Journal of the American Association for Laboratory Animal Science
Vol 50, No 1
January 2011
Pages 27–32

Perioperative Ruminal pH Changes in Domestic Sheep (*Ovis aries*) Housed in a Biomedical Research Setting

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Little information is available on normal ruminal pH values for domestic sheep (*Ovis aries*) housed in a research setting and fed a complete pelleted ration. Sheep maintained on pelleted diets undergoing surgical procedures often present with postoperative anorexia and rumen atony. To determine the relationship between diet and postoperative rumen acidosis and associated atony, we studied dietary effects on ruminal pH in an ovine surgical model. Sheep undergoing orthopedic surgical procedures were randomized into 2 diet groups. Group 1 (*n* = 6) was fed complete pelleted diet during the pre- and postoperative period, and group 2 (*n* = 6) was fed timothy grass hay exclusively throughout the study. Measures included ruminal pH, ruminal motility, and rate of feed refusal, which was monitored throughout the pre- and postoperative periods. The 2 groups did not differ significantly before surgery, and the ruminal parameters remained largely within normal limits. However, a downward trend in the strength and frequency of rumen contractions was observed in pellet-fed sheep. After surgery, the pellet-fed group showed clinical signs consistent with ruminal acidosis, supported by decreased ruminal motility, anorexia, putrid-smelling ruminal material, and death of ruminal protozoa. Intervention by transfaunation in clinically affected sheep resulted in resolution of signs. Our findings suggest that sheep fed grass hay appear to have a more stable ruminal pH, are less likely to experience anorexia and rumen atony, and thereby exhibit fewer postoperative gastrointestinal complications than do sheep on a pellet diet.

Abbreviation: VFA, volatile fatty acids.

Sheep (*Ovis aries*) typically consume a diet high in fiber consisting of fresh forage or hay, with very little energy concentrate feed. Energy concentrates contain a high density of nutrients, usually low in crude fiber (less than 18% of dry matter) and high in total digestible nutrients.[13] Energy concentrate feed provides a source of easily digestible carbohydrates, but if fed in excess can lead to ruminal acidosis.[6,25,28,29,36] Mastication of grass hay stimulates the production of copious amounts of saliva, which act to buffer the ruminal pH. Normal ruminal pH in sheep is between 6.4 and 6.8. Ruminal pH values less than 5.5 or greater than 7.0 are considered abnormal; ruminal pH of less than 5.5 is defined as subacute rumen acidosis[2,20,25,31] but typically has no clinical signs. Acute or clinical acidosis generally is seen when the ruminal pH falls below 5.0. Clinical signs may include reduced salivation, lethargy, reduced gastrointestinal motility, anorexia, and diarrhea.[37] The cause for ruminal acidosis is multifactorial and can be due to ingestion of small particle size (diameter less than 0.07 in.)[19] or highly concentrated feeds that contain rapidly fermented carbohydrates. Such diets require less chewing, resulting in decreased saliva production, which diminishes buffering capacity.[39] Ingestion of rapidly fermented carbohydrates leads to increased volatile fatty acid (VFA) production in the rumen and subsequent increased lactic acid production. This situation results in reduced ruminal pH and a shift in the ruminal fauna from predominantly gram-negative to gram-positive organisms.[9,18,26,35]

Sheep used in biomedical research often are housed indoors in elevated expanded metal flooring runs and fed a complete pelleted ration. In a research setting, disincentives associated with feeding of grass hay include clogging of floor drains and increased labor for animal care technicians. To circumvent management difficulties, pelleted feed diets have largely been adopted in large animal research settings. Complete pelleted feed diet, coupled with the stress of shipping, indoor housing, increased handling, fasting for experimental procedures, and perioperative opioids for pain management, can result in ruminal atony, inappetence, and subclinical rumen acidosis.[15,22,34,38] Clinically, this condition is manifested as ruminal atony, inappetence, and lethargy.[38]

Transfaunation consists of delivering rumen contents, containing healthy protozoa, VFA, and gram-negative bacteria, from a clinically normal ruminant into the rumen of an inappetent sheep, with the goal of repopulating the ruminal fauna and correcting the pH. Transfaunation is used widely for treatment of ruminal atony and anorexia in ruminant species.[33,38] Fresh rumen contents commonly are obtained from cows equipped with a rumen fistula. Bovine rumen contents can be used to transfaunate any ruminant species, with only minimal host specificity of the bacteria and protozoa.[7]

