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# Variation in the composition of selected milk fraction samples from healthy and mastitic quarters, and its significance for mastitis diagnosis

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Seven variables – electrical conductivity (EC), somatic cell count (SCC), N-acetyl-β-D-glucosaminidase (NAGase), lactose, protein, fat and pH – were compared in four quarter milk fractions (MF1: strict foremilk; MF2: first 12–15 ml foremilk; MF3: subsequent 40–45 ml milk; MF4: strippings) and in one cow composite milk sample (CC) per cow. The study used 142 quarters from 37 lactating cows of the German Black Pied breed. To rule out any possible effect due to management, animal physiology and analytical procedures, the collection and processing of milk samples from each cow was repeated for three consecutive days, and the means of 3-d values were used. All variables were affected significantly by milk fraction and udder health. Compared with foremilk, EC, lactose and protein levels in strippings decreased, while SCC, NAGase and fat increased. The pH of foremilk and strippings did not differ significantly in healthy or in mastitic quarters. The difference between MF1 and MF2 was significant for EC in mastitic quarters, and for SCC in healthy quarters only. In general, mastitis resulted in a significant increase in EC, SCC, NAGase and protein but in a decrease in lactose and fat contents of milk in one or more of the milk fractions studied. Comparison of cow composite milk samples from healthy and mastitic cows revealed the significance ($P<0{\cdot}01$) of udder health for EC, SCC and lactose. Of the different parameters that can distinguish between healthy and mastitic quarters or cows, EC could be used to classify 76% of quarters and 73% of cows correctly, while the lactose content permitted correct identification of 81% of quarters and 76% of cows. NAGase and pH could be used to determine the status of 73% and 61% of quarters, respectively. In general, the correlation observed in strippings was higher than in foremilk for almost all the variables studied. Surprisingly, EC, SCC, NAGase and lactose in milk from healthy quarters of mastitic cows (with at least one mastitic quarter) differed significantly ($P<0{\cdot}05$) from those from healthy quarters of cows with all four healthy quarters, indicating an inconsistent effect of mastitic quarters on neighbouring healthy quarters (quarter interdependence).

**Keywords:** Mastitis diagnosis, milk fractions, milk composition, quarter interdependence.

Despite sustained efforts in extension and education since the early 1970s, mastitis remains the health factor most affecting milk production. While cows with clinical mastitis can be readily identified by visible changes in milk composition and physical examination of the udder, subclinically mastitic quarters, which can be up to 50-times more frequent than clinically diseased glands, elude detection by these methods. Detection of subclinical mastitis is based on bacteriological examination of milk and assessment of udder inflammation. For the latter, one possible indicator is somatic cell count (SCC), which reflects the disease-combatting response of the animal to the pathogen (Schalm, 1968). Another approach is to monitor changes in the milk composition that reflect the damage to the udder epithelium and the blood-milk barrier, e.g., electrical conductivity (EC), lactose and N-acetyl-β-D-glucosaminidase (NAGase) activity. Trials worldwide have

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**Table 1.** Milk fractions collected and variables studied

| | | | Variable studied | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Milk Fraction | Type of milk sample | Quantity, ml | SCC | Bact | NAGase | EC | pH | Fat | Protein | Lactose |
| MF1 | Strict foremilk† | 5 | | | | x | | | | |
| MF2 | Foremilk I‡ | 12–15 | x | x | x | | x | | | |
| MF3 | Foremilk II | 40–45 | x | | x | x | x | x | x | x |
| MF4 | Strippings | 30–35 | x | | x | x | x | x | x | x |
| CC | Cow composite | 40–45 | x | | | x | x | x | x | x |

† Before milk ejection
‡ Aseptic collection
SCC: somatic cell count; Bact: bacteriology; EC: electrical conductivity

investigated these factors (Sheldrake et al. 1983; Fernando et al. 1985; Berning et al. 1987; Emanuelson et al. 1987; Holdaway et al. 1996 [part 2]; Hamann & Zecconi, 1998; Nogai et al. 2001), but often with varying degrees of success. Furthermore, studies have shown that milk composition varies during the process of milking. The strippings were reported to have higher NAGase activity (Berning et al. 1987; Marschke et al. 1987; Holdaway et al. 1996 [part 1]) but lower EC (Fernando et al. 1981; Holdaway et al. 1996 [part 1]) than foremilk. Similarly, it is generally accepted that strippings and strict foremilk are relatively high in SCC (Paape et al. 1985). Thus, in order to utilize effectively the measurement of various milk components to detect mastitis, the physiological variations that occur normally in different milk fractions of both healthy and mastitic quarters should be considered, along with the milk fraction in which the particular variable yields its most accurate diagnosis. Keeping these facts in mind, the present study sought to determine how the various milk components were affected by different milk fractions, by health and disease, and which milk fraction was most effective in regard to the diagnostic ability of each variable.

## Materials and Methods

### *Animals*

Thirty-seven clinically healthy German Black Pied cows, not treated antibiotically either at least 21 d prior to or during the sampling dates were included in the trial. The animals were distributed over different parities (ranging from the first to the seventh lactation) and lactation stages (up to 3 months post-calving). The animals were milked twice daily with a milking interval of 16 h between the evening (14·00) and morning (6·00) milking. Mean daily milk yield was 27·8±3·8 kg.

