*Indian Journal of Poultry Science (2024) 59(2): 205–210, Research Article*
https://doi.org./10.56093/ijps.v59i2.165179

# Comparative study on gelatinase expression in pigeon serum and breast muscles

T.C. BALAMURUGAN*, R. PRAKASH KRUPAKARAN, G. ANANDHI, C. SENTHAMIL PANDIAN, P. PERUMAL AND A. ELANGO

*Department of Veterinary Physiology and Biochemistry,*
*TANUVAS -Veterinary College and Research Institute, Salem, Tamil Nadu 636 112*

(Received on January 29, 2024; accepted for publication on May 27, 2024)

## ABSTRACT

*Balamurugan, T.C., Prakash Krupakaran, R., Anandhi, G., Senthamil Pandian, C., Perumal, P. and Elango, A. 2024. Comparative study on gelatinase expression in pigeon serum and breast muscles. Indian Journal of Poultry Science, 59(2): 205-210.*

The present study was conducted to detect the gelatinase activity by assessing matrix metalloproteinases (MMP-2 and MMP-9) in serum and tissues with use of gelatin zymography in pigeon (*Columba livia*). Experimental birds were divided into two groups, first group (n-12) was subjected to blood collection. Another group (n-12) of twelve birds was subjected for tissue collection. Tissue samples were collected properly, homogenated, filtered and analysed using gelatin zymography. All the tissue samples exhibited proteolytic activity, as they completely degraded the gelatin. Gelatin zymogram clearly showed the presence of both the active 67 kDa form and the latent 72 kDa form of MMP-2 in the tissue samples. MMP-9 expression was detected only in the breast muscle, with bands corresponding to the 82 kDa active form and the 220 kDa latent form. MMP-2 intensity was 3-5 times higher in the tissue samples compared to serum samples. It was concluded that both the active 67 kDa MMP-2 and latent 72 kDa forms of MMP-2 were present in pigeon serum and tissue samples, with higher expression levels in tissue, possibly due to the activity of intracellular MMP-2 in skeletal muscle fibers during normal homeostasis and physical activity. Although the MMP-9 gene was present in the blood, it was not expressed, while a weak active form of MMP-9 was observed in the breast muscle. The increase in uric acid levels was associated with MMP-2 expression. Additionally, serum biochemistry and hematology analysis provided baseline data for future pigeon studies.

**Keywords**: *Columba livia*, Matrix Metalloproteinases, MMP-2, MMP-9, Breast muscle

## INTRODUCTION

Skeletal muscle is a highly adaptable tissue that undergoes physiological or pathological remodeling in response to various stimuli, such as exercise, immobilization, injury, disease, or aging. The extracellular matrix (ECM), a non-cellular component found in all tissues and organs, provides a crucial structural framework for cells and initiates key biochemical and biomechanical signals required for tissue morphogenesis, differentiation and homeostasis (Frantz *et al.*, 2010). This remodeling process involves subtle or significant alterations in the structure and composition of skeletal muscle, which includes the degradation of the ECM by MMPs. Numerous studies have demonstrated that the MMPs are primary enzymes responsible for regulating ECM turnover and maintaining homeostasis (Kessenbrock *et al.*, 2010). MMPs are a family of enzymes that selectively cleave specific ECM components under both normal and pathological conditions (Wang *et al.*, 2009).

MMPs are zinc-dependent proteases (endopeptidases) that play a key role in the degradation of the ECM and tissue regeneration. The MMP family is generally classified into several including collagenases, stromelysins, gelatinases, and membrane-type metalloproteinases (Lattanzi *et al.*, 2010). In skeletal muscle, MMPs are crucial for maintaining homeostasis and the functional integrity of the tissue by degrading the ECM and facilitating the migration, differentiation, and regeneration of muscle cells. MMPs are typically produced in an inactive pro-form and must be converted in to their active forms (Rosenberg, 2002). Their activity is regulated by endogenous inhibitors known as tissue inhibitors of MMPs (TIMP) (Ulbrich *et al.*, 2021) and by an endogenous activator, extracellular matrix metalloproteinase inducer (Ulbrich *et al.*, 2021).