Although the benefits of transfaunation are acknowledged, maintaining a fistulated animal in a research environment has been perceived as impractical due to housing costs. In addition, the low volume of transfaunate that can be obtained readily from a fistulated ovine or caprine donor further questions the practicality of this treatment. Prior to the present study, the standard of care for ruminants housed indoors at our institution had been to collect ruminal contents from sheep at the conclu-

Received: 24 May 2010. Revision requested: 02 Jul 2010. Accepted: 05 Aug 2010.
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sion of an experimental study and store them at 4 °C for future use. Ongoing, unpublished studies at Colorado State University suggest that the protozoa in ruminal fluid do not survive at temperatures below 80 °F.[5] Therefore, refrigerated rumen contents likely provide only a source of VFA and do nothing to correct ruminal acidosis or restore normal protozoal activity. The precise role of the protozoa in the rumen ecosystem has long been debated and remains poorly understood.[11,40]

At the time of the present study, the changes in ruminal pH and microflora of postoperative sheep housed in a research setting and fed a complete pelleted ration were unknown. Our experience over several years suggested that many sheep enrolled in research trials and fed pelleted rations presented clinically with rumen atony, anorexia, and lethargy as sequelae to major surgical procedures. Although the specific changes that occurred in the rumen during the postoperative period were unknown, our observation was that sheep fed exclusively grass hay during the postoperative period in our facility rarely experienced anorexia and maintained an excellent appetite. The goal of the current study was to determine whether diet influenced the development of preoperative subclinical or postoperative acute ruminal acidosis in sheep managed in a research setting. Our hypothesis was that sheep fed hay would have less acidic and more normal ruminal pH, better ruminal protozoal activity, decreased rate of feed refusal, less need for transfaunation, and overall clinically fewer gastrointestinal complications.

Materials and Methods

Animals. Sheep enrolled in this study were on an IACUC-approved orthopedic protocol with approval for minimally invasive sampling of ruminal fluid. Finn–Dorset cross sheep (n = 12, female, 2 y old), weighing between 50 and 55 kg (Archer Farms, Darlington, MD) were assigned randomly to 1 of 2 study groups. One cohort of sheep (n = 6) was housed on straw bedding and exclusively fed ad libitum timothy grass hay. A second cohort of sheep (n = 6) was housed on sawdust and fed exclusively an estimated 2% body weight of a commercially available complete pelleted laboratory ration for ruminants (fed according to manufacturer's label; 5508 Rumilab LabDiet, Animal Specialties And Provisions, Quakertown, PA) twice daily for the duration of the study. All sheep were negative for *Coxiella burnetii*; vaccinated annually against ovine contagious pustular dermatitis, *Clostridium perfringes* types C and D, and tetanus; and dewormed with albendazole drench based on fecal exam results approximately 3 times yearly. Prior to use on this protocol, all sheep were housed in a paddock and fed only timothy grass hay. All sheep were transferred from other paddocks on the large animal hospital campus and had been on the premises for at least 6 mo.

Husbandry. This study was conducted at the Comparative Orthopedic Research Laboratory of the New Bolton Center, University of Pennsylvania School of Veterinary Medicine. All sheep were housed individually in 5 ft × 5 ft wire-panel stalls, according to recommendations in the *Guide for the Care and Use of Agricultural Animals in Research and Teaching*[12] as well as the *Guide for the Care and Use of Laboratory Animals*.[23] Stalls were arranged in 2 rows of 6 on concrete flooring covered with rubber stall mats. Sheep in the grass hay cohort were bedded on straw, whereas sheep in the pelleted fed cohort were bedded on sawdust. The housing barn (30 ft × 48 ft 5 in.) used natural lighting and ventilation with access to ad libitum drinking water in buckets. Rows of housing pens were separated by a 6-ft aisle down the center of the barn. All stall floors were cleaned and bedded regularly. Sheep fed hay were fed on the stall floor; sheep fed the pelleted ration were fed in individual feed buckets. This housing arrangement was used to simulate the run-style housing often used in a research setting. All sheep were in visual and olfactory contact with other sheep at all times; sheep had nose-to-nose contact with at least one other sheep at all times. All feedings were performed twice daily by laboratory personnel between 0700 and 0800 and again between 1700 and 1800. Time of feeding was documented.

Experimental design. Sheep were allowed to acclimate for approximately 15 h without feed, to keep postprandial ruminal fluid sampling times consistent throughout the study. Sheep then were assigned randomly to either the hay or pelleted feed group. After the acclimation period, all sheep underwent physical examination, including temperature, pulse, respiratory rate, and auscultation of ruminal contractions over 2 min.[21] Baseline blood work was performed consisting of glucose, urea nitrogen, creatinine, sodium, potassium, chloride, carbon dioxide, calcium, magnesium, phosphorus, cholesterol, total protein, albumin, aspartate aminotransferase, creatine kinase, γ-glutamyl transferase, and fibrinogen.

