Corn silage rumen undegradable protein content and response of growing calves to rumen undegradable protein supplement[1]

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ABSTRACT: The amount of rumen undegradable protein (RUP) in corn silage and the extent to which it is digested in the small intestine are uncertain. Three studies were conducted to determine RUP content of corn silage, and the effects of supplementing increasing concentrations of RUP on the growing performance of calves fed a corn silage diet. Experiments 1 and 2 used in situ methods to evaluate the RUP content of corn silage. In experiment 1, corn silages harvested at 37% or 42% dry matter (DM) were compared. In experiment 2, dry-rolled corn (89.4% DM) was reconstituted to 75%, 70%, 65%, and 50% DM and ensiled in mini-silos (2,265 cm^3) for 30, 90, 180, or 270 d to simulate the corn grain within corn silage, dry-rolled corn is more mature than corn grain harvested in corn silage. Experiment 3 used 60 steers (275 kg initial body weight, SD = 18) in an 83-d growing study to evaluate the effects of supplementing 0.4%, 1.7%, 3.0%, 4.2%, or 5.5% RUP (% of diet DM) on performance. In experiment 1, RUP as a % of DM was not different between the two corn silages ($P \geq 0.12$), averaging 0.59% for samples refluxed in a neutral detergent solution (NDS) and 1.8% for samples not refluxed in NDS. Dry matter digestibility (DMD) also did not differ ($P \geq 0.19$), averaging 67.4%. In experiment 2, as moisture content of the corn grain increased, DMD increased linearly ($P < 0.01$) and RUP content decreased linearly ($P < 0.01$). The DMD increased quadratically ($P = 0.02$), whereas RUP content decreased linearly ($P < 0.01$) as days of ensiling increased. In experiment 3, there were no differences in DM intake (DMI; $P \geq 0.33$) among treatments for period 1 (d 1 to 37). However, average daily gain (ADG) and gain-to-feed ratio (G:F) both linearly increased ($P < 0.01$) as RUP supplement inclusion increased. There were no differences in DMI ($P \geq 0.16$), ADG ($P \geq 0.11$), or G:F ($P \geq 0.64$) in period 2 (d 38 to 83). For the overall growing period (d 1 to 83), a linear increase was observed for ending body weight ($P = 0.01$), ADG ($P < 0.01$), and G:F ($P < 0.01$) as RUP supplement inclusion increased from 0.4% to 5.5% of diet DM. The RUP content of corn silage is lower than previously reported. Data collected suggest the crude protein within corn silage is 13% RUP, and approximately 1/2 is digestible. The moisture content of corn silage at the time of harvest and the amount of time corn silage is stored continually impact protein availability. Supplementing growing calves fed corn silage with RUP will improve performance.

Key words: corn silage, crude protein, growing cattle, in situ, rumen undegradable protein

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INTRODUCTION

Corn silage allows cattle feeders to harvest the entire corn plant at the time of greatest forage quality. When formulating cattle diets, it is important to correctly account for the crude protein (CP), rumen degradable protein (RDP), and rumen undegradable protein (RUP) content of corn silage. Because laboratory techniques designed to measure RUP values of feedstuffs are specific to either forages or concentrates (Klopfenstein et al., 2001) and corn silage is a blend of both, quantifying RUP of corn silage is difficult.

Using in situ techniques (Broderick 1994) can lead to overestimation of RUP due to the N content of microbes attached to forage particles. Mass et al. (1999) used in situ neutral detergent insoluble nitrogen to remove microbial contamination and measure RUP content of forages; this technique was further validated by Haugen et al. (2006). This technique assumes all protein not soluble in neutral detergent solution (NDS) would escape rumen degradation and all protein soluble in NDS is rumen degradable or microbial. This may not hold true for concentrate feedstuffs, such as corn grain, where a portion of the protein may be RUP but is soluble in the NDS (Klopfenstein et al., 2001; Edmunds et al., 2012).

Therefore, 3 experiments were conducted to evaluate the RUP fractions of corn silage and response of growing calves to RUP supplement when fed a corn silage diet. The first experiment evaluated the RUP content of corn silage, with and without rinsing samples with NDS to remove microbial contamination. The second experiment evaluated the RUP content of reconstituted dry-rolled corn (rDRC), which was used as a model for the corn grain within corn silage. The objectives of these experiments were to determine the RUP content and RUP digestibility of corn silage and to measure the performance response of growing calves to RUP supplement when fed a corn silage diet.

MATERIALS AND METHODS

All facilities and management procedures described in the following experiments were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee.

