Investigating the effects of a novel rumen-protected folic acid supplement on feedlot performance and carcass characteristics of beef steers

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ABSTRACT: Angus-crossbred steers (n = 180; 292 ± 18 kg) from a single ranch were used to investigate the effects of a novel rumen-protected folic acid (RPFA) supplement on feedlot performance and carcass characteristics. On d 0, steers were blocked by body weight to pens (5 steers/pen), and pens within a block were randomly assigned to dietary treatments (n = 6 pens/treatment): target intake of 0 (CON), 30 (RPFA-30), 60 (RPFA-60), 90 (RPFA-90), 120 (RPFA-120), or 150 (RPFA-150) mg RPFA·steer^{-1}·d^{-1}. Steers were weighed before feeding on d −1, 0, 55, 56, 86, 87, 181, and 182. Pen average daily gain (ADG), dry matter intake (DMI), and gain:feed (G:F) were calculated for growing (d 0 to 56), dietary transition (d 56 to 87), finishing (d 87 to 182), and overall (d 0 to 182). Liver and blood samples were collected from two steers/pen before trial initiation and at the end of growing and finishing. Steers were slaughtered on d 183, and carcass data were collected after a 48-h chill. Data were analyzed as a randomized complete block design using ProcMixed of SAS 9.4 (fixed effects of treatment and block; experimental unit of pen). Liver abscess scores were analyzed using the Genmod Procedure of SAS 9.4. Contrast statements assessed the polynomial effects of RPFA. Supplemental RPFA linearly increased plasma folate at the end of growing and finishing (P < 0.01), and linearly decreased plasma glucose at the end of growing (P = 0.01). There was a cubic effect of RPFA on liver folate at the end of growing (P = 0.01), driven by lesser concentrations for RPFA-30, RPFA-60, and RPFA-150. Growing period ADG and G:F were greatest for CON and RPFA-120 (cubic P ≤ 0.03). Transition period DMI was linearly increased due to RPFA (P = 0.05). There was a tendency for a cubic effect of RPFA on the percentage of livers with no abscesses (P = 0.06), driven by a greater percentage of non-abscessed livers in RPFA-30 and RPFA-60. Despite supplementing 1 mg Co/kg DM, and regardless of treatment, plasma vitamin B12 concentrations were low (<200 pg/mL), which may have influenced the response to RPFA as vitamin B12 is essential for recycling of folate.

Key words: folate, glucose, one-carbon metabolism, vitamin B9, vitamin B12

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INTRODUCTION

Numerous biological compounds share the basic structure and activity of folic acid, a

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water-soluble B vitamin that serves as a donor and acceptor of one-carbon units (Lucock, 2000). These natural compounds are collectively referred to as folates while the synthetic form of the vitamin is referred to as folic acid. The transfer of one-carbon units is essential for various metabolic pathways, including phosphatidylcholine synthesis, amino acid interconversion, and nucleic acid biosynthesis. A folate deficiency results in impaired cell division and protein synthesis, especially in rapidly growing tissues (Combs, 2012). Folate requirements have not been established for beef cattle, likely because folates can be synthesized endogenously by rumen microbes (Zinn et al., 1987; Schwab et al., 2006). However, rates of folate synthesis, degradation, and absorption in the ruminant gastrointestinal tract are poorly understood and could be affected by numerous dietary factors. For example, ruminal folate concentrations were greater in steers fed high-concentrate diets compared to high-forage diets (Hayes et al., 1966; Girard et al., 1994). Although minimal research has been conducted in beef cattle, folic acid supplementation has been shown to increase milk and milk protein yields in dairy cattle (Girard et al., 1995; Girard and Matte, 1998; Graulet et al., 2007). Beef production efficiency has increased markedly since the 1970s due to increased growth rates of cattle (Capper, 2011). Rapid growth driven by advancements in genetics, nutrition, and technology could increase the demand for folate to support protein and nucleic acid synthesis. If this demand exceeds the biosynthetic capacity of folate in the rumen, dietary supplementation may be warranted. Therefore, the objective of this study was to determine the effects of feeding increasing dietary concentrations of folic acid on feedlot performance, carcass characteristics, and folate status of beef steers. Vitamin B12 status was also assessed as these two vitamins are intricately linked, and a deficiency of one could lead to a secondary deficiency of the other (Herbert and Zalusky, 1962; Loughlin et al., 1964). Based on previous research indicating high degradation rates (97%) of orally supplemented folic acid in the rumen (Santschi et al., 2005), this study utilized a novel rumen-protected source of folic acid (RPFA). It was hypothesized that increasing doses of RPFA would better support the rapid growth rates of modern feedlot cattle administered growth-promoting technologies.