Prior to feeding, baseline ruminal fluid samples were obtained from all animals. Sheep were manually restrained, and an oral speculum fashioned from PVC piping (length, 6 in.; diameter, 1.5 in.) with rounded edges was placed in the sheep's mouth, and a foal nasogastric tube (length, 5 ft.; internal diameter, 1 cm) was introduced into the oral cavity and advanced over the base of the tongue and into the rumen. A 500-mL dose syringe was used to apply suction to the orogastric tube, and approximately 10 mL of ruminal contents was extracted and discarded. The first 10-mL sample was discarded to eliminate saliva contamination that would falsely elevate the pH level.[4,17,21] The dose syringe then was used to collect at least 20 mL of ruminal contents, which were placed into a 50-mL collection container kept at a temperature above 80 °F. Sheep were observed for a minimum of 10 min after this procedure to ensure that no clinical signs of respiratory distress ensued. Ruminal pH was analyzed (Accumet Basic pH Meter model AB15, Fischer Scientific, Pittsburgh, PA) within 1 min of collection, and samples were placed in a styrofoam container and kept warm at a temperature above 80 °F (HotSnapZ, South Bend, IN) until further analysis in the laboratory. All ruminal fluid samples were obtained before the morning feeding and within 1 h of the same time each day.

Feed consumption. Feed refusal was documented twice daily for all sheep. A measured amount of pelleted feed or hay was given twice daily; sheep were allowed access to this feed for 10 to 12 h before any remaining feed was measured and discarded. Feed refusal was documented by percentage of feed consumed, measured in increments of 25%.

Ruminal pH analysis. Ruminal fluid collection by using an orogastric tube can yield a pH reading that is falsely elevated by as much as 0.5 pH units, due to saliva contamination.[10,17,21] The first 10-mL sample was discarded to minimize false elevation of ruminal pH.[21] Ruminal pH was analyzed (Accumet Basic pH Meter model AB15, Fischer Scientific) within 1 min of collection. Samples were evaluated within 90 min of collection for parameters including color, consistency, odor, percentage protozoal motility, number of protozoa per low-power (10×) field, carbohydrate storage by protozoa, and anaerobic fermentation metabolism by the bacterial population.[21,38]

Ruminal fluid characteristics. ***Color.*** Sheep fed long-stem fiber, such as grass hay, typically have an olive-green color to their ruminal contents, whereas those of sheep fed a higher percentage of concentrates may have a milky-brown color. Putrefaction, occasionally observed after extended periods of anorexia, will yield a blackish-green color to rumen contents. In this study, we limited color analysis to qualitative description, in which the same observer (BHJ) documented the predominant hue of each ruminal sample.

Consistency. Ruminal fluid consistency can range from watery to more viscous in nature. Samples heavily contaminated with saliva may appear very viscous. Saliva contamination also will be reflected by an increase in pH values. Samples that appeared visibly contaminated with saliva were discarded and a second sample drawn. Assessments for saliva contamination were made at the time of sample collection to reduce the need to pass an orogastric tube a second time in an animal. Visual assessment (BHJ) in conjunction with pH values was used to rule out heavy saliva contamination.

Odor. Normal ruminal contents have an aromatic and acidic odor. A putrid or fecal odor can be indicative of an extended period of anorexia. Qualitative analysis by the same observer (BHJ) was performed on all samples and categorized as aromatic and acidic or fecal and putrid in odor. All animals were assessed at the time of sampling and feed refusal assessed in conjunction with abnormal ruminal fluid odor.

Protozoal motility. Protozoal motility generally is assessed as a percentage of progressively motile protozoa per low-power field. One drop of fresh ruminal fluid was placed on a warmed microscope slide and a coverslip placed. The same observer (BHJ) counted progressively motile protozoa compared with immobile protozoa in 10 low-power fields, and an average was obtained for each sample.

Iodine staining. Determination of the repletion of carbohydrate storage of protozoa is assessed by staining with Lugol iodine. This parameter can give an indication of the overall general health of the rumen by assessing protozoal functions. One drop of ruminal fluid and one drop of Lugol iodine were placed on a slide and a coverslip placed. The number of darkly stained protozoa compared with unstained protozoa was assessed in 10 low-power fields and an average obtained.

