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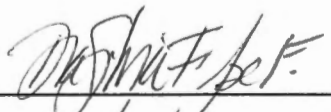
Signature 

Date 7/2/92

# SERUM IMMUNOGLOBULIN CONCENTRATION IN RESPONSE TO MATERNAL COLOSTRUM OR COLOSTRAL SUPPLEMENTATION IN DAIRY CALVES

A Thesis

Presented for the

Master of Science

Degree

The University of Tennnessee, Knoxville

Silvia Abel

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## DEDICATION

This thesis is dedicated to God as a small present for all the blessings He has given me. For being my best friend and the source of my peace and joy.

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## ABSTRACT

Thirty-three Holstein calves were removed from their dams prior to nursing and were assigned alternately to 2 L of maternal colostrum with or without 125 g colostral replacer. Calves were fed after 0 h blood sample and 12 h later. Milk replacer was fed after 24 h (4 l/d). Blood was obtained as soon as possible after birth and at 12, 24, 48, and 72 h and at 28 and 56 d of age. Maternal colostrum, colostral replacer and serum were analyzed for $IgG_1$, $IgG_2$, and IgM by radial immunodiffusion. Serum Ig concentration did not differ ($P > .10$) among treatments and increased to 24 h. Total serum Ig and $IgG_1$ concentrations were affected by BW and intake of Ig, which accounted for 76 and 72% of variation in total Ig and $IgG_1$ respectively. Significant differences due to sex were found for $IgG_2$ and IgM without differences due to BW. Negative relationship between $IgG_2$ and IgM was observed. When calves were fed good quality colostrum (total Ig > 30 mg/ml), serum $IgG_2$ concentration tended to be greater in supplemented calves ($P < .10$), while serum IgM concentration was greater in calves fed maternal colostrum. Apparent efficiency of absorption of $IgG_1$ and IgM were higher in calves fed maternal colostrum than in supplemented calves ($P < .10$).

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# PART I

# INTRODUCTION

There is a significant economic loss in the dairy industry due to high pre-weaning calf mortality. In a 1974 New York study, annual calf losses in herds with less than 100 cows averaged 15.8%; herds with more than 200 cows averaged 27.2% (26). Several studies (3, 15, 45, 46, 51, 70, 75) have shown a significant correlation between calf mortality and management of the newborn during the first days of life. Birth location, feeding management, rolling herd average, and the person caring for calves are some factors affecting calf mortality (61). High rates of morbidity and mortality are associated specifically with low immunoglobulin **(Ig)** concentration in serum of the newborn (54).

The most common causes of morbidity and mortality in young calves are pneumonia and Escherichia coli infections (colisepticemia and enteric colibacillosis; 26, 46). Since the calf is born hypo- or agammaglobulinemic (2, 6, 39, 61), the introduction into an environment where it will be exposed to microorganisms represents a period of high susceptibility to disease. Passive immunity may be acquired through transfer of Ig in colostrum (3, 6, 10, 12, 36, 72). Colostrum, the secretion from the mammary gland of the cow during the first 3 d post-parturition, plays an important role in calf health and survival (4, 5, 33, 51, 69).

Nocek et al. (51) reported that calves deprived of colostrum gained poorly and suffered severe and long

episodes of scours and high mortality. Calves fed colostrum with high total Ig (> 60 mg/ml) gained weight from birth to day 4 while those fed low total Ig (< 40 mg/ml) colostrum lost weight. Quality of colostrum (evaluated by Ig concentration) is a major factor in transfer of passive immunity to the newborn (65). Furthermore, Ig may provide local protection, which is effective in the intestinal tract following closure (2, 51). The transfer of passive immunity from the dam to its offspring through absorption of intact colostral Ig has been documented and reviewed recently (40, 64, 73).

In some circumstances, sufficient maternal colostrum may not be available for calves within 24 h after birth, or low Ig colostrum might be provided by the dam. Products containing high concentrations of Ig extracted from dried cheese whey or natural colostrum have been introduced to replace colostrum or to increase Ig content of maternal colostrum to levels recommended for feeding (29). On the other hand, feeding excess Ig may impair the development of active immunity (14, 17) and reduce efficiency of absorption (3, 57, 67). Effects of these products on acquisition of passive immunity have not been determined precisely.

# PART II

# LITERATURE REVIEW

## Transmission of Antibodies

An animal is normally exposed to a wide variety of organisms which may enter the body through the mucous surfaces of digestive, respiratory or urogenital tracts. Once an organism enters the natural barriers of the body, the presence of specific antigens initiates antibody production. This process begins regardless of the state or quantity of organism and determines whether the animal will exhibit an effect, i.e., developing the disease (6). Immunoglobulin (**Ig**) is a general term assigned to a family of high molecular weight proteins that possess electrophoretic mobility and share common physical and chemical characteristics and antigenic determinants (11, 59). They are found in the serum and other body fluids. The three Ig types found in blood and colostrum of cattle are IgG, with two subclasses, $IgG_1$ and $IgG_2$, IgA, and IgM. Each Ig has different serological activity, allotype, ability to cross membranes, and affinity for cells (11, 25).

Antigen is a substance which can stimulate a specific immune response and react specifically with antibodies or other cells (6, 25). Antibody, on the other hand, is a specific Ig produced in response to antigen (25). If production of antibody is initiated, subsequent exposure to the same antigen normally results in a greater and lasting production of antibody in blood; this process is called active immunity. Production of antibody continues

throughout life, though in gradually declining quantities unless the animal is re-exposed. The sum of past antigenic experience is reflected in the complement of antibody in the circulation (6). Passive immunity is the transfer of antibodies from an immune individual to the same or another species, thereby providing temporary resistance to disease.

**Transmission of Antibodies Before Birth**

Hypogammaglobulinemia, a condition characterized by low levels of Ig in blood, occurs in newborn ruminants, pigs, and foals. Passive immunity is acquired by consumption and absorption of colostral Ig (6, 39). Agammaglobulinemia is a complete absence of Ig. The calf is born hypo- or agammaglobulinemic due to lack of placental Ig transmission (6). Early studies (6, 34) reported that presuckling calves were negative for both agglutination and fixation tests for Brucella abortus, although their dams were positive reactors. Although these studies suggest no placental transfer of maternal Ig, others (35, 54) reported that calves are not devoid of Ig at birth, the newborn is immunocompetent, and Ig synthesis commences in the fetus (54). However, it is widely reported that Ig appear in the blood of the calf only after ingestion of colostrum (39, 61).

**Transmission of Antibodies After Birth**

Colostrum

Bovine colostrum is a combination of lacteal secretions and serum constituents, especially Ig and other serum proteins, that accumulate in the mammary gland before parturition (21). During the last trimester of gestation, Ig are transferred to colostrum, and some synthesis occurs within mammary tissue (6). All IgG and most IgM is transferred from serum, whereas IgA is synthesized predominantly (60%) by plasmocytes in the mammary gland (60, 62). Asconas et al. (1) reported that Ig passes from the blood to milk or colostrum without degradation and re-synthesis. As parturition approaches protein concentration of colostrum increases, and concentration of Ig increases to half of total protein (23). Other serum protein are incorporated into colostrum also, but at lower levels than Ig (6). Concentration of Ig in colostrum exceeds that in serum, whereas Ig concentration in milk is comparatively very low (2). Colostral Ig accounts for about 6% of first milking, decreasing rapidly to approximately .09% Ig by six milkings (3 days post-partum) (2). Concentration of Ig in colostrum average 80 to 90% IgG, 5 to 10% IgM, and 5 to 10% IgA (61).

