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Evaluation of sustained release mineral boluses as a long-term nutrient delivery method for beef cattle

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ABSTRACT

Two studies were conducted to evaluate the efficacy of sustained release mineral boluses as an alternative nutrient delivery method for beef cattle. For both studies 16 ruminally-cannulated cows were used in a completely randomized design. In study 1, we evaluated degradation rates of two bolus prototypes and cow age (2-yr-old versus 3-yr-old cows) over an 87-d study period. In study 2, we evaluated two bolus types (90-d degradation target versus 180-d degradation target), as well as two diet qualities contrasting a low-quality high-fiber forage (> 600 g/kg neutral detergent fiber and < 80 g/kg crude protein, dry matter basis) and high-quality low-fiber forage (< 500 g/kg neutral detergent fiber and> 150 g/kg crude protein, dry matter basis). For both Study 1 & 2, intake and digestion periods were conducted to evaluate cow age (study 1) or diet quality (study 2) effects on intake and rumen/reticulum function. In study 1, models containing an asymptotic effect of day and an interaction between day and bolus type were the best supported of the candidate models for bolus degradation rate. Cow age did not affect (P= 0.48) bolus degradation rates ($\widehat{\beta}$ = -0.81 ± 1.13) and degradation rates were greater (P < 0.01) for bolus prototype B compared to bolus A ($\widehat{\beta}_{\text{prototype B}}$ = -20.39 ± 1.13; $\widehat{\beta}_{\text{prototype A}}$ = -9.64 ± 0.81). Bolus degradation rate displayed an asymptotic relationship (P < 0.01) to bolus surface area for prototype A ($\widehat{\beta}$ = 5.83 ± 0.57) and a linear relationship (P < 0.01) for prototype B ($\widehat{\beta}$ = 0.001 ± 0.0001). In study 2, models containing a linear effect of day and an interaction between day and diet were the best supported of the candidate models for the degradation rate of the 90-d and 180-d prototype. In addition, both bolus protoypes displayed a diet quality × time interaction (P < 0.01) for bolus degradation rate. Cattle treated with the 90-d bolus and fed a high-quality diet had a greater (P < 0.01) degradation rate ($\widehat{\beta}_{\text{High-quality}}$ = -2.64 ± 0.08; $\widehat{\beta}_{\text{Low-quality}}$ = -1.97 ± 0.10) than the cows that were fed a low-quality diet. In contrast, cattle treated with the 180-d bolus had an inverse effect (P < 0.01), with bolus degradation rates greater ($\widehat{\beta}_{\text{Low-quality}}$ = -0.09 ± 0.007; $\widehat{\beta}_{\text{High-quality}}$ = -0.04 ± 0.005) with cows on the low-quality diet versus the high-quality diet. Across both studies, two of four bolus prototypes met target release rates at 90 days. However, bolus prototype degradation characteristics varied and were influenced by diet quality.

Abbreviations: BW, body weight; CP, crude protein; DM, dry matter; HQ, high quality; LQ, low quality; NDF, neutral detergent fiber; uNDF 240, 240-h *in vitro* digestibility; VFA, volatile fatty acids.

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1. Introduction

Mineral/vitamin supplementation has long been recognized as an important tool to increase beef cattle performance. Beef production systems worldwide are dependent upon supplemental nutrient inputs where low-quality, high-fiber, forages are the basal diet (DelCurto et al., 2000). In respect to supplemental inputs, most beef cattle producers focus on finding sources of supplemental protein (DelCurto et al., 2000). However, vitamin and mineral availability is often limited in these same scenarios. Adequate mineral nutrition plays a vital role in the physiological functions related to growth, reproduction, and immunity in cattle (Underwood and Suttle, 1999).

Producers have access to a vast array of mineral delivery systems, from single-dose delivery methods to self-fed, salt-limited, vitamin/mineral mixes. Though these systems can be useful in meeting nutrient requirements, self-fed systems are often limited by extreme variation in intake whereas, single dose delivery systems have rapid serum and liver response rates, but a short-term effect requiring multiple treatments to meet the animal's nutrient requirements (Arthington and Swenson, 2004; Jackson et al., 2020). Past research has evaluated the use of sustained release mineral boluses on mineral status along with the subsequent effects on cattle performance (Sprinkle et al., 2006; Jackson et al., 2020; McCarthy et al., 2020; Sprinkle et al., 2021). However, recent research has raised questions relative to the degradation rate of sustained release mineral boluses (Jackson et al., 2020; McCarthy et al., 2020).

Research characterizing degradation rates of sustained release mineral boluses and potential factors that may influence degradation rates are limited. Therefore, the objectives of this research were to characterize degradation rates of bolus prototypes, as well as the possible influences of cow age, and diet quality on bolus degradation. We hypothesized that bolus prototype, cow age, and diet quality can influence degradation rates of sustained release mineral boluses.

2. Materials and methods

All protocols and procedures were approved by the Montana State University Agricultural Animal Care and Use Committee (#2019-AA12). All animals used in this study were provided by the Montana Agriculture Experiment Station. This study was conducted at the Bozeman Agriculture Research and Teaching farm (45° 39' N 111° 04' W) at Montana State University in Bozeman, MT, USA.