Methylene blue. Measurement of anaerobic fermentation metabolism of the bacterial population is indicated by methylene blue reduction time. Fresh ruminal fluid (10 mL) was placed in a 15-mL conical tube, 0.5 mL 0.3% methylene blue was added, and the tube was inverted until mixed thoroughly. The samples were observed until the blue color was cleared, and the total time was documented. Typically, samples from grain-fed sheep reduce the dye in 3 to 4 min, from hay-fed animals in 5 to 6 min, and from animals with inactive bacterial populations in excess of 10 min to clear the dye.[21] Culture of ruminal bacteria and speciation is possible but was beyond the scope of this study.

Timeline. After baseline sampling and initiation of the test diets, all sheep were sampled weekly for 6 wk prior to surgery.[10,28] All samples were obtained within 1 h of the same time each day, approximately 15 h after the previous feeding[2,3] and prior to receiving their morning feeding. Before surgery, all animals were feed-fasted for 12 h with free access to water. Ruminal samples from each sheep were obtained after induction of general anesthesia on the day of surgery. Postoperatively, sheep were sampled daily for 7 d. Postoperative sampling adhered to the same collection schedule used during the preoperative period.

Surgical procedure. An equal number of sheep from both diet groups was assigned randomly to the surgical cohorts. Surgical cohorts underwent a standardized long-bone orthopedic intervention of approximately 60 min of surgery time and a total of 2 h of general anesthesia. Both test groups were given buprenorphine (0.01 mg/kg IV) at the time of induction of general anesthesia. The lateral side of the proximal forelimb was clipped, prepped with chlorhexidine solution and isopropyl alcohol, and dried. Fentanyl patches (2.5 µg/kg) were placed during surgical preparation, the surrounding area was sprayed with an aerosol adhesive (Vi-Drape, Medical Concepts Development, Woodbury, MN) covered with a breatheable, film dressing (3M Tegaderm Dressing, St Paul, MN) and a 3-in. elastic tape dressing (Elastikon, Johnson and Johnson, New Brunswick, NJ), which were left in place for 72 h.[1] All animals received flunixin meglumine (1.1 mg/kg) once daily for 3 d postoperative. All animals were assessed by a veterinarian twice daily for comfort level. Observations included percentage weight bearing on affected hindlimb, bruxism, tachypnea, and tachycardia.

Statistical analyses. Mixed-model linear regression was performed to determine the association of diet, time, pH, and the effect of the event (general anesthesia and surgery) on the 2 study populations. Data normality was confirmed with the aid of the Shapiro-Wilks test. Mixed-effects regression analysis was used to compare the preoperative ruminal pH of sheep on pelleted feed with that of those on hay. Where indicated, additional analyses using ANOVA and Students's *t* test were performed. All statistical analysis was performed with the aid of Stata 10.1 statistical software (StataCorp, College Station TX). A P value of 0.05 was used to distinguish significant from nonsignificant differences.

Results

All sheep underwent uncomplicated surgical procedures and completed the study to the endpoint. The postoperative analgesic regimen was efficacious for both cohorts.

Ruminal analyses. *Color.* The observer noted a distinct change in the color of the ruminal fluid after 1 wk of feeding the pelleted diet. Ruminal fluid turned from an olive-green color to a dark-brown color (Figure 1).

Consistency. Prior to surgery, the ruminal fluid samples from sheep on pelleted feed had less particulate or fibrous material, consistent with the composition of the pelleted feed. Ruminal fluid samples became difficult to obtain in the pellet-fed sheep at 24 to 48 h after surgery. Samples had decreased water content, and the orogastric tube became clogged more easily in these animals. The consistency of ruminal fluid from the hay-fed cohort showed no change throughout the study.

Odor. The observer noted that ruminal fluid samples from the pellet-fed cohort were less aromatic within 1 wk of consumption of the pelleted diet. The ruminal fluid from pellet-fed sheep began to take on a distinctly fecal and putrid odor 24 to 48 h after surgery. No changes in the odor of the ruminal fluid of the hay-fed cohort were noted.

Protozoal motility. During the preoperative period, samples from pellet-fed sheep showed increased numbers in ruminal protozoa. Postoperatively, preoperatively assessed protozoal motility fell by 25% to 50% in the 2 sheep with overtly decreased appetite and associated ruminal atony. Alterations in the numbers of motile protozoa were not apparent in samples from 4 the remaining pellet-fed sheep when compared with those from hay-fed sheep during the postoperative period.

Iodine staining. There was a postoperative decrease in the number of protozoa exhibiting iodine stain uptake in all animals, with no statistical difference ($P = 0.102$) noted between hay- and pellet-fed sheep.