Experiment 1—Corn Silage In Situ Measurements

Corn silage was harvested at the Eastern Nebraska Research and Extension Center (ENREC) near Mead, NE at 37% or 42% dry matter (DM) to mimic traditional corn silage harvest or a delayed harvest. Harvest began when the corn grain was at approximately ¾ milk line for the 37% DM corn silage (9/4/2014), and then delayed 2 wk coinciding with black layer formation for the 42% DM corn silage (9/16/2014). At harvest, silages were stored in large sealed silo bags (Ag-Bag, St. Nazianz, WI). After 28 d, 10 kg of each corn silage type was brought to the University of Nebraska metabolism area (Lincoln, NE), freeze-dried, and stored in dry storage until trial initiation. All analyses discussed were conducted on subsamples of this one sample taken after 28 d of fermentation.

The in situ procedure was modified from Vanzant et al. (1998). Small in situ Dacron bags (ANKOM Technology, Macedon, NY; 5 cm × 10 cm, 50 μm pore size) were labeled and weighed. Corn silage samples were ground through a 2-mm screen and 1.25 g sample was added to bags, with 16 replicate bags per sample. Dacron bags were sealed and then placed in a polyester mesh (lingerie) bag for ruminal incubation. Bags were ruminally incubated for either 20 or 30 h in 2 ruminally fistulated steers fed a 30% concentrate, 70% alfalfa hay diet. One-half of the bags were incubated in each steer. After the designated incubation time, bags were removed and frozen at –4 °C. One-half of the bags (eight bags per sample) were later thawed and taken through a simulated abomasal digestion consisting of incubating bags in a pepsin and HCl solution (1 g pepsin per L and 0.01 N HCl) maintained at 37 °C for 3 h. After simulated abomasal digestion, bags were inserted into the duodenal cannula of a steer consuming a concentrate diet. Bags were inserted into the duodenal cannula every 7 min (8 bags per d) and were retrieved from fecal matter on average 12 h after being placed in the cannula and then frozen at –4 °C. Bags that were not incubated immediately were stored at 5 °C for 1 to 3 d until duodenal insertion occurred.

Bags collected from the feces and bags from rumen incubation were machine rinsed. Rinsing consisted of 5 cycles with a 1-min agitation and a 2-min spin (Whittet et al., 2003). One-half of the bags (four bags per sample) were then refluxed in 100 °C NDS (Midland Scientific, Davenport, IA) for 1 h using an automated system (ANKOM 2000 Fiber Analyzer; ANKOM Technology) to remove microbial N contamination from the residue (Mass et al., 1999; Haugen et al., 2006). Bags were dried in a 60 °C forced-air oven for 24 h and weighed to determine DM disappearance. Concentration of N was measured in residue bags and the original corn silage sample (in duplicate) using the combustion method (AOAC, 1999; method 990.03) and an N analyzer (LECO FP-528; LECO Corp., St. Joseph, MO) to calculate CP content. The following equations were then used to determine DM digestibility (DMD), RUP as a % of CP, and RUP digestibility:

1) DMD, % = 100 × [1 – (residue remaining after rumen incubation, g of DM)/(corn silage initially incubated, g of DM)]
2) RUP, % of CP = 100 × [(residue, g of DM × % CP)/(original sample, g of DM × % CP)]
3) RUP digestibility, % = 100 × [(CP after rumen incubation, mg – CP after duodenal incubation, mg)/(CP after rumen incubation, mg)]

Data were analyzed using the mixed procedure of SAS, with experimental units consisting of four in situ bags, assigned randomly to group before rumen incubation. The model included 2 incubation times (20 and 30 h) and 2 feeds (37% and 42% DM corn silage). Steer was added as a random effect for the rumen incubated bags. It is important to note that only one steer was used for duodenal incubation; therefore, it was not added as a random effect. Significance was declared at $P \leq 0.05$ and tendencies are discussed at $P < 0.10$.

