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Original article

Ruminal fermentative parameters and blood acido-basic balance changes during the onset and recovery of induced latent acidosis in sheep

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Abstract — Four ruminal cannulated Texel wethers were used to study the long-term effects of an acidotic diet on ruminal parameters and blood acid-base status. The short-term events around feeding and the recovery of the animals were followed after this nutritional disturbance. Sheep were limit-fed consecutively a control diet H (100% hay) (one week), an acidotic diet W (60% wheat + 40% hay) (2 weeks), and again the control diet (2 weeks). Mean, minimum and maximum ruminal pH were lower, and the time and area under pH 6.0 were higher ($P < 0.001$) with the W diet than with the H diet. These pH parameters indicate a latent acidosis defined here as a subacute and maintained acidosis. Before feeding, the drop in ruminal pH with the W diet was correlated with an increase in the VFA buffering capacity (BC) ($R^2 = 0.70$) and with a decrease in the BC of both carbonic acid functions ($R^2 = 0.52$ for H_2CO_3 and 0.55 for HCO_3^-). After feeding, the acidotic diet effect on ruminal pH was not explained by variations in the BC of either of these chemical species. Ruminal lactate concentration was higher with the W diet compared to the H diet ($P < 0.001$) but remained low (< 2 mmol·L^{-1}). Total VFA concentration ($P < 0.001$), acetate ($P < 0.001$) and propionate ($P < 0.01$) proportions in the rumen decreased with the W diet, while the butyrate proportion increased ($P < 0.001$). The number of Entodiniomorphs increased with the W diet ($P < 0.001$). Most parameters showed no significant variation between the 2 weeks with the W diet ($P > 0.05$). All ruminal parameters, except for ammonia, recovered to initial levels during the H diet redistribution ($P < 0.05$) while blood parameters decreased (pH, $P < 0.05$; bicarbonates (HCO_3^-), total CO_2 content (TCO_2), base excess in whole blood (Beb) and in extra cellular fluid (Beecf), $P < 0.01$). This decrease, initiated during the distribution of the acidotic diet, suggests a mobilization of body alkaline reserves and a longer recovery time in blood than in the rumen. We observed a non-lactic but butyric latent acidosis, linked to Entodiniomorph proliferation, suggesting an intermediate stage before the onset of acute lactic acidosis.

acidosis / rumen fermentation / protozoa / blood / acid-base status

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Résumé — Modifications des paramètres fermentaires ruminaux et de l'équilibre acido-basique sanguin durant le développement d'une acidose latente provoquée sur moutons et lors de leur rétablissement. Quatre moutons Texels castrés, équipés d'une canule ruminale, ont été utilisés pour étudier les effets à long terme d'un régime acidogène sur les paramètres ruminaux et l'équilibre acido-basique sanguin. Les effets à court terme autour du repas ainsi que le rétablissement des animaux après cette perturbation nutritionnelle ont aussi été examinés. Les moutons ont reçu successivement en quantité limitée un régime témoin H (100 % foin) (une semaine), un régime acidogène W (60 % de blé + 40 % de foin) (2 semaines), et à nouveau le régime témoin (2 semaines). Les pH moyen, minimum et maximum étaient plus faibles et le temps et l'aire sous pH 6,0 plus élevés ($P < 0{,}001$) avec le régime W qu'avec le régime H. Les valeurs observées pour ces paramètres de pH sont caractéristiques d'une acidose latente définie ici comme une acidose subaiguë et prolongée. Avant repas, la chute du pH ruminal avec le régime W est corrélée avec une augmentation du pouvoir tampon (BC) des AGV ($R^2 = 0{,}70$) et avec une diminution du BC des deux fonctions de l'acide carbonique ($R^2 = 0{,}52$ pour H_2CO_3 et 0,55 pour HCO_3^-). Après repas, l'effet du régime acidogène sur le pH ruminal n'est pas expliqué par les variations du BC de l'une ou l'autre de ces espèces chimiques. La concentration du lactate ruminal est plus élevée avec le régime W qu'avec le régime H ($P < 0{,}001$) mais est restée à des valeurs faibles (< 2 $mmol \cdot L^{-1}$). La concentration en AGV totaux ($P < 0{,}001$), les proportions en acétate ($P < 0{,}001$) et en propionate ($P < 0{,}01$) dans le rumen ont diminué avec le régime W, alors que la proportion de butyrate a augmenté ($P < 0{,}001$). Parallèlement, le nombre d'Entodiniomorphes a augmenté avec le régime W ($P < 0{,}001$). La plupart des paramètres n'ont pas montré de variations significatives entre les 2 semaines de régime W ($P > 0{,}05$). Tous les paramètres ruminaux, à l'exception de l'ammoniaque, sont revenus à leur niveau initial dès la redistribution du régime H ($P < 0{,}05$), alors que les paramètres sanguins ont diminué (pH, $P < 0{,}05$; taux de bicarbonates (HCO_3^-) et de CO_2 total (TCO_2), excès de base dans le sang (Beb) et dans le fluide extracellulaire (Beecf), $P < 0{,}01$). Cette baisse, initiée durant la distribution du régime acidogène, suggère une mobilisation des réserves alcalines corporelles et un temps de rétablissement plus long dans le sang que dans le rumen. L'acidose latente butyrique et la prolifération des Entodiniomorphes observés dans cet essai pourraient être une étape intermédiaire avant le développement d'une acidose lactique aiguë.

