Relationship of Somatic Cell Count, Physical, Chemical and Enzymatic Properties to the Bacterial Standard Plate Count in Different Breeds of Dairy Goats

Chingwen Ying[1], Cheng-Bin Yang and Jih-Tay Hsu*

Department of Animal Science, National Taiwan University, No. 50, Lane 155, Kee-Lung Rd. Sec. 3
Taipei, Taiwan 106, ROC

ABSTRACT : The objective of the present study was to investigate the accuracy of mastitis diagnostic indicators for different dairy goat breeds. Biweekly milk samples were collected from individual half mammary gland of seven Saanen and seven Alpine dairy goats in the period of 40 to 120 days in milk. With threshold value set at 2.8 and 3.1 for Alpine and Saanen dairy goats, respectively, log (SPC) offered good sensitivity (0.89, 0.93), specificity (0.88, 0.95), positive predictive value (0.75, 0.85) and negative predictive value (0.95, 0.98) as a mastitis diagnostic tool. The correlations of log (SPC) with milk yield, log (SCC), ALP, LDH, Na^-, K^+ and EC were significant in Saanen dairy goats ($p<0.05$), with the highest correlation coefficient (0.653) existing between log (SPC) and log (SCC). The correlations of log (SPC) with milk yield, milk fat, milk protein, log (SCC), Na^-, K^-, EC were significant in Alpine dairy goats ($p<0.05$), with the highest correlation coefficient (0.416) existing between log (SPC) and log (SCC). There were different best-fit regression equations with different multiple diagnostic indicators for Saanen and Alpine dairy goats. In conclusion, different breeds of dairy goats may have to adapt different mastitis diagnostic parameters for a better diagnosis. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 4 : 554-559)***

Key Words : Goat Milk, Standard Plate Count, Somatic Cell Count, Lactate Dehydrogenase, Alkaline Phosphatase, Sodium, Potassium, Electrical Conductivity

INTRODUCTION

Somatic cell count (SCC) and bacterial standard plate count (SPC) have been shown to be negatively correlated ($p<0.001$) with milk yield (Zeng and Escobar, 1995) and cheese yield (Galina et al., 1996) in dairy goat. The importance of the SCC and SPC can also be shown in the quality regulation of dairy goat milk (Wilson et al., 1995; Zeng and Escobar, 1995). SCC has been successfully used in dairy cattle for monitoring mastitis (Harmon, 1994). However, SCC was not found satisfactory for diagnosis of mastitis in dairy goat (Park and Humphrey, 1986; Smith and Sherman, 1994). California Mastitis Test (CMT) was another common method in detection of mastitis in dairy cattle. However, CMT failed to accurately detect the subclinical mastitis in dairy goat (Upadhyaya and Rao, 1993; Boscos et al., 1996; Contreras et al., 1996). McDougall et al. (2001) concluded that SCC was a better predictor of mammary gland bacteriological status than CMT in both goats and sheep in early lactation. Ying et al. (2002) also found a significant positive correlation between log (SPC) and log (SCC) for Alpine dairy goats in early lactation, but not in late lactation or in commercial dairy goat herd tank milk samples. Maisi (1990) found no better performance of N-acetyl-β-D-glucosaminidase than CMT in diagnosis of dairy goat subclinical mastitis. Neutrophils and lymphocytes numbers in the goat milk have been evaluated for monitoring mammary gland infection (Dulin et al., 1983; Droke et al., 1993; Ying et al., 2002). Electrical conductivity (EC) has not been found satisfactory for correlation with SPC (Park, 1991; Ying et al., 2002). Lactate dehydrogenase (LDH), protease and alkaline phosphatase (ALP) have been evaluated in dairy cattle (Bogin et al., 1977; Kitchen, 1981) and dairy goats (Ying et al., 2002). The objective of the present study is to examine whether the relationship of the logarithm of SPC to EC, LDH, ALP, the logarithm of SCC and various milk constituents (fat, protein, lactose, K^-, Na^+) will differ for different breeds of dairy goats.