Experiment 2—Reconstituted Dry-Rolled Corn

DRC was brought from the feed mill located at the ENREC near Mead, NE to the University of Nebraska metabolism area. Using a food mixer (Model L-1000; Leland Southwest, Fort Worth, TX), different proportions of water and corn were mixed to reconstitute DRC to 50%, 65%, 70%, and 75% DM. To reach 50% DM, 20.0 kg water and 25.0 kg DRC (89.4% DM) were added to the mixer, mixed thoroughly, and then placed into an enclosed plastic tote overnight to allow the corn more time to absorb the water. The same procedure was repeated for the other DRC mixtures by varying the amount of water added; 9.4, 7.0, and 4.5 kg water was added to the 65%, 70%, and 75% DM mixtures, respectively. A 40% DM mixture was attempted by adding 31.0 kg of water; however, this was too much water and the DRC was not able to absorb it all. Reconstituting the corn was done to simulate the grain within corn silage. At corn silage harvest, the grain is drier than the forage. However, the corn kernels apparently absorb water from the forage during the ensiling process as unpublished data collected in our laboratory has shown kernels separated from ensiled corn silage to be 37% to 59% DM, depending on the DM of the corn silage, 35% and 42%, respectively. Corn kernels at corn silage harvest were 41% and 62% DM, respectively. The corn kernel continues to undergo changes from corn silage harvest until dry grain harvest (Pioneer, 2013); however, these changes are subtle. rDRC has been shown to be an appropriate model for high moisture corn (Benton et al., 2005); however, further research is warranted to determine if high moisture corn is an appropriate model for corn silage grain.

Once the DRC was reconstituted to its designated DM, it was packed into minipolyvinyl chloride silos (2,265 cm^3) using a packing density of 720 kg DM per m^3, which is representative of high moisture corn packing density used in the cattle industry (Lardy and Anderson, 2016). Silos were sealed with lids equipped with a gas release valve and stored for 30, 90, 180, or 270 d. There were three silos at each moisture level for every ensiling time (48 total silos).

On the designated day, silos were weighed, emptied, and sub-sampled for DM and CP analysis. Within 1 h of being opened, rDRC was weighed into Dacron in situ bags (ANKOM Technology; 10 cm × 20 cm, 50 µm pore size). To get 2.3 g DM content in each bag, different as-is amounts of rDRC were added to the bags based on the DM at which the corn was ensiled at. There were 4 in situ bags per steer (2) for each incubation time (2), therefore 16 bags per silo were made. Dacron bags were sealed and then placed in a polyester mesh (lingerie) bag for ruminal incubation. Bags were ruminally incubated for 20 or 30 h in 2 ruminally fistulated steers consuming a 30% concentrate diet. One-half of the bags were incubated in each steer. After the designated incubation time, bags were removed and rinsed in a washing machine. Rinsing consisted of 5 cycles of a 1-min agitation and a 2-min spin (Whittet et al., 2003). Bags were then dried in a 60 °C forced-air oven for 24 h and weighed to determine DM disappearance. The four replicated in situ bags for each treatment from each steer were then composited to have enough material for CP analysis. Residue in the bags and the original rDRC sample were measured for percent N (in duplicate) using the combustion method (AOAC, 1999; method 990.03) to calculate CP content. Rumen DMD and RUP content were calculated the same as in experiment 1.

Samples of the 50% DM rDRC ensiled for 180 d were used to estimate the fraction of protein in the grain that is not fermented in the rumen but is degraded by NDS. Forty in situ bags with 5 g of DM were ruminally incubated for 25 h in 2 ruminally fistulated cattle consuming a 30% concentrate diet, using the same protocol as described earlier for experiment 1. After the designated incubation time, bags were removed and rinsed in a washing machine using 5 cycles of a 1-min agitation and a 2-min spin (Whittet et al., 2003). Bags were then refluxed in 100 °C NDS (Midland Scientific, Davenport, IA) for 1 h. Bags were dried in a 60 °C forced-air oven for 24 h, allowed to air equilibrate for 12 h and weighed. Bags were then composited into four samples from each steer to have enough material for CP analysis.

There were 2 incubation times, 4 ensiling times, and 4 rDRC DM contents; therefore, data were analyzed as a 2 × 4 × 4 factorial using the mixed procedure of SAS. Experimental unit consisted of four in situ bags and fixed effects included rumen incubation time (20 or 30 h), ensiling time (30, 90, 180, or 270 d), corn DM (50%, 65%, 70%, or 75%), and all two-way interactions. There were linear interactions for ensiling time and corn DM for RUP content; therefore, simple effects within corn DM across days ensiled are presented. Significance was declared at $P \leq 0.05$.

Experiment 3—Supplementing RUP to Growing Cattle on a Corn Silage Diet

An 83-d growing study was conducted at the ENREC near Mead, NE using 60 crossbred steers (body weight [BW] = 275; SD = 18 kg). All steers were individually fed using the Calan gate system (American Calan, Northwood, NH). Before trial initiation, steers were limit-fed for 5 d a diet of 50% alfalfa and 50% Sweet Bran (Cargill corn milling, Blair, NE) at 2% of BW to reduce gut fill variation (Watson et al., 2013). Steers were then weighed on 3 consecutive days in the morning before being fed and the average was used as initial BW (Stock et al., 1983). On the basis of initial BW, steers were assigned randomly to 1 of 5 treatments. Cattle were fed in two pens with at least four replications of each treatment in each pen.

