

## Influences of parity, age and mineral and trace element mixture on lysozyme activity in mare's milk during early lactation period

Anas Sarwar<sup>1\*</sup>, Heinrich Enbergs<sup>1</sup>, and Erich Klug<sup>2</sup>

<sup>1</sup>Institut für Anatomie, Physiologie und Hygiene der Haustiere der Universität Bonn, Germany

<sup>2</sup>Klinik für Pferde, Tierärztliche Hochschule Hannover, Germany

---

**SARWAR, A., H. ENBERGS, E. KLUG: Influences of parity, age and mineral and trace element mixture on lysozyme activity in mare's milk during early lactation period. Vet. arhiv 71, 139-147, 2001.**

### ABSTRACT

The aim of this study was to elucidate the influences of parity, age and mineral and trace element-mixture-supplementation on the course of lysozyme activity in mare's milk during early lactation period. The interrelationship between equine milk lysozyme activity and calcium content was also tested. Lysozyme activity was measured by the modified turbidimetric method using *Micrococcus lysodeikticus* as an indicator germ. Calcium content in mare's milk was assayed using the atomic absorption method. Lysozyme activity averaged  $66500 \pm 12814$  U/ml with a range of from 3800 to 115500 U/ml in milk of 24 trotter mares during the period from the 6<sup>th</sup> to 60<sup>th</sup> day postpartum. Multiparous and old (10-14 years) mares showed significantly ( $P < 0.05$ ) higher mean values compared with their primiparous and young counterparts. Mares supplemented with mineral and trace element-mixture and not conceived during foal-heat, manifested significantly ( $P < 0.05$ ) higher initial lysozyme activities than controls. Lysozyme activity and calcium content in mare's milk was found to be strongly correlated ( $r = 0.6072$ ). These data suggest that very high lysozyme activity in milk can, above all, be considered as a protective factor for new-borns. Moreover, high concentration of lysozyme in milk can certainly be a considerable factor for the low udder infections susceptibility. The strong correlation between lysozyme activity and calcium content can be attributed to a particular stability and enhanced activity of equine milk lysozyme.

**Key words:** lysozyme, mare's milk, parity, age, mineral, trace elements, calcium

---

\* Contact address:

Dr. Anas Sarwar, Department of Anatomy, University of Agriculture, Faisalabad 38040, Pakistan, Phone: +92 41 9200161-70 Ext. 422; E-mail: anas\_sarwar@hotmail.com

## **Introduction**

Milk contains a large number of specific and non-specific immunologic factors designed to protect the newborn. They have been found to promote intestinal growth and maturation and to have immuno-modulating effects, particularly in the early period of life.

Among the non-specific immunoprotective factors, lysozyme - extraordinary bacteriolytic protein - has recently attracted renewed interest as a component of the antibacterial system, which affect the intestinal flora of the neonate, possibly also the general immune system. LATVIETIS et al. (1995) have reported that the addition of a lysozyme containing preparation in avian food significantly enhanced the T- and B lymphocytes, as well as immune factors and lysozyme in serum.

Since the immune system of newborn foal is least developed in the early postnatal period, the need for possible transfer of immunity during this time may be greatest. Thus, this study aimed to elucidate the course of lysozyme activity from the 6<sup>th</sup> to 60<sup>th</sup> day postpartum. Moreover, influences of various physiological factors such as parity, sex and supplementation of mineral and trace element-mixture are studied. Furthermore, the notion that equine milk lysozyme molecule is strongly bound to the Ca<sup>++</sup> ions (NITTA et al., 1987), was tested.

## **Materials and methods**

This study was conducted on 24 mares aged from 10-14 years maintained at a trotter farm in Münster, Germany. All mares selected for study received an additional measuring pan of bran from 6 weeks before anticipated date of parturition. Half the mares were supplemented with a mineral and trace element-mixture (Alltech Firm, Hamburg, Germany) containing metals in organic form as chelate with amino acids. Daily mineral protein intake comprised 2.0 g zinc, 1.6 g manganese, 0.6 g copper and 0.1 g magnesium. This supplementation was continued until 6 weeks postpartum post partum (p.p).

10 ml milk samples were collected in PVC tubes on alternate days from the 6<sup>th</sup> to 60<sup>th</sup> day p.p. The samples were frozen immediately after milking and stored at -20 °C until analysis. Lysozyme activity in the mare's

milk was determined by the modified turbidimetric method using *Micrococcus lysodeiktitikus* as substrate. The averages of coefficient of variance (CV) for inter- and intraassay amounted to 3.84% and 3.46%, respectively. The calcium content in mare's milk was determined by the atomic absorption photometric method.

