Effects of Supplementing Concentrates Differing in Carbohydrate Composition in Veal Calf Diets: II. Rumen Development¹

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ABSTRACT

The objective of this experiment was to examine the effects of concentrates in feed, differing in carbohydrate source, on the rumen development of veal calves. For this purpose, 160 male Holstein Friesian × Dutch Friesian crossbred calves were used in a complete randomized block design with a 5 × 2 factorial arrangement. Dietary treatments consisted of 1) a milk replacer control, 2) a pectin-based concentrate, 3) a neutral detergent fiber-based concentrate, 4) a starch-based concentrate, and 5) a mixed concentrate (equal amounts of the concentrates in treatments 2, 3, and 4). Concentrate diets were provided as pellets in addition to a commercial milk replacer. Calves were euthanized at either 8 or 12 wk of age. Plasma acetate and β -hydroxybutyrate (BHBA) were measured as indicators of rumen development. Empty rumen weight was determined, and wall samples were taken at slaughter. In most calves, a poorly developed rumen mucosa was observed. Coalescing rumen papillae with embedded hair, feed particles, and cell debris were found in all calves fed the concentrate diets. Calves fed concentrates had significantly heavier rumens than calves fed the control diet. In the dorsal location of the rumen, calves fed concentrate diets showed an increased ratio of mucosa to serosa length compared with calves fed the control diet, whereas in the ventral location only, calves fed the pectin and mixed diets showed larger ratios of mucosa to serosa length. Mucosa thickness and muscle thickness were greater in the ventral and dorsal locations of the rumen, respectively. In both locations, the NDF diet resulted numerically in the lowest mucosa thickness and highest muscle thickness among the concentrate treatments. At 8 wk, calves fed the concentrate diets had higher plasma acetate concentrations than calves on the control treatment. However, at 12 wk, only NDF-fed calves showed sig-

nificantly higher plasma acetate concentrations. The plasma BHBA concentrations of calves at 8 wk of age fed the pectin and mixed diets were higher than those of the control diet-fed calves. At 12 wk, no differences in BHBA concentrations were observed among treatments. Results of a principal component analysis indicated that, in addition to rumen volatile fatty acid concentrations, other factors were likely to affect rumen development, and that the relationships between rumen development and individual types of volatile fatty acids present in the rumen liquor were similar. Also, variations in rumen development coincided with variations in plasma acetate and BHBA concentrations.

Key words: veal calf, concentrate feed composition, rumen development, rumen development predictor

INTRODUCTION

The gastrointestinal tract of the newborn calf undergoes several anatomical and physiological changes during development until the rumen is fully functional, as in the adult ruminant. The type of diet offered has been demonstrated to affect the rate of development of the ruminant forestomachs (Brownlee, 1956). In diets for rearing calves, forages are usually a minor proportion of the diet because of the low DMI and low rates of fermentation, whereas cereals in concentrate feeds are widely used (Nocek and Kesler, 1980). Feeding calves concentrate diets should result in high concentrations of VFA in the rumen, which are necessary to stimulate a fast rumen development (Sander et al., 1959; Tamate et al., 1962).

Carbohydrates are the main source of energy for rumen microorganisms, and feeding diets differing in carbohydrate composition may result in different patterns of rumen fermentation, subsequently resulting in different VFA profiles (e.g., Bannink et al., 2006). These differences, in turn, may differentially affect rumen development.

In 1997 new European Union legislation was set for the feeding of veal calves. Details of this new legislation are described in a companion paper (Suárez et al., 2006). Based on earlier research (Blokhuys et al.,

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2000), we hypothesized that stimulating early rumen development in veal calves would be beneficial to their subsequent performance and health. In rearing calves, information on rumen fermentation of different sources of dietary carbohydrates is relatively well documented (see e.g., Maiga et al., 1994; Davis and Drackley, 1998; Lesmeister and Heinrichs, 2004), but there is limited information on their effects in veal calves. The mechanisms of rumen development are hypothesized to be similar to those in rearing calves, although the typical situation in veal calves (low DMI because of high intakes of milk replacer and potential direct interference of the milk replacer in the rumen) may complicate its predictability. Recent work by Del'Orto et al. (2002) even suggests reduced rumen development following supplementation of a veal calf diet with concentrates or sugar beet pulp, compared with milk replacer alone. The objective of this study was to gain insight into the effects of age and supplementation with concentrates differing in their carbohydrate composition on rumen development in young veal calves. In addition, this study evaluated some selected metabolites as predictors of rumen development. Animal performance and parameters of rumen fermentation are presented in our companion paper (Suárez et al., 2006).

MATERIALS AND METHODS

Animals, feeding regimens, diets, and general management are described in the companion paper (Suárez et al., 2006) and are summarized below. The ethics committee of the Dutch Institute of Animal Health and Welfare approved the experimental protocol.

