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Effect of protein supplementation on tropical grass hay utilization by beef steers drinking saline water[1]

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ABSTRACT: An experiment was conducted to assess the impact of increasing levels of supplemental soybean meal (SBM; 45.7% CP) in cattle consuming tropical grass hay (*Panicum maximum* cultivar Gatton; 7.0% CP and 81.8% NDF) and drinking low salt water (LS) or high salt water (HS). Six ruminally fistulated beef steers (BW = 375 ± 43 kg) were used in a 6-treatment, 4-period crossover experiment. Treatments were arranged as a 2 × 3 factorial, with 2 levels salt in the water (LS and HS: 786 and 6,473 mg/kg of total dissolved solids [TDS], respectively) and 3 levels of SBM (0, 0.2, and 0.4% BW/d). After 15 d of adaptation to treatments, periods consisted of 5 d for intake and digestibility determination, 1 d for monitoring ruminal fermentation, 1 d for ruminal evacuation, and 1 d for blood sampling. Supplemental SBM × water quality interactions were significant ($P < 0.05$) for most measures of intake, except for total tract digestible OM intake ($P = 0.38$) and total tract digestible NDF intake (TTDNDFI; $P = 0.32$). At greater levels of SBM, forage OM intake, NDF intake, and water intake seemed to reach a plateau in LS while this was not observed in HS. Total tract digestible OM intake increased linearly ($P = 0.01$) and TTDNDFI tended to increase ($P = 0.09$) in response to increased SBM. Digestibility of OM and NDF were not affected by treatment ($P > 0.21$). Passage rate of acid detergent insoluble ash linearly increased ($P < 0.01$) in response to SBM, although it was not affected by water quality ($P = 0.98$). Total VFA concentrations and ruminal pH were not affected ($P > 0.60$ and $P > 0.31$, respectively) by treatment. Ruminal ammonia N levels were linearly increased by SBM supplementation ($P < 0.01$) but were not affected by water quality ($P = 0.25$). However, ruminal ammonia tended ($P = 0.09$) to be greater in HS at 0.2% of SBM supplementation. No interaction was observed for plasma urea N (PUN; $P = 0.20$). Plasma urea N was affected by SBM supplementation ($P = 0.05$) and water quality ($P < 0.01$). However, PUN did not differ for 0.4% SBM supplementation ($P = 0.30$) either at LS or HS treatments. In conclusion, a high level of SBM supplementation (0.4% BW) counteracted the detrimental effect of high TDS in drinking water on low-quality forage consumption by cattle.

Key words: beef cattle, digestion, grass hay, intake, saline water, sulfate

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INTRODUCTION

In the arid and semiarid subtropics of Argentina and other parts of the world, saline water (Basán-Nickisch, 2007; FAO, 2007) and low-quality grass during the dry season can markedly reduce beef cattle performance. Protein supplementation to improve low-quality forage utilization by cattle has been well documented (Caton et al., 1988; Guthrie and Wagner, 1988; Cochran et al., 1998). Meanwhile, previous research has also shown the adverse effect of saline water on feed and water intake

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in cattle (Weeth and Hunter, 1971; Loneragan et al., 2001; Grout et al., 2006). However, the effect of the interaction between protein supplementation and saline water on low-quality forage utilization by cattle has not been studied.

When ruminants are fed low-quality forages, the kidneys play a significant role in saving and recycling N. Sheep fed low N diets showed lower plasma filtration (Leng et al., 1985; Cirio and Boivin, 1990) and greater urea reabsorption in distal convoluted tubules of the kidney (Isozaki et al., 1994; Starke et al., 2012). But kidney N reabsorption capacity may be affected by high consumption of salt. Godwin and Williams (1984) and Meintjes and Engelbrecht (2004) observed greater urinary N excretion and lower plasma urea N (**PUN**) in sheep consuming high levels of sodium chloride. Therefore, drinking of saline water, in addition to the depression in feed and water intake, could modify the ability of ruminants to handle situations with N deficient forages. Hence, we hypothesized that protein supplementation needed to maximize low-quality forage utilization is greater in cattle drinking saline water. The objective of this study was to determine the impact of increasing levels of soybean meal (**SBM**) supplementation on intake, digestion, and ruminal fermentation in beef steers fed low-quality forage, when drinking high or low salt water.

MATERIALS AND METHODS

The experimental animals were managed according to the institutional protocols approved by the Instituto Nacional de Tecnología Agropecuaria (National Institute of Agricultural Technology) for Experimental Animal Care and Use (INTA, 2013).