acidose / fermentation ruminale / protozoaires / sang / équilibre acido-basique

1. INTRODUCTION

Acute acidosis occurs after the consumption of an excessive quantity of readily fermentable carbohydrates (RFC) that rapidly alters ruminal function and can have irreversible metabolic consequences [22, 25, 45]. Ruminal perturbations include proliferation of an acid-tolerant microbial population and increased acid production (VFA and, especially, lactate) resulting in a drastic and uncompensated pH drop below about 5.0 considered as a benchmark [45]. In contrast to acute acidosis, the sequence of events leading to subacute or latent (subacute and maintained) acidosis is less well understood [22]. Latent acidosis is probably more difficult to characterize because biological parameters in the rumen fluctuate within physiological limits and are difficult to maintain. This unstable state may reflect the oscillatory behavior of the ruminal microbial population in response to diet-based fermentative hiccoughs [18].

Most prior studies of acidosis have involved its induction by carbohydrate challenge consisting of ruminal glucose infusion [7, 36] or feeding of an acidotic diet after a feed-withholding period [10, 24]. These studies focused generally on short-term (< 5 days) changes in the rumen and bloodstream. The originality of the present study was (1) to examine longer-term effects of an acidotic diet on ruminal biotic and abiotic parameters and blood acid-base status while also including short-term

dynamic events associated with feeding, and (2) to study the recovery of animals after this nutritional disturbance.

2. MATERIALS AND METHODS

2.1. Animals, diets and experimental design

Texel wethers (n = 4, 3-yr old) with an average body weight of 74.1 ± 1.3 kg at the start of the experiment were used. Each animal was fitted with a ruminal cannula (i.d. 62 mm) made of polyamide and polyvinyl chloride (Synthesia, Nogent sur Marne, France). Surgery was performed in a sterile environment under general anesthesia (Halothane, ICIU Pharma-vétérinaire, Paris, France) and under the responsibility of a licensed veterinarian with specialized training in large animal surgery. Surgical preparation of the wethers was done 3 months before the start of the study and during that time, they received a hay-only diet. Throughout the experimental period, the animals were housed in individual stalls (1.00 × 1.50 m) with automatic waterers and individual feed-bunks.

For the duration of the study, the animals were fed two equal portions at 08.00 and 20.00 h, with two different diets: a forage diet (H diet) composed of 100% chopped alfalfa hay, and a wheat rich diet (W diet) composed of 60% pelleted ground wheat (3-mm screen) and 40% hay as above. The chemical composition of the experimental feeds and diets is given in Table I. The two diets were alternatively offered according to the experimental feeding design shown in Figure 1. The animals were first adapted to the H diet ad libitum for 3 weeks

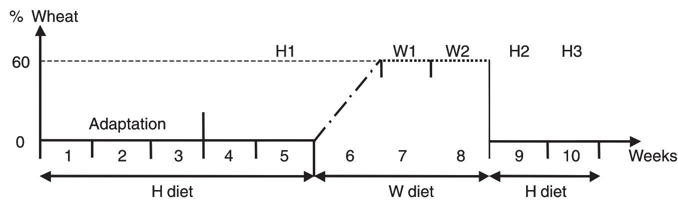


Figure 1. Experimental feeding design. H diet = 100% hay; W diet = 60% wheat + 40% hay; H1, H2, H3: H diet distributed one week before W diet, one week after and two weeks after, respectively; W1, W2: first and second weeks on W diet, respectively.

Table I. Chemical composition of experimental feed and diets (% DM).

Item	Feed		Diet[a]	
	Alfalfa hay	Wheat concentrate	H	W
Organic matter	91.2	97.2	91.2	94.8
Crude protein	14.7	9.8	14.7	11.8
NDF	45.2	15.8	45.2	27.6
ADF	32.8	2.6	32.8	14.6
Starch	0	67.9	0	40.7

[a] H diet = 100% hay; W diet = 60% wheat + 40% hay.

(weeks 1 to 3). For the rest of the experiment, the animals were fed restricted amounts (90% ad libitum) to 1.5 kg of DM per day, to ensure that the diet was ingested quickly and without orts. The H diet was offered for 2 weeks (weeks 4 and 5). The incorporation of wheat in the diet was progressive (10% per d) but quick (6 days) to reach the final acidotic W diet (week 6). The animals were maintained on the W diet for 2 weeks (weeks 7 and 8) and then returned to the H diet without transition for 2 weeks (weeks 9 and 10). The total duration of the experiment was 10 weeks and five experimental periods of one week were defined: H1, week 5 on the H diet; W1 and W2, weeks 7 and 8 on the W diet; H2 and H3, weeks 9 and 10 on the H diet.

2.2. Measurements, chemical analysis and calculations

Feed offered and refused was recorded daily to calculate DMI. Feed DM was determined by oven drying at 60 °C for 48 h and the ash content was determined by ashing samples at 550 °C for 6 h [3]. Both NDF and ADF were analyzed according to Van Soest and Robertson [54]. Crude protein was determined by the Kjeldahl method [3] and starch by an enzymatic method [19]. Water consumption was recorded daily using an individual water meter.

