GENETICAL AND SOME ENVIRONMENTAL INFLUENCES AFFECTING THE LEVEL OF LEUCOCYTE COUNTS IN THE MILK OF COWS

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636.2:591.146:591.111.1
619:636.2:618.19-002

GENETICAL AND SOME ENVIRONMENTAL INFLUENCES AFFECTING THE LEVEL OF LEUCOCYTE COUNTS IN THE MILK OF COWS

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR. F. HELLINGA
HOOGLERAAR IN DE CULTUURTECHNIEK,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG, 13 OKTOBER 1967 TE 16.00 UUR

DOOR

Y. A. AFIFI

H. VEENMAN EN ZONEN N.V. - WAGENINGEN - 1967

THEOREMS

I

Using more milking units per milker at the end of lactation will increase the risk of incidence of mastitis.

(This Thesis)

II

Leucocytes number in cattle milk can be decreased by selection.

(This Thesis)

III

For proper emptying of the udder, milking should be completed before the action of posterior pituitary hormone on udder tissue disappears.

[FOLLEY, S. J. (1956),
"Physiology and bilohemistery of lactation". Edinburgh]

IV

Increasing the milk production in subtropical countries by means of grading up to Friesian must be accompanied by improving systems of feeding and management.

[WEBSTER, C. C. and WILSON, P. N. (1966) "Agriculture in the tropics". London]

V

The combined service centres and supervised cooperatives are valuable methods for economic and social development in rural community.

[DOREEN WARRINER (1961) "Agrarian reform & community development in U.A.R." Department of Economics, London University]

VI

In countries like U.A.R. a better utilization of Egyptian clover (Trifolium Alexandrinum) during winterseason could contribute in solving the problem of livestock underfeeding during summertime.

[SENCUS (1961) published by Ministry of Agriculture (feeding department) U.A.R.]

VII

In warm countries, it is necessary to keep and store the eggs under suitable preservation conditions to prevent deterioration in egg quality from the time of laying until consumption.

[SWANSON, M.H. (1958) World's Poultry Science Journal Vol. 14 no. 1, Orr, H. L. and Snijder, E. S: (1959) Poultry Science, vol. 38. Oodwin, T. L. G. (1964) Poultry Science Vol. 43 no. 5]

VIII

Herds improvement on a national scale can be partly achieved by the use of adequate herd records which the farmers keep in their herds.

[BRADBURY, C. J. (1957) Victorian dairy farming digest, Vol. 4, Australia].

Y. A. AFIFI
Wageningen, 13 October 1967

WOORD VOORAF

Dit onderzoek werd verricht bij de Afdeling Veeteelt van de Landbouwhogeschool te Wageningen onder leiding van prof. dr. TH. STEGENGA.

Gaarne wil ik mijn welgemeende dank betuigen aan mijn hooggeachte promotor, prof. dr. TH. STEGENGA, voor zijn leiding, waardevolle adviezen en opbouwende kritiek gedurende deze studie en voor het doorlezen en corrigeren van het manuscript.

Aan de stafleden van de Afdeling Veeteelt van de Landbouwhogeschool ben ik veel dank verschuldigd voor hun medewerking tijdens deze studie, in het bijzonder aan ir. M. BEKEDAM voor zijn waardevolle adviezen en voor de vertaling van de conclusies en samenvatting in het Nederlands en aan dr. ir. R. D. POLITIEK, die altijd bereid bleek hulp en advies te geven.

Bijzonder erkentelijk ben ik dr. ir. C. J. SCHIPPER, van de 'Werkgroep Hygienische Melkwinning, T.N.O.' voor de gelegenheid geboden om het praktische deel van deze studie bij het laboratorium van de Werkgroep in Wageningen uit te voeren en voor zijn waardevolle suggesties, ir. A. J. DIJKMAN voor zijn belangstelling in deze studie, en de heren J. POST en M. J. NUNINGA voor hun vriendelijke hulp bij de praktische werkzaamheden.

Gaarne richt ik een woord van dank aan Mej. J. C. TEN BENSEL voor haar hulp, aan Mevr. E. HAAK-VAN DEN BRINK en Mej. G. H. DEKKER voor het typen van het manuscript, aan de heer W. HEIJE voor het tekenen van de figuren en aan de heer J. STELWAGEN voor zijn hulp bij het verzamelen van de monsters.

De prettige samenwerking met de heer M. H. PARDIJS, de Hoofdcontroleur van de Provinciale Melkcontrole Dienst voor Utrecht, bij de regeling met de melkcontroleurs van de plaatselijke coöperaties voor het verzamelen van de melkmonsters en bij het uitvoeren van het mastitisonderzoek en de andere enquêtes, heb ik zeer op prijs gesteld.

Gaarne memoreer ik de hulp van alle melkcontroleurs, veeverzorgers en technici die meegewerkt hebben door het geven van inlichtingen over de mechanische eigenschappen van de melkmachines en over de veeverzorging.

Ir. A. H. KRUIT, Directeur van het Melkcontrole Station Utrecht, dank ik zeer voor zijn prettige regeling van de uitvoering van dit onderzoek.

Mijn welgemeende dank betuig ik aan de heer M. KEULS van de Afdeling Wiskunde van de Landbouwhogeschool te Wageningen voor zijn waardevolle statistische adviezen, geduldige samenwerking en leiding bij het analyseren van de gegevens van deze studie met behulp van computers. Dankzij zijn hulp is deze studie voltooid.

Voor haar hulp bij het corrigeren van de Engelse tekst dank ik Mej. VAN ROSSEM, I.B.S.

Bijzonder erkentelijk ben ik de regering van de V.A.R., die de nodige middelen heeft verschaft om deze studie in Nederland uit te voeren.

Tenslotte wil ik gaarne alle leden van de Landbouwhogeschool te Wageningen en alle anderen, die op enigerlei wijze iets hebben bijgedragen om deze studie mogelijk te maken, bedanken.

CONTENTS

This thesis is also published as Mededelingen Landbouwhogeschool Wageningen 67-11 (1967)
(Communications Agricultural University Wageningen, The Netherlands)

1. INTRODUCTION AND SCOPE OF THE STUDY

It is well known that cow milk contains some leucocytes. The number is controlled by physiological and environmental factors, management and sanitation. At the same time, it has been observed and shown that heredity may have some influence on leucocytes in the milk.

Leucocytes are an important part of the body's natural defence mechanism against bacteria and other foreign irritants. Their presence in the milk in excessive number indicates stimulation of the defence mechanism against some invader. The total leucocyte count in the milk probably is the best test for detecting udder inflammation, although the cause of inflammation and type of infection must be determined by other means. Moreover most methods used in diagnosing mastitis, depend on the reaction of certain reagents with leucocytes in the milk, similar as in the Whiteside test, California mastitis test and B.M.R.[1] These methods are considered as simple field tests for the presence of mastitis. The cellular content of milk has received only limited attention from a genetic viewpoint. However, many observations indicate that there are inhereted differences in susceptibility of the animal to udder infection and mastitis. Moreover it has been investigated by other workers that there is a high genetic correlation between clinical mastitis and leucocyte counts and it is suggested that the selection for low leucocyte counts aids to the selection for greater resistance to clinical mastitis. Before using leucocytes in the milk as a selection aid against udder troubles and before suggesting possible ways of controlling mastitis, it is necessary to know in how far such methods can be related with other important economic traits, as productivity of the cow particularly milk yield and ease of milking.

The remarkable increase in using milking machines has created many problems for the dairy farmers. During recent years, increasing experimental attention has been directed to problems involved in mechanical milking, as studying the effect of various machine milking conditions on udder health and on production performance of cows.

This work has been carried out to study the following items:

1. To analyse the variation in cell counts of milk obtained from individual cows milked by machine under practical conditions. The main task is to detect to what extent genetic factors influence the variation in cell counts under this practical conditions in different seasons and in different stages of lactation. This may give more information on the question of the importance of selection in decreasing cell counts in the milk and perhaps at the same time the frequency of mastitis.

2. To study as far as possible the relation between cell counts and other important economic traits of the cow, such as milk production and ease of milking as well as the relation between leucocyte counts in the milk and clinical mastitis.

[1] Brabantse Mastitis Reactie.

3. To measure under practical conditions the influence of management and environmental factors on cell counts, such as milking vacuum level, pulsation rate, system of milking, hand or machine stripping, herd size, the ratio between number of milkers and number of milking machines used at milking time.

2. REVIEW OF LITERATURE

2.1. Leucocyte counts in normal milk

Until now opinions are divided on what is the normal number of cells in the milk of healthy cows. The cellular content of normal milk has been shown to fluctuate between wide limits. Cooledge (1918) reported approximately 900.000 cells per ml of milk from healthy animals. Cherrington et al (1933) found that milk from normal udders usually contains less than 500.000 leucocytes per c.c. of milk and Prouty (1934) reported 225.000. Plastridge et al (1939) found the average of normal milk to be 73.000 cells per ml. Chu (1949) stated that the total cell count of 100.000 per ml, which he said was generally regarded as the borderline for normal milk, was too high. He found that pathological change was present in the udder tissue, whenever the count was above 21.000 per ml. He adopted 20.000 per ml as provisional figure. MacLeod and Anderson (1952) found the average cell counts of milk from healthy cows to be 70.000 per ml. Schipper (1963) showed that the number of cells in the milk from one cow was always higher during a whole lactation period than the leucocyte number in the milk obtained from another cow under the same environmental conditions, without any indication of udder inflammation. The mean leucocyte count of one cow was $\pm$ 600.000 per ml, whereas it was $\pm$ 100.000 for another one. Smith et al (1964) reported that the mean cell count in the milk from bacteria free and clinically normal individual quarter of 46 cows in the second lactation was 765.000 per ml. He also stated there were insignificant differences between the mean counts of individual cows and quarters within cows. The mean ranged from 150.000 to 2.150.000 per ml milk.

2.2. Factors affecting leucocyte counts in milk

2.2.1. *Environmental factors*

2.2.1.1. Stage of lactation

Cell counts of the colostral milk will be higher than counts of milk taken later in the lactation cycle according to the investigation of Wayne and Macy (1933). They found that the cell counts during this period between different udder quarters of different cows ranged from 1.315.000 to 21.585.000 per c.c. MacLeod and Anderson (1952) showed that cell counts of milk of healthy cows in the first two days after parturition may be expected to be over 1.000.000 cells per ml, but after that there will be a gradual decrease in the counts until the forth week. From the first month after calving until the end of the fortyfirst week of lactation the mean cell count of milk from healthy animals remains at approximately the same level. The drying off period also affects the level of cell counts in the milk. Wayne and Macy (1933) reported that cell counts decidely higher immediately following periods of suspended milking, whereas the bacterial counts were slightly higher. He also stated that the methods of drying off the cows affects the bacterial and cell content of milk. Intermittent

and incomplete milking usually resulted in somewhat higher bacterial and cell counts during the drying off period, whereas complete cessation of milking appeared to give the most satisfactory results. NEAVE et al (1950) stated that a large number of infections occurred during the dry period and over half of these developed during the first 3 weeks after the final milking. They also reported that the method of drying off cows may have considerable influence on the amount of infection in this period. BRAUND and SCHULTZ (1963) stated that seventy per cent of quarters showed positive reactions to the California mastitis test at the end of lactation.

2.2.1.2. Time of milking

MACLEOD and ANDERSON (1952) reported that cell counts of herd milk samples from the evening milking may be expected to be slightly, but significantly higher than those in the morning milk samples. The average cell count in the morning samples was 275.000 cells per ml and that in the evening was 290.000 cells per ml.

2.2.1.3. Age of cow

CAMPBELL (1909) found that the leucocyte count and sediment was low in milk from a young cow in its first lactation period. No streptococci were found in examining the sediment. SMITH et al (1964) reported that the mean leucocyte counts in milk from healthy cows in the first lactation was 178.000 $\pm$ 10.000 and the cows means ranged from 80.000 to 410.000 per ml, whereas it was 765.000 $\pm$ 20.000 in the second lactation for the milk of another group, in which the cows means ranged from 150.000 to 2.150.000 per ml of milk. About the incidence of mastitis, LEGATES et al (1952) reported that there is a marked difference in the incidence of mastitis in first calf heifers compared to older cows. The average incidence of mastitis among the 215 heifers only was 16.7 per cent compared to 51.1 per cent for the 959 older cows. BRAUND et al (1963) stated that the incidence of mastitis increased with age and they suggested that it seemed logical that the older cows were much more apt to be positive to mastitis screening test (C.M.T., based on the number of cells in milk) than young cows as shown in the following (Table 1).

TABLE 1. Relation between incidence of mastitis and lactation period [BRAUND et al (1963)]

Lactation period	Experiment I		Experiment II	
	Total quarters	Percentage of quarters positive to C.M.T.	Total quarters	Percentage of quarters positive to C.M.T.
1	1523	13.8	3308	7.5
2	1269	25.2	1848	16.7
3	866	37.3	1547	25.7
4	673	26.4	900	26.9
5 and over	1281	48.5	1050	34.4

SCHMIDT et al (1965) reported that the incidence of infection and the presence of abnormal milk increased with advancing age, and there is a significant correlation between age and incidence of mastitis.

2.2.1.4. Machine milking

The result of special studies and surveys considered the amount of mastitis and leucocyte counts in the milk of herds milked by hand and by machine, usually were in favour of hand milking. Microscopic examination of large numbers of milk samples from machine and hand milk cows, undertaken by CONE (1944), showed that samples from machine milking contained the higher leucocyte counts and incidence of mastitis. He considered the effect of the time that the machine remained on the cows as a main factor influencing the high leucocyte counts.

2.2.1.4.1. *Vacuum level*

Raising the milking vacuum has a tendency to cause more damage to teat apex and this in turn may result in high leucocyte counts in the milk and more infection. NEAVE, SHARP, OLIVER and DODD (1957), reported that evidently teat erosions were significantly more common and severe with high vacuum, generally 2–3 times more severe. This is shown in table no. 2.

TABLE 2. The relation between teat erosion and milking vacuum [NEAVE et al (1957)]

Mean* erosion score	Number of quarters in each erosion class								
	0	0.01 – 0.25	0.251 – 0.50	0.51 – 1.0	1.01 – 1.5	1.51 – 2.0	2.01 – 10	Quarters total	Cows total
Milking vacuum									
12.5 inch Hg	33	38	20	10	5	3	1	110	35
20 inch Hg	0	16	25	20	20	9	13	110	35

* Teats examined by two persons each month throughout the first lactation period. A score of over one, signifies very distinct erosion.

WILSON (1958) claims that an inadequate vacuum reserve, resulting in wide vacuum fluctuations during milking, is an important predisposing cause of mastitis, and not the vacuum level as such. Since changing from one vacuum level to another in either direction may cause a temporary fall in milk yield and an increase in leucocytes, the latter may have increased as a result of milk being retained in the udder until the cow was accustomed to the change in vacuum and pulsation. MOBERG (1963), reported that when the machine is left overmilking, especially with a high vacuum, an inversion of the mucous membrane of the streak canal is very often the result. In the end, this will lead to acute or chronic disease conditions of the streak canal and further to infection

and mastitis. BRANDSMA et al (1965) showed that with a vacuum in excess of 40 cm of mercury there is more chance of affecting udder health. The B.M.R. test (Brabantse Mastitis Reactie) for three groups milked at three levels of vacuum 37, 46 and 63 cm of mercury showed 6.5, 10.0 and 9.9 per cent respectively.

2.2.1.4.2. *Pulsation rate*

There are few publications about the relation between pulsation rate and udder infection. But there are some reports showing that the absence of pulsations may be associated with a high level of udder infection. A report of SHINFIELD (1948)[1] mentioned that the wrong replacement for a broken part on the milking machine, resulted in no pulsations to the teat-cups milking the right side of the udder, whereas the left side received weak pulsations from a master pulsator on the engine. An examination of milk samples from half of the udder showed that there was significantly more infection (mainly streptococcus agalactia) in the glands of the right side, indicated by the following figures:

	Total quarters	quarters infected
Weak pulsations	38	11
No pulsations	38	36

Pulsations are necessary to prevent the congestion and the discomfort of continuous powerful suction on the cow's teats. However, increasing the pulsation rate to a high level will also show a harmful effect, BRATLIE et al (1963).

2.2.1.4.3. *Teat-cup liner*

The design of the teat-cup liner is known to affect the amount of mastitis. Stiff rubber liners are stated to be injurious and it is thought by some investigators that the stiff (moulded) liners are associated with more mastitis than soft (extruded) liners. DODD, OLIVER and NEAVE (1957) were able to confirm that quarters milked by the moulded liner had significantly more mastitis than those milked by extruded one. However, OLIVER et al (1957), using another type of moulded liner, could not find any more mastitis in first lactating animals than by using extruded liner. They suggested that their results may be due to the design of the liner or because only heifers were used. Other research indicated that using the wrong size of teat cup caused congestion of the teat. Teat cups with metal mouthpieces are said to cause damage to the teat.

2.2.1.4.4. *Stripping by hand or machine*

Investigations by NEAVE et al (1954) in two commercial herds, of which half the animals were stripped by hand and half by machine, showed that streptococcus mastitis was most prevalent in the cows stripped by machine and a higher proportion of the infections resutlted in mastitis (Table 3).

[1] Rep. Nat. Inst. Res. Dairying, Reading, 1948, 40.

TABLE 3. Hand versus machine stripping of machine milked cows (combined data from two herds) [NEAVE et al (1954)]

Stripping by	No. of cows	Cows infected	Quarters		Infections*			Mastitis cases detected by cowmen
			Infected	Mastitis	Total	Mastitis	%	
Hand	45	28	75	24	128	25	19.5	9
Machine	51	30	80	49	115	63	54.8	24

* Nearly all infections were caused by str. agalactia.

NEAVE et al (1954) suggested that the vacuum during machine stripping may cause damage to the lining of the teat and udder sinus.

However, later in 1957, he did not find a significant difference in the proportion of the total infections resulting in mastitis when vacuum was at 12.5 and 20 inch of mercury. OLIVER et al (1956) found more infections occurring in hand-stripped cows after they had been dried off at the end of lactation (Table 4).

TABLE 4. Infection in the dry-period of hand and machine stripped cows [OLIVER et al (1956)]

Method of stripping in previous lactation	No. of quarters not infected at drying off	No. of new infections in the dry period	Mean age of cows (lactations)	Mean yield (lb) at drying off
By hand	188	70	3.5	12.8
By machine	208	38	3.5	9.1

2.2.1.4.5. *Duration of milking*

Leaving the teat cups on the udder after the milk flow has ceased often causes discomfort to the cows and is regarded as poor practice and injurious to the teat. The best thing is to remove the teat cups as soon as the milk flow has ceased. PETERSEN (1944) stated that it is common practice for one man to milk with more milking machine units than he can handle efficiently. This results in the teat-cups being left on the udder for much longer than necessary for milking. In a study, in which 19 animals in the first lactation were regularly milked by machine for 4 min and other for 8 min throughout the lactation, DODD et al (1950) found significantly more infection and mastitis in the latter animals. In the 8 min group 16 quarters of 7 cows were infected and 8 of them developed mastitis. Whereas the corresponding figures in the 4 min group were 4 quarters of 4 cows infected and none developing mastitis.

2.2.1.5. Udder infection:

COOLEDGE (1918) reported that B. abortus infected udders gave milk with approximately five times as many leucocytes as normal cows. CHERRINGTON et al (1933) reported that leucocyte counts seem to be the most reliable index of

udder infection, because of the enormous number of leucocyte found in the milk from infected quarters, and generally high leucocyte content in milk drawn from apparently normal quarters of the same infected udders. The fact that the leucocyte content of milk remains constant after it is drawn, adds to its reliability. He found the average cell count per ml of milk from healthy cows to be 43.000, whereas the average of diseased cows was 3.000.000. PLASTRIDGE and his co-workers (1939) found milk containing haemolytic staphylococci to have an average count of over 1.000.000 cells per ml. MACLEOD et al (1953) reported that 78% of the variation in the average leucocyte counts in herd milk may be explained in terms of the percentage of mastitis animals within the herd. He stated that if the percentage of infected animals within a herd is 40 or over the average leucocyte counts in the herd milk may be expected to be 1.000.000 or over. He also found under his experimental conditions that S. agalactia infection was associated with higher leucocyte counts in the herd milk than the corresponding percentage of infection caused by organisms other than S. agalactia. This association, however, was not statistically significant. NEWBOULD and BARNUM (1959) reported that milk from cows with mastitis contains a large number of leucocytes. In acute mastitis the count may run to many millions per ml. In chronic mastitis infected quarters will shed over 500.000 per ml of milk. BAKER and BREED (1920) suggested that the decrease in milk secretion might be due to a weakening of secretory activity of gland cells, or to an increased entrance of leucocytes into the lumina of the alveoli.

2.2.1.6. Herd size

No publications were to be found on the effect of herd size on the cellular content in the milk. However there are other publications about the effect of herds size on the incidence of mastitis, which will indirectly affect the level of leucocyte in the milk. WARD (1942). RØMER (1953) and WITHERS (1956) obtained data showing that mastitis increased with the size of the herds; relatively many of the small herds were hand milked. CAZEMIER et al (1964) stated that a single evaluation of milking technique was carried out, supported by recording the time taken for several numbers of milking operations. Is was found that in herds where a mastitis problem exists, significantly less care was taken of the milking technique on an average than in herds in which few or practically no mastitis cases occurred. But difference in herd size did not result in difference in incidence of mastitis.

2.2.1.7. Hygiene

Research in this subject agrees that following a good hygienic program will reduce the transfer of micro-organisms from one udder to the next and consequently the amount of mastitis which affects the level of leucocyte counts in the milk [STABLEFORTH et al (1949), EDWARDS and TAYLOR (1949) and WILSON (1952)].

2.2.2. *Genetic factors*

As has been mentioned before, it is obvious now that there are many factors affecting the level of leucocytes in the milk, especially the physiological and environmental factors. There are also some investigations indicating that heredity may have some influence on leucocyte counts in milk and incidence of mastitis.

2.2.2.1. Differences between individual cows:

MACLEOD and ANDERSON (1952) reported that milk from healthy cows differs significantly in the average cell counts. SCHIPPER (1963) stated that the number of cells in the milk from one cow was constantly significantly higher than the number in the milk from another cow under the same conditions and without any indication of udder inflammation. SMITH and SCHULTZE (1964) showed that there were large differences between the average counts of healthy cows. The average cell counts of cows in the second lactation ranged from 50.000 to 2.150.000.

2.2.2.2. Differences between families:

MURPHY et al (1944) showed from the mastitis histories, based on leucocyte counts, cup tests and physical examination of two cow families, living under the same conditions, that there were distinct differences between the two families. He stated that heredity is definitely important in bovine udder infection and mastitis.

