Effects of feeding polyclonal antibody preparations on ruminal bacterial populations and ruminal pH of steers fed high-grain diets

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ABSTRACT: Three experiments with factorial arrangements of treatments were designed to test the efficacy of avian-derived polyclonal antibody preparations (PAP) against *Streptococcus bovis* (PAP-Sb) or *Fusobacterium necrophorum* (PAP-Fn) in reducing ruminal counts of target bacteria in beef steers supplemented or not with feed additives (300 mg of monensin/d and 90 mg of tylosin/d; MT). Feeding increasing doses of PAP-Sb in Exp. 1 or a single dose in Exp. 2 reduced *S. bovis* counts in a cubic fashion ($P = 0.014$). In Exp. 1 and 2, inclusion of MT in the diet had no effect ($P > 0.05$) on ruminal *S. bovis* counts. In Exp. 2, ruminal pH was increased ($P < 0.05$) by feeding PAP-Sb, MT, and PAP-Sb plus MT. Ruminal *F. necrophorum* counts were reduced by feeding PAP-Fn ($P = 0.002$) and MT ($P < 0.001$). Reduction in ruminal *F. necrophorum* counts was greater ($P = 0.008$) when feeding MT alone than when feeding PAP-Fn and MT together. In Exp. 3, ruminal *S. bovis* counts were not affected ($P = 0.64$) by PAP-Fn. Ruminal pH was not affected ($P = 0.61$) by feeding PAP-Fn, and the total anaerobic bacterial count was not affected ($P > 0.05$) by either PAP-Sb or PAP-Fn in Exp. 1 or Exp. 3. In conclusion, PAP of avian origin and against *S. bovis* or *F. necrophorum* were effective in reducing target ruminal bacterial populations. These PAP could be effective in preventing the deleterious effects associated with these bacteria, and possibly in enhancing animal performance.

Key words: , *Streptococcus bovis*, *Fusobacterium necrophorum*, polyclonal antibodies

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INTRODUCTION

Ionophores and antibiotics are extensively used to increase animal performance by modifying ruminal bacterial populations to obtain more efficient fermentation. However, the use of antibiotics in animal agriculture is perceived by some to be a major public health concern because antibiotics are thought to contribute to the emergence of antibiotic-resistant microorganisms (Salyers, 2002). As an alternative to antibiotics, direct-fed microbial agents have been tested to enhance animal performance, but their effectiveness is inconsistent (Ghorbani et al., 2002; Brashears et al., 2003; Elam et al., 2003). The use of naturally occurring microbial or plant extracts has also been postulated to improve animal productivity, but further development is needed to obtain efficacious natural feed additives (Newbold et al., 2001).

An alternative to feeding ionophores or direct-fed microbial agents that has not received as much attention until recently is immunization. Vaccination against ruminal *Streptococcus bovis* and *Lactobacillus* spp. led to reductions in ruminal lactate concentration and target bacteria (Shu et al., 1999). Similarly, passive immunization achieved by feeding colostrum or eggs successfully prevented bovine coronavirus diarrhea (Ikemori et al., 1997) in bottle-fed calves. Feeding avian antibodies is a possible alternative because immunoglobulin Y, the main source of immunoglobulins in avian antibodies, is resistant to heat, acid digestion, and proteolysis (Shimizu et al., 1988). Polyclonal antibody preparations (**PAP**) against various microorganisms are produced by Camas Inc. (LeCenter, MN), but they have not been tested for their ability to affect target bacteria. An objective of the present series of studies was to evaluate effects of feeding avian-derived PAP against *S. bovis* or *Fusobacterium necrophorum* on ruminal populations of target bacteria and ruminal pH of steers fed a high-grain diet supplemented or not with feed additives (**MT**, monensin and tylosin). A second objective (Exp. 2) was to evaluate effects of PAP and time postfeeding on ruminal populations of target bacteria and ruminal pH of steers fed a high-grain diet supplemented or not with MT.

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MATERIALS AND METHODS

Procedures for PAP preparation, diets, animal management, ruminal fluid collection, bacterial enumeration, and statistical analysis were the same for all 3 experiments. All animal care personnel were trained, and animals were cared for, according to the guidelines established by the University of Minnesota Institutional Animal Care and Use Committee.

Polyclonal Antibody Preparations

Polyclonal antibodies are produced under patented and proprietary procedures (Camas Inc., Le Center, MN); thus, only portions of the procedure are described herein. Immunogens were extracted from model bacteria grown under proprietary conditions to express the surface antigens that the organism uses to attach to cells. Antigens were then purified from the culture, and isolated "adherin" immunogens were made for injection into egg-laying hens with no adjuvant. The model organisms for this study were *Streptococcus bovis* (ATCC 9809) and *Fusobacterium necrophorum* (ATCC 27852). These organisms were used for the initial testing, to which wild-type organisms isolated from the rumen of normal cattle were later added. More than 600 hens were immunized per each immunogen. Eggs collected were analyzed weekly by specific ELISA test plates to monitor antibody binding.