The volume and turnover rate of the ruminal liquid phase was determined for each experimental period and each animal from a pulse dose (140 mg Cr in 50 mL water) of Cr-EDTA solution [5] injected into the rumen of each sheep via the cannula just before the morning meal. Rumen liquid samples were taken manually from the ventral sac of the rumen via the cannula using a suction pump and a rigid plastic tube (length 400 mm; i.d. 15 mm) just before the morning meal and 3, 6, 9, 12, 24 and 27 h after. After filtration through a 250-µm nylon filter, 20 mL of liquid were frozen (–20 °C) until analysis. The Cr contents of the samples were determined with an atomic absorption spectrometer (Perkin-Elmer 2380; Perkin-Elmer Courtabœuf, France) after centrifugation of the samples at 5000 × *g* for 15 min at 4 °C. The volume and the turnover rate of the ruminal liquid phase were calculated from the exponential decrease of Cr concentrations with time. After semi-logarithmic line fitting, the slope represented the fluid dilution rate (%/h), and the volume of the liquid phase in the rumen was calculated from the intercept at *t* 0.

Ruminal pH was measured continuously during each experimental period. Each wether was fitted with an indwelling pH probe. The Fisherbrand self-cleaning flat kynar pH electrode (Fisher Bioblock Scientific, Illkirch, France) had a 3-m wire extension. The wire and the probe were fixed to a 25-cm plastic stalk, ballasted by a 100-g weight to maintain the device in place in the ventral sac. The wire was thread through the cannula cover, blocked herein, and secured to the cannula. The other end of the wire was connected to a pH data logger (EL-2, Lascar Electronics Ltd., Salisbury, UK). The outlet wire and the data logger were placed in a dress handkerchief fixed to the back of the animals. Ruminal pH was recorded at 5-min intervals and data were collected weekly by connecting each logger to a PC using El-Win software (Lascar Electronics Ltd., Salisbury, UK). The following parameters were calculated for each day using the pH kinetics obtained with the indwelling probes: mean, minimum and maximum pH, time and area under pH 6.0, and time and area under pH 5.5. The calculation of the area was the difference between the pH value and pH limit (6.0 or 5.5) multiplied by the time interval (i.e. 5 min). The positive values (negative values reflect pH greater than the chosen limit of pH) were added together.

Ruminal samples (200 mL) were collected and immediately filtered as described before on two successive days per H diet week, and on three successive days per

W diet week, before (–1 h) and after the morning meal (+3 h and +6 h). All samples were analyzed individually. Two 50-mL filtrate samples were preserved under a plastic film to avoid gas exchange and were used for the titration analyses. The buffering capacity was determined by titrating one 50-mL aliquot of filtrate under continuous stirring, from its original pH to pH 2 with 1*N*-HCl and by titrating a separate, 50-mL aliquot from its original pH to pH 12 with 1*N*-NaOH. An automatic titrating unit (TitroLine alpha, Schott-Geräte, Hofheim, Germany), linked to a computer equipped with Titrisoft software (Schott-Geräte, Hofheim, Germany) was used to obtain and record the titration data and curves. The buffering capacity was calculated as $BC = \Delta H^+/\Delta pH$, in 10^{-2} moles of H^+/pH unit. The titration curves were interpreted by measuring BC values for pKa of lactate (3.7), VFA (4.8), first function (H_2CO_3: 6.2) and second function (HCO_3^-: 10.2) of carbonic acid, and ammonia (9.3).

Eight milliliters of filtrate were preserved in duplicate by adding 0.8 mL of 5% (vol/vol) orthophosphoric acid to determine VFA content by gas chromatography using 4-methylvaleric acid as the internal standard [29], and NH3 content [53]. Another 8-mL aliquot was taken for the determination of D- and L-lactic acid content using an enzymatic method (TC D-/L-lactic acid, Boehringer Mannheim 1112821, R-Biopharm, Darmstadt, Germany).

Nine millimeters of filtrate were preserved at 4 °C with 1 mL of solution made of 50% glycerol, 48% distilled water, and 2% formaldehyde for protozoa counting using a Dolfuss cell (Elvetec Services, Clermont-Ferrand, France) according to procedures described by Jouany and Senaud [30]. Protozoa were further categorized as either Entodiniomorphid or isotrichid Holotrich ciliates by skeletal plate-staining with Lugol solution.

Jugular venous whole blood was collected in syringes for blood gas collection (2 mL Blood Gas MONOVETTE®, Sarstedt, Nümbrecht, Germany) 3 h after the morning meal on the same days as ruminal sampling. The following blood parameters were determined with a pH/blood gas analyzer (IRMA SL 2000, Philips Systèmes Médicaux, Suresnes, France) immediately after sample collection: pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), bicarbonates (HCO_3^-), total carbon dioxide content (TCO_2), base excess in whole blood (Beb), base excess in extra cellular fluid (Beecf), and oxygen saturation (O_2sat). The pH, pCO_2 and pO_2 were directly measured and HCO_3^-, TCO_2, Beb, Beecf and O_2sat were calculated by the analyzer according to the Severinghaus/NCCLS formula [40].