2.1. Study 1: cow age and bolus type

Sixteen ruminally-cannulated Angus crossbred cows consisting of two age groups (2-yr of age, n = 8, 445 kg body weight; BW; 3-yr of age, n = 8; 601 kg BW) were stratified by weight within age groups and randomly allotted one of two bolus protoypes (Table 1) in a completely randomized design with a 2 × 2 factorial arrangement of treatments. Boluses were administered orally via a bolus applicator. The target degradation rate for both bolus prototypes was manufactured to be between 90 and 150 days. Each animal received two boluses at day 0 and bolus placement was mapped according to rumen reticular location on d-4 and 7. Bolus measurements were taken weekly over the course of 87-d to determine the subsequent rate of degradation. Boluses were retrieved via the ruminal cannula opening, cleaned with deionized water, and gently dried with paper towels. Boluses were then measured using a scale to assess weight (g) and digital calipers to calculate surface area (mm^2) for a cylinder. After measurements, boluses were immediately returned to the reticulum. Animal diet was designed to represent a low-quality high-fiber (> 600 g/kg neutral detergent fiber; NDF; 500 g/kg total digestible nutrients; dry matter basis; DM; Table 2) free-choice forage system, supplemented daily with a 200 g/kg crude protein (CP) range cake at 1.4 kg/hd to meet the nutrient requirements of mature, non-lactating, beef cows. Initial forage quality was obtained prior to initiation of the study by sending forage samples to a commercial laboratory for forage analysis (DairyOne, Ithaca, NY, USA) to ensure the forage met our predetermined low-quality attributes. Cattle were housed in common within a dry lot pen with no access to outside forage.

Table 1
Nutrient composition of bolus prototypes, provided by manufacturer, administered to cannulated 2- and 3-year-old beef cows (Study 1).

	Bolus A	Bolus B
Minerals, ppm		
Phosphorus	24,000	18,000
Calcium	10,000	18,000
Magnesium	101,000	99,000
Sodium	17,000	13,000
Zinc Oxide	216,240	175,620
Cupric sulfate pentahydrate	42,940	36,470
Potassium iodate	3050	3000
Cobalt carbonate	910	890
Vitamins, ppm		
Vitamin A	6450	6450
Vitamin E	77,580	75,600

*Each animal received two 80 g boluses.

2.2. Intake

A feed intake and rumen function period were conducted from d-45 to 66 of the bolus degradation study to provide support variables and information for determining bolus degradation rates. To quantify individual animal intake, animals were fitted with an electronic identification ear tag (Allflex USA, Inc., Dallas-Ft. Worth, TX, USA) and were adapted to a GrowSafe system (GrowSafe Systems Ltd., Airdrie, AB, Canada) for 14-d prior to the start of the bolus degradation study. A total of 8 pens each equipped with a GrowSafe electronic feed bunk (2 cows per pen; 1 bolus prototype per pen) were used in the feed intake and rumen function period. Each bunk was equipped with an antenna to detect animal presence. Load cells measured feed disappearance and neck bars allowed for only one animal to feed at a time. Individual animal intake was continuously recorded via wireless transfer to a data-acquisition computer. The system was monitored daily for unaccounted feed balance. If 95 % of the feed disappearance could not be accounted for over a 24-h period, the GrowSafe system would deem that period as failed. In this study no feed days were considered failed.

2.3. Rumen fill and fermentation

Ruminal liquid, DM, and indigestible NDF fill were evaluated on the final day of the intake period by performing a complete rumen evacuation 5-h post-feeding. Contents were manually evacuated by hand with the final liquid portion removed using cups and sponges. Total contents were weighed, thoroughly mixed, and subsampled in duplicate (Van Soest, 1994). The remaining ruminal contents were immediately placed back in the rumen. Rumen samples were weighed, dried in a forced-air oven at 55° C for 96-h. Dried rumen samples were then composited and ground to pass through a 1-mm screen in a Wiley Mill. Additionally, concurrent with rumen evacuations, rumen fluid samples were extracted, and pH was measured immediately then samples were stored at -20° C. Rumen fluid samples were analyzed for ammonia (NH_3) concentrations using methods described by Sigma Technical Bulletin #640, (Chaney and Marbach, 1962; Horn and Squire, 1967; Weichselbaum et al., 1969). Individual volatile fatty acid (VFA) concentrations were analyzed using a gas chromatography procedure (Baumgardt, 1964; Byers, 1979; Fritz and Schenk, 1987).

2.4. Study 2: diet quality and bolus type

Sixteen ruminally-cannulated Angus crossbred cows (age 3- to 5-yr, average weight =644 kg) were used in a completely randomized design contrasting two diet qualities (LQ and HQ) and bolus treatments (Table 3; 90-d versus 180-d sustained release target). The two diets consisted of a low-quality grass hay (LQ; > 600 g/kg NDF and < 80 g/kg CP; DM basis; Table 4) and high-quality grass/alfalfa mixed hay (HQ; < 500 g/kg NDF and > 150 g/kg CP; DM basis). The LQ diet was provided a 400 g/kg CP supplement daily at 0.91 kg/hd to meet the maintenance requirements of mature, non-lactating, beef cows (Table 4). Forage quality samples were obtained prior to initiation of the study, the mid-point, and at study completion by taking a hay core sample from each of eight bales randomly located throughout the stack of each representative diet. Hay core samples were composited by diet and sent to a commercial laboratory for forage analysis (DairyOne, Ithaca, NY, USA). Samples taken prior to the initiation of the study were used to ensure the forages met our predetermined low- and high-quality attributes. The two sustained release mineral bolus treatments were estimated by manufacturer to have a nutrient delivery span of 90 and 180-d, respectively. Bolus degradation rates were measured and calculated over a 91-d period as described for Study 1.

Table 2
Nutrient analysis of hay and supplement offered to cannulated 2- and 3-year-old beef cows (Study 1).

	Hay[1]	Supplement[2]
Dry matter, g/kg	892.4	974.4
Nutrient composition, g/kg DM basis		
Crude Protein	51.4	200.0
Acid detergent fiber	306.3	–
Neutral detergent fiber	558.8	302.0
Non-fiber carbohydrates	294.3	–
NDFD[3]	432.7	–
uNDF 240 h[4]	330.0	80.1
Total digestible nutrients	552.3	–
Ca	2.4	27.5
P	1.7	5.5
K	15.0	8.0
Na	0.4	17.5

[1] Hay was fed ad libitum.
[2] Supplement was fed daily at 1.4 kg/hd to meet nutrient requirements for mature cows. Includes 140 g/kg NPN equivalent.
[3] Neutral detergent fiber digestibility.
[4] Undigestible neutral detergent fiber based on a 240 h *in vitro*.

