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Are There Differences in Muscle Fiber Characteristics Between Normal and Dark Cutting Beef?

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What is Dark Cutting with Respect to Beef

Dark cutting is most evidently identifiable by its distinct purplish black colour. This is in comparison to the ideal “cherry red” colouring the consumer desires (Hendrick et al., 1959).

Dark cutting (left) and normal meat (right)

https://www.beachportliquidminerals.com

Typical Causes of Dark Cutting of Meat

- Dark cutting meat occurs due to the depletion of glycogen in muscles prior to slaughter (Lister, 1989).

- There are many causes of dark cutting, such as excessive activity, fighting, mixing of unfamiliar animals, lairage time (holding pens where animals are kept post transportation), shivering, transportation and handling of animals.

Implications of Dark Cutting Meat

- When detecting dark cutting meats post-mortem there is a 24-48-hrs window to grade it.
- After 24-hrs post-mortem the ultimate pH is around 5.8-6.2 generating tough meat.
- Muscles with an ultimate pH greater than 6.2 typically produce tender meat.

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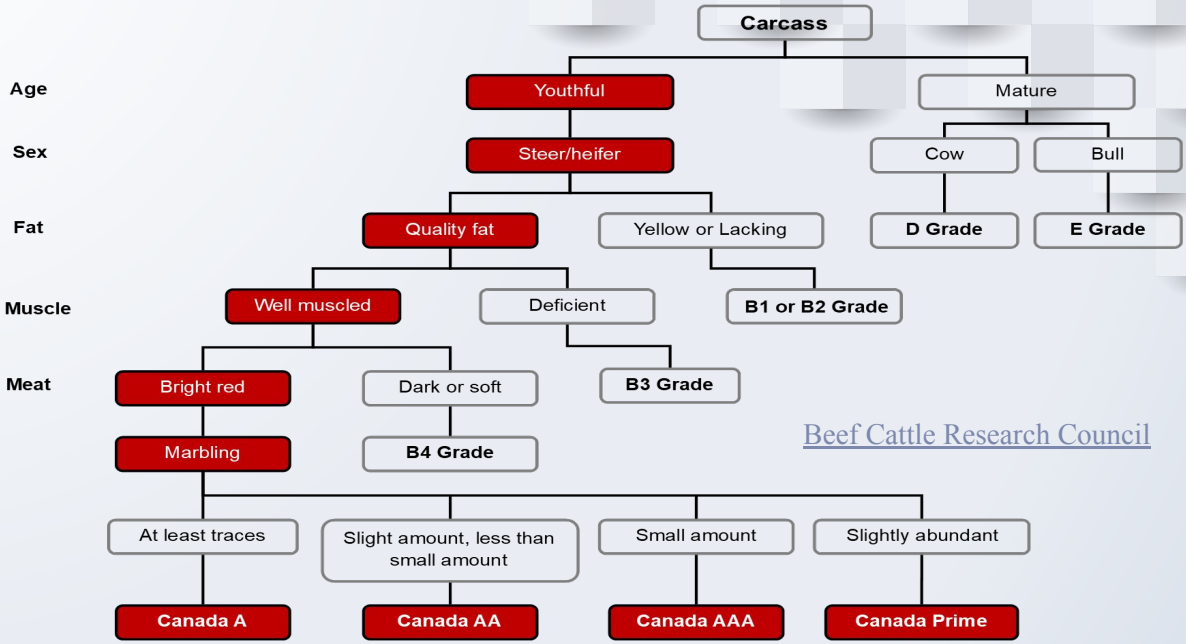
Muscle Fiber Type May Affect the Likelihood of Dark Cutting

- Dark cutting beef has more slow-twitch oxidative (type I) muscle fibers than normal beef (Zerouala & Stickland, 1991).
- Dark cutting bulls have fewer muscles exhibiting fast glycolytic characteristics than normal bulls.
- Analyzing muscle fibers characteristics may give a better perspective as to why dark cutting is occuring. Finding the number and predominant muscle fiber types can help with that.
- Slow- twitch oxidative (type I) muscle fiber types may increase the likelihood of dark cutting because they have reduced glycogen.