2.3. Statistical analyses

The results were analyzed using the GLM procedure of SAS [48] with the experimental period (n = 5) and animal (n = 4) as the main effects. In this study, time and period effects were confounded. But, since the animals were physiologically stable (adult, non-productive), restricted fed and maintained in a controlled environment, the time effect has been assumed to be negligible. Orthogonal contrasts enabled the following effects to be tested: distribution of an acidotic diet (H vs. W), adaptation to an acidotic diet (W1 vs. W2), return to a hay diet after distribution of an acidotic diet (H2 vs. H3), and comparison with the initial state (H1 vs. H2 + H3). When variables were analyzed at different times of the day (VFA, ammonia and lactate concentrations, protozoa counts, and buffering capacity), an additional analysis was performed using the repeated statement of the GLM procedure. Means were declared significant at $P < 0.05$. Correlations between pH and buffering capacities of the different constituents of the acido-basic balance were performed on 48 values (4 animals × 12 sampling days) for each sampling time.

3. RESULTS AND DISCUSSION

3.1. Effect of acidotic diet (H vs. W)

Dry matter intake (DMI) was lower with the W diet compared to the H diet, (H vs. W: $P < 0.05$; Tab. II). Reduced DMI was observed mainly the first 2–3 days of W1, during which DMI was lower for all animals. The animals then recovered their ingestion levels, except for one animal that stayed at a lower intake until the end of W2. An erratic and drastic reduction of DMI is currently observed during subacute acidosis [45, 51] for animals in production fed ad libitum. In our study, the animals were limit-fed and so not in optimal conditions to demonstrate an acidotic diet effect on feed intake. Water ingestion showed no difference between the two diets ($P > 0.05$). The turnover of the liquid phase was lower with the W diet compared to the H diet ($P < 0.01$), related to a decreased rumination [32] and/or to a weak ruminal stasis [41, 43] with high-concentrate diets.

All ruminal pH variables were affected by the acidotic diet W compared to the H diet ($P < 0.01$, Tab. III). As expected, mean, minimum and maximum ruminal pH were lower with the W diet than with the H diet. The time and area under pH 6.0 were higher with the W diet than with the H diet. The same trend was observed for the time and area under pH 5.5. In sheep fed a similar amount of starch (44%), Mackie and Gilchrist [37] observed a higher minimum pH (5.80 vs. 5.43), but the nature of starch differed in their experiment (slowly degradable maize starch vs. rapidly degradable wheat starch). Moreover, our mean ruminal pH values were higher than those reported in other studies [4, 12, 23] on steers with ad libitum access to diets containing more than 50% of rapidly degradable starch.

Latent acidosis can be defined by a mean daily pH comprised between 6.25 and 5.5, but the duration and intensity of pH decrease can vary substantially for the same mean daily pH [50]. Oetzel [43] defined latent acidosis as periods of moderately depressed ruminal pH between 5.5 and 5.0 that are between acute and chronic in duration. Also, in recent studies, the time and area under a critical pH threshold, comprised between 5.5 and 6.0, were used to describe the duration and intensity of latent acidosis, respectively [23, 35, 47] and in order to improve the characterization of the acidosis state. According to these definitions, all the pH values obtained in our experiment show that our acidotic diet model and our experimental feeding design were responsible for a latent acidosis state in all the animals.

Titrimetry is an analytical method that enables the main components of the ruminal buffering system to be identified and their buffering capacity to be measured. Also, it presents a particular interest in explaining the acido-basic imbalance involved in the acidosis state, an imbalance revealed but not explained by only measuring ruminal pH. For both diets, VFA, the two acid functions of carbonic acid and NH_3 were identified by titrimetry as the components of the ruminal acido-basic status, but not lactate (Tab. IV). Indeed, lactate does not accumulate during latent acidosis, on the contrary to acute acidosis cases [45]. Furthermore, the NH_3 BC did not show a significant variation with the two diets ($P > 0.05$) and remained low compared to the BC of the VFA and carbonic acid functions. Thus, lactate and NH_3 will not be considered in the following discussion.

As a consequence of feeding (time effect, $P < 0.001$), VFA BC increased and BC of the carbonic acid functions decreased with both diets, as observed by Fernandez et al. [20] on dairy cows fed corn silage. This may be explained by two associated mechanisms: post-prandial VFA production acidifies ruminal content with H^+ ion production, and contributes to a balance

Table II. Effect of an acidotic diet on dry matter intake, water consumption, turn-over of liquid phase and rumen volume.

	Period[a]						Statistical significance[c]			
	H diet	W diet		H diet						
Item	H1	W1	W2	H2	H3	SE[b]	H vs. W	W1 vs. W2	H2 vs. H3	H1 vs. H2+H3
Dry matter intake ($kg \cdot d^{-1}$)	1.50	1.43	1.26	1.48	1.50	0.10	*	NS	NS	NS
Water consumption (L)	4.47	3.69	3.80	4.51	3.83	0.31	NS	NS	NS	NS
Turnover rate of liquid phase ($\% \cdot h^{-1}$)	6.75	5.10	5.10	6.00	6.45	0.45	**	NS	NS	NS
Rumen volume (L)	13.25	14.86	10.69	13.14	16.76	2.49	NS	NS	NS	NS

[a] H diet = 100% hay; W diet = 60% wheat + 40% hay; H1, H2, H3: H diet distributed one week before W diet, one week after and two weeks after, respectively; W1, W2: first and second weeks on W diet, respectively.

[b] Standard error.

[c] NS: $P > 0.05$; * : $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Table III. Effect of an acidotic diet on ruminal pH parameters.

