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Evaluation of an Indwelling Ruminal Probe Methodology and Effect of Grain Level on Diurnal pH Variation in Dairy Cattle

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ABSTRACT

To evaluate a ruminal probe for recording diurnal variation in rumen pH, we fitted three ruminally fistulated cows with probes affixed to the inside of the cannula. Probes were connected to a data logger and readings were recorded hourly. The experiment was a Latin square design with cows fed three different diets: 50, 60, and 70% grain as a total mixed rations once daily. Feed offerings and refusals were recorded daily. The experimental period was 21 d. The first 6 d were for adaptation, followed by 5-d rotations through each of the diets. Daily probe readings were recorded at 4-, 6-, 8-, 12-, and 24-h intervals. At each interval, readings were recorded (precleaned), a sample of rumen fluid was taken, and pH was measured in the laboratory. As probes were removed from the rumen, probe ends were cleaned with 0.1 *N* HCl and reinserted into the rumen, and a reading was recorded (postcleaned). No protective pH probe shields were used in this experiment. There were no differences between pre- and postcleaned pH readings across cows for all diets. Mean time under pH 5.0, 5.5, and 6.0 were 0.2, 2, and 7.2 h, respectively. Diet affected length of time under a certain pH, only for readings under pH 6. Diurnal pH profiles were monophasic in nature. The degree of acidity increased after feeding and duration of nadir increased with increasing grain in the diet. Daily DMI increased but was highly variable within the first week after switching to the next higher grain level. These results indicate the use of an indwelling ruminal probe without a protective shield, cleaned, and calibrated daily can accurately measure diurnal variation of ruminal pH. In addition, transition to higher grain levels in the diet increases pH, duration of pH nadir, and daily DMI fluctuation.
(**Key words:** rumen pH, indwelling probe, grain concentration)

INTRODUCTION

Subclinical rumen acidosis is a common problem in many high producing dairy herds. This problem can affect adaptation of the ruminal environment associated with the transition period and nutritional strategies chosen for feeding dietary carbohydrate. Measuring pH can indicate whether rumen acidosis is a problem. However, to study the full implication of nutritional acidosis on overall health of the animal, more information is needed on diurnal variation in pH, including nadir and duration of nadir. The indwelling probe concept has been tested by Dado and Allen (1994) and LaCount and Nocek (1997). The critical objective is to ensure that repeated mechanical readings are representative of the true ruminal pH.

LaCount and Nocek (1997) indicated that a shield protection device located at the end of the pH probe interfered with pH determinations compared with hand sampling. In addition, different dietary regimens, as well as time in the ruminal environment, could feasibly influence the amount of mucopolysaccharide accumulation at the end of the probe. The transition to a high concentrate grain diet can have a tremendous influence on ruminal pH, nadir, and duration of nadir, as well as fluctuations of pH and DMI. As the amount of fermentable carbohydrates increases as a proportion of ration DM, production of certain acids by amylolytic bacteria increases. If acid production exceeds absorption, ruminal pH begins to decline. Certain bacteria are more tolerant to lower pH concentrations and higher lactic acid levels than others. Lactic acid utilizing bacteria (*Megasphera elsdenii*, *Selenomonas ruminanitum*, etc.) aid in protecting the rumen against accumulation by metabolizing lactic acid. Mackie and Gilcrest (1979) suggest that approximately 3 wk are required for populations of lactic acid utilizing bacteria to increase adequately to protect the rumen against rapid pH changes. They also recommend that, ideally, the increases in grain should take place every 5 to 7 d throughout a transition period. The objectives of this project were to evaluate the efficacy of an indwelling rumen probe to measure diurnal variation in pH and to determine the sensitivity of the probe

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to detect diurnal variation in cows fed a wide range of grain concentrations.

MATERIALS AND METHODS

Three multiparous ruminally cannulated cows between 120 and 170 DIM at the beginning of the study were used for this evaluation.

