Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar

Spanish Journal of Agricultural Research 2009 7(2), 330-341
ISSN: 1695-971-X

Effect of sodium bicarbonate supplementation on feed intake, digestibility, digesta kinetics, nitrogen balance and ruminal fermentation in young fattening lambs

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Abstract

Twenty-two Merino lambs (average weight=15.3 kg) were used to study the effects of inclusion of sodium bicarbonate in the concentrate on feed intake, digestibility, rate of passage, nitrogen balance and ruminal fermentation *in vivo* and *in vitro*. Lambs were allocated to two experimental groups receiving concentrate and 20 g kg^{-1} sodium bicarbonate (group *Bic*) or concentrate alone (group *Control*). Both groups received barley straw *ad libitum*. Faeces and urine were collected for 5 days to estimate digestibility, nitrogen balance and rate of passage. After slaughter (at 25 kg live weight), samples of rumen fluid were collected from each lamb to determine parameters of ruminal fermentation and to be used as inocula for batch cultures of rumen microorganisms. There were no significant differences between treatments (P>0.10) in concentrate intake, dry matter digestibility, nitrogen balance and digesta kinetics. However, straw intake was greater (P<0.05) and neutral-detergent fibre digestibility showed a tendency to be higher in the group *Bic* (P<0.10). No differences, due to the supplementation with sodium bicarbonate, were observed for *in vivo* pH, ammonia-N and volatile fatty acids concentrations (P>0.10). Results for rumen fermentation parameters determined in *in vitro* batch cultures and for fermentation kinetics estimated with the gas production technique followed a similar trend to results observed *in vivo*. Most parameters showed no significant differences between groups. Nevertheless, the extent of degradation of barley grain *in vitro* tended to be stimulated (P<0.10) by the use of sodium bicarbonate.

Additional key words: acidosis, buffer, concentrate feed, finishing lambs, *in vitro* gas production, passage rate, ruminal fermentation.

Resumen

Efecto del bicarbonato sódico sobre la ingestión, digestibilidad, cinética de la digesta, balance de nitrógeno y fermentación ruminal en corderos en crecimiento-cebo

Se utilizaron 22 corderos de raza Merina (15,3 kg de peso medio) que se distribuyeron en dos grupos, recibiendo el correspondiente pienso concentrado solo (grupo *Control*) o con 20 g kg^{-1} de bicarbonato sódico (grupo *Bic*). Para estimar la digestibilidad, el balance de nitrógeno y el ritmo de paso se recogieron muestras de heces y orina durante 5 días. Tras el sacrificio (a los 25 kg de peso) se tomaron muestras del contenido ruminal de cada cordero para determinar parámetros de la fermentación ruminal y para ser usadas en cultivos *in vitro* de microorganismos ruminales. No se observaron diferencias significativas (P>0,10) en la ingestión de concentrado, la digestibilidad de la materia seca, el balance de nitrógeno y la cinética de la digesta. Sin embargo, la ingestión de paja de cebada fue mayor (P<0,05) y la digestibilidad de la fibra neutro detergente mostró una tendencia a ser mayor (P<0,10) en el grupo *Bic*. No se observaron diferencias debidas a la inclusión de bicarbonato sódico en el pH ruminal, N-amoniacal y concentraciones de ácidos grasos volátiles *in vivo* (P>0,10). Los resultados de los parámetros de fermentación ruminal determinados mediante cultivos *in vitro* y la cinética de fermentación estimada mediante la técnica de producción de gas siguieron la misma tendencia que los resultados observados *in vivo.* La mayoría de los parámetros no mostraron diferencias significativas entre grupos. Sin embargo, el uso de bicarbonato sódico tendió a estimular la degradación del grano de cebada *in vitro* (P<0,10).

Palabras clave adicionales: acidosis, buffer, corderos en cebo, pienso concentrado, producción de gas *in vitro*, ritmo de paso.

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Received: 23-08-08. Accepted: 11-03-09.

Introduction

Fattening lambs are generally fed rations containing over 800 g kg^{-1} concentrate in order to achieve high levels of energy intake and daily weight gains (Normand *et al.*, 2001). This results in reduction of the molar proportion of acetate and increase of the molar proportion of propionate (Enemark *et al.*, 2002), which reduces methane production and enhances energy retention (Russell, 1998). However, diets containing high levels of concentrates may be also associated with digestive disorders as a result of decreases of the buffering capacity of the rumen and subsequent increases in rumen acidity (McKinnon *et al.*, 1990).