Muscle Fiber Types in Skeletal Muscles

Characteristics	Slow twitch oxidative (Type I)	Fast twitch oxidative glycolytic (Type IIa)	Fast twitch glycolytic fibers (Type IIb)
Contraction	Slow	Fast	Very fast
Diameter	Small	Intermediate	Large
Myoglobin content	High	Intermediate	Low
Mitochondria	High	Intermediate	Low
Glycogen	Low	High	High
Colour	Red	Pink	White

Canadian Beef Grading System



Beef Cattle Research Council

According to (Canadian Beef Grading Agency) "Grading is intended to place carcasses into uniform groups of similar quality, yield, and value, in order to facilitate marketing and production decisions."

Dark cutting carcasses are assigned a grade of Canada B4.

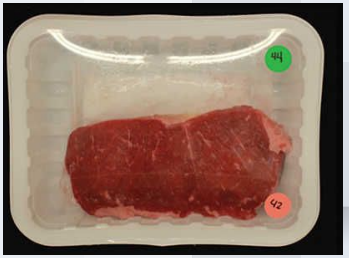
About the Canadian Beef Agency: How it's Graded?

Characteristics	How Characteristics Influence Grade/Quality of Beef
Age	Cattle over 30 months of age produce tough meat.
Sex	Bulls produce tough meat.
Muscling	The degree of muscling determines the meat yield (how much of the carcass can actually be sold to the consumer).
Fat (includes colour, texture, and cover)	Yellow is associated with beef from old cattles and so may be tough.
Marbling	Marbling makes the meat more tender and juicy.

Adapted table from Canadian Beef Grading Agency

Consumer Perception of Dark Cutting of Meats

- Consumers believe discolouration of the meat is associated with deterioration of the meat (Hendrick et al., 1959).
- The consumers look at the meat colour and degree of fat during purchasing (Troy & Kerry, 2010) .
- People connect dark cutting to having a tough and undesirable flavour (Ponnampalam et al., 2017).



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Dark Cutting of Meat - Why is this Important to the Canadian Beef Industry?

Economic loss:
Dark cutting carcasses can be discounted as much as 40%, damaging the income of the farmers (Mahmood et al., 2016).

According to (Canada's 2016/17 National Beef Quality Audit) they estimated the cost of dark cutters in Canada's beef industry at $10.6 million. In 2019, 1.4% of fed slaughter carcasses in Canada graded B4.

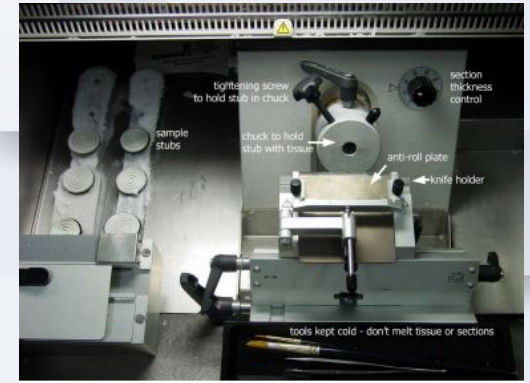
Shorter shelf life of meat:
Dark cutting beef has a shorter shelf life than normal beef due to its high pH which permits bacterial growth. (Reid et al., 2016)

Purchasing decision:
During purchasing consumers prefer to buy beef with a red cherry color because dark meat is thought to be not fresh.

Materials and Methods
Preparation of Muscle Section Slides

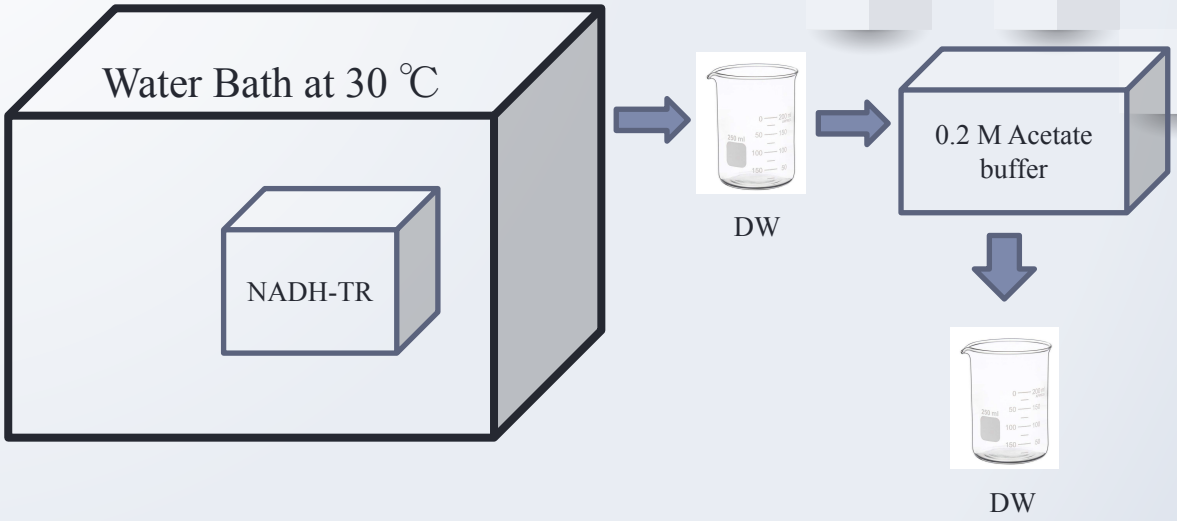
cube (b) used for muscle fiber types determination and characterization. Meat cubes were $1cm \times 1cm \times 1.5cm$.

