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ANIMAL PRODUCTION

Intake and apparent nutrient digestibility, ruminal parameters, and nitrogen balance of lambs fed with corn and forage-free diets with sodium bicarbonate

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ABSTRACT: This study evaluated forage-free diets supplemented with sodium bicarbonate (SB) based on ground flint corn on intake, apparent nutrient digestibility, ruminal parameters, and N balance. Four rumen-cannulatedDorper × Santa Inês lambs, with a body weight of 43.4 ± 0.85 kg (mean ± standard deviation) were used. The experimental design was a 4×4 Latin square. Each experimental period lasted 21 days. Experimental diets were: positive control, a diet without SB containing 400 g/kg DM of soybean hulls (CONT), and three diets based on ground flint corn, containing 10 (10SB), 20 (20SB) or 30 (30SB) g/kg DM of SB. Intake of neutral and acid detergent fiber was greater for CONT (P < 0.01) when compared to SB. Intake and apparent nutrient digestibility of dry and organic matter, ether extract, and crude protein did not differ. Intake (P = 0.01) and digestibility (P < 0.01) of non-fiber carbohydrates were greater for SB diets and neutral detergent fiber digestibility was greater (P = 0.03) for CONT. The total digestible nutrients and metabolizable energy were greater for SB (P = 0.04). Rumen concentrations of acetate (P < 0.01), isobutyrate (P < 0.01), butyrate (P < 0.01), isovalerate (P < 0.01), and total short-chain fatty acids (P < 0.01) were greater for CONT. Ruminal pH showed a quadratic (P = 0.05) response to SB levels, with greater values for 20SB. Sodium bicarbonate was efficient in increasing ruminal pH at the level of 20 g/kg DM without interfering with the intake and apparent nutrient digestibility characteristics.
Key words: buffer, high concentrate, rumen, sheep.

Consumo e digestibilidade aparente dos nutrientes, parâmetros ruminais e balanço de N de cordeiros confinados recebendo dietas com milho e sem forragem com bicarbonato de sódio

RESUMO: O presente estudo avaliou a suplementação de bicarbonato de sódio (BS) em dietas sem forragem à base de milho moído sobre o consumo e a digestibilidade aparente dos nutrientes, os parâmetros ruminais e o balanço de N. Foram utilizados quatro cordeiros Dorper × Santa Inês, com um peso corporal inicial de 43,4 ± 0,85 kg (média ± desvio padrão). O delineamento experimental foi quadrado latino 4×4. Cada período experimental durou 21 dias. As dietas experimentais foram: controle positivo, sem BS, contendo 400 g/kg de MS de casca de soja (CONT) e três dietas à base de milho moído, sem casca de soja, contendo 10 (10BS), 20 (20BS) ou 30 (30BS) g/kg de MS de BS. O consumo de fibra em detergente neutro e ácido foi maior (P < 0,01)para o CONT quando comparado com BS. O consumo e a digestibilidade aparente da matéria seca, matéria orgânica, extrato etéreo e proteína bruta não diferiram entre os tratamentos. O consumo (P = 0,01) e digestibilidade (P < 0,01) dos carboidratos não fibrosos foram superiores para as dietas contendo BS e a digestibilidade da fibra em detergente neutro foi maior para o CONT. Os teores de nutrientes digestivos totais e energia metabolizável foram superiores para as dietas contendo BS (P = 0,04). A concentração ruminal de acetato (P < 0,01), isobutirato (P < 0,01), butirato (P < 0,01), isovalerato (P < 0,01) e ácidos graxos totais de cadeia curta (P < 0,01) foram mais elevadas para o CONT. O pHruminal apresentou resposta quadrática (P = 0,05) em relação aos níveis de BS, sendo observado maior pH para os animais alimentados com a dieta 20BS. O BS foi eficiente em aumentar o pHruminal no teor de 20 g/kg MS sem interferir no consumo e na digestibilidade aparente dos nutrientes. Portanto, 20 g/kg de MS é a dose indicada de BS em dietas sem forragem para terminação de cordeiros em confinamento.
Palavras-chave: alto concentrado, ovinos, rúmen, tamponante.

INTRODUCTION

Feedlots are the most used system to decrease the production cycle and increase the quality of sheep meat (GARCIA et al., 2010). In these systems, the diets used have low forage inclusion, intending to increase energy density and, consequently, increasing the average daily gain (ADG) (WANG et al., 2020).

