MODIFICATION OF RUMEN BACTERIAL POPULATIONS FOR ENHANCED PERFORMANCE AND HEALTH

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INTRODUCTION

The significance of the symbiotic relationship between the ruminant micro-flora and host animal is not well appreciated by the casual observer. However, when one considers that, in the U.S. alone over 41,850,800 beef and dairy cows comprise the national herd, and provide over 26.3 billion pounds of beef and 170.3 billion pounds of milk (USDA, 2004ab), the economic importance of maintaining this relationship healthy is staggering. Because of demands on these ruminants to maintain production levels within economically efficient limits, use of grains in diets of U.S. ruminants is almost a necessity. However, ruminants evolved consuming diets comprised of forages. Utilization of grain in ruminant diets leads to modifications of the rumen micro-flora which cause alterations in rumen fermentation patterns, rumen and animal health (Russell and Rychlik, 2001). The development and practical application of microbial manipulation techniques to maintain efficient production levels and a healthy rumen environment has been a priority for the ruminant nutritionist. New technologies to select for or against specific microbe strains in the rumen will be characterized for their ability to permit rumen microbe manipulation consistent with future constraints on anti-microbial use, and environmental, and health implications.

MODIFICATION OF BACTERIA POPULATIONS

Alternative solutions to manipulation of rumen microbes to maintain production efficiency in feedlots and dairies include use of direct-fed microbials (**DFM**), feeding of rumen co-factors found in yeast or other products including byproduct feeds, and immunizing against certain rumen microbes. Feeding DFM is perhaps the most studied of these options.

A review published recently (Krehbiel et al., 2003) summarized studies conducted using DFM in diets of ruminant animals. The reader is referred to this review for in-depth discussion on the subject. Direct-fed microbials have been used to enhance average daily gain (**ADG**) and feed efficiency in feedlot cattle, milk production in dairy cows, and health in young ruminants; however, results have been mixed, and the mechanisms by which DFM function are unclear (Krehbiel et al., 2003). Some of the responses and proposed mechanisms of action include: detoxification of certain anti-quality plant compounds (Gregg et al., 1998), lactic acid consumption by rumen bacteria (Ghorbani et al., 2002), competitive exclusion and/or production of lactic acid (Elam et al., 2003; Huck et al., 2000), and stimulation of immune function (Krehbiel et al., 2003).

Immunization against certain rumen microbes is perhaps the most novel and least attempted method of rumen manipulation. The basis for this mechanism involves recognition of macro-molecules of non-self origin (antigens), activation of leukocytes, cytokine secretion, and engagement of mechanisms directed at removal of the pathogen. When the macrophages in the host's bloodstream encounter foreign molecules (antigens), they respond by engulfing them. This event is mediated by helper T-cells, which set a chain of reactions that leads to stimulation of B-cells. The main function of B-cells is to produce proteins called antibodies, which bind to the foreign molecule. Formation of the antibody—antigen complex signals for

the destruction of the invader via phagocytosis or activation of the complement system (Goldsby et al., 2000).

In response to a grain load challenge, sheep immunized against *Streptococcus bovis* maintained greater ($P < 0.05$) intake, and had higher ($P < 0.05$) rumen pH than sheep not immunized against *S. bovis* (Gill et al., 2000). Concentrations of L-lactate were lower ($P < 0.05$) in rumen fluid of sheep and cattle immunized against *S. bovis* (Shu et al., 1999, 2000a), and counts of *Lactobacillus spp.* and *S. bovis* were lower ($P < 0.05$) in rumen fluid contents of cattle immunized against *S. bovis* and *Lactobacillus spp.* (Shu et al., 1999). Immunized cattle had significantly greater ($P < 0.05$) dry matter intake (**DMI**; Shu et al., 1999). These observations were accompanied by the finding that antibody titers against *S. bovis* or *S. bovis* and *Lactobacillus spp.* were elevated ($P < 0.05$) in saliva, rumen fluid and blood (Shu et al., 1999, 2000a; Gill et al., 2000). A subsequent study designed to evaluate various adjuvants to enhance the efficacy of vaccines against *S. bovis* and *Lactobacillus spp.* demonstrated that neither rumen pH nor rumen lactic acid concentration was affected ($P > 0.05$) by immunization, suggesting that lactic acid did not accumulate in the rumen (Shu et al., 2000b). In this study, weight gain was not affected ($P > 0.05$) by immunization. The authors concluded that lack of lactic acid accumulation in the rumen was responsible for lack of effects on ADG.