Freeze in dry ice cooled acetone



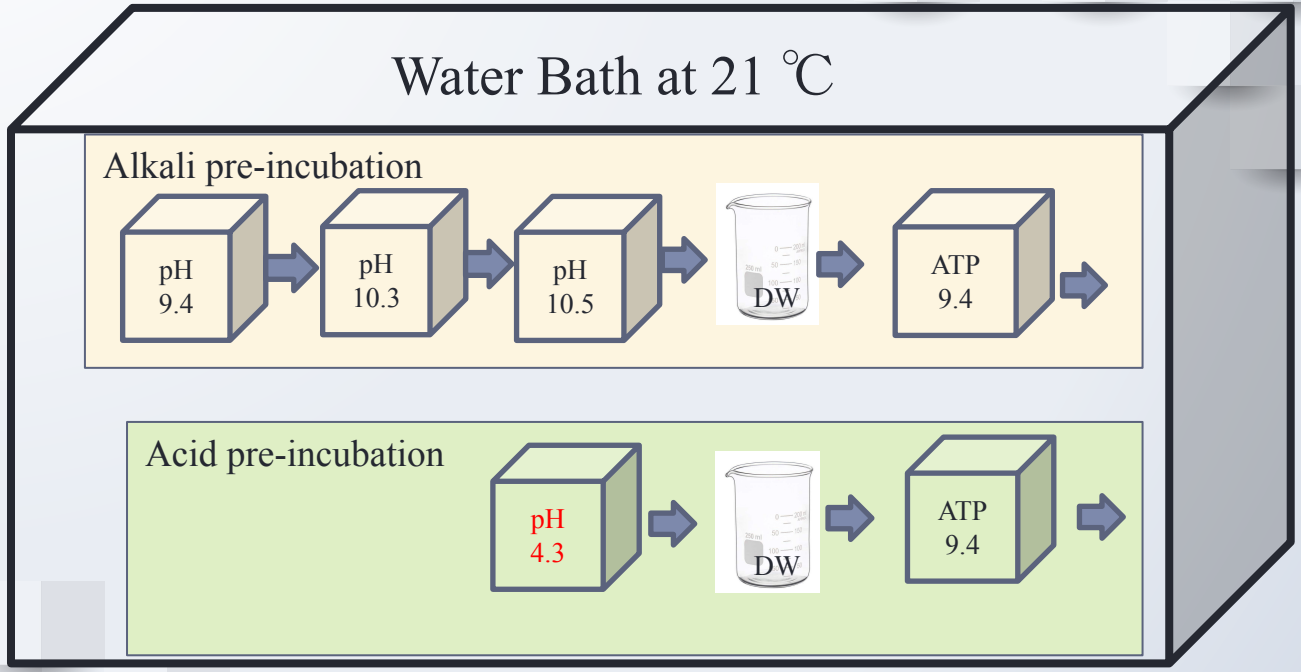
From frozen muscle cubes, 10 µm thick, transverse sections were cut in a cryostat and mounted on a dry slide glass and then stored at -80°C until staining.