2.2.2.3. Differences between sire's daughter groups:

REID (1954) has shown, there are significant differences among sires in the degree of genetic resistance to mastitis they transmit to their daughters. Among 18 Jersey heifers from one sire the incidence of mastitis was 55 per cent, whereas among 15 heifers from other sire's its frequency was less than 14 per cent. He concluded that the major factor affecting the occurrence of mastitis in this case was heredity.

2.2.2.4. Heritability of leucocyte numbers in milk and resistance to mastitis

LUSH (1950) estimated the heritability of resistance to mastitis to be 0.38. A cow was considered susceptible if it showed abnormal quarters or abnormal milk by the time they reached the age of 8 years. He reported that differences in susceptibility to mastitis apparently show a considerable genetic back ground. He stated that the selection against cows which were severely effected or have severely effected sisters or daughters should lower the incidence of mastitis. LEGATES et al (1952) also determined the heritability of resistance to mastitis to be 0.27. A cow was considered susceptible if the milk of one of the quarters contained over 500.000 leucocytes per ml of milk and demonstrable streptococcus or staphylococcus organisms. He added that using artificial insemination of sires whose dams and sisters demonstrated resistance to mastitis, presents

the most promising immediate breeding approach. YOUNG et al (1960) estimated the heritability of leucocytes in the milk. Their experiment included 422 cows terminating 682 lactations of 7 months duration or longer, during the period from November 1954 to January 1958. The results showed that the estimation was 0.38 $\pm$ 0.20 from the regression of daughter on dam and 0.23 $\pm$ 0.22 from the analysis of variance of the intra-class correlation between paternal sisters. The estimation for the repeatability of leucocytes in the milk was 0.42 $\pm$ 0.05. They also estimated the genetic correlation for clinical mastitis with leucocyte counts from the paternal sister and daughter dam regression. They were 0.80 and 0.98 respectively. From these results they suggested that many of the genes influencing clinical mastitis also influence leucocyte counts.

If this should be so, the selection for low leucocyte counts should automatically result in selection for low clinical mastitis or greater resistance to clinical mastitis. They also estimated the heritability and repeatability of clinical mastitis, defined by the percentage of lactation months in which the cow showed an abnormal appearance of the udder or its secretion. In the same study the heritability of bacterial infection was estimated (Table 5).

TABLE 5. Heritability and repeatability estimation for clinical mastitis, bacterial infection and leucocyte counts [YOUNG et al (1960)]

	Heritability estimated from		Repeatability
	Daughter-dam regression	Paternal half-sister correlation	Intra-class correlation
Clinical mastitis	0.06 $\pm$ 0.18	0.79 $\pm$ 0.21	0.31 $\pm$ 0.06
Bacterial infection	0.18 $\pm$ 0.14	0.87 $\pm$ 0.21	0.24 $\pm$ 0.06
Leucocyte counts	0.38 $\pm$ 0.20	0.23 $\pm$ 0.22	0.42 $\pm$ 0.05

O'BLENESS et al (1960) obtained a heritability estimate of 0.05 for the incidence of mastitis. This was based on the dairy farmer's information of whether the cow had produced abnormal milk previously. GAUNYA (1962) studied the influence of environmental and genetic factors on the resistance to bovine mastitis. Mastitis was quarterly diagnosed by leucocyte and bacteriological examinations. The data included 1001 mastitis histories of cows which had at least one positive test or had completed two lactations without a positive test. The degree of resistance was expressed by three methods, lactation age at which the cows get mastitis (Y_1), lactation age at which the first positive test was observed (Y_2) and a resistance-susciptible classification in which cows not designated as mastitis (Y_3). The heritability of resistance to mastitis for the first two variables ($Y_1 - Y_2$) within the herd was estimated by several ways and the following results were obtained (Table 6).

SCHMIDT et al (1965) showed that paternal half-sib heritability estimates of milk yields, number of quarters showing abnormal milk were obtained from an analysis of milking and mastitis data of 2865 Holstein cows. The within herd heritability estimates for daily milk yield was 0.353 and that for the number of

TABLE 6. The estimation of heritability for resistance to mastitis within herd [GAUNYA (1962)]

Methods of estimation	Y_1	Y_2
1. Intra-class correlation between paternal sisters	0.07	0.18
2. Intra-class correlation between maternal sisters	0.11	zero
3. Regression of daughter on dam	0.12	0.14
4. Full sib correlation	0.34	0.34
Pooled estimation	0.13 ± 0.06	0.03 ± 0.02

quarters infected with streptococcus agalactia 0.196. The heritability estimates for the number of quarters infected with organisms other than streptococcus agalactia, number of quarters infected, and number of quarters showing abnormal milk were all below 0.10. He stated also that older cows showed higher values for all indicators of mastitis.

2.2.3. *Relation between ease of milking, milk production and leucocyte count*

In the literature very few data are to be found on cell counts in the milk in relation to milk yield, ease of milking and udder conformation. The relation between these characters and mastitis is studied by many investigators.

2.2.3.1. The relation between milk rate and mastitis

MURPHY (1944) reported that fast milking cows are more easily infected than slow milking cows. MCEWEN and COOPER (1947) found a higher frequency of mastitis among fast milking cows than among slow milking ones. This finding has been verified by DODD and NEAVE (1951), by using a peak flow as a measure of the milking rate. Data on the rate of flow was obtained on three consecutive days during the fifth week after calving for 94 cows in their first lactation. When the cows were classified into five groups, according to peak flow with a class interval of one lb per minute. It was found that the frequency of cows with clinical mastitis increased from 5% in the group with the lowest peak flow (2.42 lb per min) to 44.4% in the highest peak flow group (6.79 lb per min). The results show that there is a close correlation between milk rate and incidence of mastitis. On the other hand DODD et al (1950) reported that teat erosion appeared to be correlated with slow milking cows.

2.2.3.2. Relation between milk production and susceptibility to mastitis

MURPHY et al (1944) compared two cows families to find the resistance to mastitis. He reported that the family of greater resistance was not a low milk producing family (Table 7).

He added that there was no significant difference between the two families in milk and fat production. He also stated that it was quite likely that the family of lesser resistance had the greater milk producing ability and that milk production may have been reduced, because of the irritation brought about by infection. He concluded that the family of greater resistance was able to produce as

TABLE 7. Incidence of mastitis in relation to milk production

Family No.	* Percentage of incidence of infection	Percentage of samples containing 500.000 or more leucocytes per ml milk	Average milk production lbs	Fat percentage
383	63.3	42.1	8580	4.92
247	11.8	10.3	8407	5.29

* The incidence of infection in cow families, calculated as the percentage of total milking time during which infection was present.

much milk and butterfat as the family of lesser resistance. JOAN et al (1950) stated that subclinical streptococcus and haemolytic staphylococcus infection may persist for months and either cause a gradual reduction in milk yield of the infected quarters, or it has no measurable effect.

Both types of reaction may occur simultaneously in the same udder. They also added that following penicillin treatment of clinical cases caused by streptococci and staphylococci there is little recovery in the proportionate yield of the treated quarter until the following lactation, when recovery may be complete. DODD et al (1951–1953) reported that there was a negative relationship between mastitis resistance and milk production. The fast milking cows have higher lactational milk yield, in addition they have a higher incidence of clinical mastitis and bacterial infection. However, LEGATES et al (1952) reported that the phenotypic correlations between production (fat and milk) for the first lactation and mastitis were essentially zero. The correlation between fat yields and mastitis was 0.06 and that between milk production and mastitis was 0.015. They added that the negligible phenotypic correlation between mastitis and production in the first lactation does not support the belief that mastitis is manifest most strongly in high producers. Possible genotypic and environmental interaction may occur, in which the relation might change, when cows are provided an opportunity to reach the ultimate of their producing ability. BRAUND et al (1963) reported that the percentage of positive quarters to C.M.T. reactions was higher at level of milk production below 20 lbs per day. They added that one possible interpretation of these data is that the factors causing the positive reactions have depressed the production. An other interpretation is that the lower production is a reflection af an advanced lactation which tends to increase positive reactions. Although no attempt was made to determine the interaction between production level and stage of lactation, the authors favor the latter interpretation because of the rather marked uniformity of percentage of positive quarters until the production level drops below 20 lbs per day (Table 8).

They also found a negative relation between daily milk yield and C.M.T. reactions on bulk milk. The correlation coefficient of these two variables was not statistically significant – 0.19. They reported that the herds with higher yearly production had significantly less positive reactions on bulk samples.

TABLE 8. C.M.T. in relation to milk production level [BRAUND et al 1963]

Production level lb/day	No. of cows	Per cent positive quarter C.M.T.
1. Below 20	56	43.1
2. 20–29	219	32.5
3. 30–39	431	27.2
4. 40–49	380	30.6
5. 50–59	199	31.9
6. Above 60	132	31.9

ROTHE et al (1964) showed that increase of mastitis (as measured by C.M.T. scores) caused a decrease in milk and fat production. He stated that milk and fat production were negatively correlated with bacterial counts and also with leucocyte counts. On the other hand SCHMIDT et al (1965) reported that there was a positive relation between milk yield and number of quarters infected. However the correlation coefficient between daily milk yield and mastitis organisms was statistically insignificant. In other part of his study he stated that a larger proportion of the variation in strep.-agalactia infection was also due to herd differences and that the percentage of cows showing strep.-agalactia infection decreased as the herd production increased. Whereas the percentage of cows showing strep. non-agalactia, staphylococci, and miscellaneous organisms remained about the same as the production level of the herd increased.

3. MATERIALS AND METHODS

This research was carried out in 1964 and 1965 at the Laboratory of Animal Husbandry, Agriculture University, Wageningen, The Netherlands. The practical work was done at the Laboratory of the Research group for Hygienic Milk Production T.N.O.

The research was designed to study the genetical and environmental factors affecting leucocyte counts in the milk. The following is the description of the animals used in the experiment, the way of collecting the samples and methods of preparing and counting the smears.

3.1. Material and methods used in 1964

3.1.1. *Animals*

The previous review shows that there are some physiological and environmental factors affecting leucocyte counts in the milk. To obtain reliable results in estimating the heritability of leucocyte counts in the milk, these factors should be eliminated to a large extend, especially the variance due to age, type of milking, time of milking, stage of lactation and period of lactation.

To realise this, 692 Dutch Friesian paternal sisters belonging to 15 sires kept in a large number of farms in the province of Utrecht, were selected for this investigation. This number of cows was maintained throughout the experimental period and did not include any cows sold or dried during this period. For this reason 100 cows were excluded, the starting number being 792. The cows were almost of the same age and in their fourth lactation period, nearly all

Table 9. Number of paternal sisters belonging to each sire and their distribution over the associations (cows in the fourth lactation 1964)

Sire	Number of daughters	Number of associations over which the paternal sisters were distributed
1	52	3
2	54	2
3	39	3
4	44	2
5	41	1
6	38	4
7	26	1
8	47	3
9	56	1
10	49	3
11	50	4
12	44	4
13	53	4
14	42	3
15	57	2

were spring calving and all were milked by machine. These cows were chosen according to the available records at the milk recording office in the province of Utrecht (Provinciale Melk Controle Dienst voor Utrecht). According to this selection it was impossible to find a large number of daughters belonging to one bull, the average number was about 50 daughters per sire. For the same reason the daughters of most sires were distributed among more than one milk recording association in the province of Utrecht, but the distance between them was not large. (Table 9) refers to the number of paternal sisters belonging to each bull and their distribution over the associations.

3.1.2. *Collection of milk samples*

Milk samples of individual cows were obtained in June, September and November 1964. It was impossible to collect all the milk samples in one day, because a large number of cows was distributed over several local milk recording associations. Moreover, the milk samples of the cows belonging to each association were tested once in three weeks, to registrate their milk production. To facilitate an easy way for collecting the samples, the following program was designed. The samples were collected by the milk recorders from an evening and morning milking of each cow once in every season. The milk recorders placed the samples in a special tube (10 ml) and a tablet of Potassium Bicromate (each tablet containing 25 mg Potassium Bicromate) was added to the sample for preservation. The samples were kept in a refrigerator or in a cool place during the storing period which ranged from 2–5 days until the smears were prepared for total leucocyte counts. At the end of the week on Saturday the milk samples were transported to the laboratory at Wageningen for the preparation of the smears. This work was completed in three subsequent weeks.

To investigate the effect of storage on leucocyte counts in the milk after adding Potassium Bicromate a special experiment which will be mentioned at the end of this chapter was carried out.

3.1.3. *Preparation of smears for total leucocyte counts*

3.1.3.1. Preparation of smears

The stored milk samples were placed in a water bath at 37°C for ten minutes to solve the fat, drifting to the surface of milk sample during storing. For normal distribution of the leucocytes in the milk the samples were gently shaken by an electric shaker. The smear was immediately prepared, stained and examined. Three smears were prepared for each milk sample by the following procedure.

With a certain platinum loop 0.01 ml milk was taken from the sample and spread on the surface of a square centimeter on an ordinary glass slide. Two more smears of the same sample were prepared by the same technique on the ordinary glass slide. After that the films were dried in the air and fixed by moving the object glass rapidly over the flame and stained with Newman's methyleen blue for one minute. The object glass was dried again in air and the surplus stain removed by moving the glass in a water bath and dried again.

3.1.3.2. Microscopic factor

The microscopic used for cell counts has the microscopic factor of 560.000. This factor was obtained by the following formula.

$$F = \frac{A}{G \times M}$$

A = total surface of the preparate.
G = size of the eye field of the microscope in cm^2.
M = ml of milk used to prepare the smear.

3.1.3.3. Number of microscopic fields examined

According to standard procedures the cells counted in 20 microscopic fields for every sample should be sufficient. However, in this investigation the cells were counted in 75 microscopic fields instead of 20 to find a more accurate mean leucocyte count in every sample. The number of microscopic fields to be examined is increased because the leucocyte counts in every milk sample differed from one microscopic field to another, especially when the total leucocyte counts in a milk sample are high. Increasing the number of measurements reduces the amount of variance due to special environment and this reduction of phenotypic variance represents the gain in accuracy (FALCONER 1961).

3.1.3.4. Total leucocyte counts in 1/ml milk

The average counts of 75 microscopic fields was multiplied by the microscopic factor (560.000) in order to have the counts of total leucocytes in milliliter milk. In this study we selected the method of counting the cells from the microscopic field. This method gives the true number of cells in the milk of healthy and infected cows. Whereas the WHITESIDE test, CALIFORNIA mastitis test and B.M.R., only show a positive reaction when the milk contains a high number of leucocytes, mostly not less than 500.000 cells per ml of milk. Therefore, by using these methods the exact number of leucocytes can not be obtained, particularly if the reaction is negative. Moreover, if it is positive it only indicates that the sample contains a high number of leucocytes, but it does not show the true number in 1/ml of milk. Using direct microscopic cell counts apparantly is the best method to achieve the aim of this research. In the meanwhile new methods were developed for cell estimations, such as electronic cell counting in the milk by using a special electric-apparatus. This new method could not be used in this study, because its application came too late.

3.2. MATERIAL AND METHODS USED IN 1965

This experiment was designed to study whether there is apparent differences between sire's daughter groups in the first lactation and whether it is possible to carry out the selection for decreasing cell counts in the milk or resistance to clinical mastitis at early age. 799 paternal daughters in their first lactation, belonging to 20 Dutch Friesian sires were selected for this study. As much as

possible daughters of the same bulls used in 1964 were used in this experiment as well. The experimental material was completed with daughters of other bulls. In selecting the farms in which these 799 heifers were kept, it was taken into account that in each farm daughters of at least two sires were present. Moreover, cows in their fourth lactation were included in the experiment, these cows were descendant of the same bulls used and were kept in the same farms (150 cows in fourth lactation were selected). This number of cows was maintained during the whole experimental period and did not include any cows sold or died during the experimental period. 129 cows were excluded for the above mentioned reasons and the experiment was started with 928 heifers. All animals were almost of the same age, they were in their first lactation, almost all were spring calving and all were milked by machine. The method of collecting the samples and preparing the smears for total leucocyte counts in 1965, was exactly the same as that in 1964. The daughters of most sires were distributed over more than one milk recording associations in the province of Utrecht, indicated in (Table 10).

TABLE 10. The number of paternal heifers belonging to each sire and their distribution over the associations (cows in first lactation 1965)

Sire	Number of daughters	Number of associations over which the paternal sisters were distributed
16	37	2
17	57	2
18	59	2
19	52	2
20	32	2
21	28	2
22	41	3
23	27	2
24	39	2
25	29	3
26	44	2
27	33	2
28	33	3
29	32	3
30	33	3
31	36	1
32	54	2
33	52	2
34	39	1
35	42	2

3.3. THE EFFECT OF STORAGE ON TOTAL LEUCOCYTE COUNTS IN THE MILK AFTER ADDING POTASSIUM BIOCHROMATE

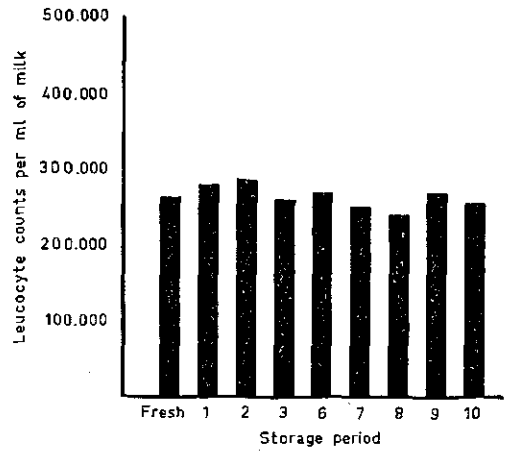
The design of the main experiment clearly indicates that it is not possible to prepare the smears from fresh milk for leucocyte counts. The milk samples were stored for periods ranging from 2–5 days before preparation. An investigation

was carried out to study the influence of storage on leucocyte counts in the milk after adding a preservative. Morning milk samples were collected from 30 cows raised at the experimental farm of the Agricultural University, Wageningen, The Netherlands. Films of the fresh milk of each sample were prepared, stained and examined, according to standard procedures, as described before. After this a tablet of Potassium Bichromate was added to ten ml of milk of each sample (each tablet containing 25 mg of Potassium Bichromate). The samples were kept in the refrigerator during ten days and films were prepared from each sample every day, except on Saturday and Sunday. Direct microscopic leucocyte counts were made from the smears and the results obtained are presented in the following table (Table 11) and (Figure 1).

TABLE 11. Effect of storage on leucocyte counts in thousands in 1/ml of milk after adding Potassium Bichromate

Sample No.	Storing period								
	Fresh milk	1st day	2nd day	3th day	6th day	7th day	8th day	9th day	10th day
1	392	467	475	392	354	437	361	324	422
2	23	45	15	30	15	7	15	23	15
3	173	166	286	211	286	309	241	233	249
4	655	761	746	851	693	475	520	700	580
5	7	15	7	38	30	0	23	15	23
6	218	241	211	271	271	294	203	233	211
7	23	30	7	15	7	0	7	0	15
8	271	248	279	271	354	346	316	392	308
9	158	196	181	158	203	264	166	203	181
10	15	15	22	7	7	15	0	7	7
11	53	37	53	133	45	45	68	60	38
12	346	309	392	264	316	286	218	279	188
13	339	369	437	279	414	324	444	369	369
14	580	618	497	459	490	444	482	490	512
15	746	791	610	655	648	603	625	505	602
16	158	279	249	349	181	203	226	264	203
17	392	467	512	399	550	414	399	429	414
18	188	181	151	151	271	195	143	241	151
19	203	166	166	173	226	203	173	233	286
20	362	264	362	286	226	324	271	316	279
21	407	300	307	227	293	274	326	307	300
22	313	327	267	300	253	220	293	367	360
23	313	267	260	267	253	313	320	347	320
24	87	80	67	33	60	27	53	80	60
25	220	327	280	240	300	207	227	253	233
26	353	487	453	393	287	293	287	367	320
27	566	440	673	493	447	427	460	560	447
28	247	253	267	227	287	240	247	267	280
29	607	513	573	373	533	447	507	473	453
30	280	387	400	300	280	240	247	393	333
Mean	263	280	286	262	270	250	242	271	254

FIG. 1. Effect of storage in refrigerators on leucocyte counts after adding potassium Bicromate.



The average cell counts of the thirty milk samples throughout ten days showed that there was a slight fluctuation and the difference in counts was very small on the succeeding days. The mean counts of all the samples are graphically shown in (Figure 1).

Inconsistant change was found throughout the ten days, the cell counts in each individual sample showed an inconsistant trend as well and differed remarkably from each other.

It was also observed that the shape and size of the nucleus of the leucocytes changed slightly during the preservation period, but at the same time the leucocytes could be easily and clearly distinguished from any other cells in the milk (plates 1 and 2). The before mentioned results justify the following conclusions.

1. This method is only suitable to count total leucocytes in the stored milk, but not to differentiate the leucocyte types.

2. Storing the samples ten days or less after adding Potassium Bichromate did not effect the cell counts. Since the storage period in the main experiment ranged between two to five days, this method was suitable for counting total leucocytes.

3.4. STATISTICAL ANALYSIS

Before proceeding with the statistical analyses of determining the heritability of leucocytes in the milk, the leucocyte counts of every cow in different seasons were transfered to the logarithms of cell counts. All the statistical analyses were calculated with aid of electronic data processing machines. Significance was determined from tables by SNEDECOR.

4. THE INFLUENCES OF ASSOCIATION, HERD, SEASON AND SIRE ON LEUCOCYTE COUNTS IN THE MILK

RESULTS AND DISCUSSION

Before continuing the study of the influence of the sire on the differences between progeny groups, it is advisible to investigate whether the differences between progeny groups are influenced by environmental factors, such as milk-recording associations, herd management etc.

4.1. Effect of association

(Results obtained by the material used in 1964)

The material and methods used in 1964 already indicated that the daughters of most sires were distributed over more than one local milk recording association (Tabel 19). Therefore the daughter groups belonging to different sires were not raised in one association. The results presented in tabel 20 show that there was a highly significant difference between progeny groups of different sires. In the following it is discussed under our experimental conditions whether these differences are caused by the influence of the association instead of the influence of the sire. To realise that accurately, the effect of sires should be eliminated, which could be achieved by comparing and carrying out an analysis of variance between associations within sires. For this part of the investigation 692 fourth lactations cows belonging to 15 sires were used. (Table 12) shows the mean leucocyte counts of progeny groups belonging to each sire, split up according to their distributions over the different associations. It can be seen that the groups of paternal sisters, differ from each other in their mean leucocyte number. However, the differences existing between progeny groups of the same sire in different associations was statistically significant in few cases only. Furthermore the difference which was significant in one season was insignificant in the other two seasons with only one exception for the groups of sire (no. 8). The differences between the average leucocyte counts of the three seasons were only significant in one case out of 12, (Table 13). On the other hand a comparison between associations, without paying attention to eliminate the effect of sires, showed highly significant differences. The F values were 4.85**, 6.47**, 4.80** in June, September and November respectively, and 6.97** for the average cell counts of the three seasons. This may be due to the effect of different sires. Accordingly, the differences between the associations could partly be explained by sire differences, since there was a seldom significant differences between associations within sire and a highly significant differences between associations overall sires. Moreover this result may be also due to the effect of increasing the number of cows and consequently the degrees of freedom, when the effect of sires within association was not eliminated.

