

BIOCHEMICAL CHANGES IN MILK IN EXPERIMENTAL ACHOLEPLASMAL MASTITIS IN GOATS

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Abstract

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To study the biochemical changes in milk during unilateral acholeplasmal mastitis, 8 lactating goats were selected, out of which 7 animals were inoculated through the right teat canal with 2 ml of *Acholeplasma laidlawii* containing 10^9 colony forming units per 1 ml. The left udder halves of these 7 goats served as controls and one goat was kept as intact control. The effects of infection were monitored over a period of 19 days. During this period one goat was killed every 3rd day after infection, while 2 animals were killed on the 19th day, i.e. one experimentally infected and one intact control. All the goats infected with *A. laidlawii* developed clinical mastitis within 24 h, which persisted till the end of the experiment. The total protein, total immunoglobulins (Ig), total phospholipids and free fatty acid contents of mastitic milk/mammary secretions showed progressive increase, whereas total lipids and glycerides decreased substantially. However, there was a marginal increase in the total cholesterol concentration.

The results indicated that *Acholeplasma* use lipolytic enzymes to degrade lipids so as to meet their needs of energy and for biosynthesis of membrane lipid bilayer during multiplication. The increase in milk protein especially Ig indicated humoral immune response to *A. laidlawii* and secretion of Ig into the mammary gland.

Goats, milk/secretion, total proteins, lipids, cholesterol, FFA, phospholipids, glycerides, immunoglobulines

Acholeplasma laidlawii has been isolated from natural cases of mastitis in association with other mycoplasmas. Despite its heterogenous nature in terms of serology, biochemistry and pathogenicity, *A. laidlawii* is considered as a saprophyte (Gourlay and Howard 1979). Although Pfutzner et al. (1979) described its pathogenicity in natural cases of bovine mastitis, yet its role in producing mastitis has remained debatable.

The present work was therefore undertaken to study biochemical alterations in milk/mammary secretions during experimentally induced acholeplasmal mastitis in goats and to assess the pathogenicity of *A. laidlawii* to mammary glands of lactating goats.

Materials and Methods

After 7 days of pre-experimental observations, eight 2.5 to 4 years old lactating goats were found to be healthy and free from subclinical mastitis. The total leucocyte count (TLC) of milk varied from 0.60 to 0.65×10^6 ml⁻¹. The milk was free from infectious agents. Two ml of 48 h culture of *A. laidlawii* containing 10^9 colony forming units (CFU) ml⁻¹ was inoculated through the right teat canal into the right mammary halves of 7 goats and the control left halves were inoculated with 2 ml sterile mycoplasma broth. In addition, one goat was kept as intact control. Any abnor-



Fig. 1. Right teat and udder half of the goat inflamed and swollen, 2 days after inoculation with *Acholeplasma laidlawii*.



Fig. 2. Right teat and udder half of a goat markedly atrophied, smaller in size 18 days after inoculation with *A. laidlawii*.

malities in the udder, changes in temperature, systemic disturbances and mastitis (assessed by California mastitis test and modified whiteside test) were recorded. Samples of milk/mammary secretions were obtained from all the goats before inoculation and every 3rd day up to 19th day after infection (DAI). TLC of milk/mammary secretions and haematological values of all the goats were also recorded. The pooled milk/mammary secretions were analysed for total lipids (Folch et al. 1957), total phospholipids (Ames 1966), total cholesterol (Zlatkis and Zak 1969), total free fatty acids (Lowry and Tinsley 1976), total glycerides (by difference), total proteins (Lowry et al. 1951) and total immunoglobulins (Oser 1955).

During the 19 days of the experiment, one goat was killed every 3rd day, while 2 goats (one goat which was experimentally infected and the other intact control) were killed on 19th DAI. Udders and their lymph nodes were examined grossly and microscopically. For microscopic examination 5 to 6 μ thick paraffin sections were stained with haematoxylin and eosin.

