Published May 5, 2017

High-sulfate water consumption determines intake and metabolic responses to protein supplementation in lambs consuming low-quality forage[1]

A. López,*† J. I. Arroquy,*†‡[2] A. V. Juárez Sequeira,†‡ N. DiLorenzo,§ M. C. Barrionuevo,† and R. A. Distel‡#

*Estación Experimental Agropecuaria Santiago del Estero, Instituto Nacional de Tecnología Agropecuaria, Jujuy 850, Santiago del Estero, Argentina; †Facultad de Agronomía y Agroindustria, Universidad Nacional de Santiago del Estero, Av. Belgrano (s) 1912, Santiago del Estero, Argentina; ‡Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina; §North Florida Research and Education Center, University of Florida, Marianna 32351; and #Departamento de Agronomía, Universidad Nacional del Sur, San Andrés 800, Bahía Blanca, Argentina

ABSTRACT: Twenty Hampshire lambs (31 ± 4 kg BW) in individual metabolism cages were used in a 10-treatment by 2-period ($n = 4$) trial to evaluate the interaction between protein supplementation and sulfate water on intake and metabolic responses when lambs were fed low-quality grass hay (*Megathyrsus maximus*; 6.4% CP, 79.5% NDF). The treatment structure was a 2 × 5 factorial: 2 water qualities (WQ; low-sulfate [LS] and high-sulfate [HS]; 442 and 8,358 mg/kg total dissolved solids, respectively) and 5 soybean meal levels (SBM; 0%, 0.25%, 0.50%, 0.75%, and 1.00% BW/d). After 15 d of adaptation, periods consisted of 5 d for determination of forage and water intake, nitrogen balance, and digestion measurements (d 16 to 20) and 1 d for blood sampling and determination of ruminal hydrogen sulfide (H_2S) concentration (d 21). Supplemental SBM × WQ interactions were significant for forage OM intake ($P = 0.04$) and total OM intake ($P = 0.04$), whereas a tendency was observed for total tract digestible OM intake ($P = 0.07$). Intake values of LS lambs were higher than those of HS lambs ($P < 0.05$) in only the first and second levels of SBM. Water intake increased linearly ($P < 0.01$) with SBM level but was not affected by WQ ($P = 0.39$). Water quality and SBM supplementation affected total tract OM digestibility (TTOMD; $P < 0.01$); LS lambs had lower TTOMD than HS lambs ($P < 0.01$). Plasma urea N increased linearly in response to SBM ($P < 0.01$) but was not affected by WQ ($P = 0.11$). Nitrogen balance was not affected by SBM × WQ interaction ($P > 0.12$), except for N utilization (N retained/N intake ratio; $P < 0.01$). Regardless of WQ, N intake ($P > 0.01$), N urine ($P > 0.01$), and N balance increased linearly ($P > 0.01$) with SBM level. Water quality adversely affected N intake and N balance, although at the highest level of SBM no differences in N balance were observed between LS and HS lambs ($P = 0.85$). No changes due to WQ were observed for either urea reabsorbed by kidneys ($P = 0.63$) or glomerular filtration rate ($P = 0.30$), but renal function was affected by SBM level ($P < 0.01$). There was a supplemental SBM × WQ interaction for ruminal H_2S concentration ($P < 0.01$) due mainly to a greater concentration from 0.25% BW SBM in HS than in LS lambs. In conclusion, these results confirmed the existence of an interaction between sulfate water and supplemental protein, which alters intake and metabolic responses when lambs are fed low-quality grass hay.

Key words: lamb, low-quality forage, nitrogen balance, sulfate, supplementation, water

J. Anim. Sci. 2017.95:2111–2120
doi:10.2527/jas2016.1264

INTRODUCTION

Low-quality forages are an important resource to sustain production of ruminants in the world, so much effort has been devoted to supplementation strategies that optimize their utilization (Cochran et al., 1998; Salisbury et al., 2004; Atkinson et al., 2010). Protein

[1]This study was conducted with funding from the Instituto Nacional de Tecnología Agropecuaria (INTA)–Programa Nacional de Producción Animal–Proyecto Alimentación de Bovinos para Carne–PNPA number 1126023, Argentina. The authors thank to Hector Miguel Fissolo (animal care and sampling) for his contributions to this study.

[2]Corresponding author: arroquy.jose@inta.gob.ar

Received December 2, 2016.
Accepted March 11, 2017.

supplementation often improves consumption and N retention in animals fed low-quality forages (Köster et al., 1996; Mathis et al., 2000; Wickersham et al., 2008b). However, the ingestion of large amounts of salt through drinking water can alter the pattern of response to protein supplementation when ruminants are fed low-quality forages. Recently, López et al. (2014) observed an interaction between protein supplementation and saline water on consumption of low-quality hay by steers, in which the level of soybean meal supplementation to reach an equivalent level of total OM intake was higher in animals drinking high-salt water compared to those drinking low-salt water.

Ruminants have efficient nitrogen-saving mechanisms to deal with shortage in dietary protein (Marini and Van Amburgh, 2003; Wickersham et al., 2004), in which kidneys play a critical role. Reductions in glomerular filtration rate and in excretion of urea N have been observed in animals fed low-protein diets (Ergene and Pickering, 1978; Tebot et al., 1998; Starke et al., 2012). However, renal hemodynamics can be modified not only by the level of dietary protein but also by the consumption of salts, altering the ability of the kidney to conserve urea N. Previous work has demonstrated that high salt intake alters nitrogen utilization by ruminants, increasing urinary N excretion and decreasing plasma urea N (Godwin and Williams, 1984; Meintjes and Engelbrecht, 2004). However, there is still a scarcity of information on potential mechanisms underlying the interaction between protein supplementation and water sulfate concentration on low-quality forage utilization.

A better understanding of the interaction between protein supplementation and water sulfates on low-quality forage consumption will improve the predictability of response to protein supplementation. Surface water and groundwater that contain sulfate in excess are common across the world and represent a major problem in livestock production systems (Weeth and Hunter, 1971), such as in arid and semiarid regions of Argentina (Basán Nickisch, 2007). Therefore, the present study was designed to determine the impact of soybean meal supplementation on intake, digestion, N balance, and renal function of lambs fed low-quality forage when drinking high- or low-sulfate water.

MATERIALS AND METHODS

The experiment was performed following the protocol approved by the Instituto Nacional de Tecnología Agropecuaria (2013) for experimental animal care and use.