MATERIALS AND METHODS

Animals and Experimental Design

All experimental procedures were approved by the Iowa State University Institutional Animal Care and Use Committee (#6-16-8292-B). One-hundred and eighty-eight newly weaned, Angus-crossbred steers were purchased from a single-source through a sale barn in Valentine, NE, and transported approximately 640 km to the Iowa State University Beef Nutrition Research Unit (Ames, IA). Upon arrival (d −18), steers were housed in partially covered concrete pens (7 steers/pen) and offered long stem grass hay top-dressed with a corn silage-based growing diet (Table 1). Hay was gradually removed during the first week to adapt steers to the trial diet. On d −11, steers were weighed, vaccinated (Bovi-Shield GOLD 5, Zoetis, Inc. Parsippany-Troy Hills, NJ), dewormed (Eprinex, Boehringer Ingelheim Animal Health USA Inc., Duluth, GA), and administered unique visual and electronic identification tags. On d 0, the 180 steers most uniform in weight (292 ± 18 kg) and disposition were blocked by body weight (BW; 6 weight blocks) and sorted into partially covered concrete pens (5 steers/pen). Pens within a block were then randomly assigned to one of six dietary treatments (n = 6 pens/treatment): target intake of 0 (CON), 30 (RPFA-30), 60 (RPFA-60), 90 (RPFA-90), 120 (RPFA-120), or 150 (RPFA-150) mg RPFA·steer^{-1}·d^{-1}. A more detailed description of supplemental RPFA treatments is provided in Table 2. Because Co is used by rumen microbes to synthesize vitamin B12 and to ensure a vitamin B12 deficiency did not influence the response to RPFA, Co was supplemented to all steers at 1 mg/kg dry matter (DM; diet analyzed 1.1 mg Co/kg DM). This concentration of supplemental Co is well above the NASEM (2016) recommendation of 0.15 mg/kg DM, but well below the maximum tolerable concentration of 25 mg/kg DM. Supplemental RPFA was delivered as part of the total mixed ration through a premix using dried distillers grains as a carrier. Five different premixes were made for each RPFA treatment; for CON, dried distillers grains replaced the RPFA premix at 5% of the diet (DM basis). Diets were mixed in ascending RPFA concentration (CON first, RPFA-150 last), and the mixer was flushed with hay between diets.

Steers were fed once daily at approximately 0800 h, and feed delivery was adjusted to allow for ad libitum feed intake. After receiving a corn silage-based growing diet for 56 d, steers were

Table 1. Ingredient and nutrient composition of diets fed to steers during the growing period (d 0 to 56) and finishing period (d 87 to 182)

	Growing	Finishing[a]
Dry matter (**DM**), %	54	76
Ingredient, % DM basis		
Corn silage	50.0	–
Cracked corn	24.1	66.1
Dried distillers grains[b]	18.9	3.0
Modified distillers grains	–	15.9
Ground hay	–	8.0
Folic acid premix[c]	5.0	5.0
Limestone	1.5	1.5
Salt	0.31	0.31
Vitamin premix[d]	0.1	0.1
Trace mineral premix[e]	0.086	0.086
Rumensin[f]	0.0135	0.0135
Analyzed composition		
Crude protein[g], %	14.0	13.5
Neutral detergent fiber[g], %	27.1	19.8
Ether extract[g], %	5.1	4.7
Co[h], mg/kg DM	1.1	1.1
Calculated composition[i]		
Net energy for gain, Mcal/kg	1.20	1.40

[a]Ractopamine hydrochloride (Optaflexx, Elanco Animal Health, Greenfield, IN) was fed at a rate of 300 mg·steer^{-1}·d^{-1} for the final 28 d of the trial.

[b]Dried distillers grains used as a carrier for micro-ingredients.

[c]For control pens, dried distillers grains replaced the folic acid premix at 5% of the diet (DM basis).

[d]Provided 2,200 IU vitamin A and 25 IU vitamin E/kg diet.

[e]Provided per kg of diet DM: 1 mg of Co (Co glucoheptonate [COPRO, Zinpro Corp., Eden Prairie, MN), 10 mg of Cu (Cu sulfate), 0.5 mg of I (calcium iodate), 20 mg of Mn (Mn sulfate), 0.1 mg of Se (sodium selenite), and 120 mg of Zn (60 mg from Zn sulfate and 60 mg from Zn amino acid complex [Availa-Zn, Zinpro Corp.]).

[f]Provided monensin (Elanco Animal Health) at 27 g/ton.

[g]Based on analysis by Dairyland, Inc., Arcadia, WI.

[h]Based on analysis by Iowa State University College of Veterinary Medicine Diagnostic Laboratory (Ames, IA).

