The Nutritive Value of Colostrum for the Calf

4. The Effect of Small Quantities of Colostral Whey, Dialysed Whey and 'Immune Lactoglobulins'

By R. ASCHAFFENBURG, S. BARTLETT, S. K. KON, J. H. B. ROY and D. M. WALKER

National Institute for Research in Dairying, University of Reading

and C. BRIGGS and R. LOVELL

Research Institute in Animal Pathology, Royal Veterinary College, Camden Town, London, N.W.1

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In our experience (Aschaffenburg, Bartlett, Kon, Terry, Thompson, Walker, Briggs, Cotchin & Lovell, 1949; Aschaffenburg, Bartlett, Kon, Walker, Briggs, Cotchin & Lovell, 1949) the factor most essential for the survival of the newborn calf is contained in the non-fatty fraction of colostrum, of which a very small quantity is sufficient to protect the calf against fatal scours. To define this factor more closely, the protective values of small quantities of colostral whey, dialysed whey and the 'immune lactoglobulins' (as defined by Smith, 1946) were compared with that of the separated colostrum from which these fractions were derived.

METHODS

Plan of experiment

The experiment was in two parts: one (autumn 1948) for the estimation of the relative effects on newborn calves of colostral whey, dialysed whey and separated colostrum; the other (spring 1950) for the comparison of the 'immune lactoglobulins' with separated colostrum. In both, the randomized block design was adopted, and one calf deprived of colostrum was included in each block. The layout was as shown in Table 1. In both experiments, eight blocks of Shorthorn bull calves were used. The calves were assigned to treatments and to pens, selected at random within each block.

Table 1

Autumn 1948		Spring 1950	
Treatment no.	Colostral fraction in first feed	Treatment no.	Colostral fraction in first feed
0	None	0	None
5	150 ml. separated colostrum	8	200 ml. separated colostrum
6	150 ml. whey	9	*c.* 14 g. 'immune lactoglobulins' in 155 ml. solution
7	200 ml. dialysed whey		

Diets

Basic diet

The calves were kept on the 'synthetic milk' (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949) for 3 weeks, the maximum daily allowance being 1 lb./10 lb. live weight, except when scouring occurred (see below).

Colostral diets

The various colostral fractions were given to the calves in their first meal within 12 hr. of birth.

Separated colostrum for autumn 1948 *experiments.* Four batches of colostrum obtained within 24 hr. of calving from Shorthorn cows were used. Each was warmed and passed twice through an ordinary cream separator. Part of each batch was stored at $-25°$, the remainder was used for the preparation of whey.

Whey. The separated colostrum was treated with rennet at 37° (1 ml. rennet extract/l.), and the whey clarified by filtration through a filter pad and through no. 3 Whatman filter-paper. Part of the whey was stored at $-25°$, the remainder was dialysed.

Dialysed whey. The whey was dialysed at 4° for 36–48 hr. in a rotating cellophane bag against continuously changing distilled water. Any protein precipitate found after dialysis was redispersed with the minimal quantity of sodium chloride. The product was stored at $-25°$.

The four batches of the different fractions were blended in equal volumes for feeding to the calves. The amount given to each calf in its initial feed contained approximately 13 g. of whey proteins.

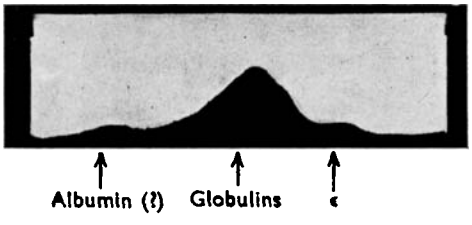

Fig. 1. Colostral globulins (batch no. 4). Descending boundary after electrophoresis for 165 min.

Separated colostrum for spring 1950 *experiments.* A similar procedure to that of the autumn 1948 experiment was adopted, and six batches instead of four were collected, this time whenever possible from the first milking only. If the quantity was insufficient, colostrum from the second milking was added. A portion of each batch was stored at $-25°$ and 'immune lactoglobulins' were prepared from the rest.