MMP-2 is a proteolytic enzyme involved in the motility, differentiation, and regeneration of skeletal muscle fibers by processing extracellular substrates (Olsen *et al.*, 2015). Dysregulated expression or activity of MMPs in various pathologies is often linked to the body's response to injury, the promotion of disease progression, and extensive tissue damage. In skeletal muscle, the incomplete understanding of MMPs and TIMPs regulation and function highlights the need for further research, which may have therapeutic potential (Alameddine, 2012). Therefore, the present study aimed to investigate the presence of MMP-2 and MMP-9 gelatinases in serum and breast muscle of pigeons under normal physiological conditions, as well as their relationship with hematological and biochemical parameters.

*Corresponding author Email: tcbalamurugan@gmail.com

## MATERIALS AND METHODS

The study was carried out at the Department of Veterinary Physiology and Biochemistry, TANUVAS-Veterinary College and Research Institute in Orathanadu, Tamil Nadu, India. The institute is situated 30 meters above sea level, at a latitude of 10.6°N and a longitude of 79.3°W.

*Experimental birds*

Experimental birds were sourced from an organized farm and divided into two groups. Each group (n-12) consists of twelve birds with similar body weight and age was subjected to blood collection. Another group (n-12) of twelve birds with similar body weight and age was subjected for tissue collection.

*Collection of tissue samples and serum*

In group I (n=12), blood samples were drawn from the brachial vein early in the morning before feeding, using a sterile 5-ml disposable syringe with a 22-gauge needle into a heparinized vacutainer. Blood for haematological analysis was collected in EDTA tubes (2 mg/mL) while samples for biochemical analysis were collected without anticoagulant. In group II (n=12), total 12 fresh pigeon tissue samples, each with a maximum diameter of 0.50 cm, were obtained during biopsy, surgery or slaughter. Tissue samples were taken from normal breast tissues, excluding tumors and the surrounding areas. The blood samples were promptly transported to the laboratory, centrifuged at 3000 rpm for 15 min, and the protein content of the serum was analyzed using a spectrophotometer (Hitachi, Model no UH5300., Japan). Protein levels were measured using the Lowry method (Lowry *et al.*, 1951), with a standard curve created from varying concentrations of bovine serum albumin (BSA). Serum samples were preserved at –80 °C in deep freezer for further analysis.

*Hematology and Serum Biochemistry*

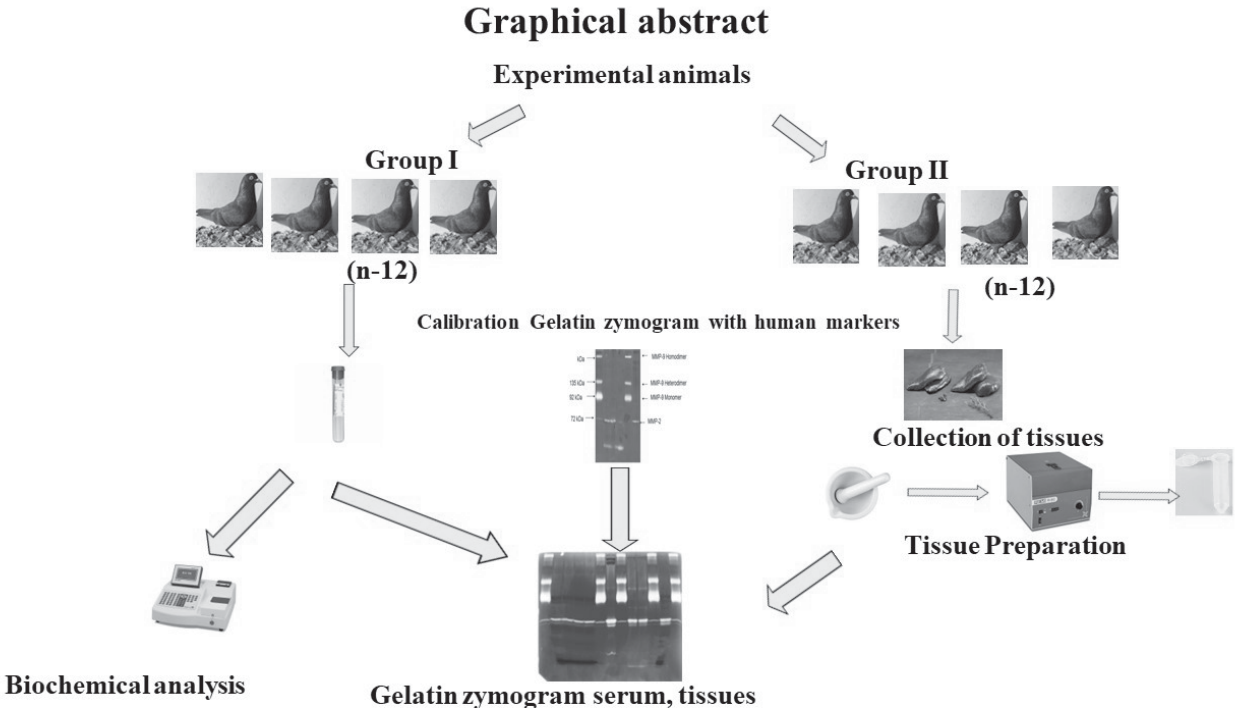
In the group I, collected serum samples were subjected to biochemical analysis for estimation of Total protein, Albumin, Globulin, Urea, Creatinine, Cholesterol, Triglycerides, Glucose and Uric acid and their concentration was measured in UV-VIS double beam spectrophotometer (Systronics, Model 2202, India).