Buffers can enhance ruminal environmental conditions by modulating acidity of the ruminal contents, preventing severe drops in pH (Le Ruyet and Tucker, 1992). Some salts, such as sodium bicarbonate, are routinely added to ruminant diets to buffer rumen pH, and have been widely used for fattening lambs.

The utilization of sodium bicarbonate has been reported to result in increases in digestibility and rate of passage, and in changes in the proportions of volatile fatty acids (Hart and Doyle, 1985; James and Wohlt, 1985). It has also been suggested that it may improve the amount and efficiency of ruminal microbial protein synthesis, which occurs independently of changes in ruminal fluid dilution rates (Mees *et al.*, 1985), and enhance bacterial uptake of ammonia (Newbold *et al.*, 1988), these effects being eventually associated to a higher feed intake and a subsequent increased daily gain (Tripathi *et al.*, 2004).

Nevertheless, available information on the effects of addition of sodium bicarbonate to lamb diets is not consistent. Thus, in addition to the positive effects mentioned above, some reports have indicated no effect or even negative effects have been observed on ruminal pH (Hadjipanayiotou, 1982; Hart and Doyle, 1985), volatile fatty acid production (James and Wohlt, 1985; Kawas *et al.*, 2007), dry matter intake (Hart and Doyle, 1985; James and Wohlt, 1985) or feed to gain ratio (Hart and Doyle, 1985). Possible explanations for this inconsistency in responses to buffer supplementation may include differences in the mode of action associated to or in addition to a change of pH (Haaland and Tyrrell, 1982) as well as the persistence of the effects. Thus, buffers could be beneficial when added to diets that cause a severe decline in pH during fermentation in the rumen, because these salts increase or stabilize the pH, improving the environmental conditions for microbial and enzymatic activity (Paggi *et al.*, 1999). Buffers may also enhance passage of starch from the rumen (Russell and Chow, 1993).

There is no much information about the use of sodium bicarbonate as buffer in the most common fattening system in Mediterranean countries, in which feedlot lambs are finished under intensive conditions over a short period of time (5-7 weeks after weaning) and slaughtered at a live body weight of 25-30 kg (Sañudo *et al.*, 1998). Therefore, recommendations on the use of dietary sodium bicarbonate in this lamb fattening system cannot be derived from information available on other fattening systems that utilize older and heavier animals.

Therefore, this experiment was conducted to study the effects of the inclusion of 20 g kg^{-1} sodium bicarbonate in the concentrate of young lambs, under a typical Mediterranean fattening system, on feed intake, digestibility, rate of passage, nitrogen balance and ruminal fermentation.

Material and methods

Animals

Twenty-two male Merino lambs (average initial age of 8-9 weeks) were divided according to their live weight (LW, 15.3 ± 0.14 kg) into two experimental groups of eleven animals each: one group was used as the control (*Control*) and the other group received concentrate supplemented with sodium bicarbonate (*Bic*). The lambs were housed in individual floor pens.

Abbreviations used: A (the asymptotic gas production), AFR (average fermentation rate), Bic (sodium bicarbonate), BW (body weight), c and d (parameters defining the fractional fermentation rate), DM (dry matter), DMD (In vitro dry matter disappearance), ED (extent of degradation), FMC (faecal marker concentration), k_1 and k_2 (estimates of the slow and fast fractional outflow rates of digesta), Lag (initial delay in the onset of gas production), LW (live weight), MNDF (microbial nitrogen flow to the duodenum), MPA (microbial purines absorbed), NDF (neutral-detergent fibre), NDFD (in vitro NDF degradation), OM (organic matter), PDE (purine derivatives excreted), SEM (standard error of the mean), TMRT (total mean retention time), TT (transit time), $t_{½}$ (time to half-asymptote), VFA (volatile fatty acids), μ (fractional rate of fermentation at $t_{½}$).

Previously, the lambs had remained stalled with their mothers, with free access to a commercial starter concentrate and alfalfa hay until the commencement of the trial. Immediately after birth, lambs had been treated with Vitasel (Lab. Ovejero, Spain) to prevent white muscle disease, and later on with Miloxan (Merial Lab., Spain) to prevent enterotoxaemia and with albendazol 2.5% Ganadexil® (Industrial Veterinaria, Spain) to control internal parasites.

The experiment was carried out in accordance with the Royal Decree 1201/2005 for the protection of animals used for experimental and other scientific purposes (BOE, 2005).

Diets

Lambs were fed barley straw and a concentrate containing barley grain, maize grain, soya bean meal, cane molasses and a mineral/vitamin mix, supplemented (*Bic*) or not (*Control*) with sodium bicarbonate (20 g kg^{-1}). Ingredients and chemical composition of the concentrates and the barley straw are presented in Table 1. All animals had free access to fresh water.