NADH-TR Staining of Muscle Section Slides

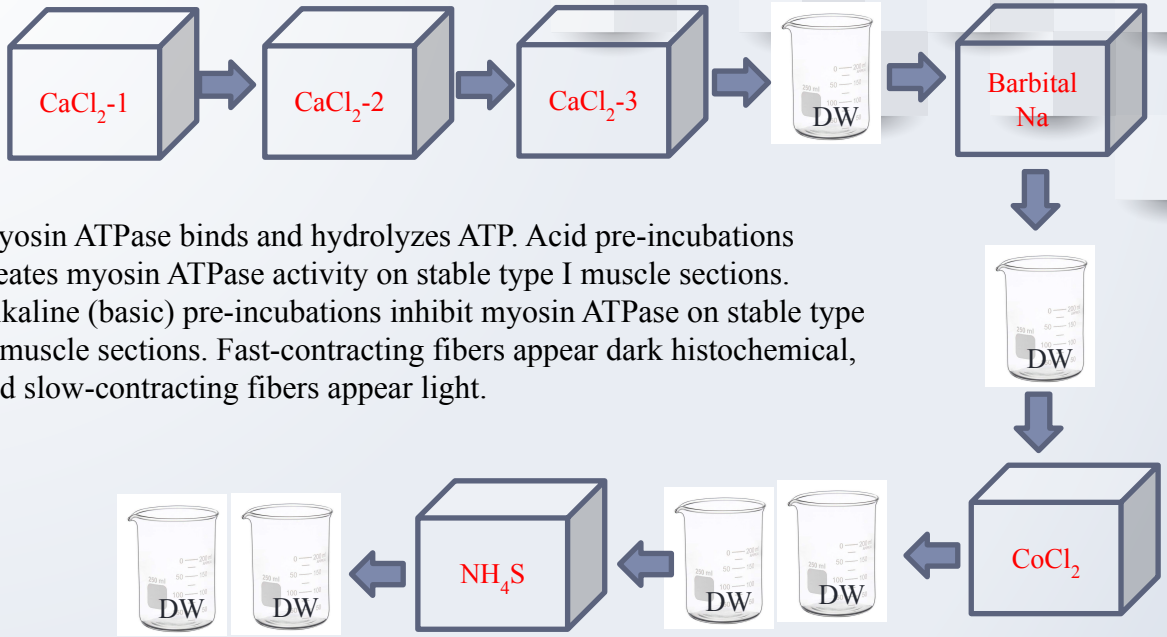


Incubate muscle sections for 20 minutes in 30°C water bath, then wash in DW (distilled water). After place in acetate buffer for 5 minutes, then wash again. Enzymatic activity releases hydrogen from the substrate, then the released hydrogen is transferred to the tetrazolium. The tetrazolium is converted to purple-blue pigment marking the site of enzyme activity. The more mitochondria a muscle fiber contains, the more intense the stain will become. The acetate buffer is used to prevent changes in the pH that may affect the biochemical activity. Type I muscle fibers turn deep blue, type II muscle fibers turn light blue or neutral in muscle sections.

Myosin ATPase Staining of Muscle Section Slides



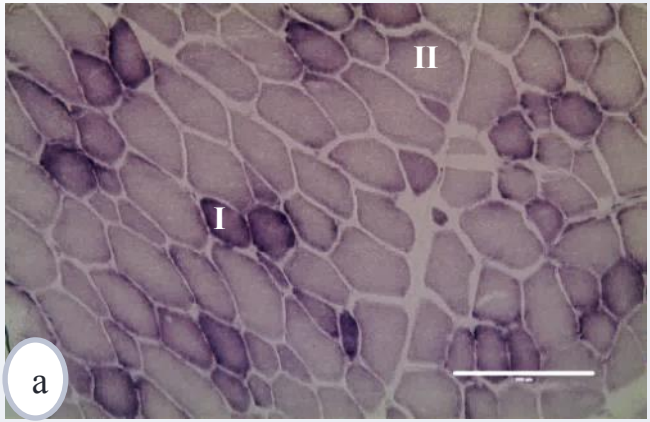
Treatment of Muscle Section Slides After Pre-incubation

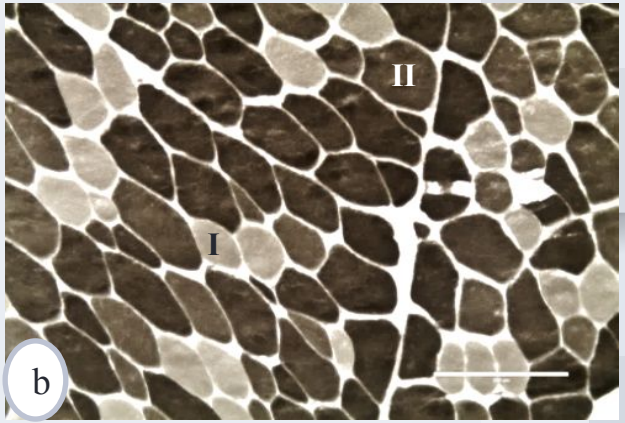


Myosin ATPase binds and hydrolyzes ATP. Acid pre-incubations creates myosin ATPase activity on stable type I muscle sections. Alkaline (basic) pre-incubations inhibit myosin ATPase on stable type II muscle sections. Fast-contracting fibers appear dark histochemical, and slow-contracting fibers appear light.