Table 3
Nutrient composition of bolus prototypes, provided by manufacturer, administered to cannulated beef cows (Study 2).

	90-d Bolus[1]	180-d Bolus[2]
Minerals, ppm		
Calcium	15,000	–
Magnesium	200	–
Sodium	900	–
Zinc oxide	800	270,000
Zinc sulphate monohydrate	900	–
Manganese oxide	100	–
Manganese sulfate monohydrate	300	58,140
Calcium iodate anhydrous	200	24,550
Cobalt carbonate	–	6000
Cobalt sulphate heptahydrate	940	–
Copper chelate	–	265,680
Vitamins, ppm		
Vitamin A	7723	1911
Vitamin D3	84	35
Vitamin E	22,400	44,181
Vitamin B6	–	421,690

[1] Each animal received two 110 g boluses.
[2] Each animal received one 100 g bolus.

Table 4
Nutrient analysis of high-quality hay, low-quality hay, and supplement offered to cannulated beef cows (Study 2).

	HQ Hay[1]	LQ Hay	Supplement[2]
Dry matter, g/kg	878.0	851.6	886.1
Nutrient composition, g/kg DM basis			
Crude Protein	177.1	72.1	463.2
Acid detergent fiber	293.1	312.3	119.5
Neutral detergent fiber	466.7	610.1	252.8
Non-fiber carbohydrates	214.4	225.7	112.1
NDFD[3]	368.5	408.0	–
uNDF 240 h[4]	301.2	381.0	109.5
Total digestible nutrients	605.5	575.8	–
Ca	9.2	2.6	27.4
P	3.1	1.4	4.6
K	35.9	16.2	10.3
Na	0.3	0.3	0.7

[1] Hay was fed ad libitum. HQ = high quality grass alfalfa hay, LQ = low quality grass hay.
[2] Supplement was fed daily at 1.4 kg/hd to meet nutrient requirements for mature cows. Includes 140 g/kg NPN equivalent.
[3] Neutral detergent fiber digestibility.
[4] Undigestible neutral detergent fiber based on a 240 h *in vitro*.

2.5. Study 2: intake and digestion

Cows were adapted to basal diets for 14-d prior to initiating the intake and digestion period. After which, a 15-d intake and digestion period corresponding to day 14–28 of the 91-d degradation study was conducted to quantify the effects of forage quality on intake, digestion, and rumen fermentation characteristics of individual cows. Animals were randomly assigned to individual pens according to their diet and bolus treatment. Forage was chopped (5−10 cm length) and provided at 0800 daily at 120 % of the average previous 3-d as-fed intake. The intake and digestion period included a 7-d pen adaption, 6-d of sample collection, 1-d collection for ruminal profile, with rumen evacuation performed on day 28.

During the 6-d sample collection period; feed, orts, and fecal output were measured for each individual animal and used to calculate total tract DM digestibility. Fecal output was estimated daily by manually removing feces from concrete floors of individual pens. Feed and orts samples were dried at 55° C for 48-h and fecal samples were dried at 55° C for 96-h in a forced air oven. Orts were then ground to pass through a 1-mm screen using a Wiley mill. Feed and ort samples were sent to a commercial laboratory (DairyOne, Ithaca, NY, USA) and analyzed for DM and NDF following a 240-h in-vitro digestibility (uNDF 240) using the Daisy^{II} Incubator (Daisy^{II} Incubator; Ankom Technology Corp., Fairport, NY, USA) to calculate ruminal fill, passage, and retention of indigestible fiber.

Immediately following the 6-d sample collection period, each cow was intra-ruminally pulse-dosed with 286.25-mg/mL of a liquid marker (Cr-EDTA) in a 250-mL aqueous solution (Udén et al., 1980) just prior to feeding or 0800. Samples were then obtained using a suction strainer (Raun and Burroughs, 1962) just prior to feeding (0-h) and at 4-, 8-, 12-, 18-, and 24-h post feeding to determine liquid kinetics and rumen fermentation characteristics. Ruminal pH, VFA, and NH_3 measurements were taken with the same procedures as described in study 1. Chromium concentration was analyzed using atomic absorption spectroscopy with a Perkin Elmer AAnalyst 300

equipped with an air/acetylene flame. Ruminal liquid volume and liquid dilution rates were estimated by regressing the natural logarithm of Cr concentrations against sampling time (Galyean, 1989). On the final day of the intake and digestion period (d-28), ruminal contents were manually evacuated 5 h post feeding via the ruminal cannula and were sampled and processed as described for Study 1 to determine liquid, DM and indigestible NDF fill (uNDF 240).

3. Statistical analysis

3.1. Degradation

We evaluated the effects of cow age and bolus type in study 1 and the effects of diet quality and bolus type in study 2 on sustained release mineral bolus degradation rates. We hypothesized that animal age and diet quality can influence the degradation rates of various bolus prototypes and degradation patterns can be described with linear or pseudothreshold models. Variables hypothesized to exhibit a pseudothreshold pattern were tested with asymptotic models by evaluating the natural log of the explanatory variable (ln[x +0.001]) (Franklin et al., 2000). We used Akaike's Information Criterion adjusted for small sample sizes (AIC_c) to evaluate support for competing models reflecting hypotheses about the effects of cow age and bolus type in study 1 and diet quality and bolus type in study 2 (Burnham and Anderson, 2002). Models with $\Delta AIC_c \leq 2$ that differed from the top model by a single parameter were excluded if confidence intervals of parameter estimates overlapped 0 (i.e., were non-informative; Arnold, 2010). Model fit was then evaluated by calculating marginal and conditional r^2 values for generalized linear mixed models (Nakagawa and Schielzeth, 2013).