TABLE 12. Mean leucocyte count of daughter groups belonging to different sires according to their distribution over different local milk-recording associations

Sire*	No. of cows in different associations	Total leucocyte counts in thousands per ml of milk in different seasons			
		June	Sept.	Nov.	Mean
1	19	167	71	213	150
	20	46	52	242	113
	13	58	102	245	135
2	17	130	124	130	128
	37	111	96	202	136
3	21	189	139	299	209
	17	144	131	214	163
4	17	233	212	322	255
	27	114	150	297	187
6	8	85	103	601	263
	7	136	119	294	183
	15	179	272	488	314
	8	292	139	335	253
8	22	285	373	571	409
	15	87	136	244	156
	10	215	348	293	285
10	5	116	135	402	218
	20	219	290	687	399
	24	298	228	557	361
11	5	184	142	555	294
	4	268	208	404	293
	16	321	447	411	393
	25	496	168	398	352
12	22	319	203	610	377
	7	284	339	586	403
	10	411	582	966	653
	5	189	261	430	293
13	15	231	269	725	408
	12	125	238	684	349
	10	662	843	733	746
	16	155	185	660	333
14	12	222	167	575	321
	19	576	593	542	570
	11	172	258	1955	795
15	40	244	308	918	490
	17	344	507	1762	871

* The table and also the statistical analysis does not include the data on the sires which daughters were raised in one association only (sires no. 5, 7 and 9).

However, the effect of association on leucocyte counts was determined from the average leucocyte counts of the three seasons. It was 3.5 percent of the total variance between daughter groups (This value was obtained from complete analysis of variance tables 25 and 27).

TABLE 13. F values between associations within sire

Sire	d.f. of associations	d.f. of cows	F values in different seasons			
			June	Sept.	Nov.	Mean
1	2	49	4.57*	.82	.73	.74
2	1	52	.41	.05	.63	.02
3	1	37	.18	.87	1.00	.55
4	1	42	2.09	.22	.01	.66
6	2	35	2.53	3.44*	2.08	1.84
8	2	44	6.35**	9.47**	5.63**	7.37**
10	2	46	5.48**	1.88	1.70	2.24
11	3	46	3.09*	.91	.19	.29
12	3	40	1.31	2.27	1.77	1.89
13	3	49	1.41	.85	.03	.56
14	2	39	1.32	1.47	.04	.32
15	1	55	.04	2.23	.81	.74

* significant ($P < 0.05$) ** highly significant ($P < 0.01$).

4.2. EFFECT OF HERD

4.2.1. *Results obtained with fourth lactation cows from material used in 1964*

To gain more information about the differences in leucocyte counts in the milk between progeny groups and the factors affecting these, an attempt was made to investigate the effect of the herd. For this purpose, selection for herds containing two or more paternal sisters was made and an analysis of variance was carried out. Table 14 presents the analysis of variance between herds within sire and association. The results obtained were inconsistant. It was found that the difference between herds within sire was sometimes significant in one association and insignificant in another. Moreover most of the significant differences between herds in one season were not always observed in the other two seasons and the mean. On the other hand, the variance between herds within all sires was significant in all seasons as it is prsented at the end of (Table 14). This result may be partly due to a large number of degrees of freedom of herds.

Table 15 presents the analysis of variance between herds within sire without eliminating the effect of associations. The results almost lead to the same explanation as mentioned above.

The results show that herd seems to have little effect, but it should be emphasized that the results obtained about the effect of the herd on leucocyte counts in the milk is not necessarily conclusive, because it is subject to several errors possible as a result of using a small number of herds.

4.2.2. *Results obtained with heifers from material used in 1965*

In 1965 a greater number of herds was used, it was 227 herds including 799 heifers. The effect of herds on leucocyte counts was calculated as a percentage of the total variance, it was 1.6, 4.1, 6.9 percent, in June, September and No-

TABLE 14. F values between herds within sire within association (4th lactation cows)

Sire	d.f. of herds in each association	d.f. of cows	F values in different seasons			
			June	Sept.	Nov.	Mean
1	1	2	.15	.47	2.53	2.31
	2	6	4.65	.92	.59	.44
	3	5	.53	1.52	3.32	2.57
2	1	2	.08	.00	.14	.86
	1	3	.42	.87	1.42	.81
3	2	3	4.84	.15	.20	.17
	5	8	.85	2.32	6.03*	5.27*
	1	3	2.24	12.96*	.01	.92
	1	2	.28	.28	.00	.00
4	4	5	.36	.80	3.39	1.19
	2	4	2.93	2.04	.02	1.32
5	2	4	3.97	14.20*	.36	3.73
7	3	4	4.64	1.53	3.31	7.42*
8	4	6	.73	1.30	.79	66.
	1	2	8.56	.08	92.20*	81.0 *
	1	2	.00	.00	.00	.00
9	8	17	2.97*	2.68*	2.39	3.55*
10	3	9	1.03	3.02	.34	1.46
11	3	5	2.64	2.18	.73	1.47
12	1	3	.62	1.04	3.98	2.10
	1	2	23.00*	5.69	9.53	12.75
13	2	3	8.18	.10	1.63	.59
	1	3	1.01	.00	.01	.14
	1	3	.00	.43	.00	.04
	1	2	.06	.02	.00	.21
Between herds within sires within associations	55	108	1.68*	1.59*	1.58*	1.78**

TABLE 15. F values between herds within sire (4th lactation cows)

Sire	d.f. of herds	d.f. of cows	F values in different seasons			
			June	Sept.	Nov.	Mean
1	8	13	1.29	.80	.93	.87
2	1	6	11.94*	3.99	.46	4.20
3	3	4	6.35	1.11	.22	.45
4	7	9	1.18	1.15	2.79	1.20
5	2	4	3.97	4.20	.36	3.73
7	3	4	4.64	1.53	3.31	7.43*
8	7	9	1.47	2.16	2.52	2.62
9	8	17	2.97*	2.68*	2.39	3.50*
10	3	9	1.03	3.02	.34	1.46
11	3	5	2.64	2.18	.73	1.47
12	5	7	1.46	1.48	1.37	1.34
13	7	10	.81	.24	.34	.17
14	2	3	11.27*	5.26	8.73	29.92*
15	7	11	1.01	2.62	3.87*	3.89*
Between herds within sires	66	111	1.66*	1.55*	1.43	1.68*

vember respectively (Table 30). The average effect of the three seasons was 3.7 percent. It is obvious that the herd has a slight influence on leucocyte counts. The values obtained also show that there was a gradual increase in herd effect during the subsequent seasons and the highest value was obtained in November. The slight influence of herds may be due to randomising daughter groups over many herds, which will decrease the herd effect on leucocyte counts.

However this point will be discussed in more detail in the part concerning the determination of the heritability of leucocytes from heifers.

4.3. Effect of season

Table 16 shows that there was an increase in mean leucocyte counts in both groups during subsequent seasons. The cell counts reached the maximum in November, however the increase in leucocyte values from June to September was not so considerable (Figure 2).

Table 16. Mean leucocyte counts in thousands for experimental animals used in 1964 and 1965

	No. of cows	Seasons			Average
		June	Sept.	Nov.	
From material used in 1964	692	211	227	525	321
From material used in 1965	799	144	243	542	310
Mean	1491	177	235	532	315

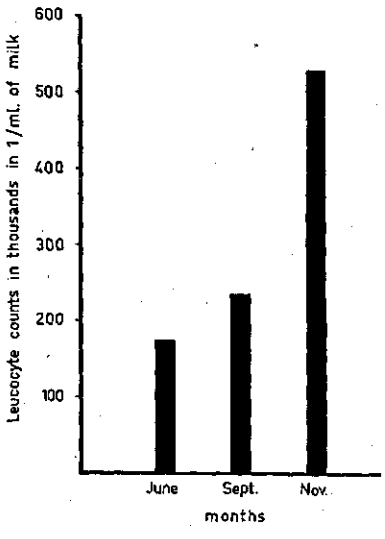


Fig. 2. Effect of season on the leucocyte counts in milk.

The high level of leucocytes in the milk in November may be mainly due to the effect of seasons. It may also have been caused by the progress of the lactation cycle and the effect of decreasing milk yields at the end of the lactation period, when some cows nearly reaching the beginning of the drying off period. These results are in agreement with the findings of BRANDSMA et al 1965 and have been demonstrated also by BRAUND et al 1963.

4.4. EFFECT OF CLINICAL MASTITIS

It is well known that cows with infected udders always produce milk with higher leucocyte counts than healthy cows [COOLEDGE (1918), CHERRINGTON et al (1933), PLASTRIDGE et al (1939), MACLEOD et al (1953) en NEWBOULD (1959)].

The results obtained with the material of 1964 showed a remarkable variation in the average leucocyte counts in the milk of progeny groups (Table 18). This variation can partly be explained in terms of the contribution of the mastitis animals within each progeny group. Table 17 shows the percentage of indicence of mastitis within different daughter groups during the fourth lactation period. This percentage was determined by the farmers information on what they had observed about the incidence of mastitis during the fourth lactation. It can be seen that the daughters groups differed from each other in this aspect and the percentage ranged from 5.5. to 26.8. This as suggests that the variance between progeny group for leucocyte counts may be partly due to differences in the in-

TABLE 17. Percentage of incidence of clinical mastitis during fourth lactation period per progeny group (material used in 1964 – cows in the fourth lactation)

Sire	Daughter number	Mean leucocyte counts in thousands per 1/ml of milk	Percentage of mastitis animals
1	52	132	7.7
2	54	132	5.5
3	39	186	23.1
4	44	221	18.2
5	41	255	14.6
6	38	256	23.7
7	26	256	19.2
8	47	284	10.6
9	56	303	26.8
10	49	326	24.5
11	50	326	20.0
12	44	432	20.5
13	53	459	17.0
14	42	562	21.4
15	57	680	22.8
Mean	692	321	18.2

TABLE 18. Comparison between daughter groups for total leucocyte counts in the milk (material of 1964 – cows in the fourth lactation)

Sire	No. of daughters	Total leucocyte counts in thousands in 1/ml of milk in each season			
		June	Sept.	Nov.	Average
1	52	90	75	233	132
2	54	120	110	166	132
3	39	166	135	256	186
4	44	173	181	309	221
5	41	120	203	444	255
6	38	173	158	429	256
7	26	210	151	407	256
8	47	196	286	369	284
9	56	173	173	565	303
10	49	211	218	549	326
11	50	316	241	442	326
12	44	301	346	648	432
13	53	293	384	700	459
14	42	323	339	1024	562
15	57	294	407	1340	680
Mean	692	211	227	525	321

cidence of clinical mastitis. However, this point will be discussed in more detail in the part concerning incidence of mastitis.

The animals used in 1965 were only heifers and the frequency of incidence of mastitis among heifers was to low to carry out such a study. Moreover the mastitis heifers were excluded at the beginning of the study.

4.5. EFFECT OF SIRE

4.5.1. *Differences between daughters groups for leucocyte counts*

4.5.1.1. Results obtained from material of 1964 (Cows in the fourth lactation)

The mean leucocyte counts in ml milk of progeny groups of fifteen sires which were in the fourth lactation are presented in table 18. It is clear that cell counts vary widely between the various daughter groups. They range from 90.000 to 323.000 in June, from 75.000 to 407.000 in September and from 166.000 to 1.340.000 in November. The general mean of total leucocyte counts for the three seasons ranged from 132.000 to 680.000 per ml of milk (Figure 3). The differences between daughter groups were statistically highly significant (Table 19).

TABLE 19. F values for total leucocyte counts in the milk between sire's daughters groups

d.f. sires	d.f. cows	Seasons			
		June	Sept.	Nov.	Average
14	677	4.13**	7.61**	5.68**	6.71**

** highly significant ($P < 0.01$).

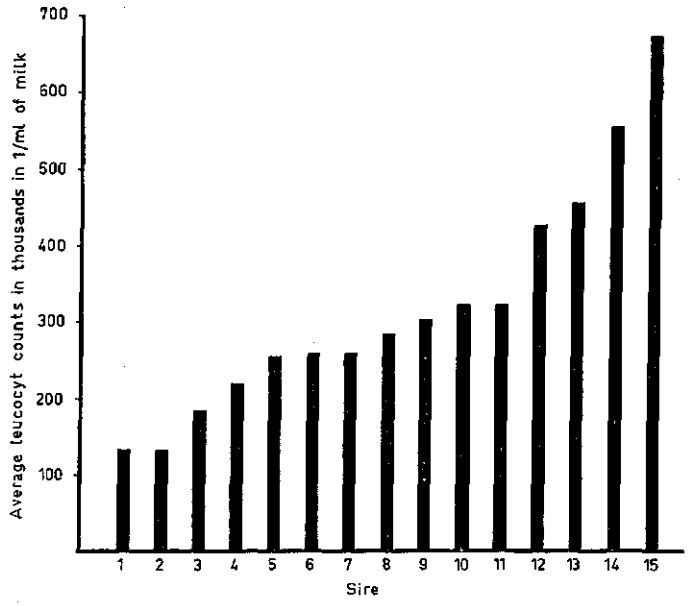


FIG. 3. Mean leucocyte counts in 1/ml of milk of daughter groups of 15 sires used 1964 (cows in the fourth lactation).

From the comparison of the frequency distribution of individuals belonging to each sire through the class interval of leucocytes in the milk shown in table 20.

TABLE 20. Frequency distribution of daughters of each sire through the class interval of average leucocyte counts in three seasons (material of 1964 – cows in the fourth lactation period)

Sire	No. of cows	Mean leucocyte counts in thousands	Class interval of leucocyte counts in milk in thousands			
			0–100 (first class)	101–500 (second class)	101–1.000 (third class)	> 1.000 (fourth class)
1	52	132	61.5	30.8	7.7	–
2	54	132	58.5	39.6	1.9	–
3	39	186	53.8	38.5	7.7	–
4	44	221	49.9	40.9	9.2	–
5	41	255	38.9	53.9	4.8	2.4
6	38	256	32.6	56.7	8.2	2.5
7	26	256	23.1	69.2	3.8	3.8
8	47	284	36.1	40.5	21.3	2.1
9	56	303	48.1	32.1	7.3	12.5
10	49	326	28.5	55.2	12.2	4.1
11	50	326	18.3	63.1	16.3	2.3
12	44	432	27.9	41.8	18.7	11.6
13	53	459	22.6	52.8	5.8	18.8
14	42	562	35.6	38.1	14.4	11.9
15	57	680	14.0	56.1	21.0	8.9

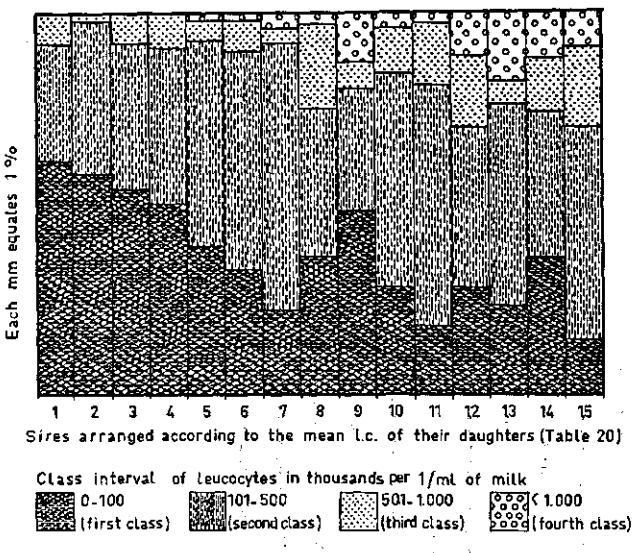


FIG. 4. Percentage distribution of daughters (4th lactation cows) of each sire through the class interval of leucocyte numbers per ml of milk (table 20).

It was observed that the sire (no. 1) whose daughters showed the lowest average leucocyte counts, had the highest percentage of his daughters, 61 per cent, in the lowest class interval ranging from zero to 100.000 cells per ml. It was also observed that the percentage distribution of the daughters of the same sire (no. 1) decreased gradualy in the successive classes, it was 30.8 per cent in the second class, ranging from 101.000 to 500.000 cells per ml and 7.7 in the third class, ranging from 501.000 to one million cells. The fourth class which cell number exceeds one million per ml did not include any individual belonging to this sire. This was contrary to the distribution of the daughters of sire (no. 15) which showed the maximum level of leucocyte counts in milk. A very low percentage of his daughters was in the lowest class, 14 percent, whereas the percentages were 56.1 and 21.0 in the second and third class respectively, moreover 8.9 percent of his daughters was in the highest class (Figure 4). In other words this means that the sire (no. 1) which showed the lowest level of leucocytes in the milk, had 7.7. percent of his daughters containing over 500.000 cells per ml of milk, whereas sire (no. 15) which provided the highest level, had 29.9 percent of his daughters containing over 500.000 cells.

The comparison between daughter groups of different sires within each a association is shown in table 21. The comparison shows that there was a remarkable difference in mean leucocyte count, especially when there were more progeny groups within each association (Figure 5).

The same material was used again for the same analyses after excluding 126 cows, showing clinical mastitis in the fourth lactation (Table 36). Comparison between sire-daughter groups for leucocyte counts of 566 healthy cows showed significant differences (Table 37). They were 1.68, 3.64**, 2.49** and 3.71** in June, September, November and average cell count of the three seasons respectively. However, this point will be discussed in more detail in the part concern-

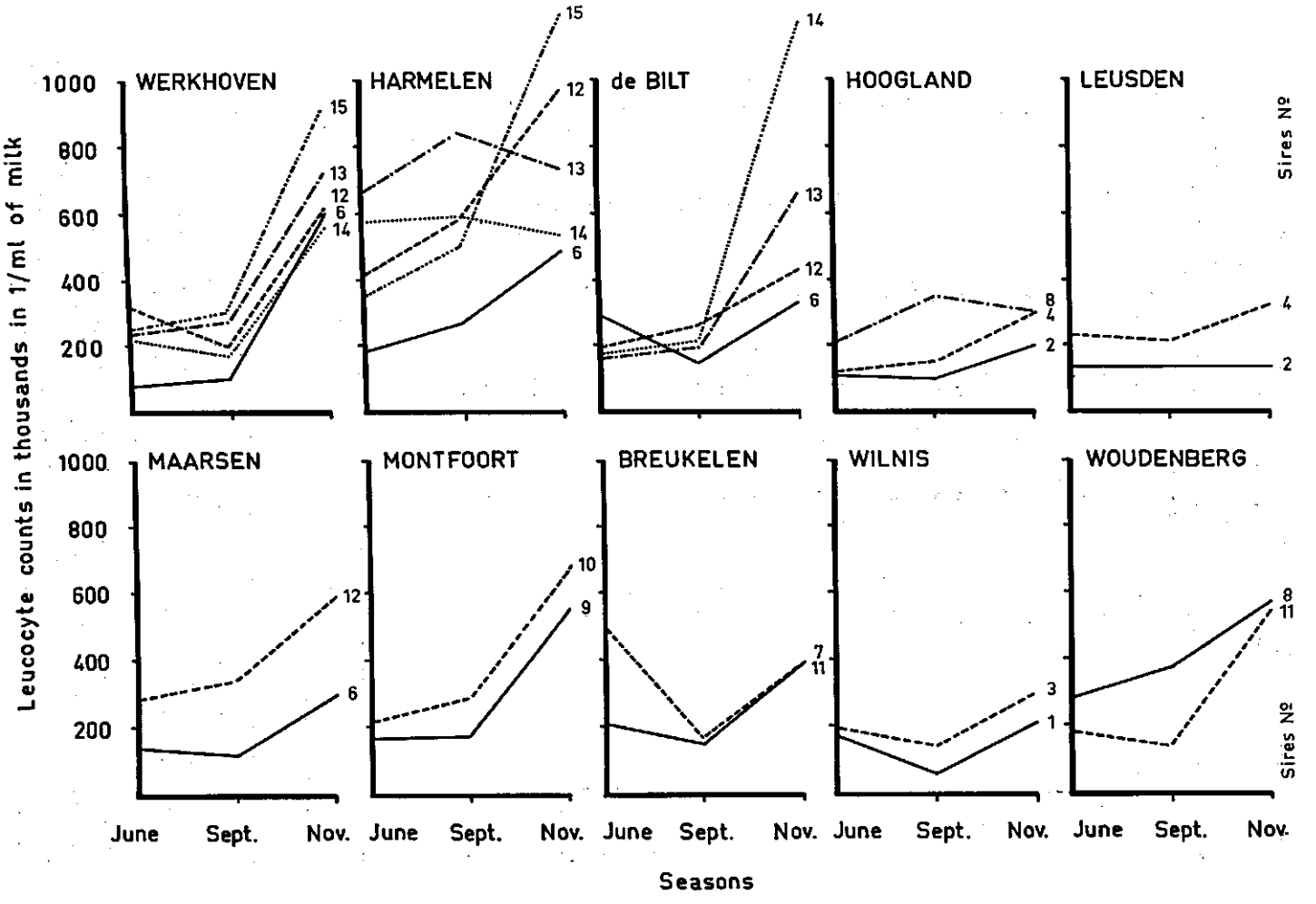


FIG. 5. Leucocyte counts in the milk of different daughter groups in the milk recording associations. (Table 21)

TABLE 21. Mean leucocyte counts for different progeny groups within each association (material of 1964 – cows in the fourth lactation)

Name of the association	Sire	Number of cows	Leucocyte counts in thousands in 1/ml of milk in different seasons			
			June	Sept.	Nov.	Mean
Werkhoven	6	8	85	103	601	263
	12	22	319	203	610	377
	13	15	231	269	725	408
	14	12	222	167	575	321
	15	40	244	308	918	490
Harmelen	6	15	179	272	488	314
	12	10	411	582	966	653
	13	10	662	843	733	746
	14	19	576	593	542	570
	15	17	344	507	1762	871
De Bilt	6	8	292	139	335	253
	12	5	189	261	430	293
	13	16	155	185	660	333
	14	11	172	258	1955	795
Hoogland	2	37	111	96	202	136
	4	27	114	150	297	187
	8	10	215	348	293	285
Leusden	2	17	130	124	130	128
	4	17	233	212	322	255
Maarssen	6	7	136	119	294	183
	12	7	284	339	586	403
Montfoort	9	56	173	173	565	303
	10	20	219	290	687	399
Breukelen	7	26	210	151	407	256
	11	25	496	168	398	352
Wilnis	1	19	167	71	213	150
	3	21	189	139	299	209
Woudenberg	8	22	285	373	571	409
	11	5	184	142	555	294

ing the relation betweeen mastitis frequency and leucocytes in the milk. The average cell counts of daughter groups after excluding the mastitis cows will also be presented there.

