

Effect of subclinical intramammary infection on somatic cell counts and chemical composition of goats' milk

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We investigated effects of subclinical intramammary infection (IMI) on milk somatic cell count (SCC) and milk composition in udder halves of dairy goats. A total of 35 mixed-age Alpine does (70 udder halves; approximately 55 kg body weight) were rotationally grazed on a mixture of vegetative forages (wheat/berseem clover, sudan grass and cowpeas). Milk samples for bacterial analysis and SCC were collected monthly from both halves from April to September, 2001. Across stages of lactation, 19–31% of udder halves became infected. The prevalence of IMI exhibited quadratic patterns through multi-peaked responses within each stage of lactation. Higher rates of IMI were observed during the early stage of lactation (19% in May) and in the late stage of lactation (31% in September). Coagulase negative *Staphylococcus* (CNS, 43.7%), *Staph. aureus* (35.4%), and *Pseudomonas aeruginosa* (12.4%) were the most prevalent pathogens. Within single-strain IMI, log SCC (6.24) was lower ($P < 0.01$) for CNS than those derived from IMI by *Staph. aureus* (6.49), *Ps. aeruginosa* (6.53) or *Serratia* spp. (6.90). Infected udder halves had a higher average SCC (4761 v. 2259×10^3 cells/ml; $P < 0.01$) than uninfected halves, but uninfected halves often had similar levels of SCC to infected halves. Daily average milk production was not significantly different between infected and non-infected goats and the relationship between IMI and SCC was not always correlated. Effective mastitis screening requires bacteriological culture since SCC was not highly correlated.

Keywords: Goats, mastitis, milk composition, somatic cell count.

Intramammary infection (IMI) increases milk somatic cell count (SCC) and reduces milk production and milk quality in dairy cows (Harmon, 1994), sheep (Watson & Buswell, 1984) and goats (Leitner et al. 2004a). However, this relationship is controversial in dairy goats (Haenlein & Hinckley, 1995; Sanchez et al. 2002).

SCC and IMI are influenced by nutrition, age, stage of lactation, level of production, stress, season and management (Eberhart, 1986; Lerondelle et al. 1992; Gonzalo et al. 1994). Of the indirect methods for detecting subclinical mastitis, milk SCC is the most widely used for dairy cattle, goats and ewes (Zeng et al. 1999; McDougall, 2001). Bacterial infection has previously been shown to increase SCC in sheep and goats (Dulin et al. 1983; Green, 1984; Bergonier et al. 2003; Moroni et al. 2005a,b).

However, there has been considerable controversy on the relationship between SCC and IMI in dairy goats (Smith & Roguinsky, 1977; Sheldrake et al. 1981; Manser, 1986; Paape et al. 2001). Milk secretion in the cow is of the merocrine type but that in the goat is an apocrine process that results in a high number of cytoplasmic particles and epithelial cells apart from leucocytes that have been counted as SCC (Dulin et al. 1983; Park & Humphrey, 1986; Paape et al. 2001). Basal values for SCC in non-infected caprine milk also vary considerably between different herds (Sheldrake et al. 1981). The present study, therefore, investigated effects of IMI status on SCC and milk composition in pastured dairy goats during early, middle and late lactation (April–September, 2001).

Material and Methods

Animals and experimental design

A total of 35 Alpine does (1–5 years old, 55 kg average body weight) were randomly selected from a herd of

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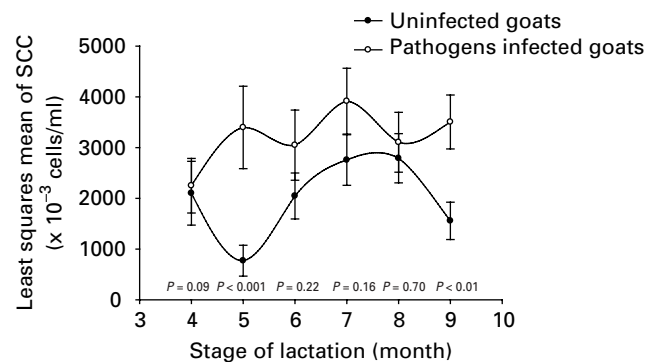
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Table 1. Distribution of bacteria and bacterial groups isolated from dairy goats ($n=35$)†

