

Frequency of concentrate supplementation for cattle fed barley straw. 2. Ruminal dilution rates, pH and metabolite concentrations

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Tellier, R. C., Mathison, G. W., Okine, E. K., McCartney, D. and Soofi-Siawash, R. 2004. **Frequency of concentrate supplementation for cattle fed barley straw. 2. Ruminal dilution rates, pH and metabolite concentrations.** Can. J. Anim. Sci. **84**: 467–479. Five ruminally cannulated crossbred steers (474 ± 30 kg) were fed diets containing 70% barley straw and 30% concentrate in an unbalanced 5 × 5 Latin square design experiment to investigate the effects of frequency of feeding concentrate (daily, alternate days or every third day) with different dietary protein concentrations (7.9 and 11.5%) on ruminal liquid and particulate dilution rates, pH and metabolite concentrations. Dilution rates of cobalt-ethylene diamine tetraacetic acid (Co-EDTA) and chromium-mordanted fibre did not differ when low- and high-protein concentrates were fed. Supplemental protein increased ($P < 0.05$) mean ruminal ammonia concentrations (3.3 vs. 1.6 mM), but had no influence on ruminal concentrations of organic acids. Frequency of concentrate feeding had no influence on dilution rate of digesta markers, pH or on mean ruminal concentrations of ammonia, lactic acid, propionic acid, isobutyric acid, valeric acid and isovaleric acid. Mean concentrations of total volatile fatty acids, acetic acid and butyric acid were, however, reduced ($P < 0.05$) when steers were fed concentrates on alternate days. The reduced ($P < 0.01$) ruminal acetic:propionic acid ratios observed in steers fed concentrates on alternate days were consistent with reduced heat productions. Positive relationships ($P < 0.05$) were obtained between heat production of steers and ruminal concentrations of isobutyric and valeric acids. It was concluded that the low-protein diet did not provide sufficient protein to meet microbial requirements and that under controlled feeding conditions cattle can be fed concentrate on alternate days or every third day without the occurrence of lactic acidosis. Additional research is warranted to examine the relationships between reduced heat production of steers fed concentrates on alternate days and ruminal concentration and production of organic acids.

Key words: Cattle, straw, protein, feeding frequency, rumen metabolites, lactic acid

Tellier, R. C., Mathison, G. W., Okine, E. K., McCartney, D. et Soofi-Siawash, R. 2004. **Fréquence des apports de concentré pour les bovins nourris de paille d'orge. 2. Taux de dilution, pH et concentration des métabolites dans le rumen.** Can. J. Anim. Sci. **84**: 467–479. Cinq bouvillons hybrides (474 ± 30 kg) canulés au rumen ont reçu un régime composé à 70 % de paille d'orge et à 30 % de concentré dans le cadre d'une expérience en carré latin 5 × 5 non équilibré qui devait préciser les effets de la fréquence à laquelle un concentré à teneur variable en protéines (7,9 et 11,5 %) était servi (tous les jours, un jour sur deux ou aux trois jours) sur les fluides, le taux de dilution des particules, le pH et la concentration des métabolites dans le rumen. Le taux de dilution du cobalt-EDTA et des fibres mordancées au chrome ne varie pas avec la teneur en protéines du concentré. Le supplément protéique accroît ($P < 0,05$) la concentration moyenne d'ammoniaque dans le rumen (3,3 c. 1,6 mM), mais n'influe pas sur la concentration des acides organiques. La fréquence à laquelle on sert le concentré n'agit pas sur le taux de dilution des marqueurs des digesta, sur le pH ni sur la concentration moyenne d'ammoniaque, d'acide lactique, d'acide propionique, d'acide isobutyrique, d'acide valérique et d'acide isovalérique dans le rumen. Toutefois, on remarque une baisse ($P < 0,05$) de la concentration moyenne des acides gras volatils, de l'acide acétique et de l'acide butyrique quand on donne le concentré aux bouvillons un jour sur deux. Le rapport plus faible ($P < 0,01$) entre l'acide acétique et l'acide propionique observé chez les sujets recevant le concentré un jour sur deux est cohérent avec la production réduite de chaleur. Il existe une corrélation positive ($P < 0,05$) entre la production de chaleur et la concentration d'acide isobutyrique et valérique dans le rumen. On en déduit que la ration peu protéique ne procure pas assez de protéines pour subvenir aux besoins de la microflore et que, dans des conditions contrôlées, on pourrait fournir du concentré un jour sur deux ou sur trois aux bouvillons, sans crainte d'acidose lactique. Il conviendrait d'entreprendre d'autres recherches pour élucider les liens entre la plus faible production de chaleur observée chez les animaux recevant le concentré un jour sur deux et la concentration ou production d'acides organiques dans le rumen.

Mots clés: Bovins, paille, protéines, fréquence de l'alimentation, métabolites du rumen, acide lactique

An Alberta study found that provision of winter feed represented approximately 33% (range 17 to 48%) of input costs in cow-calf production systems in 1998 (Alberta

Agriculture, Food and Rural Development 2003). Although feeding straw can reduce the cost of feeding pregnant cows in the winter, there are major concerns with feeding straw

Abbreviations: Co-EDTA, cobalt-ethylene diamine tetraacetic acid; **Low-1**, low-protein concentrate fed daily; **Low-2**, low-protein concentrate fed on alternate days; **High-1**, high-protein concentrate fed daily; **High-2**, high-protein concentrate fed on alternate days; **High-3**, high-protein concentrate fed every third day; **VFA**, volatile fatty acid

including low voluntary intake, low protein content, poor digestibility, low mineral and vitamin concentrations, and slow passage rate (Anderson 1978). Provision of supplemental feeds to straw-based diets to increase intakes of digestible energy and protein is desirable. However, these supplements are expensive; thus the feasibility of reducing frequency of feeding concentrates and the protein content of diets has been examined (Tellier et al. 2004).

The results obtained by Tellier et al. (2004) in a companion study raised some important questions. First, although lactate did not accumulate when the frequency of feeding concentrates was reduced to less than once daily in the study of Beatty et al. (1994), lactic acidosis is of such importance that the effect of alternate day feeding of concentrates on ruminal lactate concentrations needs to be re-examined. Second, additional information on ruminal parameters is required to aid in our understanding of why less-frequent feeding of concentrates did not negatively affect intake or digestibility of diets containing 70% straw. Third, it is important to determine if the 4% reduction in heat production in steers fed concentrates on alternate days is related to differences in metabolites available for absorption from the rumen. In this regard ruminal acetic to propionic acid ratios are of particular interest because decreases in frequencies of feeding are associated with reductions in ruminal acetic to propionic acid ratios (Froetschel et al. 1990), which in turn have been associated with improvements in efficiency of energy use (Van Soest 1994). Finally, there is the need for additional within-day data concerning ruminal metabolite concentrations to assist in development and evaluation of computer models such as those developed by Grathwol and Reichl (1995), which have incorporated information on frequency of feeding.

The objectives of this study were to test the hypotheses that: (1) lactic acid accumulations in the rumen are not excessive in steers fed concentrate with either low or high protein levels every second day or concentrate with a high protein level every third day, (2) steers were able to maintain sufficient control of the ruminal environment even with an alternate-day feeding regimen so that digestion of straw was not adversely affected, and (3) the reduction in heat production noted with steers fed concentrates on alternate days was related to ruminal concentrations of fermentation products.

Additional observations on voluntary straw intake, digestion, and heat production in steers with reduced frequency of feeding have also been obtained (Tellier et al. 2004).

MATERIALS AND METHODS

Animals and Feeds

Five crossbred steers (474 ± 30 kg) were used in the 5×5 Latin square experiment to examine the effects of frequency of feeding concentrates with straw-based diets on ruminal dilution rates and metabolite concentrations. The experiment was conducted at the Laird McElroy Environmental and Metabolic Center, University of Alberta, Edmonton Research Station, Edmonton, Alberta, Canada, with steers being cared for in accordance with the guidelines of the Canadian Council of Animal Care (1993).

Steers were offered barley straw (Lacombe, six-row) ad libitum along with five different concentrate treatments as follows: (1) low-protein concentrate fed daily (Low-1), (2) low-protein concentrate fed on alternate days at two times the daily rate (Low-2), (3) high-protein concentrate fed daily (High-1), (4) high-protein concentrate fed on alternate days at two times the daily rate (High-2), (5) high-protein concentrate fed every third day at three times the daily rate (High-3). Straw, control concentrate and high-protein concentrate contained 5.3, 14.1 and 24.6% crude protein, respectively. The amount of concentrate given was calculated to be 30% of the total as-fed feed intake of the previous week. Straw was offered ad libitum with the objective of maintaining approximately 10% weighback. Concentrates were offered at 0900 on the days that they were provided.

There was a 14-d adaptation period prior to sampling. Voluntary feed intake measurements were obtained during days 15 to 20. During this time nylon bags were also incubated in the rumen (Tellier et al. 2004). During a 72-h period between day 23 and day 31 digesta markers were administered for determination of ruminal fluid and particulate dilution times and rumen samples were taken for determination of metabolite concentrations. Additional information concerning steers and their feed is available in Tellier et al. (2004).

Liquid and Particle Markers

Cobalt-ethylene diamine tetraacetic acid, prepared according to the procedure of Uden et al. (1980) was used as a liquid marker. Co-EDTA was introduced into the rumen just before the morning feeding on the day when concentrate was offered. Samples for Co analysis were taken at 0, 2, 4, 6, 8, 10, 12, 24, 27, 30, 36 and 48 h after this dosing. Co-EDTA was also administered in the same manner 48 h after the first dose to ensure that concentrations would be sufficiently high to obtain measurements on day 3 of the period. Samples were taken through the rumen cannula at 0, 2, 4, 6, 8, 10, 12 and 24 h after this dose. Visual inspection of the graphed data confirmed that, with few exceptions, complete mixing of the marker with the ruminal contents was achieved within 2 h of administering the marker. Ruminal fluid dilution rates were calculated from $(\ln \text{ initial Co concentration} - \ln \text{ final Co concentration}) / (\text{time interval})$ when only two samples were available during the time interval and by the slope of the line of the regression of \ln Co concentrations with time when more than two samples were available during the time interval. Cobalt concentrations were too low for accurate determinations of ruminal dilution rates at 48 h, so rates were only calculated for the periods 2 to 12, 12 to 24, and 24 to 36 h after feeding. Observations available for calculation of rates in each of these time intervals were 6, 2, and 4, respectively. Estimated liquid pool size (kg) was calculated as: $(\text{g marker dose}) / (\text{extrapolated initial concentration of the marker in g kg}^{-1})$. Outflow from the rumen (kg h^{-1}) was calculated as: $\text{liquid pool size (kg)} \times (\text{fractional dilution rate h}^{-1})$.