Methylene blue. There was no statistical difference between hay- and pellet-fed groups in the time reported for samples to clear methylene blue. In addition, no significant changes were noted between pre- and postoperative values. In general, meth-

Figure 1. Ruminal fluid samples from sheep fed (left) pellets or (right) grass hay. Ruminal fluid from the pellet-fed sheep is brown in color and has less particulate matter, whereas that from grass-fed sheep is greener in color with more particulate matter.

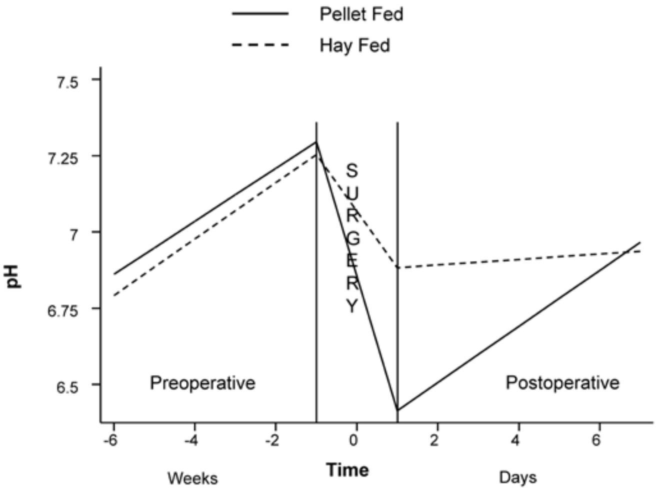

Figure 2. Mixed-effects regression model with fixed effects shows the predicted ruminal pH in pellet-fed compared with hay-fed sheep for the 6 wk prior to surgery (weeks −6 to −1), at the time of surgery (day 0), and 7 d after surgery (days 1 to 7).

ylene blue clearance times were longer than those reported in previous publications.[21]

Ruminal parameters and pH. Before surgery, the number of rumen contractions per minute was not significantly different in pellet- and hay-fed sheep; the duration and strength of the rumen contractions in the pellet-fed cohort did show a downward trend ($P = 0.584$) after 2 wk of feeding pellets. No feed refusal or significant change in ruminal pH was observed in the sheep fed a pelleted diet prior to general anesthesia and surgery.

After surgery, all 6 sheep fed the pelleted ration showed some degree of inappetence whereas those on hay did not. The onset of decreased feed intake occurred 24 to 48 h postoperatively and lasted for an average of 6 d. There was no statistically significant correlation between feed refusal and postoperative pH. Two of the 6 sheep experienced either complete anorexia or a decrease in appetite exhibited by a 50% to 75% decreased ration intake. Decreased feed consumption also correlated with rumen atony. These sheep required transfaunation with 750 mL fresh rumen contents. Before clinical signs resolved, 1 of the 2 sheep required a second transfaunation, with an additional 750 mL of fresh ruminal fluid, 24 h after the first. Serum chemistry blood work was repeated on sheep that became anorexic, but no significant changes compared with baseline blood test results were apparent.

The preoperative ruminal pH of sheep on pelleted compared with hay ration did not differ significantly ($P < 0.781$). Although the ruminal pH values of sheep fed a pelleted ration were slightly lower initially, they drifted upward toward the pH of those fed hay by 0.09 pH units per week. This initially lower pH most likely can be attributed to adaptation to a dietary change.[28] In contrast, postoperative ruminal pH was significantly ($P < 0.001$) lower in the pellet-fed sheep than the hay-fed animals when analyzed by using a mixed-effects regression model. An overview of both groups from the first preoperative measurement to the last postoperative measure shows the significant postoperative pH decrease seen in the pellet-fed group (Figure 2).

Discussion

The goal of this study was to determine whether diet influenced the development of preoperative subclinical or postoperative acute ruminal acidosis in sheep managed in a research setting. Our hypothesis was that sheep fed hay would have improved ruminal pH, better rumen protozoal activity, decreased rate of feed refusal, and less need for transfaunation, resulting in a more stable perioperative patient. We also aimed at establishing normal ruminal pH parameters for sheep fed an atypical (nonforage) ruminant diet.