*Statistics.* Data were expressed in mean  $\pm$  sd. Course of lysozyme activity was analysed with the assistance of the non linear regression test using the following function:

$$\text{Max} * \exp * (- (n * \text{DPP}) ** 20) \text{ Base}$$

The difference between mean values of different groups were tested by comparing 95% asymmetrical confidence interval. The interrelationship between lysozyme activity and calcium was ascertained with the help of correlation coefficient (r value). All computations were performed with the help of statistical computer software SPSS (SPSS Inc. USA).

## Results

Lysozyme activity in all 672 measurements ranged between 38600 and 115500 U/ml of mare's milk with an average of 66500 U/ml. Standard

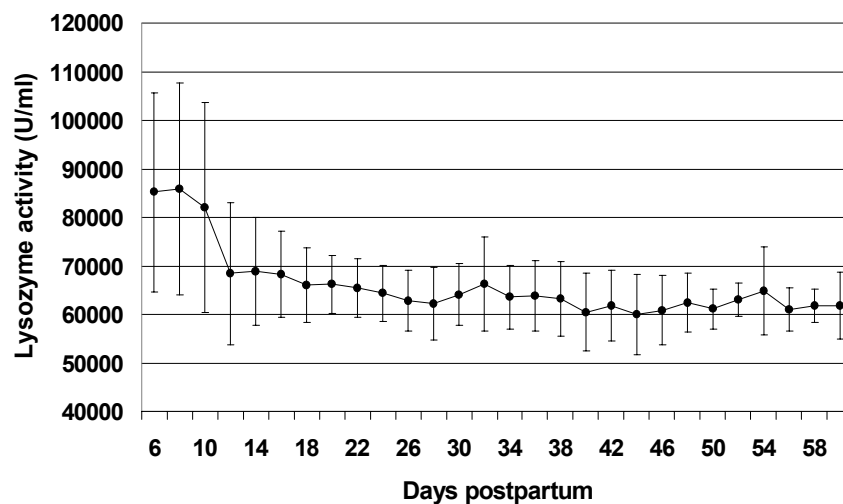


Fig.1. Course of lysozyme activities (mean  $\pm$  sd) in milk of all mares (n=24) throughout the study period

A. Sarwar et al.: Influences of parity, age and mineral and trace element mixture on lysozyme activity in mare's milk during early lactation period

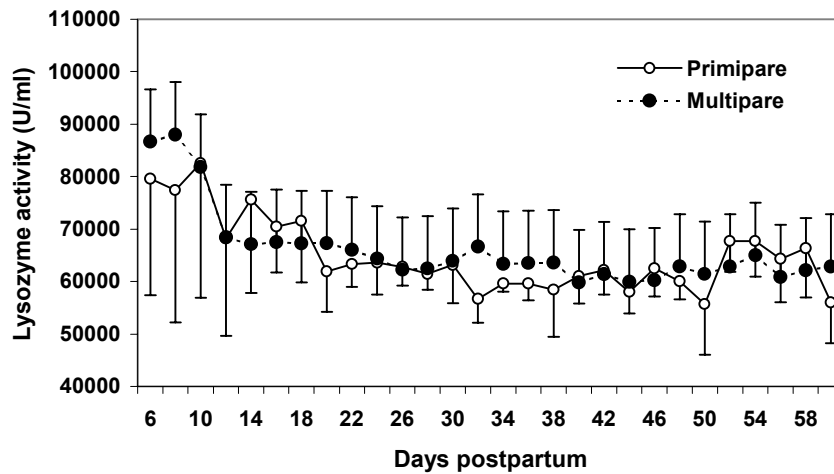


Fig. 2. Comparison between the courses of lysozyme activities (means  $\pm$  sd) in milk of primiparous (n= 5) and multiparous (n=19) mares

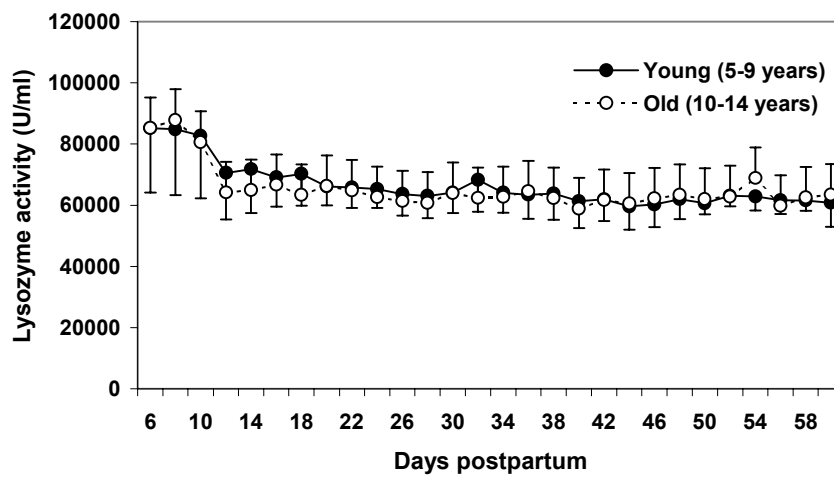


Fig. 3. Lysozyme activities (mean  $\pm$  sd) in milk of young (n=5) and old (n=19) groups of mares

