

Effects of KemTRACE® Chromium on blood parameters, GLUT4 and muscle fiber characteristics of finishing cattle¹

Introduction

Optimizing nutrient utilization for cattle fed high concentrate finishing diets requires attention to detail and good understanding of specific nutrient mode of action. This is especially important in today's high genetic potential feeder animals that are fed in conjunction with designer implant programs and in combination with beta-agonists.

Chromium has been studied for over fifty years and is considered an essential nutrient². Chromium is important for efficient nutrient utilization playing key roles in carbohydrate, lipid and protein metabolism². Generally accepted, chromium (Cr), potentiates the action of insulin³. Understanding the mode of action of chromium helps explain an animal growth response. Chromium acts to potentiate the action of insulin through optimizing the efficiency of the insulin receptor providing more glucose at the cellular level. Because the animal uses glucose in a hierarchal manner, glucose may be used for body maintenance or immune system function at the expense of providing glucose for protein accretion only. Animals fed supplemental chromium are benefiting from the additional incremental glucose availability. Other studies with chromium have shown no or limited live cattle feedlot performance but do show carcass response, suggesting the additional glucose is providing the energy needed for muscle accretion^{4,5}.

Chromium included in the diet as KemTRACE® Chromium (CrPro), has improved performance of beef cattle, expressed as body weight gain, improved feed efficiency, lower morbidity^{6,7,8}. Results from studies with feeder cattle demonstrate feeding chromium has positive effects on immune response, cytokines, and acute phase response of cattle under stress⁶. The potential effects of improved glucose metabolism on carcass characteristics has shown trends in heavier hot carcass weight and increased dressing percentage. The effect of chromium propionate on bovine muscle, intramuscular adipose and subcutaneous adipose tissue development has shown chromium enhances adipocyte differentiation greater in intramuscular adipocytes than in subcutaneous adipocytes⁹. Enhancement of adenosine monophosphate-activated protein kinase (AMPK α) and glucose transporter type 4 (GLUT4) mRNA by CrPro treatment may enhance glucose uptake in intramuscular adipocytes.

Abstract

This study evaluated supplementing KemTRACE® Chromium at varying concentrations to beef cattle fed a high concentrate diet throughout the entire finishing period. Continental crossbred steers (n = 32, 367 \pm 2.5 kg) were utilized in a complete randomized block design to evaluate the following supplemental chromium treatments: 1) 0 ppb supplemental chromium, 2) 150 ppb supplemental chromium, 3) 300 ppb supplemental chromium, and 4) 450 ppb supplemental chromium, fed in a typical high concentrate feedlot finishing diet. Treatments were formulated using KemTRACE® Chromium (CrPro). Live and carcass performance was evaluated in addition to blood glucose, insulin, serum urea nitrogen (SUN), non-esterified fatty acids (NEFA) and muscle biopsy. *Longissimus* muscle biopsy samples were obtained on d 0, 28, 56, 91, 119, and 147. Biopsies were collected at approximately 8 h prior to feeding, along with a jugular vein blood sample, and body

weight. Cattle receiving 450 ppb treatment were significantly heavier ($P < 0.05$) starting on d 56 and remained the heaviest treatment group throughout the experiment, showing also a significant linear effect ($P < 0.05$) for body weight on d 56 until d 147. There was no treatment effect nor interaction of treatment and day ($P > 0.10$) on the blood parameters measured. Muscle fiber cross-sectional area increased for all treatments from d 0 to d 147 for all three fiber types. The cattle receiving the 450 ppb treatment showed greater increase in muscle cross sectional area compared with cattle on the control treatment. Density of internalized GLUT4 on d 147 compared with d 0, were greater for 450 ppb and 300 ppb treatments. These data support the putative effects of Cr on glucose transport in peripheral tissues, and provide support for supplementation of Cr throughout the beef feedlot finishing period.