For the study preparations, approximately 200 immunized hens were randomly selected from the total group of hens used for egg collection. Eggs were collected for 3 d, and the product was made from eggs pooled from this collection. The PAP product was made using a mixture of egg protein, molasses, soy oil, and PBS at pH 7.4. At the single dose, the PAP product was fed at 2.5 mL/d. Approximately 2 mL of the egg protein was present in each 2.5-mL aliquot of the PAP product. Titers by ELISA averaged between 1:50,000 and 1:1,000,000. The preparations contained immunoglobulin Y, immunoglobulin M, and immunoglobulin A. Counts of 10^{18} antibody molecules per mL of egg protein were observed. This included approximately 10 to 20% of the preparation that was not active. Because antibodies against many bacteria are found in most eggs, commercially available egg products or eggs were tested for antibodies to specific microbes using the same protocols used to produce egg protein. Although there were small amounts of antibodies in these products and eggs to specific bacteria such as *Streptococcus* spp. or *Escherichia coli*, they did not bind specific organisms to beads coated with these antibodies. In addition, the ELISA titers did not indicate binding to the specific attachment factors.

Animals and Management

In all experiments, ruminally cannulated, crossbred steers were housed in each of 2 concrete-surfaced pens (7.3 × 9.8 m) in a total confinement barn. Pens were bedded as needed with hardwood sawdust to minimize bedding consumption. Each pen was fitted with a Calan-Broadbent System (American Calan, Northwood, NH) that accommodated 12 individual bunks (0.61 m each). Steers had free-choice access to a common water trough (0.91 m).

Table 1. Composition and analyzed nutrient content of the diet consumed by steers fed avian-derived polyclonal antibody preparations

Ingredient, % of DM	
Corn grain	82.73
Corn silage	12.73
Supplement	4.54
Supplement composition, % as fed	
Calcium carbonate	33.7
Soybean meal	22.5
Urea	22.3
Dyna-K	10.5
Trace mineral salt	8.6
Mineral oil	1.0
Vitamin and mineral mix[1]	0.8
Rumensin-80[2]	0.38
Tylan-40[2]	0.22
Nutrient content, % of DM	
CP, %	12.5
NE_g, Mcal/kg	1.39
Ca, %	0.65
P, %	0.35

[1]Composition of vitamin and mineral mix: 0.4% Zn; 0.8% Mn; 0.1% Cu; 0.002% Se; 30,000 IU vitamin A; 3,000 IU vitamin D; and 91 IU vitamin E.
[2]Composition of supplement fed to provide monensin and tylosin. A similar formulation not containing monensin or tylosin was used in each experiment.

Diets

The diet consisted of 83% dry-rolled corn grain, 13% corn silage (average particle size of 8 mm), and 4% supplement on a DM basis (Table 1). Diets were fed once daily at 0900, and the soyhull pellets with PAP or control solution were top-dressed. Each PAP or control solution was sprayed onto 120 g of soyhull pellets/d using a 10-mL syringe and hand-mixed before feeding. Steers were permitted to consume feed ad libitum. A supplement was formulated with protein, vitamins, and minerals (Table 1), with or without MT. Steers were individually fed (through a Calan-Broadbent system); feed offerings and refusals were measured daily. Samples of feed ingredients, including pellets, were collected weekly, and those of feed refusals were collected daily. Ingredient and refusal samples were composited for each study period (from 14 to 29 d). Samples were then stored frozen (–20°C) until they were analyzed for DM, CP (methods 930.15, and 990.02, respectively; AOAC, 1997), NDF, and ADF. Concentrations of NDF (with heat-stable α-amylase and sodium sulfite) and ADF were determined using a fiber analyzer (Model 200, Ankom Technology, Fairport, NY).

Experimental Design

Experiment 1. Seventeen ruminally cannulated, crossbred steers (505 ± 85 kg of BW) were used in a completely randomized design with a 4 × 2 factorial arrangement of treatments. Treatments were titrated doses of a PAP against *S. bovis* (**PAP-Sb**), and included a control, consisting of 2.5 mL of a 50:50 mixture of a PBS solution and molasses; 2.5 mL of PAP-Sb (**1**×); 5 mL of PAP-Sb (**2**×); or 7.5 mL of PAP-Sb (**3**×). Each PAP-Sb treatment was fed with or without MT at 300 mg of monensin and 90 mg of tylosin/d.

Experiment 2. Seventeen ruminally cannulated crossbred steers (643 ± 110 kg of BW) were used in a completely randomized design with a 2 × 2 factorial arrangement of treatments. Factors were inclusion or not of 2.5 mL of PAP-Sb/d, with or without MT.

Experiment 3. Seventeen ruminally cannulated crossbred steers (675 ± 113 kg of BW) were used in a completely randomized design with a 2 × 2 factorial arrangements of treatments. Two steers were removed from the experiment (1 each from MT and PAP-Fn treatments, respectively) due to conditions unrelated to the experiment. Factors were inclusion or not of 2.5 mL/d of a PAP against *F. necrophorum* (**PAP-Fn**), and with or without MT.

Sample Collection

Experiment 1. Steers were gradually adapted to the diets over a 21-d period before initiation of treatments. After the adaptation period, steers were sampled (d 0) for ruminal bacterial populations (*S. bovis*, *F. necrophorum*, and total anaerobes) in the morning, before delivery of feed and PAP treatments. A second collection took place 14 d later before feed delivery.

Experiment 2. Steers were adapted to the diet and dietary treatments for 29 d before ruminal fluid collection. On d 29, samples for measurement of ruminal bacterial populations were taken at 0 (before delivery of feed and PAP treatments) and 5.5 h postfeeding.