Results

The appetite and body temperature of the infected goats were not affected during the experiment. However, all the experimental goats developed clinical mastitis in their right udder halves, from next day after inoculation with *A. laidlawii*. The infected udder halves of all the 7 goats were inflamed, hot, tender, painful and swollen (Fig. 1) during first 3 DAI. Thereafter, the size of these udder halves decreased and remained smaller (Fig. 2) and more firm in consistency as compared to those of the uninfected ones. Between 2nd DAI and 19th DAI, there was a marked decrease in the volume of milk/mammary secretions

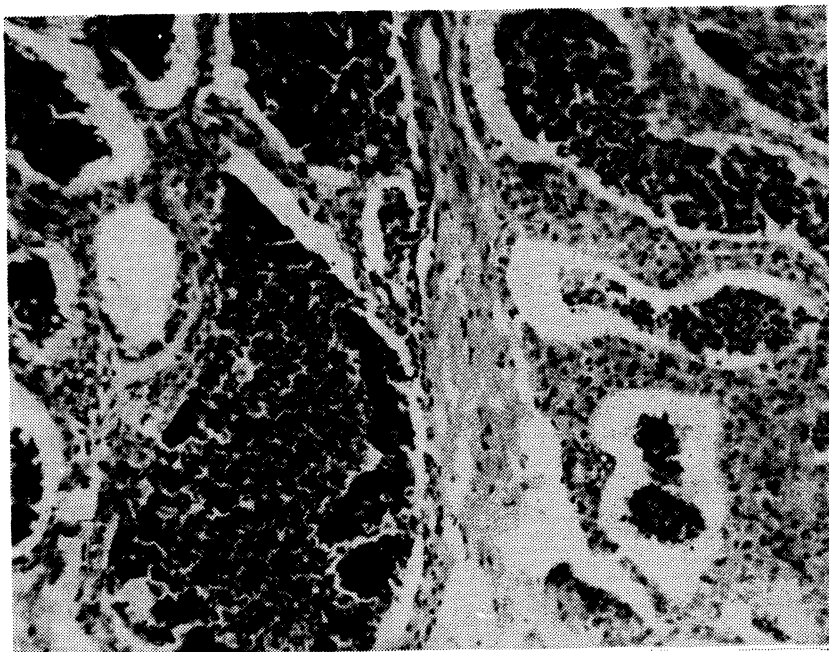


Fig. 3. Section of mammary gland showing acute diffuse purulent mastitis 3 days after inoculation with *A. laidlawii*. H.E. $\times 70$

from right infected halves and the secretions were thin, flocculent and had a yellowish tinge. On the other hand, the amount and consistency of mammary secretions/milk drawn from the control left udder halves and both the udder halves of the control goat remained unaffected. Mastitis in the right infected halves was severe during the experiment. The TLC of milk from infected halves increased from the basal value of $0.60 \times 10^6 \text{ ml}^{-1}$ to $13.97 \times 10^6 \text{ ml}^{-1}$ and $17.86 \times 10^6 \text{ ml}^{-1}$ on 3rd and 6th DAI, respectively. Thereafter these values decreased gradually ($10.50 \times 10^6 \text{ ml}^{-1}$ and $5.77 \times 10^6 \text{ ml}^{-1}$ on 9th and 12th DAI, respectively) and was $4.80 \times 10^6 \text{ ml}^{-1}$ until 19th DAI, but remained much higher than the basal values. However, the TLC in mammary secretions from uninfected left udder halves and milk from control goat was not affected.

A. laidlawii was re-isolated only from mammary secretions of all the right infected udder halves until 19th DAI. No bacteria, was detected in the milk of any goat during the experiment.

Microscopically, the infected udder halves of goats killed on 3rd and 6th DAI, showed acute diffuse purulent mastitis, characterised by marked infiltration of neutrophils in the lumina of acini (Fig. 3) and vacuolar degeneration of epithelial cells lining the acini. The animals killed on 9th, 12th, 15th, 18th and 19th DAI showed chronic interstitial mastitis characterised by atrophy of acini, proliferation of fibrous connective tissue (Fig. 4), thickening of interlobular septa, pseudolobule formation and chronic galactophoritis with calcified corpora amylacea in the lumina of lactiferous ducts (Fig. 5). The mammary lymph nodes of the infected udder halves showed reactive lymphadenitis. There were no pathological

Table 1
Changes in total protein and total immunoglobulin content in milk of control and experimental goats inoculated with *A. laidlawii*