In light of these and other results obtained with immunization, it is clear that this technology has great potential in animal agriculture, especially when considering the future trends in animal production, and the regulatory policies that might be applied as a result of public concern issues regarding the excessive use of antimicrobials in animal agriculture.

Polyclonal Antibody Preparations

Preparations of polyclonal antibodies (**PAP**) specific to various rumen bacteria are currently available. The process begins with immunization of hens (proprietary; CAMAS, Inc., Le Center, MN) against specific target bacteria (rumen dwellers, respiratory or digestive pathogens). Antibodies are extracted from eggs collected, which contain elevated titers of immunoglobulins Y (**Ig** Y), M (Ig M), and A (Ig A). Antibody preparations are further preserved with buffers and suspended in molasses. Avian antibodies are known for their ability to remain active in spite of severe environmental challenges. Activity of Ig Y was retained after being exposed to pasteurization, pH above 4, or pepsin, trypsin or chymotrypsin digestion (Shimizu et al., 1988). These characteristics make avian antibodies suitable for dosing orally into ruminant animals.

Doses of these liquid preparations (1 to 5 ml/hd/d) can be delivered via feed to the ruminant animal causing passive immunity against target bacteria. Reducing the concentration of *S. bovis* in the rumen should decrease fermentation of starch to lactic acid. Lactic acid accumulation in the rumen leads to rumen acidosis, rumen epithelium ulceration (Owens et al., 1998), infection of rumen ulcers by *Fusobacterium necrophorum*, and invasion of liver tissue by this bacterium (Nagaraja and Chengappa, 1998). Liver abscesses, which result in reduced animal performance, are the result of colonization of the liver by *F. necrophorum* (Nagaraja and Chengappa, 1998). Although by comparison, feedlot cattle are subject to greater grain loads than dairy cattle, dairy cattle are exposed to grain loads for longer periods of time, and are also susceptible to chronic acidosis, rumen ulceration and liver abscesses (Clayton et al., 1999).

MODIFICATION OF BACTERIA POPULATIONS WITH POLYCLONAL ANTIBODIES

A series of experiments was conducted to study effects of PAP against *S. bovis* (**PAPSb**) or *F. necrophorum* (**PAPFn**) on rumen populations of target bacteria. Unless otherwise specified, the common protocol for each of the experiments involved rumen fluid collection from rumen-cannulated crossbred steers prior to individually feeding (through a Calan-Broadbent System) an 85% corn grain, 15% corn silage diet containing 12.5% CP, .6% Ca, .35% P, and calculated to contain 1.38 Mcal NE_g/kg dry matter (**DM**). Rumensin (300 mg/hd/d) and Tylan (100 mg/hd/d) were included in all experiments except for when the objective was to evaluate these feed additives (**FA**). Feed additives were delivered in a supplement fed daily (454 g/hd/d), which contained FA, protein, vitamins and minerals. Steers had been adapted to consume this diet for at least 14 d prior to initiation of rumen fluid collection, and were permitted to consume feed ad libitum. A second rumen fluid collection generally took place 12 to 29 d after initiation of feeding PAP. Preparations were top-dressed either spray-dried onto soy hull pellets, or were sprayed onto soy hull pellets immediately prior to feeding.

Rumen fluid (200 ml) was collected by hand from at least three different areas of the rumen, and strained through four layers of cheesecloth. A sub-sample (48 ml) was transferred to 50-ml vials, maintained in warm water (39 °C), and immediately transported to the laboratory for enumeration of bacterial populations. Another sub-sample (100 ml) was drawn to measure pH immediately after collection.

Rumen fluid samples were transferred to an anaerobic chamber. Twenty micro liters were drawn into 96-well micro-titration plates containing 180 µl of minimal media, diluted several times (10-fold dilutions), and permitted to incubate for 48 h. Minimal media contained glucose and ammonium salts as the only carbohydrate and nitrogen source, respectively. Wells with the highest dilution were plated onto a selective Enterococcosel agar media and further incubated for another 24 h to confirm the presence of *S. bovis* in each well. Most probable number of *S. bovis* per ml of rumen fluid was confirmed with API strip reactions. Growth of *F. necrophorum* was measured after 48 h using a modified lactate media (Tan et al., 1994). The presence of *F. necrophorum* was confirmed by detection of indole production 48 h after incubation by using Kovac's reagent (Tan et. al., 1994). Most probable number of *F. necrophorum* per ml of rumen fluid was confirmed using API strip reactions. Statistical analyses were conducted as for a repeated measures design on the transformed (log 10) bacterial counts.