Diets consisted of 85% corn silage (7.7% CP) and 15% supplement (DM basis). Corn silage was grown in the same location as corn silage harvested in experiment 1, but 1 yr later. Results from experiment 1 suggest growing cattle on high corn silage diets would be metabolizable protein (MP) deficient and providing RUP supplement would increase growth. Two supplements were formulated and blended together to equal 15% of diet DM. Five different blends were evaluated with 12 steers on each treatment. The supplements included urea, minerals, vitamins A-D-E, and a finely ground corn carrier that was replaced with RUP sources (Table 1). The RUP sources were SoyPass (LignoTech USA, Rothschild, WI; 50% CP; 75% RUP as % of CP) and Empyreal (Cargill corn milling; 75% CP; 65% RUP as % of CP). SoyPass is an enzymatically browned soybean meal and

Table 1. Diets formulated for individually fed growing steers (experiment 3)

	Treatment[a]				
Ingredient	0.4	1.7	3.0	4.2	5.5
Diet composition, % of diet DM					
Corn silage	85.0	85.0	85.0	85.0	85.0
RDP supplement[b]	15	11.25	7.5	3.75	0
RUP supplement[c]	0	3.75	7.5	11.25	15.0
Protein sources, % of diet DM					
Soypass	0	2.0	3.9	5.9	7.8
Empyreal	0	1.3	2.6	3.9	5.2
Urea	1.5	1.2	0.9	0.6	0.3
Nutrient content, % of diet DM					
CP	11.7	12.5	13.3	14.2	15.0
RUP[d]	1.05	2.35	3.65	4.85	6.15
NDF[e]	37.7	37.6	37.5	37.4	37.3
Fat	3.59	3.55	3.51	3.47	3.43

[a]Amount of RUP provided by the supplement, as a % of diet DM.

[b]RDP supplement consisted of 79.2% fine ground corn, 2.9% limestone, 2.5% tallow, 9.7% urea, 2.0% salt, 3.2% dicalcium phosphate, 0.32% trace mineral premix, 0.10% vitamin A-D-E, and 0.08% monensin (Rumensin; Elanco Animal Health, Greenfield, IN).

[c]RUP supplement consisted of 52% SoyPass (LignoTech USA, Rothschild, WI), 34.7% Empyreal (Cargill corn milling, Blair, NE), 1.9% fine ground corn, 3.2% limestone, 2.5% tallow, 1.7% urea, 2.0% salt, 1.5% dicalcium phosphate, 0.32% trace mineral premix, 0.10% vitamin A-D-E, and 0.08% monensin (Rumensin; Elanco Animal Health).

[d]RUP content calculated from measured values of supplements and 10% of the CP content of the corn silage (0.767% of DM), as measured in experiment 1.

[e]NDF, neutral detergent fiber.

Empyreal is a concentrated corn gluten meal. Supplement levels consisted of 0%, 3.25%, 6.5%, 9.75%, and 13% Soypass + Empyreal (RUP sources as a % of diet DM). The supplement in these treatments supplied 0.4%, 1.7%, 3.0%, 4.2%, and 5.5% RUP (DM basis).

All steers were implanted with 36 mg zeranol (Ralgro; Merck Animal Health, Summit, NJ) on day 0 and fed ad libitum once daily at 0800 h. Feed refusals were collected weekly, weighed, and then dried in a 60 °C forced-air oven for 48 h to calculate an accurate DMI for individual steers. Interim weights were taken at 0700 h (before feeding) on days 36 and 37 and shrunk 4% to account for gut fill. At the conclusion of the study, steers were again limit-fed a diet of 50% alfalfa hay and 50% Sweet Bran (DM basis) for 5 d at 2% of BW to reduce variation due to gut fill. Weights were then collected on 3 consecutive days and averaged to calculate an ending BW.

The MP balance and RDP balance were calculated for each diet during each time period based on measured BW, average daily gain (ADG), and dry matter intake (DMI; BCNRM, 2016). Feed values are those used in Watson et al. (2017) and microbial efficiency was 11.5%. Protein and RUP content of the corn silage was modeled using data gathered in experiments 1 and 2.

