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Effect of protein and glucogenic precursor supplementation on forage digestibility, serum metabolites, energy utilization, and rumen parameters in sheep

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Abstract

Supplementation of glucogenic precursors in roughage diets may increase production responses due to improved efficiencies of nutrient utilization. Therefore, the objective of this study was to determine the effect of source of supplemental glucogenic potential (GP) on forage digestibility, serum metabolites, energy utilization, and rumen parameters of growing wethers consuming a roughage diet (8.8% crude protein, 71.4% ash-free neutral detergent fiber). Crossbred wethers (49.1 ± 4.7 kg initial BW; $n = 16$) were utilized in a 4 × 4 replicated Latin Square design with four periods of 21 d. Supplements were designed to supplement increasing amount of GP: 1) no supplementation (CON; 0 g), 2) 40 g of calcium propionate (CAP; 30 g of GP), 3) 70 g of blood meal + 100 g of feather meal (BF; 40 g of GP), or 4) combination of CAP and BF (COMBO; 70 g of GP). Total fecal and urine collection was conducted from days 13–17 to calculate digestibility estimates and urinary losses. An acetate tolerance test was administered on day 17 to determine the effect of GP on acetate clearance. Blood samples were collected on day 19 and were analyzed for serum concentrations of glucose, urea N (SUN), non-esterified fatty acids, and amino acids. Rumen fluid was collected on day 21 to determine supplementation effects on ruminal volatile fatty acid (VFA) and ammonia concentrations. Wethers receiving BF and COMBO supplementation had greatest ($P \leq 0.01$) DM and OM total tract digestibility. Supplementation did not affect ($P \geq 0.37$) NDF digestibility or digestible energy. Urinary nitrogen excretion was greatest ($P = 0.02$) for BF and COMBO. Circulating serum essential amino acid concentration was increased ($P < 0.01$) in BF and COMBO compared to CAP and CON. In addition, BF and COMBO had increased ($P < 0.01$) SUN concentrations compared to CAP and CON. Acetate half-life was not affected ($P = 0.39$) by supplementation strategy. However, area under the curve (AUC) for acetate was decreased ($P = 0.04$) with supplementation of BF and COMBO compared to CON-fed wethers. Ruminal propionate concentration was increased ($P \leq 0.01$) for wethers fed CAP and COMBO supplementation, which resulted in decreased ($P \leq 0.01$) A:P ratio. Overall, these results indicate that the increased propionate supply by providing propionate salts did not result in a protein sparing impact or increased N retention.

Key words: amino acid utilization, forage digestibility, lambs, propionate salt, protein supplementation

INTRODUCTION

Supplementation of glucogenic precursors and rumen undegradable protein (RUP) may increase production responses due to improved efficiencies of nutrient utilization. In forage-based production systems, ruminal production of acetate compared to propionate can result in imbalanced acetate:propionate ratio (McCollum and Galyean, 1985; Cronjé et al., 1991), resulting in negative modifications in energy metabolism (Mulliniks et al., 2019). Acetate:propionate ratio has been shown to decrease with supplementation of ruminal propionate precursors (Sanchez et al., 2014) or protein supplementation (DelCurto et al., 1990; Salisbury et al., 2004). Increasing glucogenic potential of the diet with RUP and calcium propionate supplementation has been shown to enhance energy metabolism in young, lactating range beef cows grazing dormant forage (Mulliniks et al., 2011). In addition, increasing post-ruminal supply of propionate increases fatty acid and acetate hindlimb uptake of growing lambs (Majdoub et al., 2003). Furthermore, Ferrell et al. (1999) reported greater digestible energy and available amino acids when a combination of energy and RUP was supplemented to a low-quality hay-based diet. The combination of RUP supplementation and post-ruminal infusion of glucose in growing wethers consuming low-quality forages has been shown to increase feed intake, rate of growth, and improve feed efficiency above RUP supplementation alone (Kempton et al., 1978). However, the additional growth response due to post-ruminal increase of glucose was only observed with the addition of RUP supplementation. Continuous duodenal infusion of glucose resulted in increased growth rate and improved feed conversion for wethers consuming a low-protein diet regardless of supplemental RUP (Leng et al.,

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1978). Our hypothesis was that providing increased amounts of glucogenic precursors would increase acetate utilization and efficiency in growing wethers on a forage-based diet. Therefore, the objective of this study was to determine the effect of supplemental glucogenic potential (GP) on forage digestibility, serum metabolites, rumen parameters, and energy utilization of a forage-based diet.