Chromium-mordanted fibre was prepared by washing ground (3-mm screen) barley straw particles in a laundry machine in 95°C water with a commercial detergent, fol-

lowed by three rinses. The residue was soaked overnight in acetone, rinsed in water until the wash water was clear, then the residue was dried at 65°C for 48 h. These washed particles were mordanted with chromium according to the procedure of Uden et al. (1980). Straw particles mordanted to chromium were administered on the day on which concentrate was offered. Samples for Cr analysis were taken at 0, 3, 6, 9, 12, 18, 26, 30, 36, 50 and 72 h after administration of the marker. Visual inspection of the graphed data indicated that complete mixing of the marker was not achieved until the 12-h sample. Dilution rates of particles were thus calculated in a similar manner as Co dilution rates on the basis of data obtained at least 12 h after administering the marker. Rates of dilution of chromium-mordanted particles were determined for 12 to 50 h for all five treatments and for 12 to 72 h and 50 to 72 h for the High-3 treatment regimen. In addition, for the Low-2 and High-2 treatments comparisons were made between the day of concentrate feeding (50 to 72 h after dosing) and the day after concentrate feeding (24 to 50 h after dosing). Ruminal particulate pool size and outflow were calculated in a similar manner as for fluid.

Ruminal pH and Organic Acids

Rumen fluid samples were taken through the rumen cannula at 0, 3, 9, 13, and 21 h after normal concentrate feeding times. Approximately 100 mL of rumen fluid was obtained using a 60-mL syringe attached to a polyethylene tube weighted with a stainless steel rumen bullet. The pH was measured immediately upon sampling with a pH meter (Expandomatics SS2, Beckman Instruments, Fullerton, CA). Rumen samples for lactate and volatile fatty acid (VFA) analysis were prepared by adding 1 mL of 25% phosphoric acid to 4 mL of rumen fluid. Samples for ammonia determination were saved in a separate vial. All samples were frozen at -20°C until they could be analyzed.

Chemical Analyses

Lactic acid and VFA analyses were performed by gas-liquid chromatography using a 30 m stable wax DA glass capillary column in a Varian Model 3600 chromatograph (Varian, Walnut Creek, CA) according to the methods described by Khorasani et al. (1996). Ammonia-N was analyzed according to the method of Fawcett and Scott (1960).

Chromium and cobalt contents in ruminal and faecal samples were determined by procedures outlined in Okine et al. (1989).

Statistical Analyses

Ruminal liquid and particle dilution rates and metabolite concentrations (including overall mean concentrations calculated for each animal within periods across all days and sampling times) were analyzed as a 5 × 5 Latin square design using the GLM procedure of SAS (SAS Institute, Inc. 1988). Treatments ($n = 5$), animals ($n = 5$) and periods ($n = 5$) were the main sources of variation. Data from the second period were not available for ruminal dilution rates. Means were separated using Student-Newman-Keul's test (SAS Institute, Inc. 1988). Comparisons between dietary protein content of con-

centrates and between frequencies of feeding concentrates were examined using the GLM procedure of SAS (SAS Institute, Inc. 1988) with only the Low-1, Low-2, High-1, and High-2 treatments being considered in these comparisons. Dilution rates and metabolite concentrations were also examined by a repeated measures analysis (SAS Institute, Inc. 1988), with time as the repeated measure. In addition, within treatments in which concentrates were fed every second or third day, the GLM procedure of SAS (SAS Institute, Inc. 1988) was used to compare dilution rates and metabolite concentrations for days on which concentrates were fed with days on which concentrates were not fed. The sources of variation in this analysis were day after concentrate feeding ($n = 2$ or 3), and animals ($n = 5$).

RESULTS

Ruminal Dilution Rates

Concentrations of Co-EDTA were too low for accurate measurements at 48 h after dosing; thus, dilution rates were only available up to 36 h. No treatment differences were detected in Co-EDTA dilution rates 2 to 24 h, 2 to 12 h or 12 to 24 h after dosing (Table 1). However, steers fed the low-protein concentrate tended ($P = 0.09$) to have a 27% higher ruminal fluid dilution rate than steers fed the high-protein concentrate during the 24- to 36-h period after dosing. Dietary treatment, dietary protein and frequency of feeding had no effect on estimated liquid pool size or daily fluid outflow.

Dietary treatment regimen, protein level and frequency of feeding concentrate had no influence on the dilution rate of chromium-mordanted particles (Table 1). Dietary treatment, dietary protein and frequency of feeding had no effect on estimated particulate pool size or ruminal dilution rate of the particles for 12 to 50 h after feeding concentrate. Fractional dilution rates of chromium-mordanted fibre in steers on the High-3 treatment were 0.027 and 0.035 h⁻¹, for the time periods 12 to 72 h and 50 to 72 h, respectively (corresponding standard errors were 0.0046 and 0.0062).

Dilution rate of Co-EDTA was also negatively correlated with ruminal acetic acid, isovaleric acid and total VFA concentrations ($r = -0.41$, -0.54 , and -0.41 , respectively). Ruminal dilution of chromium-mordanted fibre tended ($P < 0.1$) to be negatively correlated with propionate ($r = -0.40$) and total VFA concentrations ($r = -0.38$) and positively correlated ($P < 0.05$) with 48 and 72 h dry matter disappearance of straw in the rumen ($r = 0.46$ and 0.54 , respectively).

Ruminal Ammonia Concentrations

The mean ruminal ammonia concentration for all treatments and times was 2.70 mM (Table 2). When the high-protein concentrate was fed daily or on alternate days, mean ruminal ammonia concentrations were higher ($P < 0.01$) than the concentrations when the low-protein concentrate was fed (Table 2). There was a trend ($P < 0.1$) for overall ruminal ammonia concentrations to be 14% lower in steers fed concentrate on alternate days (2.28 mM) in comparison with those fed concentrates daily (2.65 mM).

Ruminal ammonia concentrations peaked 3 h after feeding whether concentrate was provided or not (Fig. 1).

Table 1. Effect of dietary regimen^z on fractional dilution rate (per h) of ruminal markers

| Hours after dosing with marker | Protein level fed daily or every 2 d contrast | | | | Frequency of feeding Concentrate contrast | | | | Individual treatments | | | | | | |
|---------------------------------|---|-------|-----------------|----------------|---|--------|-----------------|----------------|-----------------------|-------|--------------|-------|-------|-----------------|----------------|
| | Low | High | SE ^y | P ^x | 1 day | 2 days | SE ^y | P ^x | Low protein | | High protein | | | SE ^y | P ^x |
| | | | | | | | | | 1 d | 2 d | 1 d | 2 d | 3 d | | |
| <i>Cobalt-EDTA</i> | | | | | | | | | | | | | | | |
| 2 to 24 h | 0.085 | 0.092 | 0.0073 | 0.68 | 0.085 | 0.092 | 0.0073 | 0.55 | 0.084 | 0.086 | 0.086 | 0.098 | 0.077 | 0.0104 | 0.81 |
| 2 to 12 h ^w | 0.115 | 0.108 | 0.0103 | 0.62 | 0.117 | 0.105 | 0.0103 | 0.58 | 0.133 | 0.091 | 0.101 | 0.115 | 0.108 | 0.0094 | 0.17 |
| 12 to 24 h ^w | 0.068 | 0.082 | 0.0068 | 0.47 | 0.071 | 0.080 | 0.0068 | 0.90 | 0.060 | 0.076 | 0.079 | 0.084 | 0.054 | 0.0083 | 0.23 |
| 24 to 36 h ^w | 0.133 | 0.105 | 0.0078 | 0.09 | 0.123 | 0.113 | 0.0078 | 0.32 | 0.136 | 0.129 | 0.109 | 0.101 | 0.087 | 0.0098 | 0.08 |
| <i>Chromium-mordanted fibre</i> | | | | | | | | | | | | | | | |
| 12 to 50 h | 0.034 | 0.029 | 0.0032 | 0.43 | 0.031 | 0.033 | 0.0033 | 0.87 | 0.034 | 0.035 | 0.027 | 0.032 | 0.023 | 0.0030 | 0.21 |

^zLow or high-protein concentrate fed daily, every 2 d, or every 3 d.^yStandard error mean is based upon results from four animals per mean for individual treatments and eight animals per mean for contrasts.^xProbability.^wProbability of treatment, time and treatment × time effects were 0.32, <0.01 and 0.07, respectively, over the three time intervals by repeated measures analysis.**Table 2. Effect of dietary regimen^z on ruminal ammonia and lactate concentrations and ruminal pH**

