An investigation on the relationship among marbling features, physiological age and Warner–Bratzler Shear force of steer *longissimus dorsi* muscle

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Abstract Researchers nowadays have paid much attention to the relationships between tenderness and marbling, or physiological age. While the marbling was mainly evaluated qualitatively with scores or grades, and rarely related with physiological age. Present study was carried out to analyze the marbling features of *longissimus dorsi* muscle between the 12th and 13th ribs from 18, 36, 54 and 72 months old Simmental steers were quantitatively described with area and perimeter using computer vision technique. Relationship between Warner–Bratzler Shear force (WBSF), physiological age and the marbling features were examined performing regression analysis. The results revealed that WBSF positively correlated with physiological age, but negatively with marbling area and perimeter. Regression analysis showed that the relationship between the shear force and the steers' age was more close to the quadratic curve ($R^2 = 0.996$) and exponential curve ($R^2 = 0.957$). It was observed during study that marbling grew with steers age. Marbling features were in linear correlation with the steers' age, with $R^2 = 0.927$ for marbling area and $R^2 = 0.935$ for marbling perimeter. The

industries in future may speculate beef tenderness and physiological age based on the marbling features (area and perimeter), which can be determined through the online image acquisition system and image processing.

Keywords Image process · *Longissimus dorsi* · Marbling · Warner–Bratzler Shear force

Introduction

Tenderness is a top priority sensory attributes to estimate beef quality in beef industry (Cassar-Malek and Picard 2016). Evaluation of consumers on beef tenderness is subjective to personal taste, which is being affected by many factors such as breed, sex and feeding. Thus, instrumental methods have been developed to quantitatively assess the force in shearing the meat, aiming to predict the tenderness ratings. Warner–Bratzler shear force (WBSF) method is one of the most widely used methods. The WBSF of beef, especially that of bovine *longissimus lumborum* between the 12th and 13th ribs, has been considered as an important indicator of beef tenderness (Howard et al. 2013). Meanwhile, beef carcasses in the beef industry are graded based on certain classification standards, such as United States Department of Agriculture (USDA 2016) and Chinese beef quality grading (NY/T 676-2010). Among the attributes that define the quality grades, characteristics of marbling and level of carcass physiological age play a significant role.

Some researchers have made great efforts to link tenderness with marbling or physiological age. In *longissimus* muscle of highly marbled beef from Japanese Black cattle, the development of adipose tissues during the late fattening period disorganizes the structure of the intramuscular

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connective tissue and contributes to tenderization (Nishimura et al. 1999). In the sensory evaluation of longissimus muscle, Instrument marbling score explained 40% of the observed variation in panel ratings for tenderness. Steak tenderness also increased with increasing marbling degree, while tenderness differences among marbling degrees differed for steers (MA = SA > MD = MT > SM > SL = TR) and heifers (MA = SA > MD > MT > SM > SL > TR). (Emerson et al. 2013) The WBSF of longissimus muscle from yearling heifers and 2-year-old did not differ between age groups (Shackelford et al. 1995). The instrumental and sensory tenderness of the beef ribeye from Nellore steer was not influenced by physiological age (2, 4 and 6 permanent incisors) (Pflanzer and Felicio 2009). Moon et al. (2006), in Hanwoo beef females, found mature carcasses had a larger longissimus muscle area and lower marbling scores.

Nevertheless, the marbling is usually subjectively evaluated as marbling level or marbling scores, not quantitatively. Whereas the level of carcass physiological age or the month-old of steers is mainly subjectively assessed through the eruption, restoration and abrasability of incisors. Nowadays, the characteristics of marbling could be quantitatively analyzed by computer vision and image processing technique, which could be used for quick automatic grading. The cross-section cross section at the 12th–13th rib has been studied a lot since as it is the main benchmark in grading of carcasses (USDA 2016). Automatic segmentation of beef *longissimus dorsi* muscle and marbling have been developed (Jackman et al. 2009). Consumers assessment of *longissimus dorsi* beef tenderness has been correlated with image colour, marbling and surface texture features, using computer vision methodology (Jackman et al. 2010). Computer vision technique has also been used to predict beef physiological age. A trained neural network in computer vision technique was tested for predicting USDA beef physiological age grades from image features of ossification (Hattem et al. 2003).