Parameters	Days after infection									
	0	3	6	9	12	15	18	19		
n	7	7	6	5	4	3	2	1		
Total protein (g dl ⁻¹)	2.85	3.00	2.99	3.00	3.13	2.85	3.13	3.13		
Goat (C)	2.85	3.00	2.99	3.00	3.13	2.85	3.13	3.13		
Goat (E)	3.13	2.99	3.15	3.23	3.36	3.42	3.40	3.40		
Left udder half (UI)	3.13	4.11	4.70	5.29	5.00	4.70	4.41	4.41		
Total immunoglobulins (g dl ⁻¹)	0.26	0.29	0.26	0.29	0.31	0.29	0.29	0.31		
Goat (C)	0.26	0.29	0.26	0.29	0.31	0.29	0.29	0.31		
Goat (E)	0.30	0.29	0.31	0.32	0.35	0.35	0.32	0.32		
Left udder half (UI)	0.29	0.31	0.38	0.38	0.41	0.44	0.47	0.48		

n = Number of samples pooled; C = Control; E = Experimental; UI = Uninfected and I = Infected.

Table 2
Progressive change in lipid comparison of milk/mammary secretions from control and experimental goats inoculated with *A. laidlawii*

Days after infection	n	Parameters											
		Total lipids (g dl ⁻¹)		Total glycerides (g dl ⁻¹)		Total phospholipids (mg dl ⁻¹)		Total free acids (mg dl ⁻¹)		Total cholesterol (mg dl ⁻¹)			
		Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental		
0	7	3.90	3.80	3.74	3.68	65.0	66.0	62.4	65.0	38.7	40.0	40.5	
3	7	3.95	3.76	3.70	3.61	59.0	54.0	64.0	65.7	40.0	40.0	54.8	
6	6	3.87	3.83	3.70	3.66	66.3	67.0	60.0	66.3	36.8	44.1	65.4	
9	5	3.81	3.65	3.63	3.45	67.0	67.0	69.7	64.0	39.0	40.0	63.8	
12	4	3.88	3.73	3.72	3.55	59.0	67.0	65.1	64.0	40.0	41.4	49.6	
15	3	3.77	3.72	3.61	3.40	63.2	59.0	61.0	64.0	36.3	39.0	47.0	
18	2	3.88	3.76	3.75	3.59	59.0	93.3	62.4	65.1	38.7	39.0	46.0	
19	1	3.83	3.76	3.66	3.59	65.0	54.0	64.0	66.0	39.0	39.0	47.0	

n = Number of samples pooled; LU = Left udder half; RUH = Right udder half; I = Infected and UI = Uninfected.

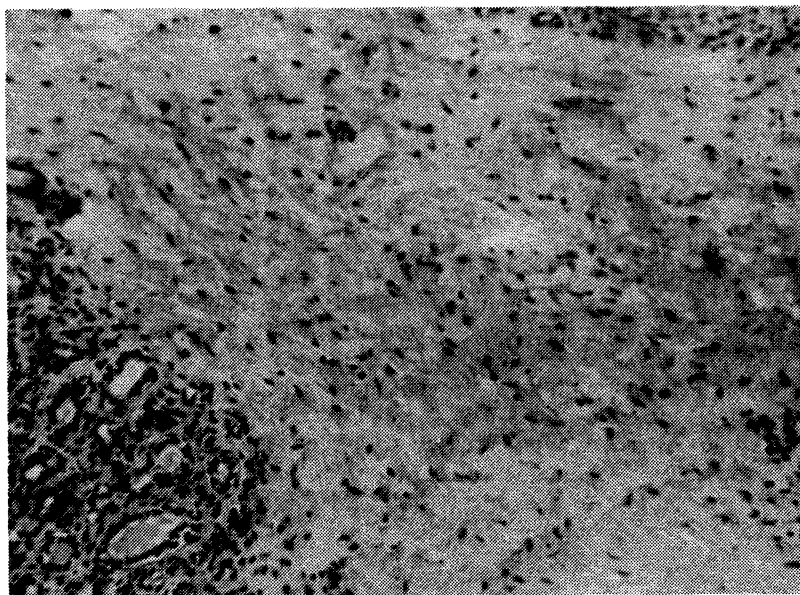


Fig. 4. Section of mammary gland showing chronic mastitis characterized by marked fibrosis and atrophy of acini, 15 days after inoculation with *A. laidlawii*. H. E. $\times 70$

changes in udder halves and their lymph nodes, both from control and non-infected left udder halves of goats.