Barley straw and concentrates were offered *ad libitum*, once a day (approx. at 09.00 h). The amount of feed offered was adjusted daily on the basis of the previous day intake, allowing refusals of 20%. Both concentrate and straw refusals were removed daily, pooled weekly for each animal and weighed and dried to determine dry matter (DM) intake.

Digestibility trial, urine collection and rate of passage

On day 20, lambs (22.5 ± 0.26 kg LW) were confined in metabolism cages fitted with specific devices to collect faeces and urine separately. After 4 days of adaptation, faeces of each animal were collected daily, weighed, mixed thoroughly and subsampled (10%), for 5 days. Aliquots from each sheep were pooled and stored at -30ºC. Pooled samples were dried to constant weight before analysis.

Urine was collected in a solution of H_2SO_4 (10%; v/v) to maintain the pH<3. Daily urine was weighed, its density was measured and a subsample (20%) was taken for each lamb for 5 days. Daily samples were pooled to form composite samples and stored at -30ºC until analysed for total nitrogen and purine derivatives.

Co-EDTA was used to estimate the liquid rate of passage, and was prepared according to the method of Udén *et al.* (1980). On day 22 of experimental trial, the animals were dosed orally with the marker (1.125 g Co-EDTA dissolved in 30 mL of water). Faeces were collected at 0, 4, 8, 12, 16, 22, 28, 34, 40, 48, 60, 72, 96, 120 and 144 h after marker administration, weighed and a subsample (10%) was taken and stored at -30ºC. Samples were dried to constant weight before analysis.

Slaughter and rumen environment

When lambs reached 25 kg of LW, they were slaughtered following standard procedures. The day before slaughter, feed was withdrawn at 20.00 h. Then, two hours prior to slaughter, feed was offered again to all lambs for 1 hour so that each had a full rumen and active fermentation at the time of slaughter. Each lamb was euthanized with an intravenous injection of barbiturate (Euta-lender®, Normon, Spain), slaughtered by exsanguination from the jugular vein and eviscerated. Total rumen contents from each slaughtered lamb were collected, mixed thoroughly and sampled. About 400 g of this mixture of rumen contents were strained through two layers of cheesecloth and the pH was measured immediately. After centrifugation (600 g at 4ºC for 10 min), a sample of 5 mL of the supernatant was acidified with 5 mL 0.2 N HCl for ammonia determination. Another 0.8 mL of the supernatant was added to 0.5 mL of a deproteinising solution (2% metaphosphoric and 0.4% crotonic acids, w/v, in 0.5 N HCl) for determination of volatile fatty acids (VFA). The samples for NH_3 and VFA were stored at -30ºC until analysed. Remaining filtered rumen fluid from twelve lambs (six from the *Control* group and six from the *Bic* group) was used as rumen inoculum for the batch cultures.

Batch cultures of rumen microorganisms

In vitro fermentation kinetics and substrate disappearance (as indicators of the degradative activity of rumen contents) of two feedstuffs, namely barley straw and barley grain, were studied by following the *in vitro* gas production technique as described by Theodorou *et al.* (1994). Chemical composition of the substrates is shown in Table 1.

Table 1. Ingredients (g kg^{-1}) and chemical composition of the concentrates, barley straw and barley grain (g kg^{-1} DM)

	Control	Bic	Barley straw	Barley grain
Ingredients				
-Barley	500	491	-	-
-Maize	230	225	-	-
-Soya bean meal	190	186	-	-
-Vitamin mineral premix	30	29	-	-
-Molasses	50	49	-	-
-Sodium bicarbonate	0	20	-	-
Chemical composition				
-Dry matter (DM, g kg^{-1})	867	870	916	896
-Crude protein	175	180	23	134
-Neutral detergent fibre	131	138	802	204
-Ash	72	76	50	41

Rumen fluid inocula were obtained separately from 12 lambs (six from the *Control* group and six from the *Bic* group), selected randomly among the lambs of each experimental group. Rumen contents were immediately transferred to the laboratory in pre-warmed thermos flasks, strained again through a double layer of muslin and kept under a flushing of CO_2.

Each feedstuff used as fermentation substrate (barley straw and barley grain) was incubated in duplicate in each of the 12 inocula. Incubations were carried out in 120 mL serum bottles in which 500 mg DM of the appropriate feedstuff was weighed out, and 10 mL strained rumen fluid and 40 mL medium were dispensed anaerobically. The medium contained buffer, macro- and micro-mineral, resazurin and reducing solutions according to Menke and Steingass (1988). Then the bottles were sealed and placed in an incubator at 39ºC. Accumulated head-space pressures and gas volumes were measured, using a pressure transducer (Bailey & Mackey, UK) and a graduated syringe, throughout the incubation period (at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72, 96, 120 and 144 h post-inoculation). The values were corrected for the quantity of substrate organic matter (OM) incubated and gas released from blank cultures (*i.e.,* rumen fluid plus buffered medium, without substrate, with and without sodium bicarbonate).

