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Cytochemical and Cytoenzymatic Architectural Properties of the Blood Cells of White Pekin Duck

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ABSTRACT

Background: A cytochemical and cytoenzymatic study involves using specialized stains to microscopically examine cellular components, including lipids and carbohydrates.

Methods: Freshly prepared blood smears from 26 apparently healthy adult ducks were used for cytochemical and cytoenzymatic analysis. The smears were stained with specific and special cytochemical stains, Periodic Acid-Schiff (PAS) for carbohydrates, Sudan Black B for lipids, acid ferrocyanide for non-heme iron and toluidine blue for mucopolysaccharides, as well as cytoenzymatic stains such as β-naphthol acetate esterase, acid phosphatase, alkaline phosphatase, β-glucuronidase and peroxidase to assess enzymatic activity.

Result: In the White Pekin duck, rare acid ferrocyanide-positive erythrocytes exhibited blue siderocyte granules with cell deformities, while leukocytes and thrombocytes were negative. Basophils strongly reacted to mucopolysaccharides, showing intense violet granules under toluidine blue, whereas other cells were non-reactive. Heterophils exhibited strong PAS positivity for carbohydrates, with eosinophils, erythrocytes and thrombocytes showing moderate positivity. Sudan black B staining revealed strong lipid positivity in eosinophils and heterophils, while other cells were showed negative for same. Cytoenzymatic analysis showed strong acid phosphatase activity in basophils, moderate activity in heterophils and weak activity in eosinophils and lymphocytes. Eosinophils and heterophils also strongly reacted to β-naphthol acetate esterase, with lymphocytes showing weak positivity. Thrombocytes were strongly positive for peroxidase and eosinophils and erythrocytes showed weak activity. Other cells remained non-reactive to β-glucuronidase, alkaline phosphatase and other enzymes, indicating distinct staining patterns across cell types.

Key words: Blood cells, Cytochemical, Cytoenzymatic, Diagnosis, White pekin duck.

INTRODUCTION

Blood tests are crucial for diagnosing haematological disorders and monitoring overall health. Blood consists of plasma and formed elements, including erythrocytes, leukocytes and thrombocytes. These components are sensitive to changes in an animal's health, often reflecting its physiological health condition (Sarkar *et al.*, 2023). Abnormalities in these elements can indicate diseases such as infections or inflammation (Khan *et al.,* 2011; Peng *et al.*, 2018).

Cytochemistry has a vital role in diagnosing and classifying haematological conditions, utilizing different staining techniques to examine cellular components like enzymes, lipids and carbohydrates (Hayhoe *et al.,* 1988). For example, Acid Ferrocyanide stains non-heme iron-containing erythrocytes, known as siderocytes (Mohd *et al.,* 2015). Sudan Black B (SBB) is used to stain lipids within heterophils, eosinophils and occasionally monocytes. Periodic acid-Schiff (PAS) staining is positive in various mammalian blood cells and helps differentiate granulocytic or megakaryocytic precursors from lymphoid precursors (Salakij *et al.*, 2019). Toluidine blue (TB), a basic dye, interacts with acid mucopolysaccharides to form red to purple metachromatic complexes, with basophils staining readily in acidic TB but not in neutral TB (Raskin, 2010). These cytochemical markers are essential for understanding immune responses and disease processes in avian species (Schwarze, 1980; Savage, 1981). Cytoenzymatic analysis, particularly the activity of enzymes such as acid phosphatase, alkaline phosphatase and peroxidase, is crucial for diagnosing specific haematological disorders in birds. The localization and activity of these enzymes within blood cells provide valuable diagnostic information,

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with enzyme distribution across different cell types offering critical insights into conditions like leukaemia and other blood-related diseases (Schwarze, 1980; Savage, 1981). These tools enhance the accuracy of veterinary diagnostics, leading to better disease management and health monitoring in poultry. For example, acid phosphatase activity in lymphocytes can indicate lymphoproliferative disorders, while alkaline phosphatase levels in neutrophils help differentiate leukemia subtypes (Kolbr *et al.*, 1958; Kaplow, 1968). Peroxidase (myeloperoxidase) serves as a marker for mammalian myeloid cells, while PAS staining helps differentiate granulocytic or megakaryocytic precursors from lymphoid precursors (Hayhoe *et al.,* 1988). Nonspecific esterase (α-naphthyl acetate esterase, ANAE) and β-glucuronidase (BG) staining of mammalian cells can also provide distinguishing staining characteristics (Raskin, 2010).

