LEVEL OF IMMUNOGLOBULINS IN THE SERUM OF THE NEWBORN DAIRY CALF

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CHAPTER I

Introduction

Incidence of calfhood diseases often determine whether a profit or loss will result from a cattle breeding enterprise. It has been estimated that 8 to 35% of all calves die as a result of disease before they reach 2 months of age. With the U.S. Department of Agriculture estimate of 42,563,000 calvings in 1964 and a value of $30 per calf, a 10% death loss could be estimated in excess of $100 million per year. Great as this loss appears to be, it is likely that permanently stunted and unthrifty animals contribute to an even greater loss. Such animals produce at a relatively low level and never reach their inherited potential performance.

Numerous diseases are responsible for calf losses, but infectious diarrhea is the major problem. It is the basic cause of more calf deaths than all other diseases combined, and it is a contributing factor in many losses attributed to other diseases. Improper feeding of newborn calves is a major predisposing cause of infectious diarrhea (Amstutz, 1965). Since the newborn calf does not receive resistance through placental transfer like some other species, it must derive its resistance via other sources.

Particular attention has been given to the role of non-specific humoral factors, including complement and conglutinin, and to specific humoral factors known as immunoglobulins or antibodies in relation to disease resistance in calves.

The transfer of maternal antibodies to the newborn ungulate was recognized as early as 1892 by Enrlich, according to Sarwar, Campbell and Petersen (1964). The concept that colostrum has special properties, distinct from those of normal milk, was developed in 1912 by Famulener, (Sarwar et al., 1964), who remarked on the high concentration of immune bodies in the early milk. Shortly after ingesting colostrum rich in immunoglobulins, increased amounts of these proteins occur in the calf's serum.

Escherichia coli is usually considered to be the basic cause of infectious diarrhea (Amstutz, 1965). Antibodies against Escherichia coli have been found to be present in colostrum and serum of calves fed colostrum milk (Gay, 1965). Immunoglobulins are secreted by the mammary gland into the colostrum, and are rapidly absorbed from the intestine of the young calf. Transfer is through the absorptive epithelial cells into the lymphatics (Comline, Roberts and Tichen, 1951). The ability of the intestinal epithelium to transport proteins is decreased approximately 56 percent during the first 24 hours after birth. This process referred to as "closure" does not appear to be absolute, since bacterial antigens may be

absorbed throughout life of the animal (Staley, 1971).

The objective of this experiment was to study possible factors that may influence the extent of immunoglobulin absorption in newborn dairy calves.

CHAPTER II

REVIEW OF LITERATURE

Disease Resistance in Neonatal Calf

The resistance of young calves to disease during early life depends partly upon active immunity produced in the calf during fetal life (Barta, 1969). This involves non-specific humoral factors, including complement and conglutinin. Complement is a group of globulins which exist in serum in an inactive form (Ingram, 1971). They may be considered to be complex proenzymes which require sequential activation. The complement system is comprised of nine distinct components designated C1, C2, C3, C4, C5, C6, C7, C8 and C9. Cell lysis by complement usually requires all nine components and is considered the prototype of complement activity. Conglutinin is naturally occurring serum protein of cattle and certain other species. Conglutinin can be detected in bovine fetal serum as early as the 110th day of gestation and the amount increases gradually until birth. Conglutinin is a serum globulin which reacts with C1, C4, C2, C3 and can fix complement, but is structurally and antigencially distinct from immunoglobulins.

Immunoglobulins are specific humoral factors produced

by the dam. The ruminant, horse, and pig do not acquire immunoglobulins via the placenta, as do the dog, rat, guinea-pig, rabbit and man, but, are born with just traces of serum antibodies. Newborn calves derive immunoglobulins via initial feedings of colostrum from which they are rapidly absorbed by the intestinal epithelium (Brambell, 1958).

Colostral immunoglobulins appear unchanged in the serum of the newborn calf within one to three hours after consuming colostrum and in effect are transported from the maternal sera to the calf conferring passive immunity (Staley, 1971). There is a gradual increase, in blood concentration, of immunoglobulins to reach a maximum at 24-36 hours (Bush et al., 1971; Butler, 1969; Klaus, Bennet and Jones and Bush, 1971). Concentration of immunoglobulins in calf's sera may approach that in the dam's colostrum and serum (Bush et al., 1971; Butler, 1969; Klaus et al., 1969).

Kruse, (1970b) reviewed recent observations of serum Ig levels (Table I) in calves born and fed on ordinary farms. The age of the calves at the time of blood sampling was estimted to be 2 to 25 days, with the great majority being less than ten days old. Caution is needed in comparison of the results since methods of analysis differed to some degree. He noted a strikingly large proportion of the calves had a low concentration of Ig in the blood serum and great individual variation was found in all investigations.

TABLE I

REVIEW ON IMMUNOGLOBULIN CONCENTRATION

					Number of calves	References
Ig concentration[1]	0-9	10-19	20-39	40-69		Gay et al., 1965,
Calves (%)	30	18	35	17	178	Scotland.
Ig concentration[2]	0-9	10-19	20-39	40-100	230	Smith, 1967
Calves (%)	15	13	22	50		England.
Ig concentration[3]	0-10	11-20	21-40	40	181	Kruse and Neimann-Sorensen, 1966,
Calves (%)	23	24	29	24		Denmark.
Ig concentration[4]	Low values				1000	G.Jonsson, personal communication,
Calves (%)	18.5					Sweden.
Ig concentration[5]	0.5	0.5-0.99	1.0-2.0		523	Kruse, unpublished observation.
Calves (%)	42.3	44.5	13.2			

[1]The relative concentration of Ig determined by a modification of the zincsulphate method of Aschaffenburg (1949). Units: 'Colorimeter reading'.

[2]Method of Aschaffenburg (1949). Units: 'Absorptiometer reading'.

[3]Method of Aschaffenburg (1949). Units: 'Extinction x 100,0·634'.

[4]Immunochemical method. Units not stated.

[5]Protein analysis and electrophoresis. Units: g Ig/100 g serum.

Protection By Immunoglobulins

The development of infectious calf diarrhea depends on an interaction between the more or less pathogenic types of E. coli involved and a number of resistance-reducing factors, such as insufficient birth hygiene, malnutrition including wrong application or no application at all of colostrum and bad housing of the newborn calves (Dam, 1968).

One of the classical symptoms of deficiency of immunoglobulins in calves is the high susceptibility to infections and the most common among them is scours (Marsh, 1968). Calf scours, a common problem during the first few days of life, is frequently fatal. Reisinger (1965) attributed 90 percent of all dairy calf diarrhea mortalities to this disease complex. Ensminger, Galgan and Sloccum (1955) ranked calf scours second in over-all importance (without regard to ages) among beef cattle diseases. Early calving in unsanitary corrals and sheds in inclement weather has increased the problem in some range areas. Blood and Henderson (1963) stated that the incidence of this disease decreases as husbandry methods are intensified, and colostrum is administered as soon as the newborn calf is able to suckle.

Gay et al. (1965) studied the immunoglobulin level of 178, 4-day old calves subjected to regular farm management procedures. Fifty-three (29.8%) were markedly or absolutely deficient in sera γ-globulins and 33 (18.5%)

possessed low globulin levels. A total of 31 calves died with colisepticaemia and 11 from other causes. All but one of the deaths from colisepticaemia were in calves whose sera were markedly deficient and the majority of these deaths were in calves considered absolutely deficient in immunoglobulins. Smith (1962) found a great variation in the levels of immunoglobulins in the blood serum of 52 calves that had spent the first two days of life with their dams, and which would have had access to colostrum. Of six of these calves with very low or no immunoglobulins, three died from bacteriemia. Very low levels of immunoglobulins also were found by Fey and Margadent (1962) in the blood serum of five out of 46 calves that had received maternal colostrum. These workers showed that 21 of 22 calves that had received colostrum, but had died of Escherichia coli bacteriemia, had no or only small amounts of immunoglobulins in their serum. This demonstrated a relationship between a γ-globulinaemia and colisepticaemia. Results obtained by Dam (1968) have shown a somewhat lower average γ-globulin level in calves which later succumbed to colisepticaemia. Smith and Orcut (1925) reported a correlation between feeding colostrum and the absence of septicemia. Wood (1955) indicated septicemia was more frequent in colostrum deprived calves, whereas a localized intestinal infection was more common in those receiving colostrum.

