# A Research Communication Brief: Gluten Analysis in Beef Samples Collected Using a Rigorous, Nationally Representative Sampling Protocol Confirms That Grain-Finished Beef Is Naturally Gluten-Free 

Shalene H. McNeill ${ }^{1}$, Amy M. Cifelli ${ }^{1, *}$, Janet M. Roseland ${ }^{2}$, Keith E. Belk ${ }^{3}$, Dale R. Woerner ${ }^{3}$ (D), Kerri B. Gehring ${ }^{4}$, Jeffrey W. Savell ${ }^{4}$ (D), J. Chance Brooks ${ }^{5}$ and Leslie D. Thompson ${ }^{5}$<br>1 National Cattlemen's Beef Association, Centennial, CO 80112, USA; smcneill@beef.org<br>2 Nutrient Data Laboratory, United States Department of Agriculture/Agricultural Research Service, Beltsville, MD 20705, USA; janet.roseland@ars.usda.gov<br>3 Department of Animal Sciences, Colorado State University, Fort Collins, CO 80523, USA; keith.belk@colostate.edu (K.E.B.); dale.woerner@colostate.edu (D.R.W.)<br>4 Department of Animal Science, Texas A\&M University, College Station, TX 77843, USA; kbgehring@tamu.edu (K.B.G.); j-savell@tamu.edu (J.W.S.)<br>5 Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX 79415, USA; chance.brooks@ttu.edu (J.C.B.); leslie.thompson@ttu.edu (L.D.T.)<br>* Correspondence: acifelli@beef.org

Received: 28 July 2017; Accepted: 20 August 2017; Published: 25 August 2017


#### Abstract

Knowing whether or not a food contains gluten is vital for the growing number of individuals with celiac disease and non-celiac gluten sensitivity. Questions have recently been raised about whether beef from conventionally-raised, grain-finished cattle may contain gluten. To date, basic principles of ruminant digestion have been cited in support of the prevailing expert opinion that beef is inherently gluten-free. For this study, gluten analysis was conducted in beef samples collected using a rigorous nationally representative sampling protocol to determine whether gluten was present. The findings of our research uphold the understanding of the principles of gluten digestion in beef cattle and corroborate recommendations that recognize beef as a naturally gluten-free food.


Keywords: beef; gluten; celiac disease; gluten sensitivity; nutrient data

## 1. Introduction

Experts recognize fresh meat such as beef as a naturally gluten-free food that is recommended as part of a healthful gluten-free diet [1-4] Beef is an important source of 10 essential nutrients including protein and key micronutrients such as iron, zinc, and B-vitamins, which are nutrients of concern for those following a gluten-free diet [5-10]. Questions have recently been raised about whether beef from conventionally-raised, grain-finished cattle may contain gluten. To date, basic principles of ruminant digestion have been cited in support of the prevailing expert opinion that beef is inherently gluten-free [11]. Although wheat, barley, and rye are common gluten-containing feed ingredients in conventional, grain-finished cattle feeds, it is well accepted that gluten proteins are hydrolyzed into individual amino acids during the ruminant digestive process. While there is general scientific consensus based on well-accepted animal physiology that meat from grain-finished beef cattle does not contain gluten, this has not been scientifically validated using current analytical methods for evaluating the gluten content of foods. Thus, gluten analysis was conducted in beef samples collected using a rigorous nationally representative sampling protocol. The findings confirm the understanding
of the principles of gluten digestion in beef cattle and corroborate recommendations that recognize beef as a naturally gluten-free food.

Celiac disease affects an estimated $1 \%$ of the population in the United States, while non-celiac gluten sensitivity is estimated to affect another $0.6 \%$ to $6 \%$ [12,13]. Thus, up to $7 \%$ of people in the U.S. may benefit from a gluten-free diet. This gluten analysis in beef provides confidence for the large number of individuals following a gluten-free diet.

## 2. Materials and Methods

### 2.1. Sampling Protocol

In order to provide accurate nutrition information to health professionals, the food industry, and consumers, the national Beef Checkoff Program and the USDA Agricultural Research Service have collaborated to conduct research resulting in updated nutrient composition data for beef retail cuts published in the USDA National Nutrient Database for Standard Reference [14]. To accurately obtain research samples representing beef retail cuts in the U.S., a rigorous nationally representative sampling protocol was developed by nutrition and meat scientists at three universities in collaboration with USDA Nutrient Data Laboratory statisticians [15-18].