After 144 h of incubation, fermentations were stopped by swirling the flasks on ice. *In vitro* dry matter disappearance (DMD; g kg^{-1}) at this end-point was estimated by filtering residues using pre-weighed sintered glass crucibles (100-160 µm pore size; Pyrex, UK). Residues were then analysed for neutral-detergent fibre (NDF) content to determine *in vitro* NDF degradation (NDFD).

Ammonia, VFA, and DM and NDF degradation

Twenty-four more cultures per substrate (2 treatments × 6 inocula [replicates] per treatment × 2 flasks per inoculum) were incubated to compare fermentation patterns when rumen fluid from either *Bic* or *Control* lambs was used as inoculum. In this case, end-point *in vitro* incubations at either 8 h (barley grain) or 24 h (barley straw) were carried out. Immediately after termination of the incubation, headspace pressure and gas volume were measured, and bottle contents were centrifuged (600 g, 4ºC, 15 min). Samples of the supernatant were taken and stored at -30ºC for ammonia and VFA determination as described above. Remaining contents of the centrifuge tube were filtered through sintered glass crucibles for subsequent determination of *in vitro* DM and NDF disappearance, as described above.

Analytical procedures

Procedures described by AOAC (2003) were used to determine DM (AOAC official method 934.01), ash (AOAC official method 942.05), and Kjeldahl nitrogen (N; AOAC official method 976.06). Neutral-detergent fibre (expressed inclusive of residual ash) was determined by the method of Van Soest *et al.* (1991), adding sodium sulphite to the solution. Only samples of concentrates and barley grain were assayed with alpha amylase.

Ammonia concentration was determined as described by Weatherburn (1967) and VFA by gas chromatography, using crotonic acid as internal standard (Ottenstein and Bartley, 1971), both in centrifuged samples.

Concentration of purine derivatives (allantoin, uric acid, xanthine and hypoxanthine) in urine was determined by HPLC according to Balcells *et al.* (1992).

Samples of faeces collected in the passage kinetics trial were digested with HNO_3 (65% w/v) in a microwave (Microwave Sample Preparation System MDS-2000, CEM Co., USA) and filtered (Whatman, UK). Concentration of Co was determined by inductively coupled atomic emission spectroscopy (ICP-AES, Perkin-Elmer Optima 2000DV, USA).

Calculations and statistical analysis

The generalized Mitscherlich model proposed by France *et al.* (2000) was fitted to the gas production profiles

$$G = A\cdot\left\{1 - e^{\left[-c\cdot(t-Lag)-d\cdot\left(\sqrt{t}-\sqrt{Lag}\right)\right]}\right\},$$

where G is the cumulative gas production (mL g^{-1} OM) at time t (h) of incubation, A (mL g^{-1} OM) represents the asymptotic gas production, c (h^{-1}) and d ($h^{-1/2}$) are parameters defining the fractional fermentation rate, and Lag (h) is the initial delay in the onset of gas production. The parameters A, c, d and Lag were estimated by an iterative least squares procedure using the non-linear regression procedure (NLIN) of the Statistical Analysis System package (SAS, 1999). For barley grain, it was shown that parameter d was not significantly different from zero, resulting in a simple exponential equation as a special case of the generalized model.

Other interesting measures were derived from the model parameters, such as time to half-asymptote ($t_{½}$, h):

$$t_{½} = \left\{\frac{-d/2 + \sqrt{\left(d^2/4 + c\cdot\left[c\cdot Lag + d\cdot\sqrt{Lag} + \ln(2)\right]\right)}}{c}\right\}^2$$

Then, fractional rate of fermentation at $t_{½}$ (μ, h^{-1}) was calculated as:

$$\mu = c + \frac{d}{2\cdot\sqrt{t_{½}}},$$

and average fermentation rate (AFR; mL gas h^{-1}), defined as the average gas production rate between the start of the incubation and the time at which the cumulative gas production was half of its asymptotic value, was calculated as:

$$AFR = \frac{A}{2\cdot t_{½}}$$

Extent of degradation (ED; g kg^{-1}) was estimated, assuming a rumen particulate outflow (k_p) of 0.0625 h^{-1}, according to the equation proposed by France *et al.* (2000):