| Hours after feeding | Protein concentration contrast for steers fed daily or every 2 d | | | | Frequency of feeding contrast for steers fed concentrate daily or every 2 d | | | | Individual treatments | | | | | | |
|---------------------------------|--|-------------------|-----------------|----------------|---|--------|-----------------|----------------|-----------------------|-------------------|--------------------|--------------------|--------------------|-----------------|----------------|
| | Low | High | SE ^y | P ^x | 1 day | 2 days | SE ^y | P ^x | Low protein | | High protein | | | SE ^y | P ^x |
| | | | | | | | | | 1 d | 2 d | 1 d | 2 d | 3 d | | |
| <i>Ammonia^w (mM)</i> | | | | | | | | | | | | | | | |
| Overall | 1.64 ^b | 3.29 ^a | 0.139 | <0.01 | 2.65 | 2.28 | 0.139 | 0.08 | 1.70 ^b | 1.59 ^b | 3.60 ^a | 2.97 ^a | 3.28 ^a | 0.20 | <0.01 |
| 0 | 1.96 ^b | 2.57 ^a | 0.139 | 0.01 | 2.47 | 2.06 | 0.139 | 0.06 | 2.10 ^{ab} | 1.82 ^b | 2.84 ^a | 2.30 ^{ab} | 2.34 ^{ab} | 1.01 | 0.04 |
| 3 | 3.24 ^b | 8.71 ^a | 0.452 | <0.01 | 6.45 | 5.50 | 0.452 | 0.16 | 3.07 ^c | 3.41 ^c | 9.83 ^a | 7.59 ^b | 6.36 ^b | 0.64 | <0.01 |
| 9 | 0.81 ^b | 2.16 ^a | 0.192 | <0.01 | 1.32 | 1.66 | 0.192 | 0.24 | 0.59 ^b | 1.02 ^b | 2.03 ^a | 2.28 ^a | 2.90 ^a | 0.27 | <0.01 |
| 13 | 0.70 | 1.33 | 0.276 | 0.13 | 1.21 | 0.82 | 0.276 | 0.34 | 0.85 ^b | 0.55 ^b | 1.58 ^{ab} | 1.09 ^b | 2.62 ^a | 0.39 | 0.02 |
| 21 | 1.47 ^b | 2.38 ^a | 0.143 | <0.01 | 2.12 | 1.72 | 0.143 | 0.07 | 1.62 ^{bc} | 1.31 ^c | 2.62 ^a | 2.14 ^{ab} | 2.81 ^a | 0.20 | <0.01 |
| <i>pH^w</i> | | | | | | | | | | | | | | | |
| Overall | 6.88 | 6.86 | 0.015 | 0.89 | 6.88 | 6.89 | 0.015 | 0.82 | 6.90 | 6.87 | 6.86 | 6.90 | 6.86 | 0.021 | 0.56 |
| 0 | 7.02 | 7.05 | 0.019 | 0.21 | 7.02 | 7.05 | 0.019 | 0.23 | 7.02 | 7.01 | 7.02 | 7.08 | 7.03 | 0.027 | 0.19 |
| 3 | 6.96 | 7.00 | 0.023 | 0.34 | 6.95 | 7.01 | 0.023 | 0.08 | 6.93 | 6.99 | 6.97 | 7.02 | 7.00 | 0.032 | 0.39 |
| 9 | 6.73 | 6.62 | 0.042 | 0.10 | 6.68 | 6.68 | 0.042 | 0.97 | 6.72 | 6.73 | 6.63 | 6.61 | 6.67 | 0.059 | 0.55 |
| 13 | 6.66 | 6.63 | 0.031 | 0.56 | 6.67 | 6.62 | 0.031 | 0.24 | 6.72 | 6.59 | 6.62 | 6.64 | 6.53 | 0.043 | 0.09 |
| 21 | 6.94 | 6.95 | 0.016 | 0.73 | 6.95 | 6.94 | 0.016 | 0.50 | 6.96 | 6.92 | 6.95 | 6.95 | 6.93 | 0.023 | 0.75 |
| <i>Lactate^w (mM)</i> | | | | | | | | | | | | | | | |
| Overall | 0.15 | 0.17 | 0.060 | 0.82 | 0.15 | 0.17 | 0.060 | 0.13 | 0.15 | 0.16 | 0.16 | 0.18 | 0.34 | 0.085 | 0.48 |
| 0 | 0.10 | 0.12 | 0.012 | 0.44 | 0.11 | 0.10 | 0.012 | 0.15 | 0.09 | 0.11 | 0.13 | 0.11 | 0.15 | 0.017 | 0.19 |
| 3 | 0.13 | 0.19 | 0.026 | 0.12 | 0.14 | 0.18 | 0.026 | 0.68 | 0.13 | 0.13 | 0.16 | 0.23 | 0.21 | 0.036 | 0.21 |
| 9 | 0.22 | 0.28 | 0.300 | 0.88 | 0.24 | 0.25 | 0.300 | 0.15 | 0.20 | 0.22 | 0.27 | 0.28 | 1.08 | 0.425 | 0.55 |
| 13 | 0.23 | 0.18 | 0.050 | 0.51 | 0.18 | 0.24 | 0.050 | 0.09 | 0.21 | 0.26 | 0.15 | 0.22 | 0.34 | 0.072 | 0.43 |
| 21 | 0.14 | 0.14 | 0.022 | 0.98 | 0.13 | 0.14 | 0.022 | 0.62 | 0.13 | 0.13 | 0.13 | 0.14 | 0.16 | 0.032 | 0.98 |

^zLow or high-protein concentrate fed daily, every 2 d, or every 3 d.^yStandard error mean is based upon five animals per mean for individual treatments and 10 animals per mean for contrasts.^xProbability.^wFor repeated measures analyses SE and probabilities of diet, time of sampling and diet × time were 0.48, <0.01, <0.01 and <0.01; 0.051, 0.56, <0.01, 0.10; and 0.209, 0.48, 0.11 and 0.65 for ammonia, pH and lactate, respectively.^{a-c} Means not followed by the same letter differ ($P < 0.05$).

There were no differences in either overall mean (1.38 vs. 1.80 mM) or peak (3.46 vs. 3.36 mM) ruminal ammonia concentrations on days when concentrate was provided compared with days when concentrate was not provided for the Low-2 diet (Fig. 1). However, at 13 h after feeding, ruminal ammonia concentrations were four times higher ($P < 0.01$) on days when steers were not fed concentrate than on days when steers were fed concentrates (0.89 vs. 0.21 mM). In contrast, on days when concentrate was fed with the High-2 diet, overall and peak ruminal ammonia concentrations were 173 and 347% ($P < 0.05$), respectively, of values on days when concentrate was not fed

(Fig. 1). When steers were given the High-3 diet, mean ruminal ammonia concentrations were 5.1, 2.6 and 2.1 mM ($P = 0.01$) on day 0, 1 and 2 after concentrate feeding, respectively (Fig. 1). On this dietary regimen, the highest ammonia concentration (11.2 mM at 3 h after feeding) occurred on the day steers were fed concentrate, and this concentration was 287% ($P = 0.01$) of the concentration on days when no concentrate was fed (3.9 mM both days).

Ruminal pH

Overall ruminal pHs were 6.90, 6.87, 6.86, 6.90 and 6.86 for the Low-1, Low2, High-1, High-2, and High-3 diets, respec-

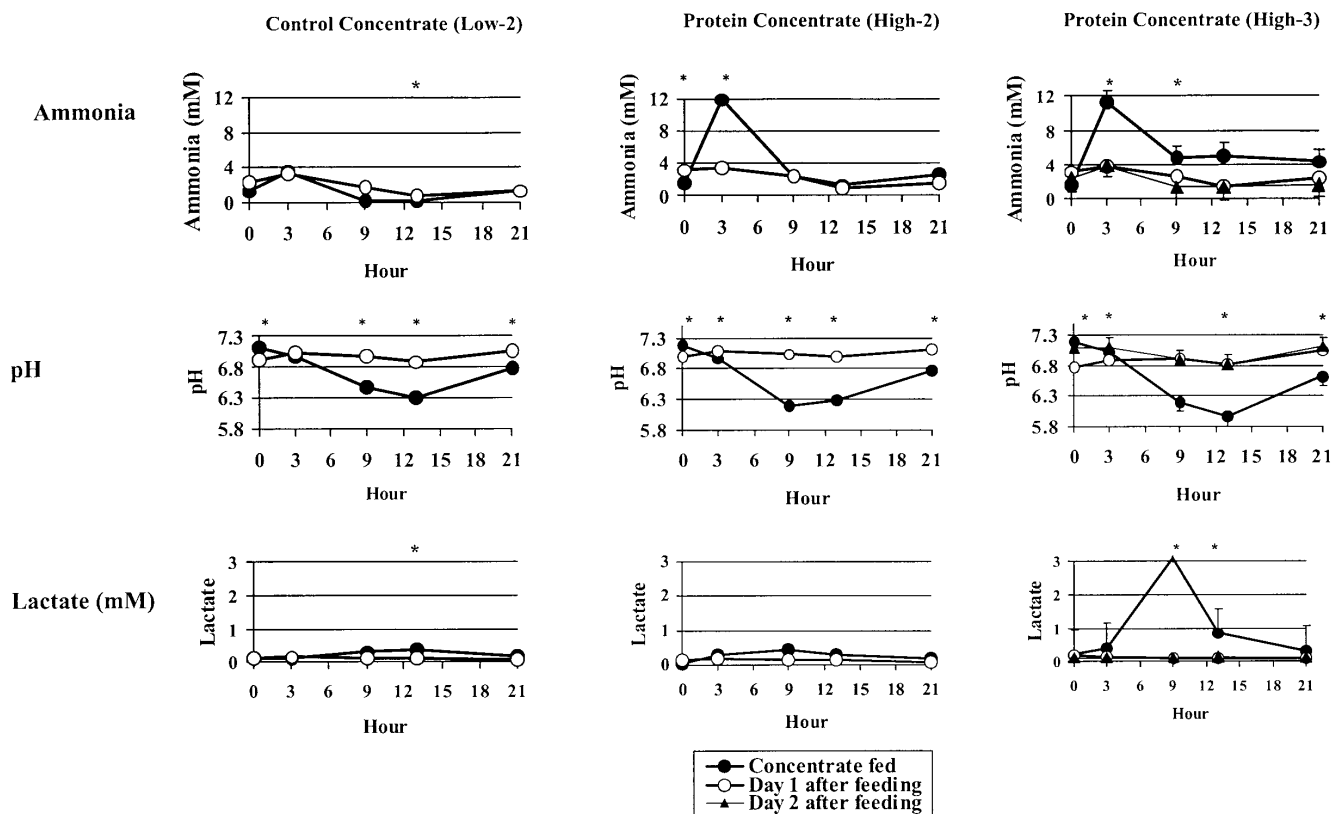


Fig. 1. Comparison of the effect of day of concentrate feeding with days after concentrate feeding (day 1 or day 2), on ruminal pH and ammonia and lactic acid concentrations. Low-2, High-2 and High-3 refer to low-protein concentrates given every second day, high-protein concentrates given every second day, and high-protein concentrates given every third day, respectively. Vertical bars are pooled standard errors. *Indicate differences ($P < 0.05$) between dietary treatments at that specific time.

tively (mean 6.88; Table 2). No differences in pH were detected between individual dietary treatments, nor did dietary protein levels or frequency of providing concentrate influence ruminal pH. Ruminal pH was, however, influenced by time after feeding ($P < 0.01$) and there was a diet \times time interaction ($P = 0.1$). The lowest pH (mean 6.62) occurred 13 h after feeding and the highest (mean 7.03) just before feeding.