### *Collection and processing of milk samples*

Each cow was sampled on three consecutive days during the routine morning milking. Details of milk sampling and the parameters studied are presented in Table 1.

### *Analytical procedures*

EC of strict foremilk was measured with a hand-held digital conductivity meter (Milku Hygiene Test-Systeme für die Landwirtschaft, D-47497 Neunkirchen-Vluyn, Germany) in the cow-shed itself, whereas in the laboratory, a microprocessor precision digital conductivity meter, model LF-539 (Wissenschaftlich-Technische Werkstätten GmbH, D-82362 Weilheim, Germany) was used to measure this variable in other milk fractions. Bacteriological examination followed microbiological procedures of the National Mastitis Council (Brown et al. 1981). The pH was assessed using a microprocessor precision digital pH/mV meter, model pH-539 (Wissenschaftlich-Technische Werkstätten GmbH). NAGase activity was measured fluorometrically (Nogai et al. 1996) using a modification of the method of Kitchen et al. (1978). SCC was determined with a Fossomatic fluoro-optical counter (Foss Electric, Hillerød, Denmark). Infrared spectrophotometry using Milkoscan (Foss Electric) was used to analyse milk samples for fat, total protein and lactose.

### *Categorization of quarter health*

Quarter health was assessed by observing the SCC and bacteriological status of foremilk (MF2) for three consecutive days following the guidelines of the International Dairy Federation (1987) but with the modification that an SCC threshold of 100 000 cells/ml was taken as an indicator of inflammation (German Veterinary Medical Society, 2002). Details are given in Table 2.

### *Statistical analysis*

Before applying statistical analysis, we assessed the mean of 3-d values for each variable from each quarter/udder representing the observation in data analysis, and calculated its coefficient of variation (CV). In general, CV ranged <5%, and two quarters where some variables showed CV >5% were excluded from the final interpretation of results. Milk SCC and NAGase activity measurements were transformed into decade logarithms (lg) as the original sets were markedly skewed. Two approaches

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**Table 2.** Defining the health status of quarters†

| | Analysis of quarter foremilk samples for bacteriology and SCC for three consecutive days | |
|---|---|---|
| Quarter health status | Pathogen | SCC, cells/ml milk |
| Healthy | Not detected (⩾twice) | All three times <100 000 |
| Latent infection | Detected (⩾twice) | All three times <100 000 |
| Mastitis (*non-specific*) | Not detected (⩾twice) | At least once >100 000 |
| Mastitis (*specific*) | Detected (⩾twice) | At least once >100 000 |

† As recommended by the German Veterinary Medical Society (2002)

**Table 3.** Quarter and udder health categorization

| Quarter health group | No. quarters, *n* | Health status of individual quarters | Udder health status of cow | |
|---|---|---|---|---|
| | | | Healthy† | Mastitic‡ |
| Ia | 47 | Healthy | x | |
| Ib | 44 | Healthy | | x |
| II | 04 | Latent infection | | x |
| III | 39 | Non-specific mastitis | | x |
| IV | 08 | Specific mastitis | | x |

† All four quarters healthy
‡ At least one quarter mastitic

were followed for data analysis. The first model was designed to investigate the effect of milk fraction and health status on the selected variables, and to study the interrelationship among different variables, expressed as correlation coefficients. For this, quarter health was categorized simultaneously taking into consideration the quarter and udder/cow health status as shown in detail in Table 3. Effects of milk fraction and quarter health status on variables were determined by two-way analysis of variances (SAS, 1985).

The second model examined the usefulness of selected variables for the differentiation of healthy and mastitic quarters/udders using discriminant function analysis (Rao, 1973). For this, healthy quarters from healthy udders (Ia) were compared with mastitic quarters (III and IV); quarters with latent infections were excluded. For each variable, different threshold settings were chosen and the values of false positive and false negative results were determined at each threshold. The threshold setting with the least probability of misclassification (minimum sum of false positive and false negatives) was taken as the critical threshold for a particular variable to differentiate healthy and mastitic quarters.

## Results

Categorization by health of quarters showed that 64·1%, 2·8%, 27·5% and 5·6% of the quarters were normally secreting glands, those with latent infections, non-specific mastitis and specific mastitis, respectively. In general, out of 142 quarters, only 12 (8·5%) were culturally positive. The organisms isolated from specific mastitis (eight quarters) included coagulase-negative staphylococci (four), *Streptococcus dysgalactiae* (two), *Escherichia coli* and *Bacillus* sp. (one each). All four latent infections were due to coagulase-negative staphylococci, a group of minor mastitis pathogens.

Tables 4–8 present the mean ± SD values for the variables in relation to milk fraction and udder health group.