[i]Based on National Academies of Sciences, Engineering, and Medicine (2016) reported net energy for gain values of feedstuffs.

transitioned over 31 d to a cracked corn-based finishing diet, which was fed for the remainder of the 182-d trial (Table 1). All steers were fed monensin at a rate of 27 g/ton (Rumensin90, Elanco Animal Health, Greenfield, IN) throughout the trial and ractopamine hydrochloride (Optaflexx, Elanco Animal Health) at a rate of 300 mg·steer^{-}1·d^{-}1 for the final 28 d of the trial. Additionally, all steers were administered an extended duration (200 d) anabolic implant (Synovex One Feedlot, Zoetis, Inc.) at trial initiation (d 0). Steers were monitored daily for illness and treated with an antibiotic if visual symptoms (anorexia, depression, lethargy, nasal discharge, etc.) were observed, and rectal

Table 2. Description of supplemental rumen-protected folic acid (RPFA) treatments delivered to steers throughout the experiment

Treatment	Dietary concentration, mg RPFA/kg dry matter	Calculated intake[a], mg RPFA·steer^{-1}·d^{-1}	Calculated dose[b], mg RPFA/kg body weight
Control	0.0	0.0	0.000
RPFA-30	3.0	31.2	0.070
RPFA-60	6.0	61.2	0.138
RPFA-90	9.0	91.8	0.205
RPFA-120	12.0	122.4	0.273
RPFA-150	15.0	153.0	0.342

[a]RPFA intake was calculated by multiplying dietary RPFA concentration by overall (d 0 to 182) dry matter intake for each treatment group

[b]RPFA dose was calculated by dividing RPFA intake by midpoint body weight (d 87) for each treatment group.

temperature was ≥40 °C. Two steers from RPFA-150 were removed from the trial (one on d 2 and the other on d 87) due to injury or poor gain; DM intake (DMI) for those pens was adjusted for the remainder of the trial to reflect one less (four instead of five) steer per pen.

Steers were individually weighed before feeding on two consecutive days at the start of the growing period (d −1, 0), end of the growing period (d 55, 56), the start of the finishing period (d 86, 87), and end of the finishing period (d 181, 182). The average of consecutive day body weights was adjusted (decreased by 4%) to account for digestive tract fill, and the adjusted average was used to calculate average daily gain (ADG) and feed efficiency (gain:feed; G:F). Pen ADG, DMI, and G:F were calculated for the growing period (d 0 to 56), transition period (d 56 to 87), finishing period (d 87 to 182), and overall (d 0 to 182). On d 182, steers were transported approximately 105 km to a commercial abattoir (National Beef, Tama, IA) and were humanely slaughtered on d 183. Hot carcass weights (HCW) were recorded at the time of slaughter. Ribeye area (REA); 12th rib backfat (BF); kidney, pelvic, and heart fat (KPH); and marbling score (MS) were collected by trained Iowa State University personnel after a 48-h chill. Dressing percent (DP) was calculated by dividing HCW by final shrunk BW, and yield grade (YG) was calculated using the USDA yield grade equation. Liver abscess scores were assessed by trained personnel using the Elanco Liver Check System (Elanco Animal Health). A liver abscess score of 0 indicates a normal, healthy liver (i.e., no abscesses); a score of A indicates a liver with one or two small abscesses or visible scars; and a score of A+ indicates a liver with one or more large, active abscesses present. Carcass-adjusted performance for the overall trial was determined by dividing HCW by average dressing percent for each treatment (CON = 63.5%, RPFA-30 = 64.2%, RPFA-60 = 63.9%, RPFA-90 = 63.6%, RPFA-120 = 63.8%, and RPFA-150 = 63.6%) to calculate adjusted final BW. One carcass (CON) was retained and was not included in the analysis of carcass characteristics, liver abscess scores, or carcass-adjusted performance.

Sample Collection and Analytical Procedures

Diet samples were collected weekly and dried in a forced-air oven at 70 °C for 48 h to determine DM content. On weigh dates, feed remaining in the bunk was collected, weighed, and sampled for DM analysis as previously described. Dry matter content of diet and feed refusals was then used to calculate pen DMI based on as-fed feed delivery and refusals. Weekly diet samples of the control diet were ground and composited for the growing and finishing period. Composites of the growing and finishing diet were sent to a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI) for analysis of nitrogen (crude protein; AOAC, 1995b; method 990.03), neutral detergent fiber (AOAC, 2005; method 2002.04) and ether extract (AOAC, 1995a; method 920.39). Composites were also sent to the Iowa State University College of Veterinary Medicine Diagnostic Laboratory (Ames, IA) for analysis of Co concentrations via inductively coupled plasma mass spectroscopy.

