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Effects of Oral Antibiotics or Bovine Plasma on Survival, Health and Growth in Dairy Calves Challenged with *Escherichia coli*

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Thirty-six Holstein bull calves were fed experimental milk replacers (454 g/day^{-1}) containing 20% crude protein and 20% fat. Milk replacers contained no additives, antibiotics (800 mg neomycin (as neomycin sulfate) and 400 mg oxytetracycline daily replacing dried whey, or 3.3% spray dried bovine plasma replacing whey protein concentrate. Calves were orally challenged with approximately 10^8 colony forming units (CFU) of enterotoxigenic Escherichia coli *on day 3. No veterinary treatments or supportive therapy were provided during the study. Calf body weight (BW) was determined weekly and average daily BW gain was calculated. Clinical scores were determined daily for fecal consistency, attitude and hydration. All clinical scores responded to oral* E. coli *challenge. Provision of antibiotics and animal plasma had no effect on fecal consistency but improved attitude and hydration scores, body weight gain and tended to reduce overall mortality. Spray dried animal plasma appears to be a viable alternative to antibiotics in calf milk replacer applications.*

INTRODUCTION

Calf milk replacers (CMR) are commercial preparations fed to calves prior to weaning to allow sale of whole milk from dairy farms. Traditionally, most CMR in certain areas of the USA have contained antibiotics (AB) to prevent or treat bacterial scours (Heinrichs *et al.*, 1995). Use of AB in CMR has recently been criticized, however, due to increasing evidence that such AB use may contribute to increased transfer of antimicrobial resistance to pathogens of medical importance (Glynn *et al.*, 1998; Dunlop *et al.*, 1998). Although the efficacy of AB in CMR applications has been established (Morrill *et al.*, 1977; Tomkins & Jaster, 1991; Quigley *et al.*, 1997), a need exists for viable alternatives to AB in the diet of young calves.

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Spray dried animal plasma (SDAP) has been shown to improve animal performance when included in the diet of young pigs (Hansen *et al.*, 1993; de Rodas *et al.*, 1995; Bergström *et al.*, 1997) and calves (Morrill *et al.*, 1995). Spray dried animal plasma contains approximately 20% IgG. Improvements in animal performance have been reported when IgG from milk or colostrum (Snodgrass *et al.*, 1982; Saif *et al.*, 1983) have been fed to calves older than 24 h of age. At least a portion of the IgG escape gastric and intestinal degradation and may contribute to the local immune response in the gut. Besser *et al.*, (1998a) reported that colostral IgG in the circulation of neonatal calves were secreted back into the intestine to assist in provision of intestinal immunity. In addition, Nollet *et al.* (1999) reported reduced incidence and severity of disease in calves fed dried bovine plasma and challenged with *Escherichia coli*. These authors concluded that improved survival and health of calves was due to the presence of glycoprotein glycans in bovine plasma.

Therefore, SDAP may be a viable alternative to AB in treating and/or preventing bacterial scours. Further, the potential specificity of IgG for viral agents and/or inhibition of pathogenic binding further makes SDAP a viable replacement for AB in CMR. The objective of this study was to evaluate the effectiveness of AB versus SDAP in CMR in response to oral challenge with *E. coli* in young calves.

MATERIAL AND METHODS

Holstein bull calves (n = 36) approximately 1 day of age were purchased from local sale barns and assigned randomly to one of three experimental CMR at a commercial research facility (Health Management Services, Tulare, CA, USA). Calves were fed one of three CMR: commercial CMR containing 20% crude protein and 20% crude fat (air dry basis; CON, CON plus 800 mg neomycin (as neomycin sulfate) and 400 mg oxytetracycline daily replacing dried whey (CAB), or CON plus 3.3% SDAP replacing 75% dried whey protein concentrate (CAP). All calves were fed 454 g of CMR daily dissolved in 3.89 l of water in two equal feedings for 21 days. A commercial calf starter (18% CP) was offered for *ad libitum* consumption from day 7 to 21; unfortunately, starter intake was not reliably measured during the trial. Therefore, intake of starter is not reported. Calves were housed in individual elevated wooden calf pens with slatted floors and no bedding. All calves were clinically examined upon arrival and once daily by a licensed veterinarian unaware of treatment assignments. Each calf was assigned a score for fecal consistency (0 = normal solid feces to 3 = severe scours), attitude (0 = normal to 2 = severe depression to moribund) and hydration (0 = no skin tenting to 2 = skin tenting for 5–10 s).