Organism†	SCC $\times 10^{-3} \pm \text{SE}$	Log SCC $\pm \text{SE}$	Infection rate, %
Coagulase-negative Staphylococci (CNS)	2451 \pm 580 ^c	6.24 \pm 0.11 ^c	43.7
<i>Staph. aureus</i>	4724 \pm 502 ^b	6.49 \pm 0.07 ^b	35.4
<i>Ps. aeruginosa</i>	4593 \pm 105 ^b	6.53 \pm 0.15 ^b	12.4
<i>Serratia</i> spp.	7949 \pm 872 ^a	6.90 \pm 0.05 ^a	2.1
Coliform	1341 \pm 0 ^d	6.12 \pm 0.0 ^d	3.3
Total infected	4761 \pm 750 ^A	6.52 \pm 0.09 ^A	—
Non-infected (78.4%)	2259 \pm 186 ^B	6.05 \pm 0.04 ^B	—

† Milk samples from half udders taken from 6 June to 10 September 2001
^{a,b,c,d} Means with different superscripts are significantly different between pathogenic bacteria ($P < 0.05$)

^{A,B} Means with different superscripts are significantly different between mean values of total infected and non-infected somatic cell counts (SCC; $P < 0.05$)

**Fig. 1.** Effect of IMI and stage of lactation on somatic cell count (SCC; $\times 10^{-3}$ cells/ml) in dairy goats ($n=35$).

44 healthy does. All does kidded between March and April. Kids were removed from does within 24 h of kidding and does were then machine milked twice a day. Milk yields were determined monthly and milk samples were collected at the same time and analysed for SCC, bacteriological status and composition (milk fat, protein, lactose and total milk solids). Mixed vegetative forages (wheat/berseem clover, sudan grass and cowpeas) were rotationally grazed from April to September 2001, with concentrate supplementation. The concentrate mix contained 74.5% rolled corn, 5% whole cottonseed, 16% soybean meal, 2% sodium bicarbonate, 0.2% dicalcium phosphate, 1.6% limestone with trace minerals and vitamin A, D, and E (Min et al. 2005).

Milk sampling

Aseptic foremilk (10 ml) and SCC samples were collected monthly after kidding from April to September 2001.

Table 2. Number and duration of persistent IMI† recorded in the experiment

Organism†	Persistent IMI	Sampling detected‡		True IMI infection rate (%)
		(no.)	period	
Coagulase-negative Staphylococci (CNS)	11	2	1–2	42.3
		2§	2–4	
		2	3–5	
		3	3–6	
		2	4–6	
		2	4–5	
		2	5–6	
		2	5–6	
		2	5–6	
		2	5–6	
		2	5–6	
<i>Staph. aureus</i>	8	3	1–3	30.8
		5	1–5	
		5§	1–6	
		2	5–6	
		3§	2–6	
		2	3–4	
		3	3–5	
		2	4–5	
		2	4–5	
		2	4–5	
		2	4–5	
<i>Ps. aeruginosa</i>	3	3§	2–5	11.5
		2§	1–5	
		2	2–3	
<i>Serratia</i> spp.	1	2	3–4	3.8
Coliform	2	2	3–4	7.8
		2	3–4	
Total	25	—	—	100

† The same pathogen was isolated two or more times from the same half of the udder

‡ The number of sampling in which the pathogens were consecutively isolated and the duration of this period

§ Bacteria were not detected on one occasion

Before the morning milking, teats were thoroughly cleaned with a cotton swab soaked in 70% ethanol. The first streams of foremilk were discarded and about 10 ml milk was collected from each half. Samples were kept on ice for a maximum of 2–4 h until bacteriological analysis. Additional samples (50 ml) were collected from each half for SCC determination and composition analysis.

Laboratory analysis

Ten microlitres of milk were plated on aesculin blood agar and examined after 24 h and 48 h incubation at 37 °C for bacterial growth. Growth of 5 or more colony forming units (cfu) of one colony type was considered positive for an IMI. Bacteria were identified according to the recommendations of the National Mastitis Council (Harmon et al. 1990). Bacterial isolates were identified by commercial micromethods (API[®]; BioMerieux, Lyon, France).

