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Colostrum supplementation and intestinal transmission of macromolecules in neonatal swine

Jerry A. McClain

To the Graduate Council:

I am submitting herewith a dissertation written by Jerry A. McClain entitled "Colostrum supplementation and intestinal transmission of macromolecules in neonatal swine." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

John P. Hitchcock, Major Professor

We have read this dissertation and recommend its acceptance:

D.A. Bemis, F.A. Draughon, F.B. Masincupp

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

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We have read this dissertation
and recommend its acceptance:

F B Masincupp

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Carolyn Minkel

Vice Provost and Dean of the Graduate School

COLOSTRUM SUPPLEMENTATION AND INTESTINAL TRANSMISSION OF MACROMOLECULES IN NEONATAL SWINE

A Dissertation
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Degree
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Jerry A. McClain
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Abstract

Three experiments were conducted utilizing 142 newborn (Hampshire X Yorkshire x Landrace) pigs. The major objectives were: 1) to examine the efficiency of a simple supplement to maternal colostrum for improving serum IgG concentration, energy status and survival rate of low-birthweight pigs; 2) to examine the effects of Ig concentrate, soybean trypsin inhibitor and L. aciodophilus on acquisition of passive immunity.

Supplementation of low-birthweight pigs, in Experiment I, tended to improve survival rate to 7 but not to 28 d of age. Neither serum IgG nor glucose were affected by supplementation. Subsequent work revealed methods to improve the efficiency of a supplemental mixture such as the one used in Experiment I. When BSA was used as a marker protein, addition of 50 $mg \cdot mL^{-1}$ of IgG concentrate to the mixture resulted in a 40 percent increase in serum BSA concentration. Incorporation of SBTI (5 $mg \cdot mL^{-1}$) into the supplement resulted in an additional increase in BSA concentration, and an improved ($P < .001$) serum concentration of PIgG as compared to only Ig concentrate and BSA in the mixture.

In Experiments II and III, incorporation of L. acidophilus into the mixtures resulted in less efficient intestinal transmission of proteins in pigs which were deprived of maternal colostrum. This effect was amplified by increasing the L. acidophilus from 10^8 to 10^{10} CFU mL^{-1}. Removal of L. acidophilus from the mixture by centrifugation did not result in a decreased concentration of BIgG. This eliminated the assumption that BIgG·L. acidophilus complexes were being

formed in great enough quantity to cause decreased BIgG. Similar reductions in serum concentrations of BIgG, as a result of feeding mixtures containing L. acidopilus, were not observed in pigs which were allowed access to maternal colostrum during the first 12 h of life. This was likely due to dilution and frequent bathing of the intestine with maternal colostrum.

From these experiments, it was concluded that increased protein concentration and SBTI had a positive effect on intestinal transmission of macromolecules in neonatal pigs which were deprived of maternal colostrum and could be used to improve supplement efficiency. However, incorporation of L. acidophilus into mixtures developed to improve passive immunity in neonatal pigs which are deprived of maternal colostrum is not recommended, since macromolecular transmission was reduced due to their presence. The mechanism by which this occurred is not clear and justifies further study.

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Introduction

The six layered placenta of the sow is efficient in preventing the transfer of maternal antibodies to fetuses. Thus, the pig is born with little if any circulating antibody, and with respect to antibody-dependent immunity, has an immunological handicap. This condition is not unique to the pig, but is shared by the newborn calf, lamb and foal. An adequate supply of maternal colostrum, prior to intestinal closure, is a simple solution to antibody deficiency in the neonate as well as a fulfillment of immediate nutritional needs. However, an adequate supply of colostrum is not always provided by the dam and situations in which the neonate is not capable of securing sufficient colostrum are not uncommon. In such situations, an alternative to maternal colostrum or a means of supplementing that which is available could prevent starvation and provide an adequate level of passive immunity.

The first study reported herein utilized low-birthweight newborn pigs and was designed to examine the effects of supplementing maternal colostrum with a mixture containing milk and immunoglobulins. Parameters measured were survival rate, weight gain and serum concentrations of immunoglobulin G and glucose. Two other studies are presented which were designed to examine the effects of milk, immunoglobulin concentrate, soybean derived trypsin inhibitor and Lactobacillus acidophilus on the intestinal transfer of macromolecules to the circulatory system of the neonatal pig.

Part I

Literature Review

A high rate of preweaning mortality continues to be a major source of waste in the swine industry. In a study, which included a total of 54 herds from the United States (48) and Canada (6), Stein et al. (1990) reported a 14.5 percent rate of mortality prior to weaning. This percentage did not include an additional 8.9 percent which were reported as dead at first observation, some of which were reported as possibly having been early mortalities rather than stillborn. Their data were collected from herds whose managers had independently chosen to use a computerized record keeping system (PigCHAMP) and may be more representative of some of the better North American producers as opposed to the average.

The pig is born into a competitive environment and must overcome numerous obstacles, including interference from littermates, in order to be assured adequate nutrition (Hartsock et al., 1976, 1977). Thus litter size and birthweight can influence opportunities for survival. It has been observed that as litter size increases, so does the number of low-birthweight pigs (Figure 1), as well as the number of pigs which do not survive to weaning (England, 1986; Pettigrew et al., 1986). However, more pigs from the large litters with a higher number of mortalities were weaned (Figure 2).

A low percentage of body fat (~1 percent) is a common characteristic of newborn pigs (Benevenga et al., 1989). During starvation, newborn pigs have the capacity to mobilize free fatty acids from lipid stores, yet the limited supply precludes lipid as a major form of reserve energy. Their low body fat coupled with sparse hair growth also tends to afford poor thermoinsulation.

Glycogen levels increase in a number of tissues during the latter stages of gestation and these stores are extensively utilized during the first hours of life.

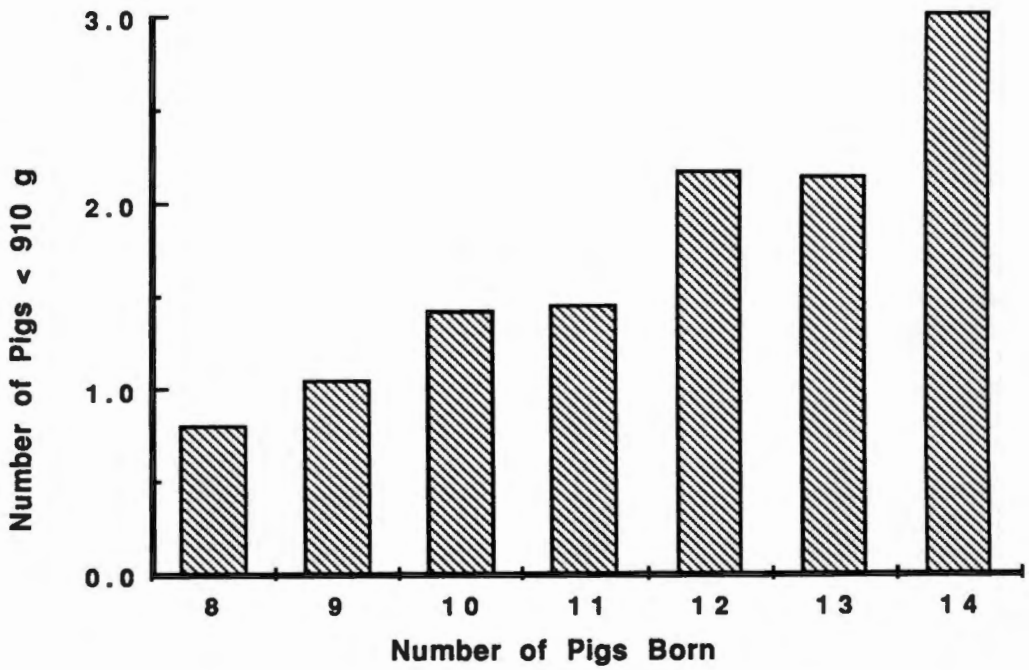

Figure 1. Occurrence of Low-birthweight Pigs in Different Sized Litters[a]

[a]adapted from England, 1986

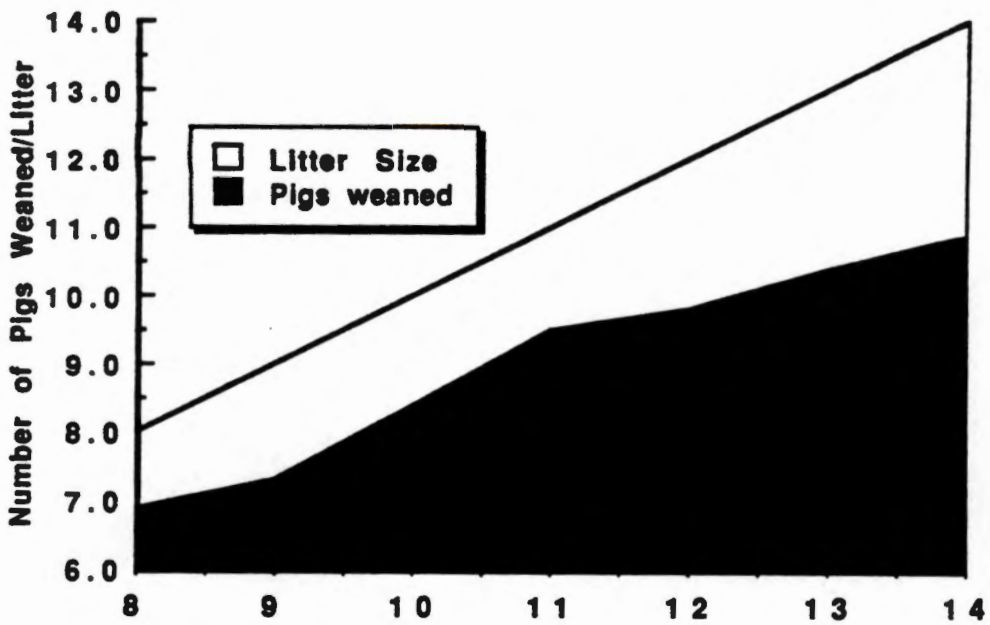

Figure 2. Relationship of Litter Size to Number of Pigs Weaned/Litter[a]

[a]adapted from England, 1986.

On a wet weight basis, liver contains as much as 200 mg $\cdot$ g^{-1} glycogen and muscle tissue contains approximately 120 mg $\cdot$ g^{-1} at birth. Blood glucose is approximately 65 mg$\cdot$ dL^{-1} at birth and increases for about 6 hours, even without feeding, after which time it is very poorly maintained. After normal feeding, glucose increases rapidly and is normally near 100 mg $\cdot$ dL^{-1}.The amount of time prior to total depletion of glycogen reserves varies with environmental and other factors , but liver glycogen will normally reach a minimal level by 12 to 18 hours postpartum. The rate of decrease in muscle glycogen is somewhat slower and a minimal level is reached between 36 and 48 hours after birth (Anderson and Wahlstrom, 1970).

The newborn pig is limited with respect to its ability to regulate body temperature (Svendsen et al., 1986). At parturition, body temperature decreases rapidly from 39 to 37^0 C , and after initiation of nursing will generally increase again after 1 to 2 hours. The critical temperature of the newborn pig is approximately 35^0 C. Thermoregulatory mechanisms improve during the first week of life and the critical temperature declines to approximately 25^0 C. Chilling of newborn pigs is not at all uncommon, and has been associated with a reduction in the acquisition of colostral immunoglobulins (Blecha and Kelley, 1981).

Factors discussed above contribute to the fragility of the newborn pig and influence survival. The relative importance of each factor, with respect to its influence on survival , would be difficult to assess in that all interact and have different levels of influence under various predisposing conditions.

Colostrum: Role in Passive Immunity

An abundance of work has been conducted with respect to the essential value of colostrum, its composition and its nutritional and immunological roles for the newborn pig (Payne and Marsh,1962; Lecce and Morgan, 1962; Hardy, 1965; Werhahn et al., 1981; Klobasa et al., 1987). Immunologically, colostrum and milk are important in two distinct ways. During the first 24 to 36 hours of life, immunoglobulin molecules are absorbed from ingested colostrum directly into the blood and lymphatic systems. Immunoglobulins which are provided by milk provide local intestinal protection until the source is withdrawn (Wilson, 1974).

Though the quantity of specific components of colostrum vary from animal to animal, depending upon age, vaccination history, nutritional status etc.; at parturition, colostrum contains approximately 15 $g \cdot dL^{-1}$ of protein. Of this protein, about 60 percent is immunoglobulin. At the onset of lactation, about 70% of the total immunoglobulin is IgG, 20 % is IgA and 10% is IgM . As lactation progresses, the relative percentages of the immunoglobulin classes change and IgA becomes the predominant immunoglobulin class (Figure 3). These changes correspond to a dramatic drop in the concentration of IgG and thus total protein (Figure 4) (Jensen and Pedersen, 1979; Klobasa et al., 1987).

This scheme, as provided by nature, is very accomodating in that IgG is well suited to systemic immunity and is present in abundance prior to intestinal closure; and IgA, which is resistant to proteolytic degradation continues to offer enteric protection following cessation of intestinal transfer of macromolecules. The importance of a continued supply of IgA, with respect to survival, has been illustrated and is likely due to the ineffectiveness of colostral derived circulating antibodies at mucosal surfaces (Wilson, 1974; Varley et al., 1986).