MATERIALS AND METHODS

All animal care and management procedures used were reviewed and approved by the University of Nebraska Institutional Care and Animal Use Committee (IACUC #1678).

Sixteen crossbred wethers (49.1 ± 4.7 kg initial BW) were utilized to determine forage digestibility, blood and rumen parameters, and acetate utilization. Wethers were sorted into 4 blocks based on initial BW in a 4 × 4 replicated Latin Square design. Wethers were assigned randomly within each period to 1 of 4 treatments to provide 0, 30, 40, and 70 g of GP: 1) control basal diet only (CON; 0 g of additional GP), 2) 40 g of NutroCal (CAP; Ca-propionate, 30 g of additional GP; Kemin Industries Inc., Des Moines, IA), 3) 70 g of blood meal and 100 g of feather meal (BF; 40 g of additional GP), or 4) combination of CAP and BF (COMBO; 70 g of additional GP). Values for CP and RUP percentages were taken from NASEM (2016). The combination of supplements for the BF treatment was 92.6% CP and 57.9% digestible RUP. Grams of GP from RUP was calculated assuming 40% of digestible RUP is glucogenic (Preston and Leng, 1987). Nutrocal contains 80% propionate which is 95% glucogenic (Steinhour and Bauman, 1988), allowing for calculation of the GP it provides. Forage provided was brome grass hay (8.8% CP, 90.9% organic matter [OM], 71.4% ash-free neutral detergent fiber [NDF_{om}], 44.8% acid detergent fiber [ADF]). Hay was ground with a tub grinder through a 2.5-cm screen and offered at a constant 2% of BW. Commercial mineral + vitamin premix (28.35 g) was offered daily to all wethers.

Periods were 21 d in length allowing for 12 d of diet adaptation, 5 d of total fecal and urine collection, and 4 d for metabolism collections. Wethers were fed forage twice daily at 0800 and 1700 h, with 50% of daily DM at each feeding. Supplementation occurred at 0730 h each day. Wethers receiving BF supplementation were adapted at levels of 40%, 60%, and 80% total supplementation on days 1–3 of each period, respectively. Feed refusals were taken daily prior to supplementation. On day 12, wethers were placed in metabolism crates at 1700 h for total fecal collection. Fecal bags were emptied and recorded at 0800 and 1700 h daily. Ten percent of each fecal collection was retained for data analysis and stored at 2.8 °C until the end of the period. Five percent of each fecal collection was composited by period and lyophilized (VirTis Freezemobile 25ES, SP Scientific, Gardiner, NY). Urine was collected daily via gravity flow into covered tubs below metabolism crates. Tubs contained 100 mL of 1 M hydrochloric acid to prevent volatilization of N and was replaced daily. At 1700 h daily, tubs were removed, weight was recorded, and 10% of total urine collection was retained and stored at 2.8 °C until further analysis. Urine was thawed and boiled to reduce water content prior to further analysis (Judy et al., 2019). Beakers filled with urine were placed into a boiling water bath (Ankom Technology, Macedon, NY) underneath a hood until urine reached consistency of a paste. Urine paste was then lyophilized (VirTis Freezemobile 25ES, SP Scientific, Gardiner, NY) and analyzed. Percentage nitrogen of urine was analyzed utilizing a LECO nitrogen analyzer (LECO, St. Joseph, MI). Energy lost from urine was analyzed with a Parr 6400 calorimeter (Parr Instrument Company, Moline, IL). Feed refusals were taken at days 10 to 15 and feed samples taken at days 12 and 19 were dried at 60 °C for 72 h to correct daily dry matter intake. Fecal, feed, and feed refusal samples were ground through a 1-mm screen of a Wiley mill and analyzed for OM, NDF_{om}, and ADF. Analysis for NDF_{om} and ADF was conducted using the beaker method (Van Soest et al., 1991). Gross energy was analyzed using a Parr 6400 calorimeter (Parr Instrument Company, Moline, IL) for individual fecal samples, composite feed samples, and composite feed refusal samples for each period. Caps containing 2.0 g of sample and 0.4 g of mineral oil sat for a minimum of 12 h prior to being bombed for determination of gross heat. Digestible energy was then calculated by subtracting the energy lost in feces from GE of feed intake (NASEM, 2016).