These experts identified a nationally representative sample of 164 beef carcasses at seven meat packing plants in six different regions. A statistically appropriate sample was selected to represent the proper proportions of yield grade, quality grade, breed, genetic type and geographic location. The carcasses were sent to the three collaborating universities for fabrication into retail cuts. Raw and cooked samples were homogenized, and composites were made for each retail cut. A chart illustrating the study sample protocol is published elsewhere [19].

### 2.2. Nutrient Analysis

Comprehensive nutrient analysis was conducted on the raw and cooked composite samples for the retail beef cuts. This included analysis for total protein and amino acids, total fat and fatty acids, cholesterol, minerals such as iron, selenium, and zinc, as well as vitamins including retinol, B-vitamins, choline, vitamin D, and vitamin E. Validated nutrient analysis methods and quality control techniques were performed throughout the study to ensure accurate nutrient data, as has been previously described [13-16]. The results of this comprehensive nutrient analysis served to update the USDA's National Nutrient Database for Standard Reference [20].

## 3. Results

In March 2015, gluten analysis was conducted on archived samples retained from the beef research described above. A total of 17 composite samples representing 17 retail beef cuts were sent to an independent laboratory for gluten analysis. Food Safety Net Services performed the gluten analysis using Veratox ${ }^{\circledR}$ (Neogen Food Safety, Lansing, MI, USA) for Gliadin R5, a validated sandwich enzyme-linked immunoassay (S-ELISA) test (Neogen Corporation and the University of Nebraska Food Allergy Research and Resource Program, Lincoln, NE, USA, 2012) distributed by Neogen ${ }^{\circledR}$ (Neogen Food Safety, Lansing, MI, USA) Corporation.

The gluten analysis results for each of the 17 composite beef samples were below the limit of detection for this test that is equivalent to less than 5 ppm of gluten (see Table 1). According to the FDA's 2013 Gluten-Free Labelling regulations, a food that is inherently free of gluten may be labelled as "gluten-free" [21-23].

Table 1. Gluten content of composite samples from 17 retail beef cuts.

| Retail Beef Cuts <br> (Raw Composite Samples) | Gluten Analysis Results (ppm *) <br> (Lowest Limit of Detection =5 ppm) |
| :---: | :---: |
| Brisket, Flat Half | $<5 \mathrm{ppm}$ |
| Clod Roast | $<5 \mathrm{ppm}$ |
| Stew | $<5 \mathrm{ppm}$ |
| Denver Cut | $<5 \mathrm{ppm}$ |
| Underblade Roast | $<5 \mathrm{ppm}$ |
| Country Style Ribs | $<5 \mathrm{ppm}$ |
| America's Beef Roast | $<5 \mathrm{ppm}$ |
| Chuck Eye Steak | $<5 \mathrm{ppm}$ |
| Top Blade Steak | $<5 \mathrm{ppm}$ |
| Mock Tender Steak | $<5 \mathrm{ppm}$ |
| Short Ribs | $<5 \mathrm{ppm}$ |
| Ribeye Steak (bone-in) | $<5 \mathrm{ppm}$ |
| Ribeye Steak (boneless) | $<5 \mathrm{ppm}$ |
| Inside Skirt Steak | $<5 \mathrm{ppm}$ |
| Outside Skirt Steak | $<5 \mathrm{ppm}$ |
| Porterhouse Steak | $<5 \mathrm{ppm}$ |
| T-Bone steak | $<5 \mathrm{ppm}$ |

* ppm = parts per million.


## 4. Discussion

These findings confirm that today's fresh beef supply from conventionally-raised cattle-the predominant type sold in grocery stores-does not contain measurable levels of gluten, and can be included in a gluten-free diet. This evidence may help individuals with gluten-related conditions avoid unnecessary dietary restriction and can provide important nutritional benefits due to the micronutrients found in beef such as iron, zinc, and B-vitamins.

A gluten-free diet is currently the only safe treatment for individuals diagnosed with celiac disease $[24,25]$. Left untreated, this genetic autoimmune disorder is associated with a wide range of symptoms, nutrient deficiencies, and serious complications. In susceptible individuals, the ingestion of the gluten protein in wheat, barley, rye, and crossbreeds of these grains damages the villi in the small intestine. This damage to the small intestine typically results in the malabsorption of vital nutrients such as iron, zinc, and B-vitamins. Anemia resulting from the malabsorption of iron and vitamin $\mathrm{B}_{12}$ is of particular concern for those with celiac disease. Avoiding or correcting nutrient deficiencies that are often present with celiac disease is a key focus of medical nutrition therapy.