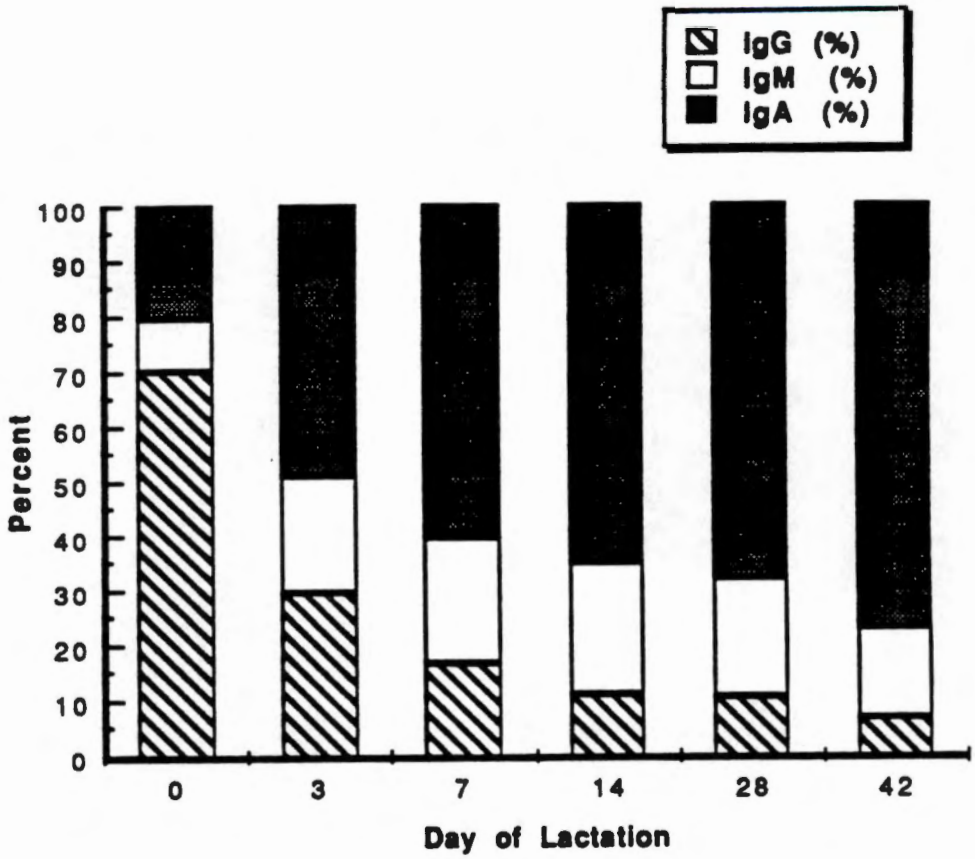

Figure 3. Relative Proportions of IgG, IgA and IgM in Porcine Whey[a]

[a]Adapted from the data of Klobasa et al., (1987) and Jensen and Pedersen, (1979).

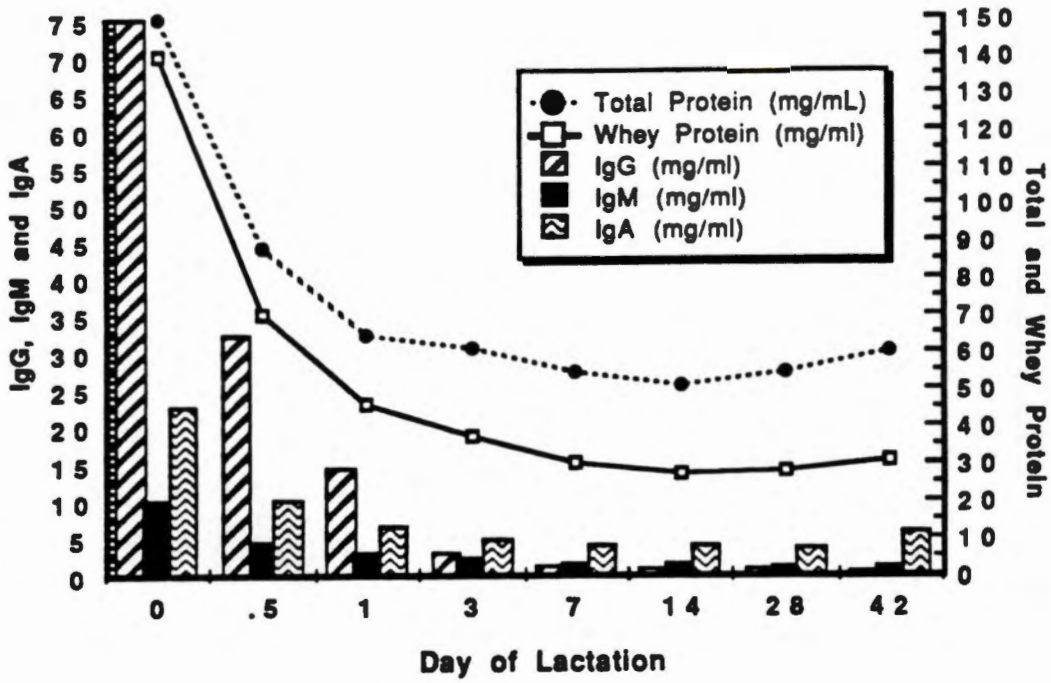

Figure 4. Concentration of Proteins During Lactation[a,b]

[a]Adapted from the data of Klobasa et al. (1987) and Jensen and Pedersen, (1979).
[b]IgG, IgM and IgA were quantitated from the whey fraction.

IgG has a half-life of approximately 14 days, IgM 5 days and IgA about 2.5 days (Curtis and Bourne, 1971). Even though newborn pigs and even fetuses as young as 55 days prepartum have been shown to be capable of antibody production (Prokesova et al., 1979, 1981;), serum immunoglobulin concentrations decrease rapidly during the first two weeks of life. Immunoglobulin synthesis in pigs which have received colostrum, is not generally detectable until 7 to 10 days of age (Allen and Porter, 1973; Wilson, 1974; Lecce, 1986) .

Traditional thought has been that the pig should receive as high a level of passive immunity as could be made possible. This has been illustrated through the various vaccination schemes directed at boosting the immunity in sows and thus passing the immunity to pigs via colostrum. However, there is convincing evidence that passively acquired IgG interferes with the development of active immunity (Drew and Owen, 1988). IgM, on the other hand, does not suppress de novo synthesis of immunoglobulins (Porter, 1986; Klobasa, 1990).

Intestinal Transmission of Macromolecules

Swine are included in Group III mammals, which unlike humans, do not transfer antibodies to their fetuses in utero. During the latter stage of lactation, immunoglobulins are transferred to colostrum, and some synthesis occurs within the mammary tissue. IgA is the only class of immunoglobulin which is synthesized predominantly by mammary cells (60%). All of the IgG and most of the IgM is transferred from serum (Bourne and Curtis, 1973). Thus it is logical that passive immunity which is afforded to the newborn pig is a reflection of the dam's exposure, and in a normal situation, with respect to antibody specificity, the immunity should be appropriate to the respective environment.

Intestinal transmission reaches a maximum from 4 to 12 hours after the first feed (Westrom et al., 1984). Transmission then rapidly declines and has generally ceased by 36 hours of age. However, uptake of macromolecules by fetal-type enterocytes does not cease at this time. The uptake of various proteins and polyvinyl pyrrolidone was shown to continue for about 3 weeks . Thus it has been demonstrated that uptake of macromolecules is not the limiting factor in cessation of transmission (Clarke and Hardy, 1971).

In a review, Staley and Bush (1985) described the transmission of macromolecules as occurring in three steps. First, the immunoglobulin binds the microvillus border, and subsequently endocytosis of the binding site and immunoglobulin occurs. The endocytosed membrane is described as having a tubular appearance. Second, there is enlargement of the tubular end piece, as a vacuole is formed. As the vacuole is enlarged, there is condensation of its contents and it breaks loose from the tubule. Third , the vacuole is transported to the cell membrane and releases its content in the lamina propria and the contents continue into the circulation.

The cessation of transfer of macromolecules from the intestine to the circulation is not fully understood. However, the effects of a number of factors on the occurrence of closure have been characterized. A small amount of colostrum (10 $mL \cdot Kg^{-1}$ $bodyweight^{-1}$) , given to newborn pigs, followed by fasting, has been shown to induce closure; while pigs which were starved for 19 to 22 hours after birth had partially lost the capacity for intestinal transmission (Westrom et al., 1984, 1985). Mature milk has been shown to induce closure more rapidly than a 5 percent glucose solution (Vellenga et al., 1988).

The development of intracellular digestion has been suggested as a possible explanation for the cessation of transmission. During the period of transmission, Brown and Moon (1979) failed to find acid phosphatase in jejunal cells during a two hour period after feeding. However , acid phosphatase was found in the cells of pigs from 1 through 10 days of age.

The intestine of the newborn pig has the capacity to absorb a variety of intact proteins and other molecules (ie., polyvinyl pyrrollidone,fluorecein isothyocyanate labelled Dextran, bovine and human albumin , horseradish peroxidase, etc.) (Lecce et al., 1961; Svendsen,et al., 1990), and it has been concluded that absorption is a nonselective process. Yet some studies have suggested at least a limited degree of selectivity. Pierce and Smith (1967) observed a greater transfer of albumin as compared to colostral IgG, and suggested that interference was due to competition between similar molecules for a common receptor within the pinocytotic vessicle. Porter (1969) concluded that secretory IgA from which the secretory-piece had been removed was absorbed, while intact secretory IgA from sow colostrum was not absorbed. The possibility of the presence of receptors for immunoglobulin binding has been investigated, but no confirmation has been presented (Staley and Bush, 1985).

Competition for binding sites or interference in transmission of antibodies by bacteria have not been thoroughly studied. However , it is well known that some microorganisms are capable of attachment to intestinal epithelial cells and there exists some evidence for competition or interference with transmission. Staley et al. (1972) monocontaminated newborn pigs with E. coli and observed a reduction in the capacity for the intestine to transport intact proteins. James et al. (1980) reported similar observations after examining ligated intestinal loops of calves which had been exposed to intestinal bacteria.

The identification of a trypsin inhibiting component in sow colostrum (Laskowski et al., 1957) led to the assumption that intestinal transmission was facilitated by the sow colostrum trypsin inhibitor (SCTI) through the prevention of proteolytic degradation. Hardy (1969) demonstrated that proteolysis of gamma globulin did indeed occur in the stomach, duodenum and ileum of the newborn pig after feeding. He also observed that colostrum reduced hydrolysis of the proteins and that this effect could be simulated to some extent by the use of a synthetic trypsin inhibitor (Trasylol). More recent studies have further characterized the presence and role of SCTI (Jensen and Pedersen, 1979; Carlsson et al., 1980; Westrom et al.,1982, 1985). Estimated levels of SCTI and its capacity to inhibit trypsin at various days of lactation are presented (Figure 5). It is interesting to note the similarities between the decline in the presence of trypsin inhibitor and immunoglobulins in colostrum (Figure 4)

The present discussion has alluded to the efficiency of colostrum for providing passive immunity to the neonatal pig, provided an adequate amount is consumed. In the development of schemes designed to supplement animals which are not capable of securing adequate nursing privileges, we should not ignore the characteristics of that product which has been provided by nature.

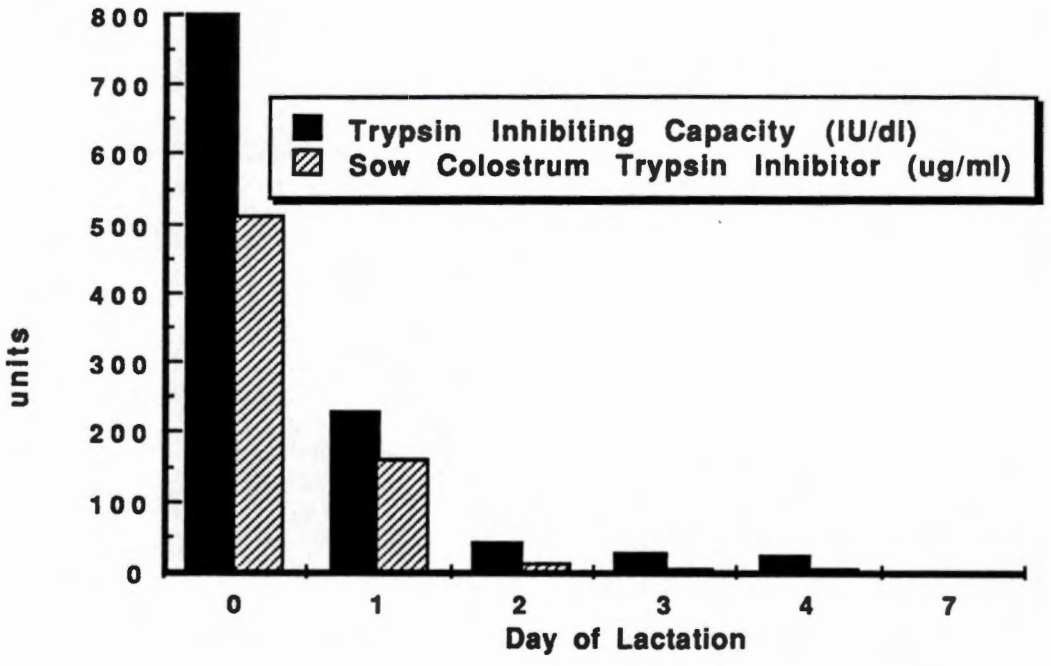

Figure 5. Inhibiting Units (IU) and Concentration of Trypsin Inhibitor in Sow Colostrum[a]

[a]Extrapolated from Westrom et al.,1982.

Naturally occurring biological products such as trypsin inhibitors from plant sources, immunoglobulins from the blood of animals in meat processing plants and whey from cheese processing are a few of the presently availabe resources. Studies designed to examine such products, with respect to their nutritional and immunological qualities, could ultimately result in a reduction of waste in the animal industry through an efficient utilization of by-products and a decrease in neonatal mortality.

PART II

COLOSTRUM SUPPLEMENTATION OF LOW-BIRTHWEIGHT PIGS

Abstract

To examine the effects of colostrum supplementation on survival of low-birthweight pigs,fifty-seven (Hampshire x Duroc x Landrace) pigs from nineteen litters born in two separate farrowing groups were utilized. The farrowing facility was visited at 12 h intervals, at which times low-birthweight (< 1.2 kg) pigs and one average sized, viable pig from each new litter were assigned to a treatment. Low-birthweight pigs either received three doses of colostrum supplement at 12 h intervals, or were simply encouraged to nurse. The supplement consisted of whole-fat milk to which 50 $mg \cdot mL^{-1}$ of immunoglobulin concentrate had been added. The concentrate contained 34 $mg \cdot mL^{-1}$ IgG. Average littermates were used as positive controls. Supplementation of low-birthweight pigs did not result in improved survival or weight gain to 28 d , as compared to littermates which were not supplemented. However, there was a tendency for improved survival to 7 d of age in supplemented pigs. Serum IgG and glucose were not affected by supplementation.