Materials & Methods

Continental crossbred steers ($n = 32$, 367 ± 2.5 kg) were obtained from a single source and processed after arrival utilizing standard feedlot processing protocols. Steers were vaccinated, treated for parasites, and given an individual ear tag for identification. Steers were transitioned to a final finishing ration (Table 1) formulated to meet or exceed dietary requirements described by the NRC (2000)¹⁰. The ration was manufactured daily, and steers were fed *ad libitum* once daily at 0800 h throughout the feeding trial. Steers were implanted on d 0 and d 88 using Synovex® Choice (Zoetis USA, Florham Park, NJ). On d 119, all steers began to receive 300 mg/hd/d of ractopamine HCl (Optaflexx™, Elanco Animal Health, Greenfield, IN) for 28 d. Animals were monitored daily for signs of illness or lameness.

A complete randomized block design was utilized. Steers were weighed 7 d and 3 d prior to initiation of the experiment. An average of the two body weights were used to stratify steers into 4 weight blocks with 4 pens in each block and 2 steers per pen. Within each block, pens were randomly assigned to treatment: 1) 0 ppb supplemental chromium, 2) 150 ppb supplemental chromium, 3) 300 ppb supplemental chromium, and 4) 450 ppb supplemental chromium. Treatments were formulated using chromium propionate from KemTRACE® Chromium (Kemin Industries, Des Moines, IA) and ground corn as a carrier. The 0 ppb supplemental chromium contained only the ground corn carrier. Treatments were administered to pens by topdressing the ration daily. Feed refusals were collected weekly and weighed to measure intake.

Table 1. Ingredient composition (% DM basis) of the experimental diets*

| Ingredients | %, DM Basis |
|--|--------------------|
| Steam-flaked corn | 75.96 |
| Alfalfa hay, chopped | 9.74 |
| Cottonseed meal | 4.58 |
| Molasses | 3.64 |
| Texas Tech University mineral supplement | 2.08 |
| Fat | 2.00 |
| Urea | 0.80 |
| Calcium carbonate | 0.75 |

*Diets were formulated to meet or exceed NRC (2000) requirements for growing – finishing beef cattle.

Results and Discussion

There was no treatment effect nor interaction of treatment and day ($P > 0.05$, Table 2) on the blood parameters measured. However, a significant day effect ($P < 0.05$) was observed for insulin, SUN, and NEFA concentrations, and a tendency for a day effect for glucose was present ($P < 0.10$). This is similar to previous research showing no difference in blood metabolites in receiving steers after 56 d of Cr-Pro supplementation⁴.

Table 2. Effects of chromium supplementation on blood serum metabolites of feedlot steers.

| Day | Glucose, mg/dL | Insulin, ng/mL | SUN, mg/dL | NEFA, mmol/L |
|------------------|----------------|--------------------|--------------------|--------------------|
| d 0 | 104.6 | 1.99 ^{wx} | 11.87 ^x | 0.29 ^x |
| d 28 | 97.9 | 2.44 ^{xy} | 11.91 ^x | 0.25 ^x |
| d 56 | 94.4 | 2.70 ^y | 17.11 ^z | 0.27 ^x |
| d 91 | 101.9 | 3.41 ^z | 14.44 ^y | 0.30 ^x |
| d 119 | 90.1 | 2.98 ^{yz} | 13.83 ^y | 0.38 ^y |
| d 147 | 86.4 | 1.44 ^w | 15.01 ^y | 0.31 ^{yx} |
| SEM | 7.48 | 0.700 | 0.864 | 0.059 |
| Treatment | | | | |
| 0 ppb | 100.5 | 2.37 | 13.70 | 0.31 |
| 150 ppb | 93.1 | 2.86 | 14.32 | 0.30 |
| 300 ppb | 90.4 | 1.98 | 14.45 | 0.28 |
| 450 ppb | 99.5 | 2.76 | 13.65 | 0.31 |
| SEM ¹ | 6.38 | 0.625 | 1.066 | 0.047 |
| P-values | | | | |
| Treatment | 0.2589 | 0.4984 | 0.8216 | 0.8441 |
| Day | 0.0701 | <0.0001 | <0.0001 | 0.0031 |
| Trt*Day | 0.4605 | 0.7551 | 0.2056 | 0.8960 |

