Effect of supplemental rumen undegradable protein and glucogenic precursors on digestibility and energy metabolism in sheep

Tasha M. King,[†] Joslyn K. Beard,[†] Mitchell M. Norman,[†] Hannah C. Wilson,[†] Jim M. MacDonald,[†] and J. Travis Mulliniks[‡,1]

[†]Department of Animal Science, University of Nebraska – Lincoln, Lincoln, NE 68583; and [‡]West Central Research and Extension Center, University of Nebraska, North Platte, NE 69101

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INTRODUCTION

Supplementation of glucogenic precursors and rumen undegradable (RUP) may increase production responses due to improved efficiencies of nutrient utilization. In forage-based production systems, ruminal production of acetate compared with propionate can result in imbalanced acetate:propionate ratio (McCollum and Galyean, 1985; Cronjé et al., 1991), resulting in negative modifications in energy metabolism. Ferrell et al. (1999) observed greater digestible energy and available amino acids when a combination of energy and RUP was supplemented to a diet of low-quality hay. Providing growing lambs with supplemental RUP consuming low-protein forage diets resulted in increased feed intake, rate of growth, and improved feed efficiency with an additional growth response observed with due to increase of postruminal glucose (Kempton et al., 1978). However, the additional growth response due to postruminal increase of glucose was only observed once RUP requirements were met. In addition, continuous duodenal infusion of glucose resulted in increased growth rate and improved feed conversion for lambs consuming a low-protein diet regardless of supplemental bypass protein (Leng et al., 1978). Increasing postruminal supply of propionate has been shown to increase fatty acid and acetate hindlimb uptake of growing lambs (Majdoub et al., 2003). Similarly, Mulliniks et al. (2011) reported that increasing glucogenic precursors with RUP and 40 g/d calcium propionate enhanced energy metabolism by increasing the rate of acetate is metabolized in young, lactating range beef cows grazing dormant forage. Our hypothesis was that providing increased levels of glucogenic precursors would increase acetate utilization and efficiency in growing lambs 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, and energy utilization of a forage 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.

Sixteen crossbred wethers (49.1 ± 4.7 kg initial BW) were utilized to determine forage digestibility and acetate utilization. Wethers were sorted into four blocks based on initial BW in a 4 × 4 replicated Latin Square design. Wethers were randomly assigned within each period to one of four treatments to provide 0, 30, 40, and 70 g of GP: 1) control (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

[1]Corresponding author: travis.mulliniks@unl.edu
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blood meal and 100 g of feather meal (BF; 92.8% crude protein [CP], 61.3% rumen undegradable protein [RUP], 40 g of GP), or 4) combination of GP and BF (COMBO; 70 g of GP). 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]) was ground with a tub grinder through a 2.5-cm screen and fed at 2% BW. An ounce of commercial mineral + vitamin premix was offered daily to all wethers.

Periods were 21 d in length allowing for 12 d of diet adaptation, 5 d of total fecal collection, and 4 d for metabolism collections. Wethers were fed brome grass hay 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 to 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 and 10% of each fecal collection was retained for data analysis and stored at 2.8°C until the end of the period. Five per cent of each fecal collection was composited by period and freeze dried. Feed refusals were taken days 10 to 15 and feed samples taken 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).

An acetate tolerance test (ATT) was conducted on day 17 to analyze acetate clearance as affected by GP of 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 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-4 centrifugal device, Millipore Corp., Burlington, MA). Filtered serum was mixed at a 5:1 ratio with 25% metaphosphoric acid containing 2 g/L or 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 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 was taken preprandial at 0730 h and 4 h postprandial at 1230 h via jugular venipuncture and saphenous venipuncture into serum separator vacuum tubes (Corvac, Kendall Healthcare, St. Louis, MO). Serum samples were analyzed for glucose, urea N (SUN), and amino acid concentrations. Glucose and SUN were also analyzed by the BORC lab of NPOD.

Statistical Analysis

Total tract digestibility data were analyzed as a Latin Square design using the MIXED procedure (SAS Inst. Inc., Cary, NC). Data were analyzed with lamb serving as experimental unit, with supplementation type and period set as fixed effects. Acetate half-lives were estimated for each animal by regressing the logarithmically transformed acetate concentrations over time (Kaneko, 1989). Area under the curves were determined for acetate and glucose using the trapezoidal summation method. Serum data were analyzed as repeated measures with time of blood collection serving as repeated factor. Significance level was set at $P \leq 0.05$.

RESULTS

Digestibility of DM and OM were greater ($P < 0.01$; Table 1) for wethers receiving BF and COMBO supplementation compared with the CAP and CON treatments. Treatments had no effect ($P = 0.93$) on NDF_{om} digestibility. Wethers on BF supplementation had greatest ($P = 0.02$) ADF digestibility, whereas CAP had the lowest ADF digestibility. Both CON and COMBO ADF digestibilities were intermediate with COMBO having similar ADF digestibility to both BF and CON ($P \geq 0.06$). Total intake of DM, OM, and ADF increased ($P < 0.01$) with increase GP supplementation, which was expected due to total intake including basal and supplementation amounts.

