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Research article

Effects of dietary supplementation of AcidBuf with different levels of salt on ruminal fermentation profile and tissue morphology of growing lambs

Ibrahim A. Alhidary

Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia

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Abstract

Forty eight 4-month-old Awassi male lambs (initial bodyweight 23.5 ± 1.3 kg) were used in a 70-day trial to evaluate the effects of supplemental AcidBuf (calcified seaweed extract) with different levels of salt (sodium chloride) on ruminal fermentation and tissue morphology of growing lambs. Animals were individually housed in shaded pens and randomly divided into 6 groups of 8 lambs each. The dietary treatments were: 1) no added supplemental AcidBuf or salt (control group; the basal diet), 2) 0.4% added AcidBuf (A+S0.0), 3) 0.4% added AcidBuf + 0.4% added salt (A+S0.4), 4) 0.4% added AcidBuf + 0.8% added salt (A+S0.8), 5) 0.4% added AcidBuf + 0.12% added salt (A+S1.2), and 6) 0.4% added AcidBuf + 1.6% added salt (A+S1.6). The basal diet was used a commercial total mixed ration, containing 1.95 Mcal M_{Em} and 13.0% CP/kg. Rumen fluid samples were collected from all lambs at 0, 2, 4 and 8 hours after feeding during day 1, 30, 60 and 70 to measure pH and fermentation profile. At the end of the study, lambs were slaughtered for evaluating the rumen papillae morphology. Lambs on the AcidBuf diets had less ($P<0.05$) pH, at after 0 and 2 h of the morning feeding and over the collection period, than those of lambs on the control diet. The length and the total surface of papillae were greater ($P<0.05$) in lambs fed only AcidBuf (A+S0.0) than those of lambs on the CON diet. Overall, these data indicate that AcidBuf and salt supplementation did not affect rumen fermentation and tissue morphology of growing lambs.

Keywords: AcidBuf; growing lamb; rumen fermentation; rumen morphology; salt

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Introduction

The total mixed rations (TMR) have been widely used for feeding ruminant animals under intensive systems, providing the majority of their nutritional requirements needed and then contributing to considerable economic gains for livestock producers, through reducing the feed losses and improving animal productivity. However, the addition of different types of grains to forage diets as TMR form can be increased the lag time and reduced the extent of fiber digestion (Allen, 1997; Enemark, 2008). This change may be caused by a decrease in the enzymatic activity of cellulose, altering in microbial strains or increases in the metabolism of readily degradable substrates, and consequently, a depression in rumen pH value which is normally greater than 5.9 (Olson, 1997). This depression in pH value (less than 5.5; Olson, 1997;

***Corresponding author:** Ibrahim A. Alhidary; Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia; E-mail: ialhidary@ksu.edu.sa

Garrett et al., 1999) may result in a high risk of sub-acute ruminal acidosis (SARA), which in turn can substantially contribute to adverse effects on the productivity and well-being of livestock and cause extreme economic losses for producers (Olson, 1997; Enemark, 2008). Economically, for example, the annual costs associated with SARA in USA have been estimated at US$ one billion (Enemark, 2008).

A variety of dietary buffers (including sodium bicarbonate, sodium bentonite, calcium carbonate and calcified seaweed) are regularly added to the total mixed rations to ameliorate ruminal pH value, increase in acetate to propionate ratio, and to improve the productivity and health status of ruminant animals (Hutjens, 1991; Allen, 1997; Enemark, 2008). Previous studies with dairy cattle have indicated that an increase in the ruminal pH values was observed from 5.5 to 6.25 when dairy cattle fed TMR diets supplemented with different sources of salt at levels of 110-225 g/d (Garry, 2002; Enemark, 2008).

