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A polyol mixture or molasses treated beet pulp in the silage based diet of dairy cows

II. The effect on the lactoperoxidase and thiocyanate content of milk and the udder health

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Abstract. The study investigated the effect of a diet containing either a polyol mixture (polyol group) or molasses (molasses group) on the lactoperoxidase (LP) and thiocyanate (SCN^-) content of milk and the udder health of dairy cows during a 12 week trial period. The control group received no extra carbohydrate feed.

On the basis of the weekly milk samples from all test animals the polyol group had on an average the highest LP content (17.8 μg/ml), the lowest SCN^- content (0.87 mg/l), and the lowest somatic cell count (152 000 cells/ml). The mean values for the molasses group were; LP: 12.6 μg/ml, SCN^-: 1.01 mg/ml and cell count: 626 000 cells/ml. The same values for the control group were 11.7 μg/ml, 0.91 mg/l and 285 000 cells/ml, respectively.

The polyol group yielded milk with an average of 51.5 % more lactoperoxidase daily than the molasses group, and 42.5 % more than the control group. These differences were, however, not found to derive from the different carbohydrate diet, because no significant change in the LP level in any group occurred during the test feeding. The LP and SCN^- contents varied considerably from one cow to the other.

The degrees of correlation between the factors tested varied markedly among the test groups. The overall values for r were as follows: LP: $SCN^- = -0.049$, LP: cell count = 0.222 and SCN^-: cell count = 0.080.

On the basis of cell content and the occurrence of mastitis cases, the polyol group had the best and the molasses group the worst udder health.

The possible effect of the LP/SCN^-/H_2O_2 antimicrobial system on mastitis resistance is evaluated.

1. Introduction

Lactoperoxidase (LP) (E. C. 1.11.1.7.) is the first enzyme identified in cow's milk. According to SHAHANI et al. (1973), its appearance was first described as early as 1881. The early observation of this heat stable hemiprotein was evidently affected by its high content in milk, where it is the most abundant en-

zyme. Peroxidase activity is found in all cow's milk, but its extent varies greatly from individual to individual and there are marked daily variations in the LP activity of milk from the same cow (KIERMEIER and KAYSER 1960, KORHONEN 1973).

Several factors have been found to affect the LP activity of milk. According to the observations of KIERMEIER and KAYSER (1960), LP activity is higher in cows of the Rotbunte Niederungsvieh breed than in Fleckvieh cows, and activity is at peak 3—5 days after parturition, gradually dropping thereafter. The latter observation has been recently confirmed (GOTHEFORS and MARKLUND 1975, KORHONEN 1977). KIERMEIER and KAYSER (1960) noted that LP activity is higher in the summer than in the winter, a fact which they consider to be due to a different diet. A feeding test showed that continual feeding with maize silage raised the peroxidase activity of milk during the test feeding period. A beet diet had no such effect on the activity.

Inconsistent results have been presented on the effects of udder infection on LP activity in milk. PATTERSON et al. (1969), TAYLOR and KITCHEN (1970) and KORHONEN (1973) found no clear correlation between the cell content of milk and LP activity, while KIERMEIER and KUHLMANN (1972) and MALIK et al. (1974) found a positive correlation.

The biological role of LP has repeatedly been linked to its antimicrobial activity (REITER and ORAM 1967, MORRISON and STEELE 1968, GOTHEFORS and MARKLUND 1975, REITER 1976). The inhibitory system catalyzed by LP requires the presence of hydrogen peroxide and thiocyanate or some other oxidigable substance (halides) (REITER et al. 1964, ORAM and REITER 1966 a, b, KLEBANOFF et al. 1966). This system is nonspecifically bactericidal or bacteriostatic and it has been found to affect numerous gram-positive and gram-negative saprophytic and pathogenic bacteria (REITER et al. 1964, KLEBANOFF et al. 1966, MICKELSON 1966, KLEBANOFF 1967, HAMON and KLEBANOFF 1973, HOOGENDOORN and MOORER 1973, BJÖRCK et al. 1975, REITER et al. 1976). The inhibitory system is also active against viruses (BELDING et al. 1970).

On the basis of this antimicrobial effect it is claimed that the LP antimicrobial system contributes in various kinds of secretions to the physiological defence mechanism against microbial infections, e.g. intestinal infections in the neonate (calves, infants) (GOTHEFORS and MARKLUND 1975, REITER et al. 1976, BJÖRCK 1977), bovine udder infections (REITER and ORAM 1967, KORHONEN 1973, REITER and BRAMLEY 1975) and dental caries in man (HOOGENDOORN and MOORER 1973, KOCH et al. 1973, HUGOSON et al. 1974).

Recently, in the Turku sugar studies MÄKINEN et al. (1975) obtained statistical evidence that a long-term xylitol diet raised the peroxidase activity of saliva and reduced the frequency of caries in man.

