The Use of Bovine Serum Protein as an Oral Support Therapy Following Coronavirus Challenge in Calves[1]

**J. D. Arthington,* C. A. Jaynes,† H. D. Tyler,‡
S. Kapil,§ and J. D. Quigley, III†**
*Range Cattle Research and Education Center
University of Florida—IFAS, Ona 33865
†American Protein Corporation, Inc., Ames, IA 50010
‡Department of Animal Sciences
Iowa State University, Ames 50010
§College of Veterinary Medicine
Kansas State University, Manhattan 66506

ABSTRACT

The objective of this experiment was to investigate the therapeutic efficacy of a supplemental bovine serum protein blend fed to calves challenged with virulent coronavirus. Twelve Holstein bull calves (approximately 3 wk of age) were allocated by initial body weight to Control (n = 5) and treated (n = 7) groups. On d 0, all calves were orally challenged with 1×10^7 plaque-forming units of virulent coronavirus isolate. Infection was allowed to progress for 24 h before treatment was started. On d 1, treated calves began receiving 160 g of dry bovine serum powder (16 g IgG) mixed into milk replacer powder (67 g) at both an a.m. and p.m. feeding. Control calves received only milk replacer powder (227 g) at both feedings. Response to coronavirus challenge and dietary treatment was monitored prior to a.m. and p.m. feeding by the collection of multiple clinical measures. Fecal consistency was decreased by coronavirus challenge but was not affected by dietary treatment. Mean daily rectal temperature and heart rate were not affected by dietary treatment. Average packed cell volume was higher in treated calves than in control (35.0 and 27.0%). Coronavirus challenge resulted in an immediate increase in respiration rate, decreasing by d 7. Control calves tended to have a greater average respiration rate compared with treated (28.7 vs. 26.8 breaths/min). Treated calves had a higher average feed intake than control (0.57 vs. 0.44 kg/d). These data suggest that bovine-serum supplemented milk replacer may decrease the severity of disease in young calves exposed to coronavirus.

(**Key words:** bovine serum, coronavirus, calf)

Abbreviation key: BCV = bovine coronavirus, **PCV** = packed cell volume.

INTRODUCTION

Over 50% of dairy calf mortality on US dairy farms is related to diarrhea (NAHMS, 1994). A common enteric pathogen infecting young dairy calves is bovine coronavirus (**BCV**). Bovine coronavirus affects both dairy and beef and is most common in calves 1 to 2 wk of age (Clark, 1993). In adult cattle, BCV has been linked to winter dysentery, a diarrheal condition affecting housed cattle (Clark, 1993). The mode of infection is often via the oral route, and both enteric and respiratory BCV infection has been shown (Reynolds, 1983). Calf morbidity rates from naturally occurring BCV infection may be as high as 15 to 25% (Langpap et al., 1979). Strict sanitation and isolation of neonatal calves can be implemented to help prevent infection; however, the exposure rate is still high on many farms. Viral infections such as BCV are not treatable with antibiotics, except to combat secondary bacterial infection. Supportive care and electrolyte therapy attempting to replace lost fluids and prevent dehydration are often the only treatments available (Clark, 1993).

Bovine serum has been shown to be an effective source of exogenous passive Ig for newborn calves (Arthington et al., 2000a,b; Quigley et al., 1998). In these studies, serum-derived Ig was offered soon after birth, when the calf's intestinal epithelium is permeable to large molecular weight proteins. As a result, the serum-derived Ig was absorbed and found to be circulating in systemic blood. In contrast, the use of bovine serum in older calves, experiencing enteric challenge, has received little attention. Quigley and Drew (2000) investigated the prophylactic effect of spray-dried bovine plasma versus antibiotics in the milk replacer of calves challenged with *Escherichia coli*. In this study, calves

offered bovine plasma had equal improvements in attitude, hydration, and BW gain to calves treated with antibiotics. In pigs, the beneficial impact of dietary plasma protein has been known since 1987 (Zimmerman, 1987). Since this time, the effect of dietary plasma on young pig performance has been linked to improved enteric health during exposure to antigens associated with normal production environments (Coffey and Cromwell, 1995) and disease-causing pathogens (Cain and Zimmerman, 1997). The objective of the current study was to evaluate the effect of dietary bovine serum on dairy calf health and performance following enteric challenge with bovine coronavirus.

