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COLOSTRUM IMMUNOGLOBULIN CONCENTRATION IN COWS: RELATIONSHIP WITH THEIR CALF MORTALITY AND WITH THE COLOSTRUM QUALITY OF THEIR FEMALE OFFSPRING

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Résumé.

CONCENTRATION EN IMMUNOGLOBULINES DU COLOSTRUM DES VACHES : RELATION AVEC LA MORTALITE DE LEURS VEAUX ET AVEC LA QUALITE DU COLOSTRUM DE LEURS FILLES. — Pendant quatre années consécutives, des échantillons de colostrum ont été prélevés au cours des trois heures qui suivent le vêlage, sur 180 vaches allaitantes d'un même troupeau. Sur les sérums colostraux, les protéines totales ont été analysées par la méthode du biuret, et les concentrations en gammaglobulines ont été déterminées par électrophorèse. Nous avons retrouvé la classique relation entre la teneur du colostrum en gammaglobulines et la mortalité des veaux. Nous avons tenté de déduire de ces données la valeur souhaitable de la concentration du colostrum et l'influence de différents facteurs physiopathologiques.

La gémelliparité, l'âge du vêlage et le niveau alimentaire en fin de gestation, quelle que soit leur influence sur la mortalité des veaux, n'entraînent pas de variation significative de la concentration du colostrum en gammaglobulines.

Au contraire, pour les mères atteintes d'infections cliniques, il y a à la fois un taux de mortalité important de leurs veaux et une plus faible concentration de leur colostrum.

Au cours des vêlages successifs, il y avait une forte répétabilité des concentrations du colostrum en gammaglobulines pour une même vache.

Il y a également une corrélation significative entre les teneurs en gammaglobulines du colostrum des mères et celles de leurs filles. Ce résultat, qui devra être vérifié sur un plus grand nombre d'animaux, suggère la possibilité d'une approche génétique du problème de la mortalité des veaux, par le biais de la sélection sur la composition du colostrum.

Introduction.

The rate of survival of calves depends significantly on their blood gammaglobulin level which is mainly determined by how early and how many colostral gammaglobulins

have been ingested, particularly at the first feed (Fey and Margadant, 1961; Gay *et al.*, 1965; Kruse, 1970; Selman *et al.*, 1971; J. Dardillat, 1973; Selman, 1973).

The quantity of colostral gammaglobulins at the first feed depends on the volume of

colostrum ingested and on its concentration of gammaglobulins. The quantities of immunoglobulins produced at the first two milkings have been studied with regard to the interval between calving and the first milking, the number of lactations, the season of calving and the breed; any significant individual variations have been noted (Kruse, 1970). In addition, Logan (1977) showed the influence of rearing conditions both on the quantities of colostrum produced at the first two milkings and on their immunoglobulin content.

In traditional rearing conditions, where the cow suckles her calf, this second factor is shown to be of prime importance by the inverse relationship between the calf mortality rate and the gammaglobulin concentrations of colostrum ingested at the first feed (Dardillat, 1974).

The present work concentrates on three main topics:

- The relationship between the immunoglobulin level of the colostrum and calf mortality at different ages, in order to try to evaluate the minimum concentration desirable in bovine colostrum.
- The influence on the concentration of the colostrum of a number of factors known to affect calf mortality: twin birth, early calving, restricted diet toward the end of the gestation period, and the cow's state of health.
- Repeatability of gammaglobulin contents of the colostrum of the same cow throughout successive calvings, and whether this is transmitted to female offspring.

Material and methods.

ANIMALS

The experiment was conducted from 1972 to 1975 on a herd from the "Institut National de la Recherche Agronomique" (INRA) consisting of one hundred suckling cows of the Salers, Aubrac and Charolais breeds and crossbreds. The calves suckled within 3 hours following birth and were fed by their mother until natural weaning occurred. Most calvings took place between January and February.

EXPERIMENTATION

Studies were based on complete records established for each cow-calf pair, consisting of yearly identification, various zootechnical measurements, and in 1973 sanitary records.

In order to evaluate the effects of a moderate restriction in diet towards the end of the gestation period, two groups of 20 cows were fed respectively with 160 and 130 kcal of metabolisable energy per kg of metabolic weight ($W^{0.75}$) during the last 80 days of gestation.

The results for 51 heifers which calved at the age of 2 were compared with those of 29 heifers calving for the first time at 3 years, and with those of 166 cows older than 3 years.