Experiment 3. Steers were adapted to the diet for at least 29 d before initiation of treatments. Ruminal fluid for pH and bacterial enumeration (*S. bovis* and *F. necrophorum*) was collected on d 0 (first day for dosing PAP-Fn) and d 12 in the morning, before delivery of feed and PAP treatments.

Ruminal Bacterial Enumerations

Quantification of S. bovis. Ruminal fluid (150 mL) was collected by hand from 3 areas in the rumen and strained through 4 layers of cheesecloth. A subsample (approximately 48 mL) was transferred to a 50-mL vial, kept at 39°C, and immediately transported to the microbiology laboratory. Vials were introduced into an anaerobic glove box (Coy Laboratories Inc., Grass Lake, MI) containing an O_2-free atmosphere of CO_2 (98%) and H_2. Vials were vortexed inside the glove box, and a 20-μL aliquot was transferred to each of 3 wells in a 96-well microtitration plate containing minimal medium (Russell et al., 1981).

Serial 10-fold dilutions were performed in triplicate to determine the most probable number (**MPN**) of *S. bovis* per milliliter of ruminal fluid (Anderson et al., 1987; Dehority et al., 1989). Minimal medium permitted selective enrichment of *S. bovis* because this bacterium can utilize ammonium salts as the only source of nitrogen (Wolin et al., 1959). Previous research in our laboratory indicated that growth of *S. bovis* in minimal medium did not differ from that in a nonselective medium, indicating that the use of a minimal medium did not inhibit the growth of *S. bovis*, and thus was suitable for use as an enrichment step.

The composition of the minimal medium was (per L): 40 mL of mineral solution 1 (0.6% K_2HPO_4), 40 mL of mineral solution 2 [0.6% KH_2PO_4, 0.6% $(NH_4)_2SO_4$, 1.2% NaCl, 0.25% $MgSO_4 \cdot 7H_2O$, 0.16% $CaCl_2 \cdot 2H_2O$], 10 mL of vitamin B solution, 5 mL of metal solution, 4.0 g of Na_2CO_3, 2 g of glucose, 0.5 g of cysteine hydrochloride, 1 mg of resazurin, and 1 mg of hemin. The vitamin B stock solution contained (per 100 mL): 20 mg each of thiamin hydrochloride, calcium D-pantothenate, nicotinamide, riboflavin, and pyridoxine hydrochloride; 10 mg of biotin; 1 mg of *p*-aminobenzoic acid; 0.5 mg of folic acid; and 0.2 mg of cyanocobalamin. The metal solution contained (per 100 mL): 50 mg of Na_4EDTA, 20 mg of $FeSO_4 \cdot 7H_2O$, 20 mg of $MnCl \cdot 4H_2O$, 2 mg of H_3BO_3, 2 mg of $CoCl_2 \cdot 6H_2O$, 1 mg of $ZnSO_4 \cdot 7H_2O$, 0.3 mg of $NaMoO_4 \cdot 2H_2O$, 0.2 mg of $NiCl_2$, and 0.1 mg of $CuCl_2 \cdot 2H_2O$. The pH of the medium was adjusted to 6.5 by adding NaOH, bottled anaerobically under oxygen-free CO_2, sealed, autoclaved, and stored at 4°C until used.

The 96-well plates were incubated inside the glove box at 39°C for 48 h. The contents of wells that demonstrated bacterial growth (visually assessed by the extent of turbidity in the well) were spread-plated (0.1 mL) onto an Enterococcosel agar medium (Becton, Dickinson, and Co., Sparks, MD) for confirmation of the presence of *S. bovis* and incubated anaerobically for 24 h at 39°C. Enterococcosel agar is a selective and differential medium for group D streptococci (such as *S. bovis*). The selectiveness is given by the presence of sodium azide and bile salts, and differentiation is achieved by the esculin hydrolysis, which produces darkening of the medium (Facklam and Moody, 1970; Anderson et al., 1987). A final (conclusive) test for confirmation of *S. bovis* was performed using API 20 Strep strips (bioMerieux, Inc., Hazelwood, MO).

Quantification of F. necrophorum. Ruminal fluid collection procedures for enumeration of *F. necrophorum* were the same as described for *S. bovis*. After ruminal fluid samples were placed inside the anaerobic glove box, a 20-μL aliquot was added to each of 3 wells of a 96-well microtitration plate containing 180 μL of modified lactate medium (Tan et al., 1994). Selectiveness of modified lactate medium is based on the presence of lactate as the only carbon source and the pres-

Table 2. Effects of feeding various doses of an avian-derived polyclonal antibody preparation against *Streptococcus bovis* (PAP-Sb) to steers consuming high-grain diets on bacterial counts (most probable number/mL of ruminal fluid) in ruminal fluid collected before (d 0) and after (d 14) PAP-Sb feeding (Exp. 1)

	Dose of PAP-Sb[1]								
Item	0		1×		2×		3×		SEM
No. of steers	4		4		4		5		
S. bovis, millions[2]	599.6		116.4		446.7		147.9		15.9
Day × dose[3]	d 0	d 14	d 0	d 14	d 0	d 14	d 0	d 14	
S. bovis, millions	363.1^{bc}	977.2^{c}	285.1^{bc}	47.6^{ab}	409.7^{c}	486.9^{c}	751.6^{c}	29.2^{a}	21.8
Fusobacterium necrophorum, thousands	94.4	69.6	84.6	99.4	97.7	17.1	48.9	14.0	27.5
Total anaerobes, billions	7.54	15.05	13.96	18.51	14.54	24.41	5.48	41.05	2.37