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However, a high level of concentrate can cause metabolic disorders, like subacuteruminal acidosis, by the accumulation of short-chain fatty acids (SCFA) and lactate that is produced during ruminal fermentation of carbohydrates, such as starch, which is the main carbohydrate present in cereal grains like corn (NAGARAJA et al., 1997).

The use of buffers can improve ruminal conditions, by increasing the outflow rate of the ruminal fluid (ROGERS & DAVIS, 1982) and improving ruminal pH (NAGARAJA et al., 1997), which can have positive effects on animal performance. A buffering agent in the ruminal fluid is SB (HEROD et al., 1978) and its use can increase the passage and fluid dilution rate of nutrients and SCFA *via* rumen as a result of increased water intake, which promotes elevation in ruminal pH (RUSSELL & CHOW, 1993). The SB supplementation in high concentrate diets for lambs resulted in greater ruminal pH and improvement of performance (SANTRA et al., 2003; TRIPATHI et al., 2004). LASKOSKI et al. (2014) evaluated SB to avoid metabolic acidosis in sheep subjected to experimental ruminal acidosis and reported that the buffer has a positive effect, reducing its severity. Conversely, GASTALDELLO JR et al. (2013) reported no effect of SB supplementation (1% DM) for feedlot lambs fed high grain diets, the authors attributed this lack of effect to the low level of SB used.

In addition to the use of buffering agents, other strategies provide greater safety for this type of diet. Soybean hulls has a high fiber content (62% of neutral detergent fiber - NDF) (NRC, 2007a), is extensively digested in the rumen (HSU et al., 1987), and has high nutritive value for sheep (TAMBARA et al., 1995; FERREIRA et al., 2011). Due to the high fiber content, the main SCFA produced is acetate (FIRKINS et al., 2006), which is a favorable fermentation pattern for the rumen environment when compared to propionate, ensuring a greater ruminal pH, and also a lower pH variation throughout the day (FERREIRA et al., 2011).

There are still controversies in the literature regarding the effects of SB on ruminal fermentation parameters. In addition, the studies evaluated the inclusion of SB with some source of roughage (SANTRA et al., 2003; TRIPATHI et al., 2004; GASTALDELLO JR et al., 2013) or a different source of cereal grain (KAWAS et al., 2005). Therefore, this study evaluated SB supplementation (10, 20 and 30 g/kg dry matter - DM) in forage-free diets for feedlot lambs and compared its chemical effect with the fiber effect of the soybean hulls on intake and apparent nutrient digestibility, ruminal parameters, and N balance.

MATERIALS AND METHODS

This study was conducted at the Department of Animal Science, at the facilities of Sheep and Goats Intensive Production System (SIPOC), “Luiz de Queiroz” College of Agriculture, São Paulo University, located in Piracicaba, São Paulo (22°42′24″ S and 47°37′53″ W), Brazil. All animal use procedures were reviewed and approved by the Animal Care and Use Committee at the same institution (CEUA/ESALQ, number 8846110320).

Animals and housing

Four ruminallycannulated and castrated Dorper × Santa Inês lambs, with an initial body weight (BW) of 43.4 ± 0.85 kg (mean ± standard deviation) and approximately 6 months old were used. They were housed in metabolism cages (1.30 × 0.55 m), with feed bunks and water troughs, in an indoor space, protected from the sun and rain. The cages had a metal structure underneath made of galvanized sheet metal that enabled the collection of urine.

Diets and experimental design

The experimental design utilized was a 4×4 Latin square, with four treatments, four periods, and four animals (REGO et al., 2019). The experiment lasted 84 days, with four periods that were 21 days each. The first 15 days were used for diet adaptation, 5 days to measure nutrient intake, urine, and feces production. Furthermore, 1 day was used to collect ruminal fluid. The adaptation was made by restriction according to DE PAULA CARLIS et al. (2021), but instead of starting with 20 g/kg of the fasting BW, we started with 18 g/kg of the fasting BW, since the diets of the present study did not contain forage.

Experimental diets were: CONT (positive control) diet without SB, containing 400g/kg DM of soybean hulls; and three forage-free diets based on ground flint corn containing 10 (10SB), 20 (20SB) or 30 (30SB) g/kg DM of SB. Diets were formulated using the Small Ruminant Nutrition System (SRNS; CANNAS et al., 2004). All experimental diets contained 25 mg/kg DM of sodium monensin (Rumensin200, Elanco Animal Health, Greenfield, USA) and were drawn up to be isonitrogenous (Table 1). Samples of diets and ingredients were collected on the day of mixing the rations and frozen at -20 ºC for later analysis.

