IOWA STATE UNIVERSITY
DEPARTMENT OF ANIMAL SCIENCE

Influence of supplementing vitamin C to yearling steers fed a high sulfur diet during the finishing period on feedlot performance and blood metabolites

A project funded in part by:

Burrough's Research Fund, Iowa State University
and
Iowa Corn Promotion Board

Danielle Pogge, Ph.D.

Stephanie Hansen, Ph.D.

Steven Lonergan, Ph.D.

January 2014

PROJECT SUMMARY

High sulfur (**S**) diets can hinder live animal performance and carcass characteristics, induce oxidative stress, and express residual effects on meat quality. The antioxidant properties of vitamin C (**VC**) may aid in negating the severity of oxidative stress and potentially recover some negative effects imposed by high S diets. The objective of this study was to examine the effects of VC supplementation during the finishing period of yearling Angus-cross steers (n = 140) on finishing performance and blood metabolites of steers receiving a 40% dry distillers grains plus solubles (**DDGS**) diet (0.55% S). Steers were blocked by initial body weight (**BW**; 950 ± 56.1 lbs), stratified within blocks by intramuscular fat (3.6% ± 0.30) determined by ultrasonography, and assigned to treatments (5 steers/pen, 7 pens/treatment). Treatments included: 1) no VC control (**CON**), 2) 5 g VC·steer^{-1}·d^{-1} (**5VC**), 3) 10 g VC·steer^{-1}·d^{-1} (**10VC**), and 4) 20 g VC·steer^{-1}·d^{-1} (**20VC**). Steers were harvested by block on three separate dates (days 91, 105, and 112). Sulfur intake linearly decreased (P = 0.01) as VC inclusion increased (59.2, 57.7, 57.0, 54.8 ± 0.79 g S·steer^{-1}·d^{-1}, for CON, 5VC, 10VC, and 20VC, respectively). As the dose of supplemented VC increased steers consumed less feed, but did not alter daily gain or final BW, indicating VC supplementation improved feed efficiency (F:G). Interestingly, plasma ascorbate (vitamin C) was greatest in the CON (1,454 µg/L), 10VC (1,304 µg/L), and 20VC (1,436 µg/L) steers compared to 5VC steers (1,186 µg/L). Under the conditions of our study, VC supplemented to a high S diet for an average of 102 days prior to harvest has limited effects on blood metabolites and ultrasound measures, but tended to increase feed efficiency of yearling steers.

INTRODUCTION

In cattle diets, greater inclusions of DDGS are often associated with greater dietary S concentrations, as the S content of DDGS can range from 0.3 to greater than 1% S (Kim, Zhang, & Stein, 2012). However, due to economic incentive, greater amounts of DDGS are being incorporated into cattle diets, thus exposing cattle across the United States to greater dietary concentrations of S than have been traditionally fed. High S diets hinder live animal performance and carcass characteristics (Zinn et al., 1997; Buckner et al., 2007; Richter et al., 2012; Pogge

and Hansen, 2013), specifically decreasing dry matter intake (**DMI**), average daily gain (**ADG**), hot carcass weight (**HCW**), and marbling score.

The role of VC as an antioxidant is well defined; however, the need for VC in finishing cattle diets has been examined less because cattle are able to synthesize ascorbate (VC) from glucose in the liver, a process not found in humans (Combs, 2008). Cattle can synthesize VC, but the quantity of circulating VC decreases in fattening cattle (Takahashi et al., 1999) and across the first 90 days of cattle being fed a 0.55% S diet (Pogge and Hansen, 2013). Additionally, the supplementation of a rumen-protected VC may enhance marbling scores of cattle receiving a 0.55% S diet (Pogge and Hansen, 2013), increase ribeye area (**REA;** Oohashi et al., 1999; Yano, 2002), and prevent the decline of circulating VC in finishing steers (Ohashi et al., 2000, Pogge and Hansen, 2013). Sulfur has been shown to be a contributing factor in the development of oxidative stress (Truong et al., 2006; Pogge and Hansen, 2013); however, it is still unclear what concentration of supplemental VC is needed to support live and carcass-based performance in cattle fed high S diets.

