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Detection of gelatinases in vanaraja poultry chicken

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Abstract

A study was undertaken to detect the presence of Matrix Metallo Proteinases (MMP) in the serum Vanaraja fowl, a native poultry breed developed by ICAR in Hyderabad. Before feeding in the early morning, serum samples were collected from three male and eight female healthy birds in a heparinized vacutainer from an organized commercial farm. Birds were properly vaccinated and deworming was done. All the serum samples were subjected to gelatin zymography (0.3%). It was revealed that only two major bands were observed at 72, 62 kDa of MMP-2 in both the groups. The intensity of latent form MMP-2 was higher than that of active form of MMP-2. Above the level of MMP-2, no band was observed corresponding to the level of MMP-9. It was further observed that there was no difference between the expression patterns of MMP-2 in both the sexes. It was concluded that the existence of serum MMP was confirmed by the presence of both the forms of active and latent form of MMP-2 and it was further inferred that MMP-9 could not be expressed in the serum of both the sexes. However the MMP 9 gene was present and it was not expressed in the serum by gelatin zymography.

Keywords: Serum, matrix metallo proteinase, gelatin zymography, vanaraja poultry chicken

Introduction

The total Poultry in the country is 851.81 Million in 2019, increased by 16.8% over previous Census. The total Backyard Poultry in the country is 317.07 Million in 2019, increased by 45.8% over previous Census ^[1]. Vanaraja is a dual-purpose chicken variety developed by the ICAR- India. Vanaraja is aimed at rural communities where it can be reared in backyard on natural, scavenged food with minimal supplementation. It produces eggs and meat based on rearing and feeding practices. Important features of this breed are multi-color feather pattern, immunity to disease, perform with less nutrition, grow faster and produce more eggs, produce brown eggs like local hens.

Extracellular matrix (ECM) macromolecules are important for creating the cellular environments required during development and morphogenesis. Enzymes responsible for collagen and other protein degradation in extracellular matrix (ECM) are matrix metalloproteinases (MMPs). Matrix metallo peptidases (MMPs), also known as matrix metallo proteinases or matrixins, are metalloproteinases that are calcium-dependent zinc-containing endopeptidases. Gelatinase A (MMP-2) and gelatinase B (MMP-9) belong to this group. They readily digest the denatured collagens, gelatins.

MMP system is important for the normal functioning of the chicken ovary. Avian bile is rich in MMP-2, enzyme implicated in the degradation of extracellular matrices such as collagens and proteoglycans and bile MMP may be evolutionarily associated with the digestion of ECM proteins that are generally resistant to digestion by conventional proteases such as trypsin, chymotrypsin, or pepsin ^[2]. In chicken, the level of MMP-2 was considerable, which helps in the timely degradation of ECM molecules from the tissue and thus facilitates morphogenesis. Till date, there is limited number of studies in MMP of avian species and their role in egg production and semen production. Thus, the objective of our study was to identify the existence of MMP in serum of chicken.

Materials and Methods

This study was conducted at the Department of Veterinary Physiology and Biochemistry, TANUVAS - Veterinary College and Research Institute, Orathanadu, Tamil Nadu, India.

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Collection and evaluation of serum

Three male and ten female healthy layer chicken vanaraja poultry chicken were selected from a commercial layer Chicken farm. Blood samples from chicken were collected in a heparinised vacutainer during early morning before feeding and they were immediately transported to the laboratory. The birds were properly vaccinated and deworming was done. The blood samples were centrifuged at 3000 rpm for 15 min. and the separated serum was analyzed for protein content by photometric estimation of blue color by using spectrophotometer [3]. The standard curve was built by using various concentrations of Bovine Serum Albumin (BSA) as standard. The serum samples were stored at -20 °C for further analysis.