4.5.1.2. Results obtained from material of 1965 (heifers)

The daughter groups, of twenty sires, in the first lactation were examined for leucocyte counts in the milk in the same way as the fourth lactation cows in the year before. All heifers used were free from mastitis. The results obtained were to a great extent similar to those of the fourth lactation cows. Table 22 shows that the leucocyte counts vary extremely wide between the different daughter groups. They ranged from 53.000 to 459.000 in June, from 98.000 to 482.000 in September and from 233.000 to 979.000 in November. The general mean of total leucocyte counts of the three seasons ranged from 171.000 to 562.000 per ml of

TABLE 22. Comparison between daughter groups for total leucocyte counts in the milk (material of 1965 – cows in the first lactation)

Sire	No. of heifers	Total leucocyte counts in thousands in each season in 1/ml of milk			
		June	Sept.	Nov.	Average
16	37	75	166	273	171
17	57	143	143	233	173
18	59	196	98	271	188
19	52	120	181	271	191
20	32	75	151	384	203
21	28	83	136	444	221
22	41	105	98	505	236
23	27	68	264	377	236
24	39	53	301	384	246
25	29	271	105	414	263
26	44	113	347	437	299
27	33	241	151	527	306
28	33	68	249	677	331
29	32	90	384	655	376
30	33	105	414	685	401
31	36	105	143	957	402
32	54	151	407	655	404
33	52	151	294	979	475
34	39	211	354	964	510
35	42	459	482	746	562
Mean	799	144	243	542	310

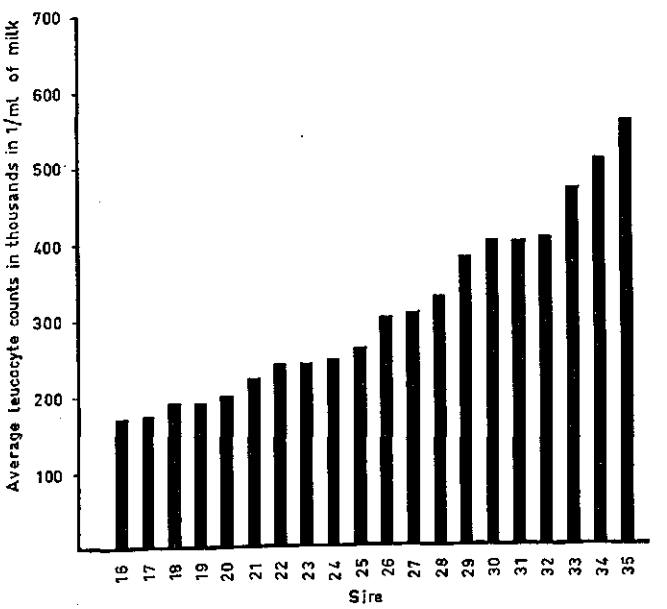


FIG. 6. Mean leucocyte counts in 1/ml of milk of daughter groups of 20 sires [heifers (1965)].

milk (Figure 6). The difference between heifer daughter groups were statistically significant (Table 23). However, the sire's influence on the variance between progeny groups after eliminating the effect of the associations (for material of 1964) and effect of herds (for material of 1965) will be discussed later (Tables 27 and 30).

TABLE 23. F-values for total leucocyte counts in the milk between daughter groups of sires in the first lactation

d.f. of sires	d.f. of heifers	Seasons			
		June	Sept.	Nov.	Average
19	779	1.84*	2.67**	4.93**	2.56**

* significant ($P < 0.05$)
** highly significant ($P < 0.01$).

4.5.2. *Sire effect on leucocyte counts at different stages of lactation*

From what is mentioned before and from the results obtained from both experiments, it could be explained that a part of these differences in leucocyte counts between daughter groups may be due to influence of the sires. In order to have an idea about the influence of sires on the total variance, the following statistical analysis has been carried out.

4.5.2.1. Results obtained from materials used in 1964

The following model was used in determining the contribution of sires and associations to the total variance of leucocyte counts (BECKER 1964).

$$Y_{ijK} = \mu + A_i + B_{ij} + e_{ijK}$$

Y_{ijK} = leucocyte counts in the milk of K^{th} cow of j^{th} association of the i^{th} sire.
μ = constant (true population average)
A_i = sire effect
B_{ij} = effect of j^{th} association in i^{th} sire.
e_{ijK} = effect of other factors within i^{th} sire in j^{th} association in K^{th} individual (undetermined variance).

TABLE 24. Analysis of variance table

Source of variation	d.f.	S.S.	M.S.	E. (M.S.)
Between sires	$S-1$	SS_s	MS_s	$\sigma^2_W + K_2\sigma^2_A + K_3\sigma^2_s$
Between associations within sires	$A-S$	SS_A	MS_A	$\sigma^2_W + K_1\sigma^2_A$
Cows within associations	$N-A$	SS_W	MS_W	σ^2_W

S = total number of sires
A = total number of associations
N = total number of cows
K_1 and K_2 = number of cows per association
K_3 = number of progeny per sire

Since there is an unequal number of cows per association and an unequal number of associations per sire the following formulas were used in computing the coefficient of K_1, K_2 and K_3 (BECKER 1964).

$$K_1 = \frac{n.. - \Sigma_i \frac{\Sigma_j n^2_{ij}}{ni.}}{A - S} \quad \text{(d.f. for associations)}$$

$$K_2 = \frac{\Sigma_i \frac{\Sigma_j n^2_{ij}}{ni.} - \frac{\Sigma_i \Sigma_j n^2_{ij}}{n..}}{S - 1} \quad \text{(d.f. for sires)}$$

$$K_3 = \frac{n.. - \frac{\Sigma_i n^2_{i.}}{n..}}{S - 1} \quad \text{(d.f. for sires)}$$

n_{ij} = number of cows per association
$n_i.$ = number of progeny per sire
$n..$ = total number of cows.

The complete analysis of variance for leucocyte counts in the milk in June, September and November was carried out by using the data obtained in each season seperately (Table 25). Moreover the same analysis was carried out for the data obtained by averaging the leucocyte counts in the three seasons for each cow (Table 25).

Since the milk of mastitis cows contains a larger number of leucocytes than healthy cows, the percentage of mastitis frequency in the progeny of each sire may be one of the causes of variation in the average leucocyte counts between daughter groups. Therefore, the same material was used again for the same analysis after excluding 126 cows, showing clinical mastitis during the fourth lactation. The results obtained are presented in table 26.

The intra class correlation for sires, associations and unknown factors was calculated from the variance components of each item as a percentage of total variance. The formula used is (Formula from KEMPTHORNE, 1957).
Intra class correlation for sires:

$$\frac{\sigma^2_S}{\sigma^2_S + \sigma^2_A + \sigma^2_W}$$

TABLE 25. Analysis of variance for leucocyte counts in the milk of cows in the fourth lactation in different seasons

Source of variation	d.f.	June			September			November			Average			E (M.S.)
		S.S.	M.S.	Variance components	S.S.	M.S.	Variance components	S.S.	M.S.	Variance components	S.S.	M.S.	Variance components	
Between sires	14	72.1	5.15	.0239	177.5	12.67	.1669	138.1	9.86	.1641	99.4	7.10	.1088	$\sigma^2_W + 24\ \sigma^2_A + 46\sigma^2_S$
Between associations within sires	23	67.5	2.93	.1167	84.3	3.66	.1389	47.8	2.08	.0243	38.7	1.68	.0431	$\sigma^2_W + 14.4\sigma^2_A$
Residual	644	815.7	1.25	1.2472	1088.5	1.66	1.6644	1135.1	1.73	1.7355	692.2	1.06	1.0584	σ^2_W

σ^2_W = variance within groups (residual)
σ^2_A = variance between associations
σ^2_S = variance between sires

TABLE 26. Analysis of variance for leucocyte counts in the milk of healthy cows (cows free from clinical mastitis) in the fourth lactation

Source of variation	d.f.	June			September			November			Average			E (M.S.)
		S.S.	M.S.	Variance components	S.S.	M.S.	Variance components	S.S.	M.S.	Variance components	S.S.	M.S.	Variance components	
Between sires	14	40.9	2.92	.000	138.9	9.92	.1943	114.7	8.19	.1674	81.8	5.84	.1049	$\sigma^2_W + 18.3\sigma^2_A + 37.5\sigma^2_S$
Between associations within sires	24	63.0	2.62	.1248	53.9	2.25	.0612	34.5	1.81	.0164	37.7	1.57	.0539	$\sigma^2_W + 12\ \sigma^2_A$
Residual	525	592.1	1.13	1.1278	794.3	1.51	1.5129	849.4	1.62	1.6180	484.8	.92	.9235	σ^2_W

σ^2_W = variance within groups (residual)
σ^2_A = variance between associations
σ^2_S = variance between sires

Intra class correlation for associations:

$$\frac{\sigma^2_A}{\sigma^2_S + \sigma^2_A + \sigma^2_W}$$

Intra class correlation for unknown factors:

$$\frac{\sigma^2_W}{\sigma^2_S + \sigma^2_A + \sigma^2_W}$$

The results obtained by using the material before and after excluding the mastitis cows are presented in table 27. The effect of sires on leucocyte counts before and after excluding the mastitis cows was on an average 9.0 and 9.7 percent of the total variance respectively. The sire effect on leucocyte counts was higher than the association effect.

TABLE 27. Variance components as percentages of total variance

	Before excluding mastitis cows				After excluding mastitis cows			
	June	Sept.	Nov.	Average	June	Sept.	Nov.	Average
Sires	1.7	8.5	8.6	9.0	0.0	11.0	9.3	9.7
Associations	8.4	7.1	1.3	3.5	10.0	3.4	0.9	5.0
Unknown factors	89.8	84.4	90.1	87.5	90.0	85.6	89.8	85.3

It is also obvious that the lower value of the sire effect was obtained in June, when most of the cows were at the beginning of their lactation period. Moreover, after excluding the mastitis cows from the material, the sire effect in June was not apparent. In September the sire effect showed the highest value, at this time most of the cows used in the present study were in the middle of the lactation stage. In November the value remained fairly constant, whereas it showed a slight decrease after excluding the diseased cows.

4.5.2.2. Results obtained with the material of 1965

In 1965 daughter groups, of twenty sires, in their first lactation period were used, all these heifers were free from clinical mastitis. In selecting the farms in which the heifers were kept, it was taken into account that each herd should include progeny of at least two sires. According to the experimental design, a complete analysis of variance for cross classification was carried out to determine the effect of sires in each season. The following model was used.

$\underline{Y}_{ijK} = \mu + \underline{a}_i + \underline{b}_j + \underline{(ab)}_{ij} + \underline{e}_{ijK}$

$\underline{Y}_{ijK}$ = leucocyte counts in milk of K^{th} cow of j^{th} sire in i^{th} herd

μ = constant

$\underline{a}_i$ = effect of i^{th} herd

TABLE 28. Equations used in estimating E (M.S.) of cross classification

Source of variation	d.f.	S.S.	M.S.	E (M.S.)
Mean	1	$Y^2N = \Sigma_i\Sigma_j\Sigma_k \overline{Y^2}_{...}$		
Herds (A)	$I^* = I - 1$	$Y^2_A{}^* = \Sigma_i (\Sigma_j\Sigma_k \overline{Y^2}_{i..}) - Y^2N$	$Y^2_A{}^*/I^*$	$K_1\sigma^2_a + K_2\sigma^2_b + K_3\sigma^2_{ab} + \sigma^2_w$
Sires (B)	$J^* = J - 1$	$Y^2_B{}^* = \Sigma_j (\Sigma_i\Sigma_k \overline{Y^2}_{.j.}) - Y^2N$	$Y^2_B{}^*/J^*$	$K_4\sigma^2_a + K_5\sigma^2_b + K_6\sigma^2_{ab} + \sigma^2_w$
Herds × Sires (A × B)	$IJ^* = IJ - 1$	$Y^2AB^* = \Sigma_i\Sigma_j (\Sigma_k \overline{Y^2}_{ij.}) - Y^2N$	Y^2AB^*/IJ^*	$K_7\sigma^2_a + K_8\sigma^2_b + K_9\sigma^2_{ab} + \sigma^2_w$
Residual (R)	$N^* = N - IJ$	$Y^2_R = \Sigma_i\Sigma_j\Sigma_k Y^2_{ijk} - \Sigma_i\Sigma_j(\Sigma_k \overline{Y^2}_{ij.})$	Y^2R/N^*	σ^2_w

where I = number of herds J = number of sires
IJ = number of classes herds by sires N = total number of individuals

* In the orthogonal case (f.i. each class of B should be represented in each class of A), subtraction of the main effects A and B in this line should give sums of squares for interaction. In the non-orthogonal case, which is more general as in our data, this subtraction should not result in the sums of squares for interaction. Therefore this subtraction has not been applied.

TABLE 29. K-values used to obtain variance components from expected mean squares

	K-values		
	σ^2_a	σ^2_b	σ^2_{ab}
Herds (A)	$K_1 = (N - M^a/N)/I^*$	$K_2 = (\Sigma_i M_i{}^{ab}/N_i - M^b/N)/I^*$	$K_3 = (\Sigma_i M_i{}^{ab}/N_i - M^{ab}/N)/I^*$
Sires (B)	$K_4 = (\Sigma_j M_j{}^{ab}/N_j - M^a/N)/J^*$	$K_5 = (N - M^b/N)/J^*$	$K_6 = (\Sigma_j M_j{}^{ab}/N_j - M^{ab}/N)/J^*$
Herds × Sires (A × B)	$K_7 = (N - M^a/N)/IJ^*$	$K_8 = (N - M^b/N)/IJ^*$	$K_9 = (N - M^{ab}/N)/IJ^*$

$M^a = \Sigma_i n^2_{i.}$ $M_j{}^{ab} = \Sigma_i n^2_{ij}$ $M_i{}^{ab} = \Sigma_j N^2_{ij}$ $M^b = \Sigma_j n^2_{.j}$ $M^{ab} = \Sigma_i\Sigma_j n^2_{ij}$

TABLE 30. Analysis of variance for leucocyte counts in the milk of heifers in different seasons

Source of variation	d.f.	June				September				November				Average			
		S.S.	M.S.	*F-value	**Variance components	S.S.	M.S.	*F-value	**Variance components	S.S.	M.S.	*F-value	**Variance components	S.S.	M.S.	*F-value	**Variance components
Herds	130	361.4	2.78		1.6	523.9	4.03		4.1	588.9	4.53		6.9	304.2	2.34		3.7
Sires	21	89.0	4.24	1.77*	1.8	157.5	7.50	2.83**	3.6	269.2	12.82	6.07**	8.7	84.6	4.03	2.76**	3.0
Herds × Sires	475	123.0	2.59		1.4	1629.2	3.43		7.4	1695.7	3.57		13.9	959.5	2.02		11.9
Residual	323	771.9	2.39		94.9	855.9	2.65		84.7	681.5	2.11		70.4	471.6	1.46		81.2

* F-values between progenies after eleminating the herd effect. ** Variance components as a percentage of total variance.

$\underline{b}_j$ = effect of j^{th} sire

$\underline{ab}_{ij}$ = interaction of sires and herds

$\underline{e}_{ijK}$ = residual effect

$\underline{a}$, $\underline{b}$, $\underline{ab}$ and $\underline{e}$ are considered independent random variables with zero mean and variance σ^2_a, σ^2_b, σ^2_{ab} and σ^2_w respectively. Elaborating formulas for expected mean squares, in case of cross classification with arbitrary numbers in the product classes, the following equations are obtained to estimate the variance components σ^2_a, σ^2_b, σ^2_{ab} and σ^2_w (Table 28). Moreover, for our experimental material the following equations are obtained to determine the *K*-values (Table 29). The results obtained by using these equations are presented in tables 30 and 31.

TABLE 31. K-values used to obtain variance components from expected mean squares

Source of variation	K-values			
	σ^2H	σ^2S	$\sigma^2H\times S$	σ^2W
Herds	6.07	1.51	1.82	1
Sires	1.70	36.04	2.06	1
Herds × Sires	1.66	1.59	1.67	1
Residual variance				1

The computations have been facilitated by the cooperation of the Mathematical Department of the Agricultural University, Wageningen, The Netherlands. Their programs, LH 348, 366 and 405 in fortran for IBM 1620, suited the experimental design of our data.

The results of the material of 1965 (heifers) also show that there was a gradual increase in sire effect in the subsequent seasons, it was 1.8, 3.6 and 8,7 percent of the total variance in June, September and November respectively. The differences between daughter groups after eliminating the effect of herds were also statistically significant. The F-values of these differences showed a gradual increase during the subsequent seasons and were 1.77*, 2.83**, 6.07** in June, September and November respectively (Table 30). The possible interpretation of the results obtained from both experiments (1964–1965) is that the sire effect on leucocyte counts in the milk is more apparent with the advancing stage of lactation, especially in the second half of the lactation cycle.

4.6. HERITABILITY OF LEUCOCYTES IN THE MILK

The previous results clearly show that part of the differences in leucocyte counts between daughter groups is due to the influence of sires. This may be true, if the differences in leucocyte counts in the milk have a genetic background. Estimating the heritability of leucocytes in the milk give a good indication of the genetic properties of this character. Moreover the magnitude of heritability expresses the proportion of the total variance that is attributable to the average

effects of additive genes, which determine the degree of resemblance between paternal sisters. In this study heritability estimates are derived from the intra class correlation coefficient. Since the components of variance allow us to construct any intra class correlation we may desire, reducing the amount of environmental variance (by subtracting the variance components due to known environmental factors from the total variance) will increase the proportional amount af additive genetic variance and give a higher value of heritability. Accordingly, the following calculations were carried out to obtain the total phenotypic variance free from the effect of association variances (for the material of 1964) and from herd variances (for the material of 1965). The model used in computing the heritability from the intra class correlation after eleminating these factors is:

$$h^2 = \frac{4\sigma^2_S}{\sigma^2_S + \sigma^2_W} \quad \text{(KEMPTHORNE 1957)}$$

σ^2_S = variance between sires
σ^2_W = residual variance.

The intra class correlation coefficient of sires was obtained by the complete analysis of variance of the data used in 1964 and 1965, which were presented in the preceding tables. The heritability for leucocyte counts in the milk in different seasons is presented in (Table 32). The results obtained show that the lowest values of heritability were found in June for fourth lactation cows as well as heifers. In September the fourth lactation cows showed a remarkable increase, particularly after excluding the 'mastitis cows', whereas the heifers showed a slight increase. In November the values of the cows in the fourth lactation was fairly the same whereas the heifers showed a marked increase. Obviously, there was a gradual increase in the value of the heritability of leucocytes in the milk during the subsequent seasons, particularly for heifers. The estimation of the heritability by using the data, representing the average leucocyte counts in the

TABLE 32. Estimation of the heritability for total leucocute counts in the milk in different seasons

	d.f. of sires	d.f. of cows	Values of heritability and their confidence intervals*	
			June	September
A. Cows in the fourth lactation period (1964)				
1. Before excluding mastitis cows	14	654	.07 (.04–.56)	.37 (.26– .97)
2. After excluding mastitis cows	14	525	.00 (–.11–.11)	.46 (.27–1.01)
B. Cows in the first lactation period (1965)	19	779	.07 (.01–.23)	.16 (.06– .36)

* Confidence interval at 95%.

three seasons of cows in the fourth lactation, before and after excluding mastitis cows, was .37 and .41 respectively. This indicates that, by using cows free from mastitis, the value of heritability of leucocyte counts in milk will slightly increase. The heifers showed lower heritability than the cows in the fourth lactation, the value was .14. Repeatability for leucocyte counts during the fourth lactation was estimated at .40 and .44 for cows in the fourth lactation, before and after excluding the 'mastitis cows'; respectively. The repeatability during the first lactation was estimated at .28.

Repeatability is the fraction of total phenotypic variance due to both additive and non additive genetic variation plus variation due to permanent environmental influence. Heritability is that fraction of total phenotypic variance due to the additive genetic variance. For this reason, repeatability is expected to be higher than heritability as found in this study. Moreover, the comparison between the results obtained from the estimation of heritability of the average leucocyte counts of each cow in the three seasons and repeatability showed that the values obtained from cows in the fourth lactation were much higher than the values obtained from heifers (Table 32). It also seems that the genetic fraction of the total variance in the fourth lactation is mainly due to additive genes, since the values of heritability were .37 and .41, whereas the values for repeatability were .40 and .44, before and after excluding the mastitis cows, respectively. The results obtained in this investigation in all seasons, except June, are in agreement with the values found by YOUNG et al (1960).

The heritability values depends on the magnitude of variance of the genetic and environmental components, a change in any one of them will affect it. According to that, we could discuss the reason of the variable values of heritability in different seasons, from the values of the variance components of each item in the subsequent months as presented in table 25. The lower values of the heritability of leucocytes in the milk obtained in June are due to the lowest magnitude of the genetic component in this season compared to the other two seasons. Moreover, these results indicate that the variance between individuals

Values of heritability and their confidence intervals*		Values of repeatability and their confidence interval	
November	From the average L.C. of the 3 seasons		
			A. Cows in the fourth lactation period (1964)
.35 (.19–.79)	.37 (.29–1.02)	.40 (.31–.98)	1. Before excluding mastitis cows
.38 (.19–.82)	.41 (.25– .97)	.44 (.28–.92)	2. After excluding mastitis cows
.44 (.19–.69)	.14 (.05–. 35)	.28 (.12–.63)	B. Cows in the first lactation period (1965)

in September and November had more genetical part, especially in the fourth lactation.

Since the highest values of heritability of leucocytes are obtained in September and November, it is advisable to use the data obtained in these two months, if we want to test progeny groups for differences in leucocyte counts in the milk. In other words, the data obtained during the second half of the lactation period of the spring calving cows.

The values of environmental components, presented in table 25, also show a noticeable fluctuation from one season to another. This may be due to the effect of stage of lactation which is connected with seasons. It was observed in this study that the number of leucocytes clearly increased in November, whereas the milk yeald of most cows decreased, because they had nearly reached the drying off period at that time. This point will be discussed in detail in the part concerning the milk production in relation to leucocyte counts in the milk. Moreover, there may be other factors having different effects on the environmental variance in different seasons.

5. THE INFLUENCES OF MASTITIS, MILK PRODUCTION AND EASE OF MILKING ON LEUCOCYTE COUNTS IN THE MILK

5.1. Material and methods

5.1.1. *Nature of data*

To obtain data on mastitis frequency, milk yield and ease of milking, a special enquiry, included these items were distributed among the owners of the cows used in 1964 and 1965. The farmers and the recorders of the milk recording associations were asked to give, as far as possible, full details on these items. The farmer had sufficient information about his cows, since nearly all animals were kept in small herds and he milked the cows himself. Moreover the recorder had regular records for each cow during the lactation periods. With this information the farmers and the recorders were able to give reliable answers. The enquiries included the following information for each cow.

