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Evaluation of Bovine Myosin Heavy Chain Isoforms and Muscle Fiber Cross-Sectional Area on the Eating Quality of 11 Different Beef Muscles

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KANSAS STATE UNIVERSITY CATTLEMEN'S DAY

Evaluation of Bovine Myosin Heavy Chain Isoforms and Muscle Fiber Cross-Sectional Area on the Eating Quality of 11 Different Beef Muscles

S. Hene, H. Ness, E. Verrill, P. Hammond, C.K. Chun, T.G. O'Quinn, and M.D. Chao

Abstract

The objective of this study was to determine the impact of muscle fiber type, diameter, and cross-sectional area (CSA) on the eating quality of 11 different beef muscles. Ten U.S. Department of Agriculture choice shoulder clod (SC), flank (F), knuckle (K), mock tender (MT), top sirloin butt (TS), brisket (B), eye of round (ER), and ribeye (R) were collected in study 1 (n = 80). In study 2, strip loin (SL), tri-tip (TT), and heel (H) were collected from ten USDA low choice beef carcasses (n = 30). Muscle fiber types, CSA, and diameters were determined. Pearson correlation analysis was performed to determine the relationship between muscle fiber type, CSA, diameter, and the eating quality of beef from previously reported studies. Correlation analysis from both studies demonstrated positive correlations between type 1 fibers and many attributes of eating quality such as juiciness and lipid flavor intensity ($P < 0.05$). Negative correlations were found between type 2A fibers and those attributes ($P < 0.05$) and between type 2X fibers and tenderness measurements ($P < 0.05$). Interestingly, a negative correlation was found between muscle fiber CSA and diameter with connective tissue amount ($P < 0.05$), and a positive correlation was found between muscle fiber CSA and diameter with tenderness measurements ($P < 0.05$) in those same studies.

Introduction

Skeletal muscle is composed of heterogeneous muscle fiber types, and there are type 1, fast twitch fibers and type 2, slow twitch fibers. Each fiber type has a unique set of characteristics influencing key factors such as energy metabolism and contractile speed in the live animal, and these same unique characteristics influence eating quality of different beef cuts. However, the exact relationship among muscle fiber types and size on beef quality has yet to be fully established. The traditional immunohistochemical method to determine muscle fiber type is labor intensive and prone to a high degree of human error, so this research is difficult.. Therefore, the objective of this study was to investigate the contribution of muscle fiber type and size on the eating quality of 11 different beef muscles.

Experimental Procedures

Eleven different beef muscles were utilized from two separate studies. In study 1, shoulder clod (SC), flank (F), knuckle (K), mock tender (MT), top sirloin butt (TS), brisket (B), eye of round (ER), and ribeye (R) were collected from 10 U.S. Department of Agriculture choice carcasses (n = 80), and each muscle was fabricated into steaks at 2 days postmortem. In study 2, strip loin (SL), tri-tip (TT), and heel (H) were collected from 10 USDA low choice carcasses (n = 30), and each muscle was fabricated into steaks at 5 days postmortem. For both studies, two cores were obtained from each sample parallel to the muscle fiber direction and frozen in a 2-methyl butane bath using liquid nitrogen immediately following fabrication.

Myofibrillar proteins were extracted from each sample and separated using sodium dodecyl-sulfate polyacrylamide gel electrophoresis with precast 6% polyacrylamide gels. Following gel electrophoresis, proteins were transferred from gels to polyvinylidene fluoride membranes. Membranes were then blocked using fluorescent blocking buffer overnight. After blocking, membranes were immunoblotted in two cocktails against 4 primary antibodies that recognize: 1) all myosin heavy chains (MyHC) isoforms; 2) type 1 only; 3) type 2X only; and 4) type 2A only. Following the primary antibody incubation, membranes were incubated with a fluorescent secondary antibody and imaged (Figure 1). The relative percentage of each fiber type was calculated by normalizing the intensity of each specific isoform against the intensity of all MyHC isoforms. A cryostat was used to collect two 10 μm cryosections per sample from the frozen cores. Slices were transferred to microscope slides and allowed to air dry. To determine cross-sectional area (CSA) and diameter, slides were incubated with primary (anti-dystrophin) and secondary antibody prior to imaging under the microscope. At 10X objective, five photomicrographs were captured. An average of 400 fibers per sample were analyzed to determine CSA and muscle fiber diameter (Figure 2). Pearson correlation analysis was conducted to determine the relationship between muscle fiber type, CSA, and diameter with the results for the eating quality of beef as determined by the trained panel that were previously reported in Chun et al. (2020) and Hammond et al. (2022).

