SOME FACTORS AFFECTING NEONATAL CALF SURVIVAL AND SUBSEQUENT GROWTH RATE

by

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DECLARATION

I declare that the work presented in this thesis has been carried out by me.

Some of the material in this thesis has already been published in the following paper:-

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SUMMARY

As in other ruminants, the transmission of passive immunity from cow to calf occurs via the colostrum in the period immediately after birth. However, work which had been carried out in the Department of Medicine of the University of Glasgow Veterinary School had shown that many calves passing through markets in the west of Scotland were hypogammaglobulinaemic and that as many as one-third of these hypogammaglobulinaemic calves were likely to die from either colisepticaemia or the effects of severe neonatal diarrhoea. Studies in the second decade of this century had shown that death was almost the inevitable sequel to colostrum deprivation, but as few farmers deliberately deprive calves of colostrum, investigations were instituted into the absorption of colostral gammaglobulins. It was shown that the absorption of the colostral gammaglobulins was related to the time of first feeding and to the concentration of gammaglobulins of the colostrum; any delay in feeding colostrum would decrease absorption of the gammaglobulins, and that absorption had ceased by 18.5 hours after birth; the absorption of colostral gammaglobulins was directly proportional to the gammaglobulin concentration of the colostrum. The present study was undertaken to examine systems of early post-natal management which would result in the acquisition of high serum concentrations of maternally-derived immunoglobulins and to examine the effect on the incidence of disease and growth rates during the first four weeks of life.

Calves were assisted to suckle colostrum to satiation as early as possible after birth. No difficulty was encountered encouraging calves to ingest a considerable weight of colostrum within an hour of birth. The quantity of colostrum varied, but was on average just over seven per cent of the calves' birth weight. For 25 calves, this intensive, immediate post-partum management regime resulted in the high serum concentrations of absorbed immunoglobulins with a mean of 27.24 ± 6.10 ZST units. No significant increase was obtained in the serum concentrations of absorbed immunoglobulins by permitting calves which had been fed to satiation immediately after birth to remain with their mothers for the first twelve hours of life and encouraging them to suckle again at twelve hours post-partum.

This regime of early, assisted suckling was extended to 100 calves and it was shown that a small proportion of calves (less than 5%) may remain severely hypogammaglobulinaemic despite ingesting an adequate quantity of colostrum. The ingestion of colostrum with very low whey immunoglobulin concentrations was responsible for this serum immunoglobulin deficiency. In the present study, loss of mammary secretions prior to parturition was the major cause of the low immunoglobulin concentration of the colostrum. The breeds of the calves which were used in this were predominantly Ayrshire, Friesian, or crosses between these two breeds, and reflected the main type and breeds of cattle in the west of Scotland. Using the data for the 100 calves assisted to suckle to satiation immediately after birth there was no significant difference in the absorption of colostral immunoglobulins between the different breeds and crosses. A small survey of calves passing through local markets revealed that over 40 per cent of the calves were severely hypogammaglobulinaemic, a proportion similar to that found in previous surveys.

Examination of the incidence of diarrhoea in calves, reared in groups of nine to fifteen, revealed that the incidence of diarrhoea was highest during the second week of life. No significant correlation could be demonstrated between the incidence of diarrhoea during the first four weeks of life and the liveweight gain over that period in seven of the eight groups of calves examined. Furthermore, there was no significant correlation between the incidence of diarrhoea during the first four weeks of life and the serum concentrations of absorbed immunoglobulins at 48 hours of age.

Eight of the 146 group-reared calves died; three individual calves and five calves, in the same group, of salmonellosis. The three individual calves which died were all severely hypogammaglobulinaemic (serum immunoglobulin concentrations of less than 10 ZST units); one calf died of a severe necrotizing pneumonia at five days of age; one calf died when only 36 hours old from the effects of very severe diarrhoea; the third calf, which had been intermittently profusely diarrhoeic from five days old died at 41 days of age. In one group of 15 calves, an outbreak of Salmonella enteritidis infection occurred. The organism was recovered from all 15 calves at some time during the outbreak, either from blood or rectal swabs. Five of the 15 calves

died; the youngest calf was only ten days old and the oldest 35 days old. The outstanding clinical features of this outbreak were pyrexia, inappetence, dullness and lethargy and weight-loss. Examination of rectal-swabs taken twice weekly revealed that the ten surviving calves rapidly cleared themselves of infection.

The behaviour patterns of one of the groups of calves which had free access to an automatic calf-feeder were studied. These studies revealed that calves with free access to an automatic calf-feeder consume much more milk-substitute than is normally offered under the traditional twice daily bucket-feeding systems with individual calves consuming as much as 16 litres per 24 hours. A highly significant positive correlation was found to exist between the mean daily consumption of milk and the liveweight gain.

The absorption of individual colostral immunoglobulins, IgG_1, IgG_2, IgM and IgA, their appearance in the lachrymal fluid and the subsequent changes during the first ten weeks of life were studied in ten calves. Very rapid absorption of colostral IgG_1 occurs when colostrum is fed soon after birth and significant concentrations of IgG_1 were detected in the serum two hours after feeding colostrum (three hours post-partum). The absorbed IgG_1 is quickly transferred from the intravascular compartment to the lachrymal secretions and appears to provide immunological protection until the local immunological cells begin to synthesise IgA at two to three weeks of age, by which time it exceeded the concentrations of both IgG_1 and IgM. The absorbed colostral IgA was only poorly transferred to the lachrymal fluid. The concentrations of IgG_2 in colostrum are extremely low and this was reflected in the low concentrations of this immunoglobulin in the serum of the calves. Throughout the first ten weeks of life the concentration of IgG_2 remained quite low.

As a result of this investigation it has been shown that it is possible to obtain high concentrations of passively transferred immunity in the newborn calf by managemental means in at least 95 per cent of calves, and that the absorbed colostral IgG_1 is rapidly transferred to at least one mucous surface.

CHAPTER 1

GENERAL INTRODUCTION AND REVIEW OF THE LITERATURE

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The clinical syndrome of profuse, watery diarrhoea in the neonatal calf which frequently results in death has been recognised for nearly two centuries. The first description of the disease appeared in "Artis Veterinariae Compendium Pathologicum" in 1799 and is attributed to Tolnay (Jensen, 1893). Knowlson (1834) discussed the White Flux and the Bloody Flux which occurred in calves and which was reputed to cause the death of approximately 25 per cent of all calves. Both conditions were much more severe in calves which were purchased at seven to ten days of age. In order to reduce those losses Knowlson advised farmers to avoid purchasing young calves but if this was necessary that they uplift the calves in their own transport. In the first half of the nineteenth century unfavourable climatic conditions and improper diet were considered important contributory factors to the disease. Dobson (1872) proposed that white-scour was a direct result of depriving calves of colostrum, although attributing the beneficial effect of the colostrum to its purgative action.

The early literature on this syndrome and its possible aetiology was reviewed by Jensen (1893). Obich (1865), Roloff (1875) and Franck (1887) are credited with proposing the possible infectious aetiology of the disease, and Obich even postulated that the infectious agent was disseminated in the diarrhoeic faeces. Other workers, such as Friedberger and Fröhner (1889) proposed that infection occurred during the birth process, gaining entry to the body via the mouth or the umbilical cord and Nocard (1886, 1902) considered that either intrauterine or umbilical infection occurred.

In 1893, Jensen (1893) reported an investigation into losses among calves on a large farm in Denmark on which nearly every newborn calf became affected by diarrhoea and on which the annual calf mortality was 50 per cent or higher. It was also Jensen (1893) who carried out the first bacteriological investigation and concluded that the disease was caused by members of the colon group of bacteria. These organisms

could be isolated regularly from the intestines, mesenteric lymph nodes, heart blood, spleen, liver and kidneys. Jensen believed that both food and bedding became contaminated by these bacteria, resulting in the subsequent infection of calves reared in this environment and claimed to be able to reproduce the disease by feeding cultures of these organisms to newborn calves. Later workers (Lovell and Hughes, 1935; Gibson, 1961) have raised the possibility that at least some of the organisms recovered by Jensen may have been members of the Salmonella group of organisms and not Escherichia coli. This association of Bacterium coli with the disease quickly led to the introduction of the term "Colibacillosis" to describe the syndrome (Poels, 1899; Van Es, 1910).

The estimations of calfhood mortality by authors such as Knowlson (1834) and Dobson (1872) were largely anecdotal with little factual basis but they indicate at least that losses could be very high. The first recorded survey of calf mortality in Britain was carried out in Scotland. Jordan (1933) reported an investigation carried out during 1931 and 1932 of 26 herds in Ayrshire, in which the mortality rate was 22 per cent. The survey was confined to heifer calves but unfortunately the author does not state the upper age limit of the survey. Smith (1934) reported a survey of 52 herds from the same area during the period 1931-34 and recorded a mortality rate of 20 per cent for dairy heifer calves of which "white scour" accounted for 40 per cent.

The first national survey of calf losses in Britain was carried out during the two year period from the beginning of 1936 to the end of 1937 and involved 355 herds in England and Wales and 47 in Scotland (Lovell and Hill, 1940). The majority of the herds in Scotland were again situated in Ayrshire. From the data supplied from the herds in England and Wales it was estimated that 5.5 per cent of all female calves died before six months of age, but in Scotland the mortality rate for calves up to six months of age was more than double that for England and Wales at 11.4 per cent. The majority of deaths occurred during the first two weeks of life.

Hector and Rowat (1948) reported the findings of a small but interesting survey of 84 herds in Dumfriesshire. The investigation

covered the period from November, 1946 to June, 1947 and it was found that 6.8 per cent of female calves died before reaching 60 days of age. The authors speculated that this mortality rate was lower than might have been anticipated from previous surveys as the farmers who cooperated in their survey were likely to be the most progressive.

Withers (1952-1953) reported a survey of 44 dairy, beef and dual-purpose herds located throughout Britain which covered a three year period from 1946 to 1949. The total number of live births on the survey farms during this period was 8720 and the annual mortality rate for female calves in England and Wales during the first six months of life was six per cent but again a considerably higher mortality rate of 11.1 per cent was recorded in the survey herds in Scotland. The annual mortality rate in beef and dual-purpose herds of 4.6 per cent was lower than that of dairy herds.

In 1962 and 1963 a survey was carried out by Leech, Macrae and Menzies (1968) which covered 1567 randomly selected farms throughout Britain and which was limited to those calves which were known to have consumed their first feed and continued until they were removed from the herd or until the end of the survey. The overall mortality rate of animals less than one year old was calculated to be 5.6 per cent and it was estimated that, during the survey year, 89,000 (2.6%) home-bred, live-born calves died within one month of being born and that 19,000 (2.4%) purchased calves died within one month of purchase. Leech and others (1968) also recorded that the mortality in south-west Scotland was more than one and a third times the national average.

Smaller, more recent surveys have continued to emphasise the regional variations in the mortality rate between south-west Scotland and England. Selman, de la Fuente, Fisher and McEwan (1971a) recorded a mortality rate of 11 per cent in dairy heifer calves during the first four weeks of life on 47 farms in the west of Scotland. Boyd (1972) surveyed 149 home-bred and 78 purchased calves on a calf rearing unit in Cheshire and recorded an overall mortality of 5.7 per cent up to four weeks of age.

Surveys of calf losses in other countries have yielded figures very similar to those recorded in Britain. In Denmark,

Ottosen (1959) estimated from the number of calves disposed of through rendering plants that the national annual calf mortality was approximately 10 per cent but recorded a mean annual mortality of 17 per cent in a survey of 211 herds. In Sweden, Jonsson and Swahn (1968) found that 2.3 per cent of 5698 calves, marketed at approximately 14 days of age through a cooperative died within four weeks of purchase. This figure is very similar to that recorded by Leech and others (1968) in Britain. Hurvell and Fey (1970) reported a similar survey of 3901 calves passing through the same cooperative and recorded a mortality of 2.5 per cent during the four weeks following purchase. In West Germany, Mayr, Kalich and Mehnert (1964) reported that the annual calf mortality rate was 10 per cent and, even at that time, this represented a loss to the agricultural industry of 57 million D marks per year.

In the United States, calf mortality has been estimated to be as high as 20 per cent in some localities (Reisinger, 1965; Amstutz, 1965). A survey of 77 dairy herds in Michigan by Oxender, Newman and Morrow (1973) revealed a calf mortality rate of 11.3 per cent between birth and 60 days of age. The authors conservatively estimated that the annual economic loss in the whole of the United States due to neonatal deaths was in excess of $ 200 million. A similar mortality rate of 10.5 per cent in calves up to six months of age was recorded in Utah (Wisniewski, Arave and Lamb, 1975). In a survey which included only the heifer calves in 247 Holstein herds in New York state, Hartman, Everett, Slack and Warner (1974) recorded a mean annual mortality rate as a result of disease between birth and three months of age of 11.8 per cent and that calves born dead or abnormal averaged 8.2 per cent to give an annual calf wastage of 20 per cent. In a similar study involving 16 dairy farms in northern California heifer calf mortality averaged 17-20 per cent between birth and three months of age (Martin, Schwabe and Franti, 1975).

In New Zealand, Cagienard (1973) stated that the calf mortality was probably high, without giving any precise figures.

All the surveys carried out in Britain have clearly demonstrated a seasonal pattern of calf mortality and that this seasonal incidence was much more marked in Scotland, and especially

south-west Scotland (Lovell and Hill, 1940; Withers, 1952c). Both Jordan (1933) and Smith (1934) recorded that the mortality rate during the late winter housing period was approximately 25 per cent, but during the autumn months it dropped to eight per cent, and Leech and others (1968) found that the mortality rate for calves born during the period May to September was 1.6 per cent compared to 5.4 per cent for calves born during January and February. Several studies have indicated breed variations in susceptibility to neonatal disease and death. Calves of the Channel Island breeds, i.e. Jersey and Guernsey calves, are reputed to have the highest mortality rates (Blakemore, Davies, Eylenberg, Moore, Sellers and Worden, 1948; Withers, 1952c; Leech and others, 1968; Oxender and others, 1973; Larouche and Black, 1973). In their farm survey Selman and others (1971a) recorded that the mortality rate for Ayrshire heifer calves was 14 per cent, but that for Friesian and Ayrshire cross Friesian calves was only six per cent. Both Withers (1952c) and Leech and others (1968) emphasised that regional differences in mortality rates might well reflect regional differences in husbandry practices and Selman and others (1971a) also suggested that different managemental methods could partially explain the apparent breed differences in their survey.

The major managemental factors which appear to influence a calf's ability to survive the neonatal period are the place of birth and the method of feeding colostrum. Calves born in byres have a much higher mortality rate than calves born in loose-boxes, which in turn have a higher mortality rate than calves born in fields (Leech and others, 1968; Selman and others, 1971a). Those calves which receive their first feed of colostrum by suckling their dam have lower mortality rates than calves which receive their first feed of colostrum by bucket or receive no colostrum at all (Lovell and Hill, 1940; Withers, 1952c; Leech and others, 1968). It has also been shown that calves which are fed colostrum within six hours of birth have a significantly lower mortality rate than those calves which did not receive colostrum until later (Oxender and others, 1973). Hartman and others (1974) found that in herds where the wife of the owner fed the calves the number of calves dying between birth and three months of age was 6.3 per cent. In herds where either the children, or the owner or manager, fed the calves the mortality rate was 8.4 per cent and 8.8 per cent respectively, but in herds where an employee fed the

calves, the mortality rate was 11.7 per cent.

During a major investigation to study the effects of different dietary regimes on the growth of young calves, Roy, Palmer, Shillam, Ingram and Wood (1955a) found that there was a positive correlation between the mortality rate of colostrum-fed calves and the length of time that a calf-house was occupied. Under their experimental conditions, the mortality rate of colostrum-deprived calves was consistently high and was independent of the length of time that the calf-house was occupied. It has also been shown that the mortality rate among the calves tends to increase with the increasing size of the adult cow herd (Leech and others, 1968; Oxender and others, 1973; Speicher and Hepp, 1973). Hartman and others (1974) found that the annual calf losses, including calves born dead, for herds under 100 cows, 100 to 200 and more than 200 cows averaged 15.8 per cent, 19.3 per cent and 27.2 per cent respectively.

The earliest national survey of calf mortality which was carried out in Britain indicated that calves were at greatest risk during the first four weeks of life and especially during the first two weeks (Lovell and Hill, 1940). The later surveys confirmed and re-emphasised this finding. Withers (1952b) estimated that of those calves which died during the first six months of life, 48 per cent died in the first week, 66 per cent in the first two weeks and 74 per cent in the first four weeks of life. The survey of Leech and others (1968) included animals up to one year of age, and they deduced that of those home-bred animals which died during the first year, 30 per cent died during the first week of life and 55 per cent died during the first four weeks of life. Other, more limited, surveys have also confirmed the first two weeks of life are the period of greatest mortality (Hector and Rowat, 1948; Oxender and others, 1973; Larouche and Black, 1973; Martin and others, 1975).

Every survey into neonatal calf mortality carried out in Britain, whether local or national, has stressed that the most important causes of this mortality have been diarrhoea, and septicaemia (Jordan 1933; Smith 1934; Lovell and Hill, 1940; Hector and Rowat, 1948; Withers, 1952b; Sellers, Smith and Wood, 1968). Following the initial association of Escherichia coli with the diarrhoea/septicaemia

syndrome by Jensen (1893), other workers confirmed this finding (Smith and Orcutt, 1925; Smith and Little, 1927; Lovell and Hughes, 1935; Briggs, 1951; Rees, 1958). There was, however, little if any attempt to distinguish the two syndromes clinically, bacteriologically or pathologically.

Gay (1965) suggested that the all-embracing diagnosis of "colibacillosis" should be divided into three syndromes on clinical and bacteriological grounds; colisepticaemia, a syndrome in which E. coli can be recovered from the internal organs and two diarrhoeic syndromes; enteric colibacillosis and enteric toxaemia, in which E. coli can only be isolated from the intestinal tract or associated mesenteric nodes.

Colisepticaemia occurs in neonatal calves which are immunoglobulin-deficient (Fey and Margadant, 1961; Gay, Anderson, Fisher and McEwan, 1965a) and rapidly results in the death of affected calves. The major clinical signs are depression, inappetence, fever, weakness and recumbency. Diarrhoea, if present at all, is scanty and a terminal event. Painful enlargement of joints and signs of meningitis are also occasionally seen (Smith, 1962; Gay, 1965; Smith and Halls, 1968a; Logan and Penhale, 1971a). Colisepticaemia is strictly time limited and rarely occurs after seven days of age (Larouche and Black, 1973). The major pathological findings are widespread petechial haemorrhages of the lungs, trachea, myocardium thymus, spleen and subendocardium. Splenic enlargement and distension of the joint capsules are also frequent post-mortem findings (Osborne, 1967b; Wray and Thomlinson, 1972). Pure cultures of E. coli can be isolated from the blood and internal organs (Smith and Halls, 1968a; Wray and Thomlinson, 1974). The serotypes of E. coli recovered from cases of colisepticaemia are those that grow rapidly in foetal calf serum (Smith, 1962) and many of the serotypes of E. coli recovered from cases of colisepticaemia in Canada, Europe and Britain are identical (Gay, 1965). It is considered that the signs and lesions of colisepticaemia are mediated by endotoxin released by the invasive E. coli (Fisher and Martinez, 1975a; Wray and Thomlinson, 1972).

The serological classification of E. coli is based on three main antigens, the somatic, or O antigen, the capsular, or K antigen

and the flagellar, or H antigen (Gay, 1965). The presence of the K antigens prevents expression of the O antigens and must be destroyed by heating before the O antigens can be agglutinated by homologous O antisera. Variation in the heat lability of the K antigen has permitted further differentiation of E. coli serotypes. There are three major classes of K antigens; the L-type K antigens are destroyed by heating at 100°C for one hour; the B-type K antigens are also destroyed by heating at 100°C for one hour, but differ from L-type K antigens in retaining the ability to combine with homologous K antisera; the A-type K antigens require to be maintained at 121°C for two and a half hours before agglutination with homologous O antisera occurs. The flagellar antigens are not very common.

Enteric colibacillosis was the term introduced by Gay (1965) to describe the syndrome in young calves of profuse diarrhoea of variable severity which occurs in the absence of an E. coli septicaemia (Gay, Anderson, Fisher and McEwan, 1965a; Fisher, Selman, McEwan and de la Fuente, 1968; Logan and Penhale, 1971a). The eventual fate of affected calves depends on the degree of physiological changes. Clinically, the syndrome is characterised by depression, profuse, yellowish-green diarrhoea of variable duration, but usually more than three days. Unlike calves with septicaemia, diarrhoeic calves frequently retain the desire to consume milk and do not become recumbent until shortly before death (Logan and Penhale, 1971a). Marked oliguria or even anuria is frequently present (Logan and Penhale, 1971a; Fisher and de la Fuente, 1972; Fisher and Martinez, 1975b). In calves affected by severe diarrhoea, weight loss is one of the outstanding clinical features, and is often accompanied by the development of sunken eyes (Blaxter and Wood, 1953; Roy and others, 1955a; Smith, 1962; Logan and Penhale, 1972; Fisher and Martinez, 1975b). Enteric colibacillosis is rarely seen after three weeks of age (Aschaffenburg, Bartlett, Kon, Terry, Thompson, Walker, Briggs, Cotchin and Lovell, 1949a; Larouche and Black, 1973).

A considerable number of detailed physiological and biochemical investigations have been carried out on calves affected by spontaneous enteric colibacillosis (Blaxter and Wood, 1953; McSherry and Grinyer, 1954; Roy, Shillam, Hawkins, Lang and Ingram, 1959; Dalton, Fisher and McIntyre, 1965; Fisher, 1965; Fisher and McEwan, 1967; Fayet, 1968; Tennant, Harrold and Reina-Guerra, 1972; Fisher

and de la Fuente, 1972; Logan and Penhale, 1972; Fisher and Martinez, 1975b). From these studies several major findings have emerged; calves with marked neonatal diarrhoea have a metabolic acidosis, a hyperkalaemia and a uraemia; they also have negative potassium and sodium balances but the plasma concentrations of sodium in diarrhoeic calves may or may not differ significantly from those of normal calves; diarrhoeic calves have significantly lower plasma concentrations of bicarbonate when compared with non-diarrhoeic controls.

Several studies have shown that marked increases in the packed cell volume can occur in calves affected by severe diarrhoea (Philips, Lewis and Knox, 1971; Fisher and de la Fuente, 1972; Logan and Penhale, 1972; Tennant and others, 1972; Boyd, Baker and Leyland, 1974). However Fisher and Martinez (1975b) found that there was no significant difference in the packed cell volume or plasma volume between healthy non-diarrhoeic calves and diarrhoeic calves. The same workers suggested that the plasma volume was maintained at the expense of fluid loss from the extravascular pool as it was shown that, in calves which died of diarrhoea, 83.7 per cent of the fluid loss came from the extravascular pool whereas only 16.3 per cent was lost from the intravascular pool. It was also demonstrated that, when fluid intakes were maintained, there was no significant difference in total fluid output between calves which died of diarrhoea and calves which survived diarrhoea or remained healthy. However, the urinary output of calves which died of diarrhoea was significantly lower than that of calves which survived diarrhoea or calves which remained normal.

As moribund diarrhoeic calves frequently have bradycardia and cardiac arrythmias can be detected electrocardiographically, primary heart failure is considered to be the immediate cause of death in calves suffering from enteric colibacillosis (Fisher, 1965). It has been shown that acidotic calves, whether induced by spontaneous, profuse diarrhoea or by the intravenous infusion of hydrochloric acid, have significantly lower concentrations of myocardial potassium than normal calves (Fisher and McEwan, 1967). These findings led Fisher and McEwan (1967) to suggest that the metabolic acidosis induced by the electrolyte disturbances as a result of continuous profuse diarrhoea caused a withdrawal of intracellular potassium ions from the myocardial cells and that this myocardial potassium depletion interfered

with cardiac depolarisation.

The pathology of enteric colibacillosis is confused. McEwan (1950) and Glantz and Kradel (1967) stated that the main pathological lesion was a gastroenteritis, but Smith (1962) was unable to detect any gross pathological differences between calves which had diarrhoea and those which remained healthy. Osborne (1967a) described a severe haemorrhagic enteritis with alternate hyperaemic and ischaemic segments of the small intestine in experimental colibacillosis induced by feeding an inoculum of five different E. coli serotypes. Osborne (1967a) suggested that the fundamental lesion in both colisepticaemia and enteric colibacillosis was a widespread thromboembolism of the microvasculature as a result of disseminated intravascular coagulation.

Gay, McKay and Barnum (1964b) described a syndrome in which calves, less than one week old and often within 48 hours of birth, developed a profuse, watery diarrhoea; became extremely depressed and rapidly prostrate. Terminally, affected calves had a weak, irregular pulse and shallow infrequent respiratory movements. Death supervened within 24 hours of onset. Bacteriological examination failed to reveal a septicaemia, but examination of the intestinal tract yielded heavy growths of pure cultures of mucoid E. coli with A-type K antigens in the upper and middle small intestine. The majority of strains belonged to three serotypes O9:K(PS274), O9:K(RVC118) and O101:K(RVC118). No pathological details were given and attempts to reproduce the syndrome experimentally were unsuccessful (Gay and others, 1964c). The name enteric toxaemia was used to describe this form of colibacillosis, as Gay (1965) considered that the non-invasive E. coli which proliferated in the anterior small intestine produced a toxin which was absorbed into the blood stream. Approximately 40 years earlier, Smith and Orcutt (1925) had reported the isolation of mucoid E. coli from the upper intestine of diarrhoeic calves, and Gay (1965) suggested that syndrome described by Gay and others (1964b) was identical to the "isocolibacillosis" described in Europe (Wramby, 1948; Ottosen, 1959).

Shortly after Gay and others (1964b) had described the enteric toxaemic syndrome of colibacillosis, Smith and Halls (1967a) reported that seven of 127 strains of E. coli which had been associated with

outbreaks of diarrhoea in calves were capable, on infusion into isolated segments of calf intestine, of producing a marked accumulation of fluid within the segments. Six of the seven positive strains could be serotyped into three groups, 09:K9, 0101:K? and 08:K? and had been isolated from calves in Belgium, Canada, Holland and the U.S.A. The seventh untypable strain, isolated in Britain, was associated with an outbreak of profuse diarrhoea and high mortality in young calves. None of 14 strains of E. coli isolated from cases of colisepticaemia produced dilatation of isolated intestinal loops. There was no difference in viability within the intestinal loops between those strains of E. coli which produced dilatation and those which did not.

Furthermore, Smith and Halls (1967a) demonstrated that oral inoculation of colostrum-fed calves less than 20 hours old with approximately 10^{10} viable organisms produced a syndrome of depression and profuse, watery diarrhoea within 9-22 hours of dosing. Many of the affected calves were moribund within 16 hours of the onset of clinical signs. Post-mortem examination of the intestinal tract revealed that abnormally high numbers of the inoculated strain were found in the anterior small intestine. No E. coli organisms were isolated from the spleen or liver. Further studies indicated that the dilating substance, or enterotoxin, was present in cell-free culture filtrates (Smith and Halls, 1967b). From these experiments, Smith and Halls (1967a) concluded that for strains of E. coli to be enteropathogenic they must produce enterotoxin and be capable of proliferation in the anterior small intestine.

Examination of porcine enteropathogenic E. coli strains revealed the existence of two different enterotoxins, a heat-labile and antigenic enterotoxin and a relatively heat-stable and apparently non-antigenic enterotoxin (Smith and Gyles, 1970a). It was considered that bovine enteropathogenic strains of E. coli produced only a heat-stable enterotoxin (Smith and Gyles, 1970b; Smith and Linggood, 1972; Moon, Whipp and Skarvedt, 1976) but recent work has shown that at least some bovine strains of enteropathogenic E. coli are capable of producing heat-labile enterotoxin (Ellis and Keinholz, 1977). The potentiality to produce enterotoxin is related to the presence of a "common K antigen" K99 which is plasmid controlled, and which is considered to facilitate adhesion by the enteropathogenic strains of

E. coli to the intestinal epithelium (Smith and Linggood, 1972; Orskov, Orskov, Smith and Sojka, 1975; Sivaswamy and Gyles, 1976b). However, not all enteropathogenic strains of E. coli possess the K99 antigen (Myers and Guinee, 1976; Moon and others, 1976).

Both types of enterotoxin produced by enteropathogenic E. coli alter fluid and electrolyte transport in the small intestine (Hamilton, Roe and Nielsen, 1977; Sack, 1975). The heat-stable enterotoxin consists of two distinct toxins of low molecular weight (1000-10,000) which have a rapid onset of action of short duration (Bywater, 1972; Sack, 1975; Burgess, Bywater, Cowley, Mullan and Newsome, 1978) and it is thought that the heat-stable enterotoxin induces intestinal secretion by increasing intestinal cyclic guanosine 5'-monophosphate concentrations (Newsome, Burgess and Mullan, 1978). This results in a net increase in water secretion into the intestinal tract (Bywater, 1973, 1975). The heat-labile enterotoxin has a high molecular weight (100,000), but has a relatively slower onset and longer duration of action than the heat-stable enterotoxin (Sack, 1975). The mode of action of heat-labile enterotoxin is identical to V. cholerae enterotoxin, stimulating the intra-epithelial adenyl cyclase/cyclic adenosine 5-monophosphate system within the small intestinal epithelial cells which results in electrolyte secretion, mainly sodium and bicarbonate ions, and water loss into the small intestine (Evans, Chen, Curlin and Evans, 1972; Guerrant, Ganguly, Casper, Moore, Pierce and Carpenter, 1973). Although it is identical to V. cholerae enterotoxin, a much larger quantity of E. coli heat-labile enterotoxin is required to produce a similar response (Sack, 1975). In one of the few studies into the physiological effects of diarrhoea induced by viable enteropathogenic E. coli organisms in the intact calf it was shown that net losses of water, sodium, chloride and potassium occurred in the distal small intestine with a compensatory but insufficient increase in water and sodium absorption in the spiral colon. There was no significant difference in the bicarbonate movement between diarrhoeic calves and normal controls (Bywater and Logan, 1974).

Logan and Penhale (1971b) reported the isolation of four enteropathogenic strains of E. coli from young calves which had been purchased at three to seven days old through two markets in the west of Scotland and which had later died of acute diarrhoea. Subsequently

they reported the experimental reproduction of the syndrome by the oral inoculation with one of these strains (Logan and Penhale, 1972). Five calves were inoculated between 39 and 48 hours of age and eight calves were less than 24 hours when challenged. All 13 calves developed severe diarrhoea within 24 hours of challenge, with weight loss, sunken eyes and oliguria as other significant clinical signs. Eleven of the 13 calves died between two and four days of being challenged. The two calves which survived were inoculated between 39 and 48 hours of age. A marked uraemia, hyperkalemia and increase in the packed cell volume were also noted. All 13 calves were colostrum-deprived but had been given an IgM preparation intravenously to prevent colisepticaemia (Penhale, Logan and Stenhouse, 1971).

The pathology of the experimentally induced enteric toxaemia has also been described (Logan, Pearson and McNulty, 1977; Pearson, McNulty and Logan, 1978a). The lesions were confined to the small intestine, from the middle to the distal third. The intestinal villi were stunted and thickened and on all villi the epithelial cells were cuboidal to columnar in structure. Occasionally, epithelial cells had been lost from the tips of a few individual villi. An increased number of neutrophil leucocytes and reticular cells were present in the lamina propria. Immunofluorescent studies revealed that adhesion of the challenge strain of E. coli was confined to the middle and distal small intestine and organisms were only found in the anterior small intestine of those calves which actually died (Logan and others, 1977).

Smith and Halls (1967a) demonstrated that only a proportion of the strains of E. coli isolated from young calves with diarrhoea is enteropathogenic and this has been confirmed in several studies. In Belgium, El Nageh (1970) and Schoenaers and Kaeckenbeeck (1973) found that seven to ten per cent of strains of E. coli isolated from diarrhoeic calves were enteropathogenic based upon their ability to cause dilatation of calf isolated intestinal loops. In Switzerland, Corboz and Becker (1973) examined 35 strains of E. coli isolated from calves less than two weeks old which had died of non-septicaemic diarrhoea and found that 14 per cent were enteropathogenic. In a study of 1004 isolates of E. coli from outbreaks of calf diarrhoea in seven states in the United States only 124 were enteropathogenic, but enteropathogenic strains of E. coli were isolated from calves in one third of the herds examined in one of the states in the survey (Myers, 1975; Myers and

Guinee, 1976). Moon and others (1976) found that 13 per cent of strains of E. coli isolated from calves in Minnesota and Montana were enteropathogenic but that these enteropathogenic strains were isolated in 24 per cent of the herds examined. In Canada, 27.5 per cent of strains of E. coli recovered from diarrhoeic calves less than two weeks old were enteropathogenic but, quite remarkably, of 100 strains isolated from non-diarrhoeic calves only one was found to be enteropathogenic (Sivaswamy and Gyles, 1976a).

Although it has now been shown that certain strains of E. coli are enteropathogenic and oral inoculation with these strains can reproduce a syndrome of diarrhoea, depression and death, Gay (1965) in his review of the role of E. coli in neonatal calf disease had concluded that there was little scientific evidence to confirm the ability of E. coli to produce diarrhoea in the young calf. Earlier, Smith (1962) had also questioned the significance of E. coli in the diarrhoeic syndrome and Dalton, Fisher and McIntyre (1960) stated that "it was impossible to eliminate the possibility of a virus such as that of mucosal disease in some cases of diarrhoea". The early reports of viruses associated with calf diarrhoea were unconfirmed (Baker, 1943; Light and Hodes, 1943), but in 1956, Brandly and McClurkin (1956) reported the recovery of a virus from two to three day old calves with diarrhoea and pneumonia in Wisconsin and the reproduction of a typical diarrhoea syndrome in colostrum-deprived calves. This virus has now been classified as an enterovirus (McClurkin, 1977).

The possibility that viruses might be implicated in the aetiology of neonatal calf diarrhoea received further impetus with the isolation of a virus, later classed as a reo-like virus, from range calves suffering from diarrhoea in Nebraska (Mebus, Underdahl, Rhodes and Twiehaus, 1969; Welch, 1971; Fernelius, Ritchie, Classick, Norman and Mebus, 1972). Following this report several other viruses have been isolated from calves with diarrhoea and these are listed in Table 1. Other microbiological agents which have also been isolated from calves with diarrhoea include Providencia stuartii (Waldhalm, Meinershagen and Frank, 1969); Chlamydia organisms (Storz, Collier, Eugster and Altera, 1971); Citrobacter (Acres, Laing, Saunders and Radostits, 1975); Cryptosporidia (Meuten, Van Kruiningen and Lein, 1974; Morin,

TABLE 1

Viruses associated with neonatal calf diarrhoea

Virus	Year Reported	Country	Ability to reproduce diarrhoea in calves	Reference
Rotavirus	1969	U.S.A.	+	Mebus and others (1969)
	1973	Australia		Turner and others (1973)
	1974	Canada		Morin and others (1974)
	1974	Britain		Woode and others (1974)
	1975	Belgium (Serology)		Zygraich and others (1975)
	1976	France		Scherrer and others (1976)
Coronavirus	1972	U.S.A.	+	Stair and others (1972)
	1974	Canada		Morin and others (1974)
	1975	Canada		Acres and others (1975)
	1975	Belgium (Serology)		Zygraich and others (1975)
	1978	Britain		Bridger and others (1978)
	1978	Denmark		Bridger and others (1978)
Parvovirus	1973	U.S.A.	+	Storz and Bates (1973)
Enterovirus	1974	U.S.A.	-	Dunne and others (1974)
Astrovirus	1978	Britain	-	Woode and Bridger (1978)
Calicivirus	1978	Britain	+	Woode and Bridger (1978)
Small cubic virus	1978	Britain	-	Almeida and others (1978)
Villous epithelial cell syncitia inducing virus	1978	U.S.A.	+	Mebus and others (1978)

Lariviere and Lallier, 1976); and Mycoplasma-like particles (McNulty, Curran and McFerran, 1975).

As the virus isolated by Mebus and others (1969) had a diameter of 60-66nm and resembled reoviruses 1, 2 and 3 and the bluetongue virus the authors described it as a reo-like virus. It was placed in the Diplornavirus group of viruses and classified as a rotavirus (Flewett, Bryden, Davies, Woode, Bridger and Derrick, 1974). Given orally to colostrum-deprived or gnotobiotic calves, diarrhoea and depression developed within 18 hours of challenge but the calves recovered spontaneously within three to four days. Diarrhoea was apparently more severe when E. coli were also present in the intestinal tract (Mebus and others, 1969). Pathologically, the lesions, stunting and thickening of the villi, are found mainly in the proximal and middle small intestine. The epithelial cells on the villi are mainly columnar to cuboidal (Mebus and others, 1971; Pearson and others, 1978b). The denudation of villi described in rotavirus infections by Mebus and others (1971) was considered to be a post-mortem artefact by Pearson and others (1978b). Rotavirus infection is thought to interfere with the glucose-coupled sodium transport and enzyme mechanisms in the small intestine (Davidson, Gall, Petric, Butler and Hamilton, 1977).

In 1972, Mebus, White, Stair, Rhodes and Twiehaus (1972) demonstrated a corona-like virus on electron-microscopical examination of diarrhoeic faeces of range calves aged five - 21 days. The physico-chemical characteristics of this corona virus have been described (Sharpee, Mebus and Bass, 1976). Experimentally, oral or intranasal inoculation of gnotobiotic or colostrum-deprived calves has resulted in diarrhoea which persisted for up to five days after an incubation period of one to five days (Mebus, Stair, Rhodes and Twiehaus, 1973a; Bridger, Woode and Meyling, 1978). Pathologically, the coronavirus could be detected by immunofluorescence on the villous epithelium of the upper, middle and lower small intestine and in both the superficial and crypt epithelium of the colon. Histological lesions were confined to the middle and lower small intestine and also the colon. In calves killed 42-48 hours after challenge the villi in both the middle and lower small intestine were shortened and the tall columnar epithelial cells were replaced by low cuboidal cells. The villous lamina propria in both areas was broader than in uninfected

controls with an increase in the number of reticulum-like cells and also congestion of the capillaries. In the spiral colon the normal tall columnar cells of the surface and crypt epithelium were replaced in areas by low cuboidal or low columnar epithelium. There was distortion of the crypt morphology and a marked reduction in the number of goblet cells within the crypts. In those calves killed at 72-96 hours after oral inoculation there was a marked recovery of villous and epithelial morphology at all levels of the intestine with very few viral particles detected by immunofluorescence (Mebus and others, 1973a; Doughri and Storz, 1977; Bridger and others, 1978).

Mebus and others (1973a) described the intestinal lesions of four colostrum-fed calves with serum neutralisation titres to coronavirus of 537, 646, 324 and 537, orally inoculated with coronavirus at four, five and thirteen days old. Although diarrhoea occurred, the lesions were much less severe than those found in similarly challenged gnotobiotic or colostrum-deprived calves, being confined to the lower small intestine and the colon. The villi were shortened and the villous epithelium was covered by low cuboidal cells. In the spiral colon the columnar epithelium of the crypts was replaced to a variable degree by cuboidal cells, but the surface epithelium retained its tall columnar structure. There were mild inflammatory changes in the colonic lamina propria with a slight infiltration of neutrophils and lymphocytes. Coronavirus was detected by immunofluorescence in the villous epithelial cells of the small intestine and the superficial and crypt epithelial cells of the colon.

In attempts to resolve this highly complex situation concerning the infectious aspect of neonatal calf diarrhoea, Acres, Laing, Saunders and Radostits (1975) examined the faeces from beef calves in a 48 cow herd at intervals up to ten days of age and Morin, Lariviere and Lallier (1976) examined 55 moribund or dead calves up to two weeks of age. Acres and others (1975) collected samples of faeces from 40 calves at one, five and ten days of age, or, as soon as possible after the onset of diarrhoea and the distribution of the enteropathogenic agents demonstrated in this survey is shown in Table 2. Thirty-two of the 40 calves became diarrhoeic before ten days of age and 11 of the 32 calves developed diarrhoea when only one day old. Eight of the

TABLE 2

Enteropathogenic agents demonstrated in the faeces of 32 diarrhoeic beef suckler calves

Infectious agent	Number of calves
* Enteropathogenic *E. coli* only	7
Rotavirus only	6
** Enteropathogenic E. coli + Rotavirus	4 (1 Normal calf)
Rotavirus + Coronavirus	2
Rotavirus + Bovine viral diarrhoea virus	1
Enteropathogenic *E. coli* + Rotavirus + Coronavirus	1
Citrobacter + Rotavirus + Bovine viral diarrhoea virus	1
No potentially enteropathogenic agent isolated	11

The following agents were not demonstrated in any of the calves or their dams:- *Salmonella* species; Infectious bovine rhinotracheitis virus; Parvovirus; Adenovirus; Parainfluenza 3 virus; *Chlamydia* species.

* Capable of causing dilatation of calf isolated intestinal loops.

** Enteropathogenic *E. coli* isolated prior to rotavirus isolation.

Ref. Acres and others, 1975.

11 calves which became diarrhoeic at one day old were excreting enteropathogenic E. coli or a Citrobacter organism when sampled at 32 hours of age. Nineteen of the 64 (30%) strains of E. coli recovered from diarrhoeic calves were enteropathogenic but only one of the 72 (1.5%) strains of E. coli isolated with calves with normal faeces were enteropathogenic. No enteropathogenic strains of E. coli were isolated from calves more than seven days old. Calves from which enteropathogenic E. coli only were isolated, were diarrhoeic for an average of 3.4 days. None of the eight strains of E. coli isolated from the adult cows was enteropathogenic.

Rotavirus was isolated mainly from calves between five and ten days old. Those six calves from which rotavirus only was isolated were diarrhoeic for an average of 5.2 days. In calves from which both enteropathogenic E. coli and rotavirus were isolated, the enteropathogenic E. coli were isolated prior to the demonstration of rotavirus. Calves from which no enteropathogenic agents were isolated were diarrhoeic for an average of 2.3 days before ten days of age and were mainly between seven and ten days old when the diarrhoea commenced. Three of the 40 calves died, when five, 12 and 47 days old. Both enteropathogenic E. coli and rotavirus were isolated from the calf which died at five days; enteropathogenic E. coli only were isolated from the calf which died at 12 days of age and which had been diarrhoeic from one day old; rotavirus was isolated at seven days of age from the calf which died when 47 days old after bouts of intermittent diarrhoea. Although there was a good correlation between the isolation of enteropathogenic E. coli and the onset of diarrhoea, the authors concluded that "there was no consistent pattern of onset or duration of diarrhoea in calves which excreted different agents".

Of the 55 dairy and beef calves less than two weeks old, examined pathologically and microbiologically by Morin and others (1976) 34 were moribund and were destroyed immediately prior to examination. The other 21 had been dead for a variable time prior to examination. No specific diagnosis was possible for 20 calves, the majority of which had been submitted dead. The diagnoses presented for the remaining 35 calves are presented in Table 3. The six calves from which E. coli only were isolated were between two and seven days of age.

TABLE 3

Pathological/aetiological diagnoses of 55 diarrhoeic calves less than two weeks old.

Diagnosis	Number of calves
E. coli	6
Cryptosporidium	5
Reo-like virus	1
Corona-like virus	2
E. coli + reo-like virus	4
Reo-like virus + corona-like virus	5
Reo-like virus + corona-like virus + *Cryptosporidium*	3
Reo-like virus + *Cryptosporidium*	2
Reo-like virus + corona-like virus + I.B.R. virus	1
Corona-like virus + *Cryptosporidium*	1
Corona-like virus + mycotic abomasitis	1
Mycotic abomasitis	3
Mycotic rumenitis + Reticulitis	1
Undetermined (Incl. 19 calves submitted dead)	20

Ref: Morin and others (1976)

Enteropathogenic strains of E. coli, as judged by the dilatation of isolated intestinal loops, were isolated from only two calves. The majority of calves in which rotavirus or coronavirus were demonstrated were between five and 14 days of age. In this aspect both studies (Acres and others, 1975; Morin and others, 1976) differ from previous studies which suggested that rotavirus infection occurred mainly during the first week of life (Mebus and others, 1971; McNulty, McFerran, Bryson, Logan and Curran, 1976; McClurkin, 1977). One interesting feature reported by Morin and others (1976) was the finding of numerous coccidia of the genus Cryptosporidium on the microvillous border of the epithelial cells in the middle and lower small intestine of five calves. The villi in the affected areas were shorter than normal, but there was minimal damage to the epithelial cells which were either normal tall columnar or at worst low columnar. A cellular infiltration of the lamina propria was present. The ages of affected calves were between five and 15 days. Previous reports of Cryptosporidiosis associated with neonatal diarrhoea had been confined to one calf (Meuten and others, 1974; Schmitz and Smith, 1975).

In a survey of 35 beef herds in western Canada, Acres, Saunders and Radostits (1977) examined faecal samples from 181 diarrhoeic and non-diarrhoeic calves less than 30 days old and isolated enteropathogenic E. coli from 11 herds (31%), rotavirus from 13 herds (37%), and both agents from 5 herds (14.3%). The overall prevalence rates for both agents were found to be similar but 81 per cent of calves excreting enteropathogenic E. coli were less than five days old, although enteropathogenic E. coli were isolated from calves between one and 11 days old. On the other hand rotavirus was demonstrated in the faeces of calves between three and 30 days old. Rotavirus could be isolated from the faeces of diarrhoeic and non-diarrhoeic calves, but enteropathogenic E. coli were isolated only from diarrhoeic calves. No attempt was made to demonstrate coronavirus, but there was no evidence of Salmonella, infectious bovine rhinotracheitis, bovine virus diarrhoea or parainfluenza 3 virus infection.

It must already be apparent that colostrum, the first milk secreted following parturition, or "that creamy oily substance called beestings" (Dobson, 1872) plays an important role in neonatal disease.

In the surveys into neonatal calf mortality both the method by and the time at which the first feed of colostrum was given had a very significant influence on whether or not calves survived the neonatal period (Lovell and Hill, 1940; Withers, 1952c; Leech and others, 1968; Oxender and others, 1973). Also, in the possible role of individual infectious agents in the aetiology of the neonatal calf diarrhoea syndrome, importance was placed upon whether calves were colostrum-fed or colostrum-deprived.

As early as 1857, Livingstone (1857) observed that suckling calves appeared to be resistant to tsetse fly attack for the first few weeks of life and Chauveau (1888) observed that lambs born to ewes which had been vaccinated against anthrax during gestation were resistant to the disease. In 1897 Jensen (1897) reported that calves were more likely to die when fed boiled milk than when fed colostrum. However, the importance of colostrum was dramatically illustrated in an experiment reported by Smith and Little in 1922; one group of ten calves was fed colostrum at birth and a second group of 12 calves was fed only milk at birth i.e. colostrum-deprived; nine of the 12 colostrum-deprived calves died, whereas all ten of the colostrum-fed calves survived the first three weeks of life (Smith and Little, 1922a). Later, Aschaffenburg, Bartlett, Kon, Roy, Sears, Ingram, Lovell and Wood (1952) convincingly demonstrated that colostrum deprivation almost invariably results in death when 22 of 24 colostrum-deprived calves, housed under identical conditions, died within three weeks of birth.

It is possible to separate serum proteins into several different components in an electrical field because of the different electrical charges on the molecules making up these components, and adult bovine serum can be separated into four major components, albumin, which migrates towards the anode of an electrical field faster than any other component, alphaglobulin, betaglobulin and gammaglobulin, the slowest migrating fraction (Irfan, 1963). Antibody activity resides within the gammaglobulin fraction (Tiselius and Kabat, 1939; Smith, 1946; Smith and Holm, 1948). The gammaglobulin fraction can be further separated into four components having immune activity, immunoglobulin G_1 (IgG_1), immunoglobulin G_2 (IgG_2), immunoglobulin A (IgA) and immunoglobulin M (IgM), (Butler, Winter and Wagner, 1971). Early workers used salt fractionation to separate serum proteins,

yielding two main fractions, pseudoglobulin and euglobulin (Howe, 1921). The pseudoglobulins were the serum proteins precipitated by sodium sulphate, and are equivalent to the gammaglobulin fraction of serum separated by electrophoresis or the immunoglobulins G_1, G_2 and A separated by immunochemistry. The euglobulin proteins were insoluble in water and consist largely of immunoglobulin M (Mukkur and Froese, 1971). Smith (1946) examined both bovine colostrum and plasma electrophoretically and introduced the term "immune lactoglobulin" to describe those proteins of colostral whey which were of low electrophoretic mobility and which were associated with the immune activity of colostrum. Smith (1946) suggested that although the colostral globulin was closely related to serum gammaglobulin they were not identical. However further studies have shown that this difference is only qualitative and the term "immune lactoglobulin" has been dropped in favour of immunoglobulin to describe those proteins of milk which possess immune activity (Rose, Brunner, Kalan, Larson, Melnychyn, Swaisgood and Waugh, 1970).

The absence of the gammaglobulin fraction in the serum of newborn calves and its appearance following the ingestion of colostrum has been repeatedly demonstrated by techniques currently available at the time of the studies, e.g. salt fractionation (Langer, 1907; Howe, 1921), electrophoresis (Jameson, Alvarez-Tostado and Sortor, 1942; Hansen and Phillips, 1947a; 1949; Smith and Holm, 1948; Polson, 1952; Pierce, 1955; Johnson and Pierce, 1959) and immunoelectrophoresis (Steck, 1962; Ward-Cox, 1968; Merriman, 1971). Generally these studies were qualitative and used merely to illustrate a normal physiological process with no attempt being made to quantitate the amount of protein absorbed.

The estimation of the concentrations of the various protein fractions in serum, including gammaglobulin, by determination of the total serum protein and electrophoresis is a universal technique but it has the major drawback of requiring sophisticated equipment and skilled technical assistance. Therefore more rapid methods of determining the concentration of gammaglobulin in serum have been sought. Three of the methods which have been described have employed precipitation of the gammaglobulin fraction by dilute salt solutions, zinc sulphate, (Aschaffenburg, 1949; Patterson, 1967; McEwan, Fisher,

Selman and Penhale, 1970b), sodium sulphite (Patterson, 1967; Stone and Gitter, 1969) and iodine agglutination (Patterson, 1967). The determination of the serum gammaglobulin concentration by refractometry has also been described (McBeath, Penhale and Logan, 1971; Reid and Martinez, 1975; Naylor and Kronfeld, 1977). The measurement of the concentration of individual immunoglobulins in the sera of young calves by the radial immunodiffusion method and their correlation with values obtained by the zinc sulphate turbidity test have also been reported (McEwan and others, 1970b).

Aschaffenburg, Bartlett, Kon, Walker, Briggs, Cotchin and Lovell (1949b) claimed that the feeding of as little as 80 ml of the non-fatty fraction of colostrum to newborn calves was sufficient to prevent death from colisepticaemia. However, Fey and Margadant (1961) employed immunoelectrophoresis semiquantitatively to demonstrate that 21 of 22 calves which had died of colisepticaemia were agammaglobulinaemic or severely hypogammaglobulinaemic; all 22 calves were said to have received colostrum. Gay, Anderson, Fisher and McEwan (1965a) found that 53 of 178 calves less than four days old which had been purchased at local markets in the west of Scotland were agammaglobulinaemic or markedly hypogammaglobulinaemic by a zinc sulphate turbidity test and that, of the agamma- or hypogammaglobulinaemic group, 36 per cent subsequently died of colisepticaemia. A total of 17.4 per cent of the calves died of colisepticaemia or from some other cause, but those that died were those calves with low serum concentrations of gammaglobulins. McEwan, Fisher and Selman (1970a) extended the survey of 415 calves and found a wide range of gammaglobulin concentrations from 0 to in excess of 60 ZST units. With two exceptions the 58 deaths due to colisepticaemia occurred within the group of calves with less than 10 ZST units of gammaglobulin. The overall mortality rate was 31.8 per cent and deaths due to other causes, mainly diarrhoea, were confined to those calves with the lowest concentrations of gammaglobulin.

Dam (1968) examined sera of 25 calves from herds where deaths due to colisepticaemia were a major problem and found no significant difference between the gammaglobulin concentration of calves which died of colisepticaemia and those which survived, although the means for both groups of 0.51g/100ml and 0.33g/100ml were very low. One calf which had a gammaglobulin concentration of 3.01g/100ml was excluded from the calculations as being abnormally high. From these findings,

Dam concluded that hypogammaglobulinaemia was of no importance in the pathogenesis of colisepticaemia.

In a farm survey of serum immunoglobulin concentrations in dairy heifer calves less than one week old, Selman and others (1971a) found that calves born in fields had significantly higher serum immunoglobulin concentrations than calves born in byres or boxes. In the same survey it was also demonstrated that the mortality rate of calves born in byres was 15 per cent but the mortality rate of calves born in fields was only three per cent. Boyd (1972) carried out a farm survey of newborn calves and also found that mortality and a high incidence of diarrhoea during the first four weeks of life were related to low concentrations of serum immunoglobulins. In a survey of calves born on the same farm during four consecutive winters, Boyd, Baker and Leyland (1974) found that calves which developed fatal diarrhoea had significantly lower concentrations of serum immunoglobulins than calves which remained normal. Logan, McBeath and Lowman (1974a) found that seven of 30 beef suckler calves which had had free access to their dams were hypogammaglobulinaemic; two of the 30 calves died, both of which were severely deficient in IgM. In another study of young calves in a beef herd, it was demonstrated that those calves which died of infectious causes during the first three weeks of life had significantly lower serum concentrations of IgG_1 than calves which remained normal (McGuire, Pfeiffer, Weikel and Bartsch, 1976). Furthermore, it has been demonstrated that calves with total serum concentrations of individual immunoglobulins, IgG, IgM and IgA of more than 30 mg/ml are less likely to develop diarrhoea than calves with serum concentrations of less than 30 mg/ml (McNulty and others, 1976).

Most of the surveys studying the relationship between serum immunoglobulin concentrations and disease have dealt with colisepticaemia and neonatal diarrhoea but Thomas and Swann (1973) examined the effect of the passively acquired serum immunoglobulin concentrations on the incidence of pneumonia. They found that the calves which had serum immunoglobulin concentrations of less than 10 ZST units on admission to a rearing unit had the highest mortality rate of seven per cent and this group required the most treatment for pneumonia. In a similar survey, Irwin (1974) correlated serum

immunoglobulin concentrations of calves on admission to a rearing unit with disease during the six weeks following admission and found that 11 per cent of calves with serum immunoglobulin concentrations of less than 20 ZST units died compared to a mortality of 1.5 per cent among calves with more than 20 ZST units. Williams, Spooner and Thomas (1975) measured the individual serum immunoglobulin concentrations of IgG_1, IgG_2, IgA and IgM of calves which were approximately two and a half weeks of age, on admission to a rearing unit, and found that low concentrations of IgG_1, IgG_2 and IgA on entry were associated with a high incidence of pneumonia at ten weeks of age. Forty-five per cent of those calves which developed pneumonia had had serum IgG_1 concentrations of less than 8 mg/ml on entry, whereas only 9.5 per cent of calves with serum IgG_1 concentrations of more than 8 mg/ml on admission later succumbed to pneumonia.

In Sweden, Hurvell and Fey (1970) demonstrated that mortality in purchased calves as a result of septicaemia, diarrhoea and pneumonia was related to low concentrations of serum immunoglobulins but a considerable proportion (16%) of calves which remained healthy were also hypogammaglobulinaemic. Bailey and McLean (1972) found that 18 per cent of calves passing through Australian markets were hypogammaglobulinaemic but did not relate this to any subsequent disease.

Surveys of calf wastage have shown that mortality is higher during the late winter housing period (Withers, 1952b; Leech and others, 1968). An examination of sera from 1,034 calves less than a week old purchased through local markets in the west of Scotland over a two year period, revealed a very pronounced seasonal variation in serum immunoglobulin concentrations with peak concentrations being present during the summer and early autumn months and minimal concentrations occurring during the months of January, February and March (McEwan and others, 1970a). Smith, O'Neil and Simmons (1967) were unable to demonstrate such a marked seasonal variation in the serum immunoglobulin concentrations of a smaller number of calves although calves born during the summer months tended to have higher immunoglobulin concentrations. However, Hurvell and Fey (1970) recorded a higher frequency of hypogammaglobulinaemia in calves born in March and April, and Boyd (1972) found that 85 calves born from October to March had significantly

lower serum immunoglobulin concentrations than 113 calves born from April to September.

Barber (1971, 1972, 1978) and Barber and MacLennan (1975) examined the total serum gammaglobulin concentrations of dairy and suckled calves and disputed this close association between gammaglobulin concentration and disease. Barber and MacLennan (1975) determined the total serum immunoglobulin concentrations of 351 single suckled beef calves. Eighty-four calves were affected by diarrhoea and 23 of these died but no relationship could be found between the serum immunoglobulin concentration and the eventual fate of the calves. Barber (1978) also found that there was no correlation between serum immunoglobulin concentration measured by a zinc sulphate turbidity test and the subsequent performance of 240 calves admitted to a rearing unit. Eighteen of the 240 calves died but 12 of these were from the same group of 40 and the author fails to comment on this very high mortality.

The physiological processes involved in the transfer of immunity from adult to offspring have been a subject of considerable interest to scientists since the latter part of the nineteenth century. Following Chauveau's (1888) observation that lambs born to ewes immunised against anthrax were resistant to the disease, Ehrlich (1892) recorded that newborn mice acquired a passive immunity to the toxins, ricin, abrin and robin via the milk from mothers immunised against these toxins.

In 1912, Famulener (1912) reviewed the early literature on maternofoetal transmission of immunity and examined the transfer of sheep haemolysins from the blood of mother goats to the kids. The main findings were that the serum of newborn kids contained no haemolysins before being permitted to suckle their mothers; that the serum of kids which received colostrum rapidly acquired a high haemolytic titre; that this transfer occurred via the colostrum; and that the ability to absorb these haemolytic antibodies was present for the first few days of life only. Unfortunately from his work with goats, Famulener (1912) assumed that in man the transfer of immunity from mother to offspring was also via the colostrum. However, Kuttner and Ratner (1923) demonstrated that the transfer of diphtheria antitoxin from mothers to newborn infants occurred through the placenta

and that human colostrum had "no significance whatever comparable to that revealed by the work of Theobold Smith and his coworkers in the feeding of the newborn calf".

Whether or not transmission of maternal antibodies to the offspring occurred before or after birth was correlated initially with the varying types of placentation found in each species, (McGirr, 1947), but Brambell (1958) concluded from a series of experiments on rabbit and rat foetuses that the degree of transfer of antibodies prior to birth is directly correlated with the development, persistence and time of withdrawal of the yolk sac into the umbilical cord. Thus in the rabbit, and guineapig in which the yolk sac is exposed to the uterine lumen during most of the gestation, transmission of antibodies occurs through the endodermal cells of the yolk sac and is not transplacental. In ruminants, horses and pigs in which the yolk sac is withdrawn into the umbilical cord early in gestation no prenatal transfer occurs and the transfer of antibodies is totally postnatal. As the yolk-sac is rudimentary in man, it is impossible for transmission of maternal antibodies to occur via the yolk sac and transfer in this species is through the placenta (Brambell, 1970). The route and duration of transmission of passive immunity in several species are shown in Table 4 (Brambell, 1970).

Much of the fundamental work on the transfer of immunity in ruminants was carried out by Smith and his associates during the decade from 1920 to 1930. Using a salt fractionation procedure, Howe (1921) showed that the serum of newborn calves contained very little globulin but that a rapid increase in the euglobulin and pseudoglobulin fractions occurred after the ingestion of colostrum. At about the same time two of Howe's colleagues, Little and Orcutt (1922) observed that calves which received colostrum with a high agglutination titre to Brucella abortus, rapidly acquired these agglutinins in the serum. Orcutt and Howe (1922) reported an excellently executed series of experiments in which, using salt fractionation, they demonstrated that the agglutinins to Brucella abortus were associated with the globulin fractions of both serum and colostrum. In the same year, Smith and Little (1922a) published their classical paper on the significance of colostrum to the newborn calf. In the following year,

TABLE 4

Route and duration of transmission of passive immunity

Species	Prenatal		Postnatal		
	Present	Route	Present	Route	Duration
Fowl	++	Yolk sac	++	Yolk sac	5d
Rat	+	Yolk sac	++	Gut	20d
Mouse	+	? Yolk sac	++	Gut	16d
Guineapig	+++	Yolk sac	0		
Rabbit	+++	Yolk sac	0		
Hedgehog	+	Unknown	++	Gut	40d
Dog	+	Unknown	++	Gut	1-2d
Cat	+	Unknown	++	Gut	1-2d
Man and Monkey	+++	Placenta	0		
Horse	0	None	+++	Gut	24 hr
Pig	0	None	+++	Gut	24-36 hr
Ruminants	0	None	+++	Gut	24 hr

Ref. Brambell (1970)

the same authors (Smith and Little, 1923) reported that calves fed serum within the first few hours of life readily absorbed Brucella abortus agglutinins from the intestinal tract. Smith (1930) demonstrated the presence of antibodies to E. coli in cows' colostrum and that the concentration of these antibodies in the colostrum varied for individual cows. Smith (1930) also demonstrated that, at parturition, the concentration of antibodies to E. coli in an individual cow's colostrum was greater than the concentration of the same antibodies in the serum. It was also shown that newborn calves could absorb antibodies to E. coli when fed milk mixed with serum from cows immunised against certain strains of E. coli (Smith and Little, 1930). After the early work of Little and Orcutt (1922) and Smith and Little (1923, 1930), the demonstration of the passive transfer of individual antibodies from colostrum to the circulation of the young ruminant by serological methods was a recognised technique and in some cases provided invaluable physiological information. Mason, Dalling and Gordon (1930) demonstrated the absorption of several heterologous antitoxins in both lambs and calves. Other marker antibodies have included diphtheria antitoxins, Haemophilus pertussis and Vaccinia virus antibodies (Smith and Holm, 1948); Trichomonas foetus antibodies (Kerr and Robertson, 1946; 1954); B. abortus antibodies and Salmonella dublin antibodies (Comline, Roberts and Titchen, 1951a), Foot and Mouth virus antibodies (Graves, 1963), and Rinderpest virus antibodies (Singh, Osman, El Cicy and Baz, 1967).

Smith and Holm (1948) not only examined the absorption of various antibodies and antitoxins, but also the catabolism of these antibodies in the young calf. They calculated the half-life of diphtheria antitoxins to be 16-20 days and the half-lives of Haemophilus pertussis and Vaccinia antibodies to be approximately 50 days. However the most significant step forward in the understanding of the physiological process of absorption was provided by Comline and others (1951a) studying the absorption of B. abortus agglutinins. Using the B. abortus agglutinins as a marker, Comline and others (1951a) demonstrated that when colostral whey was infused into the duodenum of newborn calves, the colostral immunoglobulins were absorbed through the intestinal lymphatic system and entered the peripheral circulation through the thoracic duct. Maximum concentrations of agglutinins in the thoracic duct lymph fluid occurred between two and three hours

after infusion into the duodenum. Comline and others (1951a) also demonstrated that absorption of colostral B. abortus antibodies only occurred when they were infused into the small intestine and not when infused into either the abomasum or the large intestine.

Balfour and Comline (1962) studied the absorption of bovine serum gammaglobulin labelled with radioactive iodine (I^{131}) infused into the duodenum of calves four to 17 hours of age and confirmed that absorption was primarily through the intestinal lymphatic system. Shannon and Lascelles (1968) also demonstrated that, in the unanaesthetized calf allowed to suckle colostrum, absorption of immunoglobulins occurred via the intestinal lymphatics. An increase in the immunoglobulin concentration of the thoracic duct lymph fluid was noted about 60 minutes after suckling and reached a maximum at approximately 180 minutes after suckling. However the absorption of a very small proportion of immunoglobulins through the portal system has been noted (Balfour and Comline, 1962; El-Nageh, 1967). In contrast, Kraehenbuhl and Campiche (1969) demonstrated that antibodies could be detected in the portal blood and serum within 15 to 30 minutes of infusion into the small intestine of piglets and neonatal rats and suggested that the portal system was the main route of absorption.

The neonatal rat is frequently used as an experimental animal for the study of macromolecular absorption, as the baby rat continues to absorb immunoglobulins until it is 18 to 21 days old (Halliday, 1955, 1956; Bangham and Terry, 1957). Using everted intestinal sacs of 18 day old rat ileum, Bamford (1966) provided quantitative evidence of absorption of immunoglobulins in the distal small intestine. Apparent confirmation that immunoglobulin absorption occurred predominately in the distal small intestine was given by histological techniques by which macromolecules, such as immunoglobulins, synthetic dextrans and vital dyes, could be demonstrated within the epithelial cells of the jejunum and ileum but not the anterior small intestine (Clark, 1959, 1971; Williams and Beck, 1969; Graney, 1968; Clarke and Hardy, 1969a). However, Williams and Beck (1969) also showed that the uptake of trypan blue by the epithelial cells of the distal small intestine occurred not only in the neonatal rat and ferret, but also in the neonatal rabbit and guineapig, species in which there is no post-natal transfer of immunity.

There was one other finding which lent support to the view that absorption of immunoglobulins occurred in the distal small intestine. At birth, the intestinal mucosa of the rat has an embryonic structure of tall columnar cells with many supranuclear vacuoles (Clark, 1959) and as the baby rat ages the intestinal mucosa becomes progressively more adult-like from the pylorus distally towards the ileocaecal valve. Thus in the five day old rat, the epithelial cells in the duodenum resemble those found in the adult whereas those epithelial cells in the jejunum and ileum still retain many supranuclear vacuoles (Clark, 1959). The replacement of cells takes place from the base of the villus towards the apex, and the time required for the complete replacement of vacuolated cells by mature non-vacuolated cells on each villus is approximately three days (Clark, 1959, 1971; Clarke and Hardy, 1969b). This maturation process is complete when the young rat is 18-21 days old, at the same time as the cessation of macromolecular absorption (Clark, 1959; Graney, 1968; Clarke and Hardy, 1969b).

Smith (1925) had noted the presence of vacuoles in the epithelial cells of the ileum in calves less than three days old and suggested that these vacuoles contained protein. Comline and others (1951b) confirmed that the epithelium of the ileum of calves nine to 36 hours old contained vacuoles and by specific histological staining demonstrated that these vacuoles contained protein identical to the colostral whey protein in the lumen of the ileum. Employing immunofluorescence, El-Nageh (1967) demonstrated that the uptake of bovine gammaglobulin by the intestinal epithelial cells of the young calf was maximal in the jejunum with little uptake in either the duodenum or the ileum. Hill and Hardy (1956) were also able to demonstrate vacuoles containing protein in the intestinal epithelial cells of young lambs and kids up to 36 hours old which had received colostrum. It has been shown that the small intestine of the young kid comprises two zones; the first zone included most of the duodenum and had the appearance of adult small intestine; the second zone consisted of the rest of the small intestine in which the nuclei of the epithelial cells were frequently apical and many vacuoles, often containing protein were present within the epithelial cells. The uptake of polyvinyl pyrrolidone by the epithelial cells could only be

detected in the distal 75 per cent of the small intestine. With increasing age this uptake was restricted to terminal 25 per cent of the small intestine and had almost ceased by three days of age (Clarke and Hardy, 1971).

In contrast to all the published evidence Rodewald (1970) reported that the absorption of homologous antibodies in the ten day old rat occurred in the proximal small intestine only and that little if any absorption occurred in the distal small intestine even although labelled immunoglobulin could be demonstrated within the epithelial cells of the ileum. Further studies by Rodewald (1973) confirmed this finding and indicated that the immunoglobulin appeared to be preferentially bound to the apical plasma membrane at the base of the microvilli on the luminal surface. Rodewald postulated that the immunoglobulin was then transported through the cytoplasm within coated vesicles and discharged into the extracellular space at the lateral cell surfaces.

That the absorption of the immunoglobulin occurs mainly in the proximal small intestine of the neonatal rat has been confirmed by Morris and Morris (1974, 1976b) who demonstrated that radioiodinated immunoglobulin infused into different regions of the small intestine of 12 day old rats was readily absorbed from the proximal and middle small intestine into the blood stream. There was, however, no transmission of undegraded, infused immunoglobulin from the ileum to the circulation. Later it was shown that approximately 40 per cent of the immunoglobulins presented to the epithelial cells of the proximal small intestine was transmitted as intact molecules whereas in the distal small intestine, although the infused immunoglobulins were absorbed by the epithelial cells, about 90 per cent was degraded into small molecular weight fragments and none was transmitted intact (Morris and Morris, 1977a, 1977b). The exact site of absorption of immunoglobulins within the small intestine of the ruminant has not yet been clearly defined (Hardy, 1969a; Clarke and Hardy, 1971) although histological evidence indicates that it may be the jejunum (El-Nageh, 1967; Staley, Corley, Bush and Jones, 1972).

In those species in which transfer of antibodies from the mother to the offspring occurs after birth, the capacity to absorb those antibodies is transitory (Table 4). Famulener (1912) reported

that kids deprived of the dam's colostrum for the first two days of life were unable to absorb sheep red cell haemolysins from the colostrum on the third day of life. Howe (1921), Smith and Little (1923) and Smith (1930) noted that calves fed homologous serum with a high agglutinin titre to B. abortus showed no increase in serum agglutinins when fed at 2.25, 3.25 and 18 days old. Comline and others (1951a) noted that calves which were not given colostral whey, with B. abortus agglutinins as a marker, until 63, 64 or 65 hours after birth absorbed minimal, if any, quantities of these agglutinins. Hansen and Phillips (1947b) were unable to increase the serum globulin concentration of an agammaglobulinaemic calf aged two weeks even although the colostrum was infused directly into the small intestine.

Deutsch and Smith (1957) attempted to suppress the development of gastric activity in calves with an aluminium hydroxide gel and probanthene mixture but were unable to detect the absorption of colostral globulin fed to calves 40 hours old. In a sequel to this study Smith and Erwin (1959) infused colostral globulin directly into the duodenum of calves 6, 18, and 48-60 hours old and were unable to detect any absorption of colostral globulin in those calves fed at 48-60 hours. The authors concluded that "gastrointestinal enzyme development is not the primary reason for the inability of older calves to absorb immune proteins per se".

In the unsuckled piglet, there is evidence to suggest that the ability to absorb proteins persists for at least 86 hours after birth (Lecce and Morgan, 1962) and polyvinyl pyrollidone for 65-85 hours after birth (Lecce and Morgan, 1962; Hardy, 1969b) but that suckling of milk brings about a much earlier cessation of transmission to the blood of specific antibodies (Young and Underdahl, 1950; Speer, Brown, Quin and Catron, 1959), of egg proteins (Lecce and Morgan, 1962) and of polyvinyl pyrrolidone (Lecce and Morgan, 1962; Hardy, 1969b). However, even in unsuckled piglets a significant fall in the absorption of polyvinyl pyrrolidone occurs between those piglets fed at 0-5 hours old and those fed at 15-20 hours old (Hardy, 1969b).

The physiological processes which effect the termination of the transmission of intact immunoglobulins from the gut to the circulation have not yet been fully defined. Moog (1951) observed

that there was a marked increase in alkaline phosphatase in the duodenum of the young mouse between 13 and 18 days of age, the time when cessation of absorption occurs in the young mouse. Subsequently, it was shown that this increase in alkaline phosphatase can be prematurely induced by parenterally administered steroid hormones (Moog, 1953; Moog and Thomas, 1955). Halliday (1959) noted that exogenous corticosteroids considerably reduced the absorption of antibodies by the neonatal rat, and Clark (1959) observed that intraperitoneal injections of cortisone resulted in precocious maturation of the epithelial cells and the premature cessation of macromolecular uptake by the epithelial cells in the middle region of the small intestine. Both effects took about three days to develop. Gillette and Filkins (1966) were unable to demonstrate any effect on antibody absorption when hydrocortisone was injected intramuscularly into newborn puppies, but if pregnant bitches were treated parenterally with hydrocortisone, 24 hours prior to parturition, there was a significant reduction in the puppies' ability to absorb antibodies. Having demonstrated that immunoglobulin absorption in the neonatal rat occurred in the anterior small intestine, Morris and Morris (1976a) found immunoglobulin absorption ceased in the anterior small intestine three days after the intraperitoneal injection of cortisone, but the cortisone still induced the precocious replacement of epithelial cells in the distal small intestine. Parenteral corticosterone did not induce maturation of the epithelial cells of the distal small intestine but caused a transitory, but marked, reduction in immunoglobulin transmission in the anterior small intestine. Clark (1971) demonstrated that 5-bromodeoxyuridine would inhibit cortisol-induced premature closure in the middle region of the small intestine of suckling rats.

Halliday (1959) observed that maternal deprivation led to a marked reduction in the duration of absorption in suckling rats; Clark (1971) noted, on histological examination of the intestine of suckling rats, that handling, separation from the mothers or any environmental changes, led to a reduction in the capacity of the intestinal epithelial cells to absorb macromolecules. It has also been shown that the uptake of polyvinyl pyrrolidone by the epithelial cells in the distal small intestine was much greater in neonatal guineapigs which had been well groomed than in neonatal guineapigs poorly groomed by the mothers

(Clarke and Hardy, 1970). Selman, McEwan and Fisher (1971b) observed that calves which were allowed to remain with their mothers absorbed more colostral whey immunoglobulins than those calves which were separated from their mothers at birth and fed colostrum at the same rate but maintained in isolation.

It has also been suggested that the development of a pepsin-secreting mucosa in the stomach of the young rat may also be involved in the termination of absorption. Hill (1956) demonstrated that no pepsin is secreted by the newborn rat but that when the rat is 18-21 days there is a rapid development of the pepsin secretory system and acidity of the stomach contents rapidly increases. In the newborn lamb peptic cells containing pepsinogen were shown to be present in the abomasal mucosa, but there were few parietal cells (Hill, 1956). The number of parietal cells increased over the first three days of life and pH of the abomasal contents dropped from pH 7.0 to pH 3.0. Hardy (1969c) noted that during the first 24 hours of life there was little proteolytic activity within the lumen of the calf's intestine, but considerable proteolysis occurred in the abomasum.

Having noted that 30 per cent of calves allowed free access to their dams for the first 12 to 18 hours following birth failed to absorb colostral immunoglobulins, Klaus, Bennet and Jones (1969) stated that "the defect underlying the failure of colostral globulin absorption by some calves is obscure". However, studies carried out almost simultaneously in Britain and Denmark revealed that the two factors which have most influence on the absorption of colostral immunoglobulins by the newborn calf are, the age at which colostrum is first consumed and the mass of colostral immunoglobulins presented to the calf (Selman, 1969; Kruse, 1970b).

Selman (1969) fed three groups of ten calves one feed only of a standard pool of colostrum at one, five and nine hours post-partum at 55 ml/kg bodyweight and found a significant reduction in absorption of colostral immunoglobulins between calves fed at one hour and five hours and between calves fed at five hours and nine hours. Kruse (1970b) fed 141 calves a variable volume of their own dam's colostrum once only at 2, 6, 10, 14 or 20 hours of age and demonstrated that absorption

decreased significantly with increasing age. Kruse (1970b) found that the concentration of colostral immunoglobulins in the serum of calves 24 hours old was primarily dependent on the mass of colostral immunoglobulins presented to the calf. Selman (1969) noted that there was a highly significant positive correlation between the immunoglobulin concentration of colostrum fed to calves under controlled laboratory conditions and the subsequent concentration of absorbed immunoglobulins in the calves' serum. It follows therefore that the volume of colostrum offered to the newborn calf is also very important.

Extrapolation of the data presented by Selman (1969) suggested that the intestine of the newborn calf would be impermeable to colostral immunoglobulins when it was approximately 18.5 hours old. Penhale, Logan, Selman, Fisher and McEwan (1973) examined the individual classes of immunoglobulins in the sera of calves fed colostrum once only at one, five and nine hours post-partum and found that the rate of closure for each class differed, being fastest for IgG and slowest for IgA. However, by extrapolation, Penhale and others (1973) deduced that the newborn calf was able to absorb IgG for approximately 27 hours, IgA for 22 hours, but IgM for only 16 hours.

Balfour and Comline (1962) found that the rate of absorption of immunoglobulins from the intestine of the newborn calf was influenced by the solvent in which the immunoglobulins were dissolved. The absorption of immunoglobulin was very poor when the solvent was the chloride salts of sodium, potassium, calcium or magnesium. Absorption of immunoglobulins was greatly increased when the solvent was a colostral whey solution from which the heat coaguable protein had been removed, but the addition of sodium chloride to the colostral whey solution reduced the absorption of the immunoglobulins. Further studies by Hardy (1969a) confirmed and extended these findings using both radio-iodinated polyvinyl pyrrolidone and immunoglobulin. It was found that solutions of sodium or potassium salts of lactate, pyruvate and certain other salts of lower volatile fatty acids produced absorption of immunoglobulins similar to that of colostral whey solutions and that potassium isobutyrtate was the most effective. However, some of these active compounds were found in insignificant quantities in colostrum and the lymphatic flow in the intestinal lymphatics was much reduced

by the presence of these compounds resulting in an overall reduction in the absorption of both marker compounds. Interestingly, Hardy (1969a) also found that intravenous infusions of sodium L (+) lactate promoted the absorption of a solution of radio-iodinated polyvinyl pyrrolidone in water which was infused into the duodenum.