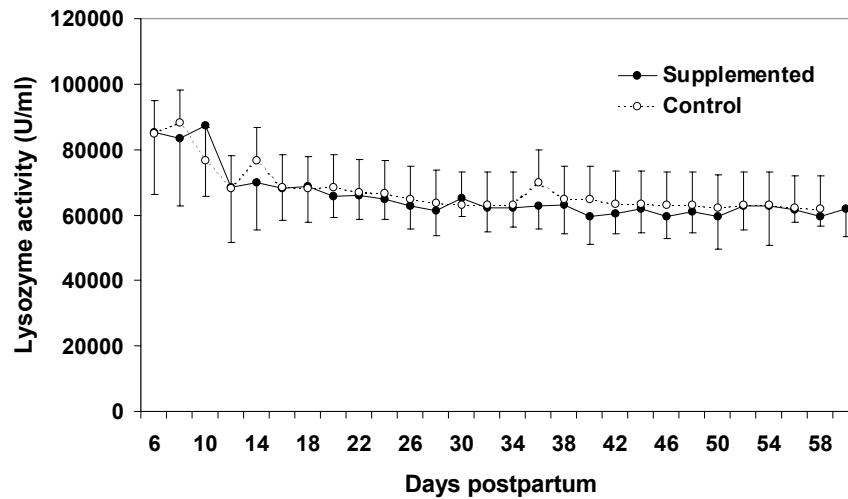


Fig. 4. Lysozyme activities (means  $\pm$  sd) in supplemented and control mares throughout study period

deviation amounted 12814 U/ml. All mares showed a strong decline in lysozyme activity (latest until 20th day p.p.). Thereafter, activities maintained a constant level (Fig. 1).

Multiparous and old (10-14 years) mares showed significantly ( $P < 0.05$ ) higher mean values compared to primiparous and young groups (Fig. 2 and 3). Mares supplemented with mineral and trace element-mixture and not conceived during foal-heat, manifested significantly ( $P < 0.05$ ) higher initial lysozyme activities compared with controls (Fig. 4). Lysozyme activity and calcium content in milk were found to be strongly correlated ( $r = 0.6072$ ).

### Discussion

Lysozyme activity averaged  $66500 \pm 12814$  U/ml with a range of from 3800 to 115500 U/ml in milk of 24 trotter mares during the period from the 6<sup>th</sup> to 60<sup>th</sup> day post partum. These results extend over the findings of

studies conducted on mare's milk by JAUREGUI-ADELL (1974), and SONNTAG (1996). However, they did not emphasise the early lactation period.

In comparison with human beings and other animal species, lysozyme activity was observed to be higher in the mare's milk. Various authors have reported a range of between 9 880 and 52000 IU/ml, with the exception of SANCHES-POZO et al. (1987), who recorded a very high mean value (235 300 IU/ml) in human milk.

Lysozyme activity in cow's milk has been extensively studied both in physiological and pathological conditions. There is common agreement that cow's milk contains a very low concentration of lysozyme (0-0.58 mg/ml). It has been suggested that the lysozyme is liberated from the broken-up neutrophils in serum, while cow's neutrophils contain an extremely low concentration of lysozyme.

RADWAN and ELMARIMI (1987), FARID et al. (1984) and ISMAIL et al. (1984) have reported a substantial rise in mastitis among different bovine species. PERSSON et al. (1992) have suggested that neutrophils are the most probable source of lysozyme in inflammation of the mammary gland. Bovine neutrophils, as in humans and other mammalian species, play a double role in inflammation: phagocytosis and destruction of micro-organisms and induction of inflammatory reaction (JAIN, 1986), for instance, by the induction of chemotaxis and by the support of phagocytosis. Hence, it is possible that a high concentration of lysozyme is released in inflammation due to high turnover rate. Lysozyme possesses not only antimicrobial characteristics but it is also important in cellular immunity by induction of chemotaxis and strengthening of the phagocytosis. In this study, lysozyme activity was found to be a hundred times higher than the values reported in serum for mares and foals. This suggests that lysozyme does not solely transfer from serum to milk by diffusion, rather, there are also additional selective mechanisms involved. KROHNEN (1973) reported a strong correlation between lysozyme activity and somatic cell count in cow's milk, which further substantiates this argument. The local production of lysozyme from the mammary epithelial cells also cannot be excluded.