### *Milk fractions*

With the exception of pH, all the variables showed significant ($P<0·05$) alterations, at least between foremilk and strippings, both in healthy and mastitic quarters (Tables 4–8). Compared with foremilk, EC, lactose and protein in strippings decreased while SCC, NAGase and fat increased. There was significantly lower EC in foremilk (MF3) than in strict foremilk (MF1) in both specific and non-specific mastitis groups but not in healthy quarters. While milk SCC fell significantly from MF2 to MF3 in healthy quarters only, NAGase activity did not change at all between these two fractions in healthy or in mastitic quarters. Cow composite milk (CC) from healthy and mastitic cows/udders showed significant differences with respect to EC, SCC and lactose, but not with respect to fat, protein and pH (Table 9).

### *Health category*

Quarters were divided into five categories on the basis of health status (Table 3). Mastitis, both at specific and non-specific levels, produced a significant ($P<0·05$) increase in EC, SCC and NAGase and a decrease in lactose content of milk in all the milk fractions studied (Tables 4–8). For strippings, increases in milk protein in both fractions were observed only for non-specific mastitis cases, and while milk fat of diseased quarters increased in foremilk, it decreased in strippings (Table 8). Milk pH did not show any significant change (Table 7).

In general, the latent infections were not associated with any significant alteration in the milk components studied.

Comparison of the two healthy quarter groups (Ia and Ib) revealed significant differences for all variables except milk protein and pH in one or more of the milk fractions studied (Tables 4–8). Quarter milk samples from Ib showed

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**Table 4.** Values of electrical conductivity in relation to milk fraction and health status of quarters

| | | Electrical conductivity, mS/cm Mean ± SD | | |
|---|---|---|---|---|
| Health group | *n*† | Strict foremilk (MF1) | Foremilk II (MF3) | Strippings (MF4) |
| Healthy (Ia) | 47 | $5{\cdot}48 \pm 0{\cdot}42^{a1}$ | $5{\cdot}41 \pm 0{\cdot}42^{a1}$ | $4{\cdot}45 \pm 0{\cdot}41^{a2}$ |
| Healthy (Ib) | 44 | $5{\cdot}71 \pm 0{\cdot}40^{bd1}$ | $5{\cdot}61 \pm 0{\cdot}33^{bc1}$ | $4{\cdot}91 \pm 0{\cdot}38^{bd2}$ |
| Latent infection (II) | 04 | $5{\cdot}43 \pm 0{\cdot}35^{ab1}$ | $5{\cdot}38 \pm 0{\cdot}36^{ab1}$ | $4{\cdot}60 \pm 0{\cdot}27^{ab2}$ |
| Non-specific mastitis (III) | 39 | $5{\cdot}96 \pm 0{\cdot}48^{c1}$ | $5{\cdot}70 \pm 0{\cdot}39^{b2}$ | $5{\cdot}11 \pm 0{\cdot}57^{c3}$ |
| Specific mastitis (IV) | 08 | $6{\cdot}03 \pm 0{\cdot}41^{cd1}$ | $5{\cdot}75 \pm 0{\cdot}31^{b2}$ | $5{\cdot}17 \pm 0{\cdot}44^{cd3}$ |

† Each single observation represents the mean of 3-d values
In columns, means with a common letter superscript do not differ significantly ($P > 0{\cdot}05$)
In rows, means with a common number superscript do not differ significantly ($P > 0{\cdot}05$)
Overall significance: per fraction: $P < 0{\cdot}01$; per health status group: $P < 0{\cdot}01$; fraction × health: NS

**Table 5.** Values of SCC in relation to milk fraction and health status of quarters

| | | SCC, log cells/ml Mean ± SD | | |
|---|---|---|---|---|
| Health group | *n*† | Foremilk I (MF2) | Foremilk II (MF3) | Strippings (MF4) |
| Healthy (Ia) | 47 | $4{\cdot}55 \pm 0{\cdot}11^{a1}$ | $4{\cdot}26 \pm 0{\cdot}31^{a2}$ | $5{\cdot}03 \pm 0{\cdot}33^{a3}$ |
| Healthy (Ib) | 44 | $4{\cdot}67 \pm 0{\cdot}16^{b1}$ | $4{\cdot}50 \pm 0{\cdot}33^{b2}$ | $5{\cdot}25 \pm 0{\cdot}38^{b3}$ |
| Latent infection (II) | 04 | $4{\cdot}63 \pm 0{\cdot}08^{ab1}$ | $4{\cdot}48 \pm 0{\cdot}16^{ab1}$ | $5{\cdot}14 \pm 0{\cdot}31^{ab2}$ |
| Non-specific mastitis (III) | 39 | $5{\cdot}17 \pm 0{\cdot}30^{c1}$ | $5{\cdot}15 \pm 0{\cdot}34^{c1}$ | $5{\cdot}83 \pm 0{\cdot}38^{c2}$ |
| Specific mastitis (IV) | 08 | $5{\cdot}55 \pm 0{\cdot}24^{d1}$ | $5{\cdot}57 \pm 0{\cdot}34^{d1}$ | $6{\cdot}18 \pm 0{\cdot}23^{d2}$ |