* Corresponding Author: J.-T. Hsu. Tel: +886-2-87321471, Fax: +886-2-27324070, E-mail: jthsu@ccms.ntu.edu.tw
[1] Department of Microbiology, Soochow University, No. 70, Lin-His Rd. Taipei, Taiwan 11102, ROC
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MATERIALS AND METHODS

Animals

Seven each of synchronized bred and kidding first lactation Alpine and Saanen dairy goats (avg. BW 50 kg) with avg. 40 days in milk were used in the present study. The animals were housed in a sheltered concrete pen and fed twice daily of lactation diet composed of 30% alfalfa hay, 41% corn, 18% soybeans, 6% molasses, 3% soybean oil and 2% vitamin and mineral premix to meet the NRC (1981) nutrient recommendation for lactating goat (BW=50 kg, 4% FCM=2 kg, DMI=1.81 kg/d). Clean drinking water

Table 1. Milk yields and milk compositions of seven Saanen and seven Alpine first lactation dairy goats examined in the present study

Item	Mean and standard deviation
Milk yield (g/milking)	535±251
Milk fat (%)	3.8±1.3
Milk protein (%)	3.7±0.6
Lactose (%)	4.1±0.6
Somatic cell count (10^3/ml)	1,086±1876
Lactate dehydrogenase (U/L)	246±407
Alkaline phosphatase (U/L)	117±209
Na^+ (ppm)	382±176
K^+ (ppm)	2,386±407
Electrical conductivity (ms/cm)	5.76±0.56

and salt block were freely accessible to the animals. Goats were milked twice daily at 8:00 and 17:00.

Sampling

Experimental period was 80 days (from 40 to 120 days in milk). Biweekly AM and PM milk samples of individual udder half of each goat were first obtained aseptically 15 ml for bacterial culturing, then the rest of milk were milked into separated container connected to the mobile milking unit (model Almatic, Alfa Laval Agri, Sweden). Aliquots of 150 ml individual udder half milk samples were saved for all other measurements.

Bacterial culturing

Milk samples were subjected to standard plate count by using Petrifilm AC (3M Co., USA) incubated at 35°C for 48 h. In the same time, milk samples were also subjected to selective culture counts of *Staphylococcus* (Medium No. 110; DIFCO, USA), and *Pseudomonas* (DIFCO No. 0927-17-1) according to FDA (1984), *Bacillus* according to Turnbull and Kramer (1991), *Streptococcus* by Columbia blood agar base (Oxoid, England) added with *Streptococcus* selective supplement SR126 (Oxoid, England) according to MacFaddin (1985), coliform by Coliform Count Petrifilm (3M Co., USA). Serial dilution method of the most probable number was employed for all the bacterial count methods. This will allow detection of one bacterial colony per gram of milk samples. Based on the selective culture counts of pathogens, we examined the sensitivity, specificity, positive predictive value, and negative predictive value for log (SPC) to serve as a suitable diagnostic method for dairy goat mastitis according to Martin et al. (1987).

Physical and chemical measurements

Using hand held electrical conductivity meter (Suntex SC-120, Taiwan), EC of individual milk sample was measured immediately after sampling from the container of milking unit. Portion of 100 ml from each sample was subjected to the automatic measuring of milk fat, milk protein, and lactose contents by Bentley 2000 (USA) and SCC by Bentley SCC 300 (USA) calibrated with goat milk standards. Bentley 2000 unit, using 2-15 nm mid-infrared wavebands conforming to AOAC (1990) method 16.034. Bentley SCC 300 used ethidium bromide to stain the DNA in somatic cells and laser based flow cytometry for automatic counting, meeting the IDF standard 148:1991. Correlation coefficient (0.43) of neutrophils/lymphocyte in relation to log (SPC) was found similar to the correlation coefficient (0.42) of log (SCC) in relation to log (SPC) for dairy goat milk of the early lactation in our previous study (Ying et al., 2002). Shailja and Singh (2002) also observed a highly positive correlation between SCC and neutrophil ($r=0.7460$; $p<0.01$) in both of cow and buffalo milk samples. Therefore, the measurements of neutrophils/lymphocyte were not performed in the present study. The rest of milk samples were used for enzymatic activity measurement of LDH (LDH SFBC kit, Art. 0736570, Roche Co., USA; Bogin and Ziv, 1973), ALP (ALP IFCC kit, Art. 0736333, Roche Co., USA; Bogin and Ziv, 1973), and concentrations of Na^+ and K^+ (AOAC, 1990). The correlation coefficients (0.44 vs. 0.41 vs. 0.43) of protease, LDH and ALP activities in relation to log (SPC) were similar to each other for dairy goat milk of the early lactation in our previous study (Ying et al., 2002). Also, the correlation coefficients (-0.33 vs. -0.35 vs. -0.32 vs. -0.33) for Na^-, K^+, P and Cl^- in relation to log (SPC) were similar in our previous study (Ying et al., 2002). To balance the laboratory work loading, the determinations of protease activity, P and Cl^- were all omitted from the present study.