$$ED = \left[\frac{c\cdot DMD}{c + k_p}\right]\cdot e^{(-k_p\cdot Lag)}$$

Methane production (CH_4; mmol g^{-1} OM) was calculated stoichiometrically according to the amounts of acetate (Ac), propionate (P) and butyrate (B) produced, following the equation proposed by Blümmel *et al.* (1997):

$$CH_4 = 0.5\cdot Ac - 0.25\cdot P + 0.5\cdot B$$

Microbial N flow to the duodenum (MNDF) was estimated from daily urinary excretion of purine derivatives as described by Chen *et al.* (1992). According to this method, the amount of microbial purines absorbed (MPA, mmol day^{-1}) corresponding to the purine derivatives excreted (PDE, mmol day^{-1}) was calculated from the following relationship:

$$PDE = 0.84\cdot MPA + (0.15\cdot BW^{0.75}\cdot e^{-0.25\cdot MPA})$$

This model corrects for the contribution of endogenous purines, which is represented by the component within brackets (taking into account the body weight, BW), and which decreases as exogenous purines become available for utilization by the animal. With the assumption that the purine:protein ratio of mixed ruminal microbes remains constant, the amount of MNDF(g day^{-1}) was calculated as follows:

$$MNDF = 70\cdot MPA/(0.83\cdot 0.116\cdot 1000) = 0.727\cdot MPA$$

where 70 is the N content of purines (g mol^{-1}), 0.83 is the digestibility coefficient for microbial purines and 0.116 is the ratio of purine N to total N in mixed microbial biomass.

A multi-compartmental model (Dhanoa *et al.*, 1985) was fitted to faecal marker excretion curves:

$$FMC = S\cdot e^{-k_1\cdot t}\cdot e^{-(N-2)\cdot e^{(k_1-k_2)t}},$$

where FMC (μg L^{-1}) is the faecal marker concentration at time t after dosing (h), k_1 and k_2 are estimates of the

slow and fast fractional outflow rates of digesta (h^{-1}), which are usually associated with the rates in the reticulum-rumen and in the caecum and proximal colon, respectively, N is the number of compartments and S is a scale parameter.

Transit time (TT, h) was calculated as:

$$TT = \sum_{i=3}^{N-1} \frac{1}{k_2 + (i-2)\cdot(k_2 - k_1)}$$

and total mean retention time (TMRT, h) as:

$$TMRT = \frac{1}{k_1} + \frac{1}{k_2} + TT$$

All data were analysed as a one-way analysis of variance, with bicarbonate addition as the only source of variation (*Control* vs. *Bic*), using the general lineal model (GLM) procedure of the Statistical Analysis System programme (SAS, 1999).

Results

There were no significant differences between groups in the dry matter intake of concentrate (P>0.10) (Table 2). On the other hand, lambs supplemented with sodium bicarbonate consumed a higher amount of dry matter of barley straw (P=0.015).

No treatment effects (P>0.10) were observed on the digestibility of DM and crude protein. However, NDF digestibility tended to be significantly higher (15%) in group *Bic* than in *Control* lambs (0.533 *vs* 0.462; P=0.080).

Total dry matter intake (809 *vs* 844 g DM·day^{-1}), average daily gain (295 *vs* 316 g day^{-1}) and feed to gain ratio (2.76 *vs* 2.72, *Control vs Bic*) were not different between treatments (P>0.10).

There were no differences between treatments for any of the parameters related to the rate of passage (k_1, k_2, TT and TMRT; P>0.10) or nitrogen balance.

Mean values of *in vivo* pH, ammonia and VFA concentrations are given in Table 3. No differences, due to the supplementation with sodium bicarbonate, were found for any of these parameters with the exception of isobutyrate concentration, which was significantly higher in the control group (1.29 *vs* 0.66 mmol L^{-1}, P<0.022). Molar proportions of VFA referred to as "others" (sum of valerate + isobutyrate + isovalerate + + caproate) were also significantly higher in the *Control* than in the *Bic* lambs (0.0612 *vs* 0.0412, respectively; P=0.027).

Parameters describing *in vitro* gas production, substrate disappearance, extent of degradation, ammonia concentration and VFA production when barley grain and barley straw were incubated, are shown in Table 4.

Extent of degradation tended to be greater (P=0.079) when barley grain was incubated with rumen inocula derived from lambs receiving bicarbonate. However, all the other variables (gas production parameters, DM and NDF disappearance, ammonia, VFA, etc.) were observed to be similar in the two experimental groups (P>0.10).

When barley straw was incubated, only production of propionate or butyrate showed a tendency (P=0.075 and 0.097, respectively) to be positively affected by the treatment. No significant effect of supplementation with sodium bicarbonate was found for any other parameter describing the fermentation of barley straw (P>0.10).