Therefore, the objective of this study was to examine the effects of four concentrations of VC supplemented for an average of 102 days prior to harvest on steer performance, ultrasound measures, and blood metabolites of steers receiving a 40% DDGS high S diet.

MATERIALS and METHODS

Study Design and Live Animal Measures

Yearling Angus-cross steers (n = 140) were purchased and transported to the Iowa State University Beef Nutrition Farm. Prior to the start of the study (day -19 or -14) ultrasound measures captured REA, percent intramuscular fat of the REA, back fat thickness (**BF**), and rump fat thickness from each steer. At the initiation of the study (March, 2012), consecutive day weights were taken and steers were blocked by initial body weight (**BW**; 950 ± 56.1 lbs), stratified within blocks by ultrasound-measured initial intramuscular fat (3.6% ± 0.30), and randomly assigned to 1 or 4 treatments (5 steers/pen, 7 pens/treatment), including: 1) no VC control (**CON**), 2) 5 g VC·steer^{-1}·day^{-1} (**5VC**), 3) 10 g VC·steer^{-1}·day^{-1} (**10VC**), and 4) 20 g VC·steer^{-1}·day^{-1} (**20VC; Table 1**). Prior to receiving the assigned study diets, steers were implanted with Component TE-IS. Single day weights were collected every 28 days and

consecutive final BW were determined over the two days immediately prior to the harvest day. Two days before harvest, in conjunction with collection of final BW, ultrasound measures (REA, percent intramuscular fat of REA, BF, and rump fat thickness) were determined.

A VC premix containing Vitashure C50 (a rumen protected ascorbic acid, 50% VC product; Balchem Corp., New Hampton, NY) and DDGS was used to introduce VC to the diet. The DDGS (POET; Jewell, IA) used in this study ranged from 0.97 to 1.04% S and 5.9 to 7.83% fat, and calcium sulfate was included in the diet to maintain a targeted total S concentration of 0.55% S. Weekly pen DMI was calculated and F:G was calculated every 28 days from steer weight gain and total DMI for each 28 days interim weight period. A 4% pencil shrink was applied to all live BW measures prior to calculation of ADG. Jugular blood was collected for the analysis of the plasma metabolites: insulin, non-esterified fatty acids (**NEFA**), and ascorbate.

Statistical Analysis

The experimental design was a randomized complete block and the data were analyzed by ANOVA using the Mixed Procedure of SAS (SAS Institute Inc., Cary, NC). The model for the analysis of the single time point data included the fixed effects of treatment and block. Repeated measures analysis included the fixed effects of treatment, block, time of sampling, and the interaction. Time was the repeated effect, pen was the experimental unit, and day 0 values were used as a covariate for all data. Three a priori single degrees of freedom contrast statements were constructed: A) no VC versus VC, B) linear effect of VC, and C) quadratic effect of VC. Significance was declared at $P \leq 0.05$ and tendencies were declared from $P = 0.06$ to 0.10.

Table 1. Ingredient composition of finishing diets (% DM basis)

Ingredient	Common Diet[1]
Corn	45.0
Corn dried distiller's grains[2,3]	40.0
Chopped hay	6.5
Corn silage	5.5
Limestone	1.4
Salt	0.3
Vitamin A premix[4]	0.1
Trace mineral premix[5]	0.035
Rumensin90[6]	0.01
Calcium sulfate[7]	0.60
Analyzed composition	
S[8], %	0.54
Calculated composition[9]	
% Lipid	5.08
Vitamin E, IU·kg^{-1} diet DM	304.3

[1]Treatments: CON: control; 5VC: 5 g vitamin C·steer^{-1}·day^{-1}; 10VC: 10 g vitamin C·steer^{-1}·day^{-1}; 20VC: 20 g vitamin C·steer^{-1}·day^{-1}

[2]Vitashure C (donated by Balchem Corp., New Hampton, NY) replaced distillers grains plus solubles (DDGS), by 0.11 to 0.43% diet DM, to achieve the target level of vitamin C per steer per day

[3]Four loads of DDGS (POET; Jewell, IA) were used during the trial, S concentrations were: 0.97, 1.04, 0.96, and 0.96% and fat content: 7.83, 6.91, 5.90, and 6.25%.