The mean daily pH on days when concentrate was fed was lower ($P < 0.01$) than the pH on days when no concentrate was fed for Low-2 (6.75 vs. 6.99), High-2 (6.72 vs. 7.07), and High-3 (6.63 vs. 6.93 and 7.03) diets (Fig. 1). The lowest pH measured at any time of the day was a pH of 5.96, which was recorded 13 h after concentrate was fed in the High-3 feeding regimen.

Lactic Acid Concentrations

The mean concentration of lactic acid in the rumen of the steers was 0.22 mM (Table 2). Dietary regimen, time and diet \times time did not influence overall lactate concentrations ($P = 0.48, 0.11$ and 0.65 , respectively), nor were any differences detected between treatments at different times after feeding. However, the numerically highest mean daily lactate concentration (1.08 mM at 9 h after feeding) occurred with the High-3 diet.

For the Low-2 diet, the mean lactate concentration on concentrate feeding days was 167% ($P = 0.02$) of the concentration on days when concentrate was not fed (Fig. 1). With this dietary regimen, peak lactate concentrations (0.38 mM) occurred 13 h after concentrate feeding, whereas peak concentrations (0.13 mM) occurred at 3 h after feeding on days when concentrates were not fed (Fig. 1). With the High-2 diet, the mean ruminal lactate concentration was 0.24 mM on days when concentrate was fed compared with 0.13 mM on days when no concentrate was fed ($P = 0.11$). When the High-3 diet was fed, mean lactate concentrations were 0.82, 0.12 and 0.10 mM ($P = 0.22$) on the day concentrate was fed, the first day after feeding concentrate and the second day after feeding concentrate (Fig. 1), respectively.

Volatile Fatty Acid Concentrations

Total Volatile Fatty Acids

The mean concentration of VFA in the rumen across all treatments was 66.7 mM. Dietary protein levels had no influence on VFA concentrations when concentrates were fed daily or on alternate days, nor were differences detected between the five individual treatments (Table 3). However, mean VFA concentrations were 8% lower ($P = 0.04$) in the rumen of steers fed concentrate every second day than in

steers fed concentrates daily (65.1 vs. 70.4 mM; Table 3). Peak VFA concentrations on the day concentrates were fed did not differ substantially even though two and three times as much concentrate was given daily with the alternate or every third day feeding schedules. Ruminal VFA concentrations were affected ($P < 0.01$) by time after feeding, with peak concentrations being observed 9 to 13 h after feeding. There was no diet \times time interaction for this parameter.

With the Low-2 diet, total VFA concentrations were higher ($P < 0.01$) on days when concentrate was fed (72.7 mM) than on days when no concentrate was fed (57.5 mM), and concentrations were much more variable (Fig. 2). On days when concentrate was not fed, the highest VFA concentration was observed at 0 h, whereas peak VFA concentration was at 9 to 13 h when concentrate was fed. Similar results were obtained with the High-2 diet (Fig. 2). With the High-3 diet, overall VFA concentrations were 77.4, 65.5 and 56.3 mM on the day concentrate was fed, the first day after feeding, and the second day after feeding, respectively ($P = 0.03$; Fig. 2). VFA concentrations were lower ($P < 0.05$) at 0 and 3 h on the second day compared with the first day after feeding concentrate.

Acetic and Propionic Acids

The mean concentrations of acetic and propionic acids in this experiment were 47.6 and 12.3 mM, respectively. Dietary protein intake did not influence either overall acetic or propionic acid concentrations in the Low-1, Low-2, High-1 and High-2 diet comparisons ($P = 0.16$ and 0.69 , respectively; Table 3). Feeding concentrate on alternate days reduced ($P = 0.02$) overall acetic acid concentrations by 8% but had no influence on propionic acid (Fig. 2, Table 3). The alternate-day feeding regimen reduced ($P < 0.1$) mean acetic acid concentrations at all times except for 9 and 13 h, but did not influence propionic acid concentrations. When Low-1, Low-2, High-1 and High-2 diets were fed, ruminal concentrations of acetic and propionic acid were higher ($P < 0.05$) on days when concentrate was offered than on days when it was not for all times after 3 h from feeding (Fig. 2).

The mean acetic:propionic acid ratio in this experiment was 3.98. Protein level in the diet of steers that were fed concentrate once daily or on alternate days had no influence on the acetic:propionic acid ratios in the rumen (Table 4). Frequency of feeding concentrate did, however, affect the acetic:propionic acid ratio, with the mean ratio being 7% higher ($P < 0.01$) in animals fed concentrate daily (4.25) in comparison with animals fed concentrate on alternate days (3.97). The overall acetic:propionic acid ratio was lower (3.82; $P < 0.01$) in steers fed the High-3 diet than in steers fed concentrate daily (High-1), but ratios did not differ between the High-2 and High-3 feeding regimens. With the High-2 diet, mean acetic:propionic acid ratio was 4.2 on days when concentrates were not fed in comparison with 3.8 on days when concentrate was fed ($P < 0.05$). When the High-3 diet was fed, acetic:propionic acid ratios were 3.6, 3.8 and 4.1 ($P < 0.01$) on the day concentrate was fed, the day after concentrate was fed and the second day after concentrate was fed (Fig. 3).

Butyric, Isobutyric, Valeric and Isovaleric Acids

Protein content of diets had no influence on the concentrations of butyric, valeric or isovaleric acids when concentrates were fed daily or on alternate days.

The mean concentration of butyric acid in the rumen of the steers was 5.0 mM (Table 4). Overall ruminal concentrations of butyric acid were 20% higher ($P = 0.01$) in steers fed concentrate daily than in steers fed concentrate on alternate days. Butyric acid concentrations on the day of concentrate feeding were 152, 179 and 169% ($P < 0.01$) of concentrations on days when concentrate was not fed for the Low-2, High-2, and High-3 diets, respectively (Fig. 3). With the High-3 diet, overall ruminal butyric acid concentrations on the days when concentrate was fed were 185% of the concentrations on the second day after concentrate feeding.

The mean concentration of isobutyric acid in the rumen of the steers in this experiment was 0.58 mM (Table 4). No differences in ruminal concentrations of isobutyrate were detected between animals fed concentrates daily and on alternate days (Table 4). Similarly, there were no differences between the five dietary regimens at any sampling time. Mean overall isobutyric acid concentrations on the day of feeding concentrate were 108, 100, and 70% of concentrations on the day after feeding for steers on the Low-2, High-2 and High-3 regimens, respectively (Fig. 3). Only with the latter diet was the difference significant ($P < 0.01$). On the second day after feeding concentrate with the High-3 diet, mean isobutyric acid concentrations were similar to those found on the days concentrates were given (0.49 vs. 0.51 mM).

The ruminal concentration of valeric acid was 0.52 mM in this experiment (Table 5). Mean valeric acid concentrations were similar in steers fed concentrates daily and on alternate days, although at 3 h after feeding concentrations were 28% higher ($P < 0.01$) in steers fed concentrate daily (Table 5). Mean overall valeric acid concentrations on the day concentrates were fed were 161, 189 and 143% ($P < 0.05$) of ruminal concentrations the day after concentrates were fed for the Low-2, High-2 and High-3 treatments, respectively (Fig. 4). On the second day after feeding concentrate with the High-3 diet, valeric acid concentrations were only 50% of concentrations ($P = 0.02$) that measured on the days on which concentrate was fed.

The mean concentration of isovaleric acid in the rumen of the steers in this experiment was 0.75 mM (Table 5). There were no differences in isovaleric concentrations between animals fed concentrates daily or on alternate days, although there was a tendency ($P = 0.06$) for concentrations of this acid to be higher in daily fed animals at 3 h after feeding (0.80 vs. 0.70 mM; Table 5). There were no differences between the five dietary regimens at any sampling time (Table 5). Mean overall isovaleric acid concentrations on the day of feeding concentrate were 120, 112 and 84% ($P < 0.05$) of ruminal concentrations the day after feeding concentrates for the Low-2, High-2 and High-3 diets, respectively (Fig. 4). On the second day after feeding concentrate with the High-3 diet, isovaleric acid concentrations were similar to those found in animals fed concentrates daily (0.61 vs. 0.67 mM).

Table 3. Effect of dietary regimen^z on ruminal total volatile fatty acid concentration and concentration of acetic and propionic acids