We used a grinder (Nogueira DPM – 4, Itapira, São Paulo, Brasil) without a sieve to ground the corn. Soybean hulls used were bran. The particle size distribution of ingredients and diets was determined by placing 100 g samples in an vibrating

Table 1 - Ingredients and chemical composition of experimental diets (g/kg DM).

Ingredients, g/kg DM	---------------Treatments[1]---------------			
	CONT	10SB	20SB	30SB
Ground flint corn	465	815	802	789
Soybean meal	101	136	139	142
Soybean hulls	400	-	-	-
Limestone	12	17	17	17
Mineral premix[2]	12	12	12	12
Ammonium chloride	5	5	5	5
Urea	5	5	5	5
Monensin, mg/kg DM	25	25	25	25
Sodium bicarbonate	-	10	20	30
---------------Chemical composition, g/kg DM---------------				
Dry matter, g/kg as-fed basis	967	963	964	959
Organic matter	941	947	936	939
Crude protein	171	154	156	160
Neutral detergent fiber	274	103	101	99
Acid detergent fiber	161	29	27	28
Non-fiber-carbohydrates	462	660	665	648
Ether extract	20	33	25	27

[1]CONT: positive control, diet without sodium bicarbonate containing 400 g/kg DM of soybean hulls; 10SB: forage-free diet based on ground flint corn containing 10 g/kg DM of sodium bicarbonate; 20SB: forage-free diet based on ground flint corn containing 20 g/kg DM of sodium bicarbonate; 30SB: forage-free diet based on ground flint corn containing 30 g/kg DM of sodium bicarbonate.
[2]Mineral premix included: 85 g/kg P; 154 g/kg Ca; 120 g/kg Na; 10 g/kg Mg; 35 g/kg S; 756 ppm Cu; 44 ppm Co; 2800 ppm Zn; 14 ppm Se; 2180 ppm Mn; 1000 ppm F; 58 ppm I.

sieve shaker (Produtest T Model, Telastem, São Paulo, Brazil) with sieves of 2.00, 0.51, 0.42, 0.297 e 0.18 mm pore sizes, during 5 min. Particle size distribution of CONT, 10SB, 20SB, 30SB, ground corn, soybean hulls, and soybean meal are presented on table 2.

Diets weremixedusing a horizontal mixer (Lucato, Limeira, São Paulo, Brazil). The additive was mixed with the mineral ingredients and, after that, was added to the mixer to compose the total mixed ration (TMR). The TMR was weighed on an electronic scale accurate to 1 g (Marte LC 100, São Paulo, Brazil) and offered *ad libitum* daily. Orts were recorded daily to calculate DMI. The previous DMI was used to calculate the amount of feed that would be offered to the animals, avoiding leftovers greater than 0.10 g/kg of daily intake.

Intake and apparent digestibility of nutrients

Between the 15th and 20th day of the experiment, the total fecal production was individually collected every day. Feces were collected using bags made of a thick fabric, similar to jeans, internally covered with canvas, and attached to the animal utilizing a harness, to avoid contamination with urine. Every day during these five days, feces and orts were weighed using an electronic scale (Marte LC 100, São Paulo, Brazil) and sampled (10% of total weigh). The samples were kept at -20 ºC for analysis of nutrients to calculate intake and apparent digestibility of nutrients.

N balance

Between the 15th and 20th day of the experiment, buckets were used under the galvanized sheet structures below the cages to collect the total urine production. To avoid ammonia volatilization, a sufficient amount of 6 N HCl was used (50 to 70 mL) to maintain the urine pH below 3.0. The urine volume that was inside the buckets was measured using graduated cylinders and 10% was sampled and kept at -20 ºC for analysis of nitrogen.

Characteristics of ruminal fluid

Ruminal fluid samples were collected on day 21 of each experimental period, at 0, 4, 8, 12, 16, 20, and 24 hours after feeding, *via* a rumen cannula. At each collection, a sample of 200 mL of ruminal fluid was obtained from each animal. The collection was done using a syringe attached to a thin hose, with two layers of nylon filter cloth (100 mesh) at the end to filter the ruminal fluid. The ruminal pH

Table 2 - Physical characterization of experimental diets and ingredients.