The exact role that colostrum plays in protection has

not been fully defined, however, various components of colostrum have been implicated. Agglutinins to the capsular "K" antigen of E. coli have been suggested as a protective factor in colostrum (Gay, 1965). Infections seem to build up in a closed community of colostrum-fed calves because certain strains against which colostrum is ineffective gradually become dominant. Therefore, in colostrum fed calves which died, few had agglutinins against the strains of E. coli associated with their dams (Ingram et al., 1956). Gay (1965) gave evidence against the specific agglutinating antibodies to the "K" antigen of E. coli in the calf. They stated that the level of specific agglutinating antibodies to the "K" antigen of the E. coli strain involved had no relationship to the susceptibility of the calf to colisepticaemia, and that the prime factor concerned was a deficiency of serum γ-globulins. Jacks and Glantz (1970) said the E. coli "O" antibodies in serum of dams were entirely 19S macroglobulins and appeared to be IgM immunoglobulins. The antibodies in colostrum and calf serum were both 7S and 19S globulins.

Characteristics of Immunoglobulins

The structure, function and occurrence of immunoglobulins in cattle are consistent with the general pattern that has been described for such molecules in more extensively studied species such as the rabbit, human, guinea-pig, and mouse. Investigations of these species have

revealed species-specific variations from the general pattern which can often be related to peculiar physiological or genetic traits.

The term, immunoglobulins, applies to a family of high molecular weight proteins that share common physicochemical characteristics and antigenic determinants. These proteins occur in the serum of other body fluids of animals and possess γ or slow β electrophoretic mobility. These proteins include all molecules with antibody activity, as well as other chemically related normal or pathological proteins (Butler, 1969). Although certain types of antibody activity are associated with particular classes of immunoglobulins, their classification is not based on the specificity of the antibody, but on the antigenic and physico-chemical characteristics of these proteins. All immunoglobulins appear to be either monomers or polymers of a four-chain (L-chains, 20,000 mole weight) and two heavy polypeptide chains (H-chins) with molecular weights varying from 50,000 to 70,000 for the different immunoglobulin classes.

Through reduction, alkylation and proteolytic enzymes, IgG can be digested to yield two "Fab" fragments and a single "Fc" fragment. The "Fab" fragment contains the NH_2-terminal half of one of the heavy chains plus one of the entire disulfide-bonded light chains, which contains 214 to 221 amino acid residues. The "Fc" fragment contains the COOH-terminal, 240 to 250 amino acids of the heavy chain, and most of the carbohydrates (Butler, Winter and

Wagner, 1971).

The COOH-terminal half of both the H-chain and L-chain fragments, or "Fc" fragment are highly constant in their amino acid sequence, whereas the NH_2-terminal half of each of these chains is considerably more variable. These variable NH_2-terminal portions contain the antibody-combining site. This site is that portion of the molecule which can combine specifically with some chemical configuration of an antigen which is called the antigenic determinant. The variability of NH_2-terminal end of both heavy and light chains is presumably related to the multitude of antibody specificities that one individual may possess. The "Fc" portion of the IgG immunoglobulin molecule cannot combine specifically with antigen, but is responsible for such properties as complement fixation, fixation to skin, and placental transfer. It carries species-specific and class-specific antigenic determinants. It can crystallize together suggesting a highly homogenous structure (Butler, 1969).

The individual immunoglobulin molecule or monomer unit possesses either two kappa (κ) or two lambda (λ) light polypeptide chains. The distribution of these two chains among immunoglobulins varies with species and in certain immunological phenomena. Five antigenically distinct classes of immunoglobulins recognized in man are referred to as IgG (γG), IgA (γA), IgM (γM), IgD (γD), and IgE (γE) Bulletin World Health Organization, 1964). Specific anti-

bodies against each of the five classes are directed primarily toward antigenic determinants located on the heavy polypeptide chains. Although the immunogenicity of the L-chains is low when a part of the intact molecule, their role in the preparation of class specific antisera cannot be ignored. The class-specific heavy chains of the different human classes are called γ for IgG, α for IgA, μ for IgM, δ for IgD and ϵ for IgE.

Three distinct classes of immunoglobulins have been identified in the bovine. IgG and IgM occur in serum and lacteal secretions and "secretory" IgA occurs mainly in lacteal secretions (Butler, 1969). IgM constitutes 10% of the total immunoglobulins in serum and colostrum. It tends to be present in higher proportions during early stages after challenge and may be designed for rapid protection (Nisonoff, 1971). IgM is apparently a more effective antibody than IgG particularly in agglutination, phage neutralization, complement fixation, and hemolysis. The primary response to brucellosis, anaplasmosis and E. coli consists of IgM (Staley, 1971). These immunoglobulins are large complex molecules, with a molecular weight of 900,000 and a sedimentation coefficient of twenty polypeptide chains, and have fast electrophoretic mobility (Tomasi, 1971).

IgG, the most abundant immunoglobulin in serum and colostrum, makes up 85-90% of the total (Butler, 1969). It is the most studied of all immunoglobulins. It occurs mostly as a monomer with a molecular weight of 160,000 and a

sedimentation coefficient of 7S. A molecule of IgG consists of two identical H chains and two identifical L chains, linked by three disulfide bonds.

In addition to class differences among immunoglobulins, smaller antigenic and physico-chemical differences in the heavy polypeptide chains within a class give rise to subclasses. Such differences are well recognized among the IgG immunoglobulins. In the human, four subclasses of IgG are recognized: IgG1, IgG2, IgG3, and IgG4. Two subclasses of IgG are recognized in the guinea pig, sheep, goat, ox, and rabbit; three are recognized in the mouse, horse, and rat. The more basic IgG molecules are called IgG2 immunoglobulins in bovine. These immunoglobulins have a mean S value of 6.6 and are plentiful in serum, but occur in low concentrations in milk, colostrum, and saliva. The subclass IgG1 consist of less basic IgG immunoglobulins which often appear more heterogeneous than IgG2 and have a mean sedimentation coefficient of 6.3S. Although there is normally no significant difference between the serum concentration of IgG1 and IgG2, IgG1 is the principal immunoglobulin of the lacteal and salivary secretions. The subclasses appear to have similar "Fab" fragments, but differ antigenically in their "Fc" fragments. The antigenic, electrophoretic, and amino acid composition and sequence differences between isolated γ-chains of IgG1 and IgG2 may reside in their "Fc" fragments (Butler, 1969).

The particular type of globulins which are important determinants of immunologic competence at most interfaces between the internal and external environments of the body, have been designated as secretory IgA. The secretory IgA antibodies are quantitatively and probably functionally dominant in all fluids bathing the organs and systems that are in continuity with the external environment-saliva, tears, colostrum, gastrointestinal secretions, and probably the fluids elaborated by the mucoid tissues of the respiratory and genitourinary tracts.

IgA is found in serum, where there is about one IgA molecule for every five or six IgG molecules, and in external secretions where the ratio of IgA to IgG is more than 20 to one. Secretory IgA has a molecular weight of 390,000 and a sedimentation coefficient of 11S, compared with 170,000 and 7S for IgA in serum. The secretory piece does not appear to contain any immunoglobulin antigenic determinants, and it has a high carbohydrate content, i.e., about 9.5 percent.

From research that has elucidated the transfer of proteins, one can hypothesize that IgA is elaborated in the "local" plasma cells and then transported in one direction into the serum, in another direction into the secretions, with the latter acquiring the secretory piece during its passage through the epithelial membranes. Thus local production in the gastrointestinal and respiratory tracts could constitute significant synthesis of serum IgA (Tomasi, 1971).

Two other immunoglobulins, IgD and IgE have been shown to occur in man but at present not in bovine.

Lacteal Secretion

During fetal life, all mammals are totally dependent on their mother for nutrients, temperature control and protection against injury and infections. At birth, this protection is terminated (Hafez, 1968). At this time, colostrum plays a very important role in ungulates providing nutrients and protection against infections, until they are able to produce their own immunity.

The first drawn milk after parturition is known as colostrum, and milk is usually not considered normal until about the fifth day after calving (Turner, 1930). During this period the total solids, especially the globulins and other proteins, are high, whereas the lactose content is lower than in normal milk. Even greater differences between normal milk and colostrum lies in their antibody titers (Smith, 1948). The blood serum of newborn calves contains very low levels of the γ-globulins, which means that the newborn calf is highly susceptible to pathogenic organisms unless given the colostrum milk, from which it quickly absorbs the disease-resisting principle. Whether or not an adequate level of immunoglobulin in the blood of a calf is obtained depends on three factors: first, the mother must yield an adequate amount of colostrum with a sufficient high concentration of immunoglobulins. Second, the calf

must have the ability to absorb the immunoglobulins efficiently. Third, man must not act so that the transfer is blocked or unduly reduced (Kruse, 1970a).