MATERIALS AND METHODS

Animals and ethical approval

The present study focused on the blood cells of White Pekin ducks raised in Mizoram, India, from April 2024 to November 2024. Blood samples were collected from the poultry farm at the College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India. The use of animals in this experiment was ethically approved by the Institutional Animal Ethics Committee (IAEC) under Approval No. CVSC/CAU/IAEC/23-24/P-19, dated October 21, 2024.

Sample collection

This study was carried out using twenty-six blood samples collected from apparently healthy White Pekin ducks, regardless of age or sex. Approximately 2 ml of blood was drawn from the wing vein of each bird and transferred to a sterile siliconized tube containing sodium citrate.

Cytochemical analysis

For cytochemical studies, the blood smears were prepared immediately after collection on grease-free slides and stained for acid ferrocyanide stain for non-heme iron (Bain 2017), toluidine stain for acid mucopolysaccharides (Bain 2017), Periodic Acid Schiff's stain for glycogen (Bain 2017) and Sudan black- B for lipid (Bain 2017), (Table 1).

Cytoenzymatic analysis

For cytoenzymatic studies, the blood smears were prepared freshly after collection on grease-free slides and stained samples were treated for acid phosphatase (Chen *et al.* 2019), alkaline phosphatase (Chen *et al.* 2019), peroxidase (Bover, 1964), α-naphthol acetate esterase (Bain, 2017), Beta-glucuronidase (Bover, 1964) (Table 1).

Stained blood smears and treated slides were examined under oil immersion using a light microscope (Olympus BX 51, Japan) to assess the cytochemical and cytoenzymatic activity of various blood cells. Key images were captured using a ProgRes C5 Cool CCD camera (D-07739 Jena, Jenoptik, Germany) for illustrative purposes.

The staining intensity was evaluated based on the activity of specific blood cells for each chemical or enzyme and graded on a scale of 0–3, where 0 = no staining/ negative, 1 = weak staining, 2 = moderate staining and 3 = intense staining.

RESULTS AND DISCUSSION

Cytochemical studies

Acid ferrocyanide staining in this study revealed positive results in mature erythrocytes (Table 2), with occasional cells exhibiting blue siderocytic granules in their cytoplasm (Fig 1A), a finding consistent with previous reports by Gupta and Singh (2012) in guinea fowl, Yadav (2012) in Kadaknath fowl and Mohd. *et al.* (2015) in Uttara fowl. Similarly, Doley *et al.* (2024) observed a sparse presence of these granules in erythrocytes of Zoar. Most of the acid ferrocyanide-positive erythrocytes in this study exhibited nuclear deformities, potentially indicating disrupted erythropoiesis, which aligns with earlier findings by Cartwright *et al.* (1975) who reported siderocytic granules as a typical feature during normoblast maturation in humans and pigs. Conversely, all leukocytes and thrombocytes were negative for acid ferrocyanide, consistent with findings by Doley *et al.* (2024) and Valenciano *et al.* (2010), who noted the rarity of siderocytic leukocytes, typically observed only in cases of feline haemolytic anaemia. Barger (2022) associated siderocytes with conditions like myeloproliferative disorders, lead toxicity and haemolytic anaemia, while Meguro *et al.* (2007) emphasized the role of non-heme iron histochemistry as a

Table 1: Different stains used for cytochemical and cytoenzymatic studies in the blood cells of white pekin duck.