Composition of colostrum during the first 72 h post-partum varies markedly from that of whole milk; colostrum is a good source of protein (particularly Ig), vitamins A, D,

E, $B_{12}$ and iron (2). Dry matter (total solids) in colostrum consists of protein, carbohydrate (mainly lactose), fat, minerals (ash) and vitamins (61). Total solids, fat and protein percent for first-milking colostrum averaged 23.9, 6.7, and 14.0%, respectively (21). After six post-partum milkings, total solids, fat, and protein dropped to 12.9, 4.0 and 3.1 %, respectively (21). It is also during this time that lactose concentration increases (53). Movement of water in digestive tract of the calf is affected by amount of dry matter in colostrum. Since Ig are absorbed intact from colostrum to blood for the first few hours after birth, water from intestinal cells may replace absorbed molecules (52). The high concentration of total solids in colostrum facilitates this osmotic transport (52). Several factors may affect total concentration of Ig in colostrum, including parity, breed, pre-partum milking, yield, and ambient temperature (61).

Studies have suggested that differences exist among dairy breeds in concentration of Ig and Ig isotype in colostrum (55). A study among the five principal dairy breeds in the U.S. reported that the Jersey breed had the highest Ig concentration and averaged 6.65%, 1.86%, and .53% IgG, IgA, and IgM, respectively (49). Holsteins had the lowest IgG concentration (4.12%) whereas Guernseys had lowest IgA (.9%) and IgM (.39%) (49). Numerous health problems and calf mortality with Guernsey calves suggest a

possible relationship with lower IgA and IgM concentrations. Guernsey calf mortality was 18.2%, while mortality in Holstein herds was 13% (32). Because small numbers of animals were used in some breeds (n = 5 for Jersey cattle), additional studies are required.

Amount of Ig transported into colostrum depends on Ig concentration in serum concentration of the dam. Serum Ig is a cumulative index of the of diseases to which the animal has been exposed. It has been reported (49) that the concentration of colostral Ig tended to increase with advancing lactation number. Muller and Ellinger (49) reported that colostral Ig was lower in first-calf heifers (5.7%) than in third (7.9%) and fourth (7.5%) lactation cows.

Amount of colostrum produced has been correlated to Ig concentration (61). High producing cows have lower Ig concentration than lower producing cows (37). Yield of colostrum, and yield of Ig at first milking after calving have large individual variation (37).

Two primary factors that determine amount of Ig absorbed by the calf are amount of colostrum fed and age at first feeding (9, 22, 38, 69). Studies have reported a linear relationship between the amount of Ig ingested (a product of colostral volume and Ig concentration), and serum Ig concentration (9, 38). Bush et al. (8) found that variation in serum Ig in calves was attributable to

differences in amount of Ig consumed per unit of body weight (BW).

Procedures for feeding colostrum have been made to improve passive transfer of Ig (38, 45, 66). Kruse (38) suggested feeding 80 to 100 g of total Ig during first hours after birth. McEwan et al. (45) reported that at least 50 g of Ig must be absorbed by a 35 kg calf to provide adequate passive immunity. Assuming an average Ig concentration of 80 g/l, 3.25 L of colostrum must be fed in the first 15 hr after birth (45). Stott et al. (67) reported that serum Ig concentration increased linearly when colostrum was fed up to 2 L and that increasing age of feeding decreased serum Ig concentration (67). Decreasing absorption with increasing age is related closely to the process of closure, wherein absorptive capacity of intestinal cells declines as age of calf increases (66). When larger amounts of Ig are fed, excess Ig may not be absorbed (67).

Bath et al. (2) suggested that calves should receive colostrum equal to 6% of BW within the first 6 hours after birth. This is equivalent to about 2.5 kg or 2.4 l for a 40.5 kg calf. For protection against heavy disease challenges, 340 to 450 g of Ig was recommended during first 24 h of life (10 to 12% of BW of first milking colostrum) (2). For the second and third day, colostrum equal to 8% of BW should be fed (2). Although these Ig will not be absorbed after closure occurs, they may provide some local

protection against microorganisms in the digestive tract (2). Total Ig ingested per unit of BW appears to be the most important factor in determining Ig concentration in serum (8). This highlights the importance of measuring Ig concentration prior to feeding. Measurement of colostral Ig allows calculation of the mass of Ig fed to the calf, or allows the producer to change the amount of colostrum fed to provide the desired Ig mass.

A common management practice is to allow the calf to nurse the cow. Stott et al. (68) reported a greater absorption of colostral Ig in suckling calves over hand-feeding calves. The difference was attributed to a labile factor present in fresh colostrum that produces and enhances absorptive capacity in intestinal epithelium and capillary permeability (68). However, such a labile factor has not been determined experimentally, and the nature of improved Ig absorption in nursed calves has not been reported.

Failure of passive immunological transfer in nursing calves has been attributed to a delay of colostral intake or to inadequate intake of Ig (22, 61). On the other hand, artificial feeding may require added management, since the lack of nursing drive and vigor in all dairy breeds may affect the time required for hand feeding (22). Large commercial enterprises may have limited time and labor available for hand feeding. Esophageal tubing is recommended when calves refuse to nurse, are sick, or cannot

nurse (2, 22, 61). Calves allowed to nurse must be assisted for better Ig absorption (22).

Surplus colostrum may be preserved conveniently for future use by freezing, brief refrigeration, or chemical treatment of fermentation and storage at ambient temperature (21).

Colostrum stored at -18 to -25$^{0}$C for at least 6 months loses few nutrients (21, 61). Freezing should be done quickly, and colostrum should be stored in small containers for better results (60). Recently, thawing frozen colostrum by properly microwave-defrosting has been suggested (31) as a safe method that maintains colostrum quality. However, care must be exercise to limit the build-up of "hot spots" that can denature Ig and other proteins (31).

Refrigeration is convenient when colostrum is in relatively high demand, such as during calving season. Minimal loss of Ig may be expected if colostrum is refrigerated for short periods (61).

Use of fermented colostrum during the first 2 d after birth is not recommended, since total Ig concentration in fermented colostrum declines by approximately 50% after 28 d of storage (61). Storage via fermentation or chemical treatment results in unavoidable nutrient losses and acceptability even though it is convenient economically (21). However, fermented colostrum can be used as a nutrient source after the first three days of life.

Colostrum can be economically stored at ambient temperature in plastic-lined containers with few handling requirements.

Intestinal Transmission of Macromolecules

Absorption of Ig through the newborn gut involves transport of Ig from the intestinal lumen to the circulation without loss of immunological activity. To accomplish this, Ig must reach the site of transport without degradation or denaturation (6, 61). At birth, several characteristics of the digestive tract of the calf differentiate it from the adult digestive system. Parietal cells, capable of secreting hydrochloric acid, are few in number at birth (6). The paucity of these cells and consequent near neutral abomasal pH prevent proteolysis when Ig are transported (6). A progressive increase in the number of parietal cells occurs during the first three days of life (6). A change in digestive enzyme activity takes place during the first hours after birth (6, 61). It has been reported that pancreatic amylase, protease and lipase activities are lowest during first 24 hr after birth, increasing during first week (6). Further, presence of a trypsin inhibitor in colostrum facilitates Ig absorption (2, 6, 61). Incorporation of soybean trypsin inhibitor into feeding of pre-colostrum piglets resulted in increased absorption of IgG before 12 h of age (41). Consequently, absence of proteolytic activity in the digestive tract and trypsin inhibitor present in colostrum may ensure that Ig reach the small intestine

without significant degradation (6, 61).