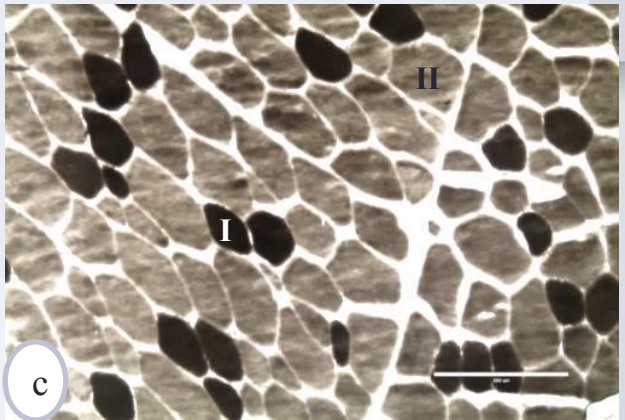
Cover slipping the tissue section preserves it for years.

Final Muscle Section Slides





Histochemistry of muscle fiber typing in LT muscle of Canada AA beef was conducted by NADH-TR (a), myosin ATPase [(alkaline at pH 10.5; (b), and acid at pH 4.3; (c)] Scale bar =200 µm.

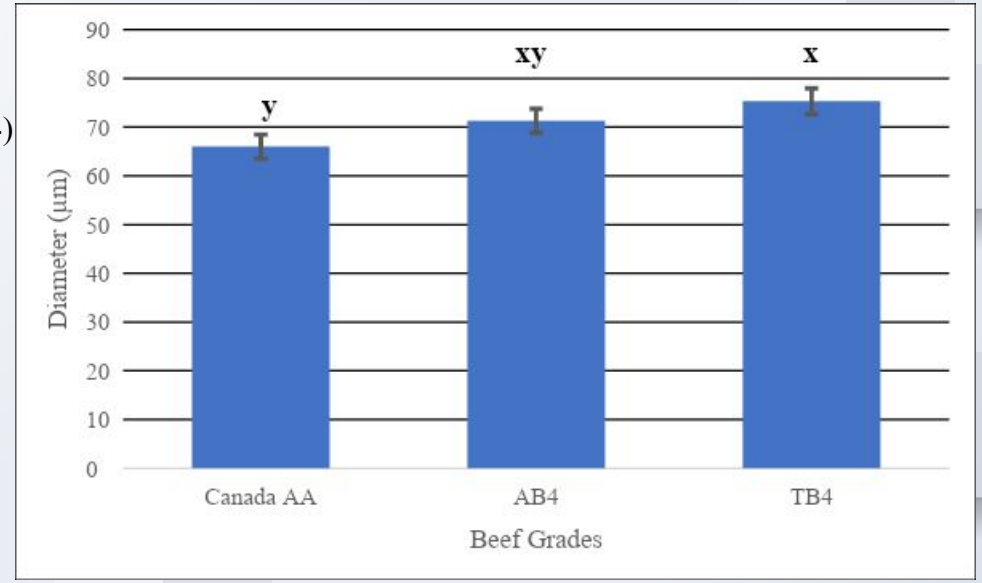


Results
Mean Muscle Fiber Diameter (µm)