Liver biopsies were performed using methods described by Engle and Spears (2000) on two steers per pen over two consecutive days (3 pens·treatment^{-1}·d^{-1}) before the start of the trial (d −5 or −4), near the end of the growing period (d 48 or 49), and near the end of the finishing period (d 177 or 178). After collection, liver samples were immediately flash-frozen in liquid N and stored at −80 °C until future analysis of folate and vitamin B12 concentrations. Blood was collected before feeding via jugular venipuncture from the same two steers per pen on d −1, 55, and 181 into tubes containing potassium EDTA (Becton Dickenson, Rutherford, NJ). Blood collection tubes were transported to the laboratory on ice before centrifugation at 1,000 × *g* for 10 min. Plasma was then removed, aliquoted, and stored at −20 °C for future analysis of glucose concentrations or −80 °C for future analysis of folate and vitamin B12 concentrations. Plasma glucose concentrations were determined using a commercially available kit (Glucose Assay, #43990901, Wako Chemical, Richmond, VA). Intra- and inter-assay CV for glucose analysis were 2.1% and 4.7%, respectively. Plasma and liver samples were sent to a commercial laboratory (Applied Biosciences, College Station, TX) for analysis of folate and vitamin B12 concentrations via radioimmunoassay. Liver samples were dried, ground with a mortar and pestle, and homogenized (0.2 g liver [DM basis] in 1 mL buffer [pH 7.2]). Samples were then centrifuged at 14,000 rpm for 30 min and the supernatant was collected for analysis. On d −5/−4, 64% of liver samples analyzed 1 ng/mL for folate. Therefore, folate concentrations are only reported for liver samples collected on d 48/49 and 177/178. Due to a high number of plasma samples analyzing below the analytical detection limit (150 pg/mL) for vitamin B12 on d −1 (89% of samples) and 55 (75% of samples), only vitamin B12

concentrations for plasma samples collected on d 181 are reported.

Statistical Analysis

Feedlot performance, carcass characteristics, blood, and liver parameters were analyzed as a randomized complete block design using the Mixed Procedure of SAS 9.4 (SAS Institute, Cary, NC). The model included the fixed effects of treatment and block with pen as the experimental unit (n = 6 pens/treatment). Initial (d 0) shrunk BW was used as a covariate in feedlot performance analyses (live and carcass-adjusted). Initial (d −1 for blood; d −5 or −4 for liver) values were used as covariates in the analysis of subsequent blood and liver sampling timepoints. A chi-square test was performed using the Frequency Procedure of SAS 9.4 to determine if the frequency of plasma B12 concentrations analyzing below the detection limit on d −1 or d 55 was different among treatments; the test was not significant ($P \geq 0.18$). Similarly, the chi-square test was not significant ($P = 0.50$) for liver folate concentrations analyzing above or below 1 ng/mL on d −5/−4. Liver abscess scores were analyzed on a pen basis using the Genmod Procedure of SAS 9.4. Pre-planned polynomial contrast statements (linear, quadratic, or cubic) were used to determine if there was a dose-response to increasing supplemental RPFA. Normality was assessed using the Shapiro-Wilk test, and outliers were determined based on Cook's D statistic and removed if >0.5. One pen (RPFA-60) was removed from d 181 plasma folate analysis, one pen (RPFA-60) was removed from liver B12 analysis for all time points, one pen (CON) was removed from d 181 liver folate analysis, and one pen (FA-120) was removed from liver folate analysis for all time points. Significance was defined as $P \leq 0.05$ and a tendency was defined as $0.05 < P \leq 0.10$.

RESULTS

Rumen Protection of RPFA

Previously conducted *in vivo* studies have shown a 10% increase in serum folate concentrations when sheep were orally supplemented unprotected folic acid and this increase was 15 times greater (150%) when sheep were orally supplemented RPFA. Assuming approximately 3% of unprotected folic acid escapes ruminal degradation (Santschi et al., 2005), then approximately 15 times more RPFA should escape ruminal degradation. Therefore, it is assumed rumen protection of RPFA is approximately 45%.

Feedlot Performance

Live animal feedlot performance for the growing (d 0 to 56), transition (d 56 to 87), and finishing (d 87 to 182) period, as well as the overall trial (d 0 to 182), is presented in Table 3.

There was a cubic effect of RPFA on BW at the end of the growing period (d 56) and growing period ADG ($P = 0.03$), driven by greater BW and ADG for CON and RPFA-120 relative to other treatments. No polynomial effects were observed for growing period DMI ($P \geq 0.78$). Thus, there was a cubic effect of RPFA on growing period G:F ($P < 0.01$), driven by greater G:F for CON and RPFA-120 relative to other treatments. There were no polynomial effects of RPFA on ADG or G:F during the transition or finishing period ($P \geq 0.40$). However, supplemental RPFA resulted in a linear increase in transition period DMI ($P = 0.05$). No polynomial effects of RPFA were observed for finishing period DMI ($P \geq 0.17$). There were no polynomial effects of RPFA on BW at the start (d 87) or end (d 182) of the finishing period, overall ADG, DMI, or G:F ($P \geq 0.15$).

Carcass Characteristics

Carcass characteristics, liver abscess scores, and carcass-adjusted feedlot performance are presented in Table 4. No polynomial effects were observed for HCW, DP, REA, MS, BF, KPH, or YG ($P \geq 0.15$). There was a tendency for a cubic effect of RPFA on the percentage of livers with a score of 0 (no abscesses; $P = 0.06$), driven by RPFA-30 and RPFA-60 having a greater percentage of livers with a score of 0 relative to other treatments. There were no polynomial effects on the percentage of livers with a score of A (one or two small abscess or abscess scars; $P \geq 0.21$) or a score of A+ (one or more large, active abscesses; $P \geq 0.18$). No polynomial effects were observed for carcass-adjusted final BW, ADG, DMI, or G:F ($P \geq 0.11$).