*Sample preparation*

A 0.50 g sample of breast tissue was weighed on a clean aluminum foil, and two milliliters of lamellar buffer were was added. The tissue was then aseptically ground using a mortar and centrifuged at 6000 rpm for 10 min at 4°C. The supernatant was collected and stored for further analysis. After homogenization, MMPs were extracted from the breast tissue following the method described by Woessner, (1995) and Gilbert *et al.*, (1997). Gelatin zymography was performed to detect both the precursor and active forms of MMP-2 and -9 in the fresh and frozen tissue samples.

*Gelatin zymography*

The samples were underwent modified SDS-PAGE, based on Laemmli's method (1970) as adapted by Heussen and Dowdle (1980), which involved adding a co-polymerizing substrate of gelatin (0.30 %, final concentration of 0.15 %) to the 0.80 % resolving gel. The samples were electrophoresed at 100 V for 20 min. Renaturation was performed using 2.50 % Triton X-100 for 3 h on a mechanical shaker with gentle agitation. The gel was then incubated in a solution containing 0.15 M NaCl, 10 mM $CaCl_2$, and 50 mM Tris (pH 7.5), for 18 h at 37°C. Subsequently, the gel was stained with 0.25%



Coomassie brilliant blue for 2 h, destained for 1 h, and finally washed with distilled water.

*Analyzing the results of gelatin zymogram*

Human capillary blood gelatinase was used as the standard marker for evaluating the zymogram bands, following the protocol established by Makowski and Ramsby (1996). Blood was collected by pricking a fingertip, and the precise weight was measured in a tarred polypropylene tube. The samples were then mixed thoroughly with a 20× volume of Laemmli buffer. These samples remained stable for 3 months at -20°C.

## RESULTS AND DISCUSSION

*Hematological profiles of pigeon*

The total erythrocyte count (TEC), packed cell volume (PCV), total leucocyte count (TLC), and haemoglobin (Hb) of the pigeons were shown in Table 1. The obtained Hb value was 14.2 and PCV value was 36.80 % and these values were within the reference range. The results of the present study on TEC, TLC, PCV and Hb were consistent with report of Shah *et al*. (2012), Gayathri *et al*. (2004), Al-Gamal (2014) and Khan *et al*. (2011). Low environmental temperatures, which are associated with a high metabolic rate, increased oxygen demand by the body, and low partial pressure of oxygen in the blood (hypoxaemia), are known to stimulate erythropoiesis (Zivot *et al*., 2018).