MATERIALS AND METHODS

The animals utilized in these experiments were cared for by acceptable practices as outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (1988). Additionally, this protocol was reviewed and approved by the Iowa State University Animal Care and Use Committee (Approval # 6-6-3245-3-B) and the Biohazards and Public Health Committee (Approval # 96-I-009-A).

Animals, Coronavirus Challenge, and Dietary Treatments

Eighteen Holstein bull calves (ave. 3 wk of age) were derived from the Iowa State University Dairy Research Herd. Calves were confined to 1.8×2.4 m, solid-sided, indoor pens at the Iowa State University Dairy Farm calf barn. Calves were screened for previous exposure to BCV by the examination of anti-BCV antibody in whole blood and viral shedding in fecal samples using methods previously described (Kapil et al., 1994; Schoenthaler and Kapil, 1999). Twelve calves tested negative for fecal viral shedding and antibody titer to BCV. These animals were selected to participate in the trial.

All calves were challenged orally on d 0 with a virulent strain of BCV known to produce moderately severe, nonfatal diarrhea (Schoenthaler and Kapil, 1999). Coronavirus was delivered orally in 10 ml of cell culture media using a 12-ml syringe connected to a length of plastic tubing held in place in the back of the oral cavity.

Before the start of the study, all calves received 227 g of a nonmedicated, commercial calf milk replacer containing 20% fat and 20% protein (APC Select, American Protein Corporation, Ames, IA) blended into 2 L of warm tap water at 0600 and 1800 h daily. Both before and after challenge, all calves were offered access to water and a commercial, pelleted calf starter (16% protein; Farmland Industries, Kansas City, MO) daily at

a level to ensure ad libitum access. Dry feed refusal was recorded daily. All calves were fasted for 24 h following BCV challenge. After fast, calves were assigned randomly to one of two dietary treatments consisting of a control (control; n = 5) and serum-fed group (treated; n = 7). Control calves continued to receive the same prestudy diet. Treated calves received 67 g of the calf milk replacer, plus 160 g of a blended commercial powder containing spray-dried, bovine serum (Lifeline; American Protein Corporation, Ames, IA) into 2 L of warm tap water, at the same times daily. The bovine serum product contained 10% Ig, 40% protein, and 0.50% fat on an as-fed basis. All liquid feed not voluntarily consumed was fed using an esophageal tube. The inclusion of the bovine serum product displaced 67 g of calf milk replacer, which resulted in a diet that provided treated calves with 32 g of additional protein and 31 g less fat at each feeding.

Clinical Measurements

Whole blood samples, for the measure of packed cell volume (**PCV**) were collected once daily at 0530, before feeding. Duplicate capillary tubes containing whole blood were centrifuged for 6 min, and the percentage of packed erythrocytes was measured directly. Objective clinical observations included rectal temperature, heart rate, and respiration rate. Respiration and heart rate were measured by using a Litman stethoscope and counting events directly for 30-s periods. Subjective clinical observations were also collected, including attitude (1 to 3 scale) and fecal consistency (1 to 4 scale) as described in Table 1. Body weights were recorded at the beginning of the study and again at d 14 following BCV challenge.

Statistical Analysis

Analysis of variance was performed using the general linear model procedure of SAS (1988) using a model for a completely randomized design. For multiple measures over time, a split-plot design was used with calf as the whole plot and time and time × treatment interactions in the subplot. Treatment means were compared using least significant differences with the appropriate error term.

RESULTS

Oral challenge with virulent bovine coronavirus resulted in a decrease in fecal consistency, which improved over the 7 d postchallenge data collection period (Figure 1). Dietary treatment with bovine serum did not influence fecal consistency (Table 2). Treated calves

Table 1. Response criteria for subjective clinical measures.[1]

| Clinical measure | Clinical score | | | |
	1	2	3	4
Attitude	Responsive	Nonactive	Moribund	N/A[2]
Fecal score	Formed	Soft	Runny	Liquid/watery

[1]Scoring was conducted by a common person throughout the study.

[2]Not included in the scoring scale.

tended ($P = 0.12$) to be more responsive following challenge than control as measured by calf attitude (Table 2). Decreased attitude score for all calves reached a peak on d 3 and improved to baseline values by d 7 (Figure 1). This improvement in calf responsiveness was reflected by a higher ($P = 0.02$) total feed intake by treated calves than control (Table 2). Although coronavirus challenge resulted in clinical morbidity, there were no instances of mortality for either treatment.