Indwelling Probe

The pH electrode was a Sensorex model 450CD flat epoxy pH probe (Sensorex Corporation, Stanton, CA) with a 2-ft wire extension and a stainless steel connector. The wire extension was threaded through a 42-cm polyflex tubing and connected by a swivel into a Plexiglas rumen cannula cover. Within the cover was mounted a swivel that allowed the probe and 35-cm support unit to rotate freely in the rumen. The cannula cover was secured onto the 10 cm i.d. Plexiglas cannula. A 20-m connecting wire was suspended by pulleys and weights over the cow to allow free movement of the cow when hooked to the cable apparatus. The other end of the cable was hooked to a pH data logger (115/230V, Microcomputer pH vision data logger 6091, Jenco Electronics, Ltd. (San Diego, CA). Separate data loggers were established for each cow to print pH readings at hourly intervals.

pH Probe Shield Evaluation

An evaluation was conducted initially to determine whether a shield was needed to protect the pH probe in the ruminal environment and the effect the shield would have on pH readings. The shield was cylindrical (5.5 cm diameter × 3.5 cm height) with a flat end and a threaded area to attach to the threaded collar around the probe. Fifty 0.2-cm holes were bored into the cylinder to allow infiltration of rumen contents. The evaluation was conducted for 96 h with two cows fed a 60% grain diet, offered once daily at 1200 h. The first 24 h, probes were inserted into the rumen with no shield, during the second 24-h period, the shield was placed on the probe; 49 to 72 h, no shield and a shield from 73 to 96 h. The resulting profiles were evaluated.

Validation

The study design was a 3 × 3 Latin square with percent grain in the diet being the main factor. The diets are shown on Table 1. Cows were assigned to percent grain in the diet such that each cow received each grain percentage over the experimental period (50, 60, or 70% grain) and such that no cow increased in grain more than 10 percentage units. The cows were acclimated for 6 d, and feed was offered at 0800 h. On d 7, probes were removed at 4-, 6-, 8-, 12-, or 24-h intervals, 3 d each (15 d total) while on each treatment. Before removal, a pH reading was recorded on the data logger. The probe was removed, cleansed with a 0.1 *N* HCl solution, reinserted into the rumen, and allowed to stabilize for 1 min, and a postcleaning pH reading was taken. In addition, a sample of rumen fluid, from the approximate location from where the probe would have been located was taken, filtered through two layers of cheesecloth, and pH was measured in the lab within 10 min after sampling.

Table 1. Ingredient composition and nutrient analysis of experiment diets.

	% Grain in total ration DM		
Ingredient	50	60	70
	% total ration DM		
Corn silage	37.5	30.0	22.5
Alfalfa silage	12.5	10.0	7.5
HMEC	11.6	16.9	20.5
Whole cottonseed	9.1	10.9	12.7
Soybean meal	11.4	13.7	13.3
Wheat midds	2.2	4.6	6.5
Soy Plus	4.9	3.0	3.2
Blood meal	1.2	0.4	0.4
Molasses	4.7	5.7	6.6
Min/Vit Suppl.[1]	4.3	4.3	6.2
Tallow	0.6	0.6	0.6
Nutrient			
DM, %	41.8	45.7	50.1
Forage DM, %	50.0	40.0	30.0
CP, %	18.3	18.1	18.1
NE_L, mcal/kg	1.72	1.76	1.80
ADF, %	23.1	21.4	19.4
NDF, %	34.3	31.8	29.7
NSC, %	38.3	40.2	41.6

[1]Calcium carbonate: 36.33, copper sulfate: 0.10, zinc sulfate: 0.36%, potassium carbonate: 6.15%, dynamate: 1.78, magox: 8.8%, salt: 17.5%, sodium bicarbonate: 18.61%, trace mineral premix: 2.18%: vitamin E 20,000: 0.97%, monocal 21: 7.27% and manganese oxide: 0.15%.