SAMPLING

Just before the first feed, during the three hours following birth, 50 ml was taken from the 4 mammary quarters and stored at -20°C . The number of samples taken was 43 in 1972, 80 in 1973, 47 in 1974, 88 in 1975, which is a total of 258 colostrums from 180 different cows. Samples were taken once from 128 cows. We were able to follow up 52 mothers for several years (32 were sampled twice, 14 three times and 6 four times).

CHEMICAL ANALYSES

After the samples have been thawed in a water-bath at 37°C , we prepared the colostrum sera by Coon's method (20 ml of colostrum + 0.2 ml of rennet + 0.1 ml of a 10% w/v solution of CaCl_2). The samples were shaken and brought to 40°C in the water-bath for 2 hours, centrifuged at 40,000 g for 20 min., and the supernatant was collected.

Total proteins (TP) were estimated by the biuret reaction using an auto-analyzer Technicon.

The gammaglobulins (γG) were separated by electrophoresis on cellulose acetate (cellogel) in a tris-veronal-Na buffer of pH 8.8 and ionic strength 0.05 for 40 min. under a 200 volts tension. Proteins were dyed with red ponceau S and the optical densities of the different fractions were measured using

a recording densitometer joined to an automatic integrator.

Electrophoresis of the colostrum wheys produced 4 peaks (albumin, β -lactoglobulins, α -lactalbumins, and γ -globulins). The peak of colostrum γ -G corresponds basically to the zone of migration of fast γ -G in blood serum (γ -G₁).

STATISTICAL ANALYSIS

1. In order to compare the γ -G concentrations of the colostrums with regard to calf survival, birth of twins, age of the mothers at calving, feeding towards the end of gestation period, state of health of the mothers or strength of the calves at birth, we calculated the mean and S.D. for the different groups, and the statistical significance of the differences was evaluated by Student's t test.

2. The statistical significances of the differences observed on the rate of mortality has been appreciated by the χ^2 test.

3. The repeatability of the γ -G content of colostrum throughout the successive calvings has been evaluated by estimating the intra-class coefficient of correlation r defined by:

$$r = \frac{\widehat{\text{Var I}}}{\widehat{\text{Var I}} + \widehat{\text{Var R}}}$$

where $\widehat{\text{Var I}}$, $\widehat{\text{Var R}}$ are the estimates of the components of the variance between individuals (Var I) and between repetitions of the measures for the same animal (Var R).

The analysis of variance aiming to estimate the components Var I and Var R has been conducted according to 2 models:

(a) the first corresponds to the simple model $X_{ij} = \mu + I_i + R_{ij}$ (i , index of individual; j , index of repetition) which takes into account the factor "individual" (I_i) and the intra-individual repetition (R_{ij});

(b) the second model also takes into account the variations linked to the years. The model is written thus:

$$X_{ij} = \mu + I_i + Y_j + R'_{ij}$$

where I_i is the effect of the i individual; Y_j is the effect of the j year; R'_{ij} is the residual effect.

Since we are dealing with no more than one data for each couple (i, j), the residual variance linked to the repetitions intra-individuals and years is estimated by the difference between the total sum of squares and the sum of squares due to the "individual" and "year" effects respectively.

4. The relationship between the γ -G content of the mothers' colostrum and those of their female offspring has been evaluated by the regression coefficient of the colostrum concentrations of the offspring over that of the mother, taking into account the particular structure of the data [Fig. 1].

The method of estimation used is an adaptation of the method described by Ollivier (1974), considering the variable of the mother as:

$$Z_i = \frac{q_i}{1 + (q_i - 1)r} (x_i - \tilde{x})$$

where q_i is the number of measures taken from the mother i , r the coefficient of repeatability of the measures, x_i, \tilde{x} the difference between the mean concentration of the mother i and the mean estimated concentration of the population \tilde{x} defined by:

$$\tilde{x} = \frac{\sum k_i x_i}{\sum k_i} \quad \text{with } k_i = \frac{q_i}{1 + (q_i - 1)r}$$

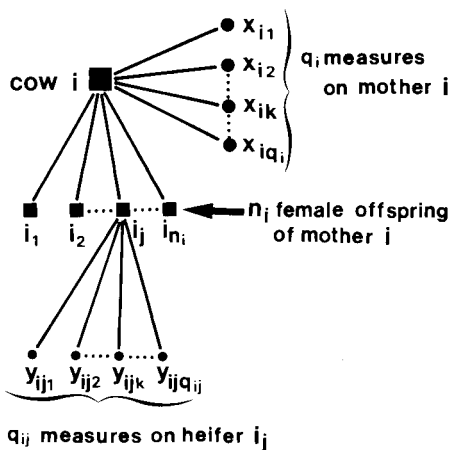


Fig. 1: Pattern of the structure of data on the relations between the cows and their female offspring.