Introduction

A reduction in the 20 to 25 percent mortality rate of pigs prior to weaning is an easily recognizable area in which efficiency of swine production can be improved. Most deaths occur during the first few days of life, and at least 50 percent of these can be attributed to starvation (England, 1986).

Inherent physiological characteristics of the newborn pig contribute substantially to its fragility. The newborn pig's body contains only 1 to 2 percent fat (Manners and McCrea, 1963), and though tissue carbohydrate stores are relatively large, the capacity for gluconeogenic activity is limited (Mersmann, 1974; Elliot and Lodge 1977; Boyd et al., 1981). The newborn pig's limited capacity to utilize internal energy reserves amplifies the importance of an external energy source.

Colostrum provides energy and passive immunity to pigs which are successful in acquiring and maintaining suckling privileges during the initial 24 to 48 h, during which time nursing patterns are being established. Weak or small pigs are at a disadvantage during this time and can easily become exhausted and die of hypoglycemia or subsequent infections due to inadequate passive immunity.

Immunoglobulin G (IgG) is the predominant Ig in sow's colostrum, approximately 80 percent, and its transfer across the intestinal epithelium is vital for passive disease protection (Porter 1969; Curtis and Bourne, 1971; Wilson, 1974). Adequate passive immunity is dependent upon sufficient intake of colostrum prior to cessation of intestinal permeability to macromolecules, which normally occurs within 24 to 36 h after birth (Speer et al., 1959; Lecce et al., 1964; Vellenga et al., 1988).

The primary objective of this study was to investigate the effects of supplementing maternal colostrum with whole-fat milk which contained porcine serum immunoglobulins on the mortality rate of low-birthweight pigs. Secondary objectives were to examine effects of supplementation on serum glucose, total protein and IgG.

Materials and Methods

Fifty-seven (Hampshire x Duroc x Landrace) pigs from 19 litters and born in two farrowing groups (July and October) were utilized. The farrowing facility was visited at 12 h intervals. Pigs which had been born during the previous 12 h were weighed, and those that weighed less than 1.2 kg were assigned to receive supplement (n=19) or as negative controls (n=19). All low-birthweight pigs were encouraged to nurse at each handling. A viable, average sized pig from each litter was selected to serve as a positive control (n=19).

The supplement consisted of whole-fat milk and 50 mg $\cdot$ mL^{-1} of Ig concentrate. Supplement was administered via stomach tube within 12 h of birth. A total of three 12 mL doses were administered at 12h intervals.

To acquire serum for separation of immunoglobulins, blood was collected from market hogs and sows at the time of slaughter. Immunoglobulins were isolated by ammonium sulfate precipitation and subsequent dialysis as described by Garvey et al.(1977). The harvested Ig concentrate was then lyophylized, pooled and stored at 4^0 C until used. The Ig concentrate contained 68 percent IgG. Other proteins were not quantitated.

Blood samples (6 mL) were collected, via the anterior vena cava, prior to the first supplemental feeding, 24 h later and immediately prior to the last supplement administration. Serum was harvested by centrifugation and stored at -44^0 C until analyzed.

Serum IgG was quantitated by a two-antibody sandwich ELISA (Harlow and Lane, 1988). Affinity purified porcine IgG was utilized as a standard (Sigma, I-4381). Conjugated anti- porcine IgG and anti-porcine IgG were purchased (Sigma, 61-9120 and 61-9100, respectively). Total serum protein and glucose

were quantitated by colorimetric procedures (Sigma procedures 540 and 510, respectively).

Treatments were assigned on a littermate basis and data were analyzed as a complete randomized block design (SAS, 1986). There were no significant block x treatment effects. Means were subjected to the Student-Newman-Keuls multiple range test.

Results and Discussion

Results are summarized in Tables 1 and 2. Initial weights were similar for supplemented and negative control pigs. Average daily gain was not improved by supplement administration, and low-birthweight pigs gained less ($P < .05$) weight than did positive control littermates.

There was a tendency for improved survival rate to 7 d in low-birthweight pigs which had been supplemented, as compared to negative control pigs. Survival of low-birthweight pigs to 28 d was not improved by supplementation, and survival rate was greater ($P < .05$) in positive control pigs than in low-birthweight pigs.

Prior to treatment, total serum protein, IgG and glucose were higher ($P < .05$) in positive control pigs as compared to the levels in low-birthweight pigs. Twenty-four h after the initial administration of supplement, total serum protein concentration in supplemented pigs was somewhat higher than in negative control pigs. Twenty-four h after the initial supplemental feeding, serum IgG concentration was a modest 2.5 mg $\cdot$ mL^{-1} higher in supplemented pigs, as compared to that in negative control pigs, and did not significantly differ. Serum glucose did not differ between treatments 24 h after the initial sampling period but remained somewhat higher in positive control pigs.

Mean concentrations of serum protein, IgG and glucose, at the initial sampling period, were higher in pigs which survived to 28 d than in pigs which died at earlier ages (Figure 1). All of these variables are indicative of the nutritional status of the pigs, and emphasize the importance of adequate colostrum at an early stage. It appears that the underlying cause for most of the deaths which occurred during the first 1 to 2 d could be attributed to inadequate

Table 1. Survival Rate and Average Daily Gain of Pigs to 28 d of Age

Treatment	Percent Survival to day: N	2	3	7	28	Initial Wt (kg)	ADG (kg)
Negative Control	19	73.7	57.9[a]	52.6[a]	47.4[a]	1.1[a]	.17[a]
Supplemented	19	89.5	79.0[ab]	79.0[ab]	57.9[a]	1.0[a]	.19[a]
Positive Control	19	94.7	94.7[b]	89.5[b]	89.5[b]	1.6[b]	.25[b]

[ab]Different superscripts within a column indicate a difference ($P < .05$)

Table 2. Serum Protein, IgG and Glucose Prior to Supplement Administration and 24 h Later

Time / Treatment	Protein ($mg \cdot mL^{-1}$)	SE[c]	IgG ($mg \cdot ml^{-1}$)	SE[c]	Glucose ($mg \cdot dL^{-1}$)	SE[c]
Initial						
Negative Control (n=19)	35.0[a]	2.1	13.9[a]	1.9	76.9[a]	5.7
Supplemented (n=19)	34.5[a]	2.7	13.2[a]	2.0	72.5[a]	5.8
Positive Control (n=19)	52.2[b]	2.4	29.9[b]	1.6	101.5[b]	6.5
+24 h						
Negative Control (n=14)	39.5[a]	2.8	17.4[a]	1.7	77.7	6.4
Supplemented (n=17)	44.3[ab]	3.6	19.9[a]	1.2	76.9	4.6
Positive Control (n=18)	50.8[b]	1.1	29.8[b]	0.8	94.4	5.6

[ab]Within each time period, different superscripts within a column indicate a difference ($P < .05$)

[c]Standard error of mean

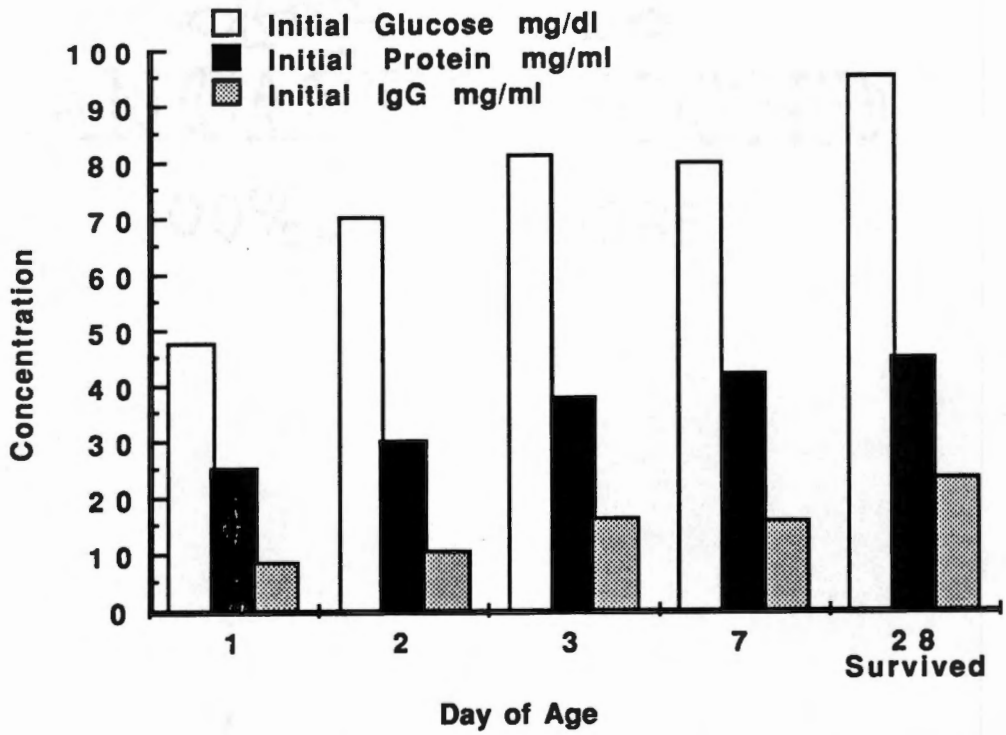

Figure 1. Initial glucose, protein and IgG of pigs which survived to 28 days in comparison to those which died at earlier ages.

nutrient intake.

Metabolic patterns of neonatal swine and the importance of acquiring glucose homeostasis have been well documented (Morrill, 1952; Mersmann, 1974). Normally, glucose levels at birth are near 65 mg· dL^{-1}, and after normal nursing, tend to level off at about 100 mg· dL^{-1}. Based upon low glucose levels in the present study, it was obvious that a number of pigs had not nursed prior to the initial sampling period.

A major purpose for supplementation is to provide a temporary source of nutrients which will allow the pig enough energy to establish a nursing pattern. Data do not suggest that this occurred in the present study. It has been suggested that intermittent feedings are undesirable and may result in increased metabolism and a more rapid reduction of an already inadequate energy supply (Svendsen et al., 1986). Though supplementation did not result in improved glucose levels at the second sampling period, no detrimental effects were observed in this study.

Providing supplementation at intervals more frequent than every 12 h, as was done in the present study, could result in an improvement in results. However, in many operations it would be difficult for workers to be available for such work at the times needed. Other management schemes which provide deprived pigs access to supplementation are possible. A number of liquid feeders designed for baby pigs are presently available. Equalizing litter size through cross-fostering has been shown to be be beneficial as well (England, 1986).

The essentiality of adequate passive immunity in a farm environment is well recognized and accepted (Lecce, 1975; Blecha and Kelley, 1981; Machedo-Neto et al.,1987). It has been shown that small amounts of colostrum

(10 mL· kg^{-1}· body $weight^{-1}$) given to newborn pigs can induce closure by 24 h of age. This emphasizes the importance of a continued source of Igs , prior to closure, in order for pigs to acquire serum antibody levels which are adequate for passive disease protection.

Supplementation of immunoglobulins in the present study did not result in a significant increase in serum IgG, though a tendency for increased serum total protein was observed. This lack of response by supplemented pigs may have been due to enhancement of closure in combination with failure to nurse adequately subsequent to supplementation. Furthermore, based upon the concentration of IgG in the supplement, the small increase in serum IgG and slightly lower glucose level of supplemented pigs 24 h after supplementation was initiated, it appears that surviving pigs which were not supplemented nursed more successfully between sampling periods than did supplemented pigs.

Implications

Supplementation of low-birthweight pigs with three 12 mL doses of milk which contained 50 mg· mL^{-1} of immunoglobulin concentrate resulted in a rate of mortality which was comparable to that of thrifty littermates to 7 d of age. However, this trend did not continue to 28 d of age at which time mortality rate of supplemented pigs was more similar to that of low-birthweight pigs which were encouraged to nurse. Weight gain, serum IgG or glucose were not affected by supplementation. Further work aimed at improving the efficiency of colostrum supplementation is needed. With respect to the present supplemental mixture, intestinal transmission of macromelcules, for providing passive immunity, and energy status are two areas in which improvements are needed.

PART III

INTESTINAL TRANSMISSION OF MACROMOLECULES

Abstract

Thirty-six pigs from six litters were removed from their dams prior to nursing and were utilized to examine the effects of various potential colostrum supplement components on intestinal transfer of macromolecules. Pigs were returned to their dams at 12 h of age and no detrimental effects were observed. Pigs were given four 12 mL feedings of supplemental mixture at 3 h intervals. Each mixture contained bovine serum albumin (BSA) as a marker. Other components were milk, immunoglobulin concentrate, soybean trypsin inhibitor and L. acidophilus. Using milk as a base, in an additive fashion, other components listed above were added to form four diets . A fifth diet was formed by adding L. acidophilus , but not soybean trypsin inhibitor. Porcine colostrum was utilized as a control. At 12 h of age, the lowest serum concentration of BSA was observed in pigs which had been fed only milk and BSA. Feeding porcine colostrum which contained the marker protein resulted in the most pronounced appearance of BSA ($P < .02$) and IgG ($P < .001$) in serum. Incorporation of SBTI had a tendency to increase absorption of BSA, and resulted in increased ($P < .001$) serum IgG . Pigs which were fed mixtures which contained L. acidophilus tended to have lower concentrations of BSA and IgG at 12 h of age as compared to comparable treatments which did not contain L. acidophilus. The initiation of nursing at 12 h of age led to an increase in serum protein , globulin and IgG in all pigs by 24 h of age. Serum concentration of IgG at 36 h of age did not differ between any treatments. These results suggest that SBTI improves intestinal transmission of macromolecules in newborn pigs which have not received colostrum.

Since the pig at birth is virtually devoid of circulating antibodies, ingestion and absorption of immunoglobulins are necesary for antibody-dependent immunity. The transfer of immunoglobulins from the intestine is facilitated by specific properties of intestinal mucosal cells which allow endocytosis and subsequent elimination of intact macromolecules into the blood and lymphatic spaces (Staley and Bush, 1985). Following the ingestion of colostrum, the capacity to transfer macromolecules rapidly declines, and closure generally has occurred by 24 to 36 h of age (Speer et al., 1959).