'*Immune lactoglobulins.*' The method used for the preparation was essentially that of Smith (1946), which is based on the removal of casein followed by dialysis of the proteins precipitated by 0·4 saturation with ammonium sulphate. The great dilution required for the removal of the casein by acid precipitation was avoided by removing it by rennet (2 ml./l. at 37°). Firm curds were produced more readily by adding calcium-chloride solution (300 mg. Ca/l. colostrum). The pH of the clear, filtered whey

was adjusted to about 6·0 with acetic acid. The precipitated proteins were separated by centrifugation, dispersed in the least amount of distilled water, and dialysed at 4° as for the whey but for 3–4 days. Dialysis was stopped when SO_4 ions were no longer detected by reaction with barium chloride. At this stage part of the globulins had precipitated out and was redispersed by the addition of sodium chloride. The product contained about 10 % protein and was stored at −25°.

Six batches were prepared, and some details of the composition of those used are given in Table 2.

Table 2. *Composition of four batches of protein fractions of colostrum used in the spring* 1950 *experiments*

	Separated colostrum			
Batch no.	Protein (g./100 ml.)	Casein as percentage of total protein	Globulin as percentage of total protein	'Immune lactoglobulins' as percentage of whey proteins
2	12·3	29·2	51·5	72·7
4	14·8	38·4	43·3	70·2
5	12·7	23·7	61·3	80·4
6	15·0	33·4	46·1	69·3

For the feeding of the experimental calves, the batches of separated colostrum or of the 'immune lactoglobulins' were blended. Calves received either 200 ml. of blended separated colostrum containing 27·4 g. of protein, of which *c.* 50 % consisted of globulins, or 155 ml. of blended lactoglobulins with a protein content of *c.* 14g.

Dr C. L. Hannay found that two of the batches of colostrum, nos. 4 and 5, had agglutinins against *Brucella abortus* to high titres, 1 : 2500 and 1 : 10,000 respectively. Similar titres were obtained when the 'immune lactoglobulins' were tested, and this suggests that the processes involved in the isolation of the globulins had not decreased the concentration of antibodies. It is assumed, but not yet proven, than any *Bacterium coli* antibodies present were also unimpaired.

A sample of the isolated whey globulins (batch no. 4) was equilibrated by dialysis against 0·055 M-sodium veronal-HCl buffer (Michaelis, 1930) of pH 8·42. After dilution with buffer to a protein content of 1 %, it was subjected by our colleague, Dr N. J. Berridge, to electrophoretic examination. Fig. 1 shows the conditions after migration for 165 min. at 10 mA. It indicates that the preparation consisted largely of globulins, though a faster moving component, probably albumin, was present. This was expected, as the centrifuged proteins precipitated by 0·4 saturation with ammonium sulphate had been dialysed without further purification.

Calves

Shorthorn bull calves were collected and managed as in earlier experiments (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949). On the arrival of a newborn calf a blood sample was taken and the globulin-turbidity test of Aschaffenburg (1949) was applied to the serum to verify that the calf had not suckled. Calves were rejected if the test was positive.

After the initial feed containing the selected fraction of colostrum all calves were fed three times daily for the first 10 days of life, and twice daily for the remainder of the period. When a calf scoured, one feed was omitted and 'synthetic milk' diluted with an equal amount of water was given at the next. The proportion of milk was gradually increased in subsequent meals until undiluted 'synthetic milk' was again given. This practice was repeated when scouring recurred. Records were kept as in the earlier experiment (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949).

RESULTS

Autumn 1948

The results for the eight blocks of Shorthorn bull calves (thirty-two animals) are summarized in Table 3.

Table 3. *Comparison of small amounts of whey and dialysed whey with separated colostrum*

Treatment no. ...	0	5	6	7
Diet given	No colostrum	150 ml. separated colostrum	150 ml. whey	200 ml. dialysed whey
Calves:				
No. used	8	8	8	8
No. died	8	3	3	3
Mean age of calves at death (days)*	4±1	10±2	11±2	7±1
Mean live-weight gain of surviving calves in 21 days (lb.)*	—	−2±3·5	−9±1·7	−9±1·9
Mean no. of days on which surviving calves scoured*	—	3±1	5±1	6±2

* Values with their standard errors of the mean. In calculating the standard errors the arrangement in blocks has been ignored.

All eight calves deprived of colostrum died, whereas only three died on each of the colostral treatments. The χ^2 test, after adjustment for continuity, shows that the difference in mortality between treatment 0 and treatments 5, 6 or 7 is significant ($P<0{\cdot}05$).