Supplementation had no effect on circulating glucose concentration ($P \geq 0.47$; Table 2) in samples taken from both jugular and saphenous veins. Addition of RUP supplementation in BF and

Table 1. Total tract digestibilities for wethers supplemented with glucogenic precursors fed a forage-based diet

	Supplementation treatment					
	CON[1]	CAP[2]	BF[3]	COMBO[4]	SEM	*P*
DM						
Total intake[5], kg	5.17[d]	5.26[c]	5.82[b]	6.07[a]	0.11	<0.01
Digestibility, %	37.4[b]	36.6[b]	43.0[a]	42.9[a]	0.98	<0.01
OM						
Total intake,kg	4.73[d]	4.84[c]	5.55[b]	5.66[a]	0.10	<0.01
Digestibility, %	42.6[b]	43.6[b]	49.8[a]	49.8[a]	1.11	<0.01
$NDFD_{om}$[6]						
Total intake, kg	3.50	3.50	3.49	3.49	0.08	0.98
Digestibility, %	44.8	45.2	45.8	45.3	1.28	0.93
ADF						
Total intake, kg	2.32[b]	2.31[b]	2.47[a]	2.48[a]	0.07	<0.01
Digestibility, %	35.6[bc]	35.4[c]	39.2[a]	38.5[ab]	1.31	0.03

[a–d]Means within a row with different superscripts differ ($P < 0.05$).

[1]CON = No supplementation.

[2]CAP = Supplementation of 40 g of NutroCal (Kemin Industries Inc., Des Moines, IA).

[3]BF = Supplementation of 70 g of blood meal + 100 g of feather meal.

[4]COMBO = Supplementation of 40 g of calcium propionate + 70 g of blood meal + 100 g of feather meal.

[5]Total intake = basal diet + supplementation.

[6]NDF_{om} = ash-free NDF.

Table 2. Impact of glucogenic precursor supplementation on serum metabolites of wethers fed a forage-based diet

	Supplementation treatment					*P*		
Measurements	CON[1]	CAP[2]	BF[3]	COMBO[4]	SEM	Trt	Time	Trt x Time
Jugular glucose mg/dL	55.4	54.1	55.8	55.8	1.93	0.87	< 0.01	0.57
Saphenous glucose mg/dL	56.7	54.8	55.5	58.0	1.84	0.47	< 0.01	0.16
Jugular SUN[5], mg/dL	11.3[b]	10.6[b]	25.9[a]	25.5[a]	1.12	< 0.01	< 0.01	0.23
Saphenous SUN, mg, dL	11.6[b]	11.2[b]	25.7[a]	25.2[a]	1.09	< 0.01	< 0.01	0.13

[a,b]Means with differing superscripts are different ($P < 0.05$).

[1]CON = No supplementation.

[2]CAP = Supplementation of 40 g of NutroCal (Kemin Industries Inc., Des Moines, IA).

[3]BF = Supplementation of 70 g of blood meal + 100 g of feather meal.

[4]COMBO = Supplementation of 40 g of calcium propionate + 70 g of blood meal + 100 g of feather meal.

[5]SUN = Serum urea N.

Table 3. Effect of supplement on acetate tolerance test for wethers consuming a forage-based diet supplemented with glucogenic precursors

	Supplementation treatment					
Acetate tolerance test response	CON[1]	CAP[2]	BF[3]	COMBO[4]	SEM	*P*
Acetate half-life, min	39	33	26	31	6	0.39
Acetate AUC[5]	298[a]	242[ab]	205[b]	228[b]	24.3	0.04
Glucose AUC	310	310	326	316	15.7	0.80

[a,b]Means with differing superscripts are different ($P < 0.05$).

[1]CON = No supplementation.

[2]CAP = Supplementation of 40 g of NutroCal (Kemin Industries Inc., Des Moines, IA).

[3]BF = Supplementation of 70 g of blood meal + 100 g of feather meal.

[4]COMBO = Supplementation of 40 g of calcium propionate + 70 g of blood meal + 100 g of feather meal.

[5]AUC = Area under curve.

COMBO increased SUN compared with CON and CAP ($P < 0.01$). A time effect was observed ($P < 0.01$) with serum concentrations being lower preprandial compared with serum concentrations taken postprandial.

Acetate half-life was not different ($P = 0.39$; Table 3) among supplemental treatments. Acetate AUC was influenced ($P = 0.04$) by supplemental treatments. Wethers fed BF and COMBO had decreased ($P \leq 0.04$) acetate AUC compared with CON wethers. Wethers fed CAP had a tendency ($P = 0.08$) to have a decreased AUC compared with CON. However, glucose AUC was not different ($P = 0.80$) among supplemental treatments.