**Table 1:** Hematological profile of pigeon (mean±SE)

| Parameter | Mean±SD | Range |
|---|---|---|
| TRBC ($\times 10^6$/µL) | 3.4±0.8 | 2.3–4.6 |
| TWBC ($\times 10^3$/µL) | 11.2±2 | 10.8–16.4 |
| PCV (%) | 36.8±1.6 | 28.6–46.2 |
| Hb (g/dL) | 14.2±1.8 | 10.2 -16.4 |
| MCV (fL) | 152.6±24 | 124.6 -186.8 |
| MCH (pg) | 44.8±2 | 32.6–56.4 |
| MCHC (g/dL) | 31.2±3 | 30.2–40.2 |
| Differential leukocyte count | | |
| Heterophils (%) | 52.4±8.2 | 42–58 |
| Lymphocytes (%) | 32.6±5.4 | 24–47 |
| Eosinophils (%) | 7.0±3.2 | 4–12 |
| Basophils (%) | 3.4±2.6 | 0-9 |
| Monocytes (%) | 4.6±2.4 | 0-13 |

Values are expressed as mean±standard deviation.
µL: microliter, g/dL: grams per decilitre, fL: femtoliters, pg: picogram

*Serum biochemistry*

The serum biochemistry of pigeons was presented in Table No. 2. Total protein content, albumin, globulin and A/G ratio were within the reference range (Al-Gamal, 2014, Khan *et al*., 2011). Awosanya *et al*. (1999) reported that blood protein levels are influenced by the quality and quantity of dietary protein. In adult pigeons, total cholesterol and triglyceride levels fall within the reference range, consistent with findings from Shah *et al*. (2004) and Gayathri *et al.* (2013).

**Table 2:** Serum biochemical profile of pigeon (mean±SE)

| Parameter | Mean±SD | Range |
|---|---|---|
| Total protein (g/dL) | 7.8±0.8 | 7.2 -10.4 |
| Albumin (g/dL) | 2.6±0.16 | 2.4- 2.8 |
| Globulin (g/dL) | 2.8±0.24 | 2.6-3.1 |
| Albumin/globulin ratio | 0.8±0.06 | 0.6-0.8 |
| Glucose (mg/dL) | 191.4±6.4 | 180.4 -210.6 |
| Cholesterol (mg/dL) | 142.4±5.8 | 126 -168 |
| Triglycerides (mg/dL) | 104.6±2.2 | 98 -116 |
| Urea (mg/dL) | 18.2 2.6 | 14.6-20.2 |
| Creatinine (mg/dL) | 0.62 | 0.46-0.78 |
| Uric acid (mg/dL) | 4.2±0.8 | 3.6-5.2 |
| AST (IU/L) | 104.2±4.8 | 54.2-108.6 |
| ALT (IU/L) | 32.6±2.6 | 24.8-44.6 |
| ALP (IU/L) | 76.8±4.4 | 74.8-136.4 |

Values are expressed as mean±standard deviation.
g/dL: grams per decilitre, mg/dL: milli grams per decilitre, IU/L: international units/litre. AST: Aspartate amino transferase, ALT: Alanine amino transferase and ALP: Alkaline phosphatase

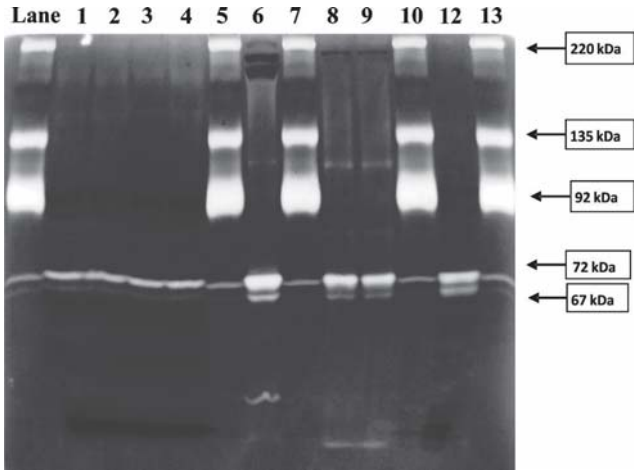
Uric acid is the main product of nitrogen metabolism in birds. Uric acid is the most important nitrogenous residue of birds (Harr, 2002). Pigeons in this study have uric acid levels slightly higher than the reference range (Zhang *et al*., 2022, Peng *et al*., 2023). Zhang *et al*. (2022) reported that the uric acid concentration in pigeon fed with complete formulated diet was 276.36 mol/L (4.65 mg/dL) and Peng *et al*. (2023) reported lower concentration of serum uric acid in breeding pigeons during winter (49-57 U mol/ L). These higher values were probably due to the high protein content of the feed. The elevated uric acid levels observed in this study suggests that the expression of MMP-2 was higher in these birds. Creatinine levels are normally low in birds (Harr, 2002). Creatinine is an important indicator of protein metabolism and kidney integrity. Creatinine, produced by the breakdown of muscle phosphocreatinine, increases with high activity, such as flying, and is influenced by diet. ALP, AST and ALT values were found to evaluate the liver function of pigeons and were within the reference range. These results were consistent with those of Al-Gamal. (2014), Khan *et al*. (2011), Shah *et al*. (2004) and Gayathri *et al.* (2013). While there are general guidelines for interpreting biochemical values in birds with unknown reference ranges, species specific ranges are preferred. Interpreting plasma biochemistry values is typically more complex than assessing a single