Statistic analysis

Data of the measured parameters were tested for correlation significance by Pearson Correlation Coefficients, and the best fit multiple regression equation identified by stepwise method using the SAS system for Windows (SAS 6.11, TS040, SAS Institute). Comparison of measured parameters between infected and uninfected udder halves within each dairy goat breed was done by general linear model (GLM) and least squares mean (LSM). The SCC and SPC measurements were transferred to logarithm before testing the correlation with other measurements.

RESULTS AND DISCUSSION

Total of 392 samples from seven Saanen and seven Alpine first lactation dairy goats in early lactation (40 to 120 days in milk) were examined in the present study. The mean and standard deviation values of milk yield, milk fat, milk protein, lactose, somatic cell count were listed in Table 1, respectively. Small group sizes of lactating goats used in the present study was due to the effort to minimize the

Table 2. Comparison of the measured parameters between infected and uninfected udder halves in Saanen and Alpine dairy goats

Item	Saanen		Alpine	
	Uninfected	Infected	Uninfected	Infected
Milk yield (g/milking)	614±260	355**±140	586±234	322**±111
Milk fat (%)	4.1±1.3	4.0±1.3	3.2±1.0	4.4**±1.6
Milk protein (%)	3.6±0.5	3.8*±0.6	3.7±0.6	4.0**±0.7
Lactose (%)	4.2±0.6	4.1±0.6	4.0±0.5	4.1±0.6
Somatic cell count (10^3/mL)	679±1,378	3,405**±3,189	746±1,096	1,366*±2,135
log (SCC)	5.3±0.6	6.4**±0.4	5.6±0.5	5.9**±0.5
Electrical conductivity (ms/cm)	5.6±0.4	5.8**±0.6	6.1±0.3	5.4**±0.8
Lactate dehydogenase (U/L)	248±489	328±425	237±380	203±87
Alkaline phosphatase (U/L)	115±232	114±343	122±166	90±63
Na^+ (ppm)	322±134	506**±330	393±129	428±154
K^- (ppm)	2,463±432	2,264**±364	2,447±310	2,117**±455
Standard plate count (cfu/ml)	1,343±13,606	19,111**±4,4635	598±3185	9,515**±27,957
log (SPC)	1.4±1.0	3.9**±0.6	1.2±1.1	3.4**±0.7

* $p<0.05$, ** $p<0.01$.

Table 3. Sensitivity, specificity, positive predictive and negative predictive values for each selected threshold of log (SPC)[1] in udder half milk samples of Alpine dairy goats

Threshold of log (SPC)	Sensitivity	Specificity	Positive predictive value	Negative predictive value
2.5	0.98	0.84	0.71	0.99
2.6	0.96	0.85	0.72	0.98
2.7	0.93	0.86	0.72	0.97
2.8	0.89	0.88	0.75	0.95
2.9	0.75	0.89	0.72	0.90
3.0	0.68	0.89	0.72	0.87

[1]SPC, standard plate counts.

animal number used for experiment according to the general consideration of animal welfare issue. Efforts of reducing variation were implemented by synchronized breeding and using solely first lactation does which can avoid the interference of lactation stage and parity. The statistics of milk fat and milk protein contents for Alpine, LaMancha, Nubian, Saanen and Toggenburg in USA from 1979 to 1992 were 3.58±0.079, 3.04±0.027; 3.81±0.047, 3.24±0.107; 4.51±0.048, 3.66±0.039; 3.47±0.063, 3.03±0.017; 3.34±0.059, 2.95±0.041, respectively (Wierschem, 1993). The milk fat and protein contents of the present study were within the reported ranges for dairy goats. The average lactose content of the present study was comparable to reported average of 4.42% (Zeng and Escorba, 1995), 4.17% (Zeng et al., 1997) and 3.98-4.73% (Upadhyaya and Rao, 1993). The Na^- and K^+ concentrations of the present study were similar to reported goat milk range of 380-580 and 1,400-2,420 ppm, respectively (Jenness, 1980; Jandal, 1996).