Results and Discussion

In study 1, flank and mock tender had the greatest relative percent of type 1 fibers, followed by shoulder clod and top sirloin butt, with brisket, ribeye, knuckle, and eye of round having the lowest ($P < 0.01$; Table 1). On the other hand, ER, R, and K had the greatest relative percent of type 2A fibers, followed by TS, B, and SC, with MT and F having the least ($P < 0.01$). However, there was only a tendency for differences in relative percent of type 2X fibers among the muscles ($P = 0.08$). In study 1, F and MT were found to have the greatest CSA followed by R, ER, SC and TS with K and B having the smallest muscle fiber area ($P < 0.01$). In study 1, F was shown to have the greatest muscle fiber diameter, followed by MT, R, ER, TS, SC, K, with B having the smallest fiber diameter ($P < 0.01$; Table 1). In study 2, there was no difference between the relative percent of type 1 fibers ($P > 0.05$). The ER had a greater relative percent of type 2A fibers than TT and H ($P < 0.05$). Finally, TT had a greater relative percent of 2X fibers than SL ($P < 0.05$) with H not differing from any of them ($P > 0.10$). In study 2, SL was found to have the greatest CSA and diameter ($P < 0.01$) with H and TT not differing between them in either CSA or diameter. It was expected that muscles used for posture and stability are more densely comprised of type 1 fibers, while muscles used

for locomotion that need quick bursts of energy and movement have a higher relative percentage of type 2 fibers.

In study 1, there was a positive correlation between fiber type 1 and initial juiciness ($r = 0.37$; $P < 0.05$; Table 2), sustained juiciness ($r = 0.39$; $P < 0.05$) and lipid flavor ($r = 0.41$; $P < 0.05$). Conversely, there was a negative correlation between fiber type 2A and initial juiciness ($r = -0.40$; $P < 0.05$) sustained juiciness ($r = -0.42$; $P < 0.05$), and lipid flavor ($r = -0.45$; $P < 0.01$). These relationships are likely due to type 1 oxidative fibers utilizing lipids for energy in comparison to type 2 fibers utilizing glycogen as an energy source. Therefore, the high lipid content in type 1 fibers likely had a positive impact on beef quality characteristics like juiciness and flavor. Furthermore, a negative correlation was seen between muscle fiber diameter and connective tissue amount ($r = -0.23$; $P < 0.05$), but a positive correlation was seen to overall tenderness ($r = 0.24$; $P < 0.05$) for study 1. In study 2, a negative correlation was seen between type 2X fiber and myofibril tenderness ($r = -0.52$; $P < 0.05$), overall tenderness ($r = -0.46$; $P < 0.05$) and WBSF ($r = -0.46$; $P < 0.05$). Furthermore, study 2 saw negative correlations between CSA and muscle fiber diameter with connective tissue content ($r = -0.45$; $P < 0.05$) and WBSF ($r = -0.60$; $P < 0.01$), but a positive correlation was to overall tenderness ($r = 0.39$; $P < 0.05$). It is known that larger muscle fibers have thinner endomysium (connective tissue) surrounding the muscle fiber. Therefore, it is possible that as muscle fiber CSA and diameter increase, the collagen fibers making up the endomysium undergo both qualitative and quantitative changes, resulting in a more tender product.

Implications

This study shows that muscles predominated by type 1 fibers will likely deliver a higher eating quality experience for consumers, while muscles with more glycolytic fibers 2A and 2X will deliver a less favorable eating experience for consumers. On the other hand, these data also demonstrated that a larger muscle fiber CSA and diameter are not necessarily a marker of negative eating quality, as muscles with those characteristics had less connective tissue and had greater tenderness scores.