	---Diets/ingredients (% retained)[1]---						
Sieve screen, mm	CONT	10SB	20SB	30SB	Ground Corn	SH	SM
2.00	12.3	30.3	28.0	24.7	26.9	1.3	1.6
0.51	34.4	33.9	29.2	31.0	29.8	54.0	44.5
0.42	28.9	17.7	19.0	20.4	21.1	27.2	33.1
0.297	7.4	3.6	5.6	4.6	5.8	5.8	7.5
0.18	4.0	2.5	3.1	2.1	3.4	4.2	3.5
Fund	13.0	12.0	15.1	17.2	13.0	7.5	9.8

[1]CONT: positive control, diet without sodium bicarbonate containing 400 g/kg DM of soybean hulls; 10SB: forage-free diet based on ground flint corn containing 10 g/kg DM of sodium bicarbonate; 20SB: forage-free diet based on ground flint corn containing 20 g/kg DM of sodium bicarbonate; 30SB: forage-free diet based on ground flint corn containing 30 g/kg DM of sodium bicarbonate; SH: soybean hulls; SM: soybean meal.

was measured with a pH meter (Digimed DM20, São Paulo, Brazil). For future determinations of SCFA (acetate, propionate, butyrate, isobutyrate, valerate and isovalerate) and ammonia N, three samples of ruminal fluid were kept in a small 2 mL plastic container (Eppendorf, São Paulo, Brazil) at -20 °C. No preservative was used in the ruminal fluid samples.

Laboratory analyses and calculations

Bromatological analysis of TMR, orts, feces, and feed ingredients were analyzed according to the Association of Official Analytical Chemistry methods as described in DE PAULA CARLIS et al. (2021), except ether extract (EE), which was analyzed using the Soxhlet method. Non-fiber carbohydrates (NFC) were estimated according to the equation: NFC (g/kg) =1000_ [NDF (g/kg) + CP (g/kg) + EE (g/kg) + ash (g/kg)]. The total digestible nutrient (TDN) content was calculated using the equation: TDN (%) = ($\%CP_{digestible} \times \%CP/100$) + ($\%EE_{digestible} \times \%EE/100$) × 2.25 + ($\%NDF_{digestible} \times \%NDF/100$) + ($\%NFC_{digestible} \times \%NFC/100$). According to NRC (1984) recommendations, the metabolizable energy (ME) was calculated: ME (Mcal/kg DM) = TDN (kg) × 0.82 × 4.4. The coefficients of apparent digestibility of nutrients were calculated by the difference between the intake of each nutrient (DM, OM, CP, EE, NDF and ADF) and its excretion, divided by the intake level of each nutrient. The N balance was calculated according to the equations: N retention (g/day) =(N_{intake}-N_{feces}- N_{urine}); N retention (% N intake) = (N_{intake}- N_{feces}- N_{urine})/N_{intake}; N retention (% N absorbed) = (N_{intake}- N_{feces}- N_{urine})/($N_{intake} \times N_{absorbed}$).

Determination and quantification of SCFA were determined according to DE PAULA CARLIS et al. (2021), using an Agilent 7890A gas chromatograph equipped with a flame ionization detector (Injector HP 7683B) and a fused-silica capillary column (19091F-112; Agilent Technologies, Santa Clara, CA, USA).

The methodology of CHANEY & MARBACH (1962) was used to evaluate the concentration of NH_3-N, by the colorimetric method, with adjustments for a microplate reader (BIO-RAD, Hercules, CA, USA), with a 550 nm absorbance filter.

Statistical analyses

The MIXED Procedure of SAS (SAS Inst., Inc., Cary, NC) was used to analyze all data. For repeated measures over time (SCFA, ruminal pH and NH_3-N concentration) the model used was $Y = \mu + A_i + P_j + T_k + E_{ijk} + H_l + (TH)_{kl} + E_{ijkl}$ where μ= overall mean, A_i= random animal effect (1 to 4), P_j=random period effect (1 to 4), T_k= fixed treatment effect (1 to 4), E_{ijk} = residual error A, H_l= fixed harvest time effect (1 to 8), $(TH)_{kl}$ = interaction between treatment and harvest time, E_{ijkl}= residual error B.

For only measures (nutrient intake, apparent digestibility, and N balance) the model used was $Y = \mu + A_i + P_j + T_k + E_{ijk}$ where μ= overall mean, A_i= random animal effect (1 to 4), P_j= random period effect (1 to 4), T_k= fixed treatment effect (1 to 4), E_{ijk}= residual erro.