Table 3. The incidence of IMI in each month of lactation in Alpine dairy goats

	Month					
	April	May	June	July	August	September
Number of animals	35	35	35	35	35	35
Number of glands infected						
New infection	13	13	6	7	10	11
Old infection	—	7	10	8	9	11
Total infected glands	13	20	16	15	19	22
Infection rate (%)	19	29	23	21	27	31
Incidence rate of new infection (%)† (new infection/gland)	—	22.8	10.5	10.9	15.8	18.3

† The incidence of new IMI was calculated as the total number of new IMI divided by the total number of uninfected glands at the previous sampling

A new IMI was diagnosed when 5 or more cfu of one colony type was isolated from a previously uninfected gland. Milk composition (percentage of fat, protein, lactose and total solids) and SCC were analysed by infrared spectroscopy (DairyLab II; Foss Food Technology, Eden Prairie MN, USA) and Fossomatic cell counter (Foss Food Technology), respectively.

Statistical analysis

SCC values were transformed to \log_{10} for statistical analysis and expressed as geometric means. SCC was analysed by least squares means techniques using the Mixed Models procedure of SAS (SAS, 1990). Effect of bacterial pathogens within IMI status was divided into five groups: coagulase negative *Staphylococci* (CNS), *Staph. aureus*, *Serratia* spp., *Ps. aeruginosa*, and coliform bacteria. Data for distribution (%) of bacteria and bacterial groups were transformed using a Logistic Regression Model (SAS, 1995) and treatment effects were established using the chi-squared procedure, as described by Min et al. (1999) and Moroni et al. (2005a, b). The relationship between SCC and the IMI status of the half udders was studied according to methodologies described by Andrews et al. (1983). The relationship between SCC, milk yield, and milk composition was analysed using a correlation coefficient procedure (SAS, 1990). When the same pathogen was isolated two or more times from the same half udder, it was considered a true-positive diagnosis of IMI (Table 2; Contreras et al. 1997). When a pathogen was isolated only once from duplicate milk samples from the same half udder, it was considered a false-positive diagnosis of IMI. The incidence of new IMI was calculated as the total number of new IMI divided by the total number of uninfected glands at the previous sampling (Table 3).

Results

IMI status was the most important factor influencing log SCC (Table 1). Infected glands during mid to late

lactation (June–September) had higher SCC than uninfected glands (4761 v. 2259×10^3 cells/ml, respectively, $P < 0.01$). However, results in Fig. 1 provide some evidence that SCC of the uninfected udder halves were not always lower than infected udder halves.

Among the 420 samples analysed, bacteria were recovered from 110 samples (26.2%). CNS accounted for 43.7% of the bacterial isolates; *Staph. aureus*, 35.4%; and *Ps. aeruginosa*, 12.4% (Table 1). SCC values were dependent on the pathogen infecting the mammary gland. CNS appeared most frequently in IMI, and log SCC (6.24) was the lowest ($P < 0.01$) among SCC from IMI caused by *Staph. aureus* (6.49), *Ps. aeruginosa* (6.53) and *Serratia* spp. (6.90; Table 1).

Of the 70 udder halves studied, 25 IMI were detected (37%). These IMI were caused by five separate pathogens (Tables 1 and 2). Two of the pathogens were *Staphylococcus* spp. Other pathogens included *Ps. aeruginosa*, *Serratia* spp. and coliform bacteria. The most prevalent isolates were CNS and *Staph. aureus*. Duration of IMI ranged from two to six consecutive samplings.

Across stage of lactation, 19–31% of udder halves were infected from April to September 2001 (Table 3). Higher rates of IMI were observed during the early stage of lactation (29% in May) and the late stage of lactation (31% in September). The prevalence of IMI in dairy goats exhibited quadratic patterns, through multi-peaked responses within stage of lactation. This coincided with incidence of new IMI. The incidence rate of new infection was highest during the early stage of lactation (23% in May) and the late stage of lactation (18% in September).