Salt (sodium chloride) and AcidBuf (calcified seaweed extract) are natural products and can be also used to regulate ruminal digestion, particularly for ruminant animals consuming high energy diets (Cruywagen et al., 2004; Beya, 2007; Bodas et al., 2007). The addition of Acidbuf and salt in ruminant diets reduces the rumen pH, increases the total volatile acid production, and improves rumen efficiency, resulting in increases in feed intake, feed efficiency, milk yield and body weight gain (Cruywagen et al., 2004; Askar et al., 2011). However, the beneficial effects of dietary supplementation with a combination acidbuf and salt to ruminants, particularly growing lambs are largely unknown. Therefore, the objective of this study was to determine whether inclusion of Acidbuf and salt in sheep diets improves ruminal fermentation and tissue morphology.

Materials and Methods

Animals and experimental design

The study was undertaken at The Experimental Farm Animal Centre, Department of Animal Production, King Saud University, Riyadh, Saudi Arabia (24°42'N and 46°44'E), and all procedures followed the Implementing Regulations of the Law of Ethics of Research on Living Creatures (Saudi Arabia National Committee of Bio Ethics) with the approval of the King Saud University Animal Ethics Committee.

Thirty days before the study commenced, forty eight growing male Awassi lambs (mean initial bodyweight 23.5 ± 3.0 kg; 4 to 5 months-old) were purchased from the local livestock market and then transported to the Experimental Station of Animal Production Department, College of Food and Agriculture Sciences, King Saud University, Riyadh. On the arrived day, lambs were immediately weighed, ear tagged, vaccinated against *clostridial* diseases and treated for internal and external parasites. Thereafter, animals were individually housed in shaded pens (1.5 m long × 1.0 m wide). Each pen was equipped with a feed trough and a 10-L plastic water bucket. Over a 30-day period prior to the commencement of the study, lambs were adapted to the pens and the basal diet. All lambs were offered a same commercial total mixed ration (WAFI, ARASCO, Riyadh, Saudi Arabia) containing 1.95 Mcal of MEm, and 13.0% CP/kg (DM basis) twice daily at 0800 and 1500 h. Water was available ad libitum.

At the beginning of the experiment (day 1), lambs were randomly assigned to one of six dietary treatments (eight animals in each treatment on the basis of weight), which were: 1) no added supplemental AcidBuf or salt (the basal diet; CON), 2) 0.4% added AcidBuf (A+S0.0), 3) 0.4% added AcidBuf + 0.4% added salt (A+S0.4), 4) 0.4% added AcidBuf + 0.8% added salt (A+S0.8), 5) 0.4% added AcidBuf + 1.2% added salt (A+S1.2), and 6) 0.4% added AcidBuf + 1.6% added salt (A+S1.2). AcidBuf was manufactured and supplied by Celtic Sea Minerals, Carrigaline, Country Cork, Ireland. At the termination of the study (d 70), lambs were firstly deprived of feed and water for 16 h and then slaughtered using the Islamic method. The stomachs were collected immediately after slaughter and weighed for further analysis.

Measurements and sampling

Feed from each treatment was sampled before the study and weekly during the study, and samples were frozen at –20°C. At the end of the study, feed samples were pooled (5%) and analyzed for nutrient composition at King Saud University laboratories. Dry Matter content was determined by drying samples in an oven at 100°C for 24 hours; while ash content was determined by incinerating samples at 550°C for 3 h in a muffle furnace. Crude protein (CP), calcium, sodium, chloride and phosphor contents were measured for each stuffed using an elemental analyzer. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to methods described by Van Soest et al. (1991) and the AOAC (method no. 973.18 C, 1990), respectively (Table 1).

Blood samples (10 ml) were collected from each lamb before the morning feeding via jugular veni-puncture on days 1, 30, 60 and 70. At each collection, 10-mL aliquots of blood were taken into the Vacutainer tubes without any additive for serum collection. Serum was obtained by centrifugation at 2,400 × g for 15 min at 4°C and then frozen at −20°C until further analysis.