In the present study, lysozyme activity maintained an initial mean value of 81524 U/ml before abruptly falling to a 20% lower level on day 12 p.p.,

on average. The base value, however, remained constant until the end of the study (60<sup>th</sup> day postpartum). HATZIPANAGIOTOU et al. (1998) also reported similar findings.

These results accord well with studies conducted in human beings. SANCHES-POZO et al. (1987) also carried out investigations from the 6<sup>th</sup> day p.p. and recorded a decline in lysozyme activity on the 12<sup>th</sup> day p.p. However, these values maintained a constant level until the end of the study (30<sup>th</sup> day p.p.). McCELLAND et al. (1978) and HENNART et al. (1991) studied the daily changes in lysozyme activity in human milk during the first week of lactation, observing a strong fall during the initial 3-4 days of lactation, and which thereafter maintained a constant level throughout the week.

When a mammal is born, it emerges from the sterile uterus into an environment where it is immediately exposed to a host of micro-organisms. Because at the time of birth the immune system of the newborn is not fully developed, the newborn is provided with specific and non-specific immune factors through mother's milk for survival (TIZARD, 1996). Higher lysozyme activities during the early postpartal period with parity and age can be attributed to time-dependent augmenting training of specific and non-specific immune mechanisms. SENFT et al. (1979) and GÖTZE et al. (1977) have also reported higher lysozyme activity in multiparous cows when compared with their primiparous counterparts.

Mares supplemented with a mineral and trace element-mixture showed significantly ( $P < 0.05$ ) higher lysozyme activity than the respective controls. No report is available for discussion of these results.

The findings of a strong correlation ( $r = 0.6072$ ) between equine milk lysozyme molecule and calcium ion testified to the notion that equine lysozyme and calcium ions are strongly bound together (HAEZEBROUCK et al., 1992; NITTA et al., 1987). Thereby, it has been proved once again that mare's milk holds a special position among mammalian species. NITTA et al. (1987) suggested that the strong binding between equine milk lysozyme and  $Ca^{++}$  ions is responsible for the particular stability and enhanced activity of equine milk lysozyme.

These data suggest that very high lysozyme activity in milk can, above all, be considered as a protective factor for new-borns. Moreover, the high concentration of lysozyme in milk can certainly be a considerable factor for low udder infections susceptibility. A strong correlation between lysozyme activity and calcium content can be attributed to a particular stability and enhanced activity of equine milk lysozyme.

### References

- FARID, A., S. A. SALIM, M. ABDEL-GAHANI, M. ISMAIL (1984): Diagnosis of bovine subclinical mastitis by determination of lysozyme level in milk. *Archiv Exper. Veterinärmed.* 38, 857-862.
- GÖTZE, P., J. MEYER, H. BUSCHMANN (1977): Untersuchungen über den Lysozymgehalt im Blut und in der Milch von gesunden und euterkranken Rindern. *Zentralb. Vet. Med. B* 24, 560-568.
- HAEZEBROUCK, P., W. NOPPE, H. VAN DAEL, I. HANSSENS (1992): Hydrophilic interaction of lysozyme and  $\alpha$ -lactalbumin from equine milk whey. *Biochem. Biophys. Acta* 1122, 305-310.
- HATZIPANAGIOTOU, A., E. RIELAND, H. ENBERGS (1998): Lysozyme activity in the milk of sucking mares during lactation. *Deutsch. Tierärztl. Wochenschr.* 105, 148-152.
- HENNART, P., F. BRASSEUR, D. L. DELOGNE-DESNEOECK, J. B. DRAMAIX, C. E. ROBYN (1991): Lysozyme, lactoferrin, secretory immunoglobulin-A content in breast milk: influence of duration of lactation, nutrition status, prolactin status and parity of mother. *Am. J. Clin. Nutr.* 53, 32-39.
- ISMAIL, M., S. A. SALEM, R. M. ARAB, R. SOLIMAN, A. S. SOLIMAN (1984): Changes in lysozyme activity in milk and its significance in the diagnosis of subclinical mastitis in goats. *Egypt Vet. Med. J.* 32, 59-65.
- JAIN, N. C. (1986): *Schalm's Veterinary haematology* 4<sup>th</sup> ed. Lea and Febiger, Philadelphia, USA.
- JAUREGUI-ADELL, J. (1974): Heat stability and reactivation of mare milk lysozyme. *J. Dairy Sci.* 58, 835-888.
- KROHNEN, H. (1973): Untersuchungen zur Bakterizidie der Milch und Immunisierung der bovinen Milchdrüse. Helsinki Veterinärmedizinische Fakultät, Diss.
- LATVIETIS, J., P. ANDERSON, U. KARKLA-KAULINS, A. BAUMANE, V. MIKELSONE (1995): Neue lysozymhaltende Präparate als Wuchs- und Immunostimulatoren in der Tierzucht. *Proc. Anim. Nutr. Conf., Latvia.* pp. 37-44.