Based on the pathogen bacterial culturing results, seven of the 28 individual udder halves were infected (infection rate 25%) in the present study. Among the seven infected udder halves, three were infected by *Staphylococcus*, the other three were infected by *Bacillus*, and one was infected by both *Staphylococcus* and *Bacillus*. *Staphylococcus* and *Bacillus* were the most often reported predominant bacteria in the goat milk (Kalogridou-Vassiliadou, 1991; Park, 1991; Egwu et al., 1994; Sung et al., 1999). The prevalence of bacterial infection of mammary gland in goats was reported in range of 17.7 to 28.7% (McDougall et al., 2000,2001; Ndegwa et al., 2001). The bacterial infection rate and predominant pathogen revealed in the present study were all reasonably similar to previous literatures. Judged by the selective culture counts of pathogens, we compared the measured parameters between infected and uninfected udder halves within each goat breed (Table 2). Infected udder halves of Saanen dairy goats showed significantly higher log (SPC), log (SCC), milk protein, EC and Na^- concentration, and lower milk yield and K^- concentration than those uninfected udder halves. Alpine dairy goats had similar responses in log (SPC), log (SCC), milk protein and K^+ concentration as Saanen dairy goats. However, in contrast to Saanen dairy goats, Alpine dairy goats had significantly higher milk fat and lower EC for infected udder halves. Baudry et al. (1997) surveyed the dairy farms in western France and found a lower milk fat content in goats with higher SCC. In contrast, Sung et al. (1999) surveyed four breeds of dairy goats in Taiwan, and found a positive correlation between percent milk fat and SCC. Stehling et al. (1986) found no effect of Staphylococcal and Streptococcal enterotoxins induced mastitis on the milk fat content of goats. In dairy cows, Hortet and Seegers (1998) concluded that there still was contradictory in the literature data regarding the changes of milk fat and protein due to mastitis. The lower EC shown in infected udder halves of Alpine goats could be related to the higher milk fat content. Woolford et al. (1998) reported a decrease of EC with the increase of milk fat content in dairy cows.

We also examined the sensitivity, specificity, positive predictive value and negative predictive value for log (SPC)

Table 4. Sensitivity, specificity, positive predictive and negative predictive values for each selected threshold of log (SPC)[1] in udder half milk samples of Saanen dairy goats

Threshold of log (SPC)	Sensitivity	Specificity	Positive predictive value	Negative predictive value
2.5	1.00	0.86	0.66	1.00
2.6	1.00	0.87	0.68	1.00
2.7	1.00	0.88	0.69	1.00
2.8	1.00	0.90	0.72	1.00
2.9	0.98	0.92	0.76	0.99
3.0	0.95	0.93	0.78	0.99
3.1	0.93	0.95	0.85	0.98
3.2	0.90	0.97	0.88	0.97
3.3	0.88	0.97	0.88	0.97
3.4	0.79	0.98	0.92	0.93
3.5	0.74	0.98	0.91	0.93

[1] SPC, standard plate counts.

as a diagnostic tool for dairy goat mastitis according to Martin et al. (1987). For Alpine goats, the threshold of log (SPC) set at 2.8 could offer a rather well mastitis diagnosis with the sensitivity, specificity, positive predictive value, and negative predictive value as 0.89, 0.88, 0.75 and 0.95, respectively (Table 3). For Saanen goats, the threshold of log (SPC) set at 3.1 could offer a reasonably good diagnosis of mastitis with the sensitivity, specificity, positive predictive value, and negative predictive value as 0.93, 0.95, 0.85 and 0.98, respectively (Table 4). It appeared that threshold of log (SPC) for mastitis diagnosis differed for Alpine and Saanen dairy goats.

For Saanen dairy goats, log (SPC) had a significant positive correlation with log (SCC), EC, LDH, ALP and Na^+ concentration and a significant negative correlation with milk yield and K^- concentration (Table 5). Among all the parameters, log (SCC) showed the highest correlation coefficient (0.653) with log (SPC). Different than the Saanen dairy goats, Alpine dairy goats showed significant positive correlation to log (SPC) with milk fat and milk protein content, but not with LDH and ALP (Table 6). Again, log (SCC) showed the highest correlation coefficient (0.416) with log (SPC) in Alpine dairy goats. There were also differences in the results of correlation analysis of measured parameters to log (SCC) between Saanen and Alpine dairy goats in the present study. Saanen dairy goats had significant negative correlation of lactose and K^- concentration with log (SCC) which was not seen in Alpine dairy goats. Alpine dairy goats had a significant positive correlation of milk protein with log (SCC) which was not the case in Saanen dairy goats.