The biochemical analysis of pooled milk/mammary secretions from right halves, inoculated with *A. laidlawii*, revealed a substantial increase in total proteins, total immunoglobulins, total phospholipids and total free fatty acids, while the total lipids and total glycerides showed a considerable decrease. There was, however, marginal increase in the level of total cholesterol (Tables 1 & 2 and Fig. 6).

The total protein content of the milk/mammary secretions from the right as well as left udder halves was 3.13 g dl^{-1} before inoculation. It increased to 4.11 g dl^{-1} and 5.29 g dl^{-1} on 3rd and 6th day post inoculation, respectively, in the mammary secretions from the infected right udder halves. Thereafter the protein content decreased gradually but still remained higher than the basal values of 3.13 g dl^{-1} (Table 1).

The total immunoglobulin content showed a tendency to increase from 0.29 g dl^{-1} before inoculation to 0.48 g dl^{-1} on 19th day PI in the mammary secretions of infected right udder halves. However, the total protein and immunoglobulin content in the milk of control goat remained unaltered (Table 1).

The total lipid content of mammary secretions from both udder halves varied between 3.83 to 3.85 g dl^{-1} before inoculation. After inoculation the total lipids decreased gradually to 2.40 g dl^{-1} on 6th DAI in the secretions of infected right udder halves. After 6th DAI, the total lipids tended to increase up to 3.16 g dl^{-1} on 19th DAI (Table 2 and Fig. 6).

The total glyceride content of the mammary secretions of infected right udder halves also decreased gradually from 3.65 g dl^{-1} before inoculation to 1.91 g dl^{-1}

on 6th DAI. It then increased gradually to 2.92 g dl^{-1} on 19th DAI. The levels of total lipids and glycerides in the milk of the control goat remained almost unaffected (Table 2 and Fig. 6).

The total phospholipid content in the mammary secretion of infected right udder halves increased significantly from 66.0 mg dl^{-1} before inoculation to 172.8 mg dl^{-1} on 6th DAI.

The free fatty acid content also showed a significant increase from 64.0 mg dl^{-1} before inoculation to 255.9 mg dl^{-1} on 6th day post infection in the secretions of infected right udder halves. However, the levels of total phospholipids and free fatty acids tended to decline thereafter upto 19th DAI but the values remained much higher than the basal values. The total phospholipids and free fatty acid content in the milk of uninfected left udder halves did not show any significant change (Table 2 and Fig. 6).

The total cholesterol content in the mammary secretions of infected right udder halves increased marginally from 40.0 mg dl^{-1} before inoculation to 65.4 mg dl^{-1} on 6th DAI and thereafter it decreased to 47.0 mg dl^{-1} on 19th DAI. However, no significant change in cholesterol content was observed in the uninfected left udder halves and in milk of control goat.

Discussion

This study clearly indicated that *A. laidlawii* was pathogenic to goat udder and caused mastitis by damaging the mammary secretory tissue. These effects have been reported to be due to binding of *Acholeplasma* to secretory cells, leading