Calf rectal temperature tended ($P = 0.06$) to be lower for treated calves than control (Table 2); however, this difference was minor and probably of no biological significance. PCV is a common measure of hydration. Calves fed bovine serum had a higher ($P < 0.01$) PCV than control (Table 2). Respiration rate was greatest on d 1 following challenge and decreased over the following 7 d (Figure 2). Respiration rate of treated calves tended to be lower ($P = 0.06$) than control (Table 2).

Starting and ending BW were similar for both treatments (Table 3). Change in BW and daily BW gain at 14 d following challenge also was similar for both treatments (Table 3).

DISCUSSION

Immunoglobulin absorption in the small intestine is not expected to occur once calves have aged beyond approximately 24 h (Stott et al., 1979). However, the integrity of the Ig protein has been shown to be at least partially resistant to digestion in the gastrointestinal tract (Brock et al., 1977). This suggests that, although not absorbed, dietary Ig may be effective against enteric antigens within the intestinal lumen. During episodes of enteric infection, orally administered immunoglobulin has been shown to protect calves against *E. coli* infection (Quigley and Drew, 2000) and could potentially be effective against other enteric pathogens, such as bovine coronavirus.

In the current study, coronavirus challenge resulted in a decrease in fecal consistency. This response improved rapidly during 7 d following challenge (Figure 1). The calves used in the current study were approximately 3 wk of age when they were challenged. Complications with coronavirus infections typically occur in calves that are 2 wk of age. The severity of disease realized in the current study may have been impacted by this additional week of age. Dietary treatment with bovine serum did not influence fecal consistency (Table 2). These results are similar to those reported by Quigley and Drew (2000), in which calves challenged with *E. coli* and fed bovine plasma also had similar fecal scores compared with control. Rotavirus is another common enteric pathogen in young calves (Torres-Medina et al., 1985). Rotavirus-challenged calves have been

Table 2. Effect of bovine serum protein on clinical measures of Holstein calves challenged with bovine coronavirus.

Clinical measure[1]	Control[2]	Treated[2]	$P =$
Fecal score, scale 1 to 4[3]	1.61 ± 0.07	1.73 ± 0.06	0.19
Attitude, scale 1 to 3[3]	1.15 ± 0.03	1.08 ± 0.03	0.12
Heart rate, beats/min	88.1 ± 1.2	86.5 ± 1.0	0.31
Rectal temperature, °C	39.0 ± 0.1	38.9 ± 0.1	0.06
Respiration rate, inhalations/min	28.7 ± 0.7	26.8 ± 0.6	0.06
Packed cell volume, %	27.0 ± 0.5	35.0 ± 0.4	<0.001
Feed intake, kg/d	0.44 ± 0.06	0.57 ± 0.05	0.02

[1]Clinical measures collected twice daily over 7 d post-challenge by a common technician.

[2]Mean ± SE. All calves were orally challenged with 1×10^7 plaque-forming units of virulent coronavirus isolate. Infection was allowed to progress for 24 h prior to the initiation of treatment. Treated calves (n = 7) received 160 g of dry bovine serum powder (16 g IgG) mixed into milk replacer powder (67 g) twice daily at 0600 and 1800. Control calves received only milk replacer powder (227 g) at both feedings. Each treatment was mixed into warm water and provided in 2-L volume feedings.

[3]Subjective measures scored as described in Table 1.

Table 3. Effect of bovine serum protein on BW gain of Holstein calves challenged with bovine coronavirus.

Measure[1]	Control[2]	Treated[2]	P =
Start weight, kg	45.1 ± 1.2	46.3 ± 1.0	0.46
End weight, kg	55.1 ± 2.6	55.7 ± 2.2	0.88
Gain, kg	10.1 ± 1.8	9.3 ± 1.5	0.75
ADG, kg/d	0.72 ± 0.13	0.67 ± 0.11	0.76

[1]Individual BW were collected before coronavirus challenge and at d 14 after challenge.