On day 3, each calf was orally challenged with approximately 10^8 colony forming units (CFU) of enterotoxigenic *E. coli*. No veterinary treatments or supportive therapy (e.g. electrolytes) was provided during the study. Calves with severe clinical signs were removed from the study and were euthanized. Although the procedures used in this study were not specifically approved by a competent animal care committee, guidelines for animal care and use were followed (Consortium, 1988) with the exception of antibiotic therapy following challenge. Calf body weight (BW) was determined weekly and average daily BW gain was calculated.

Initial BW, final BW and BW gain over the 21 day experimental period were analyzed by ANOVA using a completely randomized experimental design (SAS Institute, 1989). Daily fecal, attitude and hydration scores were analyzed as a repeated measures design using a mixed model ANOVA (SAS Institute, 1989). Calf within treatment was defined as a random variable and was used as the error term to test differences due to treatment. Single degree of freedom orthogonal contrasts were used to compare CON vs CMR containing additives and CAB vs CAP.

TABLE 1. Least squares means of animal performance in calves fed experimental milk replacers

	Treatments[a]						Contrasts[b]	
Item	CON	SE	CAB	SE	CAP	SE	1	2
No. of calves								
Day 0	12	–	12	–	12	–	–	–
Day 21	9	8.9	11	8.9	12	8.9	–	–
% Mortality[c]	25.0		8.3		0.0		0.06	NS
BW (kg)								
Day 0	33.6	0.6	33.4	0.6	32.8	0.6	NS	NS
Day 21	44.1	1.5	46.5	1.4	46.6	1.3	NS	NS
BW gain, 0–21 (g day^{-1})	497	48	619	44	665	43	0.02	NS
Fecal score[d,e]	0.74	0.09	0.72	0.08	0.62	0.08	NS	NS
Attitude score[f,g]	0.31	0.07	0.07	0.07	0.10	0.07	0.02	NS
Hydration score[f,h]	0.24	0.05	0.03	0.05	0.03	0.05	0.004	NS
Day 5 fecal sample								
E. coli, % of calves	100	0	100	0	100	0	NS	NS
K 99+, % of calves	42	15	50	15	42	15	NS	NS

[a] Treatments: CON = calf milk replacer containing no additive; CAB = calf milk replacer containing neomycin plus oxytetracycline; CAP = calf milk replacer containing 3.3% bovine plasma.
[b] Contrasts: 1 = CON vs (CAB + CAP)/2; 2 = CAB vs CAP.
[c] Analyzed by χ^2 analysis.
[d] Significant effect of day of study ($P < 0.001$).
[e] Fecal score: 0 = faces with normal consistency to 3 = severe scours.
[f] Significant interaction of day of study × treatment ($P < 0.001$).
[g] Attitude score: 0 = normal to 2 = severe depression to moribund.
[h] Hydration score: 0 = no skin tenting to 2 = skin tenting for >5 s.

RESULTS

Oral challenge with *E. coli* was effective in causing clinical signs in all calves, particularly during the first week of the study. Three of 12 calves on CON treatment and one calf on CAB treatment died during the study (Table 1). No calves on CAP treatment died. The difference between mortality of calves approached significance ($P < 0.06$) as determined by χ^2 analysis. Necropsy indicated that all calves died of colibacillosis. Fecal samples taken on day 5 indicated that all calves excreted coliforms and 42–50% of calves shed K99+ *E. coli* (Table 1).

Initial and final BW of calves did not differ among treatments; however, daily BW gain over the 21 day period was improved when calves were fed CAB or CAP ($P < 0.03$). Inclusion of antibiotics or SDAP improved BW gain by 25 and 34%, respectively, compared to calves fed CON.