The best-fitted covariance structure was the "autoregressive" (AR [1]), based on Akaike's (AIC) information criterion. The individual treatment means were generated using the LSMEANS option. Linear and quadratic orthogonal polynomials were used to evaluate the effects of SB levels on diets. One contrast was performed: CONT *vs* diets containing SB. The F test of the analysis of variance was used to determine the effects of period and interaction between treatments and periods. Effects were declared significant when $P \leq 0.05$.

RESULTS

Intake, apparent nutrient digestibility, and N balance

There was no effect of treatments on DM, organic matter (OM), EE, and crude protein (CP) intake (Table 3). When comparing CONT and SB diets, there was an effect ($P < 0.01$) of treatments on NDF and acid detergent fiber (ADF) intake, with lower values for the SB diets (mean of 0.099 and 0.035 kg/day, respectively). The NFC intake was greater ($P = 0.01$) for the SB diets (mean value of 0.587 kg/day) compared to CONT (0.407 kg/day) (Table 3). However, NDF, ADF, and NFC intake were not affected by the SB levels.

There was no effect of treatments on DM, OM, EE, and CP digestibility (Table 4). However, the NDF digestibility was lower ($P = 0.03$) for SB diets when compared to CONT, with a mean value of 77.9% (Table 4). Regarding NFC digestibility, greater values ($P < 0.01$) were observed for the diets with SB compared to CONT, with a mean value of 96.26%, while CONT had 94.3%. The values of TDN (DM) and ME (Mcal/kg DM) were greater for SB diets when compared to CONT ($P = 0.04$), with the mean values of 89.0% and 3.26 Mcal/kg DM (Table 4). There was no effect of SB levels on NDF, ADF, NFC, and TDN digestibility (Table 4). There was no effect of treatments on N balance (Table 5).

Ruminal SCFA, pH, and ammonia nitrogen

There was no interaction (treatment × sampling time) for any of the ruminal parameters evaluated. There was no effect of SB levels on SCFA concentration. However, when compared to CONT, SB diets decreased ($P < 0.01$) the ruminal concentration of acetate, isobutyrate, butyrate, isovalerate, and total SCFA (Table 6).

There was no effect of treatments on the concentration of propionate, valerate, acetate:propionate ratio, and ammonia nitrogen. The ruminal pH showed a quadratic response ($P = 0.05$) to SB levels, with a greater value observed for animals that received 20SB, which was 5.99. However, in contrast to CONT, SB diets did not affect ruminal pH.(Table 6).

DISCUSSION

Intake, apparent nutrient digestibility, and N balance

Diets that have high digestibility and low filling have their consumption controlled by the energy demand of the animal (MERTENS, 1994). Therefore, a higher DMI was expected for the CONT due to the lower TDN level and energy density of this diet when compared to the SB diets. But this did not happen. In a companion paper (VICENTE et al., 2022) we evaluated the same diets and its effects on performance, nutritional behavior, and carcass characteristics and we reported a difference in DMI between CONT and SB diets. This difference was attributed to the smaller particle size of the diet containing soybean hulls that may have increased the passage rate and also to the lower energy density of the CONT. A possible reason for the same DMI in the present experiment may be the higher total SCFA concentration of the animals that received the CONT,

Table 3 - Effect of increasing levels of sodium bicarbonate in forage-free diets on lamb nutrient intake in lambs.

Item[4]	------------------------Treatments[1]------------------------				SEM[2]	--------------------P-Value[3]------------------		
	CONT	10SB	2SB	3SB		L	Q	BS×CONT
-----------------------------------Intake, kg/d-----------------------------------								
DM	0.972	0.881	0.906	0.862	0.05	0.90	0.79	0.47
OM	0.919	0.842	0.855	0.818	0.05	0.86	0.83	0.48
EE	0.021	0.031	0.023	0.023	0.002	0.06	0.32	0.15
CP	0.160	0.129	0.131	0.133	0.008	0.84	0.98	0.17
NDF	0.272	0.130	0.086	0.083	0.02	0.16	0.47	<0.01
ADF	0.159	0.057	0.025	0.023	0.02	0.18	0.48	<0.01
NFC	0.407	0.588	0.593	0.580	0.03	0.90	0.90	0.01

[1]CONT: positive control, diet without sodium bicarbonate containing 400 g/kg DM of soybean hulls; 10SB: forage-free diet based on ground flint corn containing 10 g/kg DM of sodium bicarbonate; 20SB: forage-free diet based on ground flint corn containing 20 g/kg DM of sodium bicarbonate; 30SB: forage-free diet based on ground flint corn containing 30 g/kg DM of sodium bicarbonate.
[2]SEM: Standard error of the mean.
[3]L: linear effect; Q: quadratic effect; SB×CONT: diets containing sodium bicarbonate *vs* positive control diet.
[4]DM: dry matter; OM: organic matter; EE: ether extract; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: non-fiber carbohydrates; TDN: total digestible nutrients; ME: metabolizable energy.