Data were analyzed using the mixed procedure of SAS as a randomized block design. The experimental unit was individual animal and dietary treatment was a fixed effect. Linear and quadratic contrasts were developed to determine the effect of RUP inclusion. Significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Experiment 1—Corn Silage In Situ

There were no interactions between DM of corn silage and time incubated in the rumen ($P \geq 0.30$); therefore, main effects will be discussed. The DMD was not affected by DM of the silage or amount of time incubated in the rumen ($P \geq 0.19$; Table 2). Contrary to this study, data presented by Andrae et al. (2001) suggest that silage harvested at black layer (42% DM in this study) is less digestible then corn silage harvested at half milk line (37% in this study). Maturation of corn is a continuum and estimates of black layer are a moving target, making comparison of trials evaluating different corn silages challenging (Pioneer, 2013).

For samples that were refluxed in NDS, RUP content, as a % of DM, was not different between the high- and low-moisture corn silages and was not affected by time incubated in the rumen ($P \geq 0.12$). As a % of CP, RUP content had a tendency ($P = 0.07$) to be less for the 42% DM corn silage and also had a tendency ($P = 0.07$) to be less for corn silage incubated for 30 h compared to corn silage incubated for 20 h in the rumen. There were no differences in RUP digestibility ($P \geq 0.56$) due to DM content of corn silage or hours incubated; averaging 32.3% RUP digestibility. For samples that were not refluxed in NDS, RUP content (% of DM or % of CP) and RUP digestibility were not affected by DM of the corn silage ($P \geq 0.15$) or duration of rumen incubation ($P \geq 0.32$). Corn silage not refluxed in NDS averaged 26.5% RUP, as a % of CP, whereas corn silage refluxed in NDS averaged

Table 2. Dry matter digestibility (DMD), rumen undegradable protein (RUP) content, and RUP digestibility of 37% and 42% DM corn silage measured using in situ techniques (experiment 1)

	Treatments[a]						
	37% DM corn silage		42% DM corn silage			*P* value[b]	
Item	20	30	20	30	SEM	DM	Time
CP, %	7.2	7.2	6.5	6.5	—	—	—
DMD, %	66.1	68.9	64.6	69.8	0.85	0.84	0.19
RUP, % of DM[c]	0.67	0.64	0.51	0.53	0.02	0.12	0.87
RUP, % of DM[d]	2.1	1.8	1.7	1.6	0.19	0.15	0.32
RUP, % of CP[c]	9.3	8.9	8.9	8.4	0.04	0.07	0.07
RUP, % of CP[d]	29.2	25.1	26.8	24.7	2.81	0.64	0.73
RUP digestibility, %[c]	32.2	32.3	32.6	31.9	0.59	0.94	0.56
RUP digestibility, %[d]	45.0	34.8	35.4	34.8	3.39	0.50	0.46

[a]Treatments consisted of 37% and 42% DM corn silage ruminally incubated for 20 or 30 h.

[b]There were no interactions between corn silage DM and incubation time ($P \geq 0.30$).

[c]RUP content and RUP digestibility of corn silage were corrected for microbial attachment by refluxing in neutral detergent solution.

[d]RUP content and RUP digestibility of corn silage were not corrected for microbial attachment by refluxing in neutral detergent solution.

8.9% RUP. Digestibility of RUP also was lower for corn silage refluxed in NDS, 32.3%, compared to 37.5% for corn silage not refluxed in NDS.

Kononoff et al. (2007) reported average RUP content of 19.3% of CP and 19.9% RUP digestibility for corn silage with 10.2% CP. However, from the trial description, it is not clear if samples were refluxed in NDS and rumen incubation time is not reported. Ali et al. (2016) evaluated 15 corn silage samples with 5.5% to 8.1% CP and reported an RUP content of 29.4% of CP; these samples were not refluxed in NDS. Using a single incubation time of 12 h, Ali et al. (2012) reported average RUP content of 44.5% of CP for 20 corn silage samples not refluxed in NDS and RUP digestibility of 32.8% to 50.3%. Similarly, von Keyserlingk et al. (1996) reported average RUP content of 12 corn silage samples (6.4% to 9.8% CP) to be 26.0% of CP and RUP digestibility to be 38.5%; samples were rumen incubated for 12 h and not refluxed in NDS. Clearly, some amount of protein is removed from corn silage during the NDS reflux step. This is likely both microbial N, which is not RUP, and soluble protein from the corn grain that escaped rumen degradation and should be accounted for as RUP (Klopfenstein et al., 2001; Edmunds et al., 2012). Therefore, the RUP content of corn silage is overestimated due to microbial attachment when samples are not refluxed in NDS and when corn silage samples are refluxed in NDS, RUP content and digestibility could be underestimated.