One hundred and sixty Holstein Friesian $\times$ Dutch Friesian male calves, averaging 44.9 kg (SE = 2.8 kg) of BW were used in a complete randomized block design with a 5×2 factorial arrangement, with type of concentrate and age as the experimental factors. Dietary treatments consisted of 1) a milk replacer control (control), 2) a pectin-based concentrate, 3) a neutral detergent fiber-based concentrate, 4) a starch-based concentrate, and 5) a mixed concentrate (equal amounts of the concentrates in treatments 2, 3, and 4). Concentrate diets were provided as pellets, up to a maximum of 750 g/d of DM in addition to a commercial milk replacer. Calves were individually housed in open metal boxes (0.9 m²). Concentrates contained either beet pulp (pectin diet), soybean hulls plus corn grits (NDF diet), or corn plus barley grits (starch diet) as the main sources of pectin, NDF, and starch, respectively. Milk replacer and concentrates were supplied in separate buckets and meals were provided in equal amounts twice daily (0730 and 1830 h). At 8 or 12 wk

of the experimental period, animals were euthanatized by a T61 injection (embutramide/mebezoniumiodide/tetracain hydrochloride, Intervet International, Unterschleissheim, Germany), and immediately after slaughter the digestive tract was removed. The forestomachs and the abomasum were tied at the end of the esophagus and pylorus respectively, removed immediately, and the weight of the reticulorumen was recorded with and without its contents. The rumen was dissected along the dorsal line, and the mucosal surface was visually examined and qualitatively assessed according to the presence and density of rumen papillae as follows: 1 = poor (few papillae or short papillae) and 2 = good (numerous papillae or long papillae). The vast majority of the calves showed focal or multifocal patches of foci with coalescing and adhering papillae covered by a sticky mass of feed, hair, and cell debris, which will be referred to in this paper as "plaque." The presence or absence of plaque formation was visually assessed. For morphometrical analysis, one tissue specimen (approximately 2×2 cm) was taken from the saccus ruminis dorsalis (dorsal location) and one was taken from the saccus ruminis ventralis cranial of the pilae ruminis (ventral location), attached to small dissection plates, and fixed in 4% formaline. Each of the tissue specimens was embedded in paraffin wax, and 4 tissue cross-sections were prepared with a distance of at least 100 μ m and then stained with hematoxylin-eosin. Because the rumen mucosa showed irregular-shaped, branched, and clumped papillae, it was impossible to consistently and properly measure the length and shape of individual papillae. Consequently, to avoid any observer bias caused by nonrandom selection of suitable papillae, we decided to measure the following rumen wall parameters within each cross-section: a) ratio of mucosa length to serosa length (**RMSL**); b) mucosa thickness (**MCT**); and c) muscle thickness (**MST**). The latter 2 were scored at 3 randomly chosen sites within each cross-section. For each calf within each tissue specimen, RMSL, MCT, and MST were calculated as the average of all 4 cross-sections. The morphometric analysis was performed at a magnification of 2.5 times (Olympus microscope; Olympus Corporation, Tokyo, Japan) by using an image analysis software program (Image Pro Plus; Media Cybernetics, Silver Spring, MD).

Blood samples were taken from the jugular vein at 8 and 12 wk of age, between 3 and 5 h after the milk feeding in heparinized Vacutainers (lithium heparin tubes; Becton Dickinson BV, Alphen aan den Rijn, the Netherlands). Plasma was stored at -20°C until analysis. Acetate in plasma was determined by ion-exchange chromatography by using an Ion Pac ICE-AS1 column (Dionex system; Dionex Corporation, Sunnyvale, CA),

heptafluorobutyric acid as the eluent, and suppressed conductivity as the detection method. The BHBA was determined by using an enzymatic method (3-hydroxybutyrate dehydrogenase); BHBA is transformed into acetoacetate by the dehydrogenase enzyme in the presence of NAD. During this reaction, NAD is transformed to reduced nicotinamide adenine dinucleotide. The increase in the amount of reduced nicotinamide adenine dinucleotide is measured at 340 nm and is proportional to the amount of BHBA. Quantification was done by using a chemical standard solution.

Statistical Analysis

Empty rumen weight, rumen wall, and plasma parameters were analyzed as a complete randomized block design, in a 5×2 factorial arrangement, with diets and length of the experimental period (i.e., 8 or 12 wk of age at slaughter) as main factors. An ANOVA was performed for the continuous data according to Model 1:

$$y = \mu + \text{batch}_i + \text{diet}_j + \text{period}_k + (\text{diet} \times \text{period})_{jk} + \varepsilon_{ijkl} \quad [1]$$

where y is the dependent variable; μ is the average experimental value; batch_i is the effect of batch i ; i is 1 or 2 (2 batches of 80 calves); diet_j is the effect of dietary treatment j ; j is the control, pectin, NDF, starch, or mixed diet; period_k is the effect of period (age at slaughter) k ; k is 8 or 12 wk; $(\text{diet} \times \text{period})_{jk}$ is the effect of the interaction between diet and period; ε_{ijkl} is the error term; and l is 1, ..., 160.