The statistical significance of differences between treatments in the mean live-weight gains of calves, and in the mean number of days on which the surviving calves scoured on treatments 5–7 was determined by the t test ('Student', 1908, 1925), P values below 0·05 being regarded as significant. There were no significant differences between the colostral treatments. To take out that part of the residual error due to differences between blocks, the missing plot technique of Yates (1933) was used, and values were calculated for the calves receiving the colostral fractions that did not survive the experimental period. Analysis of covariance of live-weight gain on birth weight and analysis of variance of the number of days on which scouring occurred (x), with the values transformed $\sqrt{(x+\frac{1}{2})}$, still gave no significant differences between the colostral treatments.

Spring 1950

The results for the eight blocks of Shorthorn bull calves (twenty-four animals) are summarized in Table 4.

Table 4. *Comparison of small amounts of 'immune lactoglobulins' with separated colostrum*

Treatment no.	0	8	9
Diet given	No colostrum	200 ml. separated colostrum	*c.* 14 g.* 'immune lactoglobulins'
Calves:			
No. used	8	8	8
No. died	8	3	3
Mean age of calves at death (days)†	6±2	9±2	7±1
Mean live-weight gain of surviving calves in 21 days (lb.)†	—	−3±2·6	+1±1·5
Mean no. of days on which surviving calves scoured†	—	9±2	7±1

* Contained in 155 ml. of solution.

† Values with their standard errors of the mean. In calculating the standard errors the arrangement in blocks has been ignored.

Again all eight calves deprived of colostrum died, whereas only three died on each of the colostral treatments.

Statistical analysis on the lines already described again gave a significant difference ($P<0{\cdot}05$) between the mortality rate of calves on treatment 0 and that of calves on treatments 8 or 9, but there were no significant differences between the two colostral treatments for the mean live-weight gain and the mean number of days on which the calves scoured.

Autopsy findings

A summary of the autopsy findings is given in Table 5. Of the thirty-one calves that died, twenty-two died from a *Bact. coli* septicaemia, and eight from *Bact. coli* peritonitis and pleurisy. These are manifestations of the disease classified as colibacillosis

Table 5. *Summary of autopsy findings*

Treatment no. ...	0	5	6	7	0	8	9
Diet given ...	No colostrum	Separated colostrum	Whey	Dialysed whey	No colostrum	Separated colostrum	'Immune lacto-globulins'
Total no. of calves taken for autopsy	8	3	3	3	8	3	3
Findings at autopsy:							
Colibacillosis:							
Fatal scours including septicaemia	8	3	2	2	5	2	—
Bact. coli peritonitis and pleurisy	—	—	—	1	3	1	3
Corynebacterium pyogenes pneumonia	—	—	1	—	—	—	—

or 'fatal scours'. In the peritonitis and pleurisy cases, *Bact. coli* was invariably isolated from the heart blood and bone marrow as well as from elsewhere.

DISCUSSION

These experiments, together with previous work (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949; Aschaffenburg, Bartlett, Kon, Walker *et al.* 1949), have shown that under our conditions, the survival of the newborn calf is dependent on a factor contained in the globulin fraction of colostrum. Comparison of mortality rates indicates that in the present experiments the small quantities of the colostral fractions gave less protection than the small quantities of separated colostrum given in an earlier experiment (Aschaffenburg, Bartlett, Kon, Walker *et al.* 1949). Neither prevented scouring, and with both weight gains were subnormal. The reasons for the subnormal growth of calves given small amounts of the protective fractions of colostrum will be studied in future work.

SUMMARY

1. Shorthorn bull calves, grouped in two experiments, each of eight blocks, were given separated colostrum, or fractions of it, in the first 12 hr. of life, followed for 3 weeks by a standard diet based on dried skim milk. One calf in each block was deprived of colostrum.

2. All calves deprived of colostrum died. Twenty-five of forty calves given small quantities of separated colostrum, whey, dialysed whey or 'immune lactoglobulins' survived. These diets did not, however, prevent the calves from scouring and the calves did not gain weight normally.

3. There was a significant difference between the mortality rate of calves deprived of colostrum and that of calves receiving the colostral fractions. There was no significant difference between the performances of the surviving calves that received the colostral fractions.

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