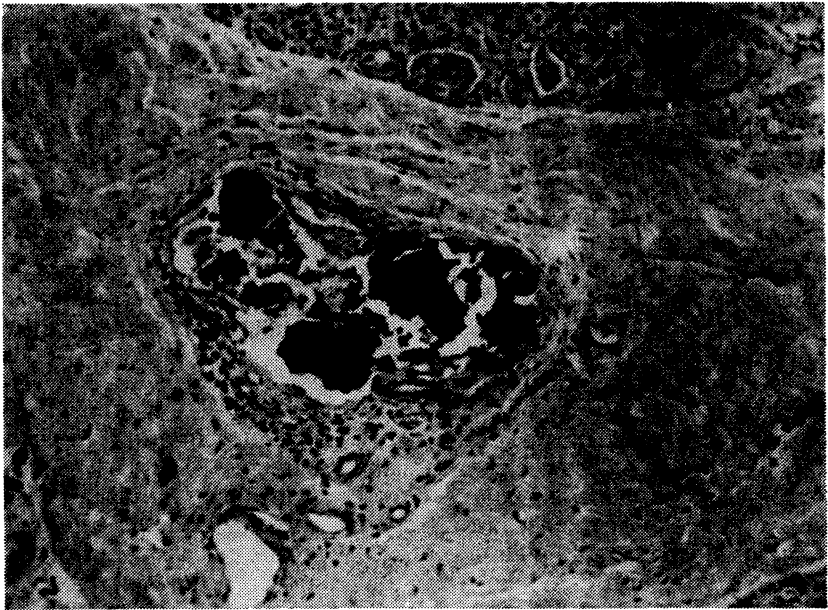


Fig. 5. Chronic mastitis with chronic galactophritis and calcified corpora amyloacea in the lumen of lactiferous duct 18 days after inoculation with *A. laidlawii*. H.E. $\times 70$

to their death as a result of local concentration of toxic and acidic metabolites H_2O_2 and also due to formation of various hydrolytic enzymes by acholeplasmas (Razin 1978).

The re-isolation of *A. laidlawii* from milk/mammary secretions even on 19th DAI confirmed that mastitis was of acholeplasmal origin. The increased TLC in milk and other gross and microscopic changes in udder of goats observed during this study were similar to those described after experimental *A. laidlawii* infection in cows (Bachvarova 1979; Pal et al. 1982, 1983; Pftzner et al. 1979; Seffner et al. 1983) and in ewes (Jones 1985). Almost similar but more pronounced changes have been observed after experimental mycoplasmal mastitis in goats due to *M. putrefaciens* (Adler et al. 1980), *M. agalactiae* subsp. *bovis* (Ojo and Ikede 1976), *M. bovigentalium* (Pal et al. 1983), *M. arginini* (Prased et al. 1985) and *M. mycoides* subsp. *capri* (Misri et al. 1988) and in experimental mastitis in sheep caused by *M. canadense* (Ball and Mackie 1986) and due to *M. mycoides* subsp. *mycoides* (LC type) (Banga et al. 1989) and in cows inoculated with *M. bovis* (Bennet and Jasper 1978; Meszaros et al. 1986).

The lipid profile of mammary secretions indicated increased cellular content due to multiplication of acholeplasmas and higher number of leucocytes. It also indicated acholeplasmal lipase activity in the milk/mammary secretions of inoculated udder halves. These changes are similar to those reported by Ragab et al. (1987), Misri et al. (1988) and Banga et al. (1989) in experimental mycoplasmal mastitis in cattle, goats and sheep due to *M. bovigentalium*, *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* (LC type), respectively. The nonspecific lipase, showing optimum activity in alkaline pH range has been observed in some species of *Mycoplasma* (Smith 1979). The moderate increase in cholesterol level in mastitic mammary secretions may be due to the fact that *A. laidlawii* membranes contributed lesser amounts of cholesterol into mammary secretions, as it is known that *A. laidlawii* as compared to mycoplasmas have lower concentration of cholesterol in their membrane. In mycoplasmal mastitis the increased cholesterol is due to hydrolysis of cholesterol esters by *Mycoplasma*. The presence of sterol esterase has also been demonstrated in *M. arthritis*, *M. gallinarum* and *A. laidlawii* (Smith 1979). The increase in total phospholipids content may be due to higher cellular contents of leucocytes, *A. laidlawii* and degenerating mammary cells in the mammary secretions. The presence of acyl COA: glycerophosphate transacylase, synthesising phosphatidic acid, the precursor of membrane phospholipids, has been demonstrated in *A. laidlawii* (Razin 1978). The increase in level of total proteins in mastitic mammary secretions may also be due to increased infiltration of cellular contents, multiplication of *A. laidlawii* and due to passage of mucilaginous secretions and albumin to milk as a result of increased capillary permeability due to mastitis (Schalm 1977). The increase in the levels of immunoglobulins could be due to immunoglobulins produced locally as a result of increased infiltration of lymphocytes or by pouring of immunoglobulins from blood stream. These findings are in accordance with findings of Schalm (1977) and Nolcross (1982). Similar increase in the levels of immunoglobulins has also been reported in infected udder halves of ewes after experimental *M. mycoides* subsp. *mycoides* (LC type) (Singh 1988).