Experimental Design

Twenty Hampshire lambs (31 ± 4 kg BW) housed in individual metabolism cages indoors were used in a 10-treatment by 2-period trial. The treatment structure was a 2 × 5 factorial, which resulted from the combination of 2 water qualities (**WQ**) and 5 levels of soybean meal (**SBM**). Water quality levels were represented by low-sulfate (**LS**) and high-sulfate (**HS**) water containing 442 and 8,358 mg/kg of total dissolved solids, respectively (Table 1). Low-sulfate water was obtained from local tap water, whereas HS was artificially prepared by adding sodium sulfate (Na_2SO_4) salt up to the target concentration. The levels of SBM were 0%, 0.25%, 0.50%, 0.75%, and 1.00% BW/d (DM basis).

Lambs were fed low-quality grass hay (*Megathyrsus maximus* cv. Gatton panic; Table 2) once daily (0700 h) at 130% of voluntary intake after all animals had consumed the SBM. Each lamb was also offered daily 6 g of a mineral mixture (composition: 27.67% calcium, 9.26% sodium, 0.62% magnesium, 0.31% phosphorus, 2,592.5 mg/kg zinc, 2,037.0 mg/kg manganese, 555.5 mg/kg copper, 30.8 mg/kg iodine, 18.5 mg/kg selenium, 6.17 mg/kg cobalt, and 0.22% monensin; RTC, Buenos Aires, Argentina). Individual water tanks of lambs were refilled each day at feeding time.

Each experimental period lasted 21 d. The first 15 d were for adapting animals to experimental conditions; d 16 to 20 were for forage and water intake, nitrogen balance, and digestion measurements, and d 21 was for blood sampling and ruminal gas sampling (only experimental period 2).

Sampling

In both experimental periods, from d 16 to 21 forage and ort samples were collected daily from each lamb just before feeding. Similarly, total fecal and total urine production were weighed daily before 0800 h. Urine was collected in a bucket containing enough 1.25 *N* HCl to keep pH below 3 to minimize N loss between collection times. Immediately following each collection, a portable pH meter multiparameter (Oakton, Vernon Hills, IL) was used to measure urine pH. Fecal (10% of daily output) and urine (5% of daily output) samples were retained and stored at −20°C until analysis. Composited water samples were also collected for each WQ and for each experimental period.

Blood samples were taken on d 21 of each experimental period at 0 and 8 h after feeding. Blood samples were collected from the jugular vein into heparinized tubes, immediately cooled on ice, and analyzed within 6 h of collection. Additionally, in period 2 a 20-mL ruminal gas sample was collected by puncture 8 h after feeding according to the technique used by

Table 1. Total dissolved solids and mineral content of drinking water

Item	LS,[1] mg/kg	HS,[1] mg/kg
Total dissolved solids	442	8358
Calcium	36	83
Magnesium	11	21
Sodium	96	3843
Sulfate	108	6363
Carbonate	207	366
Chloride	69	138

[1]LS = low-sulfate water, HS = high-sulfate water.

Gould et al. (1997), except a gas sample was extracted with a syringe and 10 mL of ruminal gas (in duplicate) were placed into alkaline water in Vacutainer for further analysis within 2 h of collection.

Laboratory Analyses and Measurements

Collected samples of feed, orts, feces, and urine were composited across days within lamb for each period. Feed, ort, and fecal samples were partially dried in a forced-air oven (55°C for 96 h), weighed, and ground (number 4 Wiley Mill; Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm screen. Partially dried ground samples of feed, orts, and feces were further dried for 24 h at 105°C for DM determination and then ashed for 3 h at 600°C for ash determination. Feed, ort, and fecal samples were analyzed for NDF and ADF with an ANKOM Fiber Analyzer 200 (ANKOM Technology, Fairport, NY) using the procedure described by Komarek (1993). Sodium sulfite was used in the NDF analysis. The NDF and ADF values reported contain residual ash. Feed, fecal, and urine samples were analyzed for total N using the procedure of Kjeldahl described by the Association of Official Analytical Chemists (1990).

The concentration of creatinine in urine and plasma and the concentration of urinary urea were determined by a kinetic method using a commercial kit (Wiener Lab S.A.I.C., Rosario, Argentina) and an automatic analyzer (BS-300 Chemistry Analyzer, Mindray, Shenzhen, China). Plasma urea N (**PUN**) concentration was analyzed by an enzymatic method kit (GT Lab, Rosario, Argentina) using a spectrophotometer (Metrolab 330, Metrolab UV Vis, Buenos Ares, Argentina). Ruminal H_2S concentration was measured spectrophotometrically (Cole-Parmer 1200, Cole-Parmer,. Vernon Hills, IL) by following the procedure provided by Leibovich et al. (2009).

Forage intake of each lamb was calculated daily by subtracting refused OM from offered OM, whereas daily water consumption was measured by subtracting refused water from offered water and adjusting by evaporative losses. Intake and total fecal output data were used to calculate the apparent digestibility of the low-quality grass hay (*Megathyrsus maximus* cv. Gatton panic), whereas nitrogen intake and nitrogen excreted through feces and urine were used to estimate nitrogen balance. Glomerular filtration rate (**GFR**) was estimated from the creatinine clearance on the basis of calculations from Hall (2010). Urea reabsorption was estimated by the difference between urea filtered by kidney and urea excreted in the urine. Urea filtered (urea tubular load) was calculated by multiplying PUN by GFR.

Table 2. Grass hay and soybean meal chemical composition

Item	Grass hay, % DM	Soybean meal, % DM
OM	89.7	94.0
CP	6.4	45.9
NDF	79.5	12.8
ADF	54.3	7.8

Statistical Analysis

Except for ruminal H_2S concentration, all data were analyzed with mixed models in InfoStat software (Di Rienzo et al., 2014) by using the following general model:

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

where y_{ijkl} is the response for period i on lamb j in SBM level k and WQ l, μ is the overall mean, p_i is a fixed effect of period i, s_j is a random effect of lamb j (nested within SBM), r_k is a fixed effect of SBM level k, c_l is a fixed effect of WQ l, $(rc)_{kl}$ is a fixed effect of the interaction between SBM level k with WQ l, and ε_{ijkl} is the random error.