Main effects and the interaction between diet and period (age at slaughter) were evaluated using the F -test. For the analysis of fractions (percentages) and binary variables (0–1), a logistic regression model was used with the same main effects as described earlier. Inferences for fractions and binary variables were based on likelihood ratio tests. Dispersion parameters were estimated from Pearson's generalized test (McCullagh and Nelder, 1989). Pairwise comparisons were carried out using the Fisher least significant differences method (for fractions and binary variables on a logistic scale). Binary variables that were unevenly distributed over the sample groups (e.g., with only zero values in one or more treatments) were analyzed parameter-free using the Fisher exact test.

In addition to the analysis described above, rumen weight and morphometric variables of the rumen wall were analyzed by analysis of covariance. The average concentrate intake during the experimental period, expressed for each calf as the deviation from the overall mean, was included as a covariable in the statistical

model. Acetate and BHBA concentrations in plasma were also analyzed by analysis of covariance. For these variables, the average concentrate intake in the last week before slaughter, likewise expressed for each calf as the deviation from the overall mean, was included in the model as a covariable. Significance was determined at $P < 0.05$ unless indicated otherwise.

Principal Component Analysis

Principal component analysis (PCA) was used to examine patterns of intercorrelations between multiple measures (Jolliffe, 1986), including the morphometric parameters of rumen development, pH, VFA, and phosphorus in the urine. Principal components produced by a PCA are linear combinations of the original measures reflecting independent characteristics (or dimensions) of the underlying correlation matrix. The loading of each measure on a principal component represents the correlation between the latent characteristic and the original measure, and thus indicates the importance of a measure for a principal component. Measures with high loadings on the same principal component of the same sign are positively correlated, and loadings of the opposite sign are negatively correlated. The first component (**PC1**) explains most of the variance (expressed in terms of the first eigenvalue); the second component (**PC2**) explains most of the remaining variation, and so forth. Measures were scaled prior to PCA (i.e., the analysis was performed on the Pearson correlation matrix). The first 2 principal components with eigenvalues equal to or larger than 1 were retained for further consideration.

In the present study, the different measures included in the PCA were all hypothesized to be associated with the level of rumen development (see below). The PCA would confirm this hypothesis if it would produce at least one component, putatively reflecting (mechanisms underlying) rumen development with high loadings from multiple measures. Two different PCA were performed. In the first PCA, a data set containing the assessed morphometric parameters of the rumen wall along with ruminal pH, lactate, and VFA concentration (acetate, propionate, and butyrate; see data presented in the companion paper by Suárez et al., 2006) was used to perform the analysis. The second PCA was applied on a data set comprising the assessed morphometric parameters of the rumen wall, the concentrations of acetate and BHBA in plasma, and the ratio of phosphorus to creatinine in the urine. The reason for grouping variables in this way was to differentiate more efficiently the real causal factors, the so-called stimulators, from the so-called predictors of rumen development. Individual VFA concentrations,

rather than total VFA, were included as variables, because each individual VFA is absorbed and metabolized to a different extent (Bergman, 1990). The phosphorus-to-creatinine ratio was included based on the assumption that urinary excretion of phosphorus is negatively related to ruminating activity (data presented by van Vuuren et al., 2004). Within both PCA, a separate PCA was carried out for the dorsal and ventral locations of the rumen, as well as with and without inclusion of the control treatment. Excluding the control treatment from the analysis (i.e., excluding data from calves with the lowest levels of VFA and morphometric parameters of the rumen wall) reduced the extent of variation in putative measures of rumen development across treatments and allowed us to examine whether patterns of intercorrelations demonstrated by the PCA reflected interindividual differences within concentrate diets, or whether they were primarily a consequence of differences between the control treatment and the other treatments. All the statistical analyses were carried out using Genstat (Genstat Committee, 2000).

RESULTS

Throughout the experimental period, calves remained healthy. Animal performance and rumen fermentation characteristics have been described elsewhere (Suárez et al., 2006). The rumen contents of calves fed concentrates were pasty in texture, whereas the contents of calves fed the control diet were liquid. The mucosa of the calves fed concentrate diets was brown to dark, and was easily removed from the rumen wall. In addition, we observed coalescing papillae with embedded hair and feed particles, defined as plaque, particularly in the ventral location of the rumen.

Rumen Mucosa Development

Table 1 presents the macroscopic evaluation of the rumen mucosa. As expected, calves fed the control diet consistently had a poorly developed rumen mucosa. In calves fed concentrate diets, the incidence of animals with poor mucosa development varied between 10 and 44%. The starch diet had a higher ($P < 0.05$) incidence (44%) of poorly developed mucosa than did the pectin and NDF diets (10 and 13%, respectively).

The rumen of a large number of calves appeared to have plaques of feed and hair firmly attached to the mucosa. The size and distribution of plaques varied considerably among animals and ranged from a few local patches with a diameter of few centimeters to confluent patches covering larger areas of the rumen. Plaque formation was hardly present in calves fed the

control diet only, but it appeared in all concentrate treatments, with incidences ranging from 6 to 100%. In the 8-wk period, calves fed the starch diet had the lowest incidence of plaque (6%; $P < 0.05$) among the concentrate-fed calves, but this increased to 73% by the end of the 12-wk period, where differences between concentrate diets in the incidence of plaque formation were no longer present ($P > 0.05$).