| Hours after feeding | Protein concentration contrast for steers fed daily or every 2 d | | | | Frequency of feeding contrast for steers fed concentrate daily or every 2 d | | | | Individual treatments | | | | | | |
|--|--|-------|-----------------|----------------|---|--------|-----------------|----------------|-----------------------|-------|--------------|-------|-------|-----------------|----------------|
| | Low | | High | | 1 day | | 2 days | | Low protein | | High protein | | | | |
| | Low | High | SE ^y | P ^x | 1 d | 2 days | SE ^y | P ^x | 1 d | 2 d | 1 d | 2 d | 3 d | SE ^y | P ^x |
| <i>Total volatile fatty acids^w (mM)</i> | | | | | | | | | | | | | | | |
| Overall | 66.4 | 69.2 | 1.60 | 0.24 | 70.4b | 65.1b | 1.60 | 0.04 | 67.6 | 65.1 | 73.2 | 65.2 | 66.4 | 2.27 | 0.13 |
| 0 | 58.2 | 60.4 | 1.97 | 0.45 | 61.2 | 57.4 | 1.97 | 0.19 | 59.2 | 57.2 | 63.3 | 57.5 | 59.9 | 2.78 | 0.57 |
| 3 | 59.0 | 61.1 | 2.01 | 0.47 | 63.6b | 56.5b | 2.01 | 0.03 | 61.8 | 56.1 | 65.3 | 56.9 | 59.3 | 2.84 | 0.20 |
| 9 | 76.4 | 82.7 | 2.68 | 0.12 | 82.8 | 76.2 | 2.68 | 0.11 | 78.8 | 73.9 | 86.8 | 78.5 | 72.8 | 3.79 | 0.14 |
| 13 | 78.2 | 82.3 | 2.27 | 0.22 | 81.9 | 78.6 | 2.27 | 0.31 | 77.4 | 79.0 | 76.5 | 78.1 | 79.9 | 3.20 | 0.32 |
| 21 | 66.6 | 67.4 | 2.07 | 0.78 | 69.7 | 64.4 | 2.07 | 0.10 | 68.5 | 64.8 | 78.9 | 64.0 | 65.6 | 2.92 | 0.47 |
| <i>Acetic acid^w (mM)</i> | | | | | | | | | | | | | | | |
| Overall | 47.6 | 49.9 | 1.11 | 0.16 | 50.8b | 46.6b | 1.11 | 0.02 | 48.5ab | 46.6b | 53.1b | 46.6b | 46.6b | 1.56 | 0.05 |
| 0 | 42.0 | 43.7 | 1.37 | 0.40 | 44.6 | 41.2 | 1.37 | 0.10 | 43.0 | 41.1 | 46.3 | 41.2 | 42.4 | 1.94 | 0.37 |
| 3 | 42.5 | 44.1 | 1.37 | 0.43 | 45.9b | 40.7b | 1.37 | 0.02 | 44.5 | 40.5 | 47.2 | 40.9 | 42.5 | 1.94 | 0.15 |
| 9 | 54.3b | 59.6b | 1.76 | 0.05 | 59.1 | 54.8 | 1.76 | 0.11 | 55.5 | 53.1 | 62.7 | 56.6 | 51.7 | 2.49 | 0.07 |
| 13 | 55.2 | 59.0 | 1.51 | 0.10 | 58.7 | 55.6 | 1.51 | 0.17 | 54.7 | 55.8 | 62.6 | 55.4 | 55.2 | 2.13 | 0.10 |
| 21 | 47.7 | 48.6 | 1.51 | 0.70 | 50.5b | 45.8b | 1.51 | 0.04 | 49.4 | 46.1 | 51.6 | 45.5 | 45.5 | 2.13 | 0.22 |
| <i>Propionic acid^w (mM)</i> | | | | | | | | | | | | | | | |
| Overall | 12.0 | 12.2 | 0.39 | 0.69 | 12.1 | 12.1 | 0.39 | 0.91 | 11.9 | 12.1 | 12.4 | 12.1 | 12.6 | 0.55 | 0.90 |
| 0 | 10.1 | 10.4 | 0.38 | 0.55 | 10.1 | 10.4 | 0.38 | 0.67 | 9.9 | 10.3 | 10.4 | 10.5 | 11.1 | 0.53 | 0.60 |
| 3 | 10.3 | 10.5 | 0.46 | 0.73 | 10.7 | 10.1 | 0.46 | 0.39 | 10.6 | 10.0 | 10.8 | 10.2 | 10.9 | 0.65 | 0.83 |
| 9 | 14.4 | 14.8 | 0.70 | 0.65 | 15.0 | 14.2 | 0.70 | 0.45 | 14.8 | 14.0 | 15.2 | 14.5 | 13.9 | 0.99 | 0.88 |
| 13 | 14.8 | 15.0 | 0.62 | 0.76 | 14.6 | 15.2 | 0.62 | 0.58 | 14.3 | 15.3 | 15.0 | 15.1 | 15.6 | 0.88 | 0.88 |
| 21 | 12.0 | 11.9 | 0.43 | 0.87 | 11.9 | 12.0 | 0.43 | 0.79 | 11.9 | 12.1 | 11.8 | 12.0 | 12.6 | 0.61 | 0.92 |

^zLow or high-protein concentrate fed daily, every 2 d, or every 3 d.

^yStandard error mean is based upon five animals per mean for individual treatments and 10 animals per mean for contrasts.

^xProbability.

^wFor repeated measures analyses SE and probabilities of diet, time of sampling and diet × time were 5.55, 0.13, <0.01 and 0.68; 3.83, 0.05, <0.01, and 0.54; and 1.36, 0.90, <0.01 and 0.85 for total volatile fatty acids, acetic acid and propionic acid, respectively.

a-c Means not followed by the same letter differ ($P < 0.05$).

Relationships with Heat Production

Using data from Tellier et al. (2004), daily heat production (kJ kg^{-0.75}) was not correlated ($r = 0.10$; $n = 24$) with daily digestible energy intake (kJ kg^{-0.75}). Similarly, heat production was not related with acetic acid, propionic acid or total VFA ruminal concentrations ($r = 0.21$, 0.09 and 0.20 , respectively). However, heat production was positively correlated ($P < 0.05$) with isobutyrate ($r = 0.41$) and valerate ($r = 0.44$) concentrations. These relationships, along with the relationship between digestible energy intake and these organic acids, are shown in Fig. 5.

DISCUSSION

Ruminal Dilution Rates

There were no effects of either protein intake or feeding frequency on dilution rate of either Co-EDTA or chromium-mordanted fibre, which is similar to results obtained by other researchers (Chase and Hibberd 1989; Hunt et al. 1989; Beaty et al. 1994).

Lactic Acid and Lactic Acidosis

Lactic acid accumulation in the rumen is of concern when high-concentrate diets are fed because such accumulations are associated with a rapid decline in pH, ruminal acidosis, microbial population shifts, and reduced fibre digestion. Rumenitis may occur if the ruminal pH decreases to 4 (Van Soest 1994). Lactic acidosis occurs when total lactic acid

concentrations are in the range of 50 to 200 mM (Nagaraja et al. 1998), although subclinical acidosis may occur with lower concentrations. In our experiment, there was no evidence of lactic- or VFA-related acidosis even on days when 6.6 kg of concentrate were fed to steers assigned to the High-3 regimen since the highest mean concentration of lactic acid observed on this treatment was 3.09 mM and ruminal pH remained above 5.8. Ruminal lactate levels greater than this have been reported even when animals receive no grain (Huntington et al. 1981). Moreover, lactic acid comprises 50 to 90% of total rumen acids in animals with severe lactic acidosis (Van Soest 1994) whereas lactic acid was less than 5% of the total organic acids in our experiment. Although there would be more concern of lactic acidosis in commercial feeding conditions, Huston et al. (1999) reported that in group-feeding situations variation in intake between cows was less with alternate day feeding than when cows were provided with concentrate daily.

Rumen Ammonia Concentrations and Microbial Requirements

Overall ruminal ammonia concentrations increased by 100% when the higher protein concentrate was fed either daily or on alternate days (Table 2). This would be expected as the high-protein concentrate contained 74% more crude protein than the low-protein concentrate and overall protein concentrations for the low- and high-protein diets of 7.9 and 11.5%, respectively.

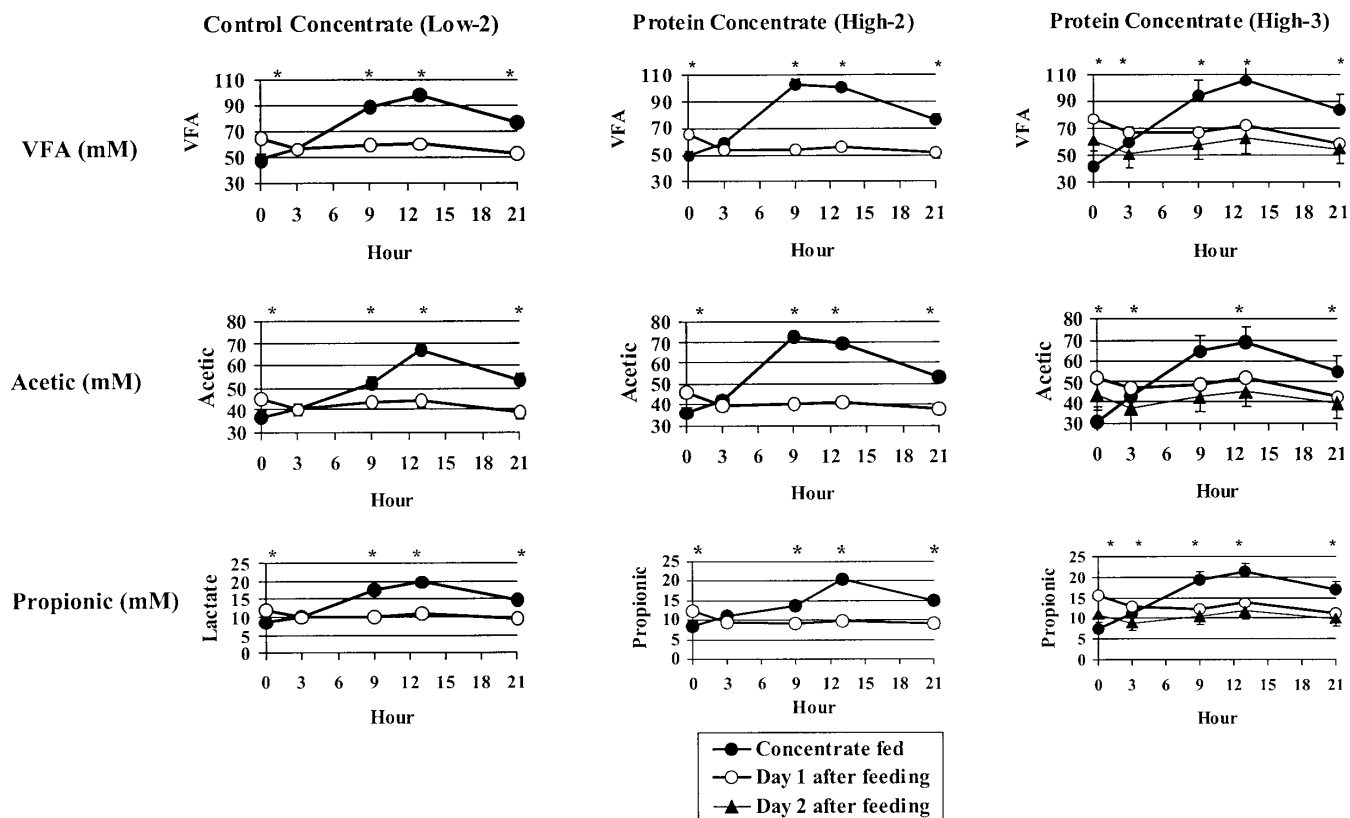


Fig. 2. Comparison of the effect of day of concentrate feeding with days after concentrate feeding (day 1 or day 2), on ruminal total volatile fatty acid, acetic acid and propionic acid concentrations. Low-2, High-2 and High-3 refer to low-protein concentrates given every second day, high-protein concentrates given every second day, and high-protein concentrates given every third day, respectively. Vertical bars are pooled standard errors. * Indicate differences ($P < 0.05$) between dietary treatments at the specific time.