In this study, the *longissimus dorsi* muscle between the 12th and 13th ribs from Simmental steers were selected to quantitatively evaluate the relationship among marbling, tenderness and physiological age. The features of marbling were quantitatively described using area and perimeter by computer vision technique. The tenderness was quantified as WBSF, while the physiological age was represented as number of permanent incisors. The present study was carried to establish a quantitative relationship, expanding the application of computer vision techniques in predicting beef tenderness and physiological age.

Materials and methods

Sample preparation and data collection

The samples were provided by Jilin province Changchun Haoyue Islamic Meat Co. Ltd (Changchun, China). After providing same feed, total 80 Simmental steers (consisted of 4 groups, 18, 36, 54 and 72 months old) were subjected to the same pre-slaughter treatment before being slaughtered in a standard way. With the help of expert butcher, steaks and *longissimus dorsi* muscle between the 12th and 13th ribs (25 mm thick, from cranial to caudal) were obtained from both sides of the carcass after 72 h post-mortem. The steaks were trimmed off from extraneous muscle and fat, allowing all white objects to be taken as marbling fat. The samples were then vacuum-packed and stored at a temperature of -20°C . Steaks from the right side were used for image acquisition while the ones from the left side were applied for WBSF analysis. The steaks were thawed for 18 h at $2-4^{\circ}\text{C}$ prior to image acquisition and WBSF analysis.

The beef images were acquired using an online acquisition system having a black background. The vision system consisted of two adjustable LED lighting systems (YX-BL25844, Yongxin Ltd, China) with a color RGB format industry high resolution (1280×960) digital camera (EM130C/M, micro-vision Ltd, China) with 8 mm C-mouth lens (aperture of 1.4 to C, AFT-0814MP, micro-vision Ltd, China), photoelectric switch (PMM18-C102NA, Hufei Ltd, China) and computer system.

Image processing

Image processing and analysis were performed similarly as described earlier (Chen et al. 2010), including graying, noise remove, image enhancement, boundary tracking (remove the black background), binaryzation, corrosion, expansion, ribeye muscle extraction, marbling area extraction, boundary detection and marbling perimeter extraction (Fig. 1).

Graying

Beef gray images were obtained by calculating the weighted average of RGB features. Graying was computed using following equation:

$$f(i, j) = 0.30R(i, j) + 0.59G(i, j) + 0.11B(i, j).$$

Fig. 1 **a** Original image of beef, **b** graying, **c** noise remove, **d** image enhancement, **e** boundary tracking (remove the black background), **f** binaryzation, **g** corrosion, **h** expansion, **i** eye muscle extraction, **j** marbling

Noise removal

To decrease the influence of noise on image quality, the median filtering method was applied to remove image noise.

Image enhancement

Bivariate Laplace algorithm was applied to enhance the image quality, especially the images' details. Following equation was used:

$$\nabla^2 f(x, y) = f(x + 1, y) + f(x - 1, y) + f(x, y + 1) + f(x, y - 1) - 4f(x, y)$$

Boundary tracking (remove the black background)

The boundary tracking method was adopted to remove the black background. The image was scanned from the upper left, setting the mutation point that was different from the background as the starting point of the boundary. Eight neighborhood method was then adopted to track the boundary, till it returned to the starting point of the boundary. All the greyscale and area of boundary line were set to 0. The processed image then displayed beef with black region whereas the area with white was its background. Then, add the new image to the image that after enhancement.

Binaryzation

Otsu automatic threshold method (Otsu 1979) was adopted for binarization processing. The similar method has also been used in previous studies (Chen and Qin 2008).

Corrosion and expansion

After binaryzation, a 3×3 structural element was used for image corrosion and expansion processing (Gonzalez and Woods 2002).