	Period[a]						Statistical significance[c]			
	H diet	W diet		H diet						
Item	H1	W1	W2	H2	H3	SE[b]	H vs. W	W1 vs. W2	H2 vs. H3	H1 vs. H2+H3
pH										
Mean	6.48	5.91	6.04	6.63	6.50	0.097	***	NS	NS	NS
Minimum	6.12	5.34	5.52	6.25	6.13	0.114	***	NS	NS	NS
Maximum	6.89	6.65	6.69	7.08	6.98	0.068	***	NS	NS	NS
Time under 6.0 (h)	0.41	12.81	11.49	0.01	0.51	1.50	***	NS	NS	NS
Time under 5.5 (h)	0	3.64	3.24	0	0.01	47.42	***	NS	NS	NS
Area under 6.0 (pH unit·h)	0.05	4.83	4.37	0	0.05	0.72	***	NS	NS	NS
Area under 5.5 (pH unit·h)	0	0.62	0.57	0	0	11.82	**	NS	NS	NS

[a] H diet = 100% hay; W diet = 60% wheat + 40% hay; H1, H2, H3: H diet distributed one week before W diet, one week after and two weeks after, respectively; W1, W2: first and second weeks on W diet, respectively.
[b] Standard error.
[c] NS: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Table IV. Effect of an acidotic diet and time relative to feeding on buffering capacity of main components of the ruminal buffering system.

	Period[a]						Statistical significance[d]			
	H diet	W diet		H diet						
Item and time relative to feeding[b]	H1	W1	W2	H2	H3	SE[c]	H vs. W	W1 vs. W2	H2 vs. H3	H1 vs. H2+H3
Buffering capacity,										
10^{-2} moles of H^+·pH $unit^{-1}$										
Lactate (pK = 3.7)	ND[e]	ND	ND	ND	ND					
VFA (pK = 4.8)										
–1h	5.77	7.37	5.98	5.41	5.65	0.28	**	**	NS	NS
+3h	9.78	9.60	9.25	8.25	9.68	0.64	NS	NS	NS	NS
+6h	8.31	8.92	7.74	7.76	8.50	0.46	NS	NS	NS	NS
NH3 (pK = 9.3)										
–1h	1.43	0.63	1.20	1.57	1.23	0.55	NS	NS	NS	NS
+3h	2.49	1.04	1.46	1.26	1.77	0.43	NS	NS	NS	NS
+6h	1.14	1.07	0.40	0.45	0.48	0.32	NS	NS	NS	NS
Carbonic acid first function (H_2CO_3, pK = 6.2)										
–1h	3.71	3.06	3.09	4.97	4.30	0.42	**	NS	NS	NS
+3h	0.38	2.14	3.02	0.33	0.78	0.32	***	NS	NS	NS
+6h	1.30	1.86	2.42	0.33	0.35	0.25	***	NS	NS	**
Carbonic acid second function (HCO_3^-, pK = 10.2)										
–1h	4. 14	2.91	3.20	4.47	3.62	0.39	*	NS	NS	NS
+3h	2.66	2.32	2.98	2.63	3.11	0.45	NS	NS	NS	NS
+6h	2.35	2.05	3. 63	2.21	3.39	0.64	NS	NS	NS	NS

[a] H diet = 100% hay; W diet = 60% wheat + 40% hay; H1, H2, H3: H diet distributed one week before W diet, one week after and two weeks after, respectively; W1, W2: first and second weeks on W diet, respectively.

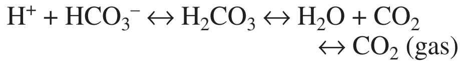
[b] –1h, +3h, +6h: sampling time around morning meal.

[c] Standard error.

[d] NS: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

[e] ND: not detected.

shift from carbonic acid to CO_2 production as follows [2]:



$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2 \leftrightarrow CO_2\ (gas)$$

The effect of an acidotic diet on VFA and carbonic acid BC varied with sampling time. Before feeding, VFA BC was higher with the W diet ($P < 0.01$), on the contrary to BC of the two functions of carbonic acid, which were lower with the W diet than with the H diet (H_2CO_3, $P < 0.01$ and HCO_3^-, $P < 0.05$, respectively). Before feeding, lower ruminal pH with the W diet than with the H diet could be explained by both an increase in VFA BC and a decrease in the BC of the carbonic acid functions. Before feeding, there was a good correlation between ruminal pH and VFA BC ($R^2 = 0.70$) and between ruminal pH and the BC of the two carbonic acid functions ($R^2 = 0.52$ for H_2CO_3 and 0.55 for HCO_3^-). After feeding, VFA BC was not modified by the acidotic W diet ($P > 0.05$), only the BC of H_2CO_3 increased ($P < 0.001$). These results can be explained by an increase in the rate of VFA absorption related to a decrease in ruminal pH observed with the W diet [15]. In response, a more rapid passage of carbonic acid from blood into the rumen was observed [52]. This dietary effect appeared only on the first function of carbonic acid because the low ruminal pH moved the carbonic acid balance in this direction. The correlation observed between ruminal pH and BC before feeding was not demonstrated after feeding probably because of the instability between the production and the absorption or transformation of the different components at the sampling time. Titrimetry measurements made before feeding may be more representative of the general state of the animal because the ruminal conditions are stable, and therefore more appropriate for demonstrating the effect of an acidotic diet on ruminal acido-basic balance.