This observation led us to investigate the effect of feeding dairy cows with a diet containing a mixture of sugaralcohols (mainly xylitol) or molasses, on the lactoperoxidase activity of milk. The same milk samples were also examined for the contents of the other factors needed in the LP system: thiocyanate and hydrogen peroxide. The results obtained were evaluated especially in relation to udder health, using the milk's cell content and the appearance of clinical udder infections as basis for the evaluation in the various test groups.

2. Material and methods

2. 1. Arrangement of the feeding trial

The trial was carried out on 24 cows (1—6 lactation periods) randomly divided into three groups each comprising eight animals which were fed for twelve weeks on one of the three experimental diets. All animals were at the same lactation stage (average 24,5 days *post partum*) and were clinically healthy. Five of the cows were Friesians and the others Ayrshires. Grass silage was fed *ad libitum*, hay 2 kg/d and barley-oat grain mixture 7—8 kg/d in the control group. The second group (molasses group) had grain concentrate with 29 % dried molasses beet pulp and the third group (polyol group) with 25 % dried beet pulp treated with mixture of sugar alcohols. Intake of sugars from molasses beet pulp was 410 g/d/animal and intake of polyols from polyol treated beet pulp was 483 g/d/animal. A stabilization period of two weeks preceded the 12-week experimental phase. More detailed information about the animals and the experimental design has been given in a previous paper (TUORI and POUTIAINEN 1977). The polyol mixture (Finnish Sugar Co., Helsinki, Finland) given to animals in the polyol group had the following average composition (per cents of the polyols): Xylitol 27.0, arabinitol 11.3, mannitol 10.0, sorbitol 8.0, rhamnitol 4.0, galactitol 3.2, reducing sugars + other sugar alcohols 36.5.

2. 2. Milk samples and their pretreatment

A milk sample was taken during the morning milking from each quarter of all cows weekly throughout the trial period. The quarter samples were combined and handled as individual samples in all analyses. 336 individual milk samples were analyzed in all.

For the determination of lactoperoxidase, fat was removed from the milk by centrifugation (20 min, 2000 r.p.m., +4° C) and casein by coagulation (0.1 % rennin addition, 30 min/37° C) and by centrifugation as above. The whey obtained was filtered through a Millipore membrane filter (pore size 0.45 μ) and the clear filtrate was used in the determination.

For the determination of thiocyanate concentration, proteins were precipitated from the milk with 20 % trichloroacetic acid (4 parts milk and 3 parts acid). The precipitate was filtered through Schleicher & Schüll Filter Nr. 595 1/2 filtration paper and the clear filtrate obtained was used in the determination.

2. 3. Determinations

2. 3. 1. Lactoperoxidase (LP)

The determination of the LP activity of milk was carried out with the following modifications (personal communication with Dr. B. Reiter) of the method of ORAM and REITER (1966 a): 3 ml of phosphate buffer (0.1 M, pH 6.7), which contained 0.01 % o-dianisidine (Sigma Chemical Co., St. Louis, Mo.), was pipetted into a glass cuvette (1 cm). 0.1 ml of the sample or standard solution was added to the cuvette. The reaction mixture was mixed and the absorbance was measured at a wave length of 460 nm. Thereafter, 0.1 ml of 9 mM H_2O_2 solution was added to the cuvette and mixed well. The reaction

mixture was held in a +37° C water bath and the change in the absorbance was measured at the same wave length exactly five minutes after the addition of hydrogen peroxide. The standard solutions for the calculation of LP concentration were made from freeze-dried lactoperoxidase (Sigma Chemical Co., St. Louis, Mo.), which according to the manufacturer had an activity of 80 units/mg of protein. In this work one unit of activity is expressed as the amount of LP needed to cause a change of 0.001 unit in the absorbance during a period of one minute. The LP content was determined from a standard curve. The results are given as the mean of two or three parallel determinations using μg/ml as the unit. All of the absorbance measurements were made using a Hitachi Perkin-Elmer 139 UV-Vis spectrophotometer.

2. 3. 2. Thiocyanate (SCN^-)

The thiocyanate content of milk was determined using the method presented by SÖRBO (1953) which is based on the formation of a red ferric thiocyanate complex according to the concentration of SCN^- ions present. The intensity of the colour of the complex was measured in a glass cuvette (1 cm) at a wavelength of 460 nm using a Perkin-Elmer spectrophotometer. The SCN^- content of the samples was determined from a standard curve which was obtained using solutions with different KSCN concentrations. The results are expressed as the mean of two parallel samples using mg/l as the unit.

2. 3. 3. Somatic cells

The number of somatic cells in the milk was determined with a FOSS-O-Matic apparatus (A/S N. Foss Electric).

2. 3. 4. Hydrogen peroxide (H_2O_2)

The Perid test (Boehringer Ag, Mannheim) was used to determine the hydrogen peroxide content of fresh whole milk. The lowest concentration registered by the test is 5 μg H_2O_2/ml of milk. All of the milk samples tested (n = 336) gave a negative result with this method.