Table 4 - Effect of increasing levels of sodium bicarbonate in forage-free diets on apparent digestibility of nutrients in lambs.

Item[4]	----------Treatments[1]----------				SEM[2]	----------P-Value[3]----------		
	CONT	10SB	20SB	30SB		L	Q	SB×CONT
----------Digestibility, %----------								
DM	89.3	90.4	91.2	92.8	0.81	0.24	0.78	0.21
OM	90.3	91.5	92.2	93.8	0.74	0.20	0.77	0.14
EE	81.8	87.8	85.3	87.5	1.10	0.89	0.31	0.06
CP	88.2	87.3	89.8	91.3	1.05	0.14	0.82	0.55
NDF	90.6	75.3	67.8	76.9	3.77	0.85	0.26	0.03
ADF	91.0	74.5	63.4	77.2	4.51	0.82	0.24	0.07
NFC	91.1	95.4	96.3	97.1	0.69	0.15	0.97	<0.01
TDN, %	87.0	91.1	88.8	90.9	0.77	0.92	0.14	0.04
ME, Mcal/kg DM	3.29	3.21	3.28	3.14	0.03	0.92	0.14	0.04

[1]CONT: positive control, diet without sodium bicarbonate containing 400 g/kg DM of soybean hulls; 10SB: forage-free diet based on ground flint corn containing 10 g/kg DM of sodium bicarbonate; 20SB: forage-free diet based on ground flint corn containing 20 g/kg DM of sodium bicarbonate; 30SB: forage-free diet based on ground flint corn containing 30 g/kg DM of sodium bicarbonate.
[2]SEM: Standard error of the mean.
[3]L: linear effect; Q: quadratic effect; SB×CONT: diets containing sodium bicarbonate *vs* positive control diet.
[4]DM: dry matter; OM: organic matter; EE: ether extract; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: non-fiber carbohydrates; TDN: total digestible nutrients; ME: metabolizable energy.

the energy gain from the metabolism of the ruminal SCFA may have compensated for the lower NDT of the CONT when compared to the SB diets.

The lower NDF and ADF intakes by the animals that received SB diets were due to the lower NDF and ADF contents of corn (10.8 and 3.1%) when compared to soybean hulls (58.6 and 41.1%), respectively (values obtained from our chemical analysis). These differences in ingredients altered the diet composition, CONT had 274 g/kg of NDF and 161 g/kg of ADF, while SB diets had mean values of 101 g/kg of NDF and 28 g/kg of ADF. The greater NFC intake of SB diets is also explained by the composition of the diet, they had an average of 658 g/kg of NFC while CONT had 462 g/kg.

The lower NDF digestibility for SB diets compared to CONT may be attributed to the lower fiber and absence of soybean hulls in the diet. This

Table 5 - Effect of increasing levels of sodium bicarbonate in forage-free diets on nitrogen balance in lambs.

Item[4]	----------Treatments[1]----------				SEM[2]	----------P-Value[3]----------		
	CONT	10SB	20SB	30SB		L	Q	SB×CONT
----------Nitrogen, g/day----------								
Intake	26.9	22.3	26.0	21.3	1.38	0.74	0.24	0.14
Fecal	3.19	2.90	2.16	2.10	0.33	0.48	0.64	0.25
Urinary	14.8	14.9	13.5	13.8	0.71	0.54	0.58	0.56
Absorbed	23.7	19.4	23.3	19.4	1.29	0.99	0.29	0.24
----------N retention----------								
g/day	8.92	4.19	7.71	7.09	1.41	0.61	0.66	0.47
% N intake	31.7	22.2	17.7	26.6	3.42	0.68	0.48	0.20
% N absorbed	35.9	23.0	17.6	25.7	4.08	0.87	0.54	0.20

[1]CONT: positive control, diet without sodium bicarbonate containing 400 g/kg DM of soybean hulls; 10SB: forage-free diet based on ground flint corn containing 10 g/kg DM of sodium bicarbonate; 20SB: forage-free diet based on ground flint corn containing 20 g/kg DM of sodium bicarbonate; 30SB: forage-free diet based on ground flint corn containing 30 g/kg DM of sodium bicarbonate.
[2]SEM: Standard error of the mean.
[3]L: linear effect; Q: quadratic effect; SB×CONT: diets containing sodium bicarbonate *vs* positive control diet.
[4]% N absorbed: percentage of N absorbed that was retained; % N intake: percentage of N intake that was retained.