Experiment 2—Reconstituted Dry-Rolled Corn

There were no two-way interactions between rumen incubation time and DM content or ensiling time ($P \geq 0.08$) for RUP content. As corn was incubated longer in the rumen (20 h vs. 30 h), RUP content decreased ($P < 0.01$; data not shown). This was expected because incubating samples in the rumen longer gives rumen microbes a greater amount of time to ferment the feed. Similarly, there were no two-way interactions between rumen incubation time and DM content or ensiling time ($P \geq 0.16$; data not shown) for DMD. For all treatments, DMD was greater for samples ruminally incubated for 30 h compared to 20 h ($P \leq 0.05$). Therefore, all results presented next are a combination of the 20- and 30-h samples. Rumen incubation time was based on estimates of passage rate and digestibility for growing cattle corn silage diets. Corn silage is also a common feed ingredient in dairy cow diets, which would have greater passage rate, and thus a shorter rumen retention time.

There was a linear interaction between corn DM and days ensiled ($P < 0.01$) for RUP as a % of CP (Figure 1). For the driest corn (75% DM), there were no differences in RUP content across all four ensiling times averaging 26.8% ($P \geq 0.27$). The RUP content of the 70% DM corn was similar at the 30 and 180 d ensiling time points averaging 24.5% ($P = 0.24$) but lower after 90 d of ensiling (19.2%; $P < 0.01$). Corn ensiled for 270 d was intermediate (21.6%) compared to that ensiled for 90 d and 30 or 180 d. The 70% DM corn had less RUP than the 75% DM corn at the 30, 90, and 270 d ensiling time points ($P \leq 0.05$). The 65% DM corn had a quadratic ($P < 0.01$) decrease in RUP content as ensiling time increased and had less RUP than both the 75% and 70% DM corn at the 90 and 180 d ensiling time points ($P \leq 0.05$). The wettest corn (50% DM)

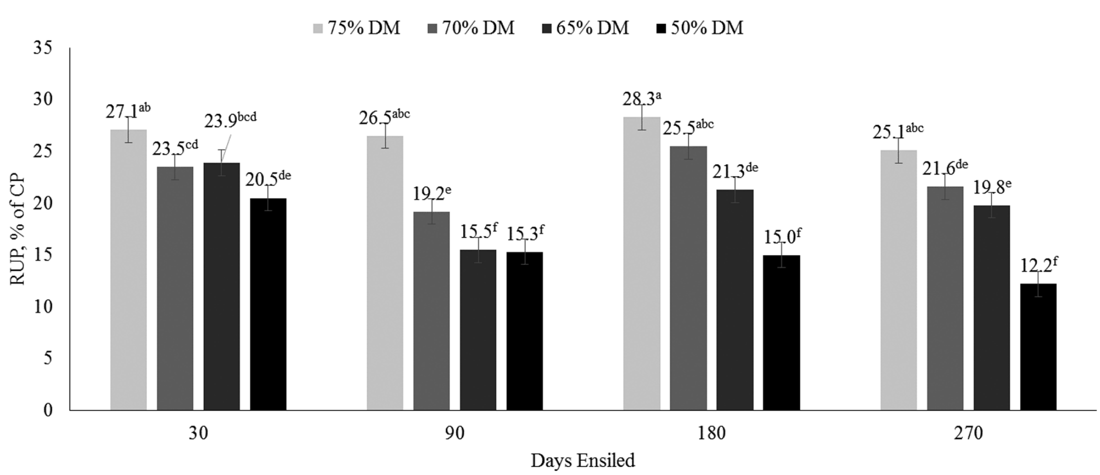

Figure 1. Effect of DM and days ensiled on RUP content of reconstituted dry-rolled corn, as a % of CP. Dry-rolled corn was reconstituted to 75%, 70%, 65%, and 50% DM and ensiled for 30, 90, 180, or 270 d (experiment 2). There was a day × DM interaction ($P < 0.01$); therefore, simple effects are shown. There was a linear increase in RUP content as DM content increased (linear contrast $P < 0.01$; quadratic contrast $P = 0.96$). There was a linear decrease in RUP content as days ensiled increased (linear contrast $P < 0.01$; quadratic contrast $P = 0.16$). [abcdef]Means without a common superscript differ ($P \leq 0.05$).

had a linear decrease ($P < 0.01$) in RUP content as ensiling time increased, and lower RUP content ($P \le 0.04$) than all other treatments at each ensiling time point except for the 65% DM corn at 90 d ($P = 0.91$) and both the 65% and 70% DM corn at 30 d ($P \ge 0.10$). Benton et al. (2005) also reported that as moisture content of rDRC and ensiling time increased, RUP content decreased.