Discussion

Sodium bicarbonate is supposed to benefit ruminants eating large amounts of readily fermentable carbohydrates and it has therefore been commonly used in commercial fattening lamb systems. It is considered innocuous to the animal and safe for the consumer without any adverse effect on the meat attributes.

The addition of sodium bicarbonate to the diet has been reported to affect, among others, ruminal pH and osmolality, volatile fatty acid production, rate of passage and voluntary feed intake. In the present experiment, dry matter intake was not significantly affected with the inclusion of bicarbonate in the diet, in agreement with the results reported by Mandebvu and Galbraith (1999) using a lower dose of sodium bicarbonate (15 g kg^{-1}). Other authors have observed a higher concentrate intake when adding this salt at similar (Phy and Provenza, 1998) or higher doses (Corcuera *et al.*, 1977).

On the other hand, despite the low proportion that barley straw represents in the diet consumed by the animals, its intake was on average 40% higher in those lambs receiving sodium bicarbonate, which could be related to the tendency to an increased NDF digestibility. Hadjipanayiotou (1982) added sodium bicarbonate to a diet of sheep fed mainly concentrates and obtained an increase in NDF digestibility (22% *vs* 15% in the present study). Contrary to our result, Hadjipanayiotou (1982) also reported improvements in the DM digestibility. However, Cooper *et al.* (1996), which included up to 40 g kg^{-1} sodium bicarbonate in their

Table 2. Mean values of feed intake, digestibility, digesta flow kinetics, nitrogen balance and microbial protein synthesis

	Control	Bic	SEM[1]	*P*[2]
Feed intake				
-Concentrate (g DM day^{-1})	795	824	15.0	0.348
-Barley straw (g DM day^{-1})	13.7	19.5	1.24	0.015
-Barley straw/total intake (g kg^{-1})	17.0	23.4	0.147	0.026
Digestibility coefficients				
-Dry matter	0.840	0.839	0.0044	0.971
-Crude protein	0.802	0.811	0.0067	0.504
-Neutral detergent fibre	0.462	0.534	0.0204	0.080
Digesta flow kinetics				
-Passage rate from rumen (k_1, h^{-1})	0.072	0.073	0.00368	0.812
-Passage rate through the caecum and proximal colon (k_2, h^{-1})	0.517	0.442	0.0427	0.396
-Transit time (TT, h)	11.4	8.4	1.31	0.265
-Total mean retention time (TMRT, h)	28.3	25.2	1.68	0.374
Nitrogen balance (g day^{-1} kg^{-1} BW)	0.740	0.666	0.02601	0.159
Microbial N flow to the duodenum (g day^{-1} kg^{-1} BW)	0.260	0.268	0.433	0.838

[1] SEM = standard error of the means. [2] *P* = level of significance

diet, did not obtain differences in NDF or DM digestibility. Other authors, however, using dehydrated alfalfa with sodium bicarbonate reported greater increases in NDF and DM digestibility (Stroud *et al.*, 1985), which may be due to the high efficiency of the forage to elevate ruminal pH (Hadjipanayiotou, 1982). It is known that the inclusion of sodium bicarbonate prevents severe decline in rumen pH, alleviating the depressive effect of a low pH on cellulolysis, and so enhancing fibre degradation (Mould *et al.*, 1983; Wedekind *et al.*, 1986).

Previous studies have shown that the use of sodium bicarbonate may result in increased rates of passage of the liquid phase of the digesta. Stokes (1983) reported a quadratic increase in the dilution rate from the rumen of sheep fed diets supplemented with increasing levels of sodium bicarbonate. In this study, although total mean retention time (TMRT) of digesta in the gastrointestinal tract was on average 11% shorter in lambs eating the sodium bicarbonate diet than in those fed the control diet, the differences were not statistically significant ($P>0.10$). Whereas some authors have attributed the changes in rate of passage to a rise in acetic acid production (Hadjipanayiotou *et al.*, 1982), others have suggested a mode of action related to a higher osmolality and water intake (Russell and Chow, 1993; Cooper *et al.*, 1996). Acetic acid concentration in rumen fluid or its production *in vitro*, when incubating rumen fluid with either barley grain or straw, did not change in lambs supplemented with sodium bicarbonate (see Tables 3, 4 and 5) but water consumption was not measured. In agreement with our results, Mees *et al.* (1985), adding 35 g kg^{-1} sodium bicarbonate to the diet, did not observe changes in liquid flow rates. The lack of response to buffer treatment in this and other studies was probably due to supplementary buffers having little effect on turnover rate in animals that already show fast digesta passage rates (Harrison *et al.*, 1975). It must be noted that the analyses of faecal Co-EDTA concentration curves are only an estimate of dilution rate (Dhanoa *et al.*, 1985). However, this method is widely accepted and leads to accurate estimate of mean retention time in the rumen, and is consistent with the current trend in animal research to use non-invasive techniques for assessing digestive parameters (Bernard *et al.*, 1998).