5.1.1.1. Mastitis frequency

The work definition of clinical mastitis was the quarters of udders being abnormal or giving abnormal milk. This includes any quarter showing discoloured milk or watery milk or clots on the strip cup. Also any quarters showing hardiness, pain, swelling or other similar abnormal conditions. The answers of these items were based on the farmer's observations during the fourth lactation period and also during the preceding lactation periods.

5.1.1.2. Milk yield

The average daily milk yield of the whole lactation of the cows in the fourth lactation in 1964 were obtained from local milk recording associations. The milk production of only 594 cows was available, whereas the production of the remaining 98 cows was not yet computed.

The cows used in 1965 were in their first lactation and the daily milk yield on the day of sampling in June, September and November was obtained.

5.1.1.3. The ease of milking

5.1.1.3.1. *According to farmer's opinion*

According to farmer's opinion about the ease of milking of the cows in their herds, the cows in the fourth lactation used in 1964 were distributed among the following classes.

1. Very slow milking cows
2. Slow milking cows
3. Normal cows
4. Fast milking cows
5. Very fast milking cows

5.1.1.3.2. *According to the estimation of milk rate*

A. The peak flow (maximum milk rate per minute) is measured for progeny groups of all A.I. bulls in The Netherlands. Consequently also for the bulls used in our experiment. The peak flow of at least 25 daughters in the first lactation is measured after 21 to 154 days from the date of calving. All the progenies tested are milked by machine, the vacuum level being 35 cm of mercury and the pulsation ratio 2:1. The milk rate obtained is corrected to 7 kg of milk per lactation. The result of this part of the progeny test was computed also with the average cell counts of sire's daughter groups.

B. To obtain more information about the relation between the milk rate and leucocyte counts in the milk, milk samples were also collected from daughters of nine other sires raised in the province of North-Holland. The milk samples were collected with the aid of the Instituut voor Veeteeltkundig Onderzoek 'Schoonoord' at Zeist. The level of leucocyte counts in the milk of each sample was tested by two methods, first by B.M.R., second by microscopic cell counts.

For statistical analysis the answers to the enquires, including the farmer's opinions were transfered to scores in figures. The manner of scoring for mastitis frequency and ease of milking was as follows.

Incidence of mastitis:

1. Score one: Cows never suffering for mastitis.
2. Score two: Cows showing clinical mastitis during the fourth lactation.

Ease of milking:

1. Score one: Cows showing very slow milking.
2. Score two: Cows showing slow milking.
3. Score three: Cows showing normal milking.
4. Score four: Cows showing fast milking.
5. Score five: Cows showing very fast milking.

All the statistical analyses were calculated with aid of Electronic Data Processing machines, using the variables lested. Significance was determined from tables by SNEDECOR.

5.2. RESULTS AND DISCUSSION

5.2.1. *Mastitis*

5.2.1.1. Mastitis frequency in relation to leucocyte counts in the milk

The percentage of incidence of mastitis among different progeny groups during the fourth lactation and also during the preceding three lactations is presented in table 33. A comparison between daughter groups for leucocyte counts as well as mastitis clearly shows that the sire (no. 1) whose daughter had the lowest level of leucocyte counts in the milk, 132.000 per ml of milk, also had a relatively lower percentage of mastitis in the fourth lactation, 7.7 per cent. On the other hand, the sire (no. 15) whose progeny showed the highest level of leucocytes, 680.000 per ml of milk, had a relatively high percentage of incidence of mastitis, 22.8 per cent. This positive relation, however, was not always found

TABLE 33. Percentage of clinical mastitis cows during lactation periods within progeny groups

Sire	No. of daughters	Mean Leucocyte counts in thousands	Diseased cows					
			In the fourth lactation only		During the period from 1st–3rd lactation		During the period from 1st–4th lactation	
			No.	%	No.	%	No.	%
1	52	132	4	7.7	4	7.6	5	9.6
2	54	132	3	5.5	4	7.4	6	11.1
3	39	186	9	23.1	4	10.2	9	23.1
4	44	221	8	18.2	9	20.1	12	27.3
5	41	255	6	14.6	4	9.7	8	19.5
6	38	256	9	23.7	8	21.0	11	28.9
7	26	256	5	19.2	5	19.2	7	26.9
8	47	284	5	10.6	5	10.6	7	14.9
9	56	303	15	26.8	9	16.1	21	27.5
10	49	326	12	24.5	14	28.5	21	42.8
11	50	326	10	20.0	6	12.0	13	26.0
12	44	432	9	20.5	8	18.2	13	29.5
13	53	459	9	17.0	4	7.5	10	18.9
14	42	562	9	21.4	6	14.2	14	33.3
15	57	680	13	22.8	13	22.8	19	33.3
	692	321	126	18.2	103	14.9	176	25.4

between all sires. It is to be noted that the sire which showed the maximum level of leucocytes in the milk, sire (no. 15), did not have the maximum value of mastitis frequency. However this value, 22.8 per cent, was comparatively higher than the percentage of mastitis frequency of lowest leucocyte groups [sire no. 1 (7.7%) and no. 2 (5.5%)] and also higher than the average value of mastitis for all experimental animals, which was 18.2 per cent (Table 33).

These results indicate that a high average cell count of daughter groups mostly predicts a high mastitis frequency (Figure 7). Moreover the correlation coefficient within sires shows that there was a highly significant positive relation between mastitis frequency and leucocyte counts in milk. It was .243** in June, .296** in September .253** in November and .312** for the average leucocyte counts in these three seasons (Table 34).

The genetic correlation between average leucocyte counts and incidence of clinical mastitis during the fourth lactation was computed by the following formula.

$$r_G = \frac{4Cov_S}{\sqrt{4\sigma^2_{S(x)}\ 4\sigma^2_{S(y)}}}$$ (MODE and ROBINSON 1959)

where, $\sigma^2_{S(x)}$ and $\sigma^2_{S(y)}$ are variance components of sires for leucocyte counts and clinical mastitis respectively; Cov_S co-variance components of sires. The components of variance and covariance of sires and associations are presented in table 35. By using the above-mentioned formula the estimates of genetic

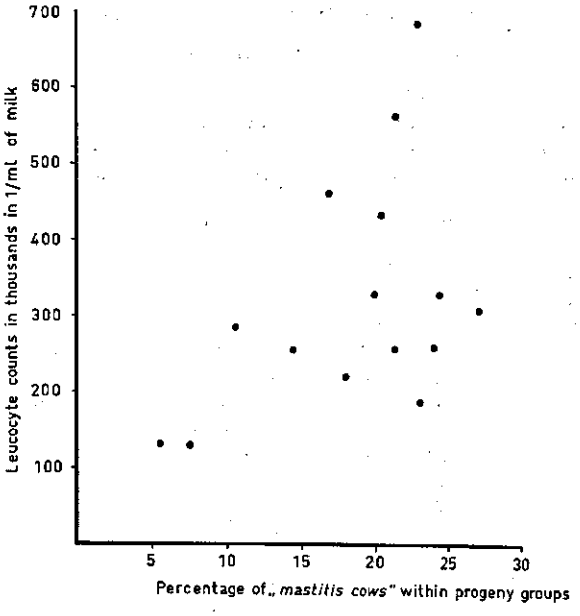


FIG. 7. Mean leucocyte counts of daughter groups and percentage of infected daughters in the fourth lactation.

TABLE 34. Correlation coefficients between leucocyte counts in the milk of fourth lactation cows in different seasons and incidence of mastitis during 4th lactation

Sire	d.f. cows	Seasons			Average
		June	Sept.	Nov.	
1	50	.201	.369**	.298*	.193
2	52	.127	.098	.018	.024
3	37	.220	.400*	.096	.130
4	42	.066	.023	.062	.049
5	39	.162	.520**	.134	.286
6	36	.121	.134	.437**	.402*
7	24	.341	.242	.055	.067
8	45	.052	.044	.036	.058
9	54	.468**	.356**	.351*	.462**
10	47	.357*	.454*	.159	.341*
11	48	.357**	.346**	.380**	.447*
12	42	.067	.087	.303*	.253
13	51	.210	.289*	.142	.285*
14	40	.564**	.434**	.503**	.599**
15	55	.243	.447**	.434**	470**
Overall sires	662	.243**	.269**	.253**	.312**

* significant ($P < 0.05$)
** highly significant ($P < 0.01$)

TABLE 35. Components of variance and covariance for average leucocyte counts and clinical mastitis

	d.f	Components of variance		Components of covariance
		Average leucocyte counts	Clinical mastitis	Leucocyte counts × clinical mastitis
Between sires	14	.1088	.0014	.0101
Between associations within sires	23	.0431	zero	zero
Residual	654	1.0600	.1466	.1092

correlation between average leucocyte counts and clinical mastitis was .83. This result is in agreement with the value found by YOUNG et al (1960). They stated that the value obtained indicates that many of the genes affecting clinical mastitis also influence leucocyte counts in milk.

From the results obtained, the remarkable variation in the average leucocyte counts in the milk between progeny groups can partly be explained in terms of percentage of mastitis animals within each group. This could be investigated by excluding the 'mastitis cows' from each daughter group as presented in table 36. Progeny groups free from mastitis always showed a lower average of cell counts in the milk than the other groups (Figure 8). The differences between the level of cells in the milk before and after excluding the mastitis cows were more

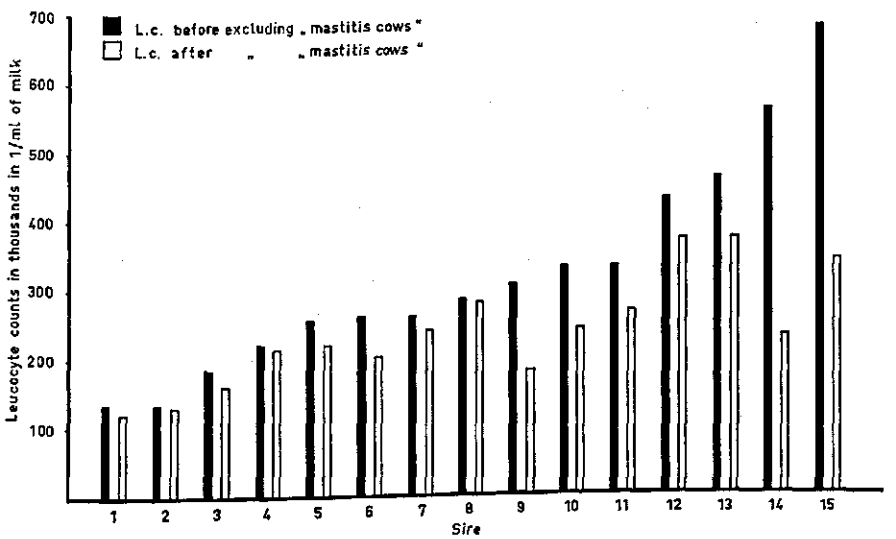


FIG. 8. Comparison between mean leucocyte counts in 1/ml of milk from all and healthy daughter groups of 15 sires used in 1964 (cows in the fourth lactation).

TABLE 36. Comparison between mean leucocyte counts in 1/ml of milk from all and healthy daughter groups of 15 sires used in 1964 (cows in the fourth lactation)

Sire	No. of daughters	No. of healthy daughters	Total leucocyte counts in thousands in 1/ml of milk								Percentage of diseased cows during fourth lactation period
			June		September		November		Average		
			All daughters	Healthy daughters	All daughters	Healthy daughters	All daughters	Healthy daughters	All daughters	Healthy daughters	
1	52	48	90	83	75	60	233	203	132	120	7.7
2	54	51	120	120	110	90	166	166	132	128	5.5
3	39	30	166	128	135	83	256	271	186	158	23.1
4	44	36	173	173	181	181	309	301	221	218	18.2
5	41	35	120	113	203	143	444	392	255	218	14.6
6	38	29	173	166	158	143	429	309	256	203	23.7
7	26	21	210	173	151	136	407	422	256	241	19.2
8	47	42	196	200	286	280	369	330	284	281	10.6
9	56	41	173	105	173	113	565	339	303	181	26.8
10	49	37	211	136	218	128	549	459	326	241	24.5
11	50	40	316	241	241	203	442	362	326	264	20.0
12	44	35	301	279	346	324	648	497	432	369	20.5
13	53	44	293	218	384	233	700	640	459	369	17.0
14	42	33	323	173	339	181	1024	324	562	226	21.4
15	57	44	294	211	407	241	1340	573	680	339	22.8
	692	566	211	168	227	170	525	376	321	238	18.2

obvious for the groups having a relatively high percentage of mastitis progeny.

The average cell counts for sire (no. 15) was 680.000 and 339.000 per ml before and after excluding the 'mastitis cows' respectively. Moreover the F-values of the cell counts in the milk between progeny groups free from mastitis showed lower values than the other groups (Table 37). However the difference in cell counts between healthy daughter groups is also significant. It is clear that mastitis frequency is one of the factors causing high cell counts in milk of progeny groups.

TABLE 37. F-values for total leucocyte counts in the milk from all and healthy daughters groups of 15 sires (material of 1964)

	d.f. sires	d.f. cows	Seasons			
			June	Sept.	Nov.	Average
All daughter groups	14	677	4.13**	7.61**	5.68**	6.71**
Healthy daughter groups	14	551	1.68	3.64**	2.49**	3.71**

** highly significant ($P < 0.01$)

5.2.1.2. Leucocyte counts in the milk of infected and healthy cows

According to the incidence of mastitis during the fourth lactation, the cows were divided into two groups. The first group included the cows showing clinical mastitis during the fourth lactation, whereas the second group included the healthy cows free from mastitis during the first four lactation periods. The results presented in tabel 38 show that infected cows gave milk with higher leucocyte counts than healthy cows in all seasons, especially in November when the count was about four times higher than the value obtained by uninfected cows (Figure 9) and (Plates 3, 4 and 5). The differences between these two groups was statistically significant (Table 39). The results obtained in this study

TABLE 38. Leucocyte counts in the milk of the healthy and 'mastitis cows' in the fourth lactation

	No. of cows	Leucocyte counts in thousands in 1/ml of milk in different seasons			
		June	Sept.	Nov.	Average
1. Healthy cows	516	136	158	331	208
2. 'mastitis cows'	126	411	501	1.274	729

TABLE 39. F-values for total leucocyte counts between healthy and 'mastitis cows' in the fourth lactation.

d.f. classes	d.f. cows	Seasons			
		June	Sept.	Nov.	Average
1	640	24.48**	27.63**	23.22**	38.11**

** Highly significant ($P < 0.01$)

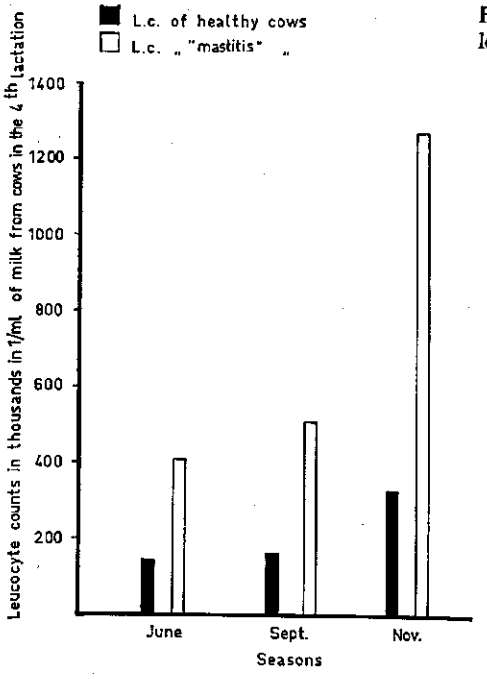


FIG. 9. Effect of incidence of mastitis on leucocyte counts in the milk.

were in agreement with COOLEDGE (1918), CHERRINGTON et al (1933), PLASTRIDGE et al (1939), MACLEOD et al (1953) and NEWBOULD (1959).

5.2.1.3. Mastitis frequency during the first four lactation periods

The history of the incidence of mastitis during the first four lactation periods, presented in tabel 33, shows that sires, which daughters showed comparatively high percentages of clinical mastitis during the preceding three lactations, mostly showed a higher percentage of clinical mastitis in the fourth lactation. The percentage of susceptible daughters varied between different progeny groups, it ranged from 7.4 to 28.5 per cent during the first three lactations, from 5.5 to 26.8 per cent in the fourth lactation and from 9.6 to 42.0 per cent during the period from the first to fourth lactation. The differences between sire daughter groups for mastitis frequency was highly significant when the data included the history of incidence of mastitis from the first to fourth lactation, whereas it was insignificant during the period from the first to third lactation or in the fourth lactation only (Table 40). The results obtained in this study were in agreement with the findings of REID (1954).

These results show that differences between progeny groups for clinical mastitis are more apparent when the data include the history of incidence of mastitis during several subsequent lactation periods. Moreover the incidence of mastitis increases with age and the older cows are much more liable to mastitis. There is a significant correlation between age and mastitis [LEGATES et al (1952),

TABLE 40. F-values for clinical mastitis between sire daughter groups in different lactation periods

d.f. of sires	d.f. of cows	In the fourth lactation only	During the period from the first to third lactation	During the period from the first to fourth lactation
14	677	1.20	1.34	2.55**

** highly significant ($P < 0.01$).

BRAUND et al (1963) and SCHMIDT et al (1965)]. Therefore the cows in early lactation periods do not show a clear picture of their susceptibility to mastitis, whereas the chance is more available in latter lactations.

This contributes in explaining the significant difference between progeny groups for clinical mastitis, when the data include the history of incidence of mastitis for the same progeny during several subsequent lactation periods.

5.2.1.4. The heritability of clinical mastitis

The data used in estimating the heritability of clinical mastitis included the history of incidence of mastitis among progeny groups from the first to fourth lactation period. The cows were considered susceptible if they showed clinical mastitis at any time during the first four lactation periods and resistant to mastitis if they were free of infection during this period. The analysis of variance for the intra-class correlation between paternal sisters is to be found (Table 41).

$$h^2 = \frac{4\sigma^2_S}{\sigma^2_S + \sigma^2_W}$$

The value obtained for the heritability of clinical mastitis was $.12 \pm .06$. This result is in agreement with the value found by O'BLENESS et al. (1960) .05, GAUNYA (1962) .13 and SCHMIDT et al. (1964) .10.

TABLE 41. Analysis of variance for clinical mastitis between paternal sisters

Source of variation	d.f.	S.S.	M.S.	E (M.S.)	Variance components
Between sires	14	6.5	.464	$\sigma^2_W + 22.6\sigma^2_A + 46\sigma^2_S$	.005
Between associations within sires	23	4.7	.204	$\sigma^2_W + 13.5\sigma^2_A$	.001
Residual	654	122.4	.188	σ^2_W	.188

σ^2_W = variance within groups (residual)
σ^2_A = variance between association
σ^2_S = variance between daughter groups of sires

5.2.2. *Milk production*

5.2.2.1. Milk production in relation to leucocyte counts in milk

5.2.2.1.1. *Results obtained with material of* 1964 *(Cows in the fourth lactation)*

The cows were divided into nine groups according to the average milk yield throughout the lactation of each cow as presented in table 42. It is seen that the mean values of the leucocyte counts fluctuated widely through the different classes of the average daily milk yields. Meanwhile, the class, including the very low producing cows (less than 13 kg), constantly showed a higher level of leucocyte counts than the other groups (Figure 10) The differences between the classes for cell counts were statistically insignificant (Table 44).

TABLE 42. Total leucocyte counts in thousands in 1/ml of milk in the classes of average daily milk yield in the fourth lactation obtained from cows records

Classes of average daily milk yield throughout the lactation period		Before excluding 'mastitis cows'					After excluding 'mastitis cows'				
		No. of cows	June	Sept.	Nov.	Average	No. of cows	June	Sept.	Nov.	Average
1.	< 13	53	297	360	760	472	42	195	170	592	319
2.	13–14	54	203	228	672	368	43	124	150	403	226
3.	14.1–15	82	289	279	563	377	60	218	172	387	259
4.	15.1–16	100	202	243	600	348	82	183	202	433	272
5.	16.1–17	101	182	184	369	245	83	140	141	284	188
6.	17.1–18	82	156	188	393	246	67	133	172	354	220
7.	18.1–19	49	250	249	680	393	38	158	158	297	204
8.	19.1–20	31	133	187	336	219	31	133	187	336	219
9.	>20	42	214	281	585	360	36	223	276	520	339

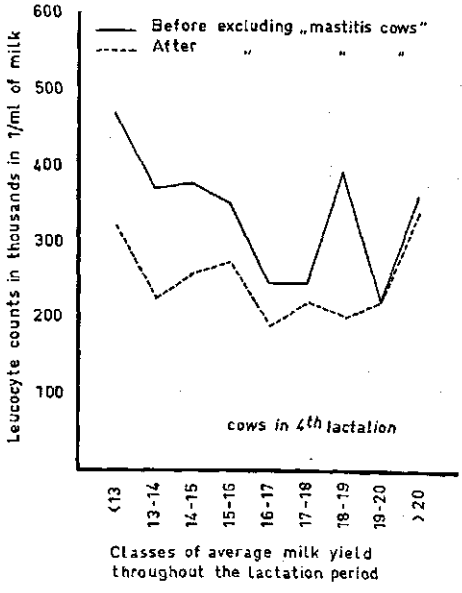


FIG. 10. Average L.c. in relation to milk-production before and after excluding 'mastitis cows'.

To investigate the relation between cell counts and milk yield of cows free from mastitis, the same classification was made after excluding the cows showing clinical mastitis during the fourth lactation (Table 42). The results obtained neither showed a clear trend between these two items. Moreover the average cell counts of the three seasons showed that very low producing cows (less than 13 kg) and very high producing cows (over 20 kg) had relatively higher cell counts than the other groups (Figure 10). To study the effect of the incidence of mastitis in both cell counts and milk production, the cows were divided into two groups. The first including the cows showing clinical mastitis during the fourth lactation, whereas the second group comprised cows free from mastitis as it is presented in tabel 43. The 'mastitis cows' showed a great increase in leucocyte counts, whereas there was a slight decrease in the average daily milk yield, only .50 kg of milk less than the healthy cows. The difference between these two groups for cell counts was statistically highly significant, whereas it was insignificant for the average daily milk yield (Table 44).

TABLE 43. *Leucocyte counts and average yield per day throughout the lactation period for healthy and mastitis cows in the fourth lactation*

	No. of cows	L.c. in thousands in different seasons				Average daily milk yield throughout the lactation kg
		June	Sept.	Nov.	Average	
Mastitis cows	112	418	509	1.178	702	15.65
Cows free from clinical mastitis	482	166	177	391	245	16.15

TABLE 44. *F-values for leucocyte counts between different classes of average daily milk production of cows in the fourth lactation*

	d.f. of classes	d.f. of cows	F-values in different seasons				Milk-production
			June	Sept.	Nov.	Average	
1. Before excluding mastitis cows	8	587	1.27	.82	.84	1.18	–
2. After excluding mastitis cows	8	473	.94	.99	1.53	1.64	–
3. Between mastitis and healthy cows groups	1	592	42.88**	44.56**	36.89**	60.15**	3.84

** highly significant ($P < 0.01$).

The possible interpretation of the results obtained is that clinical mastitis has a demonstrable effect on increasing the cell counts in the milk. In fact, many cows may still be suffering for latent mastitis after the clinical symptoms have disappeared. In the meantime, the clinical mastitis will somewhat depress the

milk yield. Generally, there is a negligible negative relation between cell counts in the milk and the average daily milk yield throughout the lactation period.