The use of radioactive isotopes has indicated that colostral immunoglobulins are not synthesized in the udder, but in the reticuloendothelial system. These are passed from the blood serum into the udder. Therefore, prior to parturition, there is a decrease in serum globulin fraction (Dixon, William and Vazquez, 1961). Larson and Gillespie (1957) have shown that the immunoglobulins and milk albumin have properties identical to the protein of blood. These proteins apparently pass unchanged from the blood into milk. However, Jacks and Glantz (1970) found that the E. coli "O" antibodies in colostral whey samples had properties different from the dams' serum. The gamma casein is similar in nature to the globulins and occurs in high levels in colostrum, which suggested that his protein also may enter the udder performed from the blood. Smith (1971) found that the hormones estrogen and progestrone are involved in the control of selective transport of IgG1 from blood serum to lacteal fluid. Kiddy et al. (1971) found that in lacteal secretions the differences in amounts of IgG, IgG1 and IgA due to weeks and cows were highly significant. A marked drop in the mean of all of these immunoglobulins occurred at parturition. Extension studies by Dixon et al. (1961) have clearly shown a concentrating mechanism present in the alveolar cells of the mammary

gland in the cow responsible for the transfer of large amounts of immunoglobulins from serum to colostrum and milk. Kruse (1970a) found no effect of season of the year on colostrum yield, Ig percent, and Ig yield. An increase of the interval between calving and the first milking (0 to 20 hours) caused a very significant drop in colostrum Ig percent. Loss of colostrum from the udder before milking increased the probability of getting colostrum with low Ig content.

Intestinal Epithelium and Absorptive Period

The place where maternal immunoglobulins are absorbed in the newborn calf is the mucosa of the small intestine (jejunum, the last portion of the duodenum, and the beginning of the ileum) and the channels of absorption of such proteins are the lymphatics of those regions (El-Nageh, 1967a). The ultrastructure of the intestinal epithelial cells of the calf has been described prior to feeding and following exposure to marker proteins (Staley et al., 1972). Ultrastructural studies have shown a characteristic cellular organelle in the intestinal absorptive epithelial cells of all species which absorb undigested proteins from the digestive tract. This organelle is a tubular system found in the apical end of the cell variously named apical tubular system, apical canaliculi, or endocytic complex. This tubular system is responsible for engulfing immunoglobulins or colostral proteins from the digestive lumen and is a

feature common to all epithelial cells which take up undigested proteins. The apical tubular system has a limited life span and is present in the absorptive cell at 24-48 hours after birth. Staley, Jones and Marshall (1968) found the retention of the tubular system in the absence of feeding (for 42 hours) and its disappearance with feeding, which lends support to the concept that this system is important in the absorption of protein macromolecules in the newborn pig. The position of the Golgi may be instrumental in regulating the ability of the cell to absorb large molecular weight proteins.

Electron microscopic studies of the luminal border of typical absorptive cells from the newborn unfed calf have well developed microvilli (brush border). The tubular system is formed by invaginations of the plasma membrane between the microvilli and may extend into the cell for two to three microns. It is through these invaginations that immunoglobulins are transported into the cell. The ends of the tubules will enlarge and form the typical colostral vacuoles. The colostral protein is then transported from the colostrum vacuole into the lymphatic in the core of the intestinal villi (Staley, 1971).

The absorptive process can be envisioned as occuring in three steps, as immunoglobulins are transported from the gut lumen into the circulation. The first step involves the engulfing of immunoglobulins by the intestinal epithelial cell. The surface membrane of the intestinal epithelial

cell between the microvilli is extremely active in invaginations and extending into the cytoplasm for some distance as tubules. Immunoglobulins enter these invaginations and are thus carried into the cytoplasm. The second step involves enlargement of the tubular end-piece to form a vacuole. Immunoglobulin or colostral protein fills and distends the vacuole, the tubular connections are lost, and the vacuole then is transported toward the basal cell membrane. Once in contact with basal cell membrane the vacuole opens and discharges its contents into the lamina propria where it passes the lymphatic endothelium into the circulation (Staley, 1971).

Pierce and Feinstein (1965) and earlier workers, have concluded that the intestinal tract of the newborn calf absorbs various types of protein indiscriminately. Bangham et al. (1968) studied the absorption of ^{131}I labeled serum and colostral proteins from the gut of the young calf by tracer and electrophoretic techniques to find out whether the calf's gut has any selectivity for its mother's proteins. Several proteins present in the labeled serum and colostrum appeared to be absorbed with equal facility.

Hardy (1969b) found that when lymph collected from the thoracic duct during the absorption of ^{131}I-γ-globulin was injected into the femoral vein, the levels of radioactivity in the blood were close to those expected if the labeled material in the lymph had been retained within the plasma. These observations suggested that ^{131}I-γ-globulin

was absorbed into the circulation of the anaesthetized young calf without significant breakdown. Gelfiltration of lymph and plasma from calves fed ^{131}I-γ-globulin has confirmed that proteolysis before and during absorption was slight, since little ^{131}I labeled material of low molecular weight was found.

Klaus et al. (1969) found that the correlation coefficient of percent colostral IgM and percent of colostral IgG absorbed was 0.94, meaning that there was no selectivity in absorption of these proteins. However, the resulting average peak serum levels of IgM in the calf were lower than either the average level in the dam's sera or colostrum (62.9% of the sera and 53.1% of the colostrum). The average IgG level in calves sera was 84.1% of the average sera level of the dam and 60.7% of the colostrum level. There was, however, a considerable variation in serum immunoglobulin concentration between calves, and the authors concluded that there was a complete lack of correlation between concentration of immunoglobulins in calf serum and colostral whey.

The "Closure Mechanism"

The mechanism whereby intestinal protein absorption ceases has been referred to as, "closure" (Lecce, Morgan and Matrone, 1964). The mechanisms of closure are not well elucidated, and they appear to vary somewhat with species intraluminal environment, length of postnatal life, and

to some degree circulating hormones. In pigs exposure to bovine colostral whey, boiled bovine colostral whey, glucose, or dialysates of colostral whey or milk will induce closure (Lecce et al., 1964). However, the calf apparently does not respond in the same manner as the pig, for closure occurs spontaneously whether or not nursing of colostrum has occurred (Fey, 1971). In attempts to maintain calves for 48 hours without feeding by blood transfusions, -globulin was absorbed at 24 hours, but not at 36 hours (Deutsch and Smith, 1957). The process of closure apparently occurs in a retrograde fashion, that is, the basal cell membrane ceases to release the envacuolated product. Transport ceases and eventually uptake by the tubule system ceases. It is then apparent that closure may only occur to a degree dependent on the absorbable material. A factor to consider is that in the presence of invading bacteria, antigens may be carried along with the microorganism as they enter the circulation. Secondly, lack of continuity of the intestinal epithelium is of obvious consequence, in that antigens brought into contact with the denuded capillary endothelium can readily pass into the circulation (Staley, 1971).

Means of enhancing globulin absorption would appear to be a key in insuring adequate levels of circulation immunoglobulins. Deutsch and Smith (1957) reported results of several attempts to increase the period of gut permeability in the newborn calf. Administration of diethylstilbestrol

and progesterone singly and in combination with each other did not alter intestinal permeability to immune proteins. Nor was permeability affected by cortisone or ACTH injections via several routes. They also treated newborn calves with $Al(OH)_3$ gel to inhibit gastric activity and prevent gastric digestion of immune proteins. The treatment did not lengthen the time of intestinal permeability. Since the fetus was known to consume quantities of amniotic fluid during the latter part of gestation, the possibility of a permeability factor in the fluid was considered. However, feeding amniotic fluid to calves failed to extend the time of intestinal permeability to immune proteins. Smith et al. (1964) injected 30 to 50 grams of γ-globulin into the amnion via a catheter through the rectal wall. Only traces of γ-globulin were found in the serum, and these amounts were no greater than would be expected from untreated calves. Also, inhibitors of deoxyribonuclease were administered to check whether deoxyribonucleic acids were influential in maintaining permeability of the gut, but the inhibitors had no effect in delaying permeability.