† Each single observation represents the mean of 3-d values
In columns, means with a common letter superscript do not differ significantly ($P > 0{\cdot}05$)
In rows, means with a common number superscript do not differ significantly ($P > 0{\cdot}05$)
Overall significance: per fraction: $P < 0{\cdot}01$; per health status group: $P < 0{\cdot}01$; fraction × health: NS

**Table 6.** Values of N-acetyl-β-D-glucosaminidase (NAGase) in relation to milk fraction and health status of quarters

| | | NAGase, log nmol/ml per min Mean ± SD | | |
|---|---|---|---|---|
| Health group | *n*† | Foremilk I (MF2) | Foremilk II (MF3) | Strippings (MF4) |
| Healthy (Ia) | 47 | $0{\cdot}34 \pm 0{\cdot}20^{a1}$ | $0{\cdot}34 \pm 0{\cdot}20^{a1}$ | $0{\cdot}46 \pm 0{\cdot}24^{a2}$ |
| Healthy (Ib) | 44 | $0{\cdot}36 \pm 0{\cdot}19^{a1}$ | $0{\cdot}36 \pm 0{\cdot}20^{a1}$ | $0{\cdot}52 \pm 0{\cdot}20^{b2}$ |
| Latent infection (II) | 04 | $0{\cdot}30 \pm 0{\cdot}04^{a1}$ | $0{\cdot}30 \pm 0{\cdot}04^{a1}$ | $0{\cdot}33 \pm 0{\cdot}08^{a1}$ |
| Non-specific mastitis (III) | 39 | $0{\cdot}57 \pm 0{\cdot}24^{b1}$ | $0{\cdot}57 \pm 0{\cdot}24^{b1}$ | $0{\cdot}76 \pm 0{\cdot}22^{c2}$ |
| Specific mastitis (IV) | 08 | $0{\cdot}59 \pm 0{\cdot}26^{b1}$ | $0{\cdot}59 \pm 0{\cdot}26^{b1}$ | $0{\cdot}80 \pm 0{\cdot}22^{c2}$ |

† Each single observation represents the mean of 3-d values
In columns, means with a common letter superscript do not differ significantly ($P > 0{\cdot}05$)
In rows, means with a common number superscript do not differ significantly ($P > 0{\cdot}05$)
Overall significance: per fraction: $P < 0{\cdot}01$; per health status group: $P < 0{\cdot}01$; fraction × health: NS

higher EC and SCC but lower lactose contents than those of Ia in all the milk fractions studied. Statistical differences were also observed for fat and NAGase, but in strippings (MF4) only. The Ib quarters presented lower levels of fat but higher NAGase activity than the Ia ones.

Comparison of different components in cow composite milk samples from healthy and mastitic udders revealed pathological changes similar to those seen on quarter level, although to a different degree. Milk from mastitic cows/udders had significantly higher EC and SCC, but lower lactose content (Table 9).

### *Diagnostic ability of selected variables*

Table 10 shows the ability of selected variables to differentiate healthy and mastitic quarters and udders

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**Table 7.** Values of pH in relation to milk fraction and health status of quarters

| | | pH Mean ± SD | | |
|---|---|---|---|---|
| Health group | *n*† | Foremilk I (MF2) | Foremilk II (MF3) | Strippings (MF4) |
| Healthy (Ia) | 47 | $6{\cdot}55 \pm 0{\cdot}08^{a1}$ | $6{\cdot}53 \pm 0{\cdot}07^{a1}$ | $6{\cdot}56 \pm 0{\cdot}08^{a1}$ |
| Healthy (Ib) | 44 | $6{\cdot}52 \pm 0{\cdot}09^{a1}$ | $6{\cdot}50 \pm 0{\cdot}09^{a1}$ | $6{\cdot}54 \pm 0{\cdot}10^{a1}$ |
| Latent infection (II) | 04 | $6{\cdot}51 \pm 0{\cdot}04^{a1}$ | $6{\cdot}48 \pm 0{\cdot}04^{a1}$ | $6{\cdot}52 \pm 0{\cdot}04^{a1}$ |
| Non-specific Mastitis (III) | 39 | $6{\cdot}54 \pm 0{\cdot}11^{a1}$ | $6{\cdot}52 \pm 0{\cdot}11^{a1}$ | $6{\cdot}56 \pm 0{\cdot}12^{a1}$ |
| Specific mastitis (IV) | 08 | $6{\cdot}61 \pm 0{\cdot}11^{a1}$ | $6{\cdot}57 \pm 0{\cdot}11^{a1}$ | $6{\cdot}61 \pm 0{\cdot}10^{a1}$ |

† Each single observation represents the mean of 3-d values
In columns, means with a common letter superscript do not differ significantly ($P > 0{\cdot}05$)
In rows, means with a common number superscript do not differ significantly ($P > 0{\cdot}05$)
Overall significance: per fraction: NS; per health: $P < 0{\cdot}01$; fraction × health: NS