$^{a–c}$Within a row, means without a common superscript letter differ, $P < 0.05$.
[1]1× = 2.5 mL of PAP-Sb; 2× = 5 mL of PAP-Sb; 3× = 7.5 mL of PAP-Sb.
[2]Least squares means for PAP-Sb effect (cubic contrast, $P = 0.014$).
[3]PAP-Sb dose × day interaction ($P = 0.04$).

ence of 3 antibiotics (bacitracin, gentamycin sulfate, and streptomycin sulfate). Wells that demonstrated bacterial growth were tested for the presumptive presence of *F. necrophorum* by adding a drop of Kovac's reagent. If indole production was observed, well contents were streaked on brain heart infusion agar plates and incubated anaerobically at 39°C for 24 h. Counts of *F. necrophorum* per milliliter of ruminal fluid were determined by the MPN technique based on triplicate 10-fold dilutions. Isolates were confirmed as *F. necrophorum* using API 20A strips (bioMerieux, Inc.).

Quantification of Total Anaerobic Bacteria. Procedures for collection of ruminal fluid for quantification of total anaerobic bacteria were the same as described above. A nonselective medium that included a variety of carbon sources was used (Bryant and Robinson, 1961). The composition of the medium was (per L): 2.5 g of glucose, 2.5 g of cellobiose, 0.5 g of soluble starch, 0.5 g of yeast extract, 10 mL of vitamin B solution, 1 mg of resazurin, 37.5 mL of mineral solution 1, 37.5 mL of mineral solution 2, 2 g of Na_2CO_3, 0.5 g of cysteine HCl, and 200 mL of centrifuged ruminal fluid (25,000 × *g*, 15 min). Compositions of mineral and vitamin B solutions were the same as described for *S. bovis* quantification. The pH of the medium was adjusted to 6.5 before autoclaving by adding NaOH. The medium was bottled anaerobically under oxygen-free CO_2, sealed, autoclaved, and stored at 4°C until used. Bacterial growth in each well was assessed visually by the extent of turbidity. Serial 10-fold dilutions were performed in triplicate to determine the MPN per milliliter of ruminal fluid.

Laboratory Methods

Ruminal pH was measured immediately using a model 345 Corning pH meter equipped with an immersible probe (Corning, Inc., Corning, NY) after ruminal contents were strained (approximately 100 mL) through 4 layers of cheesecloth. Ruminal pH measurements were conducted at 20°C.

Statistical Analyses

For the analysis of ruminal bacterial populations, data were converted before analysis using $\log_{10}$ transformation, and arithmetic means are presented. Data on populations of ruminal bacteria or pH were analyzed as a repeated measures, completely randomized design (Steel and Torrie, 1980) using the Mixed procedure of SAS (SAS Inst., Inc., Cary, NC) with a factorial arrangement of treatments (PAP or MT). Means were separated using least squares procedures when terms for main effects or interactions were significant ($P < 0.05$). Means in Exp. 1 were also separated using orthogonal polynomial contrasts to determine linear, quadratic, or cubic responses.

RESULTS

Experiment 1. Polynomial orthogonal contrasts were used to evaluate dose response (Table 2). Feeding increasing concentrations of PAP-Sb with or without MT reduced the ruminal counts of *S. bovis* in a cubic ($P = 0.014$) manner. This was due to the fact that feeding 2× had no effect ($P = 0.64$) on *S. bovis* counts relative to those in steers fed no PAP. However, feeding 1× or 3× doses for 14 d resulted in 80 and 75% lower ($P < 0.05$) ruminal counts of *S. bovis*, respectively, relative to those in steers fed no PAP. There was no ($P = 0.69$) MT × PAP-Sb interaction, but a day × dose interaction ($P = 0.04$) was observed as result of variable reductions in *S. bovis* counts 14 d after feeding PAP-Sb doses. Ruminal *S. bovis* counts before delivery of PAP-Sb (d 0) were similar ($P > 0.05$) for all doses. After 14 d of PAP-Sb feeding, ruminal counts of *S. bovis* were similar ($P = 0.79$) for 1× and 3× doses, and lower ($P < 0.05$) than those in the rumen of steers fed no PAP-Sb. When comparing ruminal *S. bovis* counts at d 0 and 14 within a given dose, a reduction ($P = 0.02$) in ruminal *S. bovis* counts was achieved only with the 3× dose. No effects ($P > 0.05$) of feeding MT with or without PAP-Sb were observed on ruminal *S. bovis* counts (Table 3).

Table 3. Effects of feeding monensin (300 mg/d) and tylosin (90 mg/d; MT) in combination with an avian-derived polyclonal antibody preparation against *Streptococcus bovis* (PAP-Sb) on ruminal bacterial counts (most probable number/mL of ruminal fluid) in steers fed a high-grain diet (Exp. 1)

	Additive[1]		
Item	None	MT	SEM
No. of steers	8	9	
S. bovis, millions	282.6	252.4	13.9
Fusobacterium necrophorum, thousands	130.7[b]	21.6[a]	1.6
Total anaerobes, billions	12.27	17.94	1.49

[a,b]Within a row, means without a common superscript letter differ, $P < 0.01$.
[1]No PAP-Sb × MT interactions were observed ($P > 0.05$).