In uninfected mammary glands (Fig. 1), SCC decreased as milk production increased (Fig. 2), reaching a minimum value of approximately 1000×10^3 cells/ml during the fourth and fifth month of lactation. This coincided with peak milk production. There was no interaction ($P = 0.41$) between IMI status (infected, not infected) by stage of lactation for SCC.

SCC was negatively correlated with daily milk yield ($P < 0.05$), milk fat ($P < 0.05$), lactose ($P < 0.01$) and total solids ($P < 0.05$; Table 4). However, results in Fig. 2 show

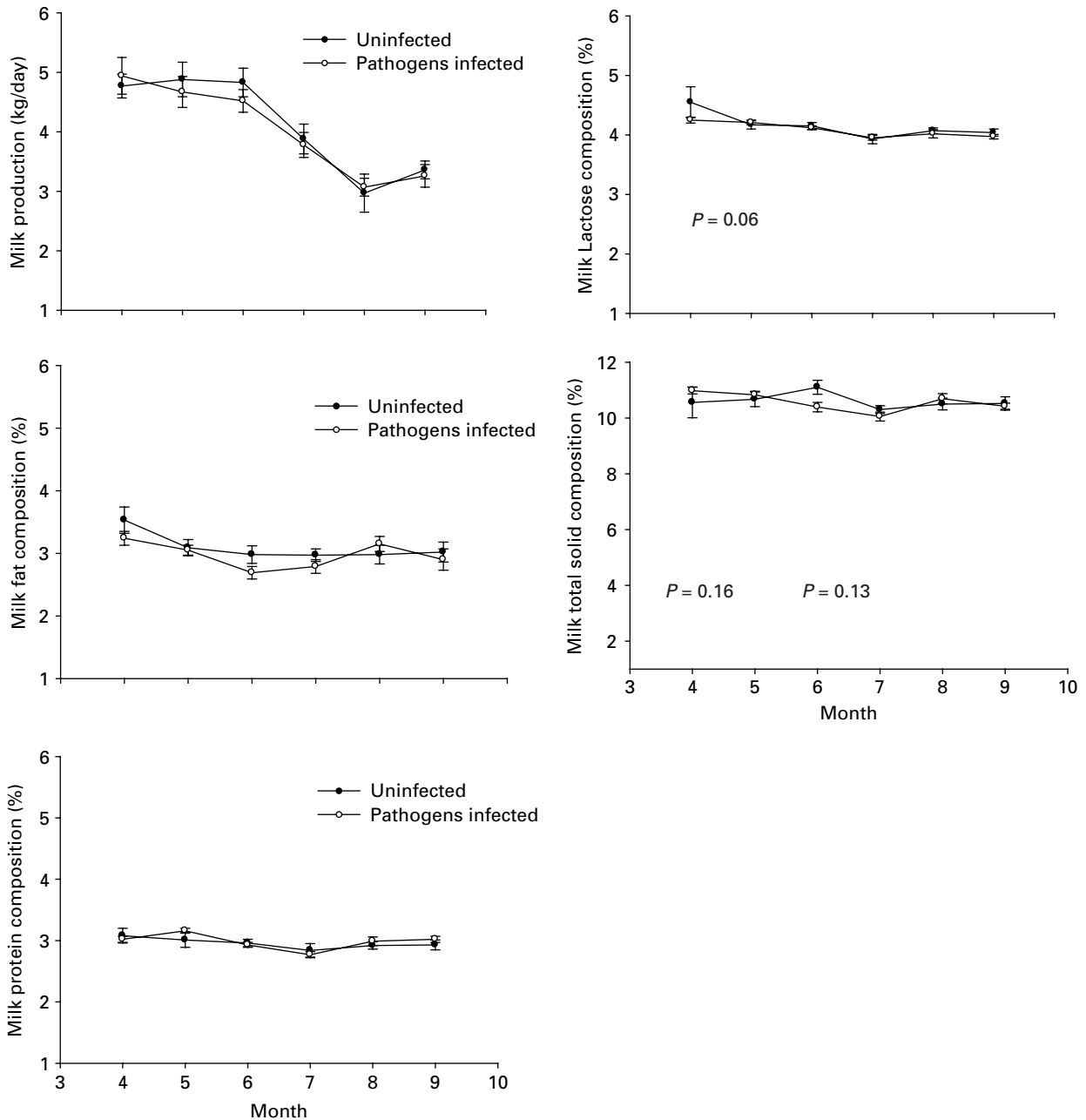


Fig. 2. Mean monthly milk chemical compositions for grazing dairy goats. Mixed vegetative forages were rotationally grazed by the goats ($n=35$). Vertical error bars represent SEM.