Blood parameters, measured 3 h after feeding, showed no significant variation between the two diets ($P > 0.05$; Tab. V), except a pO_2 decrease ($P < 0.05$). Others found small acid-base status changes in the blood during subacute acidosis induced in steers by grain challenge [8, 24]. A marked decrease in blood pH, HCO_3^-, TCO_2 and Beb were observed in ruminants during acute acidosis [8, 42, 46].

In the present study, the ruminal lactate concentration was higher on the W diet than on the H diet ($P < 0.001$) but remained at low levels (< 2 $mmol \cdot L^{-1}$) on both diets namely 60 and 40% for D and L-lactate (Tab. VI). The low concentration of lactate in ruminal content confirmed the absence of detection of lactate by titrimetry. Only a slight increase in the total ruminal lactate has been noted in animals in latent acidosis fed high starch diets (> 44% on DM basis) with concentrations below 2 $mmol \cdot L^{-1}$ in steers [9, 23], or below 10 $mmol \cdot L^{-1}$ in steers [26] and in sheep [38]. In agreement with Nocek [41] and Oetzel [43], the latent acidosis state was not characterized here by lactate accumulation. In comparison, during acute acidosis in sheep or goats, ruminal lactate concentrations can reach 26 $mmol \cdot L^{-1}$ [46], 52 $mmol \cdot L^{-1}$ [42] or more than 100 $mmol \cdot L^{-1}$ [33] with ruminal pH between 4 and 5.

In most cases, the supplementation of forage diets with readily fermentable carbohydrates involved an increase in total ruminal VFA concentration [41]. In our study, the total ruminal VFA concentration was lower with the W diet (106 $mmol \cdot L^{-1}$) than with the H diet (125 $mmol \cdot L^{-1}$) ($P < 0.001$; Tab. VI). This could be explained by a lower ruminal cellulolytic activity and thus a lower ruminal fiber digestibility with the W diet compared to the H diet [39]. It may also result from a high absorption rate of VFA with the W diet, related to the low ruminal pH [15]. The latter effect of an acidotic diet on VFA concentration has also been reported by Fulton et al. [21] during latent acidosis in steers. A decrease in VFA concentrations

Table V. Effect of an acidotic diet on pH, gas pressure, bicarbonates and CO_2 content, base excess and oxygen saturation in blood.

	Period[a]									
	H diet	W diet		H diet			Statistical significance[d]			
Item[b]	H1	W1	W2	H2	H3	SE[c]	H vs. W	W1 vs. W2	H2 vs. H3	H1 vs. H2+H3
pH	7.423	7.412	7.417	7.408	7.388	0.007	NS	NS	*	*
pCO_2 (mm Hg)	42.38	42.43	43.58	41.15	42.30	1.11	NS	NS	NS	NS
pO_2 (mm Hg)	47.80	44.30	44.15	45.33	48.25	1.42	*	NS	NS	NS
HCO_3 ($mmoles \cdot L^{-1}$)	26.85	26.20	25.70	25.18	24.70	0.52	NS	NS	NS	**
TCO_2 ($mmoles \cdot L^{-1}$)	28.05	27.38	26.90	26.33	25.90	0.53	NS	NS	NS	**
Beb ($mmoles \cdot L^{-1}$)	3.48	2.70	2.45	1.83	0.98	0.48	NS	NS	NS	**
Beecf ($mmoles \cdot L^{-1}$)	2.70	1.85	1.50	0.78	-0.05	0.54	NS	NS	NS	**
O_2sat (%)	77.50	72.98	73.50	74.95	76.85	2.10	NS	NS	NS	NS

[a] H diet = 100% hay; W diet = 60% wheat + 40% hay; H1, H2, H3: H diet distributed one week before W diet, one week after and two weeks after, respectively; W1, W2: first and second weeks on W diet, respectively.

[b] HCO_3^-: bicarbonates content; TCO_2: total CO_2 content; Beb: base excess in whole blood; Beecf: base excess in extra cellular fluid; O_2sat: oxygen saturation.

[c] Standard error.

[d] NS: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Table VI. Effect of an acidotic diet and time relative to feeding on ruminal VFA composition, lactate and ammonia concentrations.