Table 1: Mortality of the calves and levels of gammaglobulins in the colostrum (g/100 ml, X ± S.E.; between parentheses, above: number of animals, below: percentage of death).

Groups according to the number of calves per birth	Live calves	Total of dead calves	Calves dead from 0 to 2 days (a)	Calves dead from 2 days to 2 months	Calves dead after 2 months	Dead calves + live calves
(270) All calves	(204) 11.80 ± 0.25	(66) 10.29 ± 0.33(b)	(14) 9.56 ± 0.58(c) (10) 8.81 ± 0.85(d)	(44) 10.28 ± 0.42(e)	(8) 11.64 ± 0.85	
(246) Single calves	(191) 11.94 ± 0.26	(55) 10.37 ± 0.38 [22.4%]	(10) 9.99 ± 0.83 (7) 9.38 ± 1.12 [4.1%]	(37) 10.21 ± 0.48 [15.7%] (f)	(8) 11.64 ± 0.85 [3.5%]	(246) 11.59 ± 0.22
(24) Twin calves	(13) 9.78 ± 0.49(g)	(11) 9.89 ± 0.66 [46%] (i)	(4) 8.50 ± 1.20 [16.7%] (j)	(7) 10.68 ± 0.61 [35%] (k)	(0)	(24) 9.83 ± 0.39(h)

(a) Top line: total deaths from 0 to 2 days; bottom line: accidental deaths omitted.
 (b) P < 0.005; (c) P < 0.025; (d) P < 0.01; (e) P < 0.001 compared to colostrum received by all live calves.
 (f) P < 0.01; (g) P < 0.05 compared to colostrum of live single calves; (h) P < 0.025 compared to colostrum of all single calves.
 (i) P < 0.05; (j) P < 0.01; (k) P < 0.05 compared to corresponding mortality rates of single calves.

Just as in (3), the calculations can be done taking into account the variations liked to the year, or not taking them into account. When the year effect is considered, we shall use the means x_i corrected for the effects of the year estimated from the analysis of variance, and an estimation of the repeatability r obtained with this same model.

Results.

1. RELATIONSHIP BETWEEN CALF MORTALITY AND CONCENTRATION OF COLOSTRUM

γ -G levels in colostrum of cows whose calves died were compared with those whose calves survived [Table 1].

Colostrum produced by cows with dead calves contained significantly fewer γ -G than that produced by cows with living calves.

This results was very significant, both for the group of calves which died between 0 and 2 days, and which could not benefit from colostrum immunological protection, and for the group which died between 2 days and 2 months, mainly from diarrhoea and other infectious diseases. On the other hand, mothers of calves which died after 2 months had produced colostrum similar to that of mothers with living calves.

The results in Table 1, which resume the 258 colostrum samples collected after calving over a period of 4 years, suggest that the optimal level of γ -G in bovine colostrum-whey, for a good calf survival rate, is about 12 g/100 ml. Levels below 10 g/100 ml may be regarded as insufficient. In this particular

work, these samples represent 30% of the total.

2. INFLUENCE OF TWIN BIRTHS

The mortality rates observed for twin calves were significantly higher than those for single births particularly among the group of calves which died between 0 and 2 days (Table 1). Mothers of twins tend to produce less concentrated colostrum than cows with single calves; however, twin calves which died between 2 days and 2 months had not received poorer colostrum than single calves which die at the same age.

3. INFLUENCE OF THE AGE OF THE COW AT CALVING

Offspring of heifers calving for the first time at two years were compared to those of heifers at three years and those of cows.

Table 2 shows that the weights at birth of calves from 2 years-old heifers were lower than average for the breed, and significantly lower than those of calves of older mothers. Mortality rates of calves between 2 days and 2 months were similar for the three groups being considered. However, the mortality rate between 0 and 2 days was very much higher for the 2 year-old heifers. The 2 year-old heifers produced colostrum whose γ -G level was not appreciably lower than that of older mothers (Table 3).