The decrease in total lipids and glycerides indicates increased lipolysis due to lipase action of *A. laidlawii*. The presence of sterol esterase which hydrolyses sterol esters of short chain fatty acids has been demonstrated in *A. laidlawii* (Smith 1979).

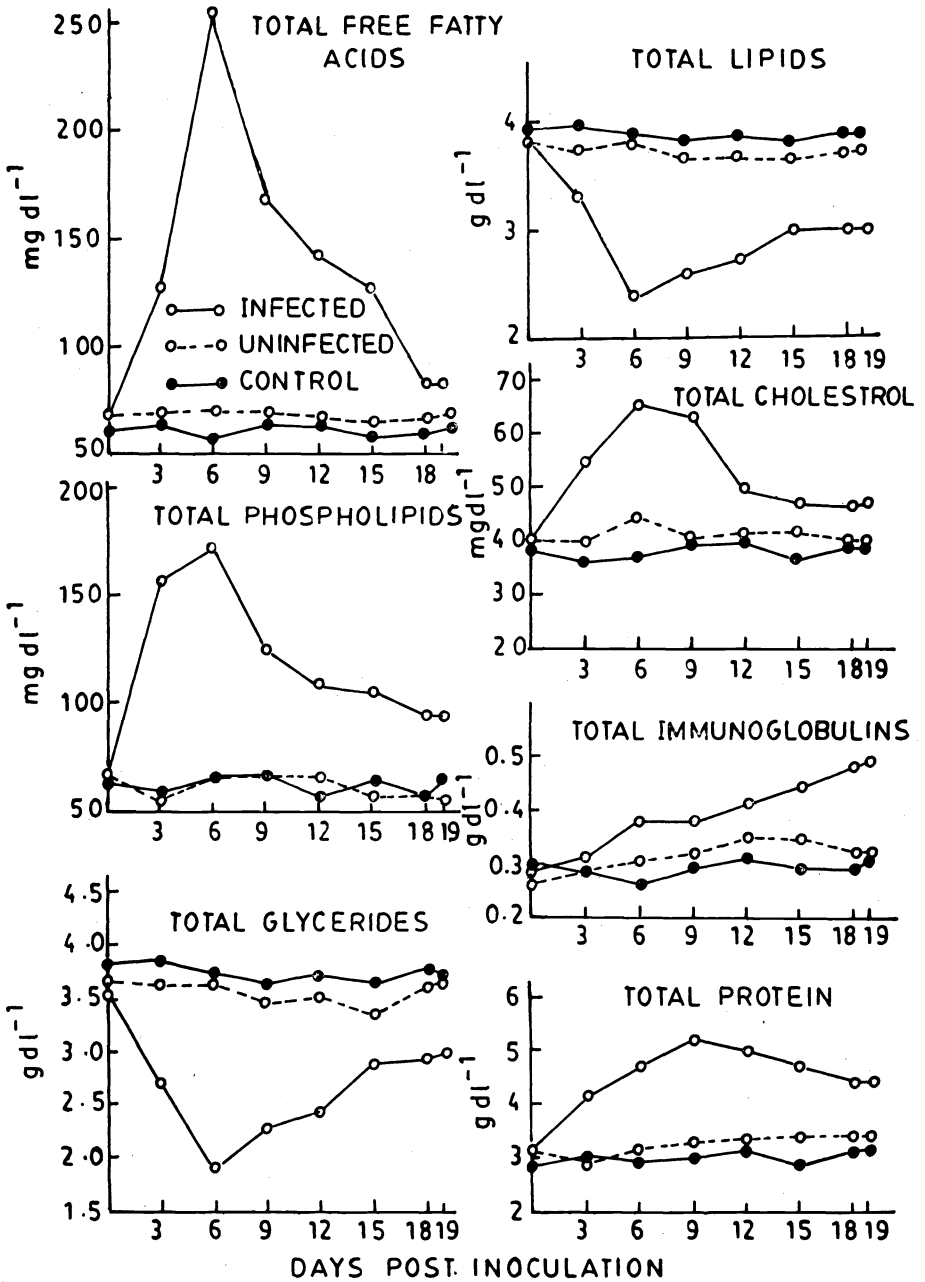


Fig. 6. Progressive changes in the chemical composition of milk/mammary secretion of goats due to experimental intramammary infection with *A. laidlawii*.