Statistical Analysis

Data to evaluate the difference between precleaning and postcleaning measurements within the probe-cleaning interval were analyzed by utilizing a model for a Latin square study. The model is listed as follows: μ = cow + period + treatment + (treatment × period) + residual. Calculations were made using the JMP procedures of SAS (SAS Institute, 1995). Across all grain treatments, probe-cleaning interval was evaluated for differences utilizing the Tukey-Kramer (Kramer, 1956; Tukey, 1991) mean separation evaluation.

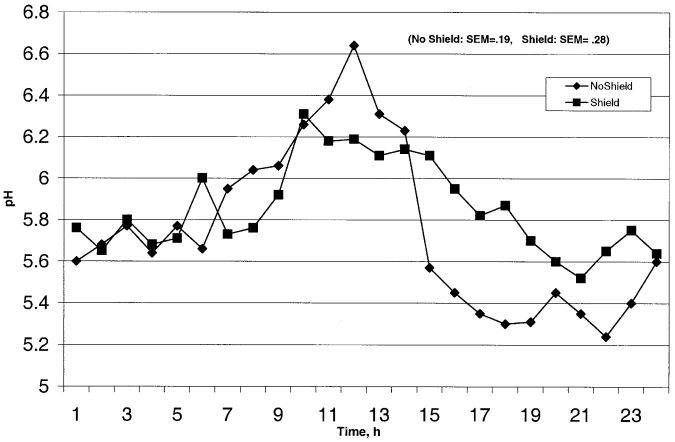

Figure 1. Effect of shield on diurnal ruminal pH.

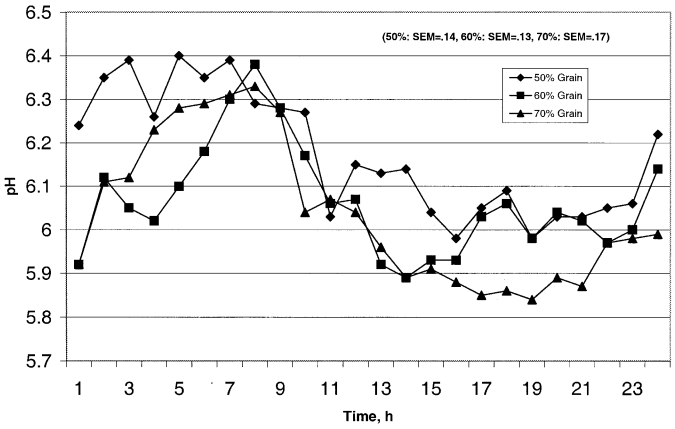

Figure 2. Effect of grain level on diurnal ruminal pH.

RESULTS AND DISCUSSION

Profiles for the unprotected probe (Figure 1) illustrated a typical drop after feeding (1.0 pH units); feed was offered at 1000 h. Ruminal pH decreased to 5.31 about 8 h postfeeding, then pH gradually increased to 6.63 about 2 h before feeding. The shielded probe exhibited a slower rate of reduction in pH postfeeding, reaching nadir at 8–9 h postfeeding, 0.3 pH units higher than the unshielded probe. A gradual increase was also seen; however, the pH only increased to 6.23, 0.4 pH units lower than the unshielded probe. Each time the shielded probe was removed, it was clogged with fibrous material. This apparently created a microenvironmental buffer around the probe, changing sensitivity so extremes in ruminal pH environment were not being detected. It may be that a more "open" protective device would allow less trapping of particles; however, the unprotected probe did not appear to be damaged or to have lodged against the rumen wall, a factor that could affect the accuracy of readings. No shield was used on the probes for subsequent experiments in this study.