Method	Purpose	Source
Acid ferrocyanide	Siderocytes	Bain (2017)
Toluidine blue stain	Acid muco-polysaccharides	Bain (2017)
Sudan black - B stain	Lipid	Bain (2017)
Periodic acid schiff's stain	Carbohydrate	Bain (2017)
Gomori's method	Acid phosphatase	Chen *et al.* (2019)
Gomori's method	Alkaline phosphatase	Chen *et al.* (2019)
Sato and selkiys's method	Peroxidase	Bover (1964)
α - naphthol acetate esterase	Esterase	Bain (2017)
Fishman and baker's method	Beta-glucuronidase	Bover (1964)

critical tool for studying degenerative diseases and pathological processes.

In this study, basophils exhibited intense positivity for acid mucopolysaccharides, while erythrocytes, thrombocytes, heterophils, eosinophils, monocytes and lymphocytes showed no reaction (Table 2). These findings align with earlier reports by Gupta *et al.* (2010) in Guinea fowls, Yadav and Singh (2012) in Kadaknath fowl, Mohd *et al.* (2015) in Uttara fowl and Shalini (2015), who noted strong reactions in fowl, moderate responses in ducks and weak to negative reactions in quail. Kumar (2019) observed weak positivity in the basophils of domestic fowl. Under toluidine blue staining, basophils appeared as round cells filled with intensely violet metachromatic granules throughout the cytoplasm (Fig 1B), similar to observations by Gupta *et al.* (2010), Yadav and Singh (2012), Mohd *et al.* (2015) and Doley *et al.* (2024). Shalini (2015) similarly reported strong granule positivity in fowl, moderate in ducks and weak to negative in quail. Raskin (2010) explained that toluidine blue, a basic dye, forms metachromatic complexes with acid mucopolysaccharides, resulting in red-purple staining. Kiehl *et al.* (1994) highlighted that toluidine blue staining could be metachromatic under acidic conditions but not at neutral pH. Ribatti (2018) attributed the metachromasia to dye polymerisation, causing a shift from blue to violet, red, or orange hues. Shao *et al.* (2013) identified glycosaminoglycans in leukocytes as primarily chondroitin sulfate with minor heparin sulfate, while Fong and Cranes (2023) linked basophil and mast cell metachromasia to highly acidic heparin molecules in their granules.

In this study, heterophils exhibited strong periodic acid-Schiff (PAS) positivity (Fig 1D), consistent with Shalini (2015), who reported similar results in ducks and quail but moderate positivity in fowl. However andreasen and Latimer (1990) and Thrall *et al.* (2004) observed PAS negativity in chicken heterophils, while Salakij *et al.* (2019) reported species-specific differences, with PAS positivity in Black kites but negativity in Black-shouldered and Brahminy kites. Eosinophils displayed moderate PAS positivity (Fig 1E), aligning with findings by Mohd *et al.* (2015) in Uttara fowl, though weaker reactions were noted by Yadav (2011) in Kadaknath fowl and Raskin (2010) in chickens. Lymphocytes showed weak PAS positivity (Fig 1F), while other cells were non-reactive (Table 2). Interestingly,