The period prior to closure may be lengthened in pigs which have been starved or fed limited amounts of glucose solution (Lecce, 1966; Vellenga et al.,1988). Unfortunately, the neonatal pig has a very low energy reserve (Mersmann, 1974; Okai et al., 1978) and can, in a relatively short time, become too weak to compete with littermates. Furthermore, the immunoglobulin concentration of colostrum decreases rapidly with normal nursing, and may decrease by as much as 50 percent after 4 h of normal nursing (Bourne, 1969). Thus delayed nursing accompanied by a weakened condition could very well lead to failure to acquire an adequate level of passive immunity.

No specific concentration of circulating immunoglobulins is necessary for adequate passive immunity. The value of passively acquired immunity depends not only upon the quantity of acquired antibodies but also upon their relationship to specific disease causing agents in the animal's immediate environment. Yet,under practical conditions, it has been clearly demonstrated that pigs with depressed immunoglobulin concentrations during the first day of life are more likely to die by 21 days of age (Blecha and Kelley,1981).

Oral solutions, consisting of a variety of components, but usually containing an energy source and immunoglobulins are a present possibility for reducing neonatal mortality due to inadequate ingestion of colostrum. With respect to the importance of passive immunity, it would be desirable to know the effects of supplemental components on the intestinal transfer of macromolecules. The aim of the present study was to investigate this question relative to a number of components which could be incorporated into a colostrum supplement. Milk, immunoglobulin concentrate (Ig concentrate), soybean derived trypsin inhibitor (SBTI), L. acidophilus and porcine colostrum were examined relative to their effects upon absorption of a marker protein, bovine serum albumin (BSA), during the first 12 h of life. The effects upon changes in IgG, total protein and total globulin also were examined at 12, 24 and 36 h of age.

Thirty-six (Duroc x Hampshire x Landrace) pigs from six litters were used in the experiment. Farrowings were attended, and the first two pigs born were allowed to remain with the sow. The next six healthy pigs born were removed prior to nursing, housed in stainless steel cages and randomly assigned to one of six treatments, which are described in Table 1.

Following parturition and 3, 6, and 9 h later, pigs were fed 12 ml of mixture, via stomach tube. Blood samples (4 ml) were taken, via the anterior vena cava, at parturition and 12, 24 and 36 h later. Blood was allowed to clot, centrifuged and the harvested serum was stored at -44^0 C until used.

Following the initial 12 h period, pigs were returned to their dams. All pigs were readily accepted by the sows and nursing was quickly initiated. No detrimental effects associated with treatment or blood sampling were observed.

Supplemental components.

To acquire serum for Ig precipitation, blood was collected from market hogs and sows at the time of slaughter. Blood was caught in pails, transferred to 250 ml centrifuge bottles, allowed to thoroughly clot and then centrifuged. Following centrifugation, serum was harvested and stored at -20^0 C until used.

Immunoglobulins were isolated from the serum by ammonium sulfate precipitation and subsequently dialyzed extensively for removal of salts as described by Garvey et al. (1977) (Appendix A). The harvested Ig concentrate was then lyophylized and stored at 4^0 C. Prior to use, the Ig concentrate was pooled. The pooled Ig concentrate contained 89 percent protein and 68 percent IgG as determined by kjeldahl and ELISA, respectively.

Table 1. Description of Treatments

Designation	Description
T1	Milk + BSA (50 $mg \cdot mL^{-1}$)
T2	Milk + BSA (50 $mg \cdot mL^{-1}$) + Ig Concentrate (50 $mg \cdot mL^{-1}$)
T3	Milk + BSA (50 $mg \cdot mL^{-1}$) + Ig Concentrate (50 $mg \cdot mL^{-1}$) + SBTI (5$mg \cdot mL^{-1}$)
T4	Milk + BSA (50 $mg \cdot mL^{-1}$) + Ig Concentrate (50 $mg \cdot mL^{-1}$) + SBTI (5$mg \cdot mL^{-1}$)+ L. acidophilus (10^6 $CFU \cdot mL^{-1}$)
T5	Milk + BSA (50 $mg \cdot mL^{-1}$) + Ig Concentrate (50 $mg \cdot mL^{-1}$) + L. acidophilus (10^6 $CFU \cdot mL^{-1}$)
T6	Porcine Colostrum + BSA (50 $mg \cdot ml^{-1}$)

Porcine colostrum was collected from sows by manual milking during parturition. The colostrum was pooled and stored at -20^{0}C until needed. Pooled colostrum contained 48 mg· mL $^{-1}$of IgG as was determined by ELISA. Other proteins were not quantitated.

Dried, whole-fat, mature milk was used in the mixtures at 20 percent solids. BSA (Fraction V) and SBTI (T-9128) were purchased (Sigma, St. Louis, Mo.).

Lyophilized L. acidophilus was purchased (American Type Culture Collection 4356) and stored at 4^0 C until it was used. For use, it was rehydrated and cultured in Man-Rogola-Sharpe (MRS) broth at 37 C in an atmosphere of 95 percent O_2 and 5 percent CO_2 . Cells were harvested by centrifugation and washed three times in .85 percent saline. Absorbance of serial dilutions of a portion of the final pellet were read on a Perkin Elmer spectrophotometer (660 nm) to establish a standard curve, and the number of colony forming units was established by the standard plate count method (Clark, et al., 1978). The viability of cells was determined prior to each use, but after transfer to the experiment site it was not possible to monitor changes in cell viability. Only L. acidophilus was in solution until moments before the first feeding at which time the remaining preweighed components were rehydrated to a volume of 48 mL.

Quantitation of serum proteins.

A two-antibody sandwich ELISA was used for quantitation of serum IgG and BSA (Harlow and Lane, 1988) (Appendix B). Antibodies to whole molecules were used in all ELISA assays. For quantitation of BSA, rabbit-anti-BSA and horseradish peroxidase conjugated rabbit-anti-BSA were purchased (United States Biochemicals, 1006 and Sigma, 61-2100, respectively).

BSA (Fraction V, Sigma) was used as a standard. For quantitation of IgG, rabbit-anti-porcine IgG and horseradish peroxidase conjugated rabbit-anti-porcine IgG were purchased (Sigma, 61-9120 and 61-9100, respectively). Purified porcine IgG was used as a standard (Sigma,I-4381). Total protein and globulin were quantitated using colorimetric procedures (Sigma, procedures 540 and 560, respectively).

Statistical analysis.

Utilizing the statistical analysis system for linear models (SAS, 1986), predetermined contrasts were used to determine the effects of treatments. To avoid confounding due to litter variations, all treatments were represented in each litter.

Results and Discussion

A low concentration of IgG was detectable in the serum of 9 of the pigs which were tested at birth. Figure 1 illustrates an ELISA of samples taken at birth, which demonstrates both the presence and absence of serum IgG. The optical densities of samples are presented in contrast to purified standard and background reaction of the primary and secondary antibodies. The sample which is illustrated represents the highest concentration of IgG (~.4 $mg \cdot mL^{-1}$) which was found in a newborn sample. In general, serum proteins of all pigs were similar at the time of birth (Table 2).

The various diets did have an effect upon absorption and serum concentration of the marker protein BSA at 12 h of age (Table 3). Feeding porcine colostrum with the marker protein resulted in the most amplified ($P < .02$) serum concentration of BSA.

The lowest serum concentration of BSA was found in pigs which were fed only milk and BSA. Addition of Ig concentrate, and then SBTI resulted in additively increased serum concentrations of BSA. Serum BSA concentrations as a result of addition of either of these individual components independently, did not differ from the preceding treatment.

L. acidophilus was incorporated into the mixtures under two conditions, in the presence and in the absence of SBTI. In each case, there was some reduction in serum BSA concentration. This reduction was particularly notable in the comparison of serum of pigs which had received mixtures that did not contain SBTI, but in either case its presence did not result in a difference.

IgG concentrations followed a pattern similar to that which was described for BSA (Table 3). As would be expected, feeding porcine colostrum resulted in

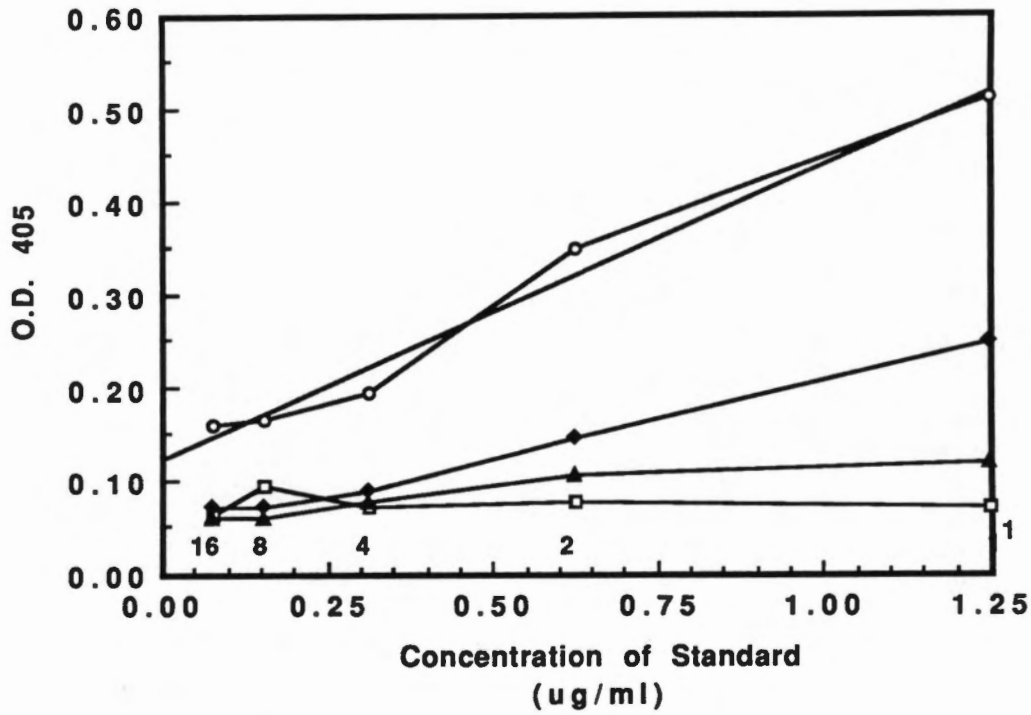

Figure 1. Serum IgG Reactivity at Birth

[1]Sample Dilution x 10^3

- Standard
- Negative Control (Primary x Secondary Antibodies)
- Birth 1 (no indication of IgG)
- Birth 2 (~.4 mg/ mL)

Table 2. Serum Protein Concentrations of Newborn Pigs Prior to Feeding
($mg \cdot mL^{-1}$)

	Variable					
Treatment	Protein	SE[a]	Globulin	SE[a]	IgG	SE[a]
T1	23.4	0.6	8.0	0.5	.05	.05
T2	22.9	1.0	7.4	1.1	.02	.02
T3	22.6	0.8	8.2	1.1	.06	.04
T4	22.5	0.7	9.0	1.0	.03	.03
T5	22.8	0.8	9.0	1.0	.06	.04
T6	22.2	0.6	8.2	0.7	.07	.07

[a]Standard Error of Mean

Table 3. Protein Concentrations in Serum at 12 Hours of Age ($mg \cdot mL^{-1}$)

				Variable							
Treatment[a]	Ig	SBTI	LA	Protein[c]	SE[b]	Globulin[d]	SE[b]	IgG[e]	SE[b]	BSA[f]	SE[b]
T1	-	-	-	25.4	0.5	6.4	0.2	-	-	4.4	0.5
T2	+	-	-	32.6	1.9	12.4	0.9	6.6	0.2	6.3	0.4
T3	+	+	-	34.8	1.8	16.7	1.4	10.3	0.5	7.7	1.2
T4	+	+	+	34.4	1.8	13.0	1.3	9.0	0.6	7.1	1.0
T5	+	-	+	29.0	1.4	9.7	0.7	5.7	0.7	4.6	0.5
T6	-	-	-	43.4	1.5	25.0	1.3	16.0	0.7	10.0	0.8

[a]All treatments contained BSA as a marker,(+) indicates the presence and (-) indicates the absence of Ig (Immunoglobulin concentrate); SBTI (Soybean trypsin inhibitor); LA (L. acidophilus 10^6 $CFU \cdot mL^{-1}$) T6=porcine colostrum, T1=milk.

[f]Standard Error of Mean

Contrasts

T1 vs T2 [c](P< .003), [d](P <.001)

T2 vs T3 [d](P <.01), [e](P<.001)

T3 vs T4 [d](P < .02), [e](P~.09)

T4 vs T5 [c](P< .02), [d,f](P<.04), [e](P<.001)

T6 vs T4 [c,d,e,](P<.001), [f](P<.02)

the highest concentration ($p < .05$) at the end of the initial 12 h period.

The incorporation of 5 $mg \cdot mL^{-1}$ of SBTI into the mixture led to an increased ($p < .001$) serum concentration of IgG as compared to the Ig concentrate and BSA fed alone. The positive effect of SBTI was somewhat inhibited ($P \sim .09$) when L. acidophilus was incorporated into the mixture, but serum IgG was still higher ($p < .001$) in each of the two groups of pigs that received mixtures which contained SBTI as compared to pigs which received mixtures that did not contain SBTI. Serum total protein and globulin concentrations increased in all pigs which were treated with specific protein mixtures during the initial 12 h period.

The initiation of nursing at 12 h of age led to subsequent increases in serum proteins by 24 h, with exception to BSA, in which concentration decreased substantially (Table 4). Pigs with lower serum IgG concentrations at 12 h experienced increases in serum IgG which, in most cases, were sufficient for alleviation of differences between treatments at 24 h. However, pigs which initially had been fed mixtures which contained L. acidophilus, but not SBTI, had a lower ($P < .05$) concentration of serum IgG at 24 h as compared to pigs which had not received either.