Table 2 shows the relative difference between the pre- and postcleaned rumen pH probe readings for the various probe-cleaning intervals. There were no differences ($P > 0.10$) between pH readings before cleaning and those after for any intervals evaluated. Overall, there were no differences ($P > 0.10$) between the pre-cleaned and laboratory samples or between postcleaned and lab samples for any of the probe-cleaning time intervals evaluated. Generally, laboratory-determined pH values tended to be higher than those taken in the cow. Approximately 10 min lapsed between sample collection and pH reading; also, samples were not gassed with carbon dioxide. These factors may have contributed to the difference.

Table 3 illustrates the pre- and postcleaned pH readings for the various cleaning intervals, stratified by percentage of grain in the diet. Except for the 12-h interval for the 50% grain level, there were no differences ($P > 0.10$) at any other probe-cleaning intervals for 50, 60, or 70% grain levels. Within cleaning period (pre- and postcleaned), there were no differences in intervals at the 50% grain level; however, at the 60 and 70% grain levels, the 8-h interval was lower than the 24-h interval. The others were not different for these, which is reflected in the overall analysis across treatments. When cows were consuming the 70% diet, the 8-h interval was lower than the 24-h interval, with the other intervals not being different from these two intervals. These results would suggest that even

Table 2. Effect of pH probe cleaning and cleaning interval on rumen pH readings.

Probe cleaning interval (h)	n	Precleaned	Postcleaned	Lab	SEM	Preclean vs. postclean
4	198	6.18	6.13^{ab}	6.23	0.06	NS
6	144	6.15	6.18^{ab}	6.13	0.05	NS
8	106	6.08	6.10^{b}	6.20	0.06	NS
12	71	6.11	6.20^{ab}	6.29	0.10	NS
24	34	6.24	6.29^{a}	6.44	0.12	NS

^{a,b}Means in the same column with different superscripts are significantly ($P < 0.05$) different.

Table 3. Effect of pH probe cleaning and cleaning interval on rumen pH readings of cows fed different percentage grains.

Probe cleaning interval (h)	n	Precleaned[1]	Postcleaned[1]	Lab[1]	SEM	Preclean vs. postclean
50% Grain diet						
4	66	6.17	6.17	6.30	0.06	NS
6	48	6.27	6.22	6.05	0.07	NS
8	36	6.08	6.22	6.20	0.10	NS
12	24	5.91	6.26	6.27	0.13	0.05
24	11	6.28	6.30	6.46	0.19	NS
60% Grain diet						
4	66	6.19	6.13^{ab}	6.24	0.06	NS
6	48	6.20	6.27^{ab}	6.22	0.08	NS
8	36	6.12	6.07^{b}	6.18	0.11	NS
12	24	6.18	6.18^{ab}	6.30	0.10	NS
24	11	6.31	6.33^{a}	6.46	0.11	NS
70% Grain diet						
4	66	6.17^{ab}	6.10^{ab}	6.16	0.05	NS
6	48	6.00^{b}	6.10^{ab}	6.12	0.08	NS
8	34	6.07^{ab}	6.03^{b}	6.23	0.07	NS
12	23	6.23^{ab}	6.15^{ab}	6.30	0.10	NS
24	12	6.32^{a}	6.34^{a}	6.59	0.15	NS

^{a,b}Means in the same columns with different superscripts are significantly ($P < 0.05$) different.

[1]pH measurements were taken while the probe was inside the rumen (precleaned), the probe was removed, cleaned with 0.1 *N* HCl then reinserted (postcleaned) and measured. While the probe was removed, a sample of rumen fluid was taken and measured in the laboratory.

through 24 h of ruminal exposure, across all diets, there was no consistent accumulation of debris (mucopolysaccharide coatings, etc.) on the probe to interfere with pH readings. Specific diets may create a slime coating that interferes with consistency in pH readings; this does not appear to have influenced the results. There was no consistent data to suggest that the probe should be cleaned more often than once daily.