Liver and Blood Metabolites

Plasma concentrations of folate, vitamin B12, and glucose at the end of the growing period (d 55) and finishing period (d 181) are presented in Table 5. Supplemental RPFA linearly increased plasma folate concentrations at the end of growing and finishing ($P > 0.01$). There was a quadratic

Table 3. Effect of rumen-protected folic acid (RPFA) supplementation to beef steers on feedlot performance during the growing (d 0 to 56), transition (d 56 to 87), and finishing (d 87 to 182) period as well as overall (d 0 to 182)

	Treatment[a]							Contrast *P*-value		
	CON	RPFA-30	RPFA-60	RPFA-90	RPFA-120	RPFA-150	SEM[b]	Linear	Quadratic	Cubic
Body weight[c], kg										
d 0[d]	292	292	292	292	292	291	–	–	–	–
d 56	389	382	384	386	388	386	2.3	0.76	0.29	0.03
d 87	447	445	444	448	449	448	3.4	0.55	0.75	0.34
d 182	608	612	607	609	611	608	7.4	0.92	0.93	0.97
Average daily gain, kg										
Growing	1.73	1.62	1.64	1.69	1.73	1.68	0.042	0.76	0.29	0.03
Transition	1.89	2.02	1.96	1.99	1.96	1.99	0.077	0.59	0.56	0.40
Finishing	1.70	1.76	1.71	1.70	1.70	1.69	0.065	0.66	0.78	0.63
Overall	1.74	1.76	1.73	1.75	1.75	1.74	0.041	0.92	0.93	0.97
Dry matter intake, kg/d										
Growing	8.2	8.3	8.2	8.4	8.2	8.2	0.13	0.78	0.78	0.86
Transition	9.5	9.7	9.7	9.8	9.8	10.1	0.19	0.05	0.99	0.42
Finishing	11.0	11.7	11.4	11.3	11.4	11.0	0.27	0.72	0.17	0.44
Overall	9.9	10.4	10.2	10.2	10.2	10.2	0.19	0.77	0.42	0.36
Gain:feed										
Growing	0.210	0.195	0.200	0.202	0.212	0.204	0.0038	0.49	0.11	<0.01
Transition	0.199	0.208	0.201	0.202	0.200	0.198	0.0070	0.59	0.59	0.64
Finishing	0.155	0.151	0.150	0.151	0.150	0.153	0.0042	0.82	0.43	0.95
Overall	0.176	0.170	0.170	0.172	0.173	0.171	0.0024	0.56	0.34	0.15

[a]Treatments (n = 6 pens/treatment): target intake of 0 (control; CON), 30 (RPFA-30), 60 (RPFA-60), 90 (RPFA-90), 120 (RPFA-120), or 150 (RPFA-150) mg RPFA·steer^{-1}·d^{-1}.

[b]Highest SEM of any treatment reported.

[c]Steers were weighed consecutively at the start of the growing period (d −1, 0), end of the growing period (d 55, 56), start of the finishing period (d 86, 87), and end of the finishing period (d 181, 182); a 4% pencil shrink was applied to the average of consecutive day body weights and the adjusted average was used to calculate average daily gain and gain:feed.

[d]Day 0 body weight used as a covariate in analysis of all other performance variables.

effect of RPFA on plasma vitamin B12 concentrations at the end of finishing (P = 0.02), driven by lesser concentrations for RPFA-60 and RPFA-90 relative to other treatments. Supplemental RPFA resulted in a linear decrease in plasma glucose concentrations at the end of growing (P = 0.01). No polynomial effects were observed for plasma glucose concentrations at the end of finishing (P ≥ 0.57).

Liver concentrations of folate and vitamin B12 at the end of the growing period (d 48/49) and finishing period (d 177/178) are presented in Table 6. There was a cubic effect of RPFA on liver folate concentrations at the end of growing (P = 0.01), driven by lesser concentrations for RPFA-30, RPFA-60, and RPFA-150 relative to other treatments. No polynomial effects of RPFA were observed for liver folate concentrations at the end of finishing (P ≥ 0.27). There were no polynomial effects of RPFA on liver vitamin B12 concentrations at the end of growing or finishing (P ≥ 0.12).