A study was designed to evaluate effect of feeding increasing doses (1X, 2X or 3X) of PAPSb in steers fed high-grain diets. There were no interactions between feeding FA and PAPSb. Feeding increasing concentrations of PAPSb with or without FA reduced rumen concentrations of *S. bovis* in a cubic ($P < 0.05$) response. This was due to the fact that feeding twice the recommended dose (2.5 ml) had no effect ($P > 0.15$) on *S. bovis* numbers. However, steers fed 2.5 or 7.5 ml PAPSb/hd/d had 80% and 75% lower ($P < 0.05$) concentrations of *S. bovis* in the rumen, respectively. When comparing rumen *S. bovis* concentrations 14 d after feeding 2.5 or 7.5 ml PAPSb/hd/d, *S. bovis* populations were 95% or 97% lower ($P < 0.05$) than those in steers fed no PAP. Feeding PAPSb had no effect ($P > 0.15$) on rumen concentrations of total anaerobic bacteria or *F. necrophorum*, but feeding FA reduced ($P < 0.01$) concentrations of *F. necrophorum* 83%.

When steers had been fed PAPSb (2.5 ml/hd/d) with or without FA for 29 d, two rumen fluid samples were collected; one pre-feeding, and one 5.5 h post-feeding to determine effects of PAPSb on rumen concentrations of *S. bovis* and rumen pH. Steers fed PAPSb had 67% lower ($P < 0.05$) rumen concentrations of *S. bovis*. Rumen pH was greater ($P < 0.05$) for cattle fed PAPSb (6.1 vs 5.7). Steers fed FA treated with PAPSb had the lowest ($P < 0.05$) concentrations of *S. bovis* (FA X PAPSb interaction P-value = 0.15), while steers fed no PAPSb or FA had the lowest ($P < 0.05$) rumen pH (FA X PAPSb interaction P-value = 0.08).

Feeding a PAP against *F. necrophorum* (PAPFn) for 12 d reduced ($P < 0.01$) concentrations of *F. necrophorum* only when FA were not fed (FA X PAP interaction P-value = 0.0003). When FA and PAPFn were fed together, *F. necrophorum* concentrations actually increased ($P < 0.01$). Feeding PAPFn alone or with FA had no effect ($P > 0.15$) on concentrations of *S. bovis* or rumen pH.

Data from a 2 X 2 Factorial design study conducted to determine effects of PAPSb or PAPFn on rumen bacteria and rumen fermentation demonstrated that either PAP was effective (PAP interaction P-value = 0.11) at reducing ($P < 0.05$) rumen concentrations of *S. bovis* measured in samples collected pre-feeding. Feeding the PAP combination tended ($P < 0.10$) to reduce concentrations of *S. bovis* in the rumen also. Feeding PAPSb had no effect ($P > 0.15$) on rumen concentrations of *F. necrophorum*. Steers fed 2.5 ml PAPFn/hd/d alone or in combination with 2.5 ml PAPSb/hd/d tended to have 75% lower ($P < 0.10$) concentrations of *F. necrophorum* in the rumen.

A study was conducted recently with steers fed a 100% corn silage based diet. The study was designed as a 5 x 5 Latin Square design to determine response to increasing doses of PAPSb (1X, 2X, 3X or 4X of 2.5 ml PAPSb/hd/d) in 50% forage diets. Rumen fluid samples were collected pre-feeding, .5, 2, and 4 h post-feeding to determine concentrations of *S. bovis*, and pH. Steers fed PAPSb had rumen concentrations of *S. bovis* reduced ($P < 0.001$) from 63% to 92%. There were cubic responses by rumen *S. bovis* populations ($P < 0.05$) and pH ($P < 0.10$) to dose. Greatest reductions in *S. bovis* populations were achieved when dosing 1X or 4X dose, while greatest rumen pH was achieved when dosing 1X dose.

A microscopic evaluation of binding between *S. bovis* and PAPSb-coated polystyrene beads (75 microns) was conducted during the first period of the 5 X 5 Latin Square study in an attempt to demonstrate visually the interaction between PAP and its target, and resistance by PAP to in vivo rumen conditions. Beads were sealed in filter bags (pore size, 25 microns), and filter bags were suspended in the rumen using mesh bags. Filter bags were retrieved, and beads recovered at the time of rumen fluid collection. Beads were Gram-stained and observed under the microscope. In addition, some beads had been coated with PAP against *Mycoplasma bovis* (**PAPMb**), recovered, visually observed under the microscope, and further challenged with *M. bovis* to determine the stability of the PAP after recovery from the rumen. A visual demonstration of beads coated with PAPSb (Figure 1) or PAPMb (Figure 3), recovered 2 or 4 h post-feeding, respectively, are presented with reference to a non-coated bead suspended in PBS buffer (Figure 2).