3.2. Surface area

To characterize the potential changes in rate of degradation over time we modeled weekly degradation rate response to estimated bolus surface area. We hypothesized that bolus degradation and surface area relationships can be described with linear, pseudo-threshold, or quadratic models. We used AIC_c to evaluate support for competing models as previously described.

3.3. Intake and digestion

The effects of bolus prototype and cow age (Study 1), forage quality and bolus protype (Study 2) on DM intake, digesta kinetics, and rumen fermentation were analyzed using an analysis of variance (ANOVA) with generalized linear model for a 2 × 2 factorial arrangement of treatments. Individual cow was considered the experimental unit (n = 4 per treatment). An Alpha of ≤ 0.05 was considered significant, and tendencies were considered at alpha ≤ 0.10. Means were separated using the Tukey method when $P \leq 0.05$. All statistical analysis were performed in R (R Core Team, 2020).

Table 5
Model selection for models evaluating the effects of day, cow age, and diet quality on bolus degradation rate of cannulated beef cows[1].

Model[2]	K[3]	AICc[4]	ΔAICc[5]	W_i[6]	r^2 m[7]	r^2 c[8]
Study 1						
ln(day) × Bolus + ln(day) × Age	6	1452.02	0.00	0.56	0.78	0.97
ln(day) × Bolus	5	1452.47	0.45	0.44	0.77	0.97
ln(day) × Age	5	1634.71	182.69	0.00	0.11	0.95
ln(day)	4	1635.90	183.88	0.00	0.10	0.95
Constant (null)	3	1854.00	401.98	0.00	0.00	0.83
Study 2- 90-d						
Day × Diet	5	904.75	0.00	1.00	0.90	0.95
Day	4	936.44	31.69	0.00	0.83	0.93
Constant (null)	3	1189.62	284.86	0.00	0.00	0.02
Study 2- 180-d						
Day × Diet	5	389.42	0.00	1.00	0.57	0.88
Day	4	419.29	29.86	0.00	0.47	0.78
Constant (null)	3	534.21	144.89	0.00	0.00	0.28

[1] Only models with Akaike weights (w_i) ≥ 0.05 are presented except for the null model.
[2] Cow is used as a random variable in all models.
[3] K = number of parameters.
[4] Akaike's information criterion adjusted for small sample size.
[5] Difference in Akaike's information criterion adjusted for small sample size compared to the best model.
[6] Akaike weight.
[7] Marginal r^2.
[8] Conditional r^2.

4. Results

4.1. Study 1: mapping

Boluses within the reticulo-rumen environment varied post administration, between the age groups. Bolus location within the 2-yr-old cows were recovered from the reticulum, cranial sac, and ventral sac, whereas all boluses recovered from the 3-yr-old cows were found consistently within the reticulum, indicating that physiological size influences bolus placement via oral administration.

4.2. Study 1: degradation rate

Models containing an asymptotic effect of day and an interaction between day and bolus type were the best supported of the candidate models for bolus degradation rate (Table 5). Models containing an interaction of day by cow age were also supported, however the parameter estimates for a day by cow age interaction ($\widehat{\beta}$ = -0.81 ± 1.13; P= 0.48) may be non-informative as confidence intervals of the effect size overlap 0. Bolus degradation rate displayed a day by bolus prototype interaction ($P < 0.01$), where bolus prototype B had a faster rate of degradation than prototype A ($\widehat{\beta}_{\text{prototype B}}$ = -20.39 ± 1.13; $\widehat{\beta}_{\text{prototype A}}$ = -9.64 ± 0.81; Fig. 1). The top model containing all supported variables had a marginal r^2 of 0.77 and a conditional r^2 of 0.97, suggesting bolus type and days in the rumen reticular environment account for 77 % of the variation associated with bolus degradation rate.

4.3. Study 1: surface area

Bolus degradation rate displayed an asymptotic relationship ($P < 0.01$) to bolus surface area for prototype A ($\widehat{\beta}$ = 5.83 ± 0.57) and a linear relationship ($P < 0.01$) for prototype B ($\widehat{\beta}$ = 0.001 ± 0.0001; Fig. 2). Bolus prototype A had a marginal r^2 of 0.44 and a conditional r^2 of 0.70, while bolus prototype B had a marginal r^2 of 0.42 and a conditional r^2 of 0.86. Therefore, surface area accounted for 44 % and 42 % of the variation associated with degradation rate for prototype A and B, respectively.

4.4. Study 1: digestibility and ruminal kinetics

There were no age or bolus effects on daily DM intake expressed as kg ($P \geq 0.18$) or g/kg BW ($P \geq 0.11$; Table 6). There was an age effect ($P < 0.01$) expressed for total DM fill suggesting that physiologically, 2-yr-old animals had less rumen capacity compared to the 3-yr-old animals. However, when DM fill was expressed as g/kg BW there was no difference ($P = 0.81$) between the age groups. Like DM fill, total liquid fill conveyed an age effect ($P = 0.02$) indicating rumen capacity differences, but when expressed as g/kg BW there was no effect ($P = 0.50$) on age. There were no age or bolus effects ($P \geq 0.43$) on the undigestible fiber percentage (uNDF 240) of the rumen contents. Rumen pH levels, acetate, butyrate, valerate, and NH_3 concentrations displayed no age or bolus effect ($P \geq 0.14$). However, propionate, isobutyrate, and acetate:propionate ratio displayed an age effect ($P \leq 0.04$), with 2-yr-old cows having greater concentrations of isobutyrate, and lower concentration of propionate compared to 3-yr-old cows. Isovalerate displayed an age × bolus