Hardy (1969a) found that lactate, pyruvate, and salts of certain lower volatile fatty acids resemble factors in colostral whey in their facilitation of the absorption of γ-globulins. However, these active compounds were not found in colostrum in significant quantities. Potassium isobutyrate was the most effective of the compounds tested, and generally accelerated absorption to a greater degree

than did colostral whey itself. Since it has been found that histamine is in relatively high concentration in colostrum, Patt, Zarkower and Eberhart (1972) studied the effect that this amine promotes transfer of ingested globulin across the intestinal epithelium. They found no significant difference in serum γ-globulin between calves that received lyophilized bovine γ-globulin and those that consumed γ-globulin and histamine.

Efficiency of Immunoglobulin Absorption by the Calf

Kruse (1970b) stated, the increase in serum Ig concentration during the first 24 hours after colostrum feeding, the change in Ig percent at the 24 hour period (Ig%24), was a function of the mass of Ig fed to the calf, the age at colostrum feeding, and the birth weight of the calf. Among these three factors the mass of Ig and the age of the calf were the two predominant factors. The absorption coefficient, expressing the absorbed fraction of a given amount of Ig, was primarily determined by the age of the calf at first feeding. Thus the absorption coefficient was reduced linearly to about half by delaying the feeding from 2 to 20 hours.

In a study by Bush et al. (1971), calves were fed known amounts of colostrum at the rate of 2.5% and 3.75% of the calves body weight during the 1st and 2nd days respectively. The peak concentration of Ig in calf sera occurred at 24 hours after birth. However, the average Ig level was

significantly lower than the average dam's sera or initial colostral Ig concentration. There was a positive linear correlation between colostral Ig consumption per unit of body weight and serum Ig concentration attained in calves. These workers maintained that 68% of the variation in blood level was accounted for by the Ig intake, but also could be due to differences in absorptive efficiency or other factors. McCoy et al. (1970) found that γ-globulin level in calves fed glucose or nothing remained at a constantly low level over a 31 hour period. Calves fed pooled colostrum at 24 hours after receiving glucose or nothing did not change in the amount of γ-globulin in the serum, suggesting that the gut was impermeable to colostral proteins by the 24th hour after birth. Selman, McEwan and Fisher (1970) reported that no significant relationship was found between total suckling time during the first 8 hours and the 48 hour serum immune globulin concentration. However, they point out that such things as suckling intensity, milking out rate, and Ig concentration of colostrum are obvious variables in an evaluation of this nature.

CHAPTER III

MATERIALS AND METHODS

Experimental Plan

Thirty cows and their calves were grouped in a randomized block design, blocking on sex and breed (Holsteins and Ayrshires). The animals for the experiment were from the dairy herd at Oklahoma State University.

All cows were bled immediately after parturition and all calves were bled at the following times: immediately after birth (pre-nursing), and at 6, 12, 18, 24, 30, 36, 42, 48, 72$\pm$ 2, 96$\pm$ 2, 120$\pm$2, 144$\pm$2, and 168$\pm$2 hours after birth. The blood samples taken were examined for serum total γ-globulin, IgG concentration and packed red blood cell volume (hematocrit).

Three pooled batches of colostrum from several cows at different times after parturition were packaged in plastic coated paper cartons and stored at -20^{o}C. The three batches of colostrum had different concentrations of total γ-globulin and were fed at two different rates according to a 2 x 3 factorial arrangement of treatments (Table II).

TABLE II

DESCRIPTION OF COLOSTRUM

Batch	Date collected	Total γ-globulin concentration g/100 g whole colostrum	IgG concentration mg/ml
1	8-14-70	0.910	10.51
2	8-29-70	2.053	18.77
3	7-29-70	3.299	29.82

There were two levels of γ-globulins, 0.3 g/$W^{.75}$ and 0.6 g/$W^{.75}$. These levels were selected in order to keep in the range of a calf's consumption.

The grams of colostrum (G) required per $W^{.75}$ for each treatment were calculated by the formula:

$$G = \frac{\text{grams } \gamma\text{-globulin required/}W^{.75}}{\text{concentration } \gamma\text{-globulin (g/100 g whole colostrum)}}$$

The pounds of colostrum (P) required for each feeding of the calf was calculated by the formula:

$$P = \frac{\text{g colostrum required/}W^{.75} \times W^{.75}}{454 \text{ g/lb.}}$$

Calves were taken from their dams immediately after birth (not allowed to nurse), dried with burlap sacks and carefully weighed. The calves were bled, then fed their allotted amount of colostrum (carefully weighed to the nearest 0.01 pound on a Toledo fan scale) via nipple bottle without loss of colostrum. Colostrum was fed every 6 hours for 8 feedings. Then starting with the next regular calf feeding, at least 6 hours after last colostrum feeding, the calf was fed whole milk at the rate of 8% of its body weight per day. This milk was from a Holstein cow and did not contain any colostrum. Records were kept on fecal ratting according to a scale: (1) firm; (2) soft, not distinct pile; (3) part watery, some solid material; (4) very watery, evidence of gas, bad diarrhea. Calves which developed

diarrhea were treated by a veterinarian who avoided injection of fluids into the bloodstream. At 2 weeks after birth the calves were weighed and recorded Appendix Tables III, IV.

The blood samples were obtained by jugular venipuncture with sterile hypodermic needles (caliber 18), one and one-half inches long. The 20 to 25 ml of blood obtained were stored in clean sterile 40 ml plastic tubes at 5°C. for no longer than 12-14 hours. The samples were free of anti-coagulants or any kind of chemicals. At the time of bleeding, blood was drawn into heparinized hematocrit tubes and was immediately subjected to centrifugation to determine packed cell volume.

Laboratory Procedures

a) Colostrum

The whole colostrum was weighed. The casein was precipitated by AOAC procedures. The superanant was decanted and total protein was determined by Kjeldahl analysis for protein and calculated on basis of whole colostrum. The total γ-globulin was determined by MicroZone electrophoresis and calculated on whole colostrum basis.

In preparation for the single radial diffusion, the whole colostrum was treated in a different manner. The batches of colostrum were centrifuged at 2,100 rpm and the fat was removed. The pH of the fat free colostral was determined and 10% Acetic acid was added to bring the pH

to 4.6. This was centrifuged and the casein free colostral whey was removed. The colostral whey was brought back to the original pH with 1N NaOH. Calculations were made to bring IgG concentration back to whole colostrum basis.

b) Serum Total Protein

Blood serum was separated by centrifugation at 2,100 rpm for 15 minutes. The serum was decanted and aliquots transferred to 5 ml and 15 ml glass test tubes, and properly labeled. The 15 ml test tube of serum was analyzed for total protein and then stored at -20°C. for further analysis.

The total protein of collected sera was determined with a Bansch and Lomb refractometer using the method described by Lecoq (1962). Samples were then stored at -20°C. until more samples were obtained, before proceeding to the next steps.

c) Total γ-globulin

Essentially the same process was used for both blood serum and colostral whey samples. The samples stored at -20°C. were thawed at room temperature and electrophoresis was performed using the MicroZone system, which was fully described by Elliott (1966). The method separates each protein fraction according to the migration rate of different molecules in a electric field. The fraction of concern here was the gamma-fraction (γ-globulins), because most immunoglobulins possess gamma mobility on electro-

phoresis (Carpenter, 1965). The actual immunoglobulin concentration in both blood serum and colostral whey was calculated as percentage of γ-globulin times the grams of total protein in the sample. This is expressed in grams per 100 ml of sample.

d) IgG

The levels of IgG in the sera of the experimental animals were assayed by means of an agar-antibody diffusion test, as described by Fahey and McKelvey (1965). In this test, specific antiserum is incorporated in an agar layer on a plate. Standard preparation of the protein to be assayed, and unknown samples are placed in wells in the agar. As diffusion occurs, a precipitin ring forms around the well. The diameter of the ring is proportional to the logarithmic concentration of the reactant being assayed.

A 2.5% solution of agar was prepared in phosphate buffered saline (PBS), pH 8.0 and allowed to equilibrate to 56°C. in a water bath. Antisera specific for IgG were diluted with PBS, and optimal dilution having been determined previously. In general, IgG antisera were diluted 1 in 9. Eight ml aliquots of diluted antiserum were heated to 56°C. Clean 3½ x 4 inch glass plates were placed in a lucite frame on a leveling table, coated around the edge with agar, and were allowed to harden. Eight ml of antiserum and 8 ml of agar were mixed and rapidly poured onto the plate. Subsequently, 30 wells were punched in the agar

layer with the aid of a template. These wells were 3 mm in diameter and 10 mm apart. Antigen samples were applied with 2 x 75 mm capillary tubes. Random wells on each plate contained standard serum, with known IgG concentration. The remainder of the wells were filled with the serum samples to be assayed.

