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EFFECTS OF ANTIBACTERIAL DRUGS ON CAMEL MILK LYSOZYME CONCENTRATION AND LACTOPEROXIDASE ACTIVITY

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ABSTRACT

The objective of this research was to discover the potential effects of antibiotics used during lactation on innate immunity factors present in milk as lysozyme and lactoperoxidase. Intramuscular administration of oxytetracycline, amoxycillin, gentamicin and enrofloxacin to lactating camels for 3 days resulted in an appreciable amount of residues in milk for 72 hours. The antibacterial drugs caused significant inhibition of activity of lactoperoxidase and lysozyme concentration in milk of camels. It is recommended to implement withdrawal time when antibacterials are used in lactating camels to preserve the properties of the milk.

Key words: Antibacterials, camel, lactoperoxidase, lysozyme, milk

Milk contains a large number of specific and non-specific immunological factors aimed at the protection of the newborn. Among the non-specific immunoprotective factors are the lysozyme and lactoperoxidase. Lysozyme, an extraordinary bacteriolytic protein, is a component of the antibacterial system and also affects the general immune system. Latvietis *et al* (1995) reported that addition of a lysozyme-containing preparation of avian food significantly enhanced the T and B lymphocytes, circulating immune factors and lysozyme in serum along with daily growth (Al-Nazawi, 2008).

Lactoperoxidase, an enzyme present in milk, is a haeme-containing glycoprotein (Isobe *et al,* 2011; Boots and Floris, 2006) thought to be an important component in the defence against the microbial activity in raw milk (Boscolo *et al,* 2007). Lactoperoxidase is found in the breast secretory epithelial cells, in the salivary, lacrimal glands of mammals and in their secretions, such as milk, saliva and tears (Boscolo *et al,* 2007). Lactoperoxidase is a part of an antimicrobial system that catalyses the oxidation of thiocyanate to the antibacterial hypothiocyanate in a hydrogen peroxide dependent reaction (Tanaka *et al,* 2003).

Many drugs or chemicals are known to activate or inhibit several body enzymes catalysing the metabolic pathways (Al-Nazawi, 2008). Systemic antibiotics are usually used in therapeutic and prophylaxis of various disease conditions and during surgical operations. Use of antibiotics may worsen enzyme activity in milk throughout the lactation period, which may affect immune system (Ihalin *et al,* 2003). Since antibiotics may be used in pregnant patients and during lactation, it is important to explore the effect of these on milk enzyme during lactation. The objective of this study was to investigate the effects of antibacterials on camel milk lysosome and lactoperoxidase activity and on udder immunity.

Materials and Methods

Animals and Treatments: Lactating camels (n=20) were divided into 5 groups equally, treated at mid lactation. Animals were treated for 3 successive days. Saline (control), oxytetracycline (5mg/kg, Norbrook, UK), amoxycillin (15mg/kg, Pfizer, UK), gentamicin (6.6mg/kg, Biomeda, UK) and enrofloxacin (2.5mg/kg, Bayer, UK) were administered intramuscularly. Milk samples were collected and stored at -30ºC until analysis.

Assay of drugs: Bacterial inhibitors were estimated by Brilliant Black Reduction Test (BR test) developed by Enterotox, Germany. This test used the endosports of *Bacillus stearothermochilus* var *calidolactis* C 953 as a test organism, and brilliant black as an indicator. The method combines the principle of agar diffusion with reduction pigment (Molina *et al,* 2003).

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The existence of bacterial inhibitor is proved when the indicator does not change colour during incubation (remains blue).

Determination of Lactoperoxidase (LPO) activity: LPO activity is determined by the (Sisecioglu *et al,* 2011). This method is based on oxidation of 2,2-Azino-bis (3-ethyl benzthiazoline-6-sulphonic acid) (ABTS) as a chromogenic substrate by means of H_2O_2 and colour compound, which occurs during reaction and gives an absorbance at 412 nm. The absorbance taken at LPO activity is defined as the amount of the lactoperoxidase oxidising 1 µmol of ABST substrate per min at room temperature. Molar absorption coefficient of ABST is used as 32400 M^{-1} cm^{-1} and specific activities are expressed as enzyme units (EU) per mg of protein (Shindler and Bardsley, 1975).

Serum Lysozyme Activity: Serum Lysozome concentrations were measured using *Micrococcus lysodiekticus* as a substrate (Lysozyme reagent kit, worthingtorn biochemical, Co. Freehold, NJ) (Al-Aknari and Homeida, 1996). The percentage changes in transmission (510 nm) per min were immediately recorded using a spectrophotometer (Hitachi, Japan). The values were compared to a standard curve, simultaneously prepared using a known concentration of egg white lysozyme.