Following either the intraduodenal infusion or oral feeding of colostrum there is a marked increase in the flow of lymphatic fluid in the intestinal lymphatic vessels (Comline and others, 1951a; Shannon and Lascelles, 1968). Hardy (1969a) demonstrated that the flow of lymphatic fluid was much greater when human serum albumin was added to colostral whey and the addition of two per cent egg albumin to the sodium or potassium salts of the lower volatile fatty acids resulted in much greater flow rates of lymphatic fluid with a much higher total recovery of labelled immunoglobulin from the intestinal tract.

During the period of intestinal permeability, the young calf has the ability to absorb from the intestinal tract a wide variety of both large and small molecules, immunoglobulins, dextrans and synthetic polymers, insulin, and albumin (Pierce, 1961; Balfour and Comline, 1962; Pierce, Risdall and Shaw, 1964; Hardy, 1969a; Hardy, 1969c). In contrast, the absorptive epithelium of the young rat appears to be highly selective between different molecules. Halliday (1955) found that both rat and mouse antibodies to S. pullorum were transmitted from the gut of the neonatal rat with equal facility, but that rabbit antibodies to B. abortus were transmitted only poorly. Antibodies to B. abortus raised in cattle were not even transmitted in detectable quantities. Furthermore, Halliday and Kekwick (1960) observed that the gut of the young rat showed selection between antibodies to different antigens produced in heterologous species and that there was also a variation in the absorption of antibodies produced at differing times in the immune response. Morris (1967, 1969) demonstrated that bovine B. abortus antibodies of the IgG_2 sub-class were more readily absorbed by the neonatal rat than antibodies of the IgG_1 sub-class.

This lack of selectivity of the calf absorptive epithelium is probably responsible for the proteinuria which is present in calves less than three days old. Smith and Little (1924) and Howe (1924) demonstrated

an obvious association between the feeding of colostrum and presence of protein in the urine of calves less than three days old. Earlier Langstein and Neuberg (1907) had observed that a proteinuria occurred in newborn calves but failed to associate this proteinuria with the feeding of colostrum. Smith and Little (1924) were unable to detect any protein in the urine of newborn calves which had been fed milk. They also noted that no protein could be detected in the urine of calves more than three days old. Deutsch and Smith (1957) concentrated urine from newborn, colostrum-fed calves and recovered a beta-lactoglobulin which gave a single peak when subjected to electrophoresis. Deutsch and Smith (1957) noted that the concentration of protein in the urine had decreased to almost undetectable levels 18 hours after the feeding of colostrum. Pierce (1959a) demonstrated that the concentration of protein in the urine of newborn calves fed colostrum in the first hours of life increased until the calf was 14-24 hours old and then gradually declined. Pierce (1961) suggested the termination of the proteinuria was due to the cessation of protein transmission by the gut rather than any alteration in renal function. Pierce (1961) also demonstrated that newborn calves fed on milk or glucose-saline and injected intravenously with gelatin developed a proteinuria of which gelatin accounted for over 50 per cent of the total protein. The renal threshold for protein molecules and fragments is 50,000 (Deutsch and Smith, 1957; Pierce, 1961). As well as the small molecular proteins like beta-lactoglobulin, the presence of degraded immunoglobulin fragments has also been demonstrated in the urine of newborn calves (Pierce, 1961; Kickhöffen, Hammer and Westphal, 1971).

The presence of immunoglobulins in colostrum had been demonstrated by salt fractionation (Howe, 1922) and by electrophoresis (Smith, 1946; Smith and Holm, 1948). Larson and Kendall (1957) examined the concentration of proteins in the serum of cows around parturition and found that the concentration of total protein was minimal at parturition and maximal about four weeks prior to and 11 weeks after parturition. By electrophoresis they demonstrated that the decrease in total protein at parturition was due to a drop in the globulin fraction. Larson (1958) showed that the decrease in serum gammaglobulins was accounted quantitatively by the increase in immune lactoglobulin of the lacteal secretions. Blakemore and Garner (1956)

observed that when radio-iodinated bovine gammaglobulin was injected intravenously into a cow ten days prior to parturition it was rapidly concentrated in the lacteal secretions.

Dixon, Weigle and Vazquez (1961) demonstrated that serum proteins were continually secreted into the udder secretions of the cow but that during the formation of colostrum the rate of transfer of gammaglobulin was 100 times greater than that of serum albumin. They estimated that the quantity of gammaglobulin which left the serum was approximately equal to that present in the colostrum at parturition. It was shown that there was little change in the concentration of gammaglobulin in the serum until three weeks prior to parturition, after which it fell sharply. The serum concentration of gammaglobulins increased rapidly in the two weeks following parturition, and then more slowly, often attaining values higher than the prepartum values. They estimated that in the period prior to parturition approximately 34g of gammaglobulin was transferred into the udder daily.

Dixon and others (1961) and Feldman (1961) made detailed histological and electronmicroscopical examinations of the mammary gland and suggested that the transfer of immunoglobulins from the serum occurred by a process of selective transudation. Dixon and others (1961) reported that early in the dry period the normally low cuboidal cells of the acinar epithelium are transformed into tall columnar cells with obvious secretory vacuoles. These findings were in direct contrast to earlier work which suggested that the presence of immunoglobulins in the colostrum could be accounted for by the plasmacytosis which occurred during the period of colostrum production, and during experimental drying-off (Campbell, Porter and Petersen, 1950). Later workers demonstrated that there was a highly selective transfer of fast gammaglobulin into the lacteal secretions from the serum during the formation of colostrum (Murphy, Aalund, Osebold and Carrol, 1964; Pierce and Fienstein, 1965) and that this fast gammaglobulin was IgG_1 (Brandon, Watson and Lascelles, 1971).

The range of chemotherapeutic agents which have been used for the treatment and prevention of neonatal calf diarrhoea is quite outstanding and includes the following; antisera, atapulgite, brilliant

green, chloramphenicol, chlorodyne, charcoal, chlortetracycline, cloxacillin, furazolidione, framycetin, kaolin, neomycin, oxytetracycline, penicillin, pectin, phthalylsulphathiazole, streptomycin, polymixin, sulphadimidine, sulphaguandine, sulphapyridine, sulphamerazine, tetracycline hydrochloride, vitamins A, D, E and K and vitamins of the B complex (Edgson, 1964). Unfortunately, the efficacy, or lack of it, of most of these compounds has been determined in field trials, a situation which is largely uncontrolled.

The early literature on the prophylactic and therapeutic use of antibiotics in neonatal calf diarrhoea was reviewed by Lassiter (1955). Oxytetracycline and chlortetracycline were the only two antibiotics for which sufficient evidence was available to draw any valid conclusions. Lassiter observed that most of the data had been accumulated from field observations and the results were often conflicting, but concluded that both chlortetracycline and oxytetracycline were probably effective in the prevention and treatment of neonatal calf diarrhoea. However, he added the further qualification that many of the studies had been conducted under conditions where diarrhoea was not a major problem.

Roy, Shillam, Palmer and Ingram (1955b) reported that in conditions of moderate "infection" chlortetracycline given orally to colostrum-deprived calves reduced the incidence of diarrhoea and resulted in significantly increased weight gains over non-supplemented colostrum-deprived controls. Ingram, Shillam, Hawkins and Roy (1958) examined the effect of penicillin and chlortetracycline on colostrum-deprived calves during the first three weeks of life. The treated calves received either 250 mg chlortetracycline for the first five days and 125 mg daily thereafter for 16 days, or 250 mg penicillin for the first five days and 125 mg daily for 16 days, or 250 mg chlortetracycline and 250 mg penicillin for the first five days and thereafter 125 mg chlortetracycline and 125 mg penicillin daily for 16 days. During the 21-day experimental period all eight colostrum-deprived control calves died, seven of colisepticaemia; three of ten chlortetracycline treated colostrum-deprived calves died; eight of ten penicillin treated calves died, all of colisepticaemia; five of ten chlortetracycline/penicillin treated colostrum-deprived calves died, four of colisepticaemia; one

of ten colostrum-fed calves died. Those calves which did not die of colisepticaemia, were diagnosed as dying of localised intestinal infection. A significant difference in weight gains could only be attributed to the chlortetracycline group by a very complicated formula. All 115 strains of E. coli recovered during the experiment were resistant to penicillin. There was a greater resistance to chlortetracycline among the E. coli strains recovered from chlortetracycline-treated calves compared with the strains recovered from non-chlortetracycline treated calves. Lang, Roy, Shillam and Ingram (1959) also studied the effects of chlortetracycline given orally at 125 mg daily for the first three weeks of life to calves which had received a small volume (400ml) of colostral whey and found that chlortetracycline significantly reduced mortality and increased the weight gain of surviving calves over non-supplemented controls. The chlortetracycline did not, however, reduce the incidence of diarrhoea in the surviving calves. In a further trial, Shillam and Roy (1963a) found that the inclusion of 125 mg chlortetracycline daily in the diet of colostrum-fed calves had no effect on the incidence of diarrhoea or on weight-gain over the first three weeks of life compared with unsupplemented controls.

Dalton, Fisher and McIntyre (1960) studied the effects of streptomycin, neomycin, oxytetracycline, penicillin, chlortetracycline, chloromycetin and phthalylsulphathiazole used prophylactically on mortality and diarrhoea in young calves which were less than seven days old at the beginning of the experiment. The drugs were given at therapeutic doses and for the 14 days of the trial. Five of the seven drugs had no significant effect on either reducing the incidence of diarrhoea or mortality compared to the untreated controls. The results produced by the remaining two drugs, oxytetracycline and chlortetracycline were equivocal. In three of four trials, chlortetracycline significantly reduced mortality and the incidence of diarrhoea and in two of three trials, oxytetracycline given orally significantly reduced the incidence of diarrhoea and mortality. It should be noted that on four of the five occasions when a significant effect was obtained with these two antibiotics the trials were conducted during the months of June or October.

In Canada, Lister and Mackay (1970) reported a trial on the effect of oral treatment of diarrhoea with an antibiotic-sulphonamide mixture which suggested that treatment with antibiotics was of value. The calves used in the trial were of a similar type used by Dalton and others (1960), being purchased at less than one week old from local markets. None of 18 treatment calves died whereas nine of 18 calves which received no medication died. The majority of calves which died had either interstitial pneumonia or bronchopneumonia and Pasteurella haemolytica was the most frequent bacterial isolate from the lungs. As well as receiving oral antibiotics the treatment group received parenteral antibiotics if the temperature exceeded 39.4°C. It is therefore likely that the reduced mortality in the treatment group was due to the control of the respiratory syndrome as there was little difference in the incidence of diarrhoea in the survivors.

In one of the few controlled field trials, Radostits, Rhodes, Mitchell, Spotswood and Wenkoff (1975) allocated 254 calves with acute neonatal diarrhoea to one of six different treatments; two groups received ampicillin orally at 12 mg/kg, one group of which was milk-starved for 24 hours but was given oral electrolytes at 150 ml/kg/24 hours; the second ampicillin treated group was given cows' whole milk; two groups of calves were treated with nifuraldezone orally and chloramphenicol parenterally; again one group was milk-starved but given electrolytes orally and the other group was allowed cows' whole milk; the remaining two groups were given no antimicrobial treatment, but one group was milk-starved and given fluids orally and the other was allowed access to cows' whole milk. No significant difference in survival rate could be found between the six different treatments regardless of the period of hospitalisation or even when the data for only those calves which were hospitalised for more than three days were analysed. The two most frequent diagnoses among the 75 calves which died were enteritis and salmonellosis. Unfortunately no individual serum immunoglobulin concentrations were given but the authors state that calves with gammaglobulin concentrations of less than 1g/100ml were difficult to treat and calves with less than 0.5g/100ml invariably died despite exhaustive treatment. Calves with gammaglobulin concentrations greater than 1.5g/100ml usually responsed quickly and did not relapse. The authors also emphasised that this regimen of treatment was extremely time consuming.

In uncontrolled studies, Acres and others (1975) found that chloramphenicol intravenously had no influence on the outcome of diarrhoeic calves less than ten days old. Similarly, Boyd and others (1974) were unable to find any significant clinical response to a range of antibiotics even although sensitivity tests on E. coli isolated from faecal swabs were carried out prior to treatment. Bywater, Palmer and Wanstall (1978) claimed that amoxycillin significantly reduced mortality and the duration of diarrhoea regardless of whether or not the E. coli were resistant to the drug in vitro.

Fisher and de la Fuente (1971) examined the effect of furazolidone and chloramphenicol on spontaneous neonatal calf diarrhoea which developed in calves with low serum immunoglobulin concentrations. The experimental animals were Ayrshire bull calves less than one week old and purchased at local markets. In two experiments the two drugs were given at therapeutic levels when diarrhoea developed and in a third experiment, the calves were given daily prophylactic doses of furazolidone. The calves were allocated to the treatment group or control groups on the basis of equal serum immunoglobulin concentrations. There was no significant reduction in either diarrhoea or mortality when the two drugs were used at therapeutic levels for treatment. No beneficial effect could be attributed to the prophylactic use of furazolidone. Generally those calves which had higher concentrations of serum immunoglobulins survived despite treatment.

The use of intravenous electrolyte therapy in the treatment of severe infantile diarrhoea of babies is a recognised and successful technique (Darrow, 1946; Hutchison, 1975; Logan and Arneil, 1978), but the usefulness of this type of treatment for neonatal diarrhoea of calves is very limited (Boyd and others, 1974). McSherry and Grinyer (1954) described the treatment of seven calves less than 21 days old suffering from diarrhoea with a balanced electrolyte solution given parenterally, but three of the seven calves died. Watt (1965) described an uncontrolled trial in which diarrhoeic calves were treated with one of four regimens, antibiotics alone (following sensitivity tests), plasma and electrolyte solutions intravenously, or a combination of antibiotics, and plasma and electrolyte solutions intravenously. Of those calves given only the electrolyte therapy (a combination of Darrow's solution (Darrow, 1946) and glucose-saline)

46.15 per cent died whereas 78 per cent of the calves treated with antibiotics alone died. In another uncontrolled field trial, Dickson (1968) suggested that a glucose-saline solution given intraperitoneally or subcutaneously was beneficial in the treatment of neonatal calf diarrhoea, although 23 per cent of the treated animals died.

Following the suggestions of Jensen (1897) and Smith and Little (1922b, 1923, 1930) that serum, given orally or parenterally, could be used as an alternative to colostrum to protect calves against the ravages of colibacillosis, a number of investigations into the value of blood, serum and plasma derived products for the protection of the neonatal calf have been reported. Dollahite (1939) found that 200 ml serum given intravenously within 24 hours of birth had little influence on the incidence of diarrhoea or on mortality. Wise and Anderson (1944) reported that a commercially available bovine serum given subcutaneously had no significant effect either prophylactically or therapeutically in neonatal calf diarrhoea. Anderson, Dupre and Lamaster (1952) described the use of lyophilised serum in the diet of young calves. MacDonald and Oakley (1961) claimed that 50 ml citrated whole blood given subcutaneously soon after birth significantly reduced the incidence of diarrhoea and reduced mortality. Lotan, Berman, Tadmor and Perk (1964) described a controlled trial comprising 254 calves in which 5 to 10g of a bovine gammaglobulin-rich fraction were given prophylactically. When calves became diarrhoeic, they were also treated by diluting the milk with water, animal carbon, dihydrostreptomycin, chloramphenicol and chlortetracycline. The authors obtained equivocal results; in the first trial, a significant reduction in mortality could be attributed to the gammaglobulin but in a second trial, there was no significant effect between the treated group and the untreated controls.

Willoughby, Butler and Thornton (1970) described a controlled trial in which 200 calves were randomly allocated to one of four treatment groups shortly after birth. The four treatments consisted of serum, albumin, a globulin preparation which was given at a dosage rate or 75-77 mg/kg bodyweight, and a globulin preparation which was given at a rate of 620 mg/kg to 26 calves and 154 mg/kg to 13 calves. Control calves were given 0.85 per cent sodium chloride solution.

Each treatment was given once only and the calves were observed for first ten days of life. There was no significant difference in the incidence of neonatal diarrhoea between individual treatment groups and the control groups. The incidence of diarrhoea was least in the group of calves which received bovine serum albumin. The attempted prophylactic use of bovine plasma has also given ambiguous results, with one group of calves receiving 250 ml plasma subcutaneously having a lower incidence of diarrhoea and mortality than a similar group receiving 500 ml plasma subcutaneously (Lister and Mackay, 1970).

As noted above Watt (1965) described the use of bovine plasma for the treatment of colibacillosis. In one treatment regime affected calves were given up to 800 ml of bovine plasma and electrolyte solutions intravenously over a prolonged period. A comparison was made between four different treatments but no controls were employed. The mortality rate of diarrhoeic calves treated with the plasma and electrolyte solutions was 7.5 per cent compared to 78 per cent for calves given antibiotics only, and 46.15 per cent for calves given only electrolyte solutions intravenously. The inclusion of antibiotic therapy to the treatment regime of plasma and electrolytes appeared to have a deleterious effect, as the mortality with this combination was ten per cent. Watt (1967) suggested that immunoglobulin component of the infused plasma was likely to be responsible for the therapeutic effect of this treatment.

In 1971, Penhale and others (1971) described the preparation of an IgM rich fraction from bovine serum. This preparation would significantly prolong the survival time of calves which died of the septicaemia/scour syndrome and although calves survived at the higher dose-rates, 1.5-2.0 g/30 kg bodyweight, the authors were unable to establish any dose/survival time relationship. Subsequently, the same workers have been able to demonstrate that 1g/30 kg of an IgM preparation given intravenously on the first day of life will protect calves from colisepticaemia in the experimental situation (Logan and Penhale, 1971c) and under field conditions (Logan, Stenhouse, Ormrod, Penhale and Armishaw, 1974b). The prophylactic use of this IgM preparation has also permitted the study of experimentally produced

colibacillosis employing enteropathogenic E. coli (Logan and Penhale, 1972; Logan and others, 1977).

It might be expected that any means by which the colostral immunity of the dam could be boosted would be of value to the newborn calf in the prevention of colibacillosis, and the intramammary vaccination of cows with a formalinized E. coli vaccine has been shown to increase the concentration of and prolong the presence of IgA in post-partum mammary secretions (Wilson, Duncan, Heistand and Brown, 1972). Sellers, Smith and Pook (1962) reported a trial employing an E. coli bacterin vaccine administered subcutaneously twice in late pregnancy, but vaccination was of no value in controlling an outbreak of diarrhoea in the young calves. Further studies revealed that an oil adjuvanted, either heat or formalin-killed E. coli vaccine elicited the best serological response in adult animals (Gay and others, 1964a). Examination of serum samples from the cows revealed that most cows possessed antibody to the O-group antigens of the test E. coli but only a limited number possessed antibody to the K antigens. Following vaccination the colostrum of the vaccinated cows had significantly higher titres to both O and K antigens of the test E. coli, but despite being born to cows with high agglutination titres to K antigens 22.9 per cent of the calves had no demonstrable K agglutinins in their serum (Gay and others, 1964a). In a field-trial, using an oil-adjuvanted, formalin-killed vaccine prepared from strains of E. coli which had been recovered from cases of colibacillosis in the herd of origin, Gay and others (1964b) were unable to demonstrate any significant reduction in mortality between calves born to vaccinated dams and calves born to control dams. Bacteriological examination revealed that many of the strains of E. coli recovered from calves which died were antigenically distinct from the strains which had been employed in the vaccine.

With the knowledge that certain strains of E. coli were enteropathogenic, Myers, Newman, Wilson and Catlin (1973) described an experiment in which 27 cows were vaccinated subcutaneously and also by the intramammary route with a known enteropathogenic strain of E. coli, B44, (Smith and Halls, 1967a,b) on two occasions prior to parturition, and 14 cows were retained as controls. All 41 calves were challenged orally with a culture of E. coli strain B44 when they

were between five and 23 hours old. All the control calves developed a profuse diarrhoea between four and 21 hours after challenge and the calves born to vaccinated cows certainly appeared to be protected to some extent from the challenge strain. However, no indication of the serum concentrations of absorbed immunoglobulins of any of the calves was given; nor the titres to the vaccinal strain in the colostrum. Myers (1976) reported the results of a field-trial involving 3,500 cows, approximately half of which were immunised twice subcutaneously with a composite vaccine of six strains of formalin-killed E. coli. There was no significant difference in the incidence of diarrhoea between untreated and treated groups but the author claimed that mortality was significantly reduced in the calves born to the vaccinated dams. However, further examination of the data provided reveals that among the vaccinated cows, two herds accounted for 35 per cent of the 60 deaths and that among the unvaccinated cows the same two herds accounted for 45 of the 99 deaths. In one herd of 260 cows there were seven deaths due to salmonellosis; three calves were born to vaccinated cows and four calves were born to control cows. No information was given about the management practices on any of the ranches nor about the passively acquired serum immunoglobulin concentrations.

Wilson and Jutila (1976a) described a similar vaccination programme in beef cows employing an enteropathogenic E. coli B44 (Smith and Halls, 1967a,b), vaccinating the cows at approximately three weeks and one week before parturition both by the intramammary route and also subcutaneously. The calves were challenged with a culture of E. coli B44 before they were 10 hours old. The data indicated that 77 per cent of calves born to vaccinated dams were strongly protected against the oral challenge with E. coli B44. Calves born to vaccinated dams had significantly (<0.05) higher anti-O and anti-K agglutinins than control calves. Only the anti-K agglutinins in the colostrum of the vaccinated cows were significantly higher than those in the control cows' colostrum. It was suggested that IgA and IgM anti-K immunoglobulins were important in the passive immunity of experimental colibacillosis (Wilson and Jutila, 1976b).

Following the identification and isolation of viruses from the faeces of diarrhoeic calves (Mebus and others, 1969; Stair and others, 1972), vaccines of attenuated strains of rotavirus and

coronavirus have been commercially developed (Mebus, White, Stair, Rhodes and Twiehaus, 1972; Mebus, White, Bass and Twiehaus, 1973b; Blackmer, 1976; Thurber, Bass and Beckenhauer, 1977). Oral vaccination of newborn ranch calves with a rotavirus vaccine was claimed to significantly reduce the incidence of diarrhoea and mortality among the calves of these herds (Mebus and others, 1972; Mebus and others, 1973). Furthermore, inoculation of cows, subcutaneously or intramuscularly, with an inactivated rotavirus vaccine increased their serum neutralising antibody titre and it was claimed that the incidence of diarrhoea in the calves born to cows treated in this way was reduced (Mebus and others, 1973). The authors suggested that the absorbed circulating antibody was not protective but that the protection resulted from the presence of increased colostral and milk antibody in the lumen of the intestine.

However, Acres and Radostits (1976) reported a trial comprising approximately 1000 cow/calf pairings in which the cows were given an enteropathogenic E. coli bacterin or a placebo in late gestation and the calves were treated with either a commercially available oral rotavirus vaccine or a placebo before they were 24 hours old. The cows were vaccinated subcutaneously twice, approximately six and three weeks prior to parturition. It was found that there were no significant differences between the immunised groups or the placebo-treated groups in either the incidence or severity of diarrhoea, or the diarrhoea-related mortality in the calves. Coronavirus was detected in faeces from calves in three of the herds and in these herds the authors suggested that the use of the rotavirus vaccine appeared to have increased the mortality rate. The authors also criticised many of the previous field-trials with oral virus vaccines because of the lack of suitable, or even any, controls. In one other field-trial in which the double blind technique was employed it was found that the use of a combined rotavirus and coronavirus oral vaccine failed to reduce the incidence of diarrhoea or mortality in herds affected by both viruses (Thurber and others, 1977).

From this review of the literature it can be appreciated that treatment of neonatal calf diarrhoea either with antibiotics, or by fluid therapy with electrolyte solutions or blood derivatives has

had only very limited, if any, success. Attempted prophylaxis by the use of vaccines prepared against the viruses and E. coli serotypes associated with this condition has also given equivocal results. Furthermore, the vaccination of cows before parturition with the same agents with a view to increasing the concentration of specific antibodies in the colostrum is likely to be of limited value because of the apparent failure to transfer these colostral antibodies to the calf in many cases. With a few notable exceptions, surveys of neonatal calf disease have shown that there is a highly positive correlation between high serum concentrations of absorbed colostral immunoglobulins and survival. Thus the experiments described in this thesis were undertaken to examine husbandry practices which would ensure maximal transfer of colostral immunoglobulins to the newborn calf, and to examine the effects, if any, of these absorbed immunoglobulins on the incidence of diarrhoea and growth rates during the first four weeks of life.

CHAPTER 2

ATTEMPTS TO PRODUCE HIGH SERUM CONCENTRATIONS OF ABSORBED IMMUNOGLOBULINS IN NEWBORN CALVES BY MANAGEMENTAL MEANS

ATTEMPTS TO PRODUCE HIGH SERUM CONCENTRATIONS OF ABSORBED IMMUNOGLOBULINS IN NEWBORN CALVES BY MANAGEMENTAL METHODS

Introduction

The importance of colostrum has been convincingly demonstrated (Smith and Little, 1922a; Aschaffenburg and others, 1952) but statements regarding the volume of colostrum that should be consumed by newborn calves are extremely vague and are confined to expressions of opinion that calves should receive sufficient colostrum as early as possible (Fey and Margandant, 1962; Loosemore, 1964; Roy, 1964; Reisinger, 1965; Fey, 1972). The major factors which influence the absorption of colostral whey immunoglobulins by the newborn calf have now been defined (Selman, 1969; Kruse, 1970b).

Although Klaus and others (1969) had suggested that there was no correlation between the serum concentrations of absorbed immunoglobulins of colostrum-fed calves and the immunoglobulin concentrations of the colostrum fed to calves, Selman (1969) demonstrated that when newborn calves were fed colostrum under standardised conditions of management there was a highly significant positive correlation between the 48-hour serum immunoglobulin concentration of the calves and the immunoglobulin concentration in the colostral whey prepared from the colostrum fed to the calves. Kruse (1970b) showed that a calf's post-colostral serum immunoglobulin concentration was directly proportional to the mass of colostral immunoglobulin fed to that calf. Both Selman (1969) and Kruse (1970b) have shown that the efficiency of absorption decreases exponentially from birth and may have ceased completely at 20 hours of age. These two factors, the mass of colostral immunoglobulins offered and the time of first feeding account for almost 80 per cent of the variation in the serum concentrations of absorbed colostral immunoglobulins of newborn calves (Kruse, 1970b).

Surveys of neonatal calf mortality have shown that calves receiving the first feed of colostrum from a bucket have significantly higher mortality rates than those which have obtained colostrum by suckling (Withers, 1952c; Leech and others, 1968). Smith and others (1967) found that low concentrations of immunoglobulins were more

common in calves that had received colostrum by bucket only" compared to those calves which had been fed colostrum by suckling or by a combination of suckling and by bucket. Selman and others (1971a) noted that calves born in loose boxes or in fields had significantly higher concentrations of immunoglobulins than calves which were born in byres. In lambs, the intensity of husbandry in the immediate post-natal period had a significant influence on the serum concentrations of absorbed immunoglobulin and mortality rates (Ducker and Fraser, 1973). Newborn lambs which were dried and assisted to suckle immediately after birth absorbed significantly more colostral immunoglobulins than lambs which were allowed to remain with their mothers but which were given no assistance to suckle.

Kaeckenbeeck and Schoenaers (1964) suggested that several small feeds of colostrum during the first 12 hours of life resulted in higher serum concentrations of antibodies than the same total volume of colostrum given as a single feed. Under standardised conditions of management Selman (1969) was unable to demonstrate any significant difference between the mean concentrations of absorbed immunoglobulins of two groups of ten calves, one group of which was fed the total allowance of colostrum on a bodyweight basis at one hour post-partum and the other group of which was fed their volume of colostrum in three equal feeds at one, five and nine hours post-partum. Selman (1969) observed that calves which were allowed to remain with their mothers absorbed significantly more colostral whey immunoglobulins than calves which separated from their dams immediately at birth. Moreover, work by the same author (Selman, 1969) indicated that the breed of a calf may influence its ability to absorb colostral whey immunoglobulins as it was shown that AyrshirexFriesian calves absorbed immunoglobulins more efficiently than pure-bred Ayrshire calves under the same standardised conditions of management. Kruse (1970c) also suggested that there was "a genetically determined variation in ability to absorb immunoglobulin".

Therefore with knowledge of the above factors various regimens of feeding colostrum to newborn calves under controlled conditions were examined to determine their effect on the subsequent serum concentration of absorbed immunoglobulins in the calves. From these experiments it

was hoped to find a managemental system of colostrum feeding which would consistently achieve high serum concentrations of absorbed colostral immunoglobulins. The opportunity was also taken to examine the effect of the breed of calf on the ability to absorb colostral immunoglobulins and to compare the serum concentrations of absorbed immunoglobulins of calves born in strictly controlled conditions with those of calves purchased at local markets when three to four days old.

1. Cows, calves, and their management

a) Cows

The cows and heifers were admitted to the Veterinary School during the last few days of gestation. With one exception the animals were all of dairy breeds and mainly Ayrshires, Friesians and crosses between these two breeds. The animals were supplied by two dealers in the west of Scotland.

The animals were aged on the number of permanent incisor teeth present and on their general appearance, and were classified as heifers or cows. Exact ageing of the animals with more than four pairs of permanent incisors was not possible.

None of the animals used showed any sign of ill health during the period of hospitalisation. The majority of animals were allowed to calve naturally and the assistance in the form of traction was applied only if it was deemed necessary.

b) Accommodation

The cows were housed in a holding byre until they were found to be in the first stage of labour, or on vaginal examination were found to be approaching this stage (see below). With the exception of the animals noted below, the cows were then transferred to a well-bedded loose-box. Frequent observation of the cows was instituted until they had calved or assistance was thought necessary. The 15 cows of Group E, (Chapter 2, Section 2) were allowed to calve in the holding byre.

c) Feeding

The animals were offered 15-20 lbs. of hay daily and were fed twice daily with 8 lbs. dairy concentrates (BOCM - Red Label Nuts, BOCM/Silcock).

d) Prediction of parturition and dam shape

The method of the prediction of parturition used was that described by Selman (1969) and the following table is taken from that thesis.

Table 5

	Consistency of Cervix	Diameter of Posterior Os of Cervix	Predicted time of Parturition
1)	Hard	3 fingers	7-15 hours
2)	Soft	3 fingers	8-15 hours
3)	Soft	4 fingers (Anterior Os, 1 finger)	4-10 hours
4)	Soft	1 inch rim remaining	2-6 hours
5)		Complete dilation	½-5 hours

The comment by Selman (1969) that some cows progressed faster than others, especially those with a soft, difficult to locate, cervix was also valid.

In his studies on the suckling behaviour of the neonatal calf Selman (1969) defined and used the terms "good shape" and "poor shape" to describe cows prior to parturition. By definition, "good shape" meant that the udder and teats were either level with or higher than the dam's xiphisternum, and "poor shape" meant that, due to the large size of abdomen and/or udder and teats, the xiphisternum was the highest part of the dam's underbelly.

e) Collection of colostrum and blood

A sample of colostrum was obtained by milking approximately 50ml of colostrum from each quarter and pooling the 200ml so obtained. The time of sampling varied but in those cows which were suckled, the sample was collected immediately after the calf finished suckling. For those cows which were left with their calves for 48 hours, the pooled sample of colostrum was collected at approximately 15 minutes post-partum. However, the dams of the calves in which colostrum feeding was delayed were machine-milked (Alfa-laval Co.Ltd., Oakfield, Cwmbran, Gwent) at some time during period between the birth and feeding of the calf. A 200ml sample of this pooled colostrum was retained. A sample of blood was collected from the jugular vein of each cow at the same time as the sample of colostrum was collected.

Prior to suckling or milking, the udder of each cow was washed with a dilute antiseptic solution (Savlon, ICI, Macclesfield, Cheshire).

f) Calves

The 282 calves described in this thesis consisted of 168 calves born at the Veterinary School and 115 calves purchased at local markets in the west of Scotland.

For those 168 calves born at the Veterinary School, every parturition was supervised as described above. The aim was to have a group of 14 or 15 calves born during a two week period, but this was not always possible. One hundred and forty five calves were reared in 12 groups. Because of the difficulty in obtaining cows at the correct stage of gestation, the number of calves in each group varied, from seven to 15 (Table 6). The calves were born over a period of 29 months from February 1971 to July 1973. The method, timing and volume of colostrum fed to each calf varied from group to group and even within groups and will be described separately in each appropriate section, but subsequently all the group-reared calves were fed a proprietary milk substitute powder (Vitamealo Calf Milk Meal, Beecham Agricultural Products, London) from an automatic calf feeder (Nursette Model 30, Fifarm-Nursette Ltd., Cupar, Fife). The remaining 26 calves were reared individually. The calves born to the dairy cows and heifers were judged to be either Ayrshires, Friesians, crosses between those two breeds, Hereford crosses or occasionally other breeds and crosses. Throughout this thesis the dam's breed is given first, e.g. AxF.xF = AyrshirexFriesian(dam)xFriesian(sire); AxH = Ayrshire(dam)xHereford(sire).

The time of parturition, initiation and cessation of colostrum suckling (where appropriate) were noted to the nearest minute for each calf. Full details are presented in Appendix 1.

The terminology defined by Cowie, Folley, Cross, Harris, Jacobsohn and Richardson (1951) for use in lactational physiology has been adhered to throughout this thesis.

g) Weighing of calves

Calves were weighed immediately prior to suckling, normally at 15 minutes post-partum and again immediately after the cessation of suckling. Weighings were carried out on a set of scales (Avery, Type 3202 CLE, Birmingham, England) weighing up to 110 lbs. in $\frac{1}{4}$ lb. divisions. Body weights were recorded to the nearest $\frac{1}{4}$ lb. These weights were

TABLE 6

Management of calves

GROUP NO.	NUMBER OF CALVES	METHOD OF FEEDING COLOSTRUM
1	11 Calves Nos. 1-11	Assisted to suckle dam to satiation once only immediately after birth; whole milk for 3 days, then onto automatic calf feeder.
2	14 Calves Nos. 12-25	Assisted to suckle dam to satiation once only immediately after birth; then directly onto automatic calf feeder.
3	14 Calves Nos. 26-39	Assisted to suckle dam to satiation immediately after birth; left with dam for 12 hours; encouraged to suckle again; then directly onto automatic calf feeder.
4	11 Calves Nos. 40-50	Assisted to suckle dam to satiation immediately after birth; left with dam for 12 hours; encouraged to suckle again; then directly onto automatic calf feeder.
5	7 Calves Nos. 51-57	Assisted to suckle dam to satiation once only immediately after birth; then directly onto automatic calf feeder.
6	10 Calves Nos. 58-67	Assisted to suckle dam to satiation once only immediately after birth; 5 calves (59,61,63,65,67) directly onto automatic calf feeder; 5 calves (58,60,62,64,66) on whole cows' milk for 4 days; then onto automatic calf feeder.
7	9 Calves Nos. 68-76	Assisted to suckle dam to satiation once only immediately after birth; 4 calves (69,71,73,75) directly onto automatic calf feeder; 5 calves (68, 70,72,74,76) on whole cows' milk for 4 days; then onto automatic calf feeder.
8	14 Calves Nos. 82-95	7 calves (82,83,84,85,89,91,95) - Assisted to suckle dam to satiation once only immediately after birth; then directly onto automatic calf feeder. 7 calves (86,87,88,90,92,93,94) - Fed 1500ml dam's colostrum at 6 hours post-partum; then directly onto automatic calf feeder.

TABLE 6 (Cont'd)

GROUP NO.	NUMBER OF CALVES	METHOD OF FEEDING COLOSTRUM
9	10 Calves Nos. 96-106	5 calves (96,97,98,103,106) - assisted to suckle dam once only immediately after birth; then directly onto automatic calf feeder. 5 calves (99,100,101,102,105) - Fed 1500ml dam's colostrum at 6 hours post-partum; then directly onto automatic calf feeder.
10	15 Calves Nos. 107-121	5 calves (111,112,113,118,120) - assisted to suckle dam once only immediately after birth; then directly onto automatic calf feeder. 5 calves (107,109,110,117,121) - allowed to remain with dam for 48 hours; then onto automatic calf feeder. 5 calves (108,114,115,116,119) - fed 1700ml dam's colostrum at 8 hours post-partum; then directly onto automatic calf feeder.
11	15 Calves Nos. 122-136	5 calves (125,129,133,134,135) - assisted to suckle dam once only immediately after birth; then directly onto automatic calf feeder. 5 calves (122,123,124,130,132) - allowed to remain with dam for 48 hours; then onto automatic calf feeder. 5 calves (126,127,128,131,136) - fed 1700ml dam's colostrum at 8 hours post-partum; then directly onto automatic calf feeder.
12	15 Calves Nos. 137-151	5 calves (141,142,144,147,148) - assisted to suckle dam once only immediately after birth; then directly onto automatic calf feeder. 5 calves (137,138,139,143,146) - allowed to remain with dam for 48 hours; then directly onto automatic calf feeder. 5 calves (140,145,149,150,151) - fed 1700ml dam's colostrum at 8 hours post-partum; then directly onto automatic calf feeder.
Individual calves	Nos. 77-81, 104,151-166, 204,224	Assisted to suckle dam to satiation once only immediately after birth; reared individually.

converted to kilograms. No calf urinated during suckling but the occasional calf did pass meconium. When this happened, the cow was prevented from ingesting it. It was collected on a sheet of paper and weighed with the calf after suckling was completed.

h) Collection of blood

A blood sample was taken from the jugular vein of each calf at 15 minutes post-partum (precolostral sample) and again at 48 hours post-partum (post colostral sample).

i) Market calves

One hundred and fifteen calves purchased at local markets in the west of Scotland were admitted to the Department of Veterinary Medicine at Glasgow University Veterinary School from October 1973 to January 1976. The calves were less than one week old on admission and most were considered to be three to four days old. They were mainly male calves of the Ayrshire or Friesian breeds or crosses between those two breeds.

A blood sample was taken from the jugular vein immediately after admission to the Veterinary School, and since this was the only sample available from these calves the serum immunoglobulin concentration of this sample was assumed to be the absorbed immunoglobulin concentration (see below).

2. Laboratory procedures

a) Preparation of serum and colostral whey

Blood was allowed to clot at room temperature for 4 hours and the serum was removed after centrifugation at 1000g for 15 minutes. An aliquot of serum was used for an immediate estimation of the serum immunoglobulin concentration using the zinc sulphate turbidity test (see below). The remainder was stored at -20°C until all the samples had been collected. Each laboratory procedure was then carried out on all samples simultaneously.

Colostral whey was prepared within 24 hours of collection by adding 0.25 ml of commercial rennet to 25 ml of colostrum in a universal bottle. The mixture was then incubated at 37°C in a water bath until it was seen to be well clotted. The sample was then centrifuged at

1000 g for 20 minutes and the almost clear whey was carefully removed with a Pasteur pipette. Care was taken at this stage not to remove any colostral fat, which formed an upper layer on the surface of the sample. Following storage at -20°C total protein estimation and paper electrophoresis were carried out on all samples at the same time.

b) Estimation of the serum immunoglobulin concentration

This was performed using the zinc sulphate turbidity test as described by McEwan and others (1970b).

A solution of zinc sulphate (208 mg $ZnSO_4.7H_2O$/litre) was prepared in a volumetric flask using carbon dioxide-free distilled water. Two matched colorimeter tubes were then taken and 6 ml of distilled water were placed into the first (control) and 6 ml of the $ZnSO_4$ solution were placed into the second (test). A sample (0.1ml) of the serum under test was delivered into each tube using an auto-zero high precision pipette (H.J. Elliot Ltd., E-mil Works, Treforest, Glamorgan). The tubes were then gently shaken and allowed to stand for a timed 30 minutes at room temperature. After this time the tubes were again shaken to ensure an even redistribution of the precipitate and inserted into an E.E.L. colorimeter (Evans Electroselenium Ltd., St. Andrews Works, Halstead, Essex), after the instrument had been set to zero with a "blank" tube containing distilled water only. A blue-green filter (Ilford No. 623) was used. The turbidity reading was found by subtracting the reading obtained for the control tube from that of the test tube and multiplying the difference by a factor of 10. This gave a result in ZST units. The value, whenever necessary, was then corrected to the nearest whole number (i.e. 0.5 = 1.0). The 48-hour absorbed serum immunoglobulin concentration was taken to be the value obtained when the pre-colostral value was subtracted from the post-colostral value.

c) Estimation of colostral whey total protein concentration

After preparation of the colostral whey as described, the total protein concentration of this whey was then measured using a Biuret reaction as described by Weichselbaum (1946).

d) Estimation of colostral whey immunoglobulin concentration

Colostral whey was prepared by the method described above. Electrophoresis of the whey was carried out using the technique described by Neil (1963). The resulting strips were evaluated automatically using a Chromoscan recording densitometer (Joyce Loebl and Company Ltd., Gateshead, England) again using the method described by Neil (1963). Two cellulose acetate strips were complated for each colostral whey sample. The mean for the two respective immunoglobulin fractions was used to determine the colostral whey immunoglobulin fractions from the previously determined colostral whey total protein concentration.

e) Immunological methods

A detailed description of the immunological methods used is given in Chapter 4.

f) Statistical methods

Throughout this work, the parametric statistical methods employed are those described by Bishop (1971); deviations of the mean are expressed as standard deviations (S.D.). The non-parametric statistical methods employed, the Friedman two-way analysis, Spearmans Ranked Correlation and the Mann-Whitney test are those described by Siegel (1956). The majority of calculations were performed on a desk top computer (Olivetti Programma 101) and the significance levels estimated using the tables of Fisher and Yates (1963).

SECTION 1

THE ABSORPTION OF COLOSTRAL WHEY IMMUNOGLOBULINS BY THE NEWBORN CALF:

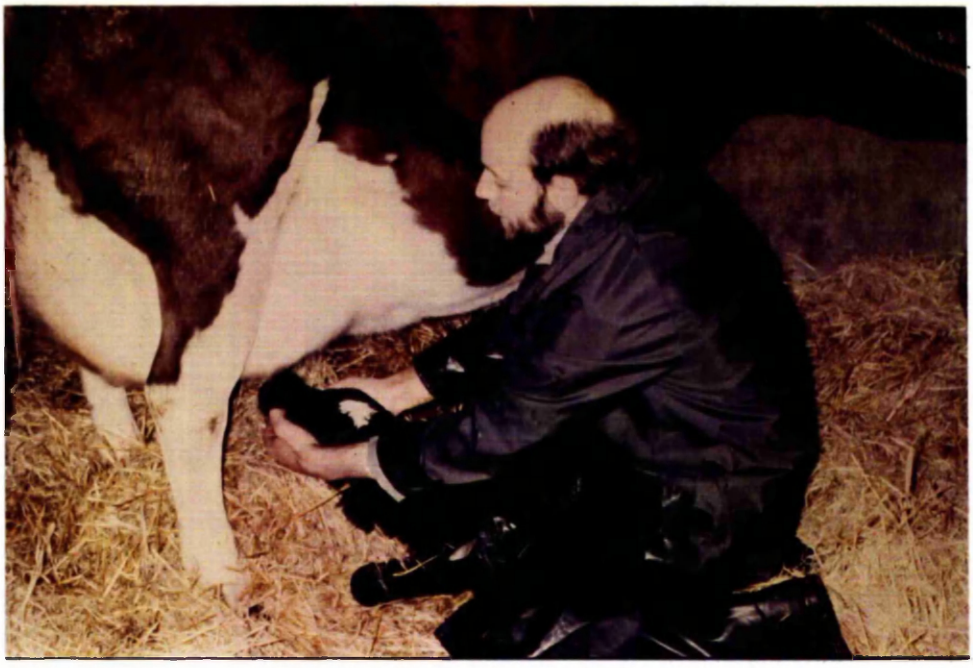
The effect of time and frequency of suckling.

Management of calves

The 50 calves of Groups 1, 2, 3 and 4 (Table 6) were divided into two treatment groups each of 25 calves on the basis of post-natal management; group A, calves 1-25 (Groups 1 and 2) and group B, calves 26-50 (Groups 3 and 4). All parturitions took place in well-bedded loose boxes. The four groups of calves (Groups 1, 2, 3, 4) were born over a six month period from February, 1971 to July, 1971; the 11 calves of Group 1 were born during a 14 day period from 22/2/71 to 7/3/71; the 14 calves of Group 2 were born during a 12 day period from 23/3/71 to 3/4/71 ; the 14 calves of Group 3 were born during a 16 day period from 11/6/71 to 26/6/71; the 11 calves of Group 4 were born during a 20 day period from 6/7/71 to 25/7/71.

Group A. Immediately after parturition, the dams were allowed to lick their calves for approximately 10-15 minutes until most of the amniotic fluid had been removed. After weighing and the collection of blood, the calves were encouraged to suckle their dams, which at this stage were always haltered. The majority of calves were unable to stand without support and had to be held in such a position to enable them to suckle (Figure 1). A teat was introduced into the mouth of each calf, and if it was slow to initiate suckling movements, a little colostrum was milked into the mouth. When the calf had emptied one quarter, it was guided to another and the point of satiation was judged to be attained when the calf no longer showed any inclination to suckle (i.e. when teat seeking advances and suckling movements were no longer made). Having suckled to satiation, each calf was immediately separated from its dam, reweighed, and placed in the separate rearing accommodation.

Group B. Immediately after parturition the 25 calves of group B were also assisted to suckle to satiation as described above. However, having been weighed after suckling each calf of group B was returned to its dam in the loose-box. Each calf was then allowed to remain with and to have free access to its dam until 12 hours post-partum. At this



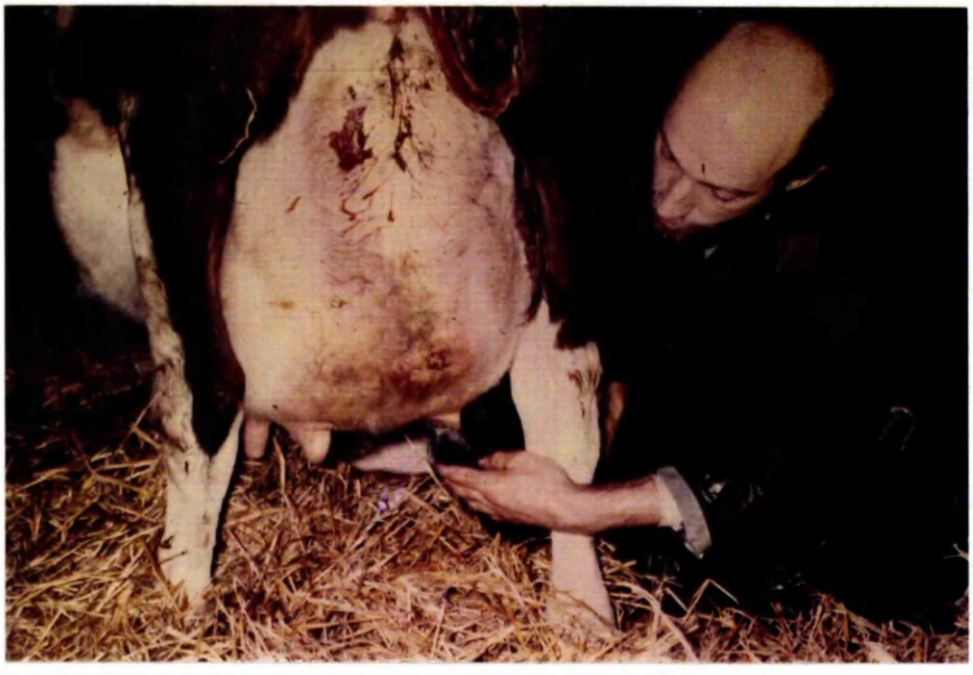


FIGURE 1 Early assisted suckling.

time, each calf was again weighed and encouraged to suckle. If a calf suckled, it was again given the opportunity to suckle to satiation and reweighed. After this second assisted suckling period, the calves of group B were separated from their dams and placed in the final rearing accommodation. During the second suckling period all the calves were ambulatory.

The pre- and post-suckling weights and the times at commencement and completion of suckling were noted for each of the 50 calves.

Results

Little difficulty was encountered in encouraging the calves to suckle during the first hour of life. Indeed on applying digital pressure to the muzzle, the majority of newborn calves immediately adopted the suckling position with the tongue, lips and mouth. Encouraging calves to suckle at 12 hours post-partum proved to be much more difficult and this was only possible with 11 of the 25 calves in group B. The calves of group B were not monitored during the 12 hours post-partum that they were allowed to remain with their dams and the number of times, if any, that a calf suckled during this period was not known; it was not clear if the reluctance of the remaining 14 was the result of their having already suckled.

The mean values for the 48-hour serum concentrations of absorbed immunoglobulin, the birth weights, the mass of colostrum ingested, the times from birth to completion of suckling, the times required for suckling to satiation and the colostral whey immunoglobulin concentrations for groups A and B are presented in Table 7. The individual results for the 50 calves are presented in Appendix 2.

In group A the 48-hour serum concentrations of absorbed immunoglobulins varied from 16 ZST units (calf 9) to 44 ZST units (calf 1) with a mean of 27.24 ± 6.10 ZST units. In group B the 48-hour serum concentration of absorbed immunoglobulins varied from 11 ZST units (calf 39) to 46 ZST units (calf 34) with the mean for all 25 calves being 29.20 ± 9.40 ZST units. No significant difference existed between these two mean values, but individually 12 calves of group B had 48-hour serum concentrations of absorbed immunoglobulins in excess of 30 ZST units compared with only eight calves of group A.

TABLE 7

The absorption of colostral whey immunoglobulins by newborn calves.

Group A - 25 calves assisted to suckle colostrum to satiation immediately after birth.

Group B - 25 calves treated similarly but allowed to remain with their dams and encouraged to suckle again at 12 hours post-partum.

	Group A	Group B
Number of calves	25	25
48 hour serum concentration of absorbed immunoglobulin - ZST units *	27.24 ± 6.10	29.20 ± 9.40
Birth weight - kg *	34.64 ± 5.34	33.45 ± 5.19
Weight of colostrum ingested at initial suckling - kg *	2.52 ± 0.73	2.46 ± 0.81
Colostral whey immunoglobulin concentration - g/100 ml *	7.31 ± 2.16	6.71 ± 1.85
Time from birth to completion of initial suckling period - minutes *	70.76 ± 16.30	66.16 ± 15.41
Time required to suckle to satiation at initial suckling period - minutes *	26.72 ± 8.59	23.16 ± 8.02
Weight of colostrum ingested at second suckling period - kg *	-	1.15 ** ± 0.75

* Mean ± S.D.

** Number of calves which suckled = 11

The weight of colostrum ingested by the 50 calves at the initial suckling period varied considerably from 0.4 kg (calf 27) to 4.1 kg (calf 16) and the mean weight of colostrum ingested at the first suckling period was 2.52 $\pm$ 0.73 kg and 2.46 $\pm$ 0.81 kg for groups A and B respectively. Although there was no significant difference in the mean birth weights of the two groups, the mean birth weight of Group A exceeded that of group B by more than 1 kg. When the weight of colostrum ingested at the first suckling period was expressed as a percentage of the birth weight the values varied from 1.0 to 12.5 per cent but the mean percentage for groups A and B was 7.28 $\pm$ 1.97 per cent and 7.46 $\pm$ 2.43 per cent respectively. As stated above only 11 calves of group B suckled at 12 hours post-partum and even in these calves the weight of colostrum ingested at this second suckling period was much less than that consumed at the first suckling period. The weight of colostrum ingested at the second suckling period varied from 0.1 kg (calf 50) to 2.3 kg (calf 26) with the mean for the 11 calves being 1.15 $\pm$ 0.75 kg.

The average time from birth to completion of suckling was just over one hour for both groups but the length of time that each calf suckled to attain satiation was considerable, being 26.72 $\pm$ 8.59 minutes for the calves of group A and 23.16 $\pm$ 8.02 minutes for the calves of group B. One calf (10) took 54 minutes to reach satiation and two calves (3, 28) required 40 minutes.

Wide individual variation occurred in the concentrations of immunoglobulins in the colostral wheys fed to the calves from 3.67g/100ml (calf 26) to 14.22g/100ml (calf 15). The mean concentrations of immunoglobulins in the whey of the colostrum suckled by the calves of groups A and B were 7.31 $\pm$ 2.16 g/100ml and 6.71 $\pm$ 1.85 g/100ml respectively. There was no significant difference between these values. No samples of colostrum were collected from the dams of the calves in group B at 12 hours post-partum and all values refer to those samples collected at the end of the first suckling period.

Three calves are worthy of further mention. Calf 9 (group A), a male Ayrshire calf, was very vigorous at birth and very keen to suckle. The dam, however, was a primiparous heifer which had very small teats and the size of the teats made prehension very difficult. As a result the calf had ingested only 0.8 kg of colostrum after 28 minutes, and

the 48-hour serum concentration of absorbed immunoglobulins was 16 ZST units. At the initial suckling period calf 27 (group B), an Ayrshire heifer calf, ingested only 0.4 kg after suckling for 25 minutes. This was due to two factors, the calf's disinterest in suckling and the dam's large puffy teats. At 12 hours post-partum the calf ingested another 0.7 kg of colostrum under supervision and the 48-hour serum immunoglobulin concentration was 23 ZST units. Calf 41 (group B) a male Ayrshire calf, suffered a prolonged parturition as a result of dystocia and considerable effort was required to revive the calf. The calf was quite languid and 94 minutes elapsed between birth and the initiation of suckling. Unfortunately the calf soon became disinterested in suckling and consumed only 0.8 kg. At 12 hours post-partum it refused to suckle at all and its serum concentration of absorbed immunoglobulins at 48 hours of age was 12 ZST units.

SECTION 2

THE ABSORPTION OF COLOSTRAL WHEY IMMUNOGLOBULINS BY NEWBORN CALVES:

The effect of different intensities of husbandry during the immediate post-natal period.

From the results obtained in Section 1 it was apparent that the early assisted feeding of colostrum resulted in high concentrations of absorbed immunoglobulin in the majority of calves. However, the supervision of every calving and the handling of calves which might still be covered in amniotic fluid was time consuming, and the opportunity was taken to compare, under controlled conditions, the very intense post-natal care of assisted suckling with two less demanding practices allowing the calf to remain with the cow for 48 hours, and separating the calf from the cow shortly after parturition and offering it colostrum at the "next-milking", a method common in the west of Scotland (Selman and others, 1971a).

Management of calves

The 15 calves in each of the Groups 10, 11, 12 (Table 6) were divided into three treatment groups each of five calves on the basis of post-natal management as follows:-

Subgroup C: five calves, born in loose-boxes. After each calf had been weighed and a blood sample collected at 15 minutes post-partum it was assisted to suckle colostrum to satiation from its own dam as described in Section 1. Each calf was then reweighed and subsequent to this single feed of colostrum, it was immediately removed to the large, straw-bedded loose-box which had been selected as the rearing accommodation.

Subgroup D: five calves, born in loose-boxes. Each calf was allowed to remain with its dam for 48 hours. No attempt was made to encourage the calves to suckle or to interfere in any way except to weigh and to collect a blood sample from each calf at 15 minutes post-partum. At 48 hours post-partum the calf was removed to the rearing accommodation.

Subgroup E: five calves, born in the holding byre. Each calf was weighed and a blood sample collected at approximately 15 minutes post-partum. It was then removed from the byre and held in separate, clean accommodation until approximately eight hours post-partum. At this time it was encouraged to suckle 1700 ml (3 pints) of its own dam's colostrum from a Rose-Miller teat funnel. Having been fed colostrum each calf was then placed in the rearing accommodation along with the other calves. Occasionally the length of time a calf remained in the byre exceeded 15 minutes but was never more than one hour.

The three groups of calves (Groups 10, 11, 12) were born over an eight month period, from December 1972 to July 1973; the 15 calves of Group 10 were born over a 21 day period from 17/12/72 to 7/1/73; the 15 calves of Group 11 were born over a 15 day period from 28/4/73 to 12/5/73; the 15 calves of Group 12 were born over a 31 day period from 26/6/73 to 26/7/73. The results for the five calves subjected to the same treatment in each of the three Groups 10, 11 and 12 were collated to form three composite groups, each of 15 calves.

Thus group C comprised 15 calves, 111, 112, 113, 118, 120 (Group 10), 125, 129, 133, 134, 135 (Group 11), and 141, 142, 144, 147, 148 (Group 12) treated as described for subgroup C.

Group D comprised 15 calves, 107, 109, 110, 117, 121 (Group 10), 122, 123, 124, 130, 132 (Group 11), and 137, 138, 139, 143, 146 (Group 12) treated as described for subgroup D.

Group E comprised 15 calves, 108, 114, 115, 116, 119 (Group 10), 126, 127, 128, 131, 136 (Group 11), and 140, 145, 149, 150, 151 (Group 12) treated as described for subgroup E.

Results

The mean values for the 48-hour serum concentrations of absorbed immunoglobulins, the colostral whey immunoglobulin concentrations and the birth weights for the three subgroups are presented in Table 8. The full individual results are presented in Appendix 2.

The 48-hour serum concentrations of absorbed immunoglobulins for the 15 calves of group C, varied from 11 ZST units (calf 144) to

TABLE 8

The absorption of colostral whey immunoglobulins by newborn calves.

Group C - 15 calves assisted to suckle colostrum to satiation immediately after birth

Group D - 15 calves allowed to remain with their dams for 48 hours - No interference

Group E - 15 calves fed 1700ml of their own dam's colostrum at eight hours post-partum

	Group C	Group D	Group E
Number of calves	15	15	15
48-hour serum concentration of absorbed immunoglobulins - ZST units *	28.13 ± 9.86	22.20 ± 11.65	15.00 ± 8.18
Colostral whey immunoglobulin concentration - g/100ml*	8.49 ± 2.21	8.20 ± 2.29	7.30 ± 3.50
Birth weight - kg*	31.45 ± 3.89	35.74 ± 4.45	31.43 ± 4.18
Weight of colostrum ingested - kg*	2.25 ± 0.63	ND	-
Volume of colostrum ingested - ml.	-		1700
Time from birth to completion of suckling - minutes*	63.13 ± 8.33	ND	494.8 ± 131.2

*Mean ± SD

ND - Not determined

44 ZST units (calf 135) with a mean of 28.13 $\pm$ 9.86 units. In group D, the minimum value was 1 ZST unit (calf 123) and the maximum value was 49 ZST units (calf 107) with a mean of 22.20 $\pm$ 11.65 ZST units. In group E, the minimum value was 2 ZST units (calves 115 and 151) and maximum value was 27 ZST units (calf 114) with a mean of 15.00 $\pm$ 8.18 ZST units. There was no significant difference in the concentrations of absorbed immunoglobulins between groups C and D or between groups D and E, but a highly significant difference ($p < 0.001$) existed between the mean 48-hour serum concentrations of absorbed immunoglobulins of groups C and E. Within each of Groups 10, 11 and 12 the mean ZST value for the five calves of subgroup C exceeded the mean ZST value of subgroup D, which, in turn, was greater than mean ZST value of subgroup E (Table 9), but no significant difference was demonstrated between the mean values.

The mean time from birth to completion of suckling by the calves of Group C was 63.13 $\pm$ 8.33 minutes. For the 15 calves of group E the length of time between birth and being fed colostrum varied from 4 hours 5 minutes (calf 114) to 13 hours 45 minutes (calf 136) with a mean of 494.8 $\pm$ 131.2 minutes (i.e. just over 8 hours). No continual observation of the calves of group D was maintained and the time to first suckling was not known.

Although the mean concentration of colostral whey immunoglobulins fed to the 15 calves of group E (7.30 $\pm$ 3.50 g/100ml) was lower than that of either group C (8.49 $\pm$ 2.21 g/100ml) or group D (8.20 $\pm$ 2.29 g/100ml) no significant difference existed between the three groups. There was also no significant difference in the birth weights between the three groups.

The results of this section tend to confirm the findings of Section 1 that the early feeding of colostrum to satiation almost always results in high serum concentrations of absorbed immunoglobulin; in group C 12 of the 15 calves had 48-hour serum concentrations of absorbed immunoglobulins of 20 ZST units or above; in group D 11 of the 15 calves had values of 20 ZST units or above; but in group E only four of the 15 calves had values of 20 ZST units or above (Appendix 2).

When the cows were assessed for shape for suckling by newborn calves, the number of cows which were considered to have a good shape

TABLE 9

48-hour serum concentrations of absorbed immunoglobulins of the three subgroups within Groups 10, 11, 12.
(ZST units)

	Calf No.	Subgroup C	Calf No.	Subgroup D	Calf No.	Subgroup E
Group 10	111	30	107	49	108	10
	112	20	109	29	114	27
	113	42	110	14	115	2
	118	40	117	27	116	17
	120	17	121	24	119	15
	Mean	29.80		28.60		14.20
	S.D.	± 11.32		± 12.78		± 9.20
Group 11	125	32	122	35	126	23
	129	28	123	1	127	22
	133	23	124	24	128	17
	134	26	130	22	131	18
	135	44	132	23	136	19
	Mean	30.60		21.00		19.80
	S.D.	± 8.17		± 12.35		± 2.59
Group 12	141	16	137	24	140	12
	142	38	138	13	145	26
	144	11	139	6	149	12
	147	28	143	15	150	3
	148	27	146	27	151	2
	Mean	24.00		17.00		11.00
	S.D.	± 10.65		± 8.51		± 9.64

for suckling was 8, 12 and 10 in groups C, D and E respectively. In dairy-type animals first calving heifers normally have a much better shape for suckling than multiparous cows (Selman, 1969), and in groups C, D and E the number of first calving heifers was 8, 11 and 9 respectively.

SECTION 3

THE ABSORPTION OF COLOSTRAL WHEY IMMUNOGLOBULINS BY NEWBORN CALVES:

The effect of variation in the concentration of immunoglobulins in the colostrum fed to newborn calves.

The relationship between the concentration of immunoglobulins in colostral whey and the subsequent serum concentration of absorbed colostral whey immunoglobulins was examined using the data for 100 calves assisted to suckle colostrum as soon as possible after birth. The 100 calves comprised the 51 calves of Groups 1, 2, 5, 6, 7, seven calves of Group 8 (82, 83, 84, 85, 89, 91, 95), five calves of Group 9 (96, 97, 98, 103, 106), five calves of Group 10 (111, 112, 113, 118, 120), five calves of Group 11 (125, 129, 133, 134, 135) and five calves of Group 12 (141, 142, 144, 147, 148) (Table 6). The remaining 22 calves (77-81, 104, 152-166 and 204) were reared individually. After each calf had been weighed and a blood sample collected at approximately 15 minutes post-partum, it was assisted to suckle colostrum to satiation from its own dam as described in Section 1. Having been fed colostrum once only each calf was immediately separated from its dam and moved to rearing accommodation. A blood sample was collected at 48 hours of age and the serum immunoglobulin concentration measured as previously described.

Results

A highly significant positive correlation ($r = 0.75$, $p =< 0.001$) was found to exist between the 48-hour serum concentrations of absorbed immunoglobulins of the calves and the concentration of immunoglobulins in the whey of the colostrum ingested by the calves (Figure 2). This highly significant positive correlation occurred despite the variations in the weight of colostrum ingested (0.8 - 4.1 kg) and the birth weights (18.1 - 46.1 kg) of the calves. There was also wide individual variation in the concentration of immunoglobulins in the whey prepared from the colostrum consumed by the calves from 1.58 g/100ml (calf 156) to 14.22 g/100ml (calf 15). Those samples of colostrum which had very low concentrations of immunoglobulins bore a greater resemblance to milk than the creamy, yellow gelatinous fluid of normal colostrum.

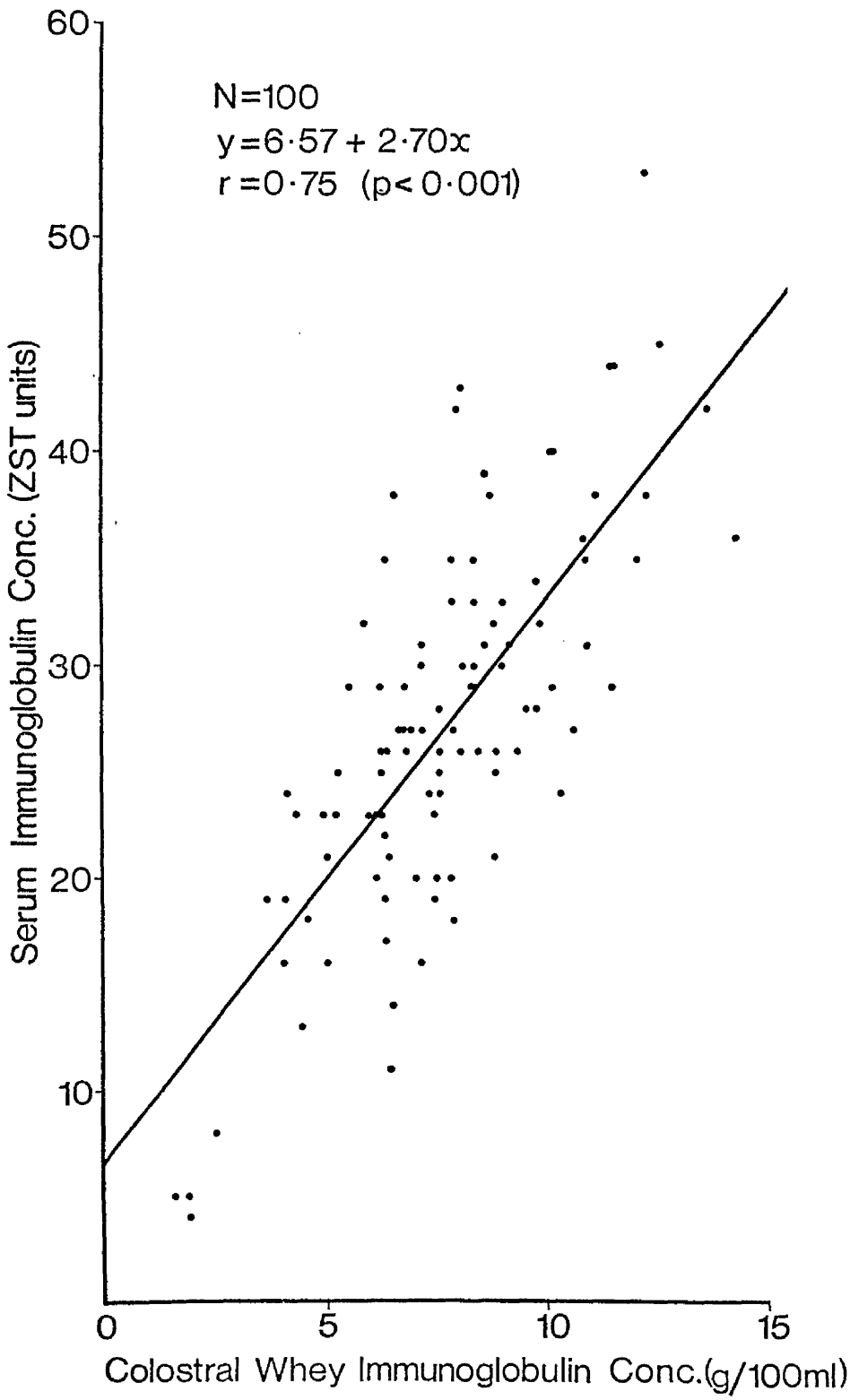


FIGURE 2 The relationship between the concentration of colostral whey immunoglobulins and the concentration of absorbed immunoglobulins of calves assisted to suckle colostrum to satiation.

The mean 48-hour serum concentration of absorbed immunoglobulins of the 100 calves was 27.17 ± 8.97 ZST units and the mean concentration of immunoglobulins in the whey of the colostrum ingested by the calves was 7.62 ± 2.49 g/100ml. The mean birth weight was 32.6 ± 5.23 kg and the mean weight of colostrum ingested was 2.29 ± 0.64 kg (Table 11). Expressed as a percentage of the birth weight the weight of colostrum ingested was 7.06 ± 1.79 per cent of the birth weight. The mean time from birth to completion of suckling by the 100 calves was 65.74 ± 22.94 minutes. Full individual results are presented in Appendix 2.

A total of 164 cows were calved under supervision to produce 168 calves; there were four sets of twins, calves 28/29, 63/64, 96/97 and 119/120. Cows of the Ayrshire breed formed the largest group of dams; there were 121 Ayrshire dams, 23 Friesian dams, 17 Ayrshirex Friesian dams, two Jersey dams and one Aberdeen Angus cross cow. Eighty-three of the 164 dams were judged to be first calving heifers.

The concentration of immunoglobulins in the colostral wheys varied enormously from 1.02 g/100ml to 14.22 g/100ml, but the mean concentration of immunoglobulins of 163 colostral wheys was 7.50 ± 2.49 g/100ml. The mean concentration of immunoglobulins in the colostral whey of the 120 Ayrshire dams was 7.39 ± 2.49 g/100ml. The 23 Friesian dams had a mean concentration of colostral whey immunoglobulins of 8.34 ± 2.38 g/100ml. The 17 AyrshirexFriesian dams had the lowest mean concentration of colostral whey immunoglobulins at 6.80 ± 2.25 g/100ml. The corresponding value for the two Jersey cows was 8.23 g/100ml (Table 10). The animal which had the lowest concentration of immunoglobulins in her colostral whey (1.02 g/100ml) was a first calving Ayrshire heifer and mother of calf 105; calf 105 (Group 9) was fed 1500 ml of colostrum at eight hours post-partum and its subsequent serum concentration of absorbed immunoglobulins at 48 hours of age was 6 ZST units. The cow with the highest concentration of immunoglobulins in her colostral whey (14.22 g/100ml) was an aged Ayrshire cow and the mother of calf 15; calf 15 (Group 2) was fed to satiation immediately after birth; it consumed 2.4 kg of colostrum and its serum concentration of absorbed immunoglobulins at 48 hours of age was 36 ZST units. The birth weights of calves 15 and 105 were 38.1 kg and 29.5 kg respectively.

TABLE 10

Breed distribution and colostral whey immunoglobulin concentration of 164 cows calved under supervision

Breed	Number of dams	Colostral whey immunoglobulin concentration (g/100ml *)
Ayrshire	121	7.39 ± 2.49+
Friesian	23	8.34 ± 2.38
Ayrshire x Friesian	17	6.80 ± 2.25
Jersey	2	8.23
Aberdeen Angus cross	1	11.43
TOTAL	164	7.50 ± 2.49+
Influence of parity		
Cows	80+	7.46 ± 2.68+
Heifers	83	7.54 ± 2.31
Ayrshire cows	69	7.19 ± 2.67
Ayrshire heifers	51	7.66 ± 2.19

* Mean ± SD

\+ No value for cow 88

The mean concentration of immunoglobulins in the colostral whey of 80 multiparous cows was 7.46 $\pm$ 2.68 g/100ml and the corresponding value for the 83 first calving heifers was 7.54 $\pm$ 2.31 g/100ml. On a breed basis, it was considered that only the Ayrshire breed comprised enough animals to make a valid comparison, there being 70 cows and 51 first calving heifers; the mean concentration of immunoglobulins in the whey of the colostrum produced by 69 Ayrshire cows was 7.19 $\pm$ 2.67 g/100ml and the corresponding value for the 51 Ayrshire heifers was 7.66 $\pm$ 2.19 g/100ml.

Six dams, the mothers of calves 62, 67, 73, 82, 115 and 156 "dripped" colostrum for several days immediately prior to parturition. The period of colostrum loss varied but was as long as six days in the case of dam 82. Once the loss had started it was usually continuous with the formation of pools of colostrum/milk in the stalls of those affected cows. The concentrations of immunoglobulins in the whey prepared from these colostra were 4.41 g/100ml (62); 1.87 g/100ml (67); 1.87 g/100ml (73); 4.32 g/100ml (82); 1.93 g/100ml (115) and 1.58 g/100ml (156). The mean concentration was 2.66 $\pm$ 1.32 g/100ml. The six animals consisted of five Ayrshire cows (62, 67, 82, 115, 156) and one first calving AyrshirexFriesian heifer (73). However, several other animals produced colostrum with concentrations of immunoglobulins in the colostral whey of less than 4 g/100ml without "dripping" colostrum including cow 105 which produced colostrum with the lowest concentration of immunoglobulins in the whey (26 - 3.67 g/100ml; 59 - 3.67 g/100ml; 79 - 2.48 g/100ml; 105 - 1.02 g/100ml; 108 - 3.58 g/100ml; 150 - 2.30 g/100ml).

SECTION 4

THE ABSORPTION OF COLOSTRAL WHEY IMMUNOGLOBULINS BY THE NEWBORN CALF:

The effect of breed.

The influence of breed on the absorption of colostral immunoglobulins by newborn calves demonstrated by Selman (1969) and Kruse (1970b) was examined under more practical farming conditions using the data for the 100 calves assisted to suckle to satiation immediately after birth described in Section 3.

Results

Pure bred Ayrshire calves formed the largest group, accounting for 43 per cent of the total. The AyrshirexFriesian calves formed 24 per cent, the Friesian calves 9 per cent, and the AyrshirexFriesianx Friesian calves formed 7 per cent of the total. Together these four breed groups accounted for 83 per cent of the 100 calves and reflect the dominant type and breeds of cattle in the west of Scotland. The mean values of the birth weights, weight of colostrum ingested, concentration of colostral whey immunoglobulins, and the 48-hour serum concentration of absorbed immunoglobulins of each breed group are presented in Table 11. The full individual results are presented in Appendix 2.

No statistically significant differences were found for any of the parameters examined between breed groups. The group of seven AyrshirexFriesianxFriesian calves had the lowest mean serum concentration absorbed immunoglobulins of 23.57 ± 11.43 ZST units. This group also ingested colostrum with the lowest mean concentration of immunoglobulins in the colostral whey of 6.87 ± 2.81 g/100ml and indeed one of the colostral wheys had an immunoglobulin concentration of only 1.87 g/100ml. The subsequent 48-hour serum concentration of absorbed immunoglobulins of the calf (73) suckling this colostrum was 5 ZST units.

All the Hereford cross calves were born to either Ayrshire or Friesian dams, there being seven Ayrshire dams and three Friesian dams. The five calves making up the mixed breeds consisted of one AyrshirexShorthorn (25), one AyrshirexFriesianxCharolais (71), one AyrshirexCharolais (80), one AyrshirexAberdeen Angus (129) and one

TABLE 11

100 calves assisted to suckle colostrum to satiation immediately after birth:

Breeds

Breed	Number of Calves	Birth Weight (kg*)	Mass of colostrum ingested (kg*)	Ig conc. of colostral whey ingested (g/100ml*)	48-hour SAIg serum absorbed Ig conc. (ZST units*)
Ayrshire	43	31.27 ± 4.92	2.24 ± 0.65	7.73 ± 2.23	26.91 ± 8.48
Ayrshirex Friesian	24	34.32 ± 5.21	2.54 ± 0.66	7.37 ± 2.70	26.75 ± 10.41
Friesian	9	35.63 ± 5.87	2.37 ± 0.69	8.24 ± 2.83	31.89 ± 6.17
Ayrshirex Friesianx Friesian	7	32.37 ± 4.25	2.00 ± 0.38	6.87 ± 2.81	23.57 ± 11.43
Ayrshire or Friesianx Hereford	10	33.43 ± 3.45	2.23 ± 0.65	7.88 ± 2.90	28.50 ± 9.83
Mixed breeds	5	33.36 ± 4.68	2.12 ± 0.29	6.96 ± 1.90	25.00 ± 4.00
Jersey	2	21.50	2.20	8.50	28.00
TOTAL 100	Mean	32.60	2.29	7.62	27.17
	S.D.	± 5.23	± 0.64	± 2.49	± 8.97

* Mean + S.D.

FriesianxAberdeen Angus calf (166).

The seven Friesian calves had the highest mean birth weight at 35.63 $\pm$ 5.87 kg and predictably the two Jersey calves had the lowest mean weight of 21.50 kg.

SECTION 5

THE ABSORPTION OF COLOSTRAL WHEY IMMUNOGLOBULINS BY THE NEWBORN CALF:

A comparison of the serum concentrations of absorbed immunoglobulins of intensively managed calves with those of "market" calves less than one week old.

The concentrations of serum immunoglobulins of 115 calves, purchased from local markets in the west of Scotland and admitted to the Veterinary School at less than one week of age, were compared to the serum concentrations of absorbed immunoglobulins of the 100 calves assisted to suckle colostrum as early as possible after birth and already described in Sections 3 and 4. As only one blood sample was collected from the "market" calves, the concentration of immunoglobulins in the serum of this sample was assumed to be the concentration of absorbed immunoglobulins.

Results

The serum concentrations of immunoglobulins of the 115 "market" calves varied from 0-50 ZST units and the mean serum concentration of immunoglobulins was 15.02 ± 11.91 ZST units. The corresponding value for the 100 calves assisted to suckle colostrum under the controlled conditions of the present experiments was 27.17 ± 8.97 ZST units. The difference between these two values is highly significant ($p<0.001$). The individual values for the "market" calves are given in Appendix 2.