The plates were incubated in closed humid boxes for 18-24 hours at $4^{o}C$. These plates were then removed and photographs were taken on each plate (Figure 1). The diameter of the precipitin rings were measured to the nearest 0.1 mm with the aid of a Bansch and Lomb magnified scale. A standard curve was prepared by plotting the immunoglobulin concentration of the standard serum samples (in mg/ml) against the size of the precipitin ring (in mm) on semi-logarithmic graph paper. The unknown serum was assayed in duplicate, and a mean value calculated. Concentration of IgG in the samples was determined by reference to the standard curve, defined by the equation: $\hat{Y} = 9.713 + 4.713X$, where $\hat{Y}$ is the corrected reading for the unknown, X is the $\log_{10}$ of concentration of IgG in unknown sample, and 9.713 and 4.713 are derived from the regression formula.

The antiserum and standard IgG serum were commercially prepared by Cappel Laboratory. To insure purified IgG in both samples, microimmunoelectrophoresis was used following the methods of (Scheidegger, 1955; Klaus, 1964). In Figure 2 rabbit antiserum to bovine IgG was placed in the trough and the standard IgG serum was placed in the bottom well.

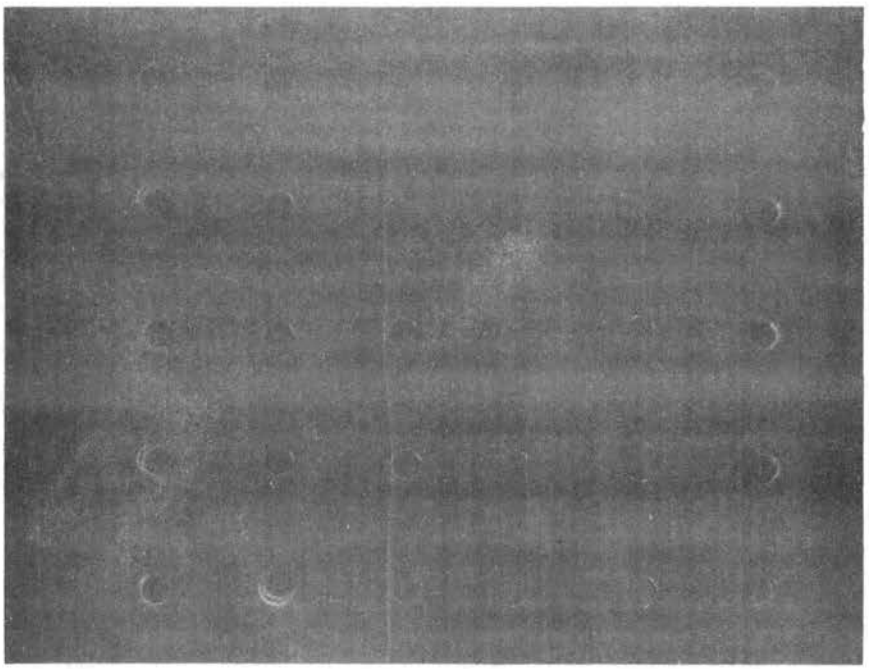

Figure 1. Single Radial Diffusion Plate for IgG

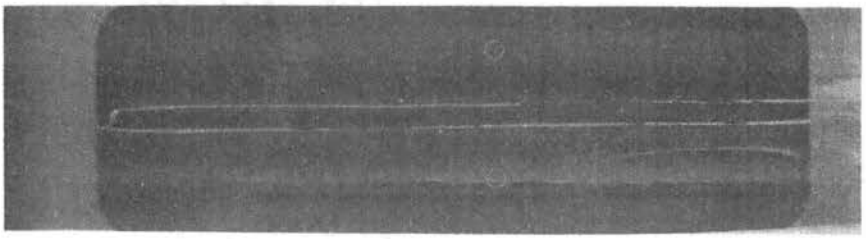

Figure 2. Microimmunoelectrophoresis for IgG Antiserum Against IgG Standard Serum

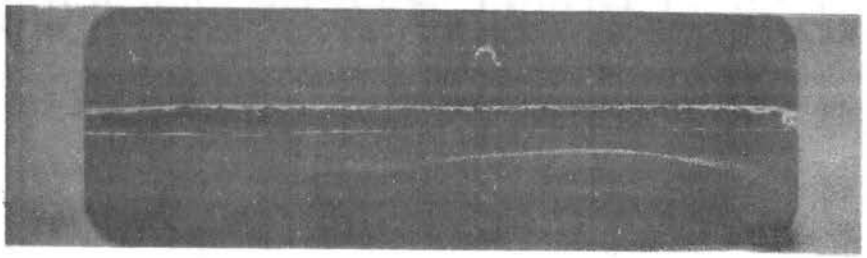

Figure 3. Microimmunoelectrophoresis for IgG Antiserum Against a Post-Colostral Serum Sample

In Figure 3 rabbit antiserum to bovine IgG was placed in the trough and a post-colostral serum sample from calf 60 was placed in the bottom well. In Figure 4 rabbit antiserum to bovine immunoglobulins was placed in the trough, a pre-colostral serum sample from calf 81 was placed in the top well and the standard IgG serum sample was placed in the bottom well. According to Butler (1969) the antiserum and standard IgG serum was pure according to this method.

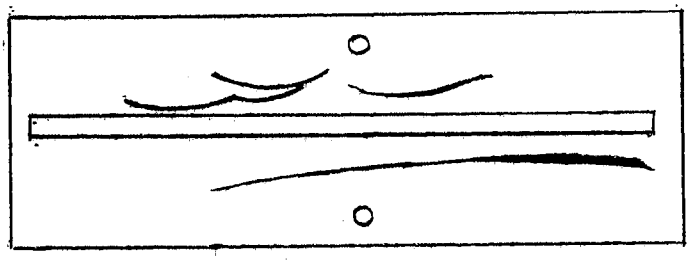

Figure 4. Microimmunoelectrophoresis for Bovine Immunoglobulin Antiserum IgG Standard Serum

CHAPTER IV

RESULTS AND DISCUSSION

There was essentially no relationship between the concentration of either total γ-globulin or the specific fraction, IgG, in the blood serum of the dams and that of their calves at birth (Appendix Table VI). Correlations (r) between dams and calves were .02 for both γ-globulin and IgG. This agrees with similar results reported by Smith et al. (1967) and Bush et al. (1971). The IgG levels in the cow serum were comparable to those found by Klaus et al. (1969), and Penhale and Christie (1969). It was noted by Dixon et al. (1961) that these values at parturition are a doubtful representation of normal adult cattle. Kiddy et al. found that IgG was lower before parturition than after calving. IgG levels in the precolostral calf serum samples also were within the same range (.007 to 4.70) as those found by Klaus et al. (1969). This low correlation gives further evidence to the fact that there is very little placental transfer in the bovine.

It is apparent from the present study and those of Pierce and Feinstein (1965), Klaus et al. (1969), and Bush et al. (1971), that the calf is not totally agammaglobulinaemic at birth. Schultz, Dunne, and Heist (1971) found

IgG containing cells at 145 days of gestation in a bacterial and viral infected fetus. Perhaps small amounts of immunoglobulins are synthesized by the bovine fetus, as has been found in human fetus (Van Furth, Schuit and Hijmans, 1965) and in rat and chicken embryos (Reade, Jenkin and Turner, 1965).

The calves had very low serum immunoglobulin concentrations prior to nursing, but in most calves the concentration increased after the ingestion of colostrum. There was a wide range in concentration at each time of sampling (Appendix Table V); however, peak concentration was reached at an average of 28 hours, with a standard deviation of 8 hours. There seemed to be little evidence of different peak times in serum concentrations due to different concentrations of γ-globulin in colostrum or feeding levels. The peak was the same for total γ-globulin (28.8 hours) for high and low feeding level; also 29.2 and 26.8 hours, respectively, for the IgG peak times (Figure 5). The overall range was from 6 to 42 hours after birth. Thereafter, immunoglobulin concentration tended to decrease until 168 hours, when the last samples were taken. It should be noted that colostrum feeding ceased at 42 hours. Therefore, through degradation, and the fact that the calf was not yet able to generate its own immunoglobulins, the serum concentration of γ-globulin and IgG would decrease.