Experimental Design

Experimental treatments were applied according to a 4-period crossover experiment. Ruminally fistulated beef steers ($n = 6$; 375 ± 43 kg BW) were assigned to individual pens (2 by 3.5 m) and fed chopped (40- by 40-mm screen) low-quality grass hay (*Panicum maximum* cultivar Gatton panic; Table 1). Treatments were arranged in a 2 × 3 factorial, which resulted from the combination of 2 levels of salt in the water and 3 levels of SBM. The levels of salt in free-available water were 786 mg/kg of total dissolved solids (**TDS**; low salt water [**LS**]) or 6,473 mg/kg of TDS (high salt water [**HS**]; Table 2), whereas the levels of SBM were 0, 0.2, or 0.4% BW/d (DM basis). Low salt water was obtained from local tap water, whereas HS was artificially prepared by adding sodium chloride (NaCl) and sodium sulfate (Na_2SO_4) salts until a target concentration was reached: 7,000 mg/kg of TDS and 3,000 mg/kg of sulfates. The level of TDS of HS was fixed to reach the threshold by which feed and water intake is limited (NRC, 2000).

Table 1. Grass hay and soybean meal chemical composition

	Grass hay	Soybean meal
Item	% of DM	
OM	87.9	92.7
CP	7.0	45.7
NDF	81.8	28.4
ADF	54.0	11.8
ADIA[1]	4.9	0.4

[1]ADIA = acid detergent insoluble ash.

The basal diet of grass hay was offered once daily (0800 h) at 130% of voluntary intake after the steers consumed all of the SBM. Steers were also fed daily 60 g of a trace mineral–salt mixture (mineral mix: 33% limestone, 1.47% copper sulfate, 0.04% calcium iodate, 0.014% cobalt carbonate, 6.67% magnesium oxide, 7.20% zinc sulfate, 0.014% sodium selenite, 3.88% iron sulfate, 2.67% calcium carbonate), formulated to meet or slightly exceed NRC (2000) recommendations. The mineral mixture was offered mixed with the SBM supplement.

Steers were subjected to each combination of water quality and SBM for four 23-d periods. Days 1 to 15 were for adaptation, 16 to 20 for forage and water intake and digestion measurements, 21 for monitoring ruminal fermentation, 22 for ruminal evacuation, and 23 for blood sampling.

Sampling

Forage and orts samples were collected just before feeding from d 16 to 20 and d 17 to 21, respectively, in each period. Fecal grab samples were collected from each steer every 6 h from d 16 to 18 to determine digestibility of the grass hay by an internal marker (acid detergent insoluble ash [**ADIA**]), advancing the sampling time 4 h each day to minimize concerns about diurnal variation in marker excretion. Composed water samples were collected for each water quality throughout each experimental period. Ruminal fluid samples were collected from each steer at 0, 4, 8, and 12 h after feeding on d 21. Ruminal fluid pH was determined using a portable pH meter with a combination electrode (Orion Research, Boston, MA) immediately following each collection. For ammonia analysis, 2 mL of ruminal fluid from each collection were acidified with 8 mL of 0.1 *N* HCl. Two milliliters of 25% (wt/vol) metaphosporic acid were also combined with 8 mL of ruminal fluid from each collection to be analyzed for VFA. Samples of ruminal fluid were kept frozen until analysis.

On d 22 of each period, ruminal evacuations were performed manually just before (0 h) and 4 h after feeding to determine ruminal liquid and solid contents. Total ruminal contents were weighed, mixed, and sampled in triplicate for determination of moisture and ADIA concentrations. The remainder of the ruminal contents was

Table 2. Total dissolved solids as well as mineral content of drinkable water

	LS[1]	HS
Item	mg/kg	
Total dissolved solids	786	6,473
Calcium	51	144
Magnesium	9	22
Sodium	196	2,026
Sulfate	240	2,890
Carbonate	235	244
Chloride	153	1,512

[1]LS = low salt water; HS = high salt water.

replaced immediately into the rumen of each animal following sample collection.

On d 23 of each experimental period blood samples were collected from the jugular vein into heparinized tubes at approximately 8 h after feeding. Blood samples were placed on ice until the analysis was performed. For collections, steers were sedated with 1 mL of xylazine (100 mg/mL; Sedomin; König, Buenos Aires, Argentina).

Forage intake was calculated by subtracting OM refused from dietary OM offered daily. Water consumption was measured by the daily change in water depth in the tank of each steer. Digestibility was estimated by using ADIA as the internal marker following the procedure described by Cochran and Galyean (1994). Similarly, solid passage rate was estimated by dividing the rate at which the internal marker was consumed (ADIA intake; kg/h) by the amount of internal marker (kg ADIA) in the rumen at each evacuation time.