Absorption of Ig has been related closely to the structure of the small intestinal epithelium (8). Absorption is defined as movement of substances from the lumen of the intestine into the blood. Apparently, normal postnatal cell re-differentiation does not take place in the villous epithelial cell of the small intestine (63). Intestinal cells maintain the endocytic complex and capacity to transport macromolecules (63).

Epithelial cells of the small intestine ingest Ig by pinocytosis, defined as the uptake of particulate matter by a cell by invagination and pinching off of the plasma membrane (25). Absorption of Ig takes place entirely in the small intestine; the most active area is the jejunum, whereas illeal epithelial cells appear to have little activity (13, 61). None is absorbed from the abomasum or large intestine (13). Transport of Ig to the circulation is not direct, but occurs via lymphatic transport (13). Immunoglobulins appear in the lymph within 1 to 2 h after the introduction of colostrum into the duodenum if feeding occurs within 27 h after birth (13).

Absorption and transport of Ig from intestinal lumen into circulation occurs by an initial binding of Ig by the microvillous border of the epithelial cell and incorporation into the cell by endocytosis. Next, the endocytosed membrane extends into the cytoplasm and form a vacuole,

followed by condensation of vacuolar contents and transportation through the cytoplasm. When the vacuole comes into contact with the basal cell membrane, the vacuole contents is exocytosed into the lamina propria which passes into the lymph (63). Within a few hours after feeding Ig appear in blood. Approximately 50% of Ig absorption may occur by 6 h postfeeding (61). Like Ig, other proteins can be transported through the intestinal epithelium; those with low molecular weights are cleared from blood by the kidneys (6).

It has not been determined precisely whether selectivity is involved during absorption and transport of proteins through intestinal epithelium. Staley and Bush (63), and Staley et al. (64) reported that calves exhibited some selectivity in absorption, which may be based on the particular protein rather than molecular weight. The authors (63) also suggested IgG receptors in the jejunum may increase absorption. Others (61) have reported that IgG is preferently absorbed and absorbed more rapidly than IgM or IgA (61). Penhale et al. (54) showed that when intravascular and extravascular distributions of Ig are considered, absorption of each Ig type differed and averaged 90% of IgG, 59% of IgM, and 48% of IgA. On the other hand, Bush and Staley (9) stated that uptake of macromolecules into cells is a non-selective process and that the proportion of different Ig classes in calf serum is a

reflection of Ig proportion in colostrum fed. Besser et al. (3) also reported no evidence of a selective Ig absorption. Further studies are needed to clarify whether absorption of Ig is a selective process.

Efficiency of Absorption

Although serum Ig increases at increasing mass of Ig fed, efficiency of Ig absorption may be negatively correlated with mass of Ig fed (3, 71). Amount of Ig absorbed by the calf is calculated by multiplying differences in concentration of Ig before and after colostrum feeding by plasma volume (28). When this value is compared with the quantity of ingested by the calf, apparent efficiency of absorption (AEA) may be determined (54, 43).

A significant negative correlation between AEA of $IgG_1$ and IgM has been reported (3). Decreasing AEA of $IgG_1$ and IgM may be due to saturation of a shared macromolecular transport mechanism through intestinal epithelium, regulation of the Ig concentration in serum, or control of Ig absorption (3).

Stott and Meneffe (71) reported greater AEA when small amounts of colostrum were fed, and suggested the presence of a selective IgM absorptive mechanism. Husband et al. (28) reported that IgM was absorbed with greater efficiency than $IgG_1$ and $IgG_2$. The authors (28) hypothesized that IgG is transported from blood to interstitial fluid more rapidly than larger IgM molecule. Therefore, the amount of IgG

measured in plasma at time of sampling may be not actually reflect the amount absorbed, thereby underestimating AEA (28).

An examination of data from computer simulations and calf feeding experiments (5) showed that absorption of $IgG_1$, $IgG_2$, and IgM was not selective in calves, and AEA averaged 46, 49, 18%, respectively. The authors (5) also concluded that the relationship between changes in serum Ig concentration and rate of absorption is not precise, and that Ig concentration in the thoracic lymph duct is a more accurate indication of AEA. Determination of a precise efficiency of absorption for each Ig class has not been conclusively determined. Several factors should be taken into account when determining AEA. When the actual plasma volume of each animal has not been measured, inaccuracy is expected. Plasma volume expands during the first hours of life of piglets and calves (45), which reduces concentration of plasma constituents. However most studies assumed a plasma volume of 5% of BW without correction for plasma expansion. Moreover, the amount transferred to blood is difficult to estimate accurately, because serum Ig concentration depends on input and output from the circulation, and total fluid volume (5, 6).

Closure

In calves, pigs and foals, the ability to transport macromolecules to the circulation occurs for a limited time

and at an increasing rate after 12 h post-partum. Staley and Bush (63) defined closure as the cessation of macromolecule transport from intestinal cells, and suggested that development of a intracellular digestive system regulated closure. This hypothesis is based on observations that acid phosphatase (and indication of lysosomal enzymes) was not present in preclosure jejunal cells of pigs, but was present at approximately 1 day of age (63). Similar observations have been made in the rodent (63). Using this hypothesis, endocytosis of Ig may continue, but Ig may be digested by lysosomal enzymes prior to exocytosis into the lymph or portal circulation. Others, however, have suggested that feeding colostrum (48, 65) or other compounds (74) stimulates pinocytosis by epithelial cells. These cells have a limited capacity specifically for endocytosis. Closure occurs when this limited capacity is exhausted. However, few data are available to identify the nature of this mechanism, and further research is required.

Closure appears to be related to time of first feeding (54, 65). Stott et al. (65) reported that feeding colostrum to calves shortly after birth reduced the period of macromolecular absorption by approximately 2.3 h. The authors (65) suggested, that colostrum stimulated pinocytosis, followed by cessation of uptake (closure). As colostrum feeding is delayed, cessation of absorption is delayed up to spontaneous closure, which occurs at

approximately 32 h (65). Penhale et al. (54) reported a progressive reduction in absorption and an inverse relationship between plasma Ig and time of colostral feeding. Rate of closure may also vary according to Ig isotype, suggesting that absorption is not indiscriminate and that each Ig class is absorbed independently (54).

Another factor associated with closure is the presence of microorganisms and site of Ig absorption. Staley and Bush (63) suggested that competition for transport between Ig and microorganisms, particularly E. coli, may interfere with Ig absorption. Closure time may be reduced by the presence of bacteria migrating along the villi, resulting in premature appearance of epithelial cells incapable of macromolecular absorption (63). Corley et al. (14) demonstrated transepithelial migration of E. coli in the newborn calf before colostral feeding. However, when E. coli was given with or after colostrum was fed, binding to the intestinal epithelium was not observed. McClain (41) reported a tendency for reduced serum IgG and total globulin at 12 h when Lactobacillus acidophilus was incorporated into milk in pre-closure piglets.

James et al. (30) suggested that rate and nature of microbial colonization of the intestine were related to closure. Calves were orally administered duodenal fluid to determine effect of susceptibility to an E. coli challenge. Serum Ig was lower in inoculated calves. Interference with

Ig absorption may have been associated with morphological changes in small intestinal epithelium which were accelerated by the presence of microorganisms or other constituents in the inoculum (30). Also, Ig may have been degraded by microorganisms within the intestinal lumen (30). The observation that inoculum reduced absorption of colostral Ig may explain causes of hypogammaglobulinemia in neonatal calves. If pinocytosis in intestinal epithelium is stimulated by early feeding of colostrum with a subsequent reduction in time of closure (65), protecting the calf from exposure to random absorption of molecules may reduce calf morbidity and mortality. Early exposure to colostrum may reduce time of closure and prevent transepithelial migration of microorganisms.