2. 4. Statistical analyses of the results

The results obtained were processed with a UNIVAC 1108 computer using the HYLPS statistical system (WETTERSTRAND et al. 1975).

3. Results

3. 1. The effect of diet on the LP content of milk

Figure 1 gives the means and ranges of the LP contents of milk samples taken weekly from the various test groups.

The means show that both before and during the test period the LP content was highest in the polyol group. The LP contents of the molasses and control

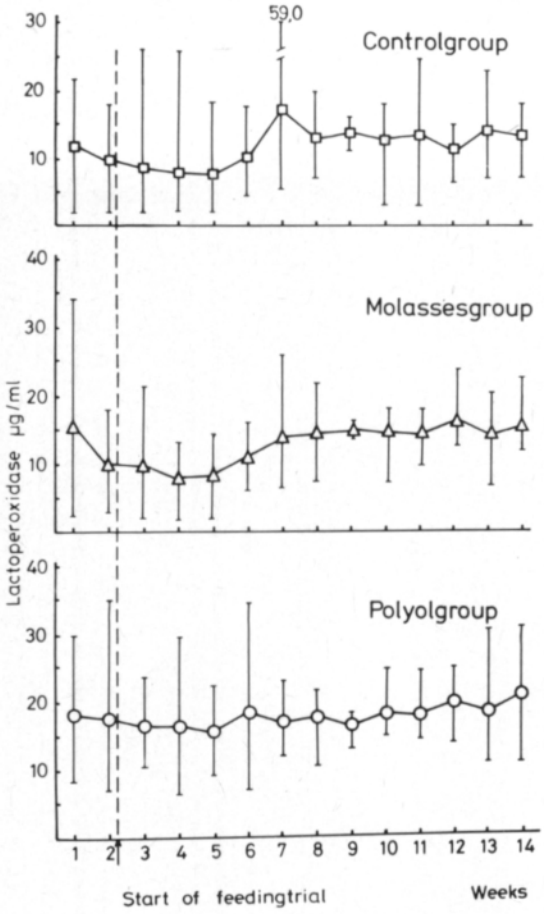

Fig. 1. The means and ranges of the lactoperoxidase content of milk in the different test groups during the trial period.

groups were on the same level compared with each other. The LP content dropped unevenly in all test groups at the beginning of the test period, but gradually began to rise during the 6th or 7th week. When the trial period ended, the LP content of the polyol and molasses groups continued to show a gradually rising trend, while the LP level in the control group seemed to remain stable.

As the figures show, the LP content of the milk from different cows fluctuates greatly. The range observed was 2.0—59.0 μg/ml. Lactoperoxidase activity was found in the milk of all cows, but usually it fluctuated greatly in the same cow from one determination time to another. Nonetheless there were clear differences in the levels of the LP content between individuals. Some cows, irrespective of group, produced milk with a LP content which was regularly above 15 μg/ml, while in other cows it was repeatedly under 10 μg/ml.

Table 1 gives the means, standard deviations, and statistical significance of the differences between the means for the LP content of the various test groups. The LP content of the polyol group was significantly ($P < 0.001$) greater than that of the other test groups, whose LP levels did not differ significantly from each other.

Table 1. The means, standard deviations of the means and significance of the differences between the means of the LP content of milk by test group.

Test group	No. of milk samples	$\overline{X}_A$* µg/ml	s**	Significance of the differences between the means
Control	110	11.7	7.3	no significance
Molasses	110	12.6	5.4	$P < 0.001$
Polyol	110	17.8	5.3	$P < 0.001$

* $\overline{X}_A$ = arithmetic mean in all tables

** s = standard deviation in all tables

3. 2. The effect of diet on thiocyanate content

The means and ranges of the SCN^- content in the various test groups during the trial period are given in Figure 2. At the beginning of the trial the SCN^- content was on an average on the same level (c. 0.45 mg/l) in the milk of all test groups, but clearly rose during 3—4 weeks from the beginning of the test period Thereafter the SCN^- level re-. mained almost unchanged to the end of the test period. The figure shows that the SCN^- content of milk varied greatly between individual cows. There was also a fluctuation in the milk of the same cow from one determination time to the other. No permanent differences in level of the kind found for the LP content were noted for thiocyanate. It was difficult to determine the presence of thiocyanate in certain milk samples with the method used, evidently because of the low content ($\lesssim$ 0.40 mg/l). The range of the SCN^- content was 0.40—2.80 mg/l.