Normal Beef (**Canada AA)**

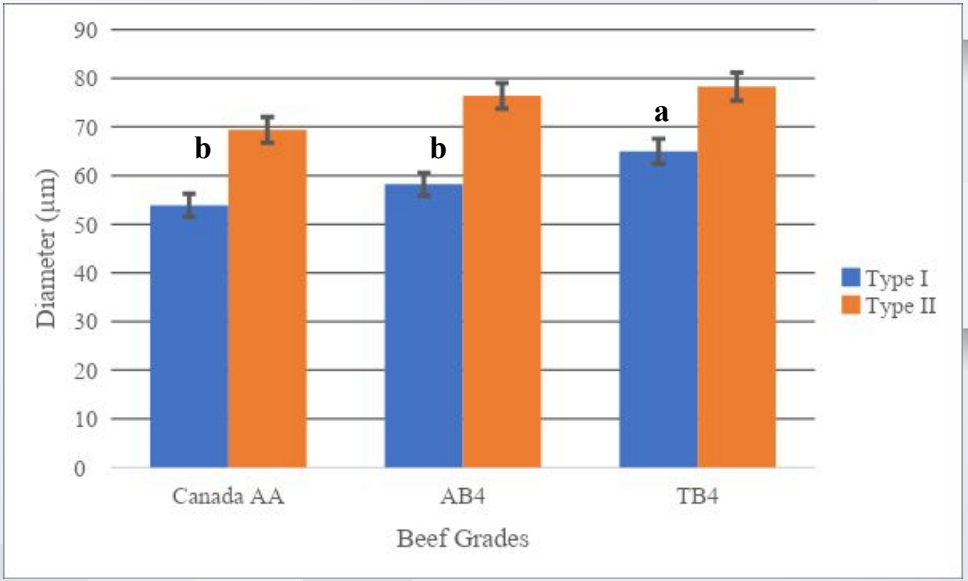
Atypical Dark Cutters (**AB4**)

Typical Dark Cutters (**TB4**)



Mean muscle fiber, is the average diameter for the above beef grades.
[x, y] Means with different letters approach difference at $p < 0.1$.
Results between mean muscle fiber type and beef grade show there is only a significant difference between TB4 beef grade and Canada AA.

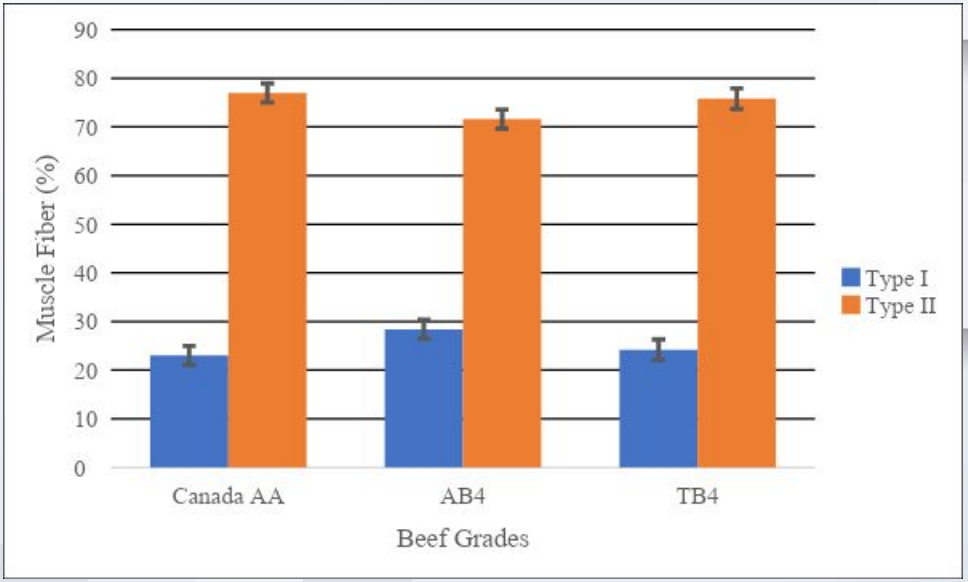
Results
Type I & Type II Muscle Fiber Diameter (µm)



[a , b, c] Means with different letters are significantly different at $p < 0.05$.