The observations reported here and the findings of Ragab et al. (1987), Misri et al. (1988) and Banga et al. (1989) in experimental mycoplasmal mastitis due to *M. bovis genitalium*, *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* (LC type) in cattle, goats and sheep, respectively, indicate that mastitis due to *Mycoplasma* and *Acholeplasma* generally cause hydrolysis of glycerides in milk and catabolise the released fatty acids for their anabolic needs.

Although *A. laidlawii* is considered to be a surface parasite, it was found to be pathogenic to lactating goat udder because it produced severe mastitis, severely damaged the mammary secretory tissue which led to agalactia and caused remarkable biochemical alterations in composition of milk.

Biochemické změny v mléce při experimentální mastitidě koz vyvolané *Acholeplasma laidlawii*

Pro studium biochemických změn v mléce bylo 7 laktujícím kozám unilaterálně inokulováno pravým strukovým kanálkem 2 ml kultury *Acholeplasma laidlawii* obsahující 10^9 životaschopných buněk (colony forming units — CFU) v 1 ml. Levá polovina mléčné žlázy těchto 7 zvířat sloužila jako kontrolní a osmá laktující koza byla použita jako kontrola intaktní. Účinek infekce byl sledován po 19 dnů. Každý třetí den po infekci bylo utraceno 1 zvíře a 19. dne i intaktní kontrola. Všechna zvířata infikovaná *A. laidlawii* jevila do 24 h po infekci příznaky klinické mastitidy, která přetrvávala až do konce pokusu. Množství celkových proteinů, celkové imunoglobuliny (Ig), fosfolipidy a volné mastné kyseliny v mléce/sekretu mléčné žlázy postižené mastitidou, vykazovala progresivní vzestup, zatímco koncentrace celkových lipidů a glyceridů podstatně poklesla. Koncentrace celkového cholesterolu jevila malý vzestup.

Výsledky naznačují, že *A. laidlawii* využívá lipolytické enzymy k degradaci lipidů nutné pro krytí vlastních energetických potřeb a pro biosyntézu membránové lipidové dvojité vrstvy během multiplikace. Vzestup koncentrace mléčných proteinů, zejména Ig, svědčí o humorální imunitní odpovědi na infekci *A. laidlawii* a sekrece Ig do mléčné žlázy.

Биохимические изменения молока при экспериментальном мастите коз, вызванном *Acholeplasma laidlawii*

С целью изучения биохимических изменений молока семи лактирующим козам односторонно инокулировали правым канальцем соска вымени 2 мл культуры *Acholeplasma laidlawii*, содержащей 10^9 жизнеспособных клеток (colony forming units — CFU) на 1 мл. Левая половина молочной железы упомянутых 7 животных стала контрольной и восьмая лактирующая коза — интактная — была использована в качестве сопоставления. Наблюдения за воздействием инфекции вели в течение 19 суток. На третьи сутки инфекции умерщвляли 1 животное и на 19 сутки — интактную лактирующую козу. У всех инфицированных *A. laidlawii* были до 1 суток после инфекции выявлены признаки клинического мастита, имеющего место до конца эксперимента. Количество общих протеинов, общие иммуноглобулины [Ig], фосфоли-

пиды и несвязанные жирные кислоты в молоке/секрете пораженной маститом молочной железы прогрессивно увеличивалось, между тем как концентрация общих липидов и глицеридов существенно понижалась. Концентрация общего холестерина незначительно повышалась.

Результаты свидетельствуют о том, что *A. laidlawii* использует липолитические ферменты для деградации липидов, необходимых для возмещения собственных энергетических потребностей и для биосинтеза мембранного липидного слоя в ходе мультипликации. Увеличение концентрации молочных протеинов, в особенности иммуноглобулинов, свидетельствует о гуморальном иммунитетном ответе на инфекцию *A. laidlawii* и секреции иммуноглобулинов в молочную железу.

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