## Colostral Supplementation

Feeding colostrum with high Ig concentration is critical to ensure adequate passive transfer. Recently, several products have been introduced to replace or supplement colostrum. These products are derived from processed cheese whey, colostral whey, serum derivates, or skim milk (27). Some are not a total replacement for colostrum, but provide antibodies specific for particular antigens. Use and value of these immunosupplements has received recent attention (16, 24, 57).

Commercial colostrum supplements should be considered as a management strategy and method for preventing failure

of passive transfer. Colostral supplements are recommended for use:

1) as a method of augmenting low quality colostrum,

2) as a digestion resistant source of Ig in the gut,

3) to prevent vertical viral infections, transmitting a disease from dam to calves, when infected colostrum is one of the means of spreading an infection [i.e., Bovine leukosis virus (27)],

4) to provide a source of specific antibodies released over prolonged periods,

5) to provide colostrum if an alternative source of colostrum is not available (27).

**Cheese Whey Derivatives**

Supplements derived from processed cheese whey are the most inexpensive and immediate sources of bovine Ig. However, due to problems with processing equipment, concentration of Ig to protective levels has been difficult. An example of this type of preparation is Colostrx$^{R}$ (Protein Technology, Inc., Minneapolis MN), which is derived from ultrafiltration of cheese whey. However, it has been suggested that the product should not be combined with colostrum, since the mixture obtained is too thick and may be excessively hyperosmotic (27).

**Colostrum Derivatives**

Colostrum derivative products (C.L. Replacer$^{R}$ Cumprem, Kenesaw, NE) are recommended for feeding within first few

hours after birth for optimal Ig absorption. The manufacturer claims that 6 to 9 g IgG/L of product are absorbed in colostrum-deprived calves (27). In addition to Ig, the product contains milk fat and protein which makes it more nutritionally acceptable. Also, L. acidophilus cultures are included to establish a non-pathogenic enteric microflora. However, a disparity was observed between claimed Ig concentration and laboratory measurement (27). Partial failure of passive transfer may be expected if this product is the only source of Ig (27).

A lyophilized whole colostrum and whey preparation, Immuno-Bac$^{R}$ (Alltech, Inc., Lexington, KY) has been marketed to completely replace colostrum if needed. Absorption of Ig from the product shown to be acceptable when fed to Jersey calves (57). However, Ig content was lower than claimed by manufacturer; therefore, amount of Ig provided by the product was less than optimal (57). Concentration of IgM in the product was lower; processing colostrum-whey ingredients used in the replacer may have destroyed IgM (57).

Colostrum derivatives are also available in a 10 g bolus form, designed specifically for goats (27). The product used for calves may require a large number of boluses to provide adequate Ig.

An oral nutritional supplement (Immuno-Dynamics Inc., Minneapolis, MN) derived from ultrafiltration of first-milking colostral whey is administered in 10 ml oral

aliquiots for three consecutive days. This product has a higher level of detectable Ig than other products, but several liters of the product is required to provide total Ig mass required by the calf (27).

**Milk Derivatives**

Only small amounts of bovine Ig are present in milk. When cows are vaccinated, milk from these cows contains larger amount of specific antibodies for prolonged period due to the extended antigenic release (27). When skim milk from cows is ultrafiltrated, a highly specific bovine Ig may be obtained (Stolle Research and Development Corporation. Lebanon, OH). Using these techniques, companies may produce milk replacers with specific antibody against K99+ E. coli or other agents (27).

**Serum Derivatives**

Serum derived products are obtained by processing exogenous circulating antibodies. Burton et al. (7) reported use of lyophilized hyperimmune equine serum as a source of antibodies for neonatal foals.

Use of immunosupplements is controversial. Several studies have shown that many products contain low Ig concentrations (16, 24). Currently, at least 5 products are available or are being formulated using whey protein. Studies evaluating efficacy of these products are needed. Research to determine extent of Ig destruction during globulin extraction from cheese whey, pasteurization,

lyophilization or processing will provide needed information to maximize Ig recovery. Immunologically functional Ig aggregates must be maintained during pasteurization and processing, and sufficient concentrations of IgM must be provided (27). Further, determination of specificity against particular antigens should be documented. Finally, maintenance of reliable Ig concentration of any colostral supplement is critical.

## Methods of Measuring Ig Concentration

Laboratory techniques for measuring Ig concentration include radial immunodiffusion (RID), refractometer, zinc sulfate turbidity test (ZST) and electrophoresis. The two most commonly used methods are RID and ZST (56). Serum electrophoresis is used more in diagnostic clinical medicine to examine various serum proteins. These methods are based upon a distinct immunologic or biochemical property of the Ig. Pfeiffer et al. (56) found no significant differences among ZST, non-commercial RID and serum electrophoresis in serum containing 30 to 50 mg Ig/ml.

### Radial Immunodiffusion

Immunodiffusion is defined as a quantitative analytical method by which components of antigen or antibody mixtures may be distinguished (25). A preparation of antigen-antibody is allowed to react and diffuse towards each other to give lines or bands of precipitate in the medium used as a support [usually a gel (25)]. There are several types of

immunodiffusion:

a) Single or simple diffusion in one dimension: an antigen solution is placed above a column of antibody incorporated in a gel,

b) Double diffusion in one dimension: antibody is incorporated into a gel in a tube, a column of gel is superimposed and the antigen placed above,

c) Double diffusion in two dimensions: the reactants are placed in wells or reservoirs in a plate of gel,

d) Radial diffusion: antigen is placed in a well in an antibody-containing gel.

Single RID relies upon specific antigen-antibody precipitation. In this method, one of the reagents (usually the antibody) is homogeneously incorporated through the entire gel, and the other reagent (usually the antigen) diffuses from a well or other starting point in the gel (59). The antigen, in relative excess compared to the antibody, passes the precipitate it forms into the gel, producing a widening precipitate as it diffuses through the gel (59). When antibody concentration is standardized in the gel, this method may be used for determination of unknown concentrations of antigen (20). Radial immunodiffusion is a sensitive method for Ig determination; quantities as little as .01 microgram can be determined with an accuracy of $\pm$ 2% (59). It also gives specific Ig class or subclass information. For quantitation of Ig in

colostrum-deprived calf serums, this method should be preferred; expensive commercial reagents needed in the preparation of RID make his method relatively high cost.

**Zinc Sulfate Turbidity**

The ZST test is a reaction-precipitation procedure, based on the biochemical properties of Ig relative to zinc sulfate. Proteins precipitated by zinc develop turbidity in solution. Absorbance produced by suspended precipitate reduces transmittance of light and can be measured spectrophotometrically at any visible wave length (56). However, variations in wavelength of the spectrophotometer affect the slope of the standard curve (56). A standard curve made from serum containing known amounts of Ig may be used to calculate Ig concentration. McEwan et al. (44) reported a significant relationship between the ZST and IgG and IgM serum Ig in newborn calves. The test consisted of a solution of zinc sulphate (208 mg/l $ZnSO_4$+ $7H_2O$) prepared in a volumetric flask using $CO_2$ free distilled water. Six ml of zinc sulfate solution were mixed with .1 ml of serum, gently shaken and allowed to stand for 60 min at room temperature. Samples then were read in a photoelectric absorptiometer. The relationship between turbidity developed by ZST and Ig concentration was linear only to approximately 30 mg/ml. Above this concentration, the increment increased exponentially. The turbidity was influenced mainly by the concentration of IgG and to a

lesser extent by IgM. Logan et al. (40) reported that some calves could have very low IgM levels and using ZST as an index of survival lead to false positives. On the other hand, Fisher and Martinez (18) reported that false positive in the ZST are unlikely unless there is hemoglobin contamination.