Based on the stepwise analysis, the best-fit regression equations for log (SPC) and log (SCC) in two different breeds of dairy goats were as follows:

Saanen:

- log (SPC)=-10.59075-0.00080×milk yield (g/milking)+0.49454×milk protein (%)+0.59607×lactose (%)+1.15047×log(SCC)+0.41561×EC(ms/cm). R^2=0.51
- log (SCC)=7.14700-0.00030×milk yield (g/milking)-0.33865×milk protein (%)-0.26660×lactose (%)+0.00019×LDH (U/L)+0.00065×Na^+ (ppm)+0.29278×log (SPC), R^2=0.57

Alpine:

- log (SPC)=-1.58256+0.39667×milk protein (%)+1.02832× log(SCC)-0.65675×EC (ms/cm), R^2=0.32
- log (SCC)=3.28510-0.00073×milk yield (g/milking)-0.06061×fat (%)+0.30598×EC (ms/cm)+0.00050×LDH (U/L)+0.00178×ALP (U/L)+0.00087×Na^+ (ppm)+0.12089×log (SPC). R^2=0.47

Similar to the finding of our previous study (Ying et al., 2002), the R^2 values for best-fit multiple regression equations were higher for log (SCC) than log (SPC). These implied that most measured parameters were more closely related to SCC than SPC. The equations reported here are intended to identify what parameters will vary closely along

Table 5. Correlation coefficients among milk variables in udder halves of Saanen dairy goats

Item[-]	Milk	Fat	Protein	Lactose	log (SCC)	EC	LDH	ALP	Na^-	K^+	log (SPC)
Milk	1										
Fat	-0.410**	1									
Protein	0.033	-0.593**	1								
Lactose	0.132	0.122	-0.441**	1							
log (SCC)	-0.406**	0.111	-0.022	-0.204*	1						
EC	0.188**	-0.529**	0.288**	-0.280**	0.089	1					
LDH	-0.212**	0.001	0.076	-0.088	0.354**	0.047	1				
ALP	-0.144*	-0.079	0.168*	-0.074	0.259**	0.026	0.908**	1			
Na^+	-0.295**	-0.074	0.286**	-0.289**	0.459**	0.366**	0.319**	0.251**	1		
K^-	0.364**	-0.275**	0.115	0.157*	-0.275**	0.199**	-0.143*	-0.129*	-0.085	1	
log (SPC)	-0.351**	-0.010	0.086	-0.001	0.653**	0.183*	0.250**	0.163*	0.423**	-0.237**	1

[-] log (SCC), logarithm of somatic cell count; EC, electrical conductivity; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; log(SPC), logarithm of standard plate count. * $p<0.05$, ** $p<0.01$.

Table 6. Correlation coefficients among milk variables in udder halves of Alpine dairy goats

Item	Milk	Fat	Protein	Lactose	log (SCC)	EC	LDH	ALP	Na^+	K^+	log (SPC)
Milk	1										
Fat	-0.406**	1									
Protein	-0.143*	0.155*	1								
Lactose	0.053	0.262**	-0.424**	1							
log (SCC)	-0.360**	0.005	0.172*	-0.141	1						
EC	0.402**	-0.774**	-0.285**	-0.239**	0.056	1					
LDH	-0.190**	0.046	0.076	-0.025	0.296**	-0.076	1				
ALP	-0.132	-0.099	0.064	-0.129	0.406**	0.088	0.884**	1			
Na^+	-0.177*	0.078	0.272**	-0.250**	0.346**	-0.034	0.366**	0.309**	1		
K^+	0.315**	-0.610**	-0.082	-0.146	0.060	0.629**	0.075	0.223**	-0.062	1	
log (SPC)	-0.314**	0.206**	0.322**	-0.048	0.416**	-0.313**	0.111	0.099	0.204**	-0.212**	1

log (SCC). logarithm of somatic cell count; EC. electrical conductivity; LDH. lactate dehydrogenase; ALP. alkaline phosphatase; log (SPC). logarithm of standard plate count. * $p<0.05$, ** $p<0.01$.

with the change of log (SCC) or log (SPC), and to reveal whether different breeds of goats will show different relationship pattern among the parameters measured.

CONCLUSION

The present study showed log (SPC) to be reasonably good indicator of mammary gland infection for both of Saanen and Alpine dairy goats in regard to the sensitivity, specificity, positive predictive value and negative predictive value for a diagnostic method. However, different threshold log (SPC) values had to be chosen for Saanen and Alpine dairy goats. The correlation coefficient of different measured parameters in relation to log (SPC) showed different pattern between Saanen and Alpine dairy goats. There were also different best-fit regression equations with different multiple diagnostic indicators for Saanen and Alpine dairy goats. Different breeds of dairy goats obviously need to adapt different mastitis diagnostic parameters for a better diagnosis.

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