Ruminal ammonia levels peaked at 3-h post-supplementation in all dietary treatments whether concentrate was provided or not (Fig. 1). Similar results were obtained by Chase and Hibberd (1989), Hunt et al. (1989) and Beaty et al. (1994). The lowered ruminal ammonia concentrations 13 h after feeding on days when the low-protein concentrate was fed in comparison with days when concentrate was not fed is consistent with increased ammonia utilization as a result of additional microbial growth. Other factors could be involved, however, since ruminal ammonia concentrations are influenced by protein degradation, urea recycling, ammonia absorption and passage as well as ammonia incorporation into microbial protein.

According to National Research Council (1996) the steers at maintenance required 544 g of degradable intake protein. Based on feed composition data, the low- and high-protein diets would have provided 281 and 430 g of ruminal degradable intake protein respectively, suggesting that the ruminal ammonia concentrations would be lower than required for optimal microbial activity. With the low-protein concentrate in our study, the mean ruminal ammonia concentration was 1.64 mM (Table 2). Much lower concentrations were measured on the day when concentrate was fed with the Low-2 diet (0.36 and 0.21 mM at 9 and 13h, respectively). These concentrations are much lower than the suggested minimum of 3 mM ammonia required to maximize growth of bacteria

in the rumen Satter and Slyter (1974), and confirm the deficiency of rumen-degradable protein with the low-protein diet. It should be noted, however, that wide ranges in ruminal ammonia concentrations have been associated with maximal ruminal microbial production and digestibility (Hoover 1986) and that the supply of available energy and carbon skeletons will influence requirements.

Even when the high-protein concentrate was provided, mean ruminal ammonia concentrations were 3.60, 2.97 and 3.28 mM for diets High-1, High-2 and High-3, respectively, which would be marginally deficient for microbial requirements according to conservative guidelines of Satter and Slyter (1974). With the High-1, High-2 and High-3 diets, mean ruminal ammonia concentrations fell to as low as 1.58, 1.09 and 2.62 mM, respectively, at 13 h after feeding concentrate (Table 2). Corresponding ammonia concentrations on days when concentrates were not fed were 0.9, 1.5 and 1.3 mM for these diets. These data suggest that the high-protein concentrate was also inadequate in terms of providing optimal ruminal ammonia concentrations.

In summary, low ruminal ammonia concentrations in steers fed the low-protein diet were consistent with the 8% reduction in acid detergent fibre digestibility with this diet (Tellier et al. 2004). Even though overall ruminal ammonia concentrations tended to be lower in steers fed concentrates on alternate days,

Table 4. Effect of dietary regimen^z on acetic acid to propionic acid ratio and concentrations of butyric and isobutyric acids

| Hours after feeding | Protein concentration contrast for steers fed daily or every 2 d | | | | Frequency of feeding contrast for steers fed concentrate daily or every 2 d | | | | Individual treatments | | | | | | |
|---|--|------|-----------------|----------------|---|-------------------|-----------------|----------------|-----------------------|--------------------|-------------------|--------------------|-------------------|-----------------|----------------|
| | Low | High | SE ^y | P ^x | 1 day | 2 days | SE ^y | P ^x | Low protein | | High protein | | | SE ^y | P ^x |
| | | | | | | | | | 1 d | 2 d | 1 d | 2 d | 3 d | | |
| <i>Acetic: propionic acid ratio^w</i> | | | | | | | | | | | | | | | |
| Overall | 4.06 | 4.16 | 0.060 | 0.27 | 4.25 _a | 3.97 _b | 0.060 | <0.01 | 4.17 _{ab} | 3.96 _b | 4.34 _a | 3.98 _b | 3.82 _b | 0.083 | <0.01 |
| 0 | 4.20 | 4.25 | 0.049 | 0.50 | 4.42 _a | 4.03 _b | 0.049 | <0.01 | 4.35 _a | 4.06 _b | 4.48 _a | 4.02 _b | 3.94 _b | 0.070 | <0.01 |
| 3 | 4.18 | 4.20 | 0.065 | 0.77 | 4.35 _a | 4.04 _b | 0.065 | 0.01 | 4.28 _{ab} | 4.07 _{ab} | 4.38 _a | 4.02 _b | 3.93 _b | 0.092 | 0.02 |
| 9 | 3.91 | 4.14 | 0.099 | 0.13 | 4.00 | 4.04 | 0.099 | 0.79 | 3.83 | 3.99 | 4.18 | 4.10 | 3.83 | 0.140 | 0.35 |
| 13 | 3.83 | 4.04 | 0.092 | 0.13 | 4.05 | 3.82 | 0.092 | 0.09 | 3.90 | 3.76 | 4.21 | 3.87 | 3.67 | 0.129 | 0.11 |
| 21 | 4.02 | 4.13 | 0.058 | 0.24 | 4.26 _a | 3.88 _b | 0.058 | <0.01 | 4.16 _{ab} | 3.89 _{bc} | 4.37 _a | 3.88 _{bc} | 3.73 _b | 0.083 | <0.01 |
| <i>Butyric acid^w (mM)</i> | | | | | | | | | | | | | | | |
| Overall | 5.0 | 5.1 | 0.19 | 0.57 | 5.5 _a | 4.6 _b | 0.19 | 0.01 | 5.3 | 4.6 | 5.7 | 4.6 | 5.3 | 0.27 | 0.06 |
| 0 | 4.3 | 4.3 | 0.23 | 0.93 | 4.7 _a | 4.0 _b | 0.23 | 0.04 | 4.6 | 4.1 | 4.8 | 3.8 | 4.6 | 0.32 | 0.26 |
| 3 | 4.4 | 4.5 | 0.19 | 0.54 | 5.0 _a | 3.9 _b | 0.19 | <0.01 | 4.8 _{ab} | 3.9 _b | 5.1 _a | 3.9 _b | 4.1 _{ab} | 0.27 | 0.02 |
| 9 | 5.7 | 6.3 | 0.33 | 0.24 | 6.6 _a | 5.3 _b | 0.33 | 0.02 | 6.4 | 5.0 | 6.9 | 5.7 | 5.5 | 0.46 | 0.10 |
| 13 | 6.2 | 6.4 | 0.33 | 0.72 | 6.6 | 5.9 | 0.33 | 0.15 | 6.4 | 6.0 | 6.9 | 5.8 | 7.3 | 0.47 | 0.21 |
| 21 | 5.0 | 5.0 | 0.20 | 0.95 | 5.4 _a | 4.7 _b | 0.20 | 0.02 | 5.4 | 4.7 | 5.5 | 4.6 | 5.5 | 0.29 | 0.11 |
| <i>Isobutyric acid^w (mM)</i> | | | | | | | | | | | | | | | |
| Overall | 0.59 | 0.60 | 0.02 | 0.73 | 0.61 | 0.58 | 0.02 | 0.36 | 0.60 | 0.58 | 0.62 | 0.57 | 0.57 | 0.03 | 0.75 |
| 0 | 0.58 | 0.62 | 0.03 | 0.32 | 0.60 | 0.60 | 0.03 | 0.97 | 0.58 | 0.59 | 0.63 | 0.62 | 0.61 | 0.04 | 0.89 |
| 3 | 0.61 | 0.62 | 0.02 | 0.24 | 0.60 | 0.55 | 0.02 | 0.11 | 0.57 | 0.64 | 0.63 | 0.56 | 0.53 | 0.03 | 0.23 |
| 9 | 0.57 | 0.61 | 0.03 | 0.40 | 0.62 | 0.54 | 0.03 | 0.07 | 0.63 | 0.56 | 0.60 | 0.52 | 0.47 | 0.04 | 0.09 |
| 13 | 0.60 | 0.59 | 0.04 | 0.77 | 0.61 | 0.58 | 0.04 | 0.45 | 0.61 | 0.59 | 0.61 | 0.56 | 0.54 | 0.05 | 0.79 |
| 21 | 0.61 | 0.62 | 0.03 | 0.87 | 0.63 | 0.59 | 0.03 | 0.34 | 0.63 | 0.59 | 0.64 | 0.59 | 0.62 | 0.04 | 0.88 |

^zLow or high-protein concentrate fed daily, every 2 d, or every 3 d.

^yStandard error mean is based upon five animals per mean for individual treatments and ten animals per mean for contrasts.

^xProbability.

^wFor repeated measures analyses SE and probabilities of diet, time and diet × time were 0.20, <0.01, <0.01 and <0.01; 0.67, and 0.06, <0.01, 0.10; and 0.08, 0.75, <0.01 and 0.03 for acetic:propionic acid ratio, butyric acid and isobutyric acid, respectively.

a-c Means not followed by the same letter differ ($P < 0.05$).

mean ruminal ammonia concentrations on days when concentrates were not fed were not too dissimilar from concentrations on days when concentrates were fed. This is consistent with the finding that frequency of feeding concentrates did not influence digestion.

Ruminal pH and Microbial Requirements

Rumen pH is an important factor that influences fibre digestion in the rumen. Normal rumen pH is in the range of pH 6 to 7, and a pH less than 6 has been implicated in reduced fibre digestibility (Van Soest 1994). The National Research Council (1996) assumes that there is no digestion of fibre when ruminal pH falls below 5.7. In our study, little effect of pH on digestion would be expected since the overall mean pH was 6.9 and mean ruminal pHs throughout the day were always above 6.5. Ruminal pH was, however, lower ($P < 0.05$) on days when concentrate was fed than on days when no concentrate was provided (Fig. 1).

The study of Beaty et al. (1994) supports our results since the lowest rumen pH they obtained in steers fed concentrate three times weekly was just below pH 6.2. Similarly, Chase and Hibberd (1989) found that lowest ruminal pHs reached on days when concentrate was provided were approximately 6.3 and 6.15 in heifers fed 2.8 kg or 4.1 kg of maize, respectively, on alternate days.

Based upon our results, and those in the literature, we conclude that it is unlikely that low ruminal pHs would adversely

affect ruminal microbial digestion of fibre when concentrate is provided on alternate days with diets containing 70% straw.

Valeric and Branched-chain Fatty Acid Concentrations and Microbial Requirements

Valeric acid and the branched-chain isobutyric and isovaleric acids have all been shown to be required by cellulolytic microorganisms in the rumen and, thus to influence microbial activity and growth in forage-based diets (National Research Council 1985). Mean concentrations of isobutyric, valeric, and isovaleric acids were 0.59, 0.52, and 0.76 mM over all treatments (Tables 4 and 5). These concentrations are low compared to the corresponding values reported Zorrilla-Rios et al. (1991) of 1.15, 0.96, and 1.45 mM acids in steers fed untreated barley straw with or without a soybean meal. Concentrations of valeric and branched-chain acids were not influenced by supplemental protein in our study although Sunvold et al. (1991) reported increased concentrations when protein supplements were fed. Branched-chain fatty acids are formed when branched-chain amino acids (e.g., valine, isoleucine and leucine) are deaminated (Yokoyama and Johnson 1988). Similarly, valeric acid can be formed from amino acids such as proline, lysine and arginine.