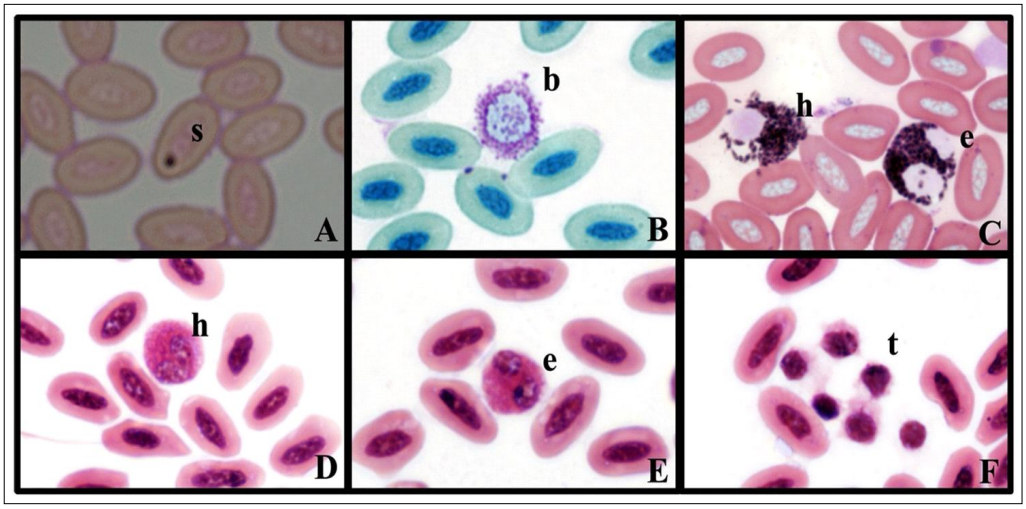

Fig 1: (A) Showing a siderocyte (S); (B) Showing a toluidine blue positive basophil (b); (C) Showing Sudan Black-B positive heterophil (h) and eosinophil (e); (D) Showing Periodic Acid-Schiff positive heterophil (h) and erythrocytes; (E) Showing Periodic Acid-Schiff positive eosinophil (e) and erythrocytes; (F) Showing Periodic Acid-Schiff positive thrombocyte (t) and erythrocytes.

Table 2: Cytochemical intensity of different blood cells in white pekin duck.

Blood cell	Acid ferrocyanide	Toluidine blue stain	Sudan black-B stain	Periodic acid schiff's stain
Erythrocyte	+	-	-	++
Heterophil	-	-	+++	+++
Eosinophil	-	-	+++	++
Basophil	-	+++	-	-
Monocyte	-	-	-	-
Lymphocyte	-	-	-	-
Thrombocyte	-	-	-	++

Gradation for intensity:

+++ Strongly positive; ++ Moderately positive; + Weakly positive; - Nonreactive.

thrombocytes exhibited moderate PAS positivity in fowl, ducks and quail, corroborating Mohd *et al.* (2015). Salakij *et al.* (2019) reported PAS-negative erythrocytes in Black-shouldered and Brahminy kites, contrasting with Chen *et al.* (2019), who found PAS-positive erythrocytes in domestic pigeons. Raskin (2010) linked PAS positivity in blood cells to glycoproteins, mucoproteins, glycolipids and glycogen, highlighting its diagnostic value for various leukemias in animals.

In this study, eosinophils showed strong positivity for Sudan Black B, characterized by black, round cytoplasmic granules (Fig 1C), consistent with findings in guinea fowl (Gupta *et al.,* 2010), Kadaknath fowl (Yadav and Singh, 2012), Uttara fowl (Mohd. *et al.,* 2015), Crested Serpent Eagles (Salakij *et al.,* 2015), domestic pigeons (Chen *et al.,* 2019) and Zoar (Doley *et al.*, 2024). However, Kumar (2019) reported only moderate positivity in domestic fowl eosinophils. Heterophils also displayed strong positivity with black elongated granules (Fig 1C), aligning with Shalini (2015) in ducks and quails but contrasting with weak positivity reported in Uttara fowl (Mohd. *et al.,* 2015), painted storks (Salakij *et al.,* 2003) and the negative results observed by Bounous and Stedman (2000) and Salakij *et al.* (2004) in other avian species. In contrast, erythrocytes, basophils, lymphocytes, monocytes and thrombocytes showed no reaction (Table 2), consistent with findings in domestic fowl, ducks, quails (Shalini, 2015) and domestic pigeons (Chen *et al.,* 2019). Exceptions include positivity in painted stork erythrocytes (Salakij *et al.,* 2003) and occasional positivity in avian monocytes (Raskin, 2010). Sudan Black B, a fat-soluble dye that stains lipid particles in myelocytic and monocytic granules (Jamal, 2020), is also useful in diagnosing various leukemias, including myeloblastic, eosinophilic, monocytic, myelomonocytic and erythroleukemia in several species (Raskin, 2010).