Ruminal H_2S concentration was analyzed as a completely randomized factorial design using InfoStat software (Di Rienzo et al., 2014). In this case the model for statistical analysis was

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

where y_{kl} is the response on H_2S concentration in SBM level k and WQ l, μ is the overall mean, r_k is a fixed effect of SBM level k, c_l is a fixed effect of WQ l, $(rc)_{kl}$ is a fixed effect of the interaction between SBM level k with WQ l, and ε_{kl} is the random error.

Linear, quadratic, and cubic orthogonal polynomial contrasts were used to partition the treatment sum of squares; the overall means obtained were assessed for significant differences at $P < 0.05$ using Fisher's test, with tendencies associated with *P*-values between 0.05 and 0.10.

RESULTS

Feed and Water Intake

Supplemental SBM × WQ interactions were present for forage OM intake (**FOMI;** $P = 0.04$), total OM intake (**TOMI;** $P = 0.04$), NDF intake (**NDFI;** $P = 0.04$), and total tract digestible NDF intake (**TTDNDFI;** $P = 0.03$), whereas there was only a tendency for total tract digestible OM intake (**TTDOMI;** $P = 0.07$; Table 3). These interactions were manifested at the lower levels of SBM supplementation (0% and 0.25% BW/d). When lambs drank LS water, FOMI and TTDNDFI followed a decreasing linear trend ($P = 0.06$ and $P < 0.05$, respectively) with increasing levels of SBM supplementation, whereas TOMI was not affected by SMB supplementation. Conversely, FOMI ($P = 0.90$) and TTDNDFI ($P = 0.79$) were not affected, whereas TOMI linearly increased ($P < 0.01$) with the addition of SBM when lambs drank HS water. It is important to note that although TTDOMI increased linearly ($P < 0.05$) with increasing levels of SBM supplementation in both WQ treatments, lambs that drank LS water had greater ($P < 0.05$) TTDOMI than lambs that drank HS water in only the first and second levels of supplemental SBM (0% and 0.25% BW/d). There was no SBM × WQ interaction for water intake (**WI;** $P = 0.60$), which tended to respond to increasing level of SBM ($P = 0.07$) in linear fashion ($P < 0.01$) but was not affected by WQ ($P = 0.39$).

Total Tract Digestibility

There were no supplemental SBM × WQ interactions for all of the digestion measures ($P > 0.52$; Table 3). Water quality and supplemental SBM affected total tract OM digestibility (**TTOMD**; $P < 0.01$ and $P = 0.01$, respectively) but had no effect on total tract NDF digestibility (**TTNDFD**; $P = 0.10$ and $P = 0.22$, respectively). Digestion of OM tended to be ($P = 0.09$) and was greater ($P < 0.05$) in HS lambs than in LS lambs for 0.50% and 0.75% BW/d of SBM, respectively. Protein supplementation linearly increased TTOMD ($P < 0.01$).

Nitrogen Balance

The measures of N balance were not affected by supplemental SBM × WQ interaction ($P > 0.12$), except for N retention as a percentage of total N intake (**N retained/N intake**; $P < 0.01$; Table 4). High-sulfate water treatment adversely affected N intake ($P < 0.01$), with lower fecal N excretion ($P < 0.01$) and N balance ($P < 0.01$). In contrast, urinary N excretion was less ($P = 0.03$) in LS lambs than in HS lambs. Nitrogen intake ($P < 0.01$), N urine ($P < 0.01$), and N balance ($P < 0.01$) lin-

Table 3. Effects of soybean meal supplementation (SBM) and water quality (WQ) on water intake (WI), forage OM intake (FOMI), total OM intake (TOMI), NDF intake (NDFI), total tract digestible OM intake (TTDOMI), total tract digestible NDF intake (TTDNDFI), total tract OM digestibility (TTOMD), and total tract NDF digestibility (TTNDFD) in lambs fed low-quality grass hay

	WQ[1]										*P*-values					
	LS				HS									SBM[3]		
	SBM[2]				SBM[2]											
Item	0	0.25	0.50	0.75	1.00	0	0.25	0.50	0.75	1.00	SEM	WQ	SBM	WQ × SBM	L	Q	C
WI, g/kg $BW^{0.75}$	153.0	147.1	157.9	173.8	194.6	141.5	123.9	159.8	170.5	202.2	16.42	0.39	0.07	0.60	<0.01	0.25	0.64
FOMI, g/kg $BW^{0.75}$	37.9	37.1	33.2	29.1	27.7	23.9	26.6	28.9	25.7	25.3	4.49	<0.01	0.86	0.04			
TOMI, g/kg $BW^{0.75}$	37.9	41.7	42.4	43.3	45.6	23.9	31.6	38.0	39.4	43.5	4.47	<0.01	0.25	0.04			
NDFI, g/kg $BW^{0.75}$	34.0	33.9	31.1	28.3	27.4	21.6	24.6	27.3	25.2	25.4	3.85	<0.01	0.97	0.04			
TTDOMI, g/kg $BW^{0.75}$	19.4	22.4	22.0	25.5	28.6	13.6	17.7	21.5	26.3	27.5	2.32	0.01	0.01	0.07	<0.01	0.98	0.97
TTDNDFI, g/kg $BW^{0.75}$	19.4	17.7	14.6	14.2	13.7	12.9	13.0	14.1	14.7	12.9	2.06	<0.01	0.86	0.03			
TTOMD, %	50.0	53.0	51.2	60.6	62.2	53.1	56.1	56.3	67.6	63.6	2.98	<0.01	0.01	0.69	<0.01	0.79	0.36
TTNDFD,%	56.1	52.0	46.4	50.9	48.2	56.2	51.9	51.3	58.9	50.0	3.21	0.10	0.22	0.52	0.19	0.56	0.14

[1]LS = low-sulfate water; HS = high-sulfate water.

[2]Supplemental amount of soybean meal as a percentage of BW per day.

[3]Main effect contrasts across supplemental amount of SBM: L = linear; Q = quadratic; C = cubic.