Rumen fluid samples (50 ml) from all lambs were collected, using an oral stomach tube and custom-designed stomach pump, at 0, 2, 6 and 8 hours after

Table 1: Nutrient composition of diets (DM basis) fed to lambs in the experiment

Item	Dietary treatmens[1]					
	CON	AB+S0.0	AB+S0.4	AB+S0.8	AB+S1.2	AB+S1.6
DM(%)	92.33	92.33	92.04	92.80	92.50	93.12
Ash, %	8.19	9.09	9.07	9.25	8.26	8.13
GE (Mcal/kg)	2.78	2.79	2.79	2.78	2.79	2.78
CP (%)	12.27	12.41	12.27	12.29	12.48	12.63
EE (%)	5.33	5.18	4.91	4.98	5.31	5.46
NDF (%)	30.57	30.45	30.76	29.43	29.90	29.39
ADF(%)	17.86	17.36	16.89	17.16	16.60	17.18
K (%)	0.68	0.68	0.64	0.65	0.69	0.67
Na (%)	0.03	0.03	0.15	0.28	0.44	0.57
Cl, (%)	0.04	0.04	0.23	0.42	0.67	0.86
S (%)	0.21	0.21	0.17	0.18	0.20	0.20
DCAD[2] (meq/kg DM)	40.58	43.75	55.42	55.95	56.16	51.13

[1]Lambs were fed different diets (n = 6) which were: 1) basal diet (CON), 2) 0.4% added Acid Buf (A+S0.0), 3) 0.4% added Acid Buf + 0.4% added salt (A+S0.4), 4) 0.4% added Acid Buf + 0.8% added salt (A+S0.8), 5) 0.4% added Acid Buf + 1.2% added salt (A+S1.2), and 6) 0.4% added Acid Buf + 1.6% added salt (A+S1.6); [2]Dietary cation-anion difference = (K + Na) - (Cl + S), mEq/kg of DM.

morning feeding on days 1, 30, 60 and 70. At each collection, rumen fluid samples were analyzed immediately for pH values, using a microprocessor pH-meter (Model pH 211, Hanna Instruments, Woonsocket, RI, USA), and then strained through four layers of cheesecloth and transferred into plastic tubes and acidified with 2 ml of concentrated sulfuric acid and frozen at −20°C for fermentation products. Rumen concentrations of volatile fatty acid (including total VFA, acetate, propionate and butyrate) were analyzed using an Agilent series gas chromatograph with HP6890 injection, 30 mm × 0.53 mm × 1.0 µm capillary column (Agilent Technologies Inc., Wilmington, DE), based on the methodology described in the document (Supelco Inc., 1975). Ammonia-N concentrations in rumen fluid and serum were analyzed according to the method described by Smith and Murphy (1993). The L-lactate, acetate and β-Hydroxybutyrate concentrations in serum were analyzed using commercial kits (BioVision Inc., Milpitas, CA).

After slaughter, rumen and reticulum tissue from all animals were immediately thawed, washed with a PBS buffer three times, and then placed in an ice bucket. Subsample of the rumen and reticulum tissues (1 cm^2; 5 replications from each animal) were taken and dehydrated with 4% formalin solution and ethanol for evaluating the rumen papillae morphology. The colour of rumen tissues was determined by using a Minolta Chroma Meter (Konica Minolta, CR-400- Japan) with a CIELAB Colour System for the colour values (L* = value designates lightness; a* and b*= colour coordinates).

The length and width of papillae were measured using a light microscope (IX71 Inverted Olympus Microscope) with software analysis (Cellsens Digital Imaging Software for Research Application), while the papillae density (number of papillae per cm^2 mucosa) was estimated by a video camera. The total surface of papillae per cm^2 mucosa was calculated following the equation described Shen et al (2004); the total surface of papillae per cm^2 mucosa = papillae length x papillae width x 2 x papillae density.

Statistical analysis

All data obtained from this study were analyzed using repeated measures and the PRO Mixed and GLM Models (SAS Institute Inc., Cary, NC). Dietary treatment (AcidBuf and salt added), animal within treatment, and day of measurement were included in the model as main effects and dietary treatment x day was included as the interaction term. Data for rumen tissue morphology were analyzed as a completely randomized design using the GLM model of SAS. The effect of the time of day (0, 2, 4 and 8 hours) was added to model for analysis of pH value and fermentation of rumen fluid. Linear and quadratic polynomial contrasts were used to evaluate the differences between dietary treatment effect and orthogonal contrast. In addition, control *vs*. dietary AcidBuf treatments and *vs.* dietary salt treatments were tested. The random variable (error term) was the animal within treatment. All data are presented as the least square mean and differences were considered significant at $P<0.05$.