Weekly BW increased throughout the study (Figure 1) but rate of increase was greater for calves fed CMR containing CAB or CAP compared to CON. Rates of BW gain were acceptable for preweaned calves fed 454 g/day^{-1} of CMR and with access to calf starter for *ad libitum* consumption and indicated that growth rates were not markedly impaired during the study.

Daily fecal scores were not significantly affected by treatment, although scores of all calves increased on the first d after oral challenge (Figure 2). Thereafter, fecal scores declined to the end of the study for all calves. By 19 days of age, fecal scores had declined to nearly 0, indicating normal fecal consistency.

Daily attitude scores (indication of animal depression) were significantly increased in calves on CON compared to other calves (Figure 3). Attitude score increased in calves

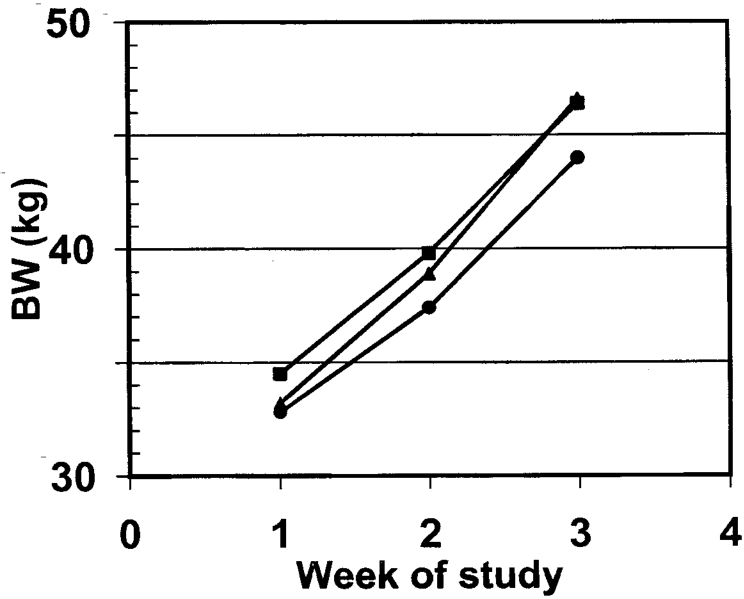

FIG. 1. Least squares means of weekly BW in calves fed control CMR (●), CMR containing AB (■), or CMR containing SDAP (▲). Standard error of weekly mean = 1.0 kg.

on CON diet by day 4 and returned to baseline by day 10. The increase in attitude score on calves fed CAP increased, but to the same degree as calves on CON. The attitude score of calves fed CMR containing CAB did not increase markedly throughout the study.

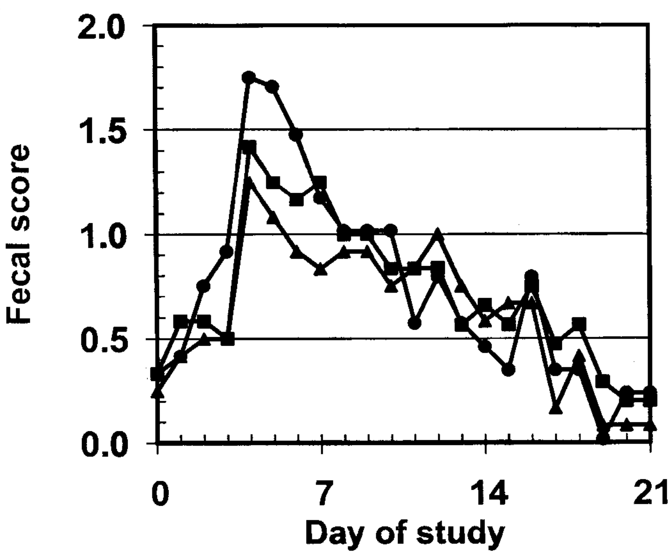

FIG. 2. Least squares means of daily fecal scores in calves fed control CMR (●), CMR containing AB (■), or CMR containing SDAP (▲). Feces was scored on a scale of 0 = normal fecal consistency to 3 = fecal consistency consistent with severe scours. Standard error of daily mean = 0.18.