[4]Vitamin A premix contained 4,400,000 IU/kg

[5]Provided per kg of diet: 30 mg Zn as $ZnSO_4$; 20 mg Mn as $MnSO_4$; 0.5 mg I as $Ca(IO_3)_2(H_2O)$; 0.1 mg Se as Na_2SeO_3; 10 mg Cu as $CuSO_4$; and 0.1 mg Co as $CoCO_3$

[6]Provided at 27 g/ton diet (donated by Elanco Animal Health)

[7]Calcium sulfate was included at an average of 0.60% diet DM (range of 0.47 to 0.67%), at the expense of DDGS, to targeted S content in the diet

[8]S content for the four treatments are repeated measures least squares mean averages throughout the entire study

[9]Lipid content was calculated from the analyzed lipid content of individual ingredients and vitamin E concentrations were calculated based on NRC values for each ingredient

RESULTS

Animal Performance

Performance data were collected thanks to funding by the Wise Burroughs Fund in the Department of Animal Science at Iowa State University. Steer performance, S intake, and

supplemental VC intake data are presented in Table 2. Based on repeated measures analysis across the experiment, DMI decreased ($P = 0.004$) as VC concentration increased in the diet, while F:G tended to increase ($P = 0.08$) with increasing VC inclusion to the diet throughout the finishing period. Vitamin C inclusion did not affect ADG ($P = 0.89$) or final BW ($P = 0.48$). No treatment by week ($P = 0.41$; DMI) or treatment by month interactions ($P = 0.95$; ADG and F:G) were observed. No differences due to VC supplementation ($P \geq 0.22$) were observed among final ultrasound data (Table 3). As designed, supplemental VC intake was different ($P < 0.01$) among treatments and VC intake demonstrated a treatment by week interaction ($P < 0.001$; data not shown). This interaction is likely due to the changing rate of VC inclusion each week, as DMI did not display a similar treatment by week interaction. Sulfur intakes closely followed DMI and linearly decreased ($P = 0.01$) with increasing concentrations of VC in the diet, and displayed a treatment by week interaction ($P < 0.001$; data not shown).

Table 2. The effect of varying concentrations of supplemental vitamin C (VC) on dry matter intake, gain, and efficiency of steers consuming a common high S (0.55% S) diet

	CON[1]	5VC[1]	10VC[1]	20VC[1]	SEM	Contrast Statements[2] *P* values		
Live Performance						Con vs. VC	Linear VC	Quad. VC
Initial weight[3], lbs	950	953	950	950	0.9	0.40	0.61	0.27
Final weight[3], lbs	1,329	1,318	1,333	1,333	7.9	0.56	0.48	0.55
DMI[4,5], lbs/d	25.08	23.85	24.40	23.34	0.326	0.004	0.004	0.65
ADG[4,6], lbs/d	3.67	3.56	3.76	3.61	0.130	0.84	0.89	0.71
F:G[4,6]	6.81	6.62	6.52	6.50	0.067	0.16	0.08	0.61
S intakes[7], g/d	59.2	57.7	57.0	54.8	0.79	0.01	0.01	0.82

[1]Treatments: CON: control; 5VC: 5 g vitamin C·steer^{-1}·day^{-1}; 10VC: 10 g vitamin C·steer^{-1}·day^{-1}; 20VC: 20 g vitamin C·steer^{-1}·day^{-1}

[2]Contrast Statements: CON vs. VC = no vitamin C vs. vitamin C; Linear VC = linear effect of vitamin C; Quad VC = quadratic effect of vitamin C

[3]A 4% pencil shrunk was applied to weights

[4]Dry matter intake, S intake, ADG, and F:G were analyzed as repeated measures

[5]Week $P < 0.001$; Treatment by week $P > 0.41$

[6]Month $P < 0.001$; Treatment by month $P \geq 0.95$

[7]Week $P < 0.001$; Treatment by week $P < 0.001$

Table 3. The effect of varying concentrations of supplemental vitamin C (VC) on ultrasound measures of steers consuming a common high S (0.55% S) diet