Laboratory Analysis

Forage, orts, fecal, and ruminal digesta samples were partially dried in a forced-air oven (96 h and 55°C), weighed, and ground (number 4 Wiley Mill; Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm screen. Ground feeds and refusal samples collected were composed on an equal weight basis across days within each water quality and period. Fecal samples were composed across day within steer and period. Ruminal digesta samples were composed by steer within each time period on an equal weight basis. Partially dried ground samples of feed, orts, and feces were dried for 24 h at 105°C for DM determination and then ashed for 3 h at 600°C for ash determination. Feed, orts, and fecal samples were analyzed for NDF and ADF with the ANKOM-Fiber Analyzer 200 (ANKOM Technology, Fairport, NY) using the procedure described by Komarek (1993). Sodium sulfite was used in the NDF analysis. Ruminal contents and fecal samples were analyzed for NDF, ADF, and ADIA using the standard procedures described by Van Soest et al. (1991). The NDF and ADF values reported contain residual ash. Feed samples were analyzed for total N using the procedure of Kjeldahl described by the Association of Official Analytical Chemists (AOAC, 1990). Frozen ruminal fluid samples were thawed at room temperature and centrifuged at 30,000 ×*g* for 10 min at 4° C, and the supernate was decanted to determine ammonia and VFA concentrations. Ruminal ammonia concentration was determined using the colorimetric procedure of Broderick and Kang (1980). Ruminal VFA were measured using a gas chromatograph (HRGC-3000C; Konik Group, Barcelona, Spain) equipped with a Zebron ZB-FFAP Capillary GC Column (15 m × 0.32 mm i.d., 0.25 μm film thickness; Phenomenex, Inc. (Torrance, CA). Oven temperature was programmed at 100°C, hold for 3 min, and increasing at 8°C/min from 100 to 230°C. The carrier gas was N_2 at 1.2 mL/min. Split ratio was 30:1. Plasma urea N concentrations were determined by the Berthelot reaction.

Statistical Analysis

Intake, digestibility, ruminal contents, passage rate and PUN data were analyzed by the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) using the following model:

$$y_{ijkl} = \mu + p_i + s_j + r_k + c_l + (rc)_{kl} + \varepsilon_{ijkl},$$

in which y_{ijkl} is the response for the period i on steer j in SBM level k and water quality l, μ is the overall mean, p_i is a random effect of period i, s_j is a random effect of steer j, r_k is a fixed effect of SBM level k, c_l is a fixed effect of water quality l, $(rc)_{kl}$ is a fixed effect of the interaction between SBM level k with water quality l, and ε_{ijkl} is random error. Treatment means were calculated using the LSMEANS option. Ruminal pH and ruminal concentrations of ammonia and VFA were also analyzed using the MIXED procedure of SAS as repeated measures (Littell et al., 1998). In this case the model of statistical analysis was

$$y_{ijklz} = \mu + p_i + s_j + r_k + c_l + t_z + (rc)_{kl} + (rt)_{kz} + (ct)_{lz} + (rct)_{klz} + \varepsilon_{ijklz},$$

in which y_{ijklz} is the response for the period i on steer j in SBM level k and water quality l at time z, μ is the overall mean, p_i is a random effect of period i, s_j is a random effect of steer j, r_k is a fixed effect of SBM level k, c_l is a fixed effect of water quality treatment l, t_z is a fixed effect of time, $(rc)_{kl}$ is a fixed effect of the interaction between SBM level k with water quality l, $(rt)_{kz}$ is a fixed effect of the interaction between SBM level k with time z, $(ct)_{lz}$ is a fixed effect of the interaction between water quality l with time z, $(rct)_{klz}$ is a fixed effect of the interaction between SBM level treatment k with water quality treatment l and time z, and ε_{ijkl} is random error. Treatment means were calculated using the LSMEANS option.

