Discrimination between barley and corn finished beef by visible and near infrared spectroscopy

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Introduction: Barley production is well suited to western Canada and provides feedstuffs (i.e., grain and silage) found in most beef finisher diets (Canfax, 2017). The recent development of low heat unit hybrid corn has, however, allowed the production of this crop in Alberta and Saskatchewan regions, providing a practical alternative to barley (Lardner, Pearce & Damiran, 2017). With the availability of both corn and barley for inclusion in finishing diets, an opportunity exists for differentiation and value addition for target markets, and branding of barley-fed beef similar to strategies already in place for corn-fed beef in eastern Canada (OCFB, 2001). Previous research has shown visible and near-infrared spectroscopy (vis-NIRS) as a successful technology to classify beef based on quality characteristics, animal feeding regime, geographical origin and species (Prieto, Pawluczyk, Dugan & Aalhus, 2017b). The objective of this study was, therefore, to test if visible and near-infrared spectroscopy (vis-NIRS) could be used to rapidly classify beef based on grain source (barley vs. corn) in finishing diets.

Material and Methods: A total of 85 in-bone ribeyes from barley (n=29) and corn (n=27) grain-fed steers from a larger study were shipped refrigerated to the Lacombe Research and Development Centre (Agriculture and Agri-Food Canada, Canada). After 15 d of ageing, two 2.5-cm steaks were fabricated from each ribeye for subsequent analyses. Sensory descriptive evaluation (9-member trained sensory panel; Prieto, Dugan, Larsen, Vahmani, & Aalhus, 2017a) and fatty acid analysis (Vahmani et al., 2017) were performed on the *longissimus thoracis* (LT), whereas colour (L^*, a^*, b^*) was measured on both LT and subcutaneous fat using a Minolta CR-300 (Minolta Canada Inc., Mississauga, ON). Vis-NIR spectra were collected on subcutaneous fat and intact (LT), and then LT was ground (Robot Coupe Blixir BX3) and scanned again. All spectra were collected at the laboratory using a portable LabSpec®4 Standard-Res spectrometer (Analytical Spectral Device-ASD Inc., Boulder, CO, USA) from 350 to 2500 nm (vis-NIR range). For the spectra collection on the intact and ground LT, the spectrometer was fitted with a 20 mm ASD fibre-optic high-intensity contact probe and scanned 50 times per reading (~5 s). When subcutaneous fat was scanned, a hand-held ASD fibre-optics pro-reflectance probe (1 m long, 3 mm optical window size) was used. Colour, fatty acids and sensory data were analyzed using the MIXED model procedure of SAS (Version 9.4 Institute Inc., Cary, NC). Grain was used as a fixed effect, and pen (and trained panellist for sensory analysis) as the random effect. Partial least square discriminant analysis (PLS-DA) was applied to the spectra regions (vis-NIRS: 350-2500 nm; vis: 350-750 nm; NIRS: 750-2500 nm) from the three tissues (fat, intact LT and ground LT) for source of grain-fed discrimination, using the software R-Project (R Development Core Team 2009).

Results: When the subcutaneous fat samples were scanned, 100% of the samples from barley-fed cattle were correctly classified, regardless of the wavelength range used for the spectra collection, and the fat samples from corn-fed steers were discriminated with 93-100% accuracy. When these calibration models were cross-validated, 100 and 93% of the fat samples from barley and corn-fed cattle, respectively, were correctly classified regardless of the region used in the analyses. Hence, 97% overall fat samples were correctly discriminated between barley and corn-fed cattle.

When the spectra were collected on intact LT, 100% of intact LT samples from both barley and corn-fed cattle were correctly classified using the NIR region. In the cross-validation, however, the highest percentage of samples correctly discriminated was found when the vis-NIR region was considered in the analysis with 96 and 85% of intact LT samples from barley and corn-fed cattle correctly classified, respectively (91% overall accuracy).

The ability of vis-NIRS to classify fat and intact LT samples based on cattle feeding regime could be partly due to differences in the sample fatty acid composition as consequence of source of grain-fed, which translated into C-H bond absorbance differences in the NIR region (950, 1200 and 1720-1760 nm; Figure 1). Indeed, Vahmani et al. (2020) observed in samples from this study, significant differences in the content of some PUFA between fat samples from barley and corn-fed cattle. Additionally, n-3 fatty acid content (0.684 vs. 0.516%), initial (6.21 vs. 5.79) and overall tenderness (6.65 vs. 6.17), and initial (5.29 vs. 4.52) and sustained juiciness (5.19 vs. 4.80) were higher ($P<0.05$) in LT from barley than corn-fed cattle, which could have contributed to the successful vis-NIRS discrimination of intact LT samples based on grain source. Although similar ($P>0.05$) L^*, a^*, and b^* colour values were found in fat and intact LT samples between barley and corn-fed steers, the vis spectra of these tissues (Figure 1) showed differences in absorbance peaks around 420, 540 and 570 nm due to grain source, related to the absorption of meat pigments and carotenoids stored in fat (Ripoll, Casasús, Joy, Molino & Blanco, 2015). Hence, potential differences in hematological pigments and carotenoids between barley and corn fat and LT samples could have also influenced the vis-NIR spectra, facilitating the discrimination of these tissues based on the source of grain-fed.

When the spectra were collected on ground LT samples, the PLS-DA accuracy in the calibration process along the different spectral regions ranged from 71 to 82%, and 66 to 85%, in barley and corn grain samples, respectively. In the cross-validation, 66 and 67% of the ground LT samples were correctly classified from barley and corn-fed cattle, respectively, using the NIR region. Overall, ground LT samples were classified with <70% overall accuracy. The lower classification accuracy for ground compared to intact LT samples could be partly related to the destruction of muscle integrity during grinding. Homogenization averages the effects of scattering by fibres (Cozzolino et al. 2002), however, this process severely disrupts the structure of the muscle, destroying and randomizing the fibre arrangement, which entails a loss of relevant information. For instance, information about colour, texture and juiciness of the muscle is lost, and initial and overall/sustainable tenderness and juiciness were characteristics distinguishing barley from corn LT samples in this study.

Conclusion: Vis-NIRS measurements on fat and intact loin can be used to accurately discriminate between corn and barley-fed beef, providing a rapid authentication tool for branded barley-fed beef for target markets.

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Table 1. Partial least square discrimination analysis of fat and meat samples from barley and corn-fed cattle.

		Correctly classified (%)								
		Fat			**Intact LT**			**Ground LT**		
		T	**Calibration**	**Validation**	***T***	**Calibration**	**Validation**	***T***	**Calibration**	**Validation**
Vis-NIR	Barley	10	100	**100**	7	96	**96**	8	82	69
	Corn		100	**93**		88	**85**		85	59
Vis	Barley	6	100	**100**	4	70	63	10	71	54
	Corn		100	**93**		66	69		66	41
NIR	Barley	9	100	**100**	6	100	93	5	79	**66**
	Corn		93	**93**		100	85		82	**67**

LT = *longissimus thoracis*

T = terms in the PLS-DA model

Figure 1. The average raw spectra for intact loin and subcutaneous fat samples from barley and corn-fed steers.