Cytoenzymatic studies

In this study, basophils displayed strong positivity for acid phosphatase (Fig 2A), contrasting with findings in guinea fowl (Gupta and Singh, 2008) and domestic pigeons (Chen *et al.*, 2019), where basophils were negative for this enzyme. Heterophils showed moderate positivity (Fig 2B), aligning with findings in rock partridges (Dönmez and Sur, 2008), avian heterophils (Claver and Quaglia, 2009) and Zoar (Doley *et al.,* 2024), although previous studies in chickens (Andreasen and Latimer, 1990) and other birds reported negative or varying reactivity. Weak positivity in eosinophils was reported in guinea fowl (Gupta and Singh, 2008), Uttara fowl (Mohd *et al.,* 2018) and domestic pigeons (Chen *et al.,* 2019). Eosinophils and lymphocytes in this study exhibited weak positivity (Fig 2C), consistent with Andreasen and Latimer (1990) in chickens, Yadav *et al.* (2015) in Kadaknath fowl and other studies highlighting acid phosphatase in avian eosinophils (Bounous and Stedman, 2000; Raskin and Valenciano, 2007). Other leukocytes, including thrombocytes and erythrocytes, were non-reactive (Table 3), consistent with observations in chickens and fowl, although acid phosphatase activity has been noted in monocytes (Raskin and Valenciano, 2007), lymphocytes (Shalini, 2015) and thrombocytes (Dönmez and Sur, 2008). Raskin (2010) highlighted the diagnostic utility of acid phosphatase activity in identifying blast cells and aiding in the diagnosis of acute myeloblastic leukemia and its subtypes, such as myelomonocytic, erythroleukemia and megakaryoblastic leukemia.

In this study, eosinophils showed moderate positivity (Fig 2D) and heterophils weak positivity for alkaline phosphatase (Fig 2E), while other blood cells were non-reactive (Table 3). Similar findings were reported by Gupta and Singh (2008a) in guinea fowl, Yadav (2011) in Kadaknath fowl and Mohd *et al.* (2018) in Uttara fowl, as well as by Doley *et al.* (2024) in Zoar. In contrast andreasen and Latimer (1990) and Raskin and Valenciano (2007) observed no alkaline phosphatase activity in chickens' heterophils and eosinophils. Chen *et al.* (2019) found

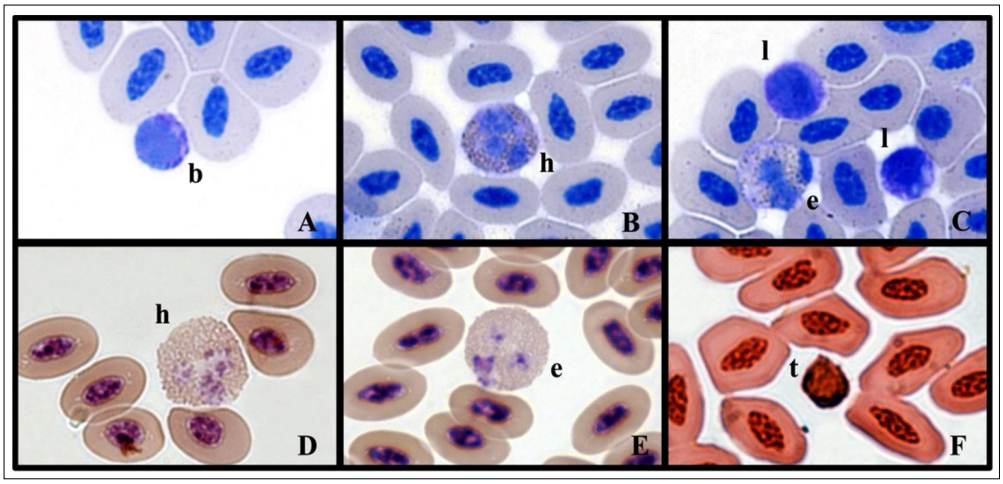

Fig 2: (A) Showing an acid phosphatase positive basophil (b); (B) Showing an acid phosphatase positive heterophil (h); (C) Showing acid phosphatase positive eosinophil (e) and lymphocytes (l); (D) showing alkaline phosphatase positive heterophil (h); (E) Showing alkaline phosphatase positive eosinophil (e); (F) Showing peroxidase positive thrombocyte (t) and erythrocytes.

alkaline phosphatase positivity in the erythrocytes of domestic pigeons, while Genovese *et al.* (2013) reported negative reactivity in avian heterophils. Previous studies by Nanba *et al.* (1977) and Elghetany *et al.* (1990) demonstrated alkaline phosphatase activity in granulocytes and specific lymphocyte subsets, with Raskin (2010) noting its potential as a diagnostic marker for myelogenous leukemia, although late-stage neutrophils in dogs and cats lacked activity.