In 20 out of the 30 calves (67%), serum immunoglobulins concentration remained below 0.5 g/100 ml of blood serum

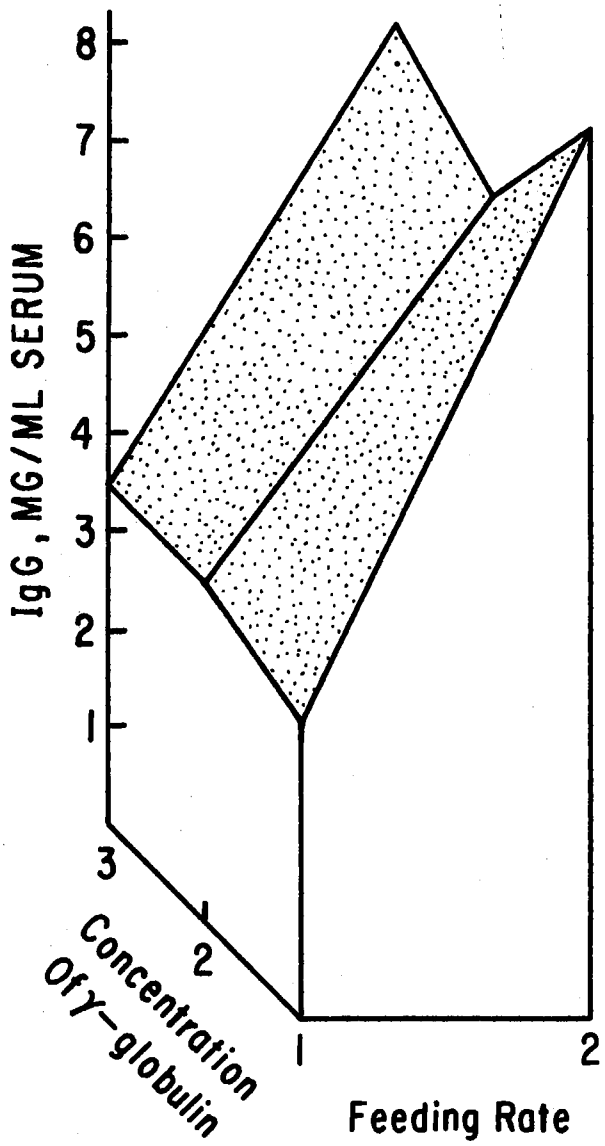

Figure 5. Graph of Serum IgG at 24 Hours

throughout the period of study. However, the results were obtained under conditions where γ-globulin intake was low, to keep the volume of colostrum at a level such that each calf could consume its allotted amount. The amount of colostral immunoglobulins consumed, at the two levels prior to 24 hours, were 22.6 g and 45.2 g per 50 kg of body weight. Similar γ-globulin values at 24 hours were reported by Bush et al. (1971) and Kruse (1970b), for calves consuming approximately the same amount of γ-globulin as fed in this trial. The serum γ-globulin and IgG values averaged over all periods were highest in calves fed colostrum with lowest concentration at the higher level, .6 $g/W^{.75}$, and lowest for the lower concentration and lower feeding level (Figure 6 and Appendix Table VII). One calf (No. 60) had an unusually large amount of γ-globulin at birth, and after feeding, serum concentration increased to a level comparable to the dam's serum. The high IgG concentration in this calf agreed with several of the values found by Klaus et al. (1969). Also, the calves with lower serum IgG values compared to values obtained by Klaus et al. (1969), and Penhale et al. (1970).

An analysis of variance was made on the data for globulin and IgG, over all periods and also for individual periods. Since there was no significant interaction ($P > .01$) between periods and treatments, particular attention to treatment effects for individual periods was justified (Appendix Table VIII). Analysis was made on

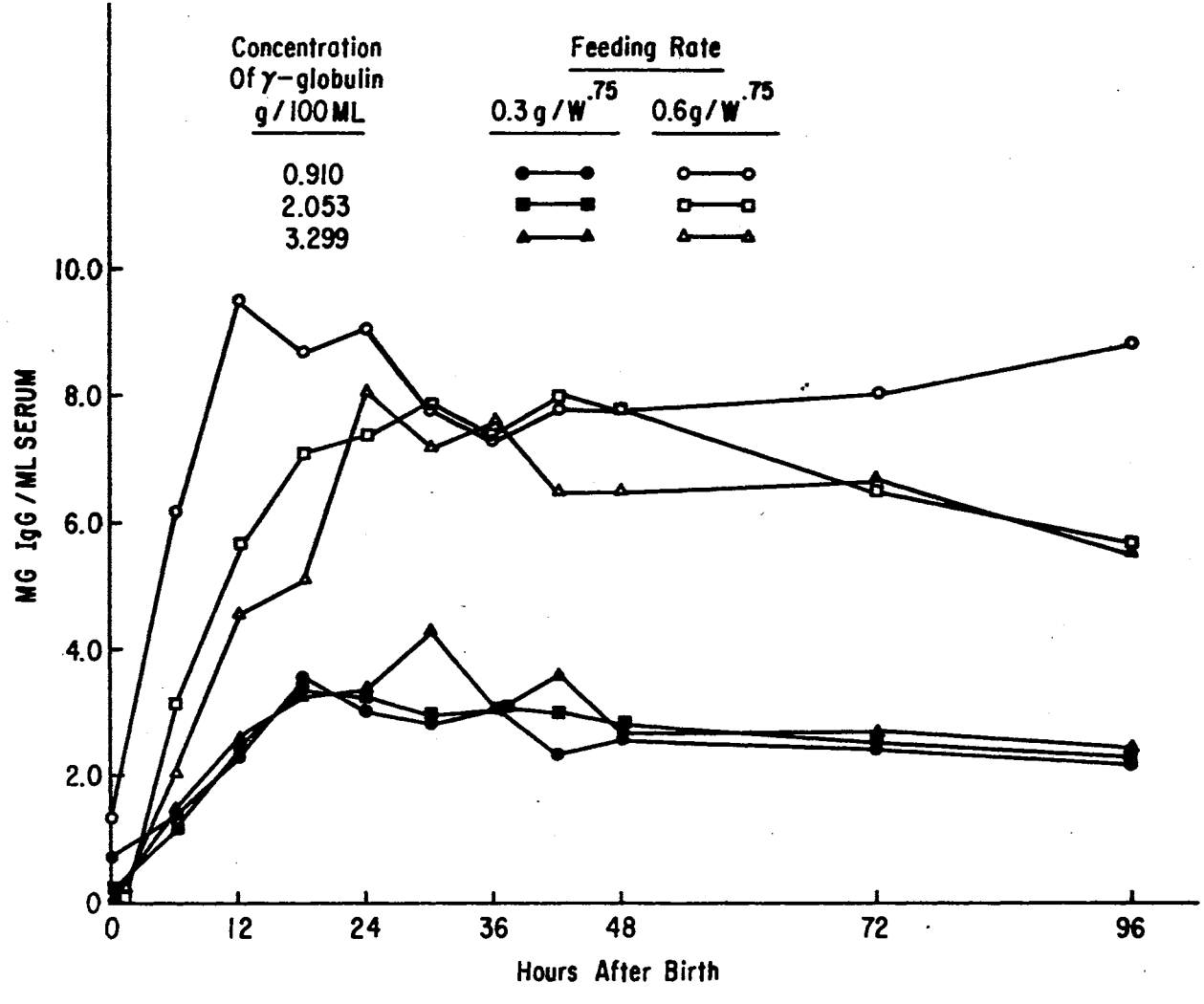

Figure 6. Graph of Mean IgG of Different Times

the data at 24 hours (Table III), which was within the range of peak absorption of immunoglobulins. It was found that there was a significant difference ($P < .01$) between intake levels in mean serum immunoglobulin concentration of both γ-globulin and IgG. The amount of colostrum fed to a 40.86 kg calf ranged from 1.06 kg at low γ-globulin concentration and high feeding level, down to .145 kg of colostrum and at the high γ-globulin concentration and low feeding level. There was no significant difference ($P > .05$) in serum concentration in colostrum. Therefore, one can assume that the increased level of other milk constituents, as immunoglobulins decreased, did not have any significant effect on the absorption of the immunoglobulins. At the concentrations of γ-globulin fed in this study, one can assume that the other constituents in colostrum did not seem to tie-up the absorptive sites for the immunoglobulins. However, one could not say whether at lower levels of immunoglobulins this would still hold true. If one would feed a lower concentrated colostrum there would probably be a problem in getting the calf to consume enough of the colostrum. In practice, a calf that consumes a small amount of colostrum may be getting as high a level of immunoglobulins as a calf that consumes a larger amount of colostrum. It depends on the γ-globulin concentration in the colostrum. There was not a significant difference ($P > .05$) between blocks. One could assume that breed or sex had little effect on the amount of immunoglobulins in