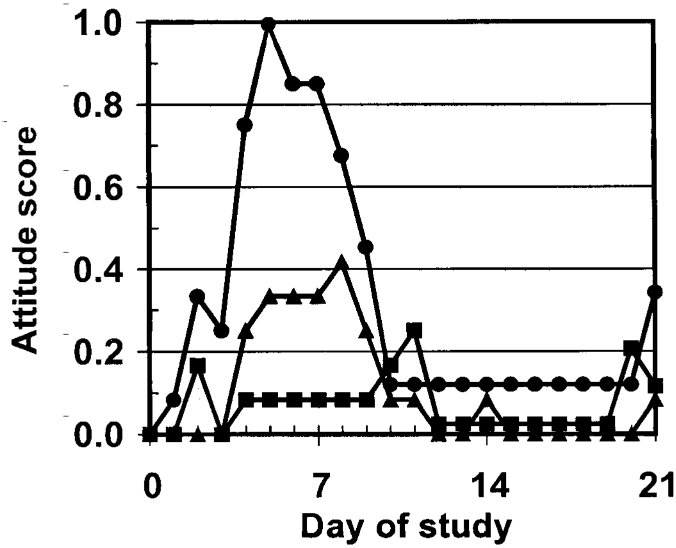

FIG. 3. Least squares means of daily attitude scores in calves fed control CMR (●), CMR containing AB (■), or CMR containing SDAP (▲). Attitude was scored on a scale of 0 = normal to 2 = severe depression to moribund. Standard error of daily mean = 0.12.

Hydration scores (Figure 4) reflected daily changes in attitude score following *E. coli* challenge. Degree of skin tenting increased in calves fed CON compared to other calves. Differences between calves on CAB and CAP treatments were not significant. Scores returned to baseline by day 10 for all treatments.

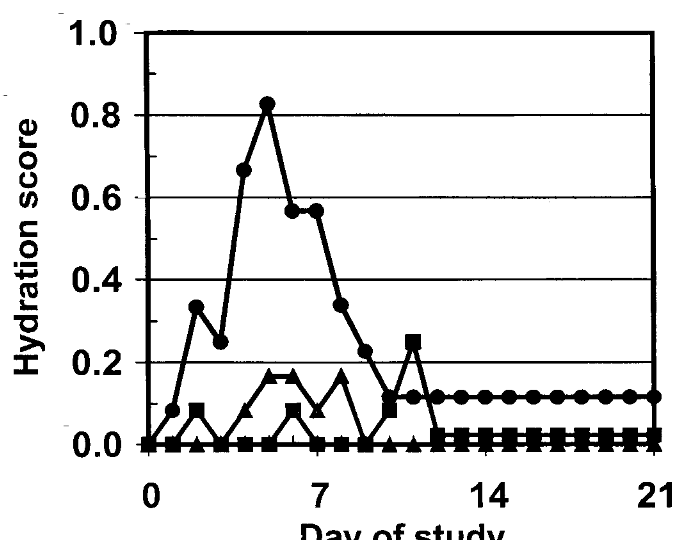

FIG. 4. Least squares means of daily hydration scores in calves fed control CMR (●), CMR containing AB (■), or CMR containing SDAP (▲). Hydration was scored on a scale of 0 = no skin tenting to 2 = skin tenting for 5 to 10 s. Standard error of daily mean = 0.10.

DISCUSSION

Oral challenge with *E. coli* resulted in typical symptoms of enteric infection, including transient depression, scours, and dehydration. Three calves died and all 36 calves showed signs of infection, indicating that the *E. coli* strain used was enterotoxigenic. Signs of disease were apparent from day 1 following infection and persisted for approximately 5 days. Calves fed CON that died or were removed from the study (n = 3) were on the study for 6.3 days. The calf fed CAP that died was on the study for 12 days. All mortality was attributed to enterotoxigenic *E. coli*. Oral *E. coli* challenge in calves is transient due to loss of receptors for the K99 pilus with increasing age, so the disease becomes less significant in older animals (Acres, 1985).