4. INFLUENCE OF THE LATE GESTATION FEEDING LEVEL

The influence of a moderate (15%) restriction in feeding level on colostrum γ -G levels

Table 2: Influence of age of mothers calving on the death rate of their calves (number of animals dead during the considered period/number of animals alive at the beginning of the same period).

Age of the calves	2 years old heifers (I)	3 years old heifers (II)	Cows older than 3 years (III)	Statistical comparison of (I) vs (II+III)
From 0 to 2 days	6/51 (11.8%)	1/29 (3.5%)	3/116 (1.8%)	$P < 0.025$
From 2 days to 2 months	7/45 (15.6%)	6/28 (21.4%)	24/163 (14.7%)	N.S.
After 2 months	1/38	0/2	7/139	N.S.
All ages	14/51 (27.5%)	7/29 (24.1%)	34/166 (20.5%)	N.S.

The mothers of twin calves have been discarded, since their distribution was different in the three groups.

Table 3: Influence of the age of mothers at calving on concentration of gammaglobulins in colostrum (between parentheses: number of samples; results in g/100 ml; $\bar{X} \pm \text{S.E.}$).

Groups of mothers related to the behaviour of calves	2 years old heifers (I)	3 years old heifers (II)	Cows older than 3 years (III)	Statistical comparison of (I) vs (II)
All mothers	(51) 10.96 ± 0.34	(29) 12.42 ± 0.30	(166) 11.63 ± 0.27	N.S.
Mothers of live calves	(37) 11.02 ± 0.40	(22) 13.34 ± 0.94	(132) 11.96 ± 0.30	$P < 0.025$

Table 4: Influence of feeding level at the end of gestation (expressed in kcal per kg metabolic weight $W^{0.75}$) on concentration of gammaglobulins (in g/100 ml of colostrum; mean \pm S.E.) and the weight of calves at birth (kg).

Feeding level	Concentration of colostrums	Death rate of calves	Weight of calves at birth
160 (19 cows)	12.28 ± 0.64	3/19 (a)	41.3 ± 1.3
130 (18 cows)	13.18 ± 0.57	2/18 (b)	39.5 ± 1.4

(a) The 3 calves died before the age of 1 month.

(b) One abortion and one death at 17 days.

None difference was significant.

The mothers of twin calves have been discarded, since their distribution was different in the two groups.

Table 5: Relation between the state of health of the mother, the concentration of gammaglobulins in colostrum (in g/100 ml; mean \pm S.E.; between parentheses; number of samples) and mortality of calves (number of dead calves during the period/number of animals present at the beginning of the period).

Health status of the mother (a)	Concentration of colostrum in globulins	Still-born	Dead in first 24 hours	Dead from disease before 3 months	Total mortality
Healthy (b) (93 cows, 98 calves)	(70) 12.60 ± 0.40	4/98 (d)	2/94	5/92 (f)	11/98
Ill (c) (13 cows, 17 calves)	(10) 9.52 ± 0.61	3/17 (e)	4/14	5/10 (g)	12/17
Statistical significance	$P < 0.001$	$P < 0.25$	$P < 0.005$	$P < 0.005$	$P < 0.005$

(a) Were considered as "ill" all the cows having been examined by a veterinarian during a period of 15 days before and 15 days after calving (mainly for mastitis or metritis).

(b) 3 pairs of twins and one triple birth: no deads. (c) 4 pairs of twins; 7 deads/8 animals.

(d) 4 calves dead during calving. (e) 3 calves dead without difficult calving.

(f) 2 calves dead from diarrhoea before 1 month, 2 dead from enterotoxemia at 2 months, 1 dead from pasteurellosis at 3 months.

(g) 5 calves dead from diarrhoea before 1 month.

was nil [Table 4]. The mortality rate of the calves and their weight at birth were not significantly modified.

5. INFLUENCE OF THE COW'S STATE OF HEALTH

Colostrum γ -G levels were compared in healthy vs diseased cows [Table 5]. The diseased cows produced a poorer colostrum than the healthy cows, and the mortality rate of their calves was significantly higher, particularly for twins.

6. RELATIONSHIP BETWEEN THE STRENGTH OF CALVES AT BIRTH AND COLOSTRUM γ -G LEVELS

The average colostrum γ -G levels of 7 mothers whose calves were clinically weak

at birth was significantly lower than that of 66 mothers with normally strong offspring (respectively 9.91 ± 1.17 vs 12.25 ± 0.35 , $P < 0.05$).