DISCUSSION

Folic acid, also referred to as vitamin B9, is an essential cofactor of several enzymes that support DNA and protein metabolism. The fully reduced form, tetrahydrofolic acid, serves as a donor and acceptor of single-carbon units (e.g., methyl and formyl groups), which are used to synthesize thymine and purines and regenerate methionine (Combs, 2012). It has generally been accepted that B-vitamin requirements of mature ruminants are met by endogenous synthesis by ruminal microorganisms (Bechdel et al., 1928). However, Girard et al. (1989) observed a 40% decrease in serum folate concentrations in dairy cows from two months postpartum to parturition, suggesting increased demand for folate to support fetal growth and milk production. Since this observation was made, several studies have reported an increase in milk and milk protein yield when exogenous folic acid was administered to lactating dairy cows (Girard et al., 1995; Girard and Matte, 1998; Graulet et al., 2007; Preynat et al., 2009). As the demand for nucleic

Table 4. Effect of rumen-protected folic acid (RPFA) supplementation to beef steers on carcass characteristics and carcass-adjusted feedlot performance

	Treatment[a]							Contrast *P*-value		
	CON	RPFA-30	RPFA-60	RPFA-90	RPFA-120	RPFA-150	SEM[b]	Linear	Quadratic	Cubic
Carcass characteristic[c]										
HCW, kg	388	394	387	388	390	386	3.95	0.53	0.59	0.74
DP, %	63.5	64.2	63.9	63.6	63.8	63.6	0.22	0.75	0.32	0.15
REA, cm[b]	85.7	84.3	84.7	84.5	84.6	85.7	1.18	0.96	0.31	0.91
MS	459	479	467	467	473	469	17.0	0.84	0.76	0.69
BF, cm	1.59	1.53	1.54	1.77	1.59	1.53	0.090	0.87	0.34	0.19
KPH, %	2.3	2.2	2.4	2.3	2.4	2.3	0.06	0.21	0.52	0.47
YG	3.51	3.57	3.53	3.76	3.6	3.45	0.126	0.97	0.18	0.40
Liver abscess score[d], %										
0	79.3	96.7	90.0	83.3	83.3	82.1	-	0.29	0.22	0.06
A	13.8	3.3	6.7	6.7	13.3	10.7	-	0.57	0.26	0.21
A+	6.9	0	3.3	10.0	3.3	7.1	-	0.27	0.37	0.18
Carcass-adjusted performance[e]										
Final BW, kg	611	613	607	610	611	607	6.3	0.69	0.93	0.81
ADG, kg	1.75	1.76	1.73	1.75	1.75	1.73	0.035	0.68	0.94	0.80
DMI, kg/d	10.0	10.3	10.2	10.2	10.2	10.1	0.18	0.76	0.39	0.40
G:F	0.176	0.170	0.170	0.172	0.172	0.171	0.0023	0.30	0.31	0.11

[a]Treatments (n = 6 pens/treatment): target intake of 0 (CON), 30 (RPFA-30), 60 (RPFA-60), 90 (RPFA-90), 120 (RPFA-120), or 150 (RPFA-150) mg RPFA·steer^{-1}·d^{-1}.

[b]Highest SEM of any treatment reported.

[c]HCW = hot carcass weight; DP = dressing percent; REA = ribeye area; MS = marbling score (small: 400, modest: 500); BF = 12th rib backfat; KPH = kidney, pelvic, and heart fat; YG = calculated yield grade.

[d]Liver abscess scores based on the Elanco Liver Check System (Elanco Animal Health, Greenfield, IN): 0 = no abscesses; A = one or two small abscesses or abscess scars; A+ = one or more large, active abscesses.

[e]Carcass-adjusted final body weight (BW; d 182) was calculated by dividing HCW by average dressing percent for each treatment (CON = 63.5%, RPFA-30 = 64.2%, RPFA-60 = 63.9%, RPFA-90 = 63.6%, RPFA-120 = 63.8%, and RPFA-150 = 63.6%); initial shrunk BW (d 0) used as a covariate in analysis of carcass-adjusted performance variables; ADG = average daily gain; DMI = dry matter intake; G:F = gain:feed.

Table 5. Effect of rumen-protected folic acid (RPFA) supplementation to beef steers on plasma concentrations of folate, vitamin B12, and glucose at the end of growing (d 55) and finishing (d 181)

	Treatment[a]							Contrast *P*-value		
	CON	RPFA-30	RPFA-60	RPFA-90	RPFA-120	RPFA-150	SEM[b]	Linear	Quadratic	Cubic
Folate, ng/mL										
d −1[c]	12.4	11.9	12.1	11.6	12.5	13.3	–	–	–	–
d 55	10.7	11.7	12.7	13.4	15.0	15.6	1.01	<0.01	0.97	0.90
d 181	14.5	15.5	20.6	17.4	19.1	19.3	1.31	<0.01	0.13	0.45
Vitamin B12, pg/mL[d]										
d 181	196	189	157	161	208	198	13.6	0.55	0.02	0.47
Glucose, mg/dL										
d −1[c]	73.5	81.1	79.7	75.5	79.6	82.3	–	–	–	–
d 55	87.1	86.6	81.7	82.5	80.1	78.6	2.68	0.01	0.83	0.99
d 181	77.3	79.3	77.0	78.9	76.8	75.3	3.28	0.57	0.58	0.99

[a]Treatments (n = 6 pens/treatment): target intake of 0 (CON), 30 (RPFA-30), 60 (RPFA-60), 90 (RPFA-90), 120 (RPFA-120), or 150 (RPFA-150) mg RPFA·steer^{-1}·d^{-1}.