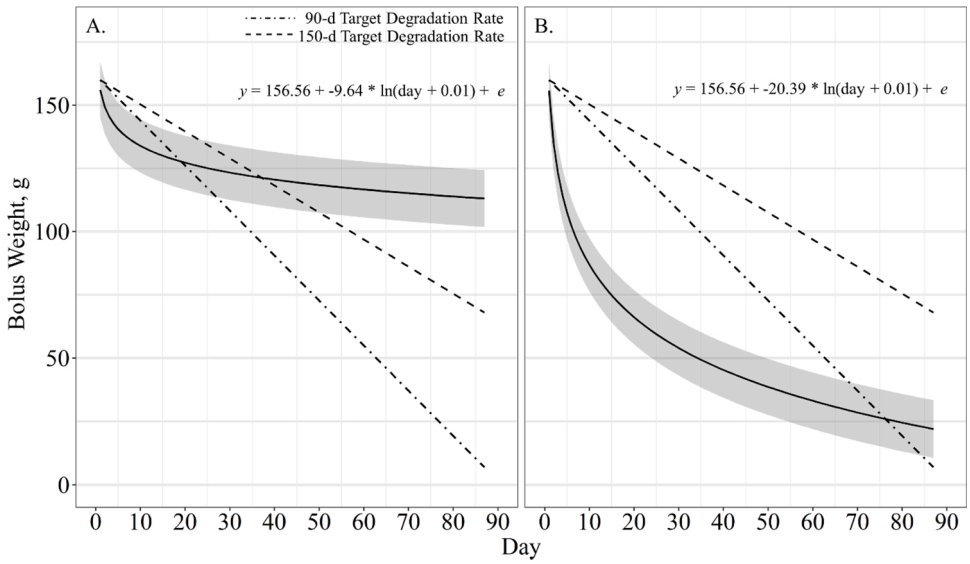

Fig. 1. Study 1: Predicted relationship (± 95 % CI represented in the shaded area) between bolus weight and length of time in the reticulo-rumen environment. Bolus prototype A (A.) versus B (B.) differed ($P < 0.01$) in rate of degradation, however both degradation curves were best described by asymptotic models. Dashed lines represent linear target degradation rate of 90 and 150 days.

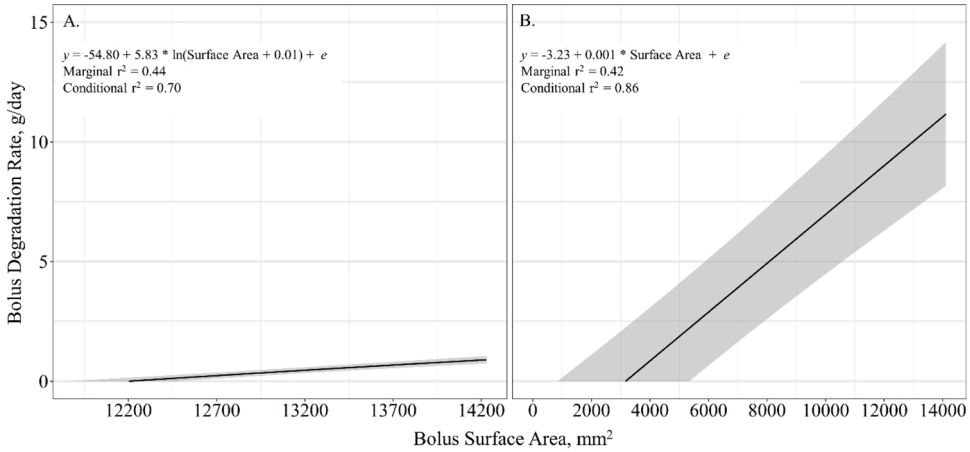

Fig. 2. Study 1: Predicted relationship (± 95 % CI represented in the shaded area) between bolus degradation rate and surface area for bolus prototype A and B.

Table 6
Animal intake, rumen fill and rumen fermentation characteristics as a function of cow age and bolus prototype of cannulated 2- and 3-year-old beef cows fed low-quality forage (Study 1).

	Bolus A[1]		Bolus B[1]		SE	*P*-value		
	2-yr-old[2]	3-yr-old[2]	2-yr-old	3-yr-old		Age	Bolus	Age*Bolus
Dry matter intake, kg/d	8.36	10.10	8.57	9.38	0.90	0.18	0.78	0.62
Dry matter intake, g/kg BW[3]	18.53	16.69	18.75	14.79	1.61	0.11	0.61	0.53
Dry matter Fill, kg	12.81	16.49	11.25	16.81	1.24	<0.01	0.63	0.46
Dry matter Fill, g/kg BW	28.08	27.44	24.59	26.35	2.30	0.81	0.34	0.61
Liquid Fill, kg	72.81	85.91	63.93	88.82	6.68	0.02	0.66	0.40
Liquid Fill g/kg BW	159.26	142.68	139.82	139.44	12.20	0.50	0.37	0.52
Rumen uNDF 240, g/kg	351.60	364.20	352.00	365.00	10.80	0.43	0.98	0.97
pH	6.46	6.66	6.22	6.33	0.13	0.28	0.93	0.72
Acetate, mol/100 mol	69.95	68.58	68.54	67.23	0.60	0.15	0.14	0.95
Propionate, mol/100 mol	16.44	17.78	17.19	18.00	0.27	<0.01	0.09	0.36
Isobutyrate, mol/100 mol	1.17	0.96	1.04	0.97	0.06	0.04	0.17	0.28
Butyrate, mol/100 mol	10.24	10.81	11.05	11.74	0.35	0.29	0.14	0.88
Isovalerate, mol/100 mol	0.79[a]	0.49[b]	0.68[ac]	0.58[bc]	0.03	<0.01	0.10	0.02
Valerate, mol/100 mol	1.41	1.39	1.49	1.49	0.10	0.90	0.60	0.92
Acetate: Propionate	4.26	3.86	3.99	3.74	0.09	0.01	0.07	0.46
Total VFA[4], mM	77.07[ab]	78.42[ab]	67.74[a]	86.70[b]	3.78	0.81	0.12	0.04
NH_3, mg/dl	3.70	4.54	3.60	4.44	0.55	0.33	0.97	1.00

[a,b,c]Means that lack common superscripts differ for age*bolus $P < 0.05$.
[1] Bolus types with a target degradation rates of 90–150 days.
[2] Cattle age groups. 2- and 3-year-old animals.
[3] BW, body weight.
[4] VFA, volatile fatty acid.

interaction ($P = 0.02$), with 2-yr old cows having greater ($P < 0.01$) isovalerate than the 3-yr old cows with bolus A, but no difference ($P = 0.12$) between the 2-yr old and 3-yr old cows with bolus B. Additionally, 2-yr-old cows had a greater ($P = 0.01$) acetate:propionate ratio compared to the 3-yr-old cows. Total VFA concentration had an age × bolus interaction ($P = 0.04$) with 3-yr-old cows treated with bolus B displaying greater ($P = 0.02$) total VFA concentrations than 2-yr-old cows treated with bolus B, but not different ($P \geq 0.22$) than animals treated with bolus A, regardless of age.