Table 3. Effects of supplemental soybean meal (SBM; 0, 0.2, and 0.4% BW) and water quality (WQ) on forage OM intake (FOMI), total OM intake (TOMI), NDF intake (NDFI), total tract digestible OM intake (TTDOMI), total tract digestible NDF intake (TTDNDFI), water intake (WI), and water intake to TOMI ratio (WI:TOMI) in beef steers fed low-quality grass hay

	Water quality[1]							*P*-value			
	LS			HS				SBM			
Items	0	0.2	0.4	0	0.2	0.4	SEM	Linear	Quadratic	WQ	SBM × WQ
FOMI, g/kg $BW^{0.75}$	46.0	55.7	55.5	33.0	40.9	48.1	4.07	0.06	0.52	<0.01	0.02
TOMI, g/kg $BW^{0.75}$	46.0	63.4	71.1	33.0	48.6	63.7	3.96	<0.01	0.51	<0.01	0.02
NDFI, g/kg $BW^{0.75}$	38.1	45.8	45.9	27.6	33.9	39.7	3.22	0.06	0.53	<0.01	0.03
TTDOMI, g/kg $BW^{0.75}$	28.5	36.2	43.1	21.1	29.5	40.9	4.50	0.01	0.85	<0.01	0.39
TTDNDFI, g/kg $BW^{0.75}$	27.4	29.2	31.6	19.7	23.3	28.9	3.99	0.09	0.79	<0.01	0.32
WI, g/kg $BW^{0.75}$	250.0	378.5	341.5	156.0	194.7	357.0	26.60	<0.01	0.54	<0.01	<0.01
WI:TOMI, g/g	5.6	6.0	4.8	4.7	4.0	5.6	0.48	0.93	0.66	<0.05	<0.01

[1]LS = low salt water; HS = high salt water.

Orthogonal polynomial contrasts (linear and quadratic) were used to partition treatment sum of squares.

RESULTS

Feed and Water Intake

Supplemental SBM × water quality interactions were significant for most measures of intake, except for total tract digestible OM intake (**TTDOMI**; $P = 0.39$) and total tract digestible NDF intake (**TTDNDFI**; $P = 0.32$). When steers drank LS, forage OM intake (**FOMI**), total OM intake (**TOMI**), NDF intake (**NDFI**), and water intake seemed to reach a plateau with the greatest level of supplemental SBM, whereas when they drank HS, protein supplementation increased all intake estimates (Table 3). Although there was no interaction, TTDOMI linearly increased ($P = 0.01$) and TTDNDFI tended to increase ($P = 0.09$) when low-quality grass hay was supplemented with SBM (Table 3). It is important to note that there were not differences between water quality in TTDOMI ($P = 0.45$) and TTDNDFI ($P = 0.27$) for the highest level of SBM supplementation. Total OM and water intake ($P < 0.01$) as well as TTDNDFI ($P < 0.05$) increased linearly in response to SBM supplementation. Meanwhile FOMI and NDFI tended ($P = 0.06$) to improve with supplemental SBM. Water quality adversely affected the consumption of water ($P < 0.01$) and forage ($P < 0.01$). However, water intake did not differ between LS and HS at the greatest level of SBM supplementation ($P = 0.58$; Table 3). Protein supplementation × water quality interactions were significant for water to TOMI ratio ($P < 0.01$). Water intake: TOMI ratio for HS was lower ($P < 0.05$) than for LS, due to a relatively low water intake of steers drinking HS at the medium level of SBM supplementation ($P < 0.01$).

Total Tract Digestibility

There was no supplemental SBM × water quality interaction for either total tract OM digestibility (**TTOMD**; $P = 0.99$) or total tract NDF digestibility (**TTNDFD**; $P = 0.96$). Supplemental SBM and water quality did not affect either TTOMD ($P = 0.64$ and $P = 0.21$, respectively) or TTNDFD ($P = 0.29$ and $P = 0.31$, respectively; Table 4).

Ruminal Contents and Solid Passage Rate

Soybean meal supplementation × water quality interaction did not affect ruminal DM content ($P = 0.34$) or passage rate ($P = 0.38$; Table 4). Ruminal DM content was affected by water quality ($P < 0.01$) with values being lower for HS treatments (Table 4). Passage rate increased linearly ($P < 0.01$) in response to SBM supplementation, although it was not affected by water quality ($P = 0.98$).

Ruminal Fermentation Profile

Treatments did not affect either total VFA concentration or molar proportions, except for propionate and butyrate (Table 5). The former decreased ($P < 0.05$) and the latter increased ($P = 0.05$) with increasing supplemental SBM. High salt water tended to increase molar proportion of propionate ($P < 0.08$). Ruminal pH was affected by sampling time ($P < 0.01$) only. The lowest pH was reached between 8 and 12 h after feeding. There was a SBM supplementation ×water quality × time interaction ($P < 0.05$) for ruminal ammonia. The interaction was primarily due to greater ammonia concentration at 8 ($P < 0.01$) and 12 ($P < 0.05$) h after feeding. Ruminal ammonia concentration increased linearly ($P < 0.01$) in response to supplemental SBM, whereas it tended to be greater ($P = 0.09$) for HS than for LS at 0.2% of SBM (Fig. 1).