[b]Highest SEM of any treatment reported.

[c]Initial (d −1) values were used as covariates in analysis of subsequent sampling timepoints.

[d]Only values for d 181 are reported because 89% of d −1 samples and 75% of d 55 samples analyzed <150 pg/mL.

acid and protein synthesis increases with bone and muscle growth, exogenous folic acid supplementation to feedlot cattle may be warranted. Previous experiments have estimated ruminal escape of unprotected folic acid to be approximately 3% (Zinn et al., 1987; Santschi et al., 2005). Therefore, exogenous folic acid supplementation to ruminants has relied on large doses delivered in the diet

Table 6. Effect of rumen-protected folic acid (RPFA) supplementation to beef steers on liver concentrations of folate and vitamin B12 at the end of growing (d 48/49) and finishing (d 177/178)

	Treatment[a]							Contrast *P*-value		
	CON	RPFA-30	RPFA-60	RPFA-90	RPFA-120	RPFA-150	SEM[b]	Linear	Quadratic	Cubic
Folate, ng/mL[c]										
d 48/49	1.59	1.10	1.16	1.43	1.44	1.20	0.146	0.58	0.45	0.01
d 177/178	2.29	3.89	2.51	2.61	2.71	2.25	0.556	0.41	0.36	0.27
Vitamin B12, ng/mL										
d –5/–4[d]	39.9	25.7	63.4	24.4	55.2	39.2	–	–	–	–
d 48/49	63.4	63.1	38.5	45.7	54.3	49.1	8.78	0.15	0.12	0.70
d 177/178	62.7	33.6	77.7	52.4	70.5	73.1	12.3	0.12	0.58	0.46

[a]Treatments (n = 6 pens/treatment): target intake of 0 (CON), 30 (RPFA-30), 60 (RPFA-60), 90 (RPFA-90), 120 (RPFA-120), or 150 (RPFA-150) mg RPFA·steer^{-1}·d^{-1}.

[b]Highest SEM of any treatment reported.

[c]Only values for d 48/49 and 177/178 are reported because 64% of d –5/–4 samples analyzed 1 ng/mL.

[d]Initial (d –5/–4) values were used as covariates in analysis of subsequent sampling timepoints.

or weekly intramuscular injections. Because weekly injections are not logistically feasible in the beef industry, the current study sought to investigate the effects of a novel rumen-protected folic acid supplement (approximately 45% rumen-protected) on beef steer feedlot performance.

Based on a cross-sectional study of 22 dairy herds in the United States (totaling 427 cows), plasma folate concentrations averaged 13.4 ng/mL without folic acid supplementation (Duplessis et al., 2020). In the current study, plasma folate concentrations averaged 10.7 and 14.5 ng/mL for CON at the end of growing and finishing, respectively. The increase in plasma folate concentrations for unsupplemented animals may reflect diet composition changes throughout the trial. Like the finishing diet in the current study, high-concentrate diets have been associated with increased concentrations of folate in the rumen (Hayes et al., 1966; Girard et al., 1994) and plasma (Duplessis et al., 2020). Supplemental RPFA linearly increased plasma folate concentrations at the end of growing and finishing, indicating some folic acid supplied by the RPFA supplement was absorbed and entered the bloodstream.

Folate and vitamin B12 are intricately linked, as the enzyme methionine synthase, which converts 5-methyl-tetrahydrofolic acid back to tetrahydrofolic acid, is vitamin B12 dependent (Loughlin et al., 1964). Because ruminal synthesis of vitamin B12 is dependent on an adequate supply of dietary Co, all steers were supplemented with 1 mg Co/kg DM (diet analyzed 1.1 mg Co/kg DM) throughout the trial. This dietary concentration is almost 6.5 times the requirement for beef cattle (0.15 mg Co/kg DM; NASEM, 2016) and should have prevented a vitamin B12 deficiency. However, plasma vitamin B12 concentrations at the end of the growing period were extremely low (<150 pg/mL), regardless of RPFA treatment. Previous research in dairy cows has indicated supplemental folic acid does not affect productive or metabolic responses when plasma concentrations of vitamin B12 are below 200 ng/mL (Girard and Matte, 2005). Although plasma vitamin B12 concentrations were near this threshold by the end of the current study (185 pg/mL), vitamin B12 status may have limited the response to RPFA (Herbert and Zalusky, 1962). The stability of vitamin B12 in serum samples stored at -80°C has been shown to linearly decrease over time (85.6%, 62.2%, and 50.5% after 1, 8, and 13 years of storage, respectively; Jansen and Beekhof, 2018). Plasma samples collected in the current trial were stored at −80°C for no more than 1 year prior to analysis so it is possible vitamin B12 concentrations were slightly greater at the time of collection.