**Table 8.** Mean ± SD values of milk lactose, protein and fat in relation to milk fraction and health status of quarters

| | | Milk constituent, Mean ± SD | | | | | |
|---|---|---|---|---|---|---|---|
| | | Lactose, % | | Protein, % | | Fat, % | |
| Health Group | *n* | Foremilk II (MF3) | Strippings (MF4) | Foremilk II (MF3) | Strippings (MF4) | Foremilk II (MF3) | Strippings (MF4) |
| Healthy (Ia) | 47 | $5{\cdot}02 \pm 0{\cdot}19^{a1}$ | $4{\cdot}52 \pm 0{\cdot}17^{a2}$ | $2{\cdot}96 \pm 0{\cdot}36^{a1}$ | $2{\cdot}40 \pm 0{\cdot}28^{a2}$ | $1{\cdot}43 \pm 0{\cdot}67^{a1}$ | $13{\cdot}21 \pm 2{\cdot}44^{a2}$ |
| Healthy (Ib) | 44 | $4{\cdot}83 \pm 0{\cdot}23^{b1}$ | $4{\cdot}37 \pm 0{\cdot}23^{b2}$ | $2{\cdot}90 \pm 0{\cdot}35^{a1}$ | $2{\cdot}48 \pm 0{\cdot}31^{a2}$ | $2{\cdot}00 \pm 1{\cdot}33^{ab1}$ | $11{\cdot}34 \pm 2{\cdot}23^{b2}$ |
| Latent infection (II) | 04 | $4{\cdot}82 \pm 0{\cdot}16^{abc1}$ | $4{\cdot}39 \pm 0{\cdot}21^{abc2}$ | $3{\cdot}37 \pm 0{\cdot}34^{a1}$ | $2{\cdot}75 \pm 0{\cdot}28^{a2}$ | $2{\cdot}21 \pm 1{\cdot}22^{ab1}$ | $12{\cdot}83 \pm 1{\cdot}11^{ab2}$ |
| Non-specific mastitis (III) | 39 | $4{\cdot}71 \pm 0{\cdot}27^{c1}$ | $4{\cdot}23 \pm 0{\cdot}30^{c2}$ | $3{\cdot}22 \pm 0{\cdot}54^{b1}$ | $2{\cdot}79 \pm 0{\cdot}53^{b2}$ | $2{\cdot}25 \pm 1{\cdot}08^{b1}$ | $11{\cdot}25 \pm 2{\cdot}05^{b2}$ |
| Specific mastitis(IV) | 08 | $4{\cdot}72 \pm 0{\cdot}22^{bc1}$ | $4{\cdot}30 \pm 0{\cdot}15^{bc2}$ | $3{\cdot}00 \pm 0{\cdot}23^{ab1}$ | $2{\cdot}63 \pm 0{\cdot}28^{ab2}$ | $2{\cdot}16 \pm 1{\cdot}78^{ab1}$ | $10{\cdot}08 \pm 2{\cdot}40^{b2}$ |

In columns, means with a common letter superscript do not differ significantly ($P > 0{\cdot}05$)
In rows, means with a common number superscript do not differ significantly ($P > 0{\cdot}05$)
Overall significance: (i) Lactose and total protein per fraction: $P < 0{\cdot}01$; per health: $P < 0{\cdot}01$; fraction × health: NS (ii) Fat per fraction: $P < 0{\cdot}01$; per health status group: $P < 0{\cdot}05$; fraction × health: $P < 0{\cdot}01$

**Table 9.** Values for various milk components in cow composite samples from healthy and mastitic udders

| | Mean ± SD values for | | |
|---|---|---|---|
| Milk Component | Healthy Udders† | Mastitic Udders‡ | Significance of Effect |
| EC, mS/cm | $5{\cdot}04 \pm 0{\cdot}41$ | $5{\cdot}43 \pm 0{\cdot}36$ | ** |
| Log SCC, cells/ml | $4{\cdot}50 \pm 0{\cdot}26$ | $5{\cdot}35 \pm 0{\cdot}52$ | ** |
| Lactose, % | $4{\cdot}84 \pm 0{\cdot}18$ | $4{\cdot}61 \pm 0{\cdot}25$ | ** |
| Protein, % | $2{\cdot}80 \pm 0{\cdot}37$ | $3{\cdot}00 \pm 0{\cdot}47$ | NS |
| Fat, % | $5{\cdot}04 \pm 1{\cdot}17$ | $4{\cdot}69 \pm 1{\cdot}39$ | NS |
| pH | $6{\cdot}56 \pm 0{\cdot}07$ | $6{\cdot}54 \pm 0{\cdot}10$ | NS |

† $n = 12$; ‡ $n = 25$; each single value represents the mean of 3-d observations
Distribution of mastitic udders with 1, 2, 3 and all 4 diseased quarters was 08, 12, 01 and 04, respectively
EC, electrical conductivity; SCC, somatic cell count
** $P < 0{\cdot}01$

(reference: SCC and culture results in MF2) by discriminant function analysis. In general, EC, NAGase and pH showed higher efficacy with strippings (MF4), and lactose with foremilk (MF3). Fat and protein did not prove to be satisfactory indicators of mastitis. The use of CC milk to differentiate healthy and mastitic udders showed high sensitivity but low specificity for EC and lactose, whereas the opposite was true for pH.