Gelatin zymography

The serum samples were analysed by the method of modified SDS-PAGE (modification of Laemmli's method, 1970) [4] carried out by Heussen and Dowdle (1980) [5] by the addition of co-polymerizing substrate of gelatin (0.3%) (final concentration was 0.15% to the resolving gel (8%). The samples were electrophoresed at 100V for 20 min. Renaturation was carried out with 2.5% Triton X-100 for 3 hrs on a mechanical shaker with a mild agitation. Then developing was done by incubating the gel in 10 mM CaCl₂, 0.15 M NaCl and 50 mM Tris pH 7.5. for 18 hrs at 37 °C. The gel was stained with 0.25% coomassie brilliant blue for 2 hrs, followed by destaining with destaining solution for 1 hr and finally the gel was washed with distilled water.

Analyzing the results of gelatin zymogram

Human capillary blood gelatinase was used as the standard marker for comparing the zymogram bands based on the procedure of Makowski and Ramsby (1996) [6]. The blood was collected from a capillary and weighed in a tarred polypropylene tube using analytical balance by using a fingerstick puncture. Samples were added with 20X volume of Laemmli buffer and thoroughly mixed. Then the aliquots were stable for 3 months at -20 °C.

Results and Discussion

On gelatin zymography, it was confirmed that MMP-2, was present in the serum samples of Vanaraja male and female birds, and the results were depicted in Fig -1. It was clearly observed that the latent form of 72 kDa MMP-2 and active form of 62 kDa MMP-2 in the serum samples of both the sexes. However, on comparing the intensity of the above said both bands, the intensity of active form of 62 kDa MMP-2 was feeble and it was lesser than 72 kDa of latent form of MMP-2. Further, the expression of latent form 220, 135 kDa of MMP-9 bands and active form of 92 kDa band were absent in both the groups. Thus, MMP-9 was absent in the serum of female and male layer chicken.

Our results were in accordance with the results of various authors [2, 7, 8]. In our laboratory, the existence of MMP-2 in giriraja fowl and chicken from commercial broiler was confirmed in the serum by gelatin zymography. Similarly, Packialakshmi *et al.*, 2014 [2] found in chicken bile, five bands at 70, 64, 58, 50, and 42 kDa were detected in gelatin zymography. The bands corresponding to 64, 50, and 42 kDa were identified as MMP2 using trypsin in-gel digestion and matrix-assisted laser desorption time-off light mass spectrometry and peptide mass fingerprinting. Further, it was confirmed that the MMP protein bands in avian bile belonged to 72 kDa type IV collagenase, also known as MMP-2, or

gelatinase A. They concluded that the bile MMP may be associated with the digestion of collagens and other extracellular matrix proteins in avian diets. Avian bile is rich in matrix metalloproteinases (MMP), the enzymes that cleave extracellular matrix proteins such as collagens and proteoglycans [2].

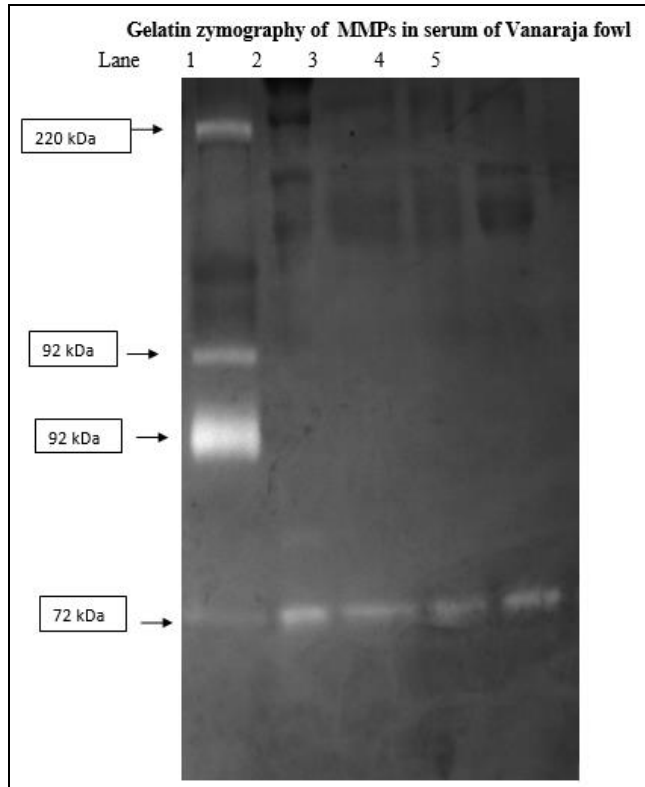
In another study by Aimes and Quigley, 1995 [9], 72-kDa gelatinase/type IV collagenase (MMP-2) can cleave type IV collagen as well as degrade denatured collagens. They also reported that both human and chicken MMP-2, free of tissue inhibitors of metalloproteinases (TIMPs) were capable of cleaving soluble, triple helical type I collagen generating the ¾- and ¼-length collagen fragments characteristic of vertebrate interstitial collagenases. Chicken MMP-2 has collagenase activity because it can denature and cleave triple helical collagens to produce characteristic three-fourths and one-fourth fragments. Hence, it is capable of denaturing and digesting fibrillar collagens. In conclusion, they showed that chicken bile constitutively secretes type IV collagenase (MMP2), which can then be modulated by their dietary constituents, and these enzymes possibly help in the digestion of ECM proteins in their diets [9].