DISCUSSION

Supplementation of RUP increased DM and OM total tract digestibility in BF and COMBO compared with CAP and CON. Increased DM and OM digestibility due to RUP supplementation has been shown in sheep consuming low-quality forages (Ferrell et al., 1999). In the current study, NDF digestibility was not influenced by treatment; however, an effect of supplementation on ADF digestibility was observed. Greatest ADF digestibility was observed in wethers receiving BF supplementation but similar ADF digestibility of wethers receiving 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 with propionic salt supplementation. In contrast, Reed et al. (2007) supplemented steers on low-quality grass hay and reported no differences in total tract digestibility between no supplement and RUP supplement groups. However, RUP-supplemented steers did have a tendency for improved ruminal ADF digestibility compared with the no supplement control.

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 circulating serum glucose concentrations increased linearly with increased consumption of glucogenic precursors. As expected, circulating SUN concentrations increased in wethers receiving RUP supplementation. Similarly, SUN concentrations were greater in wethers consuming a low-quality hay with RUP supplementation compared with no supplement (Ferrell et al., 1999).

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 GP of diets in the current study. In contrast, Mulliniks et al. (2011) observed increased clearance of acetate when increasing levels of GP were supplemented with RUP. Half-lives of acetate were similar to those observed 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. Decrease in acetate AUC for BF and COMBO compared with the CON suggests that meeting RUP requirements improved acetate utilization. A tendency for CAP to have a decreased acetate AUC compared with 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.

IMPLICATIONS

Results from this study would suggest supplementing additional glucogenic precursors in the form of RUP improved efficiency of nutrient and acetate utilization in growing lambs fed a moderate-quality hay. However, no additive effect of supplementing propionate salts and RUP (COMBO) were observed in this study. Nutrient quality of hay fed in this study has potential for increased acetate:propionate ratio, which could explain the decreased responses observed from supplementation of glucogenic precursors.

LITERATURE CITED

Cronjé, P. B., J. V. Nolan, and R. A. Leng. 1991. Acetate clearance rate as a potential index of the availability of glucogenic precursors in ruminants fed on roughage-based diets. Br. J. Nutr. 66:301–312. doi:10.1079/BJN199910033

Endecott, R. L., S. H. Cox, C. M. Rubio, C. A. Löest, D. E. Hawkins, and M. K. Petersen. 2012. Effects of supplements with increasing glucogenic precursor content on reproduction and nutrient utilization in young postpartum range cows. Livestock Sci. 145:109–118. doi:10.1016/j.livsci.2012.01.004

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

Goetsch, A. L., and M. L. Galyean. 1983. Influence of feeding frequency on passage of fluid and particulate markers in steers fed a concentrate diet. Can. J. Anim. Sci. 63:727–730. doi:10.4141/cjas83-084

Jenkins, T. C., and M. L. Thonney. 1988. Effect of propionate level in a volatile fatty acid salt mixture fed to lambs on weight gain, body composition and plasma metabolites. J. Anim. Sci. 66:1028–1035. doi:10.2527/jas1988.6641028x

Kaneko, J. J. 1989. Carbohydrate metabolism and its diseases. In: J. J. Kaneko, editor, Clinical biochemistry of domestic animals, 4th edn. San Diego (CA): Academic Press. p. 44–85.

Kempton, T. J., M. K. Hill, and R. A. Leng. 1978. The effects of varying bypass amino acid and glucose availability on lamb growth and wool growth. Proc. Aust. Soc. Anim. Prod. 12:143.

Leng, R. A., S. Economides, and F. M. Ball. 1978. The effect on growth of supplying glucose continuously into the duodenum of lambs on low-protein diets. Proc. Aust. Soc. Anim. Prod. 12:134.

Majdoub, L., M. Vermorel, and I. Ortigues-Marty. 2003. Intraruminal propionate supplementation modifies hindlimb energy metabolism without changing the splanchnic release of glucose in growing lambs. Br. J. Nutr. 89:39–50. doi:10.1079/BJN2002740

McCollum, F. T., and M. L. Galyean. 1985. Influence of cottonseed meal supplementation on voluntary intake, rumen fermentation and rate of passage of prairie hay in beef steers. J. Anim. Sci. 60:570–577. doi:10.2527/jas1985.602570x

Mulliniks, J. T., M. E. Kemp, S. H. Cox, D. E. Hawkins, A. F. Cibils, D. M. VanLeeuwen, and M. K. Petersen. 2011. The effect of increasing amount of glucogenic precursors on reproductive performance in young postpartum range cows. J. Anim. Sci. 89:2932–2943. doi:10.2327/jas.2010–3198

Preston, T. R., and R. A. Leng. 1987. Matching ruminant production systems with available resources in the tropics and subtropics. Queensland (Australia): Penambul Books.

Reed, J. J., M. R. O'Neil, G. P. Lardy, K. A. Vonnahme, L. P. Reynolds, and J. S. Caton. 2007. Effect of undegradable intake protein supplementation on intake, digestion, microbial efficiency, in situ disappearance, and plasma hormones and metabolites in steers fed low-quality grass hay. J. Anim. Sci. 85:1092–1101. doi:10.2527/jas2006-652

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2