parameter. Usually, the entire biochemical profile is utilized to evaluate the health status of the bird (Schulz *et al*., 2000).

*Gelatin zymography*

The gelatin zymogram demonstrates the presence of gelatinases in all groups, as illustrated in Fig. 1. All serum samples underwent gelatin zymography, revealed that samples from lanes 2-5 were proteolytically active, as they completely degraded the gelatin. Major bands corresponding to 72 kDa, and 67 kDa were observed, indicating the latent and active forms of MMP-2. The presence of MMP-2 was clearly evident in the serum samples, with the intensity of latent form being 3-5 times greater than that of the active form. Additionally, two more lytic bands were identified at 220 kDa, and 135 kDa, representing the proforms of MMP-9. However, neither the latent nor active forms of 92 kDa and 87 kDa were detected in any of the serum samples.

**Gelatin zymography of Pigeon Serum and Breast muscle homogenoate samples**




Lane 1, 6, 8, 11 and 13: Human capillary blood MMP marker
Lane 2 to 5- Pigeon serum sample
Lane 7, 9, 10 and 12: Pigeon breast muscle sample

**Fig.1:** Comparative Gelatin Zymogram of pigeon serum and breast muscle samples

In all the breast muscle samples (Lanes 7, 9, 10, 12), two prominent bands were observed at 72 kDa, and 67 kDa, representing the latent and active forms of MMP-2, respectively. The intensity of latent form of MMP-2 was 3-5 times greater than that of the active form. Additionally, two more lytic bands were detected at 220 kDa, and 135 kDa, indicating the proforms of MMP-9. The expression of MMP-9 was evident only in the breast muscle homogenate, with bands observed at 82 kDa, and the latent form 220 kDa visible in lanes 7, 9 and 10 and 12. However, the latent form of MMP-9 at 92 kDa was not detected in any of the tissue samples. In contrast, the active form of MMP-9 (87 kDa) was visible in lanes 9, and 10. Our findings align with those of Packialakshmi *et al*. (2014), Prakash Krupakaran *et al*. (2016) and Balamurugan and Prakash Krupakaran (2020).

Consistent with the findings of the present study, Balamurugan and Prakash Krupakaran (2020) from same laboratory reported that only the latent and active forms of MMP-2 at 72 kDa and 67 kDa MMP-2 were detected in the serum of Vanaraja fowl, with no bands indicating MMP-9 in the serum of either sex. Similarly, Prakash Krupakaran *et al*. (2016) found only two major bands corresponding to MMP-2 at 72 kDa, and 62 kDa in both male and female groups of commercial chicken. They also noticed that the intensity of latent form MMP-2 (72 kDa) was higher than that of active form (62 kDa).

In a study by Packialakshmi *et al*. (2014), five bands corresponding to 70, 64, 58, 50, and 42 kDa were detected in chicken bile using gelatin zymography. Bands at 64, 50, and 42 kDa were identified as MMP-2 through trypsin in-gel digestion and matrix-assisted laser desorption time-off light mass spectrometry with peptide mass fingerprinting. They also confirmed that the MMP protein bands in avian bile were associated with the 72-kDa type IV collagenase, commonly known as MMP-2, or gelatinase A. These authors concluded that bile MMP may play a role in the digestion of collagens and other extracellular matrix proteins in feed of birds. Avian bile is rich in MMPs, which are enzymes that degrade extracellular matrix proteins including collagens and proteoglycans.