The distribution histograms of the serum concentrations of immunoglobulins of both groups of calves are presented in Figures 3 and 4. There is an obvious difference between the two histograms; only 4 per cent of the intensively managed calves had serum immunoglobulin concentrations of less than 10 ZST units whereas 44 per cent of the "market" calves had values of less than 10 ZST units. The four intensively managed calves (67, 73, 79, 156) which had 48-hour absorbed serum immunoglobulin concentrations of less than 10 ZST units, (67, 4 ZST units; 73, 5 ZST units; 79, 8 ZST units; 156, 7 ZST units), all suckled colostrum which had very low whey immunoglobulin concentrations, 1.58 g/100ml (156), 1.87 g/100ml (67, 73) and

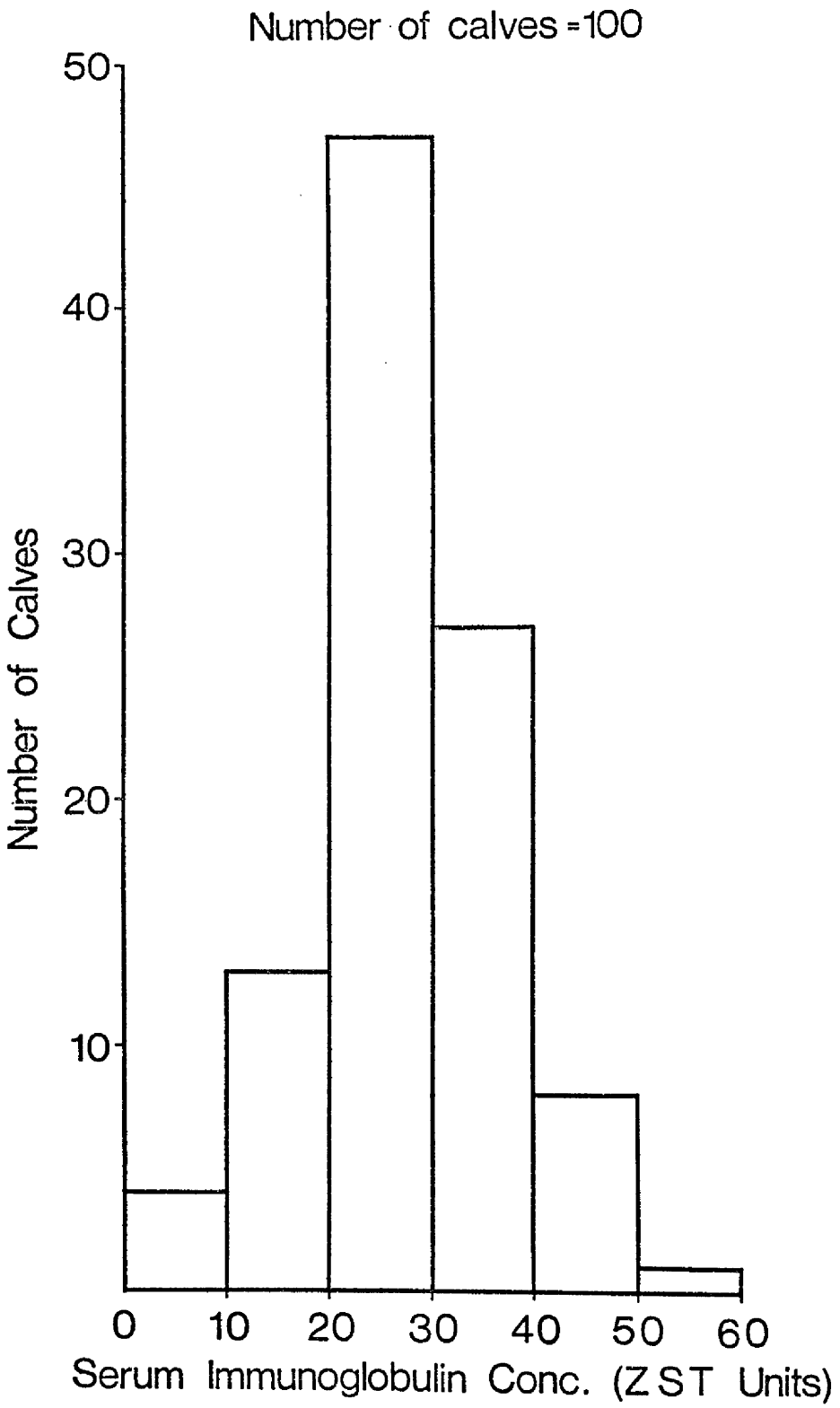


FIGURE 3 The distribution of the serum concentrations of absorbed immunoglobulins of 100 calves assisted to suckle colostrum to satiation.

2.48 g/100ml (79). Three (67, 73, 156) of the four cows had "dripped" colostrum prior to parturition. The weight of colostrum ingested by the four calves was 1.3 kg (79), 2.1 kg (73), 2.4 kg (67) and 2.5 kg (156).

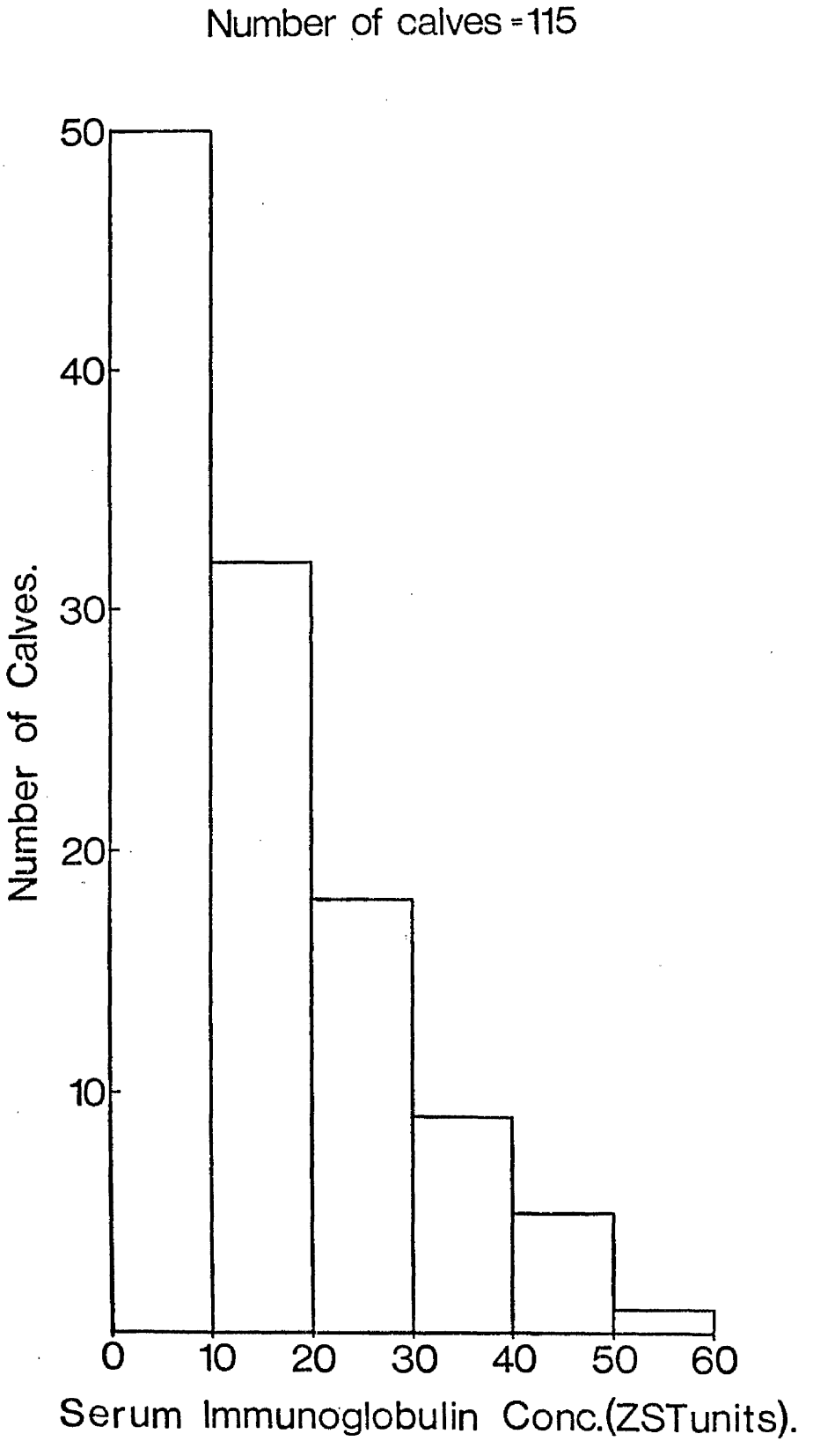


FIGURE 4 The distribution of the serum concentration of immunoglobulins of 115 "market" calves.

DISCUSSION

The zinc sulphate turbidity test has frequently been used to estimate the concentrations of immunoglobulins in the sera of newborn calves (Gay and others, 1965a, 1965b; McEwan and others, 1970a; Selman and others, 1971a,b; Boyd, 1972; Boyd and others, 1974; Logan and Gibson, 1975) and has proved to be a rapid, and quantitatively accurate method for the determination of absorbed colostral immunoglobulins. It is a technically simple test, requiring a minimum of laboratory equipment which gives consistent, repeatable results. A highly positive correlation has also been shown to exist between the zinc sulphate turbidity reaction and the total concentration of individual immunoglobulins determined by the much more sophisticated radial immunodiffusion technique (McEwan and others, 1970b; McBeath and others, 1971).

In Section 1 it was shown that no significant difference existed between the ZST values of the calves assisted to suckle colostrum to satiation once only immediately after birth (group A) and of the calves allowed to remain with their dams for 12 hours (group B). This is slightly surprising in view of the findings of Selman, McEwan and Fisher (1971b) who noted that when newborn calves were fed pooled colostrum under standardised conditions, calves which were "mothered" i.e. allowed to remain with their dams for 18 hours, had significantly higher concentrations of absorbed immunoglobulins than calves which were maintained in isolation. Although it was not known how many calves of group B suckled of their own volition during the 12 hour period that they were allowed to remain with their dams, the fact that only 11 of the 25 calves could be encouraged to suckle at 12 hours post-partum was taken as an indication that the remaining 14 had suckled at some time during this period. Certainly it has been claimed that repeated feeding of colostrum during the first 12 hours increases the efficiency of absorption (Kaeckenbeeck, Colinet and Schoenaers, 1961; Kaeckenbeeck and Schoenaers, 1964) although Selman (1969) was unable to demonstrate any significant difference in absorptive efficiency of calves fed a standard volume of colostrum as one feed at one or five hours post-partum with the same volume fed in three divided meals at 1, 5 and 9 hours post-partum. Nonetheless the ZST

values attained by the calves given colostrum in three meals were more consistent with considerably less variation.

Further examination of those calves with ZST values of 30 units or more (group A, calves 1, 2, 4, 8, 10, 15, 18, 19; group B, calves 26, 30, 31, 33, 34, 36, 40, 42, 48, 49, 50) reveals that the mean concentration of immunoglobulins in the colostral whey ingested by the eight calves of group A was 9.21 $\pm$ 2.63 g/100ml and that for the 11 group B calves was 7.71 $\pm$ 2.19 g/100ml. Only two of these 11 group B calves (26, 31) could be encouraged to suckle at 12 hours post-partum; calf 26 ingested 5.9 kg of colostrum under supervision, with a colostral whey immunoglobulin concentration at the first suckling period of 3.67 g/100ml and had a 48-hour absorbed ZST value of 34 units; calf 31 ingested 3.4 kg of colostrum with a whey immunoglobulin concentration of 10.84 g/100ml under supervision and had a 48-hour absorbed ZST value of 38 units. Thus there is some indication that the combined effects of "mothering" and the possible ingestion of more colostrum may result in higher absorbed ZST values but under practical farming conditions the additive effect of these is likely to be minimal compared with results obtained from a single large feed of colostrum.

There is little doubt that maternal deprivation has some deleterious effect on the absorption of colostral immunoglobulins in certain species. It has been shown that the separation of ten day old rats from their mothers leads to the early cessation of macromolecular absorption (Halliday, 1959) and a similar effect has also been demonstrated in newborn puppies (Filkins and Gillette, 1966). It has been suggested that maternal deprivation may result in increased levels of circulating corticosteroid as it has been noted that treatment with exogenous corticosteroid causes precocious termination of immunoglobulin absorption in species such as the rat (Halliday, 1959; Clark, 1969; Morris and Morris, 1974a, 1976a) mouse (Moog, 1953), and piglet (Payne and Marsh, 1962). Although the blood levels of these parenterally administered cortocosteroids were much higher than normal physiological concentrations, it is likely that endogenous corticosteroid also exerts some mediating effect on "closure". It has also been demonstrated that bilateral adrenalectomy of 15-18 day old rats delays the cessation of macromolecular absorption by approximately four days, but, nevertheless even in the absence of

adrenal glands "closure" proceeds normally after this delay (Daniels, Hardy and Malinowska, 1973). Gillette and Filkins (1966) were unable to demonstrate any effect on the absorption of antibodies following parenteral treatment of newborn puppies at birth with corticosteroid, but reduction in absorption occurred when corticosteroid was administered parenterally to bitches in late pregnancy. Similarly, in cows, induction of parturition with corticosteroids has been shown to reduce the absorption of colostral whey immunoglobulins by the calves produced by this technique (Husband, Brandon and Lascelles, 1973). Gillette and Filkins (1966) suggested that the difference in the effect of parenterally administered corticosteroid in young rats and puppies was due to the relatively short period of macromolecular absorption of 24-36 hours in the newborn puppy. The time required for exogenous corticosteroid to induce termination of absorption in the young rat is 2-3 days (Halliday, 1959; Clark, 1969). In consequence, given the even shorter period of macromolecular absorption of 18-27 hours in the calf (Selman, 1969; Penhale and others, 1973), it is probable that the effect of maternal deprivation will be minimal.

The results of Section 2 confirm the findings of several workers that any delay in the feeding of colostrum inevitably results in the reduced absorption of colostral immunoglobulins (Selman, 1969; Kruse, 1970b; Penhale and others, 1973). There was no significant difference between the 48-hour absorbed ZST values of the 15 calves of group C fed to satiation immediately after birth (28.13 ± 9.86 ZST units) and of the 15 calves of group D which were allowed to remain with their dams for the first 48 hours after birth (22.20 ± 11.65 ZST units). The values attained by the calves of group C were more consistent with no calf having a 48-hour ZST value of less than 10 ZST units, whereas two calves of group D had 48-hour ZST values of less than 10 ZST units (calf 123 - 1 ZST unit, calf 139 - 6 ZST units), even although the concentrations of immunoglobulins in the colostral whey were 4.95 g/100ml (calf 123) and 9.42 g/100ml (calf 139). That 11 of the 15 dams of group D were primiparous heifers may have effected early suckling by many of the calves in this group and increased absorption (Selman, 1969; Kruse, 1970c) but the mean value for the 15 calves of group D is very similar to that reported by Selman and others (1970a) for 20 calves also allowed to remain with their dams for 48 hours, but where no account was taken of any possible variation

in the concentrations of immunoglobulins in the colostral whey. Although a highly significant difference was found to exist between the mean 48-hour absorbed ZST values of the 15 calves of group C and the 15 calves of group E which were fed 1700 ml of colostrum at approximately eight hours post-partum (15.00 $\pm$ 8.18 ZST units), the values attained by the calves of group E were higher than might have been anticipated from field studies. Selman and others (1971a) recorded a mean ZST value for dairy heifer calves separated from their dams at birth, or when found, was 8.8 ZST units. In the present study every effort was made to ensure that the calves of group E ingested all the colostrum offered to them, by feeding them with a Rose-Miller teat funnel and not from a bucket.

Under natural conditions Selman and others (1970b) recorded that the time to first suckling varied from a mean of 81.4 minutes for eight calves born to beef cows to a mean of 261.1 minutes for seven calves born to dairy cows. They also noted that seven of the 30 calves observed failed to suckle at any time during the first eight hours after birth. In the present study the mean time from birth to the completion of assisted suckling for the 15 calves of group C was 63.13 $\pm$ 8.3 minutes and for the 100 calves assisted to suckle colostrum to satiation it was 65.74 $\pm$ 22.94 minutes. When Selman and others (1970b) examined the influence of dam shape on the time of first suckling, they found that the mean time to first suckling by nine calves born to "good-shaped dams" was 79.4 $\pm$ 41.8 minutes. This, of course, does not imply that the calves suckled to satiation at this first suckling period. Studying the post-lambing behaviour of Barki ewes and lambs Sharafeldin and Kandeel (1971) recorded a mean time to first suckling of 29.5 minutes. They also noted that the time to first suckling for lambs born to primiparous ewes was nearly twice that for lambs born to multiparous ewes. Ducker and Fraser (1973) demonstrated that lambs born to housed ewes and managed intensively in the period immediately after birth had significantly higher serum concentrations of absorbed immunoglobulins than lambs which had no intensive post-partum management. However, they were unable to demonstrate any difference in the serum concentrations of absorbed immunoglobulins between lambs, born indoors and managed intensively, and lambs born outdoors under natural conditions.

Although it had been suggested that no correlation exists between the concentration of immunoglobulins in colostral whey fed to calves and the subsequent serum concentrations of absorbed colostral immunoglobulins (Fey and Hunyady, 1962; Smith and others, 1967; Klaus and others, 1969; Penhale and others, 1973), a detailed investigation by Selman (1969) demonstrated that a highly significant correlation existed between the concentration of immunoglobulins in the colostral whey and the 48-hour serum concentration of absorbed colostral immunoglobulins when calves were fed on a weight-basis and at the same time post-partum. The highly significant correlation ($r = +0.75$, $p < 0.001$) between the colostral whey immunoglobulin concentration and 48-hour absorbed ZST values for 100 calves assisted to suckle to satiation immediately after birth demonstrated in Section 3 (Figure 2) confirms this finding. There are two possible reasons why the intercept on the Y-axis is 6.57 when it is known that the precolostral ZST values rarely exceed 2 ZST units (Selman, 1969). Firstly, because of the wide variation in the weight of colostrum ingested (0.8-4.1 kg) the mass of immunoglobulins presented to the calves will have varied enormously, and Kruse (1970b) has shown that more than 50 per cent of the variation in the serum concentrations of absorbed colostral immunoglobulins is directly attributable to the mass of immunoglobulins ingested by the calf. Secondly, at serum gammaglobulin concentrations of greater than 30 mg/ml, the relationship between serum gammaglobulin concentration and zinc sulphate turbidity test is not linear (McEwan and others, 1970b) and therefore the zinc sulphate turbidity test is likely to underestimate high serum immunoglobulin concentrations. It is extremely unlikely that any variation in the proportion of immunoglobulins to the total protein in the colostral whey contributed to the error as this proportion remains relatively constant (Pierce, 1962; Selman, 1969).

Four calves (67, 73, 79, 156) remained severely hypogammaglobulinaemic (less than 10 ZST units) even although all four calves were assisted to suckle to satiation and the longest time from birth to completion of suckling was 85 minutes (calf 79). The mean weight of colostrum ingested by the four calves was 2.07 ± 0.54 kg which was slightly less than the mean weight ingested by all 100 calves (2.29 ± 0.65 kg). The mean concentration of immunoglobulins in the whey prepared from the colostrum ingested by the four calves was 1.95 ± 0.37

g/100ml, only 26 per cent of the mean of 7.50 g/100ml for all 100 cows.

The problem of hypogammaglobulinaemia is not restricted to the dairy calf. Logan and others (1974a) noted that 23 per cent of single-suckled calves born indoors to beef cows which were loose-housed in the traditional Scottish court system, were hypogammaglobulinaemic, i.e. less than 5 mg/ml IgG and 1.1 mg/ml IgM (Penhale and others, 1973) and were especially deficient in IgM. By introducing a strict management regime for the supervision of parturition and by increasing the area allowed per cow, the incidence of hypogammaglobulinaemia was reduced to nil (McBeath and Logan, 1974). There was no significant increase in the mean serum concentration of IgG, but the mean serum concentration of IgM was increased by 80 per cent and that of IgA by 21 per cent. The authors suggested that these differences were due to the differential rates of intestinal "closure" for the different classes of immunoglobulin (Penhale and others, 1973). Moreover, Logan and Gibson (1975) found that 42 per cent of calves born to old beef cows (i.e. more than eight pregnancies) had serum immunoglobulin concentrations of less than 20 ZST units which the authors attributed to low pendulous udders of the dams with a consequent delay in time to first suckling. In a study of the serum concentrations of absorbed immunoglobulins in Merino and Scottish Blackface lambs, Halliday (1974) found that 12.5 per cent of twin or triplet lambs born to ewes aged seven years or more were agammaglobulinaemic when sampled at 48 hours of age. Although the author suggested this may have been due to a decline in colostrum production, it is much more likely that it was a direct result of poor udder conformation and/or poor teat shape.

The concentration of immunoglobulins in the colostral wheys fed to the 100 calves assisted to suckle to satiation varied from 1.57 g/100ml to 14.22 g/100ml and for the 162 colostral samples examined in this thesis the range was 1.02 g/100ml to 14.22 g/100ml. This wide individual variability in the immunoglobulin concentration has been noted previously (Fey and Hunyady, 1962; Selman, 1969; Kruse, 1970a; Logan, 1977) and is unrelated to feeding (Steinbach and Meyer, 1965; Logan, 1977), or to the season of the year (Selman, 1969; Kruse, 1970a).

A computer simulation study led Kruse (1970c) to suggest that newborn calves should ingest a minimum of 100g of colostral immunoglobulins within five hours of birth to reduce the possibility of hypogammaglobulinaemia. Thus, given the variation in the concentration of immunoglobulins, the yield of colostrum becomes important. Larson and Kendall (1957) recorded an average yield of 10.2 kg of colostrum for eight heifers and cows during the first 24 hours after parturition and Steinbach and Meyer (1965) found an average yield of 3.9 kg of colostrum at the first milking after parturition for 12 heifers with a range of 0.3 to 8.3 kg. Kruse (1970a) found that cows of the Danish red breed produced significantly more colostrum at first milking after parturition than cows of either the Danish black and white breed or Jersey breed. Selman (1969) demonstrated a wide variation in the volume of colostrum produced by 20 Ayrshire cows and heifers machine milked within five hours of parturition from 3.0 litres to 16.0 litres with the consequent variation in total immunoglobulin yield from 85g to 1660g. Similarly, Logan (1977) recorded a range of yields of colostrum from 22 crossbred beef cows at first milking of 100ml to 3500ml and the total production of immunoglobulins (IgG, IgM, IgA) varied from 7.33g to 236.6g.

In the present study the vast majority of cows produced sufficient colostrum to satisfy the calves, but several calves (47, 104, 144) consumed all the available colostrum without being satiated. The size and shape of the teats was the only other major factor in the present study which reduced the intake of colostrum. Several dams, especially first calving heifers (9, 70, 158), had very small teats which made prehension very difficult. Very large distended teats also made suckling difficult, as occurred in the case of calf 27 and noted previously by Logan and others (1974a).

There was no significant difference in the concentration of immunoglobulins in the colostral whey of the three major groups of breeds or crosses of cows in the present experiments, although the low numbers of Friesian dams and AyrshirexFriesian dams make it difficult to draw any firm conclusions. The 23 Friesian dams had the highest mean concentration of 8.66 ± 2.27 g/100ml. Kruse (1970a) reported that although there was no significant difference in the total yield of colostral immunoglobulins produced by either the Danish red breed

or the Danish black and white breed, a negative correlation existed between the yield of colostrum and the concentration of immunoglobulins in the whey; the Danish black and white breed had higher concentrations of colostral immunoglobulins, but yielded significantly lower volumes of colostrum than the Danish red breed. However, Logan (1977) was unable to demonstrate any correlation between the yield of colostrum and the concentration of immunoglobulins in the whey of 22 cross-bred beef cows. The mean whey immunoglobulin concentration of 7.39 ± 2.49 g/100ml in the 120 colostral samples collected from Ayrshire dams was very similar to the value of 7.53 ± 3.33 g/100ml recorded by Selman (1969) for 20 Ayrshire dams. Kruse (1970a) demonstrated that first calving heifers of the Danish red breed, but not of the Danish black and white breed, had significantly lower colostral whey immunoglobulin concentrations than cows which had had four or more calves, but, because of the lower volume of colostrum produced, first calving heifers of both breeds had significantly lower yields of immunoglobulins than multiparous cows. In the present study there was no significant difference between the colostral whey immunoglobulin concentrations of the 83 first calving heifers and the 80 multiparous cows of all breeds. Furthermore, no significant difference was found between the colostral whey immunoglobulin concentrations of 51 first calving heifers and the 69 multiparous cows of the Ayrshire breed.

Although the concentration of immunoglobulins in colostral whey appears to be unrelated to the feeding of the cows (Parrish, Wise, Hughes and Atkeson, 1948; Steinbach and Meyer, 1965; Logan, 1977), diet is likely to have a major influence on the yield of colostrum and thus on the total yield of immunoglobulin available to the newborn calf. This effect will be much more critical in beef cows kept on a minimal diet and Logan (1977) found that outwintered beef cows receiving no supplementary feeding gave significantly less colostrum than beef cows housed and fed silage ad lib. The pre-partum loss of colostrum is a major cause of low colostral whey immunoglobulin concentrations and was responsible for the very low values in four cows (67, 73, 115, 156) in the present experiments, but two other dams (79, 105) also produced colostrum with very low immunoglobulin concentrations without leaking colostrum. Pre-partum milking also reduces the immunoglobulin content of the colostrum

(Hill, Widdowson and Maggs, 1950; Aschaffenburg, Bartlett, Kon, Roy, Walker, Briggs and Lovell, 1951; Rowland, Roy, Sears and Thompson, 1953), and Kruse (1970a) was unable to define any other factors which might be responsible for low colostral whey immunoglobulin concentrations.

There are many reports of breed variation of susceptibility to neonatal disease (Blakemore and others, 1948; Withers, 1952 Leech and others, 1968; Oxender and others, 1973; Larouche and Black, 1973) although Aschaffenburg and others (1952) showed that colostrum-deprived Friesian and Dairy Shorthorn calves had similar mortality rates. Selman and others (1971b) demonstrated that FriesianxAyrshire calves absorbed significantly more whey immunoglobulins than Ayrshire calves managed under identical conditions. Therefore the findings of Section 4 in which no obvious significant difference could be demonstrated between the various breeds and crosses are interesting and suggest that the effect of any breed variation in absorptive efficiency is likely to be minimal and of little practical importance. Kruse (1970b) suggested that the poorer absorptive ability of the Danish red breed calves compared to the Danish black and white breed calves was a genetic variation. In a series of unstandardised experiments Halliday (1966) suggested that Finnish Landrace lambs had a greater absorptive efficiency than either MerinoxCheviots or Scottish Blackface lambs. However, later work by the same author (Halliday, 1971, 1974) recorded no significant difference in the immunoglobulin concentrations of two day old Merino and Blackface lambs, and that the higher serum immunoglobulin concentrations of Finnish Landrace lambs were attributable to the much smaller weight (and therefore plasma volume) of Finnish Landrace compared to Scottish Blackface, Merino and MerinoxCheviot lambs and possibly the slightly higher protein concentrations of Finnish Landrace colostrum (Halliday, 1968). Halliday (1968, 1970) also noted that the serum immunoglobulin concentrations of Blackface and MerinoxCheviot lambs were negatively correlated with litter size whereas the concentration in Finnish Landrace twins and triplets were as high as those of single lambs. This again is probably a reflection of the low weight of Finnish Landrace lambs and the volume of colostrum produced by this breed which is noted for its prolificacy and milkiness.

Examination of the two distribution histograms in Section (Figures 3 and 4) reveals that almost 44 per cent of the "market" calves were severely hypogammaglobulinaemic (less than 10 ZST units) compared to only 4 per cent of those calves assisted to suckle to satiation immediately after birth. The distribution of the serum immunoglobulin concentrations of "market" calves in this survey is virtually identical to that of Gay and others (1965a, 1965b) and McEwan and others (1970a) who also found that approximately 45 per cent of "market" calves were severely hypogammaglobulinaemic. It was also shown that almost 60 per cent of these severely hypogammaglobulinaemic calves eventually died of either colisepticaemia or neonatal diarrhoea. The majority of the "market" calves in the present study were Ayrshire bull calves but any suggestion that, because this type of calf has a very low commercial value, they receive minimal post-parturient care is refuted by the field survey of Selman and others (1971a) who demonstrated that a high proportion of dairy heifer calves retained on the farm of birth also had very low serum immunoglobulin concentrations.

The findings of Chapter 2 clearly demonstrate that the suggestion that some calves are congenitally unable to absorb colostral immunoglobulins (Fey, 1962; Gay and others, 1965a; Klaus and others, 1969) is no longer tenable and that the early feeding of colostrum to satiation will result in at least 80 per cent of calves achieving acceptable concentrations of passively acquired immunoglobulins (greater than 20 ZST units). It should be noted that a small proportion (less than 5%) of calves may remain severely hypogammaglobulinaemic despite early feeding and ingesting a considerable weight of colostrum (greater than 2 kg) because of low concentrations of immunoglobulins in the colostrum. It has also been demonstrated that second or subsequent feeds of colostrum and any effect due to breed variation are likely to be of little practical importance.

THE RELATIONSHIP BETWEEN ABSORBED IMMUNOGLOBULINS, AD LIBITUM FEEDING OF MILK SUBSTITUTE, LIVEWEIGHT GAIN, AND THE INCIDENCE OF DIARRHOEA DURING THE FIRST FOUR WEEKS OF LIFE

INTRODUCTION

In the major surveys carried out in neonatal calf mortality "scour" has been listed either as a major clinical sign or as a cause of death (Withers, 1952, 1953; Leech and others, 1968). Although overfeeding, either with whole milk or milk substitute, has frequently been cited as one of the main contributory factors of neonatal calf diarrhoea (Blaxter and Wood, 1953; Morrison, 1959; Inglis, 1960; Loosemore, 1964; Roy, 1964), Mylrea (1966) found that there was no difference in the dry matter content of faeces produced by calves on an unrestricted whole milk diet or by calves on a more conventional restricted diet. The calves on the restricted diet were fed whole milk at ten per cent bodyweight daily whilst those calves on an unrestricted diet consumed up to approximately 25 per cent of their bodyweight daily. However, using a small number of calves in a limited experiment, Wise and La Master (1968) found that calves on an unrestricted milk diet had a higher incidence of diarrhoea than calves on a restricted diet. Furthermore, Leaver and Yarrow (1972) recorded that calves, on a once-daily feeding regime, consuming 3200 ml of milk-substitute had a significantly higher incidence of diarrhoea than calves receiving only 1600 ml once daily.

The inter-relationship between housing, nutrition and the incidence of diarrhoea in the young calf received considerable impetus from the work of Roy and his colleagues who found that the incidence of diarrhoea within a calfhouse was directly proportional to the length of time the calfhouse was occupied (Roy and others, 1955a). Wood (1955) suggested that as the period of continuous occupancy progressed a "build-up of infection" occurred within the calfhouse. Roy and others (1955a) considered that this "build-up of infection" occurred at a much slower rate when calves were fed whole milk than when calves were fed a milk substitute.

Shillam, Roy and Ingram (1962a) compared the effect of whole milk and synthetic milk diets on the incidence of diarrhoea and mortality in young calves and concluded that the spray-dried skim-milk fraction of synthetic milk powders was responsible for the increased

mortality of calves fed this diet as a result of 50 per cent denaturation of the whey proteins during the drying process. Further studies confirmed that severe heat treatment of skim-milk, i.e. either 74°C for 30 minutes or 135°C for 1-3 seconds, prior to spray drying resulted in poor performances when fed to calves but that heating skim-milk to 77°C for only 15 seconds caused little denaturation of the whey proteins (Shillam and others, 1962b, 1962c). The addition of chlortetracycline (Shillam and Roy, 1963a), calcium (Shillam and Roy, 1963b), or selenium (Shillam and Roy, 1963c) had no influence on the detrimental effect of milk substitute powders based on skim-milk which had undergone severe heat treatment prior to drying. The poor response to such reconstituted milk substitute powders was associated with their poor clotting characteristics and poor protein digestion in the abomasum which resulted in an increased flow of undigested protein into the small intestine (Tagari and Roy, 1969).

The feeding of raw skim-milk directly to calves results in faeces with a very low dry matter content, an effect which appeared to be more marked in Ayrshire calves than Friesian calves (Roy, 1969). In calves fed whole skim-milk, an increased abomasal proteolysis occurs (Ternouth, 1971), but increasing the fat content of both milk substitutes and whole skim-milk reduces the incidence of diarrhoea (Roy, 1969; Roy, Stobo and Gaston, 1970; Lister, 1971; Leaver and Yarrow, 1972; Roy, Stobo, Gaston, Shotton and Ganderton, 1973). Non-milk proteins incorporated into milk substitute, such as soya bean meal (Smith, Hill and Sissons, 1970; Smith and Wynn, 1971; Roy and Ternouth, 1972; Ramsey and Willard, 1975) and fish meal protein (Huber, 1975) have been associated with a high incidence of diarrhoea in the young calf.

The type of housing has no significant effect on the incidence of diarrhoea in neonatal calves (Willet, Albright, Cunningham and Hinkle, 1968; Jorgenson, Jorgenson, Schingoethe and Owens, 1970). Similarly although there is some evidence that nipple feeding systems reduce the incidence and severity of neonatal calf diarrhoea (Hoyer and Larkin, 1954), most workers have failed to show any significant difference in the incidence of diarrhoea in calves on either open pail or nipple feeding systems (Kesler, McCarthy and Knodt, 1956; Wise and La Master, 1968; Oxender and others, 1973). The feeding of calves

once daily has become increasingly popular due to the reduction in labour costs and convenience, and it has been shown that under the same management conditions there is no significant difference in the incidence of diarrhoea in Ayrshire or Holstein calves fed milk substitute either once or twice daily (Burt, 1968; Ackerman, Thomas, Thayne and Butcher, 1969). Owen, Plum and Harris (1965) recorded a similar finding when calves were fed whole milk once or twice daily.

Although surveys have indicated that calves of the Ayrshire and Channel Island breeds are apparently more likely to die during the neonatal period (Blakemore and others, 1948; Withers, 1952c; Leech and others, 1968; Oxender and others, 1973; Larouche and Black, 1973), there is little evidence to suggest that there are any marked breed differences in the susceptibility to neonatal diarrhoea. Aschaffenburg and others (1952) noted that when deprived of colostrum there was no significant difference in either the incidence of diarrhoea or the mean age at death between Friesian or Shorthorn calves. However, Roy (1969) suggested that Ayrshire and Jersey calves were more susceptible to diarrhoea than Friesian calves and considered that this might be related to differences in the ability to digest protein.

Although the effects of milk substitute preparation, methods of feeding and type of housing on the incidence of diarrhoea in the neonatal calf have been widely studied the role played by passively acquired immunoglobulins has received little attention in many of these experiments, or has been very empirical. Thus, Roy (1969) recorded a much higher incidence of diarrhoea during the first three weeks of life in calves fed only 400ml of colostrum than in calves fed 3.4 litres of colostrum, when both groups were fed a milk substitute. Gay and others (1965a) found that although the incidence and severity of diarrhoea was not related to the concentration of serum immunoglobulins, those calves with high concentrations were unlikely to succumb to the effects of diarrhoea.

The opportunity was therefore taken to examine the relationship between the incidence and severity of diarrhoea and the serum concentrations of absorbed immunoglobulins in young calves which were reared in groups and had free access to an automatic calf feeder. Although automatic calf feeders are now widely used, there is little published work on the behaviour of calves reared in this system (Stephens, 1974; Hafez and Lineweaver, 1968), and one group of calves was observed for a continuous 24-hour period once weekly for five weeks.

MATERIALS AND METHODS

The 113 calves described in this section were born at the Veterinary School and every parturition was supervised. The aim was to have 14 or 15 calves being born during a two week period, but this was not always possible, and the 113 calves were assembled into nine groups the numbers in which varied from nine to 15 calves. They were born over a period of 25 months from June 1971 to July 1973.

Cows, calves and their management

Cows The animals referred to in this section were also those used in Chapter 2.

Briefly, the cows were Ayrshire, Friesian or AyrshirexFriesian cows or heifers admitted to the Veterinary School in late gestation. In the majority of cases, parturition took place in straw-bedded loose-boxes and were allowed to occur with the minimum of human interference. Immediately after parturition, samples of blood and colostrum were collected, and treated as described in Chapter 2.

Calves The nine groups of calves described in this section consisted of Groups 3, 4, 6, 7, 8, 9, 10, 11, 12 (Table 6).

The method, timing and volume of colostrum fed to each calf varied from group to group and even within groups and therefore each group of calves will be described separately. All calves were weighed at approximately 15 minutes post-partum as described in Chapter 2. A sample of blood was collected from the jugular vein at 15 minutes post-partum (pre-colostral sample) and at 48 hours post-partum (post-colostral sample). The serum immunoglobulin concentrations were estimated using the zinc sulphate turbidity test as described in Chapter 2. The serum concentration of absorbed immunoglobulins (ZST units) for each calf was determined by subtracting the precolostral value from the post-colostral value.

After the initial post-partum management procedures, all the groups were reared in a large, straw-bedded loose-box measuring 4.5m x 4.1m approximately and were fed milk-substitute (Vitamealo Calf Milk Meal, Beecham Agricultural Products, London) supplied from an

automatic calf feeder. Approximately 8-12 hours after the last feed of colostrum or whole milk, each calf was assisted to suckle 2-3 units of milk substitute (2-3 pints) from the automatic feeder. This was repeated approximately 12 hours later. Rarely, it was necessary to repeat this introductory training procedure for a third time.

The calves were observed at regular intervals throughout the day and once daily each calf was examined individually for the presence or absence of diarrhoea. No calf received any form of chemotherapy but care was taken to ensure that all calves appeared to be suckling the automatic feeder.

Faecal consistency The consistency of the faeces was assessed visually and classified on a score from 0 (normal) to 3 (profusely diarrhoeic). In order to determine the approximate dry matter content (DM) of each score, ten samples of each classification were collected. Two aliquots of each sample were weighed in a crucible and evaporated to constant dryness in an oven and the average dry matter content of each score was determined (Table 12). The mean values were 24.8% DM; 18.9% DM; 11.1% DM; and 4.5% DM for scores of 0, 1, 2 and 3 respectively. Previous workers have used other, but basically similar, classifications to define faecal consistency in calves. Blaxter and Wood (1953) classified faecal output as follows, 0.200g daily, normal; 200-500g daily, loose; and more than 500g daily, diarrhoeic. Logan and Penhale (1971a) classified faecal material with a dry matter content of 10% or less as diarrhoeic, as did Fisher and Martinez (1975a).

The calves were weighed once weekly on a Gascoigne calf weigher (Gascoigne, Berkeley Avenue, Reading) to the nearest pound and the value converted to kilograms. The 28-day weight of each calf was determined by calculating the average weight gained or lost per day during the seven day period in which day 28 fell and adding the appropriate sum to the previous week's weight.

Calf pencils (BOCM Calfwena pencils, BOCM/Silcock), hay, and clean water were freely available. No attempt was made to remove any soiled straw and the litter was allowed to build up, adding fresh straw when necessary. To improve drainage of the loose-box, wooden slats, approximately 9 cm high, were laid on the concrete floor, underneath the

TABLE 12

Dry Matter Content of Faeces

NORMAL FAECES

Sample No.	% Dry Matter		
	1	2	Mean
1	20.9	21.1	21.0
2	28.6	23.7	26.2
3	27.0	30.0	28.5
4	20.4	22.2	21.3
5	19.6	22.0	20.8
6	24.3	26.1	25.2
7	27.5	25.9	26.7
8	25.0	22.8	23.9
9	27.0	23.0	25.0
10	30.4	28.8	29.6
	Mean		24.83
	S.D.		± 3.09

FAECAL SCORE 1

Sample No.	% Dry Matter		
	1	2	Mean
1	18.0	19.0	18.5
2	20.0	17.1	18.5
3	18.6	19.3	18.9
4	19.1	16.0	17.5
5	21.0	24.2	22.6
6	19.0	17.0	18.0
7	12.6	17.0	14.8
8	21.8	20.0	20.6
9	26.5	27.3	26.9
10	14.5	11.6	13.1
	Mean		18.95
	S.D.		± 3.87

FAECAL SCORE 2

Sample No.	% Dry Matter		
	1	2	Mean
1	10.5	10.5	10.5
2	8.4	8.0	8.2
3	10.5	11.3	10.9
4	13.8	13.8	13.8
5	11.4	12.0	11.7
6	8.9	8.9	8.9
7	10.8	12.0	11.4
8	7.5	9.1	8.3
9	12.8	11.2	12.0
10	17.5	14.0	15.5
	Mean		11.13
	S.D.		± 2.35

FAECAL SCORE 3

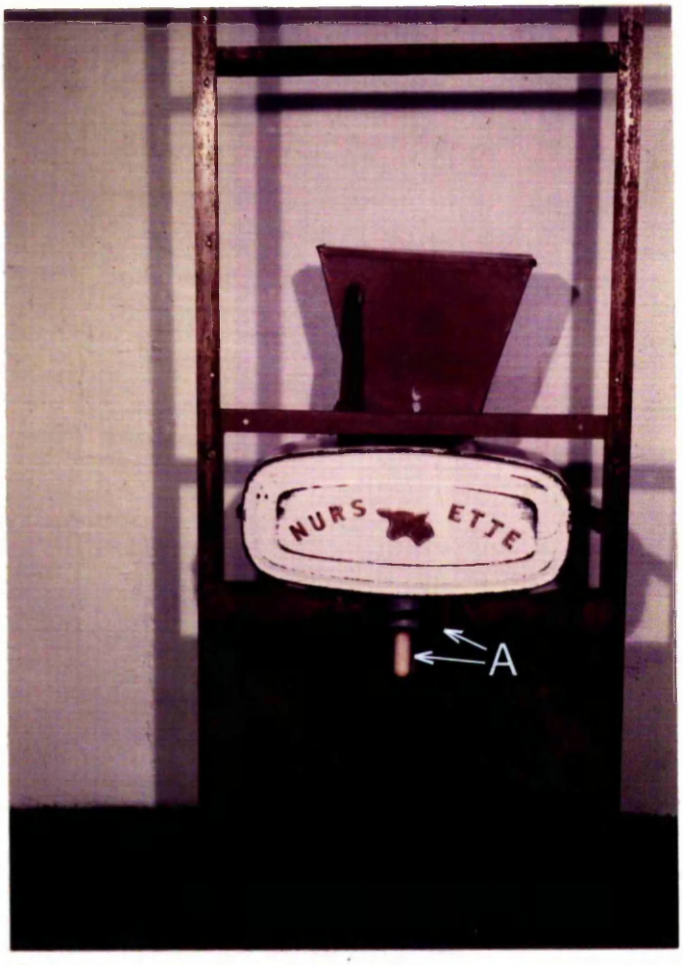
Sample No.	% Dry Matter		
	1	2	Mean
1	4.9	3.9	4.4
2	7.8	6.0	6.9
3	7.9	6.5	7.2
4	5.0	4.4	4.7
5	5.0	5.0	5.0
6	2.5	3.5	3.0
7	1.5	1.5	1.5
8	3.7	3.5	3.6
9	6.4	5.2	5.8
10	2.9	3.5	3.2
	Mean		4.54
	S.D.		± 1.79

straw. Prior to the introduction of Groups 3, 4, 6, 7, 8 and 9, the loose-box was thoroughly cleansed and fumigated with formaldehyde gas. The loose-box was not fumigated before the introduction of Groups 10, 11 and 12 but it was thoroughly cleaned and disinfected.

Automatic calf feeder The Nursette automatic calf feeder as used (Figure 5) consisted of a small water heating unit, designed to heat water to 100°F, a hopper containing dry milk substitute powder with a mechanism to dispense a set quantity of powder, and a mixing/holding bowl. The machine could supply a variable quantity of milk substitute solution. The quantity of solution supplied was determined by the position of two electrodes situated in the mixing bowl, the high and low level electrodes. In the present study, the machine was set to deliver 570 ml (1 pint) of solution at 12% total solids. When the level of solution fell below the low level electrode, the mixing cycle was initiated. The predetermined quantity of milk substitute powder was delivered from the hopper which was agitated by a vibrator motor. The powder was mixed with the remaining fluid in the holding bowl before the addition of more pre-heated water to the level of the high level electrode. The mixing cycle took approximately three minutes to complete. During the mixing cycle a stop valve was activated, which prevented the milk solution being drawn off through the rubber teat, and each freshly mixed batch of milk substitute solution had to be consumed before another mixing cycle began. The machine was cleaned thoroughly once daily. Intermittently, the mixing cycle was not initiated when the fluid in the mixing/holding bowl fell below the low level electrode. This fault was normally corrected by thoroughly re-cleaning the working parts within the mixing-bowl; ensuring that both the high and low level electrodes were scrupulously clean. Occasionally the milk substitute powder would bridge in the hopper which resulted in calves being supplied with warm water alone. This may not have been a fault in the machine or the small vibrator motor on the hopper, but was probably an inherent fault of the powder, related to its free-running properties. Bridging appeared to happen more frequently in humid weather.

Behavioural studies

The 14 calves of Group 8 (Table 6) which were reared on the automatic feeder were observed for a continuous period of 24 hours once



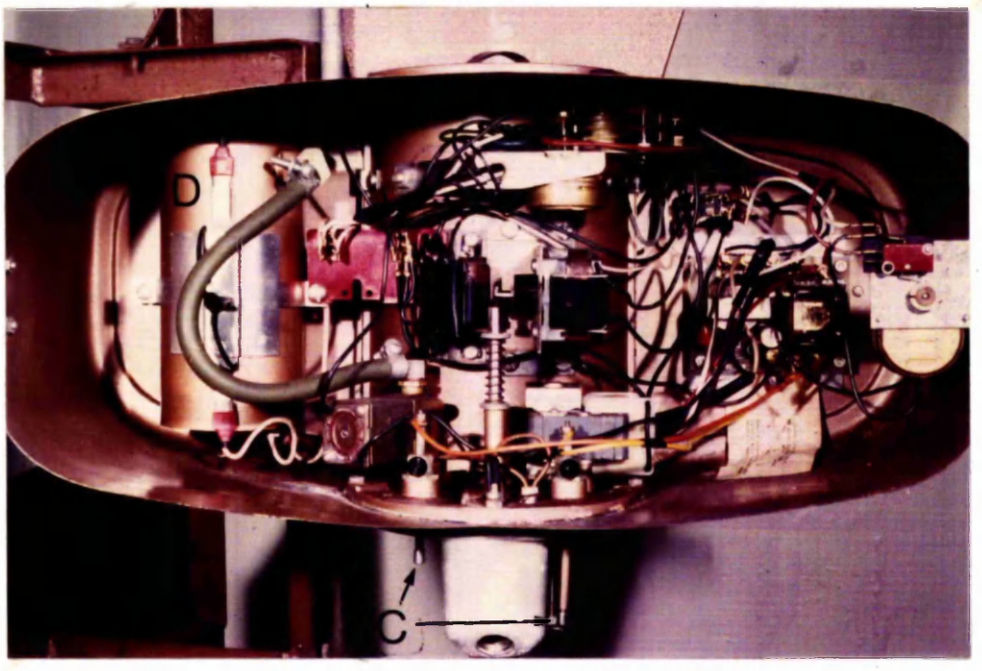


FIGURE 5 The Nursette automatic calf feeder:

A - Holding bowl and teat
B - Milk substitute powder hopper
C - High and low level electrodes
D - Water heater

weekly for five weeks. The first observation period was delayed until the youngest calf was one week old to allow complete familiarisation with the automatic feeder. The observations were carried out under continuous illumination, i.e. during the night the lights were switched on. To aid identification, all 14 calves wore numbered neck straps (Dalton Supplies Ltd., Nettlebed, Henley-on-Thames, Oxon).

As stated above the automatic calf feeder was set to deliver 570ml (1 pint) of milk substitute at 12% solids. One mixing (570 mls/ 1 pint) was deemed tobe one unit of milk substitute and the number of units consumed by each calf during a 24-hour observation period was noted to the nearest $\frac{1}{4}$ unit. To facilitate observation the clear perspex holding bowl was marked off in quarter divisions. The number of times a calf consumed milk during each 24 hour observation period was also noted. A suckling cycle was defined as that time from the beginning of suckling until the end of suckling when the calf actually moved away from the machine to perform some other activity. If a calf consumed one unit, and then was interrupted by another calf during the three minute mixing cycle, but continued to suckle when the mixing process was completed, it counted as one suckling cycle. The total machine use time was also noted. The machine was deemed to be in use from the time a calf began to suckle until the completion of the mixing cycle after the last calf had finished suckling. Thus, if there were no calves at the machine during the three minute mixing cycle, but a calf began to suckle immediately the mixing cycle was completed, it was taken that no interruption in machine use had occurred.

Laboratory procedures The laboratory procedures for the isolation of S. enteritidis from the calves of Group 10 and the agglutination tests have been described by Petrie, Selman, Grindlay and Thompson (1977).

SECTION I

THE RELATIONSHIP BETWEEN ABSORBED IMMUNOGLOBULINS, AD LIBITUM FEEDING OF MILK SUBSTITUTE, LIVEWEIGHT GAIN, AND THE INCIDENCE OF DIARRHOEA DURING THE FIRST FOUR WEEKS OF LIFE

The composition of the nine groups of calves, their immediate post-natal treatment, the incidence of diarrhoea during the first four weeks of life and the weight gains will be described separately for each group, as there was considerable variation between the groups. The weekly weights, the 28-day weights, the weaning weights and the 12-week weights are presented in full in Appendix 3.

Using the information obtained from these groups, several variables and their inter-relationships for group-reared calves were examined:

1. The relationship between the incidence of diarrhoea and age during the first four weeks of life.
2. The relationship between the incidence of diarrhoea during the first four weeks of life and time of entry to the rearing accommodation.
3. The relationship between the incidence of diarrhoea during the first four weeks of life and the serum concentration of absorbed immunoglobulins.
4. The relationship between the incidence of diarrhoea and the liveweight gain during the first four weeks of life.
5. The relationship between the feeding of whole milk for the first four days of life and the incidence of diarrhoea during the first four weeks of life.

In Group 10, the prevalence of diarrhoea and the demeanour of the first few calves born was not greatly different from what had been observed in other groups, but when the ninth calf in the group was three days old it developed a profuse diarrhoea which persisted until it died, aged ten days. Following a routine post-mortem

examination Salmonella enteritidis was isolated from the interstinal contents. A further four calves died from salmonellosis and the clinical and pathological findings of this outbreak are described in detail. The information collected on the incidence of diarrhoea in this group was not employed in the statistical analyses.

GROUP 3

There were 14 calves (26-39) in Group 3, born over a 16 day period from 11.6.71 to 26.6.71. The 14 calves were all subjected to the same immediate post-natal management treatment as follows:-

All 14 calves were born in loose-boxes. After each calf had been weighed and a blood sample collected at approximately 15 minutes post-partum, each calf was assisted to suckle colostrum to satiation from its own dam. The calves were allowed to remain and to have free access to their dams for 12 hours. At 12 hours post-partum an attempt was made to encourage each calf to suckle again. Immediately following this second suckling period, the calves were removed to the loose-box which had been selected as the rearing accommodation. Approximately eight hours after being placed in the rearing accommodation each calf was encouraged to suckle the automatic feeder.

The breeds, sex, birth and 28-day weights, 28-day faecal scores and the 48-hour serum concentrations of absorbed immunoglobulins for the 14 calves are presented in Table 13. There were 11 Ayrshire calves, two AyrshirexFriesian calves, and one AyrshirexFriesian(dam)xFriesian calf. The male to female ratio was 7:7. The mean birth weight was 33.28 ± 5.95 kg. The mean 28-day weight was 47.17 ± 7.40 kg. The mean 48-hour serum concentration of absorbed immunoglobulins was 29.86 ± 9.77 ZST units. The calves were evenly spread over the 16 day calving period, although the first two calves (26, 27) were born on the same day, four days before the next calf.

Initially, it was found to be difficult to encourage calves to suckle the automatic feeder, within six hours of being placed in the rearing accommodation. However when the first suckling was delayed until at least eight hours the calves suckled the machine willingly and few calves required assistance after the two initial introductory training periods. Each calf in Group 3 was weaned when it was approximately eight weeks old.

The overall incidence of diarrhoea in Group 3 during the first 28 days of life was low (Figure 6). One calf (32) maintained

TABLE 13

GROUP 3

CALF No.	DAY* BORN	BREED**	SEX	WEIGHT (kg) BIRTH	28DAYS	GAIN	28-DAY FAECAL SCORE	48-HOUR+ SAIg (ZST UNITS)
26	0	A	M	43.3	66.8	23.5	2	34
27	0	A	F	39.4	51.3	11.9	8	23
28	4	A	F	30.8	48.2	17.4	4	35
29	4	A	M	25.5	38.7	13.2	2	29
30	6	A	F	33.1	50.5	17.4	11	32
31	7	A	M	41.1	52.3	11.2	4	38
32	7	A	M	35.8	48.6	12.8	0	27
33	8	A	M	24.1	36.8	12.7	3	36
34	9	AxF. xF	F	25.3	41.4	16.1	6	46
35	11	AxF	F	32.7	44.5	11.8	2	21
36	11	A	M	29.4	42.2	12.8	6	43
37	13	AxF	F	32.8	43.2	10.4	8	25
38	14	A	F	34.8	45.9	11.1	9	18
39	15	A	M	37.8	50.0	12.2	22	11
			Mean	33.28	47.17	13.89		29.86
			S.D.	± 5.95	±7.40	± 3.56		± 9.77

* Day of birth in relation to first calf born

** Breed: A = Ayrshire; F = Friesian; H = Hereford
Ch = Charolais; Sh = Shorthorn; AA = Aberdeen Angus

\+ Serum concentration of absorbed immunoglobulins at 48 hours

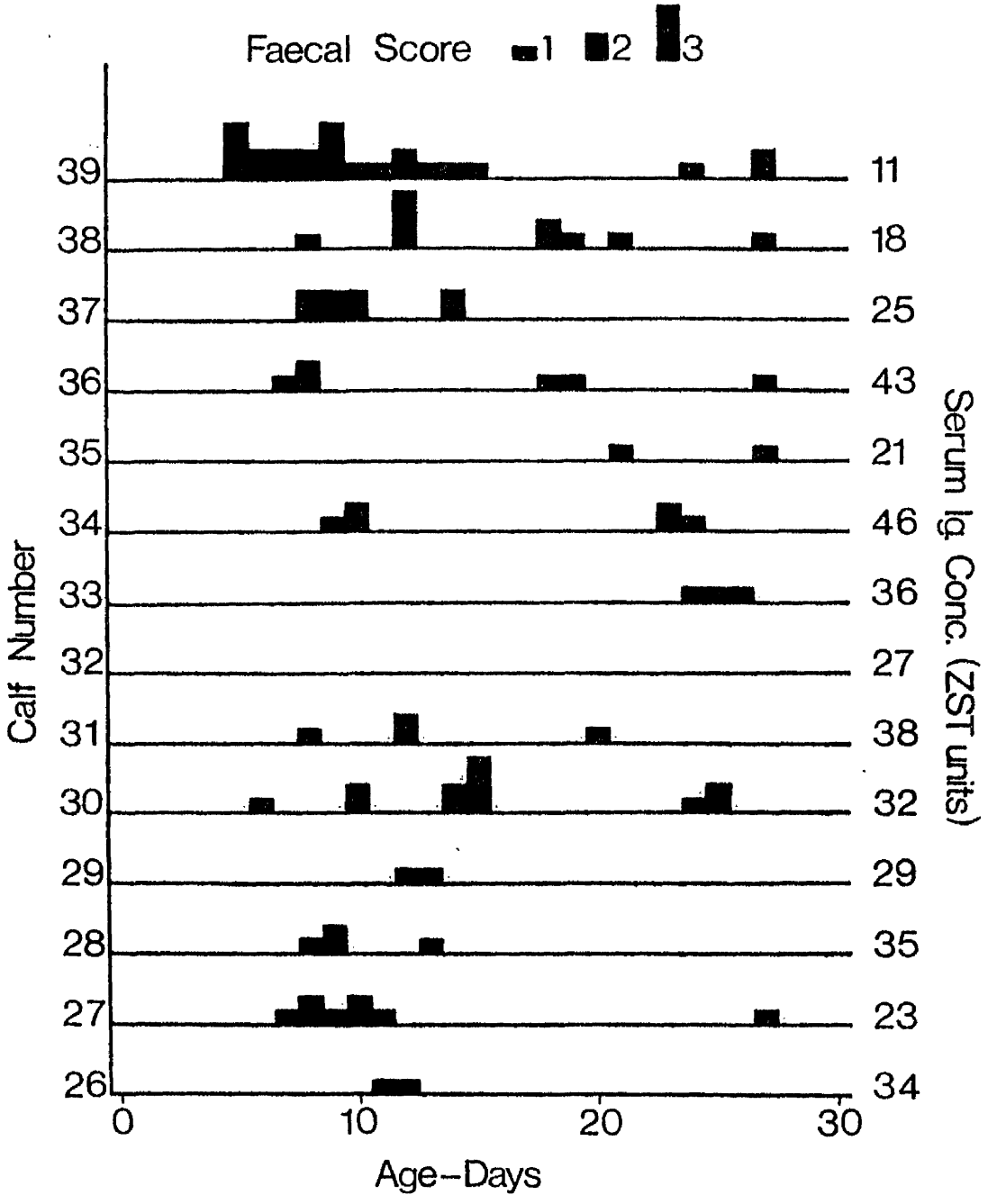


FIGURE 6 Group 3: The incidence and severity of diarrhoea during the first 28 days of life.

completely normal faeces throughout the initial 28 day period and two other calves (26, 35) produced soft faeces (faecal score 1) on only two days. Only three calves were profusely diarrhoeic (faecal score 3) at any time during the first four weeks of life. Two of the three calves (30, 38) were profusely diarrhoeic for just one day. The third calf (39) was profusely diarrhoeic (faecal score 3) on the fifth and ninth day of life and had a faecal score of 2 on days 6, 7, and 8.

There was a wide range in the weights gained over the first four weeks of life from 9.1 kg (31) to 23.5 kg (26) and the mean weight gained was 13.89 ± 3.56 kg.

GROUP 4

There were 11 calves (40-50) in Group 4, born over a 20 day period from 6.7.71 to 25.7.71. The immediate post-natal management was identical to that described for Group 3 as follows:

All calves were born in loose-boxes. After each calf had been weighed and a blood sample collected at approximately 15 minutes post-partum, each calf was assisted to suckle colostrum to satiation from its own dam. The calves were allowed to remain and to have free access to their dams for 12 hours. At 12 hours post-partum an attempt was made to encourage each calf to suckle again. Immediately following this second suckling period the calves were removed to the loose-box which had been selected as the rearing accommodation for all 11 calves. Approximately eight hours after being placed in the rearing accommodation each calf was encouraged to suckle the automatic feeder.

The breeds, sex, birth and 28-day weights, 28-day faecal scores and the 48-hour serum concentrations of absorbed immunoglobulins for the 11 calves are presented in Table 14. There were six Ayrshire calves, one AyrshirexFriesian calf, three Ayrshire(dam)xHereford calves and one AyrshirexFriesianxHereford calf. The male to female ratio was 7:4. The mean birth weight was 33.65 ± 4.31 kg. The mean 28-day weight was 47.24 ± 6.20 kg. The mean 48-hour serum concentration of absorbed immunoglobulins was 28.36 ± 9.10 ZST units.

Again no difficulty was encountered in teaching the calves to suckle the automatic feeder at the two initial introductory training periods. Each calf was weaned individually when it was eight weeks old.

The incidence of diarrhoea in Group 4 was also low (Figure 7) but no calf maintained normal faeces throughout the first four weeks of life. Seven of the 11 calves were profusely diarrhoeic at some time during the first four weeks, but only two calves (46, 47) were profusely diarrhoeic for more than one day. Two calves (40, 41) never had "diarrhoea" greater than faecal score 1, although in calf 41 this persisted from day 5 to day 12.

TABLE 14

GROUP 4

CALF No.	DAY * BORN	BREED**	SEX	WEIGHT (kg) BIRTH	28DAYS	GAIN	28-DAY FAECAL SCORE	48-HOUR+ SAIg (ZST UNITS)
40	0	AxH	M	36.1	52.3	16.2	2	36
41	2	A	M	32.7	48.2	15.5	11	12
42	5	AxH	F	29.2	47.3	18.1	11	30
43	5	A	M	35.1	49.1	14.0	16	18
44	5	AxF	F	34.0	46.4	12.4	8	22
45	7	A	F	25.7	31.8	6.1	12	25
46	14	AxH	M	34.8	49.1	14.3	19	28
47	16	A	M	35.7	49.1	13.4	18	25
48	16	AxExH	F	28.6	42.3	13.7	6	36
49	19	A	M	38.3	47.7	9.4	20	40
50	19	A	M	40.1	56.4	16.3	14	40
		Mean		33.65	47.24	13.58		28.36
		S.D.		± 4.31	± 6.20	± 3.38		± 9.10

* Day of birth in relation to first calf born

** Breed: A = Ayrshire; F = Friesian; H = Hereford
Ch = Charolais; Sh = Shorthorn; AA = Aberdeen Angus

\+ Serum concentration of absorbed immunoglobulins at 48 hours

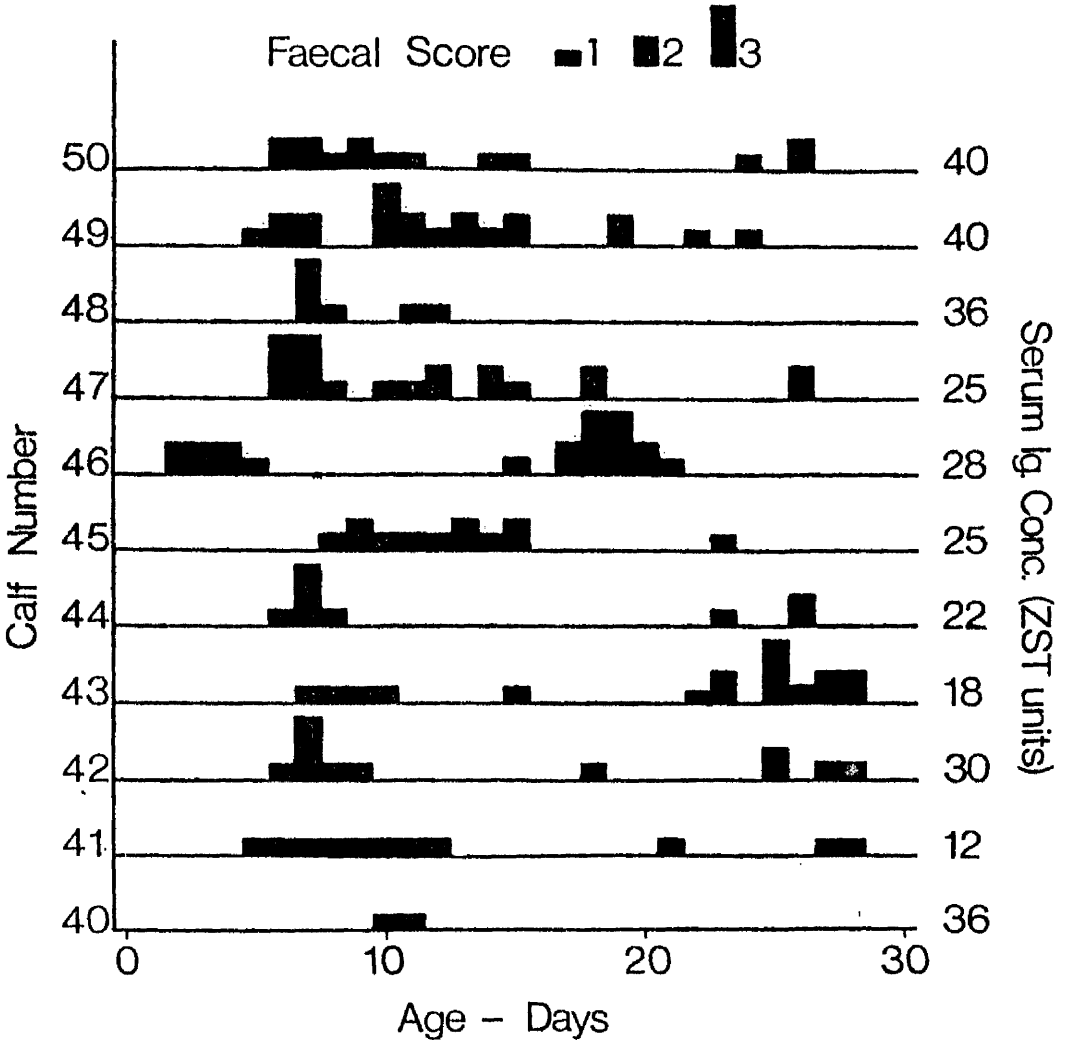


FIGURE 7 Group 4: The incidence and severity of diarrhoea during the first 28 days of life.

The weight gained by individual calves during the first four weeks was not as high as that of Group 3, with a minimum of 6.1 kg (45) and a maximum of 18.1 kg (42) but the mean weight gained, 13.58 ± 3.38 kg, was very similar.

GROUP 6

There were ten calves (58-67) in Group 6, born over a 17 day period from 12.11.71 to 28.11.71. All ten calves were born in loose-boxes and they were allocated to one of two different post-natal management treatments as follows:

Five calves (59, 61, 63, 65, 67) were weighed and a blood sample collected at 15 minutes post-partum. Then each of the five calves was assisted to suckle colostrum to satiation from its own dam. Immediately following this single feed of colostrum the five calves were removed to the loose-box which had been selected as the rearing accommodation for all ten calves. Approximately eight hours after being placed in the rearing accommodation each of the five calves was encouraged to suckle the automatic feeder.

The remaining five calves (58, 60, 62, 64, 66) were also weighed and a blood sample collected at 15 minutes post-partum. Then each of the five calves was assisted to suckle colostrum to satiation from its own dam. Subsequent to this single feed of colostrum these five calves were held in separate accommodation and fed whole cow's milk for the first four days of life at a daily rate of 10% bodyweight divided into two equal feeds, using a Rose-Miller teat funnel. After this initial four day period the five "milk-fed" calves were placed in the rearing accommodation along with the other calves and encouraged to suckle the automatic feeder approximately eight hours later.

The breeds, sex, birth and 28-day weights, 28-day faecal scores, and the 48-hour serum concentrations of absorbed immunoglobulins for the ten calves are presented in Table 15. There were four Ayrshire calves, five AyrshirexFriesian calves and one Friesian calf. The male to female ratio was 7:3. The mean birth weight was 32.62 ± 5.50 kg. The mean 28-day weight was 43.66 ± 8.54 kg. The mean 48-hour serum concentration of absorbed immunoglobulins was 24.20 ± 9.30 ZST units.

TABLE 15

GROUP 6

CALF No.	DAY* BORN	BREED**	SEX	WEIGHT (kg) BIRTH	28DAYS	GAIN	28-DAY FAECAL SCORE	48-HOUR+ SAIg (ZST UNITS)
58	0	AxF	F	34.8	56.1	21.3	3	22
59	3	AxH	F	28.8	42.3	13.5	16	19
60	5	F	M	42.0	56.3	14.3	5	27
61	8	AxF	M	41.4	51.4	10.0	5	23
62	10	A	M	32.9	39.5	6.6	10	13
63	10	A	M	25.9	33.2	7.3	14	25
64	10	A	M	31.5	44.1	12.6	11	21
65	16	A	M	26.6	37.7	11.1	1	35
66	16	AxF	M	30.5	32.8	2.3	29	14
67	16	AxF	F	31.8	43.2	11.4	14	4
		Mean		32.62	43.66	11.04		20.30
		S.D.		± 5.50	±8.54	± 5.12		±8.50

* Day of birth in relation to first calf born

** Breed: A = Ayrshire; F = Friesian; H = Hereford
Ch = Charolais; Sh = Shorthorn; AA = Aberdeen Angus

\+ Serum concentration of absorbed immunoglobulins at 48 hours

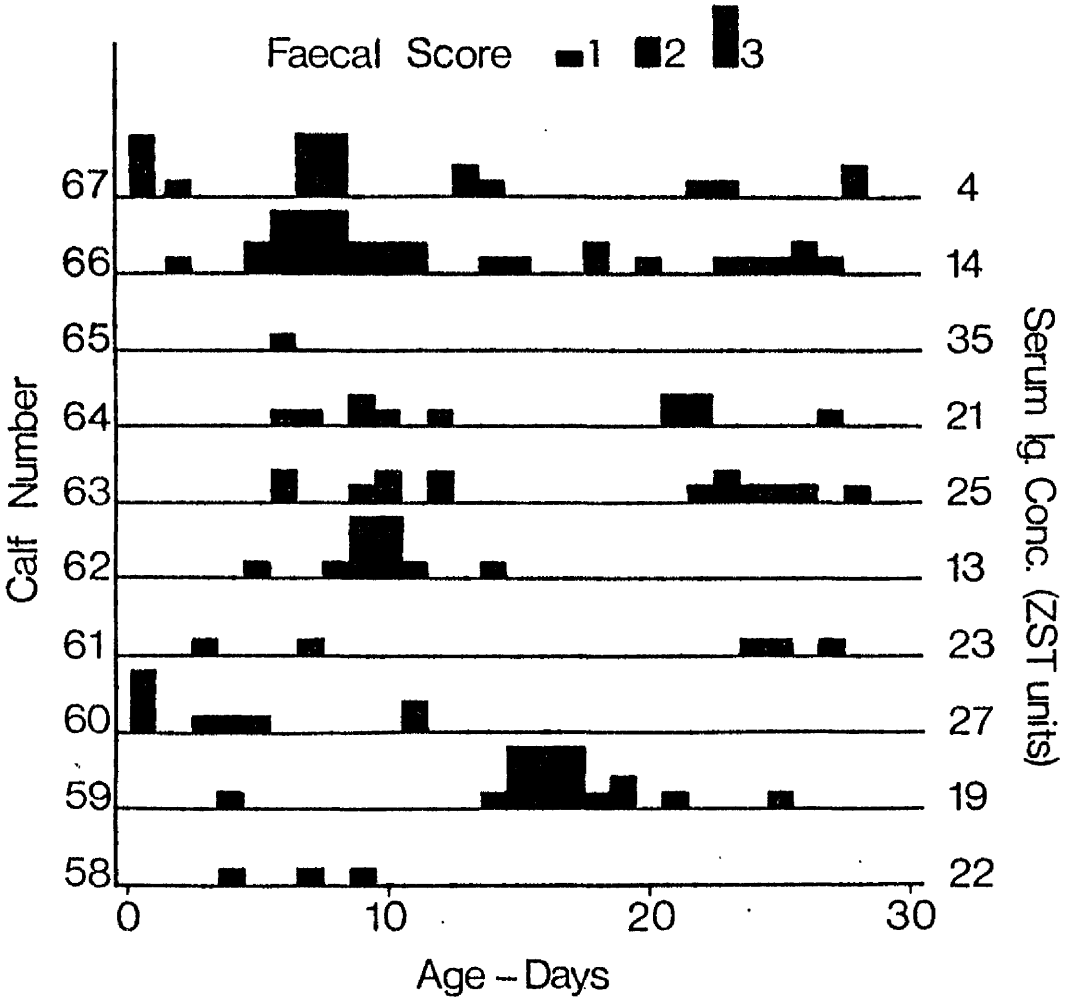


FIGURE 8 Group 6: The incidence and severity of diarrhoea during the first 28 days of life.

The first seven calves were born over an 11 day period and the last three calves were born on day 17, a six day interval between calf 64 and calf 65. The calves again learned to suckle the automatic feeder quickly. All the calves were weaned on the same day when the oldest calf (58) was 55 days old and the youngest calf (67) was 39 days old.

The incidence of diarrhoea in Group 6 during the first 28 days of life was higher than in the first two groups (Figure 8). Two calves (60, 67) developed a profuse diarrhoea within 18-24 hours of consuming colostrum, but as this initial diarrhoea lasted only two to three stools at the most it was discounted. Four calves (59, 62, 66, 67) were profusely diarrhoeic (faecal score 3) for two or more days. Calf 66 was diarrhoeic (faecal score 2 or 3) from day 5 to day 11. Calf 65 had soft faeces on one day only during the first 28 days of life.

There was wide individual variation in the weight gains over the first four weeks of life from 2.3 kg (66) to 21.3 kg (58) with a mean of 11.04 ± 5.12 kg.

GROUP 7

There were nine calves (68-76) in Group 7, born over a 12 day period from 16.12.71 to 27.12.71. All nine calves were born in loose-boxes and they were allocated to one of two different post-natal management treatments as follows:

Four calves (69, 71, 73, 75) were weighed and a blood sample collected at 15 minutes post-partum. Then each of the four calves was assisted to suckle colostrum to satiation from its own dam. Immediately following this single feed of colostrum the four calves were removed to the loose-box which had been selected as the rearing accommodation for all nine calves. Approximately eight hours after being placed in the rearing accommodation each of the four calves was encouraged to suckle the automatic feeder.

The remaining five calves (68, 70, 72, 74, 76) were also weighed and a blood sample collected at 15 minutes post-partum. Then each of the five calves was assisted to suckle colostrum to satiation from its own dam. Subsequent to this single feed of colostrum these five calves were held in separate accommodation and fed whole cow's milk for the first four days of life at a daily rate of 10% bodyweight divided into two equal feeds, using a Rose-Miller teat funnel. After this initial four day period the five "milk-fed" calves were placed in the rearing accommodation along with the other calves and encouraged to suckle the automatic feeder approximately eight hours later.

The breeds, sex, birth and 28-day weights, 28-day faecal scores, and the 48-hour serum concentrations of absorbed immunoglobulins for the nine calves are presented in Table 16. There were five Ayrshire calves, two AyrshirexFriesian calves, one AyrshirexFriesian(dam)xFriesian calf and one AyrshirexFriesianxCharolais calf. The male to female ratio was 6:3. The mean birth weight was 34.99 ± 6.02 kg. The mean 28-day weight was 44.37 ± 6.13 kg. The mean 48-hour serum concentration of absorbed immunoglobulins was 27.22 ± 11.17 ZST units.

The births of the calves were evenly spread over the 12 day period. All the calves were weaned on the same day when the oldest

TABLE 16

GROUP 7

CALF No.	DAY* BORN	BREED**	SEX	WEIGHT (kg) BIRTH	28DAYS	GAIN	28-DAY FAECAL SCORE	48-HOUR+ SAIg (ZST UNITS)
68	0	A	M	36.6	50.5	13.9	18	23
69	4	A	M	40.5	48.2	7.7	31	30
70	6	A	M	27.9	42.2	14.3	20	26
71	7	AxF.xCh	F	37.5	45.0	7.5	32	26
72	7	A	M	38.2	49.1	10.9	15	23
73	9	AxF.xF	F	29.8	Died	ND	ND	5
74	9	A	M	27.0	33.1	6.1	17	42
75	10	AxF	M	44.7	48.7	4.0	30	19
76	11	AxF	F	32.7	38.2	5.5	10	35
		Mean		34.99	44.37	8.74		25.44
		S.D.		± 6.02	± 6.13	± 3.87		± 10.35

* Day of birth in relation to first calf born

** Breed: A = Ayrshire; F = Friesian; H = Hereford
Ch = Charolais; Sh = Shorthorn; AA = Aberdeen Angus

\+ Serum concentration of absorbed immunoglobulins at 48 hours

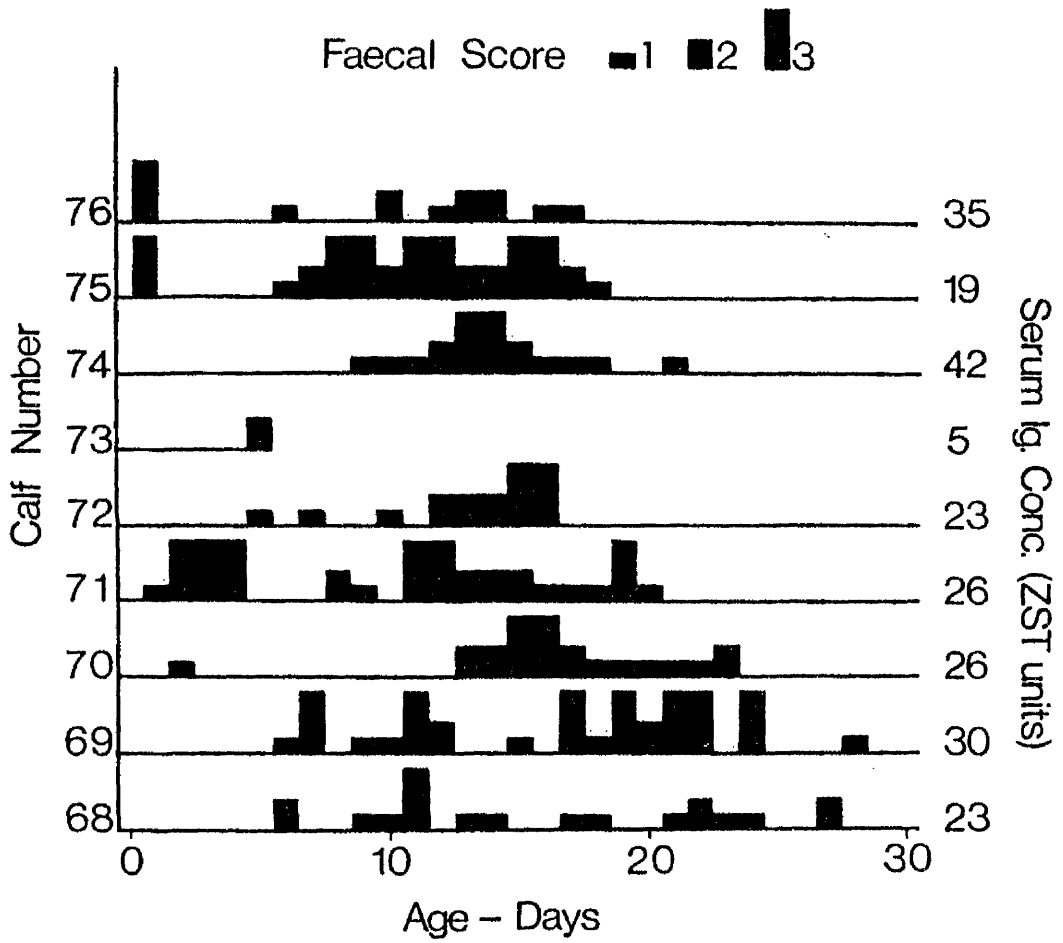


FIGURE 9 Group 7: The incidence and severity of diarrhoea during the first 28 days of life.

calf (68) was 49 days old and the youngest calf (76) was 37 days old.