There was not an interaction ($P = 0.62$) between corn DM and ensiling time for DMD; therefore, main effects are presented (Table 3). There was a linear increase ($P < 0.01$) in DMD as moisture content of the rDRC increased, going from 75.5% to 85.1%. There was a quadratic increase ($P = 0.02$) in DMD as ensiling days increased. The largest increase in DMD was from 30 to 90 d, with a 4.9% improvement ($P < 0.01$). The rDRC ensiled for 90 d had similar DMD as that ensiled for 270 d ($P = 0.19$). Benton et al. (2005) also reported that DMD increased as DM of corn decreased and ensiling time increased, with the greatest changes occurring within the first 28 d of ensiling. Other research studying the effect of moisture and ensiling time on digestibility and protein content of corn is limited, although effects of processing and particle size have been studied in depth (Galyean et al., 1981).

Samples of the 50% DM rDRC that had been ensiled for 180 d were used to estimate the amount of soluble protein that escapes rumen fermentation but is solubilized in NDS. Common protocol is to rinse forages in NDS after rumen incubation to remove microbes attached to feed particles (Mass et al., 1999; Haugen et al., 2006). However, the low neutral detergent fiber content of concentrates results in little microbial attachment (Dufour, 2017), and rinsing with NDS is not necessary. Furthermore, rinsing concentrates with NDS solublizes a portion of protein not fermented in the rumen (Klopfenstein et al., 2001; Edmunds et al., 2012). Therefore, the difference in CP content of corn grain rinsed with NDS or not after rumen incubation would be equal to soluble protein that escapes rumen fermentation but is solubilized in NDS.

For the 50% DM rDRC ensiled for 180 d, this fraction was 0.475% of DM (1.275 – 0.80 = 0.475). Assuming 59% CP in corn silage comes from the corn grain (Row et al., 2016), this means that 0.28% DM as RUP (0.475 × 0.59 = 0.28) needs to be added back to the corn silage RUP value obtained after rinsing in NDS (experiment 1). It is important to note that this would likely be lesser for grain in corn silage that is wetter than 50% DM. Data from experiment 1 showed the RUP content of the 37% DM corn silage (using the NDS technique) was 0.66% of DM (Table 2). Adding the two values (0.28 + 0.66) gives an RUP value of 0.94% of DM or 13.1% of CP. Assuming the NDS soluble protein is digestible in the intestines, the digestible RUP would be 0.49% of DM or 6.8% of CP (52% digestible). There are several assumptions in this calculation and a lack of replication of different corn silage samples, but overall it is clear that RUP content of corn silage is quite low and certainly lower than the 25% of CP that is commonly referenced (NASEM, 2016). It is also clear that neither analysis (with or without NDS) accurately measures the RUP value of corn silage. Further developments are needed.

Experiment 3—Supplementing RUP to Growing Cattle on a Corn Silage-based Diet

No differences in DMI were observed among treatments during the first (d 1 to 37) or second (d 38 to 83) half of the feeding period; nor was there a difference in DMI for the total feeding period ($P \ge 0.16$; Table 4). During the first half of the trial, ADG ($P < 0.01$) and gain-to-feed ratio (G:F) ($P < 0.01$) linearly increased as RUP inclusion increased in the diet. As RUP supplementation increased, ADG and G:F improved 44% in the first 37 d. There were no differences in ADG or G:F during the second half of the trial ($P \ge 0.11$; d 38 to 83). Numerically, there was a 9.6% linear improvement in ADG ($P = 0.11$).

Table 3. Effect of dry matter (DM) content and days ensiled on DM digestibility (DMD) of reconstituted dry rolled corn (rDRC; experiment 2) rDRC (experiment 2)[a]

	DM content of rDRC, %					*P* value[b]	
	75	70	65	50	SEM	Lin	Quad
DMD, %	75.5	78.1	81.3	85.1	0.83	< 0.01	0.82
	Number of days ensiled						
	30	90	180	270			
DMD, %	78.2	82.0	80.0	80.9	0.83	0.02	0.02

[a]Dry rolled corn (DRC) was reconstituted to 75%, 70%, 65%, or 50% DM and ensiled for 30, 90, 180, or 270 d.

[b]Linear and Quadratic orthogonal contrasts. The interaction between DM content and days ensiled was not significant for DMD ($P = 0.62$).