5.2.2.1.2. *Results obtained with material of 1965. (Cows in the first lactation)*

The heifers were classified in every season according to the daily milk yield of each cow at the time of sampling, presented in table 45. The difference between classes for leucocyte counts was highly significant in all seasons (Table 47). From figure 11 it can be seen that there was a negative relation between cell count and milk yield on the day of sampling. In September and November the class, comprising the very low producing cows, showed the highest level of cell count.

The same results were obtained when 150 cows in the fourth lactation were used in the same study (Tables 46 and 47) and (Figure 11). These cows were

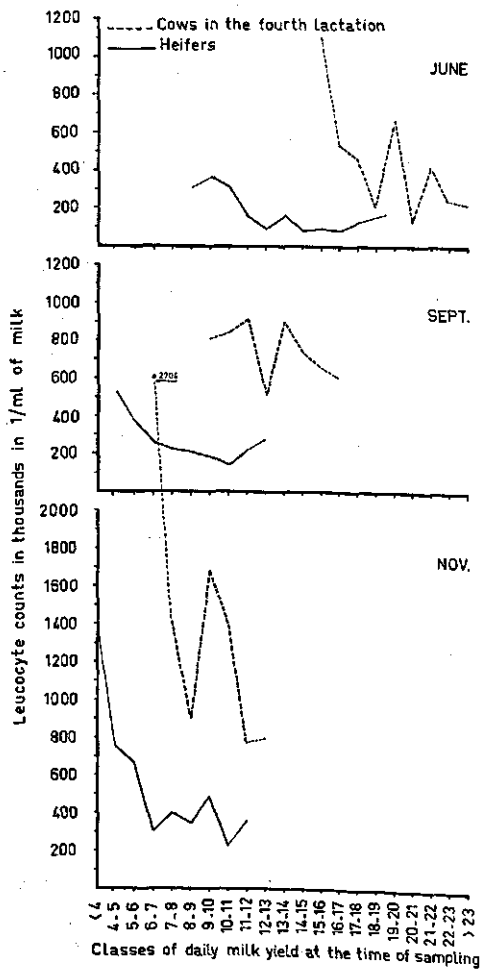


FIG. 11. Leucocyte counts in relation to milk yield on the day of sampling in different seasons (tables 45–46).

TABLE 45. Total leucocyte counts in 1/ml of milk of heifers in the classes of daily milk yield at the time of sampling in different seasons

	June			September			November		
	Classes of milk yield kg	No. of cows	L.C. in thousands	Classes of milk yield kg	No. of cows	L.C. in thousands	Classes of milk yield kg	No. of cows	L.C. in thousands
1.	<9	22	302	<6	58	537	<4	55	1476
2.	9.1–10	39	354	6.1– 7	52	371	4.1– 5	74	768
3.	10.1–11	57	309	7.1– 8	98	266	5.1– 6	115	700
4.	11.1–12	87	166	8.1– 9	126	228	6.1– 7	133	339
5.	12.1–13	121	76	9.1–10	129	213	7.1– 8	145	421
6.	13.1–14	127	151	10.1–11	126	183	8.1– 9	111	369
7.	14.1–15	111	76	11.1–12	99	138	9.1–10	75	505
8.	15.1–16	98	77	12.1–13	60	220	10.1–11	51	248
9.	16.1–17	64	76	>13	51	281	>11	40	497
10.	17.1–18	39	121						
11.	>18	34	167						
	13.5	799	144	9.5	799	243	7.1	799	542

TABLE 46. Total leucocyte counts in 1/ml of milk of cows in the fourth lactation in the classes of daily milk yield at the time of sampling in different seasons

	June			September			November		
	Classes of milk yield kg	No. of cows	L.C. in thousands	Classes of milk yield kg	No. of cows	L.C. in thousands	Classes of milk yield kg	No. of cows	L.C. in thousands
1.	<16	12	1107	<10	15	806	<7	22	2704
2.	16.1–17	14	542	10.1–11	17	851	7.1– 8	22	1416
3.	17.1–18	11	467	11.1–12	15	911	8.1– 9	16	904
4.	18.1–19	15	211	12.1–13	23	512	9.1–10	16	1680
5.	19.1–20	18	670	13.1–14	19	904	10.1–11	21	1401
6.	20.1–21	21	143	14.1–15	16	738	11.1–12	25	783
7.	21.1–22	19	444	15.1–16	14	663	>12	25	798
8.	22.1–23	15	256	>16	30	610			
9.	>23	24	233						
	20.1	149	452	13.5	149	749	9.6	147	1384

TABLE 47. F-values for total leucocyte counts in the milk between different classes of daily milk yield at the time of sampling

Lactation periods	June			September			November		
	d.f. classes	d.f. cows	F value	d.f. classes	d.f. cows	F value	d.f. classes	d.f. cows	F value
1. cows in the first lactation	10	788	3.17**	8	790	3.23**	8	790	12.36**
2. cows in the fourth lactation	8	140	2.01*	7	141	.38	6	140	2.62*

* significant ($P < 0.05$) ** highly significant ($P < 0.01$)

included in the experiment carried out in 1965, and were sired by the same bulls used at the same farms where the heifers were kept. On the other hand, the milk production on the day of sampling showed a gradual decrease during the subsequent seasons, whereas the leucocyte counts showed a remarkable increase, presented at the end of table 45 and 46 and figure 12. Moreover, in November the group, including cows in the first lactation with a daily milk production less than 4 kg showed the highest level of leucocyte counts, it was 1.476.000 cells per ml. The same result was obtained with the class, comprising cows in the fourth lactation with a daily milk production less than 7 kg, the cell count was 2.704.000 per ml of milk.

The simplest explanation of these results is that in the normal udder the number of leucocytes produced is independent of the milk secretion. Consequently the decrease in milk yield results in higher cell counts per ml of milk. The relation between milk yield and cell counts indicates this trend, especially in the fourth lactation cows (Figure 12). This implies that it is possible that cell counts in the milk rise at the end of a lactation period without an infection and eventually may show a positive reaction with C.M.T. or B.M.R. This explanation agrees with the results obtained by BRAUND et al (1963), they found that the percentage of positive quarters for C.M.T. reactions was higher at level of milk production below 20 lbs per day. JOAN et al (1950) also reported that in many cases, when the yield of previously non-infected quarters dropped below 5–6 lbs a day, the foremilk gave abnormal reactions to indirect tests, though no bacteria was found. They stated that probably such changes do not have any connection with infection, though they may be related to the method of machine milking.

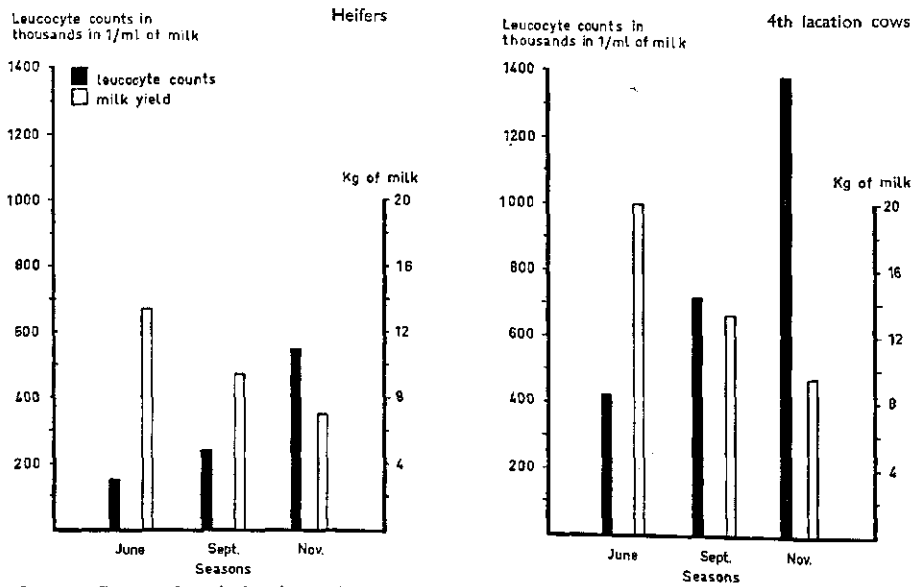


FIG. 12. Seasonal variation in L.C. and M.Y. of heifers (799 cows).
Seasonal variation in L.C. and M.Y. of cows in the 4th lactation (149 cows).

5.2.3. *Ease of milking*

5.2.3.1. Ease of milking in relation to leucocyte counts in the milk

5.2.3.1.1. *Results obtained with material of 1964. (Cows in the fourth lactation)*

The field data obtained by the farmer's own observations on the ease of milking, the cows were classified into five classes as it is presented in table 48. The difference between the classes for cell counts was insignificant (Table 49). Moreover the relation between the classes of ease of milking and leucocyte counts was not clear.

TABLE 48. Total leucocyte counts in thousands in 1/ml of milk in the classes of ease of milking for cows in the fourth lactation

Classes of ease of milking	Before excluding 'mastitis cows'					After excluding 'mastitis cows'				
	No. of cows	June	Sept.	Nov.	Average	No. of cows	June	Sept.	Nov.	Average
Very slow milking cows	24	226	211	482	306	20	160	192	465	272
Slow milking cows	88	203	211	768	394	55	136	125	306	189
Normal cows	89	324	392	580	432	75	236	230	604	357
Fast milking cows	264	233	226	459	306	222	183	170	382	245
Very fast milking cows	214	173	226	557	318	190	148	169	344	220

TABLE 49. F-values for total leucocyte counts in different classes of ease of milking (cows in the 4th lactation)

	d.f. of classes	d.f. of cows	F-values in different seasons			
			June	Sept.	Nov.	Average
1. Before excluding mastitis cows	4	674	2.31	2.50*	.71	.97
2. After excluding mastitis cows	4	557	.97	.75	1.69	1.97

* significant ($P < 0.05$)

The relation between cell counts and the ease of milking of cows free from mastitis showed almost the same results (Tables 48 and 49).

The results obtained show that differences in ease of milking cannot very well account for the large differences in leucocyte counts between cows.

5.2.3.1.2. *Results obtained with material of 1965. (heifers)*

The measured maximum milk rate per minute of the progeny of 19 sires used in 1965, and leucocyte counts of the same sire daughter groups in the first lactation are presented in tabel 50. The results obtained from data collected

TABLE 50. The measured maximum milk rate and leucocyte counts of progeny groups of different sires in the first lactation (1965)

Sire	No. of heifers	Maximum milk rate kg/min	Leucocyte counts in thousands in 1/ml of milk of heifers			
			June	Sept.	Nov.	Average
31	36	1.66	105	143	957	402
24	39	1.93	53	301	384	246
18	59	1.95	196	98	271	188
20	32	1.96	75	151	384	203
33	52	1.97	151	294	979	475
17	57	2.00	143	143	233	173
27	33	2.02	241	151	527	306
34	39	2.03	211	354	964	510
23	27	2.05	68	264	377	236
19	52	2.09	120	181	271	191
21	28	2.09	83	136	444	221
32	54	2.25	151	407	655	404
29	32	2.27	90	384	655	376
26	44	2.31	113	347	437	299
22	41	2.38	105	98	505	236
25	29	2.46	271	105	414	263
16	37	2.52	75	166	272	171
28	33	2.52	68	249	677	331
30	33	2.68	105	414	685	401

during September 1965, for progeny of nine other sires in the first lactation kept in the province of North-Holland are shown in table 51. The results do not confirm the suggestion that the increase in maximum milk rate increases the number of leucocytes in the milk. Moreover there is no clear relation between leucocyte counts and maximum milk rate (Figure 13).

TABLE 51. The measured maximum milk rate and leucocyte counts and B.M.R. tests of nine sire daughter groups in the first lactation

Sire	Maximum milk rate kg/min	Leucocyte counts		B.M.R.		
		No. of heifers	No. of leucocytes per ml milk	No. of cows positive to B.M.R.	Percentage of cows positive to B.M.R. %	B.M.R. after correction to (100^{++++}) %
1	1.74	35	82	0	0	0
2	1.81	55	414	3	5.4	4.8
3	2.15	20	120	4	20.0	8.1
4	2.47	55	203	2	3.6	2.8
5	2.50	50	52	0	0	0
6	2.57	48	173	0	0	0
7	2.62	36	241	1	2.8	1.6
8	2.89	90	83	1	1.1	0.7
9	3.10	69	128	1	1.4	1.3

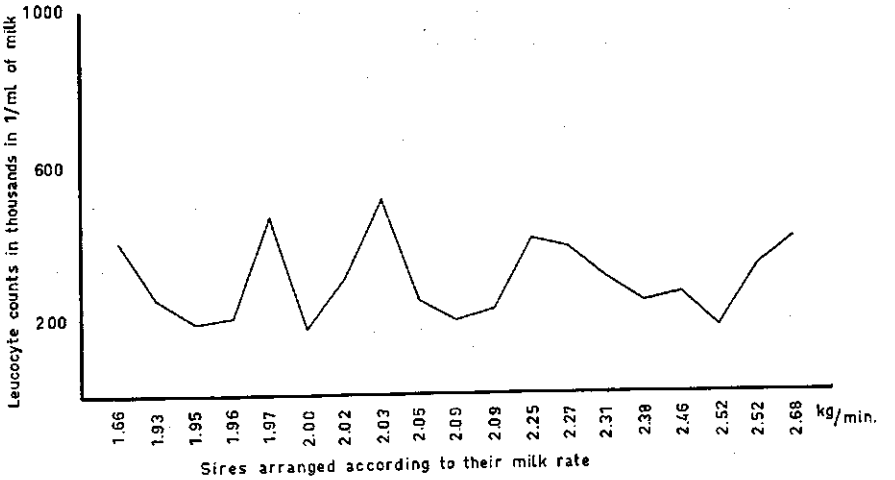


FIG. 13. Average leucocyte counts of cows in the first lactation in relation to milk rate, (Table 50).

6. THE INFLUENCES OF SOME MECHANICAL PROPERTIES OF THE MILKING MACHINE ON LEUCOCYTE COUNTS IN THE MILK

6.1. Material and methods

Technicians visited the farms in which the heifers under investigation were kept in 1965. The line vacuum level was obtained with a vacuum gauge attached to the vacuum line. They also measured the pulsation rate of each machine. They recorded the makes and types of milking machine used at each farm.

Milk samples from herd bulks were collected besides individual samples of each cow during September and November of all the farms under investigations in 1965. These samples were used to study the effect of different makes and types of milking machines on the level of leucocyte counts in herd milk.

6.2. Results and discussion

6.2.1. *Vacuum level in relation to leucocyte counts in the milk*

The relation found between milking vacuum and number of leucocytes in the milk is presented in table 52. It can be seen from the average leucocyte counts in the three seasons (Figure 14) that there was a variation in cell counts between the different classes of vacuum level until the milk vacuum exceeded 40 cm of mercury. After this vacuum level the leucocyte counts showed a remarkable increase and reached its maximum at 55 cm of mercury. However the difference between the classes of milking vacuum level for leucocyte counts was insignificant except in June (Table 53). According to this observation the cows were

Table 52. Leucocyte counts in 1/ml of milk in the classes of milking vacuum level

	Classes of milking vacuum level (cm of mercury) *	No. of heifers	Leucocyte counts in thousands in different seasons			
			June	Sept.	Nov.	Average
1	<34	93	90	233	475	266
2	34	71	128	256	685	356
3	35	59	95	249	595	313
4	36	94	226	203	286	238
5	37	72	90	264	459	271
6	38	188	151	234	482	289
7	39	40	151	248	362	256
8	40	90	68	173	723	321
9	42	43	233	264	640	379
10	45	19	452	196	768	472
11	55	24	226	520	1032	592

* In this study no cows were milked at vacuum level of 41, 43 cm of Hg etc. Moreover the cows milked at 44 and 54 cm of mercury were very few and excluded from the statistical analysis

FIG. 14. Leucocyte counts (average of the three seasons) in relation to milking vacuum.

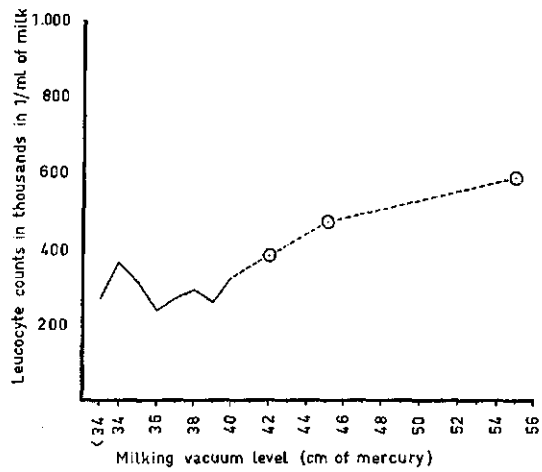


TABLE 53. F-values for leucocyte counts in the milk between different classes of milking vacuum level

d.f. of classes	d.f. of cows	Seasons			
		June	Sept.	Nov.	Average
10	782	2.58**	.88	1.71	1.43

** highly significant ($P < 0.01$).

divided into two classes based on the vacuum level, below and above 40 cm of mercury (Table 54). The group milked with a higher vacuum always showed a higher level of leucocytes than the group milked with a lower vacuum (Figure 15). Moreover the difference between them was statistically significant except in September (Table 55). The results obtained in this study agree with the findings of BRANDSMA et al (1965).

TABLE 54. Leucocyte counts in 1/ml of milk in two classes of milking vacuum level below and and over 40 cm of mercury

Classes of milking vacuum level (cm of mercury)	No. of heifers	Leucocyte counts in thousands in different seasons			
		June	Sept.	Nov.	Average
40 cm and below	707	126	233	508	288
Over 40 cm	86	300	326	815	457

TABLE 55. F-values for leucocyte counts between two classes of milking vacuum – below over 40 cm of mercury

d.f. of classes	d.f. of heifers	Seasons			
		June	Sept.	Nov.	Average
1	791	10.78**	2.16	4.75*	8.64**

* significant ($P < 0.05$) ** highly significant ($P < 0.01$)

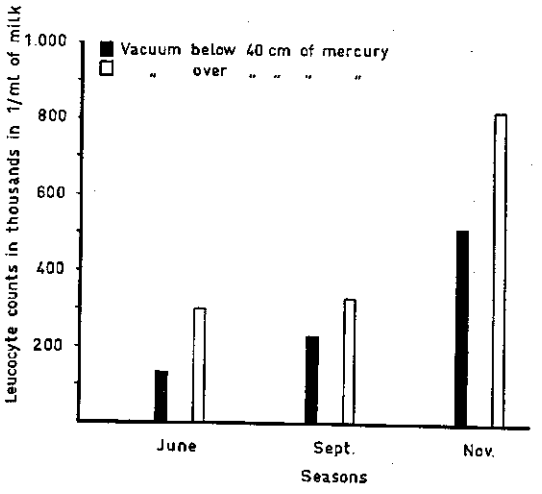


FIG. 15. Leucocyte counts in two classes of milking vacuum below and over 40 cm of mercury.

This led to the conclusion that milking cows at a vacuum level between 30 and 40 cm does not have unfavourable effect on udder health as measured by the number of leucocytes in the milk. Moreover there is no clear relation between these two factors when the vacuum level is under 40 cm of mercury. However, increasing the milking vacuum above this level tends to increase leucocyte counts in the milk, especially when the vacuum is much higher than 40 cm. In our investigation, including 793 first lactation cows, we found that 89% were milked at a vacuum level below 40 cm of mercury. A possible interpretation of these results may be that a milking vacuum over 40 cm is more liable to injure the teat apex which is mostly associated with higher leucocytes in milk. It is possible that the harmful effect of a high vacuum is more marked when the teat cups are left on the udder after the milk flow has ceased which gives more chance for blind milking. This could be observed from the data obtained in November when the milk production of the cows markedly decreased because they were approaching the drying off period. At this time the cows milked at a vacuum level of 55 cm of mercury showed the highest level of leucocyte counts in the milk, it was over one million per ml of milk. This agrees with the results of PETERSEN (1944) and MOBERG (1963). However there may be other factors than milking vacuum having a harmful effect on the udder tissue, as fluctuation in vacuum during milking, duration of milking, pulsation rate, liner design and milking technique. Generally, it is evident from the results obtained in this study and from previous investigations that a vacuum level exceeding 40 cm of mercury is liable to cause udder troubles, unless a good milking technique is practiced.

6.2.2. *Pulsation rate in relation to number of leucocytes in the milk*

Before discussing the result obtained it should be noted that technicians estimated the pulsation rate of the machines used in this study. Most herds

were milked by milking machines operating at a pulsation ratio of 1:1, but 7 herds were milked by a Westfalia milking machine at a pulsation ratio of 4:1. Heifers milked by a Westfalia machine were excluded from the experiment in order to have uniformity in the material for the pulsation ratio.

Table 56 shows that 79% of the heifers were milked at a pulsation rate from 44 tot 48 pulses per minute, while 9% were milked with less than 44 pulses per minute and 12% at over 48 pulses per minute. Increasing the pulsation rate to over 50 pulses per minute showed a remarkable increase in leucocyte counts in the milk (Figure 16). A pulsation rate below 44 pulses per minute also showed a slight increase in cell counts. The difference between pulsation rate classes for leucocytes in the milk was significant in all seasons except June (Table 57). However, from figure 16 it seems that there is no clear trend between these two items and there is no close relation between them. This result agrees with the findings of BRATLIE et al (1963).

TABLE 56. Leucocyte counts in 1/ml of milk in the pulsation rate classes

	Pulsation rate classes (Pulses per min)	No. of heifers	Leucocyte counts in thousands in different seasons			
			June	Sept.	Nov.	Average
1	<44	73	166	358	587	373
2	44	128	83	294	505	294
3	45	31	30	113	384	173
4	46	108	139	211	440	263
5	47	120	170	208	470	283
6	48	220	166	203	497	286
7	49	32	286	120	173	196
8	50	35	166	226	738	377
9	>50	25	134	602	1017	591

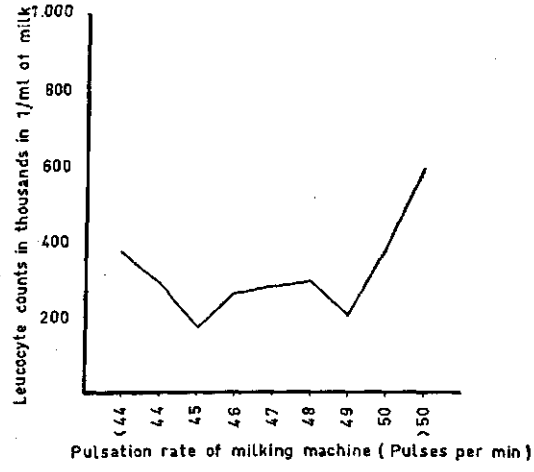


FIG. 16. Leucocyte counts (average of the three seasons) in relation to pulsation rates.

