11. Transmission electron micrograph of Omani breed thrombocyte showing nucleus (n), heterochromatin (HC) and euchromatin (EC). Myelin figures (MF), beta glycogen granules (GL), pinocytotic vessel (PV) and a large empty vacuole (V) with peripheral dense granule (DG) can be seen

The cell surface of lymphocyte was spherical, with few cytoplasmic structures visible. The nucleus was eccentric, occupying the majority of the cytoplasm in the form of a rim, which is consistent with earlier observations (Gupta and Singh, 2008; Mohd et al., 2016). The nuclei of turkey, geese, pigeon, duck, guinea fowl, and quail small lymphocytes were found to have an equal quantity of heterochromatin and euchromatin, small clear vacuoles, and multi-vascular structures (Maxwell, 1973), which were similar to the findings of this study. However, tyre-track like structure was not seen in any cell examined in the current study.

One of the most intriguing features was the significant proportion of euchromatin in the nuclei of the majority of the monocytes studied. Nuclei with more euchromatin and less stainable material are metabolically more active than those with coarse bulk of highly stained heterochromatin (Maxwell and Trejo, 1970). The presence of rough endoplasmic reticulum, which was more visible in monocytes than other white blood cells in this investigation and was consistent with prior observations, is likely associated to the high activity state according to Maxwell and Trejo (1970) and Mohd et al. (2016). As observed in the current study, the nucleus of the monocyte was kidney or bean-shaped this finding was in accord with the Gupta and Singh (2008) who reported that monocytes from guinea fowl have a kidney-shaped nucleus with a distinct nuclear membrane. Furthermore, mitochondria, lysosomes of various sizes, and dense multi-vesiculated bodies were assessed in this study, were consistent with earlier findings (Maxwell and Trejo, 1970; Salakij et al., 2014).

Similar to prior studies, the eosinophil nucleus was sometimes solitary and often bilobed, with peripheral heterochromatin and central euchromatin patterns (Maxwell and Trejo, 1970; Mohd et al., 2016). The cytoplasmic granules were spherical and thick, and they were dispersed throughout the cytoplasm. These findings were comparable to those of the Uttara fowl eosinophil, which showed circular and oval granules; nevertheless, elongated granules were identified in other bird species, such as ducks, geese, and guinea fowl (Maxwell et al., 1972; Mohd et al., 2016).

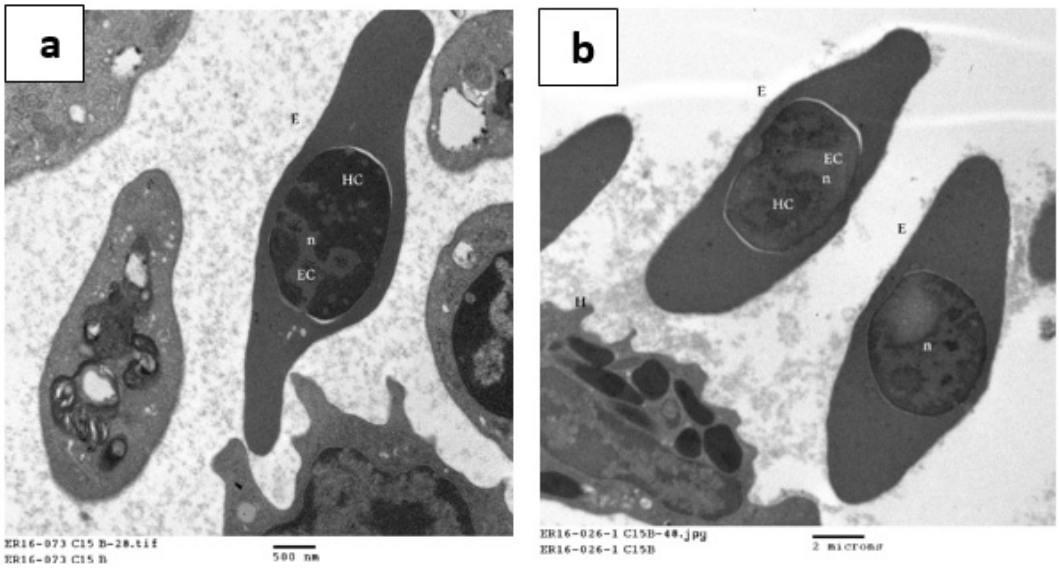

Figure 12. Transmission electron micrograph presenting erythrocyte from Cobb 500 (a) broiler and Omani local chicken (b). Nucleus (n), heterochromatin (HC) and euchromatin (EC). The cytoplasm is moderately dense because of hemoglobin content, without notable organelles. The erythrocytes have a narrower, more elongated, elliptical shape.

When compared to eosinophil, basophil has more granules, whereas heterophil has almost the same quantity. The majority of avian species had granules of smaller than 0.1-0.8 m in diameter (Maxwell and Robertson, 1998), which is similar to the findings of this study. Furthermore, chicken basophil had four types of granules, but other species such as goose, turkey, duck, Japanese quail, guinea fowl, and pigeons had three types of granules (Maxwell and Robertson, 1998). The first type of granules was electro-dense oval or elongated granules. The second type was mottled granules which is the most appendant granules. This type of granules has stippled internal structure. The third granules have a honeycomb arrangement. These three types of granules were also previously reported by (Maxwell, 1973; Maxwell and Robertson, 1998; Maxwell and Trejo, 1970; Mohd et al., 2016). The fourth type of basophil granules resembles a myelin-like according to Mohd et al. (2016).

Similar to earlier findings in Kadaknath fowl by (Yadav, 2012), the thrombocytes examined in this study displayed both oval and elongated cells with oval to round shaped eccentrically or centrally nuclei and peripherally scattered heterochromatin. There were two forms of glycogen granules in the investigated thrombocytes alfa and beta. Similarly, it was reported that beta glycogen granules were observed are in the cytoplasm of the thrombocyte (Maxwell and Trejo, 1970). However, erythrocytes in this study were oval or elliptical in shape and the cytoplasm was homogeneously stained with no cell organelles which in agreement previous studies (Mohd et al., 2016; Yadav, 2012).

Conclusion

The present study revealed that hematological parameters for local breed are similar to those of Cobb 500. This indicates that these parameters can be used as performance indicators for both breeds. The present work could contribute to understanding pathophysiological changes relevant to chicken performance.

Acknowledgement

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