The Influence of Diet and Oxidation on Calcium Retention of the Mitochondria in Fresh Beef

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Summary with Implications

Feeding dried distillers grains (DG) may influence calcium flux postmortem by disrupting the stability of the sarcoplasmic reticulum (SR) membrane, thus leading to a higher post-rigor calcium leakage, resulting in greater activation of calpains and improved tenderness. Mitochondria provide the opportunity to study calcium flux in a controlled, tightly defined environment as a model system for the SR. Cattle were finished on diets containing either 0% DG or 50% DG. Feeding DG increased proportions of PUFA in the SR and mitochondrial membrane. Oxidized mitochondria retained less calcium than non-oxidized mitochondria. Mitochondria from cattle finished on corn tended to retain more Ca than mitochondria from cattle finished on DG. These findings suggest that feeding DG in the finishing diet can possibly increase meat tenderness through altered calcium flux.

Introduction

Research at the University of Nebraska-Lincoln has found that feeding distillers grains (DG) increases polyunsaturated fatty acid (PUFA) content within the sarcoplasmic reticulum (SR) membrane. The amount of PUFA influences SR membrane fluidity and can compromise the ability of the organelle to retain calcium (its primary contents). The PUFA are highly susceptible to oxidation, which can lead to membrane collapse postmortem. If the SR membrane collapses early postmortem calcium (Ca) leakage may occur. Calcium activates proteolytic enzymes known as calpains. Calpains aide in the tenderization of meat postmortem. Therefore, altering the PUFA content in the SR membrane could lead to improved tenderness postmortem by increasing the amount of calcium available to activate calpains. Even though the SR is difficult to study, mitochondria, the secondary Ca-sequestering organelles in the muscle, are relatively easy to isolate intact and provide the opportunity to study Ca release under carefully controlled and tightly defined conditions. The objective was to isolate mitochondria from cattle fed DG and corn to determine the influence of diet and oxidation on Ca release.

Procedure

Steers (n = 48) were fed a corn-based finishing diet with or without deoiled, dried DG (50% DM basis) for 156 days. After harvest, strip loins were collected and steaks from each loin were aged for 2, 8, 14, and 21 days, powdered using liquid nitrogen, and stored at -112° F for lab analysis. Samples (n = 12) were randomly selected from each diet group for all aging periods. Mitochondria were isolated using high speed ultracentrifuge from day 2, 8, and 14. The SR was isolated from each day 2 sample. Both mitochondria and SR samples were analyzed for PUFA content using gas chromatography, and phospholipid content using thin layer chromatography. Mitochondria from days 2 and 8 were artificially oxidized using an iron and ascorbic acid mixture. Calcium measurements were performed at Ward Labs (Kearny, NE) using Inductively Coupled Plasma Optical Emission Spectra (ICP-OES).

Statistical Analysis

The Proc Glimmix procedure in SAS (SAS Institute, Inc., Cary, N.C.) was used to test the main effects of dietary treatment, aging period, PUFA content, phospholipid content, and oxidation and their interactions on the calcium retention of the mitochondria. All means were separated using the LS MEANS statement and the TUKEY adjustment with an alpha level of 0.05.

Results

In both organelles, the DG diet samples had higher 18:2 and total PUFA content

Table 1. Effect of diet on 18:2 and polyunsaturated fatty acid (PUFA) content of the SR and mitochondrial membranes.

Organelle	Fatty acid (%)	Corn	DDGS 50%[x]
SR	18:2	8[a]	17[b]
	PUFA	10[a]	17[b]
Mitochondria	18:2	9[a]	15[b]
	PUFA	15[a]	24[b]

[a,b] Means within a row with different superscripts differ *(P < 0.05)*.
[x] DDGS= dried distillers grains plus solubles

Table 2. Effect of aging on 18:2 and polyunsaturated fatty acid (PUFA) content of the mitochondrial membrane.

Aging (days)	18:2 (%)	PUFA (%)
2	12[a]	19[a]
8	9[a]	16[a]
14	15[b]	23[b]

[a,b] Means within a column with different superscripts differ (*P < 0.05*).