	Period[a]									
	H diet	W diet		H diet			Statistical significance[d]			
Item and time relative to feeding[b]	H1	W1	W2	H2	H3	SE[c]	H vs. W	W1 vs. W2	H2 vs. H3	H1 vs. H2+H3
Lactate ($mmoles \cdot L^{-1}$)										
−1h	1.20	1.42	1.93	0.95	0.89	0.19	***	*	NS	NS
+3h	0.67	1.27	1.83	0.93	0.54	0.26	***	NS	NS	NS
+6h	1.04	1.20	1.73	1.04	0.98	0.17	**	**	NS	NS
L-Lactate ($mmoles \cdot L^{-1}$)										
−1h	0.46	0.56	0.87	0.36	0.31	0.09	***	**	NS	NS
+3h	0.22	0.58	0.82	0.30	0.21	0.11	***	NS	NS	NS
+6h	0.30	0.62	0.59	0.38	0.39	0.09	**	NS	NS	NS
D-Lactate ($mmoles \cdot L^{-1}$)										
−1h	0.75	0.85	1.06	0.59	0.59	0.12	**	NS	NS	NS
+3h	0.45	0.69	1.01	0.62	0.33	0.16	**	NS	NS	NS
+6h	0.74	0.58	1.14	0.66	0.59	0.12	NS	***	NS	NS
Total VFA ($mmoles \cdot L^{-1}$)										
−1h	95.3	98.0	88.5	84.5	91.8	5.87	NS	NS	NS	NS
+3h	158.0	112.9	128.0	134.5	157.0	8.58	**	NS	NS	NS
+6h	140.0	94.1	114.8	126.5	135.4	7.96	**	NS	NS	NS
Acetate (molar %)										
−1h	68.6	61.9	62.6	66.8	67.3	0.72	***	NS	NS	NS
+3h	66.7	61.8	61.4	65.4	64.3	0.75	***	NS	NS	NS
+6h	68.4	63.1	62.6	67.2	66.8	0.93	***	NS	NS	NS
Propionate (molar %)										
−1h	17.7	17.3	17.2	18.6	18.9	0.69	NS	NS	NS	NS
+3h	21.4	19.3	19.5	21.2	22.9	0.84	**	NS	NS	NS
+6h	19.7	18.1	18.3	19.9	20.8	0.66	**	NS	NS	NS
Butyrate (molar %)										
−1h	7.9	13.9	13.7	8.0	8.1	0.69	***	NS	NS	NS
+3h	7.0	13.7	13.5	7.4	7.5	0.65	***	NS	NS	NS
+6h	7.6	13.3	13.1	7.5	7.9	0.77	***	NS	NS	NS

Table VI. (continued).

	Period[a]									
	H diet	W diet		H diet			Statistical significance[d]			
Item and time relative to feeding[b]	H1	W1	W2	H2	H3	SE[c]	H vs. W	W1 vs. W2	H2 vs. H3	H1 vs. H2+H3
Acetate/Propionate ratio										
–1h	3.89	3.67	3.74	3.61	3.61	0.15	NS	NS	NS	NS
+3h	3.12	3.31	3.27	3.12	2.85	0.16	NS	NS	NS	NS
+6h	3.48	3.60	3.52	3.40	3.25	0.15	NS	NS	NS	NS
Acetate/(Propionate+Butyrate) ratio										
–1h	2.69	2.02	2.09	2.52	2.51	0.07	***	NS	NS	NS
+3h	2.35	1.89	1.93	2.30	2.13	0.08	***	NS	NS	NS
+6h	2.51	2.03	2.08	2.46	2.34	0.09	***	NS	NS	NS
NH3 (mmoles·L^{-1})										
–1h	17.6	13.7	13.7	7.0	14.1	1.20	NS	NS	***	***
+3h	23.5	10.3	10.1	16.7	23.5	1.16	***	NS	***	*
+6h	14.6	8.6	7.2	9.5	11.9	1.26	**	NS	NS	***

[a] H diet = 100% hay; W diet = 60% wheat + 40% hay; H1, H2, H3: H diet distributed one week before W diet, one week after and two weeks after, respectively; W1, W2: first and second weeks on W diet, respectively.

[b] –1h, +3h, +6h: sampling time around morning meal.

[c] Standard error.

[d] NS: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

was observed during acute acidosis in steers [26] and in sheep [34], but these were associated with an increased lactate concentration.

Ruminal acetate ($P < 0.001$) and propionate ($P < 0.01$) proportions decreased with the W diet, while the ruminal butyrate proportion increased ($P < 0.001$) (Tab. VI). The acetate/propionate ratio was not modified ($P > 0.05$) but the acetate/(propionate + butyrate) ratio decreased with the W diet ($P < 0.001$).

The effect of dietary starch additions on the VFA profile is not clear in the literature. In some studies, the presence of starch increased the levels of propionate from 17 to 30% mainly at the expense of acetate [31, 49]. In other cases, increased propionate and butyrate proportions and decreased acetate proportions have been reported during the adaptation to high grain diets in sheep [37] and cattle [10, 24]. In agreement with our results, Eadie et al. [17] and Doreau et al. [16] observed atypical ruminal fermentation oriented towards butyrate at the expense of acetate. A high butyric fermentation in the rumen was explained by Coe et al. [10] as follows: acetate, butyrate and propionate are the main lactate metabolites in the rumen, propionate being a less favored pathway to maintain the oxydo-reduction balance. The acetate and butyrate pathways are pH dependent and the production of butyrate from lactate is maximal at low pH. Furthermore, butyrate synthesis from acetate uses hydrogen and would provide protection against increased ruminal acidity.

In agreement with other studies on steers [10, 27], the ruminal NH3 concentration measured in our experiment decreased with an increasing proportion of concentrate in the diet, and more particularly after feeding ($P < 0.001$).