Several factors influence the accuracy of ZST including anticoagulants, reagent pH, time and temperature of incubation, and degree of hemolysis. Use of serum rather than plasma is recommended to avoid effects of anticoagulant; heparin has been reported to decrease ZST (44). Also, effect of variations in fibrinogen concentration may cause samples with similar Ig concentrations to have different turbidity readings. The pH of the reagent is a key factor in ZST and must not deviate by more than .05 from 7.50. When pH is corrected by addition of acid or alkali, it may generate unsuitable reagents because ionic strength is critical (58). Moreover, when the solution is allowed to absorb $CO_2$, changes in the pH of the reagent are produced (44, 58). Time and temperature also influence ZST. The reaction is not fully complete after 1 h (44), and precision is improved if temperature is maintained at 25°C $\pm$1° (58). Others have suggested that temperature should be 20°C and the intensity of reaction should be read after 60 minutes of incubation (58).

The factor most affecting ZST is the presence of hemolysis in the serum, which may be why ZST readings generally indicate slightly higher Ig values than other methods (56). Hemoglobin binds to other serum proteins and is precipitated along with Ig by $ZnSO_4$; it also absorbs light due to its red color (56). If serum samples contain hemolysis, a correction factor should be included for Ig calculations. An alternative test (sodium sulphite turbidity test) has been suggested (18) for hemolyzed samples and those from dead calves (61). McEwan et al. (44) suggested use of a serum blank containing serum and distilled water to compensate for hemolysis.

**Refractometer**

The refractometer is an instrument for measuring indices of refraction, based on the principle that index of refraction of a solution depends on its concentration (56). The refractometer has been used to estimate Ig concentration based on concentration of total protein. Low concentrations of total protein reflect a failure in transfer of maternal Ig, since Ig are the only class of serum protein that increase markedly after colostrum ingestion (42). Assuming changes in refraction of serum or plasma are due mainly to protein content, and albumin levels are constant, the refractometer gives an indication of amount of Ig in serum (56).

Some have questioned the assumption that other serum proteins remain constant in plasma after consumption of colostrum (42, 50, 56). Serum albumin concentrations in 1 to 5 day old dairy calves vary between 1.9 and 3.4 g/100 ml, and some colostrum-deprived calves have had higher total protein values than those fed colostrum (56). Also, readings may be affected by hemoconcentration (42).

**Electrophoresis**

Electrophoresis is a method that evaluates rate of migration of charged particles through a liquid or solid medium in an electric field. Some factors may affect rate of migration, such as size and charge of particles (influenced by pH and electrolyte), viscosity of the medium and voltage applied (25). Electrophoretic mobility and separation determinations, measured in $cm^2$ $vol^{-1}$ $sec^{-1}$, can be determined in absence or presence of a supporting medium, i.e., agar, paper, or starch (25). The method of transporting antigen electrophoretically through an antibody-containing gel, immunoelectrophoresis (IEP), both unidimensionally and bidimensionally, is based on the same principle as single RID. At constant antibody concentration throughout the gel, the total surface area of precipitate obtained is proportional to the antigen concentration used (59). The IEP method has been reported to be a reliable method for determining Ig concentration. Pfeiffer et al. (56) analyzed serum samples on cellulose acetate strips, and

Ig was calculated by relating percent area recorded as protein. It was then converted to mg/ml by multiplying Ig percent by total concentration of protein. The Ig method was repeatable, but tended to underestimate actual content in serum with high Ig concentrations. Values were underestimated by 5 mg/ml when Ig concentrations were between 40-50 mg/ml. One advantage of this method is that serum standards are not required, and samples can be analyzed individually. However, interpretation of the area of Ig may be subjective.

**Colostrometer**

Fleenor and Stott (19) developed a practical field method for measuring Ig concentration in bovine colostrum. The method is based on the linear relationship between colostral specific gravity and Ig concentration. In this study, 14 colostrum samples were collected from nursed and unnursed cows within 24 h postpartum and 15 colostrum samples from unnursed cows immediately postpartum. Samples were assayed for specific gravity by a hydrometer calibrated in increments of .002, with a range of 1.00 to 1.220. Colostrum samples were also assayed for major constituents and Ig. Data were analyzed by regression and an equation was developed to estimate colostral Ig concentration from specific gravity. Specific gravity was related to total solids, total protein, and Ig concentration (19). Components such as fat, nonprotein nitrogen, and casein were

unrelated to specific gravity. Although lactose and albumin were related to specific gravity, they had small coefficients of variation, whereas Ig had the largest coefficient of variation of all constituents. Therefore, authors (19) concluded that Ig was the primary factor in variation of colostral specific gravity.

Incorporating this information into a conventional hydrometer, a colostrometer was developed. Although the colostrometer is useful for estimating relative quality of Ig in colostrum, it is not a quantitative technique (19).

Three quality levels were proposed for evaluating colostrum: poor, moderate, and excellent (0-20, 21-50, and > 51 mg/ml of total Ig, respectively) (19). The cutoff between poor and moderate was determined by assigning a specific gravity reading slightly higher than the average specific gravity of whole milk. For moderate and excellent, the cutoff is based on the fact that a cow should yield 2 kg of colostrum with a minimum of 50 mg Ig/ml (37, 45). It has been recommended that only colostrum within the excellent zone should be fed to pre-colostral neonates as soon as possible after birth (19).

Limitations of the colostrometer have been reported. Mechor and Grohner (47) reported that temperature alters colostrometer readings; colostrum samples at lower temperature have increased density which results in an overestimation of Ig concentration. The authors (47) do not

recommend use of the colostrometer for estimating Ig in colostrum. On the other hand, it has been recommended continued use of the colostrometer, measuring the colostrum only when its temperature is close to body temperature (J. D. Quigley, personal commmunicantion).

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# PART III

## SERUM IMMUNOGLOBULIN CONCENTRATION IN RESPONSE TO MATERNAL COLOSTRUM OR COLOSTRAL SUPPLEMENTATION IN DAIRY CALVES

## Introduction

Efforts to decrease calf morbidity and mortality have led to changes in management of newborn calf. Since the calf is born hypo- or agammaglobulinemic (1, 5, 11, 16), absorption of adequate amounts of colostral immunoglobulins (Ig) is essential for acquisition of passive immunity (1, 5, 17).

Among factors that influence passive transfer of colostral Ig, age at first feeding and mass of Ig ingested are most important (4, 6, 8, 13, 21). However, in some circumstances, sufficient maternal colostrum may not be available for calves within 24 h after birth, or colostrum containing low Ig may be provided by the dam. Products containing high concentrations of Ig extracted from dried cheese whey or natural colostrum have been introduced; these products are designed to replace colostrum or to increase Ig content (9). On the other hand, feeding excess Ig may impair the development of active immunity (16, 18) and reduce efficiency of absorption of Ig (2, 15, 20). Effects of these products on acquisition of passive immunity have not been determined precisely. Objective of this study was to determine effects of a colostral replacer on serum Ig concentration and efficiency of absorption when fed to supplement maternal colostrum.