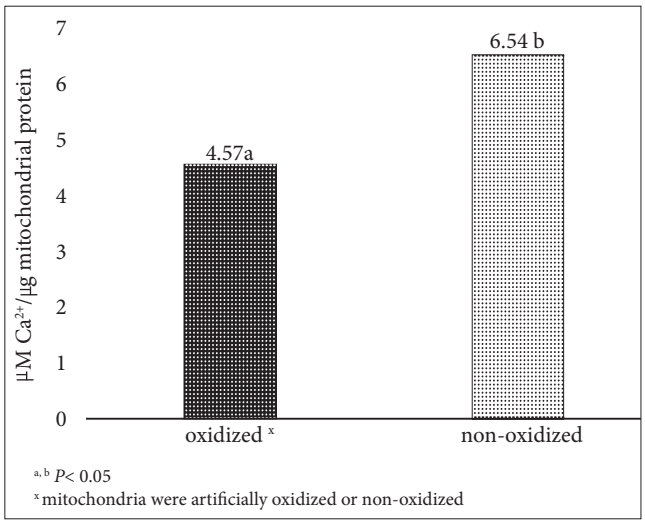

Figure 1. Effect of Oxidation on Mitochondrial Calcium Retention.

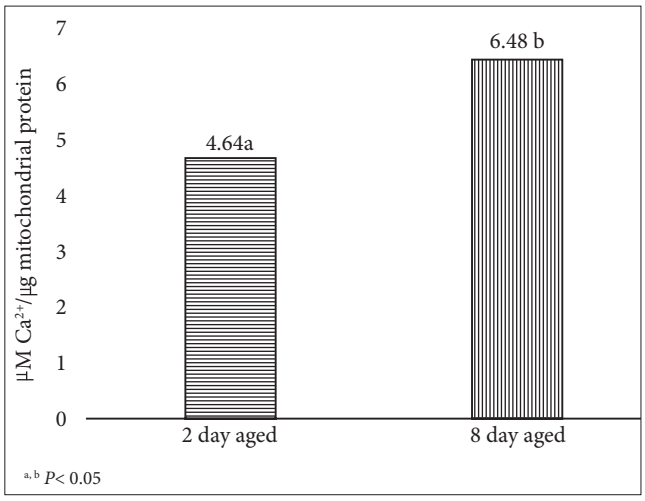

Figure 2. Effect of Aging on Mitochondrial Calcium Retention.

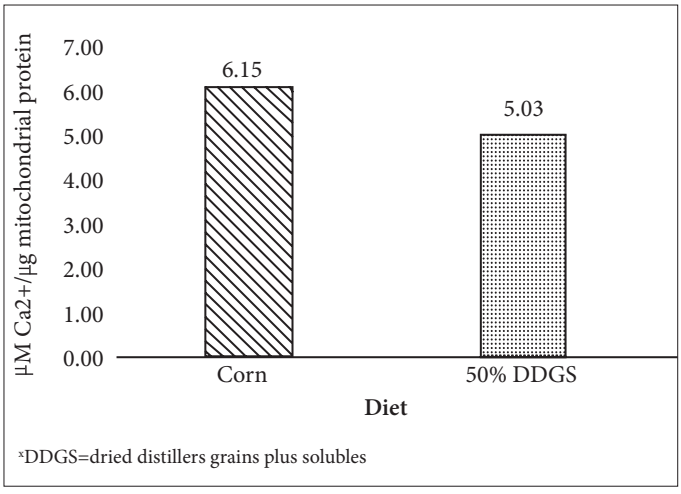

Figure 3. Effect of Diet on Mitochondrial Calcium Retention.

($P < 0.01$) compared to corn samples (Table 1), which was consistent with previous studies. Day 14 mitochondrial lipids had higher 18:2 ($P < 0.01$) and total PUFA ($P < 0.05$) contents compared to day 2 and 8 mitochondrial lipids (Table 2). This was expected because during aging of meat oxidation propagates the formation of additional trans double bonds in other fatty acids, creating more unsaturated fatty acids. Phospholipid contents (phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol) of the mitochondria and SR were unaffected by diet ($P > 0.10$). Oxidized mitochondria retained significantly less Ca than non-oxidized ($P < 0.01$) mitochondria (Fig. 1). This supported the hypothesis that as oxidation increases, the membrane stability decreases, thus allowing calcium to leak from the organelle. Day 2 mitochondria retained significantly less Ca than day 8 ($P < 0.01$) mitochondria (Fig. 2), which is opposite of the results that were expected, because the total PUFA content was not significantly different between the two aging periods. Overall, mitochondria from cattle finished on corn tended ($P = 0.08$) to retain more Ca than mitochondria from cattle finished on DG, which supported the hypothesis (Fig. 3).

Conclusion

Results indicate that greater PUFA content deposited in organelles may affect Ca flux by increased susceptibility to oxidation. A DG diet may influence Ca flux and ultimate tenderness by this mechanism.

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