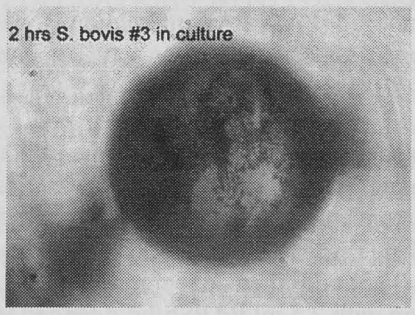

Figure 1. Polystyrene bead coated with a polyclonal antibody preparation against *Streptococcus bovis* recovered from the rumen 2 h post-feeding, and Gram-stained. Gram staining and masses of bacteria are observed in the upper 3/4 surface of the bead.

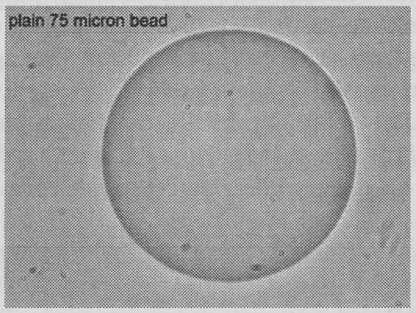

Figure 2. Polystyrene bead not coated suspended in PBS buffer, provided here as a visual reference.

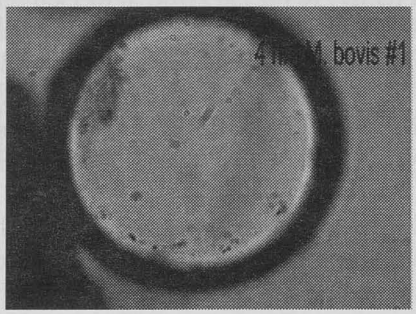

Figure 3. Polystyrene bead coated with a polyclonal antibody preparation against *Mycoplasma bovis* recovered from the rumen and Gram-stained 4 h post-feeding. Bead appears to bind some debris, but lack of Gram staining indicates absence of rumen bacteria binding.

Effects on Rumen pH, Volatile Fatty Acids, and Ammonia

Data from a 2 X 2 Factorial design study conducted to determine effects of PAPSb or PAPFn on rumen bacteria and rumen fermentation demonstrated that, except for pH measured 6 h post-feeding, rumen pH of steers fed PAP against *S. bovis* and against *F. necrophorum* (**PAPFn+Sb**) was greater ($P < 0.05$) throughout a 24-h collection period (Figure 4). Rumen pH of steers fed either PAP was greater 2 h post-feeding (Figure 4). In addition, rumen pH of steers fed PAPFn tended ($P < 0.10$) to be greater even at 4 h post-feeding. These patterns of rumen pH revealed that acid load at critical times post-feeding may be reduced with PAP feeding. Additionally, these data demonstrated that beyond 4 h post-feeding, single

PAP had no effect on rumen pH. Steers were fed once daily in this study. It would be of interest to discover whether effects of feeding single PAP on rumen pH hold for cattle fed more than once daily.

Concentrations of volatile fatty acids (**VFA**) in rumen fluid from samples collected concurrent with pH measurements did not ($P > 0.15$) differ. Lactic acid concentrations were not detectable. It is quite possible that feeding PAP may exert its effects through enhanced VFA absorption from the rumen, which may be reflected by greater rumen pH. Although the possibility exists that modulated rates of VFA production, caused by modified numbers of rumen bacteria, lead to more evenly distributed VFA production over time. Regardless, it is expected that greater rumen pH, at least temporarily, may support improved rumen health, enhanced VFA absorption, and greater performance.