Rumen Weight and Morphometric Parameters

Empty rumen weights and rumen wall morphometric parameters are presented in Table 2. Empty rumen weights at slaughter were affected by diet and period ($P < 0.001$). An interaction between diet and period also was observed ($P < 0.01$). Averaged by treatments, empty rumen weights varied between 563 and 1,423 g and between 734 and 1,788 g for the 8- and 12-wk periods, respectively. Calves fed the concentrate-based diets had higher empty rumen weights compared with calves fed the control diet ($P < 0.001$). At the end of the 8-wk period, calves fed the pectin diet had the heaviest rumen weight (1,423 g) and those fed the starch diet had the lowest rumen weight (894 g). The interaction between diet and period was mainly caused by the large increase in rumen weights of calves fed the starch diet from the 8- to 12-wk period (894 vs. 1,531 g, respectively).

The RMSL in the dorsal location of the rumen of concentrate-fed calves was consistently higher compared with calves fed milk replacer only ($P < 0.01$), but among the concentrate diets, no significant differences were observed. The RMSL was smaller ($P < 0.001$) at 12 wk than at 8 wk. In the ventral location of the rumen, the RMSL was higher compared with the dorsal rumen wall location and was not affected by period. Calves fed the mixed and pectin diets had higher RMSL than calves fed the control diet ($P < 0.05$).

In the dorsal location, MCT was decreased compared with that observed in the ventral location of the rumen (595 to 853 μm for the dorsal location and 863 to 1,399 μm for the ventral location, respectively). In the dorsal location of the rumen, the MCT did not differ among the concentrate treatments. However, calves fed the mixed and starch diets had higher MCT than calves fed the control diet ($P < 0.05$). In the ventral location, a diet effect was observed for MCT in the ventral location ($P < 0.001$). Calves fed the starch and pectin diets had increased MCT compared with calves on the NDF and control diets, respectively ($P < 0.05$). Moreover, the MCT of calves fed the mixed diet was greater than the MCT in calves fed the control diet ($P < 0.05$).

Although not compared statically, the MST in the dorsal location of the rumen wall was increased com-

Table 1. Effects of period (age at slaughter) and diet (supplemented concentrates differing in carbohydrate source)¹ on the incidence of poor development of rumen mucosa and plaque formation (macroscopic)

Variable	Diet					Period	
	Control	Pectin	NDF	Starch	Mixed	8 wk	12 wk
Poor development of mucosa, % of calves	100 ^a	10 ^c	13 ^c	44 ^b	29 ^{bc}	38	41
Plaque, % of calves							
8 wk	6 ^b	44 ^a	67 ^a	6 ^b	56 ^a		
12 wk	0 ^b	80 ^a	94 ^a	73 ^a	100 ^a		

^{a-c}Means in the same row with different superscripts differ ($P < 0.05$; nonparametric analysis, Fisher's exact tests).

¹Diets were as follows: control = milk replacer; pectin = pectin-based concentrate; NDF = NDF-based concentrate; starch = starch-based concentrate; mixed = mixed concentrate (equal amounts of the pectin-, NDF-, and starch-based concentrates).

pared with that observed in the ventral location (means across treatments were 1,692 and 1,322 μm for the dorsal and ventral locations, respectively). Furthermore, in the dorsal location only a period effect ($P < 0.05$) was observed, whereas in the ventral location both diet ($P < 0.01$) and period effects were present ($P < 0.05$). In both locations, the MST decreased with increasing length of the experimental period from 8 to 12 wk, respectively. In the ventral location of the rumen, calves fed the NDF diet had a significantly increased MST compared with calves fed the starch, mixed, and control diets, respectively.

The analysis of covariance of the rumen wall morphometric parameters demonstrated that a significant part of the variation in the RMSL and MCT in the dorsal location of the rumen was explained by the within-treatment variation in concentrate intake (estimates of regression coefficients: 3.25 ± 0.93 SE, $P <$

0.001, and 454 ± 239 SE, $P < 0.06$, for RMSL and MCT, respectively).