^{wxyz} Means within same row with different superscripts differ for effect of day ($P < 0.05$)

¹Pooled standard error of the mean

Table 3. Effects of chromium supplementation on fiber cross-sectional area (μm^2) of *longissimus* biopsies of feedlot steers.

| Treatment | Type I | Type IIA | Type IIX |
|------------------|----------------------|------------------------|------------------------|
| 0 ppb | | | |
| d 0 | 2994 ^{fg} | 4030 ^{ghi} | 5830 ^{hij} |
| d 28 | 2978 ^{fg} | 4320 ^{fghi} | 6729 ^{efghi} |
| d 56 | 2988 ^{fg} | 4355 ^{efgh} | 5544 ^{ij} |
| d 91 | 3186 ^{efg} | 5304 ^{abcd} | 7361 ^{bcdefg} |
| d 119 | 4361 ^a | 5510 ^{abc} | 8330 ^{abc} |
| d 147 | 3947 ^{abcd} | 5563 ^{abc} | 7391 ^{bcdefg} |
| 150 ppb | | | |
| d 0 | 2707 ^{gh} | 3816 ^{hi} | 5723 ^{hij} |
| d 28 | 2324 ^h | 3422 ⁱ | 5483 ^j |
| d 56 | 2909 ^{fg} | 4239 ^{fghi} | 6579 ^{fghij} |
| d 91 | 3679 ^{bcde} | 5034 ^{bcdef} | 6177 ^{ghij} |
| d 119 | 4447 ^a | 6142 ^a | 8566 ^{ab} |
| d 147 | 3671 ^{bcde} | 5354 ^{abcd} | 7880 ^{abcd} |
| 300 ppb | | | |
| d 0 | 2854 ^{gh} | 4240 ^{fghi} | 5600 ^{ij} |
| d 28 | 3185 ^{efg} | 4490 ^{defgh} | 6942 ^{efgh} |
| d 56 | 3077 ^{fg} | 4945 ^{cdefg} | 7348 ^{bcdefg} |
| d 91 | 2650 ^{gh} | 4713 ^{cdefgh} | 7536 ^{bcdef} |
| d 119 | 3410 ^{def} | 5085 ^{abcde} | 6820 ^{efgh} |
| d 147 | 3616 ^{cde} | 5286 ^{abcde} | 8262 ^{abcd} |
| 450 ppb | | | |
| d 0 | 2665 ^{gh} | 3953 ^{hi} | 5898 ^{hij} |
| d 28 | 2702 ^{gh} | 4430 ^{defgh} | 6686 ^{efghi} |
| d 56 | 2677 ^{gh} | 3935 ^{hi} | 5506 ^{ij} |
| d 91 | 3168 ^{efg} | 4983 ^{cdef} | 7250 ^{defg} |
| d 119 | 4241 ^{ab} | 5936 ^{ab} | 8888 ^a |
| d 147 | 4133 ^{abc} | 6065 ^a | 6997 ^{defgh} |
| SEM ¹ | 294.2 | 482.8 | 638.9 |
| P-values | | | |
| Treatment | 0.7326 | 0.9485 | 0.9293 |
| Day | <0.0001 | <0.0001 | <0.0001 |
| Trt*Day | <0.0001 | <0.0001 | <0.0001 |

^{abcde fghij} Means within same row with different superscripts differ for effect of interaction of day and treatment ($P < 0.05$)

¹Pooled standard error of the mean

An interaction between treatment and day ($P < 0.05$) was observed for cross-sectional area for all three fiber types. For the fiber type distribution, there were interactions of treatment and day present ($P < 0.05$). This data supports the increase in live weight and HCW. Table 3 shows fiber area for each treatment over time.

For the density of GLUT4, there was a treatment by day interaction ($P < 0.05$). The density of GLUT4 decreased from d 0 to d 147 for all treatments, but the density in samples from the control treatment decreased more than the three treatments receiving chromium (Figure 2). Internalization of GLUT4 was also measured, as exhibited in Figure 3. There was not a treatment by day interaction ($P > 0.05$). However, there were significant treatment and day effects ($P < 0.05$). There was a greater density of internalized GLUT4 on d 147 compared with d 0, and the 450 ppb and 300 ppb treatments had greater densities of internalized GLUT4 than the control treatment ($P < 0.05$).