Protein concentrations at 36 h of age are shown in Table 5. No treatment difference existed. However, there was a continued tendency for lower ($P \sim .06$) IgG in pigs which had been fed L. acidophilus, but not SBTI, in contrast to pigs which had not been fed either.

Transmission of the marker protein, BSA, was most efficient when fed in combination with porcine colostrum. Previous studies also have demonstrated the capacity for colostrum to enhance macromolecular transmission (Westrom et al., 1985), whereas mature milk has been found ineffective (Vellenga et al.,

Table 4. Protein Concentrations in Serum at 24 Hours of Age ($mg \cdot mL^{-1}$)

Treatment[a]	Ig	SBTI	LA	Variable							
				Protein[c]	SE[b]	Globulin[d]	SE[b]	IgG[e]	SE[b]	BSA[f]	SE[b]
T1	-	-	-	46.5	2.0	24.7	1.7	18.1	1.8	2.7	0.4
T2	+	-	-	48.5	2.0	29.5	2.9	19.7	1.7	3.5	0.5
T3	+	+	-	49.3	3.2	30.3	1.9	20.3	1.4	3.6	0.5
T4	+	+	+	45.0	4.2	27.6	1.8	18.5	1.3	3.0	0.5
T5	+	-	+	41.5	2.2	23.3	1.9	14.0	1.5	3.1	0.6
T6	-	-	-	55.4	3.4	30.4	2.0	20.7	2.2	5.4	0.7

[a]All treatments contained BSA as a marker,(+) indicates the presence and (-) indicates the absence of Ig (Immunoglobulin concentrate); SBTI (Soybean trypsin inhibitor); LA (L. acidophilus 10^6 $CFU \cdot mL^{-1}$) T6=porcine colostrum, T1=milk.

[b]Standard Error of Mean

Contrasts

[c,f]T6 vs T4 is different ($P < .02$)

[d,e]T2 vs T5 is different ($P < .05$)

Table 5. Protein Concentrations in Serum at 36 Hours of Age ($mg \cdot mL^{-1}$)

				Variable							
Treatment[a]	Ig	SBTI	LA	Protein	SE[b]	Globulin	SE[b]	IgG	SE[b]	BSA	SE[b]
T1	-	-	-	47.2	2.0	32.0	2.9	17.8	1.3	1.9	0.3
T2	+	-	-	51.4	1.8	33.5	2.0	20.7	1.6	2.1	0.2
T3	+	+	-	50.7	2.7	27.4	2.2	18.6	1.4	2.4	0.2
T4	+	+	+	54.9	1.7	27.4	2.4	18.5	0.9	2.2	0.2
T5	+	-	+	52.4	3.9	27.9	2.2	19.0	0.9	1.9	0.4
T6	-	-	-	57.9	2.6	32.1	2.7	18.9	1.2	3.7	0.4

[a]All treatments contained BSA as a marker,(+) indicates the presence and (-) indicates the absence of Ig (Immunoglobulin concentrate); SBTI (Soybean trypsin inhibitor); LA (L. acidophilus 10^6 $CFU \cdot mL^{-1}$)
T6=porcine colostrum, T1=milk.
There were no treatment differences ($p > .05$)

[b]Standard Error of Mean

1988).

Interestingly, even though more BSA (50 mg· mL^{-1}) than IgG was fed (35 mg · mL^{-1}), more IgG was absorbed. Since the addition of the Ig concentrate (Tmt 2) resulted in an increased BSA concentration of nearly 40 percent as compared to BSA fed alone; it appears that the proteins had a sparing effect upon one another. Conversely, since BSA concentration did not increase as much as that of IgG, even when SBTI was added, it appears that BSA is slightly more susceptible to degradation. This is in accord with Westrom et al. (1985) whose data demonstrated an increase in absorption of fluorescein-isothyocyanate-labelled dextran as increased amounts of BSA were fed. Data from work with newborn calves also indicated that higher concentrations of immunoglobulins have a positive effect upon the rate as well as the quantity of macromolecular transmission (Stott and Fellah, 1983).

Shortly following parturition, sow colostrum has a high capacity for the inhibition of trypsin which apparently facilitates an increased efficiency in the intestinal transmission of intact macromolecules (Carlsson et al., 1974; Jensen and Pedersen, 1979; Westrom et al., 1982). In the present study, incorporation of SBTI for the same purpose, did result in increased ($p < .05$) absorption of IgG before 12 h of age. BSA absorption also increased by more than 20 percent as a result of adding SBTI to the mixture.

Lactobacilli and products in which they are utilized have been studied extensively in humans and other animals (Fuller, 1989). Fermentation products as well as products containing viable cells are available for swine in all phases of production, and in general, the effects of such products on health and performance are well documented (Cole and Newport, 1987; Lessard and Brisson, 1987; Fuller, 1989). Yet, nothing seems to be known with respect to the

effects of L. acidophilus on intestinal transmission of macromolecules in the preclosure pig.

In the present study there was a tendency for reduced serum IgG and total globulin at 12 h as a result of L. acidophilus having been incorporated into the mixtures which were fed. Though these results are not conclusive, they suggest that, if fed at a higher level, L. acidophilus might cause significant reductions in intestinal transmission of intact molecules.

The naturally born animal is readily contaminated with a variety of microbial strains at parturition (Savage, 1987), and Lactobacilli have been isolated from feces of pigs, as young as 4 h of age, at 10^4 CFU· g^{-1} (Muraldihara et al., 1977). Acknowledgment of the normal presence of large numbers of cells in the digestive tract makes it difficult to imagine that the dose of L. acidophilus which was fed, once in the tract, was a major source of interference. However, Lactobacilli are known to possess a number of proteases and to require several amino acids for growth (Peterson and Marshall, 1990). Thus it is possible that the formation of immunologically unrecognizable peptides and (or) complexes occurred during the time in which the complete mixtures were in solution. This is somewhat supported by the lack of a decrease in total serum protein (Table 3) as compared to the reduced concentration of IgG. Currently, work in this laboratory is being conducted to examine the effects of different levels of L. acidophilus and to better characterize the effects upon transmission of macromolecules.

It is important to consider the effects of supplemental mixtures upon subsequent antibody absorption, since it would not be deirable to hasten closure and thus inhibit acquisition of maternal antibodies if a nursing pattern is established (Vallenga et al., 1988). All of the pigs in this study continued to

absorb IgG after initiation of nursing at 12 h of age (Figure 2). However, the highest mean IgG concentration observed (20.7 mg· mL^{-1}), in the present study, was approximately 10 mg· mL^{-1} lower than normally nursed pigs (not littermates) with which we have worked at the same age and from the same herd (unpublished data). Therefore, it appears that a degree of closure had occurred which was sufficient to reduce the final level of passive immunity which was acquired. Part of this could possibly be attributed to a reduction in colostral immunoglobulins durng the 12 h treatment period. It has been observed that IgG concentration can decrease by as much as 50 percent after 4 h of normal nursing (Bourne, 1969).

Implications

These data do not clearly characterize the influence which L.acidophilus have upon the intestinal transfer of macromolecules in the newborn pig. However, they do indicate a tendency for a serum reduction of immunologically recognizable molecules as a result of feeding mixtures of which they were a part. Further work is needed to more clearly characterize the effects of L. acidophilus in the neonatal pig during the period of intestinal permeability to macromolecules. The results do clearly indicate that SBTI enhances intestinal transmission of macromolecules in newborn pigs which have not received maternal colostrum. It appears that SBTI could be utilized to improve the efficiency of products developed to provide passive immunity to colostrum deprived pigs.

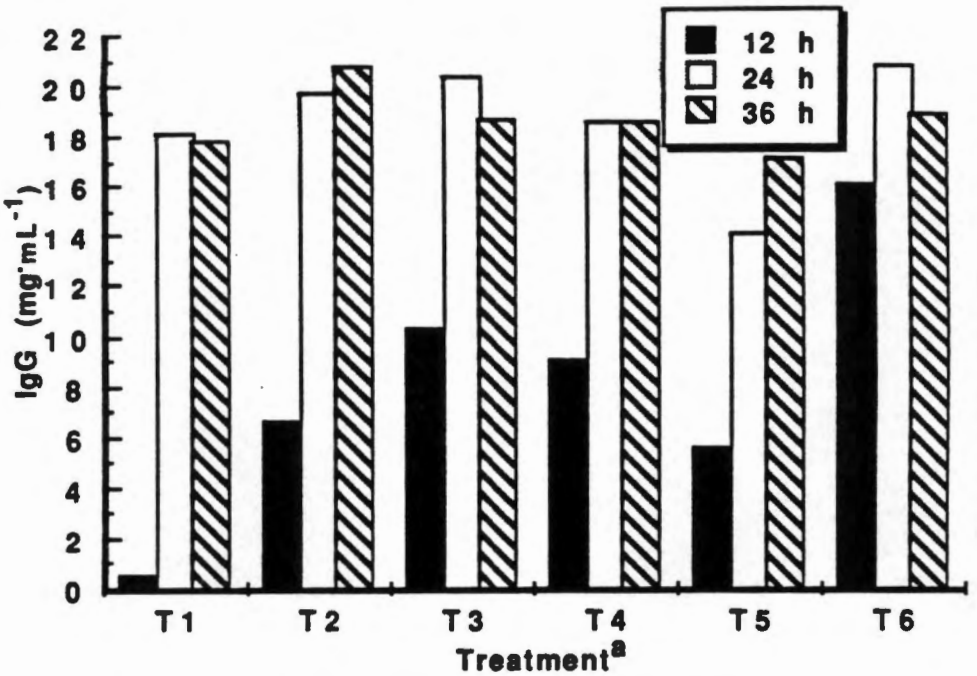

Figure 2. Serum IgG From 12 to 36 Hours

[a]Treatment descriptions are presented in Table 1.

PART IV

EFFECTS OF *LACTOBACILLUS ACIDOPHILUS* ON INTESTINAL TRANSFER OF BOVINE IgG IN NATURALLY AND ARTIFICIALLY MAINTAINED NEWBORN PIGS

Abstract

Forty-nine newborn (Duroc x Hampshire x Landrace) pigs were utilized to examine the effects of adding 0, 10^8, or 10^{10} CFU · mL^{-1} of L. acidophilus to bovine colostrum on the intestinal transfer of bovine and porcine IgG. At 0, 3, 6 and 9 h, 12 mL of bovine colostrum mixtures were administered to artificially maintained pigs, which received only bovine colostrum for 12 h, and to naturally maintained pigs which had full access to maternal colostrum. Pigs which received only maternal colostrum served as controls. At 12 h , bovine IgG (BIgG) concentration tended to be lower ($P < .07$) in serum of artificially maintained pigs which were fed bovine colostrum with 10^8 CFU · mL^{-1}, and was lower ($P < .001$) in serum of pigs which were fed bovine colostrum which contained 10^{10} CFU · mL^{-1}, as compared to the concentration in the serum of pigs which had been fed normal bovine colostrum. Serum content of BIgG was lower, in naturally maintained pigs which were fed bovine colostrum which contained L. acidophilus , but differences were not significant. Serum concentrations of porcine IgG (PIgG) were not affected by feeding L. acidophilus. However, there was a tendency for lower ($P < .1$) serum PIgG in naturally maintained pigs which had been fed bovine colostrum as compared to controls. Artificially maintained pigs had lower ($P < .001$) serum PIgG at 36 and 48 h than pigs which had nursed during the initial 12 h . These data indicate that the strain of L. acidophilus which was used can interfere with intestinal transmission of BIgG in pigs which are deprived of maternal colostrum.

Introduction

Though porcine colostrum and milk both have multifunctional roles, their immunological functions can be divided into two major phases which parallel physiological changes in the neonate. Colostrum is necessary, at an early stage, for the passive acquisition of systemic immunity . This role is well suited to colostrum, which contains a high concentration of immunoglobulins and protease inhibitors which facilitate the intestinal transfer of intact macromolecules (Svensen et al., 1971; Westrom et al., 1982).

After 24 to 36 h, the neonatal pig's capacity for intestinal transfer of macromolecules is lost (Speer et al., 1959), and the immunoglobulin concentration of colostrum has decreased (Jensen and Pedersen, 1979). After 7 d of lactation, sow's milk has an immunoglobulin concentration of approximately 6.5 mg · mL^{-1} (Curtis and Bourne, 1971). This continued source of immunoglobulins is important in that it offers a degree of protection against organisms which may cause enteric disease (Wilson, 1974; McCallum et al., 1977).

Situations in which sow's colostrum or milk is not available, or situations in which it needs to be supplemented are certainly not uncommon. In such situations, a reasonable goal would be to offer immunological and nutritional quality that is comparable to that which is provided by the dam.

It is possible that a degree of enteric protection can be provided to animals which are deprived of their dam's colostrum or milk by including Lactobacilli in their diets, and consequently it is included in some formulations for neonatal and young pigs. Though Lactobacilli historically have been considered to be influential in creating enteric stability, reports relative to their

efficiency in controlling undesirable organisms have indicated somewhat inconsistent results (Muraldihara et al., 1977; Shahani et al., 1980; Itoh and Freter, 1989).

In a previous study, we observed a slightly lower concentration of serum IgG, in pigs 12 h of age, which had been fed a mixture which contained L. acidophilus (10^6 CFU ml^{-1}) as compared to that observed in pigs which had been fed a similar mixture not containing L. acidophilus. The purpose of the present study was to determine if L. acidophilus would have similar effects when added to bovine colostrum and to determine if the response could be amplified by an increase in the number of cells. Furthermore, we wanted to determine if the effect was due to some interference in the intestine or to the presence of cells in colostrum prior to feeding. Total serum protein and globulin, bovine IgG (BIgG) and porcine IgG (PIgG) concentrations were examined in experimental pigs at 12, 36 and 48 h of age. Changes in PIgG from birth to 28 d of age are discussed.

Materials and Methods

Forty-nine pigs from seven litters were used in the study. Farrowings were attended, and the first seven pigs born in each litter were removed from the sow prior to nursing. Pigs were housed in stainless steel cages until a complete treatment group had been born. They were then randomly assigned to one of seven treatments (Table 1).