BHBA and Acetate in Plasma

Plasma concentrations of BHBA and acetate are presented in Table 3. Results represent average values in the last week before slaughter (at 8- and 12-wk, respectively). The plasma acetate concentration was affected by diet ($P < 0.001$) but not by period. An interaction between diet and period ($P < 0.05$) also was observed. In the 8-wk-period group, concentrations of acetate for calves fed concentrate diets ranged from 0.09 to 0.14 mmol/L and were higher than those observed in calves fed the control diet (0.06 mmol/L; $P < 0.001$). In addition, the plasma acetate concentration in calves fed the pectin diet (0.14 mmol/L) was higher ($P < 0.05$) than that of calves fed the NDF (0.10 mmol/

Table 2. Effects of period (age at slaughter) and diet (supplemented concentrates differing in carbohydrate source) on rumen weight and morphometric variables of rumen wall, determined at slaughter¹

Variable	Diet					SEM	<i>P</i>			Period		SEM
	Control	Pectin	NDF	Starch	Mixed		D	P	D $\times$ P	8 wk	12 wk	
Rumen weight (g)							***	***	**			
8 wk	563 ^d	1,423 ^a	1,234 ^{ab}	894 ^c	1,117 ^b	76.3						
12 wk	734 ^c	1,738 ^{ab}	1,788 ^a	1,531 ^b	1,726 ^{ab}	76.3						
Rumen dorsal location												
Ratio of mucosa length to serosa length	3.0 ^b	5.1 ^a	4.5 ^a	4.5 ^a	4.8 ^a	0.2	***	***	NS	4.8	4.1	0.1
Mucosa thickness, μm	595 ^b	730 ^{ab}	691 ^{ab}	799 ^a	853 ^a	61.5	*	NS	NS	787	680	38.9
Muscle thickness, μm	1,797	1,579	1,851	1,621	1,610	86.1	NS	*	NS	1,786	1,597	54.5
Rumen ventral location												
Ratio of mucosa length to serosa length	4.0 ^b	7.2 ^a	5.7 ^{ab}	6.2 ^{ab}	8.6 ^a	1.0	*	NS	NS	6.1	6.5	0.6
Mucosa thickness, μm	863 ^c	1,399 ^a	968 ^{bc}	1,320 ^a	1,177 ^{ab}	94.1	***	NS	NS	1,107	1,184	59.5
Muscle thickness, μm	1,294 ^b	1,334 ^{ab}	1,507 ^a	1,292 ^b	1,181 ^b	66.8	**	*	NS	1,383	1,268	42.3

^{a-c}Means in the same row with different superscripts differ ($P < 0.05$). NS = not significant.

¹Diets were as follows: control = milk replacer; pectin = pectin-based concentrate; NDF = NDF-based concentrate; starch = starch-based concentrate; mixed = mixed concentrate (equal amounts of the pectin-, NDF-, and starch-based concentrates). D = diet; P = period; D $\times$ P = interaction.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3. Effects of period (age at slaughter) and diet (supplemented concentrates differing in carbohydrate source) on rumen weight and morphometric variables of rumen wall on plasma acetate and BHBA concentrations in the last week before slaughter¹

Variable	Diet					SEM	P			Period		SEM
	Control	Pectin	NDF	Starch	Mixed		D	P	D × P	8 wk	12 wk	
Acetate, mmol/L							***	NS	*	0.10	0.11	0.005
8 wk	0.06 ^c	0.14 ^a	0.10 ^b	0.09 ^b	0.12 ^{ab}	0.010						
12 wk	0.09 ^b	0.11 ^{ab}	0.13 ^a	0.11 ^{ab}	0.11 ^{ab}	0.010						
BHBA, mmol/L							*	**	**	0.10	0.09	0.0038
8 wk	0.09 ^c	0.14 ^a	0.09 ^{bc}	0.09 ^{bc}	0.11 ^b	0.008						
12 wk	0.09	0.09	0.08	0.09	0.09	0.008						

^{a-c}Means in the same row with different superscripts differ ($P < 0.05$).

¹Diets were as follows: control = milk replacer; pectin = pectin-based concentrate; NDF = NDF-based concentrate; starch = starch-based concentrate; mixed = mixed concentrate (equal amounts of the pectin-, NDF-, and starch-based concentrates). D = diet; P = period; D × P = interaction.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

L) or the starch (0.09 mmol/L) diet, respectively. The observed interaction between diet and period was due to a decrease in acetate concentration in calves fed the pectin diet (0.14 vs. 0.11 mmol/L) and in those fed the NDF diet (0.10 vs. 0.13 mmol/L) from wk 8 to 12, respectively.

Plasma BHBA concentrations also were affected by diet ($P < 0.05$) and period ($P < 0.01$). Moreover, an interaction between diet and period was observed ($P < 0.01$). The plasma BHBA concentration varied between 0.09 and 0.14 mmol/L. In the 8-wk period, the plasma BHBA concentration of calves fed the pectin diet was higher than those of calves fed other diets ($P < 0.05$). The observed diet × period interaction was caused by a decrease in the BHBA concentrations of calves fed the pectin and mixed diets (0.14 and 0.11 vs. 0.09 and 0.09) in week 8 and 12, respectively.

PCA

Results of the PCA for pH, VFA, and the morphometric variables are presented in Table 4. Eigenvalues of the first 2 components were always greater than 1, and they explained 53.0 and 52.7% of the total variation when histological measures of the dorsal and ventral locations of the rumen, respectively, were included in the analysis. Following PCA of the complete data set, the loading patterns on PC1 were similar in both locations. The ruminal concentrations of acetate, propionate, and butyrate, together with the RMSL, all loaded negatively, and the concentration of ruminal lactate loaded positively on PC1 (Table 4). Thus, across all treatment groups a high ruminal concentration of VFA, but not of lactate, coincided with high levels of RMSL and vice versa. The RMSL and MCT also carried high and same-sign loadings on PC2 (Table 4), together with the level of ruminal lactate in the ventral location.