An acetate tolerance test (ATT) was conducted on day 17 to analyze acetate clearance rates and glucogenic potential of the diets as affected by supplemental treatments. Jugular catheters were inserted the morning of the ATT, through which a 20% acetic acid solution was infused at 1.25 mL/kg of BW. Blood samples were then collected (~7 mL) –1, 0, 1, 3, 5, 7, 10, 15, 30, 60, and 90 min relative to infusion. Samples were placed in Corvac serum separator tubes, cooled, and centrifuged at 2,000 × *g* at 4 °C for 20 min. Serum was collected and stored at –20 °C for later analysis of acetate, insulin, and glucose concentrations. Serum was filtered with a centrifugal filter device for 100 min at 4 °C at 5,000 × *g* for deproteinization (Amicon Ultra-2 centrifugal device, Millipore Corp., Burlington, MA). Filtered serum was mixed at a 5:1 ratio with 25% metaphosphoric acid containing 2 g/L of 2-ethyl butyric acid as an internal standard. Acetate concentration was analyzed via gas chromatography adapted from the method of Goetsch and Galyean (1983). The half-life of acetate was calculated as the time required for a 50% decrease from peak serum concentration (Kaneko, 1989). Serum samples were analyzed for glucose concentration by lab in the Biomedical and Obesity Research Core (BORC) of the Nebraska Center for Prevention of Obesity Diseases (NPOD). Serum acetate and glucose area under the curves (AUC) were calculated using the trapezoidal method.

On day 19, a blood sample (~ 7 mL) was taken preprandial at 0730 h and 4 h post-prandial at 1230 h via venipuncture from the jugular vein and the saphenous vein found in the hindlimb into serum separator vacuum tubes (Corvac, Kendall Healthcare, St. Louis, MO). Serum samples were analyzed for glucose, urea N (SUN), non-esterified fatty acids (NEFA), and circulating amino acid (AA) concentrations. Glucose and SUN were also analyzed by the BORC lab of NPOD. Amino acid concentrations were analyzed using the EZ:faast For Free (Physiological) Amino Acid Analysis kit (Phenomenex, Torrance, CA) for gas chromatography (GC). Serum samples were analyzed for NEFA concentration utilizing the WAKO HR Series NEFA-HR(2) (FUJIFILM Wako Diagnostics U.S.A., Mountain View, CA). The intra- and interassay CV were, respectively, 5.7% and 3.4% for serum

NEFA, 3.0% and 4.1% for serum glucose, 2.7% and 4.6% for SUN, and 4.0% and 4.7% for serum AA.

Rumen samples were collected 4 h post-prandial at 1230 h on day 21. A sample of contents (40 mL) were collected through oral lavage, snap frozen in liquid nitrogen, and stored at –20 °C until analysis. Samples were thawed and centrifuged at 5,000 × *g* for 20 min prior to analysis for VFA and ruminal ammonia concentration. For analysis of VFA concentration, 2.0 mL were pipetted into test tubes. To each test tube, 0.5 mL of ice cold 25% meta-phosphoric acid/crotonic acid solution was added and then vortexed. Test tubes were then refrigerated at 4 °C for 30 min and then centrifuged at 10,000 × *g* for 15 min. Tuberculin syringes are filled with 3.0 mL of supernatant and filtered through a filter-tip syringe into a GC vial and analyzed for VFA concentration. For ruminal ammonia concentration, 40 µL of rumen fluid plus 40 µL of H_2O were dispensed into plastic test tubes. Phenol reagent was added at 2.5 mL followed by 2.0 mL of alkaline hypochlorite reagent. Tubes were then vortexed and incubated in a 37 °C water bath for 10 min. Then 300 µL was pipetted from each tube into the wells of a microtiter plate and absorbance was read on each plate at 550 nm. A standard curve was calculated using linear regression, where x is the absorbance and y is the concentration. Sample absorbances were applied to standard curve calculation to determine concentration.

Statistical Analysis

Total tract digestibility and rumen parameters data were analyzed as a Latin Square design using the MIXED procedure (SAS Inst. Inc., Cary, NC). Wethers were blocked by weight into light and heavy blocks. Data were analyzed with wether serving as experimental unit, with supplementation type and period as fixed effects. Wether within period served as a random effect. Acetate half-lives were estimated for each animal by regressing the logarithmically transformed acetate concentrations over time (Kaneko, 1989). Area under the curves was determined for acetate and glucose using the trapezoidal summation method. Serum data were analyzed as repeated measures with time of blood collection serving as a repeated factor with an autoregressive covariate structure. Treatment by location (jugular vein and the saphenous vein found in the hindlimb) and treatment by time interactions for serum samples were not significant ($P > 0.05$) and were removed from the model. Significance level was set at $P \leq 0.05$.