TABLE III

ANALYSIS OF VARIANCE ON TOTAL γ-GLOBULIN AND IgG IN BLOOD SERUM AT 24 HOURS

Source	d.f.	Sum of squares	Mean squares	F value	Expected mean square
			Total γ-globulin		
Blocks	4	0.3371	0.0843	0.213	
Feeding level	1	0.5045	0.5045	12.771	$\sigma_\epsilon^2+5\,\sigma_{FxC}^2+15\,\sigma_F^2$
Concentration	2	0.0270	0.0135	0.343	$\sigma_\epsilon^2+5\,\sigma_{FxC}^2+10\,\sigma_C^2$
Feed x concentration	2	0.0964	0.0482	1.220	$\sigma_\epsilon^2+5\,\sigma_{FxC}^2$
Error	20	0.7901	0.3950		σ_ϵ^2
			IgG		
Blocks	4	35.45	8.86	0.854	
Feeding level	1	185.85	185.85	17.902	$\sigma_\epsilon^2+5\,\sigma_{FxC}^2+15\,\sigma_F^2$
Concentration	2	1.94	0.97	0.094	$\sigma_\epsilon^2+5\,\sigma_{FxC}^2+10\,\sigma_C^2$
Feed x concentration	2	5.40	2.70	0.260	$\sigma_\epsilon^2+5\,\sigma_{FxC}^2$
Error	20	207.63	10.38		σ_ϵ^2

the calf serum. However, due to the small number of experimental units and the fact that only a small sample of each breed was represented, one might question the validity of this estimate. The calves were born in the fall, winter and spring so that the season and management were confounded in the blocks.

By reference to the expected mean squares, (Table III) it was possible to estimate the amount of total variance attributable to different factors. Since the variance due to concentration and to interaction was essentially zero, the fraction of total variance attributable to feeding level could be estimated. Thus, about 50% of the variation in blood serum immunoglobulins was attributed to the level of feed. Bush et al. (1971), however, found that 68% of the variation in blood values were attributable to the amount of immunoglobulin consumed. The large amount of variation in blood γ-globulin and IgG levels may be partially accounted for by the weight of the calf and the low intake level of immunoglobulins. If a malabsorption problem exists, an experiment designed to block on body weight and level of immunoglobulin intake would give more evidence toward this problem.

The efficiency of absorption of the γ-globulin and IgG intake at 0, 6, 12 and 18 hours after birth was found by calculating the amount present in serum at 24 hours after birth, using McEwan, Fisher and Selman (1968) blood plasma volume in the calf. The overall efficiency for γ-globulin

and IgG were 33.2±17.4% and 66.3±31.0%, respectively (Appendix Table IX). The abosorption coefficients for the low feeding level were 29.2±11.0% for total γ-globulin and 57.7± 27.5% for IgG. For the high level of feeding it was 37.3± 21.1% for total γ-globulin and 74.9%±45.5% for IgG. The absorption coefficients for the low concentration of γ-globulin and 60.4±30.% for IgG. For the intermediate concentration it was 31.9±8.9% for total γ-globulin and 70.9±32.2% for IgG. For the high concentration it was 33.0±10.2% for total γ-globulin and 75.9±25.7% for IgG. Pierce and Feinstein (1965) found that although bovine colostrum contains IgM, IgA and electrophoretically fast IgG, slow IgG is virtually absent, and thus does not appear in the serum of the suckling calf. This accounts for the wide difference in the absorption efficiency of total γ-globulin and IgG. The total γ-globulin values obtained were within the same range (43%) as those found by Kruse (1970a,c). The IgG values for absorption were very similar (60%) to those found by Staley et al. (1971). One calf (No. 60) had a very high absorptive efficiency, i.e., 99%. This could mean that this calf generated its own immunoglobulins.

Blood serum immunoglobulin concentration in the calves and incidence of diarrhea did not seem to be related in this experiment, in contrast to reports by Fey and Margadent (1962), Gay et al. (1965) and Smith (1962). There did not seem to be any evidence of a trend of increased incidence of diarrhea for either feeding levels or concentrations

of immunoglobulin in the colostrum. However, after colostrum feeding was discontinued, the incidence of diarrhea increased.

There seemed to be a trend toward a higher packed red blood cell volume (10% higher) as the calves approached a diarrhea stress condition. This could be explained by the loss of body fluids through diarrhea.

In considering the relationship of blood serum immunoglobulins to disease resistance in this experiment, the results should be weighed with the facts: with the low levels of immunoglobulins fed, there was a low level of immune globulins attained; the number of experimental units was small; and no control of exposure to pathogenic organisms was performed.

CHAPTER V

SUMMARY AND CONCLUSIONS

Serum γ-globulin and IgG was measured in calves fed colostrum in a 2 x 3 factorial arrangement of treatment. Concentrations of total γ-globulin and IgG ranged from .056 to .414 g/100 ml, respectively. There was a low correlation between the blood samples of the cows and calves. Peak immunoglobulin concentration serum of the calves occurred at an average time of 28 hours after birth with little difference in treatments. Twenty calves were hypogammaglobulinaemic throughout the period of study. This could be accounted for by the low feeding level. There was a significant difference ($P < .01$) of serum γ-globulin and IgG for feeding level, but not ($P > .05$) for the concentration γ-globulins. Fifty percent of the variation in serum values was due to feeding level. There was a wide variation in absorption efficiency (33.2$\pm$17.4%) for γ-globulin and (66.3$\pm$31.0% for IgG. These results support the conclusion that the immunoglobulin content of a cow's colostrum is the most important aspect in level of immunity obtained by a calf.

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APPENDIX

TABLE IV

GENERAL INFORMATION ABOUT CALVES USED IN THE EXPERIMENT

Block	Animal No.	Body Weight kg Birth	Body Weight kg 14-day	Breed	Sex	Date of Birth	Treatment Group
1	33	43.13	41.77	Hol.	M	10- 9-70	1
2	352	39.95	38.59	Hol.	F	10-30-70	1
3	84	38.59	37.68	Ayr.	M	2- 7-71	1
4	357	34.50	34.50	Ayr.	F	11- 6-70	1
5	389	28.60	27.24	Ayr.	F	2- 8-71	1
1	41	39.93	40.41	Hol.	M	10-29-70	3
2	373	49.49	46.76	Hol.	F	12- 7-70	3
3	37	29.96	29.51	Ayr.	M	10-19-70	3
4	369	39.04	34.50	Ayr.	F	12- 1-70	3
5	399	36.32	35.87	Ayr.	F	4-25-71	3
1	72	41.77	39.04	Hol.	M	1- 5-71	5
2	355	37.68	41.77	Hol.	F	11- 5-70	5
3	87	31.78	28.60	Ayr.	M	2-17-71	5
4	350	27.24	27.69	Ayr.	F	10-25-70	5
5	397	36.32	36.77	Ayr.	F	3-30-71	5

TABLE IV (Continued)

Block	Animal No.	Body Weight kg Birth	Body Weight kg 14-day	Breed	Sex	Date of Birth	Treatment Group
1	60	47.22	46.76	Hol.	M	12-10-70	2
2	370	42.22	40.41	Hol.	F	12- 3-70	2
3	36	38.59	37.68	Ayr.	M	10-16-70	2
4	368	37.22	36.32	Ayr.	F	11-30-70	2
5	395	37.68	38.59	Ayr.	F	3-16-71	2
1	51	47.67	47.22	Hol.	M	12- 1-70	4
2	351	43.13	41.77	Hol.	F	10-28-70	4
3	61	42.68	40.41	Ayr.	M	12-11-70	4
4	372	31.33	30.42	Ayr.	F	12- 6-70	4
5	390	34.05	34.05	Ayr.	F	2-12-71	4
1	69	44.04	39.95	Hol.	M	1- 2-71	6
2	365	39.95	35.41	Hol.	F	11-23-70	6
3	94	34.50	32.68	Ayr.	M	3- 1-71	6
4	361	36.77	36.32	Ayr.	F	11-19-70	6
5	381	38.13	34.50	Ayr.	F	1- 6-71	6