The incidence of diarrhoea in Group 7 was very high (Figure 9) Two calves (75, 76) developed a profuse diarrhoea within 18-24 hours of consuming colostrum. Again this diarrhoea had a duration of two to three motions at the most. One calf (71) was profusely diarrhoeic (faecal score 3) on the second, third and fourth day of life. This was extremely uncommon, as diarrhoea, excluding the post-colostral diarrhoea, rarely occurred in any calf before the fifth day of life. Calf 71 was also diarrhoeic (faecal score 2 or 3) for five days from day 11 to day 15. One calf (69) was intermittently profusely diarrhoeic from day 7 to day 24. Only one calf (76) was not profusely diarrhoeic on any day during the first 28 days of life.

There was wide individual variation in the weight gains over the first four weeks of life from 4.0 kg (75) to 14.3 kg (70), with a mean weight gain of 8.74 ± 1.37 kg.

Calf 73, an AyrshirexFriesianxFriesian heifer calf became tachypnoeic (65/min) and hyperpnoeic when only 30 hours old. A tachycardia was also present but no cardiac murmurs could be detected on auscultation. The inspiratory and expiratory sounds were very harsh but no adventitious sounds were detectable. The calf remained extremely dull and was unwilling or unable to suckle the automatic feeder unless assisted. The calf became progressively dyspnoeic and died when five days old. Post-mortem examination revealed an extensive exudative interstitial pneumonia with marked consolidation of both lungs. Extensive areas of suppuration and necrosis were associated with these areas of consolidation and a pleurisy was also present. Bacteriological examination of the lung tissue revealed a pure culture of Staphylococcus pyogenes. It is probable that this calf suffered from an inhalation pneumonia although there was nothing to indicate that this may have occurred. The serum concentration of absorbed immunoglobulins at 48 hours of age was 7 ZST units.

GROUP 8

There were 14 calves (82-95) in Group 8, born over a 13 day period from 25.3.72 to 6.4.72. All 14 calves were born in loose-boxes but for colostrum feeding the calves were allocated to one of two different post-natal management treatments as follows:

Seven calves (82, 83, 84, 85, 89, 91, 95) were weighed and a blood sample collected at 15 minutes post-partum, and then each of the seven calves was assisted to suckle colostrum to satiation from its own dam. Immediately following this single feed of colostrum the calves were removed to the loose-box which had been selected as the rearing accommodation for all 14 calves.

The remaining seven calves (86, 87, 88, 90, 92, 93, 94) were separated from their dams at 15 minutes post-partum when they were weighed and a blood sample collected. They were then held in separate, clean accommodation until approximately six hours post-partum when they were allowed to suckle 1500ml of their own dam's colostrum from a Rose-Miller teat funnel. The colostrum had been milked from the dams either by hand or by machine. Immediately after this delayed colostrum feeding the calves were placed in the rearing accommodation along with the other calves and encouraged to suckle the automatic feeder approximately eight hours later.

The breeds, sex, birth and 28-day weights, 28-day faecal scores, and the 48-hour serum concentrations of absorbed immunoglobulins for the 14 calves are presented in Table 17. There were nine Ayrshire calves, two AyrshirexFriesian calves, one AyrshirexFriesianxFriesian calf and one AyrshirexHereford calf. The male to female ratio was 7:7. The mean birthweight was 29.54 ± 3.37 kg. The mean 28-day weight was 41.47 ± 5.14 kg. The mean 48-hour serum concentration of absorbed immunoglobulins was 26.29 ± 10.80 ZST units.

With 14 calves born over a 13 day period this group had the most compact calving pattern. All the calves were weaned on the same day when the oldest calf (82) was 53 days and the youngest (95) was 41 days old.

TABLE 17

GROUP 8

CALF No.	DAY* BORN	BREED**	SEX	WEIGHT (kg) BIRTH	28DAYS	GAIN	28-DAY FAECAL SCORE	48-HOUR+ SAIg (ZST UNITS)
82	0	AxF	M	32.2	52.7	20.5	11	23
83	1	AxF. xF	M	32.5	42.9	10.4	15	43
84	1	A	M	29.6	39.2	9.6	13	39
85	2	AxH	F	34.0	47.6	13.6	9	31
86	2	A	M	24.5	39.9	15.4	22	22
87	2	F	F	30.0	45.1	15.1	12	24
88	3	A	M	34.3	46.6	12.3	16	22
89	6	A	F	29.0	34.4	5.4	22	45
90	6	A	M	27.7	41.3	13.6	19	15
91	6	A	F	26.0	38.4	12.4	14	24
92	7	A	F	31.2	39.2	8.0	12	14
93	9	A	F	30.0	38.7	8.7	15	18
94	11	AxF	F	29.6	40.8	11.2	10	12
95	12	A	M	22.9	33.8	10.9	23	36
		Mean		29.54	41.47	11.94		26.29
		S.D.		± 3.37	± 5.14	±3.71		± 10.80

* Day of birth in relation to first calf born

** Breed: A = Ayrshire; F = Friesian; H = Hereford
Ch = Charolais; Sh = Shorthorn; AA = Aberdeen Angus

\+ Serum concentration of absorbed immunoglobulins at 48 hours

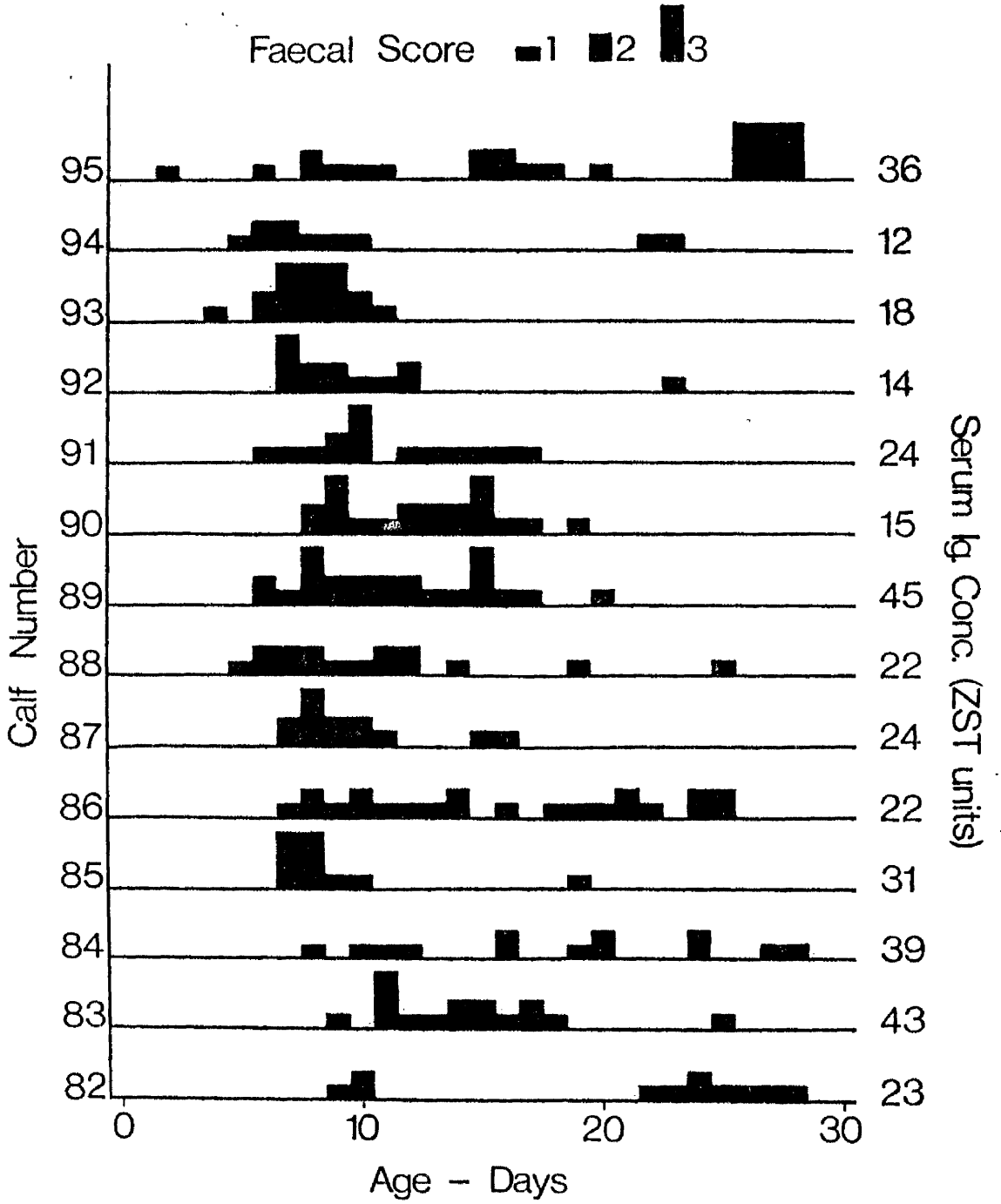


FIGURE 10 Group 8: The incidence and severity of diarrhoea during the first 28 days of life.

The incidence of diarrhoea over the first 28 days of life was quite low (Figure 10). Only nine calves were profusely diarrhoeic (faecal score 3) and only five of these were profusely diarrhoeic for two or more days. Most of the diarrhoea occurred between five and 20 days of age, but calf 95 was profusely diarrhoeic on day 26, 27 and 28.

The weight gains over the first 28 days of life ranged from 5.4 kg (89) to 20.5 kg (82) and the mean weight gain was 11.94 ± 3.71 kg.

GROUP 9

There were ten calves (96-103, 105, 106) in Group 9, born over a 17 day period from 1.7.72 to 16.7.72. All ten calves were born in loose-boxes but for colostrum feeding the calves were allocated to one of two post-natal management treatments as follows:

Five calves (96, 97, 98, 103, 106) were weighed and a blood sample collected at 15 minutes post-partum, and then each of the five calves was assisted to suckle colostrum to satiation from its own dam. Immediately following this single feed of colostrum the calves were removed to the loose-box which had been selected as the rearing accommodation for all ten calves.

The remaining five calves (99, 100, 101, 102, 105) were separated from their dams at 15 minutes post-partum when they were weighed and a blood sample collected. They were then held in separate, clean accommodation until approximately six hours post-partum when they were allowed to suckle 1500ml of their own dams' colostrum from a Rose-Miller teat funnel. The colostrum had been milked from the dams either by hand or by machine. Immediately after this delayed colostrum feeding the calves were placed in the rearing accommodation along with the other calves and encouraged to suckle the automatic feeder approximately eight hours later.

The breeds, sex, birth and 28-day weights, 28-day faecal scores, and the 48-hour serum concentrations of absorbed immunoglobulins for the ten calves are presented in Table 18. There were nine Ayrshire calves and one AyrshirexFriesian(dam)xFriesian calf. The male to female ratio was 8:2. The mean birth weight was 31.86 ± 3.34 kg. The mean 28-day weight was 46.20 ± 7.79 kg. The mean 48-hour serum concentration of absorbed immunoglobulins was 22.10 ± 9.89 ZST units.

The first eight calves were born over a nine day period, but there was an interval of a week between calf 103 and calf 105. All the calves were weaned on the same day when the oldest calf (96) was 51 days and the youngest (106) was 35 days old.

TABLE 18

GROUP 9

CALF No.	DAY* BORN	BREED**	SEX	WEIGHT (kg) BIRTH	28DAYS	GAIN	28-DAY FAECAL SCORE	48-HOUR+ SAIg (ZST UNITS)
96	0	A	M	30.5	34.1	3.6	10	23
97	1	A	M	31.5	43.7	12.2	8	19
98	2	A	M	34.9	56.4	21.5	15	20
99	6	A	M	34.6	53.9	19.3	8	8
100	4	A	F	29.3	48.2	18.9	16	29
101	7	AxF.xF	M	37.5	51.4	13.9	28	20
102	7	A	M	30.9	46.4	15.5	39	31
103	8	A	M	33.8	51.6	17.8	15	27
104	13	A	F	18.1	ND	ND	ND	16
105	15	A	M	29.5	42.7	13.2	35	6
106	16	A	F	26.1	33.6	7.5	10	38
		Mean		31.86	46.20	14.34		22.10
		S.D.		± 3.34	±7.79	±5.56		± 9.89

* Day of birth in relation to first calf born

** Breed: A = Ayrshire; F = Friesian; H = Hereford
Ch = Charolais; Sh = Shorthorn; AA = Aberdeen Angus

\+ Serum concentration of absorbed immunoglobulins at 48 hours

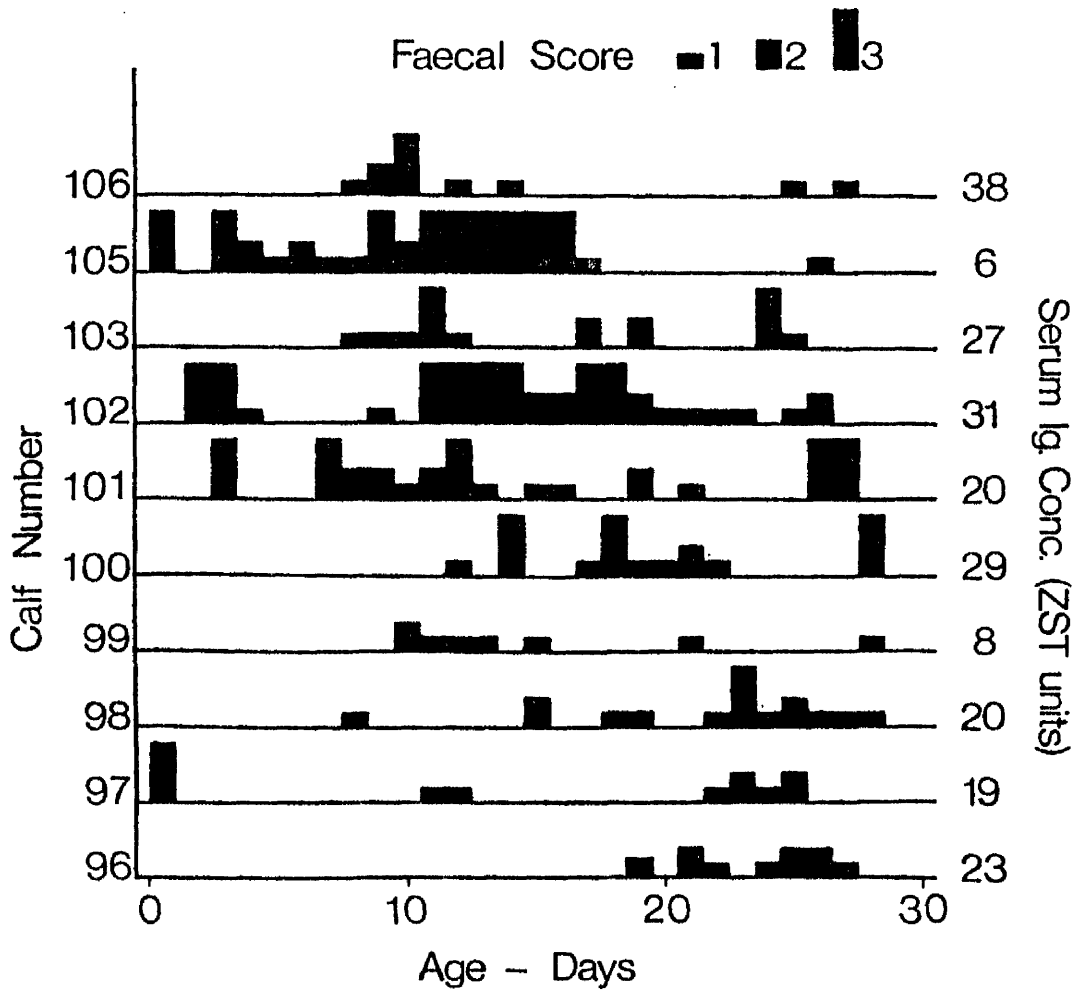


FIGURE 11 Group 9: The incidence and severity of diarrhoea during the first 28 days of life.

The incidence of diarrhoea for the ten calves is presented in Figure 11. Persistent, profuse diarrhoea occurred in two calves (102, 105). Calf 102 was profusely diarrhoeic (faecal score 3) for six of the eight days between 11 and 18 days of age, and on the remaining two days the faecal score was 2. Between nine and 16 days of age calf 105 was profusely diarrhoeic for seven days and had a faecal score of 2 on the other day. Two calves (97, 105) developed a profuse diarrhoea 18-24 hours after the ingestion of colostrum.

The weight gains over the first 28 days of life varied from 3.6 kg (96) to 21.5 kg (98) with a mean of 14.34 ± 1.76 kg. The two calves (102, 105) with the most persistent diarrhoea gained 15.5 kg (102) and 13.2 kg (105) respectively during the first four weeks of life.

Two calves are worthy of further mention. Calf 104 was not included in the group, as shortly after birth its mother lay on top of it and fractured its left femur. The fracture was repaired and the calf was reared individually. This calf is also interesting in that the ZST value of the precolostral serum sample was 15 units. An Ayrshire heifer calf was born on 5.7.72 and would have become calf 99. This calf developed a very profuse, watery diarrhoea within 24 hours of birth, rapidly collapsed and died when approximately 36 hours old. Colostrum feeding had been delayed in this calf; it was fed 1500 mls of its dam's colostrum (whey immunoglobulin concentration 6.99 g/100ml) at seven hours post-partum and the concentration of absorbed immunoglobulins in a serum sample collected at 24 hours of age was 5 ZST units.

GROUP 10

There were 15 calves (107-121) in Group 10, born over a 22 day period from 17.12.73 to 7.1.73. The 15 calves were allocated to one of three different post-natal management treatments as follows:

Subgroup C comprised five calves (111, 112, 113, 118, 120) which were born in loose-boxes. After each calf had been weighed and a blood sample collected at 15 minutes post-partum, it was assisted to suckle colostrum to satiation from its own dam. Subsequent to this single feed of colostrum, these five calves were immediately removed to the loose-box which had been selected as the rearing accommodation for all 15 calves.

Subgroup D comprised five calves (107, 109, 110, 117, 121) which were born in loose-boxes and allowed to remain with their dams for 48 hours. No attempt was made to encourage the calves to suckle or to interfere in any way except to weigh and to collect blood samples from the calves at 15 minutes post-partum. After 48 hours with their dams, the calves were removed to the rearing accommodation.

Subgroup E comprised five calves (108, 114, 115, 116, 119) each of which was born in the holding byre. After each calf had been weighed and a blood sample collected at 15 minutes post-partum it was removed from the byre and held in separate, clean accommodation until approximately eight hours post-partum. At this time it was allowed to suckle 1700ml of its dam's colostrum from a Rose-Miller teat funnel. Having been fed colostrum the calves were then placed in the rearing accommodation along with the other calves.

All 15 calves were encouraged to suckle the automatic feeder approximately eight hours after being placed in the rearing accommodation.

The breeds, sex, birth weights, 28 day weights and the 48-hour serum concentrations of absorbed immunoglobulins for the 15 calves of Group 10 are presented in Table 19. There were nine Ayrshire calves, one AyrshirexFriesian calf, two AyrshirexFriesian(dam)xFriesian calves,

TABLE 19

GROUP 10

CALF No.	SUB-GROUP[++]	DAY BORN[*]	BREED[**]	SEX	WEIGHT (kg) BIRTH	28DAYS	GAIN	28-DAY FAECAL SCORE	48-HOUR[+] SAIg (ZST UNITS)
107	D	0	AxCh	F	27.3	35.5	8.2	7	49
108	E	3	A	M	27.5	35.3	7.8	28	10
109	D	3	F	M	34.1	38.7	4.6	12	29
110	D	7	A	F	31.4	44.0	12.6	18	14
111	C	8	A	F	30.2	37.3	7.1	10	30
112	C	10	A	M	27.5	31.6	4.1	11	20
113	C	10	A	F	30.0	35.0	5.0	27	42
114	E	12	A	M	31.8	43.2	11.4	29	27
115	E	14	A	M	32.9	Died	ND	ND	2
116	E	15	A	F	39.3	35.5	-3.8	21	17
117	D	16	AxCh	F	41.6	48.6	7.0	31	27
118	C	17	AxF	F	31.4	Died	ND	ND	40
119	E	21	AxF.xF	M	24.8	Died	ND	ND	15
120	C	21	AxF.xF	F	25.7	Died	ND	ND	17
121	D	21	A	M	39.8	46.2	6.4	18	24
			Mean		31.68	39.17	6.4		24.20
			S.D.		± 5.15	± 5.46	±4.2		± 12.70

* Day of birth in relation to first calf born

** Breed: A = Ayrshire; F = Friesian; H = Hereford
Ch = Charolais; Sh = Shorthorn; AA = Aberdeen Angus

\+ Serum concentration of absorbed immunoglobulins at 48 hours

++ Subgroup based on immediate post-natal management

two AyrshirexCharolais calves and one Friesian calf. The male to female ratio was 9:6. The mean birth weight was 31.68 $\pm$ 5.16 kg. The mean 48-hour serum concentration of absorbed immunoglobulins was 24.20 $\pm$ 12.70 ZST units. The mean values for the three subgroups were, subgroup C, 29.80 $\pm$ 11.32 ZST units, subgroup D, 28.60 $\pm$ 12.78 ZST units, subgroup E, 14.20 $\pm$ 9.20 ZST units.

The incidence and severity of diarrhoea and the fate of the calves in Group 10 over the ten week period from the birth of the first calf (107) is shown in Figure 12. The day on which the first calf was born was taken as day 0. The incidence of diarrhoea among the eight calves born up to day 14 was negligible except for calf 108. Calf 108 had shown episodes of dullness from three days of age but its faeces had been of normal consistency until days 8 and 9 when it had a faecal score of 1. For the next four days it was persistently diarrhoeic (faecal score 2).

The first calf to become seriously ill (115) was born on day 14. On the morning of its third day of life (day 17) it was found to be dull and had a profuse, yellow diarrhoea (faecal score 3). This profuse diarrhoea persisted from day 17 to day 24, and with the continuation of this severe diarrhoea the calf became weaker, eventually being able to suckle the automatic feeder only when assisted. Soiling of the hair under the chin with saliva was noted on day 21 and this continued until death. By day 22 weight loss was visually apparent and the eyes had receded within their orbits. On days 23 and 24 the calf was unable to stand and lay in sternal recumbency. It died on the evening of day 24, aged ten days. At death the calf weighed only 24.1 kg, 10 kg less than its birth weight. During the seven day illness the calf's rectal temperature never exceeded 103.4^{o}F and blood was never seen in the faeces. At necropsy Salmonella enteritidis was isolated from the liver, kidneys, spleen and intestines.

During the period that calf 115 was profusely diarrhoeic, several of the calves born before calf 115 were also profusely diarrhoeic, (faecal score 3), notably calves 108, 110 and 114. Of the calves born after calf 115, profuse diarrhoea did not develop until about four days after the death of calf 115 (calves 117, 119, 120, 121). Between days

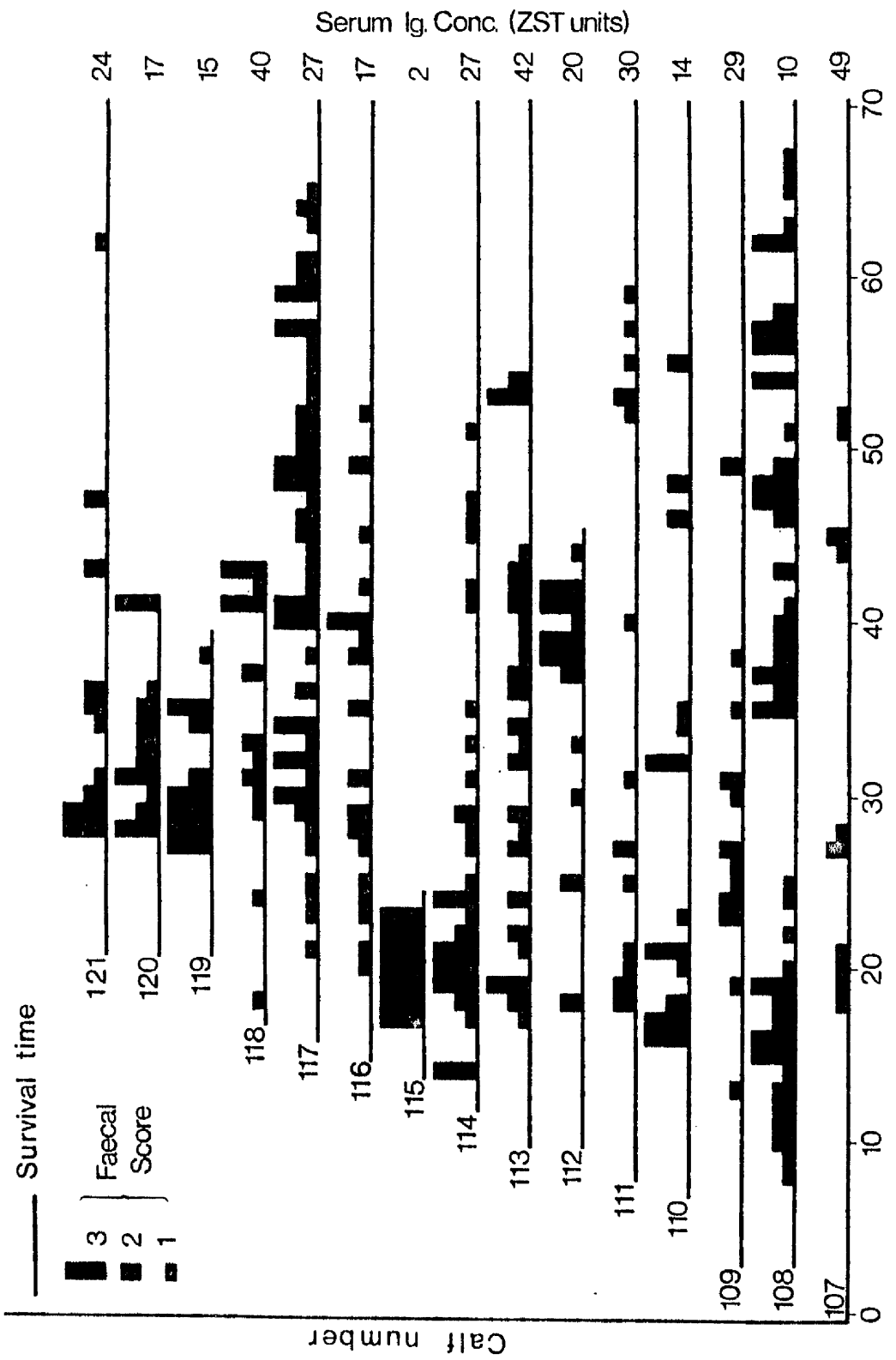


FIGURE 12 Group 10: The incidence and severity of diarrhoea during the outbreak of salmonellosis.

24 and 43 diarrhoea was prevalent throughout the group, but never more than 60 per cent of the calves were diarrhoeic (faecal score of 2 or 3) on any one day. Diarrhoea was mainly confined to the calves born after calf 115. After day 43, the incidence of diarrhoea decreased and was confined mainly to calves 108 and 117. A careful daily examination of each calf's faeces failed to reveal any evidence of dysentery.

After the death of calf 115, the daily rectal temperature of each calf was recorded and the mean values are presented in Figure 13. On day 35, 11 days after the death of calf 115 the mean rectal temperature was 104.9°F and five calves had a rectal temperature in excess of 106°F (calves 109, 112, 114, 118, 119). All 14 calves were very dull, and inappetent, with very little milk substitute powder being used on day 35. On the following day the appetite of the group improved dramatically and although four calves had rectal temperatures in excess of 105°F, none had a rectal temperature above 106°F.

Between day 35 and day 45 a further four calves (112, 118, 119, 120) died. Calf 112 died on day 45, aged 35 days; calf 118 died on day 43, aged 26 days; calf 119 died on day 39, aged 18 days; calf 120 died on day 41, aged 20 days. Although all four calves were profusely diarrhoeic (faecal score 3) at some time during their life this was not a consistent clinical finding and only two (118, 120) were profusely diarrhoeic on any of the three days prior to death (Figure 12). Diarrhoea was most severe in calf 119 which was profusely diarrhoeic on five days between days 26 and 34 although it survived until day 39. The rectal temperatures of these four calves were considerably higher than the ten calves which survived (Figure 13). Soiling of the hair under the chin by slimy saliva was noted in all four calves. All showed obvious weight loss and terminally they became very lethargic, spending most of their time lying down. They were unable or unwilling to suckle the automatic feeder unless assisted. On the last two days of life, calves 118 and 120 became tachypnoeic (respiratory rate, 60-90 per minute), had a frequent spontaneous cough and a bilateral mucopurulent nasal discharge.

Full post-mortem examinations were carried out on calves 112, 118, 119 and 120. The calves were dehydrated and the contents of their

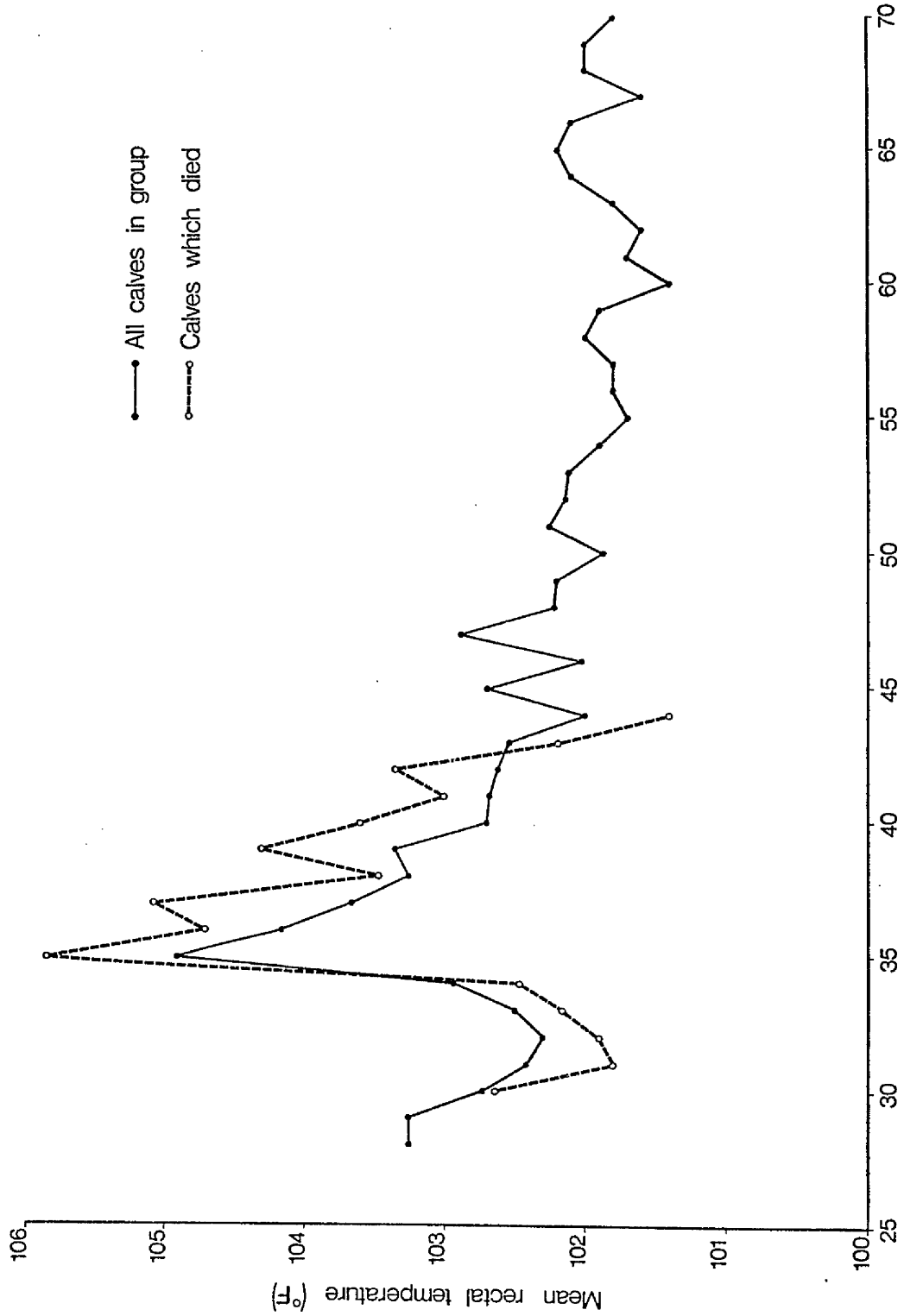


FIGURE 13 Group 10: The mean rectal temperatures during the outbreak of salmonellosis.

large and small intestines were watery, yellow and foetid. Dysentery was not present. The mesenteric lymph nodes were swollen and congested. The histological findings were similar. In each there was an enteritis characterised by necrosis and erosion of the surface epithelium with a moderate infiltration of mononuclear cells, lymphocytes and plasma cells in the interstitial lamina propria and submucosa. Necrotic foci were present in the submucosa and were also found in the mesenteric lymph nodes, the liver and the spleen. Three of the calves (112, 118 and 120) had an acute exudative pneumonia. The apical and cardiac lobes of the lungs were consolidated, as was the anterior border of the diaphragmatic lobes. The consolidated tissue was sharply defined from the normal lung, deep red, and was of greater volume than normal. On section, individual lobules were clearly delineated due to thickening of the interlobular septa by oedema fluid. The cut surface of consolidated lobules was mostly dark red in colour but scattered about on the surface were raised, reddish brown or yellowish patches which produced a mottled effect. Histologically, the lumina of the alveoli contained oedema fluid, variable amounts of fibrin, bacteria and red blood cells. Groups of alveoli packed with inflammatory cells in various stages of disintegration corresponded to the raised yellowish patches seen on gross examination. The bronchi and bronchioles contained only a few polymorphs and some cell debris and the lining epithelia were intact.

Salmonella enteritidis was isolated from the intestines, liver, spleen and mesenteric lymph node of each fatal case. Lung tissue from two (112 and 120) of the three calves which had pneumonia was cultured and a mixed flora of Gram-positive and Gram-negative coccabacilli but no salmonellae were isolated. However, S. enteritidis was recovered from the bronchial lymph node of calf 112.

The bacteriological examination of rectal swabs taken twice weekly was instituted after the death of calf 115. S. enteritidis was isolated from rectal swabs taken from 13 of the remaining 14 calves during the outbreak but on each sampling day at least 20 per cent of the calves were negative (Table 20). Eleven of the 14 calves had positive rectal swabs on day 36; ten of the 14 calves had positive rectal swabs on day 39; ten of 12 calves had positive rectal swabs on

TABLE 20

GROUP 10: Bacteriological examination of rectal swabs, blood and loose-box effluent for *S. enteritidis*.

CALF No.	DAY BORN	TIME OF SAMPLING (days after birth of first calf)											
		29	32	36	39	43	46	50	53	37	60	63	67
107	0	-	-	-	-	+	-	-	-	-	-	-	-
108	3	-	-	+	+ B-	+ B-	+	-	-	-	-	-	-
109	3	-	+	+	+	+	+	-	-	-	-	-	-
110	7	-	-	+	-	- B-	+	-	-	-	-	-	-
111	8	-	-	+	+	+	+	-	-	-	-	-	-
112	10	-	-	-	+ B+	+ B-		Died day 45					
113	10	+	+	+	+	+	+	-	-	-	-	-	-
114	12	-	+	+	+	+	+	-	-	-	-	-	-
116	15	-	-	+	-	+	+	+	+	-	-	-	-
117	16	-	-	-	-	- B+	-	-	-	-	-	-	-
118	17	-	-	+	+	+ B+		Died day 43					
119	21	-	-	+	+			Died day 39					
120	21	-	-	+	+ B+			Died day 41					
121	21	-	+	+	+	+	+	-	-	-	-	-	-
	EFFLUENT	N.S.	N.S.	N.S.	N.S.	N.S.	+	-	+	-	+	-	-

+ = *S. enteritidis* recovered from rectal swab. - = No *S. enteritidis* recovered. N.S. = Not sampled.
B+ = Blood culture positive for *S. enteritidis*. B- = Blood culture negative for *S. enteritidis*.

day 43; eight of ten calves had positive rectal swabs on day 46. Calf 113 had positive rectal swabs on days 29 (first sampling day), 32, 36, 39, 43 and 46 but was negative on every sampling day after that. This calf was profusely diarrhoeic on only two days during its first 60 days of life. S. enteritidis was recovered from rectal swabs taken from calf 107 on only one occasion (day 43). The organism was never recovered from any of the rectal swabs taken from calf 117 although it was one of the two calves which were persistently diarrhoeic. However the organism was isolated from a blood sample taken on day 43 (Table 20).

Single positive rectal swabs were found on days 50 and 53 and thereafter no positive rectal swabs were obtained despite twice weekly sampling for a further five weeks (i.e. until day 87). Nine of the ten surviving calves were slaughtered at 10 months of age and at this time attempts to recover salmonellae from the intestines, bile and mesenteric lymph nodes proved unsuccessful.

Swabs taken of the loose-box effluent from day 46 onwards were positive on only three occasions (days 46, 53 and 60). The last positive effluent swab was obtained one week after the last calf was found to be positive despite sampling for a further four weeks and the loose-box not being cleaned out.

The agglutination titres of both the sera and the colostral whey from the 15 cows are presented in Table 21. Five dams had flagellar (H) serum titres of 1/320 or more at the time of parturition. The highest (1/1280) occurred in cow 108 which also had a colostral whey flagellar titre of 1/1280. Somatic (O) agglutinin titres were much lower in both the sera and colostral wheys of the cows. No H or O agglutinins were present in any of the precolostral serum samples of the 15 calves, but post-colostral serum samples from 12 calves were positive for flagellar agglutinins and seven post-colostral samples were found to contain O agglutinins. The highest flagellar agglutination titre (1/320) was attained by two calves (107 and 119).

The flagellar agglutinin titres in the convalescent serum samples of the calves are presented in Table 22. Flagellar agglutinins were detected in the sera of only three of the surviving calves despite the recovery of S. enteritidis from either rectal swabs or blood taken

TABLE 21

GROUP 10: S. enteritidis flagellar and somatic agglutination titres of calf post-colostral serum, maternal colostral whey and maternal serum.

Calf No.	Sub-Group	Day Born	48-Hour SAIg (ZST units)	Agglutination titres: Calf post colostral serum titres*		Dam colostral whey titres		Dam serum titres		Fate of Calf
				H	O	H	O	H	O	
107	D	0	49	1/320	1/40	1/640	1/80	1/40	1/20	Recovered
108	E	3	10	- **	-	1/1280	1/80	1/1280	1/40	"
109	D	3	29	1/40	-	1/160	1/80	1/160	1/80	"
110	D	7	14	1/20	-	1/160	1/40	1/80	1/80	"
111	C	8	30	1/10	1/10	1/160	1/20	1/320	1/80	"
112	C	10	20	1/40	1/20	1/40	1/20	1/80	1/40	Died day 45
113	C	10	42	1/20	1/20	1/80	1/40	1/40	1/40	Recovered
114	E	12	27	1/80	1/20	1/320	1/40	1/20	-	"
115	E	14	2	-	-	1/20	-	1/40	1/20	Died day 24
116	E	15	17	1/20	-	1/80	-	1/80	1/20	Recovered
117	D	16	27	1/40	-	1/160	1/40	1/320	1/80	"
118	C	17	40	1/160	1/160	1/640	1/320	1/160	1/160	Died day 43
119	E	21	15	1/320	1/20	1/640	1/40	1/640	1/20	Died day 39
120	C	21	17	1/80	-	1/640	1/40	1/640	1/20	Died day 41
121	D	21	24	-	-	1/160	1/40	1/80	1/40	Recovered

* Precolostral H and O agglutination titres all negative.

** - = Negative.

TABLE 22

GROUP 10: S. enteritidis flagellar agglutination titres of convalescent serum.

Calf No.	Time of Sampling (days from birth of first calf)						
	38	45	52	59	66	73	80
107	-*	-	-	-	-	-	-
108	-	-	-	-	-	-	-
109	1/10	1/160	1/320	1/160	1/160	1/160	1/80
110	-	-	-	-	-	-	-
111	-	-	-	-	-	-	-
112	-	1/5120	D.				
113	-	-	-	-	-	-	-
114	-	-	-	-	-	-	-
115	D.						
116	-	-	-	1/80	1/1280	1/640	1/320
117	-	-	-	1/160	1/20	1/80	1/20
118	-	D.					
119	-	D.					
120	-	D.					
121	-	-	-	-	-	-	-

* Negative

D. 112 - Died day 45
115 - Died day 24
118 - Died day 43
119 - Died day 39
120 - Died day 41

from all ten calves. One calf (112) is worthy of further attention. This calf consumed colostrum with whey titres of 1/40 (H) and 1/20 (O) and its 48-hour H and O agglutinin titres were only 1/40 and 1/20 respectively. However by 45 days of age, the day of its death, calf 112 had an H agglutinin titre of 1/5120, although it had been negative one week earlier. This was the highest value attained by any calf.

GROUP 11

There were 15 calves (122-136) in Group 11, born over a 15 day period from 28.4.73 to 12.5.73. The 15 calves were allocated one of three different post-natal management treatments as follows:

Subgroup C comprised five calves (125, 129, 133, 134, 135) which were born in loose-boxes. After each calf had been weighed and a blood sample collected at 15 minutes post-partum, it was assisted to suckle colostrum to satiation from its own dam. Subsequent to this single feed of colostrum, these five calves were removed to the loose-box which had been selected as the rearing accommodation for all 15 calves.

Subgroup D comprised five calves (122, 123, 124, 130, 132) which were born in loose-boxes and allowed to remain with their dams for 48 hours. No attempt was made to encourage the calves to suckle or to interfere in any way except to weigh and to collect blood samples from the calves at 15 minutes post partum. After 48 hours with their dams the calves were removed to the rearing accommodation.

Subgroup E comprised five calves (126, 127, 128, 131, 136) each of which was born in the holding byre. After each calf had been weighed and a blood sample collected at 15 minutes post-partum it was removed from the byre and held in separate, clean accommodation until approximately eight hours post-partum. At this time it was allowed to suckle 1700ml of its own dam's colostrum from a Rose-Miller teat funnel. Having been fed colostrum the calves were then placed in the rearing accommodation along with the other calves.

All 15 calves were encouraged to suckle the automatic feeder approximately eight hours after being placed in the rearing accommodation.

The breeds, sex, birth and 28-day weights, 28-day faecal scores, and the 48-hour serum concentrations of absorbed immunoglobulins for the 15 calves are presented in Table 23. In this group there were ten calves sired by beef bulls, six by a Hereford bull, two by an Aberdeen-Angus bull and one by a Shorthorn bull. There were only five Ayrshire,

TABLE 23

GROUP 11

CALF NO.	SUB-GROUP++	DAY* BORN	BREED**	SEX	WEIGHT (kg) BIRTH	28DAYS	GAIN	28-DAY FAECAL SCORE	48-HOUR+ SAIg (ZST UNITS)
122	D	0	AxH	M	42.0	62.2	20.2	4	35
123	D	2	AxE x F	M	36.8	50.4	13.6	1	1
124	D	6	AxSh	F	34.0	53.1	19.1	14	24
125	C	6	F	M	30.6	40.8	10.2	1	32
126	E	7	F	M	29.5	45.0	15.5	5	23
127	E	8	AxH	F	29.1	39.9	10.8	4	22
128	E	9	AxH	F	35.6	50.6	15.0	7	17
129	C	9	AxAA	F	29.3	39.2	9.9	7	28
130	D	10	FxAA	F	34.6	41.9	7.3	16	22
131	E	11	A	F	27.2	42.7	15.5	25	18
132	D	12	FxAA	M	29.7	37.2	7.5	17	23
133	C	12	AxF. xF	M	36.5	43.1	6.6	4	23
134	C	13	FxH	F	31.4	41.3	9.9	16	26
135	C	14	AxH	M	40.3	48.1	7.8	12	44
136	E	14	FxH	M	38.9	47.7	8.8	10	19
			Mean		33.70	45.54	11.85		23.80
			S.D.		± 4.57	±6.57	± 4.36		± 9.47

* Day of birth in relation to first calf born

** Breed: A = Ayrshire; F = Friesian; H = Hereford
Ch = Charolais; Sh = Shorthorn; AA = Aberdeen Angus

\+ Serum concentration of absorbed immunoglobulins at 48 hours

++ Subgroup based on immediate post-natal management

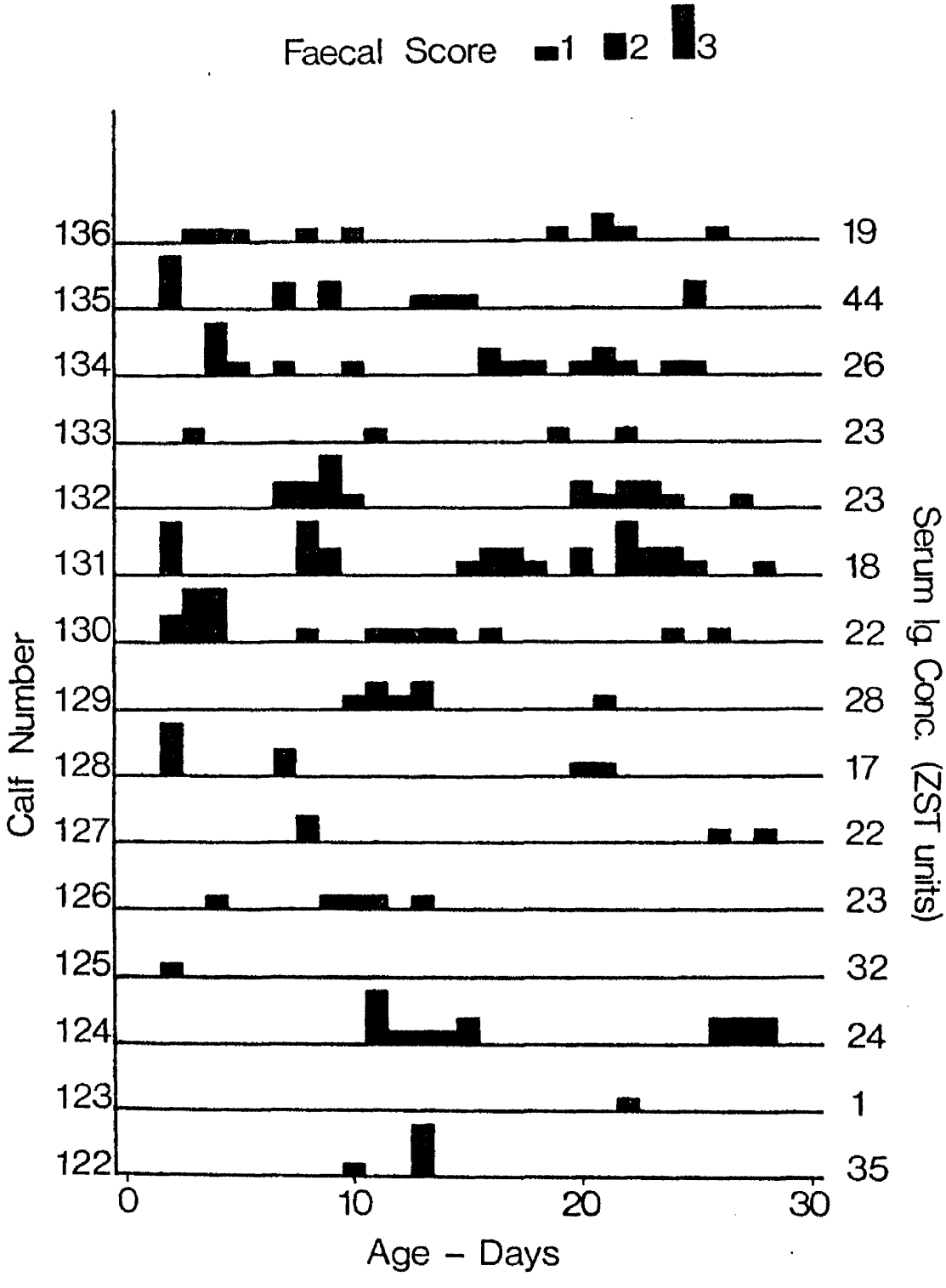


FIGURE 14 Group 11: The incidence and severity of diarrhoea during the first 28 days of life.

Friesian or AyrshirexFriesian calves. The male to female ratio was 8:7. The mean birth weight was 33.70 ± 4.57 kg. The mean 28-day weight was 45.54 ± 6.57 kg. The mean 48-hour serum concentration of absorbed immunoglobulins was 23.80 ± 9.47 ZST units. The mean values for the three subgroups of calves were, subgroup C, 30.60 ± 8.17 ZST units, subgroup D, 21.00 ± 12.35 ZST units, subgroup E, 19.80 ± 2.59 ZST units.

The calving pattern in this group was also very compact. All the calves were weaned on the same day when the oldest calf (122) was 48 days old and the youngest calf (136) was 34 days old.

The incidence of diarrhoea during the first 28 days of life for each calf is presented in Figure 14. Four calves were profusely diarrhoeic during the first four days and one, calf 130, had a faecal score of 2 on day 2 and 3 on days 3 and 4. Thereafter its maximum daily faecal score was 1 on eight of 24 days. Calf 123 (subgroup D) which had a serum concentration of absorbed immunoglobulins of 1 ZST unit at 48 hours of age had a maximum faecal score of 1 and that only on day 22.

The weight gains over the first 28 days of life varied from 6.6 kg (133) to 20.2 kg (122) with a mean of 11.85 ± 4.36 kg.

GROUP 12

There were 15 calves (137-151) in Group 12 born over a 31 day period from 26.6.73 to 26.7.73. The 15 calves were allocated to one of three different post-natal management treatments as follows:

Subgroup C comprised five calves (141, 142, 144, 147, 148) which were born in loose-boxes. After each calf had been weighed and a blood sample collected at 15 minutes post-partum, it was assisted to suckle colostrum to satiation from its own dam. Subsequent to this single feed of colostrum, these five calves were removed to the loose-box which had been selected as the rearing accommodation for all 15 calves.

Subgroup D comprised five calves (137, 138, 139, 143, 146) which were born in loose-boxes and allowed to remain with their dams for 48 hours. No attempt was made to encourage the calves to suckle or to interfere in any way except to weigh and to collect blood samples from the calves at 15 minutes post-partum. After 48 hours with their dams the calves were removed to the rearing accommodation.

Subgroup E comprised five calves (140, 145, 149, 150, 151) which were born in a byre. After each calf had been weighed and a blood sample collected at 15 minutes post-partum it was removed from the byre and held in separate, clean accommodation until approximately eight hours post-partum. At this time it was allowed to suckle 1700ml of its own dam's colostrum from a Rose-Miller teat funnel. Having been fed colostrum the calves were then placed in the rearing accommodation.

All 15 calves were encouraged to suckle the automatic feeder approximately eight hours after being placed in the rearing accommodation.

The breeds, sex, birth and 28-day weights, 28-day faecal scores, and the 48-hour serum concentrations of absorbed immunoglobulins for the 15 calves are presented in Table 24. There were two Ayrshire calves, three Friesian calves, three AyrshirexFriesian calves, three AyrshirexFriesian(dam)xFriesian calves and four calves sired by a

TABLE 24

GROUP 12

CALF No.	SUB-GROUP[++]	DAY BORN*	BREED**	SEX	WEIGHT (kg) BIRTH	28DAYS	GAIN	28-DAY FAECAL SCORE	48-HOUR+ SAIg (ZST UNITS)
137	D	0	AxH	F	35.4	47.0	11.6	0	24
138	D	0	FxH	F	42.7	56.3	13.6	14	13
139	D	5	F	M	34.2	47.3	13.1	4	6
140	E	6	F	M	32.7	46.8	14.1	6	12
141	C	11	AxF	M	32.5	51.2	18.7	11	16
142	C	12	FxH	F	30.1	45.8	15.7	6	38
143	D	14	A	M	37.0	45.0	12.0	8	15
144	C	15	AxF	F	27.0	39.9	12.9	20	11
145	E	17	AxF.xF	M	28.8	39.0	10.2	41	26
146	D	19	AxF.xF	F	35.5	49.6	14.1	13	27
147	C	19	AxF	M	36.6	53.2	16.6	3	28
148	C	25	AxH	F	32.3	43.7	11.4	15	27
149	E	26	A	M	31.8	42.7	10.9	16	12
150	E	29	AxF.xF	M	33.2	44.6	11.4	36	3
151	E	30	F	M	28.4	26.4	-2.0	48	2
			Mean		33.21	45.23	12.29		17.33
			S.D.		± 3.98	± 6.97	±4.58		± 10.49

* Day of birth in relation to first calf born

** Breed: A.= Ayrshire; F = Friesian; H = Hereford
Ch = Charolais; Sh = Shorthorn; AA = Aberdeen Angus

+ Serum concentration of absorbed immunoglobulins at 48 hours

++ Subgroup based on immediate post-natal management

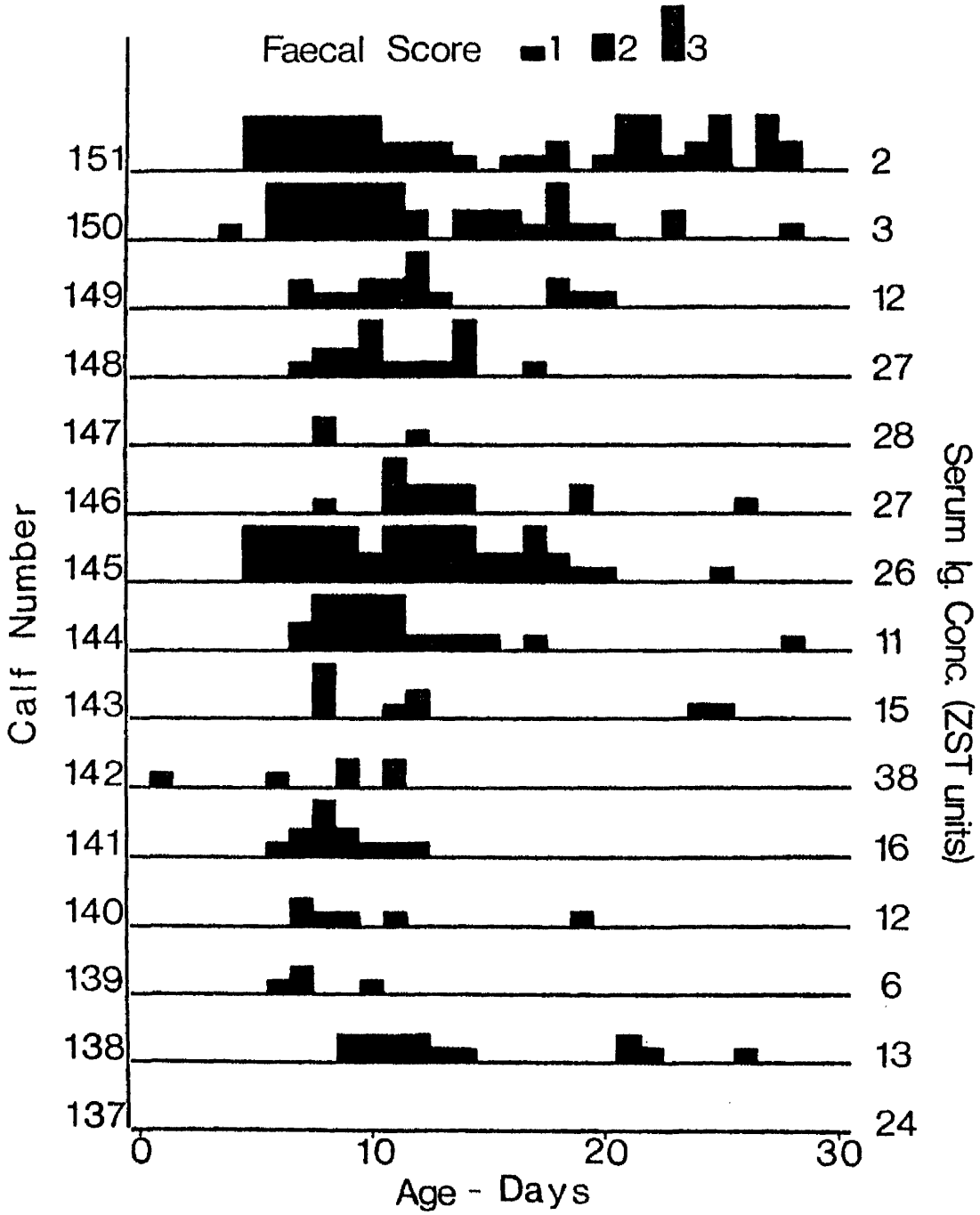


FIGURE 15 Group 12: The incidence and severity of diarrhoea during the first 28 days of life.

Hereford bull. The male to female ratio was 9:6. The mean birth weight was 33.21 ± 3.98 kg and the mean 28-day weight was 45.23 ± 6.97 kg. The mean 48-hour serum concentration of absorbed immunoglobulins was 17.33 ± 10.49 ZST units. This value was significantly lower than the mean value obtained for Groups 3 and 4 (p 0.01) but not for any of the other groups. The mean values for the three subgroups of calves were, subgroup C, 24.00 ± 10.65 ZST units, subgroup D, 17.00 ± 8.51 ZST units, subgroup E, 11.00 ± 9.64 ZST units.

The calving pattern in this group was very wide and it took over four weeks to assemble the 15 calves. Because of this the calves were weaned in four lots when the calves were between 44 and 52 days of age.

The incidence of diarrhoea during 28 days of life for each calf is presented in Figure 15. Two calves (145, 151) in this group had the highest 28 day faecal scores of all the group reared calves and four of the calves (144, 145, 150, 151) had persistent, profuse diarrhoea. Calf 144 was profusely diarrhoeic (faecal score 3) from eight to 11 days of age. Between five and 18 days of age, calf 145 had a faecal score of 3 on ten days and faecal score of 2 on four days. Calf 150 was profusely diarrhoeic (faecal score 3) on six consecutive days between five and ten days of age. During the first 28 days of life calf 151 had a faecal score of 3 on ten days and a faecal score of 2 on six days. This calf continued to be intermittently, profusely diarrhoeic for a further 13 days, when it died aged 41 days. At post-mortem examination the carcase was emaciated; slight ascites was present; the liver showed gross evidence of fatty degeneration and there was congestion of the lungs. No salmonellae spp. were isolated. The concentration of absorbed immunoglobulins at 48 hours of age had been 3 ZST units.

In this group the range of weight gains over the first 28 days were very wide from a loss of 2.0 kg (151) to a gain of 18.7 kg (141). The three other calves which had been persistently diarrhoeic, 144, 145, 150 gained 12.9, 10.2, and 11.4 kg respectively. The mean weight gained during the first 28 days was 12.29 ± 4.58 kg.

1. The relationship between the incidence of diarrhoea and age during the first four weeks of life.

From examination of Figures 6, 7, 8, 9, 10, 11, 14, 15 illustrating the incidence and severity of diarrhoea it can be seen that very few calves were diarrhoeic during the first four days of life, but that both the prevalence and the severity increased thereafter and appeared to be maximal during the second week of life. It also appeared that those calves born towards the end of a group were more diarrhoeic than earlier born calves. Both these possible variables were analysed for eight groups of calves (Groups 3, 4, 6, 7, 8, 9, 11, 12) using the Friedman two-way analysis (Siegel, 1956). The incidence of marked diarrhoea (faecal score 2 or 3) per calf during the first four weeks of life varied between the groups (excluding Group 10 and calf 73) from 1.7 days diarrhoea per calf for the 14 calves of Group 3 to 6.3 days diarrhoea per calf for the eight calves of Group 7. The mean incidence of diarrhoea in the 97 group-reared calves during the first four weeks of life was 3.7 days per calf.

To compute the analysis the calves of each group were divided into blocks of two or three calves in relation to their chronological entry to the rearing accommodation. The first 28 days of life were divided into four seven-day blocks. The daily faecal score of each calf for each of the seven days was summed and added to the seven-day block scores of the other calves within the block to give a total seven-day faecal score for each block of two or three calves.

In each of the eight groups analysed, the highest seven-day faecal score occurred from day 8 to day 14, i.e. during the second week of life. However, in seven of the eight groups the seven-day faecal score during the second week of life was not significantly different from the seven-day faecal scores of the three other seven-day blocks, i.e. the first, third or fourth week of life. In the remaining group (Group 12, calves 137-151) the seven-day faecal score during the second week of life was significantly greater than in any of the other three weeks ($0.02 > p > 0.01$). For the eight calves of

Group 7 the seven-day faecal scores of weeks two and three were much greater than those of weeks one and four, and almost approached statistical significance ($0.05 > p > 0.02$).

In four of the eight groups (Groups 7, 8, 9, 12), the second highest seven-day faecal score occurred from day 15 to 21, i.e. the third week of life. The second highest seven-day faecal score occurred during the first week of life in Groups 4 and 6 and during the fourth week of life in Group 3. In the remaining group, Group 11, the highest seven-day faecal score occurred during the second week of life, but weeks one and four had the equal second highest seven-day faecal score. Group 11 had however a very low incidence of diarrhoea overall.

There was some evidence that, for those calves born early in the formation of a group, the time of maximum prevalence of diarrhoea could be delayed. In Groups 6 and 7, the highest seven-day faecal score of the first block of calves born (calves 58, 59 in Group 6; calves 68, 69 in Group 7), occurred during the third week of life. In Group 9 the highest seven-day faecal score of the first block of calves (calves 96, 97) occurred during the fourth week of life.

2. The relationship between the incidence of diarrhoea during the first four weeks of life and time of entry to the rearing accommodation.

The impression was gained when the calves were being examined daily, that not only the prevalence but also the severity of diarrhoea was much more marked in those calves born towards the end of a group. However, the last block of calves had the highest 28-day faecal scores in only five of the eight groups of calves (Groups 3, 4, 6, 11, 12). In the remaining three groups of calves (Groups 7, 8, 9) other blocks of calves had the highest 28-day faecal scores; in Group 7, the second of the four pairs of calves (calves 70, 71) had the highest 28-day faecal score followed by the first pair (calves 68, 69); in Group 8, the third of the five blocks of calves (calves 88, 89, 90) had the highest 28-day faecal score; in Group 9 the fourth of the five blocks of calves (calves 102, 103) had the highest 28-day faecal score followed by the last pair of calves (calves 105, 106). Conversely, the first blocks of calves had the lowest 28-day faecal scores in only four of the eight groups of calves (Groups 4, 6, 9, 11).

Statistical examination by the Friedman two-way analysis revealed that a significant difference between the blocks of calves within each rearing group only occurred in Group 12 (calves 137-151, $0.02 > p > 0.01$). But even in this group although the last block of calves added to the rearing accommodation (calves 149, 150, 151) had the highest 28-day faecal score, the third block of calves (calves 143, 144, 145) had the second highest 28-day faecal score.

In Group 11, the 28-day faecal scores increased from the first block of three calves through to the last block of three calves, but there was no significant difference between the blocks of calves. In Group 7, calf 75, which together with calf 72 formed the third block of the group, suffered a prolonged period of profuse diarrhoea (Figure 9), but the second block of calves (70, 71) had the highest combined 28-day faecal score. The last block in this group (calves 74, 76) in fact had the lowest 28-day faecal score.

3. The relationship between the incidence of diarrhoea during the first four weeks of life and the serum concentration of absorbed immunoglobulins.

The correlation between the absorbed serum immunoglobulin concentration and the incidence of diarrhoea during the first 28 days of life was examined using Spearman's ranked correlation. To compute the analysis, the individual daily faecal scores of each calf over the first four weeks of life were summed to give the 28-day total for that calf and compared with its serum concentration of absorbed immunoglobulins at 48 hours of age.

Eight of the 97 calves in the eight groups (Groups 3, 4, 6, 7, 8, 9, 11, 12) analysed had 28-day faecal scores of 30 or above. Three of these, 105 (Group 9), 150, 151 (Group 12), which had 28-day faecal scores of 35, 36 and 48 respectively, had 48-hour serum concentrations of absorbed immunoglobulins of 6, 3 and 2 ZST units respectively. In contrast, the other five calves, 69, 71 and 75 (Group 7), 102 (Group 9) and 145 (Group 12) which had 28-day faecal scores of 31, 32, 30, 39 and 41 respectively, had 48-hour serum concentrations of absorbed immunoglobulins of 30, 26, 19, 31 and 26 ZST units. Diarrhoea was not an inevitable sequel to low ZST values; calf 123 (Group 11) which had a 48-hour serum concentration of absorbed immunoglobulins of only 1 ZST unit, had a total 28-day faecal score of 1; during the first 28 days of life, the consistency of the faeces on 27 days was considered to be normal and on just one day, day 22, the faeces were given a score of 1 (Figure 14).

The results for each of the eight groups were analysed separately, but no significant correlation was found to exist between the total faecal score for the first 28 days of life and the concentration of absorbed serum immunoglobulins at 48 hours of age.

4. The relationship between the incidence of diarrhoea and liveweight gain during the first four weeks of life.

The difference between the birth weight of individual calves and their weight at 28 days of age varied considerably from a loss of 2 kg (calf 151) to a maximum gain of 23.5 kg (calf 26). There were also variations between the groups in the mean liveweight gains over the first 28 days of life from 8.74 ± 3.87 kg (Group 7) to 14.34 ± 5.56 kg (Group 9). The mean liveweight gain for the 97 group-reared calves in Groups 3, 4, 6, 7, 8, 9, 11, 12, over the first 28 days of life was 12.28 ± 4.40 kg. The individual weekly weights and the liveweight gains to 28 days, weaning and 12 weeks are given in Appendix 3.

The relationship between the 28-day faecal score and weight gain during the first 28 days of life for each calf was examined by Spearman's ranked correlation. No significant correlation existed between the gain in weight over the first 28 days of life and the 28-day faecal score in seven of the eight groups of calves. However a negative correlation was found to exist between the 28-day weight and the 28-day faecal score for the 15 calves of Group 12 ($p < 0.05$). Although Group 7 had the highest incidence of diarrhoea (6.3 days per calf) and the lowest mean liveweight gain (8.74 ± 3.87 kg) of the eight groups there was no statistical correlation between the 28-day faecal scores and the liveweight gains of the individual calves. The weight gained or lost during each of the individual weeks in the first four weeks of life was compared with the weekly faecal score for the 14 calves of Group 8, but again there was no significant correlation determined.

The loss of weight or the failure to gain weight only occurred when diarrhoea was continually profuse (faecal score 3) over a period of days. This is best exemplified by calf 151 (Group 12) which was intermittently profusely diarrhoeic from five days of age. Its birth weight was 28.4 kg, but its weight at 28 days of age was only 26.4 kg, a loss of 2 kg.

Several other calves had poor weight gains which could be related to episodes of diarrhoea over the first four weeks of life.

Calf 66 (Group 6) had lost 2.3 kg of its birth weight by 11 days of age, having been diarrhoeic (faecal score 2 for 4 days and faecal score 3 for 3 days, Figure 8) from five to 11 days of age and at 28 days of age it had only gained 2.3 kg over its birth weight.

Calf 75 (Group 7) gained only 4.0 kg over the first 28 days of life, but from four to 11 days of age it lost 2.7 kg, having had diarrhoea for five of the seven days (faecal score 2 for 2 days and faecal score 3 for 3 days, Figure 9). During the following week despite having diarrhoea for six days (faecal score 2 for 3 days and faecal score 3 for 3 days) it gained 1.8 kg.

Calf 69 (Group 7) lost 0.5 kg between 17 and 24 days of age, having had diarrhoea for five days (faecal score 3 for 4 days and faecal score 1 for 1 day) during that period, and it gained only 7.7 kg over the first four weeks of life.

Calf 71 (Group 7) which suffered profuse diarrhoea (faecal score 3) on the second, third and fourth day of life, lost 1.6 kg during the first seven days and thereafter gained weight only slowly to achieve a gain of 7.5 kg over the first 28 days of life.

Calves 102 and 105 (Group 9) both lost weight during periods of profuse diarrhoea but had 28-day weight gains of 15.5 kg and 13.2 kg respectively.

In contrast, several calves had poor weight-gains during the first four weeks of life, although they were not severely affected by diarrhoea during that period. Calf 62 (Group 6) had a 28-day faecal score of 10, having only had diarrhoea on six of the first 28 days of life, and then only 4 days at faecal score 1 and 2 days at faecal score 3. During the first 28 days of life calf 62 gained only 6.6 kg compared to the group average of 11.04 ± 5.12 kg.

Calf 76 (Group 7) had a 28-day faecal score of 10, but it gained only 5.5 kg during the first four weeks of life, the second poorest gain of any calf in the group.

Calf 96 (Group 9) gained only 3.5 kg during the first four weeks of life and yet had a faecal score of only 10, the second lowest in the group. During the same period its twin, calf 97, gained 12.2 kg.

5. The relationship between the feeding of whole milk for the first four days of life and the incidence of diarrhoea during the first four weeks of life.

Most manufacturers of milk-substitute powders recommend that calves should be fed whole milk for the first days, possibly in self-protection and also in an attempt to ensure that farmers feed adequate quantities of colostrum during this period. It may also be partly due to the regulations of the various milk marketing boards in Britain, which require that colostrum and milk is excluded from milk supplied to the boards for a minimum of four days post-partum. . The opportunity was taken to determine if the feeding of milk-substitute from the first day of life had any influence on the incidence of diarrhoea during the first four weeks of life in calves which were fed colostrum to satiation as early as possible after birth.

This relationship was examined using the 18 calves of Groups 6 and 7. Group 6 consisted of five calves (59, 61, 63, 65, 67) fed whole cow's milk for eight feeds at approximately 12 hour intervals after the initial single feed of colostrum, and five calves (58, 60, 62, 64, 66) which were introduced directly to the automatic calf feeder after they had been assisted to suckle colostrum to satiation once only. Group 7 consisted of only nine calves, because of the difficulty in obtaining cows at the correct stage of gestation over a suitably short period. As a result four calves (69, 71, 73, 75) were fed whole milk for eight feeds subsequent to the initial assisted feed of colostrum and five calves (68, 70, 72, 74, 76) were introduced directly to the automatic feeder. Unfortunately one of the four substitute-fed calves (calf 73) died, leaving only three solely milk-substitute fed calves in Group 7.

The incidence and severity of diarrhoea for each calf in the two groups are shown in Figures 8 and 9. To determine if the feeding of whole milk had any influence on the incidence of diarrhoea during the first four weeks of life, the total 28-day faecal scores for the whole milk fed group and milk-substitute fed group within both Group 6

and Group 7 were analysed by the Mann-Witney test (Siegel, 1956). In Group 6, there was no significant difference in the 28-day faecal scores between either the whole milk fed calves or the milk-substitute fed calves. In Group 7, the group with the highest overall incidence of diarrhoea, the three milk-substitute calves had significantly higher 28-day faecal scores ($p < 0.02$).

SECTION 2

STUDIES ON THE BEHAVIOUR AND GROWTH RATES OF A GROUP OF CALVES REARED ON AN AUTOMATIC CALF FEEDER

The opportunity was taken to observe the behaviour and the suckling patterns of a group of calves which had free access to an automatic calf feeder. Group 8 (Tables 6 and 17) was chosen for this study as the 14 calves were born over a relatively short period of 13 days. The breed, sex, birth weights and 48-hour serum concentrations of absorbed immunoglobulins of the 14 calves are shown in Table 17. The group consisted of nine Ayrshire calves, three AyrshirexFriesian calves, one Friesian calf and one AyrshirexHereford calf. The birth weights varied from 22.9 kg to 34.0 kg and the 48-hour serum concentrations of absorbed immunoglobulins varied from 12 to 45 ZST units.

The frequency and severity of diarrhoea by members of this group has already been examined in the previous section but profuse, watery diarrhoea was only noted in eight calves, and then only for a maximum of three days during the first 28 days in calf 93 when it was 7, 8 and 9 days old. Diarrhoea was noted occasionally beyond the first 28 days of life (Figure 10).

None of the calves in this group had any difficulty learning to suckle the automatic feeder and most of the calves had become accustomed to the machine within two days of entry to the group. A social hierarchy developed within the group which was not related to the age of the calf although the most dominant calf (82) was the oldest calf. This calf consumed the greatest volume of milk on four of the five 24-hour observation periods, and had the highest mean volume over the five observation periods. Domination was shown by pushing away any calves at the feeder and starting to suckle. On occasions calf 82 would insert himself between the machine and other calves without taking any milk himself but preventing access to the teat by other calves. Another form of domination was what appeared to be deliberate harassment of other calves by butting calves which

were at the feeder and those calves engaged in other activities, such as eating calf pencils.

Competition for the single teat at the automatic feeder usually took the form of "head pressing" i.e. trying to remove the incumbent calf from the teat by exerting lateral force to the head and neck region. More aggressive competition was seen when a calf put its head underneath the calf suckling and attempting to lift the successful calf up and away from the teat. The most aggressive competition was employed by the most dominant calves, such as calf 82, and took the form of "battering ram" tactics. The less aggressive forms of competition were countered by the successful calf swinging round and standing along side the machine, as a calf suckling a cow would do, thus reducing the force which could be exerted by the competing calf. The other method used was for the incumbent calf to swing its hindquarters round towards the competing calf, allowing the competing calf less room to manoeuvre. Often, however, these defensive attempts failed, especially with two evenly matched calves, and each calf might have to settle for one suck about. Normally the time taken to empty the bowl was about one minute, but when there was competition, the time to finish one mix could be as long as six minutes.

Various sounds were generated by the automatic feeder while the mixing cycle was in operation and when the mixing process was complete a very obvious "click" was audible. Having finished off one mix several of the more dominant calves would often move away from the feeder to engage in other activities during the mixing cycle but when the "click" occurred at the completion of the mixing cycle, they would return to the feeder and displace less successful calves, which had begun to play with the teat during the mixing cycle.

Those calves which were subservient to dominant calves or which were unsuccessful would suckle the mixing bowl or occasionally might suckle the ear of the successful calf. One of the least dominant calves, 93, required only to be threatened by a more dominant calf to give up the teat, and if removed from the teat, very rarely challenged the successful calf. This calf was also very slow, often taking up to six minutes to suckle one mixing even if unmolested. During the last three observation periods its social rank appeared to

improve and it consumed considerably more milk. Although calf 89 consumed the lowest mean volume of milk over the five observation periods it did not appear to be dominated to any great extent.

As well as ear-sucking, other forms of non-nutritious suckling were noted. This most commonly took the form of suckling the preputial area of other calves and was carried out by both dominant and subservient calves.

Calf pencils and hay were freely available from the first week and rumination by several calves was noted during the first observation period, i.e. when some of the calves were only one week old. The ruminating action was unlike that of adult cattle and had a very fast circular motion and resembled much more the ruminating action of sheep. However, in the first two observation days, resting was rarely accompanied by rumination. During these resting periods calves tended to lie with heads tucked round on their flanks almost under the hind leg. Occasionally there were "play-periods" when as many as ten calves would run round the loose-box obviously enjoying themselves. Vocalization was a very rare feature in this group or any of the other groups of calves and only occurred during the occasional "play-periods".

No coughing was noted in the first observation period and during the second observation period only an occasional cough was noted but principally among the older calves. In the last three 24-hour observation periods the frequency of coughing increased and reached a peak during the fourth and fifth observation period when 55 coughs per hour per 14 calves were noted. The frequency of coughing was not consistent throughout the day, e.g. between 5 am and 6 am of the fifth observation period when most calves were resting there were only 15 coughs per hour, but between 8 pm and 9 pm there were 55 coughs per hour per 14 calves.

From these observations on a group of calves reared on an automatic calf feeder the following factors were determined:

1. The volume of milk substitute consumed by calves with free access to an automatic calf feeder.

2. The frequency of suckling by calves with free access to an automatic calf feeder.

3. The relationship between frequency of suckling and time of day for calves with free access to an automatic calf feeder.

4. The relationship between the volume of milk substitute consumed and liveweight gain of calves with free access to an automatic calf feeder.