Results of the PCA for plasma acetate and BHBA, the urine phosphorus-to-creatinine ratio, and the morphometric variables are presented in Table 5. Similar to the previous analysis, eigenvalues of the first 2 factors in the dorsal and ventral rumen locations were greater than 1. The cumulative percentages of the total variation explained by the first 2 factors were 55.8 and 53.8% when histological measures of the dorsal and ventral locations of the rumen, respectively, were included in the analysis (Table 5). In the PC1 produced by the PCA of the complete data set, variables with high and same-sign loadings included the relative mucosa length and MCT, together with the acetate and BHBA in plasma. The PC1 also had high loadings for the phosphorus-to-creatinine ratio in the urine, but with signs opposite those for the morphometric measures and for the acetate and BHBA in plasma. Thus, across treatment groups, high levels for the morphometric measures of rumen development were associated with high concentrations of acetate and BHBA in plasma but with low phosphorus-to-creatinine ratios in the urine, and vice versa. In particular, the MCT exhibited cross-loading between the PC1 and PC2 when the complete data set was analyzed (Table 5).

In both PCA, excluding the control treatment did not greatly change the loading patterns in the ventral location of the rumen. When morphometric data from the dorsal rumen were analyzed, however, the loading patterns changed dramatically after excluding observations from the control treatment (Tables 4 and 5).

DISCUSSION

Rumen Tissue Morphology

Although not defined as plaque, other researchers have reported observations similar to ours. In calves

Table 4. Loadings on the first and second principal components (PC1 and PC2, respectively) extracted by PC analysis of histological measurements of dorsal and ventral rumen development, pH, and VFA in the ruminal fluid recorded in calves fed concentrate diets differing in carbohydrate source¹

Variable	Dorsal rumen location				Ventral rumen location			
	Complete data set		Without control		Complete data set		Without control	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Ratio of mucosa length to serosa length	-0.59	-0.53	-0.58	-0.47	-0.60	-0.51	-0.35	-0.72
Mucosa thickness, μm	-0.25	-0.85	-0.65	-0.61	-0.38	-0.67	-0.27	-0.79
Muscle thickness, μm	0.23	-0.39	0.10	-0.32	0.09	-0.17	0.16	0.08
Rumen pH	0.44	0.28	0.49	-0.32	0.48	0.47	-0.34	0.45
Acetate in rumen fluid, mmol/L	-0.80	0.31	0.63	-0.07	-0.79	0.32	-0.53	0.27
Propionate in rumen fluid, mmol/L	-0.82	0.06	0.22	-0.34	-0.80	0.13	-0.50	-0.08
Butyrate in rumen fluid, mmol/L	-0.69	0.13	0.24	-0.39	-0.69	0.15	-0.52	-0.08
Lactate in rumen fluid, mmol/L	0.61	-0.27	-0.53	0.64	0.52	-0.60	0.71	-0.37
Eigenvalue	2.82	1.42	1.80	1.47	2.76	1.46	1.65	1.57
Variance explained, %	35.25	17.77	22.53	18.43	34.53	18.21	20.6	19.6

¹Loadings greater than 0.50 are indicated in bold. Results of the analyses are presented with (complete data set) and without the control treatment (milk replacer only: without control) included.

fed concentrate only, Bull et al. (1965) and Nocek et al. (1984) found clumping of papillae with embedded hair and feed particles. Likewise, but in steers fed diets containing high levels of concentrate (>90%), Haskins et al. (1969) described similar observations in the rumen mucosa. In the present study, the incidence of plaque formation in calves fed concentrate diets varied between 6 and 67% at 8 wk of age, and between 73 and 100% at 12 wk of age. The lower incidence of plaque at 8 wk, and the subsequent rise at 12 wk, shown by calves fed the starch diet corresponds to the increase in concentrate intake from the 8- to 12-wk period in the starch group (Suárez et al., 2006).

To facilitate the comparison of empty rumen weights of concentrate-fed calves with those found in the literature and to avoid the confounding effect of different ages and concentrate intakes, data on empty rumen weights gathered from the literature were subse-

quently recalculated and expressed in grams per kilogram of BW. The results are shown in Figure 1. In the present study, empty rumen weights (g/kg of BW) of calves fed concentrate diets ranged from 10.6 to 16.8 g/kg of BW and were in line with data reported by Klein et al. (1987), Greenwood et al. (1997), and Anderson et al. (1987a,b) for rearing calves of similar age fed only with concentrate, and with data reported by Cozzi et al. (2002) for veal calves fed coarse solids. However, empty rumen weights were slightly lower than those reported by Stobo et al. (1966) and Nocek et al. (1984), respectively. As already mentioned, dry feed intake stimulates rumen growth, and dietary factors such as the level of milk or milk replacer feeding (Jenny et al., 1982; Huber et al., 1984), the level of fiber (influenced by the source of NDF), and the physical nature of the dry feed (Morrill, 1992; Beharka et al., 1998) have been reported to affect dry feed intake and conse-