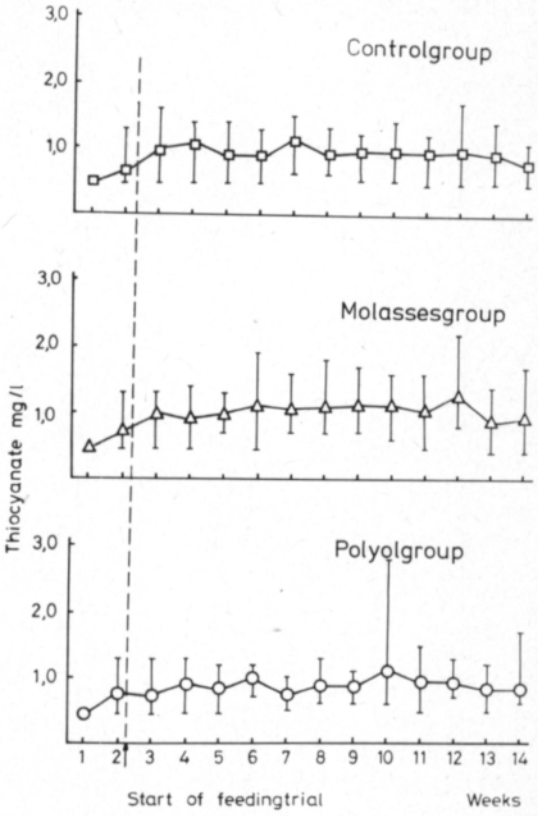

Fig. 2 The means and ranges of the thiocyanate content of milk in the different test groups during the trial period.

Table 2 gives the means, standard deviations, and statistical significance of the differences between the means for the SCN^- content in the various test groups. The results show that the concentration was highest in the molasses group, and lowest in the polyol group. The difference between these two groups was very significant. There was no significant difference between the polyol and control groups, however.

Table 2. The means, standard deviations of the means and significance of the differences between the means of the SCN^- content of milk by test group.

Test group	No. of milk samples	$\overline{X}_A$ mg/l	s	Significance of the differences between the means
Control	102	0.91	0.31	$P < 0.05$
Molasses	101	1.01	0.38	no significance
Polyol	101	0.87	0.34	$P < 0.01$

3. 3. *The effect of milk yield on the LP and SCN^- content*

The correlation between milk yield and LP content during the trial period is given in Figure 3, which shows the average milk yield per day per cow and the average LP content for the various test groups. The figure indicates that the amount of milk produced per day gradually dropped in all groups because the maximum milk yield occurred at the beginning of the trial period. There were clear differences in the average milk yield of the test groups. The control group had the best yield (20.6 l/d/cow) and the molasses group the poorest (18.6 l/d/ cow), the difference being statistically significant (TUORI and POUTIAINEN 1977). The polyol group yielded 19.3 litres per day per animal. On the basis of the figure there seems to be an inverse correlation between milk yield and the LP content, i.e. while the former dropped during the test period, the latter rose.

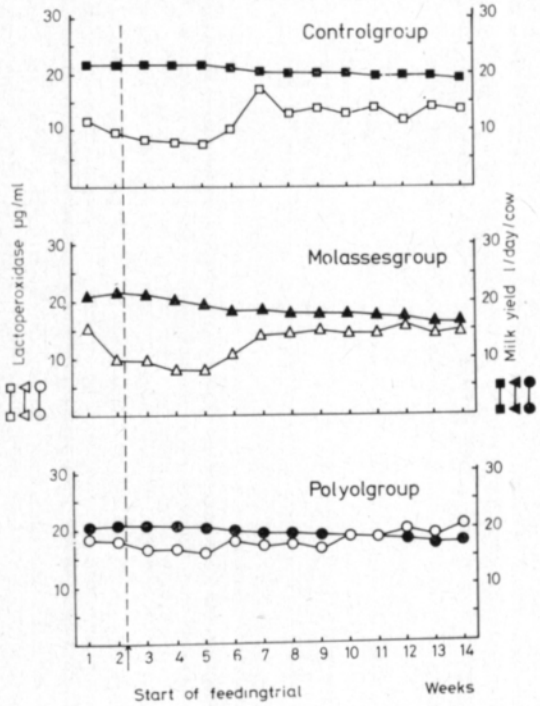

Fig. 3. The means of the lactoperoxidase content of milk and the milk yield in the different test groups during the trial period.

This correlation was confirmed through correlation analysis, and the results of this analysis are given in Table 3. The correlation is negative in all groups, and the overall correlation is highly significant ($P < 0.001$). An equally strong inverse correlation was found between milk yield and the SCN^- content, also shown in Table 3. This correlation can be explained by the physiological dilution/concentration effect, since thiocyanate evidently is secreted passively from the blood into the milk according to changes in the permeability of the udder tissue. During the normal lactation period secretion is small, but the SCN^- content rises towards the end of the lactation period as the udder's permeability increases and the milk yield decreases. An udder infection also increases the thiocyanate content of milk, as noted on the basis of milk samples taken from cows with a clinical mastitis.