Concentrations of ammonia nitrogen (**ammonia-N**) in rumen fluid samples collected concurrent with pH measurements followed patterns that were almost completely opposite to rumen pH curves. During the first 2 h post-feeding, rumen ammonia-N concentrations were lower ($P < 0.05$) for steers fed either PAP alone (PAP X sampling time interaction P-value = 0.03). Rumen ammonia-N concentrations were lower ($P < 0.05$) for steers fed PAPFn+Sb at 0, 2, 4 and 9 h post-feeding. Concentrations of rumen ammonia-N are the result of deamination and ammonia absorption processes. Although other rumen fermentation data are not yet analyzed to provide a more complete picture of effects of feeding PAP on rumen fermentation, it is known that absorption of ammonia from the rumen is increased as pH reaches 6.5. Because of greater rumen pH maintained in steers fed PAPFn+Sb, it is likely that ammonia absorption from the rumen may have played a role in lower ammonia-N concentrations. Regardless of the mechanism that may lead to lower rumen ammonia-N concentrations in the rumen of steers treated with both PAP, rumen ammonia-N beyond 2 h post-feeding was in concentrations lower than those required to maintain maximum microbial growth (5 mg NH_3-N/100 ml; Satter and Slyter, 1974). This finding may have implications on animal performance (see section on feedlot performance below). Concentrations of rumen ammonia-N were only observed to be below this threshold beyond 15 or 21 h post-feeding for steers fed no PAP or PAPSb, respectively.

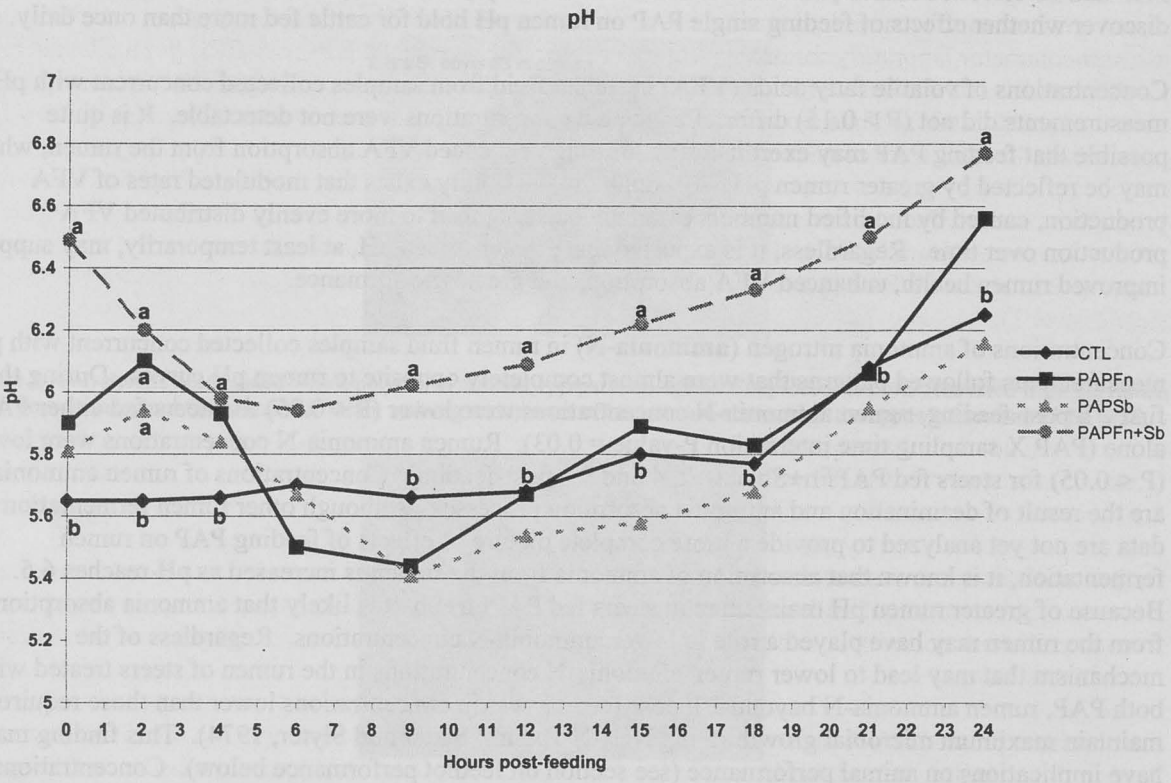

Figure 4. Effects of feeding a polyclonal antibody preparation against *Streptococcus bovis*, *Fusobacterium necrophorum* or both on rumen pH measured pre-feeding, and up to 24 h post-feeding. Within sampling time, [a,b] designates differences ($P < 0.05$) between control and respective antibody treatment.