Table 5. Loadings on the first and second principal component (PC1 and PC2, respectively) extracted by PC analysis of histological measurements of dorsal and ventral rumen development, acetate and BHBA in plasma, and the phosphorus-to-creatinine ratio in urine recorded in calves fed concentrate diets differing in carbohydrate source¹

Variable	Dorsal rumen location				Ventral rumen location			
	Complete data set		Without control		Complete data set		Without control	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Ratio of mucosa length to serosa length	-0.80	-0.24	-0.59	-0.46	-0.73	-0.37	-0.57	-0.63
Mucosa thickness, μm	-0.52	-0.75	-0.87	-0.22	-0.52	-0.66	-0.33	-0.79
Muscle thickness, μm	0.24	-0.73	-0.53	0.10	-0.06	-0.56	0.26	-0.21
Plasma acetate concentration	-0.61	0.05	-0.31	0.07	-0.61	0.46	-0.66	0.40
Plasma BHBA concentration	-0.53	0.21	0.09	-0.70	-0.66	0.47	-0.74	0.38
Ratio phosphorus:creatinine in urine	0.54	-0.48	-0.43	0.74	0.55	-0.10	0.25	-0.11
Eigenvalues	1.92	1.43	1.68	1.30	1.91	1.32	1.55	1.38
Variance explained, %	32.03	23.79	28.00	21.71	31.80	21.96	25.84	22.97

¹Loadings greater than 0.50 are indicated in bold. Results of the analyses are presented with (complete data set) and without the control treatment (milk replacer only: without control) included.

Figure 1. Relationship between DMI and empty rumen weight (g/kg of BW).

quently rumen growth, in young calves. Therefore, correction for differences in dry feed intake should be implemented when comparing results of forestomach growth.

In the present research, calves fed the starch diet had lower empty rumen weights at 8 wk than those fed the other concentrate treatments, which may be a result of the lower concentrate intake of the calves (Suárez et al., 2006). However, the pectin treatment resulted in the highest empty rumen weights at a low concentrate intake. Simple linear regressions were performed to clarify the relationship between the increase in empty rumen weight and the anatomical constituents of the rumen wall in the dorsal and ventral rumen locations. The MCT and MST were considered as independent variables. Results from the linear regressions showed that, although significant, only a small part of the variation in empty rumen weight was explained by the MST in the dorsal location and the MCT in the ventral location of the rumen, accounting for 9.4% ($P < 0.01$) and 3.0% ($P < 0.01$) of the variation, respectively. The estimate of the regression coefficient for MCT was positive ($0.18 \text{ g}/\mu\text{m} \pm 0.07 \text{ SE}$), whereas the estimate of the regression coefficient for MST was negative ($-0.22 \text{ g}/\mu\text{m} \pm 0.09 \text{ SE}$). When treatment effects on the incidence of plaque formation (Table 1) were compared with those on empty rumen weights (Table 2), the results suggested that a considerable part in the variation in empty rumen weights was due to variation in the extent of plaque formation.

BHBA and Acetate in Plasma

Plasma BHBA and acetate concentrations were measured in view of their potential suitability as

quick, noninvasive indicators of rumen development. Plasma acetate concentrations were always higher for concentrate treatments than for milk only. The concentration of BHBA in plasma significantly decreased as calves aged, and at the end of the 12-wk period, plasma BHBA concentrations in concentrate-fed calves were not different from those fed the control diet. Other researchers have observed an increase in plasma BHBA with age (Quigley et al., 1991; Quigley and Bernard, 1992; Greenwood et al., 1997). The increased supply of milk replacer and, except for the starch diet, the decreased intake of concentrate over time (see Suárez et al., 2006) may explain the conflicting results. In addition, in the present study plasma BHBA was measured in peripheral blood samples. Thus, the values reported here also include any BHBA converted from butyrate and acetate in the liver (see Bergman, 1990). To better analyze the relationship between plasma acetate and BHBA concentrations, these latter variables (considered to be quick, noninvasive predictors of ruminal activity) were included in a PCA together with those reflecting morphometric development of the rumen.