The marked differences in LP content found among the different test groups are not, however, due only to the amount of milk produced. This is found when the amount of lactoperoxidase secreted in the milk is calculated on the basis of the daily milk yield. A cow in the polyol group produced an average of 336.0 mg of lactoperoxidase per day during the test period, while the LP yield in the molasses group was 222.5 mg, and that in the control group 235.8 mg. The cows in the polyol group thus had a clearly greater secretion of LP into the milk

than those in the other groups. Nevertheless, feeding the cows with polyol pulp did not seem to be responsible for this effect, since during the test period the LP level remained nearly the same as during the stabilization period. In the control group, however, the LP level had clearly risen being on an average nearly double. Evidently factors other than diet caused the difference in the LP levels between the various test groups.

Table 3. The correlation (r value) between milk yield (l/d/cow) and LP and SCN^- contents in the different test groups.

Test group	LP	SCN^-
Control	−0.257**	−0.127
Molasses	−0.369***	−0.096
Polyol	−0.117	−0.317**
All groups	−0.263***	−0.277***

Degree of significance:
*** = $P < 0.001$
** = $P < 0.01$

3.4. Udder health of the cows in the various test groups

The udder health of the cows was followed during the trial by making weekly determinations of the number of somatic cells in the individual milk samples. In questionable cases a microbiological analysis was made of the milk samples from each quarter in order to identify possible pathogenic bacteria in the udder Any cases of mastitis found were immediately treated with antibiotics.

Figure 4 shows the means and ranges of the cell content of milk in the various test groups during the trial period. At the beginning of the trial the average cell content was highest in the molasses group and lowest in the polyol group. During the test period the weekly means for cell content varied in all test groups, while the level remained almost unchanged. The figure shows that the cell count fluctuated very markedly in the milk of different

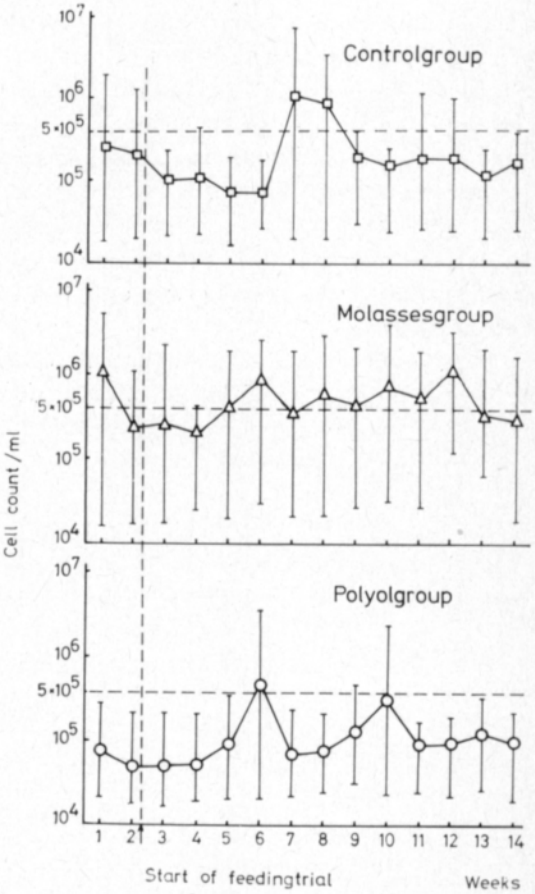

Fig. 4. The means and ranges of the cell content of milk in the different test groups during the trial period.

cows. The greatest fluctuationswere found in the molasses group and the smallest in the polyol group. If the limit in determining udder health is set at 500 000 cells/ml of milk, then the figure shows that there were clear differences among the various test groups. The mean cell content of the polyol group exceeds the limit only once, while the mean of the control group exceeds 500 000 cells/ml twice and the molasses group eight times. In this connection one should note that the high cell content (> 1 million cells/ml) of individual milk samples most often, though not always, correlated with a clinically confirmed mastitis case. Subclinical infections or other secretion disturbances thus evidently have had an effect on the cell count.

Table 4 gives the mean cell contents and their ranges as well as the statistical significance of the differences between the means for the various test groups. On the basis of the means, the polyol group differs highly significantly from the molasses group, but for the control group the difference is slight. The cell contents of the control and molasses groups, on the other hand, differ significantly.

Table 5 presents observations on mastitis cases found in the various test groups from the beginning of the new lactation period to the end of the test period. The basis for evaluation is the cell content of milk samples for each cow (> 500 000 cells/ml) and the appearance of clinically confirmed udder infections. The table shows that after parturition but before the test period, the molasses group had two cases of mastitis and the polyol group one. In addition there were cows (2—3) in the molasses and control groups whose milk samples contained more than 500 000 cells/ml. During the test period the molasses group was found to have three cases of mastitis (in different cows) and the control and polyol groups each had one case. In the molasses group the milk of six cows contained over 500 000 cells/ml, and this content was found in 27 samples. In the control group the corresponding figures were 3 cows and 8 samples, while in the polyol group only one cow was found to have samples with over 500 000 cells/ml.