1. The volume of milk substitute consumed by calves with free access to an automatic calf feeder.

The volume of milk substitute consumed by each individual calf during the five 24-hour observation periods is shown in Table 25. There was a marked individual variation with the mean volume of milk substitute consumed per calf over the five observation periods ranging from 6.51 litres to 13.64 litres. Over the five observation periods the lowest volume consumed per 24-hour period was 4.12 litres (calf 84) and the highest was 16.05 litres (calf 82), both during the first observation period.

There was remarkably little week-to-week variation in the volume of milk substitute consumed by individual calves during each of the five 24-hour observation periods. During the first 24-hour observation period, calf 84 consumed the lowest of all the 24-hour values at 4.12 litres, but over the next four 24-hour observation periods its mean 24-hour consumption was 7.49 litres. The three youngest calves, 93, 94, 95, had quite low intakes during the first 24-hour observation period of 4.97, 6.82, and 4.83 litres respectively. This may have been partly due to a lack of familiarisation with the automatic feeder and partly due to competition from calves who had already been on the automatic feeder for up to three weeks.

Calf 89, which had the lowest mean 24-hour consumption of 6.51 litres over the five observation periods, was an Ayrshire heifer which had weighed 29 kg at birth, only the fifth lowest in group and had in fact the highest 48-hour serum concentration of absorbed immunoglobulins of 45 ZST units.

TABLE 25

The volume of milk consumed per 24-hour observation period.

CALF No.	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	MEAN ± SD
82	16.05*	13.78	9.66	15.76	12.93	13.64 ± 2.58
83	7.39	7.67	10.23	7.81	7.81	8.18 ± 1.16
84	4.12	7.95	7.81	5.82	8.38	6.82 ± 1.80
85	8.95	10.51	8.10	7.10	7.81	8.49 ± 1.31
86	6.96	11.93	11.08	9.38	9.80	9.83 ± 1.89
87	10.51	10.65	10.94	11.51	11.51	11.02 ± 0.47
88	12.07	10.37	7.95	8.38	9.52	9.66 ± 1.65
89	5.26	6.25	9.23	5.54	6.25	6.51 ± 1.59
90	4.97	11.08	8.81	9.52	11.65	9.20 ± 2.63
91	7.24	10.09	9.94	8.24	10.23	9.15 ± 1.33
92	6.53	8.24	5.97	7.53	11.51	7.95 ± 2.17
93	4.97	7.24	10.23	9.52	10.37	8.47 ± 2.32
94	6.82	9.23	10.09	8.81	9.80	8.95 ± 1.29
95	4.83	11.22	5.54	10.80	9.94	8.47 ± 3.04
Mean	7.62	9.73	8.97	8.98	9.82	litres
S.D.	± 3.32	± 2.07	± 1.71	± 2.57	± 1.80	

* litres.

2. The frequency of suckling by calves with free access to an automatic calf feeder.

The number of suckling cycles for each individual calf during the five 24-hour observation periods is shown in Table 26. The mean number of suckling cycles of each calf over the five 24-hour observation periods ranged from 6.6 to 11.4. As with the volume of milk substitute consumed, there was a wide variation of suckling cycles over the five observation periods from 4 (calf 92, week 3) to 16 (calf 82, week 1; calf 87, week 4). The range of the mean number of suckling cycles per 24-hour observation period for the group was 8.1 (week 5) to 10.7 (week 4).

The mean number of suckling cycles of calf 89 over the five 24-hour observation periods was 8.4. This was only the sixth lowest even although this calf had the lowest average 24-hour intake of 6.51 litres. During the third observation period calf 82, which had the highest mean consumption, suckled only six times, compared with the 16 times on the first week but this was also the 24-hour observation period during which it consumed only 9.66 litres, the lowest of its five 24-hour intakes.

TABLE 26

The number of suckling cycles per 24-hour observation period.

CALF No.	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	MEAN ± SD
82	16	11	6	12	8	10.6 ± 3.85
83	8	7	9	15	7	9.2 ± 3.35
84	8	6	11	9	6	8.0 ± 2.12
85	9	6	8	7	10	8.0 ± 1.58
86	8	9	10	15	8	10.0 ± 2.92
87	8	11	10	16	12	11.40 ± 2.97
88	9	8	6	7	5	7.0 ± 1.58
89	7	10	12	7	6	8.4 ± 2.51
90	8	9	9	8	6	8.0 ± 1.22
91	10	8	10	14	10	10.4 ± 2.19
92	6	7	4	9	7	6.6 ± 1.82
93	7	8	14	12	8	9.8 ± 3.03
94	10	9	11	9	7	9.2 ± 1.48
95	11	11	10	10	13	11.0 ± 1.22
Mean	8.93	8.57	9.28	10.71	8.07	
S.D.	± 2.43	± 1.74	± 2.61	± 3.24	± 2.37	

3. The relationship between frequency of suckling and time of day for calves with free access to an automatic calf feeder.

One of the most striking features of this study into the suckling behaviour of a group of calves on an automatic feeder was the marked lull in activity which occurred in the early morning between 3 am and 6 am. This reduction in the use of the machine took place despite the calves being housed under conditions of continuous illumination. The mean hourly volume of milk-substitute consumed and the number of calves suckling during the five 24-hour observation periods are presented in Figure 16. The hourly consumption of milk-substitute by each individual calf during the five weekly observation periods is given in Appendix 3.

During the second observation period, this early morning reduction in activity was much less noticeable, with seven calves consuming 24 units between 3 am and 5 am. Of these 24 units consumed, calves 84 and 88 took 6 and 5 units respectively, and even on this morning, the machine was not suckled by any calf from 5.05 am to 6.05 am. During the fourth 24-hour observation period no calf used the machine for a period of 3 hours 8 minutes between 2.30 am and 6 am.

The total time that the machine was in use, either being suckled or mixing during each of the five observation periods is shown in Table 27. The mean length of time that the machine was in use over the five 24-hour observation periods was 14 hours 43 minutes, with a maximum of 18 hours 32 minutes (week 2) and a minimum of 11 hours 1 minute (week 5).

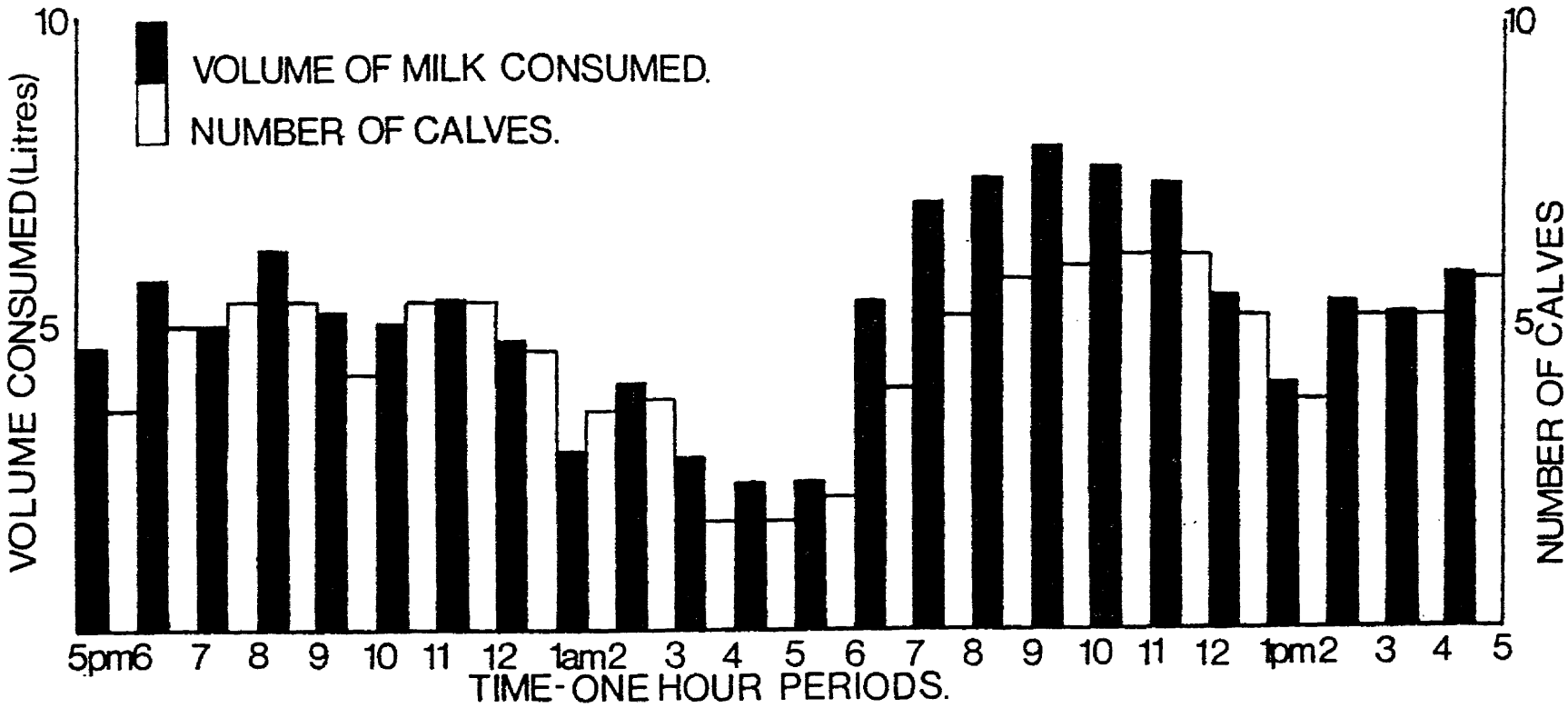


FIGURE 16 The mean hourly volume of milk-substitute, supplied from an automatic calf feeder, consumed during five weekly 24-hour observation periods.

TABLE 27

The total machine use time per 24-hour observation period

WEEK 1	12 hours	22 minutes
WEEK 2	18 hours	32 minutes
WEEK 3	14 hours	12 minutes
WEEK 4	15 hours	8 minutes
WEEK 5	11 hours	1 minute
MEAN	14 hours	43 minutes

4. The relationship between the volume of milk-substitute consumed and the liveweight gain of calves with free access to an automatic calf feeder.

The relationship between liveweight gain and the volume of milk-substitute consumed was examined for the 14 calves of Group 8. The 14 calves were weighed once weekly at 2 p.m. on the afternoon before the five weekly 24-hour observation periods were started at 5 p.m.

The weekly liveweights for the 14 calves for this four week period are presented in Table 28. There was a wide range of total liveweight gains over the 28 days from 9.3 kg to 23.7 kg. Moreover, a wide variation in weekly liveweight gains occurred not only between calves, 0.5 kg (calf 92) to 7.7 kg (calf 88) but also between weeks for the same calf. Between day 25 and day 32, calf 92 gained only 0.5 kg, but between day 39 and day 46, it gained 4.5 kg. Calves 82 and 86 had the most consistent weekly liveweight gains, 5.93 ± 0.82 kg/week (calf 82) and 4.78 ± 0.61 kg/week (calf 86). The mean daily liveweight gains over the 28 day period, calculated by dividing the total liveweight gain by 28, varied from 0.34 kg/day (calf 93) to 0.85 kg/day (calf 82).

A highly significant positive correlation ($r = 0.83$, $p<0.001$), was found to exist between mean volume of milk-substitute consumed during the five 24-hour observation periods and the mean daily liveweight gains (Figure 17). A highly significant positive correlation ($r = 0.82$, $p<0.001$) was also demonstrated to exist between the mean volume of milk-substitute consumed during the five 24-hour observation periods and the total liveweight gain over the 28 days covered by the five observation periods (Figure 18).

TABLE 28

Weekly liveweight, total liveweight gain, mean weekly and mean daily liveweight gain of the 14 calves of Group 8

CALF No.	LIVEWEIGHT (kg)					LIVEWEIGHT GAIN (LWG)		
	DAY 18*	DAY 25	DAY 32	DAY 39	DAY 46	Total LWG over 28 days	Mean weekly LWG (kg)	Mean daily LWG (kg)
82	44.1	50.9	55.9	61.4	67.8	23.7	5.93 ± 0.82	0.85
83	37.3	39.5	45.5	47.7	51.4	14.1	3.53 ± 1.79	0.50
84	35.0	38.2	40.0	41.8	47.3	12.3	3.08 ± 1.75	0.44
85	42.7	45.5	48.6	50.0	54.5	11.8	2.95 ± 1.27	0.42
86	32.7	36.8	42.3	46.8	51.8	19.1	4.78 ± 0.61	0.68
87	37.7	41.8	46.4	50.9	53.6	15.9	3.98 ± 0.88	0.57
88	37.3	40.0	47.7	42.7	56.4	19.1	4.78 ± 2.17	0.68
89	28.2	31.8	34.1	35.5	39.5	11.3	2.83 ± 1.19	0.40
90	34.1	36.4	40.0	44.5	49.1	15.0	3.75 ± 1.07	0.54
91	30.5	34.5	38.2	39.1	42.7	12.2	3.05 ± 1.44	0.44
92	33.2	37.3	37.8	41.4	45.9	12.7	3.18 ± 1.82	0.45
93	34.5	35.5	37.7	39.1	44.1	9.6	2.40 ± 1.80	0.34
94	31.8	34.1	37.3	40.9	45.5	13.7	3.43 ± 0.95	0.49
95	23.8	24.7	28.8	33.8	35.6	11.8	2.95 ± 1.92	0.42

* Day of weighing in relation to birth of calf 82 - born on day 0.

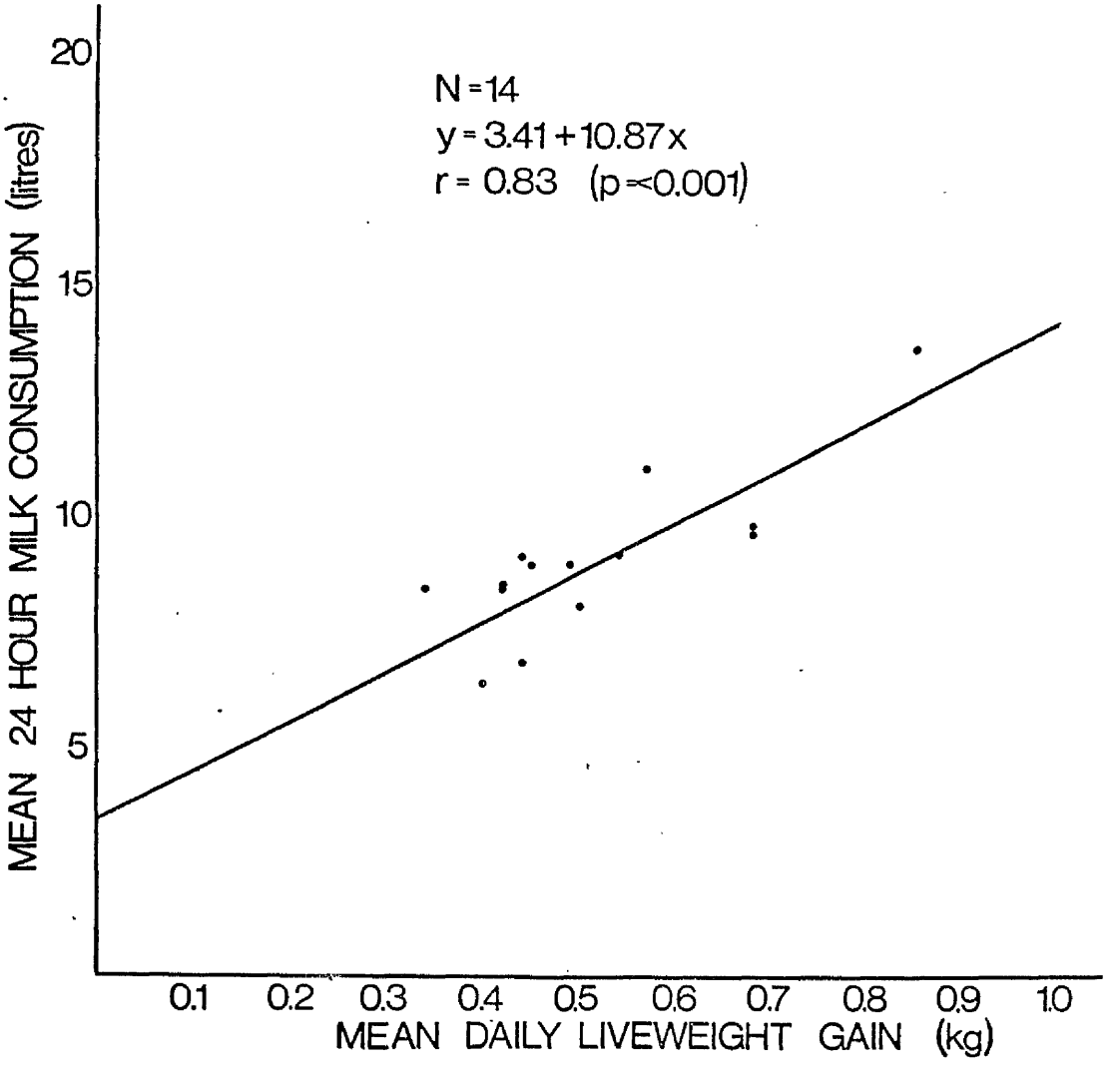


FIGURE 17 The relationship between the mean daily volume of milk-substitute consumed and the mean daily liveweight gain.

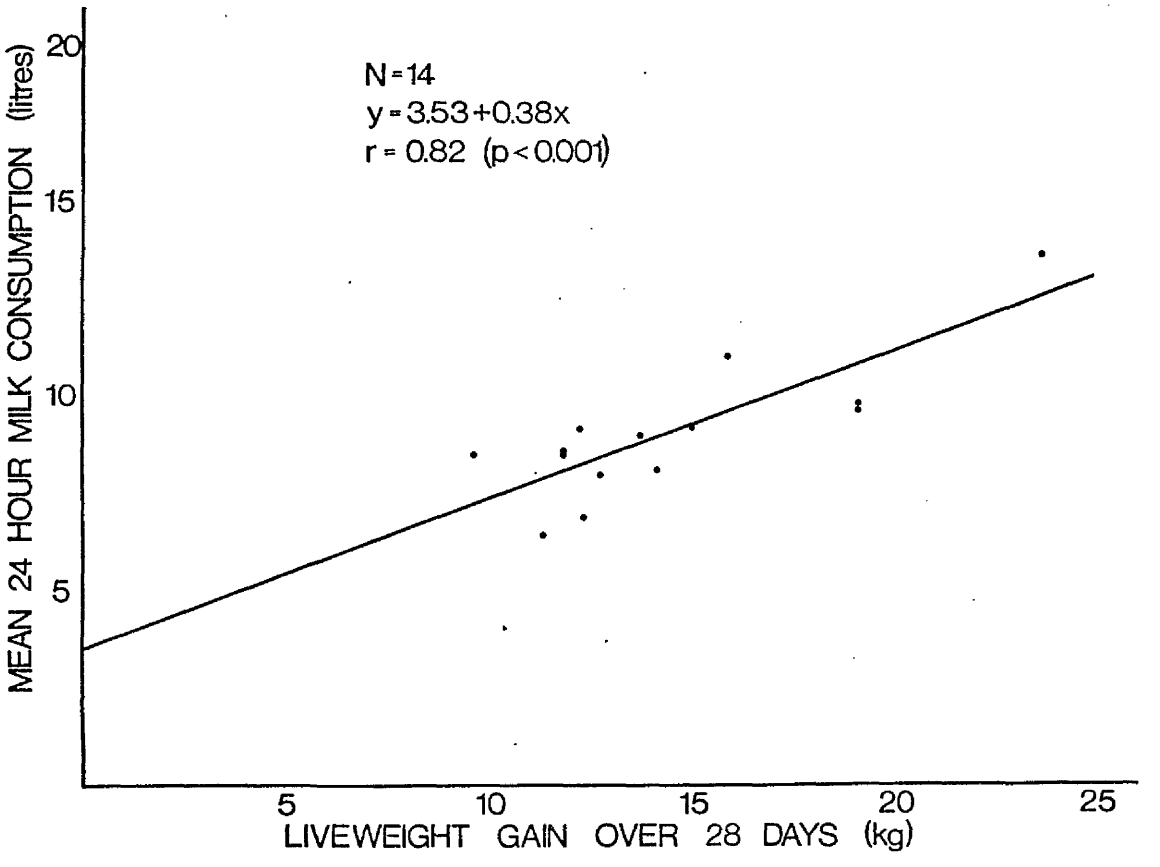


FIGURE 18 The relationship between the mean daily volume of milk substitute consumed and the total liveweight gain over 28 days.

DISCUSSION

Excluding the five calves of Group 10 which died from salmonellosis, only three of 131 group-reared calves (including the calves of Group 1, Group 2 and Group 5) died, a mortality of 2.3 per cent. Diarrhoea was the major clinical sign in two of the three calves. One calf (Group 9) died within 36 hours of birth, having developed a profuse, watery diarrhoea when approximately 24 hours old. Although no bacteriology was carried out, it is possible that this calf was suffering from enteric toxaemia as described by Gay (1965) caused by an enterotoxigenic *E. coli* (Smith and Halls, 1967a). Calf 151 (Group 12) died, aged 41 days, after suffering profuse diarrhoea intermittently from five days of age. No salmonella organisms were isolated, and no histological examination of the intestine was carried out. Calf 73 (Group 7) died, aged five days, of a severe necrotising pneumonia. All three calves had serum concentrations of absorbed immunoglobulins of less than 10 ZST units; calf 151, 3 ZST units; calf 73, 7 ZST units; the third calf (Group 9) had a serum concentration of absorbed immunoglobulins of 5 ZST units at 24 hours of age.

Surveys of calfhood disease have generally confined themselves to the mortality rates of young calves, rather than the precise causes of death, as the diagnoses have been based upon farmer/stockman observation (Lovell and Hill, 1940; Withers, 1952, 1953; Leech and others, 1968). The signs of ill-health have of necessity been poorly defined, broad terms such as scouring, coughing, off-feed, fever, incoordination, or other clinical signs of which scouring was the predominant clinical sign occurring more than twice as frequently as any other sign (Leech and others, 1968). Nevertheless, Leech and others (1968) estimated that 9 per cent of all calves scoured. A field-study carried out in Canada found an overall incidence of diarrhoea during the first ten days of life of 24.5 per cent, although during the period January to March this figure increased to 38 per cent (Willoughby and others, 1970). Unfortunately the authors fail to define the term diarrhoea.

The collection of the total daily faecal output during physiological studies of calf diarrhoea has permitted the accurate quantitation of daily faecal output and faecal dry matter content

(Blaxter and Wood, 1953; Mylrea, 1966; Logan and Penhale, 1972; Fisher and de la Fuente, 1972; Logan and Penhale, 1971a; Fisher and Martinez, 1975b; Logan and others, 1977). From these studies calves producing faeces with a dry matter content of 10 per cent or less and a total daily output of more than 500g have been classed as diarrhoeic (Logan and Penhale, 1971a; Fisher and Martinez, 1975b; Logan and others, 1977). Logan and Penhale (1972) also noted that the faeces of calves which were severely diarrhoeic had a dry matter content of five per cent or less. In the present study faeces with a score of 2 had a mean dry matter content of 11.1 per cent and faeces with a score of 3 had a mean dry matter content of 4.5 per cent. It is unlikely that a farmer/stockman would be too concerned by a calf passing faeces with a score of 1.

In the present study, the incidence of diarrhoea was maximal between five and 16 days of life for all groups except Group 11, although a significant difference between weeks could only be demonstrated in Group 12. This is later than the period of maximal incidence of diarrhoea between two and seven days recorded by Logan and Penhale (1971a,b) and Logan and others (1974b). However in studies of spontaneous diarrhoea in "market" calves purchased at three to seven days of age, the maximum period of diarrhoea occurred between five and 13 days of age (Logan and Penhale, 1971c) or between 10 and 15 days of age (Dalton and others, 1960), ages more consistent with those of the present study. Many of the studies into the aetiology of neonatal calf diarrhoea have been confined to the first two weeks of life (Acres and others, 1975; Morin and others, 1976), and most investigators agree that by 21 days of age calves which have not succumbed are generally voiding normal faeces (Aschaffenburg and others, 1949a; Dalton and others, 1960; Gay, 1965). With a few exceptions this was confirmed in the present study.

The failure to demonstrate any correlation between the incidence of diarrhoea and the serum concentrations of absorbed immunoglobulins confirmed the observations of other workers (Gay and others, 1965a; McEwan and others, 1970a; Hurvell and Fey, 1970), although it has been repeatedly demonstrated that high levels of circulating absorbed immunoglobulins almost invariably protect calves from the ultimate effect of severe diarrhoea (Fey and Margadant, 1962; Gay

and others, 1965a,b; McEwan and others, 1970a; Boyd, 1972; Penhale and others, 1973). However, in one experimental study, in which the calves were challenged with an enterotoxigenic strain of E. coli, before being fed colostrum, high circulating levels of absorbed immunoglobulins failed to prevent death from marked diarrhoea in two calves (Logan and others, 1977).

In the present study the lack of correlation between the incidence of diarrhoea during the first four weeks of life and the serum concentrations of absorbed immunoglobulins, may have been influenced by several factors. The overall incidence of diarrhoea (faecal score 2 or 3) for the 97-group-reared calves (excluding Group 10 and calf 73) during the first four weeks of life was 3.7 days per calf. This varied between the groups with Group 7 having the highest value of 6.3 days diarrhoea per calf and Group 3 having the lowest value of 1.7 days diarrhoea per calf. This low incidence of diarrhoea may have been partly due to the use of clean rearing accommodation for every group, and in part to the relatively high mean serum concentrations of absorbed immunoglobulins. Roy and others (1955a) demonstrated that there is a highly significant correlation between the length of time a calf-house had been occupied and the incidence of diarrhoea amongst the calves housed. Although the incidence of moderate to severe diarrhoea during the first 28 days for the 15 calves of Group 12 was only 5.0 days per calf, compared to 6.3 days per calf for Group 7 and 5.1 days per calf for Group 9, it was the only group in which the incidence of diarrhoea during the second week of life was significantly greater than the other three weeks. Group 12 was also the only group in which the last calves added to the rearing accommodation had a significantly greater incidence of diarrhoea than the calves born earlier. Group 12 had the widest calving spread of 31 days, with the result that calves 150, 151 were placed in the rearing accommodation more than four weeks after calves 137 and 138 had been born. This prolonged calving period may have allowed a build up of infection in the rearing accommodation as described by Wood (1955).

The calves of Group 12 also had the lowest mean concentration of absorbed immunoglobulins at 17.33 ZST units which may also have contributed to an increase in the weight of infection within the rearing accommodation. The mean serum concentrations of absorbed

immunoglobulins between the groups varied from 17.33 ± 10.49 ZST units for the 15 calves of Group 12 to 29.86 ± 9.77 ZST units for the 14 calves of Group 3, which had the lowest mean incidence of diarrhoea during the first four weeks of life of 1.7 days per calf. Group 7 which had the highest incidence of diarrhoea had a mean serum concentration of absorbed immunoglobulins of 25.44 ± 10.35 ZST units. The mean ZST values of all three subgroups in Group 12 were lower than the corresponding means in Groups 10 and 11. The low values of subgroups D and E, which made the major contribution to the low overall mean of Group 12, can be explained in terms of delayed and insufficient feeding of colostrum. Calf 144 (subgroup C) was born to an 18-month-old heifer and required considerable traction, which may have depressed the efficiency of absorption through increased circulating steroid levels.

The role of circulating immunoglobulins in enteric disease has yet to be fully defined. Parenterally administered colostral whey or gammaglobulin preparations have little influence on the outcome of the diarrhoeic syndrome, but they will prevent colisepticaemia (Fey and others, 1963; Willoughby and others, 1970; Logan and Penhale, 1971a). Experimental evidence suggested that passively transferred colostral antibody against rotavirus was not protective in the calf, or the lamb (Woode and others, 1975: Snodgrass and Wells, 1976). In contrast Macnulty and others (1976) demonstrated that calves with high circulating levels of absorbed immunoglobulins were protected against rotavirus infection and later this was also confirmed in lambs (Snodgrass and Wells, 1978). A significant negative correlation has been demonstrated between the serum immunoglobulin concentrations and the mean daily faecal output (Fisher and de la Fuente, 1972; Fisher, Martinez, Trainin and Meirom, 1975).

It has been suggested that there is a negative correlation between serum immunoglobulin concentrations and mortality from salmonellosis (Irwin, 1974; Fisher and others, 1976) but no such relationship could be demonstrated in the outbreak of salmonellosis which affected Group 10. Also, there was no correlation between either the flagellar or somatic titres of the serum samples collected at 48 hours of age and the 48-hour serum concentrations of absorbed immunoglobulins. On the basis of five experimental calves, Fisher and others (1976) suggested that protection against neonatal salmonellosis

from absorbed colostral immunoglobulins was non-specific and was dependent on high circulating concentrations of IgM. Certainly as salmonellosis in calves is invariably septicaemic (Smith and Halls, 1968b) the importance of the IgM class of immunoglobulins is not unexpected (Hobbs, 1974).

One of the outstanding clinical signs of severe neonatal diarrhoea in the calf is loss of weight (Blaxter and Wood, 1953; Dalton and others, 1960; Fisher and de la Fuente, 1972; Fisher and Martinez, 1975b). In the present experiments loss of weight or failure to gain weight only occurred over a short period when diarrhoea was marked. Excluding the calves affected by salmonellosis, only one calf (151) lost weight over the first 28 days of life and it was intermittently profusely diarrhoeic from five days of age. Under more controlled physiological experiments Fisher and Martinez (1975b) were unable to demonstrate any significant difference between the weight gains of diarrhoeic calves which survived and healthy non-diarrhoeic calves, although a highly significant difference in weight gains was shown between diarrhoeic calves which died and the healthy non-diarrhoeic calves. In seven of the eight groups of calves there was no correlation between the total faecal score over the first 28 days of life and weight gained during that period. In Group 12 there was a negative correlation between these two parameters ($p < 0.05$) but even in this group the mean weight gain over the first 28 days was considerably higher than that of Group 7. Excluding Group 10, Group 7 which had the highest mean incidence of diarrhoea also had the lowest mean weight gain during the first 28 days of life at 8.74 ± 5.56 kg.

The highly significant positive correlation between the volume of milk consumed and the daily liveweight gain for the 14 calves of Group 8 has demonstrated that in situations where the incidence of diarrhoea is low, the major constraint to growth is the amount of food consumed. Roy and others (1958) calculated that the volume of whole milk required for maintenance of body weight was 2.15 to 3.34 litres per day for calves whose initial birth weight varied from 27.3 to 45.4 kg. For calves of similar body weight the volume of reconstituted synthetic milk employed in their experiments required to maintain body weight varied from 1.77 to 2.89 litres. In the present study, by extrapolation, the volume of reconstituted milk substitute required to

maintain body weight was 3.41 litres. This value compares not unfavourably with the figures of Roy and others (1958) given the variation in body weights. Variations in both the fat and carbohydrate content of the milk substitute powder may also have contributed to the difference in value. It has also been suggested that calves obtaining milk substitute from automatic feeders have poor weight gains and reduced efficiency of food conversion in comparison with calves on bucket-feeding to appetite twice daily (Bakker, 1967, Cited by Roy, 1977).

The suggestion that overfeeding with milk during the neonatal period is largely anecdotal (Blaxter and Wood, 1953; Inglis, 1960; Loosemore, 1964) but Roy and others (1958) put forward experimental evidence to show that there was a tendency for a higher incidence of diarrhoea to occur in calves consuming greater volumes of milk; as did Wise and La Master (1968) and Leaver and Yarrow (1972). However, Mylrea (1966) demonstrated that calves between nine and 38 days of age could consume up to 26 per cent of their body weight daily of whole milk offered ad lib without being predisposed to diarrhoea. In the present study calf 82 consumed a mean of 13.64 litres per day but had a total 28-day faecal score of 11 and calf 87 consumed a mean of 11.02 litres/day with a 28-day faecal score of only 12.

There is little doubt that the use of milk substitute powders is more likely to predispose calves to diarrhoea than whole milk (Roy and others, 1955b; Shillam, Roy and Ingram, 1962a) and that the dried skim milk powder is the constituent responsible for this increased incidence of diarrhoea. The more severe the heat treatment of skim milk prior to spray drying the greater the denaturation of the protein and the higher the incidence of diarrhoea (Shillam and others, 1962b; 1962c). The severe heat treatment of the skim milk alters the clotting properties of the reconstituted milk substitute powder and results in increased amounts of undigested protein nitrogen passing from the abomasum into the duodenum (Tagari and Roy, 1969).

The voluntary intake of milk substitute supplied from an automatic calf feeder appears to be directly related to the dry matter content of the milk supplied as Hafez and Lineweaver (1968) noted that both the frequency of suckling and the time spent suckling decreased on increasing the dry matter content of reconstituted milk substitutes.

The dry matter content of milk substitute supplied by the automatic calf feeder used in the present experiments was not monitored but one study has shown that dry matter content of the milk substitute furnished by an automatic feeder designed to supply milk at 8.5 per cent dry matter varied from 1.3 per cent to 24.7 per cent (Riggot and Quarmby, 1971). Occasionally in the present study the milk powder became bridged in the holding hopper of the feeder resulting in the calves being supplied with warm water.

Hafez and Lineweaver (1968) recorded that Hereford and Holstein calves, aged one to six weeks and penned alone or in pairs, suckled a Nursette feeder on average 12.3 (Hereford) and 23.4 (Holstein) times per day. Similarly, Stephens (1974), who observed groups of 15 Friesian bull calves on a Nursette automatic feeder for a three hour period between 2 and 5 p.m. twice weekly recorded that each of his observed calves suckled between five and seven times per six hours. If these three hour observation periods were representative of the whole day, an assumption which is not necessarily true, Stephen's calves would have been suckling 20-28 times per day, figures comparable with those of Hafez and Lineweaver but considerably higher than the seven to 11 times per day recorded in the present observations. The natural suckling activity of calves has been studied on several occasions and the recorded number of suckling cycles per 24 hours has been remarkably similar with means of four to eight suckling cycles per day. The average duration of each suckling cycle recorded in these studies was approximately nine minutes (Peterson and Woolfolk, 1955; Walker, 1962; Hutchison, Woof, Mabon, Saleke and Robb, 1962; Hafez and Lineweaver, 1968; Ewbank, 1969).

One of the most interesting findings during the present observations was the marked reduction in suckling activity between 2 a.m. and 6 a.m. when most of the time was spent either resting or ruminating. This reduction in activity occurred despite the conditions of continuous illumination. A similar but less marked and less consistent lull in activity occurred during the afternoon. Hafez and Lineweaver (1968) failed to note any diurnal variation in the suckling activity of their calves, and postulated that this may have been due to the calves being maintained under continuous illumination. Observations on natural suckling behaviour have shown that peaks of suckling activity occur at approximately six hour intervals, but two major suckling periods occur

around midnight and at approximately 5 a.m. Observing 22 beef cows and calves, in New Zealand, at grass, for ten 24 hour periods between August and February, Walker (1962) recorded only eight suckling episodes between 1.30 a.m. and 3 a.m., and six of the eight times occurred when the calves were very young. During the present observations the time of maximum suckling activity took place between 5 a.m. and 8 a.m.

Not surprisingly, Hafez and Lineweaver (1968) found that calves consuming milk of 19.5 per cent dry matter gained weight more quickly than calves supplied with milk of 6.5 per cent dry matter; even although the calves fed the 6.5 per cent dry matter milk suckled more frequently in an attempt to satisfy their dry matter intake requirement. Other workers (Walker, 1962; Ewbank, 1969) failed to find any correlation between the number of suckling cycles and daily live weight gain of calves suckling cows naturally. Hutchison and others (1962) who studied Zebu cows and calves in Tanganyika found that calves which suckled more frequently, had poorer live weight gains, but made the comment that these particular calves were nursed by cows who were yielding very little milk, and this increased frequency of suckling was a response to hunger.

Salmonellosis in calves is recognised as an important and increasingly widespread problem in Britain (Hughes, Gibson, Roberts, Davies, Davies and Sojka, 1971), but only a few detailed clinical descriptions of outbreaks have been published (Craig, Davies and Massey, 1941; Rankin, Newan and Taylor, 1967; Nazer and Osborne, 1977). The major clinical features which differentiated the outbreak of S. enteritidis infection, which affected the calves of Group 10, from other forms of neonatal diarrhoea were high fever, lethargy and inappetence. Eleven days after the death of the first calf, five of the 14 calves had rectal temperatures in excess of 106°F and the mean rectal temperature of all 14 calves was 104.9°F. Over the next 14 days the mean rectal temperature gradually fell to normal. The mean rectal temperatures of the last four calves which died were considerably higher than that of the survivors. On the day of maximum pyrexia, the group as a whole was almost completely inappetent, with very little milk powder being consumed. The other striking clinical feature was

lethargy and dullness, not only of the calves which eventually died but also of those which survived. Dysentery was never recorded despite careful daily clinical examination, although this is commonly associated with the disease in calves (Gibson, 1961; Hughes and others, 1971; Richardson, 1975).

Weight loss was particularly marked in those calves which died, probably from a combination of diarrhoea, especially in the case of calf 115 which was profusely diarrhoeic for seven days, and inappetence or inability to compete for the single teat on the automatic feeder. Of the ten surviving calves, the liveweight gains were poor and indeed, weight loss was severe in some cases. This was best illustrated by calf 116, which weighed 39.3 kg at birth, but gradually lost weight over the first three weeks of life, until at 24 days of age it weighed only 34.1 kg. However, by 28 days of age its weight had increased to 35.5 kg although it did not regain its birth weight until it was 46 days old.

Two days before the day of maximum pyrexia and nine days after the death of calf 115 S. enteritidis was isolated from only four calves, but four days later, the organism was isolated from 11 calves. On the next two sampling days ten of 14 calves and ten of 12 calves had positive rectal swabs. Thereafter the faecal excretion of the organism fell off dramatically, with only one calf having positive rectal swabs 16 and 19 days after the day of maximum pyrexia. Thus, in this outbreak, S. enteritidis infection behaved in a manner similar to that described for S. dublin infection (Field, 1959; Osborne and others, 1974) with the calves apparently clearing themselves of infection completely.

If one accepts the suggestion that a significant flagellar titre is one in excess of 1/320 (Clarenburg and Vink, 1949; Field, 1959; Sojka and others, 1974), then only two of the surviving ten calves could retrospectively be considered as definitely having been infected with S. enteritidis. Only three calves developed antibodies to the flagellar antigens in their convalescent sera but on each weekly sampling day only one calf had an agglutination titre of 1/320 or above. The highest flagellar agglutination titre encountered was 1/5120 found in a serum sample collected from calf 112 shortly before

it died at 35 days of age. This observation would support the view of Osborne and others (1974) that high agglutinin titres are merely evidence of past infection and are not necessarily an indication of resistance.

The source of the infection was never positively identified. However, as the main source of infection for calves would appear to be the adult carrier cow (Field, 1959; Thomas and Harbourne, 1972; Richardson, 1973), and as five of the 15 dams had serum flagellar titres of 1/320 or greater, it is possible that one of the dams was a non-clinical excretor. The most likely source of the infection was cow 108 which had a serum flagellar titre of 1/1280 at parturition. Its calf had also shown episodes of dullness, lethargy and intermittent diarrhoea from an early age. It is equally possible that calf 115, which was born in the byre which held cow 108 and the other seropositive cows, may have become infected immediately after birth as a result of being born into a contaminated environment. Infection would then have gradually built-up in the rearing accommodation through contamination of coats, bedding, or the single teat on the automatic feeder (Osborne and others, 1974).

CHAPTER 4

AN EXAMINATION OF IMMUNOGLOBULINS IN THE LACHRYMAL FLUID OF NEWBORN CALVES

AN EXAMINATION OF IMMUNOGLOBULINS IN THE LACHRYMAL FLUID OF NEWBORN CALVES

Introduction

Tiselius and Kabat (1939) clearly demonstrated that antibody activity resided within the gammaglobulin fraction of serum. Later Smith and Holm (Smith, 1946; Smith and Holm, 1948) showed that antibody activity of bovine serum and colostrum was also closely associated with the gammaglobulin component. Advances in protein biochemistry have permitted the identification of at least four protein fractions with immune activity in bovine serum and secretory fluids (Murphy, Aalund and Osebold, 1965; Pierce and Feinstein, 1965; Milstein and Feinstein, 1968; Butler, 1969; Mach, Pahud and Isliker, 1969).

The basic immunoglobulin structure consists of four polypeptide chains, two heavy chains and two light chains held together by disulphide bonds. Each immunoglobulin class has antigenically distinct heavy chains. The light chains can be divided into two groups, either kappa or lamda, which are immunogenically distinct. All immunoglobulin classes have either kappa or lamda light chains, but each immunoglobulin molecule has only one type of light chain (Roitt, 1974).

At a symposium held in 1970, a nomenclature, based on the W.H.O. nomenclature for human immunoglobulins (WHO, 1965), was proposed for the immunoglobulins of the domesticated bovidae (Butler, Winter and Wagner, 1971). The major antigenically distinct classes of immunoglobulins, identified by the heavy chain determinants, were accepted as IgG, a heterogeneous group of immunoglobulins consisting predominately of IgG_1 and IgG_2, IgA and IgM. The term free secretory component was also proposed for the small protein molecule, bovine glycoprotein-a. There is evidence that a bovine homocytotropic immunoglobulin analogous to human IgE also exists (Wells and Eyre, 1970; Hammer, Kickhofen and Schmid, 1971; Doyle, 1972; Jarrett, 1973).

In the normal adult cow the concentration of IgG in serum is approximately 20 mg/ml (Butler and others, 1971) with subclass IgG_1 accounting for slightly more than half of the total IgG (Butler, 1969; Nansen, 1970; Mach and Pahud, 1971). Both IgG_1 and IgG_2 molecules

have the basic immunoglobulin structure, similar sedimentation coefficients of 6.5-7S and equal molecular weights of approximately 150,000 - 163,000 daltons (Butler and others, 1971). Minor differences in the amino acid sequences of the C terminal ends of the heavy chains exist between the two sub-classes; a threonine residue on the heavy chain of IgG_1 being replaced by a methionine residue on the heavy chain of IgG_2 (Milstein and Feinstein, 1968). IgG_2 carries a greater net positive charge than IgG_1 and thus migrates more slowly towards the anode of a basic agar gel electrophoretic field than IgG_1. The molecules of both sub-classes contain 2-3 per cent carbohydrate (Butler and others, 1971). An examination of 11 breeds of cattle revealed that, in a high proportion of animals, the heavy chains of IgG_2 carry an allotype A1, absent from the heavy chains of IgG_1 (Blakeslee, Butler and Stone, 1971). Using a radio-labelled isotope technique, Nansen (1970) calculated the half-lives of bovine IgG_1 and IgG_2 to be 9.6 and 17.7 days respectively, but in four animals less than 10 months old, Pedersen (1973) recorded half-lives of 21.3 and 24.6 days for IgG_1 and IgG_2 respectively. Sasaki, Davis and Larson (1976), also employing isotope labelled techniques found that the half-life of IgG_1 in cows was reduced to 4.6 days in the period immediately before and after parturition. During the same period the half-life of IgG_2 was 12.1 days. MacDougall and Mulligan (1969) determined the half-life of radio-labelled IgG_1 in calves to be 18 days. Calculations of the half-life of IgG from the decrease in absorbed colostral IgG have usually given values of 16 to 32 days (Husband, Brandon and Lascelles, 1972; Porter, 1972; Logan, Penhale and Jones, 1973; Sasaki and others, 1977a).

Although bovine IgG_1 and IgG_2 are related immunogenically and physico-chemically, they differ in many biological functions and activities. Variations have also been noted in the biological functions of the two ovine and caprine IgG sub-classes, and in these species the IgG sub-classes have been the subject of more detailed studies than bovine IgG_1 and IgG_2.

One of the major differences between bovine IgG_1 and IgG_2 is the selective transfer and accumulation of IgG_1 in colostrum which occurs in the late stages of gestation (Murphy, Aalund, Osebold and Carrol, 1964; Pierce and Feinstein, 1965; Sasaki and others, 1976). Only the IgG_1 sub-class is active in complement mediated reactions

(Feinstein and Hobart, 1969; Pearson and Lloyd, 1972; Beh, 1973). A similar finding has also been noted for ovine IgG_1 (Feinstein and Hobart, 1969; Rhee, Broad and Jonas, 1970; Esteves, Sant'anna, dos Santos Annes and Binaghi, 1974; Grant, Adams and Miller, 1975; Stevenson and Elliott, 1978) and caprine IgG_1 (Micusan and Borduas, 1977). However Feinstein and Hobart (1969) presented evidence to suggest that ovine IgG_2 may be able to fix homologous complement at physiological concentrations.

The production of sub-class IgG_1 anti-hapten and anti-carrier antibodies in sheep and goats is much more rapid and results in higher serum concentrations than sub-class IgG_2 antibodies (Margni, Castrelos and Paz, 1973; Micusan and Borduas, 1977). Similarly in sheep experimentally infected with Fasciola hepatica serum antibodies of the IgG_1 sub-class to the parasite appeared more quickly and reached higher concentrations than those of the IgG_2 sub-class (Movsesijan, Jovanovic, Aalund and Nansen, 1975). There is a tendency for the concentration of sub-class IgG_2 antibodies to specific antigens to increase as the concentration of sub-class IgG_1 antibodies declines (Margni and others, 1973) and Williams and Green (1976) reported that the serum concentration of bovine IgG_2 increased around the time of parturition, a period when the serum concentration of IgG_1 is lowest.

Both classes of ovine and caprine IgG are involved in haemagglutination reactions but IgG_1 is much more efficient (Kaplan and Freeman, 1971; Esteves and others, 1974; Micusan and Borduas, 1977). Antibodies of the IgG_1 sub-class produce heterologous passive cutaneous anaphylactic and reverse Arthus reactions but only sub-class IgG_2 antibodies are capable of producing homologous passive cutaneous anaphylaxis (Esteves and others, 1974; Micusan and Borduas, 1977). Although it is known that an IgG antibody produces homologous passive cutaneous anaphylaxis in the mouse, rat and guinea pig (Spiegelberg, 1974), the possibility that the sheep and goat IgG_2 fractions employed by both Esteves and others (1974) and Micusan and Borduas (1977) were contaminated with IgE antibody cannot be overlooked.

In a study of sheep antibodies produced in response to guinea pig Bence-Jones protein, Stevenson and Elliott (1978) found that only antibody of sub-class IgG_2 mediated K-cell cytoxicity. Earlier, Grant, Adams and Miller (1975) reported that ovine K-cell dependent cytotoxic antibody was mediated by both IgG_1 and IgG_2 sub-classes.

In both goats and sheep macrophage cytophilic activity appears to be confined to the IgG_2 sub-class (Watson, 1976; Borduas and Micusan, 1977). These cytophilic antibodies are considered to fulfil the function of opsonisation (Spiegelberg, 1974). Nansen (1970) noted that cows with pyogenic infections had increased synthesis and higher serum concentrations of IgG_2 than normal controls. In a further study (Nansen, 1972) it was found that 14 per cent of a group of 93 cows with primary pyogenic infections were IgG_2 deficient compared with only 1 per cent of 100 cows which had non-infectious conditions. It has also been found that whereas no variation in serum concentrations of IgG_1 occurs with age, dairy cows over six years old have significantly higher serum concentrations of IgG_2 than cows less than six years old (Williams, Maxwell and Spooner, 1975).

The wide differences in biological activity between ovine IgG_1 and IgG_2 led Esteves and others (1974) to doubt if they ought to be classified together despite their physico-chemical similarities.

Bovine IgA was first isolated and characterised by Mach, Pahud and Isliker in 1969 (Mach and others, 1969), although an immunoglobulin with an immunoelectrophoretic position corresponding to human IgA had been noted before that by other workers (Murphy and others, 1964; Murphy and others, 1965; Gough, Jenness and Anderson, 1966) and the identification of a 10S globulin in bovine serum had been described (Sullivan, Prendergast, Antunes, Silverstein and Tomasi, 1969). The concentration of IgA in the serum of adult cows is approximately 0.3 mg/ml (Mach and Pahud, 1971).

Although bovine IgA has the basic immunoglobulin structure with a sedimentation coefficient of 7S, a high proportion of bovine serum IgA exists in the dimeric form (Butler, 1971; Mach and Pahud, 1971), the two basic 7S molecules being joined by a cysteine-rich polypeptide J chain (Morrison and Koshland, 1972; Komar, Abson and Mukkur, 1975). J chain is necessary for the <u>in vivo</u> polymerisation of IgA (Parkhouse and Della Corte, 1973). Bovine secretory IgA, an IgA dimer attached to one molecule of secretory component and normally secreted at mucous surfaces, has a sedimentation coefficient of 11S and a molecular weight of 385,000 daltons (Butler and others, 1971). Unlike many other species, about 20 per cent of the IgA in bovine

serum is secretory IgA (Mach and Pahud, 1971). The carbohydrate content of the IgA molecule is between six and ten per cent (Butler and others, 1971) and the half-life of bovine serum IgA, calculated from the decline in plasma concentrations of absorbed colostral IgA, is approximately three days (Husband and others, 1972; Porter, 1972; Logan and others, 1973).

IgA is known to have anti-viral activity in the absence of complement (Tomasi and Grey, 1972) and the anti-viral activity in bovine nasal secretions to parainfluenza-3 virus has been ascribed to IgA (Morein, 1970, 1972). Beh (1973) reported that IgA prepared from milk whey was devoid of complement fixing activity but had agglutinating activity. It has been suggested that human and porcine secretory IgA are bacteriolytic in the presence of complement and lysozime (Adinolfi, Glynn, Lindsay and Milne, 1966; Hill and Porter, 1974). However, Eddie, Schulkind and Robbins (1971) failed to show any potentiation of the bacteriolytic effect of lysozime on Salmonella typhimurium by rabbit secretory IgA. In most species IgA is the predominant immunoglobulin secreted at mucosal surfaces (Tomasi and Grey, 1972). Secretory IgA antibodies are known to inhibit the adherence of bacteria to mucosal surfaces (Williams and Gibbons, 1972) and Brandtzaeg (1973) considered that they formed the body's "first line of defense" against infection.

Secretory component is a glycoprotein with a molecular weight of 75,000 daltons and a carbohydrate content of five per cent (Mach, 1970; Butler and others, 1971). Although normally bound to a dimer of IgA, abundant free secretory component is found in cow's milk, a common source of the protein (Mach, 1970). Unbound secretory component has not been found in bovine serum (Mach and Pahud, 1971). It is thought that secretory component stabilizes the IgA molecule in the presence of denaturing agents and protects it from proteolysis (Steward, 1971; Jerry, Kunkel and Adams, 1972).

Brandtzaeg (1973) proposed that free secretory component was synthesised in epithelial cells lining glands and conjugation of the secretory component and IgA occurred within the epithelial cell before release into the lumen of the gland. However, in an examination of the IgA secretory system of the rabbit mammary gland, Kraehenbuhl, Racine and Galardy (1975) were unable to confirm that the conjugation of IgA and secretory component occurred within the epithelial cells.

Bovine IgM is a macroglobulin with a sedimentation coefficient of 19S and a molecular weight of 900,000-103,000 daltons (Butler and others, 1971). Historically, macroglobulins with immune activity were first noted in the sera of horses and cattle (Heidelberger and Pedersen, 1937; Kabat and Mayer, 1961). The carbohydrate content of the bovine IgM molecule is 10-12 per cent (Butler and others, 1971) and the half-life, calculated from the decline in plasma concentrations of absorbed colostral IgM, is four days (Husband and others, 1972; Porter, 1972; Logan and others, 1973). The concentration of IgM in adult serum is approximately 2-5 mg/ml (Mach and Pahud, 1971).

As similarity in structure, physiochemical and immunochemical reactions exists between bovine and human IgM (Mehta, Reichilin and Tomasi, 1972; Beale and Buttress, 1972), it can be assumed that the bovine IgM molecule is also a pentamer, consisting of five basic 7S units and a J chain (Metzger, 1970; Komar and Mukkur, 1975). As with IgA, J chain is necessary for the in vivo polymerisation of IgM (Parkhouse and Della Corte, 1973). A smaller single unit 7S molecule of bovine IgM also occurs in the serum at low concentrations (Butler and Maxwell, 1972) and increased proportions of this single unit form are found in some protozoan diseases (Masseyeff, Blondel and Mattern, 1972).

Antibodies of the IgM class are involved in agglutination reactions (Robbins and others, 1965), complement fixation reactions (Spiegelberg, 1974) and complement mediated cytotoxic reactions (Grant and others, 1975; Stevenson and Elliott, 1978). They are the first antibodies to be synthesised in an immune response. Murphy, Osebold and Aalund (1966) investigated the antibody response in cattle to Anaplasma marginale and found that the early complement mediated antibody which first appeared between 20-30 days post-infection was solely IgM. Four to five days later complement mediated IgG_1 antibodies were detectable but, even at the maximum response, IgG_1 antibodies only accounted for about one quarter of the total complement mediated antibody. IgM agglutinating antibodies were also synthesised early in the response but IgG agglutinating antibodies were not detected until approximately 18 months after infection. The immunological response of adult cattle to contagious bovine pleuropneumonia organisms is almost identical (Pearson and Lloyd, 1972). Rose and Roepke (1964) found

that the early agglutinating antibodies produced following Brucella abortus vaccination had very high molecular weights and were likely to be IgM antibodies. In contrast, Beh (1973) found little agglutinating or complement fixing activity in an IgM fraction prepared from the serum of a cow vaccinated 16 days earlier with B. abortus strain 19.

Thus the ox has an immune system in which the major immunoglobulin classes are similar physiochemically, functionally and immunogenically to those of man and other non-ruminant species and which provides it with immunological protection. However, differences between the human and bovine immune systems exist. In man, secretory IgA is the major immunoglobulin secreted in colostrum (Tomasi and Grey, 1972) whereas IgG_1 is the predominant immunoglobulin of bovine colostrum. Also, in man, secretory IgA is the major immunoglobulin secreted at mucous surfaces, but in the ox the respective roles of secretory IgA and IgG at mucous surfaces may differ.

Of all the external secretions of the cow, colostrum contains the highest concentration of immunoglobulins. As early as 1946, Smith (1946) had noted that the bovine plasma protein fraction with immune activity could be separated into two major components; gammaglobulin, (IgG_2 in present day terminology), an electrophoretically-slow protein, and T-globulin (IgG_1 in present day terminology), an electrophoretically-faster protein. From a study on the variation in the mobility of these two plasma globulins and colostral globulin, Smith (1946) implied that immune globulin of colostrum was similar to T-globulin (IgG_1). It was later shown that the colostral gammaglobulin was derived directly from serum and that with approaching parturition a marked concentration of this gammaglobulin occurred within the udder as compared to other serum proteins (Askonas and others, 1954; Larson and Gillespie, 1957; Larson, 1958; Dixon and others, 1961; Blakemore and Garner, 1956; Garner and Crawley, 1958). Johnson and Pierce (1959) found that most of the colostral gammaglobulin had a sedimentation coefficient of 6.45S. Subsequently, it was shown that the electrophoretically-fast 7S serum globulin (IgG_1) was selectively transferred to colostrum from serum with the almost complete exclusion of the electrophoretically-slow 7S serum gammaglobulin (IgG_2) (Murphy and others, 1964; Pierce and Feinstein, 1965).

Johnson and Pierce (1959) noted that a small proportion of the total colostral immunoglobulin consisted of an 18S macroglobulin and the presence of IgM in colostrum was confirmed by Murphy, Aalund, Osebold and Carrol (1964). By analogy with the immunoelectrophoretic position of human IgA, Murphy and others (1964) considered that a bovine IgA component was also present in colostrum. However, the presence of secretory IgA was only confirmed some time later by Mach and others, (1969).

Thus the four major immunoglobulin classes present in serum are also found in colostrum but there is a marked selective transfer of IgG_1 which accounts for 87 per cent of the total colostral whey immunoglobulin (Mach and Pahud, 1971). The concentrations of colostral whey and serum immunoglobulins recorded by various groups of workers have been tabulated by Roesti and Fey (1975), but those given by Mach and Pahud (1971) were as follows: IgG_1 - 75 mg/ml; IgG_2 - 1.9 mg/ml; IgA - 4.4 mg/ml; IgM - 4.9 mg/ml. Brandon, Watson and Lascelles (1971) found that in the two to three weeks prior to parturition the concentration of serum IgG_1 fell abruptly by more than 50 per cent, whilst the serum concentrations of IgG_2, IgM, IgA and albumin remained virtually unchanged. Sasaki and others (1976) determined that this drop in the serum concentration of IgG_1 could not by itself account for the accumulation of IgG_1 in the colostrum, but that during the period of colostrum formation the synthesis of IgG_1 was greatly increased and there was a marked reduction in its half-life.

In an attempt to elucidate this selective transport mechanism further, Hammer, Kickhofen and Malchow (1969) studied the <u>in vitro</u> absorption of an electrophoretically-fast IgG (IgG_1) isolated from bovine colostrum and an electrophoretically-slow IgG (IgG_2) isolated from serum on to mammary gland epithelial cells collected six to 21 days prior to the donor calving and found that the electrophoretically-fast IgG was preferentially bound to the epithelial cells. Further studies with dispersed mammary cells and radio-iodinated immunoglobulins have shown that the surfaces of these cells possess specific sites for binding IgG_1 and IgG_2 immunoglobulins, the number of sites being dependent on the stage of lactation and pregnancy. During mid-lactation the binding sites per cell for IgG_1 and IgG_2 are 9,000 and 2,400

respectively, with both types of binding sites having similar association constants. With the onset of colostrum formation, the number of specific IgG_1 binding sites per cell increases. Approximately one week prior to parturition, new binding sites appear on the cell surfaces which have a very strong affinity for sub-class IgG_1 molecules. The ratio of IgG_1: IgG_2 binding sites per cell is similar to the 7:1 ratio of IgG_1:IgG_2 concentrations found in colostrum and milk (Sasaki, Larson and Nelson, 1977b).

Following parturition a rapid decrease in the concentrations of all colostral immunoglobulins occurs within 48 hours (Porter, 1972), and one week after parturition the concentrations of all four immunoglobulins in whey are similar to those of mid-lactation milk, with mean concentrations of 0.35 mg/ml of IgG_1, 0.06 mg/ml of IgG_2, 0.04 mg/ml of IgM and 0.05 mg/ml of IgA (Mach and Pahud, 1971). As the number of IgG_1 binding sites on mammary cells collected in mid-lactation exceeded those of IgG_2 and because of the small amount of serum albumin found in milk, Sasaki and others (1977b) suggested that the selective mechanism, so outstanding during the colostrum formation, prevailed throughout lactation. A selective concentration of IgG_1 over IgG_2 has also been noted in the non-lactating mammary gland of non-pregnant, multiparous cows (Smith, Conrad and Porter, 1971).

The manner by which mucous surfaces are protected from infection has been of interest for some considerable time and the early work on the protection of mucous surfaces by the production of specific antibodies has been reviewed by Pierce (1959b). In this excellent review Pierce dealt with the published work on the alimentary, respiratory, urinary and reproductive tracts and mammary gland up to the late 1950's.

The possible existence of a local immune system in the alimentary tract was first suggested by Besredka (1919) following his work with Shigella bacilli in rabbits. Subsequently, Davies (1922) reported that significant agglutination titres to Shigella organisms could be demonstrated in the saline extracts of stools from human patients affected by Shigella dysentery, and that these coproantibodies could be detected during the first week of infection. Following the experimental oral infection of guinea pigs with Vibrio cholera, Burrows, Elliot and Havens (1947) demonstrated the presence of specific antibodies

in their stools, but there was no correlation between the concentration of specific antibodies in the serum and resistance to oral challenge with the organism (Burrows and Ware, 1953). In man the production of coproantibodies can also be induced by the oral administration of killed V. cholera (Freter, 1962) and coproantibody can be demonstrated in the stools of naturally infected, acutely ill cholera patients (Freter, De, Mondal, Shrivastava and Sunderman, 1965). This cholera coproantibody is predominately secretory IgA (Northrup, Bienenstock and Tomasi, 1970) and in the clinically normal adult human secretory IgA is the major immunoglobulin in intestinal secretions (Tomasi and Grey, 1972). The density of IgA plasma cells greatly exceeds the density of IgM and IgG plasma cells at all levels of the intestine in man (Crabbe and Heremans, 1966).

The concentrations of the four major bovine immunoglobulin classes in gastro-intestinal secretions were first reported by Mach and Pahud (1971) who examined secretions collected from the abomasum and the small and large intestines of Simmental cattle of various ages. Those authors recorded mean values of 24 mg/100ml of IgA, 25 mg/100ml of IgG_1 and 6 mg/100ml of IgG_2. Only traces of IgM were detected. Curtain, Clark and Dufty (1971) examined the intestinal secretions for IgG_1 and IgG_2 only in one 20-month-old Hereford heifer and recorded concentrations of 8.29 mg/ml of IgG_1 and 0.760 mg/ml of IgG_2 in the small intestine. Newby and Bourne (1976) determined the relative concentrations of immunoglobulins recovered from the proximal metre of the small intestine of four 18-month-old cattle. They found that IgG_1 was the predominant immunoglobulin accounting for over 50 per cent of the total immunoglobulin content. IgA formed only 20 per cent of the total immunoglobulin and even less when wall-associated immunoglobulins were examined. IgG_2 formed about 15 per cent of the total immunoglobulin content. IgM, if detected, was present only in very low concentrations.

Using a technique in which class-specific anti-sera were conjugated to horseradish peroxidase, Newby and Bourne (1976) stated that IgG-staining plasma cells predominated in the duodenal lamina propria of adult cattle although no absolute figures were given, nor was any differential count of IgG_1 or IgG_2 staining cells attempted.

Only small numbers of IgA-staining plasma cells were seen. Curtain and others (1971) examined the IgG_1 and IgG_2 immunocyte population in the intestinal tract of one adult and found that IgG_1 plasma cells accounted for 90 per cent of the total. Allen and Porter (1975) detected numerous IgA and IgM-containing cells in the jejunum of four 12-24 month old animals by immunofluorescent techniques, but the IgA cells predominated, with only very few cells staining for IgG_1.

The presence of antiviral activity due to antibodies in the nasal secretions of man was demonstrated many years ago (Burnet, Lush and Jackson, 1939; Francis, 1940, 1943). However it is only relatively recently that a similar finding has been demonstrated in calves (Gutekunst, Paton and Volenec, 1969; Gates, Cesario, Ebert, Kriel, Wulff, Poland, Gutekunst and Chin, 1970; Frank and Marshall, 1971; McKercher, Saito, Franti, Wada and Crenshaw, 1972). In both natural and experimental infections of calves with parainfluenza-3 virus this antibody activity has been attributed to IgA (Morein, 1970; 1972). Quantitatively secretory IgA is the major immunoglobulin in nasal secretions, accounting for 90 per cent of the total immunoglobulin concentration (Mach and Pahud, 1971; Duncan, Wilkie, Hiestand and Winter, 1972; Table 29). However, examination of the immunoglobulin bearing cells in the nasal, tracheal and pharyngeal mucosa of adult cattle has revealed a predominance of IgG_1 staining cells (Morgan and Bourne, 1978; Curtain and others, 1971).

In an examination of the immunoglobulins in the lachrymal secretions of 20 adult cows of the Red Danish breed, Pedersen and Nansen (1972) recorded the following mean values: IgG_1, 0.139 ± 0.06 mg/ml; IgM, 0.378 ± 0.37 mg/ml; IgA, 2.5 ± 0.72 mg/ml. They were unable to determine a mean concentration for IgG_2, as in most samples the concentration fell below their lower limit of detection of 0.06 mg/ml. The values determined by Mach and Pahud (1971); Duncan and others (1972); Butler, Maxwell, Pierce, Hylton, Asofsky and Kiddy (1972b) are given in Table 29. Secretory IgA isolated from tears and nasal secretions exists in two polymeric forms, 11S-IgA and 15S-IgA (Butler, 1971; Komar and others, 1975) and both lachrymal and nasal secretory IgA possess antigenic determinants absent from colostral secretory IgA (Butler, 1971).

TABLE 29

Concentrations of immunoglobulins in lachrymal fluid, nasal secretions and saliva

REFERENCE	YEAR	BREED/TYPE	No.OF ANIMALS	IgG mg/ml	IgG_1 mg/ml	IgG_2 mg/ml	IgM mg/ml	IgA mg/ml
LACHRYHAL FLUID								
Mach and Pahud	1971	Simmental; cows	10	-	0.30	0.12	0.006	2.60
				Range -	(0.20-0.50)	(0.03-0.38)	(0.004-0.01)	(1.55-3.10)
Duncan and others	1972	Holstein-Friesian; heifers, 2-3 years old. (Results of 9-12 weekly samples)	4	-	$0.13 \pm 0.07^{*}$	$0.05 \pm 0.04^{*}$	$0.07 \pm 0.12^{*}$	$0.87 \pm 0.33^{*}$
Pedersen and Nansen	1972	Red Danish Milkbreed; cows, 2-10 years old	20	-	$0.139 \pm 0.06^{*}$	0.024^{**}	$0.378 \pm 0.37^{*}$	$2.50 \pm 0.72^{*}$
Butler and others	1972b	Holstein-Friesian, cows (Results of 10 weekly samples pre- and post-partum)	6	Prepartum -	$0.54 \pm 0.08^{+}$	ND	ND	$3.88 \pm 0.49^{+}$
				Postpartum -	$0.48 \pm 0.07^{+}$	ND	ND	$3.75 \pm 0.58^{+}$
NASAL SECRETIONS								
Mach and Pahud	1971	Simmental; cows	10	-	0.04	0.025	Traces	1.95
				Range	(0.03-0.05)	(0.01-0.04)		(1.40-2.30)
Duncan and others	1972	Holstein-Friesian; heifers, 2-3 years old. (Results of 9-12 weekly samples)	4	-	$0.19 \pm 0.15^{*}$	$0.08 \pm 0.08^{*}$	0.23 ± 0.13	$1.73 \pm 0.85^{*}$
SALIVA								
Mach and Pahud	1971	Simmental; cows	10	-	0.03	0.01	0.01	0.56
				Range	(0.02-0.08)	(0.005-0.025)	(0.004-0.02)	(0.40-0.70)
Duncan and others	1972	Holstein-Friesian; heifers, 2-3 years old. (Results of 9-12 weekly samples)	4	-	$0.02 \pm 0.01^{*}$	$0.01 \pm 0.00^{*}$	Not detected	$0.35 \pm 0.26^{*}$
Butler and others	1972b	Holstein-Friesian, cows (Results of 10 weekly samples pre- and post-partum)	6	Prepartum -	$0.05 \pm 0.01^{+}$	ND	ND	$0.30 \pm 0.04^{+}$
				Postpartum -	$0.03 \pm 0.01^{+}$	ND	ND	$0.17 \pm 0.05^{+}$

* Mean $\pm$ S.D. $^{+}$ Mean $\pm$ S.E.

** Mean of 6 animals; in remaining 12 examined, concentration was less than minimal detectable.

ND - Not determined.

In bovine saliva, the total concentration of immunoglobulins is low, approximately 0.5 mg/ml, 90 per cent of which is secretory IgA (Mach and Pahud, 1971; Butler and others, 1972; Duncan and others, 1972). It has been suggested that the low immunoglobulin content of bovine saliva may be directly related to the presence of a rumen and ruminal bacteria (Porter and Noakes, 1970), but as Mach and Pahud (1971) pointed out the daily production of salivary IgA must be several grams, given the large volumes of saliva produced by the cow. The in vitro production of secretory IgA and free secretory component by isolated bovine salivary gland tissue has been demonstrated (Mach and Pahud, 1971; Butler and others, 1972). Cells producing IgG and IgM were also identified.

The use of the single radial immunodiffusion technique has permitted the determination of concentrations of the individual immunoglobulin classes in the serum of neonatal calves following the absorption of colostrum (Klaus and others, 1969; Porter, 1972; Husband and others, 1972; Logan and others, 1973; Roesti and Fey, 1975; Table 30). By these techniques it has also been possible to demonstrate the presence of low concentrations of immunoglobulins in pre-colostral serum. IgG_1 is normally the major immunoglobulin of pre-colostral sera but occasionally either IgA or IgM predominates (Husband and others, 1972).

Considerable interest has been shown in the ontogeny of the immune systems at mucous surfaces and the immunoglobulin concentration of various external secretions of the young calf. In the intestinal tract Porter, Noakes and Allan (1972) estimated the concentrations of immunoglobulins present in secretions collected from the mid-jejunum of young preruminant calves one to six weeks old by the use of Thiry-Vella loops and double re-entrant canulae. IgM was the predominant immunoglobulin at a concentration of 65.66 mg/100ml, followed by IgG_2, 52.0 mg/100ml; IgA, 38.33 mg/100ml; and IgG_1, 6.65 mg/100ml. In contrast, Newby and Bourne (1976) examined the immunoglobulin content in secretions from the proximal metre of the small intestine of seven calves aged between two and 14 weeks of age; at two weeks of age IgG_1 formed almost 100 per cent of the total immunoglobulin content; at approximately three weeks of age IgG_2 and IgA were detected for the first time. The relative proportion of IgA increased up to 14 weeks

TABLE 30

Concentrations of immunoglobulins in the serum of colostrum-fed calves

REFERENCE	YEAR	BREED/TYPE	No.OF ANIMALS	AGE AT SAMPLING	IgG mg/ml *	IgG_1 mg/ml *	IgG_2 mg/ml *	IgM mg/ml	IgA mg/ml *
Klaus and others	1969	Mixed	10	Precolostrum	1.2 ± 0.5	-	-	0.1 ± 0.0	-
				48 hours	22.6 ± 18.2	-	-	1.16 ± 0.9	-
McBeath and others	1971	Dairy Calves	24	7 days	6.25 ± 0.80+	-	-	0.97 ± 0.13+	-
		"Market" Calves	32	7 days	7.26 ± 0.65+	-	-	0.75 ± 0.08+	-
		Suckler Calves	24	7 days	14.20 ± 1.25	-	-	1.98 ± 0.22+	-
Husband and others	1972	Mixed	7	Precolostrum	-	0.70 ± 0.55+	0.16 ± 0.07+	0.97 ± 0.66+	0.29 ± 0.26+
				48 hours	-	14.43 ± 1.18	1.70 ± 0.30	4.65 ± 1.34+	2.02 ± 0.81
Porter	1972	Ayrshire	5	2-3 days	-	43.16 ± 12.43	1.52 ± 0.70	3.5 ± 1.47	1.70 ± 0.18
Logan and others	1973	Not stated	16	24 hours	14.35 ± 5.59	-	-	3.23 ± 2.56	2.35 ± 1.76
Braun and others	1973	Not stated	10	Precolostrum	0.12	-	-	0.14	-
			10	Postcolostrum	10.5	-	-	0.17	-
Sawyer and others	1973	Not stated	100	Precolostrum	0.16	-	-	0.11	-
			23	24 hours	7.55	-	-	1.75	-
Logan and others	1974a	Blue-Greyx Charolais	30	24 hours	30.90 ± 17.28	-	-	2.48 ± 2.08	2.59 ± 1.68
McBeath and Logan	1974	Blue-Greyx Charolais	27	24 hours	28.36 ± 9.17	-	-	4.47 ± 3.15	3.14 ± 2.36
Roesti and Fey	1975	Simmental	23	Precolostrum	0.8 ± 0.7	-	-	0.2 ± 0.3	0.1 ± 0.1
			24	? 12 hours	11.1 ± 6.0	-	-	1.4 ± 1.0	0.7 ± 0.3
McGuire and others	1976	Beef Calves	18-20	7 days	-	37.5 ± 13.3	0.6 ± 0.3	3.0 ± 2.0	-
Molla	1976	Holsteinx Friesian	6	Precolostrum	-	0.32 ± 0.48	0	0.36 ± 0.46	0.06 ± 0.138
				24 hours	-	20.09 ± 9.8	1.62 ± 1.14	4.62 ± 1.79	5.40 ± 4.02
Logan and others	1978	Suckler Calves	6	48 hours	21.5 ± 2.6+	-	-	4.03 ± 0.93+	2.04 ± 0.57+
Halliday and others	1978	Blue-Grey Beef Calves	56	48 hours	-	27.05 ± 1.62+	1.10 ± 0.11+	2.1 ± 0.19+	-
		FriesianxHereford Beef Calves	53	48 hours	-	21.60 ± 1.88+	1.05 ± 0.10+	1.55 ± 0.17+	-

* mg/ml mean ± standard deviation.

\+ mean ± standard error.

of age and in two calves examined at 14 weeks of age IgA accounted for 15 per cent and 64 per cent of the total immunoglobulin content. Very low concentrations of IgM were detected and then only in two of the seven calves. Unfortunately neither group of workers gave any indication of the passively acquired immune status of the calves used in these experiments.

From these studies in both the calf and the adult cow it is evident that IgG_1, if not the predominant immunoglobulin, is found in relatively high concentrations in bovine intestinal secretions. The origin of this IgG_1 has been the subject of much discussion (Newby and Bourne, 1976; Curtain and others, 1971; Cripps, Husband and Lascelles, 1974; Beh and Lascelles, 1973; Allen and Porter, 1975). The two possibilities considered most frequently are the presence of specific immunocytes in the mucosa synthesising IgG_1 and the translocation of the immunoglobulin from serum. Newby and Bourne (1976) found that in calves up to 14 weeks of age as much as 60 per cent of the intestinal IgG_1 was derived from serum. Similarly, in sheep it has been determined that most of the IgG_1, IgG_2 and IgM present in intestinal secretions is derived from serum (Cripps and others, 1974). Earlier, Curtain and others (1971) suggested that the majority of IgG_1 was synthesised locally by IgG_1 plasma cells in the lamina propria.

There is general agreement that most of the immunoglobulin-associated lymphoid cells in the intestine are found in the lamina propria between the crypts with only a few immunocytes occurring in the villous lamina propria (Allen and Porter, 1975; Newby and Bourne, 1976). Immunofluorescent studies of the intestines of young preruminant calves between the second and sixth weeks of life by Porter and co-workers revealed that the apical cytoplasm of the crypt epithelial cells stained for both IgA and IgM. They could find little evidence of either IgG_1 or IgG_2 in the apical cytoplasm of the crypt epithelial cells. Examination of the lamina propria of six five week-old preruminant calves revealed that IgA staining cells accounted for 70 per cent of the class-specific immunocytes at all levels of the intestine. IgM staining cells formed approximately 23 per cent of the total. Only a few IgG_1 and IgG_2 plasma cells were detected. The density of all immunocytes in the lamina propria decreased from the duodenum towards the colon. Free secretory component was only found

in the apical cytoplasm of the crypt epithelial cells and never in the lamina propria (Porter and others, 1972; Allen and Porter, 1975).

In the very young calf, studies have been much more limited. Allen and Porter (1975) detected almost equal numbers of IgA and IgM staining immunocytes in the jejunal lamina propria of a four day-old calf but Newby and Bourne (1976) were unable to detect any IgA plasma cells in the lamina propria of a one week old calf. Butler and others (1972b) carried out in vitro synthetic studies with tissue cells from the ileum of a one-week old calf and failed to detect the production of any immunoglobulins.

Morgan and Bourne (1978) determined the concentration of immunoglobulins and the number of plasma cells in the respiratory tract of young calves one to six weeks old. Throughout the period of examination, IgG_1 was the predominant immunoglobulin in both nasal and tracheal washings especially during the first week of life. Absolute concentrations of immunoglobulins were not determined because of the method of sample collection. The relative concentration of IgG_1:IgA was greatly reduced by the time the calves were six weeks old. Initially the concentration of IgM was higher than the concentration of IgA but by three weeks of age the concentration of IgA exceeded that of IgM. The number of immunocytes staining for IgA, IgG_1 and IgM in the lamina propria were similar and were detectable in all sites at one week of age. IgG_2 staining cells only became detectable at two weeks of age but only in very small numbers.

In the case of lachrymal secretions, Sullivan and others (1969) were unable to detect any immunoglobulins in the tears of calves collected immediately after birth and prior to suckling colostrum but IgG_1 was readily detected in tears collected 24 hours after suckling colostrum. A similar finding was noted for saliva collected before and after ingesting colostrum. Smith, Wells, Burrells and Dawson (1976) were unable to detect any immunoglobulins in the precolostral tears of five pairs of twin lambs. After suckling naturally IgG was detected in the lachrymal secretions of all ten lambs at 24 hours of age. No separation of IgG into IgG_1 and IgG_2 was attempted. IgM and IgA were not detected until the lambs were two to three weeks old, but by 21 days of age the mean concentration of IgA exceeded the mean

concentrations of both IgG and IgM.

Unlike the immune system of man, where it has been demonstrated that secretory IgA is the major immunoglobulin secreted at all mucous surfaces (Tomasi and Grey, 1972; Spiegelberg, 1974) in the cow, the relative contribution of IgG_1 and secretory IgA to the total immunoglobulin concentration of external secretions is still the subject of debate. In the young calf especially, controversy exists over both the principal class of immunoglobulin involved and the source of immunoglobulin present in the intestinal secretions. In the major studies carried out so far (Porter, Noakes, Allan, 1972; Allen and Porter, 1975; Newby and Bourne, 1976) no attention was given to the passively acquired immune status of the calves. There has been little standardisation in the age, management and disease status, if any, of the calves under experiment. Discrepancies have also existed in the technical methods employed to determine the concentration of immunoglobulins in the external secretions and in the immunohistological techniques. Therefore the opportunity was taken to determine the serum concentrations of the four major immunoglobulins in calves for a period of time from birth and to examine the immunoglobulin content of secretions from one readily accessible mucous surface, the conjunctiva.