Table 4. Effects of soybean meal supplementation (SBM) and water quality (WQ) on daily intake of N, fecal and urinary excretion of N, and balance of N in lambs fed low-quality grass hay

	WQ[1]											*P*-values					
	LS				HS										SBM[3]		
	SBM[2]				SBM[2]												
Item	0	0.25	0.50	0.75	1.00	0	0.25	0.50	0.75	1.00	SEM	WQ	SBM	WQ × SBM	L	Q	C
N intake, g/d	5.1	10.7	14.5	19.7	22.4	3.0	9.3	13.3	18.4	22.1	1.23	<0.01	<0.01	0.45	<0.01	0.35	0.99
N fecal, g/d	2.7	3.5	3.8	3.5	3.4	1.6	2.4	3.0	2.8	3.3	0.45	<0.01	0.26	0.12	0.08	0.21	0.51
N urine, g/d	4.1	6.6	9.6	12.4	14.8	4.8	6.8	10.5	14.2	14.8	0.86	0.03	<0.01	0.39	<0.01	0.58	0.26
N balance, g/d	−1.7	0.5	1.1	3.8	4.2	−3.4	0.1	−0.3	1.4	4.0	0.80	<0.01	<0.01	0.32	<0.01	0.83	0.36
N retained/N intake, %	−49.1	0.0	6.8	19.2	18.9	−104.1	−2.9	−2.3	6.7	18.2	13.12	<0.01	<0.01	<0.01			

[1]LS = low-sulfate water; HS = high-sulfate water.

[2]Supplemental amount of soybean meal as a percentage of BW per day.

[3]Main effect contrasts across supplemental amount of SBM: L = linear; Q = quadratic; C = cubic.

early increased as the level of SBM supplementation increased, but the N excreted in the feces was not affected ($P = 0.26$). Importantly, N balance was lower in HS than LS lambs without SBM supplementation (−3.4 and −1.7, respectively; $P = 0.05$), although it did not differ between LS and HS lambs at the highest level of SBM supplementation (4.0 and 4.2, respectively; $P = 0.85$). Although N retained/N intake responded linearly ($P < 0.01$) with a quadratic trend ($P = 0.08$) in LS lambs and in a cubic relation in HS lambs ($P = 0.03$) to increasing level of supplemental SBM, the greatest response occurred with the first increment in SBM supplementation (0.25% BW/d).

Plasma Urea Nitrogen

There was no supplemental SBM × WQ interaction for PUN ($P = 0.38$; Table 5). Water quality had no effect on PUN ($P = 0.11$), whereas it linearly increased as the level of SBM supplementation increased ($P < 0.01$).

Urine Output and Renal Function

With the exception of urinary urea concentration (**UUC**; $P < 0.03$), supplemental SBM × WQ interactions did not affect urine output ($P = 0.25$), urinary urea as a percentage of the total urinary nitrogen (**UUN/UN**; $P = 0.86$), urea reabsorbed by the kidneys ($P = 0.61$), or GFR ($P = 0.37$). The interaction observed for UUC was originated at the highest level of SBM supplementation as a result of a greater concentration of urea in urine ($P < 0.05$) in lambs drinking LS compared to those drinking HS. Urine output was greater ($P < 0.03$) in HS lambs than in LS lambs, whereas UUN/UN, urea reabsorption, and GFR were not affected by WQ ($P > 0.30$). The results of increasing levels of SBM supplementation were a reduction at decreasing rate in urea reabsorption (quadratic; $P = 0.08$) and a linear increase in urine output and GFR ($P < 0.01$).

Sulfur Intake and Ruminal H_2S Concentration

Sulfur water intake (**SWI**) and sulfur total intake (**STI**) tended to be affected by a supplemental SBM × WQ interaction ($P = 0.10$ and $P = 0.08$, respectively; Table 6). As expected, SWI and STI increased linearly ($P < 0.01$) in HS lambs, but they did not in LS lambs as the level of supplemental SBM increased ($P = 0.98$ and $P = 0.11$, respectively). Ruminal H_2S concentration was affected by a WQ × SBM interaction ($P < 0.01$). The interaction was due to a marked increase in H_2S concentration at 0.25% BW/d of SBM supplementation in lambs drinking HS. Hydrogen sulfide concentration reached a plateau at the intermediate level of SBM supplementation in HS lambs (quadratic relation; $P < 0.01$).

Table 5. Effects of soybean meal supplementation (SBM) and water quality (WQ) on urinary output, plasma urea nitrogen (PUN), urinary urea concentration (UUC), urinary urea as a percentage of the total urinary nitrogen (UUN/UN), urea reabsorbed by kidney (Urea reabs.), and glomerular filtration rate (GFR) in lambs fed low-quality grass hay

	WQ[1]										*P*-values					
	LS				HS									SBM[3]		
	SBM[2]				SBM[2]											
Item	0	0.25	0.50	0.75	1.00	0	0.25	0.50	0.75	1.00	SEM	WQ	SBM	WQ × SBM	L	Q	C
Urine Output, g/kgBW$^{0.75}$	33.3	27.4	31.2	41.3	48.9	33.6	23.2	45.3	52.8	63.1	7.31	0.03	0.03	0.25	<0.01	0.23	0.32
PUN, mg/dL	29.0	38.0	41.7	43.7	48.2	30.2	37.7	40.7	50.7	54.7	3.65	0.11	<0.01	0.38	<0.01	0.66	0.76
UUC, g/dL	1.3	3.4	3.5	3.9	4.0	1.5	3.2	3.2	3.4	3.1	0.34	<0.01	<0.01	0.03			
UUN/UN, %	67.7	63.9	70.4	78.0	77.0	67.2	67.1	65.5	76.1	74.5	6.10	0.58	0.45	0.86	0.12	0.70	0.41
Urea reabs., %	50.8	34.7	28.7	28.4	19.6	43.7	34.8	25.3	23.6	27.3	5.16	0.63	<0.01	0.61	<0.01	0.08	0.61
GFR, mL/min	24.2	40.8	35.4	48.7	47.2	30.9	31.1	29.4	47.8	43.0	4.44	0.30	<0.01	0.39	<0.01	0.77	0.51

[1]LS = low-sulfate water; HS = high-sulfate water.

[2]Supplemental amount of soybean meal as a percentage of BW per day.

[3]Main effect contrasts across supplemental amount of SBM: L = linear; Q = quadratic; C = cubic.

DISCUSSION

Our results confirm that HS drinking water can depress utilization of low-quality forage by ruminants and that the potential to stimulate intake via protein supplementation is greater in animals drinking saline water. In this regard, TTDOMI increased approximately 30% and 15% in HS and LS lambs, respectively, when they were supplemented with 0.25% BW/d of SBM. It is generally accepted that RDP should fall between 7% and 13% of digestible OM intake (Hollingsworth-Jenkins et al., 1996; Köster et al., 1996; Cochran et al., 1998) to maximize forage utilization; however, higher levels of RDP may be needed when animals drink saline water. In the current experiment, HS lambs could match TTDOMI of LS lambs at 0.50% BW/d of SBM supplementation, which represented a RDP to TTDOMI ratio of about 17.5% (calculated from data in NRC, 2000), a value higher than the above range of reference (7% to 13%).