Table 6 - Effect of increasing levels of sodium bicarbonate in forage-free diets on ruminal parameters in lambs.

Item[4]	----------------Treatments[1]--------------				SEM[2]	----------------P-Value[3]----------------				
	CONT	10SB	20SB	30SB		L	Q	SB×CONT	H	T×H
----SCFA, mM----										
Acetate	44.6	35.4	31.5	35.0	1.06	0.87	0.07	<0.01	<0.01	0.96
Propionate	33.0	31.8	25.4	28.3	1.76	0.44	0.25	0.24	<0.01	1.00
Isobutyrate	0.62	0.41	0.36	0.48	0.02	0.15	0.06	<0.01	<0.01	0.91
Butyrate	8.50	5.38	4.72	4.28	0.31	0.08	0.83	<0.01	0.13	1.00
Isovalerate	1.18	0.84	0.60	0.87	0.06	0.88	0.06	<0.01	0.10	0.98
Valerate	1.70	1.41	1.66	1.79	0.10	0.16	0.79	0.70	0.05	0.95
Total	89.6	64.1	63.4	70.5	2.78	0.36	0.51	<0.01	<0.01	0.99
A:P	1.65	1.76	1.85	1.41	0.09	0.21	0.24	0.91	0.29	1.00
pH	5.81	5.77	5.99	5.87	0.04	0.32	0.05	0.43	<0.01	1.00
NH_3-N, g/dL	14.5	14.2	13.4	14.8	0.75	0.81	0.54	0.82	0.74	0.91

[1]CONT: positive control, diet without sodium bicarbonate containing 400 g/kg DM of soybean hulls; 10SB: forage-free diet based on ground flint corn containing 10 g/kg DM of sodium bicarbonate; 20SB: forage-free diet based on ground flint corn containing 20 g/kg DM of sodium bicarbonate; 30SB: forage-free diet based on ground flint corn containing 30 g/kg DM of sodium bicarbonate.
[2]SEM: Standard error of the mean.
[3]L: linear effect; Q: quadratic effect; SB×CONT: diets containing sodium bicarbonate *vs* positive control diet; H: effect of hour after feeding, T×H: interaction between treatment and hour after feeding.
[4]SCFA: short chain fatty acid concentration; A:P: acetate to propionate ratio; NH_3-N: ammonia concentration.

ingredient has a high ruminal fermentation and digestibility of the fibrous fraction (HSU et al., 1987) since it has high amounts of cellulose (average of 43.0% DM) and hemicellulose (average of 18.0% DM) and low lignin (average of 2.6% DM) (IPHARRAGUERRE & CLARK, 2003) .

The greater NFC digestibility for the SB diets compared to CONT may be associated with its greater corn content, considering that the main NFC of corn is starch, which can be present between 70 and 80% of its composition (ROSTAGNO et al., 2000) and only 9.0% of NDF (NRC, 2007a). In addition, NAKAMURA & OWEN (1989) reported a greater passage rate with soybean hulls replacing corn, which may have also happened in the present study and contributed to lower NFC digestibility in CONT.

The greater TDN and ME values for the SB diets are explained by the greater amount of corn in these diets, which has a greater energy density when compared to soybean hulls, 88 and 77% of TDN and 3.4 and 3.9 Mcal/kg of digestible energy, respectively (NRC, 2007b).

Degradability and energy status of the animal (KEBREAB et al., 2002), dietary protein quantity, nitrogen constitution of protein and relation with other nutrients (FIRKINS & REYNOLDS, 2005) can affect nitrogen utilization by ruminants. However, in the present study, there was no effect of treatments on nitrogen balance. The diets were formulated to be isonitrogenous. The animals had a similar CP intake, and the digestibility of this fraction did not differ, which can explain the absence of effect.

Ruminal SCFA, pH, and ammonia nitrogen

The lower ruminal concentration of acetate for SB diets was due to the lower NDF content of these diets (Table 1). The higher fiber and NDF digestibility for CONT (Table 4) increased the concentration of acetate, since it is the main SCFA resulting from fiber fermentation (FIRKINS et al., 2006). The inclusion of soybean hulls replacing corn has consistently increased the ruminal concentration of acetate (CUNNINGHAM et al., 1993; IPHARRAGUERRE et al., 2002; FERREIRA et al., 2011).