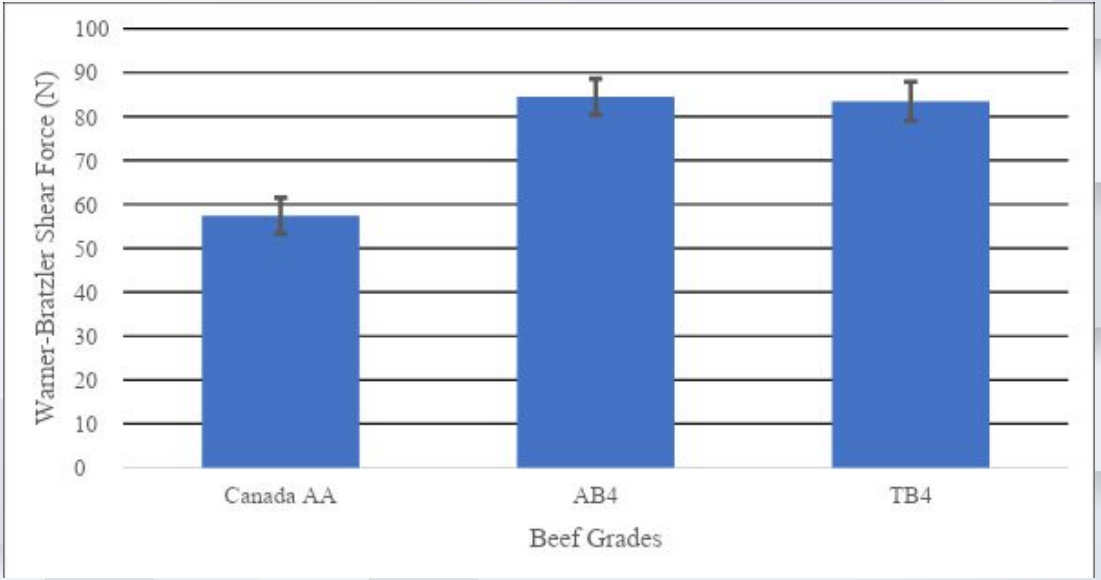
Results show that dark cutting carcasses TB4 had a greater type I muscle fiber diameter than atypical dark cutting and normal beef.

Results
Type I & Type II Muscle Fiber Types (%)



The results for both muscle fiber type (%) showed that dark cutting had no effect.

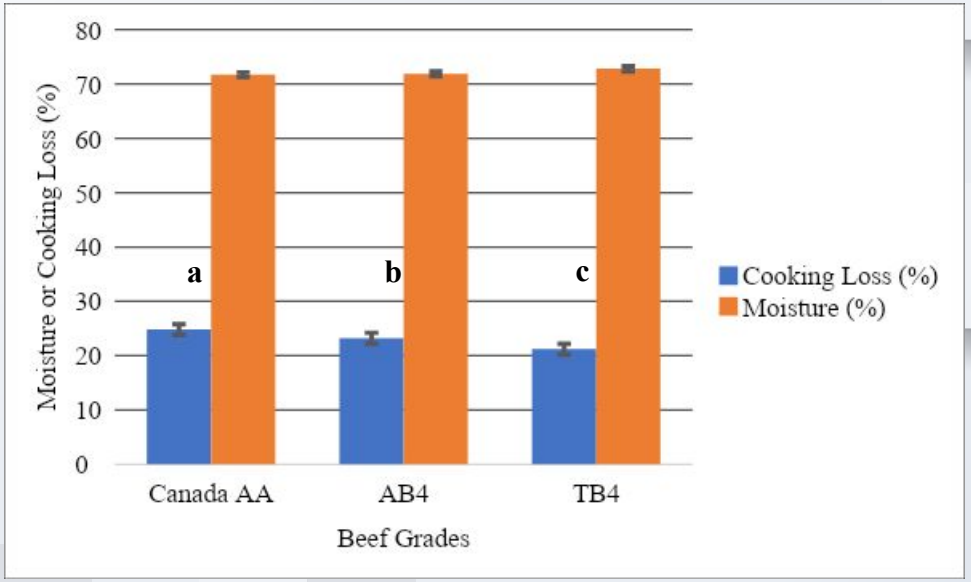
Results
Warner-Bratzler Shear Force (N)



Warner-Bratzler shear force is a test that measures the amount of force needed to cut through meat. It is a standard test conducted to measure tenderness of meat.

Dark cutting beef (AB4 and TB4) was tougher than normal beef (Canada AA).

Results
Moisture (%) in Raw Meat & Cooking loss (%)



[a, b, c] Means with different letters are significantly different at $p < 0.05$.

The percentage of cooking loss did not have a significant difference between the grades. Canada AA was slightly higher in moisture percentage.

Conclusion

- The diameter of type I muscle fibers was increased in the high pH typical dark cutters because of their high ultimate pH which preserved the protein.
- Protein that is not denatured (is preserved) will hold more water than protein that is denatured.
- In theory, when a muscle fiber has a small diameter and less glycogen it makes the meat most likely to have a higher ultimate pH.

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