MATERIALS AND METHODS

Cows, calves and their management

a) Cows The cows were managed as described in Chapter 2. After parturition samples of blood and colostrum were collected. The serum and colostral whey were prepared and stored until necessary as described in Chapter 2.

b) Calves The ten calves employed in this chapter consisted of five group-reared calves, calves 83, 85 and 93 (Group 8), calves 98 and 106 (Group 9), and calves 77, 79, 81, 204 and 224 (Table 6) which were reared individually. Nine of the ten calves were born in loose-boxes and assisted to suckle colostrum from their own dam as soon as was practical after parturition as described in Chapter 2. The tenth calf (93, Group 8) was born in a loose-box and mothered by its dam for 15 minutes at which time the cow was removed from the calving box. During this 15 minute mothering period the calf was not permitted to suckle its dam. The calf was then fed 1500ml of colostrum milked from its own dam at six hours post-partum. The five group-reared calves had free access to an automatic calf feeder (Nursette Model 30) supplying milk substitute (Vitamealo calf milk meal). The remaining five calves were housed individually and were fed the same milk-substitute by bucket at a rate of 10% bodyweight per day divided into two feeds. All the calves were weighed at 15 minutes post-partum and samples of blood were collected at the following times; prior to feeding with colostrum, and at 2, 4, 8, 12, 16, 20, 24, 30, 36 and 48 hours after being fed colostrum. Thereafter, samples of blood were collected every second day until the calves reached six weeks of age and then once weekly until they were ten weeks old. The serum was prepared as described in Chapter 2 and stored at -20^{o}C until required.

Tears were collected from the calves immediately before the collection of each blood sample using blunt-tipped Pasteur pipettes. Approximately 1 ml was collected from each eye without any chemical stimulation. The time required to collect this volume of tears was approximately 15-20 minutes. No ocular bleeding was ever observed whilst collecting tears and testing each sample with Labstix (Ames Company, Miles Laboratories, Slough) failed to reveal any contamination. The tears were stored without further treatment at -70^{o}C until required.

Laboratory procedures

Isolation of immunoglobulins and preparation of antisera

The methods followed for the preparation of bovine immunoglobulins and antisera are essentially those described by Fey, Pfister, Messerli, Sturzenegger and Grolimund (1976). Initially, bovine IgG_1, IgG_2 and IgM were purchased from Miles Laboratories (Miles Laboratories Ltd., Stoke Poges, Slough) for the immunisation of rabbits.

a) Serum

Blood was collected from adult cows at slaughter. The blood was allowed to clot at 4°C overnight and the serum harvested after centrifugation. Serum from a number of cows was pooled and stored at -20°C until required.

b) Colostrum

Colostrum was obtained from cows immediately after parturition. Colostral whey was prepared by the addition of rennet as described in Chapter 2. After careful separation the whey was stored at -20°C until required.

c) Saliva

Saliva was collected from adult cows by the following method. The animals were injected with an appropriate volume of xylazine (Rompun, Bayer UK Ltd., Eastern Way, Bury St. Edmunds, Suffolk) a sedative which tends to cause salivation. The saliva was collected in a strong polythene nose bag in which holes were cut to enable the animal to breath. The collected saliva was immediately filtered through gauze milk filters (J.J. Blow, Chatsworth Road, Chesterfield) and sufficient sodium azide was added to give a 0.02% solution to prevent microbial growth. It was then refiltered through Whatman No. 1 filters (Whatman Ltd., Springfield Mill, Maidstone, Kent) and stored at -20°C until required.

d) Gel filtration chromatography

This was routinely carried out in 2.5 x 100 cm columns (Pharmacia (Great Britain) Ltd., Prince Regent Road, Hounslow, Middlesex) using Sepharose 6B (Pharmacia) as the bed material.

Sepharose 6B is sold as a slurry which obviates the necessity for de-fining and de-aeration required with other gel filtration bed materials.

Columns were packed with the aid of a packing column. A hydrostatic head of 20 cm was then applied and the column run until a constant level was achieved. The applied samples were eluted by downward flow, maintaining a constant hydrostatic head of 20 cm by use of a Mariotte flask. The eluates from the column were monitored at OD280 nm (8300 Uvicord II, LKB Instruments Ltd., LKB House, 232 Addington Road South, Croyden, Surrey) collected by an automatic fraction collector (7000 Ultrorac Fraction Collector, LKB Instruments).

Occasionally Sephadex G200 (Pharmacia) was used as the bed material in accordance with the manufacturer's instructions.

Buffer: The eluant buffer for all gel filtration chromatography was TRIS-HCl 0.1 M pH 8.6 to which 0.005% thimerosal was added to prevent microbial growth.

e) Ion exchange chromatography

Ion exchange chromatography was carried out using DEAE cellulose (De 52 microgranular Preswollen, Whatman). The gel was washed several times in the acid part of the phosphate buffer (NaH_2PO_4 0.01M) until the desired pH was attained. The gel was then suspended in the final buffer, e.g. phosphate buffer 0.01M, pH 7.4.

Care was taken to remove the fines and de-aerate the slurry before packing into a K25/30 column (Pharmacia) with the aid of a packing column. The column was packed to a constant level with a hydrostatic head of 25 cm. The column was top loaded and eluted by downward flow, the eluates being monitored as for gel filtration chromatography. The proteins were eluted by a stepwise increase in buffer molarity at constant pH.

f) Immunoelectrophoresis

Immunoelectrophoresis was performed according to the method of Scheidegger (1955) in 0.06 M barbital buffer pH 8.6. Agarose (Litex, International Enzymes Ltd., Hanover Way, Windsor) was used for all gel precipitation methods except the radial immunodiffusion test. Agarose

has the advantage of reducing immunoelectrophoretic endosmosis (Williams and Chase, 1968). The 1.5% agarose was dissolved in barbital buffer in a boiling water bath and divided into aliquots of 20-25 ml in stoppered universals. The electrophoresis was carried out in a Shandon electrophoresis chamber (Shandon Scientific Co., London) at a constant current of 20 ma. and allowed to run for approximately 4 hours or until it was considered that the bromophenol blue marked albumin had progressed sufficiently. The precipitin arcs were developed with appropriate antisera in a moist chamber at room temperature. The plates were examined and recorded at various times up to 48 hours.

g) Immunodiffusion

Immunodiffusion was carried out according to the method of Ouchterlony (1958) in 1% agarose. Small petri dishes were used and a layer of 1.5 - 2mm of the agarose was run into the petri dishes on a horizontal table. Wells were cut using a template and removed by aspiration. Again the precipitin arcs were allowed to develop with appropriate antisera in a moist chamber and read at various times up to 48 hours.

h) Radial immunodiffusion

Radial immunodiffusion was carried out according to the method described by Mancini, Carbonara and Heremans (1965).

1.5% Special Agar-Noble (Difco Laboratories, Detroit, USA) was made up in barbital buffer pH 8.6, ionic strength 0.02 with 0.02% sodium azide as a bacteriostatic. Aliquots were stored in tightly capped universal bottles. When required the solidified gel was melted in a boiling water bath, then allowed to cool to 56°C. To the gel an appropriate volume of undiluted antiserum which had also been warmed to 56°C was added using pipettes and beakers which had been prewarmed. The plates were poured immediately.

The antiserum gel mixture was poured into a mould formed by two photographic plates separated by a U-shaped frame, approximately 1 mm thick and held together by bulldog clamps. One of the glass plates was siliconised and after solidification the clamps were removed and the siliconised plate was slid off. Wells were punched in the agar using a 14 gauge hypodermic needle and a template. The punched out agar was

removed by aspiration.

The wells were filled with an appropriate volume of test serum using a microsyringe (Hamilton, Toronto), and incubated in a moist chamber at room temperature for an appropriate period of time. This varied with immunoglobulin class concerned; 48 hours for IgG_1, IgG_2 and IgA and 72 hours for IgM. The standard curve was drawn by plotting the squares of the diameters of the rings for four known concentrations of antigen. The diameters were measured using a binocular microscope equipped with a measuring eyepiece (x 10). To estimate the concentration of antigen in the unknown samples, only those samples which had precipitation rings which fell between the maximum and minimum rings of the known concentrations were measured. Those unknown samples which had precipitation rings greater or less than the standard solutions were re-estimated on Mancini plates with the appropriate dilution of antiserum. Each sample was determined three times. The standard curves for each immunoglobulin were drawn from known dilutions of a pooled serum sample which had been previously calibrated against a reference serum sample kindly supplied by Professor Fey, Bern.

i) Immunisation procedures

Animals immunised were rabbits, guinea-pigs and goats.

Goats - Goats were immunised intramuscularly with 5-10 mg protein emulsified in Freund's complete adjuvant (Difco) and repeated at 2-4 weeks with 5-10 mg protein emulsified in Freund's incomplete adjuvant (Difco). The animals were bled 2-4 weeks later.

Rabbits - Rabbits were immunised according to the protocol suggested by Herbert (1973).

Guinea-pigs - Guinea-pigs were immunised according to Binaghi, Oriol and Boussac-Aron (1967) as suggested by Fey and others (1976). Although guinea-pigs produce antibodies of high specificity, the small volume of serum which can be harvested is a major disadvantage.

In order to render the antisera class specific each antiserum was absorbed with soluble antigens as follows:-

IgG_1 - Antiserum to bovine IgG_1 was rendered class specific by absorption with IgG_2 at 2 mg/ml of antiserum.

IgG_2 - Antiserum to bovine IgG_2 was rendered class specific by absorption with IgG_1 at 2 mg/ml of antiserum.

IgM - Guinea-pig antiserum to bovine IgM was rendered class-specific by absorption with IgG (1 mg/ml) and foetal calf serum (0.2 ml/ml).

IgA - Guinea-pig antiserum to bovine IgA was rendered class-specific by absorption with IgG at 1 mg/ml.

j) Isolation of IgG_2

IgG_2 was prepared from bovine serum as described by Fey and others (1976). Bovine serum was diluted to 2g% with phosphate buffered saline (PBS) and under constant stirring saturated ammonium sulphate was added slowly until 33% saturation was reached. Stirring was continued for 3 hours at room temperature. Following centrifugation, the supernatant was discarded and the precipitate was dissolved in distilled water. Re-precipitation with ammonium sulphate at 33% saturation was repeated a further twice. After the third precipitation with $(NH_4)_2SO_4$, the precipitate was dissolved in PBS and dialysed against running tap water for 2-4 hours. It was then dialysed against PBS until SO_4 ions were no longer detected with 1% BaCl.

The ammonium sulphate precipitated globulin was dialysed against 0.01 M phosphate buffer pH 7.4 and an ion exchange chromatography performed on DEAE cellulose. The breakthrough peak contained IgG_2 and the leading fraction was used to prepare antiserum after concentration with carbowax (Gurr, London). The specificity of the antiserum following absorption is shown in Figure 19.

k) Isolation of IgG_1

The starting material for the isolation of IgG_1 was colostral whey which had been precipitated with saturated $(NH_4)_2SO_4$ as described for serum. The ammonium sulphate precipitated colostral globulin was dialysed against 0.02M phosphate buffer pH 6.3. An ion exchange chromatography was carried out on DEAE cellulose with the initial

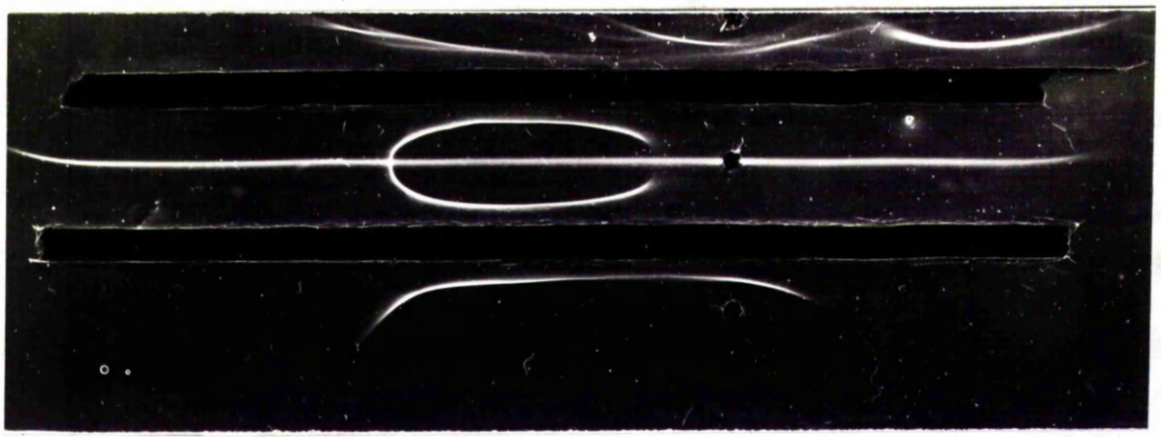


FIGURE 19 Specificity of absorbed goat anti-bovine IgG_2 serum:- Immunoelectrophoresis: Top well, bovine serum; top trough, anti-total bovine serum; middle well, bovine IgG_2; bottom trough, absorbed anti-bovine IgG_2; bottom well, bovine IgG_2.

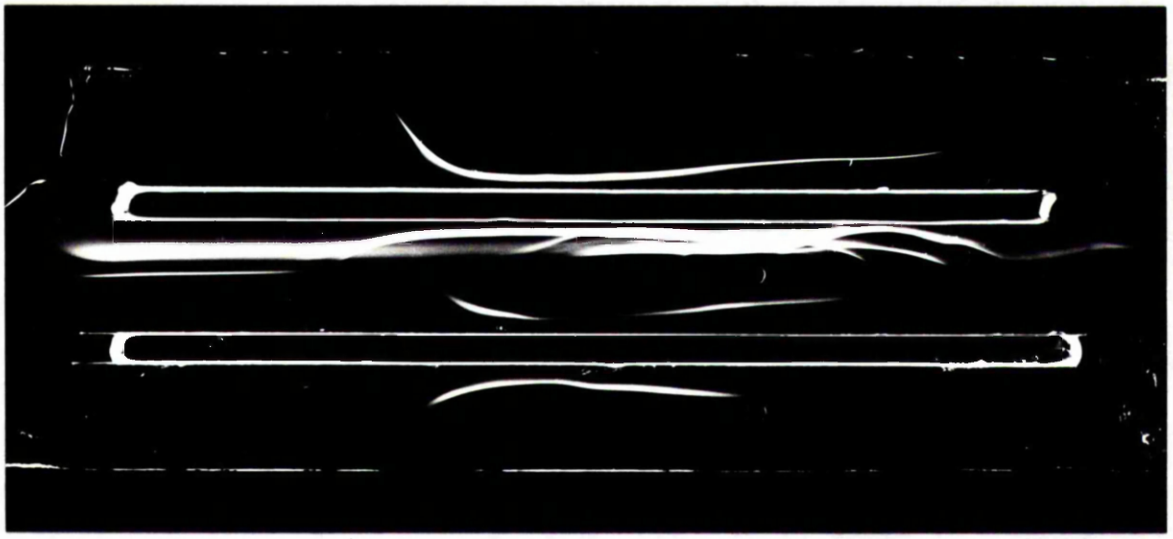


FIGURE 20 Specificity of absorbed goat anti-bovine IgG_1 serum:- Immunoelectrophoresis: Top well, bovine IgG_1; top trough, anti-total bovine serum; middle well, bovine serum; bottom trough, absorbed anti-bovine IgG_1; bottom well, bovine IgG_1.

buffer being 0.02M phosphate pH 6.3 and a step wise gradient elution carried out with 0.05 M and 0.01 M phosphate pH 6.3 buffers. IgG_1 was eluted with the 0.05 M buffer. Several 0.05 M eluates were pooled and re-chromatographed to obtain a purer sample. The specificity of the anti IgG_1 serum following absorption is shown in Figure 20.

l) Isolation of IgM

Pooled bovine serum was dialysed extensively against 0.01 M $KH_2 PO_4$ (pH 4.75) for two days (Mukkur and Froese, 1971). The precipitate obtained after centrifugation was washed with the buffer. The precipitate was dissolved in 0.01 M acetate buffer pH 5.4 containing 0.15 M Na Cl; the final volume being 20% of the initial serum volume. To precipitate residual IgG, 0.1 M Zn SO_4 was added drop-wise until the final concentration was 25 mM. The solution was stirred for 2 hours at room temperature and then centrifuged at 8000 rpm. To remove excess zinc ions from the supernatant 1% tetrasodium EDTA was added and left standing for 2 hours. The excess EDTA was removed by dialysing against PBS (Fey and others, 1976). The solution was then concentrated and applied to a Sepharose 6B column and eluted with 0.1 M TRIS-HCl 1M NaCl at pH 8.6. The first peak was found to contain IgM. This protein fraction was used to immunise both guinea-pigs and rabbits and the specificity of the antiserum after absorption is shown in Figure 21.

m) Isolation of IgA

The filtered saliva was concentrated 100 fold by positive pressure dialysis (142 mm UF cell; Millipore (UK) Ltd., Millipore House, Abbey Road, London), and the concentrated saliva was fractionated on Sepharose 6B with 0.1 M Tris-HCl, 1 M NaCl at pH 8.6.

It was found that the F2 peak was enriched IgA and this was used initially for immunisation of rabbits. Frequently the F1 peak failed to react with any antisera and may have been aggregated molecules or mucus. After absorption with bovine IgG antiserum produced to this enriched fraction showed a line of complete identity to anti-bovine secretory IgA serum kindly supplied by Dr. J.P. Mach, Lausanne (Figure 22). Later, in an attempt to obtain a purer sample of IgA, the enriched fractions of IgA were treated as follows. The protein solution was dialysed against 0.002 M phosphate buffer pH 7.2. This was then added

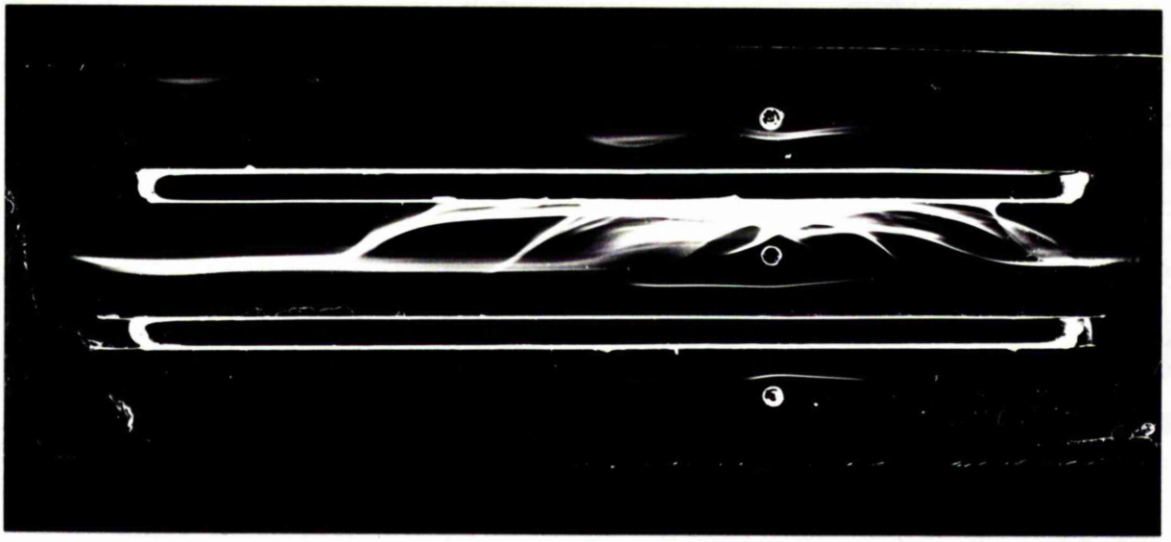


FIGURE 21 Specificity of absorbed rabbit anti-bovine IgM serum:- Immunoelectrophoresis: Top well, bovine IgM; top trough, anti-total bovine serum; middle well, bovine serum; bottom trough, absorbed anti-bovine IgM; bottom well, bovine IgM.

to a slurry of DEAE cellulose equilibrated with the same buffer. Adsorption of the protein onto the cellulose was carried out under gentle stirring overnight. The slurry was then washed twice with one volume of 0.02 M phosphate buffer pH 7.2 on a Buchner funnel. The DEAE cellulose was then suspended in the same buffer and a column poured. IgA was eluted by a 0.06 M phosphate buffer pH 7.0. This eluate was concentrated and dialysed against PBS to remove the phosphate ions. To three volumes of the protein solution one volume of 100 mM Zn SO_4 was added. It is stirred at room temperature for 2 hours and centrifuged at 3000 rpm. Excess zinc ions are removed by adding 1% tetrasodium EDTA and dialysed against PBS to remove excess EDTA (Fey and others, 1976).

Antiserum to this bovine IgA was produced in rabbits and guinea-pigs and the purity of the antisera is shown in Figure 23.

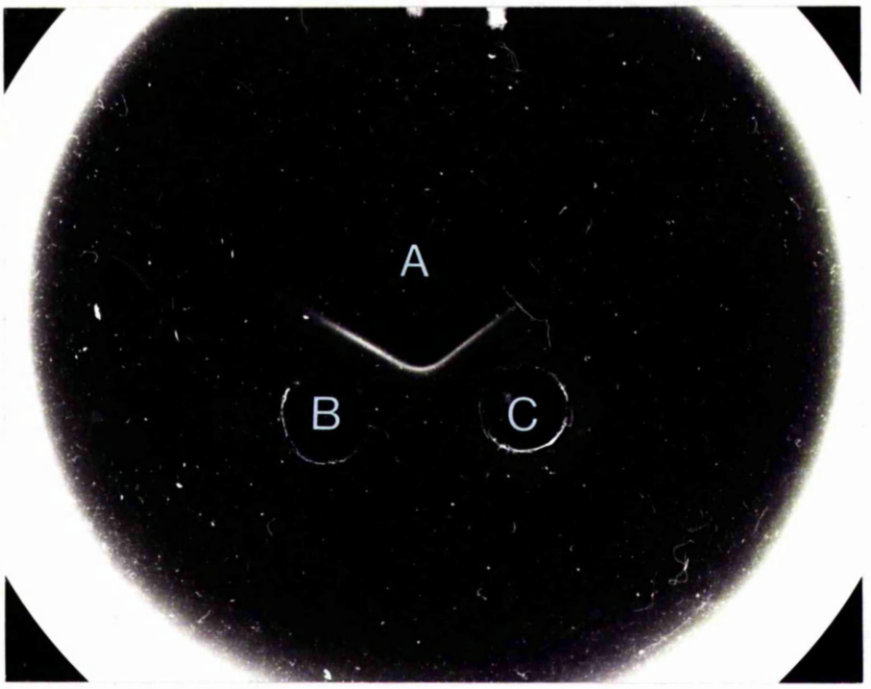


FIGURE 22 Immunodiffusion:- Line of complete identity between "Swiss" bovine secretory IgA antiserum and an absorbed antiserum to salivary secretory IgA enriched fraction raised in rabbits: Well A, secretory IgA enriched fraction from bovine saliva; well B, anti-bovine secretory IgA kindly supplied by J.P. Mach; well C, absorbed rabbit anti-bovine secretory IgA.

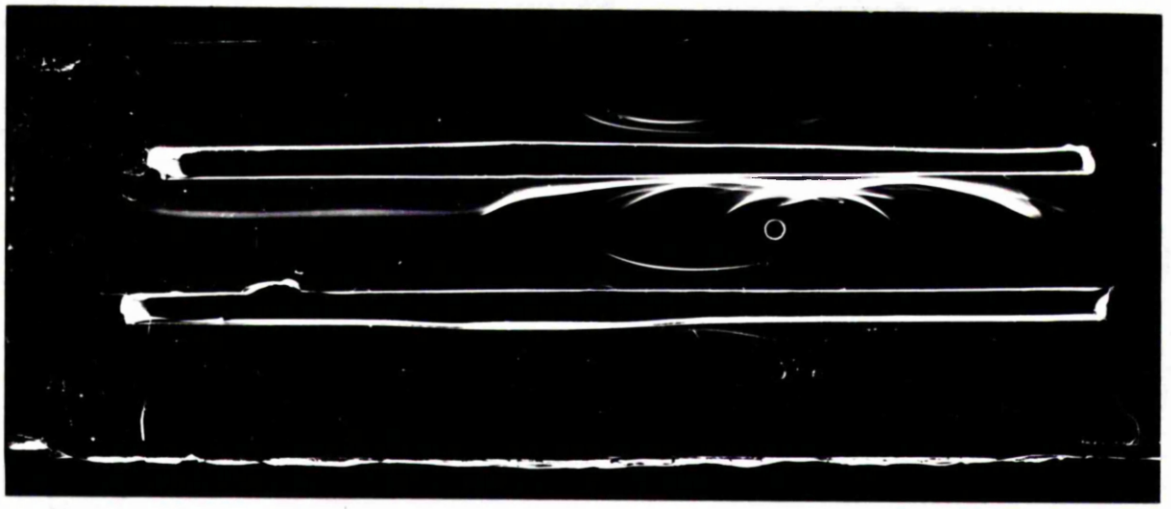


FIGURE 23 Specificity of absorbed guineapig anti-bovine secretory IgA serum:- Immunoelectrophoresis: Top well, secretory IgA enriched fraction; top trough, anti-total bovine serum; middle well, bovine serum; bottom trough, absorbed guineapig anti-bovine IgA serum; bottom well, secretory IgA enriched fraction.

RESULTS

Clinical findings

The breed, birthweight and sex of each of the ten calves are given in Table 31. As stated in the Materials and Methods calves 77, 79, 80, 204 and 224 were reared in small individual boxes, which had been cleaned out prior to use but which had not been thoroughly disinfected. The remaining five calves (83, 85, 93, 98, 106) were reared in two groups of 14 and 10 calves (Groups 8 and 9), which were housed in large straw-bedded loose boxes. The calves were examined for diarrhoea at least once daily during the first six weeks of life. Throughout the first ten weeks of life calves 79 and 104 were considered to be clinically normal, although calf 79 had soft but not diarrhoeic faeces on several days during this period. Calf 77 was never diarrhoeic but between five and six weeks of age its respiratory rate increased; it developed a cough and on one day (38 days old) was markedly pyrexic ($106^{\circ}F$). On auscultation an occasional rhonchus was detected on the right side of the thorax. Treatment with oxytetracycline (Terramycin, Pfizer Ltd.) was given intravenously for three days after which time the calf appeared normal. Calf 80 was diarrhoeic (faecal score 2 or 3) on five days between nine and 15 days old, but was otherwise clinically normal. Between four and ten days of age calf 224 was diarrhoeic (faecal score 2 or 3) on five days. At eight days of age it developed a marked bilateral conjunctivitis. This was treated with chloramphenicol ophthalmic ointment (Chloromycetin Ophthalmic Ointment, Parke Davis and Company) for two days and the eyes were clinically normal by ten days of age.

All five group-reared calves were diarrhoeic at some time during the first four weeks of life but never for any extended period of time. Calf 83 was diarrhoeic (faecal score 2 or 3) for four days between 11 and 17 days of age. Calf 85 was diarrhoeic (faecal score 3) for two days when it was seven and eight days old. Diarrhoea was most severe in calf 93 which was diarrhoeic for five days (faecal score 3 for 3 days; faecal score 2 for 2 days) between six and ten days of age. Calf 98 was diarrhoeic (faecal score 2 or 3) on only three days during the first four weeks of life and calf 106 was diarrhoeic when it was nine (faecal score 2) and ten (faecal score 3)

TABLE 31

Concentration of immunoglobulins in precolostral and postcolostral (48 hours of age) serum of ten newborn calves.

Calf No.	Breed*	Sex	Birth weight (kg)	Weight of colostrum ingested (kg)	Immunoglobulin Concentration				48-hour SAIg + (ZST units)
					IgG_1	IgG_2	IgA	IgM	
77	A	F	33.2	2.3	Pr.0	0	0	0	34
					P. 31.2	0.94	5.28	1.06	
79	AxH	F	32.4	1.3	Pr.0.90	0.026	0.056	0.48	8
					P. 12.4	0.28	0.54	0.82	
80	AxCh	M	39.3	1.8	Pr.0	0	0	0	26
					P. 29.40	1.22	1.91	1.45	
83	AxF	M	32.5	2.3	Pr.0	0	0	0	43
					P. 41.50	1.10	6.30	1.20	
85	AxH	F	34.0	1.7	Pr.0	0	0	0	31
					P. 28	0.71	2.28	3.40	
93	A	F	30.0	1500ml**	Pr.0.098	0.010	0	0.156	18
					P. 16.00	0.42	1.17	2.00	
98	A	M	34.9	1.8	Pr.0.08	0	0	0	20
					P. 22.80	0.58	0.92	1.09	
106	A	F	26.1	1.8	Pr.0.048	0.02	0	0.138	34
					P. 28.38	0.56	1.29	2.86	
204	AxF.xF	F	29.8	2.3	Pr.1.0	0.024	0.060	0.048	27
					P. 33.96	0.42	1.11	0.94	
224	BGxAA	M	32.6	1.7	Pr.0.032	0.08	0	0	18
					P. 18.48	0.44	0.33	0.20	

*Breed: A = Ayrshire; F = Friesian; H = Hereford
AA = Aberdeen Angus; BG = Blue Grey

+ Serum concentration of absorbed immunoglobulins at 48 hours.

** Fed at 6 hours post-partum. Pr.- Pre colostrum. P.- 48 hours post-partum.

days old. Although several other calves in the Groups 8 and 9 were pyrexic and tachypnoeic at different times between six and ten weeks of age and were treated with oxytetracycline intravenously, none of the five calves from which lachrymal fluid was collected was so affected or treated.

Serology

The 48-hour serum absorbed immunoglobulin concentrations (48H SAIg) measured by the zinc sulphate turbidity test for the ten calves are also given in Table 31. The calves were allocated to one of four groups based on the 48-hour serum concentration of absorbed immunoglobulins determined by the zinc sulphate turbidity test: Group A, one calf (83) with a 48H SAIg of 43 ZST units; Group B, five calves (77, 80, 85, 106, 204) with a 48H SAIg of approximately 30 ZST units (mean 30.40 ± 3.78 ZST units); Group C, three calves (93, 98, 224) with a 48H SAIg of approximately 20 ZST units (mean 18.66 ± 1.15 ZST units); Group D, one calf (79) with a 48H SAIg of less than 10 ZST units.

Serum immunoglobulins

The concentrations of IgG_1, IgG_2, IgA and IgM in the precolostral serum and in the serum collected at 48 hours of age (post-colostral) are presented in Table 31. The individual results for each of the ten calves are presented in Appendix 4. A highly significant correlation ($r = 0.964$, $p < 0.001$) was found to exist between the 48-hour concentration of absorbed colostral whey immunoglobulins (ZST units) and the sum of the 48-hour concentrations of the four classes of immunoglobulins (Figure 24). A highly significant correlation ($r = 0.933$, $p < 0.001$) was also found to exist between the 48-hour concentration of absorbed colostral whey immunoglobulins (ZST units) and the 48-hour serum concentration of IgG_1 (Figure 25). A significant correlation ($r = 0.791$, $p < 0.01$) was found to exist between the 48-hour concentration of absorbed colostral whey immunoglobulins (ZST units) and the 48-hour serum concentration of IgA (Figure 26). There was no correlation between the 48-hour concentration of absorbed colostral whey immunoglobulins (ZST units) and either the 48-hour serum concentration of IgG_2 ($r = 0.686$, $p < 0.02$) or the 48-hour serum concentration of IgM ($r = 0.442$).

IgG_1 The absorption of colostral IgG_1 and the subsequent changes in the serum concentrations of the four groups during the first ten weeks of life are shown in Figure 27. Low concentrations of IgG_1 (0.032-1.00 mg/ml) were detected in the pre-colostral serum of six of

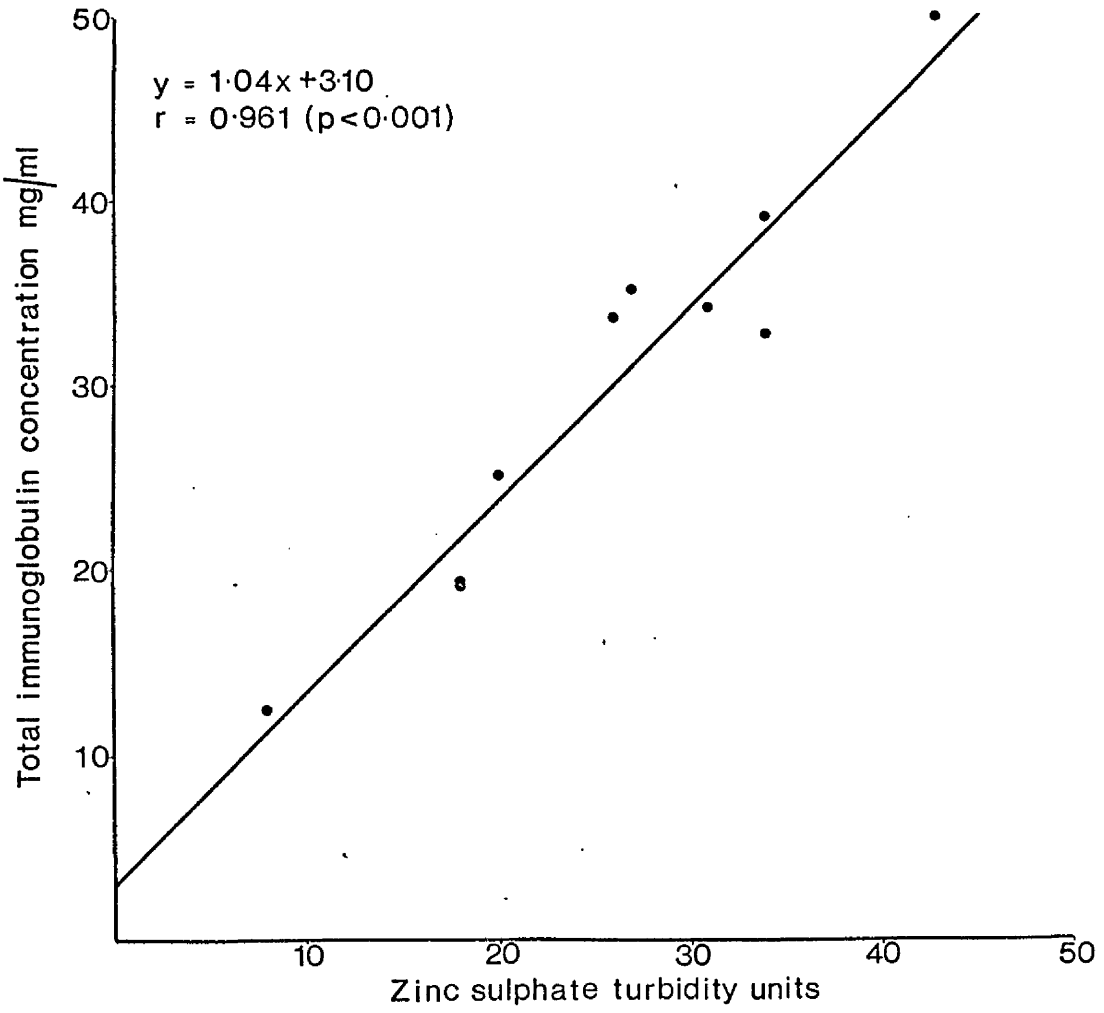


FIGURE 24 The relationship between the serum immunoglobulin concentration (ZST units) and the sum of the individual immunoglobulins (IgG_1, IgG_2, IgM and IgA).

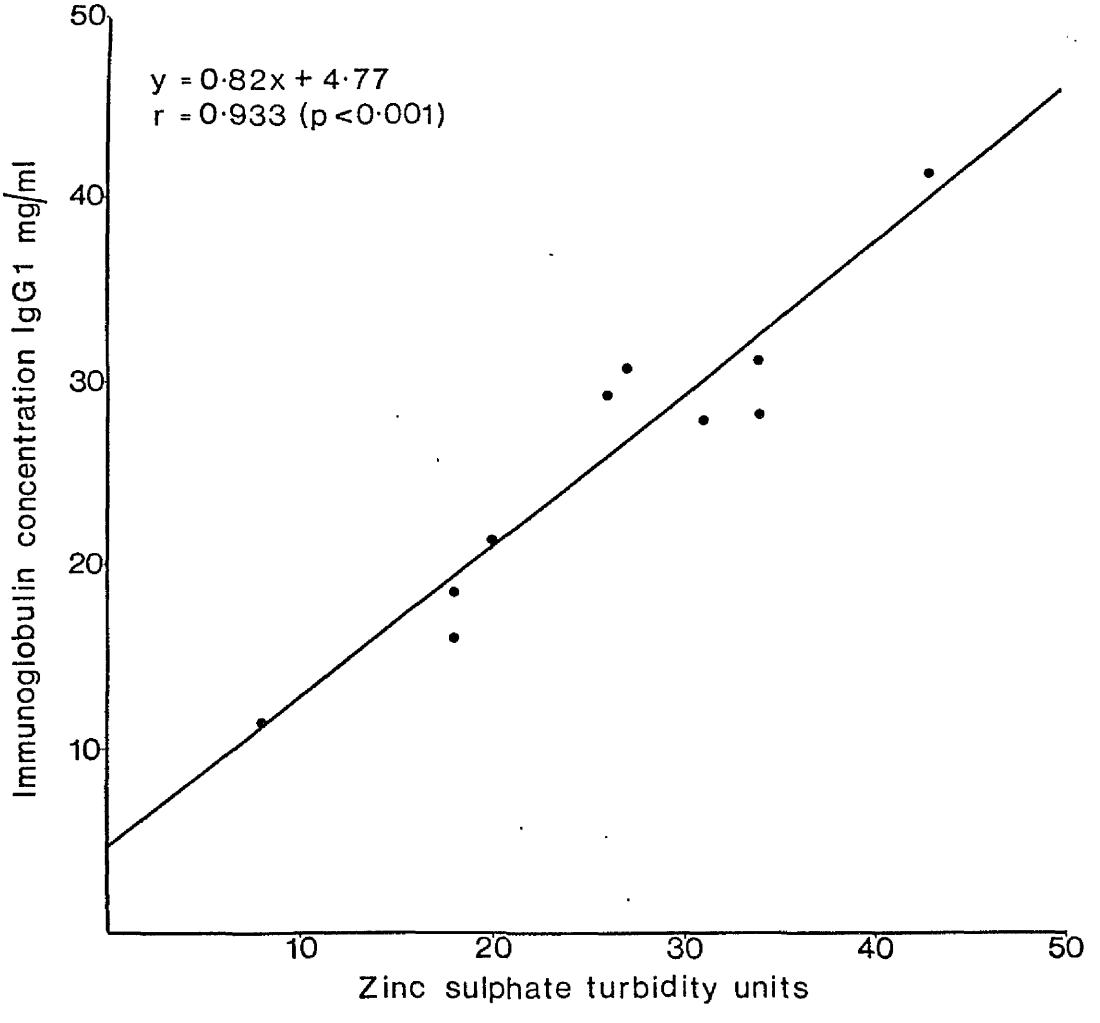


FIGURE 25 The relationship between the serum immunoglobulin concentration (ZST units) and the serum concentration of IgG_1.

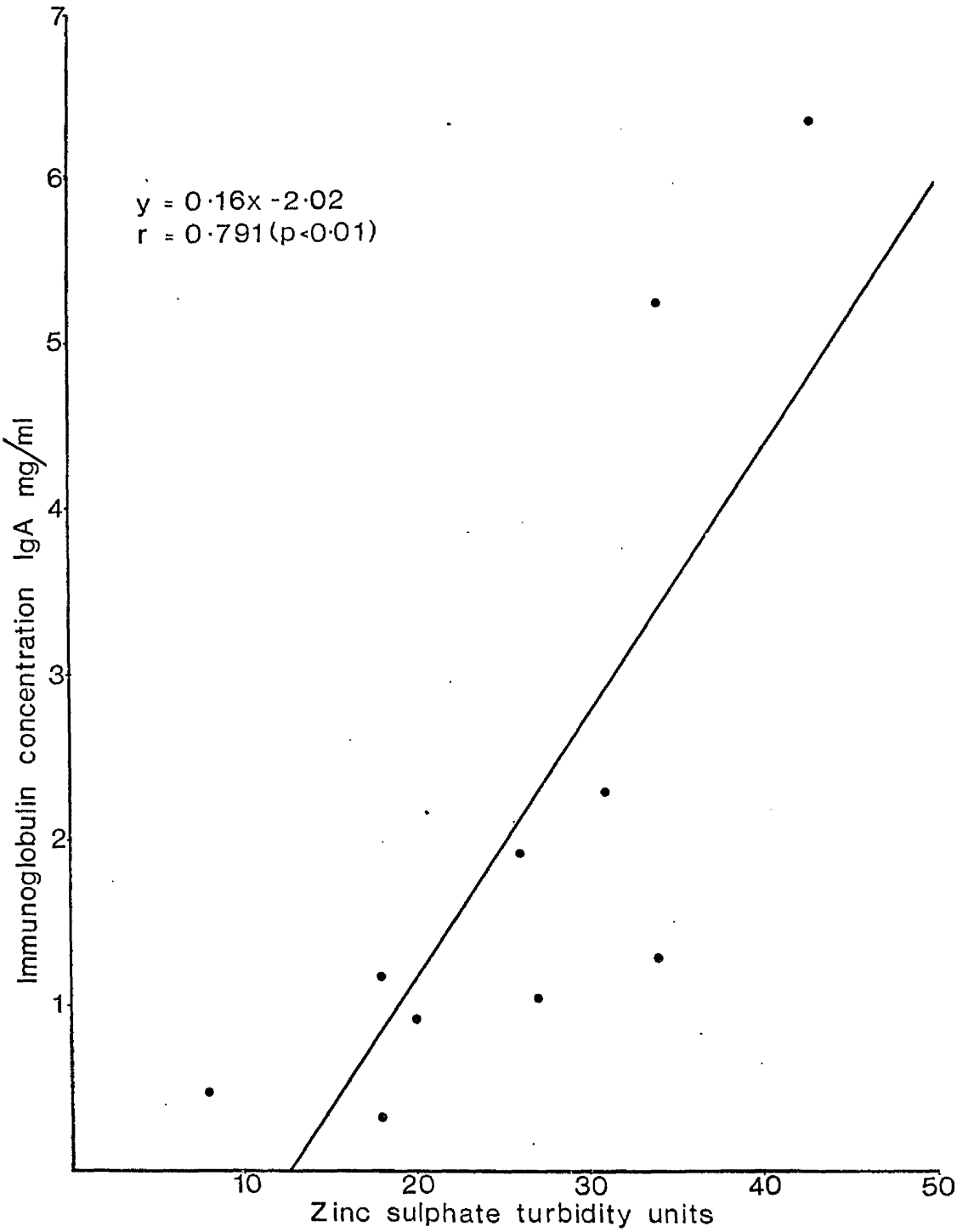


FIGURE 26 The relationship between the serum immunoglobulin concentration (ZST units) and the serum concentration of IgA.

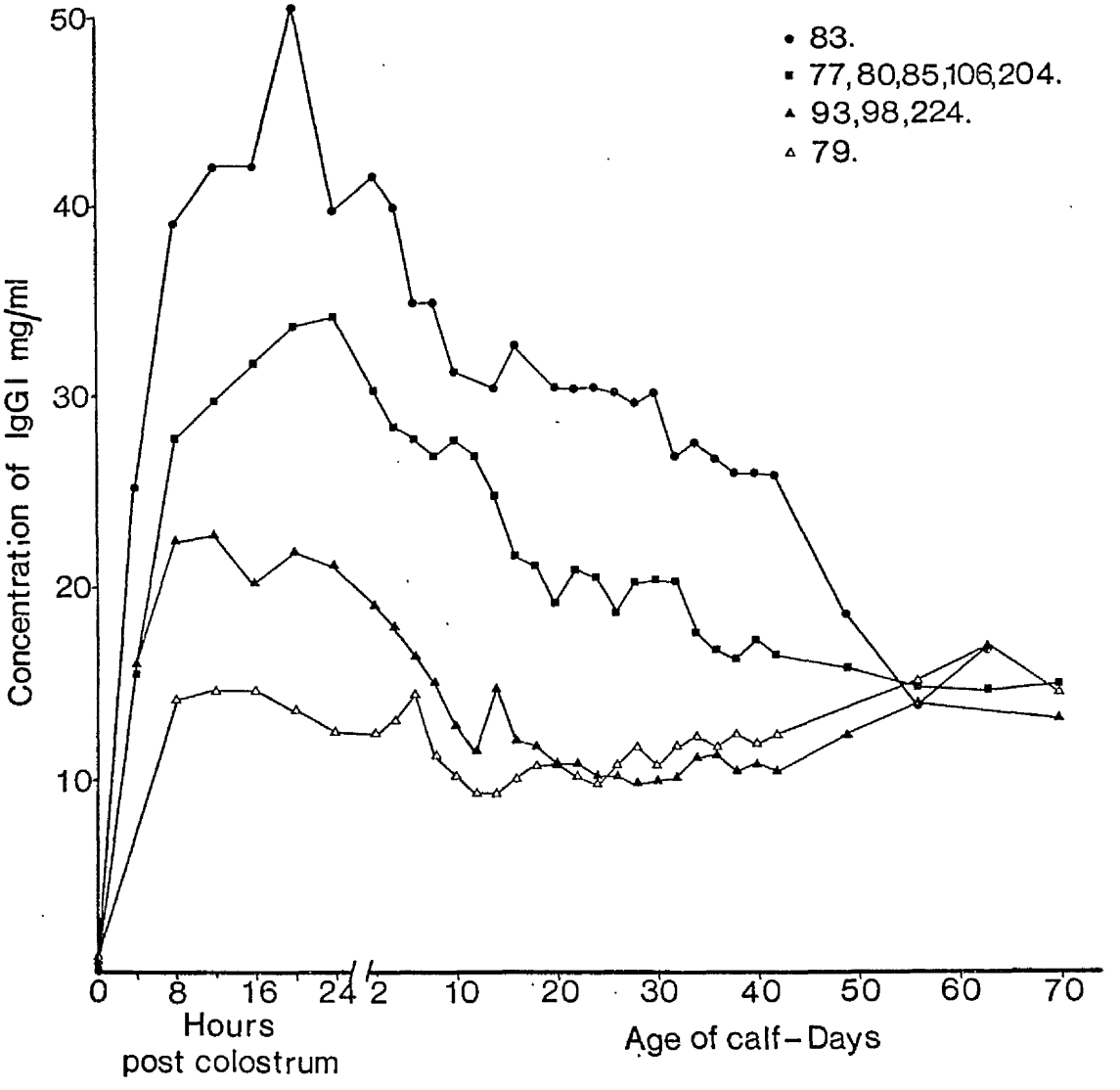


FIGURE 27 The absorption of colostral IgG_1 by newborn calves and the subsequent changes in the serum concentrations during the first ten weeks of life.

the ten calves. Following the ingestion of colostrum a very rapid increase in the serum concentration of IgG_1 occurred and in the four calves sampled at two hours post-colostrum concentrations of 3.7-14.5 mg/ml IgG_1 were detected. Maximum concentrations of IgG_1 occurred at 16-24 hours after the feeding of colostrum and fell thereafter. The concentrations of IgG_1 in the serum of the four calves 79, 93, 98 and 224 increased rapidly to about eight hours post-colostrum but then only very slowly until 20 hours post-colostrum when they began to decline.

In Groups A, B and C, there was a very sharp decrease in the serum concentrations of IgG_1 during the first three weeks of life, but only a slow decline in the IgG_1 serum concentration of calf 79 (Figure 27). After 21 days of age the IgG_1 serum concentration of calf 79 began to steadily increase. At eight weeks of age the serum concentrations for the four groups were approximately equal (mean 14.18 ± 1.12 mg/ml). The half-lives of each class of absorbed colostral immunoglobulin are presented in Table 32. Using the mean serum concentrations of IgG_1 of the ten calves at 2 and 8 days of age the half-life of absorbed colostral IgG_1 was 21.5 days, although wide variation between the four groups occurred (16.9 days to 43.8 days).

<u>IgG_2</u> The serum concentrations of absorbed IgG_2 were uniformly low and the mean values for the ten calves are given in Figure 28. An increase in the concentration of IgG_2 began to occur at three weeks of age but at ten weeks of age the mean concentration was still only 1.96 mg/ml. The half-life of absorbed colostral IgG_2 was found to be 28.5 days (Table 32).

<u>IgA</u> The absorption of colostral IgA followed a similar pattern to the absorption of IgG_1 (Figure 29). Calf 83 attained the highest 48-hour serum concentration of IgA, 6.3 mg/ml. IgA was detected in the pre-colostral serum of only two calves (79, 0.056 mg/ml; 204, 0.06 mg/ml). It was detected in the serum of the nine calves sampled at four hours post-colostrum (0.33-3.36 mg/ml). A rapid decline in the serum concentration of IgA occurred and in calf 79 it was not detected from day four to day 24. At about this time the concentration of serum IgA also began to increase in the other three groups. The half-life for absorbed colostral serum IgA was found to be 2.64 days (Table 32).

TABLE 32

The half-lives of absorbed colostral immunoglobulins.

	IgG_1	IgG_2	IgA	IgM
			(days)*	
Group A Calf 83	23.6	ND	3.65	14.7
Group B Calves, 77, 80 85, 106, 204.	32.9	ND	2.5	6.95
Group C Calves, 93, 98, 224.	16.9	ND	2.0	6.6
Group D Calf 79.	43.8	ND	ND	5.0
All ten calves.	21.5	28.5	2.64	6.95

* Determined from mean serum values at 2 and 8 days of age.

ND Not determined.

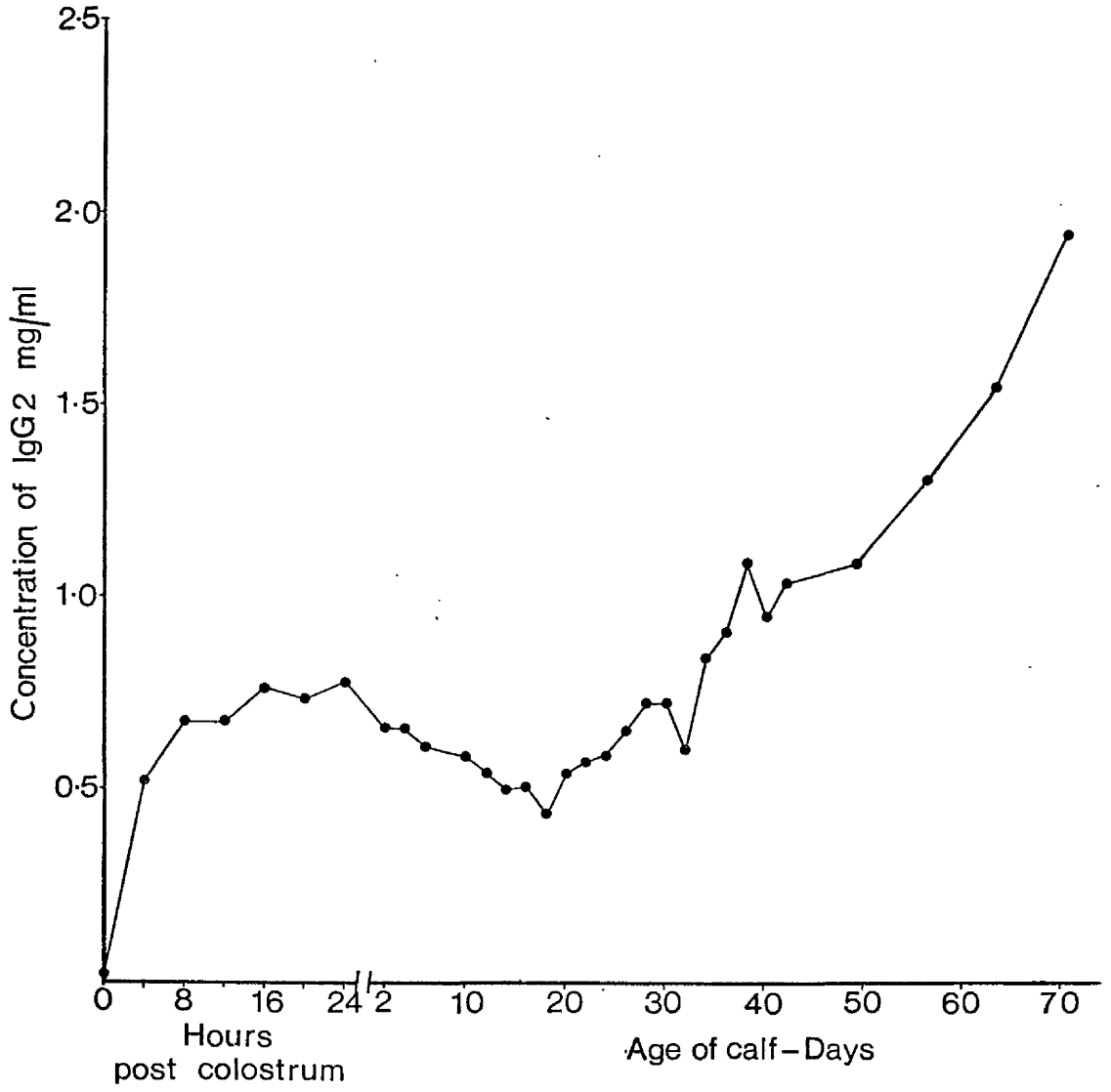


FIGURE 28 The absorption of colostral IgG_2 by newborn calves and the subsequent changes in the serum concentrations during the first ten weeks of life.

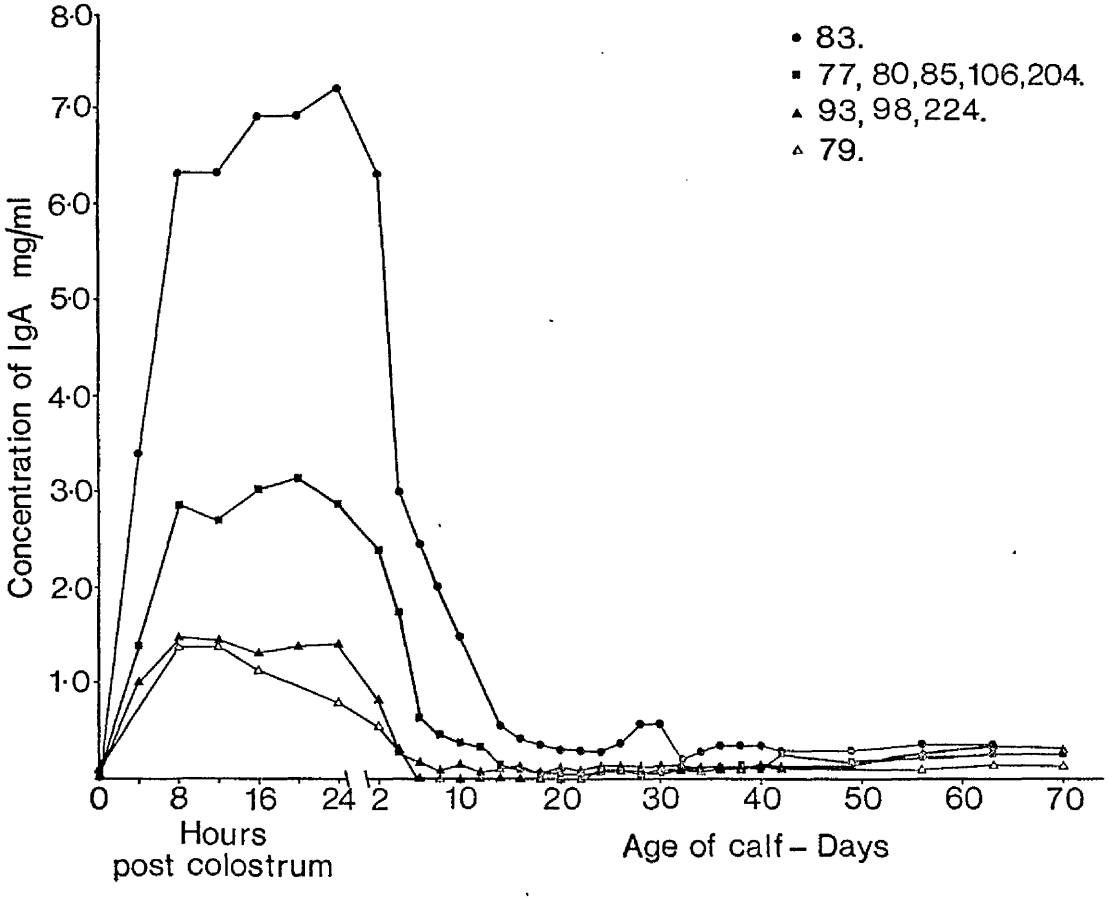


FIGURE 29 The absorption of colostral IgA by newborn calves and the subsequent changes in the serum concentrations during the first ten weeks of life.

IgM The absorption of colostral IgM and the varying serum concentrations during the first ten weeks of life are presented in Figure 30. Low concentrations of IgM (0.048-0.480 mg/ml) were detected in the pre-colostral serum of four calves. Calf 83 which attained the highest 48-hour serum concentrations of IgG_1 (41.5 mg/ml) and IgA (6.3 mg/ml) had a low 48-hour serum concentration of IgM (1.28 mg/ml). The half-life of absorbed colostral IgM was found to be 6.95 days, but for calf 83 it was 14.7 days (Table 32).

Lachrymal fluid

IgG_1 Detectable concentrations of IgG_1 were found in the lachrymal fluid either at the same sampling time or the sampling time following its detection in serum. IgG_1 (0.028 mg/ml) was detected in the pre-colostral lachrymal fluid of calf 79 only. Low concentrations of IgG_1 (0.002-0.264 mg/ml) were detected in six of the remaining nine calves at four hours post-colostrum and in all ten calves at eight hours post-colostrum (mean, 0.163 mg/ml). The concentrations of IgG_1 in lachrymal fluid were maximal at 24 hours post-colostrum (mean 0.377 mg/ml) but remained uniformly low at approximately 1-2 per cent of serum concentrations. The mean values for all ten calves over the first ten weeks of life are presented in Figure 31. A significant correlation ($r = 0.76$, $p < 0.01$) existed between the 48-hour serum concentration of IgG_1 and 48-hour concentration of IgG_1 in lachrymal fluid (Figure 32) and between the mean serum and lachrymal fluid concentrations of IgG_1 for all ten calves throughout the ten week period ($r = 0.71$, $p < 0.001$) (Figure 33).

IgG_2 During the ten week period of examination, no IgG_2 was detected in the lachrymal fluid of any calf.

IgA The mean concentration of IgA in the lachrymal fluid of all ten calves is presented in Figure 34. In one calf (77) IgA was detected in the lachrymal fluid at every sampling time from two hours post-colostrum. This calf had the second highest concentration of serum IgA during the first 48 hours of life. IgA was present in the lachrymal fluid of calf 79 from four to 48 hours post-colostrum but it then fell below the lower limit of detection until the calf was 14 days old. In three further calves (80, 98, 106), IgA was first

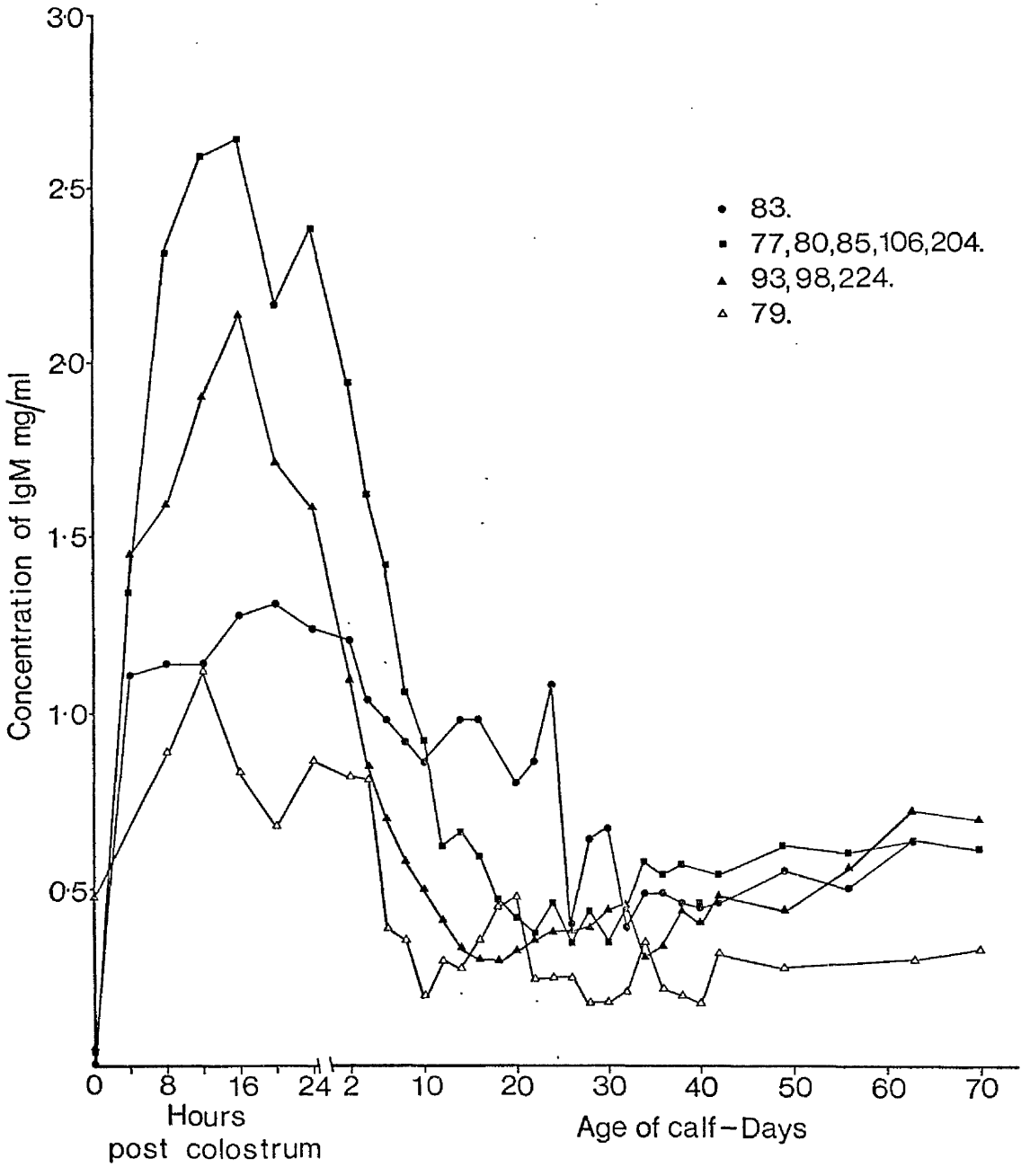


FIGURE 30 The absorption of colostral IgM by newborn calves and the subsequent changes in the serum concentrations during the first ten weeks of life.

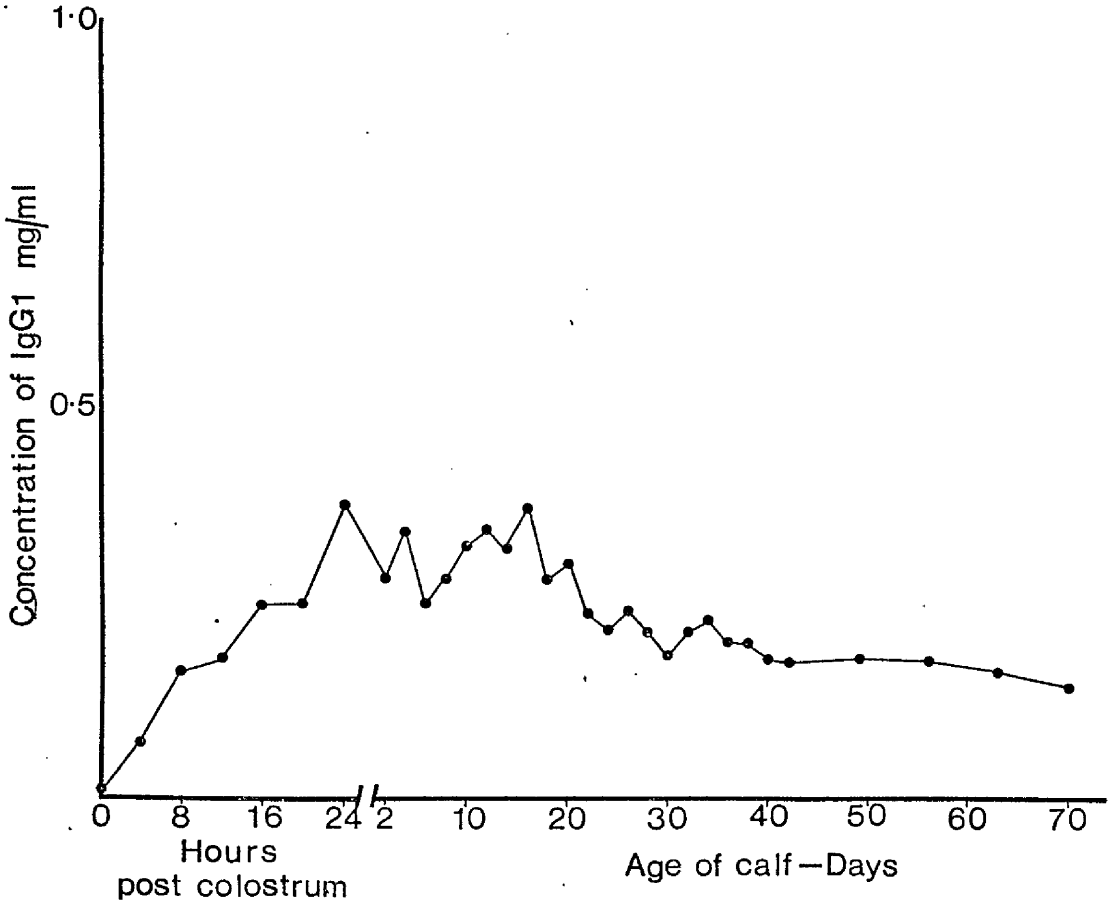


FIGURE 31 The mean concentration of IgG_1 in the lachrymal fluid of ten colostrum-fed calves during the first ten weeks of life.

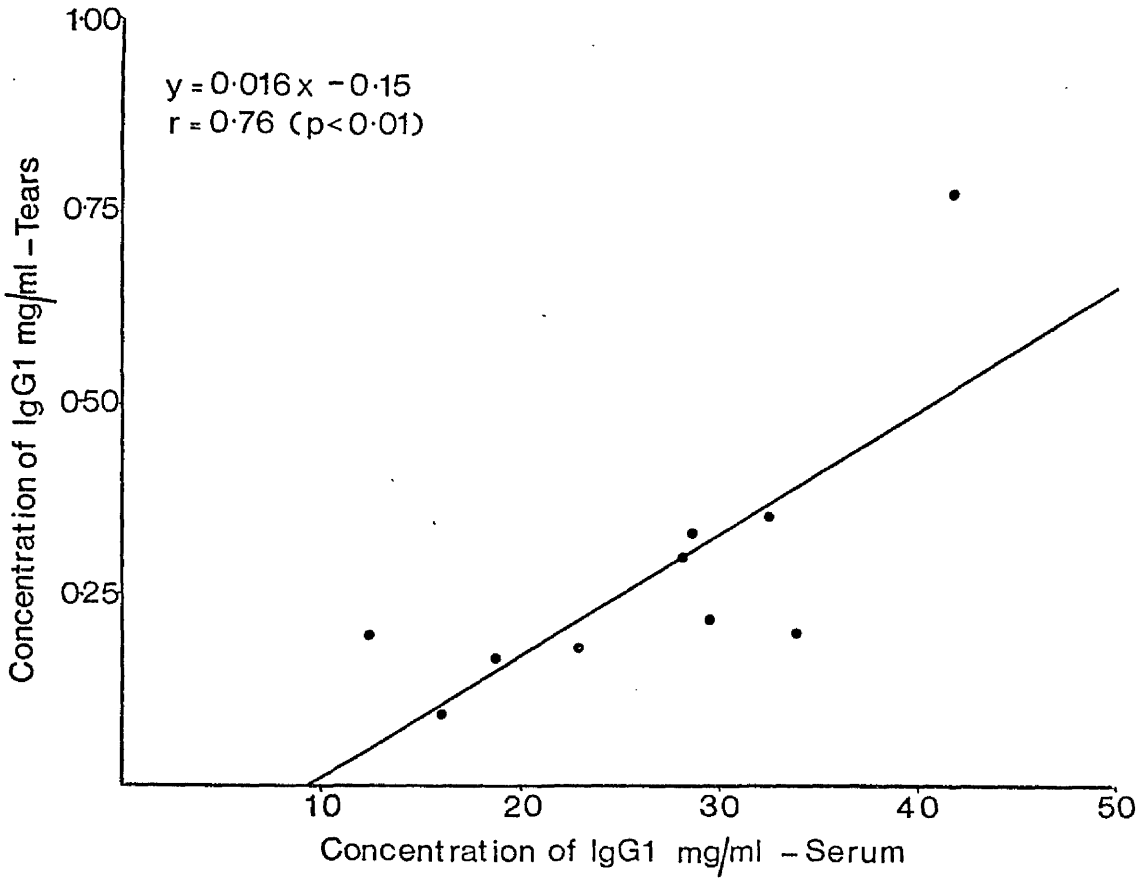


FIGURE 32 The relationship between the concentration of IgG_1 in serum and lachrymal fluid of ten calves at 48 hours of age.

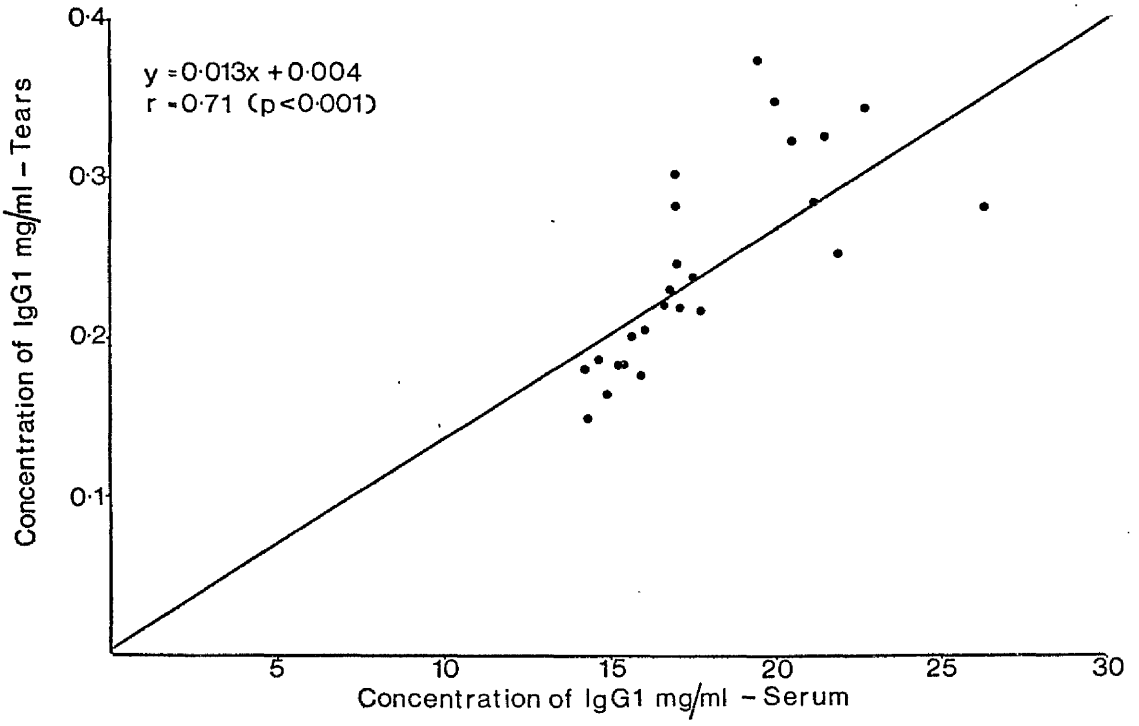


FIGURE 33 The relationship between the mean serum concentration of IgG_1 and the mean concentration of IgG_1 in lachrymal fluid of ten calves during the first ten weeks of life.

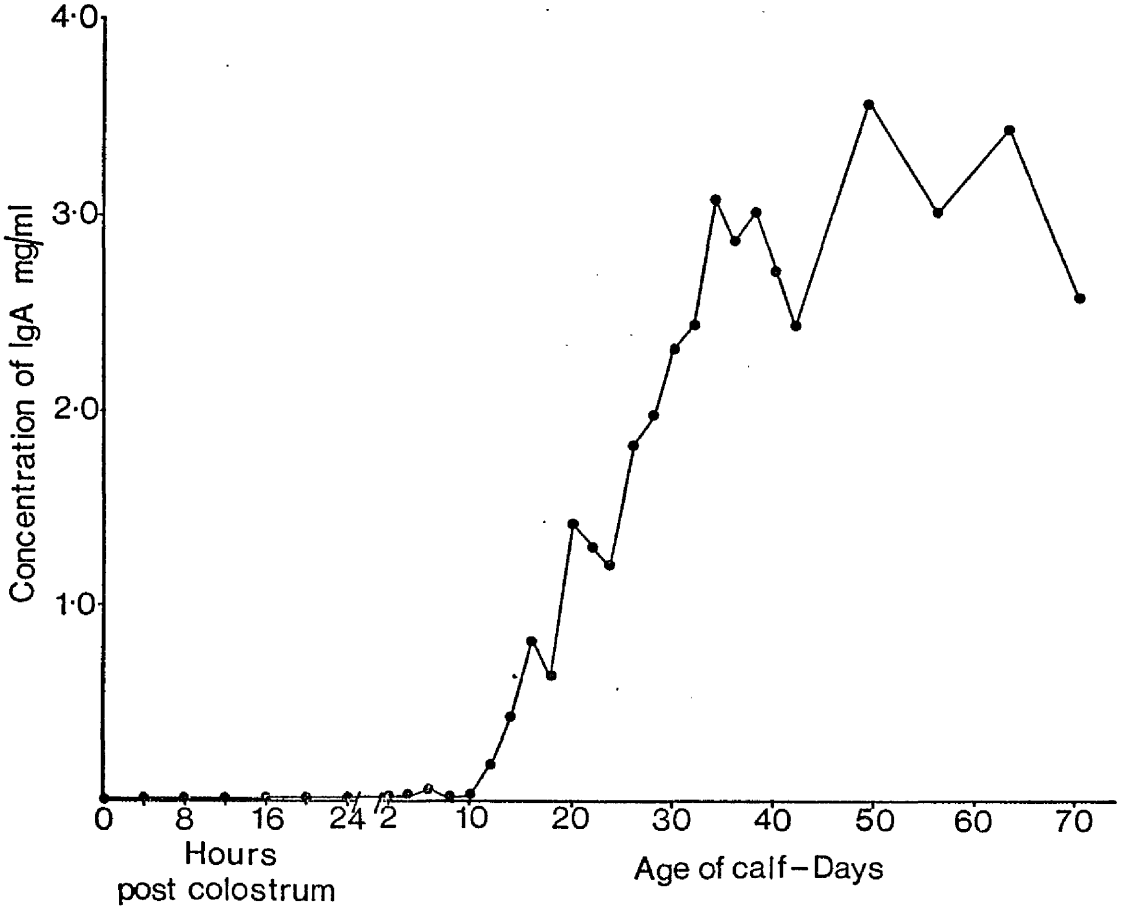


FIGURE 34 The mean concentration of IgA in the lachrymal fluid of ten colostrum-fed calves during the first ten weeks of life.

detected during the first week of life, although in one calf (98) a low concentration (0.040 mg/ml) was transiently found at 20 hours post-colostrum. In the remaining five calves lachrymal IgA became detectable at various times during the second week of life but in one calf (204) it was not detected until 14 days of age. In the case of calf 83 which attained the highest 48-hour serum concentration of IgA, IgA was first detected in the lachrymal fluid at 12 days of age. During the fourth week of life the concentration of lachrymal IgA increased rapidly and after 42 days the mean concentration was always in excess of 2 mg/ml.

IgM The mean concentration of IgM in the lachrymal fluid of all ten calves is presented in Figure 35. During the first week of life IgM was detected in tears of only two calves but, excluding the three calves in which IgA was detected during the first 48 hours of life, IgM was present in the lachrymal fluid either before or at the same time as IgA. The latest detection of IgM occurred at 12 days of age and initial concentrations varied from 0.002 mg/ml to 0.342 mg/ml. As with IgA, IgM was transiently detected (0.010 mg/ml) in the tears of calf 98 at 20 hours post-colostrum but then not again until four days of age. In calf 106 IgM was detected continuously from 24 hours of age.