Results

Serum biochemical and rumen fermentation profiles

Differences between treatments in the serum and ruminal concentrations of biochemical variables measured in the study are presented in Table 2. With regards to treatment effect, the addition of AcidBuf and salt, at any level, had no effects ($P>0.05$) on the measured fermentation and biochemical variables compared with the control group. However, a quadratic response was observed ($P<0.05$) in the serum concentrations of L-lactate and β-hydroxybutyrate, as

dietary salt level increased. In addition, there were day and time of measurement effects on the ruminal concentrations of total VFA, acetate, L-lactate and ammonia-N ($P < 0.05$; Table 2).

pH values of ruminal fluid

The pH value of ruminal fluid was not affected (P>0.05) by dietary treatments across groups Lambs had similar rumen fluid pH at the various collection times (Table 3). However, lambs treated with the AcidBuf had less (P<0.05) pH after 0 and 2 h of the feeding and over the collection period, than those lambs in the control diet. In addition, a quadratic response was observed (P<0.05) in pH of rumen fluid, as dietary salt level increased (Table 3).

Colour components of ruminal and reticulum tissues

No treatment differences were observed in colour component data for both rumen and reticulum tissues. In addition, adding either AcidBuf or salt to lamb diets had no effects (P>0.05) on the colour component (LAB value) of rumen and reticulum when compared with un-supplemented lambs (Table 4).

Rumen tissue morphology

The length and the total surface of papillae were affected (P = 0.02 and 0.04, respectively) by dietary treatments. Lambs fed the A+S0.0 diet had a greater (P= 0.02) PL when compared with lambs fed the CON and A+S0.8 diets (Table 5). A similar pattern was also occurred in TSP which was greater in the A+S0.0 lambs group (1236.6 mm^2/cm^2) than those lambs fed the CON, A+S0.8 and A+S1.2 diets (P = 0.04; Table 5). Moreover, the addition of AcidBuf (the 0.4% AcidBuf diets) resulted in an increase in the density and total surface when compared with those of un-supplemented lambs (the basal diet; P<0.05; Table 5).

Discussion

The objective of the current study was to determine whether inclusion of Acidbuf and salt in lamb diets improves rumen fermentation and morphometric measurements. AcidBuf and salt, as natural feed ingredients, can be used to regulate feed intake, rumen buffering capacity and acid-base balance, particularly ruminant animals consuming high energy diets (Allen, 1997; Poppi et al., 2000; Yang and Beauchemin, 2007). However, the addition of AcidBuf with different levels of salt in the current study increased the level of DCAD, as dietary salt level increased in the diets (range from 40.58 to 56.16 meq/ kg DM). These levels of DCAD used in the current study are within the reference range of DCAD used previously in lamb (Las et al., 2007), dairy cattle (Vagnoni and Oetzel, 1998) and in beef cattle diets (West et al., 1992).

In the current study, the addition of AcidBuf with different levels of salt had no effect on ruminal pH and fermentation products measured in the rumen and serum. These are consistent with previous studies indicating that there is not a beneficial of dietary AcidBuf and/or salt on ruminal fermentation and their products in sheep (Phillips et al., 2015) or dairy cattle (Cruywagen et al., 2007). In contrast, Cruywagen et al (2004) observed that the rumen pH and VFA concentrations in rumen were increased as the dose of AcidBuf increased in dairy cattle. In addition, adding different types of salt to dairy cattle diets improved buffering capacity in term of an increased rumen pH and ammonia concentration (Solorzano et al., 1989).