### *Correlation between variables*

Correlation coefficients between selected variables in different milk fractions are given in Table 11. In general, somewhat higher correlations were observed in strippings than in foremilk.

## Discussion

Milk composition is affected by a wide array of factors, both endogenous and exogenous, physiological and pathological. Breed, age and lactation stage are the main physiological factors. Feeding also influences milk composition markedly. In the present trial, these factors were controlled as much as possible in that all cows were subjected to identical management practices at a single farm, belonged to the same breed, and were in the same stage of (early) lactation.

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**Table 10.** Evaluation of selected variables, electrical conductivity (EC), N-acetyl-β-D-glucosaminidase (NAGase) and lactose, by discriminant function analysis in differentiating healthy† and mastitic‡ quarters and udders

| | | | Detection of diseased quarters/udders, % | | Detection of healthy quarters/udders, % | | |
|---|---|---|---|---|---|---|---|
| Variable | Milk fraction | Threshold value§ | False negative | Positive prediction | False positive | Negative prediction | Overall efficiency, % |
| EC, mS/cm | MF1 | 5·70 | 25·53 | 74·47 | 36·18 | 63·82 | 69·15 |
| | MF3 | 5·85 | 57·45 | 42·55 | 08·51 | 91·49 | 67·20 |
| | MF4 | 4·80 | 19·15 | 80·85 | 29·79 | 70·21 | 75·54 |
| | CC | 5·10 | 12·0 | 88·0 | 58·34 | 41·66 | 72·97 |
| NAGase, nmol/ml per min | MF2 | 3·30 | 44·69 | 55·31 | 14·89 | 85·11 | 70·22 |
| | MF3 | 3·20 | 46·81 | 53·19 | 17·02 | 82·98 | 68·09 |
| | MF4 | 4·90 | 38·30 | 61·70 | 14·89 | 85·11 | 73·41 |
| Lactose, % | MF3 | 4·85 | 21·28 | 78·72 | 17·02 | 82·98 | 80·85 |
| | MF4 | 4·30 | 31·92 | 68·08 | 10·64 | 89·36 | 78·72 |
| | CC | 4·85 | 12·0 | 88·0 | 50·0 | 50·0 | 75·66 |
| pH | MF2 | 6·65 | 72·34 | 27·66 | 10·64 | 89·36 | 58·51 |
| | MF3 | 6·61 | 72·34 | 27·66 | 08·51 | 91·49 | 59·57 |
| | MF4 | 6·65 | 70·22 | 29·78 | 08·51 | 91·49 | 60·63 |
| | CC | 6·63 | 80·0 | 20·0 | 08·33 | 91·67 | 43·23 |

† Represents healthy quarters (group 1a), $n=47$
‡ Represents both non-specific and specific mastitis (groups III plus IV), $n=47$
§ Threshold where total error was found to be minimum irrespective of the proportion of false positive and false negative results
MF1 = strict foremilk; MF2 = foremilk I; MF3 = foremilk II; MF4 = strippings; CC = cow composite milk sample

**Table 11.** Correlation coefficients among various milk components of different milk fractions

For MF2, MF3 and MF4, $n=142$; for CC, $n=37$

| Combination | MF2 | MF3 | MF4 | CC |
|---|---|---|---|---|
| SCC : NAGase | 0·45 | 0·44 | 0·50 | —† |
| SCC : EC | 0·52 | 0·41 | 0·58 | 0·56 |
| NAGase : EC | 0·43 | 0·35 | 0·56 | — |
| Lactose : SCC | — | −0·59 | −0·48 | −0·56 |
| Lactose : NAGase | — | −0·50 | −0·59 | — |
| Lactose : EC | — | −0·66 | −0·62 | −0·66 |

† Not observed
SCC, somatic cell count; NAGase, N-acetyl-β-D-glucosaminidase; EC, electrical conductivity

### *Variation in milk composition throughout milking*

Despite many trials having assessed compositional changes in different milk fractions during milking, the absolute values from these publications are difficult to compare because the definition of these fractions varies greatly, especially in the cases of foremilk (sampled with or without udder preparation) and strippings (which may be obtained with or without oxytocin administration and may comprise varying volumes). Thus, it seemed more logical to compare the general pattern followed by a variable during milking. Applying this scheme to our results showed that different variables over the milking process followed similar tendencies to those described earlier (Fernando et al. 1985; Berning et al. 1987; Marschke et al. 1987; Aoki et al. 1992; Holdaway et al. 1996; Nogai et al. 2001).

Interrelationships between different variables were examined by calculating the correlation coefficients (Table 11). In general, our findings reflected those in earlier publications (Fernando et al. 1985; Berning et al. 1987; Timms & Schultz, 1987; Nielen et al. 1992). However, some authors report divergent results (Fernando et al. 1985; Emanuelson et al. 1987; Berning & Shook, 1992). Such discrepancies can be attributed mainly to different fraction definitions and levels of udder infection in herds sampled.