Following parturition and 3, 6 and 9 h later, pigs were fed 12 mL of the appropriate mixture of bovine colostrum, via stomach tube. After the initial feeding, pigs which were to be allowed access to porcine colostrum were returned to the sows. Blood samples (4 mL) were taken, via the anterior vena cava, at parturition and 12, 36 and 48 h later. Blood was allowed to clot, centrifuged and the harvested serum was stored at -44° C until analyzed.

Bovine colostrum was from one milking of a single source. Portions of the colostrum were stored frozen in several containers until it was needed. L. acidophilus was added to the colostrum immediately prior to the first feeding; thus, the cells were present in the colostrum for the remainder of the feeding period.

L. acidophilus was purchased in a lyopholyzed state (American Type Culture Collection, 4356). Cells were rehydrated, cultured and subcultured in Man-Rogalla-Sharpe (MRS) broth at 37^0 C in an atmosphere of 95 percent O_2 and 5 percent CO_2. Cells were harvested by centrifugation and washed three times in .85 percent saline. Upon completion of the last wash, the pellets were resuspended in a small quantity of saline. This slurry was then added to a volume of saline which was eventually brought to 48 mL of solution at an optical density which indicated 10^8 or 10^{10} CFU· ml^{-1} as determined by the standard

Table 1. Description of Treatments[a]

Designation	Description
T1	Bovine colostrum
T2	Bovine colostrum + L. acidophilus (10^8 CFU· mL^{-1})
T3	Bovine colostrum + L. acidophilus (10^{10} CFU· mL^{-1})
T4	Maternal colostrum + Bovine colostrum
T5	Maternal colostrum + Bovine colostrum + L.acidophilus (10^8 CFU · mL^{-1})
T6	Maternal colostrum + Bovine colostrum + L. acidophilus (10^{10} CFU · mL^{-1})
T7	Maternal colostrum

[a]L. acidophilus was added to bovine colostrum prior to the first feeding, and pigs were given 12 mL when a complete treatment group was collected and again 3, 6 and 9 h later.

plate count method (Clark et al.,1978). The cells were then centrifuged again, and the pellet and saline were stored at 4° C until used, at which time the saline was poured off and bovine colostrum was added. Due to a lack of cooperation from the sows as to farrowing time, cells could not be harvested in a precise phase of growth, and storage time in pellet form varied.

A two-antibody sandwich ELISA was used for quantitation of porcine and bovine IgG (Harlow and Lane, 1988). Antibodies were purchased (Sigma, St. Louis, Mo.). Total protein and globulin were quantitated by colorimetric procedures (Sigma, procedures 540 and 560, respectively).

Statistical analysis were performed utilizing the statistical analysis system (SAS, 1986). A series of predetermined contrasts were used to assess effects of the various treatments. To avoid confounding due to between litter variation, all treatments were represented in each litter.

Results and Discussion

Mean serum concentrations of total protein and globulin, at birth, were 22.6 and 7.8 mg · mL^{-1}, respectively, and were very similar in all pigs. Serum IgG (bovine or porcine) was not quantitated at birth; however, serum which had been collected at birth was used as a control on each ELISA plate. Though indication of a very low concentration of PIgG occassionaly appeared, concentrations were too low to be meaningful relative to the concentrations of standard which were being used.

A summary of the results in pigs 12 h of age is presented in Table 2. Concentrations of BIgG in the serum of artificially maintained pigs which were fed bovine colostrum was lowered ($P < .001$) by adding L. acidophilus (10^{10} CFU · mL^{-1}) to the colostrum prior to feeding. A reduction occurred also when 10^8 CFU· mL^{-1} were added, but the difference was not as pronounced when compared to pigs which had been fed bovine colostrum in which cells were not added. Treated pigs which were allowed to nurse absorbed less ($P < .0001$) BIgG than pigs which did not nurse. There was a tendency for reduced serum BIgG as a result of adding L.acidophilus to bovine colostrum which was fed to naturally maintained pigs, and those which received L. acidophilus at 10^{10} CFU·mL^{-1} had a serum BIgG concentration which was 1.4 mg · mL^{-1} lower than naturally maintained pigs which received only bovine colostrum.

Serum concentrations of PIgG in nursed pigs which were fed bovine colostrum were not affected by the addition of L. acidophilus. However, PIgG was somewhat lower in nursed pigs which were fed bovine colostrum as

Table 2. Serum Proteins at 12 Hours of age
($mg \cdot mL^{-1}$)

				Variable							
Treatment[a]	BC	MC	LA	Protein[b]	SE[1]	Globulin[c]	SE[1]	PIgG[d]	SE[1]	BIgG[e]	SE
T1	+	-	-	32.5	0.9	21.1	0.9	__._	_._	13.4	.8
T2	+	-	10^8	31.6	2.0	18.9	1.1	__._	_._	11.8	.3
T3	+	-	10^{10}	28.9	1.2	17.4	1.2	__._	_._	10.2	.4
T4	+	+	-	57.2	5.1	47.5	2.2	32.9	2.9	9.4	.4
T5	+	+	10^8	60.1	2.7	48.8	2.9	33.8	2.9	9.1	.8
T6	+	+	10^{10}	59.8	2.5	50.8	1.0	34.6	1.9	8.0	.7
T7	-	+	-	51.2	2.4	40.7	1.2	33.6	1.7	_._	_

[a]Treatment (first 12 h of life): BC=bovine colostrum; MC=maternal colostrum; LA=L. acidophilus in CFU mL^{-1}

[1]Standard Error of Mean

Contrasts

[e]T1 vs T2 (P~.06)

[e]T1 vs T3 is different (P < .0005)

[e]T2 vs T3 (P~ .068)

b,c,d,[e]T1, 2 & 3 vs 4, 5 & 6 is different (P <.0001)

[b]T4, 5 & 6 vs 7 is different (P < .02)

[c]T4, 5 & 6 vs 7 is different (P <.001)

[d]T4, 5 & 6 vs 7 is different (P < .09)

compared to the serum concentration in pigs which received only maternal colostrum.

As would be expected, total protein and globulin concentrations were higher ($P < .0001$) in nursed pigs than in pigs which had received only bovine colostrum. In artificially maintained pigs, total serum protein and globulin were somewhat lower in those which were fed bovine colostrum containing L. acidophilus, as compared to artificially maintained pigs which were fed only bovine colostrum, but they did not differ.

Serum protein concentrations in pigs 36 and 48 h of age are summarized in Tables 3 and 4, respectively. Pigs which were not allowed access to maternal colostrum during the initial 12-h period absorbed a substantial amount of PIgG prior to 36 h, but concentrations remained lower ($P < .0001$) than concentrations of nursed pigs. L. acidophilus did not affect the serum concentrations of PIgG regardless of whether or not pigs had nursed during the initial 12 h of life. However, nursed pigs which had been fed bovine colostrum had lower ($P < .01$) serum concentrations of PIgG as compared to nursed pigs which had not been fed bovine colostrum.

By 48 h of age, all protein concentrations had decreased in each group of pigs. Nursed pigs which had been fed bovine colostrum had lower ($P < .05$) concentrations of PIgG than nursed pigs which had not received bovine colostrum. Pigs which had nursed during the initial 12 h period maintained higher ($P < .05$) concentrations of PIgG than did pigs which had been deprived of maternal colostrum during that time.

Addition of L. acidophilus to bovine colostrum, prior to feeding, resulted in decreased serum concentrations of BIgG in unsuckled pigs to which it was fed, as compared to pigs which received only bovine colostrum. Feeding the same mixtures to pigs which were given free access to maternal colostrum

Table 3. Serum Proteins at 36 Hours of Age ($mg \cdot mL^{-1}$)

Treatment[a]	BC	MC	LA	Variable							
				Protein[b]	SE[1]	Globulin[c]	SE[1]	PlgG[d]	SE[1]	BlgG[e]	SE[1]
T1	+	-	-	52.6	2.2	35.2	2.4	18.1	1.7	10.2	.6
T2	+	-	10^8	51.2	5.1	36.2	1.8	21.7	1.8	7.8	.6
T3	+	-	10^{10}	50.2	1.3	33.2	2.4	19.4	2.1	6.5	.5
T4	+	+	-	49.9	4.2	37.2	2.4	26.2	2.0	6.3	.5
T5	+	+	10^8	49.8	4.1	39.3	1.6	26.5	1.5	6.4	.4
T6	+	+	10^{10}	50.8	3.3	37.9	2.3	27.2	2.2	5.9	.2
T7	-	+	-	47.3	2.5	36.3	1.8	32.5	1.8	_.	._

[a]Treatment (first 12 h of life): BC=bovine colostrum; MC=maternal colostrum; LA= L. acidophilus in CFU mL^{-1}

[1]Standard error of mean

Contrasts

[c,d]T1, T2 & T3 vs T4, T5 & T6 is different ($P < .0001$)

[e]T1 vs T2 & T3 is different ($P < .001$)

[d]T4, T5 & T6 vs T7 is different ($P < .05$)

Table 4. Serum Proteins at 48 Hours of Age
($mg \cdot mL^{-1}$)

				Variable							
Treatment[a]	BC	MC	LA	Protein[b]	SE[1]	Globulin[c]	SE[1]	PIgG[d]	SE[1]	BIgG[e]	SE[1]
T1	+	-	-	45.7	2.6	32.3	2.5	16.9	1.5	8.7	.6
T2	+	_	10^8	42.4	1.2	31.2	2.0	20.5	1.6	6.9	.5
T3	+	-	10^{10}	41.8	2.6	30.4	2.2	18.1	1.7	6.1	.5
T4	+	+	-	52.3	3.2	35.9	3.0	24.6	1.8	5.6	.2
T5	+	+	10^8	50.6	4.7	35.7	2.1	23.5	1.2	5.8	.4
T6	+	+	10^{10}	47.8	4.2	37.3	3.3	26.8	2.2	5.3	.3
T7	-	+	-	46.3	2.3	37.6	2.3	29.5	1.4	_._	._

[1] Standard error of mean

[a]Treatments (first 12 h of life): BC=bovine colostrum; MC=maternal colostrum; LA=L. acidophilus in $CFU \cdot mL^{-1}$

Contrasts

[b,c,d,e]T1, T2 & T3 vs T4 , T5 & T6 is different ($P < .05$)

[d]T4, T5 & T6 vs T7 is different ($P < .01$)

resulted in some decrease in serum concentrations of BIgG, but the differences were not as pronounced.

The fact that significant differences in serum concentrations of BIgG did not exist in nursed pigs which were fed the mixtures was probably due to the generally lower ($P < .0001$) absorption of BIgG in these pigs as compared to pigs which had received only bovine colostrum. This reduced absorption was possibly due to dilution of BIgG by maternal colostrum and could, in part, have been a function of preferential absorption of PIgG, which was discussed by Leary and Lecce, 1979.

It seems logical to assume that since addition of L. acidophilus to bovine colostrum caused a reduction in serum concentrations of BIgG in pigs to which it was fed, its presence in the gut would likewise have resulted in decreased absorption of PIgG in nursed pigs, if the reduction was due to interference. Interestingly, this did not hold true as there were no effects on serum concentrations of PIgG.

In an attempt to understand more clearly the cause of the reduced serum concentrations of BIgG in pigs which had been fed bovine colostrum containing L. acidophilus, we proceeded on the hypothesis that BIgG and cells were forming immunologically inactive or unabsorbed complexes. To test this, we mixed bovine colostrum and L. acidophilus in the same manner in which it had been used originally, but worked only with the higher concentration of cells (10^{10} CFU $\cdot$ mL^{-1}), and equal volumes of the same colostrum with no cells added. After 4 h of incubation, aliquots of each sample were centrifuged in order to remove cells and any proteins to which they were bound. This was accomplished with three sets of samples. A comparison of BIgG activity was then examined by ELISA (Figure 1). No substantial loss of BIgG was indicated.

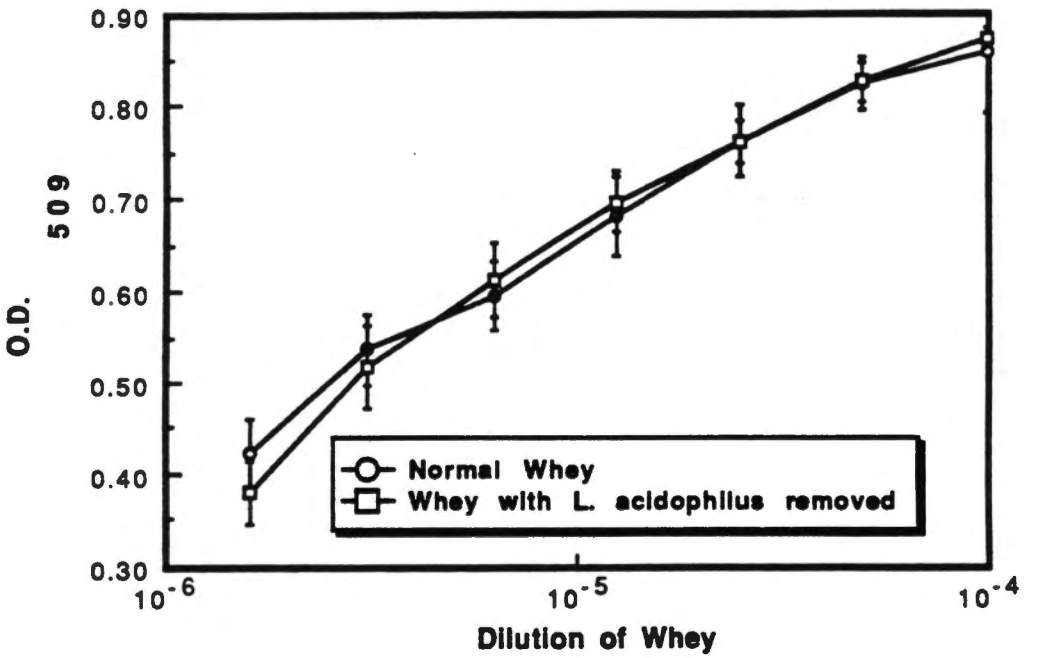

Figure 1. Reactivity of BigG After Removal of L. acidophilus

When affinity purified bovine and porcine IgG were examined, using various dilutions of cells added to the same concentrations of IgG, reactivity did not change.