Cows

The concentrations of immunoglobulins in the dams' sera and colostral wheys are presented in Table 33. The mean serum concentration of IgG_1 was 10.23 ± 1.87 mg/ml. Wide individual variations occurred in the serum concentration of IgG_2 with a mean concentration of 7.81 ± 3.96 mg/ml. The mean concentration of serum IgA was 0.63 ± 0.69 mg/ml and the mean serum concentration of IgM was 2.96 ± 1.51 mg/ml. Very high concentrations of IgG_1 were found in colostral whey, the mean being 97 ± 24.29 mg/ml. A highly significant correlation ($r = 0.915$, $p < 0.001$) was found between the concentration of IgG_1 in colostral whey and the gammaglobulin concentration of the colostral whey estimated by electrophoresis (Figure 36). The mean concentration of IgG_2 in colostral whey was 2.90 ± 1.51 mg/ml, significantly lower ($p < 0.01$) than the serum concentration of IgG_2. There was a highly significant difference between the mean concentration

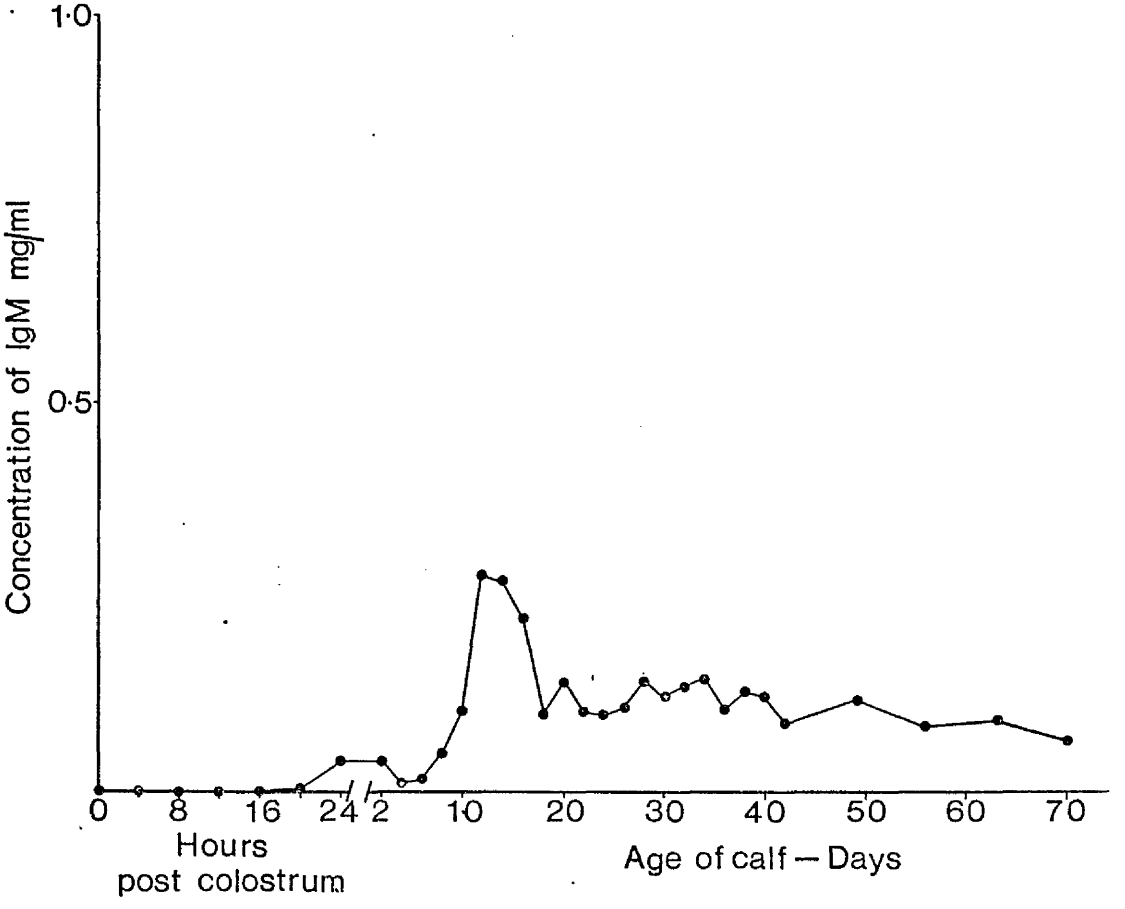


FIGURE 35 The mean concentration of IgM in the lachrymal fluid of ten colostrum-fed calves during the first ten weeks of life.

TABLE 33

The concentrations of immunoglobulins in the serum and colostral whey of the ten dams.

Cow No.	Serum				Colostral whey			
	IgG_1	IgG_2	IgA	IgM	IgG_1	IgG_2	IgA	IgM
	(mg/ml)				(mg/ml)			
77	12.70	12.20	0.50	3.80	124	4.50	11.86	9.48
79	12.30	6.75	0.36	3.60	54	1.15	3.70	3.28
80	12.00	13.40	0.62	4.32	78	6.00	9.74	4.32
83	12.14	1.16	0.51	1.14	76	1.90	5.14	4.08
85	9.35	9.30	0.36	4.66	98	1.15	3.72	4.36
93	8.00	1.40	0.36	0.80	94	3.00	7.04	8.80
98	8.80	8.80	0.26	1.56	84	2.70	5.14	3.50
106	9.00	8.00	0.36	2.80	127	2.00	8.10	9.90
204	10.00	8.25	0.41	4.90	120	3.00	6.00	7.00
224	8.00	8.80	2.60	2.04	114	3.55	5.20	0.44
Mean	10.23	7.81	0.63	2.96	97.0	2.90	6.56	5.51
S.D.	± 1.87	±3.96	±0.69	±1.51	±24.29	±1.51	±2.65	±3.12

of IgA (6.56 ± 2.64 mg/ml) in whey and the mean concentration in serum ($p < 0.001$). Although the mean concentration of IgM (5.51 ± 3.12 mg/ml) in colostral whey was higher than that of serum, the difference was not significant.

No correlation existed between the concentration of any class of immunoglobulin in the ten colostral wheys and the 48-hour serum concentration of the same class of immunoglobulin in the calves' sera, nor between the gammaglobulin concentrations of the colostral wheys and the 48-hour ZST values.

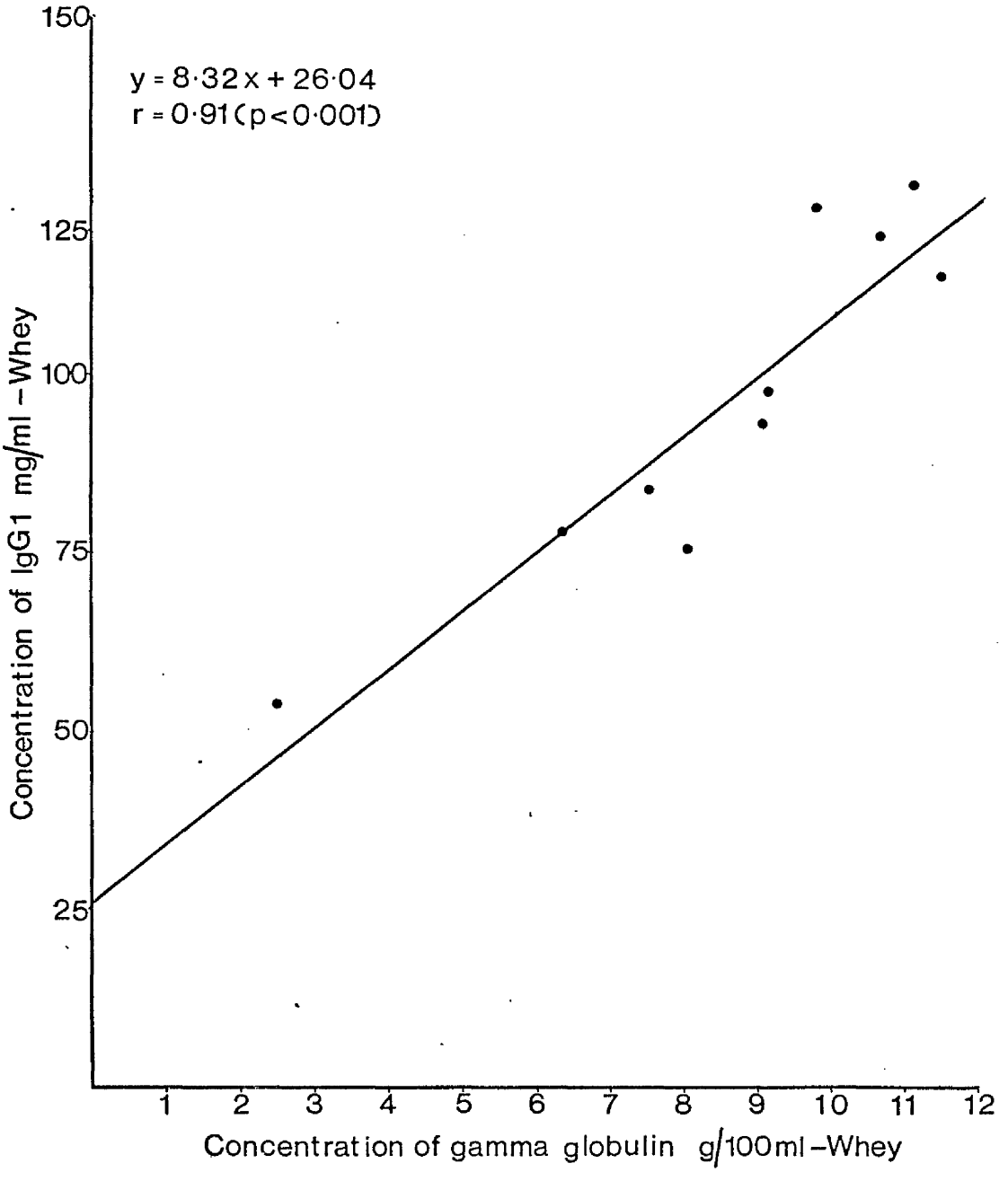


FIGURE 36 The relationship between the concentration of gammaglobulin and IgG_1 in bovine colostral whey.

DISCUSSION

While the newly born calf is generally considered agammaglobulinaemic at birth, several authors have reported the presence of low concentrations of IgG and IgM in the sera of pre-colostral neonatal calves (Porter, 1972; Husband and others, 1972; Penhale and others, 1973). In the present study six of the ten calves had low levels of immunoglobulins in the pre-colostral sera. Two calves had IgG_1 and IgG_2 in their precolostral serum and two calves had both IgG sub-classes and IgM in the precolostral serum. In the remaining two calves all four immunoglobulin classes, IgG_1, IgG_2, IgM and IgA, were detected in the precolostral serum. Of the four immunoglobulin classes, IgA is detected least frequently in precolostral serum. However, Brandon and Lascelles (1971) noted its presence in thoracic duct lymph fluid of calves collected prior to feeding with colostrum. Husband and others (1972) detected measureable concentrations of IgA in the precolostral sera of two of seven calves and Molla (1978) recorded its presence in the precolostral serum of one of six calves. The source of such immunoglobulins is uncertain but they may represent the initiation of the calf's immune responses as suggested by Husband and others (1972) since the foetus is competent in certain immune responses from five months of age (Schultz, 1973). In general, however, significant levels of serum immunoglobulins are not produced by the calf until it is between two to three weeks of age and protection against neonatal disease in the interim period has been clearly shown to depend on the presence of adequate amounts of maternal immunoglobulins acquired through ingestion of colostrum (Gay and others, 1965a,b; McEwan and others, 1970a).

The concentrations of absorbed colostral immunoglobulins in the serum of newborn calves are often determined by the zinc sulphate turbidity test described by McEwan and others (1970b) as used in Chapter 2. This assay has previously been shown to correlate well with calf serum IgG and IgM levels (McEwan and others, 1970b; McBeath and others, 1971) and in the present experiments it has also been shown to correlate with the total sum of the individual concentrations of IgG_1, IgG_2, IgA and IgM present in post-colostral sera. It also correlated well with the individual 48-hour serum concentrations of IgG_1 and IgA

but not with the 48-hour concentrations of IgG_2, nor, in contrast to the findings of McEwan and others (1970b), with the concentrations of IgM.

The degree of precipitation of immunoglobulins by salt solutions varies with both the concentration and nature of salt solution used (Williams and Chase, 1967) and zinc sulphate solutions selectively precipitate bovine IgG (Butler, 1971; Cambier and Butler, 1974; Fey and others, 1976). Therefore the highly significant correlation found between the concentrations of IgG_1 and the values obtained by the ZST test is not unexpected. Both IgA and IgM are relatively soluble in zinc salt solutions (Butler, 1971; Cambier and Butler, 1974) and will not be fully precipitated in the zinc sulphate turbidity test. However, the significant correlation between the serum concentrations of IgA and the ZST values may be related to the significant positive correlation shown to exist between the concentrations of IgG_1 and IgA in colostrum (Butler, Kiddy, Pierce and Rock, 1972a), although no such correlation was demonstrated in the present study. The concentration of IgG_2 in post-colostral serum is very low and thus even comparatively large variations in the concentrations of IgG_2 and IgM are likely to be masked by the preponderance of the precipitate due to IgG_1, making statistical correlation with the ZST results uncertain. Williams and others (1975) examined sera from calves approximately three weeks of age and found very significant correlations between the concentrations of each of the four immunoglobulin classes and ZST values, which is surprising given both the short half-lives of IgM and IgA, approximately five and three days respectively, and their solubility in zinc salt solutions.

Estimations of individual serum immunoglobulin concentrations showed that maximum serum concentrations of all four immunoglobulin classes occurred between 16 and 24 hours after the ingestion of colostrum, times which are similar to those recorded by Husband and others (1972) but earlier than those noted by Logan, McMurray, O'Neill, McParland and McRory (1978). The serum concentrations of immunoglobulins at 48 hours of age fall within the range of those determined by other workers (Table 30). The differences in the maximum serum concentrations can be explained by the variations in both the amounts of colostrum ingested and the times of first feeding (Selman, 1969; Kruse, 1970b). The lack of correlation between the concentrations of individual immunoglobulins in the colostral whey

and the subsequent concentrations of these immunoglobulins in the calves' sera at 48 hours of age has been noted previously (Klaus and others, 1969; Penhale and others, 1973; McGuire, Pfeiffer, Weikel and Bartsch, 1976) and was probably also due to factors noted above.

Following the ingestion of colostrum, the serum IgG_1 concentrations increased rapidly in all ten calves; significant concentrations of IgG_1 were detected in the serum of the four calves fed within an hour of birth and sampled at two hours after colostrum feeding (three hours post-partum). This is slightly earlier than the four hours post-partum when Logan and others (1978) first detected immunoglobulins in the sera of their calves. An examination of the thoracic duct lymph fluid of 13 calves which were fed colostrum at six to eight hours post-partum, revealed that substantial concentrations of all four immunoglobulin classes first appeared in the lymph between 30 minutes and three hours after colostrum feeding (Brandon and Lascelles, 1971). In the majority of their calves (8/13) the concentration of immunoglobulins in lymph fluid increased only slightly during the first hour after feeding, but increased rapidly during the second hour and reached a maximum four hours after feeding.

In the case of the four calves (79, 93, 98 and 224, Groups C and D), which had the lowest ZST values, the serum concentrations of IgG_1 increased at the same rate as in the other calves over the first eight hours after ingesting colostrum, but thereafter no further significant increase occurred. This may indicate that only a proportion of the absorptive capacity of the small intestine was utilised. The concentration of IgG_1 (54 mg/ml) in the colostral whey ingested by calf 79 was almost half that of the mean for all ten cows. In the case of calf 93, two factors may have combined to reduce the total amount of colostral IgG_1 absorbed; the calf was not fed until six hours post-partum and then it was only fed 1500ml of colostrum. The poor absorption of the colostral IgG_1 by calf 224 cannot be easily explained in terms of either delayed feeding or small amounts of colostral IgG_1. However the concentration of IgM in the colostral whey from cow 224 was only 0.44 mg/ml and the concentration of IgM in the serum of calf 224 at 48 hours of age was only 0.2 mg/ml, less than the suggested minimum (0.8 mg/ml) for protection against colisepticaemia (Penhale and others, 1970).

Penhale and others (1973) found that the time of intestinal closure after birth varied between the different classes of immunoglobulin from 16 hours for IgM to 27 hours for IgG. The estimated closure time for IgA was 22 hours. Logan and others (1974a) noted that calves may have adequate serum concentrations of one immunoglobulin class but may be deficient in another class, frequently IgM. This deficiency was attributed to the early closure of the intestine to IgM molecules but as has been demonstrated above it may also be a result of low immunoglobulin concentrations in the colostrum. Thus the final serum concentrations of colostrum derived maternal immunoglobulins may therefore depend upon both the quantity of colostral immunoglobulin presented to the calf and the rate of closure for that specific immunoglobulin class in the intestine.

The half-lives of the four classes of immunoglobulins calculated from the decrease in absorbed colostral immunoglobulins in the present study, IgG_1, 21.5 days; IgG_2, 28.5 days; IgA, 2.64 days; IgM, 6.95 days, accord well with values determined by similar methods by other workers (Husband and others, 1972; Porter, 1972; Logan and others, 1973; Sasaki and others, 1977a). The apparent half-lives determined by this method have generally been longer than those calculated from radio-isotope studies, as Sasaki and others (1977a) calculated that the half-life of IgG_1 in the neonatal calf was 11.5 days by isotope studies, but 19.9 days by the method used above. There are two possible explanations for the very long apparent half-life of 43.8 days determined for IgG_1 in calf 79; either significant amounts of endogenous IgG_1 were being synthesised or, as in man, the fractional catabolic rate of IgG_1 is directly proportional to its serum concentration (Hobbs, 1974). The endogenous production of both IgG_1 and IgG_2 is thought to occur between eight and 16 days of age (Husband and others, 1972). However in the present study, the mean concentration of IgG_2 at ten weeks of age was only 1.96 mg/ml, considerably less than the concentration found in adults. McGuire and others (1976) also noted that the serum concentration of IgG_2 remained below 1.0 mg/ml until two months of age, but even at three months the mean concentration was still only 1.8 mg/ml. In colostrum-deprived lambs synthesis of IgG_2 only begins at three to four weeks of

age and as late as five to six weeks in colostrum-fed lambs (Varela-Diaz and Soulsby, 1972; Pearson and Brandon, 1976; Verdouw-Chamalaun, Noordzij and Goudsward, 1977). A temporary deficiency of IgG_2 during the first few weeks of life has also been demonstrated in goats (Micusan, Boulay and Borduas, 1976). It is unlikely that IgG_2 contributes significantly to the immunological protection of the calf during the neonatal period.

The serum concentrations of IgA at 48 hours of age were much higher than those normally found in adult bovine serum, but they declined rapidly. Husband and others (1972) found that the calf only began to synthesise significant quantities of IgA when approximately nine weeks old. In the present study, the serum concentration of IgA was less than 0.4 mg/ml in almost every case between two and six weeks of age but thereafter began to increase gradually. In human babies significant concentrations of serum IgA become detectable during the fourth week of life (West, Hong and Holland, 1962; Stiehm and Fudenberg, 1966). In colostrum-deprived lambs IgM is the first of the four immunoglobulin classes to be detected in the serum at approximately eight days of age (Cole and Morris, 1971). In calves the endogenous production of IgM also begins during the second week of life (Ingram and Malcolmson, 1970; Husband and others, 1972).

IgG_1 was detected in tears collected prior to the ingestion of colostrum of only one calf (79). All four immunoglobulin classes were present in the pre-colostral serum of this calf with the concentration of IgG_1 being 0.90 mg/ml. Smith and others (1976) were unable to detect any immunoglobulins in the tears of lambs collected before ingestion of colostrum but the mean concentration of 0.28 mg/ml IgG at 24 hours of age was only slightly lower than the 0.377 ± 0.17 mg/ml IgG_1 attained in the present study. Although these authors did not separate IgG into its two sub-classes, it is highly probable that they were measuring IgG_1. The highest concentration of IgG in the tears of lambs occurred at three days whereas in this study the highest mean concentration of IgG_1 occurred at 24 hours. Subsequently the concentration of IgG_1 in the tears of the calves fell slowly. Pedersen and Nansen (1972) demonstrated a significant positive correlation between the concentration of IgG_1 in the serum and tears of adult cows. This relationship has now been confirmed for the concentration of

passively acquired serum IgG_1 and the concentration of IgG_1 in lachrymal fluid at 48 hours of age. A highly significant correlation also existed between the mean serum concentration of IgG_1 and the mean concentration of IgG_1 in tears throughout the ten week period. The mean concentration of IgG_1 of 0.189 ± 0.08 mg/ml in lachrymal fluid of the ten calves after 30 days of age was similar to those found in adult cows by other workers (Table 29).

When calf 224 developed a conjunctivitis at eight days of age, a marked increase in the concentration of IgG_1 occurred from 0.156 mg/ml on day 6 to 0.378 mg/ml on day 8. The eyes were clinically normal on day 10 and this was reflected in concentration of IgG_1 which had fallen to 0.156 mg/ml on this day. In an examination of the immunoglobulins in the tears produced in experimentally induced infectious kerato-conjunctivitis, Pedersen (1973) found that concentration of IgG_1 decreased because of the increased production of tears but that the total IgG secreted increased. The increased concentration of IgG_1 in tears of calf 224 on day 8 is probably indicative of an increased capillary permeability.

The virtual absence of IgM and IgA from the tears of the calves during the first ten days of life is almost identical to the situation that occurs in lambs (Smith and others, 1976). In lambs both IgM and IgA only became detectable in the lachrymal fluid when the lambs were between two and three weeks old but by 21 days of age the concentration of IgA exceeded that of both IgM and IgG. In the present study, the mean concentration of IgA at 14 days of age (0.437 ± 0.266 mg/ml) was higher than the mean concentration of IgG_1 (0.323 ± 0.212 mg/ml) and IgM (0.273 ± 0.184 mg/ml). Thereafter the concentration of IgA increased rapidly and from 30 days of age was always in excess of 2 mg/ml, which is similar to the concentration found in adult cows (Table 29). Arora, Tripathi, Killinger and Myers (1977) examined lachrymal fluid from calves of different ages, from a few days to 33 weeks old. IgA was only detected in the tears of calves more than three weeks old and the concentrations of IgA found in the tears of older calves were much lower than the values found in the present study. They were unable to detect IgM in any samples.

The highest mean concentration of IgM (0.28 mg/ml) occurred on day 12 and the concentration of IgM exceeded that of IgA on days 8, 10 and 12. It is of interest to note that IgM immunocytes predominate in Harderian glands of chickens during the first four weeks of life. From four to ten weeks of age IgG and IgA immunocytes are present in equal numbers and thereafter IgA immunocytes predominate (Albini, Wick, Rose and Orlans, 1974). No study of the immunocytes of calf lachrymal glands appears to have been carried out, but Aitken and Survashe (1977) found moderate plasma cell infiltration of both the Harderian and lachrymal glands of two adult cows, without differentiating the plasma cell types with specific immunoglobulin antisera. The majority of plasma cells in human, presumably adult, lachrymal glands stain for IgA, with only the occasional IgG and IgM plasma cells being present (Franklin, Kenyon and Tomasi, 1973).

Smith and others (1976) also examined the nasal secretions of newborn lambs for immunoglobulins before and after ingesting colostrum and over the first 14 days of life recorded concentrations similar to those found in lachrymal fluid. The dramatic increase in the concentration of IgA which occurred at 21 days in lachrymal fluid was not so apparent in the nasal secretions. During the first six weeks of life the peak concentration of IgG in the nasal and tracheal washings was approximately 0.8 mg/ml. The relative concentration of IgG_1 decreased from the first week of life to six weeks of age. At one week of age the concentration of IgM was higher than the concentration of IgA but from three weeks of age the concentration of IgA exceeded that of IgM.

Although IgA was detected in the lachrymal fluid of two calves (77, 79) during the first 48 hours of life, IgG_1 was the predominant immunoglobulin of bovine tears for at least the first ten days of life. There are several mechanisms by which the passively acquired IgG_1 may be transferred into lachrymal secretions. As IgG_1 was present in the tears of all ten calves by eight hours post-colostrum, the possibility of local production occurring within the lachrymal gland can be discounted. The two most probable explanations are the simple diffusion of IgG_1 from the intravascular compartment into the lachrymal fluid or the selective transfer of this immunoglobulin from the blood.

Quantitatively IgG_1 is the predominant passively acquired immunoglobulin in neonatal calf serum and, as 40 to 45 per cent of the total IgG_1 occurs extravascularly (Pedersen, 1973), its appearance in secretions by a simple diffusion process is not unexpected. The fact that a significant positive correlation was found to exist between the concentration of IgG_1 in tears and the serum IgG_1 concentration at 48 hours of age also suggests that a simple diffusion process was occurring. Bovine IgG_2 has an intravascular:extravascular distribution ratio similar to that of bovine IgG_1 (Pedersen, 1973), and if these immunoglobulins are transferred to the lachrymal fluid by simple diffusion the relative proportion of serum IgG_2 appearing in tears should be similar to that of IgG_1. This method of approach, however, is obviated because of the low serum concentrations of passively acquired IgG_2 attained. The concentration of IgG_1 in the lachrymal fluid was approximately 1 to 1.5 per cent of the serum concentration. At 48 hours of age the mean serum concentration of IgG_2 was 0.664 ± 0.31 mg/ml and 1 per cent of this is less than the minimum concentration of IgG_2 (0.008 mg/ml) which could be detected in the present study.

The intravascular:extravascular distribution of bovine IgA has not been determined, but in man approximately 55-60 per cent of IgA is found extravascularly (Tomasi and Grey, 1972). In man the intravascular:extravascular distribution ratio of IgM is 4:1 and a similar distribution has been postulated for bovine IgM (Penhale and others, 1973). It is therefore unlikely that IgM would be transferred to lachrymal fluid by transudation.

Under the experimental conditions employed in the present study the minimum detectable concentration of IgA was 0.01 mg/ml. The maximum serum IgA concentration attained was 7.2 mg/ml (calf 83) and if IgA was diffusing at a similar rate to IgG_1, the corresponding concentration of IgA in lachrymal fluid would have been 0.072 mg/ml, significantly above the minimum concentration detectable. However IgA was not detected in the lachrymal fluid of calf 83 at any time during the first 48 hours. IgA was detected in the lachrymal fluid of two calves (77, 79) during the first 48 hours post-partum. In calf 77 it was noted as early as two hours post-colostrum; at two hours post-colostrum the concentration of IgA in the lachrymal fluid

represented 6.8 per cent of the serum concentration. Excluding this sample, the concentration of IgA in the lachrymal fluid during the first 48 hours of life was, on average, 1.08 per cent of the serum IgA concentration. During the same period the concentration of IgA in the lachrymal fluid of calf 79 was approximately 3.5 per cent of the serum IgA concentration. Calf 79 was the only calf in which any immunoglobulins (IgG_1 - 0.028 mg/ml) were detected in lachrymal fluid prior to the ingestion of colostrum and in which low concentrations of all four immunoglobulins were found in the pre-colostral serum. There is little doubt that the IgA detected in the tears of calf 79 was derived from the serum, as no IgA could be detected in the tears from four to 14 days of age and no IgA was detected in the serum between six and 24 days of age.

This very poor transfer of absorbed colostral IgA to at least one mucous surface confutes the suggestion of Porter (1972) that the short half-life of absorbed IgA was directly related to its selective transport onto mucous surfaces. However as bovine colostral IgA consists mostly of 11S molecules (Butler, 1971) it is possible that the extravascular:intravascular distribution of IgA is similar to that of IgM with only small quantities, if any, diffusing from the intravascular compartment.

The possibility that IgG_1 is selectively transferred to lachrymal fluid cannot be overlooked. It has been established that IgG_1 is selectively transported from serum into the colostrum of cows (Sasaki and others, 1977b), sheep (Watson and Lascelles, 1973) and goats (Micusan and Borduas, 1976). This selective transport is a function of the Fc fragment of IgG_1 molecules, as the transfer of IgG_1 Fc fragments from serum to colostrum is six times greater than the corresponding transport of IgG_2 Fc fragments (Micusan and Borduas, 1976). It has also been suggested that, compared to either IgM or IgA, colostral IgG is taken up selectively by the absorptive cells of the calf's intestine (Penhale and others, 1973).

A study of the immunoglobulins in the lachrymal secretions of adult cows demonstrated that the IgG_1:albumin ratio of lachrymal fluid was six times greater than that of serum. In the same study,

the lachrymal concentration of IgG_2 could be determined accurately in only six of the 20 cows sampled. In these six animals, the concentration of IgG_2 in the lachrymal fluid was 0.33 per cent of the serum IgG_2 concentration, whereas the concentration of IgG_1 in the lachrymal fluid was 0.92 per cent of the serum concentration of IgG_1. In the remaining 14 cows the concentration of IgG_2 in the lachrymal fluid was less than the minimum detectable of 0.6 mg/100ml (Pedersen and Nansen, 1972). The transport of both IgG sub-classes into lachrymal secretions has been investigated in cattle aged four-to-ten months with radio-labelled IgG_1 and IgG_2 and at that age it was shown that IgG_1 was selectively transported into lachrymal fluid (Pedersen, 1973).

In the present study it was not possible to determine if there was any selective transfer of absorbed colostral IgG_1. This would have required the simultaneous determinations of albumin concentrations in the serum and lachrymal fluid, or the use of radio-labelled IgG_1 and IgG_2. However, in view of the above mentioned studies it is tempting to suggest that there is every possibility of the selective transfer of absorbed colostral IgG_1 from blood to mucous surfaces.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION AND CONCLUSIONS

The present study has confirmed and extended the findings of earlier workers, notably Selman (1969) and Kruse (1970a; 1970b) on the absorption of colostral immunoglobulins by the newborn calf. In the vast majority of calves, early assisted suckling to satiation results in high circulating concentrations of absorbed colostral immunoglobulins. The mean weight of colostrum ingested by 100 calves assisted to suckle to satiation immediately after birth was 2.29 ± 0.63 kg and this represented 7.1 per cent of their birthweight. As the time from birth to the completion of suckling was 65.74 ± 22.94 minutes, it demonstrates that the newborn calf has no difficulty ingesting large quantities of colostrum in the immediate post-partum period. Although the presence of the dam has been shown to enhance the absorption of colostral immunoglobulins under strictly controlled conditions (Selman and others, 1971b), allowing calves to remain with their dams for the first 12 hours of life, after being assisted to suckle to satiation immediately after birth, failed to produce a significant increase in the concentrations of absorbed immunoglobulins in the blood at 48 hours of age. However, there was some evidence that mothering and the opportunity to ingest more colostrum during this 12 hour period may result in higher serum concentrations of absorbed immunoglobulins in the individual calf (Chapter 2, Section 1), but the extra effort involved outweighs any practical advantage.

Kruse (1970c) suggested that the possibility of hypogammaglobulinaemia could be minimized by feeding at least two kilograms of colostrum within five hours of birth, but in the present study, it has been demonstrated that a small number of calves (less than 5%) may remain severely hypogammaglobulinaemic (less than 10 ZST units) despite ingesting this quantity of colostrum within 90 minutes of birth. Colostrum with low whey immunoglobulin concentrations was responsible for this serum immunoglobulin deficiency. As noted by Kruse (1970a), this is most commonly caused by prepartum loss of colostrum in the last days of gestation. However, in the present study, two animals, both first calving heifers, produced colostrum with very low concentrations of immunoglobulins in the whey, without dripping colostrum prior to parturition. No significant difference

could be demonstrated between the immunoglobulin concentrations of the whey prepared from the colostrum of first calving heifers and multiparous cows. The mean concentration of immunoglobulins in the whey prepared from the colostrum of 163 cows was 7.50 $\pm$ 2.49 g/100ml. Logan (1977) has shown that the level of feeding has a direct effect on the volume of colostrum produced in beef cows, and therefore on the total mass of immunoglobulins available for the newborn calf. As the single-suckled calf gives only a low financial return, beef-cows are, of necessity, frequently maintained on less than adequate diets, and the inevitable low serum concentrations of passively acquired immunoglobulins may be a major factor in the outbreaks of neonatal diarrhoea which frequently occur in single-suckled calves (Woode and others, 1975). Because of their intensive management, low colostrum yields are unlikely to be a significant problem in dairy cows. In fact, the possibility of dilution of the colostral immunoglobulins in the high-yielding, "steamed-up" dairy cow should not be overlooked.

The small survey of the serum immunoglobulin concentrations of calves less than one week old which were being sold through markets in the west of Scotland revealed that approximately 40 per cent were severely hypogammaglobulinaemic. The proportion of hypogammaglobulinaemic calves is very similar to those found in previous surveys covering the same geographical area (Gay and others, 1965a; 1965b; Fisher and others, 1968; McEwan and others, 1970a). Furthermore, a survey of dairy heifer calves retained on the farms of birth in same region, showed that a similar proportion of these animals were also hypogammaglobulinaemic (Selman and others, 1971a) and indicated that their immediate post-natal husbandry was no better than that given to the surplus male calves. The results of the present survey demonstrate the difficulty in communicating basic scientific information to the farming community.

Examination of the incidence of diarrhoea in group-reared calves during the first four weeks of life revealed that it was highest during the second week of life. Although it has been frequently demonstrated that marked diarrhoea is accompanied by weight loss (Roy and others, 1955a; Dalton and others, 1960; Fisher and Martinez, 1975b), no significant correlation could be shown between the incidence of diarrhoea during the first four weeks of life and the liveweight gain over that period in seven of eight groups of calves. Furthermore

there was no significant correlation between the incidence of diarrhoea during the first four weeks of life and the serum concentrations of absorbed immunoglobulins at 48 hours of age in any of the eight groups of calves. These findings may have been due to the high serum concentrations of passively acquired immunoglobulins, and the use of clean accommodation for each group which may have prevented a "build-up of infection" (Roy and others, 1955a; Wood, 1955). The one group (Group 12), in which there was a significant correlation between the liveweight gain and the incidence of diarrhoea during the first four weeks of life had the lowest mean concentration of absorbed immunoglobulins (17.33 ± 10.49 ZST units), and the longest assembly period (31 days).

The calves with access to the automatic feeder ingested much more milk substitute than is normally offered under the traditional twice daily bucket-feeding system. A highly significant positive correlation was demonstrated between volume of milk consumed and both the daily liveweight gains and the total liveweight gain over the period of milk-feeding. There was no evidence to suggest that these high daily intakes of milk predisposed the calves to diarrhoea and, thus, confirmed the findings of Mylrea (1966).

Several important results emerge from the study on the absorption and redistribution of the individual classes of immunoglobulins. Very rapid absorption of colostral IgG_1 occurs when colostrum is fed soon after birth. Significant concentrations of IgG_1 were detected in serum at two hours post-colostrum feeding (three hours post-partum) in four calves. Serum concentrations of all four classes of immunoglobulins reached a maximum between 16 and 24 hours after the feeding of colostrum. The absorbed IgG_1 was rapidly transferred from the intravascular compartment to the conjunctival mucous surface, and in significant concentrations. This IgG_1, which may reach the lachrymal fluid either by simple transudation or selective transfer, provides immunological protection until the mucous surfaces of the eye begin to synthesise significant quantities of secretory IgA at two to three weeks of age. A similar increase in the concentration of lachrymal IgA at approximately the same age has also been demonstrated in lambs (Smith and others, 1976). During the immediate post-partum period, absorbed colostral IgA is only poorly transferred to the

lachrymal fluid, and the short half-life of absorbed IgA is not associated with its transfer to mucous surfaces as was suggested by Porter (1972). The concentrations of IgG_2 in colostral whey are extremely low and this was reflected in the low concentrations of this immunoglobulin in the post-colostral sera of the calves. Even at ten weeks of age the serum concentration of IgG_2 was still very low, being only 20 to 25 per cent of the concentration in adult serum. This is identical to the situation which pertains in lambs (Varela-Diaz and Soulsby, 1972; Pearson and Brandon, 1976; Verdouw-Chamalaun and others, 1977), and almost certainly this is related to the biological functions of IgG_2.

This study has not completely resolved the role of passively acquired serum immunoglobulins in neonatal calf diarrhoea, but it has been shown that early ingestion of colostrum results in the rapid appearance of IgG_1 at one mucous surface. This process is likely to be repeated at all mucous surfaces throughout the body, including the intestine, and is thus likely to provide that mucous surface with immunological protection. Certainly de Rham and Isliker (1977) have shown that bovine IgG_1 is only very slowly degraded by the action of trypsin, and suggested that any IgG_1 which reached the intestine, either by active secretion or transudation, would retain its antibody activity.

The importance of the early ingestion of colostrum was illustrated by Logan and others (1977) who reported that two calves, which were experimentally inoculated with an enterotoxigenic E. coli orally at one and two hours of age before receiving colostrum at three and four hours of age, were not protected despite acquiring high serum concentrations of absorbed immunoglobulins. Three control calves, which were challenged with the same strain of E. coli two hours after being fed colostrum at five hours of age, did not develop diarrhoea and remained normal throughout the experimental period. The authors suggested that, to prevent adhesion of the pathogenic E. coli to the intestinal epithelium, immunological protection must be present before infection. The present study has demonstrated that the newborn calf can ingest approximately seven per cent of its birthweight of colostrum within an hour of birth. This mass of colostrum will provide the intestinal tract with an immediate immunological barrier against challenge, and will effect the maximal absorption of these colostral immunoglobulins, resulting in high serum immunoglobulin concentrations in at least 95 per cent of calves.

APPENDICES

APPENDIX I

CALF NO.	GROUP	BREED*	SEX	BIRTH WEIGHT (kg)	MASS/VOLUME OF COLOSTRUM INGESTED	COLOSTRAL WHEY PROTEIN CONCENTRATION (g/100ml)		TIME (minutes)		SERUM IMMUNOGLOBULIN CONCENTRATION (ZST units)		
						TOTAL PROTEIN	GAMMAGLOBULIN	Required to suckle to satiation	From birth to completion of suckling	Precolostrum	48 hours	Absorbed
1	1	F	M	44.5	2.8 kg	15.54	11.41	24	65	2	46	44
2	1	A	F	35.3	3.2 kg	13.58	9.80	34	83	1	33	32
3	1	A	M	39.1	3.1 kg	10.24	6.23	40	87	1	27	26
4	1	AxF	M	36.4	2.9 kg	7.44	5.83	25	65	1	33	32
5	1	F	M	29.9	2.0 kg	8.40	6.19	23	87	1	30	29
6	1	AxF	M	46.1	3.1 kg	8.38	6.09	29	75	1	24	23
7	1	A	M	38.2	3.5 kg	8.80	6.78	29	74	1	27	26
8	1	A	F	28.9	1.6 kg	11.22	8.31	25	90	1	34	33
9	1	A	M	30.6	0.8 kg	8.66	5.09	28	60	0	16	16
10	1	AxF	M	28.4	2.5 kg	12.02	8.99	54	115	2	35	33
11	1	A	M	36.5	1.9 kg	11.64	7.82	21	63	1	21	20
12	2	AxF	F	32.9	3.0 kg	9.48	6.35	22	52	2	31	29
13	2	A	F	30.1	2.4 kg	12.10	8.31	16	55	2	31	29
14	2	A	M	43.2	2.9 kg	9.36	6.45	29	76	1	22	21
15	2	AxF	M	38.1	2.4 kg	18.78	14.22	35	100	1	37	36
16	2	A	F	31.9	4.1 kg	9.04	5.93	33	69	0	23	23
17	2	F	F	32.1	2.1 kg	6.50	4.08	26	55	2	26	24
18	2	AxH	M	35.5	2.4 kg	10.36	7.07	22	72	1	32	31
19	2	AxF	F	39.6	3.4 kg	11.22	8.04	15	64	2	32	30
20	2	A	M	32.8	2.3 kg	7.64	5.26	15	48	2	27	25
21	2	AxF.xF	M	37.7	1.5 kg	11.64	8.83	17	50	2	28	26
22	2	A	F	25.5	1.7 kg	10.74	6.31	34	73	1	20	19
23	2	AxF	F	34.4	3.0 kg	9.60	6.13	25	67	1	21	20
24	2	A	M	28.5	2.0 kg	9.32	6.64	22	52	1	28	27
25	2	AxSh	M	29.8	2.3 kg	9.32	6.70	25	72	0	27	27
26	3	A	M	43.3	3.6 + 2.3 kg	5.64	3.67	39	12 hours	3	37	34
27	3	A	F	39.4	0.4 + 0.7 kg	10.32	7.35	25	12 hours	2	25	23
28	3	A	F	30.8	3.0 + 1.3 kg	9.40	6.66	40	12 hours	2	27	35
29	3	A	M	22.5	1.3 + 0.8 kg	9.40	6.66	15	12 hours	1	30	29
30	3	A	F	33.1	2.3 + 0 kg	13.22	8.85	10	12 hours	2	34	32

APPENDIX I

CALF NO.	GROUP	BREED*	SEX	BIRTH WEIGHT (kg)	MASS/VOLUME OF COLOSTRUM INGESTED	COLOSTRAL WHEY PROTEIN CONCENTRATION		TIME		SERUM IMMUNOGLOBULIN CONCENTRATION		
						TOTAL PROTEIN (g/100ml)	GAMMAGLOBULIN	Required to suckle to satiation (minutes)	From birth to completion of suckling	Precolostrum	48 hours (ZST units)	Absorbed
31	3	A	M	41.1	2.3 + 1.1 kg	15.40	10.84	25	12 hours	1	39	38
32	3	A	M	35.8	3.1 + 1.8 kg	8.64	5.21	24	12 hours	2	29	27
33	3	A	M	24.1	2.8 + 0 kg	8.40	5.23	30	12 hours	1	37	36
34	3	AxF.xF	F	25.3	2.3 + 0 kg	12.00	7.03	16	12 hours	1	47	46
35	3	AxF	F	32.7	2.7 + 0 kg	9.28	6.23	39	12 hours	1	22	21
36	3	A	M	29.4	2.7 + 0 kg	16.00	10.86	19	12 hours	0	25	43
37	3	AxF	F	32.8	2.2 + 0.7 kg	9.94	6.67	35	12 hours	0	25	25
38	3	A	F	34.8	3.0 + 0 kg	6.80	4.74	20	12 hours	1	19	18
39	3	A	M	37.8	2.2 + 2.3 kg	6.72	4.30	21	12 hours	1	12	11
40	4	AxH	M	36.1	3.0 + 0 kg	9.00	5.94	18	12 hours	3	39	36
41	4	A	M	32.7	0.8 + 0 kg	9.28	6.17	18	12 hours	3	15	12
42	4	AxH	F	29.2	2.2 + 0 kg	12.24	8.58	15	12 hours	0	30	30
43	4	A	M	35.1	2.7 + 0 kg	7.80	4.79	17	12 hours	1	19	18
44	4	AxF	F	34.0	3.2 + 1.4 kg	7.26	4.65	15	12 hours	1	23	22
45	4	A	F	25.7	1.3 + 0 kg	9.92	7.13	25	12 hours	2	27	25
46	4	AxH	M	34.8	2.5 + 0 kg	9.80	6.83	20	12 hours	1	29	28
47	4	A	M	35.7	2.2 + 0.2 kg	7.54	5.42	24	12 hours	1	26	25
48	4	AxF.xH	F	28.6	3.3 + 0 kg	9.80	7.70	24	12 hours	0	36	36
49	4	A	M	38.3	3.0 + 0 kg	11.98	8.25	23	12 hours	3	43	40
50	4	A	M	40.1	3.5 + 0.1 kg	12.00	7.91	22	12 hours	2	42	40
51	5	F	M	39.1	3.3 kg	12.00	8.27	19	49	1.5	30.5	29
52	5	A	F	24.6	2.7 kg	13.96	8.39	32	63	0.5	35.5	35
53	5	A	M	34.2	2.5 kg	13.96	10.12	10	50	1	40	40
54	5	AxF	M	36.3	2.8 kg	16.40	12.16	25	55	0.5	53.5	53
55	5	AxF	F	34.6	2.3 kg	11.20	7.79	30	152	0	33	33
56	5	F	M	27.6	1.6 kg	8.66	5.48	20	70	0.75	29.75	29
57	5	A	F	32.6	2.2 kg	11.20	7.84	30	64	0.5	18.5	18
58	6	AxF	F	34.8	1.5 kg	8.60	6.30	15	36	0	21.5	22
59	6	AxH	F	28.8	1.7 kg	6.10	3.67	18	41	5.5	24.5	19
60	6	F	M	42.0	3.3 kg	9.60	6.85	17	45	0	27	27

APPENDIX I

CALF NO.	GROUP	BREED*	SEX	BIRTH WEIGHT (kg)	MASS/VOLUME OF COLOSTRUM INGESTED	COLOSTRAL WHEY PROTEIN CONCENTRATION (g/100ml)		TIME (minutes)		SERUM IMMUNOGLOBULIN CONCENTRATION (ZST units)		
						TOTAL PROTEIN	GAMMAGLOBULIN	Required to suckle to satiation	From birth to completion of suckling	Precolostrum	48 hours	Absorbed
61	6	AxF	M	41.4	2.4 kg	9.60	6.21	23	48	0	23	23
62	6	A	M	32.9	1.7 kg	6.24	4.41	17	51	0	13	13
63	6	A	M	25.9	1.7 kg	12.44	8.80	12	49	1	26	25
64	6	A	M	31.5	2.0 kg	12.44	8.80	14	42	1.5	22	21
65	6	A	M	26.6	2.2 kg	11.16	7.83	20	56	0	35	35
66	6	AxF	M	30.5	1.4 kg	9.64	6.49	21	46	0	14	14
67	6	AxF	F	31.8	2.4 kg	3.16	1.87	23	60	0.5	4.5	4
68	7	A	M	36.6	1.6 kg	9.96	7.55	19	81	1.5	24.5	23
69	7	A	M	40.5	2.8 kg	12.32	8.29	28	58	0.5	30.5	30
70	7	A	M	27.9	1.9 kg	11.68	8.01	20	87	0	26	26
71	7	AxF.xCh	F	37.5	2.4 kg	10.00	7.51	26	76	0	26	26
72	7	A	M	38.2	3.2 kg	8.72	5.88	22	61	0.5	23.5	23
73	7	AxF.xF	F	29.8	2.1 kg	3.20	1.87	13	54	0.5	5.0	5
74	7	A	M	27.0	2.8 kg	11.46	7.94	14	60	0.5	42	42
75	7	AxF	M	44.7	3.8 kg	6.10	4.02	31	59	0.5	19	19
76	7	AxF	F	32.7	2.4 kg	9.04	6.30	20	47	0.5	35.5	35
77	-	A	F	33.2	2.3 kg	13.40	9.74	23	65	0.5	34	34
78	-	A	F	32.3	2.1 kg	11.40	7.53	20	58	0.5	28.5	28
79	-	AxH	F	32.4	1.3 kg	3.74	2.48	40	85	2	10	8
80	-	AxCh	M	39.3	1.8 kg	9.94	6.32	30	70	0.5	26.5	26
81	-	AxF	F	23.5	2.2 kg	8.92	6.53	48	100	1	39	38
82	8	AxF	M	32.2	2.5 kg	6.20	4.32	17	36	0	23	23
83	8	AxF.xF	M	32.5	2.3 kg	10.70	8.00	28	83	0	43	43
84	8	A	M	29.6	1.7 kg	10.20	7.54	26	81	0.5	39.5	39
85	8	AxH	F	34.0	1.7 kg	12.26	9.09	16	59	0	31	31
86	8	A	M	25.5	1500 ml	11.32	8.50	-	360	0.5	22	22
87	8	F	F	30.0	1500 ml	8.64	5.96	-	454	0.5	24.5	24
88	8	A	M	34.3	1500 ml	-	-	-	360	1.5	23.5	22
89	8	A	F	29.0	1.8 kg	16.84	12.51	20	52	0	44.5	45
90	8	A	M	27.7	1500 ml	8.50	5.95	-	360	1	16	15

APPENDIX 1

CALF NO.	GROUP	BREED*	SEX	BIRTH WEIGHT (kg)	MASS/VOLUME OF COLOSTRUM INGESTED	COLOSTRAL WHEY PROTEIN CONCENTRATION (g/100ml)		TIME (minutes)		SERUM IMMUNOGLOBULIN CONCENTRATION (ZST units)		
						TOTAL PROTEIN	GAMMAGLOBULIN	Required to suckle to satiation	From birth to completion of suckling	Precolostrum	48 hours	Absorbed
91	8	A	F	26.0	2.6 kg	14.65	10.30	33	75	0	24	24
92	8	A	F	31.2	1500 ml	13.68	9.56	-	370	0	14	14
93	8	A	F	30.0	1500 ml	14.00	9.00	-	360	0	18	18
94	8	AxF	F	29.6	1500 ml	13.16	8.80	-	360	1	13	12
95	8	A	M	22.9	1.4 kg	14.52	10.88	20	45	0	36	36
96	9	A	M	30.5	2.3 kg	10.10	7.41	19	99	0.5	23	23
97	9	A	M	31.5	2.2 kg	10.10	7.41	14	113	1.0	19.5	19
98	9	A	M	34.9	1.8 kg	12.08	7.49	30	74	1	21	20
99	9	A	M	34.6	1500 ml	8.64	4.95	-	360	0.5	8.5	8
100	9	A	F	29.3	1500 ml	12.92	8.21	-	360	0.5	29.5	29
101	9	AxF.xF	M	37.5	1500 ml	9.04	6.20	-	360	0.5	20.5	20
102	9	A	M	30.9	1500 ml	14.64	9.91	-	360	1.0	31.5	31
103	9	A	M	33.8	1.9 kg	10.60	6.90	21	69	0.5	27.5	27
104	-	A	F	18.1	1.0 kg	5.27	4.02	17	61	15	31	16
105	9	A	M	29.5	1500 ml	2.08	1.02	-	360	0.5	6.5	6
106	9	A	F	26.1	1.8 kg	16.10	11.04	20	72	0	38	38
107	10	AxCh	F	27.3	-	14.04	9.78	-	48 hours	1	50	49
108	10	A	M	27.5	1700 ml	6.50	3.58	-	305	1	10.5	10
109	10	F	M	34.1	-	11.82	8.29	-	48 hours	2.5	31.5	29
110	10	A	F	31.4	-	7.38	5.05	-	48 hours	0.5	14.5	14
111	10	A	F	30.2	2.8 kg	11.28	7.09	22	62	0	30	30
112	10	A	M	27.5	1.6 kg	9.50	6.97	20	55	1	21	20
113	10	A	F	30.0	2.6 kg	18.43	13.56	25	60	1	42.5	42
114	10	A	M	31.8	1700 ml	11.40	8.59	-	245	0.5	27.5	27
115	10	A	M	32.9	1700 ml	3.70	1.93	-	570	0.5	2.5	2
116	10	A	F	39.3	1700 ml	9.12	6.46	-	585	0	17	17
117	10	AxCh	F	41.6	-	9.84	7.31	-	48 hours	1	27.5	27
118	10	AxF	F	31.4	3.4 kg	14.80	10.04	25	65	2	42	40
119	10	AxF.xF	M	24.8	1700 ml	9.30	6.32	-	515	1	16	15
120	10	AxF.xF	F	25.7	1.5 kg	9.30	6.32	15	68	1.5	18.5	17

APPENDIX 1

CALF NO.	GROUP	BREED*	SEX	BIRTH WEIGHT (kg)	MASS/VOLUME OF COLOSTRUM INGESTED	COLOSTRAL WHEY PROTEIN CONCENTRATION (g/100ml)		TIME (minutes)		SERUM IMMUNOGLOBULIN CONCENTRATION (ZST units)		
						TOTAL PROTEIN	GAMMAGLOBULIN	Required to suckle to satiation	From birth to completion of suckling	Precolostrum	48 hours	Absorbed
121	10	A	M	39.8	-	15.90	11.58	-	48 hours	0	24	24
122	11	AxH	M	42.0	-	14.67	10.40	-	48 hours	3	38	35
123	11	AxF.xF	M	36.8	-	7.00	4.95	-	48 hours	2	3	1
124	11	AxSh	F	34.0	-	13.80	9.48	-	48 hours	0.5	24.5	24
125	11	F	M	30.6	1.4 kg	11.97	8.77	35	75	1	33	32
126	11	F	M	29.5	1700 ml	9.80	6.18	-	433	1	24	23
127	11	AxH	F	29.1	1700 ml	15.90	9.55	-	395	1	23	22
128	11	AxH	F	35.6	1700 ml	15.90	10.96	-	481	0	16.5	17
129	11	AxAA	F	29.3	2.3 kg	14.88	9.75	24	71	0	28	28
130	11	FxAA	F	34.6	-	17.88	11.57	-	48 hours	2	24	22
131	11	A	F	27.2	1700 ml	9.84	6.00	-	555	1.5	19.5	18
132	11	FxAA	M	29.7	-	14.26	9.38	-	48 hours	1	24	23
133	11	AxF.xF	M	36.5	1.9 kg	7.72	5.18	30	80	1.5	24.5	23
134	11	FxH	F	31.4	1.9 kg	12.75	9.32	20	55	1.5	27	26
135	11	AxH	M	40.3	3.2 kg	15.00	11.49	20	50	0.5	44.5	44
136	11	FxH	M	38.9	1700 ml	14.10	10.55	-	825	0.5	19.5	19
137	12	AxH	F	35.4		7.80	5.18	-	48 hours	1.5	25.5	24
138	12	FxH	F	42.7	-	7.83	6.12	-	48 hours	1.5	14.5	13
139	12	F	M	34.2	-	13.22	9.42	-	48 hours	1.0	6.5	6
140	12	F	M	32.7	1700 ml	9.96	7.12	-	520	1.5	13.5	12
141	12	AxF	M	32.5	1.5 kg	9.96	7.13	40	70	1	17	16
142	12	FxH	F	30.0	2.8 kg	12.21	8.69	20	62	0.5	38.5	38
143	12	A	M	37.0	-	9.22	6.19	-	48 hours	1.0	16	15
144	12	AxF	F	27.0	2.1 kg	9.72	6.42	15	60	1.5	12.5	11
145	12	AxF.xF	M	28.8	1700 ml	10.86	8.33	-	525	0.5	26.5	26
146	12	AxF.xF	F	35.5	-	11.70	8.23	-	48 hours	1.5	28.5	27
147	12	AxF	M	36.6	2.7 kg	13.32	9.52	17	59	1.5	29.5	28
148	12	AxH	F	32.3	2.0 kg	10.80	7.16	25	55	2	29	27
149	12	A	M	31.8	1700 ml	14.82	10.23	-	480	1	13	12
150	12	AxF.xF	M	33.2	1700 ml	4.20	2.30	-	480	0.5	3.5	3

APPENDIX I

CALF NO.	GROUP	BREED *	SEX	BIRTH WEIGHT (kg)	MASS/VOLUME OF COLOSTRUM INGESTED	COLOSTRAL WHEY PROTEIN CONCENTRATION (g/100ml)		TIME (minutes)		SERUM IMMUNOGLOBULIN CONCENTRATION (ZST units)		
						TOTAL PROTEIN	GAMMAGLOBULIN	Required to suckle to satiation	From birth to completion of suckling	Precolostrum	48 hours	Absorbed
151	12	F	M	28.4	1700 ml	16.38	11.50	-	495	2	4	2
152	-	J	F	20.5	1.4 kg	18.00	11.99	20	200	1.5	36.5	35
153	-	J	M	22.5	3.0 kg	8.00	5.01	25	71	1	22	21
154	-	AxF.xF	M	34.5	2.0 kg	11.44	7.29	25	45	0.5	24.5	24
155	-	A	M	31.6	2.6 kg	13.84	10.08	25	60	2	31	29
156	-	A	M	33.3	2.5 kg	2.90	1.58	15	40	2	7	5
157	-	AxF	M	31.0	1.5 kg	15.20	11.44	43	64	0.5	29.5	29
158	-	A	F	35.2	2.5 kg	7.54	4.93	17	37	1	24	23
159	-	AxF	M	30.9	2.3 kg	11.80	8.49	33	51	0	26	26
160	-	F	M	36.3	2.3 kg	16.68	12.24	21	40	0.5	38.5	38
161	-	A	M	29.8	1.8 kg	12.12	8.57	21	46	0.5	31.5	31
162	-	F	M	38.6	2.5 kg	14.68	10.86	19	42	1	36	35
163	-	AxH	F	33.2	2.0 kg	14.98	10.88	23	46	0.5	31.5	31
164	-	AxF	F	35.8	3.5 kg	9.50	6.27	18	42	0.5	25	25
165	-	FxH	F	36.8	3.2 kg	12.76	8.98	23	55	0.5	30.5	30
166	-	FxAA	M	30.9	1.8 kg	6.30	4.53	28	50	2	20	18
204	-	AxF.xF	F	29.8	2.3 kg	15.30	10.61	18	85	2	29	27
224	-	AAxBG	M	32.6	1.7 kg	14.60	11.43	20	98	0	18	18

* Breeds : A = Ayrshire; F = Friesian; H = Hereford;
Ch = Charolais; Sh = Shorthorn; AA = Aberdeen Angus;
B.G. = Blue-Grey.

APPENDIX 2

TABLE 1

Data regarding the breed, sex and the serum concentrations of Immunoglobulins of the 25 calves of Group A.

Calf	Breed	Sex	Serum immunoglobulin concentration (ZST units)		48 hour serum concentration of absorbed immunoglobulins (ZST units)
			Pre-colostral (15 minutes post-partum)	Post-colostral (48 hours post-partum)	
1	Fr	M	2	46	44
2	A	F	1	33	32
3	A	M	1	27	26
4	AxFr	M	1	33	32
5	Fr	M	1	30	29
6	AxFr	M	1	24	23
7	A	M	1	27	26
8	A	F	1	34	33
9	A	M	0	16	16
10	AxFr	M	2	35	33
11	A	M	1	21	20
12	AxFr	F	2	31	29
13	A	F	2	31	29
14	A	M	1	22	21
15	AxFr	M	1	37	36
16	A	F	0	23	23
17	Fr	F	2	26	24
18	AxH	M	1	32	31
19	AxFr	F	2	32	30
20	A	M	2	27	25
21	AxFrxFr	M	2	28	26
22	A	F	1	20	19
23	AxFr	F	1	21	20
24	A	M	1	28	27
25	AxSh	M	0	27	27

A = Ayrshire; Fr = Friesian; H = Hereford;
Sh = Shorthorn.

APPENDIX 2

TABLE 2

Data regarding the total protein and immunoglobulin concentrations of the colostral whey fed to the 25 calves in Group A.

Calf	Colostral whey total protein concentration (g/100 ml)	Whey immunoglobulin concentration (g/100 ml)	48 hour serum concentration of absorbed immunoglobulins (ZST units)
1	15.54	11.41	44
2	13.58	9.80	32
3	10.24	6.23	26
4	7.44	5.83	32
5	8.40	6.19	29
6	8.38	6.09	23
7	8.80	6.78	26
8	11.22	8.31	33
9	8.66	5.09	16
10	12.02	8.99	33
11	11.64	7.82	20
12	9.48	6.35	29
13	12.10	8.31	29
14	9.36	6.45	21
15	18.78	14.22	36
16	9.04	5.93	23
17	6.50	4.08	24
18	10.36	7.07	31
19	11.22	8.04	30
20	7.64	5.26	25
21	11.64	8.83	26
22	10.74	6.31	19
23	9.60	6.12	20
24	9.32	6.64	27
25	9.32	6.70	27
Mean	10.44	7.31	27.24
S.D.	± 2.65	± 2.16	± 6.11
S.E.	± 0.53	± 0.43	± 1.22

APPENDIX 2 TABLE 3

Data regarding the birth weight and weight of colostrum ingested by the 25 calves in Group A.

Calf	Birth weight of calf (kg)	Weight of colostrum ingested (kg)	48-hour serum concentration of absorbed immunoglobulins (ZST units)
1	44.5	2.8	44
2	35.3	3.2	32
3	39.1	3.1	26
4	36.4	2.9	32
5	29.8	2.0	29
6	46.1	3.1	23
7	38.2	3.5	26
8	28.9	1.6	33
9	30.6	0.8	16
10	28.4	2.5	33
11	36.5	1.9	20
12	32.9	3.0	29
13	30.1	2.4	29
14	43.2	2.9	21
15	38.1	2.4	36
16	31.9	4.1	23
17	32.1	2.1	24
18	35.5	2.4	31
19	39.6	3.4	30
20	32.8	2.3	25
21	37.7	1.5	26
22	25.5	1.7	19
23	34.4	3.0	20
24	28.5	2.0	27
25	29.8	2.3	27
Mean	34.64	2,52	27.24
S.D.	± 5.34	± 0.73	± 6.11
S.E.	± 1.07	± 0.15	± 1.22

APPENDIX 2

TABLE 4

Data regarding the time required for newly born calves in Group A to suckle their dams to satiation and the total time from birth to completion of suckling.

Calf	Time required to suckle to satiation (mins)	Time from birth to completion of suckling (mins)	48 hour serum concentration of absorbed immunoglobulins (ZST units)
1	24	65	44
2	34	83	30
3	40	87	26
4	25	65	32
5	23	87	29
6	29	75	23
7	29	74	26
8	25	90	33
9	28	60	16
10	54	115	33
11	21	63	20
12	22	52	29
13	16	55	29
14	29	76	21
15	35	100	36
16	33	69	23
17	26	55	24
18	22	72	31
19	15	64	30
20	15	48	25
21	17	50	26
22	34	73	19
23	25	67	20
24	22	52	27
25	25	72	27
Mean	26.72	70.76	27.24
S.D.	± 8.59	± 16.30	± 6.11
S.E.	± 1.72	± 3.26	± 1.22

APPENDIX 2

TABLE 5

Data regarding the breed, sex and the serum concentrations of immunoglobulins of the 25 calves of Group B.

Calf No.	Breed	Sex	Serum immunoglobulin concentration (ZST units)		48 hour serum concentration of absorbed immunoglobulins (ZST units)
			Pre-colostral (15 minutes post-partum)	Post-colostral (48 hours post-partum)	
26	A	M	3	37	34
27	A	F	2	25	23
28	*A	F	2	37	35
29	*A	M	1	30	29
30	A	F	2	34	32
31	A	M	1	39	38
32	A	M	2	29	27
33	A	M	1	37	36
34	AxFrxFr	F	1	47	46
35	AxFr	F	1	22	21
36	A	M	0	25	25
37	AxFr	F	0	25	25
38	A	F	1	19	18
39	A	M	1	12	11
40	AxH	M	3	39	36
41	A	M	3	15	12
42	AxH	F	0	30	30
43	A	M	1	19	18
44	AxFr	F	1	23	22
45	A	F	2	27	25
46	AxSh	M	1	29	28
47	A	M	1	26	25
48	AxFr	F	0	36	36
49	A	M	3	43	40
50	A	M	2	42	40

A = Ayrshire; Fr = Friesian; H = Hereford;
Sh = Shorthorn.
* Twins.

APPENDIX 2

TABLE 6

Data regarding the total protein and the immunoglobulin concentrations of the colostral whey fed to the 25 calves of Group B under supervision immediately after birth and the final 48-serum concentration of absorbed immunoglobulin of these 25 calves.

Calf	Colostral whey total protein concentration (g/100 ml)	Whey immunoglobulin concentration (g/100 ml)	48 hour serum concentration of Absorbed immunoglobulins (ZST units)
26	5.64	3.67	34
27	10.32	7.35	23
28	9.40	6.66	29
30	13.22	8.85	32
31	15.40	10.84	38
32	8.64	5.21	27
33	8.40	5.23	36
34	12.00	7.03	46
35	9.28	6.23	21
36	16.00	10.86	43
37	9.94	6.67	25
38	6.80	4.74	18
39	6.72	4.30	11
40	9.00	5.94	36
41	9.28	6.17	12
42	12.24	8.58	30
43	7.80	4.79	18
44	7.26	4.65	22
45	9.92	7.13	25
46	9.80	6.83	28
47	7.54	5.42	25
48	9.80	7.70	36
49	11.98	8.25	40
50	12.00	7.91	40
Mean	9.91	6.71	29.20
S.D.	± 2.57	± 1.85	± 9.40
S.E.	± 0.51	± 0.37	± 1.88

APPENDIX 2

TABLE 7

Data regarding the birth weight and weight of colostrum ingested by the 25 calves of Group B under supervision immediately after birth.

Calf	Birth weight of calf (kg)	Weight of colostrum ingested (kg)	48 hour serum concentration of absorbed immunoglobulins (ZST units)
26	43.3	3.6	34
27	39.4	0.4	23
28	30.8	3.0	35
29	25.5	1.3	29
30	33.1	2.3	32
31	41.1	2.3	38
32	35.8	3.1	27
33	24.1	2.8	36
34	25.3	2.3	46
35	32.7	2.7	21
36	29.4	2.7	43
37	32.8	2.2	25
38	34.8	3.0	18
39	37.8	2.2	11
40	36.1	3.0	36
41	32.7	0.8	12
42	29.2	2.2	30
43	35.1	2.7	18
44	34.0	3.2	22
45	25.7	1.3	25
46	34.8	2.5	28
47	35.7	2.2	25
48	28.6	3.3	36
49	38.3	3.0	40
50	40.1	3.5	40
Mean	33.45	2.46	29.20
S.D.	± 5.19	± 0.81	± 9.40
S.E.	± 1.04	± 0.16	± 1.88

APPENDIX 2 TABLE 8

Weight of colostrum ingested under supervision at 12 hours post partum by calves in Group B and the total weight of colostrum ingested under supervision.

Calf	Weight of colostrum ingested at 12 hours post partum (kg)	Total weight of colostrum ingested under supervision (kg)
26	2.3	5.9
27	0.7	1.1
28	1.3	4.3
29	0.8	2.1
30	-	2.3
31	1.1	3.4
32	1.8	4.9
33	-	2.8
34	-	2.3
35	-	2.7
36	-	2.7
37	0.7	2.9
38	-	3.0
39	2.3	4.4
40	-	3.0
41	-	0.8
42	-	2.2
43	-	2.7
44	1.4	4.6
45	-	1.25
46	-	2.5
47	0.2	2.4
48	-	3.3
49	-	3.0
50	0.1	3.6
Mean	1.15	2.97
S.D.	± 0.75	± 1.18
S.E.	± 0.23	± 0.24

APPENDIX 2 TABLE 9

Data regarding the time required for newly born calves in Group B to suckle their dams to satiation and the total time from birth to completion of suckling of the initial feed post-partum.

Calf	Time required to suckle to satiation (mins)	Time from birth to completion of suckling (mins)	48 hour serum concentration of absorbed immunoglobulins (ZST units)
26	39	64	34
27	25	57	23
28	40	95	35
29	15	83	29
30	10	65	32
31	25	65	38
32	24	70	27
33	30	82	36
34	16	58	46
35	39	81	21
36	19	52	43
37	35	57	25
38	20	50	18
39	21	64	11
40	18	66	36
41	18	113	12
42	15	56	30
43	17	60	18
44	15	55	22
45	25	70	25
46	20	60	28
47	24	74	25
48	24	53	36
49	23	41	40
50	22	63	40
Mean	23.16	66.16	29.20
S.D.	± 8.02	± 15.41	± 9.40
S.E.	± 1.63	± 3.08	± 1.88

APPENDIX 2

TABLE 10

The absorption of colostral whey immunoglobulin by newborn calves:-

Intensity of management Group C

15 Calves aided to suckle to satiation immediately after birth

Calf No.	Sex	Breed	Birth Weight (kg)	Immunoglobulin concentration of colostral whey (g/100ml)	Weight of colostrum ingested (kg)	48-hour serum concentration of absorbed immuno-globulins (ZST units)
111	F	A	30.2	7.09	2.8	30
112	M	A	27.5	6.97	1.6	20
113	F	A	30.0	13.56	2.6	42
118	F	AxF	31.4	10.04	3.4	40
120	F	AxF.xF	25.7	6.32	1.5	17
125	M	F	30.7	8.77	1.4	32
129	F	AxAA	29.3	9.75	2.3	28
133	M	AxF.xF	36.6	5.18	1.9	23
134	F	FxH	31.4	9.32	1.9	26
135	M	AxH	40.3	11.49	3.2	44
141	M	AxF	32.5	7.13	1.5	16
142	F	FxH	30.1	8.69	2.7	38
144	F	AxF	27.1	6.42	2.1	11
147	M	AxF	36.6	9.52	2.7	28
148	F	AxH	32.3	7.16	2.1	27
Mean			31.45	8.49	2.25	28.13
S.D.			± 3.89	± 2.21	± 0.63	± 9.86

APPENDIX 2

TABLE 11

The absorption of colostral whey immunoglobulin by newborn calves :-

Intensity of management Group D

15 Calves allowed to remain with their dams for 48 hours - No interference

Calf No.	Sex	Breed	Birth Weight (kg)	Immunoglobulin concentration of colostral whey (g/100ml)	Weight of colostrum ingested (kg)	48-hour serum concentration of absorbed immuno-globulins (ZST units)
107	F	AxCh	27.3	9.78	ND**	49
*109	M	F	34.1	8.29	ND	29
110	F	A	31.4	5.05	ND	14
117	F	AxCh	41.6	7.31	ND	27
121	M	A	39.8	11.58	ND	24
122	M	AxH	42.0	10.40	ND	35
123	M	AxF.xF	36.8	4.95	ND	1
124	F	AxSh	34.0	9.48	ND	24
130	F	AxAA	34.6	11.57	ND	22
132	M	FxAA	29.7	9.38	ND	23
137	F	AxH	35.4	5.18	ND	24
138	F	FxH	42.7	6.12	ND	13
139	M	F	34.2	9.42	ND	6
143	M	A	37.0	6.19	ND	15
146	F	AxF.xF	35.5	8.23	ND	27
Mean			35.74	8.20		22.20
S.D.			± 4.45	± 2.29		± 11.65

** ND - Not determined.

* Cow treated for mastitis - Clots in all four quarters.

APPENDIX 2

TABLE 12

The absorption of colostral whey immunoglobulin by newborn calves :-

Intensity of management Group E

15 Calves fed 1700ml of their own dam's colostrum at 8 hours post-partum

Calf No.	Sex	Breed	Birth Weight (kg)	Immunoglobulin concentration of colostral whey (g/100ml)	Volume of colostrum ingested (ml)	48-hour serum concentration of absorbed immuno-globulins (ZST units)
108	M	A	27.5	3.58	1700 ml	10
114	M	A	31.8	8.59	1700 ml	27
115	M	A	32.9	1.93	1700 ml	2
116	F	A	39.3	6.46	1700 ml	17
119	M	AxF.xF	24.8	6.32	1700 ml	15
126	M	F	29.5	6.18	1700 ml	23
127	F	AxH	29.1	9.55	1700 ml	22
128	F	AxH	35.6	10.96	1700 ml	17
131	F	A	27.2	6.0	1700 ml	18
136	M	FxH	38.9	10.55	1700 ml	19
140	M	F	32.7	7.12	1700 ml	12
145	M	AxF.xF	28.8	8.33	1700 ml	26
149	M	A	31.8	10.23	1700 ml	12
150	M	AxF.xF	33.2	2.30	1700 ml	3
151	M	F	28.4	11.50	1700 ml	2
Mean			31.43	7.30		15.00
S.D.			± 4.18	± 3.05		± 8.18

APPENDIX 2

TABLE 13

Individual data for 100 calves assisted to suckle colostrum to satiation once only immediately after birth:- Breeds

43 Ayrshire calves

Calf No.	Birth weight (kg)	Weight of colostrum ingested (kg)	Immunoglobulin concentration of colostral whey consumed (g/100 ml)	48-hour serum concentration of absorbed immunoglobulins (ZST units)
2	35.3	3.2	9.80	32
3	39.1	3.1	6.23	26
7	38.2	3.5	6.78	26
8	28.9	1.6	8.31	33
9	30.6	0.8	5.09	16
11	36.5	1.9	7.82	20
13	30.1	2.4	8.31	29
14	43.2	2.9	6.45	21
16	31.9	4.1	5.93	23
20	32.8	2.3	5.26	25
22	25.5	1.7	6.31	19
24	28.5	2.0	6.64	27
52	24.6	2.7	8.39	35
53	34.2	2.5	10.12	40
57	32.6	2.2	7.84	18
62	32.9	1.7	4.41	13
63	25.9	1.7	8.80	25
64	31.5	2.0	8.80	21
65	26.6	2.2	7.83	35
68	36.6	1.6	7.55	23
69	40.5	2.8	8.29	30
70	27.9	1.9	8.01	26
72	38.2	3.2	5.88	23
74	27.0	2.8	7.94	42
77	33.2	2.3	9.74	34
78	32.3	2.1	7.53	28
84	29.7	1.7	7.54	39

APPENDIX 2

TABLE 13 (Cont'd)

Ayrshire calves (cont'd)

Calf No.	Birth weight (kg)	Weight of colostrum ingested (kg)	Immunoglobulin concentration of colostral whey consumed (g/100 ml)	48-hour serum concentration of absorbed immunoglobulins (ZST units)
89	29.0	1.8	12.51	45
91	26.0	2.6	10.30	24
95	22.9	1.4	10.88	36
96	30.5	2.3	7.41	23
97	31.5	2.2	7.41	19
98	34.9	1.8	7.49	20
103	33.9	1.9	6.90	27
104	18.1	1.0	4.02	16
106	26.1	1.9	11.04	38
111	30.2	2.8	7.09	30
112	27.5	1.6	6.97	20
113	30.0	2.6	13.56	42
155	31.6	2.6	10.08	29
156	33.3	2.5	1.58	5
158	35.2	2.5	4.93	23
161	29.8	1.8	8.57	31
Mean	31.27	2.24	7.73	26.91
S.D.	± 4.92	± 0.65	± 2.23	± 8.48
S.E.	± 0.75	± 0.09	± 0.34	± 1.29

APPENDIX 2

TABLE 13 (Cont'd)

24 AyrshirexFriesian calves

Calf No.	Birth weight (kg)	Weight of colostrum ingested (kg)	Immunoglobulin concentration of colostral whey consumed (g/100 ml)	48-hour serum concentration of absorbed immunoglobulins (ZST units)
4	36.4	2.9	5.83	32
6	46.1	3.1	6.09	23
10	28.4	2.5	8.99	33
12	32.9	3.0	6.35	29
15	38.1	2.4	14.22	36
19	39.6	3.4	8.04	30
23	34.4	3.0	6.13	20
54	36.3	2.8	12.16	53
55	34.6	2.2	7.79	33
58	34.8	1.5	6.30	22
61	41.4	2.4	6.21	23
66	30.5	1.4	6.49	14
67	31.8	2.4	1.87	4
75	44.7	3.8	4.02	19
76	32.7	2.4	6.30	35
81	23.5	2.3	6.53	38
82	32.2	2.5	4.32	23
118	31.4	3.4	10.04	40
141	32.5	1.5	7.13	16
144	27.0	2.1	6.42	11
147	36.6	2.7	9.52	28
157	31.0	1.5	11.44	29
159	30.9	2.3	8.49	26
164	35.8	3.5	6.27	25
Mean	34.32	2.54	7.37	26.75
S.D.	± 5.21	± 0.66	± 2.70	± 10.41
S.E.	± 1.06	± 0.13	± 0.55	± 2.12

APPENDIX 2

TABLE 13 (Cont'd)

Calf No.	Birth weight (kg)	Weight of colostrum ingested (kg)	Immunoglobulin concentration of colostral whey consumed (g/100 ml)	48-hour serum concentration of absorbed immunoglobulins (ZST units)
7 AyrshirexFriesianxFriesian calves				
21	37.7	1.5	8.83	26
73	29.8	2.1	1.87	5
83	32.5	2.3	8.00	43
120	25.7	1.5	6.32	17
133	36.6	1.9	5.18	23
204	29.8	2.3	10.61	27
154	34.5	2.4	7.29	24
Mean	32.37	2.00	± 6.87	± 23.57
S.D.	± 4.25	± 0.38	± 2.81	± 11.43
S.E.	± 1.61	± 0.14	± 1.06	± 4.32
9 Friesian calves				
1	44.5	2.8	11.41	44
5	29.9	2.0	6.19	29
17	32.1	2.1	4.08	24
51	39.1	3.3	8.27	29
56	27.6	1.6	5.48	29
60	42.0	3.3	6.85	27
125	30.6	1.4	8.77	32
160	36.3	2.3	12.24	38
162	38.6	2.5	10.86	35
Mean	35.63	2.37	8.24	31.88
S.D.	± 5.87	± 0.69	± 2.83	± 6.17
S.E.	± 1.96	± 0.23	± 0.94	± 2.06

APPENDIX 2

TABLE 13 (Cont'd)

Calf No.	Birth weight (kg)	Weight of colostrum ingested (kg)	Immunoglobulin concentration of colostral whey consumed (g/100 ml)	48-hour serum concentration of absorbed immunoglobulins (ZST units)
10 Hereford cross calves				
18	35.5	2.4	7.07	31
59	28.3	1.7	3.67	19
85	34.0	1.7	9.09	31
134	31.4	1.9	9.32	26
135	40.3	3.2	11.49	44
142	30.1	2.8	8.69	38
148	32.3	2.1	7.16	27
163	33.2	2.0	10.88	31
165	36.8	3.2	8.98	30
79	32.4	1.3	2.48	8
Mean	33.43	2.23	7.88	28.50
S.D.	± 3.45	± 0.65	± 2.90	± 9.83
S.E.	± 1.09	± 0.20	± 0.92	± 3.11
5 Other crosses				
25	29.8	2.3	6.70	27
71	37.5	2.4	7.51	26
80	39.3	1.83	6.32	26
129	29.3	2.3	9.75	28
166	30.9	1.8	4.53	18
Mean	33.36	2.12	6.96	25.0
S.D.	± 4.68	± 0.29	± 1.90	± 4.00
S.E.	± 2.09	± 0.13	± 0.85	± 1.79

APPENDIX 2

TABLE 13 (Cont'd)

Calf No.	Birth weight (kg)	Weight of colostrum ingested (kg)	Immunoglobulin concentration of colostral whey consumed (g/100 ml)	48-hour serum concentration of absorbed immunoglobulins (ZST units)
2 Jersey calves				
152	20.5	1.4	11.99	35
153	22.50	3.0	5.01	21
Mean	21.5	2.20	8.50	28
TOTAL 100 CALVES				
Mean	32.59	2.29	7.61	27.17
S.D.	± 5.22	± 0.63	± 2.49	± 8.97
S.E.	0.52	± 0.06	± 0.25	± 0.89

APPENDIX 2

TABLE 14

Serum immunoglobulin (Ig) concentration of 115 "market" calves.