TABLE 57. F-values for leucocyte counts in the milk between different pulsation rat classes

d.f. of classes	d.f. of cows	Seasons			
		June	Sept.	Nov.	Average
8	763	.98	2.32*	2.48*	2.01*

* significant ($P < 0.05$)

6.2.3. *Makes and types of milking machines in relation to leucocytes in milk*

A survey of the average leucocyte counts in milk of herds grouped according to the makes of milking machine is presented in table 58. In the present study 9 makes of machine were used over 244 herds. The herds milked by milking machine (no. 3) showed on an average a higher cell count in the milk than the other herds. However this difference was statistically insignificant (Table 59). Another survey was carried out to study the effect of the types of milking machine on cell counts in the milk. The herds were divided into two groups, the first including the herds milked by a standing type machine and the second by a suspended type (Table 58). The type of machine did not show any effect on cell counts in the milk, the difference between the groups was statistically insignificant (Table 59).

TABLE 58. Leucocyte counts in 1/ml of milk in the groups of makes and types of milking machine

	No. of herds	No. of cows	L.c. in thousands in 1/ml of milk		
			Sept.	Nov.	Average
MAKES OF MILKING MACHINE					
1	46	1167	512	1039	776
2	80	2041	633	1032	829
3	48	1156	618	1476	1047
4	15	334	603	904	753
5	7	150	444	1122	783
6	9	230	693	1100	896
7	7	193	580	821	701
8	6	150	535	851	693
9	6	124	475	986	731
TYPES OF MILKING MACHINE					
1. Standing type	132	3268	565	1168	866
2. Suspended type	92	2287	632	1032	836

TABLE 59. F-values for total leucocyte counts in the milk between different groups of makes and types of milking machine

	d.f. groups	d.f. herds	Sept.	Nov.	Average
Makes of milking machine	8	215	.28	1.24	.77
Types of milking machine	1	222	.76	1.04	.13

7. THE INFLUENCE OF THE FARM MANAGEMENT ASPECTS ON LEUCOCYTE COUNTS IN THE MILK

7.1. Material and methods

Technicians visited each dairy farm from which data were used in 1965 and recorded the following information about the herd management.

1. Milking routine followed, concerning the ratio between number of milkers and number of milking units they handle.
2. The way of stripping practiced at the farm, e.g. stripping by hand or machine.
3. Number of cows in each herd.

During September and November, samples from herd milk were collected from each farm for cell counts.

7.2. Results and discussion

7.2.1. *Milking routine (Man/machine ratios)*

The milking routine, concerning the ratio between the milkers and bucket-units they use, differed from herd to herd. From the inquiry carried out for this study, it appeared that in 31 per cent of the herds one milker handled only one bucket unit, in 36 per cent of herds one milker handled two bucket units, in 24 per cent, two milkers handled two bucket units and in 9 % two milkers handled three bucket units. The results presented in table 60 show that the leucocyte level was slightly lower in the milk of herds milked by the system of one milker handling one bucket. However, the difference between the mean leucocyte counts of the four groups was statistically insignificant (Table 61). Since the more frequent systems of milking routine are one unit per man (31 %) and two units per man (36 %), it seems more useful to compare the effect of these two systems on the leucocytes level in the milk. The results indicated a higher average cell count in the milk samples from herds milked by the system of one man handling two units, especially in November (Figure 17).

Moreover, the difference between these two systems for cell counts in the milk was highly significant in November, but insignificant in September. A comparison of the average cell counts of the two seasons showed a significant difference between the two systems (Table 61). The lower level of cell counts obtained by using one unit per man may be due to the fact that this system gives the milker available time to watch the milk flow and to remove the teat cups as soon as the cow is milked out. On the other hand, in November the milk production of most cows decreases, because they are approaching the drying off period and consequently they need shorter time than before to be milked out. At this time using the system of one man handling two units will increase the risk of over milking. This happens because the milker cannot return to the cow when she is milked out, as he is still busy with the milking routine work, such as washing the udder, taking the fore milk, putting the teat cups of the other unit on the teat and stripping. This interpretation could be

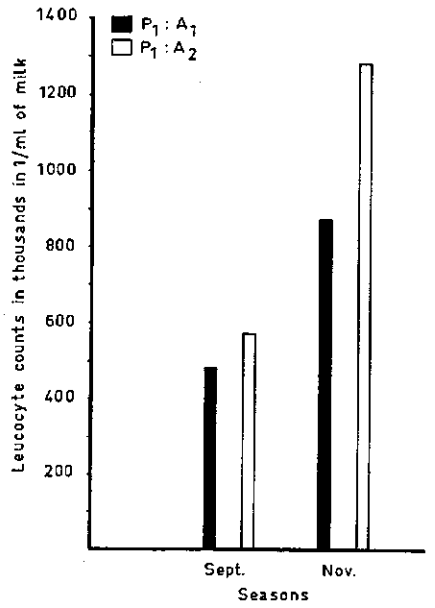


FIG. 17. The effect of man/machine ratios on cell counts in the milk.

verified by using the simple mathematical formula in (Machine milking, 1959, Bulletin no. 177, page 90). This formula describes the extent to which the number of cows milked per hour is affected by man, cow and machine in any milking installation.

$$P = \frac{60}{AWT} = \frac{60N}{UT}$$

where

P = cows milked per hour
AWT = available work time (min) (Time available for the milker to do the routine work on each cow).
UT = unit time (This includes the total milking time and the time the unit is idle in respect of that cow).
N = the number of units used.

If the available work time, as it is estimated by CLOUGH (1958), CHETWYND (1956) and ROBERTS (1958) is 2 minutes per cow, the milker cannot milk more than 30 cows per hour no matter how many units he operates.

$$P = \frac{60}{AWT} = \frac{60}{2} = 30$$

By using the second part of the formula, $\frac{60N}{UT}$, the number of cows milked per hour depends on the number of units per milker and the milk yield of the cows at each milking. In this way it is possible to compare the number of cows

milked per hour by the two milking routine systems $P_1:A_1$ and $P_1:A_2$ in September and November. Considering the average milk yield per cow per milking to be about 7 kg in September, the total milking time is about 4 minutes and the time needed for the unit to be idle for the cow is about one minute. The number of cows milked per hour by each system is:

1. one unit per milker

$$P = \frac{60N}{UT} = \frac{60 \times 1}{4 + 1} = 12 \text{ cows/hour}$$

2. Two units per milker

$$P = \frac{60N}{UT} = \frac{60 \times 2}{4 + 1} = 24 \text{ cows/hour}$$

Considering the average milk yield per cow per milking to be about 5 kg in November, the total milking time is about 3 minutes per cow and the time the unit is idle on the udder one minute. The number of cows milked per hour by each system is

1. One unit per milker

$$\frac{60 \times 1}{3 + 1} = 15 \text{ cows/hour}$$

2. Two units per milker

$$\frac{60 \times 2}{3 + 1} = 30 \text{ cows/hour}$$

The above mentioned shows that by using the system of one man handling two buckets ($P_1:A_2$) the number of cows milked per hour increases compared to ($P_1:A_1$). In autumn the milk yield decreases and machine time shortens. The number of cows milked per man per hour may rise up to 30 by using the system ($P_1:A_2$). But realising that 2 minutes is the minimum time needed per cow for routine work, (this time is independent of the system of milking), it appears that there is no time left for the milker to watch the milk flow. This besides that not all cows requiring equal time to be milked out, may easily result in some other irregularites occurring, e.g. insufficient controll and overmilking. This may contribute to the higher cell count in herds where one man handles two buckets particularly in the autumn. Therefore it is advisable, when the milk routine system is two units per man, to change this system to one unit per milker at the end of the lactation period. The new system will provide the milker with available time to watch the milk flow of each cow and to take out the teat cups as soon as the cow is milked out.

7.2.2. *Stripping by hand or machine*

The influence of stripping by hand or machine on the level of cell counts in the milk is presented in table 60. The results obtained in September did not show any influence of machine stripping on cell counts compared to hand stripping. However, in November the machine stripping showed a high level of cell counts in the milk and the difference between the two was highly significant (Table 61).

TABLE 60. Leucocyte counts in 1/ml of milk with different systems of machine milking

Systems of machine milking	No. of herds	L.C. in thousands in 1/ml of milk		
		Sept.	Nov.	Average
NO. OF MILKERS (P) TO NO. OF APPARATUSES (A) HANDLED IN MILKING				
P:A				
1:1	67	482	866	670
2:2	51	663	1077	866
2:3	20	753	1085	919
1:2	79	572	1281	926
HAND STRIPPING VERSUS MACHINE STRIPPING				
Hand stripping	146	644	851	747
Machine stripping	71	591	1303	947
NO. OF COWS PER HERD				
<15	17	407	1446	926
15	7	354	731	535
16	6	580	1107	844
17	10	279	1228	753
18	10	467	904	678
19	6	557	1243	896
20	15	731	1009	866
21	11	655	896	776
22	12	474	764	610
23	9	768	1401	1085
24	13	716	1017	791
25	17	648	1002	821
26	9	678	1665	1175
27	14	874	1469	1175
28	15	542	1062	798
29	6	738	1266	1009
30	9	316	1152	731
31	7	535	942	738
>31	34	716	1024	866

The possible interpretation is that efficient machine stripping during the earliest lactation period does not effect the leucocyte level in herd milk. The significant difference for leucocytes occurring in November by hand and machine stripping, may be partly due to the milking routine practiced at the farm. In using the system of one man handling one bucket, hand stripping is mostly

TABLE 61. F-values for leucocyte counts in the milk between different systems of milking

	d.f. groups	d.f. herds	Seasons		
			Sept.	Nov.	Average
A. MILKING ROUTINE					
1. No. of milkers to no. of milking apparatuses	3	213	1.71	2.45	2.17
2. $P_1:A_1$ versus $P_1:A_2$ only	1	144	1.19	6.86**	5.89*
B. HAND STRIPPING VERSUS MACHINE STRIPPING	1	215	.43	10.69**	4.27*
C. NO OF COWS PER HERD	18	208	.94	.72	.66

* significant ($P < 0.05$)
** highly significant ($P < 0.01$)

practiced, because the milker has sufficient time left for hand stripping. On the other hand, when one man handles two buckets, machine stripping is applied, because the milker has to milk more cows in a certain time (POLITIEK, 1963). The preceding study on man/machine ratios in relation to leucocyte counts in the milk shows that using the system of one man handling two buckets results in higher cell counts in herd milk than the other system, especially in November. Poor management and inefficient milking at this time may subject the cows to over milking, especially those approaching the drying off period. It seems that machine stripping as such does not greatly affect the leucocyte level in the milk and the system of management and milking routine followed, when machine stripping is practice, is more effective. Therefore it is advisable to follow a good milking technique and management when machine stripping is practiced, especially at the end of lactation period.

7.2.3. *Number of cows per herd*

The results presented in table 60 show that there was no relation between the number of cows per herd and leucocyte level in the milk. The correlation coefficient between these two items for the average cell counts in the two months was insignificant and the value was .17. The difference between the various groups of herd size for leucocyte counts in milk was insignificant (Table 61).

It seems that the level of leucocyte counts in herd milk is more influenced by other factors, like the degree of efficiency of milking and management, than by the number of cows per herd.

8. GENERAL DISCUSSION AND CONCLUSION

The present work revealed some interesting results. It is well demonstrated that there was a significant difference in cell counts between progeny groups of different sires which were free from mastitis during the fourth lactation (Table 37). The heritability of leucocyte number obtained by using this material was on an average .41. Nearly similar results were obtained when heifers were used, especially at the end of the lactation period (Table 32). Thus it seems that leucocyte counts in the milk are influenced by genetic factors, and selection against sires which daughters show relatively high cell counts, should lower the leucocyte level in the milk. The differences between normal progeny groups (free from mastitis) in leucocyte counts in the milk may be due to several factors. One of them is that progeny groups perhaps differ from each other in leucocyte counts in all tissues including the blood. There is no information available on the question whether the leucocyte counts in the blood of cattle are also influenced by genetic factors. There are publications on this subject in mice. CHAI (1957) has shown that leukemia is heritable in mice. During the course of a genetic study of variability in body size, it was discovered that the small strains of mice were leukopenic. The total white blood cell counts averaged 8380 in the large strain and 2320 in the small one. He added that in so far as leukopenia in the small mice is concerned, the present evidence indicates that it is a heritable character and possibly determined by a small number of genetic factors. In fowl, GHANY et al (1963) reported that the leucocyte counts in the blood of the native breed (FAYOUMI) were slightly higher than that in the imported breed (Rhode Island Red). The values were 85.000 and 81.000 per ml respectively. It will be interesting to investigate whether there are also genetic differences in the number of leucocytes in the blood of cattle and to study the relation between cell counts in the blood and milk.

The difference in average cell counts between daughter groups of different sires, before excluding the mastitis cows, may be considered to a certain extent as a result of differences in mastitis frequency between the groups. This could be verified by excluding the cows which had been suffering for mastitis. The results obtained showed that the level of cell counts in the milk of healthy progeny was lower than the level obtained before excluding the 'mastitis cows'. The range in the differences for cell counts between progeny groups was also reduced (Figure 8). However, the differences in average cell counts between progenies, after excluding the 'mastitis cows', was still existing (Table 37). This argument is in favour of the view that the differences in cell counts between progeny groups are not only the results of differences in clinical mastitis frequency. There may be other factors affecting the level of cell counts in milk, such as sub-clinical mastitis, stage of lactation, milk production and management particularly milking technique.

The lowest values of heritability of leucocytes were obtained in June, when the spring calving cows were at the beginning of the lactation period. The highest

values of heritability were found in September and November, when the cows were in the middle and at the end of lactation period respectively (Table 32). This result will have a great value in increasing the accuracy with which the sire's breeding value may be estimated on the basis of the progeny test. The accuracy of the progeny test is expressed by the regression formula based on the heritability of the character and number of progeny per sire.

$$b = \frac{N\,0.25h^2}{1 + (N-1)0.25h^2}$$ [ROBERTSON and RENDEL (1950)]

where

b = Repeatability
N = Number of daughters per sire
h^2 = Heritability of the character

To maintain 70 per cent of accuracy in the present work in all seasons, the following number of daughters per sire are needed in each season (Table 62).

The results presented in table 62 show that there is a wide variation in the number of daughters per sire needed at different seasons to reach 70% of accuracy. The higher the heritability, the lower the number of cows needed per sire. Moreover, under the conditions of this study the heifers had lower values of heritability than fourth lactation cows in all seasons except November.

TABLE 62. Number of daughters per sire needed in each season to achieve 70% of accuracy in the progeny test for leucocyte counts in the milk

	b %	Seasons					
		June		September		November	
		h^2	No. of progeny per sire	h^2	No. of progeny per sire	h^2	No. of progeny per sire
Progeny in 4th lactation	70	.07	135	.37	25	.35	25
Progeny in 1st lactation	70	.07	135	.16	55	.44	20

Generally one could come to the conclusion that it is necessary to decide the proper time during the lactation period at which the progeny test should eventually be carried out. A suitable time apparently is when the highest value of heritability of leucocytes in milk is obtained. Also the results of progeny tests to estimate the sire's breeding value for leucocytes in the milk are valid only under the same environmental conditions as those where the test is made.

High cell counts is the most important symptom of incidence of mastitis. The background of this study was to investigate, at least partly, in how far differences in cell counts between progeny groups were related to susceptibility for clinical mastitis under practical conditions. It was demonstrated from the present

results that daughter groups of sires which had a relatively high average cell count, mostly showed a high mastitis frequency (Table 33). Moreover, the healthy progenies (free from mastitis) of sires no. 1 and 2, showing the lowest level of cell counts, also had the lowest percentage of mastitis cows (Table 36). The correlation coefficient between leucocyte counts in the milk and clinical mastitis was highly significant (Table 34). Furthermore there was a highly genetic correlation between clinical mastitis frequency and leucocyte counts within progeny groups. The value was .83. A similar result was obtained by YOUNG et al (1960), they came to the conclusion that many of the genes which influence clinical mastitis also influence leucocyte counts in the milk. This correlation is more or less self evident because mastitis, at least clinical mastitis, is almost identical to high leucocyte counts in the milk.

From the previous discussion and what was mentioned before, it may be concluded that under the optimum conditions of farm management, selection against sires whose progenies produce milk with higher cell counts than the usual number in the milk of healthy cows, could partly play a role in reducing the susceptibility to clinical mastitis to a certain extent. On the other hand, it should be kept in mind that this research does not investigate the relation between cell counts in the milk and mastitis frequency caused by bacterial mammary infection such as streptococcal, staphylococcal and coli infection. Consequently, there is no definite answer to the question whether a low average cell count in the milk of the daughter group means that this group is less susceptible to mastitis. Especially after the finding of SCHALM (1964) that coliform mastitis is a disease of the normal leucocyte-free mammary gland. He observed a too rapid fall in the leucocyte number after the initial inflammatory response and before all Aerobacter aerogens (one type of coliform mastitis) were destroyed. When this happened a second attack of acute mastitis developed. In some glands a chronic infection resulted and then acute mastitis appeared, whenever the leucocyte numbers fell below 200.000 per ml of foremilk. He concluded that mammary quarters producing milk with cell counts of 300.000 to 500.000 per ml of foremilk were found to have a high degree of protection against experimental coliform mastitis.

A low value of heritability for clinical mastitis, .12, may be expected, since general health is affected by many external conditions. By this value, depending on mass selection only against clinical mastitis, will lead to a very slow genetic improvement per generation. An effective way of controlling mastitis could be achieved by paying attention to herd management to prevent the stress on the teat as much as possible, using a suitable method of hygiene to prevent the spread of organisms from cow to cow, selecting against cows severely affected or having severely affected daughters or sisters and in favour of sires whose dams and sisters demonstrate resistance to mastitis. This selection may contribute slightly in the control of mastitis.

It is important to know the relation between leucocyte counts and milk production. The present work does not show a clear relation between these two traits. Using cows in the fourth lactation and free from mastitis showed that

very low producing cows (less than 13 kg) and very high producing cows (over 20 kg) had relatively higher cell counts than the other groups (Figure 10). However, it seems more appropriate to discover this relation during the first lactation. In the present study it seems that there was a negative relation between cell counts and milk yield when heifers were used (Figure 11). It should be taken into account, however, that the daily milk yield of heifers in this study represents the milk production at the time of sampling and not the average daily milk yield of whole lactation. A detailed study is needed to investigate this relation during the first and subsequent lactations.

It was interesting to find that increasing the milk vacuum over 40 cm of mercury was associated with higher leucocyte counts in the milk, especially near the end of lactation period (Table 54). This may be due to a greater risk of injuring the teat apex when the vacuum is much higher than the optimum and this will affect the level of cell counts in the milk. The same result is obtained when the pulsation rate is less than 44 pulses per minute or over 50 (Figure 16). It appears that it is necessary that the milk vacuum level in the line and pulsation rate must be carefully inspected by experts and to be corrected if they are higher than the optimum level. This inspection will lead to detect the faults in milking machine equipment and to make sure that it stays in good working order.

Farm management, especially milking routine is related to leucocyte counts in the milk. Applying the system of one milker handling two milking units, gives milk with high leucocyte counts, especially at the end of the lactation period. When the milk routine system, two units per milker, is applied it may be better to change this system at the end of lactation period to one unit per man to prevent over milking. However, applying this suggestion depends on the economic situation of the farm, because changing the system from $P_1:A_2$ to $P_1:A_1$ will need more labour or more time for milking the same number of cows. On the other hand, it should be kept in mind that the best milking system will fail, if the proper milking technique is not applied.

SUMMARY

An investigation was carried out to study the influence of genetic and some environmental factors on the number of leucocytes in the milk. The study included four investigations.

1. To investigate in how far the genetic factors influence the variation in cell counts of milk obtained from individual cows milked by machine under practical conditions in different herds, seasons and stages of lactation.
2. To investigate as far as possible the relation between cell counts in the milk and clinical mastitis and other economic traits of cows, viz. milk yield and ease of milking.
3. The influence of some mechanical properties of the milking machines on leucocyte counts in the milk, e.g. milking vacuum, pulsation rate and makes of milking machines.
4. The effect of farm management aspects on the level of leucocytes in the milk, viz. milking routine, way of stripping and number of cows per herd.

This study was continued for two years. The first year 692 paternal daughters in the fourth lactation descending from 15 Dutch Friesian sires were used. In the second year 799 heifers descending from 20 Friesian sires were included in the study. The cows were raised in the province of Utrecht and distributed among several milk recording associations and kept in large number of farms. All cows were spring calving and milked by machine. Three milk samples were collected from each cow, one in the months of June, September and November. The leucocyte count was determined by counting the cells from the microscopic field. The history of incidence of mastitis during the first four lactation periods was estimated with the aid of milk recording associations supplied by the farmers. The measurements of milking vacuum and pulsation rate were carried out by technicians. A summary of the results obtained follows.

1. The effect of milk recording association and herd on leucocyte counts in the milk was on an average 3.5 and 3.7 per cent of the total variance respectively (Tables 27 and 30).
2. Seasons which were narrowly related with stage of lactation, showed a great influence on leucocyte counts in the milk. The highest level of cell counts in the milk was obtained in November when most of the cows had almost reached the drying off period (Figure 2).
3. Leucocyte counts in milk varied between different daughter groups (Figures 3 and 6). The differences between them for cell counts were statistically significant (Tables 19 and 23).
4. The sire effect on leucocyte counts was 9.0 per cent of the total variance during the fourth lactation, whereas it was 3.0 per cent during the first lactation. There was an increase in sire effect during the subsequent seasons. When cows in the fourth lactation were used the estimated values of sire effect were 1.7, 8.5 and 8.6. in June, September and November respectively. When

only heifers were used the values were 1.8, 3.6 and 8.7 for the same months respectively (Tables 27 and 30).