The increases in length, total surface and density of papillae of lambs fed diets supplemented with AcidBuf and salt were observed in the current study. The changes of rumen papillae characteristics (e.g. size, number and distribution) are mainly dependent on

Table 2: Mean concentrations (± SE) of ruminal and serum measures in lambs fed diets supplemented with AcidBuf and different levels of salt

Analyte, unit	Dietary treatments[1]						SE.
	CON	A+S0.0	A+S0.4	A+S0.8	A+S1.2	A+S1.6	
Ruminal (m*M*)							
Total VFA	83.27	84.09	81.78	82.67	84.25	83.16	5.54 [D, P]
Acetate	48.30	47.77	47.83	48.25	48.47	48.03	2.33[D, P]
Propionate	22.38	22.80	22.03	22.42	21.95	22.15	1.15 [P]
Butyrate	9.98	10.09	9.61	9.92	10.26	9.95	0.56 [P]
L-Lactate	0.27	0.26	0.28	0.25	0.26	0.29	0.03[D, P]
Ammonia-N	10.05	9.91	11.06	9.75	10.96	9.76	1.84[D, P]
Serum (μ*M*)							
Acetate	36.45	35.61	36.53	36.35	35.67	36.44	0.94[D]
L-Lactate	0.75	0.76	0.73	0.75	0.74	0.73	0.03[D, Q]
β-Hydroxybutyrate	9.15	8.48	9.04	8.65	9.58	8.87	0.86[D, Q]
Ammonia-N	89.13	94.67	86.30	92.54	93.09	88.25	8.34

[1]Values are for lambs (n = 48) fed either CON = the basal diet (control group); A+S0.0 = 0.4% added AcidBuf; A+S0.4 = 0.4% added AcidBuf + 0.4% added salt; A+S0.8 = 0.4% added AcidBuf + 0.8% added salt; A+S1.2 = 0.4% added AcidBuf + 1.2% added salt; or A+S1.6 = 0.4% added AcidBuf + 1.6% added salt. Blood and rumen fluid were collected on days 1, 30, 60 and 70; [D] Day effect (P<0.05); [P] Time collection effect (P<0.05).

Table 3: Effects of dietary supplementation of AcidBuf and salt on pH values of ruminal fluid of growing lambs

Time (h)	Dietary treatments[1]						SE.
	CON	A+S0.0	A+S0.4	A+S0.8	A+S1.2	A+S1.6	
00:00	6.39	6.10	6.19	5.96	6.29	6.02	0.14 [A, D, Q]
02:00	6.04	5.85	5.95	5.91	6.02	5.78	0.17 [A, D, Q]
04:00	5.95	5.83	5.68	5.97	5.94	5.88	0.13
08:00	5.82	5.90	5.75	5.95	5.84	6.09	0.11 [D, S]
Overall	6.07	5.92	5.85	5.88	5.94	5.94	0.19 [A, P]

[1]Values are for lambs (n = 48) fed either CON = the basal diet (WAFI); A+S0.0 = 0.4% added AcidBuf; A+S0.4 = 0.4% added AcidBuf + 0.4% added salt; A+S0.8 = 0.4% added AcidBuf + 0.8% added salt; A+S1.2 = 0.4% added AcidBuf + 1.2% added salt; or A+S1.6 = 0.4% added AcidBuf + 1.6% added salt. Rumen fluid were collected on days 0, 30, 60 and 70; [A] dietary AcidBuf response (P<0.05), [D] Day effect (P<0.05); [Q] Dietary salt level quadratic response (P<0.05); [P] Time collection effect (P<0.05); [S] Dietary salt response (P<0.05).

Table 4: Effects of dietary supplementation of AcidBuf and salt on colour values of rumen and reticulum tissues of lambs

Color components[2]	Dietary treatments[1]						SE.
	CON	A+S0.0	A+S0.4	A+S0.8	A+S1.2	A+S1.6	
Rumen							
L*	29.27	32.97	33.01	34.80	32.24	29.82	3.04
a*	2.54	2.84	3.29	3.66	3.35	3.01	0.54
b*	7.11	8.31	10.10	9.73	10.05	7.72	1.42
Reticulum							
L*	38.08	40.46	38.11	39.30	42.51	37.05	3.11
a*	2.71	2.91	3.15	2.99	3.00	3.06	0.57
b*	7.92	7.67	8.82	8.37	9.10	7.20	1.52