7. REPEATABILITY OF γ -G LEVELS IN COLOSTRUM DURING SUCCESSIVE CALVINGS

7.1. Model (a) without consideration of the year effect.

The analysis was performed only on the group of cows supposed to have nothing in common (unidentified mothers). Among 62 cows, in which 135 analyses had been made, 49 cows were measured at least twice. Variance analysis is detailed in Table 6.

The estimate of repeatability was near 0.20, significantly different of zero ($P < 0.05$).

Table 6: Variance analysis of colostrum globulin levels, following the factors "individual" and "repetition of intra-individual measures".

Source of variation	Degrees of freedom	Observed mean square	Expected mean square	F
Between individuals (I)	61	14.7783	Var R+kVar I	1.535 (b)
Between measures intra-individual (R)	73	9.6252	Var R	

(a) k is the Kempthorne coefficient defined as:

$$k = \frac{1}{p-1} \left(q_0 - \sum_i \frac{q_i^2}{q_0} \right)$$

where p =number of individuals measured; q_i, q_0 =the number of measures performed respectively on individual i and on the whole population.

k is then a mean weighted number of measures for each individual; in the present case, $k=2.172$; $Va R=9.6252$; $Var I$ is then 2.3728.

(b) It is possible to compute $r=0.198$ with $\sigma r=0.112$, $P < 0.05$.

Table 7: Variance analysis of colostrum globulin levels, taking into account the factors "individual" and "year".

Source of variation	Degrees of freedom	Observed mean square	Expected mean square	F
Between years (A')	3	88.8239	—	14.27 $P < 0.01$
Between individuals (I')	61	15.39126	Var R'+k'Var I' (a)	2.47 $P < 0.01$
Residual (R')	70	6.2226	Var R'	

(a) $k'=2.1475$; $Var I'=4.2694$; $Var R'=6.2226$; $r'=Var I'/(Var I'+Var R')=0.407$ with $\sigma r=0.100$.

If the analysis was limited to the subgroup of 49 females whose γ -G level in colostrum had been measured at least twice, similar results were obtained (estimated repeatability $r=0.202$).

7.2. Model (b) taking into account the year effect.

The analysis was performed on the same group of 62 cows with 135 colostrum analysis on 4 years [Table 7].

When the influence of the "year" effect was deduced, there was a sensible increase in the estimated repeatability ($r=0.41$, $P<0.01$, vs 0.20 in the preceding model).

8. RELATION BETWEEN THE COWS AND THEIR FEMALE OFFSPRING

8.1. With no correction for the "year" effect.

This has been computed through the estimation of the weighted regression of the colostrum concentration of the 32 heifers over that of their mothers.

Considering a repeatability of 0.20, the estimated regression coefficient mother-spring was $\beta=0.290$; the S.D. was $\sigma\beta=0.108$. This coefficient is then significantly different of zero.

8.2. With correction for the "year" effect.

The correction applied was:

year 1972 =	-1.87
year 1973 =	+1.28
year 1974 =	-1.55
year 1975 =	+2.14

Considering an estimated repeatability of 0.41, the estimated regression coefficient was $\beta=0.239$ with a S.D. $\sigma\beta=0.137$.

The preceding conclusion remains valid, but it is preferable to consider this last coefficient β .

Discussion.

The results obtained confirm on the whole that variations in mortality and γ -G levels in maternal colostrum-when they are related. One factor is the quantity of γ -G in the colostrum offered to the calf at its first feed. How-

ever this factor, which affects the calf survival, does not take into account all the observations made in the course of this work.

Persistent hypo γ -globulinaemia in calves cannot be explained only in terms of insufficient quantity. Indeed, even poor colostrum contains a sufficient amount of γ -G to avoid the acute hypo γ -globulinaemia observed sometimes in calves which have been suckled early. One might try to explain these observations in terms of an insufficient supply of colostrum produced by the mother, which is the case for some heifers (Kruse, 1970), or for cows which are reared in particularly hard conditions (Logan, 1977). But in the herds studied here, no cases of low milk production were observed. Another possible explanation could be that the appetite of the calf is low, as has been observed by Selman *et al.* (1971). A defective digestive system leading to inadequate absorption of γ -G is also a possible explanation, or an increase in protein catabolism, but there is insufficient experimental evidence to support these last two hypotheses.