No differences in ruminal pH *in vivo* due to buffer addition were observed (see Table 3), in agreement with other results reported in the literature (Hadjipanayiotou, 1982; Hart and Doyle, 1985; Russell and Chow, 1993; Khorasani and Kennelly, 2001; Kawas *et al.*, 2007). Nevertheless, it should be mentioned that, in the current experiment, values of pH were only measured immediately after slaughter, and diurnal variations in ruminal pH were not recorded.

Published results about changes in VFA production in response to sodium bicarbonate administration are

Table 3. Mean values of pH, ammonia and volatile fatty acids (VFA) concentrations in rumen fluid

	Control	Bic	SEM[1]	*P*[2]
pH	5.46	5.21	0.150	0.415
Ammonia (mg L^{-1})	168	121	21.7	0.393
VFA (mmol L^{-1})				
-Acetate	57.0	56.9	5.37	0.992
-Propionate	52.1	55.2	5.03	0.767
-Butyrate	10.4	10.7	1.42	0.930
-Others[3]	7.1	4.9	0.69	0.123
-Total VFA	126.6	127.7	12.03	0.965
-Acetate/propionate (mol mol^{-1})	1.12	1.05	0.029	0.245

[1] SEM = standard error of the means. [2] *P* = level of significance. [3] Calculated as the sum of isobutyrate, isovalerate, valerate and caproate.

inconsistent, with some showing increases (Thomas and Hall, 1984; Hart and Doyle, 1985; Khorasani and Kennelly, 2001) and others decreases (James and Wohlt, 1985) or no changes (Mees *et al.*, 1985; Kawas *et al.*, 2007). No significant effects were detected in the present experiment, with the exception of the molar proportion of the sum of valerate + isobutyrate + isovalerate + caproate. Some authors have observed higher acetate and lower propionate molar proportions related, respectively, to greater fibre degradation (Van Soest *et al.*, 1991), and to an increased ruminal dilution rate and resulting washout of soluble carbohydrates from the rumen (Russell and Chow, 1993).

The use of sodium bicarbonate as a dietary additive did not affect the acetate to propionate ratio in the rumen, showing a low value characteristic of high intakes of concentrate (starchy) feeds, as suggested by Khorasani and Kennelly (2001). It is noteworthy that these low ratios (<1.5) fell into the range (from 0.9 to 4) previously reported by other authors (Woods and Luther, 1962). A negative effect of sodium bicarbonate on propionate production would have been very advantageous in fattening lambs because when in excess, propionate might be a precursor for the synthesis of odd-numbered and methyl branched chain fatty acids, whose proportions are high in soft adipose tissues (Normand *et al.*, 2001).

Although some studies have suggested that sodium bicarbonate can enhance the rate of ammonia utilization by the rumen bacteria (Newbold *et al.*, 1988), other researchers working with sheep (Stokes, 1983) and cattle (Khorasani and Kennelly, 2001) have shown no effect on ruminal ammonia levels, which is in line with the results observed in this experiment. Some studies also suggested that sodium bicarbonate supplementation may enhance microbial protein synthesis (Harrison *et al.*, 1975) as result of changes in the dilution rate (Newbold *et al.*, 1988). In the present experiment no differences in nitrogen balance or flow of microbial protein from the rumen were estimated from purine derivatives excretion, which agrees with the lack of significant variations found in the digesta kinetics.

Characterization of *in vitro* ruminal fermentation when sodium bicarbonate is included in the diet has been reported for cattle and sheep (Le Ruyet and Tucker, 1992; Cobos-Peralta *et al.*, 2005), but available information for young fattening lambs is particularly scarce. Results for rumen fermentation characteristics obtained by the *in vitro* batch cultures and the gas production technique followed a similar pattern to results obtained *in vivo*. Sodium bicarbonate had no significant effect on parameters such as ammonia or VFA production, although in incubations there was a tendency for higher production of propionic and butyric acids with barley straw.