It is probable that the effect of the acidotic W diet on the fermentative profile described above was a consequence of microbial modifications in the rumen. The total protozoa count increased with the W diet ($P < 0.001$, Tab. VII) and was related to an increase in Entodiniomorph numbers, which represented the majority (90%) of the protozoa population. An increase in the percentage of rapidly degradable starch in the diet generally favors the development of protozoa up to a certain level [14], above which the protozoa concentration falls [38]. In our case, the amount of rapidly degradable starch (40%) ingested by limit-fed sheep appeared to be in favor of the proliferation of the Entodiniomorph population. Entodiniomorphs are able to engulf large quantities of starch [11, 55]. Sequestered from bacteria, starch is fermented by protozoa to VFA rather than lactate and at a slower rate than that of bacteria, thereby reducing the risk of acidosis [1, 6]. Moreover, starch fermentation by protozoa leads to higher butyrate production [55], as observed in our study. However, an eventual role of bacteria cannot be ruled out to explain the increase in butyrate proportion and low lactate concentrations on diet W. Indeed, *Butyrivibrio fibrisolvens* produces butyrate as a primary fermentation end product [28]. Moreover, ruminal lactate-utilizing bacteria, such as *Megasphaera elsdenii*, a major producer of butyrate from lactate [13], may also have prevented lactate accumulation. Increased numbers of these bacteria have been observed in grain-adapted sheep [37] and cattle [44].

3.2. Adaptation to W diet (W1 vs. W2)

Most parameters showed no significant variations between W1 and W2 ($P > 0.05$). The acidosis state was therefore maintained in our animals, without deterioration, during the 2 weeks, W1 and W2, of the acidotic diet, in agreement with the definition of latent acidosis (subacute and maintained). Few studies have reported the effect of an acidotic diet over such a long period as in our study. In many cases, subacute acidosis studies lasted only a few days [24, 36]. However,

Table VII. Effect of an acidotic diet and time relative to feeding on ruminal protozoa number.

	Period[a]									
	H diet	W diet		H diet			Statistical significance[d]			
Item and time relative to feeding[b]	H1	W1	W2	H2	H3	SE[c]	H vs. W	W1 vs. W2	H2 vs. H3	H1 vs. H2+H3
Total protozoa ($\times 10^3 \cdot mL^{-1}$)										
–1h	173	286	394	86	59	76	**	NS	NS	NS
+3h	118	333	453	81	88	76	**	NS	NS	NS
+6h	129	376	315	86	73	51	***	NS	NS	NS
Holotrichs ($\times 10^3 \cdot mL^{-1}$)										
–1h	3.69	5.02	3.38	4.10	3.64	0.58	NS	NS	NS	NS
+3h	8.83	4.27	4.06	4.08	10.18	0.93	**	NS	***	NS
+6h	2.26	2.50	2.62	1.72	2.12	0.55	NS	NS	NS	NS
Entodiniomorphs ($\times 10^3 \cdot mL^{-1}$)										
–1h	169	281	391	82	55	76	**	NS	NS	NS
+3h	109	329	449	77	78	76	***	NS	NS	NS
+6h	127	373	313	84	71	51	***	NS	NS	NS

[a] H diet = 100% hay; W diet = 60% wheat + 40% hay; H1, H2, H3: H diet distributed one week before W diet, one week after and two weeks after, respectively; W1, W2: first and second weeks on W diet, respectively.
[b] –1h, +3h, +6h: sampling time around morning meal.
[c] Standard error.
[d] NS: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

in agreement with our results, Brown et al. [8] observed no significant changes in ruminal and blood acido-basic status on steers maintained for two 2 weeks on a 50% concentrate diet after subacute acidosis challenge. Coe et al. [10] reported a constant ruminal pH and fermentative pattern between two weeks for steers on an all-cereal diet while the protozoa population decreased.

3.3. Recovery after latent acidosis (H1 vs. H2 + H3) and adaptation to a hay diet after two weeks on an acidotic diet (H2 vs. H3)

Dry matter intake, abiotic and biotic ruminal parameters, except NH_3 concentration ($P < 0.001$), recovered to initial levels (H1 vs. H2 + H3, $P > 0.05$) often as early as the first week (H2) following an acidotic diet distribution (H2 vs. H3, $P > 0.05$). However, blood parameters decreased between H1 and H2 to H3 (pH, $P < 0.05$; HCO_3^-, TCO_2, Beb and Beecf, $P < 0.01$). This decrease was initiated during the distribution of the acidotic diet, and continued after stopping it. The decrease in blood pH could be the reflection of a progressive mobilization of the body alkaline reserves (bases and blood bicarbonates) during acidosis and of a longer time period required for these reserves to return to their initial levels in the blood than in the rumen. High cereal diets reduced saliva input and therefore bicarbonate entry into the rumen [32]. In response, a higher proportion of bicarbonate must be derived from the blood to maintain ruminal pH levels [24]. Data on recovery periods after acidosis are lacking, even if it is an important point to consider for setting up experiments with the Latin square design in the future. Animals should have recovered to their initial state before being subjected to a second treatment. The sparse information that is available on this aspect in the literature concerns acute acidosis challenges, with return to normal pH and lactic acid levels in the rumen of sheep after 3 days or more [33, 42].

4. CONCLUSION

Our results demonstrate the effect of an acidotic diet on ruminal acido-basic balance. The low ruminal pH characterizing latent acidosis may be mainly due to modifications in carbonic acid and volatile fatty acid buffering capacity. The long-term effect of an acidotic diet on the alkaline reserves in the blood suggests a longer recovery period than is seen from the ruminal parameters. Ruminal fermentation profiles indicate not lactic but butyric latent acidosis in relation with Entodiniomorph proliferation and could be an intermediate stage on the way to acute acidosis. The latent acidosis observed in these studies appears to be one of the possible variants of this state but it is difficult to generalize especially with productive animals. Further integrative research including ruminal and blood biological parameters is needed to better understand and characterize the various aspects and evolution of latent acidosis.

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