RESULTS

Wethers consuming CON consumed a greater quantity of forage resulting in greater ($P = 0.02$; Table 1) forage DM intake compared to their supplemental counterparts. Total DM and ADF intake increased ($P < 0.01$) with the addition of protein (BF and COMBO) to the diet compared to CON and CAP. Total OM intake was increased ($P < 0.01$) with increasing glucogenic potential with wethers fed COMBO diet having the greatest OM intake. Wethers receiving BF and COMBO supplementation had greater ($P < 0.01$) DM and OM total tract digestibilities compared to the CAP and CON treatments. Supplemental treatments did not influence

Table 1. Effect of protein and glucogenic precursor supplementation on intake, total tract digestibilities, and digestible energy for wethers fed a forage-based diet

Measurement	Treatment[1]				SEM	*P*-value
	CON	CAP	BF	COMBO		
Dry matter						
Forage intake, kd/d	1.01[a]	0.99[b]	0.98[b]	0.99[b]	0.02	0.02
Total intake[2], kg/d	1.03[d]	1.05[c]	1.16[b]	1.21[a]	0.02	< 0.01
Digestibility, %	37.4[b]	36.6[b]	43.0[a]	42.9[a]	0.98	< 0.01
Organic matter						
Forage intake, kd/d	0.94	0.94	0.94	0.94	0.02	0.94
Total intake, kg/d	0.95[d]	0.97[c]	1.10[b]	1.13[a]	0.02	< 0.01
Digestibility, %	42.6[b]	43.6[b]	49.8[a]	49.8[a]	1.11	< 0.01
NDF_{om}[3]						
Forage intake, kd/d	0.68	0.67	0.66	0.67	0.02	0.08
Total intake, kg/d	0.70	0.70	0.70	0.70	0.02	0.98
Digestibility, %	44.8	45.2	45.8	45.3	1.28	0.93
Acid detergent fiber						
Forage Intake, kd/d	0.46	0.46	0.46	0.46	0.01	0.97
Total intake, kg/d	0.46[b]	0.46[b]	0.49[a]	0.50[a]	0.01	< 0.01
Digestibility, %	35.6[bc]	35.4[c]	39.2[a]	38.5[ab]	1.31	0.03
Digestible energy, Mcal/kg	1.69	1.74	1.63	1.65	0.05	0.37

[a–d]Means within a row with different superscripts differ ($P < 0.05$).
[1]Supplements were designed to supply increasing levels of glucogenic potential (GP): (1) basal diet only (CON; 0 g of GP), (2) 40 g of NutroCal (CAP; Ca-propionate, 30 g of GP; Kemin Industries Inc., Des Moines, IA), (3) 70 g of blood meal and 100 g of feather meal (BF; 40 g of GP), or (4) combination of CAP and BF (COMBO; 70 g of GP).
[2]Total intake = basal diet plus supplementation and mineral.
[3]NDF_{om} = ash-free neutral detergent fiber.

(P = 0.93) NDF_{om} digestibility. However, supplementation type did influence (P = 0.03) ADF digestibility. Wethers supplemented with protein (BF or COMBO) had greater (P = 0.02) ADF digestibility than CAP supplemented wethers. However, ADF digestibility was not different (P = 0.54) between CON- and CAP-supplemented wethers, BF- and COMBO-supplemented wethers, or COMBO- and CON-supplemented wethers. Forage OM and ADF intake did not differ ($P \geq 0.94$) among treatments. However, forage NDF intake tended (P = 0.08) to be greater for CON-supplemented wethers compared to their supplemental counterparts. Digestible energy was not influenced (P = 0.37) by increasing glucogenic potential of the diet. In addition, urinary energy loss was not influenced (P = 0.71; Table 2) by supplemental treatments. However, urinary nitrogen loss was increased (P = 0.02) with BF and COMBO having greater losses compared to CAP and CON.

Circulating serum glucose concentration was not influenced ($P \geq 0.47$, Table 3) by supplementation. The addition of RUP supplementation in BF and COMBO resulted in increased ($P < 0.01$) circulating SUN compared to CON and CAP. Serum NEFA concentrations were not influenced (P = 0.27) by supplemental treatments. No treatment by time interactions were observed ($P > 0.05$) for serum concentrations of glucose, SUN, or NEFA.