## Materials and Methods

### Animal Assignments and Experimental Design

Thirty-three Holstein calves (19 bulls and 14 heifers) were assigned alternately at birth to one of two treatments in a completely randomized experimental design. Treatments were maternal colostrum (**MC**) and MC supplemented with 125 g of a colostral replacement product consisting of a blend of lyophilized bovine colostrum and dried whey (**CS**; Immuno-Bac; Alltech, Inc., Nicholasville, KY).

### Animal Management

Pregnant cows were housed on a drylot until approximately 7 d prepartum, and were moved to 5 x 3 m stalls bedded with sawdust until calving. Some cows calved more than 7 d before expected; calving occurred in a drylot or pasture. Newborn calves remained with the dam for a limited time to allow the dam to clean and stimulate the calf; however, calves were not allowed to nurse. Within 30 to 40 min calves were moved to the calf barn, weighed, navels were dipped in an iodine solution (7%) and placed in individual pens (2.5 x 1.5 m) bedded with shavings. Pens were cleaned daily, and shavings were replaced every other week.

### Feeding Management

Maternal colostrum was hand-milked from the dam shortly after parturition, transported to the calf barn, weighed, and separated into two 2 L bottles. Surplus colostrum was

stored at 4$^0$C or -20$^0$C. When < 4 L was obtained, previously frozen or refrigerated colostrum was added. Frozen colostrum was thawed with warm water. Colostrum was fed after the 0 h blood sample was collected and 12 h later. Colostral replacer was added to 2 L of MC and mixed thoroughly prior to feeding. After 24 h, all calves were fed commercial milk replacer (15% fat, 22% protein) at approximately 0800 and 1700 h to weaning at 56 d. A commercial calf starter was offered from 3 to 56 d. Water was available at all times.

**Feeding Sampling and Analysis**

A 10 ml sample of MC was stored at -20$^0$C prior to analysis of $IgG_1$, $IgG_2$, IgM, and IgA by radial immunodiffusion (VMRD, Inc., Pullman, WA). Dilutions (10:1) for $IgG_1$ analysis were made with .9% saline. Colostral replacer was reconstituted 10:1 with tap water, diluted with .9% saline, and analyzed as for colostrum. After 22 h of incubation at room temperature, precipitant rings were measured using an ocular micrometer on a stereomicroscope (American Optical, Buffalo, NY).

**Blood Sampling and Analysis**

Blood (10 ml) was collected by jugular venipuncture into evacuated containers without anticoagulant for separation of serum as soon as possible after birth (0 h) and 12, 24, 48 and 72 h thereafter. Additional samples were collected at 28 and 56 d of age. Blood was allowed to

coagulate and serum was collected by centrifugation (3000 *xg*). Samples were stored at -20$^0$C prior to analysis of $IgG_1$, $IgG_2$, and IgM by RID as described for colostrum, but without dilution. Intake of colostrum, colostral replacer and milk replacer were recorded at each feeding. Incidence of scours, respiratory infections, and other illnesses was reported daily.

**Statistical Analysis**

Serum Ig at 12, 24, 48 and 72 h was analyzed as a split-plot experimental design using the model : $Y_{ijk} = \mu + T_i + A(T)_{ij)} + H_{ik} + TH_{ik} + e_{ijk}$ where $Y_{ijk}$ = serum Ig; $T_i$ = effect of $i^{th}$ treatment; $A(T)_{ij)}$ = effect of $j^{th}$ animal within the $i^{th}$ treatment; $H_k$ = effect of $k^{th}$ hour after birth; $TH_{ik}$ = effect of treatment and hour interaction; $e_{ijk}$ = residual. Animal within treatment was used as error term to test treatment. Analyses were conducted using a mixed model algorithm (3). Apparent efficiency of absorption was calculated as: AEA = [Serum Ig concentration (g/l) x (.07 L blood / kg BW) / Ig intake (g)]. Plasma volume was estimated as 7% of BW (10). Serum Ig concentration at 28 and 56 d of age were analyzed as a completely randomized design using analysis of variance. Declaration of significance was $P < .05$ unless otherwise noted.

## Results and Discussion

Body weight ranged from 26.3 to 50.8 kg (Table 1). Average age at first feeding was 1.9 h (SE = .2) indicating that colostrum was consumed prior to initiation of closure. Ten calves (4 MC, 6 CS) refused colostrum during the first or second feeding. Refusal averaged .57 kg (SE = .1) and was consumed in an additional feeding 2 to 4 h later. No symptoms of respiratory diseases were observed during the study. Five calves (2 MC, 3 CS) were treated for scours for more than one day during the study; eight others were treated for scours for one day only.

Concentration of total Ig in MC did not vary between treatments ($P > .10$) and ranged from 5.5 to 96.2 mg/ml. Average concentration of Ig in MC (56.9 mg/ml) is considered good quality as defined by Fleenor and Stott (7). Distribution of Ig isotypes in MC averaged 85.2, 6.5, 3.5, and 5.0% for $IgG_1$, $IgG_2$, IgM, and IgA, respectively.

Means of Ig concentration in colostral replacer are in Table 1. Colostral replacer was obtained in two lots, which contained 14.3 and 8.5% total Ig. Large variation of Ig concentration in this and a previous study (15) indicates clearly the need for accurate measurement of Ig content in colostral supplement prior to use.

Intake of total Ig or isotype (Table 2) did not differ by treatment ($P > .10$). Total Ig intake exceeded 100 g by 12 h, which has been proposed as the minimum required for

**TABLE 1. Descriptive statistics of BW, immunoglobulin concentration of maternal colostrum (MC), colostral replacer (CR) and age at first blood sample and feeding.**

| Item | n | Min | Max | Mean | SE |
|---|---|---|---|---|---|
| BW, kg | 33 | 26.3 | 50.8 | 39.2 | .4 |
| MC total Ig, mg/ml | 33 | 5.5 | 96.2 | 56.9 | .5 |
| MC $IgG_1$, mg/ml | 33 | 4.6 | 76.6 | 48.5 | 1.6 |
| MC $IgG_2$, mg/ml | 33 | .2 | 7.4 | 3.6 | .1 |
| MC IgM, mg/ml | 33 | .6 | 5.7 | 1.9 | .1 |
| MC IgA, mg/ml | 33 | .1 | 6.4 | 2.9 | .2 |
| CR Total Ig, % | 2 | 8.4 | 14.2 | 11.4 | 2.9 |
| CR $IgG_1$, % | 2 | 6.7 | 11.7 | 9.2 | 2.5 |
| CR $IgG_2$, % | 2 | .7 | 1.2 | 1.1 | .2 |
| CR IgM, % | 2 | .4 | .5 | .4 | .1 |
| CR IgA, % | 2 | .5 | .8 | .6 | .2 |
| Age at first sampling, h | 35 | .7 | 4.0 | 1.9 | .2 |
| Age at first feeding, h | 35 | .2 | 2.8 | .5 | .1 |

**TABLE 2. Least squares means of immunoglobulin intake[1] at 0 and 12 h in calves fed maternal colostrum (MC) or MC plus 125 g of colostral replacer (CS).**

| Item, g | MC | CS | SE |
|---|---|---|---|
| 0 h | | | |
| $IgG_1$ | 91.8 | 113.4 | 10.8 |
| $IgG_2$ | 7.2 | 8.6 | .8 |
| IgM | 4.0 | 4.3 | .5 |
| Total | 103.1 | 126.2 | 11.5 |
| 12 h | | | |
| $IgG_1$ | 182.1 | 227.9 | 21.4 |
| $IgG_2$ | 14.3 | 17.1 | 1.5 |
| IgM | 8.1 | 8.5 | 1.0 |
| Total | 204.5 | 253.6 | 22.8 |

[1]Calves were 1.9 h of age at 0 h feeding.

acceptable passive transfer when measured at 24 hr after birth (16). Therefore, it is likely that calves fed MC obtained adequate Ig without supplementation.