Acknowledgments

The Beef Checkoff.

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Table 1. Relative muscle fiber type percentage, muscle fiber cross-sectional area (CSA), and diameter measurements of 11 beef muscles from 2 studies (n = 110)

Muscle[1]	Type 1, %	Type 2X, %	Type 2A, %	CSA, μm²	Diameter, μm
Study 1					
TS	28.85^{c}	34.52	36.64^{ab}	2.02^{bc}	1.57^{bcd}
R	25.19^{cd}	33.16	41.52^{a}	2.25^{ab}	1.65^{abc}
B	25.43^{cd}	38.15	36.42^{ab}	1.37^{d}	1.29^{e}
F	48.87^{a}	32.83	18.29^{c}	2.38^{a}	1.71^{a}
K	21.94^{d}	37.48	40.45^{a}	1.84^{c}	1.50^{d}
MT	45.6^{a}	33.98	20.43^{c}	2.38^{a}	1.68^{ab}
ER	18.59^{d}	39.20	42.08^{a}	2.13^{abc}	1.61^{abcd}
SC	35.70^{b}	33.82	30.48^{b}	1.98^{bc}	1.53^{cd}
[2]SEM	3.30	2.90	3.00	0.13	0.04
P-value	<0.01	0.08	<0.01	<0.01	<0.01
Study 2					
H	21.93	31.93^{ab}	46.69^{b}	2.34^{b}	1.67^{b}
SL	18.19	25.45^{b}	56.36^{a}	3.88^{a}	2.15^{a}
TT	17.12	35.61^{a}	47.27^{b}	2.68^{b}	1.82^{b}
[2]SEM	3.50	3.10	3.60	0.20	0.07
P-value	0.5	<0.05	<0.05	<0.01	<0.01

[a-d] Values within a column without a common superscript differ significantly at $P < 0.05$.

[1]TS = Top sirloin butt; R = Ribeye; B = Brisket; F = Flank; K = Knuckle; MT = Mock tender; ER = Eye of round; SC = Shoulder clod.

[2]Standard error of the mean.

Table 2. Correlation coefficient (r) of muscle fiber types, muscle fiber cross-sectional area (CSA) and diameter with eating quality measurements evaluated by trained panels

	Type 1	Type 2X	Type 2A	CSA (μm^2)	Diameter (μm)
Study 1					
Initial juiciness	0.37**	-0.04	-0.39**	0.16	0.17
Sustained juiciness	0.39**	-0.05	-0.42**	0.17	0.18
Myofibrillar tenderness	0.27*	-0.24*	-0.24*	0.14	0.20
Connective tissue content	-0.03	0.05	0.00	-0.12	-0.23*
Lipid flavor intensity	0.41**	-0.03	-0.45**	0.06	0.09
Overall tenderness	0.15	-0.08	-0.12	0.18	0.24*
Warner-Bratzler shear force	-0.09	0.03	0.13	-0.03	-0.08
Study 2					
Initial juiciness	0.15	-0.06	-0.07	0.00	0.02
Sustained juiciness	0.14	0.01	-0.12	-0.04	-0.02
Myofibrillar tenderness	0.15	-0.52**	0.34	0.33	0.33
Connective tissue content	-0.10	0.44*	-0.31	-0.45*	-0.44*
Lipid flavor intensity	-0.16	0.22	-0.06	-0.22	-0.20
Overall tenderness	0.13	-0.46*	0.30	0.39*	0.39*
Warner-Bratzler shear force	-0.13	0.50*	-0.34	-0.60**	-0.61**

* $P < 0.05$.
** $P < 0.01$.