*Expression of MMP-2 in breast muscle*

In this study, the levels of MMP-2 in breast muscle samples were found to be 3-5 times higher than those in serum samples. Additionally, MMP-2 expression exceeded that of MMP-9. This could be attributed to MMP-2's role as a key component of the intracellular proteolytic system in normal skeletal muscle, particularly in fast-twitch type II fibers. Similar findings were reported by Olsen *et al*. (2015), who observed MMP-2 in skeletal muscle fibers. It has been suggested that MMP-2 functions as a proteolytic enzyme involved in the motility, differentiation and regeneration of skeletal muscle fibers by processing extracellular substrates (Olsen *et al*., 2015). MMP-2 expression is also influenced by the physical activity of muscle fibers. Supporting this, Carmeli *et al*. (2005) found that high-intensity exercise is necessary to enhance MMP-2 expression in skeletal muscle, particularly in muscles with high proportion of fast fibers. Moreover, high-intensity exercise led to an increase in both MMP-2 mRNA and protein levels in muscles with a high percentage of type II fast-twitch fibers such as the gastrocnemius and quadriceps superficialis (Carmeli *et al*., 2005). In conclusion, intracellular MMP-2 in skeletal muscle fibers is active during normal homeostasis and is influenced by physical activity.

Furthermore, MMP-2 has been identified as a crucial for effective muscle regeneration, with both

MMP-2 and MMP-14 playing roles in muscle fiber maturation by regulating angiogenesis (Miyazaki *et al.*, 2011). Intracellular MMP-2 (Olsen *et al.*, 2015) is also linked to muscle fiber atrophy under various physiological and pathological conditions (Carmeli *et al.*, 2004). This function is likely due to MMP-2's ability to hydrolyze sarcomeric proteins including troponin I, myosin light chain-1, titin, and á-actinin (De Coux *et al.*, 2015).

*Expression of MMP-9 in breast muscle*

In this study, major bands were observed at 220 kDa, and 135 kDa in breast muscle samples, corresponding to the proforms of MMP-9. However, the 92 kDa latent form of MMP-9 was not observed in the tissue sample (lane 12), while the 82 kDa MMP-9 was present in tissue samples (lanes 7, 9, and 10). The expression of MMP-9 was lower than that of MMP-2 in these tissue samples, likely because MMP-9 is was not directly related to muscle function or intense exercise. Consistent with our findings, Carmeli *et al*. (2005) reported that exercise did not affect MMP-9 levels in skeletal muscle. However, elevated levels of active MMP-9 protein have been associated with skeletal muscle hypertrophy, and regulating MMP-9 levels may hold therapeutic potential for muscle diseases, including Duchenne muscular dystrophy (Dahiya *et al.*, 2011).

This study suggests that gelatinase activity expression may vary between individual bird. In skeletal muscle, MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, and MMP-14 facilitate myoblast migration (Nishimura *et al.*, 2008) while MMP-7, MMP-9, and MMP-14 are also involved in cell fusion (Morgan *et al.*, 2010). Therefore, a balance between MMPs and their physiological inhibitors, the tissue inhibitors of metalloproteinases (TIMPs), is crucial for maintaining tissue homeostasis. The upregulation of MMPs and/or TIMPs has been linked to vascular growth and enlargement in endurance-trained individuals, as well as to inflammation and muscle regeneration in injured or diseased muscles (Alameddine, 2017). Lastly, MMP-2 and MMP-9 may play a role in muscular dystrophies and muscle atrophy.

## CONCLUSION

It was concluded that both the 67 kDa active form and the 72 kDa latent form of MMP-2 were present in pigeon serum and tissue samples, higher MMP-2 expression in tissue samples than in serum. This could be attributed to the fact that intracellular MMP-2 is active in skeletal muscle fibers during normal homeostasis and is affected by physical activity. While the MMP-9 gene was detected in the blood, it was not expressed in serum; however, a faint active band of MMP-9 was observed in the breast muscle. This suggests that MMPs, which are involved in ECM remodeling, play a crucial role in tissue growth and morphogenesis. Additionally, increased uric acid levels were linked MMP-2 expression. Serum biochemistry and hematology analysis also provided baseline data for further studies on pigeons.