USE OF ANTIBODIES AGAINST PROTEOLYTIC BACTERIA IN THE DAIRY COW

A preparation of polyclonal antibodies specific to three strains of resident proteolytic bacteria (*Clostridium stricklandi and C. aminophilum, and Peptostreptococcus anaerobius*) was used in 28 multiparous dairy cows, paired by days in milk (**DIM**; > 50 d) and allocated to one of two dietary treatments, to determine the impact of short-term feeding PAP against proteolytic bacteria on rumen fermentation and yield of milk and milk components. Reducing the concentration of proteolytic bacteria in the rumen should decrease amino acid digestion and production of ammonia from the rumen. Thus, the original nature of a proportion of dietary protein (up to 9% of that fed; Russell, 1996) may be retained for intestinal digestion—a process that is relatively more efficient as it prevents excessive ammonia loss, and may lead to a better amino acid profile presented for production processes.

A PAP against rumen proteolytic bacteria in a soy hull-based carrier pellet (14 cows) or the soy-hull-based carrier pellet (14 cows) was top-dressed for two 14-d treatment feeding periods in a switch-back design; each period was preceded by a 14-day baseline measurement period. Otherwise, diets were formulated to contain 1.72 Mcal NE_l/kg DM, 17.5% CP, .65% Ca and .35 % P. Diets were mixed in a total-mixed-ration feeding cart and delivered once daily.

On the last day of each period, rumen fluid was removed via gastric tube for evaluation of rumen pH, rumen ammonia and volatile fatty acid concentrations. A sample of this fluid was preserved and shipped to a sub-contractor laboratory for quantification of rumen proteolytic bacteria via PCR gel methodologies. Cows were divided into early or late lactation groups according to the point at which DMI tends to be stabilized (NRC, 2001). Thus, cows considered to be in late lactation were beyond 140 DIM; those with fewer than 140 DIM were considered to be in early lactation.

When cows in early lactation were treated with PAP, they tended ($P < 0.13$) to have greater yield of milk and 4% fat-corrected milk (Table 1). Milk yield, corrected to a 4% fat content, tended to increase when cows were treated with PAP due to a small, numerical increase in fat content, and a 1.5% increase in milk yield (Table 1).

When early lactation cows were treated with PAP, the response to treatment, as defined by the change in milk yield relative to the average milk yield in the preceding period, was 0.77 kg/d. This response was larger ($P = 0.06$) than that experienced when cows were fed the carrier. Apparently, increased (1.5%) milk output when PAP was fed was accompanied by a similar increase (1.7%; $P < 0.05$) in DMI (Table 1).

Neither milk fat nor protein concentration was affected ($P > 0.15$) by feeding PAP in early lactation cows. However, concentration of milk solids was reduced ($P < 0.05$) when PAP was fed (Table 1). Neither somatic cell count nor milk urea nitrogen were affected ($P > 0.15$) when feeding PAP to early lactation cows (Table 1).

Milk yield, expressed as 4% fat-corrected milk or as the change in milk yield relative to the average milk yield in the preceding period, was not affected ($P > 0.15$) by feeding PAP to late lactation cows (Table 1). Similarly, milk fat, protein or somatic cell counts were not affected ($P > 0.15$) by feeding PAP to late lactation cows. However, concentration of milk solids and that of milk urea nitrogen were reduced ($P < 0.05$) when PAP was fed (Table 1).

Feeding a PAP specific against proteolytic bacteria had no effect on absolute concentrations or proportions of each VFA measured. Similarly, rumen ammonia concentrations or pH were not affected by feeding a PAP specific against proteolytic bacteria.

Table 1. Effects of feeding a polyclonal antibody preparation against rumen proteolytic bacteria to cows in early or late lactation

	Item	Control	Polyclonal antibody	SE	P-value
Early lactation	Cows	8	8		
	Milk, kg/d	44.54	45.20	0.25	0.13
	4% FCM [a], kg/d	38.00	39.12	0.44	0.13
	Response [b], kg/d	-0.01	0.77	0.23	0.06
	DMI, kg/d	20.97	21.34	0.04	0.002
	Fat, %	2.98	3.09	0.06	0.24
	Protein, %	2.83	2.78	0.03	0.30
	SCC [c], x1000	160.50	151.50	23.98	0.81
	Solids, %	5.76	5.69	0.017	0.03
	MUN [d], mg/dL	10.59	10.16	0.24	0.25
Late lactation	Cows	19	19		
	Milk, kg/d	36.86	37.09	0.45	0.73
	4% FCM [a], kg/d	33.26	33.75	0.65	0.61
	Response [b], kg/d	0.71	0.97	0.46	0.70
	DMI, kg/d	21.38	21.36	0.17	0.93
	Fat, %	3.40	3.46	0.086	0.60
	Protein, %	3.06	3.02	0.018	0.15
	SCC [c], x1000	145.90	130.93	22.96	0.66
	Solids, %	5.66	5.58	0.026	0.05
	MUN [d], mg/dL	10.96	9.88	0.19	0.001

[a] Milk corrected to 4% fat content.
[b] Milk yield change relative to preceding (baseline) period.
[c] Somatic cell counts.
[d] Milk urea nitrogen.