4.5. Study 2: degradation rate

Each bolus prototype was rated at a specific nutrient release rate of 90 or 180-d. Models containing a linear effect of day and an interaction between day and diet were the best supported of the candidate models for the degradation rate of the 90-d prototype (Table 5). Bolus degradation for the 90-d prototype displayed a day by diet interaction ($P < 0.01$), where cattle fed a high-quality diet had a faster rate of degradation than cattle fed a low-quality diet ($\widehat{\beta}_{\text{High-quality}} = -2.64 \pm 0.08$; $\widehat{\beta}_{\text{Low-quality}} = -1.97 \pm 0.10$; Fig. 3A). The top model containing all supported variables had a marginal r^2 of 0.90 and a conditional r^2 of 0.95, suggesting that diet quality and

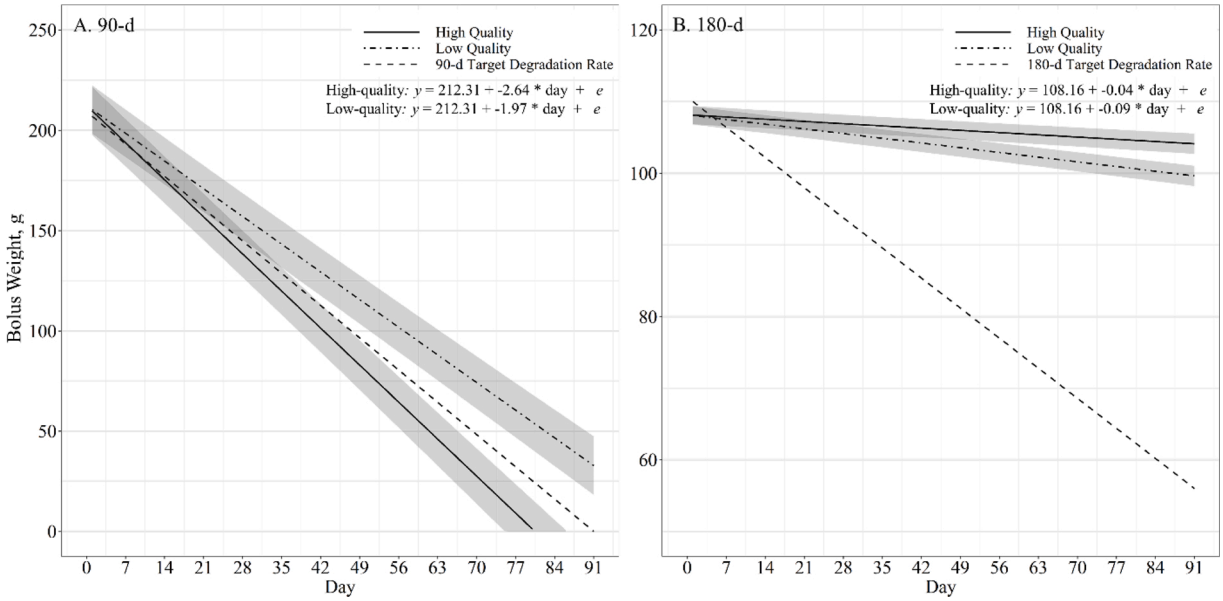

Fig. 3. Study 2: Predicted relationship (± 95 % CI represented in the shaded area) between bolus weight, prototype, diet quality and length of time in the reticulo-rumen environment for 90-d (A.) and 180-d (B.) bolus prototypes. Degradation curves were best described by linear models. Dashed line represents linear target degradation rate for 90 and 180 days.

days in the rumen reticular environment account for 90 % of the variation associated with bolus degradation rate. Similarly, for the 180-d bolus prototype models containing a linear effect of day and an interaction between day and diet was the best supported of the candidate models. Bolus degradation for the 180-d prototype displayed a day by diet interaction ($P < 0.01$), where cattle fed a low-quality diet had a faster rate of degradation than cattle fed a high-quality diet ($\widehat{\beta}_{\text{Low-quality}} = -0.09 \pm 0.007$; $\widehat{\beta}_{\text{High-quality}} = -0.04 \pm 0.005$; Fig. 3B). The top model containing all supported variables had a marginal r^2 of 0.57 and a conditional r^2 of 0.88, suggesting that diet quality and days in the rumen reticular environment account for 57 % of the variation associated with bolus degradation rate.

4.6. Study 2: surface area

Estimated surface area of the 90-d bolus displayed no effect ($P = 0.16$) on bolus degradation rate. However, this could be attributed to boluses not maintaining shape integrity over time, making surface area calculations difficult to interpret. The relationship between

Table 7
Animal intake, digestibility, and liquid kinetics as a function of diet quality and bolus prototype of cannulated beef cows fed low-quality and high-quality forages (Study 2).