The pattern observed for FOMI with increasing levels of SBM supplementation largely explains the differences in TTDOMI discussed above. Up to 0.50% BW/d of SBM supplementation, LS lambs decreased (14%) FOMI, whereas HS lambs increased (21%) FOMI in comparison to unsupplemented conditions. A possible explanation may be the difference in NDFI between LS and HS lambs when unsupplemented with SBM. It has been stated that a positive effect of protein supplementation can be expected when forage intake is below 12.5 g/kg BW (Mertens, 1994; Ferrell et al., 1999). In the current experiment, NDFI was 14.4 and 9.1 g/kg BW in LS and HS lambs, respectively, at 0% BW/d SBM supplementation. Although protein supplementation stimulates N-deficient forage intake in beef cattle (Bodine et al., 2000; Mathis et al., 2000; Bandyk et al., 2001), similar studies with sheep are consistent with our results (Bohnert et al., 2002; Currier et al., 2004; Salisbury et al., 2004; Atkinson et al., 2010). For example, Chandrasekharaiah et al. (2012) observed that FOMI decreased linearly with increasing level of SBM supplementation in sheep fed finger millet straw (4.3% CP). Moreover, in a recent study, FOMI by wethers was not affected by supplementation with urea or SBM, whereas steers fed the same low-quality forage (4.7% CP) showed enhanced intake when supplemented with urea or SBM (McGuire et al., 2013). The authors attributed these differences in part to a higher NDFI in unsupplemented lambs. This discrepancy between species appears to be related to the greater ability of sheep to consume forage with high levels of NDF as a result of lower retention time of the digesta in rumen (Riaz et al., 2014). These results and arguments may explain why FOMI increased in response to SBM supplementation

only in lambs drinking HS water. On the other hand, the decline in feed intake due to high sulfate in drinking water (particularly high in sulfates) observed in the present study is consistent with results from previous studies (Weeth and Hunter, 1971; Weeth and Capps, 1972; Ward and Patterson, 2004). Like the results of López et al. (2014), TOMI was 36% lower in HS than LS lambs when the low-quality forage used in the present study was unsupplemented with SBM.

The mechanisms by which sulfates in drinking water affect feed intake remain unclear. Some authors have suggested that the H_2S produced by sulfate-reducing bacteria (**SRB**) may adversely affect ruminal motility, increasing retention time and digestibility of ruminal DM content (Bird, 1972; Kandylis, 1984; Drewnoski et al., 2014), with a consequent reduction in feed intake (Loneragan et al., 2001; Uwituze et al., 2011; Drewnoski and Hansen, 2013). However, in our study ruminal H_2S concentrations do not explain the decrease in TTDOMI observed in HS lambs at 0% and 0.25% BW/d SBM supplementation, suggesting that alternative intermediate metabolites from SRB in the ruminal fluid (e.g., HS^-) could have depressed TTDOMI. Although ruminal pH was not recorded, the formation of H_2S from sulfide (pH-dependent process) may have been inhibited because of the fibrous nature of the diet at low levels of SBM supplementation (Richter, 2011; Drewnoski et al., 2014; Morine et al., 2014). To our knowledge, this is the first time that a WQ × protein supplementation interaction has been reported for ruminal hydrogen sulfide concentration in vivo. However, further research is needed to better understand the mechanisms involved in H_2S production to generate efficient supplementation strategies when ruminants are fed low-quality forages.

The effect of protein supplementation on low-quality forage digestion is somewhat variable in the literature, with studies reporting improvements in both TTOMD and TTNDFD (Arroquy et al., 2004; Wickersham et al., 2004, 2008b; Chandrasekharaiah et al., 2012) or only in TTOMD (Bandyk et al., 2001; Sanson et al., 2003; Schauer et al., 2010), whereas no differences were observed by others (Salisbury et al., 2004; Wickersham et al., 2008a). In the current experiment, the increase observed in TTOMD can be largely attributed to a direct effect of highly digestible SBM because increases in protein intake did not improve TTNDFD. At the same time, HS treatment positively altered TTOMD, which could be related to an increase in the retention time of ruminal DM content as explained for the intake above but insufficient to affect fiber digestibility.

Consistent with previous studies (Godwin and Williams, 1984; Holter and Urban, 1992; López et al., 2014), it was observed in the current experiment that increasing the protein content of the diet stimulated

Table 6. Effects of soybean meal supplementation (SBM) and water quality (WQ) on sulfur water intake (SWI), sulfur total intake (STI), and ruminal hydrogen sulfide (H_2S) concentration in lambs fed low-quality grass hay

	WQ[1]										*P*-values						
	LS				HS									SBM[4]			
	SBM[2]				SBM[2]												
Item	0	0.25	0.50	0.75	1.00	0	0.25	0.50	0.75	1.00	SEM[3]	WQ	SBM	WQ × SBM	L	Q	C
SWI, mg/kg $BW^{0.75}$	5.3	5.0	5.4	5.9	6.5	315.7	286.1	374.3	399.7	464.2	33.40	<0.01	0.13	0.10	0.02	0.49	0.61
STI,[5] mg/kg $BW^{0.75}$	47.4	70.1	87.1	108.4	125.4	341.7	340.1	450.8	495.9	581.6	35.90	<0.01	<0.01	0.08	<0.01	0.63	0.69
H_2S[6]	1.2	1.2	1.2	1.2	1.7	1.6	2.7	3.3	2.9	2.9	0.20	<0.01	<0.01	0.01			
H_2S,[7] mg/kg	(17.0)	(15.5)	(17.4)	(15.8)	(50.1)	(38.9)	(489.8)	(2089.3)	(977.2)	(955.0)							

[1]LS = low-sulfate water; HS = high-sulfate water.

[2]Supplemental amount of soybean meal as a percentage of BW per day.

[3]$n = 2$.

[4]Main effect contrasts across supplemental amount of SBM: L = linear; Q = quadratic; C = cubic.

[5]Sulfur content of diet ingredients was estimated from NRC (2000).

[6]Data for H_2S were log transformed to meet statistical assumptions of equal variance.