that daily average milk production was not significantly different between infected and non-infected goats, with no interaction ($P=0.87$) between IMI and stage of lactation. This suggests that the interrelationship between IMI and milk yield may not be correlated and an increase in SCC may be independent of IMI. Effects of mammary gland infection status in alpine dairy goats were not significantly different ($P>0.05$) for milk fat, protein and total solids (Fig. 2). There was no significant interaction

($P=0.44-0.69$) between IMI status and stage of lactation for milk chemical composition (fat, protein, lactose and total solids).

Average percentage of lactose tended to be lower ($P=0.06$) in milk from infected udder halves than from uninfected udder halves during early and mid stages of lactation (April–June; Fig. 2). Average milk production ($P<0.01$), percent fat ($P<0.01$), protein ($P<0.02$) and lactose ($P<0.01$) decreased as lactation progressed (Fig. 2).

Table 4. Correlation coefficient (R^2) matrix for measured parameters from dairy goats ($n=35$)

	<i>nt</i>	SCC‡ (log)	Milk yield (kg/d)	Fat (%)	Protein (%)	Lactose (%)	Total solids (%)
SSC	206	1.00					
Milk yield	208	-0.15*	1.00				
Fat	214	-0.14*	0.05	1.00			
Protein	210	0.07	-0.04	0.52***	1.00		
Lactose	208	-0.19**	0.28***	2.00**	0.14*	1.00	
Total solids	205	-0.17**	0.09	0.84**	0.69***	0.44***	1.00

† Number of observations

‡ Somatic cell count

Statistical significance: * $P < 0.04$; ** $P < 0.01$; *** $P < 0.001$; values without asterisks are not significant ($P > 0.05$)

Discussion

Similarly to results obtained by others (Contreras et al. 1997; Moroni et al. 2005a,b), the present study showed a high percentage of IMI caused by CNS and *Staph. aureus* and a low percentage of IMI caused by other pathogens (Gram-negative bacteria). SCC was negatively correlated with daily milk yield, milk fat, lactose and total solids. However, daily average milk production and certain milk components were not significantly different between infected and non-infected goats.

In the present study, milk collected from infected glands had higher SCC than uninfected glands. The prevalence of IMI in dairy goats exhibited quadratic patterns, through multi-peaked responses within stage of lactation. This is a similar trend with the incidence of the IMI status. Higher rates of IMI were observed during the early stage of lactation (29% in May) and the late stage of lactation (31% in September). This is a similar trend to another study in goats (Bergonier et al. 2003). However, Fig. 1 shows that the SCC of healthy udder halves (uninfected) was not always lower than that of infected udder halves. Studies have shown similar results where a controversy exists between the relationship of SCC and IMI (Smith & Roguinsky, 1977; Sheldrake et al. 1981; Paape et al. 2001). It has been shown that SCC increased with increasing stage of lactation, but there were no significant difference in SCC between infected and uninfected udder halves at the end of lactation period (Moroni et al. 2005c). This demonstrates that effective mastitis screening of dairy goats requires bacteriological culture of milk samples since SCC were not highly correlated with IMI.

Dairy goat studies in the USA (Haenlein & Hinckley, 1995) and in France (Cremoux, 1998) show the seasonal nature of SCC, which increased at the beginning and the end of lactation where SCC was above the legal limits (1113×10^3 cells/ml). Similarly, results from uninfected Alpine dairy goats in the present study showed large seasonal variations in SCC from 1000 to 3000×10^3 cells/ml. These results were much higher than in previous reports (Haenlein & Hinckley, 1995). Results from the current study and a recent review article (Bergonier et al. 2003) demonstrated that SCC in goats consistently