Feeding increasing doses of PAP-Sb had no effect ($P > 0.05$) on ruminal *F. necrophorum* or total anaerobic bacterial counts (Table 2). Feeding MT with or without PAP-Sb reduced ($P < 0.01$) counts of *F. necrophorum* in ruminal fluid (Table 3), but had no effect ($P > 0.05$) on ruminal counts of total anaerobes.

Experiment 2. There were no interactions ($P > 0.05$) between MT or PAP-Sb and sampling time on populations of *S. bovis* or pH. Results demonstrated no MT × PAP-Sb interaction ($P = 0.149$) for ruminal *S. bovis* counts or pH ($P = 0.087$; Table 4). Feeding MT had no effect ($P > 0.05$) on ruminal *S. bovis* counts or pH. Feeding PAP-Sb reduced ($P = 0.045$) ruminal *S. bovis* counts and increased ($P = 0.014$) ruminal pH (Table 4).

Experiment 3. Because a PAP-Fn × MT interaction ($P < 0.001$) existed, results are expressed as interaction means (Table 5). Counts of *F. necrophorum* were reduced ($P < 0.01$) in steers fed MT, PAP-Fn, or both compared with steers fed no PAP or MT. When MT and PAP-Fn were fed together, ruminal *F. necrophorum* counts were greater ($P = 0.008$) than those observed when feeding MT alone. Reductions ($P < 0.01$) of 96 or 82% in ruminal counts of *F. necrophorum* were achieved when MT or PAP-Fn were fed, respectively. No effects ($P > 0.05$) on ruminal *S. bovis* counts or ruminal pH were observed when PAP-Fn or MT was fed.

Table 4. Effects of feeding an avian-derived polyclonal antibody preparation (PAP) against *Streptococcus bovis* (PAP-Sb) on ruminal *S. bovis* counts and pH in steers fed a high-grain diet (Exp. 2)[1]

	PAP treatment		
Item	None	PAP-Sb	SEM
No. of steers	8	9	
S. bovis, millions of MPN/mL of ruminal fluid[2]	243.3[b]	79.2[a]	14.3
Ruminal pH	5.67[a]	6.08[b]	0.10

[a,b]Within a row, means without a common superscript letter differ, $P < 0.05$.
[1]No PAP-Sb × MT interactions were observed ($P > 0.05$).
[2]MPN = most probable number.

DISCUSSION

Effects of PAP on Ruminal S. bovis. Ruminal counts of *S. bovis* observed in our experiments were similar to those reported by previous studies with feedlot cattle fed high-grain diets (Coe et al., 1999; Shu et al. 1999; Dehority, 2003). Our results indicated (Exp. 1 and 2) that PAP-Sb was effective in reducing counts of target bacteria in the rumen when fed at the 1× or 3× dose. The cubic response by *S. bovis* counts to PAP-Sb was due to the fact that the 2× dose (5 mL of PAP-Sb/d) had no effect on ruminal *S. bovis* counts. It is not clear why the 2× dose did not affect ruminal *S. bovis* counts, but it could be due to a phenomenon of antibody-to-antibody agglutination at greater antibody titers. This theory, however, would not explain why feeding the 3× dose did reduce ruminal *S. bovis* counts. It is possible that feeding the 3× dose yielded sufficiently greater antibody binding sites to overcome antibody-to-antibody agglutination. We have also observed this effect with the 2× dose in a separate experiment (DiLorenzo et al., 2005).

The extent of *S. bovis* count reduction was 80 or 75% for the 1× or 3× dose, respectively, and was comparable to a 79% reduction in ruminal *S. bovis* counts obtained by feeding 250 mg of virginiamycin/d to steers consuming a high-grain diet (Coe et al., 1999). Reductions in ruminal *S. bovis* counts of 99% were reported for steers vaccinated (i.m.) against *S. bovis* 16 h after an abrupt change from a forage diet to a high-grain diet (Shu et al., 1999). Differences observed in the magnitude of response by ruminal *S. bovis* counts between the study conducted by Shu et al. (1999) and our study are probably due to substantial differences in methodology approaches. Feeding PAP-Sb is considered a passive immunization method, because antibodies against *S. bovis* are produced by immunized hens and then fed to steers. The immunization method followed by Shu et al. (1999) led to active immunization, because steers were exposed to the antigen and adjuvant to develop their own immune response (Ghaffar, 2004).

When 2.5 mL of PAP-Sb/d (1× dose) was fed (Exp. 2) for 29 d, a 67% reduction in ruminal *S. bovis* counts was observed when compared with steers fed no PAP-Sb. This reduction was lower than that achieved in Exp. 1 (80%) with the same dose. Lower ruminal *S. bovis* counts in Exp. 2 as well as lack of differences in ruminal *S. bovis* between 0 and 5.5 h postfeeding in Exp. 2 could be because counts of *S. bovis* in the rumens of animals adapted to high-grain diets do not tend to deviate much from 10^7/mL of ruminal fluid (Dehority, 2003). Because Exp. 1 was conducted for 14 d after adaptation to eating high-grain diets and Exp. 2 was conducted for 29 d after adaptation, a longer period of exposure of steers in Exp. 2 to the high-grain diet may have led to differences in ruminal *S. bovis* counts and their magnitude of re-