Serum Ig concentrations at 12, 24, 48 and 72 h are in Table 3. No measurable Ig concentration was found in serum of prefed calves. Lab analyses of several 0 h samples showed small precipitant rings, which were lower than minimum standards (.11, .12, .08, and .05 mg/ml for $IgG_1$, $IgG_2$, IgM, and IgA, respectively). Stott et al. (21) and Husband et al. (10) also reported low serum Ig concentration in prefed calves due to lack of placental transfer of Ig (5, 15).

No treatment effect was observed in serum Ig concentration ($P > .10$), which may be related to maternal Ig intake. Maternal colostrum used in this study was of a superior quality; when MC was supplemented with 125 g of colostral replacer, 11.4 g of total Ig was provided. The additional amount of Ig may have saturated the limited number of cell receptors in the calf's intestinal epithelium resulting in unabsorbed Ig (15, 18, 21). By 12 h, serum Ig concentration was increased ($P < .01$) in both treatment (Table 3) and had exceed minimum serum Ig required for adequate passive transfer according to Penhale et al. (14). Concentration of all Ig isotypes increased to 24 h, then varied to 72 h, which suggests that absorption was maximized at the second feeding. Serum concentration at 12 and 24 h

**TABLE 3. Concentration of serum immunoglobulin in calves fed maternal colostrum (MC) or MC supplemented with 125 g colostral replacer (CS).**

| Item, mg/ml | MC | CS | SE |
|---|---|---|---|
| $IgG_1$ | | | |
| 12 h | 10.0 | 10.4 | 1.5 |
| 24 h | 15.8 | 14.9 | 1.5 |
| 48 h | 13.5 | 13.7 | 1.5 |
| 72 h | 14.0 | 16.0 | 1.5 |
| $IgG_2$ | | | |
| 12 h | 1.2 | 1.4 | .2 |
| 24 h | 1.5 | 1.9 | .2 |
| 48 h | 1.6 | 1.5 | .2 |
| 72 h | 1.4 | 1.5 | .2 |
| IgM | | | |
| 12 h | 1.3 | 1.4 | .2 |
| 24 h | 1.9 | 1.5 | .2 |
| 48 h | 1.6 | 1.4 | .2 |
| 72 h | 1.4 | 1.3 | .2 |

were similar to those reported by Stott et al. (20) and Quigley et al. (15), but lower than those reported by Boyd (4). Other studies (12, 18) have reported that maximum concentration of IgG is reached earlier than IgM. In the present study peak Ig concentrations were reached approximately at 24 h after birth regardless of Ig isotype. General pattern of colostral Ig absorption in the calf is characterized by an increase of serum Ig concentration after colostral feeding up to a peak, followed by a decline in Ig concentration with increasing age (16). Variation observed in serum $IgG_1$ concentration after 24 h may have been related to differences among calves in rate of exchange between plasma and extravascular fluid, and rates of removal by secretion or catabolism (4, 10, 14). Rates of removal of Ig may be related to molecular weight (3); $IgG_1$, is removed more rapidly than IgM (163,000 vs $10^6$ MW) (10).

Factors other than treatment that may have influenced serum Ig concentration were evaluated using stepwise regression analyses. Serum Ig concentration at 0, 12, 24, 48, and 72 h were used as depended variables, and BW, intake of each Ig and total Ig, sex, and age as independent variables. Quadratic and cubic terms were included in each analysis.

Equation predicting total Ig was: Total Serum Ig = $35.596 + .138 * S - .0002 * S^2 - 1.655 * BW + .019 * BW^2 - .005 * H^2$; where S = total Ig Intake; H = $IgG_2$ intake; $r^2$ =

.78. Negative quadratic response of total serum Ig to total Ig intake probably reflected a saturation of absorptive capacity, which may occurs when amount of Ig offered is in excess of intestinal epithelial capacity (15, 18, 21). Negative effect of BW was probably related to increasing plasma volume at increasing BW.

Equation predicting serum $IgG_1$ was: Serum $IgG_1$ = 36.519 + .129 * G - 2.5E-3 * $G^2$ - 1.68 * BW + .019 * $BW^2$; G = intake of $IgG_1$; $r^2$ = .72. Similar relationship between serum $IgG_1$, intake of $IgG_1$, and BW has been reported (20).

Differences due to sex were significant ($P < .01$) in regression of serum $IgG_2$ and IgM concentration without differences in BW. Equation predicting serum $IgG_2$ for heifers was: Serum $IgG_2$ = .162 + .222 * H - .0001 * $H^3$ - .006 * T - .112 * M + .002 * $M^2$; where H = intake of $IgG_2$, T = age at first feeding and M = intake of IgM; $r^2$ = .80. Negative effect of time on serum $IgG_2$ is probably related to closure (19). Negative relationship between serum $IgG_2$ and intake of IgM may be related to saturation of a shared macromolecular transport mechanism across calf's intestinal epithelium (18).

Equation predicting serum $IgG_2$ for bulls was: $IgG_2$ = -.24 + 7.63E-6 * $BW^3$ + .012 * G - 2.28E-5 * $G^2$; where G = $IgG_1$ intake; $r^2$ = .61.

Equation predicting serum IgM concentration in heifers was: Serum IgM = .279 - .001 * $H^2$ - .011 * T + .008 * G +

.064 * M; where $H^2$ = intake of $IgG_2$ squared, G = intake of $IgG_1$, and M = intake of IgM; $r^2$ = .74. Again, a negative relationship between $IgG_2$ and IgM was observed. On the other hand, positive linear relationship between intake of $IgG_1$ and serum IgM differed from previous studies (18, 21) which reported that serum IgM concentration tended to decrease when colostral Ig concentration was lower. It is not clear why different results were observed when $IgG_2$ and IgM were related.

Equation predicting serum IgM for bulls was: Serum IgM = 1.61 - 4.0E-3 * T - 2.995E-3 * $BW^2$ + 5.18E-5 * $BW^3$ - 2.0E-8 * $G^3$ + .2512 * M; $r^2$ = .65.

To determine if supplementing colostrum with lower Ig concentration increased serum Ig concentration, MC was classified by its Ig content. Fleenor et al. (7) previously identified classes of colostrum: poor (total Ig <= 20 mg/ml, fair (21 to 50 mg/ml), and good ( > 50 mg/ml). Cutoff between poor and moderate was based on specific gravity of whole milk, assigning to poor quality a slightly higher than average specific gravity of normal milk. Whole milk has a specific gravity that corresponds to an Ig concentration of 14.16 mg/ml (7). Cutoff between moderate and good was based upon earlier findings that, to insure adequate passive immunity, a cow should yield 2 kg of colostrum with at least 5% Ig (50 mg/ml) (7). In the present study, only two samples (one MC, one CS) contained less than 20 mg/ml.

