Journal of Animal Research: v.3 n.1 p.43-45. June, 2013

Breed effect on serum lysozyme activity in indigenous breeds of Sheep

Shanker Dayal[1*], Pushpendra Kumar[2] and Purushottam Kaushik[3]

[1, 2]DLFM, ICAR Research Complex for Eastern Region, Patna, Bihar, INDIA
[3]Department of Veterinary Public health, Bihar Veterinary College, Patna, INDIA

[*]Corresponding Author: S Dayal, Email: antudayal@gmail.com

Received: 11 December 2012; Accepted: 10 May 2013

ABSTRACT

Serum lysozyme is widely known for its immuno-protective action. The level is an index of macrophage function and this reflects the status of Reticulo-endothelial (RE) system in the body. It is a ubiquitous bacteriolytic enzyme present in the body fluids and tissues. It acts as an important antimicrobial component in the serum and body fluids. Therefore, the present study was undertaken to detect the effect of breed on mean serum lysozyme activity. A total of 275 animals of same age, sex and season of birth belonging to three breeds of indigenous sheep viz. Chokla, Malpura, and Muzaffarnagri were included under the present investigation. The serum lysozyme level in Chokla, Malpura and Muzaffarnagri was determined by Lysoplate assay method. Least square analysis was done to study the association of breed as well as genotype with mean serum lysozyme activity. There was significant difference (Pd≤0.05) of serum lysozyme activity among the breeds. Among all the three breeds, Chokla showed highest mean serum lysozyme activity. The mean serum lysozyme activity for Chokla, Malpura and Muzaffarnagri breed of sheep was 3.13±0.13 µg/ml, 2.39±0.14 µg/ml and 2.51±0.18 µg/ml, respectively.

Keywords: Sheep, breed, lysozyme

Lysozyme acts as an important antimicrobial component in serum and body fluids. The role of lysozyme as an antibacterial agent appears to be mediated through its direct bacteriolytic action as well as via stimulatory effect on macrophage phagocytic function. The lysozymes are 1, 4, â-N-acetyl muramidase that enzymatically degreades a glycosidic linkage between C-1 of N-acetyl muramic acid and C-4 of N-acetylglucosamine in the bacterial peptidoglycan component of cell wall (Phillips, 1966). Serum lysozyme activity reflects the homeostatic expression of reticulo-endothelial system, which is one of the most fundamental defense mechanism against infections (Lie, 1980). Moreover, several studies have provided evidences that lysozyme can be used as index of macrophage functional status (Di Luzio, 1979). Determination of serum and / or urinary lysozymes is useful in the diagnosis of several diseases or as a marker substance in their development (Jolles and Jolles, 1983). Serum lysozyme level has been intensively used in diagnosis of several diseases like leukemia, sarcoidosis etc. Urinary lysozyme measurement is helpful

in evaluating the patient with renal diseases especially those associated with tubular dysfunction (Jolles and Jolles, 1984). The presence of enzyme in the cerebrospinal fluid has been claimed to be a sensitive index of inflammatory and neoplastic diseases of central nervous system (Di Lorenzo *et al.*, 1977). Lysozyme shows an interspecies or breed variation in swine, cattle and sheep (Sotirov *et al.*, 2005). Importance of this antimicrobial factor in innate immunity and breed related differences in various species has motivated us to investigate the serum lysozyme concentration in sheep breeds of India.

MATERIALS AND METHODS

A total of 275 sheep of three breeds namely Chokla (100), Malpura (100) and Muzaffarnagri (75) of same age, sex and season of birth were randomly selected for the present investigation. 2 ml blood was collected from jugular vain in 10 ml tubes. The blood was allowed to clot for one hour at room temperature (25 C) and the samples were centrifuged at 4000 rpm for 10 min and then serum was collected in separate tube carefully. Serum lysozyme level in each animal was determined using 'lysoplate' assay method (Lie *et al*. 1986) with some modifications. Lyophylized *Micrococcus lysodeikticus* was used as substrate in the assay. Fifty ml of 1% agarose dissolved in phosphate buffer (0.07 M Na2HPO4 and NaH2PO4, pH = 6.2) were mixed with 2.5 mg *Micrococcus lysodeicticus* at 67°C. This mixture was poured out in a glass plate. After solidifying at room temperature 32 wells were made (5 mm diameter). 10 µl serum were poured out in 26 wells. Six standard dilutions (from 50-1.562 µg/ml) of lysozyme (Sigma, USA) were also poured in 6 wells. The samples were incubated for 17h at 37°C and lytic diameters were measured. The concentrations (after $\log_2$ transformation) of the known standards were regressed on the diameter of the lysed zones around these standards. The slope of the curve and intercept were determined. The lysozyme concentration of the unknown sera samples were determined by regression equation, $Y = bx + c$ Where, Y- Concentration of the unknown sample, b- Slope of regression equation, c- Intercept of regression equation and x- Diameter of lysed zone around unknown sample. The differences of the lysozyme activity among the various breeds were analyzed by least squire analysis.