PCA

First PCA. It has been demonstrated that at birth, the rumen is undeveloped and its growth depends on the consumption of dry feed (Flatt et al., 1958; Huber, 1969). More specifically, the VFA (acetate, butyrate, and propionate) resulting from fermentation of dietary carbohydrates were identified as the main promoters of forestomach development (Sutton et al., 1963a). Propionate and butyrate exert a larger stimulatory effect

on the development of the rumen papillae than does acetate (Sander et al., 1959; Tamate et al., 1962). Sutton et al. (1963b) observed that propionate and butyrate are the main promoters of tissue growth in relation to the higher metabolization of these acids on absorption through the rumen mucosa. In the present study, the loading patterns of PC1 extracted by a PCA of the complete data set (i.e., with the control treatment included) clearly demonstrate a positive relationship between the VFA concentrations in rumen liquid (acetate, propionate, and butyrate) and morphometrical rumen development (RMSL and MCT; see Table 4). Moreover, this pattern of interrelationships was consistently maintained regardless of the rumen location (dorsal or ventral). Results of this PCA did not support the notion that individual VFA may differ in their ability to stimulate mucosa development. Loadings of individual concentrations of VFA in the rumen fluid on the PC1 were highly similar. This might be explained by assuming either that the variation in dietary carbohydrate composition was not large enough to elicit sufficient differences in mucosa growth, or that in the present experiment the response in mucosal development was mediated by factors other than the VFA concentration alone. Correspondingly, the fact that the RMSL and MCT also had high loadings on PC2 (see Table 4) strongly suggests that the development of the ruminal wall is indeed also mediated by factors other than the VFA. Low ruminal pH, a high lactate concentration, ruminitis, and decreased rumen motility are generally associated with ruminal acidosis, a fermentative disorder normally related to diets containing large amounts of highly digestible carbohydrates or lacking an adequate level of effective fiber (Nocek, 1997; Owens et al., 1998). Results in the present study confirm these findings, because in PC1, lactate and pH were interrelated and were negatively correlated with rumen mucosal development in both locations of the rumen. However, it must be pointed out that this negative correlation was no longer present when the control treatment was excluded from the analysis. This means that these patterns of intercorrelations apparently depended largely on the differences between the control treatment and the other treatments, rather than on differences among or interindividual differences within the concentrate diets. In contrast to Dell'Orto et al. (2002), who reported reduced papillae length and epithelial thickness in the rumen of veal calves supplemented with corn silage or sugar beet pulp compared with milk replacer alone, the present results clearly confirm that papillae length and epithelial thickness are larger upon supplementation with solid feed.

Second PCA. Quigley et al. (1991) and Quigley (1996) have demonstrated that in young calves, the rumen epithelium has the capacity to absorb and metabolize VFA from an early age onward. Consequently, in rearing calves, increased rumen VFA concentrations are associated with high plasma BHBA concentrations. As a result, BHBA has been widely used as a quick indicator of rumen development in rearing calves (Quigley et al., 1991; Quigley and Bernard, 1992). In addition, because of rumination activity, adult ruminants normally excrete small amounts of phosphorus in the urine, with most phosphorus being eliminated through the feces. Therefore, the phosphorus-to-creatinine ratio in the urine has recently been proposed as a potential parameter to study the rumen development in calves (van Vuuren et al., 2004). The analysis of our findings confirmed the proposed relationships between rumen development and the physiological parameters used to evaluate this characteristic in the present study. Across all treatments, plasma acetate and BHBA concentrations were positively correlated and the phosphorus-to-creatinine ratio was negatively correlated with the morphometric parameters of rumen development (RMSL and MCT). Similar to the first PCA considered, MCT had high loadings on both PC1 and PC2, which is consistent with the idea that the mechanisms underlying rumen development in calves also involve factors other than VFA. Notably, in contrast to the PCA involving VFA in the rumen fluid, the loading pattern linking plasma levels of acetate and BHBA and morphometric measures of rumen development in the ventral region with the same underlying characteristic was largely preserved after exclusion of the control treatment from the analysis (Table 5). This suggests that within or between concentrate diets, or both, there was meaningful covariation among these measures.

CONCLUSIONS

Feeding concentrates differing in the carbohydrate composition to veal calves promoted rumen development compared with calves fed milk replacer only. Calves fed concentrate diets had heavier rumens than calves fed the control diet; however, a high incidence of coalescing rumen papillae with embedded hair and feed particles was observed in calves fed the concentrates. In most calves, a poorly developed rumen mucosa was observed. In the dorsal location of the rumen, calves fed concentrate diets showed an increased RMSL when compared with calves fed the control diet, whereas only calves fed the pectin and mixed diets showed a larger RMSL in the ventral location. Regardless of the dietary treatment, the MCT was greater in

the ventral than in the dorsal location of the rumen; in contrast, the MST was greater in the dorsal than in the ventral rumen location. In both locations, the NDF diet resulted numerically in the lowest MCT and highest MST among the concentrate treatments. In the companion paper (Suárez et al., 2006), we demonstrated that variation in the carbohydrate composition of the concentrates led to variation in the total VFA concentrations and their molar proportions. Although variation in the carbohydrate composition caused variation in rumen development, the latter was generally small. In addition, the common physical nature of the diets may have prevented the expression of gross anatomical differences in rumen morphology. The PCA strongly suggested that variation in the VFA concentration correlates, to some extent, with the development of the rumen, and that individual VFA, including acetate, propionate, and butyrate, were similarly related to rumen development. The variation in rumen development also coincided with the variation in plasma concentrations of acetate and BHBA.

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