Oral SDAP provided approximately 3 g of oral IgG per day. Although some of the IgG are digested (Besser *et al.*, 1998a), the resistance of Ig to enzymatic digestion by trypsin and chymotrypsin (Brock *et al.*, 1977) suggests that at least a portion of the oral IgG reach the small intestine.

The efficacy of IgG from colostrum to protect animals from *E. coli* infection is well documented. Neonatal piglets, lambs, and calves fed colostrum and challenged with *E. coli* did not exhibit clinical signs, particularly when colostrum was obtained from dams vaccinated against K99+ *E. coli* (Morgan *et al.*, 1978; Sojka *et al.*, 1978; Gregory *et al.*, 1983; Valente *et al.*, 1988). Others report differing degrees of infection, depending upon strain of *E. coli*, age and breed and susceptibility of the animal to infection (Duchet-Suchaux *et al.*, 1982; Wray *et al.*, 1989). Logan *et al.* (1974) challenged neonatal calves with a preparation of *E. coli* at 24 h of age. Calves fed colostrum remained healthy, whereas calves fed colostral preparations containing individual Ig survived but developed severe diarrhea and dehydration. All colostrum-deprived calves challenged with *E. coli* died.

Spray dried animal plasma has been used as an ingredient in CMR formulations (Morrill *et al.*, 1995; Quigley & Bernard, 1996). Morrill *et al.* (1995) reported improved animal performance (growth and efficiency) when calves were fed CMR containing bovine plasma but not porcine plasma to day 42. Besser *et al.* (1988a) reported significant transfer of circulating IgG_1 into the intestine and that the Ig retained significant functional activity (Besser *et al.*, 1998b). The authors calculated that calves absorbed 100 g of IgG from colostrum would subsequently secrete 1–4 g of IgG into the intestine daily from day 1 to 14. In a subsequent study, the authors (Besser *et al.*, 1988b) injected calves with IgG containing high rotavirus antibody titers. The injected IgG was protective in response to an oral challenge with rotavirus, suggesting transfer of Ig into the intestine and retention of immunological activity.

In addition to IgG, SDAP may also provide other immunological components that may reduce the incidence and severity of enteric disease. Nollet *et al.* (1999) fed calves 2 l of milk three times per day with spray dried bovine plasma at 0, 10 or 25 gl^{-1}. Calves were orally challenged with 10^{10} CFU of *E. coli* at 12 to 24 h of age. Spray dried bovine plasma was pasteurized (50°C for 15 min) to destroy IgG activity. Calves fed 25 gl^{-1} of bovine plasma were protected from enteric challenge. Calves fed 10 gl^{-1} showed moderate signs of enteric disease (anorexia, scours, depression) and one calf died. All calves fed 0 g of bovine plasma died within 7 days of challenge due to colibacillosis.

Antibiotics in CMR have been shown to reduce the incidence of scours and improve BW gains in calves (Kiser, 1976; Morrill *et al.*, 1977; Tomkins & Jaster, 1991; Quigley *et al.*, 1997). Our study confirms these findings. In light of the increasing concerns related to subtherapeutic use of antimicrobials in animal feeds (Glynn *et al.*, 1998), the need for viable alternatives to AB in CMR is clear.

Supplementation of CMR with AB remains a common practice. The National Dairy Heifer Evaluation Project reported that 63.1% of US producers reported feeding CMR to calves prior to weaning; 52.7% reported using CMR containing AB (Heinrichs *et al.*, 1995). More producers in the Northeastern, Midwestern and Southeastern USA reported using CMR containing AB (>70%) compared to the Western USA (50.1%). Clearly, the use of AB in CMR has been widely accepted by the industry.