	CON[1]	5VC[1]	10VC[1]	20VC[1]	SEM	Contrast Statements[2] *P* values		
Initial						Con vs. VC	Linear VC	Quad VC
Rump fat, %	0.29	0.28	0.31	0.30	0.015	0.89	0.47	0.99
IMF[3], %	3.58	3.61	3.59	3.62	0.068	0.72	0.71	0.99
Back fat, in	0.25	0.25	0.25	0.25	0.117	0.94	0.98	0.88
REA[4], in^2	10.55	10.76	10.64	10.98	0.16	0.21	0.11	0.77
End								
Rump fat, %	0.59	0.58	0.59	0.57	0.019	0.87	0.64	0.67
IMF, %	5.37	5.24	5.47	5.02	0.181	0.57	0.23	0.37
Backfat, in	0.54	0.54	0.56	0.53	0.019	0.79	0.85	0.41
REA, in^2	14.56	14.49	14.65	14.80	0.162	0.63	0.22	0.70

[1]Treatments: CON: control; 5VC: 5 g vitamin C·steer^{-1}·day^{-1}; 10VC: 10 g vitamin C·steer^{-1}·day^{-1}; 20VC: 20 g vitamin C·steer^{-1}·day^{-1}

[2]Contrast Statements: CON vs. VC = no vitamin C vs. vitamin C; Linear VC = linear effect of vitamin C; Quad VC = quadratic effect of vitamin C

[3]Percent intramuscular fat of the ribeye

[4]Ribeye area

Blood Metabolites

Plasma and serum metabolites are presented in Table 4. Final plasma ascorbate concentrations showed a quadratic response to supplemented VC ($P = 0.02$), in which the 5VC cattle exhibited lesser ($P \leq 0.01$) plasma ascorbate (1,186.2 µg/L ± 64.8) compared to the CON (1,454.0 µg/L) and 20VC cattle (1,436.4 µg/L), but were not different ($P = 0.21$) from the 10VC cattle (1,304.2 µg/L). The CON cattle tended to have a greater plasma VC ($P = 0.08$) and lesser plasma insulin ($P = 0.07$) concentration compared to VC supplemented cattle. Increasing VC inclusion did not affect serum NEFA ($P = 0.12$).

Table 4. The effect of varying concentrations of supplemental vitamin C (VC) on blood metabolites of steers consuming a common high S (0.55% S) diet

	CON[1]	5VC[1]	10VC[1]	20VC[1]	SEM	Contrast Statements[2] *P* values		
						CON vs. VC	Linear VC	Quad VC
Plasma metabolites								
Ascorbate[3], µg/L	1,454.0	1,186.2	1,304.2	1,436.4	64.8	0.08	0.53	0.02
Insulin[3], µg/L	1.28	1.65	1.85	1.69	0.19	0.07	0.23	0.11
HOMA-IR[4]	11.09	13.86	15.14	18.74	2.76	0.17	0.09	0.88
Serum NEFA[3], µEq/L	189.69	175.18	170.82	228.58	19.6	0.93	0.12	0.11

[1]Treatments: CON: control; 5VC: 5 g vitamin C·steer^{-1}·day^{-1}; 10VC: 10 g vitamin C·steer^{-1}·day^{-1}; 20VC: 20 g vitamin C·steer^{-1}·day^{-1}

[2]Contrast Statements: CON vs. VC = no vitamin C vs. vitamin C; Linear VC = linear effect of vitamin C; Quad VC = quadratic effect of vitamin C