5. In using cows in the fourth lactation, the level of cell counts in the milk of healthy progeny (cows never showing mastitis during the fourth lactation) was lower than the level obtained before excluding the cows suffering for mastitis. The decrease was more obvious in the progeny including many cows with a mastitis history during the fourth lactation (Figure 8).
6. The healthy progeny also showed a significant difference for cell counts in the milk (Table 37). The sire effect on the variation between daughter groups for leucocyte counts was more apparent after excluding the 'mastitis cows' except in June when the effect of sire was not obvious (Table 27).
7. Heritability estimates for leucocytes in the milk were obtained from paternal sister correlations. The values obtained by daughter groups in the fourth lactation were higher than the values from heifers, they were .37 and .14 respectively. However, in November the heifers showed a higher heritability, the value was .44. The lowest values were obtained in June when the spring calving cows were in the first half of the lactation period, whereas the highest values were in September and November when the cows were in the middle and the end of the lactation period respectively. Estimating the heritability of cell counts obtained from progeny groups in the fourth lactation after excluding the 'mastitis cows' showed a slight increase in the values obtained in all seasons except June (Table 32).
8. Repeatability for leucocyte counts in the milk was estimated at .40 and .28 by using progeny groups in the fourth and first lactation respectively.
9. The daughter groups of sires which had a relatively high average cell count mostly showed a high 'mastitis frequency'. However, this positive relation was not always present (Table 33).
10. The correlation coefficient within sires shows that there was a highly significant correlation between mastitis history and leucocyte counts in the milk (Table 34). The estimated genetic correlation was .83 for clinical mastitis with leucocyte counts (Table 35).
11. The 'mastitis cows' produced milk with higher leucocyte counts than healthy cows (Figure 9). The difference between them for leucocyte counts was statistically significant (Table 39).
12. The heritability estimate for clinical mastitis obtained from paternal sister correlation by using data concerning the history of incidence of mastitis during the first four lactation periods was .12.
13. The differences between the classes of average daily milk yield for cell counts of cows in the fourth lactation were statistically insignificant. Using cows in the fourth lactation and free from mastitis showed the same results (Table 44). However, very high producing cows and very low producing cows showed relatively higher cell counts than the other groups (Figure 10). In using heifers, the classes containing the very low producing cows at the time of sampling, showed the highest level of cell counts in September and November (Figure 11). Moreover the differences between the classes of daily milk yield on

the day of sampling were significant for cell counts (Table 47).

14. During the subsequent seasons, which were related with the stage of lactation, the daily milk yield showed a gradual decrease, whereas leucocyte counts in milk showed a remarkable increase (Figure 12). Moreover, in November at the end of lactation period the heifers group, showing the lowest daily milk (less than 4 kg) had the highest cell counts in the milk (Table 45). The same results were obtained when cows in the fourth lactation were used (Table 46).
15. The effect of incidence of mastitis was more pronounced on cell counts in the milk than on milk production. The cell counts of the 'mastitis cows' increased considerably, whereas the milk production decreased slightly compared to normal cows (Tables 43 and 44).
16. The relation between leucocyte counts in the milk and ease of milking did not demonstrate a clear trend (Figure 13). The differences between the different classes of ease of milking for cell counts were insignificant (Table 49).
17. Increasing the milking vacuum over 40 cm mercury tends to increase leucocyte counts in the milk (Figure 15). The difference for leucocyte counts between the cows milked below and over 40 cm was statistically significant (Table 55).
18. Increasing the pulsation rate over 50 pulses/minute showed a remarkable increase in leucocyte counts. A pulsation rate below 44 pulses/minute showed a slight increase in cell counts (Figure 16). The differences for leucocyte counts between the classes of pulsation rate were significant (Table 57).
19. The makes of milking machine had no effect on cell count in the milk. The differences between the groups milked with different makes were insignificant (Table 59).
20. Milking routine (man/machine ratios) practiced in the farm, greatly affected the cell counts, especially at the end of the lactation period. The system, one milker handling two units showed a higher value of cell counts in November than the system, one man handling one unit (Figure 17). The difference in cell counts between these two systems was only significant in November, when most of the cows reached the drying off period (Table 61).
21. In September, machine stripping did not show any influence on the cell counts compared to hand stripping. However, in November machine stripping showed a high value of cell counts and the difference between the two systems of stripping was significant (Tables 60 and 61).
22. There was no relation between number of cows per herd and level of leucocytes in the milk (Tables 60 and 61).

ACKNOWLEDGEMENT

The present work was carried out in the Department of Animal Husbandry, Agricultural University, Wageningen. The Netherlands, under the supervision of Prof. Dr. Th. STEGENGA. The writer wishes to express his wholehearted gratitude to his promotor Prof. Dr. TH. STEGENGA for his guidance, valuable advice and stimulating criticism throughout the work as well as for reading and correcting the manuscript. I feel greatly indebted to the staff members of the Department of Animal Husbandry for their co-operation during this work in particular to Ir. M. BEKEDAM for his useful advice and for translating the conclusion and summary in Dutch, to Dr. Ir. R. D. POLITIEK who never failed to give help and advice whenever asked for.

I also wish to mention my special gratitude to Dr. Ir. C. J. SCHIPPER 'Werkgroep Hygienische Melkwinning, T.N.O.' for providing the facilities to do the practical work at the laboratory of Workgroup at Wageningen and for his useful suggestions, to Ir. A. J. DIJKMAN for his special interst in the study, to Mr. J. POST and Mr. M. J. NUNINGA for their kind help during the practical work.

I wish to thank Miss J. C. TEN BENSEL for her kind assistance, Mrs. E. HAAK-VAN DEN BRINK and Miss G. H. DEKKER for their kind effort in typing the manuscript, Mr. W. HEIJE for drawing the figures and Mr. J. STELWAGEN whose help in collecting the milk samples was very much appreciated.

The author gratefully acknowledges the fine co-operation of Mr. M. H. PARDIJS 'De Hoofdcontroleur van de Prov. Melkcontrole Dienst voor Utrecht' for his arrangement with the milk recorders of the local associations for collecting the milk samples, for conducting the mastitis survey and the other inquiries. My sincere thanks are due to all milkrecorders, herdsmen, and technicians who co-operated in supplying information concerning the mechanical properties of milking machines and herd management. I am very grateful to Ir. A. H. Kruit 'Directeur van het Melkcontrole Station Utrecht' for his good arrangement to carry out this survey.

The writer is greatly indebted to Mr. H. KEULS of the Department of Mathematics of the Agricultural University, Wageningen for his useful statistical advice, patient co-operation and guidance in analysing the data of this work with the aid of electronic data processing machines, With his help this work has been accomplished.

I want to express my gratefulness to Miss VAN ROSSUM 'I.B.S.' for her help in correcting the English text.

I am very indebted to the Government of the U.A.R. for providing the facilities to carry out this work in the Netherlands.

Finally I am very grateful to the members of the Agricultural University at Wageningen and to all others who have contributed in any way to make this study possible.

SAMENVATTING

Normale rundermelk bevat leukocyten. Het gehalte aan leukocyten, in het vervolg aangeduid als celgehalte, varieert. Dit onderzoek heeft betrekking op de variatie in celgehalten en op de faktoren, erfelijke en niet-erfelijke, die hierop van invloed kunnen zijn. Het onderzoek omvat vier delen:

1. Het bepalen van de mate van invloed van erfelijke faktoren op de variatie in celgehalte van melk van onder normale omstandigheden op verschillende bedrijven gehouden melkkoeien, welke machinaal worden gemolken en waarbij tevens het seizoen en het laktatiestadium in aanmerking worden genomen.
2. Het onderzoeken van het verband tussen het celgehalte en klinische mastitis en andere van economische betekenis zijnde eigenschappen, zoals de melkproduktie en de melksnelheid.
3. Het onderzoeken van de invloed van enige technische bijzonderheden van de onder praktijkomstandigheden gebruikte melkmachines, zoals het fabrieksmerk, het vacuum en de pulsatiesnelheid op het celgehalte.
4. Het effekt van de omstandigheden op het melkveehouderijbedrijf, zoals het aantal melkkoeien, de methode van melken en de wijze van namelken, op het celgehalte.

Het onderzoek is uitgevoerd in 1964 en 1965. De onderzochte koeien werden gehouden op een groot aantal bedrijven met melkcontrole in de provincie Utrecht.

In 1964 waren ter beschikking 15 groepen paternale halfzusters in de vierde laktatie, totaal omvattend 692 koeien van het F.H.-ras. In 1965 had het onderzoek betrekking op 20 groepen paternale halfzusters, totaal 799 dieren, nu in de eerste laktatie en eveneens van het F.H.-ras.

Alle dieren hadden gekalfd in het voorjaar en werden machinaal gemolken. Van elke koe zijn drie melkmonsters onderzocht. De monsters zijn genomen in juni, september en november.

Het celgehalte is bepaald door tellen onder een microscoop. Over het optreden van mastitis tijdens eerste vier laktaties is navraag gedaan bij de veehouders door de controleurs van de verenigingen voor melkcontrole.

De metingen van het vacuum en van de pulsatiesnelheid van de melkmachines zijn uitgevoerd door adviseurs voor machinaal melken van het Melkcontrolestation Utrecht (MCSU).

Het onderzoek heeft de volgende resultaten opgeleverd:

1. Het effekt van het gebied – in dit onderzoek samenvallend met de verenigingen voor melkcontrole als geografische eenheden – op het celgehalte kon worden geschat op 3,5% van de totale variantie.

Het effekt van het bedrijf op het celgehalte is geschat op 3,7% van de totale variantie (Tabellen 27 en 30).

2. Het seizoen, grotendeels identiek met het laktatiestadium, bleek veel invloed te hebben op het celgehalte. Het celgehalte bleek het hoogst te zijn bij de in

november genomen melkmonsters; die in het onderzoek betrokken koeien zijn in deze maand in het laatste stadium van de laktatieperiode (Fig. 2).

3. Het celgehalte varieerde tussen de verschillende dochtergroepen van de stieren (Figuren 3 en 6). Deze verschillen bleken significant te zijn (Tabellen 19 en 23).
4. Het effekt van de vader der onderzochte koeien op het celgehalte was gemiddeld 9,0% van de totale variantie bij koeien in de vierde laktatie; het was 3,0% bij koeien in de eerste laktatie. Verder was er een stijging van het effekt van de vader in de opeenvolgende seizoenen. Bij koeien in de vierde laktatie steeg dit effekt nl. van 1,7% in juni tot 8,5% in september en 8,6% in november. Bij koeien in de eerste laktatie zijn deze percentages achtereenvolgens 1,8; 3,6 en 8,7 (Tabellen 27 en 30).
5. Het celgehalte van de melk van koeien in de vierde laktatie, waarbij nimmer klinische mastitis werd waargenomen, was lager dan van koeien waarbij dit wel het geval was. Deze bevinding was duidelijker naarmate in de groepen afstammelingen van stieren meer koeien voorkwamen waarbij klinisch mastitis was waargenomen (Fig. 8).
6. Ook na uitsluiting van koeien, waarbij eerder klinisch mastitis is waargenomen, bleken er significante verschillen te bestaan tussen de groepen dochters van de stieren in celgehalte van de melk (Tabel 37). Dit stiereffekt was zelfs duidelijker, behoudens bij de in juni genomen melkmonsters, toen het stiereffekt niet duidelijk was (Tabel 27).
7. De schattingen van de erfelijkheidsgraad van de celgehalten zijn verkregen uit correlaties tyssen de paternale groepen halfzusters. De waarden zijn hoger bij koeien in de vierde laktatie dan bij die in de eerste laktatie en bedroegen 0,37 resp. 0,14. In november echter bleek de erfelijkheidsgraad bij koeien in de eerste laktatie 0,44 te bedragen. De laagste waarden werden verkregen uit de waarnemingen in juni, wanneer de in het voorjaar kalvende koeien in het eerste deel van de laktatie zijn. De schattingen van de erfelijkheidsgraad zijn hoger bij de waarnemingen in september en november. Schattingen van de erfelijkheidsgraad bij koeien in de vierde laktatie na uitsluiting van dieren waarbij klinisch mastitis werd waargenomen, leverde een geringe stijging van de erfelijkheidsgraad op in alle maanden van onderzoek uitgezonderd juni (Tabel 32).
8. De herhaalbaarheid van het celgehalte in melk werd berekend op 0,40 en 0,28 uit dochtergroepen in de vierde, resp, de eerste laktatie.
9. Bij de dochtergroepen van stieren met een relatief hoog celgehalte viel ook dikwijls een hoge 'mastitis frequentie' op. Deze positieve relatie was echter niet steeds aanwezig (Tabel 33).
10. Er bleek een sterk significante correlatie binnen dochtergroepen te bestaan tussen het celgehalte van de melk en de 'mastitis historie' als indicatie voor de gevoeligheid voor mastitis. (Tabel 34). De genetische correlatie tussen het celgehalte en klinische mastitis kon worden geschat op 0,83 (Tabel 35).
11. De z.g. 'mastitis koeien' (koeien die eerder mastitis hadden gehad), produceerden melk met een hoger celgehalte dan gezonde koeien (Fig. 9). Het verschil is significant (Tabel 39).

12. De schatting van de erfelijkheidsgraad van klinische mastitis uit de correlatie tussen paternale halfzusters bedroeg 0,12. Deze schatting werd verkregen uit gegevens omtrent het optreden van mastitis tijdens de eerste 4 laktaties.
13. Er is geen significant verschil aangetoond tussen de celgehalten in melk van koeien tijdens de 4e laktatie bij indeling volgens melkproduktie per dag. Dit resultaat veranderde niet door uitsluiting van koeien die eerder mastitis hadden gehad (Tabel 44). Echter, de melk van hoog produktieve zowel als van laag produktieve dieren bleek meer cellen te bevatten dan de melk van koeien met een normale produktie (Fig. 10).

Wat de koeien in de eerste laktatie betreft, bleken de ten tijde van het nemen van de melkmonsters laag produktieve dieren de hoogste celgehalten te vertonen in september en november (Fig. 11). Ook waren de verschillen tussen de celgehalten significant bij indeling van koeien in de eerste laktatie in klassen volgens de melkproduktie ten tijde van het nemen van de melkmonsters (Tabel 47).

14. In de opeenvolgende seizoenen, nauw samengaand met het vorderen van de laktatie, daalde de melkproduktie geleidelijk en steeg het celgehalte opvallend (Fig. 12). Bovendien bleken koeien in de eerste laktatie met de laagste dagelijkse melkproduktie (lager dan 4 kg) het hoogste celgehalte in de melk te hebben (tabel 45). Analoge resultaten zijn gevonden bij koeien in de vierde laktatie (Tabel 46).
15. Het effekt van het optreden van mastitis was groter op het celgehalte van de melk dan op de melkproduktie. De celgehalten bij de z.g. 'mastitis koeien' waren aanzienlijk hoger, terwijl de melkproduktie slechts in geringe mate lager was (Tabellen 43 en 44).
16. Er was geen duidelijk verband waarneembaar tussen het celgehalte van de melk en de melkbaarheid (Fig. 13). De verschillen tussen de klassen bij indeling van de dochtergroepen volgens de vererving van de melkbaarheid van de vader, waren niet significant (Tabel 49).
17. Verhoging van het vacuum van de melkmachine boven 40 cm Hg resulteerde in een tendens tot stijging van het celgehalte (Fig. 15). Het verschil in celgehalte tussen koeien, gemolken met een vacuum beneden resp., boven 40 cm Hg was significant (Tabel 55).
18. Het celgehalte steeg opmerkelijk wanneer de pulsatiesnelheid groter werd dan 50 slagen per minuut. Een snelheid lager dan 44 slagen per minuut gaf een geringe stijging van het celgehalte te zien (Fig. 16). Het verschil in celgehalte tussen de klassen, ingedeeld volgens pulsatiesnelheid was significant (Tabel 57).
19. Het fabrieksmerk van de melkmachines had geen effekt op het celgehalte. De waargenomen verschillen tyssen de groepen, gemolken met verschillende merken, waren niet significant (Tabel 59).
20. De werkwijze bij het melken – het aantal apparaten per melker – bleek veel invloed te hebben op het celgehalte, vooral tegen het einde van de laktatieperiode. De werkwijze van 2 apparaten per melker gaf aanleiding tot hogere celgehalten in november dan de werkwijze van 1 apparaat per melker (Fig. 17).

Het verschil in celgehalte bij deze twee werkwijzen was alleen significant in november, dus tegen het einde van de laktatie (Tabel 61).

21. In vergelijking met namelken met de hand bleek machinaal namelken geen enkele invloed te hebben op het celgehalte bij de monsters genomen in september. In november evenwel werden hoge celgehalten waargenomen bij machinaal namelken. Het verschil met namelken met de hand was significant (Tabellen 60 en 61).
22. Tenslotte bleek er geen verband waar te nemen tussen het aantal koeien per bedrijf en het celgehalte van de melk (Tabellen 60 en 61).

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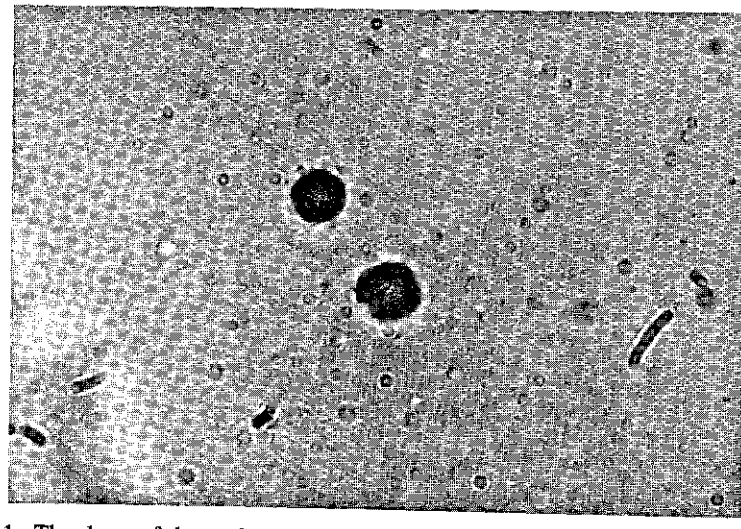

PLATE 1. The shape of the nucleus of leucocytes in the fresh milk.

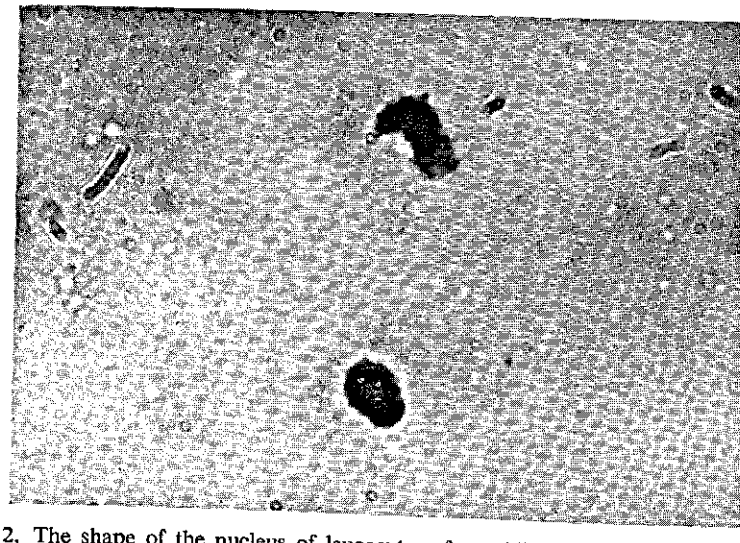

PLATE 2. The shape of the nucleus of leucocytes after adding Potassium Bichromate and storing the milk sample for 8 days.

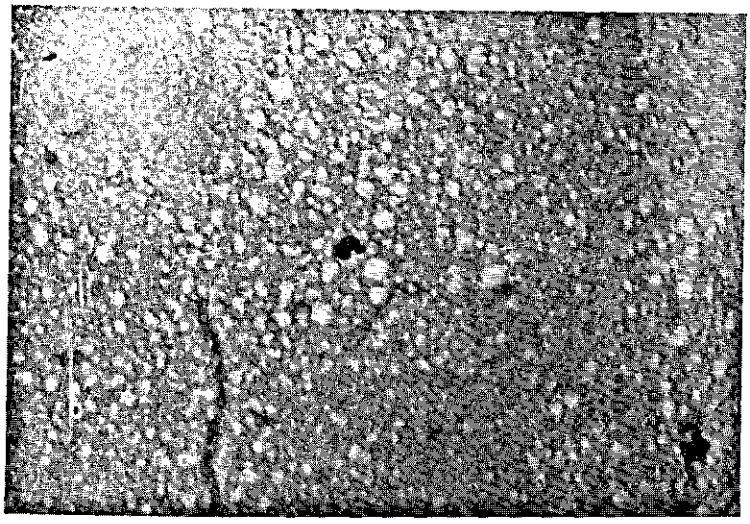

PLATE 3. Milk sample taken from healthy cow showing a few number of leucocytes.

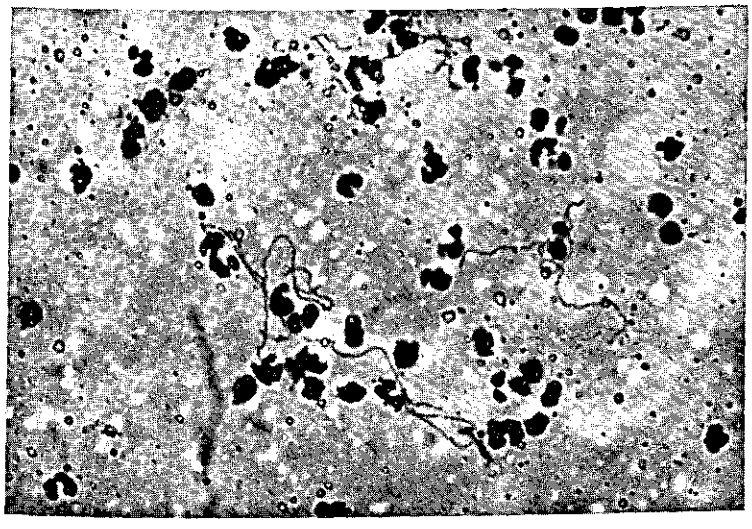

PLATE 4. Milk sample taken from a cow suffering from 'mastitis' showing numerous leucocytes and Pathogenic bacteria.

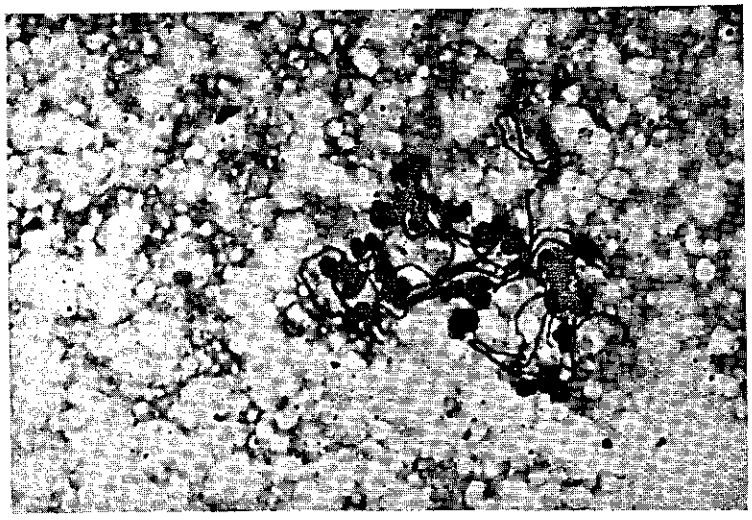

PLATE 5. Photomicrograph of milk from an infected udder showing leucocytes clustered around bacteria.