[7]Means were back transformed.

water intake. However, an unexpected response was the lack of effect of WQ on water intake, suggesting that the level of 6,363 mg/kg sulfate in water was below the taste discrimination threshold under present experimental conditions (Digesti and Weeth, 1976). It is important to note that the experiment was conducted at mild temperatures (21.9°C, 30.0°C, and 14.1°C for mean, maximum, and minimum daily temperatures, respectively), which reduces water requirements and possibly lessens the effect of sulfates on water intake (Ray, 1989; Loneragan et al., 2001).

In general, protein supplementation has been shown to increase N retention and N utilization efficiency in ruminants consuming low-quality forages (Bohnert et al., 2002; Swanson et al., 2004; McGuire et al., 2013). Chandrasekharaiah et al. (2012) showed a quadratic increase in N balance and N retention in response to SBM supplementation in sheep fed a low-quality forage–based diet (4.3% CP). A similar response was observed in the current experiment regardless of WQ, although a high level (1.00% BW/d of SBM) of protein supplementation was needed to maximize N balance and N utilization efficiency in lambs drinking HS water. Previous research has shown that the availability of drinking water can alter the efficiency of N utilization in ruminants (Silanikove, 2000; Ghassemi Nejad et al., 2014), highlighting the role of the kidneys in urea N conservation (Marini and Van Amburgh, 2003; Marini et al., 2004; Meintjes and Engelbrecht, 2004). Urea is the most abundant nitrogen compound in the urine of mammals (Huntington et al., 2001; Dijkstra et al., 2013); therefore, renal urea handling plays a critical role in N economy, particularly in animals fed low-protein diets (Starke et al., 2012). Considering that water intake did not differ between HS and LS lambs at all levels of SBM supplementation, the results of the current experiment suggest that alterations in N balance were a consequence of depression in FOMI, rather than an effect of WQ on renal function. This argument is consistent with the fact that PUN, UUN/UN, urea reabsorption, and GFR were not altered by WQ. Since urea is freely filtered in the glomeruli, the product of GFR and PUN determines the theoretical maximum urea excretion by the kidneys (Hall, 2010), although the final urea excretion is defined by the efficiency of reabsorption in the renal tubules. Some authors suggest that kidney N reabsorption capacity is affected by salt consumption (Godwin and Williams, 1984; Meintjes and Engelbrecht, 2004); however, in the current experiment the ability to retain N was modified only by SBM level. The lambs drinking high-salt water urinated more than LS lambs, which would indicate a process of osmotic diuresis attributable to increased load of solute in the renal tubules. In rats it has been documented that osmotic diuresis increases the abundance of specific urea transporters (**UT-A1**) in the inner medullary collecting duct (Kim et al., 2005). Although the regulation mechanisms are not well understood, the concentration of urea in the tubular fluid appears to be 1 of the factors that regulate the expression of these urea transporters (Sands, 1999; Kim et al., 2005). Starke et al. (2012) concluded that urea reabsorption in the kidney was increased via an upregulation of UT-A1 mRNA expression in goats fed a diet deficient in protein. Therefore, in the current experiment a potential greater abundance of urea transporters in HS lambs could have facilitated urea reabsorption in a situation where the tubular urea concentration was low because of relatively low water reabsorption.

The results of this experiment showed that protein supplementation improved N retention in lambs consuming low-quality forage and drinking HS water. However, higher levels of protein supplementation were needed to maximize digestible OM intake and N utilization efficiency in HS lambs than in LS lambs. This result confirms the existence of an interaction between HS water and supplemental protein that alters the response to low-quality forage utilization by sheep.

LITERATURE CITED

Arroquy, J. I., R. C. Cochran, M. Villareal, T. A. Wickersham, D. A. Llewellyn, E. C. Titgemeyer, T. G. Nagaraja, D. E. Johnson, and D. Gnad. 2004. Effect of level of rumen degradable protein and type of supplemental non-fiber carbohydrate on intake and digestion of low-quality grass hay by beef cattle. Anim. Feed Sci. Technol. 115:83–99. doi:10.1016/j.anifeedsci.2004.02.005

Association of Official Analytical Chemists. 1990. Official methods of analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Atkinson, R. L., C. D. Toone, T. J. Robinson, D. L. Harmon, and P. A. Ludden. 2010. Effects of ruminal protein degradability and frequency of supplementation on nitrogen retention, apparent digestibility, and nutrient flux across visceral tissues in lambs fed low-quality forage. J. Anim. Sci. 88:727–736. doi:10.2527/jas.2009-2246

Bandyk, C. A., R. C. Cochran, T. A. Wickersham, E. C. Titgemeyer, C. G. Farmer, and J. J. Higgins. 2001. Effect of ruminal vs. postruminal administration of degradable protein on utilization of low-quality forage by beef steers. J. Anim. Sci. 79:225–231. doi:10.2527/2001.791225x

Basán Nickisch, M. 2007. Manejo de los Recursos Hídricos en Zonas Áridas y Semiáridas para Áreas de Secano. Inst. Nac. Tecnol. Agropecu., Buenos Aires.

Bird, P. R. 1972. Sulphur metabolism and excretion studies in ruminants: X. Sulphide toxicity in sheep. Aust. J. Biol. Sci. 25:1087–1098.

Bodine, T. N., H. T. Purvis, C. J. Ackerman, and C. L. Goad. 2000. Effects of supplementing prairie hay with corn and soybean meal on intake, digestion, and ruminal measurements by beef steers. J. Anim. Sci. 78:3144–3154. doi:10.2527/2000.78123144x

Bohnert, D. W., C. S. Schauer, and T. DelCurto. 2002. Influence of rumen protein degradability and supplementation frequency on performance and nitrogen use in ruminants consuming low-quality forage: Cow performance and efficiency of nitrogen use in wethers. J. Anim. Sci. 80:1629–1637. doi:10.2527/2002.8061629x

Chandrasekharaiah, M., A. Thulasi, and K. T. Sampath. 2012. Effect of different rumen degradable nitrogen levels on microbial protein synthesis and digestibility in sheep fed on finger millet straw (*Eleucine coracana*) based diet. Small Rumin. Res. 102:151–156. doi:10.1016/j.smallrumres.2011.07.005

Cochran, R. C., H. H. Köster, K. C. Olson, J. S. Heldt, C. P. Mathis, and B. C. Woods. 1998. Supplemental protein sources for grazing cattle. In: 9th Florida Rumin. Nutr. Symp., Gainesville. p. 123–136.