[2]Mean ± SE. All calves were orally challenged with 1×10^7 plaque-forming units of virulent coronavirus isolate. Infection was allowed to progress for 24 h before the initiation of treatment. Treated calves (n = 7) received 160 g of dry bovine serum powder (16 g IgG) mixed into milk replacer powder (67 g) twice daily, at 0600 and 1800. Control calves received only milk replacer powder (227 g) at both feedings. Each treatment was mixed into warm water and provided in 2-L volume feedings.

shown to respond favorably to dietary treatment with exogenous Ig derived from colostrum of vaccinated cows. In one study, calves fed colostrum containing anti-rotavirus antibody did not become diarrheic, compared with calves fed normal colostrum, which experienced diarrhea for over 7 d following challenge (Castrucci et al., 1984). In contrast to the current study and that reported by Quigley and Drew (2000), these calves began consuming the anti-rotavirus colostrum at birth, thereby allowing some Ig absorption. Similarly, newborn pigs were protected against *E. coli* challenge-induced diarrhea when orally dosed with egg yolk-based antibody solutions obtained from immunized hens (Yokoyama et al., 1992). The bovine-serum material used in the current study was not tested for titers against BCV. Nevertheless, BCV is widespread in cattle populations (Clark, 1993), suggesting that the presence of coronavirus-specific antibody titers would be likely in pooled bovine blood.

Calf responsiveness was measured by subjectively scoring calf attitude (Table 1). Trated calves tended (P = 0.12) to be more responsive following challenge than control (Table 2). Decline in attitude peaked on d 3 and improved to baseline values by d 7 (Figure 1). Similar improvements in calf attitude have been reported in *E. coli*-challenged calves treated with oral bovine plasma (Quigley and Drew, 2000). The improvement in calf responsiveness in the current study was reflected by a higher (P = 0.02) total feed intake by treated calves than by control (Table 2). Improved feed intake is a commonly reported response in early-weaned pigs offered plasma-fortified starter diets (Gatnau et al., 1991; Kats et al., 1994) and may be related to enteric health in both pigs and calves.

Calf rectal temperatures were consistently higher (P < 0.01) at the 0600 h data collection than at the 1800 h collection. This may be a result of the normal diurnal glucocorticoid response in farm animals, for which peak excretion occurs in the morning hours (Breazile, 1988).
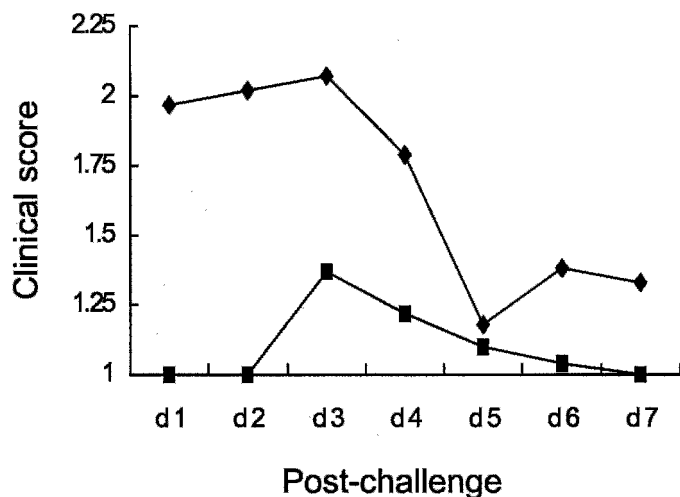

Figure 1. Attitude (◆) and fecal score (■) of Holstein bull calves challenged with coronavirus. Measures were scored subjectively two times daily using a common technician. Scoring system is described in Table 1. SEM = 0.08 and 0.21 for attitude and fecal score, respectively. Main effect of time, P < 0.01 for both attitude and fecal score.
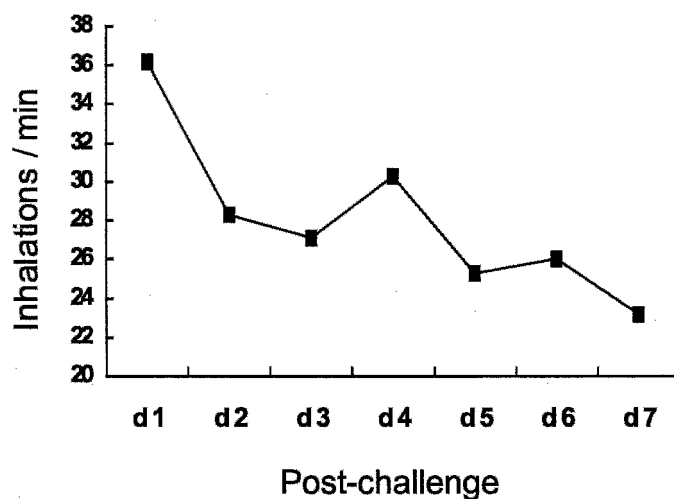

Figure 2. Respiration rate of Holstein bull calves challenged with coronavirus. Inhalations were counted by a common technician using a Litman stethoscope for 30-s periods. SEM = 2.9 inhalations/min. Main effect of time, P < 0.01.