Acetic, Propionic and Butyric acids

The main energy supply for the ruminant animal is the acetic, propionic and butyric acids derived from ruminal fer-

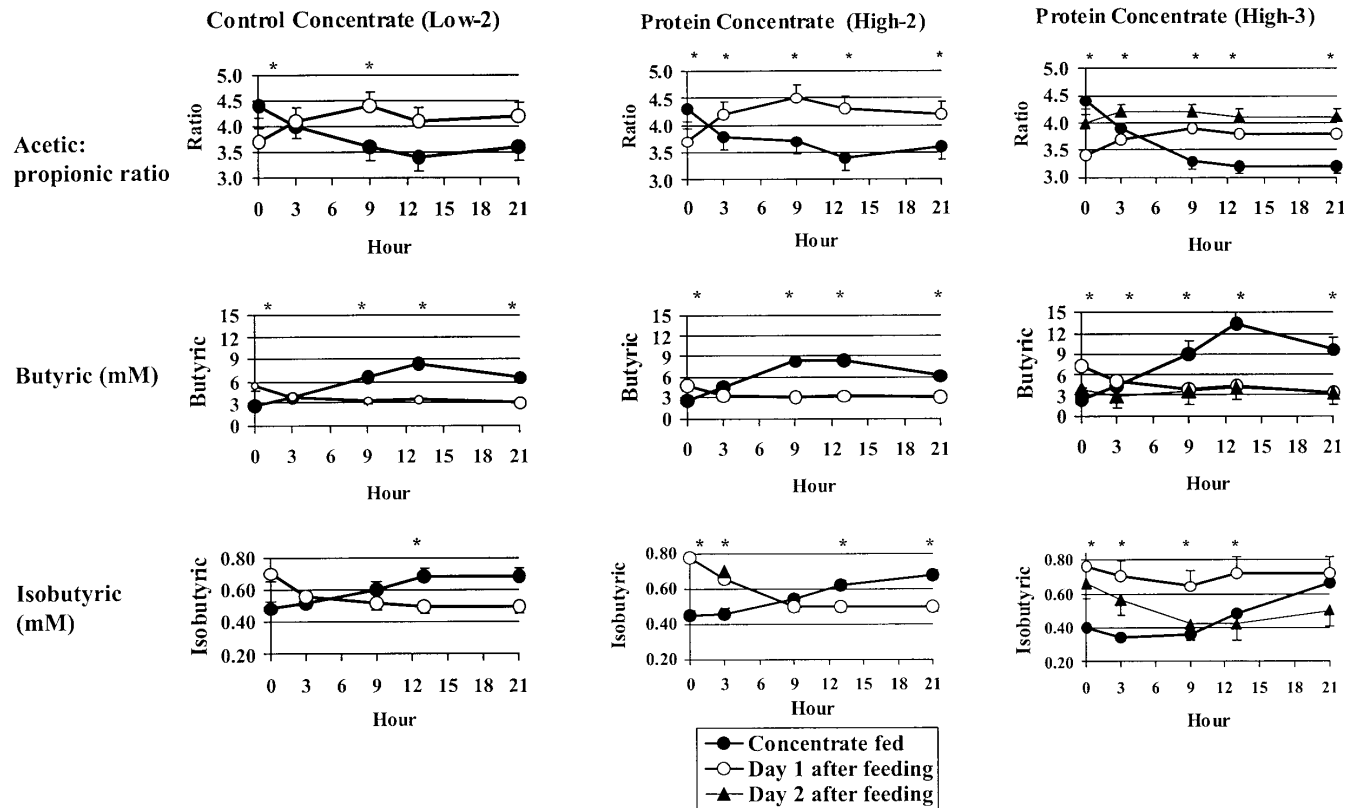


Fig. 3. Comparison of the effect of day of concentrate feeding with days after concentrate feeding (day 1 or day 2) on ruminal acetic propionic acid ratios and butyric and isobutyric acid concentrations. Low-2, High-2 and High-3 refer to low-protein concentrates given every second day, high-protein concentrates given every second day, and high-protein concentrates given every third day, respectively. Vertical bars are pooled standard errors. * Indicate differences ($P < 0.05$) between dietary treatments at that specific time.

mentation. Total VFA and acetic acid concentrations were not affected by protein content of the diet, but were higher ($P < 0.05$) in animals fed concentrate daily than in steers fed concentrate on alternate days (Table 3). Our results are supported by those of Knox and Ward (1961), Chase and Hibberd (1989) and Froetschel et al. (1990) but are at variance with those of Hunt et al. (1989) and Collins and Pritchard (1992).

Propionic acid concentrations were not influenced by protein supplementation or frequency of concentrate feeding (Table 3), which is similar to the results of Collins and Pritchard (1992).

Acetic:propionic acid ratios were not influenced by protein content of the diet (Table 4). This agrees with results of Zorrilla-Rios et al. (1991) but differs from those of Sunvold et al. (1991). In our study, mean acetic:propionic acid ratios were 7% lower ($P < 0.01$) when concentrates were fed on alternate days (Table 3). This result is similar to the 9% nonsignificant decrease observed by Chase and Hibberd (1989) and with suggestions of Froetschel et al. (1990). The acetic:propionic acid ratio in our study was also lower on days when concentrate was provided than on days when no concentrate was provided (Fig. 3). This would be expected because feeding concentrates shifts fermentation end products towards propionate whereas feeding fibrous feeds shifts

fermentation end products towards acetate (Owens and Goetsch 1988).

Butyric acid concentrations were unaffected ($P = 0.57$) by protein (Table 4), which agrees with other results obtained with straw-based diets (Fike et al. 1995; Zorrilla-Rios et al. 1991) but not with those obtained by Sunvold et al. (1991) or Hunt et al. (1989). Butyric acid concentrations were reduced ($P = 0.01$) in animals fed concentrates on alternate days (Table 4). This contrasts with results of Hunt et al. (1989), Chase and Hibberd (1989) and Collins and Pritchard (1992) who could not detect any effect on butyric acid concentrations when concentrates were fed daily or on alternate days.

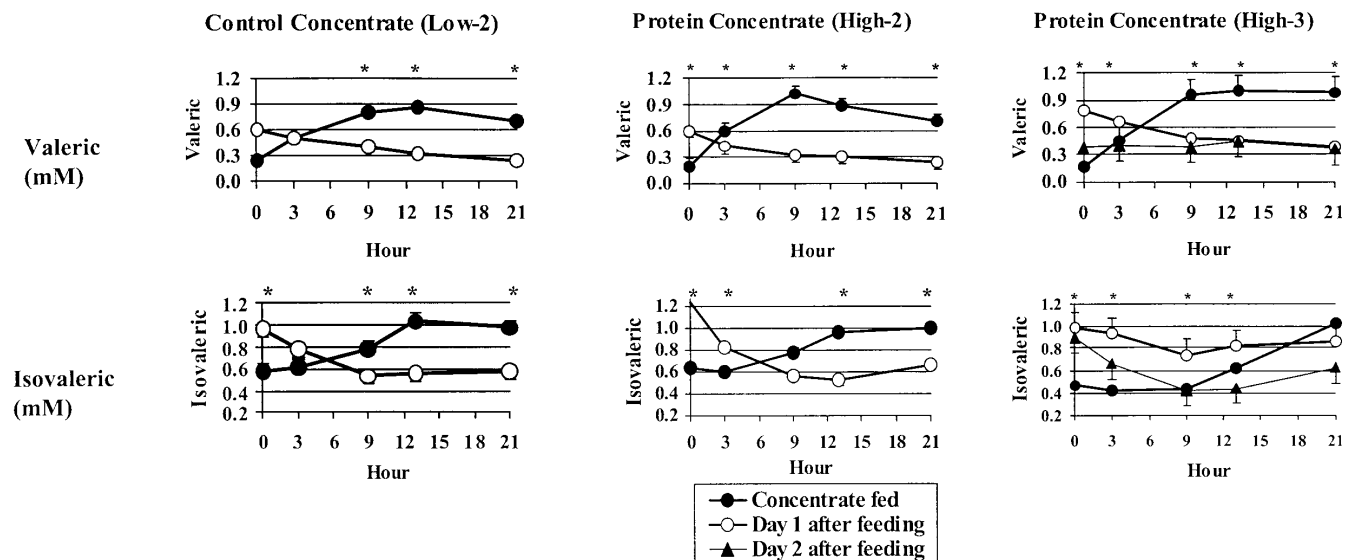
Concentrations of acetic, propionic and butyric acid were higher on days when concentrates were fed than on days when they were not fed (Figs. 2 and 3). This would be expected since additional carbohydrate was fermented on days on which concentrates were fed.