Duong *et al.* (2003) [10] explained that MMP-2 Plays an essential role in producing epithelial-mesenchymal transformations in the avian embryo. It was expressed as neural crest cells that detach from the neural epithelium, and also expressed in the sclerotome and in the dermis at the time that the EMT is initiated. These patterns suggested that MMP-2 plays a role in cell motility during the EMT and during later morphogenesis.

Ozigit *et al.* 2005 [11] found that MMP-2 and MMP-9 was more intense and extensive in ascitic broilers than in the controls by Immunostaining. However, a decrease in intensity was seen with increasing age both in normal and ascitic chickens. The presence of MMP-9 enzyme was negatively correlated with the presence of TIMP-1 enzyme. It is suggested that MMP-2 and MMP-9 enzymes might play a role in the permeability increase of vessel walls by the destruction of the basement membranes in the salt-induced experimental ascites syndrome in broiler chickens [11].

In another study, presence of both forms of MMP-2, latent and active, in the oviduct of growing chickens. Expression of MMP-2 at the protein level increased during maturation while the activity of this metalloproteinase decreased in the oviduct. This may indicate the existence of mechanisms that regulate the activity of this enzyme in the chicken oviduct. Both protein expression and activity of MMP-9 declined markedly in the oviduct during maturation of chickens. A significant amount of MMP-9 present between 10 and 14 weeks of age strongly suggests its participation in the processes occurring at the early stages of the oviduct development [12].

In another study of zymography by Godbert *et al.*, 2008 [13], found that the precursor of chicken matrix metalloprotease 2 (pro-MMP-2; 72KDa) as a complex with TIMP-2 (tissue inhibitor of metalloproteinases) was present in egg white, yolk, all along the oviduct and in the liver of laying hens. The fact that MMP-2 was found as a proform in fresh eggs suggested that the activity of this metalloprotease was regulated under specific conditions during embryonic development. Hence, both active and latent form of MMP-2 was present in Vanaraja chicken serum samples. There was no significant difference found in between sexes. It was further inferred that MMP-9 could not be expressed in the serum of both the sexes.



Lane 1. Human capillary blood gelatinases as markers
 Lane 2. Vanaraja male
 Lane 3. Vanaraja male
 Lane 4. Vanaraja female
 Lane 5. Vanaraja female

Fig 1: Gelatin zymography of MMPs in serum of Vanaraja fowl (15 microliters of serum in each well)

Conclusion

It was concluded that, both active and latent form of MMP-2 was present in Vanaraja chicken serum samples. It was further inferred that MMP-9 could not be expressed in the serum of both the sexes. However the MMP 9 gene was present and it was not expressed in the serum by gelatin zymography. Gelatinases have major role of in various physiological activities, in embryonic development, in the extracellular matrix remodelling required for follicle growth, development and regression, in development of endometriosis and in producing epithelial–mesenchymal transformations in the embryo. Further work should be carried out to find out the role of MMP in egg production and their role in fertilization process of female birds.

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