Table 4. Effects of supplemental soybean meal (SBM) and water quality (WQ) on total tract OM digestibility (TTOMD), total tract NDF digestibility (TTNDFD), ruminal content, and passage rate in beef steers fed low-quality grass hay

	Water quality[1]							*P*-value			
	LS			HS				SBM			
Items	0	0.2	0.4	0	0.2	0.4	SEM	Linear	Quadratic	WQ	SBM × WQ
TTOMD, %	60.7	56.3	60.4	64.6	60.6	64.3	5.46	0.95	0.39	0.21	0.99
TTNDFD, %	63.0	52.9	55.9	65.5	57.6	58.7	5.65	0.25	0.28	0.31	0.96
Ruminal content, g/kg $BW^{0.75}$	105.9	101.7	92.3	99.1	84.0	85.1	7.68	0.24	0.76	<0.01	0.34
Passage rate, %/h	1.90	2.40	3.40	1.22	2.85	3.60	<0.01	<0.01	0.79	0.98	0.38

[1]LS = low salt water; HS = high salt water.

Plasma Urea Nitrogen

Soybean meal supplementation × water quality interaction was not present for PUN ($P = 0.20$). Plasma urea N linearly increased in response to SBM supplementation ($P < 0.05$; Table 5), whereas HS increased PUN compared with LS ($P < 0.01$). Plasma urea N was ($P < 0.01$) or tended ($P = 0.06$) to be greater for HS compared with LS for the first and second level of supplemental SBM, respectively, whereas it was not affected by water quality at 0.4% of SBM supplementation ($P = 0.30$).

DISCUSSION

The major aim of this experiment was to investigate the potential for an interaction between protein supplementation (i.e., SBM level) and water quality (i.e., LS vs. HS) relative to their effect on low-quality forage and water intake as well as ruminal fermentation and total tract digestion. Significant interactions between SBM and water quality were observed for FOMI, TOMI, and NDFI, indicating that water quality differentially affects the response to protein supplementation. Additionally, protein supplementation and water quality also exerted an independent impact on feed and water intake such as was observed in previous works. In general, providing supplemental protein to cattle consuming low and medium quality grass hay improves forage utilization (Köster et al., 1996; Bandyk et al., 2001; Wickersham et al., 2004). On the other hand, high concentrations of salts in drinking water (especially sulfates) reduce feed and water intake in cattle (Weeth and Hunter, 1971; Harper et al., 1997; Loneragan et al., 2001). In general, in our experiment we observed a similar response (i.e., 20%) in forage intake independent of water quality when SBM supplementation increased from 0.0 to 0.2% BW. At greater levels of supplementation (0.4% BW), forage intake seemed to reach a plateau in LS, whereas it kept increasing at the same pace in steers consuming HS. In fact, forage intake increased almost 40% when it was supplemented with the greatest level of SBM in steers drinking HS. The plateau observed in LS was an expected response because we selected SBM levels above ruminal degradable protein (**RDP**) requirements to maximize forage utilization. Mathis et al. (1999) observed a plateau for FOMI when SBM was fed at 0.16% BW to beef cows consuming low-quality forage.

Protein supplementation has been observed to stimulate low-quality forage intake basically by increasing digestion and passage rate. Wickersham et al. (2004) observed that TTOMD as well as TTNDFD and rate of passage increased linearly when RDP was supplemented to steers fed low-quality grass hay. In our study, increased forage intake in response to SBM supplementation is largely explained by an increase in rate of passage, because there were no differences in total tract digestibility or ruminal contents. The lack of effect on TTOMD and TTNDFD may have been due to the fact that the increase in passage rate was probably similar to the increase in digestion rate, to the quality of the basal forage used, or to a combination of both. The response of fiber digestion to protein supplementation is somewhat variable in the literature. Mathis et al. (2000) conducted 3 experiments in which they assessed the effects of supplemental RDP on intake of medium to low-quality forages by beef steers. They observed effects of protein supplementation on FOMI, TOMI, and TTOMD when forage CP was 4.3%, whereas when forage CP was greater (i.e., 5.9 or 8.1%), they did not observe any response to RDP supplementation. A range of 6 to 8% CP in the basal forage is considered to be the threshold for a response to protein supplementation (DelCurto et al., 2000). In our study, forage CP (7.0%) and NDF (81.8%) were greater than those in forages used in other experiments (Köster et al., 1996; Mathis et al., 1999; Bandyk et al., 2001). Therefore, in our study it appears that ruminal N availability may not have been limiting fiber digestion. In their studies with low-quality forages, Köster et al. (1996), Mathis et al. (1999), and Bandyk et al. (2001) reported ruminal ammonia values lower than 0.7 m*M* when no protein was supplemented, while we measured 2.55 m*M* in the treatment with 0% SBM. On the other hand, the increase in the rate of passage in our experiment was similar to