	90[1]		180[1]			*P*-value		
	LQ[2]	HQ[2]	LQ	HQ	SE	Diet	Bolus	Diet*Bolus
Dry matter intake, kg/d	14.20	13.74	13.52	14.74	1.27	0.77	0.90	0.52
Dry matter intake, g/kg BW[3]	21.79	21.37	20.60	23.55	1.94	0.53	0.80	0.40
Dry matter digestibility, g/kg	570.70	583.70	553.50	607.50	24.20	0.19	0.89	0.41
Dry matter fill, kg	13.98	12.64	15.82	11.91	1.17	0.04	0.64	0.30
Dry matter fill, g/kg BW	21.50	19.35	24.25	19.15	1.73	0.06	0.48	0.41
uNDF 240 fill, kg	4.62	5.33	5.36	5.54	0.54	0.43	0.39	0.63
uNDF 240 fill, g/kg BW	7.14	8.11	8.26	8.93	0.90	0.38	0.30	0.19
uNDF 240 passage, %	2.92	2.87	2.26	2.95	0.26	0.24	0.30	0.19
uNDF 240 retention, hr	34.48	36.62	44.89	24.88	3.36	0.26	0.22	0.10
Liquid fill, kg	103.64	100.87	106.19	90.22	8.04	0.27	0.62	0.43
Liquid fill, g/kg BW	160.36	153.88	162.34	145.62	12.61	0.38	0.81	0.69
Liquid passage, %	3.52	2.82	3.65	3.14	0.55	0.30	0.68	0.86
Liquid turnover, hr	29.51	37.06	31.89	32.50	4.19	0.35	0.80	0.70
Liquid flow, L/hr	3.64	2.76	3.74	2.89	0.52	0.12	0.82	0.98

[1] Bolus type of 90-d degradation rate or 180-d degradation rate.
[2] Hay quality offered to animals. Low quality (LQ) or high quality (HQ).
[3] BW, body weight.

bolus degradation and surface area for the 180-d bolus prototype was not evaluated due to the bolus failing to meet the degradation rate criteria, therefore was terminated after 91 days.

4.7. Study 2: intake, digestibility, and liquid kinetics

There were no diet or bolus effects ($P \geq 0.19$) on DM intake and DM digestibility (Table 7). However, there was an effect ($P = 0.04$) of diet quality on total DM fill suggesting that animals on the low-quality diet had greater rumen fill. Furthermore, DM fill expressed as g/kg BW tended ($P = 0.06$) to be greater for the low-quality treatment. There were no effects ($P \geq 0.22$) of diet quality or bolus prototype observed for liquid fill, undigestible fiber content and passage rates (uNDF 240), liquid passage, liquid turnover, and flow rates.

4.8. Study 2: fermentation characteristics

Ruminal pH was not influenced ($P = 0.25$) by diet quality. However, all other rumen fermentation characteristics, including individual VFA and ammonia concentrations, displayed a diet × hour interaction ($P < 0.01$; Fig. 4) suggesting that ruminal fermentation is highly influenced by the quality of the diet. Specifically, 4-h total VFA concentrations were greater ($P = 0.02$; Fig. 4A) for animals on the high-quality diet. The ratio of acetate to propionate was observed to be greater ($P < 0.01$) for cattle fed the high-quality diet at all hours (Fig. 4B). Ammonia concentrations were found to be greater ($P < 0.01$) for the high-quality diet at all hours except for 12-h (Fig. 4C). Acetate concentrations were greater ($P \leq 0.03$) for cattle fed the high-quality diet at 4-, 8-, and 12-h (Fig. 4D). However, cows on the low-quality diet had greater ($P < 0.01$) acetate concentrations at 0-, 18-, and 24- h suggesting that time post feeding influences acetate levels by diet quality. Propionate showed greater ($P < 0.01$) concentrations for the low-quality diet treatment at all hours (Fig. 4E). Butyrate concentrations were greater ($P < 0.01$) for animals fed the low-quality diet at 4-, 8-, and 12-h post feeding (Fig. 4F). The branch chain fatty acids (isobutyrate and isovalerate) and valerate displayed greater ($P \leq 0.04$) concentrations for cattle offered the high-quality diet at all hours (Fig. 4G–I).

5. Discussion

Ruminant livestock production on a worldwide basis regularly occurs in areas not suitable for cultivation. In addition, the forage resources in these areas are often limited in nutrient availability requiring supplemental inputs (DelCurto and Olson, 2010). Therefore, nutrient delivery payout is an important factor to vitamin and mineral administration. Current nutrient delivery methods for trace minerals and vitamins include salt-limited mineral mixes, single-dose delivery methods, and, when possible hand-fed supplement programs. However, the salt limited approach has high intake variation and the single-dose delivery methods are limited by short-term payout length (Hersom and Thrift, 2018; Jackson et al., 2020). Our studies evaluated an additional supplement delivery tool that could be used in combination with other delivery methods to meet the nutritional needs of ruminant livestock in extensive management systems.

5.1. Study 1: cow age and bolus type

Despite differences in intake and rumen capacity, cow age did not influence bolus degradation. However, age did effect bolus placement post administration. In this study, boluses not found in the reticulum 4-d post administration were placed in the reticulum after recovery, where they were consistently recovered for the remainder of the study. Though this study did not examine degradation rates as a factor of ruminal reticular location, research has shown that boluses retained in the reticulum had higher rates of release than those in the rumen, due to the small reticular compartment creating friction between individual boluses resulting in higher rates of erosion (Riner et al., 1982). In our study, boluses were always found in the reticulum for 3-yr-old cows, however, bolus locations varied for 2-yr-old cows. Thus, if boluses were retained in the rumen, cow age may have influenced bolus degradation based on bolus location (reticulum vs. rumen). However, typically dense objects in the rumen tend to migrate to and stay in the reticulum over time (Ward and Ducharme, 1994).

Bolus prototype was the primary variable that effected rate of degradation with bolus B having a greater degradation rate than bolus A. For both bolus A and B, best-fit degradation models included an asymptotic effect on days post administration. Bolus B met the target degradation at 90-d while bolus A displayed overall degradation that did not meet the manufacturer's specifications. Although bolus B met the 90-d criteria, 60 % of bolus weight was delivered in the first 30 days post administration. To further explain the asymptotic degradation rates observed for both boluses in this study, we regressed weekly bolus degradation with weekly bolus surface area. Changes in bolus degradation overtime was explained, in part, by bolus surface area with $r^2 > 0.40$.