RESULTS AND DISCUSSION

Serum lysozyme activity vary significantly ($P \leq 0.5$) among the indigenous breed of sheep (table 1). The mean serum lysozyme activity was higher in Chokla than the Malpura and Muzaffarnagri breeds of sheep. Chokla is having 25% more serum lysozyme activity then the Malpura and Muzaffarnagri. The mean serum lysozyme activity for Chokla, Malpura and Muzaffarnagri breed of sheep was 3.13±0.13µg/ml, 2.39±0.14 µg/ml and 2.51±0.18 µg/ml, respectively. Range of serum lysozyme activity was 0.03- 7.27 in Chokla, 0.30-4.13 in Malpura and 0.03-5.56 in Muzaffarnagri breed of sheep. The mean serum lysozyme activity of all sheep breed was 2.68±0.09µg/ml.

Table 1: Effects of breed on serum lysozyme activity.

S. No.	Breed	Serum Lysozyme Activity(µG/ML)	Range(µG/ML)
1.	Chokla	3.13±0.13[a]	0.03 to 7.27
2.	Malpura	2.39±0.14[b]	0.30 to 4.13
3.	Muzaffarnagri	2.51±0.18[b]	0.03 to 5.56

*Different superscript indicates significant difference (Pd”0.05) in serum lysozyme activity

Sotirov *et al.,* 2006 also reported the mean serum lysozyme activity in the range of 0.2 to 5 µg/ml in different breeds of sheep. The lysozyme activity in the present study is also quite comparable to serum lysozyme activity in cattle *i.e*. 3.16 µg/ml and 2.26 µg/ml in Rathi and Tharparkar breeds but lower than the mean serum lysozyme activity of buffalo i.e. 27.35 µg/ml (Sahoo, 2007).

CONCLUSION

Present study revealed significant difference (Pd”0.05) of serum lysozyme activity among three indigenous sheep breeds namely Chokla, Malpura, and Muzaffarnagri. Among all the three breeds, Chokla showed highest mean serum lysozyme activity. The mean serum lysozyme activity for Chokla, Malpura and Muzaffarnagri breed of sheep was 3.13±0.13 µg/ml, 2.39±0.14 µg/ml and 2.51±0.18 µg/ml, respectively.

ACKNOWLEDGEMENT

We thank Director, CSWRI, Avikanagar for providing Blood samples from indigenous breed of sheep to carry out this work.

REFERENCES

Di Lorenzo, N., Palma, L. and Ferrante L. 1977. Cerebrospinal fluid lysozyme activity in patients with central nervous system tumours. *Neurochirurgia.* **20**(1): 19-22.

Di Luzio, N.R. 1979. Lysozyme activity : an index of macrophage functional status. In Lysozymes in Biology and Pathology 6. J.T. Dingle, P. J. Jacques and J., H., Shaw (eds). North Holland Publishing Company. Amsterdam New York Oxford: 447-462.

Jolles, J. and Jolles, P. 1983. Lysozymes’ esterase activity. *FEBS Letters.* **162**(1): 120-122.

Jolles, P. and Jolles, J. 1984. What’s new in lysozyme research? Always a model system, today as yesterday. *Molecular and Cellular Biochemistry,* **63**(2):165-189.

Lie, O. 1980. Genetic variation in the serum lysozyme activity in cattle. *Acta Veterinaria Scandinavica,* **21:**448-450.

Lie, O., Syed, M. and Solbu.1986. Improved Agar plate assays of bovine lysozyme and hemolytic complement activity. *Acta Veterinaria Scandinavica*, **27**:23 – 32.

Phillips, D.C. 1966. The three-dimensional structure of an enzyme molecule. *Scentific American.* **215**(5): 78-90.

Sahoo, N. R. 2007. Molecular characterization of serum lysozyme gene in riverine buffalo. Ph.D Thesis submitted to IVRI, deemed University.

Sotirov, L., Dimitrov, I. and Djorbineva, M. 2005. Serum lysozyme concentrations in different sheep breeds. *Bulgarian Journal of Veterinary Medicine,* **8**(2):83-89.

Sotirov, L., Semerdjiev, V., Maslev, T. and Gerchev, G. 2006. Breed and age related differences in lysozyme concentrations and complement activity in rams. *Trakia Journal of Sciences.* **4**(3):20-24.