Conclusions

The objective of this study was to evaluate feeding varying concentrations of chromium to beef cattle fed a high concentrate diet throughout the finishing phase utilizing live animal and carcass performance. There were no significant differences in measured blood parameters, glucose, insulin, serum urea nitrogen, (SUN) or NEFA's. Fiber cross-sectional area increased for all treatments from d 0 to d 147 for all three fiber types. However, there was a greater amount of increase for the 450 ppb treatment compared with the control treatment. The density of GLUT4 decreased from d 0 to d 147 for all treatments, but the density in samples from the control treatment decreased more than the three treatments receiving chromium. There was a greater density of internalized GLUT4 on d 147 compared with d 0, and the 450 ppb and 300 ppb treatments had greater densities of internalized GLUT4 than the control treatment.

The importance of GLUT4 to skeletal muscle homeostasis has been established¹¹. When GLUT4 is disrupted in mouse skeletal muscle, there is a drastic reduction in glucose transport, insulin sensitivity, and glucose tolerance. Supplementation with CrPro has been shown to increase insulin sensitivity in beef cattle¹². The increase in fiber cross-sectional area coupled with the increase of internalized GLUT4 transporters in the 450 ppb CrPro treatment suggests that CrPro supplementation increases the insulin sensitivity and glucose tolerance to allow for continued growth of skeletal muscle fibers even when GLUT4 is translocated to the interior of the cell. The results of this study indicate feeding 450 ppb chromium throughout the entire finishing period maximizes feedlot performance and optimizes carcass muscle accretion.

References

1. Kemin Internal Document, 16-00043.
2. Perchova, A., Pavlata, L. 2007. Chromium As An Essential Nutrient: a Review. *Veterinari Medicina*, 52, 1:1-18.
3. The Role of Chromium in Animal Nutrition. National Research Council. 1997. ISBN: 978-0-309-06354-8.
4. Sanchez-Mendoza, B., A. Montelongo-Terriquez, A. Plascencia, N. Torrentera, R.A. Ware, and R.A. Zinn. 2014. Influence of feeding chromium-enriched enzymatically hydrolyzed yeast on growth performance, dietary energetics and carcass characteristics in feedlot cattle under conditions of high ambient temperature. *Journal of Applied Animal Research*, 43:4, 390-395.
5. Kneeskern, S.G., A.C. Dilger, S.C. Loerch, D.W. Shike, and T. L. Felix. Effects of chromium supplementation to feedlot steers on growth performance, insulin sensitivity, and carcass characteristics. 2015. *J. Animal Sci.* 2016.94 doi:10.2527/jas2015-9517.
6. Bernhard, B.C., N.C. Burdick, W. Rounds, R.J. Rathman, J.A. Carroll, D.N. Finck, M.A. Jennings, T.R. Young, and B.J. Johnson. 2012. Chromium supplementation alters the performance and health of feedlot