Currier, T. A., D. W. Bohnert, S. J. Falck, and S. J. Bartle. 2004. Daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage: I. Effects on cow performance and the efficiency of nitrogen use in wethers. J. Anim. Sci. 82:1508–1517. doi:10.2527/2004.8251508x

Digesti, R. D., and H. J. Weeth. 1976. A defensible maximum for inorganic sulfate in drinking water of cattle. J. Anim. Sci. 42:1498–1502. doi:10.2527/jas1976.4261498x

Dijkstra, J., O. Oenema, J. W. van Groenigen, J. W. Spek, A. M. van Vuuren, and A. Bannink. 2013. Diet effects on urine composition of cattle and N_2O emissions. Animal 7:292–302. doi:10.1017/S1751731113000578

Di Rienzo, J. A., F. Casanoves, M. G. Balzarini, L. Gonzalez, M. Tablada, and C. W. Robledo. 2014. InfoStat version 2014. http://www.infostat.com.ar (Accessed 16 June 2016.)

Drewnoski, M. E., and S. L. Hansen. 2013. Effect of delaying the feeding of high sulfur until 28 days after adaptation to finishing diet on cattle intake, gain, and ruminal hydrogen sulfide concentrations. Livest. Sci. 155:230–235. doi:10.1016/j.livsci.2013.05.014

Drewnoski, M. E., D. J. Pogge, and S. L. Hansen. 2014. High-sulfur in beef cattle diets: A review. J. Anim. Sci. 92:3763–3780. doi:10.2527/jas.2013-7242

Ergene, N., and E. C. Pickering. 1978. The effects of reducing dietary nitrogen and of increasing sodium chloride intake on urea excretion and reabsorption and on urine osmolality in sheep. Q. J. Exp. Physiol. 63:67–76. doi:10.1113/expphysiol.1978.sp002416

Ferrell, C. L., K. K. Kreikemeier, and H. C. Freetly. 1999. The effect of supplemental energy, nitrogen, and protein on feed intake, digestibility, and nitrogen flux across the gut and liver in sheep fed low-quality forage. J. Anim. Sci. 77:3353–3364. doi:10.2527/1999.77123353x

Ghassemi Nejad, J., J. D. Lohakare, J. W. West, and K. I. Sung. 2014. Effects of water restriction after feeding during heat stress on nutrient digestibility, nitrogen balance, blood profile and characteristics in Corriedale ewes. Anim. Feed Sci. Technol. 193:1–8. doi:10.1016/j.anifeedsci.2014.03.011

Godwin, I. R., and V. J. Williams. 1984. Renal control of plasma urea level in sheep: The diuretic effect of urea, potassium and sodium chloride. Exp. Physiol. 69:49–59. doi:10.1113/expphysiol.1984.sp002795

Gould, D. H., B. A. Cummings, and D. W. Hamar. 1997. In vivo indicators of pathologic ruminal sulfide production in steers with diet-induced polioencephalomalacia. J. Vet. Diagn. Invest. 9:72–76. doi:10.1177/104063879700900113

Hall, J. E. 2010. Guyton and Hall textbook of medical physiology. 12th ed. Elsevier, Philadelphia. p. 1120.

Hollingsworth-Jenkins, K. J., T. J. Klopfenstein, D. C. Adams, and J. B. Lamb. 1996. Ruminally degradable protein requirement of gestating beef cows grazing native winter sandhills range. J. Anim. Sci. 74:1343–1348. doi:10.2527/1996.7461343x

Holter, J. B., and W. E. Urban. 1992. Water partitioning and intake prediction in dry and lactating Holstein cows. J. Dairy Sci. 75:1472–1479. doi:10.3168/jds.S0022-0302(92)77904-1

Huntington, G., M. Poore, B. Hopkins, and J. Spears. 2001. Effect of ruminal protein degradability on growth and N metabolism in growing beef steers. J. Anim. Sci. 79:533–541. doi:10.2527/2001.792533x

Instituto Nacional de Tecnología Agropecuaria. 2013. Guía para cuidado y uso de animales de experimentación. http://inta.gob.ar/documentos/manuales-sobre-cuidado-y-supervisación-y-uso-de-animales. (Accessed 10 April 2015.)

Kandylis, K. 1984. Toxicology of sulfur in ruminants: Review. J. Dairy Sci. 67:2179–2187. doi:10.3168/jds.S0022-0302(84)81564-7

Kim, D., J. D. Klein, S. Racine, B. P. Murrell, and J. M. Sands. 2005. Urea may regulate urea transporter protein abundance during osmotic diuresis. Am. J. Physiol. Renal Physiol. 288:F188–F197. doi:10.1152/ajprenal.00200.2004

Komarek, A. R. 1993. A filter bag procedure for improved efficiency of fiber analysis. J. Anim. Sci. 76(Suppl.1):250. (Abstr.)

Köster, H. H., R. C. Cochran, E. C. Titgemeyer, E. S. Vanzant, I. Abdelgadir, and G. St-Jean. 1996. Effect of increasing degradable intake protein on intake and digestion of low-quality, tallgrass-prairie forage by beef cows. J. Anim. Sci. 74:2473–2481. doi:10.2527/1996.74102473x

Leibovich, J., J. T. Vasconcelos, and M. L. Galyean. 2009. Effects of corn processing method in diets containing sorghum wet distillers grain plus solubles on performance and carcass characteristics of finishing beef cattle and on in vitro fermentation of diets. J. Anim. Sci. 87:2124–2132. doi:10.2527/jas.2008-1695

Loneragan, G. H., J. J. Wagner, D. H. Gould, F. B. Garry, and M. A. Thoren. 2001. Effects of water sulfate concentration on performance, water intake, and carcass characteristics of feedlot steers. J. Anim. Sci. 79:2941–2948. doi:10.2527/2001.79122941x

López, A., J. I. Arroquy, A. V. Juárez Sequeira, M. García, M. Nazareno, H. Coria, and R. A. Distel. 2014. Effect of protein supplementation on tropical grass hay utilization by beef steers drinking saline water. J. Anim. Sci. 92:2152–2160. doi:10.2527/jas.2013-7138

Marini, J. C., J. D. Klein, J. M. Sands, and M. E. Van Amburgh. 2004. Effect of nitrogen intake on nitrogen recycling and urea transporter abundance in lambs. J. Anim. Sci. 82:1157–1164. doi:10.2527/2004.8241157x