Table 5. Effects of feeding an avian-derived polyclonal antibody preparation against *Fusobacterium necrophorum* (PAP-Fn) and feeding monensin (300 mg/d) and tylosin (90 mg/d; MT) for 12 d on ruminal *F. necrophorum* and *Streptococcus bovis* counts and pH in steers fed a high-grain diet (Exp. 3)

	Treatment[1]				
Item	None	MT	PAP-Fn	MT + PAP-Fn	SEM
No. of steers	4	3	3	5	
F. necrophorum, thousands of MPN/mL of ruminal fluid[2]	113.74[c]	4.30[a]	20.78[b]	15.45[b]	0.12
S. bovis, millions of MPN/mL of ruminal fluid	97.2	79.8	117.3	135.4	3.3
Ruminal pH	6.18	6.13	6.35	6.24	0.26

[a–c]Within a row, means without a common superscript letter differ, $P < 0.01$.
[1]PAP-Fn × MT interaction was observed ($P < 0.001$).
[2]MPN = most probable number.

sponse to feeding PAP-Sb between the 2 experiments. Other studies revealed no differences in counts of starch-utilizing bacteria in the rumen of steers fed high-grain diets between 0 and 12 h postfeeding after a 12-d adaptation period (Leedle et al., 1982).

When considerable quantities of starch enter the rumen after feeding, the numbers of starch-utilizing bacteria increase as starch is rapidly hydrolyzed, peaking at 16 h postfeeding (Leedle et al., 1982). We chose to sample ruminal fluid 5.5 h postfeeding to correspond with lowest pH observed during 24-h pH measurements for steers fed high-grain diets (Cooper et al., 2002). When immunizing against *S. bovis* using an intramuscular vaccine, Shu et al. (1999) observed reduced counts of ruminal *S. bovis* 16 h after challenging grazing steers with a high-grain diet; however, 24 h after the challenge, no differences were found in ruminal *S. bovis* counts between immunized and nonimmunized steers. In Exp. 2, ruminal *S. bovis* counts were not affected by time postfeeding, agreeing with the observations of Leedle et al. (1982). Further research needs to be conducted to elucidate diurnal fluctuations of *S. bovis* in response to feeding PAP-Sb or MT, or under dietary changes.

In contrast to previous observations by several authors (Dennis et al., 1981; Nagaraja et al., 1981; Coe et al., 1999), feeding a supplement formulated to deliver 300 mg of monensin/d and 90 mg of tylosin/d did not affect the population of ruminal *S. bovis* in Exp. 1. A reduction of 60% in ruminal *S. bovis* concentrations was reported when 250 mg of monensin/d plus 90 mg of tylosin/d were fed to steers on a high-grain diet (Coe et al., 1999). We also observed a 67% reduction in *S. bovis* counts due to PAP-Sb feeding compared with steers fed no PAP-Sb; however, no effects on *S. bovis* were observed due to MT feeding during Exp. 2. Although some of these differences may be attributable to the size and breed of experimental animals (Coe et al., 1999 used 288- to 318-kg Holstein steers), differences may be due to response to monensin by *S. bovis* strains. Dennis et al. (1981) conducted an extensive study to determine the minimum inhibitory concentrations of monensin needed to impair the growth of most lactate-producing bacteria of the rumen. Interestingly, 2 of the 3 strains of *S. bovis* tested were sensitive to monensin, indicating some of the lowest minimum inhibitory concentrations of all species and strains tested. However, the third strain tested (*S. bovis* 124) was resistant to monensin at the concentrations tested (48 μg/mL of medium). This resistant strain of *S. bovis* is considered one of the exemptions to the general belief that monensin inhibits gram-positive bacteria (Westley, 1977; Nagaraja et al., 1981). Dawson and Boling (1983) indicated that monensin-resistant bacteria might be found in greater numbers in rumens of animals fed monensin-supplemented diets than those not fed monensin. However, this phenomenon was not necessarily associated with altered fermentation patterns. It is possible that differences in *S. bovis* response to MT feeding in our experiments was caused by differential response of *S. bovis* strains to continuous monensin feeding.

No effect on ruminal *S. bovis* counts was observed when PAP-Fn was fed indicating a specificity of this PAP to *S. bovis*. The specificity of avian antibodies was discussed by other authors (Shimizu et al., 1988; Ikemori et al., 1992) and their conclusions support our observations indicating that avian antibodies are highly specific.

Effects on Ruminal F. necrophorum. Ruminal counts of *F. necrophorum* were not affected by feeding PAP-Sb, confirming the specificity of avian antibodies to *F. necrophorum*. However, an antagonistic effect was apparent between MT and PAP-Fn. When MT was fed without PAP-Fn, counts of ruminal *F. necrophorum* were lowest. Counts of ruminal *F. necrophorum* did not differ between steers fed PAP-Fn alone or in combination with MT, but were lower than those in steers fed no PAP-Fn. The antagonistic effect of PAP-Fn when fed in combination with monensin and tylosin could be due to an alteration of ruminal microbial population equilibrium as yet undefined. Further studies regarding changes in ruminal bacterial population under the diets and treatments tested should be conducted to better understand this interaction.