Diet and Diurnal Variation in pH

Mean diurnal pH profiles by diet are shown in Figure 2. Feeding corresponds to the 8-h time point. Typically, profiles were monophasic with a drop in pH after feeding, a gradual reduction until nadir (3 to 8 h postfeeding), then either a sustaining of nadir, and (or) a gradual increase until feeding. The rate and degree of pH decline postfeeding and the duration of nadir increased with percentage of grain in the diet. Although there were individual differences among cows, the data generally illustrate that cows receiving higher grain diets maintain a lower pH for longer periods of time than cows receiving more forage.

Table 4 shows the effect of grain percentage on hours below specific pH thresholds. There is no difference ($P > 0.10$) among grain levels on mean hours per day below pH 5 and 5.5. There was a tendency for cows fed the 60 and 70% grain diets to have slightly more hours under pH 5 and 5.5. Cows fed 60 and 70% grain diets had more hours during the day less than pH 6.0 compared with cows consuming diets with 50% grain (8.3, 8.5, and 5.4 h, respectively). The average pH, less than pH 5, was similar for cows receiving the 50 and 60% grain diets and much lower for those receiving the 70% grain diets; however, there were very few numbers and they were extremely variable (Table 5). Average pH greater than 5 but less than 5.5 was 5.29 for cows receiving the 50% grain diet, which was slightly higher compared with those receiving 60 and 70% grain diets. Mean ruminal pH values greater than

Table 4. Effect of percent grain in the diet on hours daily ruminal pH is below 5.0, 5.5, and 6.0.

	Mean h/d below pH			
% Grain	5.0	5.5	6.0	SE
50	0.17	1.8	5.4^{b}	0.07
60	0.25	2.0	8.3	0.07
70	0.20	2.2	8.5^{a}	0.09

^{a,b}Means in the same columns with different superscripts are significantly ($P < 0.05$) different.

Table 5. Effect of percent grain in the diet on mean ruminal pH within specific ranges.

	< 5.0			> 5.0, < 5.5			> 5.5, < 6.0		
% Grain	n	$\overline{X}$	SE	n	$\overline{X}$	SE	n	$\overline{X}$	SE
50	5	4.87	0.10	23	5.29	0.02	27	5.72	0.02
60	7	4.86	0.06	24	5.11	0.22	41	5.70	0.02
70	9	4.16	0.69	27	5.10	0.20	48	5.74	0.20

5.5 and less than 6.0 were similar across the three diets. However, there were only 27 measurements for the 50% diet and 48 measurements for those consuming 70% grain in this pH range. These numbers support the general contention that as grain level increases in the diet, the severity of pH depression, both frequency and duration, increases.

Ruminal pH Adaptation

The diurnal variations of pH 24 h before and after the dietary change are illustrated in Figures 3, 4, and 5. Consuming the 50% grain diet had a typical monophasic reduction in pH postfeeding, a stabilization phase, and an increase in pH (Figure 3). The day after receiving 60% grain, ruminal pH patterns became more variable and generally lower than the 50% grain diets. Particularly noticeable is the postfeeding stabilization period that sustained itself below 5.7 for approximately 5 h in contrast to readings on the previous day. Ruminal pH was maintained at pH 6.0 and above during this period of time. Daily DMI 7 d before and after the dietary switch is illustrated in Figure 6. Intake was stable between 19.5 and 20.0 kg before the change. The day after, intake increased to 21.8 kg, then dropped to 19.5 kg by d 6, but increased to 21.3 kg by d 7.

Cows fed the 60% diet developed a more stable pattern after being on the diet for approximately 21 d (Figure 4). When cows were switched from 60 to 70% grain, basic profiles were very similar. However, the drop in pH postfeeding was 1.0 pH unit compared to 0.6 for the 60% grain diets. Rumen pH remained below 5.5 for 3 h before increasing, then stabilized and rebounded above 6.0 by 10 h postfeeding. Cows consuming the 60% grain stabilized DMI between 20.9 and 21.8 kg after being on the diet for 21 d. The day after changing to 70% grain, DMI was extremely variable, ranging from 19.7 to 23.1 kg (Figure 6). When cows were switched from a 70% to a 50% grain diet (Figure 5), postfeeding pH profiles (12 to 23 h) were very similar. A major difference was the reduction in pH following feeding. Cows fed a diet of 50% grain showed only a 0.4 unit reduction in pH after feeding, and extremely variable during the entire day. Dry matter intake decreased 3.6 kg the day after switching from a 70 to 50% grain TMR, and stayed low.