Supplemental RPFA linearly decreased plasma glucose concentrations at the end of the growing period. Serum folate concentrations have been inversely associated with insulin resistance in humans (Li et al., 2017), and folic acid supplementation improved insulin sensitivity in lactating dairy cows (Girard et al., 2019). Therefore, the decrease in circulating glucose concentrations observed in the current study could indicate an increase in glucose uptake by peripheral tissues in response to insulin signaling. Vitamin B12 also supports energy metabolism as a cofactor of methylmalonyl CoA mutase, which catalyzes the last step in converting propionyl-CoA to succinyl-CoA for entry into the citric acid cycle (Flavin and Ochoa, 1957; Eggerer et al., 1960). This reaction is important in ruminants as propionate, produced by ruminal fermentation of dietary carbohydrates, is the primary

gluconeogenic precursor. Graulet et al. (2007) investigated the effects of dietary supplements of folic acid (0 or 2.6 g/d) and vitamin B12 (0 or 0.5 g/d) on the metabolism of dairy cows in early lactation. When folic acid and vitamin B12 were supplemented together, plasma glucose concentrations were increased, but when folic acid was supplemented alone, plasma glucose concentrations decreased (Graulet et al., 2007). A weekly intramuscular injection of folic acid (320 mg) alone or in combination with vitamin B12 (10 mg) tended to decrease whole-body glucose rate of appearance, which represents the sum of glucose available from portal absorption, glycogenolysis, and gluconeogenesis (Duplessis et al., 2017). Alternatively, a weekly intramuscular injection of folic acid (160 mg) and vitamin B12 (10 mg) increased whole-body glucose availability in lactating dairy cows (Preynat et al., 2009). These data further illustrate the complex interrelationship between folate and vitamin B12. By the end of the current trial, there were no effects of RPFA on plasma glucose concentrations, potentially due to an improvement in vitamin B12 status and increased capacity for gluconeogenesis or increased insulin resistance in late-stage finishing steers (Smith, 2017).

The liver represents a major storage site for folates and vitamin B12 in the body (Combs, 2012). In contrast to the linear effects of RPFA observed in plasma, there was a cubic effect of RPFA on liver folate concentrations at the end of growing driven by greater concentrations for CON, RPFA-90, and RPFA-120 relative to other treatments. A cubic effect of RPFA was also observed for growing period performance, driven by greater BW, ADG, and G:F for CON and RPFA-120 relative to other treatments. A similar dose-response to the one observed herein has been observed in growing pigs, where pigs supplemented with 0 or 12.5 mg folic acid/kg DM were more efficient than pigs supplemented with 2 or 5 mg folic acid/kg DM (Wang et al., 2020). Numerous studies have reported no effect of folic acid supplementation on DMI, BW, or body condition score of dairy cows (Girard et al., 1995; Preynat et al., 2009; Duplessis et al., 2017; Girard et al., 2019). Very limited research has been conducted regarding folic acid supplementation on beef cattle growth. Zinn et al. (1987) observed no effect of B-vitamin supplementation to crossbred beef calves (116 kg) on ADG, DMI, or F:G for the first 56 d in the feedlot. However, it is impossible to determine the specific effects of folic acid in this study as multiple B-vitamins (folic acid and vitamin B12, among others) were supplemented at once.

There was a linear effect of RPFA on transition period DMI in the current study, driven by the greatest DMI for RPFA-150. The transition period was designed to prevent rapid changes in dietary energy concentration and subsequent ruminal acidosis, which is thought to be the primary cause of liver abscesses in cattle (Nagaraja and Chengappa, 1998). The current study is the first to investigate the effects of RPFA on liver abscesses in cattle to the authors' knowledge. In humans and mice, a folate deficiency has been associated with liver injury and nonalcoholic fatty liver disease (Pogribny et al., 2013; Sid et al., 2017; Xia et al., 2018). Steers supplemented RPFA at 30 or 60 mg/d tended to have a greater percentage of livers with no abscesses (93.4%) compared to steers receiving no supplemental RPFA (79.3%). While liver abscesses are often associated with high-energy diets fed to beef cattle in feedlots, Holstein steers have been shown to have a greater incidence of liver abscesses (28.3%) compared to beef steers and heifers (15.0%; Amachawadi and Nagaraja, 2016). With an increasing number of dairy influenced cattle being fed for beef production, future research should investigate the effects of folic acid supplementation on liver abscess development in these animals.

B-vitamin supplementation has been vastly ignored in the field of ruminant nutrition due to the ability of rumen microorganisms to synthesize these vitamins endogenously. However, it is difficult to estimate B-vitamin supply in ruminants as it includes dietary sources that escape ruminal destruction or utilization and the amount synthesized by ruminal microorganisms (Girard and Graulet, 2021). It was hypothesized that increasing supplemental RPFA would help support the DNA and protein synthesis required for the growth of steers in the current study. Supplemental RPFA did increase plasma folate concentrations throughout the study but did not improve feedlot performance, possibly due to a lack of vitamin B12 to support cellular utilization of folate. Further dose titration studies are warranted utilizing lower doses of RPFA than those tested herein.

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