## REFERENCES


Akerejola, O.O., Umuna N.N. and Denis, S.M. 1980. Serum biochemical levels of cattle in Northern Nigeria. *Nigerian Veterinary Journal*, **9**: 26-31.

Alameddine, H. 2017. The matrix metalloproteinase and tissue inhibitors of metalloproteinase balance in physiological and pathological remodeling of skeletal muscles. In: Chakraborti, S. and Dhalla, N. (eds), *Proteases in Physiology and Pathology*, Springer, Singapore. pp. 49-76. https://doi.org/10.1007/978-981-10-2513-63

Alameddine. H.S. 2012. Matrix metalloproteinases in skeletal muscles: friends or foes? *Neurobiology of Disease*, **48**(3): 508-518.

Al-Gamal, M.A. 2014. Blood biochemical profile of young and adult racing pigeons (*Columba livia domestica*) in Egypt. *Middle East Journal of Applied Sciences*, **4**(3): 528-538.

Awosanya, B., Joseph, J.K., Apata, D.F. and Ayoola, M.A. 1999. Performance, blood chemistry and carcass quality attributesm of rabbits fed raw and processed pueraria seed meal. *Tropical Animal Science Journal*, **2**: 89–96.

Balamurugan, T.C and Prakash Krupakaran, R. 2020. Detection of gelatinases in Vanaraja poultry chicken. *Journal of Entomology and Zoology Studies*, **8**(1): 1025-1027.

Carmeli, E., Moas, M., Lennon, S. and Powers, S.K. 2005. High intensity exercise increases expression of matrix metalloproteinases in fast skeletal muscle fibres. *Experimental Physiology*, **90**(4): 613-619.

Carmeli, E., Moas, M., Reznick, A.Z. and Coleman, R. 2004. Matrix metalloproteinases and skeletal muscle: A brief review. *Muscle and Nerve*, **29**(2): 191-197.

Dahiya, S., Bhatnagar, S., Hindi, S.M., Jiang, C., Paul, P.K., Kuang, S. and Kumar, A. 2011. Elevated levels of active-matrix metalloproteinase-9 cause hypertrophy in skeletal muscle of normal and dystrophin-deficient mdx mice. *Human Molecular Genetics*, **20**(22): 4345-4359.

De Coux, A., Lindsey, M.L., Villarreal, F., Garcia, R.A. and Schulz, R. 2014. Myocardial matrix metalloproteinase-2: Inside out and upside down. *Journal of Molecular and Cellular Cardiology*, **77**: 64-72.

Frantz, C., Stewart, K.M. and Weaver, V.M. 2010. The extracellular matrix at a glance. *Journal of Cell Science*, **123**(24): 4195-4200.

Gayathri, K.L., Shenoy, K.B. and Hegde, S.N. 2004. Blood profile of pigeons (*Columba livia*) during growth and breeding. *Comparative Biochemistry and Physiology*, **138**: 187-192.

Gilbert, S.J., Wotton, P.R. and Tarlton, J.F. 1997. Increase expression of promatrix metalloproteinase-9 and neutrophil elastase in canine dilated cardiomyopathy. *Cardiovascular Research*, **34**: 377-383.

Harr, K.E. 2002. Clinical chemistry of companion avian species: A Review. *Veterinary Clinical Pathology*, **31**: 140-151.

Heussen, C. and Dowdle, E.B. 1980. Electrophoretic analysis of plasminogen activators in polyacrylamide gels containing sodium dodecyl sulfate and copolymerized substrates. *Analytical Biochemistry*, **102**(1): 196–202.

Kessenbrock, K., Plaks, V. and Werb, Z. 2010. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell*, **141**(1): 52-67.

Khan, B.Y.A., Ali, F., Saeed, M.Q. and Iqbal F.A.M. 2011. A study on serum biochemistry and haematological profiling of Blue Rock Pigeon (*Columba livia*) in Multan (Punjab, Pakistan) *Pakistan Journal of Zoology,* **43**(5): 1012-1014.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* **227**: 680–685.

Lattanzi, K., Plaks, V. and Werb, Z. 2010. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell,* **141**: 52–67.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry,* **193**(1): 265–275.

Makowski, G.S. and Ramsby, M.L. 1996. Calibrating gelatin zymograms with human gelatinase standards. *Analytical Biochemistry,* **236**(2): 353–356.