Kiser (1976) suggested that AB are commonly used in dairy calf production to compensate for poor colostrum feeding practices, hygiene and management. Although significant educational efforts have been made in the past 25 years to improve these practices, the USDA estimated in 1991 that over 40% of dairy heifer calves fail to acquire sufficient passive immunity. These data suggest the need for techniques to reduce the severity of enteric infections to improve calf health, growth, and efficiency. Management of AB on dairy farms to maximize response while limiting the risk of transmission of antimicrobial resistance may be inadequate (Goodger *et al.*, 1993). Removal of AB from CMR may reduce the spread of microbial resistance to AB and the possible transfer of AB resistance to pathogens of medical importance. However, alternatives to antimicrobial compounds will remain important to producers rearing young calves. Use of bovine plasma in the diets of ruminant animals must be carefully scrutinized. Strict procedures are necessary to minimize the risk of transmission of disease causing agents from ruminants to ruminants. Current regulations allow the feeding of bovine plasma to ruminant animals in most, but not all, countries.

In conclusion, calves challenged with an enteropathogenic strain of *E. coli* showed less severe symptoms when they were fed either neomycin/oxytetracycline or bovine IgG from SDAP. Spray dried animal plasma appears to be a viable alternative to AB in CMR formulations.

REFERENCES

Acres, S. D. (1985) Enterotoxigenic *Escherichia coli* infections in newborn calves: A review, *Journal of Dairy Science* **68**, 229–256.

Besser, T. E., Mc Guire, T. C., Gay, C. C. & Pritchett, L. C. (1988a) Transfer of functional immunoglobulin G (IgG) antibody into the gastrointestinal tract accounts for IgG clearance in calves, *Journal of Virology*, **62**, 2234–2237.

Besser, T. E., Gay C. C., McGuire, T. C. & Evermann, J. F. (1988b) Passive immunity to bovine rotavirus infection associated with transfer of serum antibody into the intestinal lumen, *Journal of Virology*, **62**, 2238–2242.

Bergström, J. R., Nelssen, J. L., Tokach, M. D., Goodband, R. D., Dritz, S. S., Owen, K. Q. & Nessmith Jr W. B. (1997) Evaluation of spray-dried animal plasma and select menhaden fish meal in transition diets of pigs weaned at 12 to 14 days of age and reared in different production systems, *Journal of Animal Science*, **75**, 3004–3009.

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

Consortium (1988) Guide for the care and use of agricultural animals in agricultural research and teaching. Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, Champaign, IL, USA.

de Rodas, B. Z., Sohn, K. S., Maxwell, C. V. & Spicer, L. J. (1995) Plasma protein for pigs weaned at 19 to 24 days of age: Effect on performance and plasma insulin-like growth factor I, growth hormone, insulin, and glucose concentrations, *Journal of Animal Science*, **73**, 3657–3665.

Duchet-Suchaux, M., Bertin, A., De Rycke, J. & Lechopier, P. (1982) Experimental *Escherichia coli* diarrhoea in colostrum deprived lambs, *Annales de Recherches Veterinaires*, **13**, 259–266.

Dunlop, R. H., Mc Ewen, S. A., Meek, A. H., Clarke, R. C., Black, W. D. & Friendship, R. M. (1998) Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada, *Preventive Veterinary Medicine*, **34**, 283–305.

Glynn, M. K., Bopp, C., Dewitt, W., Dabney, P., Mokhtar, M. & Angulo, F. J. (1998) Emergence of multidrug-resistant salmonella enterica serotype typhimurium DT104 infections in the United States, *New England Journal of Medicine*, **338**, 1333–1338.

Goodger, W. J., Troutt, F., Wallace, L. & Williams, C. (1993) Management factors affecting antibiotic residues and the establishment of antibiotic resistance in dairy calves, *Bovine Practitioner*, **27**, 135–140.

Gregory, D. W., Cardella, M. A. & Myers, L. L. (1983) Lamb model in the study of immunity to enteropathogenic *Escherichia coli* infections, *American Journal of Vetinary Research*, **44**, 2073–2077.