These observations indicate that the cows in the molasses group had clearly the largest number of udder infections, and the poorest udder health, whereas

Table 4. The means, ranges and significance of the differences between the means of the cell content of milk by test group.

Test group	No. of milk samples	$\overline{X}_A$ cells/ml	Ranges cells/ml	Significance of the differences between the means
Control	112	285,804	15,000 — 8,317,000	→ $P < 0.025$
Molasses	112	626.625	12,000 — 6,438,000	→ $P < 0.2$ → $P < 0.001$
Polyol	112	152,402	19,000 — 4,385,000	

the udder health of the polyol group can be considered better than that of the other groups. Since the same types of differences were already noted during the stabilization period, the possible effects of a different diet on udder health cannot, however, be demonstrated with certainty.

Table 5. Number of mastitis cases[1]) and cows whose milk cell content was > 500,000 cells/ml, and the corresponding number of milk samples in the different test groups from the beginning of the lactation period to the end of the test period.

Test group	Beginning of lactation + stabilization period	Stabilization period (2 weeks)		Test period (12 weeks)		
	Mastitis cases No. of cows (n = 8/ group)	Cell content of milk > 500 000 cells/ml		Mastitis cases No. of cows (n = 8/ group)	Cell content of milk > 500 000 cells/ml	
		No. of samples (n = 16/ group)	No. of cows (n = 8/ group)		No. of samples (n = 96/ group)	No. of cows (n = 8/ group)
Control	0	2	2	1	8	3
Molasses	2	3	3	3	27	6
Polyol	1	0	0	1	3	1

[1]) Clinically confirmed udder infection in one or more quarters.

3.5. The correlation between LP, SCN^- and cell contents

On the basis of the character of the reaction of the $LP/SCN^-/H_2O_2$ inhibitory system, there are certain forms of interdependence among these factors which are known to exist in *in vitro* conditions, i.e. in chemically determined reaction mixtures (REITER et al. 1964, HOGG and JAGO 1970 a, b, REITER et al. 1976). Since these intercorrelations in milk are understood only to a limited extent (KORHONEN 1973), correlation analysis was used to determine the interdependence between the LP and SCN^- contents determined from milk, and the interdependence between these factors and the milk cell content. Table 6 presents the degrees of correlation between these factors in the various test groups, and also the total correlation coefficients calculated for all the samples.

The results show that there is a weak negative correlation between the LP and SCN^- contents of milk in all samples, while the correlation for the polyol and molasses groups is even weakly positive. On the other hand, the LP content correlates positively with the cell content, the correlation being highly significant for the samples of the control group and for all samples. However, this correlation is only slightly positive for the polyol and molasses groups. The correlation between the SCN^- and cell contents also varies from one test group to the other. In the polyol group the correlation is highly positive, while there is no clear dependence when it is calculated for all the groups.

Table 6. The correlation (r value) between the LP, SCN^- and cell contents of milk in the different test groups.

Factor	Polyol group n = 112		Molasses group n = 112		Control group n = 112		All groups n = 336	
	LP	SCN^-	LP	SCN^-	LP	SCN^-	LP	SCN^-
LP content	1.000		1.000		1.000		1.000	
SCN^- content	0.076	1.000	0.019	1.000	−0.087	1.000	−0.049	1.000
Cell content	0.081	0.318**	0.141	−0.051	0.578***	0.037	0.222***	0.080

Degree of significance:
*** = $P < 0.001$
** = $P < 0.01$

4. Discussion

In several studies diet has been found to have an effect on the LP activity of cow's milk (KIERMEIER and KAYSER 1960, SYVÄOJA and VIRTANEN 1968, SKVORTSOV and KUDRIN 1976). The study of KIERMEIER and KAYSER (1960) showed that a maize silage diet increases, and a beet diet correspondingly decreases the peroxidase activity of milk. According to them, the dependence of activity on feed was most likely the result of the peroxidases contained in the feed which were secreted into the milk. In this study the diets of the test groups did not include feedstuffs which are known to have a high peroxidase activity. The diet varied only with regard to carbohydrates, which were presumed to affect the LP activity. Even though there was a statistically significant difference in the LP contents of milk in the different test groups on the basis of means (Table 1), other observations do not reinforce the supposition that the diet used had any effect on the LP activity. If one compares the LP level during the test period with the level during the stabilization period, one notes that in this respect there is a clear change only in the control group, in which the LP level on an average nearly doubled. Surprisingly, the LP level of the polyol group was markedly higher even during the stabilization period than that of the other groups. There was no significant change in the level during the test period, from which it can be concluded that a polyol pulp diet did not have the expected effect on LP activity.

The metabolism of polyols in ruminants has not been fully elucidated. POUTIAINEN et al. (1976) noted that the rumen microbes of the cow weakly fermented xylitol and arabinitol *in vitro*. When a polyalcohol mixture was infused into the rumen, it was, however, obvious that the animal absorbed and metabolized polyalcohols. Even though it is thus possible that polyols are secreted as such into the milk, or that they generally affect the cow's enzyme activities, it is probable that the metabolic effects of ingesting polyols on secretory enzymes are most clearly seen in monogastric animals and in man, as noted by MÄKINEN et al. (1975). In this connection it can be mentioned that the addition of xylitol to milk was not found to have any effect on the LP activity under *in vitro* conditions.