USE OF ANTIBODIES AGAINST STARCH OR LACTATE UTILIZERS IN THE FEEDLOT

During two consecutive years, Angus crossbred steer calves (267 kg) were stratified by weight, and assigned to one of sixteen (year 1) or twelve pens (year 2) in a study designed to determine the impact of feeding PAPSb or PAPFn on performance and carcass characteristics of steers fed high-grain diets. Pens

were randomly assigned to one of four dietary treatments resulting from a factorial arrangement of treating diets with a PAP against *S. bovis* or *F. necrophorum*. Dietary treatments consisted of mixing a dose PAPSb or PAPFn or PAPFn+Sb in a soy hull-based carrier pellet (120 g) or the soy-hull-based carrier pellet (120 g) in diets based on corn grain (50:50 high moisture corn:dry rolled corn), corn silage, and a supplement. Diets were formulated to contain 1.38 Mcal NE_g/kg DM, 12.5% CP, .65% Ca, and .35% P. The supplement was formulated to meet protein, vitamin, and mineral requirements, and to supply an ionophore (100 mg laidlomycin propionate/hd/d). Steers were implanted with a trenbolone acetate-based implant initially, and according to projected slaughter date (within 85 to 100 days before slaughter). Steers were marketed when 65% of the steers in the pen reached choice grade as assessed visually.

Feeding either PAP enhanced ($P < 0.05$) feed conversion (analyzed as kg gain/kg feed DM; PAP interaction P-value = 0.04). Feeding PAPSb tended ($P < 0.10$) to improve carcass gain-adjusted feed conversion (PAP interaction P-value = 0.12). These findings are significant because it is the first time that manipulation of bacterial populations with the use of oral antibodies led to effects on feedlot performance (Table 2). Although it is speculative at this point, effects of feeding PAPFn+Sb may be negated by rumen environment conditions which result in lower ammonia-N concentrations, thereby leading to lower bacterial yields.

Dressing percentage of steers fed PAPFn was lower ($P < 0.05$) than that of steers fed no PAPFn (Table 2). Only subcutaneous fat was affected by feeding PAP. Steers fed PAPSb had carcasses with greater ($P < 0.05$) fat depth than those of steers fed no PAP, while those fed PAPFn had carcass fat that tended ($P < 0.10$) to be greater than that of steers fed no PAP (PAP interaction P-value = 0.03; Table 2). No other carcass traits were affected by feeding PAP.

Incidence of liver abscesses was not collected during year 1 due to implications of the US-Canadian border closure in response to Canada's only BSE case on kill schedule. During year 2, liver abscess incidence was lower ($P < 0.05$) for steers fed PAPFn with or without PAPSb than those fed no PAP and PAPSb (Table 2).

Table 2. Effects of feeding a polyclonal antibody preparation against *Streptococcus bovis* or *Fusobacterium necrophorum* or both on feedlot performance and carcass characteristics of steer calves (two-year data; liver abscess incidence data only for year 2)

	Treatment [a]				
Item	Control	PAPSb	PAPFn	PAPFn+Sb	SE
No. of pens	7	7	7	7	
Initial BW [b], kg	265	266	267	267	
Final BW, kg	539	544	545	543	2.6
Adjusted [c] final BW, kg	539	543	540	538	2.4
ADG, kg	1.71	1.74	1.74	1.73	0.02
Adjusted [c] ADG, kg	1.70	1.72	1.71	1.69	0.02
DMI, kg/d	9.46	9.32	9.39	9.37	0.10
DM required/kg gain, kg	5.58 [d]	5.38 [e]	5.40 [e]	5.44 [de]	0.002
Adjusted [c] DM required/kg gain, kg	5.56	5.41	5.52	5.54	0.002
Carcass characteristics					
Hot weight, kg	338	341	339	337	1.5
Dressing [f], %	62.8	62.6	62.2	62.2	0.20
Ribeye area, cm^2	78.52	79.16	78.90	79.87	0.58
Fat depth, cm	1.27 [d]	1.50 [e]	1.45 [de]	1.40 [d]	0.05
Marbling score [g]	510	522	512	520	8.88
Kidney, pelvic and heart fat, %	2.13	2.15	2.13	2.17	0.02
Abscessed livers [hf], %	20.0	28.6	13.9	5.6	