These results lead to the belief that the effects of L. acidophilus on immunoglobulin absorption occurred within the intestine, even though this is not supported by any differences in serum concentrations of PIgG. However, conditions under which nursed and unnursed pigs were maintained differed greatly. Thus, differences in factors such as feeding frequency, volume, and immunoglobulin concentration may have had substantial influence on the effects of the L. acidophilus. Unfortuanately, in retrospect, the present study did not lend itself to an adequate assessment of the effects of these factors.

Previous studies have demonstrated that E. coli which were bound to intestinal epithelial cells reduced the capacity for intestinal transfer of proteins (Staley et al., 1972; James et al., 1980). Various strains of L. acidophilus have been shown to be capable of adhering to intestinal cells (Mayra et al., 1983; Conway et al.,1987). Interference with intestinal transfer is a possible explanation for the decreased absorption of BIgG observed in the present study if we assume that the frequent bathing of the intestine of nursed pigs with maternal colostrum did not allow such interference. In support of this idea, since no maternal colostrum was consumed by artificially maintained pigs during the initial 12 h , L. acidophilus was a greater percentage of the intestinal contents , and each introduction of bovine colostrum provided the intestine with a fresh supply of cells.

Pigs which were fed only bovine colostrum for the first 12 h of life had lower serum concentrations of PIgG at 36 and 48 h of age as compared to pigs which nursed during this period. This is explained by the nonspecific initiation of closure by bovine colostrum, an occurrence which has been well documented

in a number of studies in which various nutrient sources were examined (Payne and Marsh, 1962; Lecce et al., 1964; Frenyo, 1987; Vellenga et al., 1988).

Though not a major focus of this study, it is interesting to note the changes which occurred in serum PIgG concentrations in pigs at stages up to 28 d of age (Figure 2). Since L. acidophilus did not affect PIgG, for illustrative purposes data were pooled according to the form(s) of colostrum which were received by pigs during the initial 12 h of life. PIgG decreased in a somewhat parallel fashion in pigs at ages up to 7 d. After 7 d of age, serum PIgG decreased only in the pigs which previously had the higher concentrations. This suggests a more rapid development of active antibody production in the pigs which initially had lower serum PIgG concentrations. This was likely due to a lack of specific antibodies to antigens present in the particular environment, based upon the lower initial serum concentration of IgG, and may reflect also a lack of specificity of bovine immunoglobulins for common porcine antigens which were present. Similar observations have been discussed by Drew and Owen (1988).

Implications

Adding 10^8 CFU· mL^{-1} of L. acidophilus (ATCC 4356) to bovine colostrum prior to feeding resulted in decreased serum concentration of BIgG in pigs which were deprived of maternal colostrum and a more amplified reduction was observed when 10^{10} CFU· mL^{-1} were added. Removal of L. acidophilus from bovine colostrum by centrifugation did not result in a decreased concentration of bovine IgG, therefore, it is assumed that the effects of L. acidophilus were exhibited within the intestine. However, results from this study

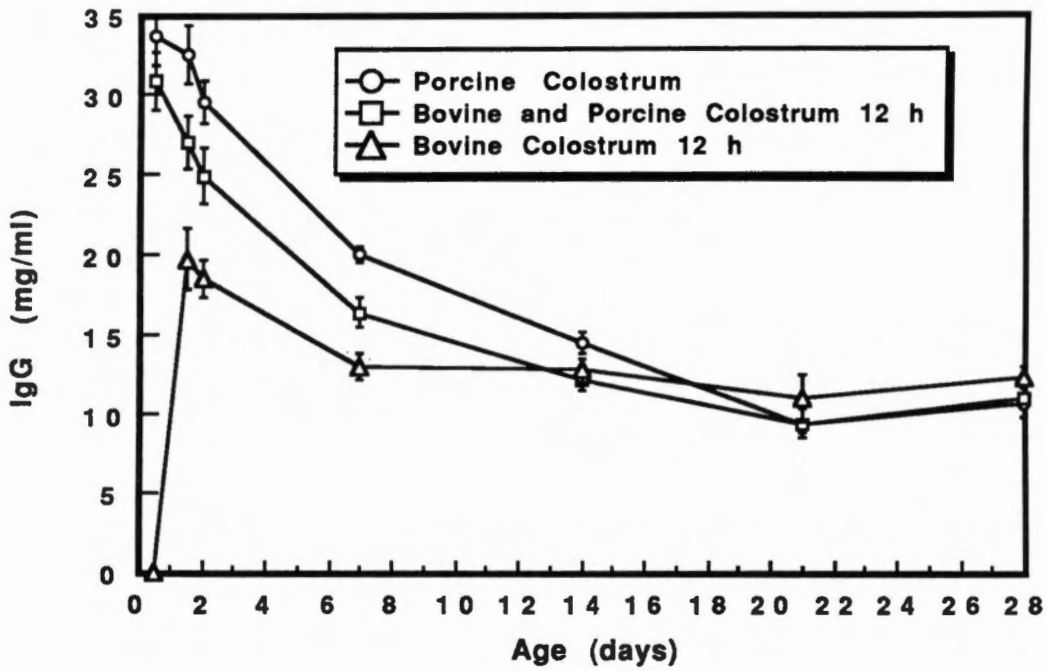

Figure 2. Concentrations of Serum PIgG From 12 Hours to 28 Days of Age

offer no explanation relative to the mechanism by which the observed reduction in transmission occurred. Based upon these results, incorporation of L. acidophilus into mixtures developed to provide passive immunity to neonatal pigs deprived of maternal colostrum can not be recommended.

Part V

General Discussion and Summary

General Discussion

There are a number of factors involved in the high rate of porcine neonatal mortality. These factors are not limited to but include nutritional deficiency, crushing by the dam, hypothermia and inadequate passive immunity (Hartsock and Graves, 1976; Blecha and Kelley, 1981; England, 1986; Stein et al., 1990).

The ultimate goal of work presented in the present dissertation is a reduction in porcine neonatal mortality, particularly that which is associated with inadequate nutrient intake and lack of passive immunity. As an approach to this goal we supplemented low-birthweight, sow-reared pigs, at 12 h intervals, with three 12 mL doses of bovine milk which contained 50 $mg \cdot mL^{-1}$ of Ig concentrate (Experiment I). Efficiency of the supplemental mixture for providing passive immunity and energy to pigs was examined by quantitating serum IgG and glucose concentrations. Information from this experiment provided a basis for subsequent work by revealing areas in which the supplemental mixture needed improvement. The purpose of Experiment II was to investigate the effects of Ig concentrate, soybean trypsin inhibitor and L. acidophilus on transmission of macromolecules and level of passive immunity when incorporated into a mixture such as that which was used in Experiment I. Experiment III was designed to further examine an observation made in Experiment II, which suggested that intestinal transmission of macromolecules was inhibited as a result of incorporation of L. acidophilus into the mixtures.

In Experiment I, there was a tendency for improved survival rate, to 7 d of age, of supplemented (79%) low-birthweight pigs as compared to those which

were not supplemented (52.6%). This trend did not continue through 28 d of age and mortality rate of supplemented pigs (57.9%) was similar to that of pigs which were not supplemented (47.4%). Though improvement in survival rate to 7 d of age can be perceived as "a step in the right direction", no economic gain can be realized.

Data collected in Experiment I indicated that the supplemental mixture needed to be more efficient at providing energy and passive immunity. This was illustrated by a lack of increase in serum IgG and glucose in supplemented pigs as compared to low-birthweight pigs which were not supplemented.

It is possible that improvement in energy status could be made by more frequent administration of the supplement than the 12 h intervals which were used. However, this would be time consuming and in many operations would require the presence of a farm employee at times which could interfere with other responsibilities.

To minimize cost and maximize utilization, supplements need to be delivered directly to the deprived animals. Therefore it would be desirable to have the capacity to economically and with a reasonable amount of labor, provide an efficient supplement, which at small, fairly infrequent doses, would result in reduced mortality and morbidity. However, this does not preclude the utilization of liquid delivery systems, which under proper management schemes could provide deprived animals access to an adequate supply of nutrients. Systems which are presently available vary in their degree of sophistication from the simple "poultry waterer" to more elaborate centralized, automatic delivery systems. Cost, labor and lack of demonstrated efficiency are likely deterents to many producers with respect to utilization of such systems.

It is possible that incorporation of an energy-rich component into a supplemental mixture would improve its efficiency as an energy source. Work with medium-chain triglycerides has been somewhat encouraging. Benevenga et al. (1989) observed that 12 mL of medium-chain triglyceride given to newborn pigs, which were subsequently starved for 12 h, had a sparing effect upon liver glycogen stores. However, in a second trial the decrease in disappearance of liver glycogen was not observed. Success at providing a utilizable, energy-rich nutrient which will spare stored energy, could ultimately result in decreased mortality by allowing pigs to maintain adequate energy to overcome factors which interfere with nursing.

Svendsen et al. (1990) reported that low-birthweight (< 1.0 Kg) pigs had a greater capacity for intestinal transmission of macromolecules than did pigs which weighed more than 1.0 Kg at birth. This observation leads to the assumption that the low concentration of serum IgG which was observed in low-birthweight pigs in Experiment I was the result of lack of colostrum ingestion rather than reduced capacity for transmission. However, this does not offer an explanation for the lack of increase in serum IgG concentration of pigs which were administered supplement which contained 34 $mg \cdot mL^{-1}$ of IgG.

A number of factors have been shown to influence intestinal permeability to macromolecules. In work which was conducted utilizing newborn calves, it was suggested that elevated corticosteroids suppressed intestinal permeability to IgG_1 (Stott et al., 1976); however the contrary also has been reported (Stott and Reinhard, 1978). Svendsen et al. (1990) , working with pigs, observed that intestinal transmission of macromolecules was greater in pigs which had lower concentrations of cortisol at birth. Therefore, we should not overlook the stress

which was related to handling as well as competition from littermates and other environmental factors as possible factors in causing the low serum concentration of IgG which was observed in Experiment I.

To equalize stress between treaments, in the present study, a substance which contained no immunoglobulin could have been administered to those pigs which were not supplemented. However, one could also postulate that any fluid which was given could serve to reduce the intestinal concentration of IgG which was ingested via maternal colostrum by increasing the volume of fluid in the intestine. In support of the latter, ingested mixtures which contained high protein concentrations, as compared to lower concentrations, were shown to have a positive influence on rate and quantity of macromolecular transmission (Stott and Fellah, 1983; Westrom et al., 1985). Ideally, with an adequate number of pigs, each situation could be examined within many litters.

When milk, from the same pooled source as used in Experiment I, was fed in combination with the marker protein, BSA, mean serum concentration of BSA at 12 h of age was 4.4 $mg \cdot mL^{-1}$. By incorporating more protein, in the form of 50 $mg \cdot mL^{-1}$ of Ig concentrate, mean serum concentration of BSA increased to 6.3 $mg \cdot mL^{-1}$. These results support the idea that higher protein concentration increases macromolecular transmission.

At least two factors could have been involved in the observed increase in serum concentration of BSA; proteolytic activity and/or increased efficiency in absorption. Since proteolytic activity is relatively low in the newborn pig (Pond and Maner, 1984), the increase in protein concentration of the mixture may have exceeded the protein digestive capacity of the neonates, simply leaving

more intact molecules available for absorption. Conversely, Burton and Smith (1977) suggested that increased protein concentration enhanced intestinal pinocytotic activity. In a more specific observation, Smith et al. (1979) noted that vacuolization was enhanced by PIgG but not by human IgG, and that the transmission of human IgG to serum was dependent on the quantity of porcine IgG in the intestine. This suggests stimulation by PIgG in the Ig concentrate as a possible factor in the observed increase in serum BSA.

In addition to low proteolytic activity in the newborn pig, hydrolysis of protein to constituent amino acids is effectively inhibited during the first 24 to 36 h by trypsin inhibitor which is present in sow colostrum (Laskowski et al., 1957; Jensen and Pedersen, 1979). Incorporation of soybean trypsin inhibitor (5 $mg \cdot mL^{-1}$) into mixtures which were used in Experiment II resulted in improved transmission of macromolecules to serum of treated pigs, and could possibly result in an improvement in the acquisition of passive immunity via supplementation schemes such as the one which was used in Experiment I.

In Experiments II and III, incorporation of L. acidophilus into mixtures resulted in decreased transmission of PIgG (from Ig concentrate), BSA and BIgG in pigs which were not allowed access to maternal colostrum for the first 12 h of life. However, in Experiment III, incorporation of L. acidophilus into bovine colostrum did not influence the concentration of PIgG in the serum of pigs which were allowed to nurse during the first 12 h of life.

The mechanism by which L. acidophilus influenced intestinal transmission of intact macromolecules in pigs which were deprived of maternal colostrum for the first 12 h of life is not clear. We did observe that availability of BIgG was not reduced as a result of binding to L. acidophilus, since removal of

L. acidophilus from mixtures by centrifugation did not result in decreased IgG concentration. This led us to hypothesize that a form of interference had occurred within the intestine. However, this theory was not supported since absorption of PIgG by nursed pigs which were fed mixtures containing L. acidophilus did not decrease.

Previous studies have suggested that interference by bacterial cells does occur. Staley et al. (1972) contaminated newborn pigs with E. coli (055) and observed that the E. coli did bind to intestinal epithelial cells and that capacity for intestinal transfer of proteins was reduced. Similar observations have been made in work which utilized newborn calves (James et al., 1980). L. acidophilus has also been shown to adhere to intestinal cells (Mayra et al., 1983; Conway et al., 1987). Interference with transmission appears to be a possibility for the reduced serum concentration of BIgG in pigs which were not allowed access to maternal colostrum in Experiment III.

The preceding observations fail to offer insight as to why absorption of PIgG in nursed pigs was not influenced by feeding mixtures containing L. acidophilus. It is very possible that dilution of L. acidophilus by maternal colostrum and the frequent bathing of the intestine as pigs nursed were factors involved in prevention of the negative effects, in contrast to pigs deprived of maternal colostrum which were fed every3 h.