Of the 16 serum amino acids (AA) measured, 11 AA were increased ($P < 0.01$; Table 4) with the addition of RUP in the diet with no differences in AA concentrations between BF and COMBO. However, circulating serum concentrations of alanine were reduced (P = 0.01) in wethers consuming BF and COMBO compared to CON and CAP. Serum methionine concentrations were greater ($P < 0.01$) for CON and CAP-fed wethers than BF-fed wethers. Circulating serum concentrations of tryptophan were reduced (P = 0.04) for wethers consuming only the basal diet compared to wethers receiving additional GP. Total circulating serum AA concentrations were greater ($P < 0.01$) in wethers consuming BF and COMBO supplemental treatments. Inclusion of RUP supplement in BF and COMBO resulted in greater ($P < 0.01$) serum concentration of essential amino acids (EAA) compared to CAP and CON. However, serum concentrations of non-EAA were not influenced (P = 0.40) by supplemental treatments. Glucogenic, ketogenic, and gluco-ketogenic AA were increased ($P < 0.01$) in wethers fed BF and COMBO.

Serum acetate half-life was not influenced (P = 0.39; Table 5) by supplemental treatments. However, acetate AUC was influenced (P = 0.04) by supplemental treatments. Wethers fed BF and COMBO supplements had decreased ($P \leq 0.04$) acetate AUC compared to wethers fed CON diet. However, wethers fed CAP had a tendency (P = 0.08) to have a decreased acetate AUC compared to wethers fed CON. However, glucose and insulin AUC were not different (P = 0.80 and 0.84, respectively) among supplemental treatments.

Rumen ammonia concentration was affected ($P < 0.01$; Table 6) by supplementation. Wethers fed BF had greater ($P < 0.01$) ruminal ammonia concentration compared to wethers fed CAP and CON and tended (P = 0.10) to be greater than wethers fed COMBO diet. Control and CAP supplemental treatments did not differ (P = 0.84) in ruminal ammonia concentration and were less ($P < 0.01$) than COMBO. Total VFA concentration had a tendency (P = 0.10) to be impacted by supplement. Ruminal acetate concentration was not influenced (P = 0.61) by supplemental treatments. However, supplementation had an effect ($P < 0.01$) on ruminal propionate concentration. Wethers receiving CAP and COMBO had greater ($P < 0.01$) ruminal propionate concentration than CON and BF. Control and BF did not differ (P = 0.66) in propionate concentration. In addition, ruminal butyrate concentration did not differ (P = 0.76) among supplementation treatments.

Table 2. Effect of protein and glucogenic precursor supplementation on urinary energy and nitrogen losses in wethers consuming a forage-based diet

Urinary loss	Treatment[1]				SEM	*P*-value
	CON	CAP	BF	COMBO		
Urinary energy, Mcal	0.80	0.98	1.08	1.04	0.19	0.71
Urinary nitrogen, g	42.0[b]	56.4[b]	106.0[a]	88.4[a]	15.8	0.02

[a,b]Means with differing superscripts are different ($P < 0.05$).
[1]Supplements were designed to supply increasing levels of glucogenic potential (GP): (1) basal diet only (CON; 0 g of GP), (2) 40 g of NutroCal (CAP; Ca-propionate, 30 g of GP; Kemin Industries Inc., Des Moines, IA), (3) 70 g of blood meal and 100 g of feather meal (BF; 40 g of GP), or (4) combination of CAP and BF (COMBO; 70 g of GP).

Table 3. Effect of protein and glucogenic precursor supplementation on circulating serum metabolites of wethers consuming a forage-based diet

	Treatment[1]					*P*-values		
Measurements	CON	CAP	BF	COMBO	SEM	Trt	Time[2]	Trt x Time
Glucose, mg/dL	55.4	54.1	55.8	55.8	1.93	0.87	< 0.01	0.57
SUN[3], mg/dL	11.3[b]	10.6[b]	25.9[a]	25.5[a]	1.12	< 0.01	< 0.01	0.23
NEFA[4], mg/dL	3.06	2.86	2.79	2.73	0.12	0.27	< 0.01	0.45

[a,b]Means with differing superscripts are different ($P < 0.05$).
[1]Supplements were designed to supply increasing levels of glucogenic potential (GP): (1) basal diet only (CON; 0 g of GP), (2) 40 g of NutroCal (CAP; Ca-propionate, 30 g of GP; Kemin Industries Inc., Des Moines, IA), (3) 70 g of blood meal and 100 g of feather meal (BF; 40 g of GP), or (4) combination of CAP and BF (COMBO; 70 g of GP).
[2]Time = blood sample was taken pre-prandial at 0730 and 4 h post-prandial at 1230 h.
[3]SUN, serum urea N.
[4]NEFA, non-esterified fatty acids.