Ruminal pH can be difficult to assess accurately due to diurnal changes, which can range from 0.5 to 1.0 pH units.[24,36] Ruminal pH also can vary due to its distinct topography within the various compartments, which are divided by the rumen pillars into a dorsal and ventral sac and then further divided into the caudoventral and caudodorsal blind sacs.[17] In our study, these fluctuations were minimized by sampling sheep at consistent intervals.[16,20,27] Saliva contamination is another concern when sampling orogastrically. Alternatives to orogastric sampling, such as rumenocentesis, are not without complications.[30] Rumenocentesis by repeated percutaneous needle puncture can increase the likelihood of abscess formation in the peritoneal cavity or result in peritonitis.[10,32] Samples that are obtained by rumenocentesis are often of very limited volume and only permit pH assessment, without evaluation of ruminal fluid color, odor, or microflora populations.[16] Submersible telemetry units that can be placed into the rumen to measure continuous pH changes are often bulky and may require a rumen fistula for insertion of pH sensors and their retrieval due to drift of calibration over time. Units created for small ruminants do exist[32] and were explored for this investigation. These small units can be placed orally by using a balling gun. However due to unpredictable drift of the pH sensors and their limited battery life, these units were not a viable option for the present study.

Our preoperative observations showed that the 2 feed cohorts did not differ significantly and that the ruminal parameters remained largely within normal limits. However, we did note a downward trend ($P = 0.584$) in the strength and frequency of rumen contractions in pellet-fed sheep. A shift in bacterial populations from lactic acid-producing bacteria (*Streptococcus bovis* or *Lactobacillus*) in the pellet-fed sheep likely occurred preoperatively, but the pH balance was maintained because there were also lactate-consuming bacteria.[26] With the stress of preoperative fasting, the lactate consumers were more susceptible and died, but the lactate producers continued to produce acid. The hay-fed sheep likely had a smaller population of lactate producers before surgery, resulting in decreased lactic acid production.[18,26,31,35] Given the postoperative data from the pellet-fed cohort in the present study, we conclude that these sheep apparently were more prone to developing gastrointestinal complications after exposure to stress, such as perioperative fasting, general anesthesia, and surgical interventions.

All animals recovered from surgery and general anesthesia. After surgery, sheep in the pellet-fed group showed clinical signs consistent with rumen acidosis, supported by decreased rumen motility, anorexia, putrid-smelling rumen material, and death of rumen protozoa. We also observed that the pellet-fed sheep experiencing postoperative anorexia had a concurrent decrease in water consumption. As a result, the rumen contents of these sheep were considerably drier than those fed hay. Given the desiccation of the ruminal contents and subsequent blockage of the tube during ruminal fluid collection, saliva contamination may have been increased. Therefore, postoperative pH values may have been elevated falsely whereas clinical signs were clearly indicative of an acute episode of ruminal acidosis. This apparent discrepancy may explain why many of the pH readings remained within the low-normal range of 5.5 to 5.8 and why there was no statistically significant correlation between feed refusal and postoperative ruminal pH.

Therefore, a reasonable explanation is that the aforementioned diurnal pH variations, slight variations within the compartment of the rumen sampled, effects of sampling time after feeding, and potential saliva contamination may have contributed to elevating (albeit falsely) the pH of the ruminal fluid.[4,10,21,32] It is clinically significant that all pellet-fed animals developed some degree of anorexia 24 to 48 h postoperatively and that lasted for an average of 6 d. Intervention by transfaunation resulted in clinical improvement in the 2 animals most severely affected.

A significant ($P = 0.011$) increase in protozoal numbers was observed in the rumen samples from pellet-fed sheep and may be related to the carbohydrate content and digestibility of the pelleted diet.[8,14,18] Alternatively, an increase in the protozoal fauna might be explained by the difference in rumen fill between the 2 cohorts under investigation.[7] Grass-hay diets result in more fibrous and bulkier rumen mats, providing more rumen fill, compared with pelleted diets. Although the importance of the protozoal population and associated morphology is still highly debated[11], death of ruminal protozoa, rumen atony, and anorexia are important clinical findings. These clinical signs all occurred in our cohort fed a pelleted diet. Their presence typically warrants timely intervention such as transfaunation to resolve the clinical signs.

A complete pelleted ration is not considered to be a concentrate feed, but any dramatic change in feed can predispose ruminants to gastrointestinal upset.[25,29] Ruminal acidosis is more likely to occur in animals that are unadapted to either an easily digestible feed with high starch content or to feed with small particle size (less than 0.07 in.), such as pelleted feed.[19] Small particle size results in decreased mastication and decreased saliva production and therefore decreases buffering capacity. The decrease in buffering capacity in conjunction with diets with a higher percentage of carbohydrate result in increased cleavage of starches by bacteria and an increase in VFA and lactic acid, thereby decreasing ruminal pH. Rumen papillae, which are responsible for absorption of VFA, are unadapted and unable to absorb the increased VFA that are produced. As a result, the pH of the rumen remains low. The change in the pH of the rumen typically causes shifts in the bacterial and protozoal populations.[31]

Although this orthopedic procedure required general anesthesia, sheep were recovering within 3 h of induction. Several surgical models in sheep require general anesthesia times upward of 7 or 8 h (for example, cardiothoracic research models often necessitating the need for cardiopulmonary bypass). Because deleterious effects on appetite and rumen motility can be seen with relatively short fasting and general anesthesia times, we consider it crucial to monitor ruminal health during the perioperative period.