This theory suggests that calves are more intensely susceptible to stress stimuli in the evening hours when a greater glucocorticoid-associated stress response may occur. Calf rectal temperature tended ($P = 0.06$) to be lower for treated calves than for control; however, this difference was minor (0.2°C) and probably of no biological significance. Increased PCV is a common result of hemoconcentration, which is an indicator of an animal's level of hydration. Calves fed bovine serum had a higher ($P < 0.01$) mean PCV than control (Table 2). Although values for both treatments are within the normal erythrocyte PCV range for cattle (24.0 to 46.0%; Schalm, 1986), the reason for the observed treatment difference is difficult to explain. Splenic contraction, resulting from excitement, can cause an increase in PCV, even in dehydrated cattle that have recently consumed water (Bianca, 1970). Although the difference in PCV was small (8.0%), it may reflect a difference in how treatments were affecting the anxiety of animals during handling and sample collection.

Respiration rate was greatest on d 1 following challenge, decreasing over the following 7 d (Figure 2). Respiratory infection has been reported in experiments with calves challenged with an enteric coronavirus (Reynolds, 1983). This rapid increase in respiration may likely be the result of an associated respiratory infection following challenge. Moreover, calves may have aspirated some of the challenge material when the oral inoculant was delivered. Treated calves tended ($P = 0.06$) to have a lower mean respiration rate than control (Table 2).

The inclusion of bovine plasma as an ingredient in calf milk replacers has been investigated (Morrill et al., 1995). Although unchallenged, calves in this study experienced increased growth when offered bovine plasma containing calf milk replacer. In the current study, starting and ending BW were similar for both treatments (Table 3). Feed intake decreased ($P < 0.01$) following BCV challenge and progressively increased during the 7-d postchallenge period. Perhaps the most biologically significant observation of the current study is the increased feed intake experienced by treated calves (Table 2). Although change in BW and daily BW gain was similar for both treatments (Table 3), feed intake was more closely correlated with calf gain for treated calves than with control ($R^2 = 0.92$ and 0.65 for treated and control calves, respectively), suggesting less variability in the efficiency of treated calves to convert feed into added BW.

In the current study, calves fed bovine-serum-fortified calf milk replacer were more responsive, tended to have a less pronounced increase in respiration, and had greater total feed intake than control. Further investigation into the use of bovine serum as a dietary therapeutic aid for calves experiencing enteric challenge is warranted.

REFERENCES

Arthington, J. D., M. B. Cattell, and J. D. Quigley, III. 2000a. Effect of dietary IgG source (colostrum, serum, or milk-derived supplement) on the efficiency of Ig absorption in newborn Holstein calves. J. Dairy Sci. 83:1463–1467.

Arthington, J. D., M. B. Cattell, J. D. Quigley, III, G. C. McCoy, and W. L. Hurley. 2000b. Passive immunoglobulin transfer in newborn calves fed colostrum or spray-dried serum protein alone or as a supplement to colostrum of varying quality. J. Dairy Sci. 83:2834–2838.

Bianca, W. 1970. Effects of dehydration, rehydration and overhydration on the blood and urine of oxen. Br. Vet. J. 126:121–133.

Breazile, J. E. 1988. The physiology of stress and its relationship to mechanisms of disease and therapeutics. Vet. Clinics North America: Food Anim. Pract. 4:441–480.

Brock, J. H., F. R. Arzabe, F. Ortega, and A. Pineiro. 1977. The effect of limited proteolysis by trypsin and chymotrypsin on bovine colostral IgG1. Immunology 32:215–219.

Cain, C. M., and D. R. Zimmerman. 1997. Effect of spray-dried plasma (SDP) on fecal shedding of hemolytic *Escherichia coli*. (HEC) and rotavirus by pigs in a segregated early weaned (SEW) environment. J. Anim. Sci. 75(Suppl. 1):61. (Abstr.)