Table 4. Effect of protein and glucogenic precursor supplementation on serum amino acid concentrations of wethers consuming a forage-based diet

Amino acid, nMol/mL	Treatment[1]				SEM	*P*-value
	CON	CAP	BF	COMBO		
Alanine	4.76[a]	4.40[a]	3.75[b]	3.60[b]	0.33	< 0.01
Glycine	9.10[b]	9.58[b]	11.31[a]	11.72[a]	0.84	< 0.01
Valine	6.42[b]	6.10[b]	16.86[a]	15.85[a]	1.13	< 0.01
Leucine	1.34[b]	1.26[b]	3.70[a]	3.47[a]	0.26	< 0.01
Isoleucine	1.44[b]	1.38[b]	1.90[a]	1.84[a]	0.17	< 0.01
Threonine	1.95[b]	1.88[b]	2.65[a]	2.62[a]	0.22	< 0.01
Serine	1.95[b]	2.12[b]	3.50[a]	3.76[a]	0.14	< 0.01
Proline	1.30[b]	1.33[b]	2.47[a]	2.41[a]	0.18	< 0.01
Asparagine	0.91[b]	0.88[b]	1.22[a]	1.17[a]	0.10	< 0.01
Methionine	0.20[a]	0.19[a]	0.16[b]	0.17[ab]	0.02	0.04
Phenylalanine	0.89[b]	0.91[b]	1.22[a]	1.31[a]	0.08	< 0.01
Glutamine	6.50	5.50	6.99	6.67	0.95	0.17
Ornithine	1.47[b]	1.50[b]	2.53[a]	2.42[a]	0.36	< 0.01
Lysine	4.44[b]	4.20[b]	5.12[a]	5.28[a]	0.45	< 0.01
Histidine	2.03	1.91	2.28	2.12	0.29	0.34
Tyrosine	0.91	0.96	1.00	1.05	0.08	0.13
Tryptophan	1.23[b]	1.35[a]	1.35[a]	1.37[a]	0.08	0.04
Total AA	131.5[b]	125.8[b]	150.9[a]	148.1[a]	9.43	< 0.01
Essential AA	19.45[b]	18.96[b]	34.78[a]	33.66[a]	2.34	< 0.01
Non-Essential AA	113.2	107.9	117.3	115.6	7.91	0.40
Glucogenic AA	36.69[b]	36.01[b]	52.48[a]	51.66[a]	3.72	< 0.01
Ketogenic AA	5.76[b]	5.51[b]	8.80[a]	8.75[a]	0.67	< 0.01
Gluco-Ketogenic AA	4.40[b]	4.53[b]	5.39[a]	5.49[a]	0.33	< 0.01

[a,b]Means within a row with different superscripts differ ($P < 0.05$).
[1]Supplements were designed to supply increasing levels of glucogenic potential (GP): (1) basal diet only (CON; 0 g of GP), (2) 40 g of NutroCal (CAP; Ca-propionate, 30 g of GP; Kemin Industries Inc., Des Moines, IA), (3) 70 g of blood meal and 100 g of feather meal (BF; 40 g of GP), or (4) combination of CAP and BF (COMBO; 70 g of GP).

Table 5. Effect of protein and glucogenic precursor supplementation on acetate tolerance test for wethers consuming a forage-based diet

Measurement	Treatment[1]				SEM	*P*-value
	CON	CAP	BF	COMBO		
Acetate half-life, min	39	33	26	31	6	0.39
Acetate AUC[2]	298[a]	242[ab]	205[b]	228[b]	24	0.04
Glucose AUC	310	310	326	316	15	0.80
Insulin AUC	32	33	37	32	5	0.84

[a,b]Means with differing superscripts are different ($P < 0.05$).
[1]Supplements were designed to supply increasing levels of glucogenic potential (GP): (1) basal diet only (CON; 0 g of GP), (2) 40 g of NutroCal (CAP; Ca-propionate, 30 g of GP; Kemin Industries Inc., Des Moines, IA), (3) 70 g of blood meal and 100 g of feather meal (BF; 40 g of GP), or (4) combination of CAP and BF (COMBO; 70 g of GP).
[2]AUC, area under curve.

Ruminal acetate to propionate (A:P) ratio was affected ($P < 0.01$) by supplement. Wethers fed CAP had a reduced ($P < 0.01$) A:P ratio than those receiving BF or CON but did not differ ($P = 0.58$) in A:P ratio from COMBO. Control and BF treatments did not differ ($P = 0.77$) in A:P ratio.