Therefore, two categories were established to determine effects of colostrum quality. Data were separated into poor (<= 30 mg/ml) and good (>= 30 mg/ml) categories for analysis. It is recognized that the selection of 30 mg Ig/ml colostrum was somewhat arbitrary; however, this figure allows sufficient numbers in each group to allow statistical evaluation. Table 4 contains least squares means of serum Ig concentration, segregated within group. No treatment effects were observed in calves fed poor quality colostrum. Among calves fed good quality colostrum, serum $IgG_2$ tended to be greater in calves fed CS, while serum IgM were greater in calves fed MC. Quigley et al. (15) also reported no increase in serum IgM concentration, although MC was supplemented by a non-maternal colostrum source. Maternal colostrum in that study averaged 33.4 mg/ml. Apparently, excessive Ig concentration may have caused inhibition in colostral IgM absorption. A similar observation has been reported (20).

When Ig apparent efficiency of absorption (**AEA**) was evaluated according to quality of colostrum fed, no significant differences due to treatment were observed (Table 5). Higher AEA of $IgG_1$ and IgM ($P < .10$) were observed in calves fed MC. This pattern of increasing Ig intake but decreasing AEA may be related to a physiological limitation to the mass of Ig that can be absorbed as reported previously (2, 15, 18). Stott and Meneffee (18)

**Table 4. Least squares means of serum immunoglobulin concentration by quality[1] in calves fed maternal colostrum (MC) or MC plus 125 g of colostral supplement (CS).**

| | Poor | | | | Good | | | |
|---|---|---|---|---|---|---|---|---|
| Item | MC | CS | SE | *P*[2] | MC | CS | SE | *P* |
| $IgG_1$ | | | 1.7 | NS[a] | | | 1.1 | NS |
| 12 h | 5.8 | 9.4 | | | 11.9 | 10.5 | | |
| 24 h | 8.7 | 11.4 | | | 19.0 | 15.7 | | |
| 48 h | 9.0 | 11.1 | | | 15.4 | 14.4 | | |
| 72 h | 7.3 | 12.8 | | | 17.0 | 16.8 | | |
| $IgG_2$ | | | .2 | NS | | | | |
| 12 h | .9 | 1.2 | | | 1.2 | 1.5 | .1 | + |
| 24 h | 1.2 | 1.4 | | | 1.7 | 2.0 | | |
| 48 h | 1.3 | 1.3 | | | 1.8 | 1.6 | | |
| 72 h | 1.0 | 1.4 | | | 1.6 | 1.5 | | |
| IgM | | | .1 | NS | | | | |
| 12 h | 1.1 | 1.5 | | | 1.4 | 1.4 | .1 | * |
| 24 h | 1.3 | 1.7 | | | 2.1 | 1.5 | | |
| 48 h | 1.2 | 1.6 | | | 1.7 | 1.4 | | |
| 72 h | 1.1 | 1.5 | | | 1.5 | 1.3 | | |

[1]Quality of colostrum: Poor = total Ig in MC <= 30 mg/ml (n=31); Good = total Ig in MC > 30 mg/ml (n=98).

[2]Probability of a significant treatment effect.

[a]$P > .10$.

*$P < .05$.

+$P < .10$.

**TABLE 5. Least squares means of apparent efficiency of absorption[1] (AEA) by quality[2] at 24 h in calves fed maternal colostrum (MC) or MC plus 125 g of colostral supplement (CS).**

| AEA, % | MC | CS | SE | $P^3$ |
|---|---|---|---|---|
| $IgG_1$ | 27.1 | 18.8 | 2.5 | * |
| $IgG_2$ | 31.7 | 55.1 | 20.0 | NS[a] |
| IgM | 68.0 | 49.7 | 7.2 | + |
| Poor[3] | | | | |
| $IgG_1$ | 35.2 | 19.6 | 6.3 | NS |
| $IgG_2$ | 38.2 | 23.2 | 8.8 | NS |
| IgM | 58.6 | 38.5 | 11.1 | NS |
| Good[3] | | | | |
| $IgG_1$ | 23.9 | 18.6 | 2.8 | NS |
| $IgG_2$ | 30.0 | 60.0 | 26.1 | NS |
| IgM | 71.1 | 51.3 | 9.0 | NS |

[1]Calculated as: $\frac{\text{Serum Ig (g/l) * (BW,kg * .07 l/kg)}}{\text{g Ig Intake}}$

[2]Quality of colostrum: Poor = total Ig in MC <= 30 mg/ml (n=31); Good = total Ig in MC > 30 mg/ml (n=98).

[3]Probability of a significant treatment effect.

*$P < .05$

+$P < .10$

NS$P > .10$

a$P > .10$

reported a similar phenomenon of greater efficiency of IgM absorption when colostral Ig content was lower. The authors (18) proposed a selective IgM absorptive mechanism to explain this finding. It is also possible that intake of IgM in calves fed CS was not enhanced by the colostral replacer product. Some problems have been reported with the viability of IgM in colostral replacer products (9). Processing, storage and reconstitution may affect structure of the IgM molecule (15).

Among classes of Ig, $IgG_1$ had the lowest AEA. These data correspond with data reported by Husband et al. (10) and Quigley et al. (15). It has been hypothesized that IgG is transported from blood to interstitial fluid more rapidly than larger IgM molecules. Therefore, the amount of IgG measured in serum at time of sampling may be not actually reflects the amount absorbed, thereby underestimating AEA (10).

Concentrations of serum Ig concentration at 28 and 56 d of age are in Table 6. No significant difference due to treatment was found between groups.

## Conclusions

Within a range of colostral Ig fed in this study, there was no benefit of supplementation with CS. A negative relationship between intake of $IgG_2$ and serum IgM may suggest competition for a shared macromolecule transport mechanism through intestinal epithelium. Supplementing high

**TABLE 6. Least squares means of serum Ig concentration at 28 and 56 d of age in calves fed maternal colostrum (MC) or MC plus 125 g of colostral supplement (CS).**

| Item | MC | SE | CS | SE | $P^1$ |
|---|---|---|---|---|---|
| 28 d | | | | | |
| $IgG_1$ | 10.50 | 1.18 | 9.27 | 1.22 | NS[a] |
| $IgG_2$ | 1.01 | .06 | .89 | .07 | NS |
| IgM | .58 | .03 | .630 | .03 | NS |
| 56 d | | | | | |
| $IgG_1$ | 8.44 | .63 | 7.68 | .63 | NS |
| $IgG_2$ | 1.34 | .10 | 1.39 | .10 | NS |
| IgM | .65 | .03 | .65 | .03 | NS |

[1]Probability of a significant treatment effect.

[a]$P > .10$.

Ig colostrum may actually impair absorption of IgM and supplementing high Ig colostrum may reduce AEA of $IgG_1$ and IgM.

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# VITA

Silvia F. Abel Francisco was born in Santiago, Dominican Republic, on December 19, 1965. Her parents are Alfredo Abel and Lucila Francisco. She was raised in Santiago and graduated from Liceo Experimental UNPHU in June 1982. In August of the same year she attended The Universidad Nacional Pedro Henriquez Urena where she received the degree of Doctor in Veterinary Medicine on December 1988. After graduation she worked in small animal veterinary hospital. In 1989 she was awarded with a Laspau-Fulbright scholarship. In August 1990 she began graduate studies at the University of Tennessee where she obtained a Master Degree in Animal Science in August 1992. The author will return to her native country after graduation and will be part of the faculty staff at The Universidad Nacional Pedro Henriquez Urena, Santo Domingo.