Convincing evidence for the existence of receptors, which are specific for IgG, on intestinal cells of neonatal rats has been presented (Jones et al., 1972; Wallace and Rees, 1980). To the authors knowledge, there has been no confirmation of similar receptors on intestinal cells of the pig. However, evidence for a degree of selectivity in absorption has been presented (Pierce and Smith, 1967; Porter, 1969). Leary and Lecce (1979) observed that PIgG

was absorbed preferentially in the presence of other molecules and proposed specific receptors as a possible mechanism. As previously mentioned, Smith et al. (1979) observed that vacuolization was enhanced by PIgG but not by human IgG. These observations lend some support to the consideration that a preference for intestinal absorption of PIgG as compared to BIgG may have been a factor related to the lack of influence of L. acidopholus on transmission of PIgG in nursed pigs. This is mentioned only as a hypothesis, since any inference such as this goes well beyond the scope of the present study and is an area which is open for further investigation.

Bovine colostrum was utilized in Experiment III, not to examine its efficency for providing passive immunity to neonatal pigs, rather to afford a marker protein (BIgG) which could be distinguished from the IgG of maternal origin. However, due to the availability of bovine products, as opposed to porcine colostrum, concentrated bovine whey and colostrum are often used as supplements for neonatal pigs. Conversely, McCallum et al. (1977) reported that bovine colostrum provided excellent passive protection to colostrum-deprived neonatal pigs but reported less than satisfactory results with immunoglobulins fractionated from bovine serum.

In conclusion, supplementation of low-birthweight pigs with three 12 mL doses of mature bovine milk containing 50 $mg \cdot mL^{-1}$ of Ig concentrate did not improve survival, increase serum IgG nor glucose. However, subsequent work suggests that transmission of IgG can be improved by incorporation of soybean trypsin inhibitor to the mixture. This is likely due to a reduction in proteolytic degradation of the molecules, similar to colostrum's capacity to inhibit trypsin. Results also suggest that a higher concentration of protein facilitates increased intestinal transmission of intact macromolecules. This is possibly attributed to

exceeding the protein digestive capacity of the neonate and/or stimulation of the pinocytotic process. Improvement in the energy status of deprived neonates is needed. It is likely that this can be accomplished through different management schemes or through utilization of energy-rich nutrient sources. More work in this area is well justified. It was observed that addition of L. acidophilus to supplemental mixtures resulted in decreased transmission of intact macromolecules to serum of colostrum-deprived, limit fed pigs prior to cessation of the period of absorption. Formation of complexes involving macromolecules and L. acidophilus was considered but not assumed to be the cause, since concentration of IgG did not decrease when L. acidophilus was removed from the mixtures by centrifugation. The mechanism by which L. acidophilus decreased macromolecular transmission is by no means clear and is an area for further study.

Summary

Three experiments were conducted utilizing 142 newborn (Hampshire X Yorkshire x Landrace) pigs. The major objectives were: 1) to examine the efficiency of a simple supplement to maternal colostrum for improving serum IgG concentration, energy status and survival rate of low-birthweight pigs; 2) to examine the effects of Ig concentrate, soybean trypsin inhibitor and L. aciodophilus on acquisition of passive immunity.

Supplementation of low-birthweight pigs, in Experiment I, tended to improve survival rate to 7 but not to 28 d of age. Neither serum IgG nor glucose were affected by supplementation. Subsequent work revealed methods to improve the efficiency of a supplemental mixture such as the one used in Experiment I. When BSA was used as a marker protein, addition of 50 $mg \cdot mL^{-1}$ of IgG concentrate to the mixture resulted in a 40 percent increase in serum BSA concentration. This indicated that increased protein concentration resulted in increased macromolecular transmission and can be a factor in provision of passive immunity via supplementation. Incorporation of SBTI (5 $mg \cdot mL^{-1}$) into the supplement resulted in an additional increase in BSA concentration, and an improved ($P < .001$) serum concentration of PIgG as compared to only Ig concentrate and BSA in the mixture. This suggested a reduction in proteolytic degradation due to the presence of SBTI. In conjunction with the observed increases in serum IgG, serum total globulin also increased ($P < .001$) as a result of addition of Ig concentrate and was further increased ($P < .01$) by incorporation of SBTI.

In Experiments II and III, incorporation of L. acidophilus into the mixtures resulted in less efficient intestinal transmission of proteins in pigs which were deprived of maternal colostrum. This effect was amplified by increasing the L. acidophilus from 10^8 to 10^{10} CFU mL^{-1}. Removal of L. acidophilus from the mixture by centrifugation did not result in a decreased concentration of BIgG. This eliminated the assumption that BIgG·L. acidophilus complexes were being formed in great enough quantity to cause decreased BIgG. This led to the assumption that reduced transmission resulted from a form of interference within the intestine. Similar reductions in serum concentrations of BIgG, as a result of feeding mixtures containing L. acidophilus, were not observed in pigs which were allowed access to maternal colostrum during the first 12 h of life. This was likely due to dilution and frequent bathing of the intestine with maternal colostrum.

From these experiments, it was concluded that increased protein concentration and SBTI had a positive effect on intestinal transmission of macromolecules in neonatal pigs which were deprived of maternal colostrum and could be used to improve supplement efficiency. However, incorporation of L. acidophilus into mixtures developed to improve passive immunity in neonatal pigs which are deprived of maternal colostrum is not recommended, since macromolecular transmission was reduced due to their presence. The mechanism by which this occurred is not clear and justifies further study.

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APPENDIXES

Appendix A

Preparation of Immunoglobulin Concentrate

Preparation of Immunoglobulin Concentrate

I. Blood collection and separation of serum

(A.) Materials

1. Pails for blood collection
2. 250 mL centrifuge bottles
3. Centrifuge
4. Containers (sterile) suitable for storage of serum
5. Funnel
6. Metal spatula or applicator stick

(B.) Blood collection. Blood for the present work was collected from market weight hogs and sows at the time of slaughter. If sufficient assistance was available to adequately restrain the animal, with the use of a large funnel, blood was collected directly into centrifuge bottles. However, if animals could not be sufficiently restrained, it was found less difficult and somewhat safer to collect the blood in pails and transfer it to the centrifuge bottles. The transfer had to be made promptly in order to avoid problems with clot formation.

(C.) Procedure for harvesting serum. (Garvey et al., 1977)

1. Allow the blood to stand for one hour at room temperature for clot formation.
2. If clots are stuck to the container sides, carefully separate with metal spatula or applicator stick.
3. Move the blood to a refrigerator or cooler and permit clot contraction. In the present work, centrifugation was generally started within two hours of collection. However, the procedure

suggests that clots be allowed to contract 12 to 24 hours.

4. Decant the serum to clean centrifuge bottles and centrifuge at 1000 X g for 30 minutes at 4^0 C (2500 rpm with a 14 cm radius).

5.Decant centrifuged serum to desired containers for storage.

II. Immunoglobulin precipitation and dialysis. (Garvey et al., 1977)

(A.) Materials

1. $(NH_4)_2SO_4$ solution, saturated at room temperature
2. NaOH, 2 N
3. Borate buffered saline
4. Saline
5. pH meter
6. Magnetic stir plate and bar
7. Refrigerated centrifuge
8. Dialysis tubing (mwco: 6-8000)
9. Large beaker or jar for dialysis
10. Container large enough to accomodate the quantity of serum to be precipitated + the ammonium sulfate solution
11. Container and stand to accomodate dropwise addition of ammonium sulfate solution to serum
12. 10% Barium chloride

(B.) Precipitation procedure.

It is essential that in steps 1 through 7 all reagents are used and all procedures are performed at room temperature. Saturation of the ammonium sulfate solution is temperature dependent but at room temperature, requires ~750 g/liter of water.

1. Adjust the pH of the saturated solution to 7.8 by addition of 2 N NaOH, this should be done just prior to precipitation since ammonia will be released upon prolonged standing.
2. With constant stirring, slowly add (dropwise) a volume of saturated solution which is equal to one-half the volume of serum. Continue to stir the solution for an additional 2 to 3 hours to avoid trapping of components other than gamma globulins in the precipitate.
3. Centrifuge the suspension at room temperature for 30 minutes at 1400 x g (about 3000 rpm with a radius of 14 cm).
4. Dissolve the precipitate in enough saline to restore the solution to the original volume.
5. Repeat steps1 through 4; for the third precipitation, repeat steps 1 through 3.
6. Dissolve the third precipitation in borate buffered saline to a volume which will allow easy removal of the solution to dialysis tubing.
7. Remove the ammonium sulfate by dialysis against borate-buffered saline.

(C.) Dialysis procedure.

1. Assemble , in the cold, the dialysis jar with buffer and the magnetic stirrer.
2. Cut a piece of tubing of appropriate size.
3. Wet the tubing with distilled water and tie two knots in one end. Fill the tubing with distilled water and test for leaks.
4. Wash the tubing for about 30 minutes by placing it in a beaker of distilled water. Change the water 2 or 3 times.
5. Pour the solution to be dialyzed into the tubing.
6. Eject the air above the solution by squeezing this section of tubing. Tie the open end of tubing. To allow the uptake of water, leave a space above the surface of the liquid approximately equivalent to the volume of material in the tubing.
7. Fasten the end of the tubing to a glass rod to suspend it or fasten it to the side of the jar. If the tubing floats more than is desired a weight can be attached to the end.
8. Operate the magnetic stirrer at a speed resulting in gentle movement of the buffer.
9. Change the dialyzate every 8 to 16 hours. To test for sulfate ions, take a small sample of the dialyzate, make it acidic by addition of a drop of hydrochloric acid. Add a few drops of 10% barium chloride; if a a white turbidity or precipitate forms, further dialysis is needed. A negative test indicates that dialysis is complete. At this point the precipitate is ready for lyophilization.

(D.) Lyophilization (general considerations)

1. Empty precipitate into a container suitable for the freeze-dryer to be used. A thin layer to increase exposed surface area will decrease time required for the drying process.

2. Precipitate should be solidly frozen prior to any vacuum being applied.

3. Follow specific operating instructions for the dryer being used.

4. For this work, the shelf temperature was initially set to -40^0 C .

5. After 12 hours, the shelf temperature was increased to -20^0 C .

6. If probes are available to monitor sample temperature, dryness of the sample can be estimated without shut down. The product is generally dry when the shelf temperature is equivalent to the product temperature, condenser temperature is < -84 and the vacuum is < 10 millitorr.

Appendix B

The Two-Antibody Assay

The Two-Antibody Sandwich Assay

I. Quantitating antigens using the two-antibody sandwich.

(A.) General Procedure (Harlow and Lane, 1988)

1. Prior to the assay, both antibody preparations need to be purified and one must be labeled. Affinity purified antibodies (labeled and unlabeled) of a common source are available from a number of commercial sources.

2. For most applications a polyvinylchloride (PVC) microtiter plate is suitable.

3. Bind the unlabeled antibody to the bottom of each well to be used by adding 50 ul of antibody solution (20 ug/mL in PBS). PVC will bind approximately 100 ng per well. Incubate for at least 2 hours at room temperature in a humid environment. (We incubated overnight).

4. Wash the wells two times with phosphate buffered saline (PBS). A 500 mL squirt bottle is convenient for washing. The antibody solution or washes can be removed by briskly flicking the plate above a suitable waste container. * Do not allow the wells to become completely dry between any steps, this increases cross reactivity (John Norman, personal communication).

5. Any remaining sites for protein binding on the microtiter plate must be saturated by incubating with blocking buffer. Fill wells to the top and incubate for two hours to overnight at room temperature. (See note 1)

6. Wash the wells twice with PBS.

7. Add 50 ul of the antigen solution to the wells and incubate for two hours at room temperature. For quantitation, the antigen solution should be titrated (See note 2). Incubate for at least two hours at room temperature.

8. Wash the plate four times with PBS.

9. Add the labeled second antibody. The concentration of labeled antibody to be added can be determined in preliminary experiments (usually 1: 2-4000) . For quantitation, the second antibody should be used in excess. All dilutions should be done in blocking buffer.

10. Incubate for two hours or more at room temperature.

11. Wash three times with PBS.

12. Add 50 ul of substrate solution to each well. Determine optical density at 5, 10 and 15 minute developing times (See Note 3).

(B.) Notes

1. Poor blocking results in problems with excessive background. For quantitation of porcine IgG, we used 5% wt/vol nonfat dry milk, mixed in PBS and blocked overnight. For quantitation of bovine IgG and BSA, we used 10 mM Tris, .9% NaCl and .05% Tween 20 in distilled water at pH 7.4.

2. If the antigen solution is too concentrated (in excess), accurate quantitation will not be possible. Our initial dilutions of antigen varied from 1: 100 for newborn samples to 1: 1200 for more concentrated samples. Ten to 11 subsequent (2x) dilutions were accomplished in the wells. If reductions in optical densities are not observed as samples are diluted on the plate, it is probable that

further dilution of the antigen is needed.

3. Horseradish peroxidase conjugated antibodies were used in the present work. A Peroxidase substrate kit (Bio Rad, Richmond, CA) was used for quantitation. The substrate, in the presence of peroxidase turns an intense blue-green color and absorbance can be determined at 415 nm.

Vita

Jerry A. McClain was born February 23, 1952 in Hohenwald, Tennessee and completed his public education there. Shortly following graduation from high school in 1970, he entered the Army and did a tour of duty in Vietnam. After release from active duty he began working for Goodyear in Union City, Tennessee and enrolled in classes at The University of Tennessee at Martin. It was there that he met Trudi Counce, of Counce Tennessee and they were married in 1973. Their first daughter, Itaska (Snookey) McClain, was born in 1976. He received a B. S. in Education in 1977 and the following August moved to Counce, Tennessee and started teaching in McNairy County, Tennessee. During the six years living in that area, Trudi and he had the privilege of caring for seven foster children who were all eventually reunited with relatives. He and his family moved to Knoxville in pursuit of more education. Their second daughter, Laura (Doodles) McClain, was born in 1986. He received a B. S. in 1986, M. S. in 1988 and was granted the Ph. D. degree in Animal Science in 1991.