Statistical analysis: Results are expressed as mean ± SD and presence of significant differences among means of the groups were determined using one way ANOVA with a Tukey-Kramer Posy-test for significance. Values were considered significant when ($P < 0.05$).

Results

Intramuscular administration of antibacterials to lactating camels resulted in the presence of antibiotic in milk indicated by positive Brilliant Black Reduction Test (BR test) for up to 60 hours (Table 1) was compared to saline controls. The results of the effect of antibacterials on lactoperoxidase and lysosome are shown in table 2. All antibacterials significantly ($P<0.05$) exhibited inhibitory effects on both lactoperoxidase and lysozymes.

Discussion

Administration of oxytetracycline, amoxycillin, gentamicin and enrofloxacin to camels by intramuscular route resulted in an appreciable amount of drug residues in milk. Milk is suspension of fat droplet in aqueous phase in which lactose, inorganic salts and proteins are dissolved. The passage of antibacterials from systemic circulation into milk is determined by the extent of binding to plasma proteins, pH of solvent, degree of lipid solubility and degree of ionisation (pka) of the drug (Booth, 1982). Most of antibacterials used in the study are highly lipid soluble with an alkaline pka, therefore expected to be found in milk being more acidic than blood (Baggot, 1983). Furthermore, camel milk has higher acidic nature than bovine, consequently higher concentration of antibacterials is expected to be found in camel milk.

Table 1. Residues of drugs in camel milk after intramuscular administration of antibacterials (n=4 each).

Name of Antibacterials Drug	Time after last injection of drug in hours					
	12	24	36	48	60	72
Control (saline)	-	-	-	-	-	-
Oxytetracycline	+	+	+	+	+	-
Amoxycillin	+	+	+	+	+	-
Gentamicin	+	+	+	+	+	-
Enrofloxacin	+	+	+	+	+	-

Table 2. Effects of antibacterials on the activity of lactoperoxidase and concentration of lysozymes in milk of camels (n=4 each).

Name of Antibacterials Drug	Lactoperoxidase Activity (µg/ml)	Lysozyme Activity (µg/ml)
Saline (control)	6.23 ± 0.86	8.42 ± 0.32
Oxytetracycline	*3.10 ± 0.36	*5.13 ± 0.24
Amoxycillin	*3.41 ± 0.41	*4.92 ±0.26
Gentamicin	*3.22 ± 0.32	*5.51 ± 0.22
Enrofloxacin	*3.12 ± 0.33	*4.81 ± 0.25

* Significant ($P<0.05$).

Administration of antibiotics have produced inhibitory effect on milk lactoperoxidase. Similarly *in vitro* inhibitory effect of amikacin, ceftriaxone, ceftazidime, teicoplanin and prednisolone on lactoperoxidase in milk have been noticed (Sisecioglu *et al,* 2011). *In vivo* inhibitory effect of anaesthetic drugs like ketamine and bupivacaine on lactoperoxidase during lactation was also demonstrated (Uguz and Ozdemir, 2005). Lactoperoxidase is present in milk of different species in varying concentration (Reiter, 1985). Lactoperoxidase acts in a system together with thiocyanate and hydrogen peroxide by catalysing the peroxidation of thiocyanate to putative antimicrobial hypothiocyanite (Aune and Thomas, 1977). The antimicrobial property of lactoperoxidase gives it a potential application in the preservation of raw milk under ambient conditions (Pruitt and Kamau, 1991).

Injection of antibacterials effectively decreased lysozyme levels in milk. Appreciable amounts of lysozyme were found in camel milk during 60 days postpartum (Sarwar and Enbergs, 2005). Serum lysozyme activity was significantly decreased in rats treated with dexamethasone and bacteriostatic agents. Serum lysozyme activity is considered to be an index of macrophage functions (Currie and Eccles, 1976). Similar studies showed that suppression of macrophage activity with methyl palmitate was associated with reduction of lysosome enzyme release in serum while activation of macrophages with glucan was associated with a marked release of the enzyme in serum (Koskoshis and Di Luzio, 1979). Furthermore, poly-D-glutamic acid was able to increase macrophages intracellular lysosome and subsequently increased phagosome-lysome fusion (Al-Aknari and Homeida, 1996). Such mechanism involving phagosome-lysosome interaction occur in case of antibacterial inhibitory activities.

Camel milk does not sour quickly as compared to cow and goat milk. It is consumed fresh and no need to be boiled. These properties are due to presence of bactericidal and viricidal substances such as lactoperoxidase and lysozyme. Administration of antibacterial will interfere with these properties. Therefore, it is recommended to implement strict withholding time for antibacterials given to lactating camels.

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