It is well documented that those following a gluten-free diet are at increased risk of multiple vitamin and mineral deficiencies for a number of reasons [7-9]. For example, gluten-free flours and grain products such as breads, pastas, and cereals are not subject to the same enrichment and fortification standards as wheat-based products. Thus, many gluten-free grain products contain lower levels of iron and B-vitamins such as thiamin, riboflavin, niacin, and folate. This further increases the risk of iron and B-vitamin deficiencies in individuals following a gluten-free diet.

## 5. Conclusions

Knowing whether or not a food contains gluten is vital information for individuals with celiac disease and non-celiac gluten sensitivity. The approach described in this report can serve as a model for others interested in substantiating the gluten-free nature of their products. The publication of results from gluten analyses such as this can help to further inform health professionals and the food industry and ultimately benefit those who must avoid gluten in their diets.

To our knowledge, this is the first effort to conduct gluten analysis in a nationally representative sample of beef. The rigorous sampling protocol and validated enzyme-linked immunoassay used for this analysis provides scientific evidence to support current recommendations that recognize beef as
an inherently gluten-free food that can be enjoyed in a healthful gluten-free diet. This understanding is important since beef is a source of many vital nutrients such as iron, zinc, and B-vitamins that are of concern for those following a gluten-free diet. Encouraging gluten-restricting individuals to enjoy beef as part of a healthful gluten-free diet may reduce unnecessary dietary restriction, and improve diet satisfaction and nutrient adequacy.

Acknowledgments: This project is funded by the Beef Checkoff. The authors thank the researchers at USDA Nutrient Data Laboratory for their assistance with nutrient data validation and analysis, Larry Douglass for his statistical analysis, and the meat scientists at Colorado State University, Texas A\&M University, and Texas Tech University for their collaboration and involvement in these studies.
Author Contributions: S.H.M. and A.M.C. devised the gluten analysis study and wrote and prepared the manuscript; J.M.R. contributed to the analysis of the nutrient data and validation; K.E.B., D.R.W., K.B.G., J.W.S., J.C.B. and L.D.T. contributed to the concept of the overall nutrient data study, designed the sampling protocol, and performed the sample collection, preparation, and analysis.
Conflicts of Interest: S.H.M. and A.M.C. are employed by the National Cattlemen's Beef Association.