DISCUSSION

Protein supplementation has been shown to increase intake and digestibility of low-quality forages (Owens et al., 1991) and increase rate of fermentation and microbial protein flow to the small intestine (Kunkle et al., 2000). Supplementation of RUP in both BF and COMBO increased DM and OM total tract digestibility compared to treatments without RUP supplementation. The similar total tract OM digestibility between CAP and CON agrees with results by others that observed no differences in OM or NDF digestibility when supplementing with varying glucogenic precursor sources (Vanhatalo et al., 2003; Sanchez et al., 2014). Increased DM and OM digestibility with RUP supplementation has been shown in sheep consuming low-quality forages (Ferrell et al., 1999). In the current study, NDF digestibility was not

Table 6. Effect of protein and glucogenic precursor supplementation on volatile fatty acids and ammonia concentration of wethers consuming a forage-based diet

Measurement	Treatment[1]				SEM	*P*-value
	CON	CAP	BF	COMBO		
Total VFA[2], mMol	49.7	58.1	45.5	56.3	3.91	0.10
Acetate, %	70.6	54.7	69.2	55.4	2.34	0.61
Butyrate, %	6.7	5.5	6.6	5.3	0.26	0.76
Propionate, %	20.3[b]	37.9[a]	20.1[b]	36.1[a]	1.51	< 0.01
A:P ratio[3]	3.51[a]	1.56[b]	3.47[a]	1.63[b]	0.10	< 0.01
Ammonia, mg/dL	5.30[b]	5.17[b]	9.70[a]	8.62[a]	0.46	< 0.01

[a,b]Means with differing superscripts are different ($P < 0.05$).
[1]Supplements were designed to supply increasing levels of glucogenic potential (GP): (1) basal diet only (CON; 0 g of GP), (2) 40 g of NutroCal (CAP; Ca-propionate, 30 g of GP; Kemin Industries Inc., Des Moines, IA), (3) 70 g of blood meal and 100 g of feather meal (BF; 40 g of GP), or (4) combination of CAP and BF (COMBO; 70 g of GP).
[2]VFA, volatile fatty acid.
[3]Acetate:propionate ratio.

influenced by supplemental treatments. In agreement, Sawyer et al. (2012) reported that protein supplementation (RUP or RDP) did not influence NDF digestibility of a low-quality forage. In contrast to NDF digestibility in the current study, supplementation did influence ADF digestibility. Greatest ADF digestibility was observed in wethers consuming the BF supplementation but was not different from wethers fed COMBO. Wethers receiving no supplementation (CON) had an ADF digestibility intermediate to COMBO and CAP. This suggests that the RUP has a greater effect on ADF digestibility compared to propionic salt supplementation only.

Urinary N excretion was increased in wethers receiving BF and COMBO compared to CON and CAP. In agreement, Salisbury et al. (2004) reported increased urinary N excretion in lambs receiving supplemental RUP compared to their counterparts receiving no supplement. In contrast to the results in this study, postruminal infusion of propionate in sheep consuming a forage diet has resulted in greater N retention as a result of increased urea N recycling (Kim et al., 1999). Similarly, Agarwal et al. (2015) reported a N retention increase of 10% to 16% of apparent digested N when wether lambs were supplemented with sodium propionate. While not statistically significant, the numerical decrease between BF and COMBO is similar to results from Ørskov et al. (1999) who observed reductions in urinary N excretion when glucose was infused intragastrically.

Serum glucose concentrations were similar among all treatments in this study. In agreement, Jenkins and Thonney (1988) reported no difference in plasma glucose concentration with increasing GP of diet. In contrast, Mulliniks et al. (2011) reported that circulating serum glucose concentrations increased linearly with increased consumption of glucogenic precursors in young, lactating range cows grazing low-quality forage. Reed et al. (2007) reported no difference in blood glucose concentrations among steers being supplemented with increasing levels of protein. However, Reed et al. (2007) did observe an increase in SUN due to protein supplementation. As expected and due to increased N intake in the current study, circulating SUN concentrations were greater in wethers receiving RUP supplementation.