increases with stage of lactation. Average SCC in dairy goats (>2000 to $<4000 \times 10^3$ cells/ml) is much higher than in ewes ($<2000 \times 10^3$ cells/ml). It is reported that most new subclinical infections in cows occur during the first 3 months of lactation (Rainard & Poutrel, 1982). Recently, Moroni et al. (2005c) reported that somatic cell score continuously increased with increasing days in milk (day 0–290). However, large variations in the prevalence of IMI were observed across stage of lactation suggesting that a larger sample size is needed in order to better assess the dynamics of udder health during this period. Infections in one udder half appears to have an influence on the adjacent udder half by the observation of elevated SCC in the non-infected half (Dulin et al. 1983; Moroni et al. 2005b). In addition, milk from healthy goat udders tends to have elevated SCC for physiological reasons and this depends on stage of lactation (Haenlein et al. 2002). This result differs from that of Davis et al. (2004) in dairy cattle, where no increase in SCC was observed in healthy quarters of infected cows.

Across stage of lactation, 19–31% of udder halves were infected from April to September and this is similar to findings of Contreras et al. (1995) and McDougall et al. (2002). The prevalence of halves with IMI was much higher in SCC for dairy goats (35.5%) than for sheep (19.0%; McDougall et al. 2002). In addition, in Italian Alpine dairy goats, IMI occurrence was 40.2% during a complete production season (January–November 2000; Moroni et al. 2005c).

Mastitis-causing bacteria can be mainly divided in two categories: contagious pathogens, which include *Streptococcus agalactiae* and *Staph. aureus*, and environmental pathogens, which include Gram-negative bacteria and *Streptococcus* species other than *Str. agalactiae* (Eberhart et al. 1979; Eberhart, 1986). In the present study, CNS (43.7%), *Staph. aureus* (35.4%), and *Ps. aeruginosa* (12.4%) were the most prevalent isolates. CNS were the most frequently isolated bacteria and have been shown to be the predominant bacteria isolated from the bovine streak canal (Harmon et al. 1995). Within single-strain IMI, CNS appear most frequently, and log SCC (6.24) was lower in CNS infected goats than goats infected by *Staph. aureus* (6.49), *Ps. aeruginosa* (6.53) and *Serratia* spp.

(6–90). However, among CNS, *Staph. epidermidis* is generally associated with the highest values of SCC in both ewes and goats (Bergonier et al. 2003). Most CNS isolates (60–80%) produced alpha, beta, or synergistic haemolysis and these haemolytic patterns induced significantly higher SCC than non-haemolytic isolates (Bergonier et al. 2003). These data suggest that *Staph. aureus*, *Ps. aeruginosa* and *Serratia* spp. cause a much greater immune response and could potentially lead to more damage to udder tissue (Moroni et al. 2005b). This trend was similar to results from other studies with dairy ewes, cows and goats (Reneau, 1986; Upadhyaya & Rao, 1993; Gonzalez-Rodriguez et al. 1995; McDougall et al. 2002). In addition, Bergonier et al. (2003) noted that CNS was the most prevalent *Staphylococcus* spp. ranging from 25 to 93% of those isolated.

IMI increases milk SCC and reduces milk yield and milk quality in dairy cows (Harmon, 1994), sheep (Watson & Buswell, 1984) and goats (Leitner et al. 2004a). This relationship, however, is controversial in dairy goats (Haenlein & Hinckley, 1995; Sanchez et al. 2002). In the present study, SCC was negatively correlated with daily milk yield, milk fat, lactose and total solids. However, average daily milk production was not significantly different between infected and non-infected goats, possibly due to the stage of lactation and the dominance of seasonal breeding of goats (Haenlein, 2002). In addition, the bacteriological status (infected or uninfected) of goat udders did not affect daily milk production and milk protein concentrations in the current study. The results are in contrast to those in the literature (Leitner et al. 2004a, b) which report increased milk protein concentrations when dairy goats have IMI. Across stage of lactation, overall percentage of lactose tended to be lower in milk from infected half udders than for uninfected halves during early lactation. The decrease in lactose concentration in the infected halves is consistent with findings in sheep (Leitner et al. 2003) and goats (Leitner et al. 2004a, b). Average milk production, concentrations of fat, protein, lactose and total solids decreased as lactation progressed.

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