[1] values are for lambs (n = 48) fed either CON = the basal diet (WAFI); A+S0.0 = 0.4% added AcidBuf; A+S0.4 = 0.4% added AcidBuf + 0.4% added salt; A+S0.8 = 0.4% added AcidBuf + 0.8% added salt; A+S1.2 = 0.4% added AcidBuf + 1.2% added salt; or A+S1.6 = 0.4% added AcidBuf + 1.6% added salt; [2] L* = Lightness; a* = Redness; b* = Yellowness.

Table 5: Effects of AcidBuf supplementation with different levels of salt on morphometric measurements of the rumen papillae of lambs

Measurement[2], unit	Dietary treatments[1]						SE.
	CON	A+S0.0	A+S0.4	A+S0.8	A+S1.2	A+S1.6	
PL, mm	5.33[b]	7.59[a]	5.69[b]	5.32[b]	5.36[b]	6.99[ab]	0.79 [Q, T]
PW, mm	1.21	1.11	1.10	1.11	1.08	1.20	0.12
PSA, cm^2	21.10	29.69	20.88	19.85	19.95	29.24	8.39 [Q]
PD, n/cm^2	59.53	79.33	76.00	69.00	81.33	69.06	13.25 [A]
TSP, mm^2/cm^2	787.8[b]	1236.6[a]	954.4[b]	814.9[b]	937.8[b]	1117.5[ab]	156.9 [A, T]
WSC, mm	0.09	0.08	0.06	0.08	0.07	0.09	0.008
LP, mm	0.32	0.29	0.26	0.28	0.25	0.31	0.03
WE, mm	0.52	0.49	0.42	0.45	0.39	0.50	0.08
SM, mm	0.83	0.98	0.85	0.89	0.86	0.96	0.11

[a,b] Within a row, means without a common superscript differ (P < 0.05); [1] values are for lambs (n = 48) fed either CON = the basal diet (WAFI); A+S0.0 = 0.4% added AcidBuf; A+S0.4 = 0.4% added AcidBuf + 0.4% added salt; A+S0.8 = 0.4% added AcidBuf + 0.8% added salt; A+S1.2 = 0.4% added AcidBuf + 1.2% added salt; or A+S1.6 = 0.4% added AcidBuf + 1.6% added salt; [2] LP = lamina propria; PD = papillae density; PL = papillae length; PSA = papillae surface area; PW = papillae width; SM = submucosa; TSP = total surface of papillae; WE = width of epithelium; WSC = width of stratum corneum; [A] dietary AcidBuf response (P < 0.05); [Q] Dietary salt level quadratic response (P<0.05); [S] Dietary salt response (P<0.05); [T] Dietary treatment effect (P<0.05).

species, age and the diet ingredients (including forage to concentrate ratio and energy content of the diet). The increased size and number of papillae allow to absorb more ruminal fermentation products, mainly VFA, across the rumen epithelium, which in turn cause an increase transport rate of ruminal fermentation products the and improvements in both efficiency of rumen buffering capacity and nutrient digestions (Shen et al., 2005; Steele et al., 2012; Blanco et al., 2015).

Conclusions

Under the conditions of the present study, the results indicate that the inclusion of dietary AcidBuf, alone or in combination with different levels of salt in growing lambs diets had no additional consistent benefits on rumen fermentation and morphology. Furthermore, the variations in responses (rumen fermentation and morphological profiles) to AcidBuf and different levels of salt supplementation occurred in the current study indicate that further studies are

required to define the effects of AcidBuf and salt supplementation clearly to detect possible interactions and monitor the response of growing lambs when fed these two supplements. This will allow the development of a response gradient for varying amounts of added AcidBuf and salt being fed growing lambs to ascertain the amount which is the most beneficial for production. It should also be considered to identify salt sources and animal conditions in which AcidBuf and salt supplementation would be most advantageous regarding productivity variables, availability and cost for the sheep industry.

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