When cows were increased in grain intake, DMI increased, and pH decreased dramatically. These events have the potential to predispose the cow to an acidotic state, which would in turn reduce subsequent DMI. The fluctuations in pH most likely creating the fluctuations in DMI. Greater fluctuations in pH and DMI occur as grain level increases. However, by 21 d, both

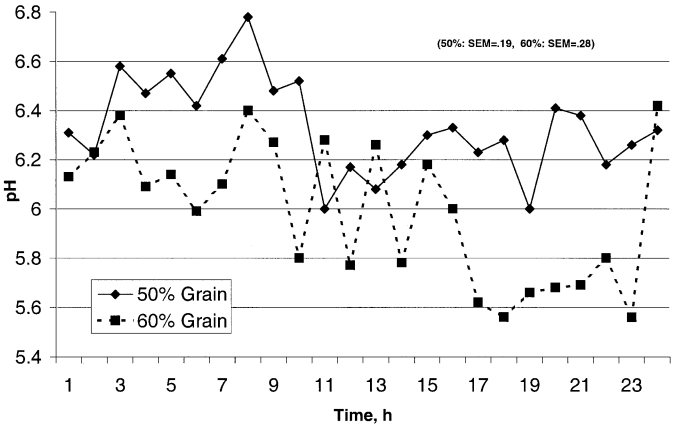

Figure 3. Effect of changing from 50 to 60% grain on diurnal ruminal pH.

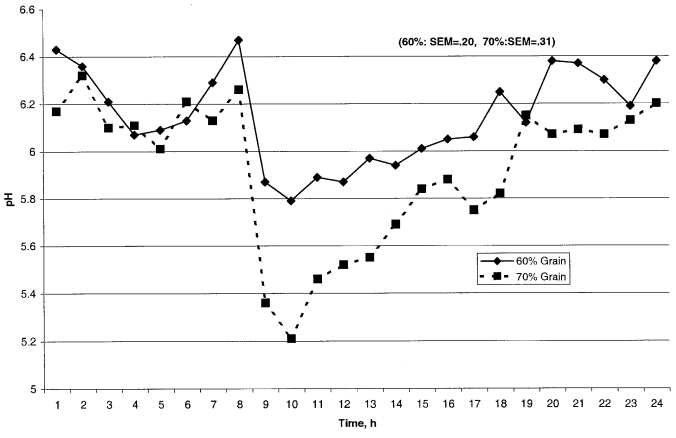

Figure 4. Effect of changing from 60 to 70% grain on diurnal ruminal pH.

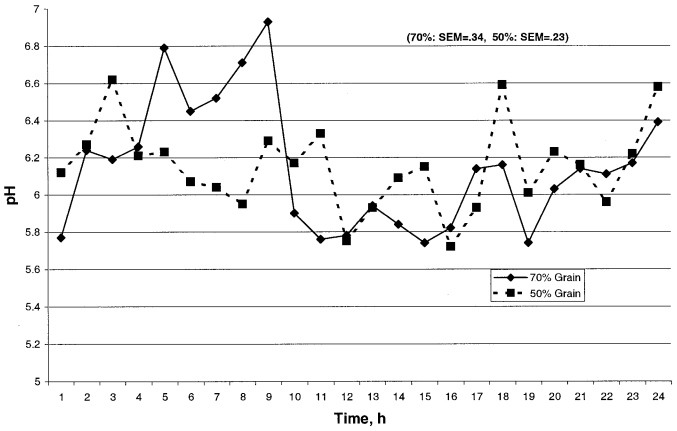

Figure 5. Effect of changing from 70 to 50% grain on diurnal ruminal pH.