Lactoperoxidase is a secretory enzyme which, when it occurs in milk, is evidently synthesized in the udder tissue (TAYLOR and KITCHEN 1970). The

factors which affect the biosynthesis of LP are not, however, known. KIERMEIER and KAYSER (1960) noticed that the breed of cow has an effect on the peroxidase activity of milk, which shows that genetic determinants are probable. These might partly explain the large, permanent differences of LP activity in the milk of different cows which were noticed in this and several other earlier studies. On the other hand, the LP activity of the milk of the five Friesian cows in this study did not, on an average, differ from the LP level of the milk from Ayrshire cows.

Calculated on the basis of the daily secretion of LP the cows in the polyol group produced on an average 51.5 % more lactoperoxidase in their milk per day during the test period and the cows in the molasses group 42.5 % more LP than the cows in the control group. This observation is hard to explain, because the physiological conditions did not differ markedly among the various test groups. The marked differences between the individuals in the LP production further reinforce the concept of the role of genetic factors.

These observations raise the question of the significance of LP synthesis and content on activating the $LP/SCN^-/H_2O_2$ antimicrobial system in milk. Even though there was LP activity in all milk samples, it was continuously very low in certain cows, and a sudden, transient drop was also found in cows whose LP level was normally high. No reason for these fluctuations could be found. It is evident that a certain LP activity is needed in milk in order to activate the inhibitory system, since milk is known to contain factors which weaken the inhibitory system such as catalase and reducing compounds (WRIGHT and TRAMER 1958, BJÖRCK 1977).

According to BJÖRCK et al. (1975) and REITER et al. (1976), cow's milk does, however, normally contain a sufficient amount of lactoperoxidase to enable the antimicrobial system to function. The contents of SCN^- and H_2O_2 are, on the other hand, considered to be critical factors for the functioning of the system. According to BJÖRCK (1977), the physiological SCN^- content of milk is sufficient to ensure a bactericidal effect if an equimolar amount of hydrogen peroxide is present.

According to the results of this study, the SCN^- content of milk varied among the test groups on an average between 0.87 and 1.01 mg/l (Table2), but variations among individuals were considerably larger. These averages correspond to the results obtained by PYSKA (1974), but are lower than those presented in several other studies (VIRTANEN 1963, LAWRENCE 1970, HOPPE et al. 1971, KORHONEN 1973). It is acknowledged that it is possible to affect the SCN^- content of milk through diet (VIRTANEN 1963), but since in this study the animals in the same group had an identical diet, the variations noted among individuals cannot be explained on this basis. Udder infections have been noted (KORHONEN 1973) to increase the SCN^- content of milk, and this observation is further strengthened in this study (Table 6).

Not a single milk sample was found to contain hydrogen peroxide with the method used (minimum limit 5 μg H_2O_2/ml). The sensitivity of the test is, however, so slight that whatever low contents (2—4 μg/ml) were found in freshly cannulated milk (REITER 1976) were not registered. According to the latest observations (KORHONEN and REITER 1977) the PMN leucocytes of milk

produce considerable amounts of hydrogen peroxide when phagocytizing casein micelles or bacteria *in vitro*. Thus we can assume that the conditions may exist for the active functioning of the LP inhibitory system *in vivo* in the udder. There are indications of this when a comparison is made of the LP contents (Figure 1) and cell contents (Figure 4 and Table 4), on the one hand, and the appearance of udder infections (Table 5) in the various test groups, on the other. The cows in the polyol group had on an average the highest LP content, the lowest SCN^- content, the lowest cell content and the smallest number of clinical or subclinical udder infections. Since the results of the molasses group are in diametric contradiction to these, the observations may be regarded as an indication that the LP inhibitory system is active *in vivo* and inhibits microbial infections in the udder. Udder pathogenic bacteria are sensitive to the effect of the LP system *in vitro* (REITER 1976), but no *in vivo* tests have been carried out. According to REITER (REITER and BRAMLEY 1975, REITER 1976), there is, however, indirect evidence that the LP system is active in cows. This view is supported e.g. by the previous results of KORHONEN (1973) on the relationship between LP activity and the SCN^- content, and the occurrence of udder infections.

As a summary of the results presented above it appears that a diet with polyols or molasses in the amounts used here did not have any noticeable effect on the LP activity of cow's milk. Individual differences in the LP activity may results from genetical and physiological factors. There seems to be a certain interdependence between LP content and the occurrence of udder infections, which suggests that the LP inhibitory system in milk is important for bovine udder health. Confirmation, of this view will, however, require *in vivo* tests on the activity of the LP system in the udder. In addition it would be interesting to determine whether it would be possible to activate the LP system *in vivo* and/or strengthen it using a suitable diet.