The precise reason for the significant postoperative decrease in pH and ensuing inappetence is clearly multifactorial yet associated with perioperative exposure to various stress factors discussed in this study. Our findings show that sheep fed a grass-hay diet have a more stable ruminal pH and are less likely to experience anorexia and rumen atony. Sheep in a surgical biomedical research setting are more suitable candidates, with fewer postoperative gastrointestinal complications, when they are fed a typical diet of grass hay. Therefore adjusting the dietary options of sheep exposed to shipping and research related stressors may alleviate some of the commonly seen gastrointestine-related complications. A more physiologically appropriate diet consisting of adequate longstem fiber (grass hay) for ruminants in research settings should strongly be considered as part of comprehensive animal welfare.

Acknowledgments

We thank Dr Raymond Sweeney for assisting with the design of this project and Dr F Claire Hankenson for her mentoring and editorial assistance.

References

1. **Ahern BJ, Soma LR, Boston RC, Schaer TP.** 2009. Comparison of the analgesic properties of transdermally administered fentanyl and intramuscularly administered buprenorphine during and following experimental orthopedic surgery in sheep. Am J Vet Res **70:**418–422.
2. **Beauchemin KA, Yang WZ.** 2005. Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage. J Dairy Sci **88:**2117–2129.
3. **Bhandari SK, Ominski KH, Wittenberg KM, Plaizier JC.** 2007. Effects of chop length of alfalfa and corn silage on milk production and rumen fermentation of dairy cows. J Dairy Sci **90:**2355–2366.
4. **Bramley E, Lean IJ, Fulkerson WJ, Stevenson MA, Rabiee AR, Costa ND.** 2008. The definition of acidosis in dairy herds predominantly fed on pasture and concentrates. J Dairy Sci **91:**308–321.
5. **Callan RJ.** 2006. Personal communication: ruminal fluid microorganism viability after storage.
6. **Castillo C, Hernandez J, Mendez J, Llena J, Pereira V, Lopez-Alonso M, Benedito J.** 2006. Influence of grain processing on acid-base balance in feedlot steers. Vet Res Commun **30:**823–837.
7. **Dehority BA.** 1978. Specificity of rumen ciliate protozoa in cattle and sheep. J Eukaryot Microbiol **25:**509–513.
8. **Dennis SM, Arambel MJ, Bartley EE, Dayton AD.** 1983. Effect of energy concentration and source of nitrogen on numbers and types of rumen protozoa. J Dairy Sci **66:**1248–1254.
9. **Doetsch RN, Robinson RQ.** 1953. The bacteriology of the bovine rumen: a review. J Dairy Sci **36:**115–142.
10. **Duffield T, Plaizier JC, Fairfield A, Bagg R, Vessie G, Dick P, Wilson J, Aramini J, McBride B.** 2004. Comparison of techniques for measurement of rumen pH in lactating dairy cows. J Dairy Sci **87:**59–66.
11. **Elsden SR.** 1945. The fermentation of carbohydrates in the rumen of the sheep. J Exp Biol **22:**51–62.
12. **Federation of Animal Science Societies.** [Internet]. 2010. Guide for the care and use of agricultural animals in research and teaching, 3rd ed. [Cited 14 Apr 2010]. Available at: http://www.fass.org/docs/agguide3rd/Ag_Guide_3rd_ed.pdf
13. **Food and Agricultural Organization of the United Nations.** [Internet]. The use of concentrate feeds in livestock production [Cited 14 Apr 2010]. Available at: http://www.fao.org/ag/againfo/programmes/en/lead/toolbox/Refer/fcrpsec1.pdf.