Miyazaki, D., Nakamura, A., Fukushima, K., Yoshida, K., Takeda, S. and Ikeda, S.I. 2011. Matrix metalloproteinase-2 ablation in dystrophin- deficient mdx muscles reduces angiogenesis resulting in impaired growth of regenerated muscle fibers. *Human Molecular Genetics,* **9**(20): 1787-1799.

Morgan, J., Rouche, A., Bausero, P., Houssaýni, A., Gross, J., Fiszman, M.Y. and Alameddine, H.S. 2010. MMP-9 over expression improves myogenic cell migration and engraftment. *Muscle and Nerve,* **42**(4): 584-595.

Nishimura, T., Nakamura, K., Kishioka, Y., Kato-Mori, Y., Wakamatsu, J. and Hattori, A. 2008. Inhibition of matrix metallo proteinases suppresses the migration of skeletal muscle cells. *Journal of Muscle Research and Cell Motility,* **29**(1): 37-44.

Olsen, H.E., Solli, A.I., Hafstad, A., Winberg J.O. and Uhlin-H. L. 2015. Intracellular MMP-2 activity in skeletal muscle is associated with type II fibers. *Journal of Cellular Physiology,* **230**(1): 160-169.

Packialakshmi, B., Liyanage, R., Rasaputra, K.S., Lay, J.O. and Rath, N.C. 2014. Isolation and characterization of chicken bile matrix metalloproteinase. *Poultry Science,* **93**: 1495-1502.

Peng, J., Huang, W., Liang, Y., Zhang, W., Zhang, Y., Yang, M., Zheng, S., Lv, Y., Gou, Z., Cheng, C., Gao, H., Wang, W., Peng, J. and Huang, Y. 2023. Optimal dietary energy and protein levels for breeding pigeons in the winter "2 + 3" lactation pattern. *Poultry Science*, **102**(10):102964. https://doi.org/ 10.1016/j.psj.2023.102964.

Prakash Krupakaran, R., Balamurugan, T.C., Lakshmi, R.D., Sheeba, A. and Perumal, P. 2016. Detection of Matrix Metalloproteinase -2 in layer chicken. *Journal of Cell and Tissue Research,* **16**(2): 5575-5578.

Rosenberg, G. 2002. Matrix metalloproteinases in neuroinflammation. *Glia,* **39**: 279–291.

Schulz, J.H., Bermudez, A.J., Tomlinson, J.L., Firman, J.D. and He, Z. 2000. Blood plasma chemistries from wild mourning doves held in captivity. *Journal of Wildlife Diseases,* **36**(3): 541–545.

Shah, S.N., Ganayi, B.A., Shahnawaz, M., Bhat, S.A., Shergojry, S.A., Bantigegnna, D. and Molla, A. 2012. Seasonal influence on some blood and biochemical parameters of pigeons (*Columba livia*) in Rajasthan, India. *Comparative Clinical Pathology,* **22**: 581-583.

Ulbrich, P., Khoshneviszadeh, M., Jandke, S., Schreiber, S. and Dityatev, A. 2021. Interplay between perivascular and perineuronal extracellular matrix remodeling in neurological and psychiatric diseases. *European Journal of Neuroscience,* **53**: 3811–3830.

Wang, W., Pan, H.Y., Murray, K., Jefferson, B.S. and Li, Y. 2009. Matrix metalloproteinase-1 promotes muscle cell migration and differentiation. *American Journal of Pathology,* **174**: 154-174.

Woessner, F.J. 1995. Quantification of matrix metalloproteinases in tissue samples. *Methods in Enzymology,* **248**: 510-528.

Zivot, A., Lipton, J.M., Narla, A. and Blanc, L. 2018. Erythropoiesis: insights into pathophysiology and treatments in 2017. *Molecular Medicine,* **24**(1): 11.

Zhang, R., Ma, H., Han, P., Li, Y., Sun, Y., Yuan, J., Wang, Y., Ni, A., Zong, Y., Bian, S., Zhao, J. and Chen J. 2022. Effects of Feed Systems on Growth Performance, Carcass Characteristics, Organ Index, and Serum Biochemical Parameters of Pigeon. *Poultry Science,* **101:** 102224.