- cattle during the receiving period and enhances their metabolic response to a lipopolysaccharide challenge. *J. Animal Sci.* 90:3879-3888.
7. Bernhard, B.C., N.C. Burdick, R.J. Rathman, J.A. Carroll, D.N. Finck, M.A. Jennings, T.R. Young, and B.J. Johnson. 2012. Chromium supplementation alters both glucose and lipid metabolism in feedlot cattle during the receiving period. *J. Animal Sci.* 90:4857-4865.
 8. Burdick, N.C., Bryan C. Bernhard, Jeffrey A. Carroll, Ryan J. Rathman, Bradley J. Johnson. 2011. Enhancement of the acute phase response to a lipopolysaccharide challenge in steers supplemented with chromium. *Innate Immunity* 18 (4) 592-601.
 9. Tokach, J. Rebecca, Ribeiro, R. B., Flavio, Chung, Ki Yong, Rounds, Whitney and Johnson, J. Bradley. 2015. Chromium Propionate Enhances Adipogenic Differentiation of Bovine Intramuscular Adipocytes. *Front Vet Sci.* 2015; 2: 26. Published online 2015 Sep 8. doi: 10.3389/fvets.2015.00026.
 10. Nutrient Requirements of Beef Cattle: Seventh Revised Edition: Update 2000. DOI: 10.17226/9791.
 11. Zisman, A., O.D. Peroni, E.D. Abel, M.D. Michael, F. Mauvais-Jarvis, B.B. Lowell, J.F. Wojtaszewski, M.F. Hirshman, A. Virkamaki, L.J. Goodyear, C.R. Kahn, and B.B. Kahn. 2000. Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nat. Med.* 6:924-928.
 12. Spears, J.W., C.S. Whisnant, G.B. Huntington, K.E. Lloyd, R.S. Fry, K. Krafka, A. Lamptey, and J. Hyda. 2012. Chromium propionate enhances insulin sensitivity in growing cattle. *J. Dairy Sci.* 95:2037-2045.
 13. Kemin Internal Document, IMG-KANA-00082.
 14. Kemin Internal Document, IMG-KANA-00081.

Appendix

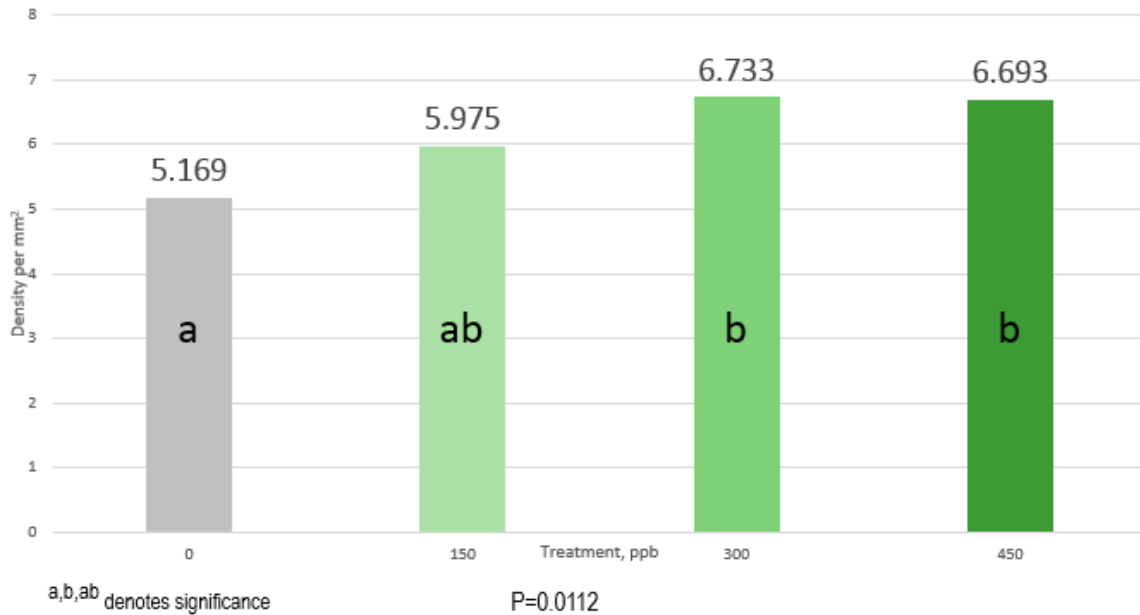


Figure 1. The effect of incremental chromium supplementation on internalized GLUT 4, per mm² in the *longissimus* muscle