Table 5. Effects of increasing amount of soybean meal (SBM) and water quality (WQ) on ruminal pH, ruminal ammonia, plasma urea nitrogen, total ruminal VFA concentration, and proportions in beef steers fed low-quality grass hay

	Water quality[1]							*P*-value			
	LS			HS				SBM			
Item	0	0.2	0.4	0	0.2	0.4	SEM	Linear	Quadratic	WQ	SBM × WQ
pH	6.92	6.67	6.57	6.73	6.67	6.63	0.09	0.16	0.70	0.32	0.30
Ammonia N, m*M*	1.70	7.75	15.48	3.39	12.28	14.03	1.66	<0.01	0.34	0.25	0.23
PUN,[2] mg/dL	20.5	28.5	38.5	31.0	34.3	41.5	5.2	0.02	0.64	<0.01	0.20
Total VFA, m*M*	99.7	116.5	112.6	105.5	100.1	110.1	9.4	0.43	0.89	0.60	0.54
Percentage of ruminal VFA,[3] mol/100 mol											
ACE	81.5	81.9	81.2	81.0	81.5	81.4	0.4	0.93	0.16	0.35	0.47
PROP	13.9	13.4	13.4	14.5	13.6	13.8	0.4	0.03	0.06	0.08	0.78
BUT	3.40	3.64	3.99	3.43	3.65	3.57	0.12	0.05	0.67	0.32	0.29
ISOBUT	0.43	0.42	0.55	0.43	0.48	0.47	0.06	0.12	0.58	0.81	0.34
VAL	0.24	0.21	0.28	0.18	0.27	0.22	0.03	0.25	0.81	0.46	0.24
ISOVAL	0.44	0.48	0.57	0.40	0.56	0.54	0.07	0.12	0.61	0.94	0.68

[1]LS = low salt water; HS = high salt water.

[2]PUN = plasma urea N.

[3]VFA: ACE = acetate; PROP = propionate; BUT = butyrate, ISOBUT = isobutyrate; VAL = valerate; ISOVAL = isovalerate.

those reported by other researchers (Guthrie and Wagner, 1988; Bodine et al., 2000; Wickersham et al., 2004). We observed a marked increase in the rate of passage in response to increasing level of SBM supplementation, which would partially explain the concurrent increase in forage intake. Egan and Moir (1965) stated that protein supplementation stimulates passage rate and intake by increasing gastrointestinal motility. However, passage rate was not affected by water quality, so it does not seem to explain the interaction between FOMI, TOMI, and NDFI with water quality. On the other hand, it is important to note that even though there was no interaction between either TTDOMI or TTDNDFI and water quality, no differences were observed between LS and HS in TTDOMI or TTDNDFI at the greatest level of SBM supplementation. The greater proportional increases in TTDOMI compared to TOMI observed in this study seems to have been due to SBM supply per se rather than a combined effect with TTOMD. The addition of SBM did not increase TTDNDFI in LS but did in HS (approximately 47% compared to the control). But the potential to stimulate OM intake via RDP has limits (Mathis et al., 1999; Moore et al., 1999). Köster et al. (1996) observed that FOMI increased quadratically with increasing protein supplementation of low-quality forage. They concluded that TTDOMI was maximized when RDP to TTDOMI ratio was about 11%, although RDP requirements vary with inherent characteristics of forage. Therefore, it is generally accepted that RDP should fall in the range of 7 to 13% of the organic matter digestibility OMD (Hollingsworth-Jenkins et al., 1996; Cochran et al., 1998) to achieve maximum forage utilization. In the present study, the lowest content of RDP was about 6% of TTDOMI. Therefore, our results suggest that saline water might alter RDP's requirements to maximize forage utilization. However, it is unlikely that these levels of ruminally available N had not been enough to meet the needs of ruminal microorganisms. Ruminal ammonia concentration and RDP to TTDOMI ratio were far above the values proposed by other authors (Cochran et al., 1998; Satter and Slyter, 1974; Hollingsworth-Jenkins et al., 1996). We have calculated RDP to TTDOMI ratios of 13 and 18% for 0.2 and 0.4% BW of SBM supplementation, respectively.