Ruminal Fermentation and Heat Production

A major purpose of this component of the research was to examine possible mechanisms for the reduction in heat production of the steers fed concentrates on alternate days (Tellier et al. 2004). The reduced overall concentrations of total VFAs and acetic acid in the rumen of steers fed concentrates on alternate days (Table 3) might suggest that reduced VFA production

Table 5 Effect of dietary regimen^z on concentrations of valeric and isovaleric acids

| Hours after feeding | Protein concentration contrast for steers fed daily or every 2 d | | | | Frequency of feeding contrast for steers fed concentrate daily or every 2 d | | | | Individual treatments | | | | | | |
|--|--|------|-----------------|----------------|---|-------------------|-----------------|----------------|-----------------------|-------------------|-------------------|-------------------|-------------------|-----------------|----------------|
| | Low | High | SE ^y | P ^x | 1 day | 2 days | SE ^y | P ^x | Low protein | | High protein | | | | |
| | | | | | | | | | 1 d | 2 d | 1 d | 2 d | 3 d | SE ^y | P ^x |
| <i>Valeric acid</i> ^w (mM) | | | | | | | | | | | | | | | |
| Overall | 0.51 | 0.52 | 0.02 | 0.61 | 0.53 | 0.50 | 0.02 | 0.40 | 0.51 | 0.50 | 0.54 | 0.50 | 0.54 | 0.03 | 0.78 |
| 0 | 0.39 | 0.40 | 0.02 | 0.70 | 0.38 | 0.41 | 0.02 | 0.34 | 0.35 | 0.42 | 0.40 | 0.40 | 0.44 | 0.06 | 0.47 |
| 3 | 0.54 | 0.60 | 0.03 | 0.18 | 0.64 _a | 0.50 _b | 0.03 | 0.01 | 0.59 _{ab} | 0.50 _b | 0.69 _a | 0.51 _b | 0.51 _b | 0.04 | 0.03 |
| 9 | 0.65 | 0.68 | 0.04 | 0.59 | 0.70 | 0.64 | 0.04 | 0.24 | 0.71 | 0.60 | 0.70 | 0.67 | 0.61 | 0.06 | 0.53 |
| 13 | 0.60 | 0.61 | 0.04 | 0.85 | 0.62 | 0.59 | 0.04 | 0.56 | 0.61 | 0.59 | 0.63 | 0.59 | 0.63 | 0.06 | 0.96 |
| 21 | 0.46 | 0.45 | 0.03 | 0.89 | 0.44 | 0.47 | 0.03 | 0.38 | 0.44 | 0.47 | 0.43 | 0.47 | 0.57 | 0.04 | 0.11 |
| <i>Isovaleric acid</i> ^w (mM) | | | | | | | | | | | | | | | |
| Overall | 0.76 | 0.79 | 0.03 | 0.44 | 0.78 | 0.76 | 0.03 | 0.72 | 0.77 | 0.74 | 0.79 | 0.78 | 0.71 | 0.04 | 0.67 |
| 0 | 0.76 | 0.88 | 0.04 | 0.06 | 0.79 | 0.86 | 0.04 | 0.29 | 0.75 | 0.77 | 0.83 | 0.94 | 0.78 | 0.06 | 0.24 |
| 3 | 0.73 | 0.78 | 0.03 | 0.28 | 0.80 | 0.70 | 0.03 | 0.06 | 0.75 | 0.70 | 0.85 | 0.71 | 0.67 | 0.05 | 0.13 |
| 9 | 0.73 | 0.68 | 0.04 | 0.37 | 0.75 | 0.66 | 0.04 | 0.19 | 0.81 | 0.66 | 0.69 | 0.67 | 0.53 | 0.06 | 0.08 |
| 13 | 0.80 | 0.72 | 0.04 | 0.19 | 0.75 | 0.77 | 0.04 | 0.77 | 0.90 | 0.80 | 0.71 | 0.74 | 0.63 | 0.05 | 0.20 |
| 21 | 0.77 | 0.84 | 0.03 | 0.23 | 0.80 | 0.80 | 0.03 | 0.96 | 0.76 | 0.78 | 0.84 | 0.83 | 0.83 | 0.05 | 0.75 |

^zLow or high-protein concentrate fed daily, every 2 d, or every 3 d.^yStandard error mean is based upon five animals per mean for individual treatments and ten animals per mean for contrasts.^xProbability.^wFor repeated measures analyses SE and probabilities of diet, time of sampling and diet × time were 0.08, 0.78, <0.01 and < 0.01; 0.10, 0.67, < 0.01, and <0.01 for valeric acid and isovaleric acid, respectively.^{a-c}Means not followed by the same letter differ ($P < 0.05$).**Fig. 4.** Comparison of the effect of day of concentrate feeding with days after concentrate feeding (day 1 or day 2), on ruminal valeric and isovaleric acid concentrations. Low-2, High-2 and High-3 refer to low-protein concentrates given every second day, high-protein concentrates given every second day, and high-protein concentrates given every third day, respectively. Vertical bars are pooled standard errors. * Indicate differences ($P < 0.05$) between dietary treatments at that specific time.

was the cause of the reduced heat production. This is highly unlikely, however, since digestibilities of dry matter and fibre were not reduced by alternate day feeding (Tellier et al. 2004) and dilution rate of liquid in the rumen was numerically 20% greater ($P = 0.44$) in steers fed concentrates on alternate days. Greater dilution rates would be expected to result in lowered ruminal concentrations of VFAs (correlation coefficients between VFA concentration and cobalt and chromium dilution rates were 0.41 and -0.38 , respectively).

The 7% decrease in ruminal acetic:propionic acid ratio in animals fed concentrates on alternate days (Table 4) suggests the animals were absorbing a VFA mixture containing an increased percentage of propionic acid. Propionate is gluconeogenic and can improve efficiency of energy use in ruminants (Van Soest 1994); thus a reduced acetic:propionic acid ratio is consistent with the improved energetic efficiency and reduced heat production which we observed in animals fed concentrate on alternate days (Tellier et al. 2004). When results

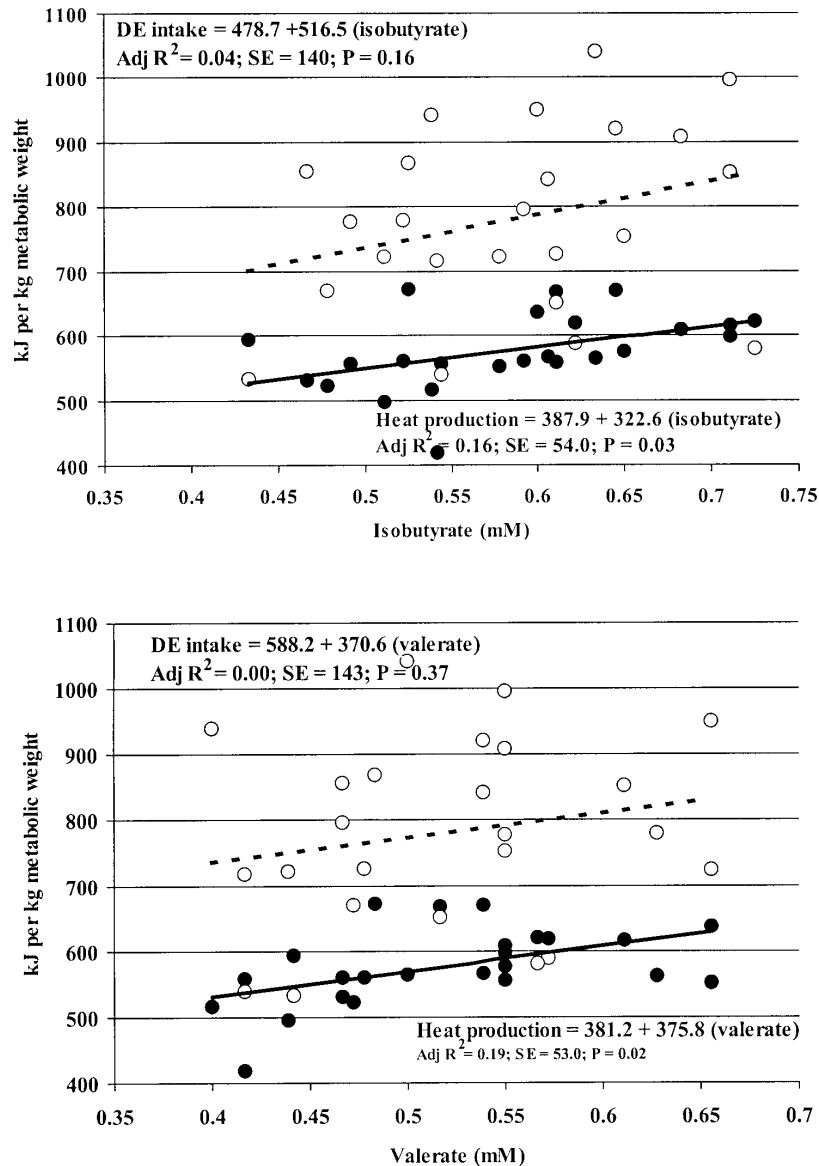


Fig. 5. Relationships between ruminal concentrations of organic acids and heat production ($n = 24$) (\bullet , $\text{kJ kg}^{-0.75}$) and digestible energy (DE) intake (\circ , $\text{kJ kg}^{-0.75}$) as reported by Tellier et al. (2004).

from individual steers were examined, however, there was no significant relationship between heat production ($\text{kJ kg}^{-0.75}$) and ruminal acetic or propionic acid concentrations or with acetic to propionic acid ratios. Measurements of heat production may, however, have been too variable to detect significant relationships when individual animals rather than treatment means were used. Indeed, when relationships between means for the five dietary treatments were examined, corresponding correlation coefficients for acetic acid, propionic acid and acetic:propionic acid ratios were 0.95, -0.06 and 0.97, supporting the suggestion that differences in the proportion of propionic acid in the mixture of absorbed VFAs may have been a factor influencing heat production.

Significant relationships were observed between heat production and ruminal concentrations of isobutyrate and valerate (Fig. 5). The significance of these relationships is questionable, however, since somewhat similar but non-

significant relationships occurred between digestible energy intake and heat production. However isobutyric acid is gluconeogenic and combinations of isobutyric, 2-methyl butyric, isovaleric and valeric acids have increased productivity of cattle as well as growth hormone concentrations (Andries et al. 1987). Additionally, increases in plasma insulin concentrations have been noted in cattle fed these acids (Coutinho et al. 1987). Such effects are consistent with a change in heat production in animals.

CONCLUSIONS AND IMPLICATIONS

Ruminal pH measurements and lactic acid concentrations suggested that there was little likelihood of clinical acidosis with alternate-day or even every third day concentrate feeding under this controlled feeding situation, although there would be more concern in herd-feeding situations. Reduced frequency of concentrate feeding did not have a major effect on ruminal pH, or

on ammonia and volatile fatty acid concentrations although overall mean total VFA, acetic acid and butyric acid concentrations were lower ($P < 0.05$) in animals fed concentrate on alternate days. With the Low-2 diet ruminal ammonia concentrations did not differ between days on which concentrates were fed and days when concentrates were not fed, and with the exception of 3 h after feeding, the same was true with the High-2 diet. This was undoubtedly a major reason why ruminal straw disappearance and *in vivo* digestibility were not influenced by feeding frequency. Ruminal ammonia concentrations in steers fed the low protein diet never reached levels sufficient for maximal microbial production in the rumen, therefore supplemental dietary protein was required for optimal rumen function, no matter what the frequency of feeding. The 7% increase in digestible energy content of the high-protein diet (Tellier et al. 2004) should be considered when assessing the economics of protein supplementation of straw-based diets.

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