Hansen, J. A., Nelssen, J. L., Goodband, R. D. & Weeden, T. L. (1993) Evaluation of animal protein supplements in diets of early-weaned pigs, *Journal of Animal Science*, **71**, 1853–1862.

Heinrichs, A. J., Wells, S. J. & Losinger, W. C. (1995) A study of the use of milk replacers for dairy calves in the United States, *Journal of Dairy Science*, **78**, 2831–2837.

Kiser, J. S. (1976) A perspective on the use of antibiotics in animal feeds, *Journal of Animal Science*, **42**, 1058–1072.

Logan, E. F., Stenhouse, A., Ormrod, D. J. & Penhale, W. J. (1974) The role of colostral immunoglobulins in intestinal immunity to enteric colibacillosis in the calf, *Research Veterinary Science*, **17**, 290–301.

Morgan, R. L., Isaacson, R. E., Moon, H. W., Brinton, C. C. & To, C. C. (1978) Immunization of suckling pigs against enterotoxigenic *Escherichia coli*-induced diarrheal disease by vaccinating dams with purified 987 or K99 pili: protection correlates with pilus homology of vaccine and challenge, *Infection and Immunity*, **22**, 771–777.

Morrill, J. L., Dayton, A. D. & Mickelsen, R. (1977) Cultured milk and antibiotics for young calves, *Journal of Dairy Science*, **60**, 1105–1109.

Morrill, J. L., Morrill, J. M., Feyerherm, A. M. & Laster, J. F. (1995) Plasma proteins and a probiotic as ingredients in milk replacer, *Journal of Dairy Science*, **78**, 902–907.

Nollet, H., Laevens, H., Deprez, P., Sanchez, R. Van Driessche, E. & Muylle, E. (1999) The use of non-immune plasma powder in the prophylaxis of neonatal *Escherichia coli* diarrhoea in calves, *Zentralblatt fur Veterinarmedizin Reihe A*, **46**, 185–196.

Quigley, J. D., III & Bernard, J. K. (1996) Milk replacers with or without animal plasma for dairy calves, *Journal of Dairy Science*, **79**, 1881–1884.

Quigley, J. D., III, Drewry, J. J., Murray, L. M. & Ivey, S. J. (1997) Body weight gain, feed efficiency, and fecal scores of dairy calves in response to galactosyl-lactose or antibiotics in milk replacers, *Journal of Dairy Science*, **80**, 1751–1754.

Saif, L. J., Redman, D. R., Smith, K. L. & Theil, K. W. (1983) Passive immunity to bovine rotavirus in newborn calves fed colostrum supplements from immunized or nonimmunized cows, *Infection and Immunity*, **41**, 1118–1131.

SAS Institute (1989) *SAS/STAT User's Guide*, Version 6, 4th edn, SAS Institute Inc., Cary NC, USA.

Snodgrass, D. R., Stewart, J., Taylor, J., Krautil, F. L. & Smith, M. L. (1982) Diarrhoea in dairy calves reduced by feeding colostrum from cows vaccinated with rotavirus, *Research Veterinary Science*, **32**, 70–73.

Sojka, W. J., Wray, C. & Morris, J. A. (1978) Passive protection of lambs against experimental enteric colibacillosis by colostrum transfer of antibodies from K99 vaccinated ewes, *Journal of Medical Microbiology*, **11**, 493–499.

Tomkins, T. & Jaster, E. H. (1991) Preruminant calf nutrition, *Veterinary Clinics of North America Food Animal Practice*, **7**, 557–576.

Valente, C., Fruganti, G., Tessi, B., Ciorba, A., Cardaras, P., Floris, A. & Bordoni, E. (1988) Vaccination of pregnant cows with K99 antigen of enterotoxigenic *Escherichia coli* and protection by colostrum in newborn calves, *Comparative Immunology, Microbiology and Infectious Diseases*, **11**, 189–198.

Wray, C., McLaren, I. & Pearson, G. R. (1989) Occurrence of 'attaching and effacing' lesions in the small intestine of calves experimentally infected with bovine verocytotoxic *E. coli*, *Veterinary Record*, **125**, 365–368.