### *Milk fractions*

The fraction-related changes are thought to result from a complex series of interactions between physiological milk emission and the physicochemical properties of the milk constituents. Two phenomena seem important in this context. On the one hand, the oxytocin released naturally during the milking process leads to increased permeability of the blood-milk barrier and hence to increased influx of $Na^+$ and $Cl^-$ into the milk with a simultaneous efflux of lactose and $K^+$ into the blood serum (Linzell & Peaker, 1971; Allen, 1990). The altered Na : K ratio adversely affects the protein release (Questel & Kaplan, 1970; Ledbetter & Lubin, 1977), which would explain the decreases of lactose and protein in strippings

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(MF4). On the other hand, because of capillarity, corpuscular elements like leucocytes and fat globules tend to remain in the alveolar lumen and are expelled towards the end of milking, which explains the increase in these components in strippings (MF4). While fat globules are part of the epithelial secretions, alveolar leucocytes are considered the cells that have most recently entered the milk, and are therefore the most active ones. Milk fat is known to inhibit EC and leads to a decrease of the latter in strippings (Prentice, 1962).

Since NAGase is produced in part by polymorphonuclear cells, an excretion pattern similar to that of SCC was expected. However, unlike SCC, NAGase activity did not decrease from MF2 to MF3, and this was reflected in the relatively low correlation between these two components. Fraction-dependent correlation between SCC and NAGase has been described previously and attributed to various factors such as dilution effects, age-dependent secretion of NAGase from leucocytes and possible NAGase linking to fat globules. Elevated levels in strippings are thought to be caused by the absence of milk dilution in combination with large-scale movement of white blood cells into this fraction owing to milking stimulus (Paape et al. 1985). Some authors also suggest the mechanical stress during milking on the teat as one possible cause for the rise in this enzyme in strippings (Berning et al. 1987; Dulin et al. 1987; Holdaway et al. 1996; Nogai et al. 2001).

### *Health categorization*

Mastitis is known to affect milk composition. Increasing SCC, NAGase and EC, and decreasing lactose in milk from diseased quarters was expected and has been documented previously, even at fraction level.

The pattern of change in variables associated with inflammation (SCC, NAGase and EC) differed from that of the classical milk quality constituents (lactose, fat and protein). In the latter, the original pattern continued but at different levels. Inflammation indicators, however, seemed to lose their physiological patterns (Tables 4–6), as is shown, for example, by the fact that the increase of SCC and NAGase activity from foremilk to strippings was more pronounced in diseased quarters. The elevated gradient of change in these inflammation indicators in mastitic quarters is thought to be caused by pathophysiological processes such as a higher attraction of leucocytes to the infection site and a greater leakage of blood constituents into alveolar milk (strippings) under the influence of physiological release of oxytocin during milking, through the already damaged epithelium of mastitic quarters (Linzell & Peaker, 1971).

Mastitis-related changes in lactose, fat and protein were less marked. When considering the means of SCC, NAGase activity and EC in MF1 and MF2 (the fractions usually used for mastitis diagnosis) it became clear that the seriousness of mastitis cases was relatively moderate, with an average SCC of 5·5 lg cells/ml for specific mastitis. Thus, damage to the milk-blood barrier and concomitant changes in secretory constituents were also expected to be less pronounced. The literature shows that controversy still surrounds the concentration changes due to disease in most of these quality components. While it is generally accepted that lactose decreases, total milk protein may increase (Auldist et al. 1995), remain unaltered (Mitchell et al. 1986; Rogers et al. 1989) or even decrease (Lee et al. 1991). These differences are thought to be due in part to the milk fraction in question but are mainly attributed to the fact that 'total protein' consists of different fractions. Among these, those originating from blood such as bovine serum albumin and immunoglobulins increase as an inflammatory response, and those synthesized in the mammary gland such as casein, lactalbumin and lactoglobulins decrease owing to impaired alveolar secretion. Thus the type and amount of pathological response plays an important role in the total protein value (Kitchen, 1981). The present results should be evaluated in the same manner.

There are also contradictory reports on the fat content of mastitic milk. Kitchen (1981) and Auldist et al. (1995) report a lower fat concentration in the milk from cows with subclinical mastitis whereas Rogers et al. (1989) observed no clear effect of mastitis on milk fat. Conversely, Mitchell et al. (1986) report an increase in fat concentration due to mastitis. While decreases in milk fat suggest decreased synthesis as a result of epithelial damage and/or the lipolytic action of leucocyte enzymes (Azzara & Dimmick, 1985), the higher fat contents could be explained by a strong reduction in milk yield rather than by a decreased fat synthesis, suggesting thus an only apparent increase in the concentration of fat (Schultz, 1977). Our results (Table 8) showed that, in mastitic quarters, fat levels in foremilk (MF3) were higher than in healthy ones, while fat levels in strippings were lower. Although differences in foremilk values were not continuous and elude proper interpretation, milk fat in MF4 decreased constantly, confirming the observations of the papers cited above.

Finally, there were no conclusive mastitis-related changes in pH. This inertia has been documented extensively (Oshima & Yoshida, 1988; Holdaway et al. 1996).