Calf No.	Serum Ig conc. (ZST units)	Calf No.	Serum Ig conc. (ZST units)	Calf No.	Serum Ig conc. (ZST units)
Paisley 22/10/73					
399	5	403	2	407	14
400	42	404	2	408	0
401	16	405	32	409	8
402	9	406	6	410	1
Paisley 7/1/74					
189	2	196	12	204	18
190	38	197	2	205	6
191	6	198	8	232	10
192	19	199	6	233	9
193	6	200	29	234	18
194	25	201	11	235	12
195	12	202	19	236	23
		203	3	237	16
Kilmarnock 8/10/74					
121	5	125	19	129	42
122	30	126	22	130	37
123	17	127	9	131	50
124	22	128	31	132	33
Paisley 7/1/75					
141	11	145	5	149	2
142	7	146	39	150	23
143	2	147	3	151	25
144	21	148	0		

APPENDIX 2

TABLE 14 (Cont'd)

Calf No.	Serum Ig conc. (ZST units)	Calf No.	Serum Ig conc. (ZST units)	Calf No.	Serum Ig conc. (ZST units)
Ayr 8/1/75					
152	10	157	3	162	19
153	21	158	17	163	19
154	16	159	25	164	0
155	13	160	26	165	27
156	1	161	3	166	22
Paisley 14/2/75					
134	19	136	17	137	5
135	7				
Paisley 7/10/75					
299	17	303	29	307	44
300	36	304	29	308	15
301	19	305	32	309	43
302	15	306	2	310	42
Paisley 8/1/76					
320	16	323	21	325	3
321	18	324	23	326	9
322	21				
Ayr 20/1/76					
273	8	277	7	283	3
274	8	278	8	284	6
275	1	279	11	285	7
276	2	280	3	286	9
275	14	281	6	287	5
276	14	282	3	288	4

APPENDIX 3

CALF WEIGHTS

TABLE 1

GROUP 1

Calf No.	1	2	3	4	5	6	7	8	9	10	11
Date of Birth	22/2/71	25/2/71	26/2/71	26/2/71	1/3/71	1/3/71	3/3/71	4/3/71	6/3/71	6/3/71	7/3/71
Birth Weight	44.5	35.3	39.1	36.4	29.9	46.1	38.2	28.9	30.6	28.4	36.5
Date/Weight (kg)											
26/2/71	47.3	35.9	-	-	-	-	-	-	-	-	-
5/3/71	53.6	41.4	42.3	38.6	32.3	48.2	40.0	29.5	31.8	-	-
12/3/71	60.9	46.4	46.8	44.1	40.5	52.7	45.5	34.5	36.4	33.2	37.3
18/3/71	62.7	48.2	47.7	47.3	40.9	56.4	49.1	37.7	40.0	39.1	42.3
25/3/71	64.5	48.2	50.9	49.1	42.7	56.8	53.6	45.0	43.2	42.7	43.6
1/4/71*	72.7	50.9	53.6	53.6	50.0	60.0	58.2	45.5	46.4	49.1	43.6
8/4/71	71.8	52.3	53.6	54.5	50.0	60.0	58.2	47.3	48.2	49.1	46.8
15/4/71	78.2	59.1	60.0	60.0	55.0	65.5	62.7	50.9	54.5	52.7	51.4
22/4/71	86.4	63.6	63.6	64.5	58.2	70.5	68.2	56.4	59.1	58.2	58.2
29/4/71	95.0	67.3	69.1	70.5	64.5	75.5	75.5	58.6	64.5	61.8	63.2
6/5/71	102.7	73.2	75.0	76.4	70.9	83.6	79.1	65.0	70.0	68.6	68.2
13/5/71	111.8	78.6	81.8	83.6	79.1	90.9	84.5	70.5	75.9	73.2	73.6
20/5/71	116.4	82.7	89.5	89.1	86.4	95.5	88.6	75.0	84.1	76.8	78.2
27/5/71	128.2	89.1	95.5	96.4	94.5	104.5	96.4	79.5	90.9	84.1	87.3
3/6/71	113.6	95.5	99.1	100.0	97.3	110.0	101.4	86.8	95.5	88.2	90.9
Wt.at 28 days	63.6	48.2	51.4	50.0	44.5	60.5	58.2	45.5	46.4	49.1	45.0
Wt.at weaning	72.7	50.1	53.7	53.7	50.0	60.2	58.2	45.6	46.4	49.1	43.8
LWG+ birth to weaning	28.2	14.8	14.6	17.3	20.1	14.1	20.0	16.7	15.8	20.7	7.3
Days on milk	39	35	34	34	32	32	30	29	29	26	25
Daily LWG-kg/day Birth to weaning	0.72	0.42	0.43	0.51	0.63	0.44	0.67	0.58	0.54	0.80	0.29
Wt.at 12 wks.	114.4	82.7	90.5	90.1	91.0	100.6	95.3	79.5	90.9	85.3	88.8

+ Live weight gain

* All calves weaned 3/4/71

GROUP 2

Calf No.	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Date of Birth	23/3/71	25/3/71	25/3/71	27/3/71	27/3/71	28/3/71	28/3/71	31/3/71	31/3/71	31/3/71	31/3/71	1/4/71	1/4/71	3/4/71
Birth Weight	32.9	30.1	43.2	38.1	31.9	32.1	35.5	39.6	32.8	37.7	25.5	34.4	28.5	29.8
Date/Weight (kg)														
25/3/71	32.7	-	-	-	-	-	-	-	-	-	-	-	-	-
1/4/71	32.7	32.7	46.4	37.3	35.0	32.7	36.4	40.5	30.9	36.8	24.5	36.4	-	-
8/4/71	38.2	36.8	53.6	45.0	38.6	37.3	41.8	40.9	37.3	38.2	27.3	37.3	31.8	33.2
15/4/71	44.5	37.3	55.9	50.5	41.8	40.9	44.5	41.4	36.4	38.2	29.1	37.7	32.3	33.2
22/4/71	50.0	39.1	60.9	55.5	45.0	43.6	52.7	42.7	40.0	43.2	33.2	40.9	35.5	38.2
29/4/71	51.8	41.8	64.5	59.1	48.2	46.4	54.5	46.4	41.8	47.7	37.3	40.9	36.4	41.4
6/5/71*	56.4*	48.2*	72.3*	65.5*	50.9*	49.5*	58.2*	48.2*	49.1*	52.7*	37.7*	45.9*	37.3*	42.7*
13/5/71	56.8	52.7	72.7	64.5	52.7	51.9	62.3	51.4	45.5	54.1	40.0	48.6	38.6	42.3
20/5/71	60.0	55.0	78.2	66.4	54.5	53.6	65.0	54.5	47.3	57.7	40.9	49.1	39.5	44.5
27/5/71	65.9	58.2	84.1	73.6	60.0	60.0	69.1	59.1	52.3	62.7	46.4	54.5	43.6	48.2
3/6/71	72.7	64.1	93.6	81.4	65.9	67.3	78.2	64.1	56.8	66.8	50.9	58.6	48.2	52.7
10/6/71	79.1	68.2	102.7	87.7	70.5	71.8	82.7	70.5	61.8	73.6	53.6	64.1	50.0	57.7
17/6/71	83.2	72.3	110.9	95.0	77.3	79.1	90.9	76.4	67.3	79.1	56.8	71.4	55.5	62.7
24/6/71	90.0	79.1	122.3	103.2	82.3	87.3	103.2	85.0	73.6	89.1	67.3	78.6	60.0	68.2
1/7/71	ND	ND	125.0	108.2	89.1	93.2	108.2	92.7	78.2	93.2	73.2	85.9	66.4	70.9
Wt.at 28 days	48.6	39.1	60.9	56.4	45.9	45.0	53.6	44.1	41.4	46.8	36.8	40.9	36.4	41.8
Wt.at weaning	56.4	48.2	72.3	65.5	50.9	49.5	58.2	48.2	49.1	52.7	37.7	45.9	37.3	42.7
LWG birth to weaning	23.5	18.1	29.1	27.4	19.0	17.4	22.7	8.6	16.3	15.0	12.2	11.5	8.8	12.9
Days on milk	44	42	42	40	40	39	39	39	36	36	36	35	35	33
Daily LWG-kg/day Birth to weaning	0.53	0.43	0.69	0.68	0.47	0.45	0.58	0.22	0.45	0.42	0.34	0.33	0.25	0.39
Wt.at 12 wks.	82.0	72.3	110.9	97.3	78.7	82.6	96.2	80.0	72.7	87.6	65.8	78.6	60.0	68.9

* All calves weaned 6/5/71

APPENDIX 3

TABLE 2

GROUP 3

Calf No.	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Date of Birth	11/6/71	11/6/71	15/6/71	15/6/71	17/6/71	18/6/71	18/6/71	19/6/71	20/6/71	22/6/71	22/6/71	24/6/71	25/6/71	26/6/71
Birth Weight(kg)	43.3	39.4	30.8	25.5	33.1	41.1	35.8	24.1	25.3	32.7	29.4	32.8	34.8	37.8
Date/Weight (kg)														
17/6/71	51.4	41.8	33.2	28.6	-	-	-	-	-	-	-	-	-	-
21/6/71	58.6	46.4	36.8	31.8	38.6	47.3	41.8	25.9	30.9	33.6	32.7			
1/7/71	60.9	49.1	38.2	33.2	40.5	47.7	45.5	30.9	32.7	39.1	35.5	36.8	38.6	44.5
8/7/71	65.9	50.9	44.1	37.3	44.1	49.5	46.8	32.3	36.4	40.0	34.5	36.4	41.4	42.7
15/7/71	69.1	52.7	49.1	39.1	49.1	51.8	42.3	35.0	38.2	41.8	38.2	40.0	40.0	44.1
22/7/71	74.5	50.5	51.4	47.7	53.2	53.6	51.8	39.1	44.1	45.0	42.7	42.7	45.0	47.7
29/7/71	78.6	55.0	57.3	52.3	57.7	58.2	55.9	45.9	45.5	47.3	43.2	44.5	47.3	52.7
5/8/71	85.9*	60.9*	63.2*	58.6*	62.7	65.0	58.2	49.5	49.5	51.8	50.5	49.5	50.0	55.5
12/8/71	91.4	63.6	63.6	59.1	65.0*	69.1*	61.8*	57.7*	55.5*	59.5	54.5	54.1	57.7	58.6
19/8/71	101.8	72.3	75.0	67.3	70.5	73.2	67.3	58.2	56.8	61.8*	59.1*	60.0*	63.6*	63.6*
26/8/71	109.5	77.3	80.5	77.3	78.6	84.1	74.5	62.7	61.4	67.7	67.7	68.2	67.7	67.3
2/9/71	119.1	84.1	90.0	85.5	86.8	93.6	79.5	70.5	66.8	78.6	74.1	76.4	77.3	66.4
9/9/71	126.8	88.6	97.3	90.9	93.2	100.0	82.7	77.3	72.3	83.6	80.9	83.2	83.2	70.9
16/9/71	136.4	94.5	100.9	97.3	100.0	110.0	91.8	85.5	77.3	92.7	88.6	88.2	89.1	81.4
23/9/71	141.8	102.7	109.1	104.1	107.3	114.5	100.0	91.8	ND	ND	94.5	ND	96.4	90.5
Wt.at 28 days	66.8	51.3	48.2	38.7	50.5	52.3	48.6	36.8	41.4	44.5	42.2	43.2	45.9	50.0
Wt.at weaning	85.9	60.9	63.2	58.6	65.0	69.1	61.8	57.7	55.5	61.8	59.1	60.0	63.6	63.6
LWG birth to weaning	42.6	21.5	32.4	33.1	31.9	28.0	26.0	33.6	30.2	29.1	29.7	27.2	28.8	25.8
Days on milk	55	55	51	51	56	55	55	54	53	58	58	56	55	54
Daily LWG-kg/day Birth to weaning	0.77	0.39	0.63	0.64	0.57	0.51	0.47	0.62	0.57	0.50	0.51	0.49	0.52	0.48
Wt.at 12 wks.	120.2	84.7	95.2	89.4	93.2	94.5	84.0	79.6	74.4	90.1	86.4	88.2	90.1	84.0

* Each calf weaned when approximately 8 weeks old

GROUP 4

Calf No.	40	41	42	43	44	45	46	47	48	49	50
Date of Birth	6/7/71	8/7/71	11/7/71	11/7/71	11/7/71	13/7/71	20/7/71	22/7/71	22/7/71	25/7/71	25/7/71
Birth Weight(kg)	36.1	32.7	29.2	35.1	34.0	25.7	34.8	35.7	28.6	38.3	40.1
Date/Weight (kg)											
15/7/71	43.2	33.6	32.7	36.4	35.5	-	-	-	-	-	-
22/7/71	46.8	36.8	35.9	39.5	36.8	26.4	35.5	-	-	-	-
29/7/71	49.1	41.4	39.1	44.1	40.0	27.3	37.3	37.3	31.8	41.8	45.0
5/8/71	52.7	47.7	45.5	47.7	45.0	30.0	43.2	41.4	33.2	42.3	45.5
12/8/71	55.5	50.0	48.6	50.0	47.7	32.3	44.5	41.4	38.2	44.5	50.0
19/8/71	60.0	53.6	55.0	57.7	52.3	36.4	50.0	48.6	41.4	45.0	55.0
26/8/71	65.9	60.5	59.5	63.2	58.6	39.5	54.5	52.7	47.7	49.5	57.3
2/9/71	71.8*	66.8*	65.0*	72.7*	63.6*	45.0*	61.4	58.6	56.4	52.7	59.1
9/9/71	77.3	70.0	70.5	75.0	69.1	49.5	69.5	65.0	56.4	56.8	63.6
16/9/71	81.4	76.4	77.3	83.2	75.9	55.0	71.8*	67.7*	61.8*	58.2*	69.5*
23/9/71	91.8	79.5	84.1	90.9	80.5	60.0	74.5	72.3	68.2	60.9	73.2
30/9/71	90.9	87.3	89.1	96.8	87.3	65.9	81.8	81.8	74.1	63.6	78.6
7/10/71	95.5	93.6	94.5	105.5	93.2	71.8	88.2	86.4	79.1	69.1	86.4
14/10/71	ND	ND	100.0	110.9	95.5	75.5	90.9	88.6	83.6	63.6	89.1
21/10/71	ND	ND	106.4	117.3	97.7	81.8	97.3	102.7	90.0	70.0	97.7
Wt.at 28 days	52.3	48.2	47.3	49.1	46.4	31.8	49.1	49.1	42.3	47.7	56.4
Wt.at weaning	71.8	66.8	65.0	72.7	63.6	45.0	71.8	67.7	61.8	58.2	69.5
LWG birth to weaning	35.7	34.1	35.8	37.6	29.6	19.3	37.0	32.0	33.2	19.9	29.4
Days on milk	58	56	53	53	53	51	58	56	56	53	53
Daily LWG-kg/day Birth to weaning	0.62	0.61	0.68	0.71	0.56	0.38	0.64	0.57	0.59	0.38	0.55
Wt.at 12 wks.	91.4	87.3	91.4	100.5	89.8	70.1	90.1	88.6	83.6	68.2	92.8

* Each calf weaned when approximately 8 weeks old

APPENDIX 3

TABLE 3

GROUP 5

Calf No.	51	52	53	54	55.	56	57
Date of Birth	24/8/71	25/8/71	25/8/71	26/8/71	27/8/71	30/8/71	31/8/71
Birth Weight (kg)	39.1	24.6	34.2	36.3	34.6	27.6	32.6
Date/Weight (kg)							
26/8/71	40.5	25.0	34.1	-	-	-	-
2/9/71	48.2	30.5	42.7	41.8	39.5	39.5	35.5
9/9/71	57.3	35.0	46.4	46.4	41.4	35.0	41.8
16/9/71	62.3	39.1	46.8	54.5	43.2	37.7	43.6
23/9/71	67.3	41.4	50.9	57.7	46.4	40.0	44.5
30/9/71	72.3	46.8	56.4	60.9	49.1	43.6	46.4
7/10/71	78.2	46.8	59.1	64.5	52.3	48.6	49.1
14/10/71 *	89.1*	51.8*	62.7*	72.3*	55.5*	53.6*	45.5*
21/10/71	93.2	51.4	62.3	72.3	51.8	57.7	45.5
28/10/71	97.3	55.0	68.2	71.8	54.5	58.6	50.5
4/11/71	105.5	60.9	71.8	77.3	56.8	65.1	56.8
11/11/71	110.5	65.0	75.5	83.2	61.8	70.9	57.7
18/11/71	116.4	69.5	77.3	88.2	66.8	73.6	61.4
25/11/71	128.6	75.9	82.3	95.5	70.5	80.9	66.8
Wt.at 28 days	65.9	38.6	46.8	57.7	46.8	41.8	45.5
Wt.at weaning	89.1	51.8	62.7	72.3	55.5	53.6	45.5
LWG birth to weaning	50.0	27.2	28.5	36.0	20.9	26.0	12.9
Days on milk	51	50	50	49	48	45	45
Daily LWG-kg/day Birth to weaning	0.98	0.54	0.57	0.73	0.44	0.58	0.28
Wt.at 12 wks.	114.7	68.9	77.0	88.2	67.3	77.8	63.4

* All calves weaned 14/10/71

GROUP 6

Calf No.	58	59	60	61	62	63	64	65	66	67
Date of Birth	12/11/71	15/11/71	17/11/71	20/11/71	22/11/71	22/11/71	22/11/71	28/11/71	28/11/71	28/11/71
Birth Weight (kg)	34.8	28.8	42.0	41.4	32.9	25.9	31.5	26.6	30.5	31.8
Date/Weight (kg)										
18/11/71	40.5	31.8	41.8	-	-	-	-	-	-	-
25/11/71	46.8	36.4	49.1	44.5	32.7	26.4	31.4	-	-	-
2/12/71	49.5	38.2	50.0	46.8	33.2	27.3	34.5	28.2	30.5	32.7
9/12/71	54.1	40.9	51.4	48.6	34.5	29.1	38.2	30.9	28.2	34.5
16/12/71	60.9	42.7	56.4	50.0	37.7	31.8	41.4	34.1	30.5	39.1
23/12/71	66.8	50.0	56.8	53.2	40.5	33.6	45.0	35.0	31.8	40.9
29/12/71	70.9	51.4	59.5	56.4	44.1	38.2	50.0	40.0	33.6	44.5
6/1/72 *	76.8*	55.5*	65.9*	60.9*	47.3*	40.9*	53.6*	41.8*	35.5*	47.3*
13/1/72	82.7	59.5	68.6	70.0	54.5	47.3	60.0	46.8	40.9	46.8
20/1/72	86.4	63.2	73.6	73.6	57.7	51.8	64.5	50.0	43.6	52.7
27/1/72	93.2	69.1	80.9	80.0	64.5	59.1	72.7	54.5	48.2	56.8
3/2/72	101.8	77.3	88.2	89.1	73.6	66.4	80.9	56.4	54.5	63.6
10/2/72	109.5	83.2	95.5	95.5	78.6	71.4	88.2	60.0	58.2	68.6
17/2/72	120.5	91.4	104.1	105.0	86.8	78.2	94.5	65.0	64.5	73.2
24/2/72	125.9	94.5	110.0	89.5	89.5	83.6	96.4	65.5	66.4	77.3
Wt.at 28 days	56.1	42.3	56.3	51.4	39.5	33.2	44.1	37.7	32.8	43.2
Wt.at weaning	76.8	55.5	65.9	60.9	47.3	40.9	53.6	41.8	35.5	47.3
LWG birth to weaning	42.0	26.7	23.9	19.5	14.4	15.0	22.1	15.2	5.0	15.5
Days on milk	55	52	50	47	45	45	45	39	39	39
Daily LWG-kg/day Birth to weaning	0.76	0.51	0.49	0.41	0.32	0.33	0.49	0.39	0.13	0.40
Wt.at 12 wks.	102.9	80.6	94.4	98.2	83.3	75.3	91.8	65.5	65.3	75.0

* All calves weaned 6/1/72

APPENDIX 3

TABLE 4

GROUP 7

Calf No.	68	69	70	71	72	73	74	75	76
Date of Birth	16/12/71	20/12/71	22/12/71	23/12/71	23/12/71	24/12/71	25/12/71	26/12/71	27/12/71
Birth Weight (kg)	36.6	40.5	27.9	37.5	38.2	29.8	27.0	44.7	32.7
Date/Weight (kg)									
16/12/71	36.4	-	-	-	-	-	-	-	-
23/12/71	39.5	41.8	29.5	37.3	-	Died	-	-	-
29/12/71	40.9	42.3	31.8	35.9	37.7	-	29.1	45.5	33.2
6/1/72	46.8	45.9	32.7	39.1	40.5	-	28.2	42.7	33.6
13/1/72	50.0	45.5	37.7	40.9	42.7	-	30.0	44.5	35.5
20/1/72	54.5	49.1	42.3	44.5	48.6	-	32.7	46.8	36.8
27/1/72	55.5	53.6	45.5	49.5	51.8	-	33.6	50.0	39.1
3/2/72	62.3	56.4	51.4	54.5	59.5	-	34.5	58.2	45.5
10/2/72	64.5	60.5	55.5	56.4	61.4	-	36.8	60.5	45.5
17/2/72	69.5	64.5	57.7	62.3	63.6	-	40.0	65.0	49.5
24/2/72	73.6	65.5	62.7	65.5	68.6	-	40.9	67.7	52.7
2/3/72*	80.5*	72.7*	69.1*	72.7*	77.3*	-	46.4*	75.0*	55.9*
9/3/72	87.7	74.1	74.5	76.9	82.7	-	53.6	81.8	61.4
16/3/72	94.1	81.4	80.5	82.3	90.0	-	54.5	87.3	66.4
23/3/72	101.8	87.3	89.1	86.4	97.7	-	60.0	95.5	71.8
Wt.at 28 days	50.5	48.2	42.2	45.0	49.1	-	33.1	48.7	38.2
Wt.at weaning	62.3	56.4	51.4	54.5	59.5	-	34.5	58.2	45.5
LWG birth to weaning	25.7	15.9	23.5	17.0	21.3	-	7.5	13.5	12.8
Days on milk	49	45	43	42	42	-	40	39	39
Daily LWG-kg/day Birth to weaning	0.52	0.35	0.55	0.40	0.51	-	0.19	0.35	0.33
Wt.at 12 wks.	87.7	78.2	77.9	82.3	90.0	-	56.1	90.8	69.5

* All calves weaned 2/3/72

GROUP 8

Calf No.	82	83	84	85	86	87	88	89	90	91	92	93	94	95
Date of Birth	26/3/72	27/3/72	27/3/72	28/3/72	28/3/72	28/3/72	29/3/72	1/4/72	1/4/72	2/4/72	4/4/72	6/4/72	6/4/72	6/4/72
Birth Weight (kg)	32.2	32.5	29.6	34.0	25.5	30.0	34.3	29.0	27.7	26.0	31.2	30.0	29.6	22.9
Date/Weight (kg)														
30/3/72	34.5	34.5	32.7	36.8	28.2	34.5	36.8	-	-	-	-	-	-	-
6/4/72	38.6	38.2	34.5	40.0	30.9	34.5	35.5	29.1	31.4	28.6	30.9	30.9	29.5	22.9
13/4/72	44.1	37.3	35.0	42.7	32.7	37.7	37.3	28.2	34.1	30.5	33.2	34.5	31.8	23.8
20/4/72	50.9	39.5	38.2	45.5	36.8	41.8	40.0	31.8	36.4	34.5	37.3	35.5	34.1	24.7
27/4/72	55.9	45.5	40.0	48.6	42.3	46.4	47.7	34.1	40.0	38.2	37.8	37.7	37.3	28.8
4/5/72	61.4	47.7	41.8	50.0	46.8	50.9	52.7	35.5	44.5	39.1	41.4	39.1	40.9	33.8
11/5/72*	67.8	51.4	47.3	54.5	51.8	53.6	56.4	39.5	49.1	42.7	45.9	44.1	45.5	35.6
18/5/72	68.2	57.3	52.3	57.3	57.3	55.9	57.7	42.3	50.9	46.4	51.8	46.4	46.4	39.7
25/5/72	74.5	63.6	57.7	62.7	67.3	60.9	62.7	46.4	56.8	54.1	59.1	51.8	51.8	46.5
1/6/72	78.6	70.0	60.9	66.8	71.4	66.8	71.8	51.4	60.0	58.6	65.0	56.4	56.4	52.4
8/6/72	85.5	76.4	65.5	75.0	75.5	72.3	77.3	56.4	66.4	64.5	71.8	60.0	61.8	58.8
15/6/72	92.7	83.6	72.3	79.1	85.5	76.4	84.6	84.5	70.5	71.4	75.9	65.0	66.8	64.7
22/6/72	99.5	90.5	78.6	85.5	89.5	83.2	92.7	68.6	76.8	76.8	84.1	69.5	74.1	70.6
29/6/72	106.8	96.8	85.9	92.3	95.5	90.0	100.9	73.6	82.7	83.6	89.1	73.2	80.0	78.2
Wt.at 28 days	52.7	42.9	39.2	47.6	39.9	45.1	46.6	34.4	41.3	38.4	39.2	38.7	40.8	33.8
Wt.at weaning	71.4	56.4	51.4	57.3	56.4	58.2	59.1	41.8	52.7	46.8	52.7	46.4	49.1	44.6
LWG birth to weaning	39.2	23.9	21.8	23.3	30.9	28.2	24.2	12.8	25.0	20.8	21.5	16.4	19.5	21.7
Days on milk	51	50	50	49	49	49	48	45	45	45	44	42	40	40
Daily LWG-kg/day Birth to weaning	0.77	0.48	0.44	0.48	0.63	0.58	0.52	0.28	0.56	0.46	0.49	0.39	0.49	0.54
Wt.at 12 wks.	95.6	87.5	75.9	83.6	88.4	81.2	91.5	70.1	78.5	78.8	86.2	72.1	80.0	78.2

* All calves weaned 16/5/72

APPENDIX 3

TABLE. 5

GROUP 9

Calf No.	96	97	98	99	100	101	102	103	104	105	106
Date of Birth	1/7/72	1/7/72	3/7/72	7/7/72	5/7/72	8/7/72	8/7/72	9/7/72	14/7/72	16/7/72	17/7/72
Birth Weight (kg)	30.5	31.5	34.9	34.6	29.3	37.5	30.9	33.8	18.1	29.5	26.1
Date/Weight (kg)											
6/7/72	31.8	34.1	38.2	-	-	-	-	-	-	-	-
13/7/72	33.6	40.0	44.1	41.8	37.3	41.4	35.5	37.3	ND	-	-
20/7/72	33.2	42.3	51.4	46.8	41.8	42.7	42.3	40.9	ND	30.9	26.8
27/7/72	31.8	41.8	53.6	49.1	45.0	46.8	40.5	45.5	ND	31.8	27.3
3/8/72	37.3	46.4	58.6	53.6	48.2	50.9	45.5	50.9	25.9	38.6	28.6
10/8/72	41.8	48.6	56.4	56.4	50.9	52.3	47.7	50.9	ND	38.6	32.3
17/8/72	45.5	52.3	62.7	59.5	55.9	56.8	51.8	53.2	ND	45.5	34.5
24/8/72*	50.5	58.2	66.8	66.8	56.8	60.0	55.5	55.9	35.0	48.2	39.5
31/8/72	54.1	65.0	71.8	75.5	61.4	68.6	63.6	65.9	37.7	55.5	44.5
7/9/72	58.6	70.5	74.5	76.8	66.4	73.6	68.2	68.6	43.2	60.5	47.3
14/9/72	64.5	74.1	80.5	81.8	67.7	78.2	73.6	74.1	46.8	65.9	52.3
21/9/72	70.5	80.5	86.8	89.5	72.3	81.8	77.7	79.5	52.3	71.8	54.1
28/9/72	77.3	88.2	94.5	94.5	78.2	90.0	85.5	87.3	56.8	78.2	59.1
5/10/72	80.5	92.7	96.4	95.5	83.6	96.4	88.6	94.1	63.6	82.7	65.9
12/10/72	83.2	97.7	102.3	100.0	89.5	103.2	93.2	100.9	68.6	88.2	72.3
Wt.at 28 days	34.1	43.7	56.4	53.9	48.2	51.4	46.4	51.6	ND	42.7	33.6
Wt.at weaning	47.7	53.6	65.5	62.3	54.1	59.1	54.5	55.5	ND	49.1	37.3
LWG birth to weaning	17.2	22.1	30.6	27.1	24.8	21.6	23.6	21.6	ND	19.6	11.2
Days on milk	51	51	49	45	47	44	44	43	ND	36	35
Daily LWG-kg/day Birth to weaning	0.34	0.43	0.62	0.60	0.53	0.49	0.54	0.50	ND	0.54	0.32
Wt.at 12 wks.	72.4	82.3	91.2	94.5	77.3	91.8	87.3	90.2	64.4	85.0	69.5

* All calves weaned 21/8/72

GROUP 10

Calf No.	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121
Date of Birth	17/12/72	20/12/72	20/12/72	24/12/72	25/12/72	27/12/72	27/12/72	29/12/72	31/12/72	1/1/72	2/1/73	3/1/73	7/1/73	7/1/73	7/1/73
Birth Weight (kg)	27.3	27.5	34.1	31.4	30.2	27.5	30.0	31.8	32.9	39.3	41.6	31.4	24.8	25.7	39.8
Date/Weight (kg)															
22/12/72	27.7	26.8	34.5	-	-	-	-	-	-	-	-	-	-	-	-
29/12/72	28.6	28.2	35.9	32.7	30.0	27.3	30.5	-	-	-	-	-	-	-	-
5/1/73	31.4	30.0	37.7	34.5	30.9	28.6	31.4	32.7	27.3	37.3	42.7	33.6	-	-	-
12/1/73	34.1	33.6	37.7	36.8	33.6	28.6	33.2	34.6	Died	36.8	41.4	33.2	24.6	25.5	39.6
19/1/73	39.1	35.9	39.1	43.2	36.4	33.6	34.1	40.5	-	34.6	45.5	35.0	24.6	24.6	38.2
26/1/73	42.7	36.8	39.1	45.9	38.6	30.9	35.5	43.2	-	34.1	46.8	34.1	Died	24.1	38.6
2/2/73	47.3	38.6	41.4	49.1	42.7	Died	39.1	50.0	-	37.3	50.0	Died	-	Died	45.5
9/2/73	50.5	41.4	43.2	54.6	45.5	-	42.3	55.5	-	38.2	55.5	-	-	-	48.2
16/2/73*	54.1	43.6	45.0	60.9	50.5	-	46.8	56.8	-	40.5	51.4	-	-	-	51.4
23/2/73	58.6	47.7	48.6	67.3	55.5	-	51.4	64.1	-	41.4	55.5	-	-	-	55.0
2/3/73	61.4	51.4	51.8	72.3	58.2	-	55.0	68.2	-	45.5	59.1	-	-	-	60.0
9/3/73	68.6	57.3	57.7	82.3	64.5	-	59.5	75.9	-	51.4	65.5	-	-	-	66.4
16/3/73	74.1	60.9	62.7	90.0	68.6	-	66.4	81.4	-	55.5	70.0	-	-	-	72.3
23/3/73	76.4	66.4	65.9	94.5	73.2	-	70.5	86.4	-	59.1	74.5	-	-	-	78.2
30/3/73	ND	ND	ND	ND	ND	-	ND	96.4	-	66.4	84.5	-	-	-	86.4
6/4/73	ND	ND	ND	ND	ND	-	ND	103.6	-	72.7	90.9	-	-	-	95.0
Wt.at 28 days	35.5	35.3	38.7	44.0	37.3	31.6	35.0	43.2	-	35.5	48.6	-	-	-	46.2
Wt.at weaning	51.8	42.7	44.1	57.3	48.2	-	44.5	56.8	-	39.5	56.4	-	-	-	50.0
LWG birth to weaning	24.5	15.2	10.0	25.9	18.0	-	14.5	25.0	-	0.2	14.8	-	-	-	10.2
Days on milk	56	53	53	49	48	-	46	44	-	41	40	-	-	-	35
Daily LWG-kg/day Birth to weaning	0.44	0.29	0.19	0.53	0.38	-	0.32	0.57	-	0.005	0.37	-	-	-	0.29
Wt.at 12 wks.	70.2	59.9	61.3	91.3	70.6	-	69.3	86.4	-	62.2	81.6	-	-	-	88.8

* All calves weaned 11/2/73

APPENDIX 3

TABLE 6

GROUP 11

Calf No.	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136
Date of Birth	28/4/73	30/4/73	4/5/73	4/5/73	5/5/73	6/5/73	7/5/73	7/5/73	8/5/73	9/5/73	10/5/73	10/5/73	11/5/73	12/5/73	12/5/73
Birth weight (kg)	42.0	36.8	34.0	30.6	29.5	29.1	35.6	29.3	34.6	27.2	29.7	36.5	31.4	40.3	38.9
Date/Weight (kg)															
4/5/73	48.6	38.6	-	-	-	-	-	-	-	-	-	-	-	-	-
11/5/73	51.4	45.9	40.5	34.5	32.7	31.8	35.9	29.1	33.6	28.2	31.8	36.8	33.2	-	-
18/5/73	57.7	49.1	43.6	36.4	36.8	35.5	36.4	32.7	35.0	32.7	35.0	40.0	33.6	43.6	41.8
25/5/73	61.8	48.2	47.7	37.3	40.0	37.7	39.5	35.0	37.3	35.5	36.4	41.8	35.5	42.7	43.2
1/6/73	63.2	52.3	52.7	40.9	45.0	39.5	46.8	40.0	42.3	37.3	38.6	44.5	38.2	45.5	43.2
8/6/73	67.3	57.7	57.7	44.1	44.1	40.0	49.1	38.6	43.2	43.6	37.3	43.2	40.0	45.9	45.0
15/6/73*	75.9*	65.9*	61.4*	47.7*	52.3*	48.6*	55.9*	49.1*	50.9*	46.4*	45.0*	51.8*	42.7*	54.1*	54.1*
22/6/73	82.7	72.3	65.5	52.7	58.6	51.4	60.5	51.8	55.5	50.9	49.1	57.3	45.9	59.5	56.9
29/6/73	85.0	79.5	70.5	56.8	62.3	54.5	65.9	53.6	59.5	54.1	55.0	62.7	47.3	64.5	59.5
6/7/73	92.3	86.4	74.1	61.8	69.5	59.5	70.5	58.6	65.5	58.2	58.6	68.6	50.5	70.9	64.5
13/7/73	98.2	90.9	80.0	67.3	76.8	65.9	76.8	63.2	69.5	63.6	61.8	72.3	57.3	74.5	70.0
20/7/73	107.3	95.5	85.0	73.2	81.8	70.5	81.8	68.2	75.5	69.1	69.1	77.7	55.9	80.9	75.0
27/7/73	119.1	103.6	90.9	80.5	80.9	75.0	90.0	76.4	83.6	77.3	76.4	86.4	60.0	89.5	84.1
3/8/73	125.5	108.2	97.3	88.2	98.2	78.6	94.5	81.8	88.2	82.3	80.9	94.1	66.4	95.9	90.0
10/8/73	133.2	114.5	102.7	93.6	103.6	84.1	101.4	90.9	94.5	90.0	85.9	100.0	72.3	103.2	97.3
Wt,at 28 days	62.2	50.4	53.1	40.8	45.0	39.9	50.7	39.2	41.9	42.7	37.3	43.1	41.3	48.1	47.7
Wt,at weaning	75.9	65.9	61.4	56.8	52.3	48.6	55.9	49.1	50.9	46.4	45.0	51.8	42.7	54.1	54.1
LWG birth to weaning	33.9	29.1	27.3	17.0	22.8	19.4	20.2	19.9	16.2	19.2	15.2	15.2	11.3	13.8	15.2
Days on milk	48	46	42	42	41	40	39	39	38	37	36	36	35	34	34
Daily LWG-kg/day Birth to weaning	0.71	0.63	0.65	0.40	0.56	0.49	0.52	0.51	0.43	0.52	0.42	0.42	0.32	0.41	0.45
Wt,at 12 wks.	107.3	99.0	90.9	80.5	81.8	75.9	91.4	78.6	86.6	80.9	80.5	93.2	66.4	95.5	90.9

* All calves weaned 15/6/73

GROUP 12

Calf No.	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151
Date of Birth	26/6/73	26/6/73	1/7/73	2/7/73	7/7/73	8/7/73	10/7/73	11/7/73	15/7/73	15/7/73	15/7/73	21/7/73	22/7/73	25/7/73	26/7/73
Birth Weight (kg)	35.4	42.7	34.2	32.7	32.5	30.0	37.0	27.0	28.8	35.5	36.6	32.3	31.8	33.2	28.4
Date/Weight (kg)															
29/6/73	36.6	47.3	-	-	-	-	-	-	-	-	-	-	-	-	-
6/7/73	41.8	50.9	38.2	34.5	-	-	-	-	-	-	-	-	-	-	-
13/7/73	41.4	51.4	39.1	35.5	38.2	36.4	39.5	28.6	-	-	-	-	-	-	-
20/3/73	45.0	54.5	40.9	39.1	37.3	38.6	40.0	30.0	31.8	35.5	39.1	-	-	-	-
27/7/73	48.6	57.7	44.5	45.0	43.2	40.5	40.5	32.7	31.4	36.8	43.6	33.2	34.1	34.5	30.5
3/8/73	58.6	59.5	51.4	48.2	49.5	44.5	42.3	35.5	35.9	40.5	46.8	32.7	34.1	34.5	30.0
10/8/73	60.9	62.7	56.8	53.2	53.6	47.3	45.5	40.0	37.7	44.5	50.9	34.1	35.4	32.7	28.2
17/8/73	67.7*	64.1*	59.1*	58.6*	55.9	50.5	49.5	39.1	42.7	53.2	55.0	38.2	40.0	37.3	29.1
24/8/73	75.9	72.3	68.2	63.2	62.7*	55.9*	55.5*	43.2*	50.0	55.5	65.0	45.9	45.0	44.5	25.9
31/8/73	83.2	78.2	73.6	65.0	66.8	60.0	60.0	47.3	56.8*	57.3*	69.1*	45.9	50.5	46.4	27.3
7/9/73	91.4	80.9	81.4	69.5	71.4	65.9	65.5	50.9	56.4	65.0	74.5	50.9*	56.4*	53.6*	Died
14/9/73	97.7	84.5	87.3	75.9	75.9	66.8	72.7	54.5	60.5	70.5	81.8	55.9	62.3	55.9	-
21/9/73	105.9	87.7	95.5	81.4	79.5	63.6	77.3	58.6	63.6	75.5	87.7	60.5	67.3	59.1	-
28/8/73	114.5	92.3	102.3	87.7	84.5	66.8	86.4	62.7	67.3	80.9	95.5	65.0	73.2	60.9	-
Wt,at 28 days	47.0	56.3	47.3	46.8	51.2	45.8	45.0	39.9	39.0	49.6	53.2	43.7	42.7	44.5	26.4
Wt,at weaning	67.7	64.1	59.1	58.6	62.7	55.9	55.5	43.2	56.8	57.3	69.1	52.3	56.4	53.6	Died
LWG birth to weaning	32.3	21.4	24.9	25.9	30.2	25.9	18.5	16.2	28.0	21.8	32.5	20.0	24.6	20.4	-
Days on milk	52	52	47	46	48	47	45	44	49	47	47	50	49	46	-
Daily LWG-kg/day Birth to weaning	0.62	0.41	0.53	0.56	0.63	0.55	0.41	0.37	0.57	0.46	0.69	0.40	0.50	0.44	-
Wt,at 12 wks.	102.7	84.5	96.4	83.2	85.5	80.5	86.4	65.9	78.2	87.7	103.6	75.0	85.5	72.7	-

* Each calf weaned when approximately 7 weeks old

TABLE 7

Amount of milk-substitute consumed by the 14 calves of Group 8 during the five 24-hour observation periods

(1 unit = 1 pint/570ml)

	P.M.												A.M.									P.M.		
	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-1	1-2	2-3	3-4	4-5
Calf 82																								
Wk. 1	3	1.75	-	2	-	-	2.5	2.5	-	2.5	-	-	-	3.5	-	-	2	4	-	1	0.5	2.5	1	-
2	0.5	-	-	-	-	-	2.25	-	3	3.5	1	-	-	-	2.25	-	3	-	1.75	-	2	1.5	3.5	-
3	-	-	-	3	-	2	1	-	-	-	-	-	-	-	-	-	7.25	-	-	-	3.75	-	-	-
4	-	2	3	-	6	-	1	1	-	-	-	-	-	-	8	-	-	2.5	-	3.25	-	-	1	-
5	0.25	3	-	6.25	-	-	-	-	0.5	4	-	-	-	-	3.75	-	-	5	-	-	-	-	-	-
Calf 83																								
Wk. 1	-	-	-	-	-	-	-	0.5	-	2	-	-	-	-	0.5	0.5	1.5	1	-	1	-	-	5	-
2	-	2.5	1.75	-	-	-	-	-	-	-	3	-	-	-	-	0.25	-	2	-	-	-	4	-	-
3	-	-	4.5	-	-	-	1	-	2	-	-	-	-	-	1.25	6.25	-	-	-	0.5	-	1.5	-	1
4	-	-	1.5	-	-	2	1	-	-	0.75	-	-	-	-	-	-	3	-	-	1.25	-	1.75	1	1.5
5	2.25	-	-	0.5	-	5.75	-	-	-	-	-	-	-	-	-	1.5	-	2.75	1	-	-	-	-	-
Calf 84																								
Wk. 1	-	-	-	-	-	-	1.5	-	-	-	-	-	1	-	0.25	0.5	3	-	-	-	-	0.5	-	0.5
2	0.5	-	-	0.5	-	-	-	-	-	-	6	-	-	-	-	-	2	-	-	-	-	-	5	-
3	3	-	-	-	1.5	1.25	-	0.5	-	-	-	0.5	-	-	-	-	-	0.5	1	-	0.5	-	-	5
4	1	-	-	1	-	2	-	-	-	-	-	-	-	-	-	4	1	-	-	-	-	-	0.5	0.75
5	-	-	0.5	-	7.75	-	-	-	-	-	-	-	0.75	-	-	0.5	-	4.75	-	-	-	-	-	0.5
Calf 85																								
Wk. 1	-	1	-	-	-	0.5	-	1	-	2	-	-	-	-	5	-	-	-	3.75	-	-	1.5	1	-
2	-	3.5	-	-	-	-	-	6	-	-	-	-	-	2	-	-	1.75	-	-	4.25	1	-	-	-
3	-	2	-	-	1.5	-	-	-	-	-	-	-	3	2.75	-	0.5	-	3.5	-	0.5	-	-	0.5	-
4	-	0.75	0.5	-	-	-	-	-	4	-	-	-	-	-	1.5	-	3.25	-	-	0.75	-	1.75	-	-
5	-	-	-	3	2	-	-	-	-	0.5	-	-	-	-	3	1	-	1	-	-	2	0.5	-	0.75
Calf 86																								
Wk. 1	-	-	-	-	0.5	1.5	-	2	-	0.75	-	-	3	-	-	-	-	-	2.5	2	-	-	-	-
2	-	-	-	4.5	-	-	-	-	-	-	4	1	-	-	-	-	3	1	4	-	-	-	0.5	3
3	3	-	1	-	-	3.25	-	-	-	-	-	-	4	-	0.75	1	-	2	3	-	-	-	0.5	1
4	-	-	-	3	1	1	-	-	0.75	-	-	-	-	3	-	-	1.75	-	-	3	3	-	-	-
5	3	-	-	-	2.75	-	1	-	-	-	-	-	-	-	-	6.5	-	-	1	-	1	-	2	-
Calf 87																								
Wk. 1	1	2.5	-	-	2.5	-	-	-	-	1.5	-	-	-	-	-	5.5	-	-	2	1	-	-	2.5	-
2	-	-	4	-	-	-	0.75	-	-	3.75	-	-	-	-	-	4.5	-	3.25	-	-	-	2	-	0.5
3	4	-	-	-	2	-	2	-	-	-	-	-	-	-	-	4.25	-	2.5	-	2	-	-	2.5	-
4	-	4	-	-	3	1	0.5	0.25	-	-	-	-	-	-	1.5	1.75	2	1.5	-	0.5	-	2.5	1.25	-
5	-	-	1.5	3	-	1.25	-	1	-	-	-	-	3	-	0.75	2	-	1.75	0.5	1	2	-	-	2.5
Calf 88																								
Wk. 1	5	-	-	-	-	-	1	-	-	0.5	6	-	-	-	-	-	-	1	2.5	-	-	2.25	-	3
2	-	-	6	1	-	0.25	-	-	-	-	-	5	-	-	-	1	-	4	-	1	-	-	-	-
3	-	-	-	-	-	1	-	-	-	3	1	-	-	-	3.5	-	-	-	2.5	-	-	-	-	3
4	-	1.25	-	2	-	4	-	-	-	-	-	-	-	4	-	-	-	3	-	-	-	-	0.5	-
5	-	5	-	-	-	2.25	-	-	1	-	-	5.5	-	-	-	-	-	-	3	-	-	-	-	-

TABLE 7(cont'd)

	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-1	1-2	2-3	3-4	4-5
Calf 89																								
Wk. 1	-	0.5	1	-	-	-	-	-	1	1	-	-	-	1	-	-	2	-	-	2.75	-	-	-	-
2	-	0.5	0.25	1	-	-	-	-	-	0.75	-	1	1	1	-	3	-	-	-	1	1	-	-	0.5
3	-	-	2	-	-	-	2	3	-	-	-	0.5	-	1.75	-	-	-	0.5	2	-	2.5	1	-	-
4	-	-	-	-	-	1	1	-	2	-	-	-	-	-	-	-	0.75	-	1	-	3	1	-	-
5	-	-	-	-	-	-	0.5	3	-	-	-	-	-	-	-	1.5	-	-	3	-	-	-	-	3
Calf 90																								
Wk. 1	-	0.5	0.5	-	-	-	-	2	-	-	-	-	1	-	-	1	-	2.25	-	-	-	1.5	-	-
2	-	1	-	2.25	0.5	-	-	4.25	-	-	-	-	-	-	1	5.25	-	-	-	4.75	-	0.5	-	-
3	-	3	0.5	-	-	0.5	1	-	-	-	-	-	-	-	5	-	-	-	-	4.5	-	-	-	1
4	4	-	-	4	-	-	-	-	-	-	-	-	-	-	3.5	2.25	-	-	1	2	-	-	-	-
5	-	-	2	-	3.5	1.75	-	-	-	-	-	-	-	1.75	4	-	-	-	-	-	-	7.5	-	-
Calf 91																								
Wk. 1	-	-	1.5	1	-	1	-	-	1	-	-	-	1	-	2	-	-	1	-	2	1	-	-	1.25
2	2.5	1	-	-	-	0.5	-	-	-	-	-	-	-	3	2.5	-	-	-	4.75	2	-	-	-	1.5
3	2	-	-	3	1	-	0.5	-	1	-	-	-	-	2	-	-	4.25	-	0.5	-	1.25	-	2	-
4	2	-	-	1	1	-	-	0.75	0.75	1.25	-	-	-	1.5	-	-	-	0.5	3	2.25	-	-	-	1
5	-	-	0.5	-	0.25	3.25	2	-	1	-	-	-	3	-	-	-	5	-	1	-	-	-	2	-
Calf 92																								
Wk. 1	-	1.5	-	-	-	-	2.5	-	-	-	-	-	-	-	-	2	2	-	-	-	-	-	0.5	3
2	-	4	-	-	-	1	0.5	-	1	1	-	3	-	-	-	-	-	-	-	-	4	-	-	-
3	-	3	-	-	-	-	-	3	-	-	-	-	-	-	4	-	-	-	-	-	-	-	0.5	-
4	-	-	1	1	1.5	-	-	-	-	4	-	-	-	-	-	-	2	-	-	-	-	1	-	2.75
5	-	-	-	0.5	-	-	5	2	-	-	-	-	-	-	1.25	0.5	1	1	5	-	-	-	4	-
Calf 93																								
Wk. 1	-	-	0.5	-	-	-	1	-	2	-	-	1.25	-	-	-	-	0.5	-	-	-	2.5	1	-	-
2	-	-	-	-	-	0.5	-	-	0.75	2	-	-	-	-	-	-	1	2	2	-	-	-	-	4.5
3	-	2	-	1	2	-	3	1	-	-	2	-	-	-	1.5	-	1.5	-	1	2.5	-	-	-	0.5
4	-	-	0.25	-	1.5	-	4	-	-	-	-	-	1	-	-	2.5	0.25	3.5	2	-	-	-	1.75	-
5	2.5	-	-	-	-	-	6.5	-	-	-	-	-	-	-	-	1	5	0.25	1	-	-	-	2	-
Calf 94																								
Wk. 1	-	-	1	-	-	0.5	-	1	-	-	1.75	-	-	0.5	-	1	-	1	2.25	-	-	1.5	1.5	1
2	-	-	-	-	-	-	-	-	2.25	1	-	-	-	2.75	-	-	3.25	-	-	-	2	1	-	3
3	-	3	-	1	-	1	-	1	-	-	-	-	-	1	2	-	0.75	4	-	-	-	2.5	-	1.5
4	-	-	5.25	-	-	-	2.5	-	-	-	-	-	-	4.5	-	-	-	-	3.25	-	-	-	-	-
5	-	-	0.5	3.25	0.75	-	-	-	1.5	-	-	-	-	3.5	-	-	4	0.5	-	-	-	-	1	2.25
Calf 95																								
Wk. 1	-	-	1	-	-	-	1	0.5	-	-	-	-	1	-	2	-	-	0.75	-	1.25	-	-	-	1
2	1.5	0.5	-	0.25	1	-	-	2.75	1	-	-	-	-	4.25	1.25	-	-	1.75	1.5	-	-	1	3	-
3	-	-	-	2	-	1.5	-	1	-	-	-	-	-	1.5	-	-	0.25	-	1.5	-	1	1	-	-
4	-	-	1	1.5	2.5	-	-	2	-	-	-	-	-	2.5	-	2.5	-	1.25	1.75	-	-	4	-	-
5	-	1	-	2.5	-	2.75	-	-	-	-	0.5	3.5	-	-	0.25	1.5	2	-	-	0.5	2	1	-	1

APPENDIX 4

Calf 77

	Serum (mg/ml)				Tears (mg/ml)		
	IgG_1	IgG_2	IgA	IgM	IgG_1	IgA	IgM
Pre-colostrum	0	0	0	0	0	0	0
2 hours *	3.70	0.11	0.410	0.48	0	0.028	0
4 " *	4.32	0.84	2.16	0.72	0	0.044	0
8 " *	30.69	0.99	5.58	1.18	0.190	0.028	0
12 " *	ND	ND	ND	ND	ND	ND	ND
16 " *	31.44	0.99	4.72	1.84	0.428	0.044	0
20 " *	31.80	0.92	4.42	0.951	0.266	0.028	0
24 " *	36.0	0.97	4.72	1.12	0.552	0.060	0
2 days	31.2	0.94	5.28	1.06	0.352	0.060	0
4 "	32.4	0.89	2.80	0.83	0.44	0.044	0
6 "	27.36	1.24	1.52	0.58	0.530	0.028	0
8 "	27.36	1.08	1.12	0.62	0.488	0.030	0
10 "	30.50	1.12	1.00	0.39	0.468	0.090	0.068
12 "	27.80	0.97	0.91	0.35	0.372	0.478	0.300
14 "	27.90	1.02	0.10	0.33	0.352	0.900	0.254
16 "	20.88	0.94	0.12	0.29	0.352	0.832	0.118
18 "	23.04	0.94	0.08	0.25	0.292	0.610	0.048
20 "	24.15	1.26	0.14	0.27	0.300	0.500	0.010
22 "	21.42	1.09	0.08	0.19	0.266	0.420	0.032
24 "	ND	ND	ND	ND	0.266	0.840	0.184
26 "	14.60	0.52	0.14	0.18	0.532	0.610	0.084
28 "	14.88	0.56	0.14	0.19	0.196	0.610	0.072
30 "	ND	ND	ND	ND	ND	ND	ND
32 "	19.15	0.40	0.10	0.10	0.132	0.700	0.046
34 "	15.30	0.78	0.14	0.30	0.132	0.610	0.046
36 "	12.45	0.85	0.19	0.27	0.312	0.820	0.082
38 "	13.00	1.03	0.14	0.25	0.240	1.040	0.220
40 "	16.20	0.78	0.14	0.42	0.258	1.240	0.248
42 "	16.20	0.88	0.25	0.19	0.142	0.700	0.082
49 "	16.20	0.91	0.23	0.45	0.132	1.240	0.082
56 "	13.10	1.49	0.34	0.38	0.210	1.240	0.072
63 "	13.10	1.04	0.25	0.17	0.118	1.70	0.056
70 "	14.60	1.4	0.25	0.49	0.118	1.240	0.023

* Post-colostrum.

APPENDIX 4

Calf 79

	Serum (mg/ml)				Tears (mg/ml)		
	IgG_1	IgG_2	IgA	IgM	IgG_1	IgA	IgM
Pre-colostrum	0.90	0.026	0.056	0.48	0.028	0	0
2 hours *	ND	ND	ND	ND	ND	ND	ND
4 " *	ND	ND	ND	ND	0.156	0.032	0
8 " *	14.10	0.29	1.36	0.89	0.208	0.048	0
12 " *	14.6	0.25	1.36	1.12	0.220	0.032	0
16 " *	14.6	0.30	1.12	0.83	0.248	0.032	0
20 " *	13.6	0.27	0.44	0.68	0.156	0.010	0
24 " *	12.55	0.24	0.78	0.87	0.294	0.032	0
2 days	12.4	0.28	0.54	0.82	0.196	0.032	0
4 "	13.10	0.27	0.30	0.81	0.196	0	0
6 "	14.4	0.11	0	0.39	0.280	0	0.100
8 "	11.3	0.16	0	0.36	0.236	0	0.358
10 "	10.2	1.11	0	0.20	0.236	0	0.220
12 "	9.35	0.18	0	0.30	0.180	0	0.220
14 "	9.35	0.21	0	0.28	0.260	0.390	0.220
16 "	10.00	0.23	0	0.36	0.220	0.490	0.328
18 "	10.70	0.24	0	0.45	0.292	0.390	0.152
20 "	10.70	0.29	0	0.48	0.240	0.490	0.152
22 "	10.20	0.38	0	0.25	0.248	0.435	0.152
24 "	9.80	0.46	0.062	0.25	0.248	0.390	0.104
26 "	10.70	0.40	0.062	0.25	0.122	0.300	0.066
28 "	11.65	0.59	0.048	0.18	0.224	1.29	0.172
30 "	10.70	0.37	0.082	0.18	0.248	1.29	0.104
32 "	11.70	0.45	0.082	0.21	0.248	1.95	0.116
34 "	12.25	0.15	0.062	0.35	0.050	0.84	0.028
36 "	11.70	0.56	0.082	0.22	0.172	1.29	0.028
38 "	13.35	0.76	0.082	0.20	0.308	1.16	0.066
40 "	12.9	0.81	0.102	0.18	0.152	1.60	0.066
42 "	13.35	0.94	0.082	0.32	0.284	0.90	0.104
49 "	ND	ND	ND	ND	ND	ND	ND
56 "	15.00	0.61	0.082	0.28	0.192	1.04	0.072
63 "	16.60	0.69	0.102	0.30	ND	ND	ND
70 "	14.55	0.81	0.102	0.33	0.224	1.28	0.092

* Post-colostrum.

APPENDIX 4

Calf 80

	Serum (mg/ml)				Tears (mg/ml)		
	IgG_1	IgG_2	IgA	IgM	IgG_1	IgA	IgM
Pre-colostrum	0	0	0	0	0	0	0
2 hours *	ND	ND	ND	ND	ND	ND	ND
4 " *	12.25	0.44	1.43	0.76	0.002	0	0
8 " *	25.25	0.94	2.71	2.10	0.082	0	0
12 " *	22.92	1.1	3.28	1.45	0.116	0	0
16 " *	22.10	1.2	3.55	2.00	0.156	0	0
20 " *	29.40	1.37	3.36	1.89	0.202	0	0
24 " *	24.96	1.27	3.54	1.45	0.236	0	0
2 days	29.40	1.22	1.91	1.45	0.218	0.040	0
4 "	29.28	1.19	0.672	1.45	0.728	0.132	0
6 "	29.50	1.04	0.184	1.65	0.260	0.576	0
8 "	24.70	1.05	0.078	0.98	0.202	0	0.028
10 "	23.60	0.99	0.140	1.00	0.148	0	0.140
12 "	22.20	0.95	0.062	0.70	0.540	0.296	0.594
14 "	21.50	0.71	0.078	0.46	0.248	0.310	0.678
16 "	21.50	0.71	0.078	0.44	0.236	0.480	0.392
18 "	ND	ND	ND	ND	0.260	0.620	0.265
20 "	19.50	0.74	0.062	0.35	0.236	0.543	0.190
22 "	20.80	0.69	0.078	0.35	0.152	0.420	0.156
24 "	ND	ND	ND	ND	ND	ND	ND
26 "	18.90	0.92	0.058	0.37	0.214	0.780	0.180
28 "	18.90	1.02	0.040	0.37	0.116	0.840	0.140
30 "	18.90	0.99	0.078	0.39	0.122	0.06	0.092
32 "	26.50	0.67	0.180	0.62	0.172	1.48	0.158
34 "	18.25	0.75	0.087	0.46	0.176	1.70	0.158
36 "	18.25	0.99	0.078	0.54	0.152	1.55	0.124
38 "	15.75	0.75	0.078	0.49	0.152	1.83	0.140
40 "	18.90	1.20	0.058	0.57	0.132	1.43	0.066
42 "	18.90	1.18	0.078	0.59	0.104	1.05	0.066
49 "	15.20	1.12	0.078	0.51	0.104	2.72	0.102
56 "	13.65	1.12	0.078	0.59	ND	ND	ND
63 "	14.00	1.24	0.078	0.68	0.120	1.69	0.018
70 "	15.50	1.40	0.098	0.76	0.080	2.72	0.026

* Post-colostrum.

APPENDIX 4

Calf 83

	Serum (mg/ml)				Tears (mg/ml)		
	IgG_1	IgG_2	IgA	IgM	IgG_1	IgA	IgM
Pre-colostrum	0	0	0	0	0	0	0
2 hours *	12.14	1.16	0.51	1.14	ND	ND	ND
4 " *	25.20	1.20	3.36	1.11	0.176	0	0
8 " *	39.00	1.09	6.30	1.14	0.312	0	0
12 " *	42.00	0.88	6.30	1.14	0.312	0	0
16 " *	42.00	1.20	6.90	1.28	0.222	0	0
20 " *	50.40	1.23	6.90	1.31	0.336	0	0
24 " *	39.60	1.20	7.20	1.24	0.288	0	0
2 days	41.50	1.10	6.30	1.2	0.780	0	0
4 "	39.60	1.09	3.00	1.04	0.252	0	0
6 "	34.80	0.94	2.45	0.98	0.400	0	0
8 "	34.80	0.94	2.00	0.92	0.400	0	0
10 "	31.20	0.91	0.96	0.86	0.760	0	0
12 "	ND	ND	ND	ND	0.900	0.10	0.342
14 "	30.30	0.74	0.54	0.98	0.660	0.52	0.368
16 "	33.50	0.77	0.42	0.98	0.898	2.30	0.530
18 "	ND	ND	ND	ND	ND	ND	ND
20 "	30.30	0.36	0.31	0.80	0.479	1.84	0.296
22 "	30.30	0.74	0.29	0.86	0.528	2.59	0.328
24 "	30.30	0.74	0.28	1.08	0.210	1.24	0.130
26 "	30.00	0.86	0.36	0.40	0.440	2.04	0.156
28 "	29.45	0.86	0.56	0.64	0.426	3.61	0.156
30 "	30.00	0.84	0.56	0.67	0.184	3.36	0.092
32 "	26.50	0.81	0.20	0.39	0.276	3.30	0.192
34 "	27	0.89	0.28	0.49	0.516	6.68	0.334
36 "	26.5	1.00	0.34	0.49	0.584	7.40	0.334
38 "	25.95	1.14	0.34	0.46	0.340	7.20	0.204
40 "	25.95	1.16	0.34	0.45	0.276	6.60	0.216
42 "	25.65	1.08	0.28	0.46	0.288	2.44	0.100
49 "	18.5	1.25	0.28	0.55	0.220	4.80	0.108
56 "	13.8	1.10	0.34	0.50	0.240	4.80	0.040
63 "	16.55	2.22	0.34	0.64	0.136	4.90	0.020
70 "	ND	ND	ND	ND	ND	ND	

* Post-colostrum.

APPENDIX 4

Calf 85

	Serum (mg/ml)				Tears (mg/ml)		
	IgG_1	IgG_2	IgA	IgM	IgG_1	IgA	IgM
Pre-colostrum	0	0	0	0	0	0	0
2 hours *	ND	ND	ND	ND	ND	ND	0
4 " *	25.95	0.43	1.62	2.06	0.264	0	0
8 " *	32.50	0.71	3.00	2.96	0.332	0	0
12 " *	32.25	0.71	2.64	3.60	0.338	0	0
16 " *	40.00	0.79	2.08	3.70	0.314	0	0
20 " *	ND	ND	ND	ND	ND	ND	ND
24 " *	38.75	0.72	2.81	3.53	0.618	0	0
2 days	28.00	0.71	2.28	3.40	0.300	0	0
4 "	30.00	0.61	0.91	2.29	0.442	0	0
6 "	30.00	0.56	0.40	2.42	0.228	0	0
8 "	ND	0.62	0.40	1.20	0.368	0.012	0.036
10 "	27.10	0.68	0.312	0.82	0.532	0.112	0.182
12 "	27.10	0.51	0.248	0.78	0.424	0.400	0.464
14 "	22.40	0.40	0.198	0.66	0.202	0.680	0.330
16 "	21.00	0.38	0.198	0.57	0.368	0.806	0.194
18 "	21.00	0.40	0.198	0.53	0.218	1.05	0.080
20 "	11.00	0.22	0.098	0.52	0.470	4.32	0.272
22 "	21.65	0.39	0.124	0.53	0.260	3.45	0.080
24 "	19.75	0.40	0.098	0.48	0.260	1.28	0.060
26 "	20.25	0.42	0.081	0.42	0.246	1.64	0.046
28 "	21.00	0.42	0.134	0.42	0.206	2.32	0.070
30 "	19.30	0.44	0.076	0.276	0.220	2.70	0
32 "	18.50	0.50	0.080	0.36	0.346	4.64	0
34 "	19.30	0.59	0.080	0.39	0.376	2.94	0.132
36 "	18.50	0.51	0.080	0.39	0.080	1.68	0.080
38 "	ND	ND	ND	ND	0.094	2.10	0.120
40 "	16.60	0.62	0.100	0.41	0.280	2.20	0.108
42 "	14.80	0.44	0.076	0.39	0.170	3.25	0.120
49 "	15.50	0.74	0.130	0.47	0.180	3.74	0.042
56 "	14.80	1.02	0.176	0.35	ND	ND	ND
63 "	16.00	1.22	0.302	0.58	0.136	4.31	0.040
70 "	14.80	1.48	0.424	0.58	0.158	3.20	0.040

* Post-colostrum.

APPENDIX 4 Calf 93

	Serum (mg/ml)				Tears (mg/ml)		
	IgG_1	IgG_2	IgA	IgM	IgG_1	IgA	IgM
Pre-colostrum	0.098	0.010	0	0.156	0	0	0
2 hours *	ND	ND	ND	ND	ND	ND	ND
4 " *	19.75	0.42	1.43	2.34	0	0	0
8 " *	20.02	0.50	1.91	2.60	0.068	0	0
12 " *	20.25	0.50	1.69	2.34	0.208	0	0
16 " *	19.30	0.59	1.69	2.34	0.388	0	0
20 " *	19.30	0.30	1.63	2.34	0.208	0	0
24 " *	18.50	0.42	1.55	2.55	0.388	0	0
2 days	16.00	0.42	1.17	2.00	0.096	0	0
4 "	15.50	0.59	0.325	1.45	0.124	0	0
6 "	13.75	0.40	0.260	1.13	0.104	0	0
8 "	11.80	0.33	0.124	0.64	0.164	0	0
10 "	11.80	0.22	0.184	0.57	0.268	0.024	0.198
12 "	11.30	0.36	0.084	0.52	0.104	0.052	0.198
14 "	18.40	0.17	0.080	0.46	0.104	0.184	0.412
16 "	10.90	0.24	0.076	0.44	0.186	1.12	0.244
18 "	13.60	0.16	0.056	0.37	0.208	1.34	0.064
20 "	8.90	0.12	0.160	0.37	0.164	1.45	0.094
22 "	9.85	0.20	0.060	0.38	0.104	1.12	0.094
24 "	9.85	0.22	0.084	0.49	0.080	1.02	0
26 "	9.85	0.20	0.076	0.47	0.128	3.64	0.198
28 "	8.00	0.30	0.084	0.52	0.288	4.83	0.270
30 "	10.90	0.40	0.124	0.52	0.176	4.48	0.182
32 "	10.25	0.44	0.216	0.52	0.114	3.80	0.160
34 "	ND	ND	ND	ND	0.142	5.20	0.148
36 "	ND	ND	ND	ND	0.112	4.83	0.122
38 "	ND	ND	ND	ND	0.152	5.56	0.140
40 "	12.35	0.52	0.142	0.49	0.080	4.68	0.130
42 "	12.35	0.57	0.142	0.49	0.088	5.38	0.140
49 "	12.05	0.75	0.176	0.47	0.132	5.20	0.156
56 "	13.05	0.95	0.410	0.52	0.176	5.78	0.166
63 "	15.50	1.14	0.464	0.79	0.132	4.92	0.140
70 "	13.05	1.42	0.410	0.70	0.152	4.68	0.150

* Post-colostrum.

APPENDIX 4

Calf 98

	Serum (mg/ml)				Tears (mg/ml)		
	IgG_1	IgG_2	IgA	IgM	IgG_1	IgA	IgM
Pre-colostrum	0.08	0	0	0	0	0	0
2 hours *	ND	ND	ND	ND	ND	ND	ND
4 " *	19.25	0.46	1.28	1.90	0.016	0	0
8 " *	30.12	0.67	1.85	1.98	0.074	0	0
12 " *	28.44	0.67	1.85	3.06	0.096	0	0
16 " *	21.96	0.74	1.63	3.61	0.102	0	0
20 " *	21.96	0.69	1.79	2.36	0.390	0.040	0.010
24 " *	21.96	0.69	1.79	1.90	0.196	0	0
2 days	22.80	0.58	0.920	1.09	0.184	0	0
4 "	23.00	0.47	0.370	0.85	0.308	0.044	0.002
6 "	19.85	0.54	0.210	0.74	0.196	0.044	0.008
8 "	18.70	0.49	0.134	0.54	0.076	0.044	0.008
10 "	13.90	0.39	0.088	0.43	0.184	0.060	0.094
12 "	ND	ND	ND	ND	ND	ND	ND
14 "	14.50	0.46	0.096	0.32	0.252	0.400	0.186
16 "	13.40	0.46	0.110	0.28	0.156	0.282	0.060
18 "	9.90	0.33	0.110	0.24	0.168	0.380	0.060
20 "	12.30	0.47	0.096	0.37	0.252	0.588	0.138
22 "	11.30	0.50	0.110	0.37	0.184	0.680	0.122
24 "	11.30	0.64	0.110	0.37	0.356	1.62	0.228
26 "	ND	ND	ND	ND	ND	ND	ND
28 "	10.75	0.64	0.096	0.37	0.212	0.995	0.322
30 "	8.90	0.71	0.110	0.45	0.196	1.33	0.398
32 "	9.90	0.67	0.134	0.41	0.252	1.62	0.266
34 "	11.80	0.82	0.134	0.28	0.406	3.40	0.242
36 "	12.90	0.74	0.110	0.30	0.212	3.57	0.108
38 "	9.90	0.74	0.110	0.45	0.308	3.40	0.170
40 "	9.85	0.67	0.096	0.28	0.308	2.35	0.127
42 "	ND	ND	ND	ND	ND	ND	ND
49 "	12.30	0.74	0.110	0.41	0.420	5.86	0.188
56 "	13.05	2.34	0.110	0.59	0.212	4.18	0.094
63 "	12.30	2.34	0.146	0.64	0.172	2.10	0.046
70 "	13.05	5.40	0.184	0.68	0.158	2.52	0.073

* Post-colostrum.

APPENDIX 4

Calf 106

	Serum (mg/ml)				Tears (mg/ml)		
	IgG_1	IgG_2	IgA	IgM	IgG_1	IgA	IgM
Pre-colostrum	0.048	0.02	0	0.138	0	0	0
2 hours *	ND	ND	ND	ND	ND	ND	ND
4 " *	15.00	0.36	1.03	2.44	0.112	0	0
8 " *	22.80	0.51	1.36	3.85	0.108	0	0
12 " *	30.84	0.61	2.94	3.51	0.200	0	0
16 " *	30.12	0.62	2.94	3.36	0.268	0	0
20 "	37.68	0.54	2.80	3.10	0.302	0	0
24 " *	34.80	0.59	1.32	3.10	0.670	0	0.044
2 days	28.38	0.56	1.29	2.86	0.336	0.030	0.044
4 "	25.25	0.38	0.892	2.58	0.720	0.106	0.104
6 "	22.40	0.38	0.492	1.96	0.260	0.062	0.066
8 "	25.25	0.41	0.310	1.96	0.182	0.024	0.063
10 "	24.35	0.48	0.200	1.84	0.228	0.024	0.092
12 "	ND	ND	ND	ND	ND	ND	ND
14 "	23.05	0.41	0.176	1.38	0.762	0.062	0.115
16 "	22.40	0.43	0.112	1.06	0.938	0.132	0.249
18 "	19.38	0.38	0.060	0.60	0.742	0.552	0.095
20 "	18.36	0.64	0.082	0.52	0.624	0.310	0.097
22 "	ND	ND	ND	ND	0.422	0.472	0.087
24 "	19.00	0.72	0.112	0.52	0.390	1.116	0.185
26 "	ND	ND	ND	ND	0.228	1.720	0.128
28 "	19.85	0.90	0.140	0.90	0.320	2.75	0.193
30 "	ND	ND	ND	ND	0.408	3.34	0.228
32 "	17.40	0.84	0.140	0.74	0.354	3.9	0.285
34 "	17.40	1.36	0.162	1.26	0.288	4.25	0.276
36 "	16.80	1.26	0.162	0.98	0.214	2.84	0.150
38 "	16.80	1.64	0.260	0.98	0.228	3.10	0.135
40 "	ND	ND	ND	ND	ND	ND	
42 "	16.20	1.44	0.062	0.90	0.200	1.66	0.126
49 "	16.20	2.28	0.162	1.07	0.200	2.75	0.112
56 "	15.00	2.00	0.224	1.04	0.160	2.03	0.124
63 "	13.40	2.76	0.288	1.09	0.320	4.27	0.427
70 "	ND	ND	ND	ND	ND	ND	ND

* Post-colostrum.

APPENDIX 4

Calf 204

	Serum (mg/ml)				Tears (mg/ml)		
	IgG_1	IgG_2	IgA	IgM	IgG_1	IgA	IgM
Pre-colostrum	1.0	0.024	0.060	0.048	0	0	0
2 hours *	14.50	0.12	0.300	0.200	0	0	0
4 " *	20.65	0.37	0.690	0.70	0	0	0
8 " *	28.15	0.70	1.46	1.47	0.072	0	0
12 " *	31.31	0.74	1.82	1.80	0.156	0	0
16 " *	34.83	0.88	1.96	2.30	0.198	0	0
20 " *	35.70	0.86	1.89	2.62	0.190	0	0
24 " *	36.00	1.21	1.96	2.20	0.204	0	0
2 days	33.96	0.42	1.11	0.94	0.204	0	0
4 "	24.88	0.74	0.560	0.95	0.128	0	0
6 "	29.27	0.62	0.420	0.48	0.120	0	0
8 "	30.39	0.66	0.244	0.52	0.372	0	0
10 "	32.64	0.60	0.154	0.58	0.308	0	0.038
12 "	29.47	0.60	0.120	0.65	0.134	0	0.094
14 "	28.21	0.62	0.078	0.48	0.204	0.200	0.094
16 "	22.84	0.66	0.078	0.61	0.266	0.530	0.102
18 "	20.80	0.61	0.078	0.48	0.082	0.244	0.038
20 "	22.84	0.66	0.044	0.44	0.090	0.42	0.104
22 "	19.89	0.62	0.060	0.39	0.062	0.22	0.010
24 "	22.84	0.68	0.044	0.39	0.072	0.50	0.010
26 "	20.80	0.68	0.060	0.41	0.236	2.37	0.166
28 "	25.50	0.68	0.060	0.30	0.090	0.90	0.048
30 "	22.84	0.68	0.060	0.39	0.060	1.00	0.010
32 "	19.38	0.72	0.078	0.39	0.060	0.90	0.026
34 "	18.05	0.74	0.060	0.49	0.120	1.96	0.102
36 "	17.13	0.81	0.078	0.52	0.174	1.34	0.038
38 "	19.38	0.74	0.098	0.57	0.090	1.91	0.102
40 "	ND	ND	ND	ND	0.204	2.65	0.140
42 "	16.32	1.05	0.098	0.60	0.072	1.25	0.028
49 "	16.21	0.98	0.118	0.60	0.082	2.37	0.120
56 "	16.21	1.30	0.180	0.62	0.120	2.51	0.028
63 "	16.21	1.38	0.364	0.63	0.160	3.89	0.048
70 "	ND	ND	ND	ND	ND	ND	ND

* Post-colostrum.

APPENDIX 4

Calf 224

	Serum (mg/ml)				Tears (mg/ml)		
	IgG_1	IgG_2	IgA	IgM	IgG_1	IgA	IgM
Pre-colostrum	0.032	0.08	0	0	0	0	0
2 hours *	4.35	0.27	0.220	0.11	0	0	0
4 " *	9.00	0.22	0.330	0.11	0.020	0	0
8 " *	14.60	0.37	0.710	0.20	0.186	0	0
12 " *	19.60	0.69	0.800	0.30	0.156	0	0
16 " *	19.60	0.52	0.620	0.46	0.142	0	0
20 " *	24.20	0.48	0.670	0.43	0.232	0	0
24 " *	23.00	0.55	0.840	0.30	0.328	0	0
2 days	18.48	0.44	0.330	0.20	0.166	0	0
4 "	18.48	0.39	0.150	0.25	0.114	0	0
6 "	15.72	0.36	0.084	0.25	0.156	0	0
8 "	14.50	0.33	0.032	0.56	0.378	0	0.010
10 "	ND	ND	ND	ND	0.156	0	0.020
12 "	11.75	0.27	0.032	0.30	0.138	0.175	0.026
14 "	11.75	0.28	0.124	0.26	0.192	0.730	0.068
16 "	11.75	0.28	0.167	0.22	0.138	1.29	0.032
18 "	ND	ND	ND	ND	ND	ND	ND
20 "	11.20	0.70	0.130	0.27	0.176	4.34	0.058
22 "	11.20	0.57	0.106	0.35	0.164	3.22	0.010
24 "	9.75	0.88	0.124	0.29	0.114	2.80	0.010
26 "	10.65	1.28	0.120	0.31	0.094	3.38	0.010
28 "	10.65	1.35	0.140	0.31	0.114	1.73	0.014
30 "	10.15	1.42	0.132	0.35	0.068	2.40	0.026
32 "	ND	ND	ND	ND	ND	ND	ND
34 "	10.65	1.60	0.148	0.34	0.106	3.50	0.020
36 "	9.75	1.53	0.132	0.38	0.076	3.40	0.020
38 "	11.20	1.96	0.132	0.43	0.106	3.40	0.026
40 "	10.15	1.86	0.132	0.48	0.062	1.85	0.026
42 "	8.75	1.86	0.124	0.48	0.248	5.44	0.026
49 "	ND	ND	ND	ND	ND	ND	ND
56 "	ND	ND	ND	ND	ND	ND	ND
63 "	ND	ND	ND	ND	ND	ND	ND
70 "	ND	ND	ND	ND	ND	ND	ND

* Post-colostrum.

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