Once N requirements of the ruminant have been met, additional AA can contribute to protein accretion or be oxidized (Lobley, 1992). Amino acids are estimated to contribute 5%–7% of glucose produced through gluconeogenesis by the ruminant (Engelking, 2015). Due to the lack of glucose absorbed through the small intestine of a ruminant, gluconeogenesis is a continual process occurring in the liver of ruminants in constant need of substrates. In a review, Bell et al. (2000) states that in high-producing dairy cows, protein catabolism is greater than 1,000 g/d to meet the need for amino acids and glucose. Infusion of casein as a glucogenic precursor source resulted in increased essential AA and branch chained AA in lactating dairy cows (Vanhatalo et al. 2003). Similar results were reported in the current study with increased circulating serum essential and glucogenic AA concentrations in wethers receiving BF and COMBO supplementation. Optimal metabolizable energy balance was reported when a combination of glucose + casein was infused in ewes during late gestation (Barry and Manley, 1985). This suggests that a balance of glucose and N may need to be met to maximize AA efficiency. Infusing low levels of glucose into fasted steers decreased urinary N excretion, suggesting that inclusion of glucose or a glucogenic precursor would create a protein sparing effect (Ørskov and MacLeod, 1990). This protein sparing effect may act in two ways: by allowing AA or N to go towards accretion instead of energy or prevent catabolism of muscle for energy. Although both BF and COMBO had greater AA concentrations than CAP, the addition of calcium propionate to COMBO did not further improve the AA utilization above BF in the current study.

Acetate clearance can be used as an indication of the GP of a diet and reveals efficiency of oxidative metabolism (Cronjé et al., 1991). Acetate half-life was not influenced by supplemental treatments in the current study. In contrast, Mulliniks et al. (2011) reported increased clearance rate of acetate when increasing levels of GP were supplemented. Acetate half-life in the current study was similar to those reported in previous studies where animals were consuming low-quality forage diets (Cronjé et al., 1991; Endecott et al., 2012). However, acetate half-life has been reported to be as quick as 10 min (Preston and Leng, 1987), approximately 2.5 to 3 times quicker than reported in this current study, suggesting that opportunities exist to increase oxidative metabolism. The decrease observed in acetate AUC for BF and COMBO compared to the CON suggests that meeting RUP requirements improved acetate utilization. A

tendency for CAP to have a decreased acetate AUC compared to CON suggests that the increased GP of the diet will improve acetate uptake, but RUP requirements may need to be met to improve acetate utilization. The tendency for improved acetate utilization with increasing levels of GP and no change in circulating serum glucose concentration suggests that wethers can maintain glucose concentration in circulating serum even with increased dietary GP (Kaneko, 1989).

Total VFA concentration tended to be increased in CAP and COMBO fed wethers. Similarly, Sanchez et al. (2014) did not observe a difference in total VFA concentration when supplementing propionate as calcium propionate or *Propinobacterium* in heifers fed low-quality hay. Calcium propionate supplementation in the current study resulted in greater concentration of ruminal propionate. This resulted in a reduced A:P ratio for CAP and COMBO supplemental treatments. Other studies supplementing propionate have also shown an increase in ruminal propionate production and decrease of A:P ratio (van Houtert and Leng, 1993; Sanchez et al., 2014). Although not observed in the current study with BF supplementation, others have observed an increase in ruminal propionate concentrations and decrease A:P ratio in supplementation of RUP to ruminants consuming roughages (DelCurto et al., 1990; Salisbury et al., 2004).

Ruminal ammonia N concentration was greatest for BF and COMBO, which is to be expected due to the nature of the protein supplementation resulting in greater N intake. Increased rumen ammonia N due to protein supplementation has been previously observed in protein supplementation on low-quality forages (Salisbury et al., 2004; Reed et al., 2007). DelCurto et al. (1990) supplemented steers consuming roughage with varying levels of protein and energy and observed an increase in ruminal ammonia N concentration for those being supplemented with high levels of protein. Sanchez et al. (2014) observed a tendency for ruminal ammonia concentration to be decreased in diets supplemented with a propionate source compared to the control. In contrast, the current study had similar ruminal ammonia concentrations between CON and CAP at 5.30 and 5.17 mg/dL, respectively.

IMPLICATIONS

Results from this study would suggest that supplementing protein increases circulating serum concentrations of glucogenic AA, which can be utilized in gluconeogenesis increasing supply of glucose. Increasing glucogenic precursors with rumen undegradable protein resulted in improved efficiency of nutrient and acetate utilization in growing wethers fed a moderate-quality forage. Providing propionate salts as a supplement in wethers consuming moderate-quality forage resulted in an increased ruminal propionate concentration resulting in a decreased A:P ratio. However, the increased propionate supply by providing propionate salts did not result in a protein sparing impact or increased N retention.

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