In this study, thrombocytes showed strong positivity for peroxidase (Fig 2F), consistent with Santos *et al. (*2003) in roadside hawks, but differing from Salakij *et al.* (2004) in Greater and Lesser Adjutants, Chen *et al.* (2019) in domestic pigeons and Salakij *et al.* (2019) in Black-shouldered Kites, Brahminy Kites and Black Kites. Eosinophils and erythrocytes in White Pekin ducks showed weak peroxidase positivity, similar to Bounous and Stedman (2000), Gupta and Singh (2008), Bonadiman *et al.* (2009) and Mohd *et al.* (2018), but contrasting with Chen *et al.* (2019) and Salakij *et al.* (2015), who found erythrocytes in pigeons and Crested Serpent Eagles negative for peroxidase. All other blood cells in this study were non-reactive, aligning with Bounous and Stedman (2000), Salakij *et al.* (2004) and Gupta and Singh (2008a), who reported no peroxidase activity in avian heterophils, lymphocytes, basophils and monocytes. Raskin (2010) noted peroxidase activity in the matrix of eosinophilic granules but not the crystalloid core, emphasizing its diagnostic value for myeloblastic and myelomonocytic leukaemia in various animal species.

In this study, eosinophils and lymphocytes showed weak positivity for β-glucuronidase (Fig 3B, 3C), consistent with Yadav and Singh (2015) in Kadaknath fowl, but contrasting with Salakij *et al.* (2004), who found no reactivity in the eosinophils of Greater and Lesser Adjutants. Other blood cells were non-reactive for this enzyme (Table 3), aligning with Salakij *et al.* (2004), who also reported no reactivity in erythrocytes, heterophils and thrombocytes, while basophils were positive and monocytes weakly positive. Yadav and Singh (2015) noted that basophils, lymphocytes and monocytes in Kadaknath fowl were negative, differing from Salakij *et al.* (2015), who observed

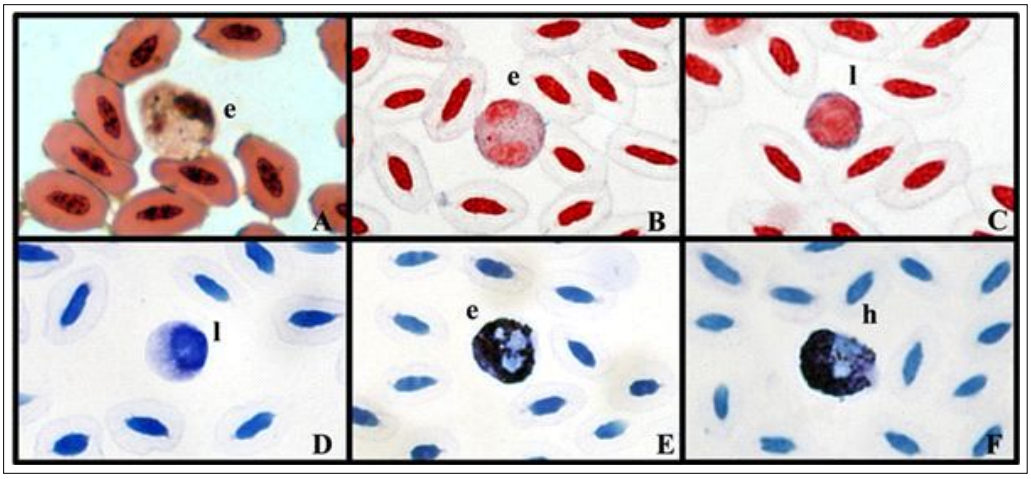

Fig 3: (A) Showing a peroxidase positive eosinophil (e) and erythrocytes; (B) Showing â -glucuronidase positive eosinophil (e); (C) Showing â -glucuronidase positive lymphocyte (l); (D) Showing alpha naphthyl acetate esterase positive lymphocyte (l); (E) Showing alpha naphthyl acetate esterase positive eosinophil (e); (F) Showing alpha naphthyl acetate esterase positive heterophil (h).