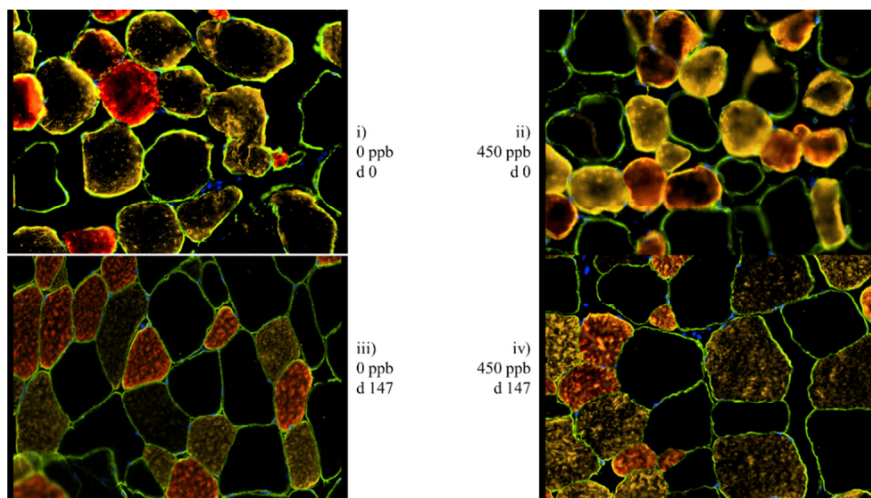


Figure 2. Immunohistochemical staining of *longissimus* muscle biopsies of feedlot steers supplemented with chromium propionate throughout the feeding period. Images i-iv depicts immunohistochemical staining for fiber type and cross sectional area. Blue denotes nuclei, green signifies the fiber sarcolemma, red represents type I fibers, yellow designates type IIA fibers, and type IIX fibers are fibers without color¹³.

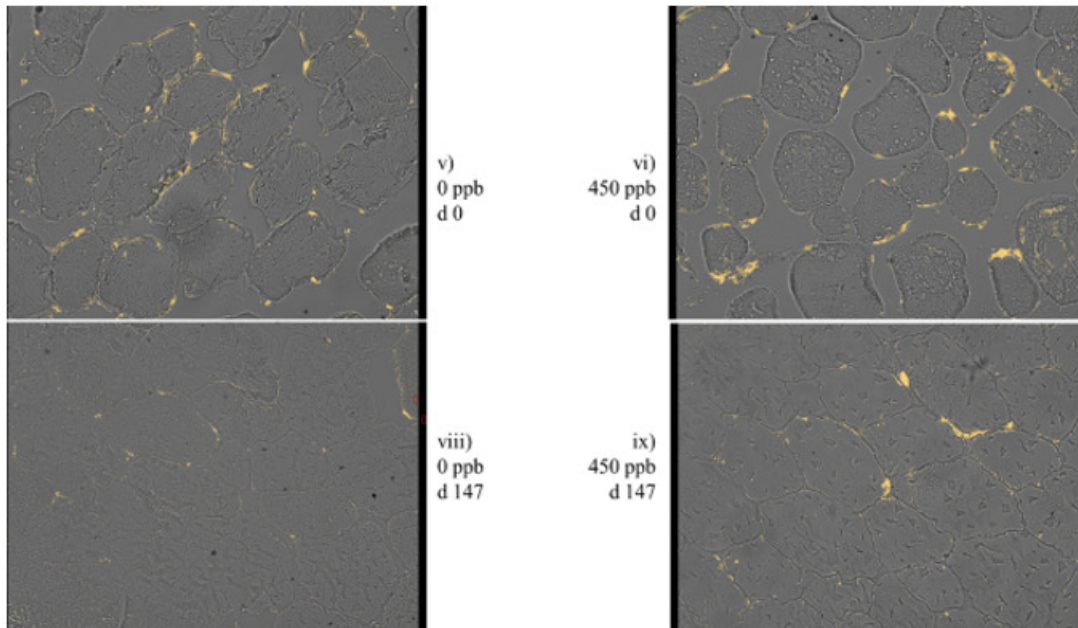


Figure 3. Immunohistochemical staining of *longissimus* muscle biopsies of feedlot steers supplemented with chromium propionate throughout the feeding period. Images v-vi depict immunohistological staining for GLUT4 density (yellow). Internalization was judged by proximity of the GLUT4 to the fiber membrane¹⁴.