Feeding MT in Exp. 1 reduced ruminal *F. necrophorum* counts by 83%. In Exp. 3, a reduction of 96% in ruminal *F. necrophorum* counts relative to control was

observed for MT, compared with the 82% reduction relative to control with PAP-Fn. Similar reductions (98%) in ruminal *F. necrophorum* counts were found when 250 mg of monensin/d plus 90 mg of tylosin/d were fed to steers on a high-grain diet (Coe et al., 1999). Feeding 90 mg of tylosin/d caused a reduction of 89% in ruminal *F. necrophorum* counts in steers fed a high-grain diet for 17 d (Nagaraja et al., 1999). Differences in the magnitudes of reduction in our studies between MT and PAP-Fn could be attributed to differences in the mode of action. Tylosin is a macrolide antibiotic and acts by directly interfering with bacterial protein synthesis, whereas antibodies are thought to bind with the bacterium, impairing reproduction.

Fusobacterium necrophorum is inhibited at a pH below 5 (Coe et al., 1999). The fact that the lowest pH found in our experiments was 5.43 (control steers, Exp. 2) indicates that inhibition of *F. necrophorum* due to low ruminal pH was not likely to have occurred in any of the experiments.

Effects on Ruminal pH. In spite of a reduction in ruminal counts of *F. necrophorum* in response to feeding PAP-Fn, no effects on ruminal pH were observed indicating no major impact of this bacterium on ruminal pH despite its role in ruminal lactate use. Similar findings were reported by Nagaraja et al. (1999).

Feeding monensin and tylosin did not affect ruminal pH in Exp. 3. These results agree with previous studies reporting no effects on ruminal pH when monensin or tylosin or both were fed in high-grain diets (Zinn, 1988; Coe et al., 1999; Nagaraja et al., 1999). The lack of effect of tylosin on ruminal lactate or total VFA concentration in steers fed high-grain diets might have resulted in the lack of effect on ruminal pH (Nagaraja et al., 1999). Feeding PAP-Sb increased ruminal pH in Exp. 2; likely due to a reduction in ruminal *S. bovis* counts (Owens et al., 1998).

Effects on Total Anaerobic Bacteria. Total anaerobic bacteria were not affected by PAP-Sb or MT in Exp. 1. Dawson and Boling (1983) evaluated the response by calves fed diets containing monensin and observed similar total anaerobic bacterial counts to calves not fed monensin. The complexity of the ruminal microbial ecosystem is such that, in general, when groups or species of microorganisms are decreased in numbers, other groups or species develop because of substrate availability; a relatively constant number of total bacteria for a given diet is thus maintained. Variations in ruminal bacterial counts are expected, however, with different diets, levels of intake, and postprandial sampling times (Leedle et al., 1982; Dehority and Orpin, 1988). Products that affect total anaerobic bacterial counts are not commonly used in production agriculture. Day-to-day stability in total anaerobic bacteria numbers was documented previously (Leedle et al., 1982). Maintaining total anaerobic bacteria at similar numbers as those found in control steers is a reflection of the innocuous effect of feeding MT or PAP-Sb on total anaerobic bacterial counts.

IMPLICATIONS

Feeding a polyclonal antibody preparation against *Streptococcus bovis* or *Fusobacterium necrophorum* reduced ruminal counts of the target bacteria in steers fed high-grain diets. The polyclonal antibody preparation did not seem to cross-react within the 2 bacterial species tested, indicating some specificity of avian antibodies. Because *S. bovis* and *F. necrophorum* play a key role in the development of ruminal acidosis and abscessed livers, respectively, reducing the ruminal counts of these bacteria could be effective in preventing these deleterious conditions and enhancing animal performance.

LITERATURE CITED

Anderson, K. L., T. G. Nagaraja, J. L. Morrill, T. B. Avery, S. J. Galitzer, and J. E. Boyer. 1987. Ruminal microbial development in conventionally or early-weaned calves. J. Anim. Sci. 64:1215–1226.

AOAC. 1997. Official Methods of Analysis. 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Brashears, M. M., M. L. Galyean, G. H. Loneragan, J. E. Mann, and K. Killinger-Mann. 2003. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given *Lactobacillus* direct-fed microbials. J. Food Prot. 66:748–754.

Bryant, M. P., and I. M. Robinson. 1961. An improved nonselective culture medium for ruminal bacteria and its use in determining diurnal variation in numbers of bacteria in the rumen. J. Dairy Sci. 44:1446–1456.

Coe, M. L., T. G. Nagaraja, Y. D. Sun, N. Wallace, E. G. Towne, K. E. Kemp, and J. P. Hutcheson. 1999. Effect of virginiamycin on ruminal fermentation in cattle during adaptation to a high concentrate diet and during an induced acidosis. J. Anim. Sci. 77:2259–2268.

Cooper, R. J., C. T. Milton, T. J. Klopfenstein, T. L. Scott, C. B. Wilson, and R. A. Mass. 2002. Effect of corn processing on starch digestion and bacterial crude protein flow in finishing cattle. J. Anim. Sci. 80:797–804.

Dawson, K. A., and J. A. Boling. 1983. Monensin-resistant bacteria in the rumens of calves on monensin-containing and unmedicated diets. Appl. Environ. Microbiol. 46:160–164.

Dehority, B. A. 2003. Rumen Microbiology. 1st ed. Nottingham Univ. Press, Nottingham, UK.

Dehority, B. A., and C. G. Orpin. 1988. Development of, and natural fluctuations in, rumen microbial populations. Pages 151–183 in The Rumen Microbial Ecosystem. P. N. Hobson, ed. Elsevier, Barking, UK.