In the present study we placed the healthy quarters into two different groups: group Ia, those in healthy udders (all four quarters secreting normally), and group Ib, those in diseased ones (at least one quarter not secreting normally). Significant differences were found between these two groups for all variables except protein and pH, in most cases in all fractions, indicating that healthy quarters of mastitic udders functioned at a different metabolic level. This observation stands in contrast to the previously assumed independence of quarters. However, evidence for quarter interdependence regarding milk yield and composition has been accumulating recently, showing

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that quarters with SCC of <100 000 cells/ml do react when neighbouring quarters become mastitic (Woolford, 1985; Hamann et al. 1998, 2002). As seen in the present trial, there were fewer compositional changes in healthy quarters of diseased udders than in infected quarters.

### *Evaluation of milk constituents in mastitis diagnosis*

Evaluation of different variables by discriminant function analysis showed clearly that the diagnostic ability of a variable depends considerably on the milk fraction. In general, greater differences in the concentrations of different variables between healthy and mastitic quarters towards the end of milking suggest that the ability of a variable to discriminate between healthy and mastitic quarters might be greater with strippings than with foremilk. In the present study, the same was true for EC, NAGase, SCC and pH (Table 10). There is some evidence in support of this suggestion with respect to EC (Fernando et al. 1982; Aoki et al. 1992), SCC (Holdaway et al. 1996), and NAGase (Berning, 1987; Marschke, 1987; Aoki et al. 1992).

EC is widely accepted as an indicator for both cow-side and in-line mastitis diagnosis, and MF1 is used for cow-side testing. Here, the MF1 led to a better discrimination than the MF3 fraction. Mean EC in healthy (Ia) and specifically mastitic (IV) quarters differed by 0·55 mS/cm (10·04%) in MF1, by 0·34 ms/cm (6·28%) in MF3, and by 0·72 mS/cm (16·18%) in MF4 (Table 4). A similar trend has been reported by others (Woolford & Williamson, 1982; Holdaway et al. 1996). This trend is probably due to the fact that MF1 (strict foremilk) represents the small amount of residual milk that remains undiluted in the teat cistern until milk ejection at the following milking (Woolford & Williamson, 1982). Under practical conditions, using strict foremilk, i.e., sampling without udder preparation, still remains a viable alternative for cow-side testing. However, with systems designed to monitor EC in-line, udder preparation for milking would instead most often result in the measurement of EC on foremilk obtained after milk ejection. Therefore, for more accurate results we may have to rely heavily on milk obtained at the end of milking, i.e., strippings.

Mastitis-related changes in secretory pattern were observed for NAGase (Table 6). The increase in absolute NAGase activity between foremilk and strippings was much lower in healthy quarters (38·23%) than in quarters with non-specific mastitis (53·31%) and specific mastitis (58·03%). Discriminant function analysis revealed that the ability of NAGase to differentiate healthy and mastitic quarters was higher in strippings (73·41%) than in the foremilk fractions MF2 (70·22%) and MF3 (68·09%). This agrees closely with the findings of Holdaway et al. (1996), who also report 29% misclassifications for NAGase activity of foremilk in differentiating healthy quarters and quarters infected by major pathogens.

Lactose led to consistently good discrimination in all fractions studied, even in CC samples. These findings support the observations of Renner (1975) but contradict those of Fernando et al. (1985) and Holdaway et al. (1996), who found lactose to be a relatively poor indicator of mastitis. A possible explanation for this inconsistency is the difference in the definition of mastitis; the latter works define mastitis only bacteriologically, thus disregarding non-specific mastitis cases. In the present study we considered both bacteriological findings and SCC.

Finally, pH was found not to be a good indicator of mastitis. It is the only variable that showed marked differences between the expressiveness of quarter and cow composite samples. These results confirmed earlier observations (Oshima & Yoshida, 1988; Holdaway et al. 1996).

This study showed that significant changes in milk composition could also be found in CC samples (Table 10). In the special case of lactose, this fraction gave a surprisingly good indication of udder health. This is a promising result indicating a possibility for an economical estimator of udder health. Yet it must not be forgotten that SCC on the quarter level (MF2) is the basis of proper mastitis diagnosis, and that a discriminatory ability of approximately 75% still means that about a quarter of mastitis cases passed undetected. It is therefore necessary to rank lactose, along with the other variables studied here, for what they are: an auxiliary in the estimation of udder health, but not precise tools for mastitis diagnosis.

In conclusion, significant variation occurs in milk composition during milking, and the pattern of variation differs between healthy and mastitic quarters. In general, greater differences in the levels of milk components between healthy and mastitic quarters and a better correlation between different variables towards the end of milking suggest the superiority of strippings over the foremilk in mastitis diagnosis. The significant difference in the levels of different variables in CC milk samples from healthy and mastitic udders supports the use of CC milk for identifying mastitic cows. However, this estimation cannot substitute for the cytobacteriological analysis of foremilk samples. Significant differences were found in healthy quarters from healthy and mastitic udders, which supports the theory of the interdependence of quarters, and indicates an inconsistent effect of mastitic quarters on the milk from neighbouring healthy quarters. This should be kept in mind in the evaluation of the diagnostic ability of these mastitis indicators in order to prevent large numbers of false positive results.

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