pH and DMI have stabilized. When shifting from 70 to 50% grain, initially there is a reduction and variation in DMI; however, after the initial reduction, variation is low. As summarized by Nocek (1997), intake patterns are the most important indicator of subclinical acidosis. Fulton et al. (1979a) evaluated the effect of increasing concentrate from 35 to 90% for cattle fed either dry rolled corn or hard winter wheat. Intake profiles for cattle fed corn appeared to be consistent, indicating cattle were adjusting to high grain diets. However, cattle consuming wheat diets did not demonstrate increased intake. There was considerable variation in intake patterns in both groups, but the variation was not as great for cattle consuming corn until the 90% level was reached, at which time intake decreased dramatically. Cattle fed wheat diets experienced acidosis, with a dramatic decrease in intake for a few days. The cattle recovered and ate again, but were not able to adjust to the wheat. Extreme variation in diurnal pH will cause cattle to go off feed. Cattle that were fed wheat were unable to reduce their ruminal acid load by slowing their eating rate. Cattle began eating again when ruminal pH reached a level >5.6. In another study, Fulton et al. (1979b) showed that when ruminal pH was manually adjusted to >5.6 for cows consuming wheat diets, intake patterns were more similar to those consuming corn. Therefore, it appears that for beef cattle, ruminal pH should be maintained >5.6 to minimize intake reduction associated with subclinical acidosis. Nordlund et al. (1995) suggested similar ruminal pH thresholds for dairy cattle. The present study would support that as ruminal pH is reduced as a result of increased grain intake. Dry matter intake is quite varied for up to a week postchange. However, pH appears to decrease in diurnal variation, with a resultant stabilization of DMI as the animal adapts to the increased grain percentage. Although this adaptation process appears to take place, the long-term physiological effects of high grain levels are most likely deleterious and require further evaluation.

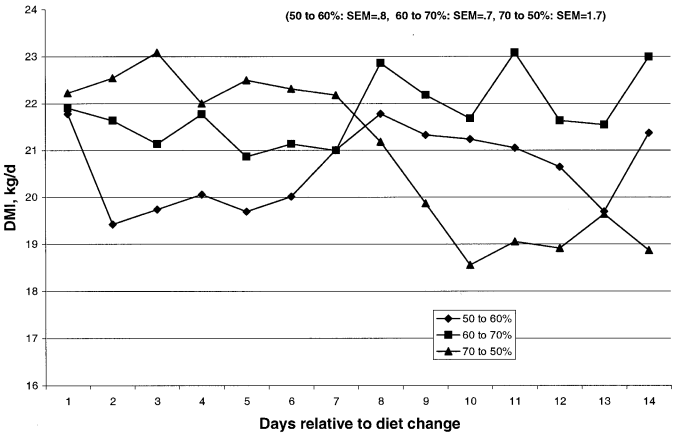

Figure 6. Effect of changing grain levels on daily DMI profiles.

CONCLUSIONS

The indwelling probe methodology described in this experiment can be used to accurately measure diurnal variation in ruminal pH. A probe shield should not be used unless it has spaces large enough to allow particulate matter to flow freely around the face of the probe. Cleansing and standardization of the probe once daily is adequate to maintain sensitivity. Cows consuming 50, 60, or 70% grain diets exhibited different diurnal ruminal pH profiles, with the 70% grain diet having a greater decrease in pH postfeeding and maintaining a longer duration under pH 6 compared with cows being fed a 50% grain diet. Cows switched from 50 to 60 and from 60 to 70% grain in one day exhibited an increased amount of variation in diurnal pH and daily intake for at least 1 wk after the switch. However, cows appeared to adapt with time, and variation in DMI pattern was reduced.

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