Ribeye muscle extraction

After the above processing, the largest connected region with 0 grey value was the ribeye muscle. The eye muscle was extracted using components labeling algorithm, as follows:

1. Scan pixel, starting from the upper left of the binary image, to the lower right corner of the image;
2. If the grey value of scanned pixel was 0, label the connected region was labeled as K and counted as A_K of pixel with "0" grey value. Similarly, next connected region was labelled as $K + 1$ and count A_{K+1} .
3. Comparing A_{K+1} and A_K . If $A_{K+1} > A_K$, retain the $k + 1$ connected region, and reset the grey value of the k connect region to 255. If $A_{K+1} < A_K$, retain the k connected region, and reset the grey value of the $k + 1$ connect region to 255.
4. Until scanning to the lower right corner, the retained connected region was the largest connected region, as well as the ribeye muscle (as shown in Fig. 1i).

Marbling area extraction

Edge detection algorithm was used to extract the marbling area. The marbling area was demonstrated after preserving the image contour with 0 grey value and resetting the rest of the pixels in the area to 255, the marbling area was demonstrated (as shown in Fig. 1j).

Parameters determination of marbling area

The actual marbling area value (A_t) was calculated first by performing image calculation, calculating the actual area (K_a , cm^2) of each pixel and then by counting the numbers (N_a) of the pixels with "255" grey value in the marbling area. Equation given was used for calculating actual marbling area,

$$A_t = K_a \times N_a.$$

The actual marbling perimeter (C_t) was calculated by preserving the image contour with 0 grey value and resetting the rest of the pixels in the area to 255. The actual length (K_c , cm) of each pixel was calculated by performing image calculation along with counting the numbers (N_c) of the pixels with “0” grey value in the marbling area. Values of K_c and N_c were used to calculate actual marbling perimeter using equation,

$$C_t = K_c \times N_c.$$

WBSF analysis

The WBSF analysis was performed similarly as described earlier (Geesink et al. 1995; Wheeler et al. 1994). Beef samples were heated in retort pouches in a 75 °C water bath to a core temperature of 70 °C, then chilled 24 h at 4 °C. Six round cores with a diameter of 1.27 cm were drilled parallel to the fiber orientation. Cores with obvious connective tissue defects were discarded. Shear force determinations were conducted on a TMS-PRO (FTC Co., USA) equipped with a WBSF head, with a crosshead speed of 200 mm/min. The shear force of the beef sample was represented as the arithmetic mean value of 6 cores.

Statistical analysis

SPSS 18.0 was used to perform statistical analysis. For Analysis of Variance (ANOVA), the data was analyzed at a significant level of $P \leq 0.05$.

Results and discussion

The results of WBSF, marbling area and perimeter of ribeye muscle in different age groups and the regression analysis are presented in Table 1 and Fig. 2. For the group of same age, the WBSF was all in linear correlation with marbling areas or marbling perimeter ($P < 0.001$). It was moreover observed that WBSF linearly decreased with increasing marbling area or perimeter value of ribeye muscle. The same negative correlation between marbling level and WBSF has also been reported by Xiong et al. (2007). In all 3 groups having different marbling level, the one having highest marbling level resulted lowest WBSF.

WBSF was in positive correlation with steers' age. The WBSF increased at an inconsistent rate with the growth of steers' age. The mean value increased 50% from 18 to 72 months, it was also observed that the change increased was slowly for 18–36 months, while for 36–72 months it changed rapidly. The WBSF of the ribeye muscle from 18 months group was significantly lower than that from 54

Table 1 The shear force, marbling area and perimeter of the ribeye muscle with different physiological age, and regression analysis ($Y = \text{Warner-Bratzler Shear force}$)