The lower concentration of iso acids for SB diets when compared to CONT was also due to the presence of soybean hulls in CONT since it is an ingredient with considerable protein content (CP = 13%) (NRC, 2007a), thus the degradation of amines and proteins in the rumen causes an increase in the concentration of these acids. With some SCFA in greater concentrations, the concentration of total SCFA was also greater for CONT. IPHARRAGUERRE & CLARK (2003) in a review of soybean hulls in dairy cow feed reported that the inclusion of soybean hulls results in a fermentation that maintains or increases the total SCFA concentration, which is in agreement with the data of the present study. SARWAR et al.

(1992) and PANTOJA et al. (1994) proposed that the highly digestible NDF present in soybean hulls may allow greater rumen fermentation, possibly resulting in greater concentrations of SCFA.

Greater ruminal pH for animals that received 20SB is justified by the action of SB, which is considered a rumen buffer. ROGERS& DAVIS (1982) reported a greater dilution rate of rumen fluid with the inclusion of SB, tending to reduce the retention time of DM and decrease the amount of total SCFA produced in rumen per unit of DMI. With a greater dilution rate, the starch flow through the rumen is greater, which would decrease the fermentation of this carbohydrate, with less starch to be fermented in the rumen, the production of propionate would be lower, justifying the change in ruminal pH, as proposed by RUSSELL & CHOW (1993). The positive effect of SB found in this study is in agreement with those reported by SANTRA et al. (2003), who also found a positive effect of SB on ruminal pH, which was greater for the groups that received the additive. Conversely, the level of 10SB may have been insufficient to provide alteration in rumen fluid dilution rate, not altering the ruminalpH. GASTALDELLO JR et al. (2013) reported no effect of the addition of SB on the ruminal pH of lambs, this is in agreement with the results of the present experiment since they used a fixed dose of only 10 g/kg (the same as the 10SB), which may have been insufficient. Regarding the 30SB level, it may be that this level has exceeded the optimal dose. The buffering capacity can be influenced by the quantity of CO_2, because the reaction depends on this component ($CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$). Maybe the rumen environment was already with a high amount of CO_2 and the buffer failed to change the ruminal pH.

The lack of effect between CONT and SB diets for ruminal pH showed that SB had a similar effect as soybean hulls fiber content, the buffer was efficient in the rumen environment when associated with forage-free diets. Therefore, the similarity between CONT and SB diets is a positive result in this experiment. It is worth mentioning that ruminal pH bellow 5.6 is often used as reference standards for chronic acidosis (COOPER et al., 1997).

CONCLUSION

In conclusion, sodium bicarbonate supplementation in forage-free diets with high flint corn inclusion was efficient in maintaining a ruminal pH above the value considered chronic acidosis and when compared to the treatment containing soybean hulls they did not differ. This indicated that the chemical effect of the buffer was similar to the physical effect provided by the fiber of the soybean hulls, which shows that it can be an option in forage-free diets for lambs. The total short chain fatty acid concentration was lower for the treatment supplemented with sodium bicarbonate, but this must not be looked at individually but together with the other positive results, specially the higher ruminalpH. Among the sodium bicarbonate levels, the 20 g/kg DM level was the best, since he showed a greater value for ruminal pH, which enables greater safety in forage-free diets for lambs.

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AUTHORS' CONTRIBUTIONS

Ana Carolina Silva Vicente: conceptualization, methodology, investigation, validation, formal analysis, data curation, writing-original draft; Matheus S. P. Carlis, Isabela J. dos Santos, Adrielly L. A. da Silva, Paulo Césae G. Dias Júnior, Nathalia R. Eckermann, Terezinha T. de Souza, Daniel M. Polizel, Marcelo Baggio: investigation, writing-review & editing; Janaina S. Biava, Alexandre V. Pires: methodology, writing-review & editing, visualization; Evandro M. Ferreira: conceptualization, methodology, validation, formal analysis, writing-review & editing, supervision, funding acquisition.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The experiments were conducted in accordance with laws and regulations and approved by the Ethics Committee on the Use of Animals (CEUA / ESALQ), protocol No. 8846110320.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analysis, or interpretation of the data; in the writing of the manuscript, and in the decision to publish the results.

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