Table 4. Effects of rumen undegradable protein (RUP) supplement in corn silage-based growing diets on steer performance (experiment 3)

	Treatments[a]						*P* value[b]	
Item	0.4	1.7	3.0	4.2	5.5	SEM	Lin	Quad
Initial BW, kg	274	275	274	276	274	5.36	0.99	0.86
Ending BW, kg	367	378	376	392	389	7.55	0.01	0.17
Days 1 to 37								
Interim BW, kg	314	321	323	331	331	6.68	0.03	0.26
DMI, kg/d	7.0	7.2	7.3	7.4	7.1	0.25	0.68	0.33
ADG, kg	1.06	1.24	1.35	1.50	1.54	0.10	<0.01	0.06
G:F	0.150	0.172	0.185	0.201	0.216	0.010	<0.01	0.11
MP balance	–152	–122	–69	–31	+1	—	—	—
RDP balance	+153	+128	+98	+69	+34	—	—	—
Days 38 to 83								
DMI, kg/d	7.7	8.5	8.3	8.4	8.3	0.32	0.22	0.16
ADG, kg	1.15	1.25	1.14	1.33	1.26	0.06	0.11	0.28
G:F	0.149	0.147	0.137	0.158	0.152	0.007	0.64	0.86
MP balance	–166	–63	+35	+68	+126	—	—	—
RDP balance	+168	+151	+112	+78	+40	—	—	—
Days 1 to 83								
DMI, kg/d	7.3	7.8	7.8	7.9	7.7	0.27	0.34	0.19
ADG, kg	1.11	1.25	1.23	1.40	1.39	0.05	<0.01	0.03
G:F	0.152	0.160	0.158	0.177	0.181	0.005	< 0.01	0.22
MP balance	–164	–96	–14	+21	+82	—	—	—
RDP balance	+160	+139	+105	+73	+37	—	—	—

[a]All cattle were fed 85% corn silage with a combination of rumen degradable and RUP supplements to supply 0.4%, 1.7%, 3.0%, 4.2%, or 5.5% RUP in the diet (DM basis). The RUP source was a blend of SoyPass (LignoTech USA, Rothschild, WI) and Empyreal (Cargill corn milling, Blair, NE).

[b]Linear and Quadratic orthogonal contrasts.

Throughout the entire trial (d 1 to 83), as supplemental RUP inclusion increased from 0.4% to 5.5%, a linear increase was observed in ending BW ($P = 0.01$). With no differences in DMI ($P \geq 0.19$), averaging 7.7 kg/d, and a linear increase in ADG ($P < 0.01$), G:F also linearly improved ($P < 0.01$) as RUP inclusion increased from 0% to 5.5%. Over the entire trial G:F was improved by 19% for the 5.5% RUP compared to 0.4% RUP treatment. This is consistent with data presented by Hilscher et al. (2019), reporting a linear increase in both ADG and G:F as supplemental RUP was increased in a corn silage growing diet from 0% to 4.2% (DM basis). Felix et al. (2014) also reported increased ADG, final BW, and G:F in corn silage growing diets supplemented with RUP sources (dried distillers grains with solubles or soybean meal) compared to diets supplemented with only urea.

The MP balance (supply minus requirement) for the first 37 d increased from –152 to +1 g/d as RUP inclusion increased from 0.4% to 5.5% (Table 4). For the overall feeding period (d 1 to 83), MP balance increased from –164 to +82 g/d as RUP inclusion increased from 0.4% to 5.5%. As cattle become more mature, there is less protein in their gain, resulting in lower MP requirements (NASEM, 2016). The improvement in feed efficiency in the first 37 d may be due to meeting the greater amino acid requirements of younger, lighter calves. This partially explains the large improvement in G:F with RUP supplementation during the first 37 d of the trial and less improvement during the last 46 d. The model prediction of MP supply for the first period (d 1 to 37) shows that a gain response would be expected up to the 5.5% supplemental RUP treatment. The gain and efficiency responses would reflect that as there was an increase from the 4.2% to the 5.5% RUP level. Although the performance data for the second period (d 38 to 83) are not as clear, the overall 83-d results support the model projection with maximum gain response at the 4.2% level of RUP supplementation.

Supplementing bypass protein to growing cattle on a corn silage diet linearly increased ADG and ending BW by meeting MP requirements. Once MP requirements are met, excess MP provided from RUP can be used as energy. Corn silage has very low RUP content and increased moisture coupled with increased ensiling time can further decrease RUP of corn silage. Therefore, growing cattle on a corn silage diet will have improved performance when supplemented with an RUP source. Data gathered from all three experiments suggest the RUP content of corn silage is approximately 1% of DM, of which

50% is digestible. This results in less than 7% of the CP in corn silage being available to growing animals as digestible RUP and strong gain responses by these animals to RUP supplementation. For the first 30 d on a corn silage diet growing calves should be supplemented with an RUP source at 3.0% or more of diet DM, if maximum ADG is the goal.

Conflict of interest statement. None declared.

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