Table 3: Cytoenzymatic intensity of different blood cells in white pekin duck.

Blood cells	Acid phosphatase	Alkaline phosphatase	Peroxidase	β-glucuronidase	Periodic acid schiff's stain
Erythrocyte	-	-	+	-	-
Heterophil	++	+	-	-	+++
Eosinophil	+	++	+	+	+++
Basophil	+++	-	-	-	-
Monocyte	+	-	-	-	-
Lymphocyte	-	-	-	+	+
Thrombocyte	-	-	+++	-	-

Gradation for intensity:

+++ Strongly positive; ++ Moderately positive;+ Weakly positive; - Nonreactive.

weak positivity in erythrocytes of Crested Serpent Eagles and Shikras. Salakij *et al.* (2003) found weak or no staining in heterophils of Painted Storks, while thrombocytes were positive. Genovese *et al.* (2013) reported positivity in avian heterophils and Salakij *et al.* (2015) noted positivity in heterophils, basophils, lymphocytes and thrombocytes of Crested Serpent Eagles, with weak positivity in monocytes. Furthermore, Salakij *et al.* (2019) observed β-glucuronidase positivity in lymphocytes and thrombocytes of Black-shouldered Kites, with variability in other kite species. Haskins *et al.* (1984) associated β-glucuronidase deficiency with mucopolysaccharidosis VII (Sly syndrome), leading to glycosaminoglycan accumulation in tissues.

In this study, eosinophils and heterophils exhibited strong positivity for α-naphthol acetate esterase (ANAE) (Fig 3E, 3F), consistent with Salakij *et al.* (2015) in Crested Serpent Eagles and Shikras, while Shalini (2015) reported weak positivity in fowl eosinophils and weak to negative reactions in ducks. Lymphocytes showed weak positivity (Fig 3D), in line with Ergün *et al.* (2004) in ostriches, Oznurlu *et al.* (2012) in pigeons and Shalini (2015) in quails. All other cell types were non-reactive (Table 3), aligning with Dönmez and Sur (2008), who found negative results in erythrocytes and positive reactions in heterophils of Rock Partridges, as well as Salakij *et al.* (2004), who reported negative results in heterophils of Lesser and Greater Adjutants. These findings contrast with Salakij *et al.* (2003), who observed strong ANAE positivity in heterophils of Painted Storks, highlighting interspecies variability. According to Pinkus *et al.* (1979), ANAE activity is mainly associated with T lymphocytes and is a crucial marker in diagnosing lymphoproliferative disorders such as leukemia and lymphomas, helping differentiate T and B cell populations and providing insights into disease progression and the rapeutic responses.

CONCLUSION

The cytochemical and cytoenzymatic studies conducted on the blood cells of White Pekin ducks revealed distinct staining patterns, providing valuable insights into the biochemical properties of these cells. Acid ferrocyanide positivity was seen only on abnormal erythrocytes, while basophils strongly reacted to acid mucopolysaccharides with intense violet granules. The PAS stain highlighted intense glycogen positivity in heterophils and moderate positivity in eosinophils, erythrocytes and thrombocytes. Sudan Black B staining revealed lipid-rich granules in eosinophils and heterophils, with no reaction in other blood cells. Cytoenzymatic analysis demonstrated varied enzyme activities, with strong acid phosphatase activity in basophils, alkaline phosphatase activity in eosinophils and peroxidase positivity in thrombocytes. Additionally, weak β-glucuronidase and strong α-naphthol acetate esterase activities were observed in eosinophils and heterophils, providing a detailed enzymatic profile that contributes to the understanding of these cells' functional roles in avian species.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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