An important factor not taken into account in studies of protein supplementation of low quality forages is water consumption. Because of the close relationship between the consumption of water and food, it is reasonable to expect a decrease in DMI with water intake restriction (Silanikove, 1992). Some research indicates that restrictions greater than 30% in water intake decrease voluntary DMI (Weeth et al., 1968; Utley et al., 1970; Burgos et al., 2001). High amounts of sulfate reduces water intake and hence the consumption of food (Weeth and Hunter, 1971; Digesti and Weeth, 1976; Grout et al., 2006). Patterson et al. (2003) conducted a study in which food and water intake of growing steers decreased quadratically and linearly, respectively, in response to increases TDS and sulfates concentration in drinking water. They concluded that water with 7,000 mg/kg TDS and 4,600 mg/kg sulfates reduce animal health and performance. Other factors such as digestion and renal protein excretion may also be affected by water restriction (Weeth et al., 1970; Van der Walt et al., 1999; Burgos et al., 2001). Similarly to Weeth and Hunter (1971) and Grout et al. (2006), we observed a reduction of 40 and 28% in water and TOMI, respectively, in HS without SBM relative to LS without SBM supplementation. However, at the great-

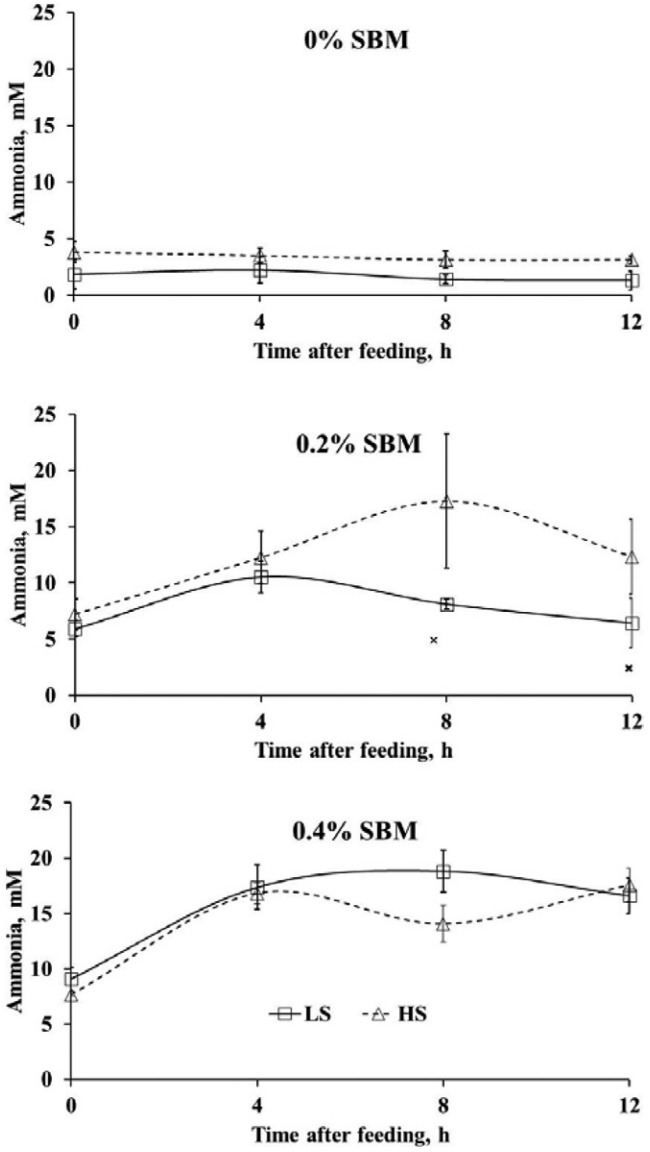

Figure 1. Effect of water quality on ruminal ammonia concentration after feeding for 3 levels of soybean meal (SBM) supplementation: 0, 0.2, and 0.4% BW/d. Effects: SBM linear, $P < 0.01$; SBM quadratic, $P = 0.34$; water, $P = 0.25$; SBM × water, $P = 0.23$; and SBM × water × Time, $P < 0.05$. "x" indicates significant difference ($P < 0.05$) between low salt water (LS) and high salt water (HS) for each sampling time within level of SDM supplementation. Error bars represent ±1 SEM.