[a] Polyclonal antibody preparations against *Fusobacterium necrophorum* (PAPFn), *Streptococcus bovis* (PAPSb) or both (PAPFn+Sb).
[b] Obtained after withholding feed and water for 16 hours.
[c] Adjusted using hot carcass weight divided by the average dressing percentage (62.8).
[d,e] Means with uncommon superscripts differ ($P < 0.05$).
[f] Effect of PAPFn ($P < 0.05$).
[g] 400 = slight, 500 = small, 600 = slightly abundant.
[h] Data collected only during year 2.

SUMMARY AND CONCLUSIONS

Based on the literature, and data presented herein, antibodies derived from avian sources targeted at specific rumen bacteria affect target bacteria within at least a 4-h window post-feeding in cattle fed 85% or 50% grain diets. The rumen and its highly reactive environment appear not to affect antibody activity as it regards modification of target rumen bacteria populations. Although, it is not clear from these data whether only target bacteria are affected.

In high-grain diets, effects of feeding a polyclonal antibody against *Streptococcus bovis* are enhanced by feeding Rumensin, and, perhaps more specifically, Tylan. Effects of feeding a polyclonal antibody against *Fusobacterium necrophorum* may actually be negated by feeding Rumensin and Tylan. More research is required into this interaction to determine proper dosing and choice of feed additive supplementation under commercial conditions.

It is clear that polyclonal antibody preparations may interact with each other in the rumen. Feeding a polyclonal antibody preparation against *Fusobacterium necrophorum* reduced concentrations of *Streptococcus bovis*, but feeding a polyclonal antibody preparation against *Streptococcus bovis* did not reduce concentrations of *Fusobacterium necrophorum*. Because of these interactions, and other effects in the rumen, it is also expected that feeding polyclonal antibody preparations against these bacteria may also affect other bacteria populations; the implications of which are presently unknown.

Feeding polyclonal antibody preparations against *Fusobacterium necrophorum*, *Streptococcus bovis*, or both had profound effects on rumen pH and rumen ammonia. Effects on rumen pH appear to be positive, at least as it concerns feeding single antibody preparations. However, it remains to be determined whether feeding cattle twice or more daily will have sustained effects on rumen pH, and whether these effects on rumen pH readily translate to enhanced performance. There is some early indication that feeding both preparations results in reduced rumen ammonia-N concentrations, which may have implications on bacterial yields, and performance.

Polyclonal antibody preparations against rumen proteolytic bacteria hold promise as a method to manipulate rumen ammonia, reduce rumen proteolysis, increase bypass protein value, and improve milk yields. Further work is required to elucidate what level of modulation occurs on proteolytic bacteria, and the resulting gain in rumen bypass protein value.

Feeding a polyclonal antibody preparation against *Streptococcus bovis* resulted in improved feed efficiency measured using live weight gain. Improvements in feed efficiency measured using carcass-adjusted gain were more moderate. Regardless, these improvements would result in reductions in feed cost in the order of $6.75 to $9.00/hd (272 kg gain, and feed cost of $165.38/Ton DM). Similarly, feeding a polyclonal antibody preparation against *Fusobacterium necrophorum* resulted in marked reductions in liver abscess incidence. It is not clear from these studies how these preparations may be interacting, but it appears that, at the doses tested, feeding a polyclonal antibody preparation against *Fusobacterium necrophorum* negates effects on performance observed when feeding a polyclonal antibody preparation against *Streptococcus bovis*. However, effects of feeding a polyclonal antibody preparation against *Fusobacterium necrophorum* on liver abscess incidence appear to be independent of this potentially negative interaction.

In conclusion, a method exists for development, preservation, and delivery of functional polyclonal antibody preparations in diets of ruminants. These preparations are effective, perhaps due to their resistance to low pH, high temperatures, and proteolysis, in reducing populations of target bacteria and modulating rumen pH. Feedlot performance or milk yield may be improved by effects of modulating

populations of bacteria that produce compounds which hinder rumen fermentation (i.e., lactic acid) or reduce substrate utilization by host (i.e., feed protein). Further work is required in this field to fine-tune doses of antibodies, delivery systems, and to prevent possible negative interactions with existing feed additives

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