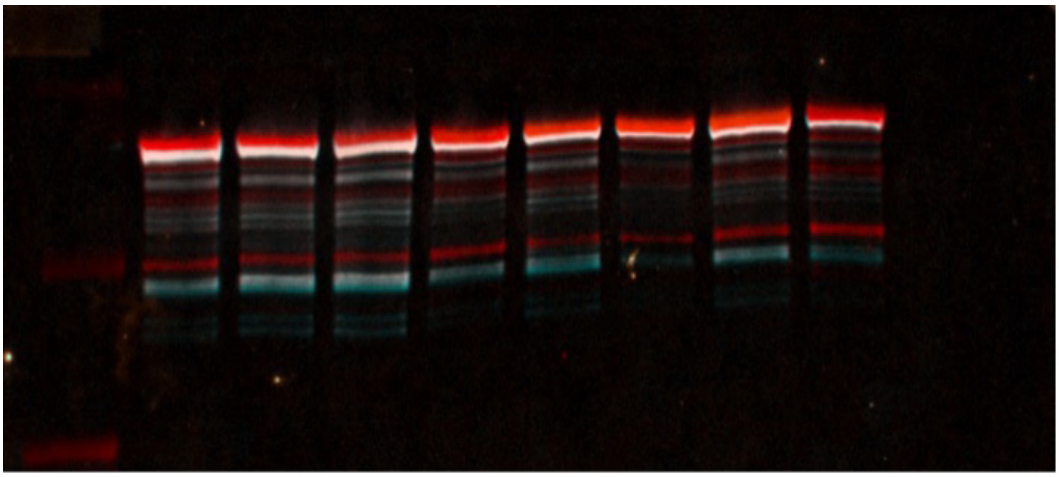

Figure 1. Cocktail 1 myosin heavy chain isoform immunoblot.

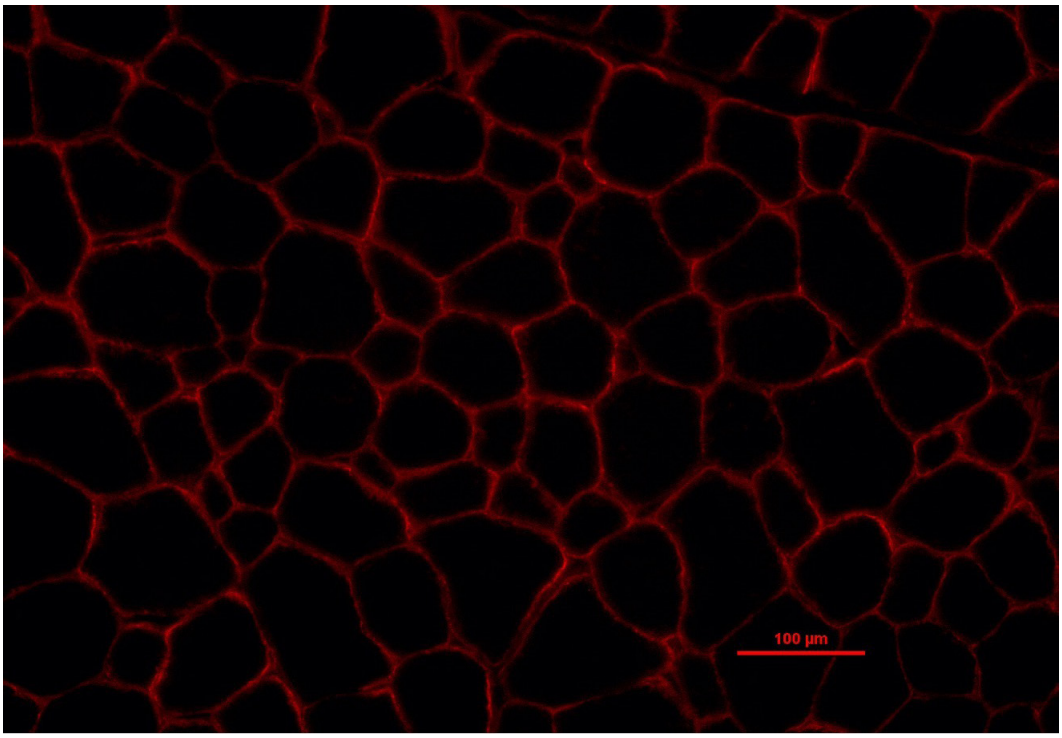

Figure 2. Representative muscle fiber cross sectional area (CSA) and diameter.