14. **Franzolin R, Dehority BA.** 1996. Effect of prolonged high-concentrate feeding on ruminal protozoa concentrations. J Anim Sci **74:**2803–2809.
15. **Galyean ML, Lee RW, Hubbert ME.** 1981. Influence of fasting and transit on ruminal and blood metabolites in beef steers. J Anim Sci **53:**7–18.
16. **Garrett EF, Pereira MN, Nordlund KV, Armentano LE, Goodger WJ, Oetzel GR.** 1999. Diagnostic methods for the detection of subacute ruminal acidosis in dairy cows. J Dairy Sci **82:**1170–1178.
17. **Geishauser T, Gitzel A.** 1996. A comparison of rumen fluid sampled by ororuminal probe versus rumen fistula. Small Rumin Res **21:**63–69.
18. **Goad DW, Goad CL, Nagaraja TG.** 1998. Ruminal microbial and fermentative changes associated with experimentally induced subacute acidosis in steers. J Anim Sci **76:**234–241.
19. **Heinrichs J, Kononoff, P.** 1996. Evaluating particle size of forages and TMRs using the New Penn State Forage Particle Separator. University Park (PA): Department of Dairy and Animal Science of The Pennsylvania State University.
20. **House JK, Smith BP, Fecteau G, VanMetre DC.** 1992. Assessment of the ruminant digestive system. Vet Clin North Am Food Anim Pract **8:**189–202.
21. **House JK, Smith BP, VanMetre DC, Fecteau G, Craychee T, Neves J.** 1992. Ancillary tests for assessment of the ruminant digestive system. Vet Clin North Am Food Anim Pract **8:**203–232.
22. **Hungate RE.** 1975. The rumen microbial ecosystem. Annu Rev Ecol Syst **6:**39–66.
23. **Institute for Laboratory Animal Research.** 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academies Press.
24. **Keunen JE, Plaizier JC, Kyriazakis L, Duffield TF, Widowski TM, Lindinger MI, McBride BW.** 2002. Effects of a subacute ruminal acidosis model on the diet selection of dairy cows. J Dairy Sci **85:**3304–3313.
25. **Kleen JL, Hooijer GA, Rehage J, Noordhuizen JP.** 2003. Subacute ruminal acidosis (SARA): a review. J Vet Med A Physiol Pathol Clin Med **50:**406–414.
26. **Mackie RI, Gilchrist FMC.** 1979. Changes in lactate-producing and lactate-utilizing bacteria in relation to pH in the rumen of sheep during stepwise adaptation to a high-concentrate diet. Appl Environ Microbiol **38:**422–430.
27. **Mould FL, Kliem KE, Morgan R, Mauricio RM.** 2005. In vitro microbial inoculum: a review of its function and properties. Anim Feed Sci Technol **123-124:**31–50.
28. **Nagaraja TG, Titgemeyer EC.** 2007. Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. J Dairy Sci **90:**E17–E38.
29. **Nordlund KV.** 2003. Factors that contribute to subacute ruminal acidosis. Preconvention seminar 7: dairy herd problem investigation strategies. American Association of Bovine Practitioners 36th Annual Conference, 15–17 Sep 2003. Columbus, OH.
30. **Nordlund KV, Garrett EF.** 1994. Rumenocentesis: a technique for collecting rumen fluid for the diagnosis of subacute rumen acidosis in dairy herds. Bovine Practitioner **28:**109–112.
31. **Owens FN, Secrist DS, Hill WJ, Gill DR.** 1998. Acidosis in cattle: a review. J Anim Sci **76:**275–286.
32. **Penner GB, Aschenbach JR, Gabel G, Oba M.** 2009. Technical note: evaluation of a continuous ruminal pH measurement system for use in noncannulated small ruminants. J Anim Sci **87:**2363–2366.
33. **Rager KD, George LW, House JK, DePeters EJ.** 2004. Evaluation of rumen transfaunation after surgical correction of left-sided displacement of the abomasum in cows. J Am Vet Med Assoc **225:**915–920.
34. **Ruckebusch Y.** 1983. Pharmacology of reticuloruminal motor function. J Vet Pharmacol Ther **6:**245–272.
35. **Russell JB, Hino T.** 1985. Regulation of lactate production in *Streptococcus bovis*: a spiraling effect that contributes to rumen acidosis. J Dairy Sci **68:**1712–1721.
36. **Rustomo B, AlZahal O, Odongo NE, Duffield TF, McBride BW.** 2006. Effects of rumen acid load from feed and forage particle size on ruminal pH and dry matter intake in the lactating dairy cow. J Dairy Sci **89:**4758–4768.
37. **Slyter LL.** 1976. Influence of acidosis on rumen function. J Anim Sci **43:**910–929.
38. **Smith BP.** 2009. Large animal internal medicine: diseases of the alimentary system. In: Murray MJ, Guard C, editors. Large animal internal medicine, 4th ed. New York (NY): Mosby.
39. **Sudweeks EM, Ely LO, Mertens DR, Sisk LR.** 1981. Assessing minimum amounts and form of roughages in ruminant diets: roughage value index system. J Anim Sci **53:**1406–1411.
40. **Williams AG.** 1986. Rumen holotrich ciliate protozoa. Microbiol Rev **50:**25–49.