## References

1. Understanding Celiac Disease. Available online: http://www.eatright.org/resource/health/diseases-andconditions / celiac-disease/understanding-celiac-disease (accessed on 22 June 2015).
2. Gluten-Free Diet: What can I eat? Available online: http:// celiac.org/live-gluten-free/glutenfreediet/foodoptions/ (accessed on 22 June 2015).
3. Gluten-Free Diet: Basic Diet Choices. Available online: http:/ /www.csaceliacs.org/basic_gf_food_choices.jsp (accessed on 22 June 2015).
4. GIG's Gluten-Free Diet and Drug Instruction. Available online: https:/ /www.gluten.org/resources / diet-nutrition/gigs-gluten-free-diet-and-drug-instruction/ (accessed on 22 June 2015).
5. McNeill, S.H. Inclusion of red meat in healthful dietary patterns. Meat Sci. 2014, 98, 452-460. [CrossRef] [PubMed]
6. Niewinski, M.M. Advances in celiac disease and gluten-free diet. J. Am. Diet. Assoc. 2008, 108, 661-672. [CrossRef] [PubMed]
7. Shepherd, S.J.; Gibson, P.R. Nutritional inadequacies of the gluten-free diet in both recently-diagnosed and long-term patients with coeliac disease. J. Hum. Nutr. Diet. 2013, 26, 349-358. [CrossRef] [PubMed]
8. Theethira, T.G.; Dennis, M. Celiac disease and the gluten-free diet: Consequences and recommendations for improvement. J. Dig. Dis. 2015, 33, 175-182. [CrossRef] [PubMed]
9. Wierdsma, N.J.; van Bokhorst-de van der Schueren, M.A.; Berkenpas, M.; Mulder, C.J.; van Bodegraven, A.A. Vitamin and mineral deficiencies are highly prevalent in newly diagnosed celiac disease patients. Nutrients 2013, 5, 3975-3992. [CrossRef] [PubMed]
10. Zanovec, M.; O'Neil, C.E.; Keast, D.R.; Fulgoni, V.L.; Nicklas, T.A. Lean beef contributes significant amounts of key nutrients to the diets of U.S. adults: NHANES 1999-2004. Nutr. Res. 2010, 30, 375-381. [CrossRef] [PubMed]
11. Gluten Intolerance and Grain Fed Meat. Available online: https://www.bestfoodfacts.org/glutenintolerance/ (accessed on 22 June 2015).
12. National Institutes of Health Consensus Statement on Celiac Disease. Available online: http:/ / consensus. nih.gov / 2004/2004CeliacDisease118PDF.pdf (accessed on 22 June 2015).
13. Volta, U.; Caio, G.; De Giorgio, R.; Henriksen, C.; Skodje, G.; Lundin, K.E. Non-celiac gluten sensitivity: A work-in-progress entity in the spectrum of wheat-related disorders. Best Pract. Res. Clin. Gastroenterol. 2015, 29, 477-491. [CrossRef] [PubMed]
14. USDA Nutrient Data Set for Retail Beef Cuts from SR, Release 3.0. Available online: https:/ /www.ars.usda. gov / ARSUserFiles / 80400525/Data/Meat/Retail_Beef_Cuts03.pdf (accessed on 17 June 2015).
15. Acheson, R.J.; Woerner, D.R.; Martin, J.N.; Belk, K.E.; Engle, T.E.; Brown, T.R.; Brooks, J.C.; Luna, A.M.; Thompson, L.D.; Grimes, H.L.; et al. Nutrient database improvement project: Separable components and proximate composition of raw and cooked retail cuts from the beef loin and round. Meat Sci. 2015, 110, 236-244. [CrossRef] [PubMed]
16. Desimone, T.L.; Acheson, R.A.; Woerner, D.R.; Engle, T.E.; Douglass, L.W.; Belk, K.E. Nutrient analysis of the beef alternative merchandising cuts. Meat Sci. 2013, 93, 733-745. [CrossRef] [PubMed]
17. Martin, J.N.; Brooks, J.C.; Thompson, L.D.; Savell, J.W.; Harris, K.B.; May, L.L.; Haneklaus, A.N.; Schutz, J.L.; Belk, K.E.; Engle, T.; et al. Nutrient database improvement project: the influence of USDA quality and yield grade on the separable components and proximate composition of raw and cooked retail cuts from the beef rib and plate. Meat Sci. 2013, 95, 486-494. [CrossRef] [PubMed]
18. West, S.E.; Harris, K.B.; Haneklaus, A.N.; Savell, J.W.; Thompson, L.D.; Brooks, J.C.; Pool, J.K.; Luna, A.M.; Engle, T.E.; Schutz, J.S.; et al. Nutrient database improvement project: the influence of USDA quality and yield grade on the separable components and proximate composition of raw and cooked retail cuts from the beef chuck. Meat Sci. 2014, 97, 558-567. [CrossRef] [PubMed]
19. Lean Matters: Chronicling Beef's Change from Gate to Plate. Available online: http:/ /www.beefresearch. org/CMDocs/BeefResearch/Nutrition/LeanMatters_Web.pdf (accessed on 24 August 2017).
20. USDA National Nutrient Database for Standard Reference, Release 27. Available online: https: / /www.ars.usda.gov/northeast-area/beltsville-md/beltsville-human-nutrition-research-center/nutrient-data-laboratory/docs/sr27-home-page/ (accessed on 22 June 2015).
21. Food Labelling; Gluten-Free Labelling of Foods. Available online: https://www.federalregister.gov/articles/ 2013/08/05/2013-18813/food-labeling-gluten-free-labeling-of-foods (accessed on 22 June 2015).
22. Food Allergen Handbook, 9th ed. Available online: http://www.neogen.com/FoodSafety/pdf/ AllergenHandbook_12.pdf (accessed on 17 June 2015).
23. Codex Alimentarius, International Food Standards, Coded Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten, Codex Standard 118. Available online: http:/ /www.codexalimentarius.org/ standards/list-of-standards/ (accessed on 22 June 2015).
24. Celiac Disease Evidence-Based Nutrition Practice Guideline 2009. Available online: http:/ / www.andeal. org/topic.cfm?cat=3677 (accessed on 22 June 2015).
25. Rubio-Tapia, A.; Hill, I.D.; Kelly, C.P.; Calderwood, A.H.; Murray, J.A. American college of gastroenterology clinical guidelines: Diagnosis and management of celiac disease. Am. J. Gastroenterol. 2013, 108, 656-676. [CrossRef] [PubMed]