From the present results, it seems that for colostrum sampled within 3 hours after calving, a level equal to or greater than 12 g/100 ml can be considered adequate and a level lower than 10 g/100 ml is suspect. The range between normal and abnormal values appears to be relatively narrow. Thus the differences observed in colostrum composition do not appear to explain entirely the extent of the blood γ -G level variations in 48 hours-old calves (J. Dardillat, 1973).

Furthermore, the mortality rate observed between 0 and 2 days (abortions, still-births, dystocia, non-viable calves, calves crushed by their mother) also appears to be associated with the production of weak colostrum. Insufficient colostrum immunity cannot explain these mortalities, since the experimental calves entirely deprived of colostrum survived well beyond the age of 48 hours.

The twin calves have ingested colostrum significantly weaker in γ -G than have the single calves [Table 1]. This could be an explanation for the generally higher mortality rate observed for twins (Mauleon, 1974). But if we compare the dead calves only, the dead twin calves have had the same colostrum than single dead calves. Among the calves remained alive, the twins have had a very poor colostrum compared to the single calves.

These results led us to conclude that the relation between the γ -G concentration of colostrum and the rate of survival is not only due to the immunological protection afforded to the calves, but also to the fact that γ -G colostrum concentration reflects the physiological status of the mother, and therefore of the foetus.

It might be thought that the heifers which calve early at 2 years suffer, like the mothers of twins, from "physiological overburden" which leads both to a high offspring mortality and to the production of a deficient colostrum. It has in fact been observed that the colostrum is about normal and that the excessively high mortality rate is essentially connected with calving difficulties. Under the conditions in which the herd was reared, early calving at two years does not seem to affect the survival abilities of the calves, the higher death rate being due to difficult calving (Legendre, 1974) nor does it seem to affect the physiological condition of the mother. A 15% restriction in the late gestation feeding level did not affect maternal production in the short time either. The influence of feeding levels variations, within reasonable limits, is very difficult to demonstrate, both in mortality rate where results diverge (J. Dardillat *et al.*, 1969; Legendre, 1974) and in colostrum γ -G levels, where effects are generally nil. On the whole, up till now, the influence of general rearing conditions, open air or winter stabling (Logan, 1977) or calving season (Kruse, 1970) on colostrum γ -G levels has not been demonstrated. The large range of individual variations in colostrum levels found by different authors can explain this failure as well as the fact that it is easier to demonstrate the influence of individual physiological factors. The influence of breed has in fact been shown by Kruse (1970). In the present work,

we have observed that the state of health of the mother has a significant effect on the colostrum levels and the calves' mortality (diseased cows, which only represent 12% of the whole, were responsible for half of the mortality rate).

The repeatability from year to year of colostrum composition, joined to the fact that a poor colostrum seems to signify an inability to produce normally resistant offspring (even if a good colostrum pool is given) leads to envisaging the elimination of mothers with weak colostrum, particularly when they are in a precarious health state.

Our results suggest also that there is some heritability of colostrum γ -G concentration. Although this assumption is based on a limited number of animals, and that it could be biased by other factors (for instance infectious diseases common to mother and offspring) [Foulley and Ménéssier, 1974], experimental studies in mice (Biozzi *et al.*, 1970) give it some likelihood. More data are needed to state on its interest as a selection criterion.

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Summary.

Samples of colostrum were taken from 180 suckling cows (beef cows) from the same herd, during the 3 hours immediately after calving over a period of 4 consecutive years.

Total proteins in colostrum-whey were estimated by the biuret method, and concentrations of γ -globulins determined by electrophoresis.

The significance of the close relationship between calf mortality and γ -globulin levels in the maternal colostrum was studied. An attempt was made to define levels suitable for the health of the calves.

Twin birth, age at calving and food intake towards the end of the gestation period, what-

ever their effect on calf mortality rate, did not bring about any significant variation in the levels of colostral γ -globulins.

On the other hand, however, cows suffering from various infections had lower levels of colostral γ -globulins, and produced a higher calf mortality rate.

A high degree of repeatability in γ -globulin levels was observed in colostrums produced by the same cow during successive calvings.

A correlation between colostral γ -globulin levels of a particular cow and those of her female offspring was also found. This result, which should be controlled on a larger number of animals, suggests the possibility of an approach of the problem of calf mortality, using a sanitary and genetic selection designed to reduce the number of cows producing colostrum with low levels of γ -globulins.

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