The extent of degradation of barley grain tended to be stimulated slightly ($P<0.10$) in animals receiving the buffer. On the other hand, no significant differences were detected when barley straw was incubated. This lack of effect of sodium bicarbonate on *in vitro* NDF degradability in contrast to the improvement observed in *in vivo* NDF digestibility could be accounted for by the highly buffered incubation medium ("artificial saliva", McDougall, 1948) used in the *in vitro* batch cultures. Nevertheless, it is noteworthy that extent of degradation of straw estimated from the fermentation kinetics was very low (<0.12) when ruminal fluid from young lambs fed a diet rich in concentrates was used as inoculum for the mixed ruminal microorganisms cultures. In contrast, extent of degradation of barley grain can be considered within a normal range. Thus, whereas amylolytic activity is normal in rumen fluid of these

Table 4. *In vitro* gas production parameters (A, Lag, $t_{½}$ and μ), average fermentation rate (AFR), extent of degradation (ED), ammonia concentration, volatile fatty acids (VFA) and methane production, and dry matter (DM-D) and neutral-detergent fibre (NDF-D) disappearance after 8 and 144 h incubation for barley grain and barley straw

	Control	Bic	SEM[1]	*P*[2]
Barley grain				
A (mL)	236	239	10.7	0.942
Lag (h)	0.125	0.000	0.0410	0.321
$t_{½}$ (h)	4.85	4.15	0.583	0.255
μ (h^{-1})	0.154	0.168	0.0148	0.349
AFR (mL h^{-1})	25.9	28.9	1.33	0.458
ED (g g^{-1})	0.633	0.670	0.0075	0.079
Ammonia (mg L^{-1})	43	58	4.7	0.226
VFA (mmol g^{-1} OM)				
-Acetate	15.3	14.2	0.62	0.501
-Propionate	23.0	23.2	0.80	0.909
-Butyrate	3.6	2.8	0.402	0.500
-Others[3]	2.0	1.5	0.253	0.383
-Total VFA	43.9	41.8	1.11	0.491
-Acetate/propionate (mol mol^{-1})	0.70	0.61	0.0334	0.319
Methane (mmol g^{-1} OM)	3.75	2.71	0.371	0.300
DM-D_{8h} (g g^{-1})	0.770	0.755	0.0085	0.285
NDF-D_{8h} (g g^{-1})	0.176	0.153	0.0122	0.253
DM-D_{144h} (g g^{-1})	0.996	0.921	0.0050	0.299
NDF-D_{144h} (g g^{-1})	0.484	0.525	0.0226	0.538
Barley straw				
A (mL)	171	171	11.6	0.994
Lag (h)	13.7	9.7	2.08	0.357
$t_{½}$ (h)	44.7	32.1	5.19	0.239
μ (h^{-1})	0.036	0.044	0.0037	0.264
AFR (mL h^{-1})	2.74	2.89	0.138	0.877
ED (g g^{-1})	0.089	0.118	0.0012	0.441
Ammonia (mg L^{-1})	128	145	6.3	0.239
VFA (mmol g^{-1} OM)				
-Acetate	9.2	11.6	0.93	0.388
-Propionate	6.6	8.8	0.46	0.075
-Butyrate	1.2	1.6	0.09	0.097
-Others[3]	1.5	1.5	0.19	0.996
-Total VFA	18.5	23.5	1.25	0.159
-Acetate/propionate (mol mol^{-1})	1.32	1.31	0.090	0.966
Methane (mmol g^{-1} OM)	3.55	4.39	0.402	0.483
DM-D_{24h} (g g^{-1})	0.309	0.345	0.0180	0.509
NDF-D_{24h} (g g^{-1})	0.210	0.236	0.0203	0.680
DM-D_{144h} (g g^{-1})	0.582	0.637	0.0137	0.141
NDF-D_{144h} (g g^{-1})	0.540	0.598	0.0167	0.214

[1,2,3] See Table 3.

lambs, cellulolytic activity seems to be depressed to a significant extent. Feeding bicarbonate was not sufficient to neutralize this depressing effect, although estimated extent of degradation and measured DM and NDF disappearance at 24 and 144 h were always numerically higher, without reaching statistical significance, when straw was incubated in ruminal fluid from lambs of the *Bic* group.

From the results shown here, it can be concluded that the addition of 20 g kg^{-1} sodium bicarbonate to concentrate fed to young fattening lambs can improve the intake of straw and NDF digestibility. However, the mode of action of this buffer additive remains unclear. Further studies will be necessary to test the hypothesis that the lack of a stronger effect in this kind of young animals may be probably related to the short duration of the finishing period under the typical Mediterranean lamb fattening system.

Acknowledgements

Raúl Bodas gratefully acknowledges receipt of a scholarship from the Ministry of Education and Science (MEC, Spain), under the programme FPU. We thank Dr. R. I. Richardson (University of Bristol, UK) for his careful review of the manuscript.

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