5.2. Study 2: forage quality and bolus type

Forage quality was a factor affecting the rate of bolus degradation, with the cows on the 90-d and 180-d bolus treatment. Animals offered the HQ diet along with the 90-d bolus had greater rates of degradation than those offered a LQ diet. Studies suggest that forage structure, quality and digestibility is highly influenced by seasonality (Ganskopp and Bohnert, 2001) and in turn requires changes in microbial activity and fermentation within the rumen reticular environment to utilize different fiber structures (Krause et al., 2003). Though degradation rates of the 180-d bolus were also influenced by diet quality, the failure of these boluses in meeting their nutrient

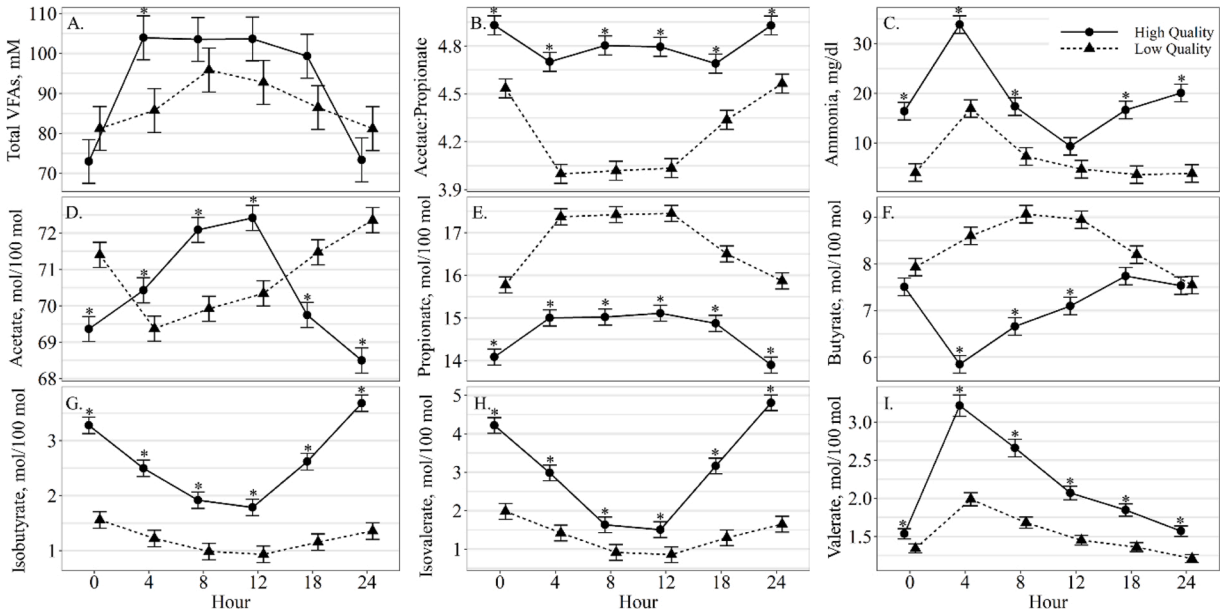

Fig. 4. Study 2: Effects of diet quality on individual VFA concentrations, acetate:propionate ratio, and ammonia concentration with a diet × hour interaction ($P < 0.01$). Treatments include high-quality and low-quality diets. * Denotes statistical difference within hour ($P < 0.05$).

delivery criteria renders the observation irrelevant.

In an effort to explain the influence of forage quality on bolus degradation, we conducted a feed intake, digestibility, and rumen fermentation trial to characterize differences due to forage quality. Our base rations met the CP and NDF criteria for low and high-quality forages. However, DM intake, total tract digestion, and many of the ruminal kinetic measurements were similar among the two diets. We believe that this could be explained in part by our choice of comparing a grass hay (LQ) to a grass alfalfa mix hay (HQ). The actual estimate for NDF digestibility for the grass alfalfa mix hay was less than the NDF digestibility of the grass hay. In turn, this may relate to the low digestibility of stems in legumes compared to stems in grasses (Van Soest, 1994). Fiber digestion rate and ruminal fill limitations may have limited intake and total tract digestion for both diets (Galyean and Defoor, 2003). However, ruminal volatile fatty acids and ammonia differed among the two diets suggesting differing ruminal environments and substrate base microbial populations. Contrary to expectations, the LQ diet had greater propionate and, as a result, had a lower acetate: propionate ratio than HQ diets, further suggesting microbial and associated end-product differences. Ruminal fill was greater for LQ diets and, although observational the hay mat layer of the reticulo-rumen environment was compacted and dense compared to the grass alfalfa mixed diet. All these factors likely contributed to the differences in bolus degradation rates among base diets.

In contrast to Study 1, surface area was not found to be related to bolus degradation rates. All four prototypes had unique characteristics in degradation rates. These characteristics are likely impacted by bolus composition, heat, pressure, and mechanical processes in creating the bolus prototypes.

6. Implications

Two of the four boluses that were tested had degradation rates that hit the target payout rate at 90 days. However, one was best described by an asymptotic curve, with approximately 60 % of its nutrients being delivered in the first 30 days post administration. The 90-d bolus administered in study 2, displayed a uniform rate of degradation represented by a linear model. Animals offered the HQ diets with the 90-d bolus had an increased rate of bolus degradation compared to cows on the LQ 90-d bolus treatment. Results from this research underscore the need to characterize degradation rates of sustained release mineral boluses that might be considered for commercial use in the ruminant livestock industries. By understanding factors that influence bolus degradation rates, livestock managers can more precisely mange the nutrient needs of ruminant livestock.

Authors statement

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Declaration of Competing Interest

Authors report no declaration of competing interest.

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