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SELOSTUS

Sokerialkoholiseoksella käsitelty tai melassoitu juurikasleike lypsylehmillä säilörehuruokinnalla

II. Polyoli- ja melassiruokinnan vaikutuksesta lehmänmaidon laktoperoksidaasi- ja tiosyanaattipitoisuuteen sekä utareterveyteen

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Kokeessa tutkittiin ruokinnan vaikutusta lehmänmaidon laktoperoksidaasin (LP) ja tiosyanaatin (SCN^-) pitoisuuteen syöttämällä 24 lypsylehmälle (19 Ayrshire, 5 friisiläistä) 12 viikon ajan dieettiä, joka erosi eri koeryhmissä (8 eläintä/ryhmä) hiilihydraattisisällön suhteen. Vertailukautta edelsi kahden viikon vakiointikausi, jolloin kaikilla lehmillä oli sama dieetti. Vertailukaudella ryhmä I (polyoliryhmä) sai päivittäin polyoliseosta leikkeenä 483 g eläintä kohti. Ryhmä II (melassiryhmä) sai päivittäin sokereita melassileikkeen muodossa 410 g eläintä kohti. Ryhmä III (vertailuryhmä) ei saanut ylimääräistä hiilihydraattirehua. Kaikilta koeeläimiltä otettiin viikottain maitonäytteet, joista analysoitiin LP- ja SCN^--pitoisuus ja vety-

peroksidin esiintyminen sekä somaattisten solujen lukumäärä lehmien utareterveyden arvioimiseksi. Saatujen tulosten perusteella arvioitiin LP/SCN^-/H_2O_2-antimikrobisen systeemin mahdollista vaikutusta utareterveyteen.

Analyyseissä saatiin seuraavat tulokset:

LP-pitoisuus oli erittäin merkitsevästi ($P < 0.001$) suurempi polyoli- ($\bar{x} = 17.8$ µg/ml) kuin melassi- ($\bar{x} = 12.6$ µg/ml) ja vertailuryhmässä ($\bar{x} = 11.7$ µg/ml). Polyoliryhmän lehmät tuottivat maidossa päivittäin 51.5 % enemmän laktoperoksidaasia kuin melassiryhmän ja 42.5 % enemmän kuin vertailuryhmän lehmät. Polyoliryhmän LP-pitoisuus oli suurin jo vakiointikaudella eikä merkittävää muutosta, mikä olisi johtunut erilaisesta ruokinnasta, havaittu tässä eikä muissa ryhmissä vertailukauden aikana. Eri lehmillä esiintyi kuitenkin suuria eroja LP-tasossa ja ne näyttivät olevan pysyviä.

SCN^--pitoisuus oli pienin polyoliryhmässä ($\bar{x} = 0.87$ mg/l) ja suurin melassiryhmässä ($\bar{x} = 1.01$ mg/l) eron ollessa hyvin merkitsevä ($P < 0.01$). Vertailuryhmän SCN^--pitoisuus oli $\bar{x} = 0.91$ mg/l. SCN^--pitoisuus vaihteli huomattavasti eri lehmillä. Keskiarvojen perusteella nousi SCN^--pitoisuus vähäisesti kaikissa ryhmissä koekauden aikana. Missään maitonäytteessä ei havaittu vetyperoksidia (≥ 5 µg H_2O_2/ml).

Solupitoisuus oli alhaisin polyoliryhmässä ($\bar{x} = 152\,000$ solua/ml) ja korkein melassiryhmässä ($\bar{x} = 626\,000$ solua/ml) eron ollessa erittäin merkitsevä ($P < 0.001$). Vertailuryhmän solupitoisuus oli $\bar{x} = 285\,000$ solua/ml. LP- ja solupitoisuuden välinen korrelaatioaste vaihteli eri koeryhmissä, mutta oli keskimäärin $r = +0.222$ ($P < 0.001$). SCN^-:n ja solupitoisuuden välillä oli selvä korrelaatio vain polyoliryhmässä ($r = +0.318$) ($P < 0.01$). LP- ja SCN^--pitoisuuden välillä oli heikko negatiivinen korrelaatio. Solupitoisuuden ja kliinisten utaretulehdustapausten perusteella oli polyoliryhmän lehmillä paras ja melassiryhmän lehmillä huonoin utareterveys.

Tulosten perusteella voidaan todeta, että polyolien ja melassin syöttö kokeessa käytettyinä määrinä kolmen kuukauden ajan ei vaikuttanut havaittavasti maidon LP- tai SCN^--pitoisuuteen. Toisaalta tulokset viittaavat siihen, että LP:n katalysoimalla antimikrobisella systeemillä on merkitystä utareterveydelle, sillä maidon LP-pitoisuuden ja utaretulehdusten esiintymisen välillä havaittiin käänteinen suhde.