A. Sarwar et al.: Influences of parity, age and mineral and trace element mixture on lysozyme activity in mare's milk during early lactation period

- McCELLAND, D. B. L., J. McGRATH, R. R. SAMSON (1978): Antimicrobial factors in the human milk: studies of concentration and transfer to the infant during the early stages of lactation. *Acta. Paediat. Scand. (Suppl.)* 271, 1-20.
- NITTA, K., H. TSUGE, S. SUGAI, K. HIMAZAKI (1987): The calcium binding property of equine lysozyme. *FEBS Letter* 223, 405-408.
- PERSSON, B., L. V. L. BOHN, H. ANDERSEN (1992): Quantitative determination of immunoglobulins, lysozyme and lactoferrin in the teat and udder of the dry cow during endotoxin-induced inflammation. *J. Vet. Med. B.* 39, 165-175.
- RADWAN, Y. A., A. A. ELMARIMI (1987): The use of lysoplate test in the diagnosis of subclinical mastitis. *Bull. Ani. Health Prod. Afr.* 35, 100-135.
- SANCHES-POZO, A., J. LOPEZ-MORALES, J. IZQUIERDO, A. MARTINEZ-VALVERDE, A. GIL (1987): Protein composition of human milk in relation to mother's weight and socio-economic status. *Human Nutr. Clin. Nutr.* 41C, 115-125.
- SENFT, B., F. MEYER, G. ERHARDT (1979): Untersuchungen über die Lysozymkonzentration in der Milch schwarzbunter Kühe. 30<sup>th</sup> Annual Meeting, EAAP, Harrogate.
- SONNTAG, C. A. (1996): Konzentration verschiedener Inhaltsstoffe der Stutenmilch im Verlauf der Laktation unter besonderer Berücksichtigung der Lysozymaktivität. Leipzig Uni., Vet. Med. Fak., Diss.
- TIZARD, I. R. (1996): Immunity in the fetus and newborn. In: *Veterinary Immunology – An Introduction*. 5<sup>th</sup> ed. W. B. Saunders Company. USA.

Received: 23 February 2001

Accepted: 21 June 2001

---

**SARWAR, A., H. ENBERGS, E. KLUG: Utjecaj učestalosti ždrijebljenja, dobi te minerala i mikroelemenata na lizozimsku aktivnost kobiljeg mlijeka tijekom rane laktacije. *Vet. arhiv* 71, 139-147, 2001.**

**SAŽETAK**

Cilj ovog istraživanja je bio utvrditi utjecaj učestalosti ždrijebljenja, dobi te minerala i mikroelemenata na lizozimsku aktivnost kobiljeg mlijeka tijekom rane laktacije. Lizozimska aktivnost je određena modificiranom turbidimetrijskom metodom uz pomoć bakterije *Micrococcus lysodeikticus*. Količina kalcija je određena atomskim apsorpcijskim spektrofotometrom. Lizozimska aktivnost u mlijeku iznosila je  $66500 \pm 12814$  U/ml u rasponu od 3800 do 115500 U/ml tijekom razdoblja od 6. do 60. dana nakon ždrijebljenja. U mlijeku multiparnih i starih kobila (u dobi od 10-14 godina) utvrđena je veća lizozimska aktivnost u odnosu na aktivnost u mlijeku mladih kobila. Veća aktivnost utvrđena je u mlijeku kobila prihranjivanih mješavinama minerala i mikroelemenata. Pozitivna korelacija utvrđena je između lizozimske aktivnosti i sadržaja kalcija ( $r = 0,6072$ ). Podaci ukazuju da velika aktivnost lizozima u mlijeku može imati zaštitnu ulogu za ždrebad. Osim toga velika koncentracija lizozima može imati veoma važnu ulogu u otpornosti mliječne žlijezde prema različitim infekcijama.

**Ključne riječi:** lizozim, kobilje mlijeko, ždrijebljenje, dob, minerali i mikroelementi, kalcij

---