TABLE V

PEAK ABSORPTION PERIODS AND CALF PERFORMANCE

Calf No.	Time of Maximum Ig Level (hrs.)	Maximum Ig Level in Blood (gm/100 ml)	Time of Maximum IgG Level (hrs.)	Maximum IgG Level in Blood mg/ml	Diarrhea Rating Avg.	Diarrhea Treatment
33	24	.396	24	2.929	1.90	day 8
352	36	.322	24	4.740	1.77	day 11
84	18	.374	18	3.512	1.00	
357	30	.405	36	3.159	2.50	days 7-8
389	6	.418	18	4.343	1.52	
41	30	.450	30	3.651	1.76	days 9 & 10
373	30	.423	24	4.850	2.44	days 6-10
37	36	.430	30	3.943	2.07	day 8
369	42	.288	24	2.139	2.38	days 3-6
399	24	.474	42	4.880	1.29	
72	18	.422	30	2.454	2.60	days 8-9
355	24	.393	30	3.035	1.83	days 2 & 4
87	36	.554	24	4.614	2.69	day 6
350	42	.361	30	7.819	1.07	
397	36	.492	18	5.164	1.00	

TABLE V (Continued)

Calf No.	Time of Maximum Ig Level (hrs.)	Maximum Ig Level in Blood (gm/100 ml)	Time of Maximum IgG Level (hrs.)	Maximum IgG Level in Blood mg/ml	Diarrhea Rating Avg.	Diarrhea Treatment
60	24	1.596	24	21.402	2.07	days 9-10
370	24	.685	12	16.631	2.25	days 2-5
36	24	.533	24	6.579	1.74	days 7 & 8
368	18	.342	18	5.497	2.26	days 4-6
395	42	.556	24	6.057	1.0	
51	24	.628	42	7.885	1.85	days 5-7
351	30	.765	42	10.135	1.48	day 3
61	18	.629	30	8.257	2.18	days 2-8
372	24	.547	30	8.108	1.6	
390	24	.546	30	8.877	1.18	
69	36	.691	42	7.139	2.34	
365	42	.731	24	12.936	1.89	days 3 & 4
94	42	.658	36	8.681	2.1	day 6
361	24	.537	30	7.529	2.2	day 3
381	36	.393	30	7.214	1.89	days 7 & 8

TABLE VI

COW-CALF COMPARISON

Calf No.	Cow No.	γ-globulin		IgG		Hematocrit	
		Cow	Calf	Cow	Calf	Cow	Calf
		g/100 ml		mg/ml		%	
33	977	1.172	.163	14.29	.088	37.0	33.5
352	026	.945	.216	11.34	.079	44.0	43.5
84	997	1.172	.180	10.54	.059	29.0	45.0
357	094	1.534	.170	12.46	.059	37.0	42.5
389	948	1.888	.202	16.23	.088	40.0	48.0
41	144	1.160	.249	9.83	.207	44.0	40.0
373	640	2.368	.202	22.38	.072	54.0	39.5
37	875	1.581	.165	15.45	1.331	50.0	36.0
369	593	2.166	.129	18.35	.059	41.0	42.0
399	615	3.045	.265	20.52	.065	41.0	39.0
72	102	1.440	.197	19.88	.054	38.0	45.0
355	077	1.876	.241	15.06	.442	34.0	40.0
87	488	2.013	.193	15.75	.263	44.0	40.0
350	135	1.809	.220	21.29	.065	50.0	40.0
397	789	2.077	.226	15.89	.007	37.5	40.0
60	996	1.846	.414	14.71	4.694	32.0	43.0
370	864	1.775	.206	13.07	2.034	36.5	42.5
36	884	1.512	.277	12.59	.059	35.0	39.0
368	802	1.988	.245	17.46	.065	45.0	40.0
395	721	2.343	.270	15.89	.136	40.5	42.0
51	862	1.782	.333	15.04	.396	48.0	40.0
351	011	2.520	.144	22.09	.150	40.5	30.0
61	950	1.011	.056	14.65	.065	46.0	43.0
372	115	1.836	.258	18.63	.088	49.0	41.0
390	776	2.044	.257	14.67	.072	43.5	51.5
69	003	.844	.234	13.95	.054	34.0	37.5
365	521	2.135	.228	24.51	.128	40.0	34.0
94	773	2.844	.184	17.74	.010	37.5	46.0
361	869	1.675	.164	20.91	1.086	48.5	42.5
381	791	1.904	.188	24.96	.079	38.0	41.5

TABLE VII

MEAN SERUM IMMUNOGLOBULIN

	Treat-ment	Period (hours after birth) 0	6	12	18	24	30	36	42	48	72	96	120	144	168	Mean
ϒ-globulin g/100 ml	1	.187	.258	.305	.293	.318	.340	.308	.327	.291	.315	.319	.338	.342	.328	.305
IgG mg/ml	1	.075	1.455	2.318	3.262	3.012	2.851	3.019	2.368	2.632	2.414	2.245	2.315	1.922	2.042	2.281
ϒ-globulin g/100 ml	2	.283	.623	.676	.686	.733	.652	.630	.650	.754	.647	.652	.640	.664	.602	.635
IgG mg/ml	2	1.397	6.233	9.554	8.744	9.111	7.828	7.337	7.804	7.883	8.053	8.917	7.061	6.675	6.623	7.373
ϒ-globulin g/100 ml	3	.202	.265	.301	.277	.352	.382	.344	.372	.337	.344	.282	.368	.367	.319	.322
IgG mg/ml	3	.347	1.209	2.595	3.398	3.416	3.001	3.012	3.022	2.836	2.584	2.441	2.386	2.398	2.082	2.481
ϒ-globulin g/100 ml	4	.210	.249	.397	.532	.566	.528	.509	.498	.554	.520	.480	.479	.510	.440	.466
IgG mg/ml	4	.155	3.241	5.810	7.177	7.462	7.943	7.406	8.068	7.89	6.545	5.77	6.235	6.219	7.019	6.210
ϒ-globulin g/100 ml	5	.216	.249	.303	.339	.391	.365	.404	.362	.363	.337	.316	.341	.280	.331	.328
IgG mg/ml	5	.180	1.544	2.671	3.316	3.40	4.388	3.015	3.644	2.722	2.827	2.461	2.297	2.512	2.517	2.678
ϒ-globulin g/100 ml	6	.199	.341	.409	.464	.539	.521	.526	.559	.493	.524	.553	.539	.500	.473	.474
IgG mg/ml	6	.289	2.133	4.647	5.145	8.189	7.296	7.602	6.574	6.504	6.759	5.644	5.406	5.293	5.192	5.477

TABLE VIII

ANALYSIS OF VARIANCE FOR ALL PERIODS

Source	d.f.	Sum of squares	Mean squares	F value
		γ-globulin		
Blocks	4	2.690	0.673	1.6508
Feed	1	4.479	4.479	10.9930
Concentration	2	0.493	0.246	0.6049
Feed x concentration	2	0.803	0.401	0.9853
Error A	20	8.148	0.407	
Period	13	1.866	0.143	20.4427
Feed x period	13	0.356	0.027	3.9048
Concentration x period	26	0.163	0.006	0.8940
Feed x conc. x period	26	0.160	0.006	0.8767
Error B	312	2.190	0.007	
		IgG		
Blocks	4	282.58	70.64	0.805
Feed	1	1575.26	1575.26	17.950
Concentration	2	40.38	20.19	0.230
Feed x concentration	2	93.12	46.56	0.530
Error A	20	1755.50	87.76	
Period	13	752.53	57.89	41.107
Feed x period	13	131.33	10.10	7.174
Concentration x period	26	60.56	2.33	1.654
Feed x conc. x period	26	51.86	1.99	1.416
Error B	312	439.36	1.41	

TABLE IX

ABSORPTIVE EFFICIENCY

Calf No.	γ-globulin absorptive efficiency %	IgG absorptive efficiency %
33	46.73	17.00
352	19.79	79.24
84	32.30	40.54
357	13.78	37.38
389	15.21	38.45
41	31.40	63.37
373	31.70	107.90
37	23.70	18.66
369	18.55	44.51
399	39.66	84.04
72	36.51	46.05
355	29.71	39.15
87	50.80	88.32
350	24.21	75.86
397	23.74	84.07
60	120.39	147.35
370	47.59	49.95
36	24.94	30.75
368	5.81	30.22
395	19.78	49.62
51	30.46	73.21
351	48.12	96.16
61	42.27	76.89
372	26.69	75.15
390	27.33	69.51
69	43.60	73.42
365	34.52	137.91
94	34.58	78.47
361	35.62	64.95[b]
381	17.36[a]	70.84

[a]Mean 33.2% standard deviation 17.4%

[b]Mean 66.3% standard deviation 31.0%

VITA

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