Marini, J. C., and M. E. Van Amburgh. 2003. Nitrogen metabolism and recycling in Holstein heifers. J. Anim. Sci. 81:545–552. doi:10.2527/2003.812545x

Mathis, C. P., R. C. Cochran, J. S. Heldt, B. C. Woods, I. E. Abdelgadir, K. C. Olson, E. C. Titgemeyer, and E. S. Vanzant. 2000. Effects of supplemental degradable intake protein on utilization of medium to low-quality forages. J. Anim. Sci. 78:224–232. doi:10.2527/2000.781224x

McGuire, D. L., D. W. Bohnert, C. S. Schauer, S. J. Falck, and R. F. Cooke. 2013. Daily and alternate day supplementation of urea or soybean meal to ruminants consuming low-quality cool-season forage: I—Effects on efficiency of nitrogen use and nutrient digestion. Livest. Sci. 155:205–213. doi:10.1016/j.livsci.2013.05.015

Meintjes, R. A., and H. Engelbrecht. 2004. Changes in the renal handling of urea in sheep on a low protein diet exposed to saline drinking water. Onderstepoort J. Vet. Res. 71:165–170. doi:10.4102/ojvr.v71i3.255

Mertens, D. R. 1994. Regulation of forage intake. In: G. C. Fahey Jr., editor, Forage quality, evaluation, and utilization. Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc. Am., Madison, WI. p. 450–493.

Morine, S. J., M. E. Drewnoski, and S. L. Hansen. 2014. Increasing dietary neutral detergent fiber concentration decreases ruminal hydrogen sulfide concentrations in steers fed high-sulfur diets based on ethanol coproducts. J. Anim. Sci. 92:3035–3041. doi:10.2527/jas.2013-7339

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Ray, D. E. 1989. Interrelationships among water quality, climate and diet on feedlot performance of steer calves. J. Anim. Sci. 67:357–363. doi:10.2527/jas1989.672357x

Riaz, M. Q., K. H. Südekum, M. Clauss, and A. Jayanegara. 2014. Voluntary feed intake and digestibility of four domestic ruminant species as influenced by dietary constituents: A meta-analysis. Livest. Sci. 162:76–85. doi:10.1016/j.livsci.2014.01.009

Richter, E. L. 2011. The effect of dietary sulfur performance, mineral status, rumen hydrogen sulfide, and rumen microbial populations in yearling beef steers. MS Thesis. Iowa State Univ., Ames.

Salisbury, M. W., C. R. Krehbiel, T. T. Ross, C. L. Schultz, and L. L. Melton. 2004. Effects of supplemental protein type on intake, nitrogen balance, and site, and extent of digestion in whiteface wethers consuming low-quality grass hay. J. Anim. Sci. 82:3567–3576. doi:10.2527/2004.82123567x

Sands, J. M. 1999. Regulation of renal urea transporters. J. Am. Soc. Nephrol. 10:635–646.

Sanson, D. W., S. I. Paisley, and R. J. Gaebe. 2003. Utilization of mature low-quality grass hay by lambs and steers supplemented with soybean meal products. Prof. Anim. Sci. 19:290–296.

Schauer, C. S., M. L. Van Emon, M. M. Thompson, D. W. Bohnert, J. S. Caton, and K. K. Sedivec. 2010. Protein supplementation of low-quality forage: Influence of frequency of supplementation on ewe performance and lamb nutrient utilization. Sheep Goats Res. J. 25:66–73.

Silanikove, N. 2000. The physiological basis of adaptation in goats to harsh environments. Small Rumin. Res. 35:181–193. doi:10.1016/S0921-4488(99)00096-6

Starke, S., A. S. Muscher, N. Hirschhausen, E. Pfeffer, G. Breves, and K. Huber. 2012. Expression of urea transporter is affected by dietary nitrogen restriction in goat kidney. J. Anim. Sci. 90:3889–3897. doi:10.2527/jas.2011-4262

Swanson, K. C., H. C. Freetly, and C. L. Ferrell. 2004. Nitrogen balance in lambs fed low-quality brome hay and infused with differing proportions of casein in the rumen and abomasum. J. Anim. Sci. 82:502–507. doi:10.2527/2004.822502x

Tebot, I., S. Faix, M. Szanyiova, A. Cirio, and L. Leng. 1998. Micropuncture study on urea movements in the kidney cortical tubules of low protein fed sheep. Vet. Res. 29:99–105.

Uwituze, S., G. L. Parsons, K. K. Karges, M. L. Gibson, L. C. Hollis, J. J. Higgins, and J. S. Drouillard. 2011. Effects of distillers grains with high sulfur concentration on ruminal fermentation and digestibility of finishing diets. J. Anim. Sci. 89:2817–2828. doi:10.2527/jas.2010-3401

Ward, E. H., and H. H. Patterson. 2004. Effects of thiamin supplementation on performance and health of growing steers consuming high sulfate water. Proc. Am. Soc. Anim. Sci. West. Sec. 55:375–378.

Weeth, H. J., and D. L. Capps. 1972. Tolerance of growing cattle for sulfate-water. J. Anim. Sci. 34:256–260. doi:10.2527/jas1972.342256x

Weeth, H. J., and J. E. Hunter. 1971. Drinking of sulfate-water by cattle. J. Anim. Sci. 32:277–281. doi:10.2527/jas1971.322277x

Wickersham, T. A., R. C. Cochran, E. C. Titgemeyer, C. G. Farmer, E. A. Klevesahl, J. I. Arroquy, D. E. Johnson, and D. P. Gnad. 2004. Effect of postruminal protein supply on the response to ruminal protein supplementation in beef steers fed a low-quality grass hay. Anim. Feed Sci. Technol. 115:19–36. doi:10.1016/j.anifeedsci.2004.03.005

Wickersham, T. A., E. C. Titgemeyer, R. C. Cochran, E. E. Wickersham, and D. P. Gnad. 2008a. Effect of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. J. Anim. Sci. 86:3079–3088. doi:10.2527/jas.2007-0325

Wickersham, T. A., E. C. Titgemeyer, R. C. Cochran, E. E. Wickersham, and E. S. Moore. 2008b. Effect of frequency and amount of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. J. Anim. Sci. 86:3089–3099. doi:10.2527/jas.2007-0326