est level of SBM supplementation, there were no differences in water intake between steers drinking HS or LS. Increasing protein content of the diet has been reported to elicit water intake (Holter and Urban, 1992; NRC, 2000). According to Godwin and Williams (1984), total water intake shows a curvilinear increase with increasing nitrogen intake. These authors suggested that urine production is increased in response to increasing N intake. This may be due to the diuretic effect of increased protein intake (Dinn et al., 1998) through an increased glomerular filtration rate (Eriksson and Valtonen, 1982; Godwin and Williams, 1984). Results of our experiment suggest that high levels of protein supplementation can, to some extent, reverse the effect of TDS and sulfates on water intake. As water intake:TOMI ratio remained constant, increases in water intake resulted in an increase in TOMI. These results support Wilson and Hindley (1970) and Wilson and Dudzinski (1973) studies, in which they suggested that the reduction in DMI is attributable to low consumption of HS instead of a lower salt tolerance. Wilson and Dudzinski (1973) conducted a study in which they assessed the influence of not only the concentration but also the volume of saline water (1.5 and 2% sodium chloride) consumed on food intake by sheep. Saline water decreased food intake, except when animals increased saline water consumption. In our study, increases in saline water and sulfates intake did not alter animal health. Waters with high sulfates content have the potential to generate polioencephalomalacia (**PEM**; McAllister et al., 1997), due to the production of a toxic gas (hydrogen sulfide [H_2S]) from the reduction of sulfates by ruminal bacteria. However, we did not observe any symptoms characteristics of PEM in the present study. One possible explanation may be that H_2S production had not been high enough to induce PEM. The amount of gas in the rumen increases with diets high in nonfiber carbohydrates and low in fiber (Gould et al., 1997; Gould, 2000), where ruminal pH is generally lower. We recorded pH > 6.60, which may have inhibited the formation H_2S from sulfide decreasing the accumulation of gas in the rumen (Richter, 2011).

The lack of response in total VFA concentration in our study may have been due to the fact that TTOMD was not affected by increasing levels of SBM supplementation (McCollum and Galyean, 1985; DelCurto et al., 1990).

Ruminal ammonia levels and PUN increased linearly with increasing SBM supplementation, which is in concordance with the literature (Bodine et al., 2000; Bandyk et al., 2001; Muscher et al., 2010). However, some important considerations should be taken into account with respect to the effect of water quality. Although ruminal ammonia concentration was not affected by water quality, PUN was increased. When the animal body needs to save water, urea reabsorption in the kidney increases. Reduction in the amount of urea loss through urine was observed when water intake was restricted (Leng and Szanyiová, 1987; Van der Walt et al., 1999). Previous works highlighted the importance of the kidney as a mechanism to conserve N (Leng and Szanyiová, 1987; Faix et al., 1988; Marini et al., 2004). In a recent experiment, Starke et al. (2012) observed that N reabsorption in goat increased in response to a low protein diet by an increase in the expression of specialized urea transporters localized in the kidney. In our study, the high concentration of PUN in HS may have been due to restriction of water intake, which resulted in a lower removal of urea through the urine. However, when water intake did not

differ (at the greatest level of SBM), PUN was similar between LS and HS. The level of PUN is an important factor in determining the amount of urea recycled to the rumen (NRC, 2000; Wickersham et al., 2008). At ruminal ammonia concentration lower than 4 m*M*, PUN is positively correlated with the amount the urea-N recycled to the rumen (Lapierre and Lobley, 2001). Previous work has demonstrated the importance of N recycling when low-quality forage is fed (Wickersham et al., 2008, 2009). Bandyk et al. (2001) conducted an experiment in which assessed the effects of ruminal vs. postruminal administration of casein on utilization of low-quality forages in beef steers. They attributed the improved forage utilization to recycling of postruminal infused protein. In our study, although N recycling was not measured, the greater concentration of ruminal ammonia observed for HS over LS both at 0.2% SBM may have been as a result of recycling mechanisms. Because of the importance of urea recycling in providing ruminally available N in animals consuming low quality forages and the potential effect that HS has on the retention of N in the kidney, this issue requires more investigation in the future.

According to our results, high levels of SBM supplementation (0.4% BW) counteracted the detrimental effect of saline water on low-quality forage consumption by cattle, thereby showing the existence of an interaction between water quality and supplemental protein for total and forage intake. However, such an interaction was not evident for digestible OM intake. Observed values of PUN suggest that high levels of salt in water decreases urinary N excretion, which may increase N recycling to the rumen. However, the mechanism by which protein supplementation could alleviate the negative effects of salty water remains unclear and will be an objective of future research directions.

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