Dehority, B. A., P. A. Tirabasso, and A. P. Grifo Jr. 1989. Most-Probable-Number procedures for enumerating ruminal bacteria, including the simultaneous estimation of total and cellulolytic numbers in one medium. Appl. Environ. Microbiol. 55:2789–2792.

Dennis, S. M., T. G. Nagaraja, and E. E. Bartley. 1981. Effects of lasalocid or monensin on lactate-producing or -using rumen bacteria. J. Anim. Sci. 52:418–426.

DiLorenzo, N., R. K. Gill, F. Diez-Gonzalez, J. E. Larson, and A. DiCostanzo. 2005. Response by steers fed high-forage diets to oral doses of a polyclonal antibody preparation against *Streptococcus bovis* on target bacteria populations and rumen fermentation products. J. Anim. Sci. 83(Suppl. 1):51. (Abstr.)

Elam, N. A., J. F. Gleghorn, J. D. Rivera, M. L. Galyean, P. J. Defoor, M. M. Brashears, and S. M. Younts-Dahl. 2003. Effects of live cultures of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Propionibacterium freudenreichii* on performance, carcass, and intestinal characteristics, and *Escherichia coli* O157 shedding of finishing beef steers. J. Anim. Sci. 81:2686–2698.

Facklam, R. R., and M. D. Moody. 1970. Presumptive identification of group D Streptococci: The bile-esculin test. Appl. Microbiol. 20:245–250.

Ghaffar, A. 2004. Immunology – Chapter Fourteen: Immunization. Available: http://www.med.sc.edu:85/ghaffar/immunization.htm Accessed Sept. 17, 2004.

Ghorbani, G. R., D. P. Morgavi, K. A. Beauchemin, and J. A. Z. Leedle. 2002. Effects of bacterial direct-fed microbials on ruminal fermentation, blood variables, and the microbial populations of feedlot cattle. J. Anim. Sci. 80:1977–1986.

Ikemori, Y., M. Kuroki, R. C. Peralta, H. Yokoyama, and Y. Kodama. 1992. Protection of neonatal calves against fatal enteric colibacillosis by administration of egg yolk powder from hens immunized with K99-piliated enterotoxigenic *Escherichia coli*. Am. J. Vet. Res. 53:2005–2008.

Ikemori, Y., M. O. K. Umeda, F. C. Icatlo, Jr., M. Kuroki, H. Yokoyama, and Y. Kodama. 1997. Passive protection of neonatal calves against coronavirus-induced diarrhea by administration of egg yolk or colostrum antibody powder. Vet. Microbiol. 58:105–111.

Leedle, J. A. Z., M. P. Bryant, and R. B. Hespell. 1982. Diurnal variations in bacterial numbers and fluid parameters in ruminal contents of animals fed low- or high-forage diets. Appl. Environ. Microbiol. 44:402–412.

Nagaraja, T. G., T. B. Avery, E. E. Bartley, S. J. Galitzer, and A. D. Dayton. 1981. Prevention of lactic acid acidosis in cattle by lasalocid or monensin. J. Anim. Sci. 53:206–216.

Nagaraja, T. G., Y. D. Sun, N. Wallace, K. E. Kemp, and C. J. Parrot. 1999. Effects of tylosin on concentrations of *Fusobacterium necrophorum* and fermentation products in the rumen of cattle fed a high-concentrate diet. Am. J. Vet. Res. 60:1061–1065.

Newbold, C. J., C. S. Stewart, and R. J. Wallace. 2001. Developments in rumen fermentation—The scientist's view. Pages 251–279 in Recent Advances in Animal Nutrition. P. C. Garnsworthy, and J. Wiseman, ed. Nottingham Univ. Press, Nottingham, UK.

Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1998. Acidosis in cattle: A review. J. Anim. Sci. 76:275–286.

Russell, J. B., W. G. Botje, and M. A. Cotta. 1981. Degradation of protein by mixed cultures of rumen bacteria: Identification of *Streptococcus bovis* as an actively proteolytic rumen bacterium. J. Anim. Sci. 53:242–252.

Salyers, A. A. 2002. An overview of the genetic basis of antibiotic resistance in bacteria and its implications for agriculture. Anim. Biotechnol. 13:1–5.

Shimizu, M., R. C. Fitzsimmons, and S. Nakai. 1988. Anti-*E. coli* immunoglobulin Y isolated from egg yolk of immunized chickens as a potential food ingredient. J. Food Sci. 53:1360–1366.

Shu, Q., H. S. Gill, D. W. Hennessy, R. A. Leng, S. H. Bird, and J. B. Rowe. 1999. Immunization against lactic acidosis in cattle. Res. Vet. Sci. 67:65–71.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., NY.

Tan, Z. L., T. G. Nagaraja, and M. M. Chengappa. 1994. Selective enumeration of *Fusobacterium necrophorum* from the bovine rumen. Appl. Environ. Microbiol. 60:1387–1389.

Westley, J. W. 1977. Polyether antibiotics: Versatile carboxylic acid ionophores produced by *Streptomyces*. Adv. Appl. Microbiol. 22:177–223.

Wolin, M. J., G. B. Manning, and W. O. Nelson. 1959. Ammonium salts as sole source of nitrogen for the growth of *Streptococcus bovis*. J. Bacteriol. 78:147.

Zinn, R. A. 1988. Comparative feeding value of supplemental fat in finishing diets for feedlot steers supplemented with and without monensin. J. Anim. Sci. 66:213–227.