Castrucci, G., F. Frigeri, M. Ferrari, V. Cilli, F. Caleffi, V. Aldrovandi, and A. Nigrelli. 1984. The efficacy of colostrum from cows vaccinated with rotavirus in protecting calves to experimentally induced rotavirus infection. Comp. Immun. Microbiol. Infect. Dis. 7:11–18.

Clark, M. A. 1993. Bovine coronavirus. Br. Vet. J. 149:51–70.

Coffey, R. D., and G. L. Cromwell. 1995. The impact of environment and antimicrobial agents on the growth response of early weaned pigs to spray-dried porcine plasma. J. Anim. Sci. 73:2532–2539.

Gatnau, R., D. R. Zimmerman, T. Diaz, and J. Johns. 1991. Determination of optimum levels of spray-dried porcine plasma (SDPP) in diets for weanling pigs. J. Anim. Sci. 69(Suppl. 1):369. (Abstr.)

Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 1988. Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, Champaign, IL.

Kapil, S., A. M. Trent, and S. M. Goyal. 1994. Antibody responses in spiral colon, ileum, and jejunum of bovine coronavirus-infected neonatal calves. Comp. Immunol. Microbiol. Infect. Dis. 17:139–149.

Kats, L. J., J. L. Nelssen, M. D. Tokach, R. D. Goodband, J. A. Hansen, and J. L. Laurin. 1994. The effect of spray-dried porcine plasma on growth performance in the early weaned pig. J. Anim. Sci. 72:2075–2081.

Langpap, T. J., M. E. Bergeland, and D. E. Reed. 1979. Coronaviral enteritis of young calves: Virologic and pathologic findings in naturally occurring infections. Am. J. Vet. Res. 40:1476–1478.

Morrill, J. L., J. M. Morrill, A. M. Feyerherm, and J. F. Laster. 1995. Plasma proteins and a probiotic as ingredients in milk replacer. J. Dairy Sci. 78:902–907.

National Animal Health Monitoring Service (NAHMS). National Dairy Heifer Evaluation Project. 1994. Dairy Heifer Morbidity, Mortality, and Health Management Focusing on Preweaned Heifers. USDA-APHIS-VS.

Quigley, J. D., III, and M. D. Drew. 2000. Effects of oral antibiotics or bovine plasma on survival, health and growth in dairy calves challenged with *Escherichia coli*. Food Agric. Immunol. 12:311–318.

Quigley, J. D., III, D. L. Fike, M. N. Edgerton, J. J. Drewery, and J. D. Arthington. 1998. Effects of a colostrum replacement product derived from serum on immunoglobulin G absorption by calves. J. Dairy Sci. 81:1936–1939.

Reynolds, D. J. 1983. Coronavirus replication in the intestinal and respiratory tracts during infection in calves. Ann. Rech. Vet. 14:445–446.

SAS User's Guide: Statistics, Version 6 Edition. 1988. SAS Inst., Inc., Cary, NC.

Schalm, O. W. 1986. Cattle: Normal hematology with comments on disease. Paages 178–207 in Schalm's Veterinary Hematology. 4th ed. N. C. Jain, ed. Lea and Febiger, Philadelphia, PA.

Schoenthaler, S., and S. Kapil. 1999. Development and application of a bovine coronavirus antigen detection enzyme-linked immuno-sorbent assay. Clin. Diag. Lab. Immunol. 6:130–136.

Stott, G. H., D. B. Marx, B. E. Menefee, and G. T. Nightengale. 1979. Colostral immunoglobulin transfer in calves. I. Period of absorption. J. Dairy Sci. 62:1632–1638.

Torres-Medina, A., D. H. Schlafer, and C. A. Mebus. 1985. Rotaviral and coronaviral diarrhea. Vet. Clinics North America: Food Anim. Pract. 1:471–493.

Yokoyama, H., R. C. Peralta, R. Diaz, S. Sendo, Y. Ikemori, and Y. Kodama. 1992. Passive protective effect of chicken egg yolk immunoglobulins against experimental enterotoxigenic *Escherichia coli* infection in neonatal piglets. Infect. Immunol. 60:998–1007.

Zimmerman, D. R. 1987. Porcine plasma proteins in diets of weanling pigs. ISU Swine Res. Rpt. ASL-R482.