[3]Jugular blood was drawn prior to feeding 2 days before harvest

[4]Homeostasis model assessment, insulin resistance

DISCUSSION

Performance and Blood Metabolites

Increasing the inclusion rate of VC tended to increase feed efficiency of steers, evident by a decrease in DMI of approximately 0.66 to 1.76 lbs/day with no differences in ADG or final BW among the four treatments. Pogge and Hansen (2013) reported no influence of VC supplementation on feed efficiency, but cattle were less efficient when dietary S was greater than 0.34%. However, amongst poultry and swine, increases in feed efficiency have been observed with VC supplementation, specifically in stressful situations such as heat stress and weaning (de Rodas et al., 1998; Sahin et al., 2003). While indicators of stress were not evaluated in the present study, Pogge and Hansen (2013) have reported a 0.55 % S diet may be involved in the development of oxidative stress in cattle, indicated by an increased ratio of oxidized-to-reduced glutathione in the liver. Bottje and Carstens (2009) indicated an increased ratio of oxidized-to-reduced glutathione corresponded to a less feed efficient animal. Pogge and Hansen (2013) reported the supplementation of VC (10 g·steer^{-1}·day^{-1}) to the 0.55% S diet decreased the ratio of oxidized-to-reduced glutathione. Because glutathione and VC share a regenerative relationship, VC may be alleviating some of the oxidative stress occurring in steers consuming a high S diet, which may help explain the tendency to improve feed efficiency in the present study.

Currently, the NRC (1996) does not specify a daily VC requirement cattle because of their ability to synthesize VC from glucose in the liver. Plasma ascorbate concentrations of healthy beef cattle, across all aspects of production, range from 2,400 to 4,700 µg/L (Smith et al., 2009), and they are as low as 294 to 742 µg/L in finishing steers consuming a 0.55% S diet (Pogge and Hansen, 2013). Takahashi et al. (1999) noted that plasma ascorbate concentrations decreased during the fattening period and suggested that this decrease may be related to the consumption of VC as a means to control the development of oxidative stress. Similar to this response, Pogge and Hansen (2013) reported a sharp decrease in plasma ascorbate during the initial 90 days of finishing in steers consuming a 0.55% S diet; however, when VC was included in the diet the decline in plasma ascorbate was prevented. In the present study, all cattle were fed a high S diet and no decreases in plasma ascorbate were noted during the finishing period; however, it is unknown how plasma ascorbate concentrations of steers consuming a high S diet may have compared with ascorbate concentrations in steers consuming a low S diet because such a diet was not examined in the present study.

Padilla et al. (2007) observed a linear increase in plasma ascorbate of fattening beef cows as the content of a supplemented rumen by-pass VC source increased. In the present study, it was hypothesized that plasma ascorbate concentration would be least in the non-supplemented CON cattle, and would linearly increase as the VC inclusion rate increased. However, this hypothesis was not supported by the experimental data. Despite supplementation of a rumen-protected VC source to three of the four treatment groups, the un-supplemented CON cattle exhibited the greatest plasma ascorbate concentration. While the reason for the unexpected plasma ascorbate differences is unknown, it may be related to individual animal variation or may be the result of changes in endogenous production of VC due to VC supplementation. Tsao and Young (1990) reported exogenous supplementation of VC to mice decreased the production of VC by the liver, and authors suggested the production of endogenous VC might be directly related to the concentration of VC in the portal blood. Interestingly, plasma ascorbate concentrations in the present study were markedly greater than those measured by Pogge and Hansen, 2013. The results of the current study and those of Pogge and Hansen (2013) suggest a potential for a threshold for circulating ascorbate, which may be influenced by the age of the cattle at the time of exposure to a high S finishing diet. The differing responses of plasma ascorbate

concentrations and marbling scores between the current study and Pogge and Hansen (2013) may suggest a certain plasma ascorbate concentration is necessary to support marbling.

NEFA concentrations were not different between treatments, and Oohashi et al. (1999) similarly reported no difference in NEFA concentration of cattle supplemented with 50 g·steer^{-1}·day^{-1} of L-ascorbic acid-2-phosphate for the entire finishing period compared to those supplemented for a portion (early or later) or not receiving supplemental VC at all during the finishing period. Interestingly, the supplementation of VC in the present study tended to increase plasma insulin concentrations by approximately 0.37 to 0.57μg/L compared to CON steers.

In conclusion, results of this study suggest that the supplementation of 5 to 20 g VC·steer^{-1}·day^{-1}, during the later finishing period (91 to 112 d prior to harvest), to yearling steers consuming a high S diet tended to improve feed efficiency; however, limited effects were noted on blood metabolites and only a tendency to affect other carcass characteristics. However, further research is required to determine the exact mechanism by which VC supplementation is altering feed efficiency.

Literature Cited

Bottje, W. G. and G. E. Carstens. (2009). Association of mitochondrial function and feed efficiency in poultry and livestock species. *Journal of Animal Science,* 87(E. Suppl.):E48-E63.

Buckner, C. D., Erickson, G. E., Mader, T. L., Colgan, S. L., and Karges, K. K. (2007). Optimum levels of dry distillers grains with solubles for finishing beef steers. *Nebraska Beef Cattle Reports*, paper 68.

Combs, Jr., G. F. (2008). The vitamins: Fundamental aspects in nutrition and health. 3rd ed. Elsevier Acad. Press, Philadelphia, PA.

de Rodas, B. Z., C. V. Maxwell, M. E. Davis, S. Mandali, E. Broekman, and B. J. Stoecker. (1998). L-ascorbyl-2-polyphosphate as a vitamin C source for segregated and conventionally weaned pigs. *Journal of Animal Science,* 76:1636-1643.

Kim, B. G., Zhang, Y., and Stein, H. H. (2012). Sulfur concentration in diets containing corn, soybean meal, and distillers dried grains with solubles does not affect feed preference or growth performance of weanling or growing-finishing pigs. *Journal of Animal Science,* 86:1579-1587.

Nutrition Research Council. (1996). Nutrient Requirements of Beef Cattle 7th Rev. ed. Natl. Acad. Press, Washington, D.C.

Ohashi, H., H. Takizawa, and M. Matsui. 2000. Effect of vitamin C on the quality of Wagyu beef. *Research Bulletin of the Aichi-ken Agricultural Research Center,* 32:207-214.

Oohashi, H., H. Takizawa, and H. Morita. (1999). Effects of vitamin C administration on the meat quality in Japanese black steers. *Research Bulletin of the Aichi-ken Agricultural Research Center* (Jpn.), 31:454-252.

Padilla, L., T. Matsui, K. Shibano, H. Katamoto, and H. Yano. (2007). The effect of vitamin C supplementation on plasma concentration and urinary excretion of vitamin C in cattle. *Journal of Animal Science,* 85:3367-3370.

Pogge, D. J. and Hansen, S. L. (2013). Supplemental vitamin C improves marbling in feedlot cattle consuming high sulfur diets. *Journal of Animal Science,* 91:4303-4314.

Richter, E. L., Drewnoski, M. E., and Hansen, S. L. (2012). Effects of increased dietary sulfur on beef steer mineral status, performance, and meat fatty acid composition. *Journal of Animal Science,* 90:3945-3953.

Sahin, K., N. Sahin, and O. Kucuk. (2003). Effects of chromium and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature (32°C). *Nutrition Research,* 23:225-238.

Smith, S. B., H. Kawachi, C. B. Choi, C. W. Choi, G. Wu, and J. E. Sawyer. (2009). Cellular regulation of bovine intramuscular adipose tissue development and composition. *Journal of Animal Science,* 87(E. Suppl.):E72-E82.

Takahashi, E., T. Matsui, S. Wakamatsu, N. Yuri, Y. Shiojiri, R. Matsuyama, H. Murakami, S.

Tanaka, S. Torii, and H. Yano. (1999). Serum vitamin C concentration in fattening and fattened beef cattle. *Animal Science Technology* (Jpn), 70:119-122.

Tsao, C. S. and M. Young. (1990). Effect of exogenous ascorbic acid intake on biosynthesis of ascorbic acid in mice. *Life Science,* 45:1553-1557.

Truong, D. H., Eghbal, M. A., Hindmarsh, W., Roth, S. H., and O'Brien, P. J. (2006). Molecular mechanisms of hydrogen sulfide toxicity. *Drug Metabolism Reviews,* 38:733-744.

Yano, H. (2002). The effect of vitamin C supplementation on fattening performance and carcass characteristics in beef cattle. *Shokuniku ni kansuru Josei Kenkyu Chosa Seika Hokokusho* (Jpn.). 20:154-158.

Zinn, R. A., Alvarez, E., Mendez, M., Montano, M., Ramirez, E., and Shen, Y. (1997). Influence of dietary sulfur level on growth performance and digestive function of feedlot cattle. *Journal of Animal Science,* 75:1723-1728.