Month-old	Shear force (N)			Marbling area (cm ²)			Marbling perimeter (cm)			X = Marbling area			X = Marbling perimeter		
	Max	Min	Mean $\pm$ SD	Max	Min	Mean $\pm$ SD	Max	Min	Mean $\pm$ SD	Regression equation	R ²	Sig.	Regression equation	R ²	Sig.
18	78.17	44.17	63.27 $\pm$ 11.19 ^c	36.75	25.15	29.68 $\pm$ 3.35 ^a	47.16	40.37	43.23 $\pm$ 1.82 ^b	$y = 159.32 - 3.24x$	0.94	0	$y = 311.78 - 5.75x$	0.878	0
36	93.45	53.3	67.73 $\pm$ 11.41 ^c	34.07	24.72	30.19 $\pm$ 3.41 ^a	47.34	39.31	44.46 $\pm$ 2.29 ^{ab}	$y = 162.76 - 3.15x$	0.886	0	$y = 289.29 - 4.98x$	0.987	0
54	107.87	64.87	80.62 $\pm$ 11.94 ^b	36.34	22.64	31.02 $\pm$ 3.59 ^a	49.03	40.84	45.69 $\pm$ 2.07 ^a	$y = 181.86 - 3.26x$	0.963	0	$y = 338.52 - 5.65x$	0.961	0
72	139.17	81.88	99.39 $\pm$ 19.73 ^a	36.94	19.75	31.72 $\pm$ 5.84 ^a	49.98	38.17	46.16 $\pm$ 3.93 ^a	$y = 205.88 - 3.36x$	0.986	0	$y = 330.40 - 5.01x$	0.992	0

^{a,b,c}Indicate the results of significance test: different alphabet, $P < 0.05$; same alphabet or included, $P > 0.05$

Fig. 2 The marbling area **a** and perimeter values **b** of ribeye muscle was related with Warner–Bratzler Shear force

to 72 months group, whereas a non-significant difference was found from the ribeye muscle obtained from 36 months group.

Regression analysis showed that the relationship between the shear force and the steers' age was more close to the quadratic curve ($R^2 = 0.996$) or exponential curve ($R^2 = 0.957$), but it wasn't linear. The results are in line with Wulf et al. (1996), who stated that animal age was positively correlated with shear force across all cuts, within the sirloin and round from Limousin steers. *Longissimus dorsi* steaks from more mature cows had an increased WBSF (Xiong et al. 2007). Tenderness scores for the older Hanwoo beef females were lower than for younger and intermediate groups. The physiological age level was significantly correlated with WBSF (Moon et al. 2006). The shear force in *longissimus thoracis* taken from Qinchuan steers increased by 37.49% with the physiological age from 0 to 8 permanent incisors (Wang et al. 2016).

The marbling area and perimeter of ribeye muscle were in positive correlation with steers' age. The fat in beef with increasing age also increased, resulting more intramuscular fat content. Marbling features were in linear correlation with the steers age, with $R^2 = 0.927$ for marbling area and $R^2 = 0.935$ for marbling perimeter. The marbling area of ribeye muscle between different age groups were found to be non-significant ($P > 0.05$). The marbling perimeter value of 18 months beef was significantly different from that of 54 months beef or 72 months beef ($P < 0.05$), but similar with that of 36 months beef. The correlation between marbling and physiological age are in line with previous studies. Animal age was found to be significantly correlated with marbling level of Limousin steers (Wulf et al. 1996) and Hanwoo beef females carcass (Moon et al. 2006). Growth stage had influenced the marbling development in *longissimus* muscle from beef cattle (Kern et al. 2014). After trimming process, the marbling perimeter value of 18 months beef was significantly different from that of 54 months beef or 72 months beef (Hall et al.

2016). Pflanzner and de Felicio (2011) analyzed the intramuscular fat content of rib steaks of Nellore steer (grouped by physiological age, 2, 4 or 6 permanent incisors) using chemical method. They found that physiological age had significant influence on the intramuscular fat content of rib steaks, and thus had a further impact on marbling level. In most studies, the marbling was rarely specified as marbling area and marbling perimeter, whereas marbling scores or marbling level had only been described by individual's subjective evaluation. However, after use of computer vision technique, the beef marbling could be evaluated using parameters such as area and perimeter. The *longissimus* muscle area and marbling score could be predicted on-line using MARC beef carcass image analysis system (Shackelford et al. 2003). Area and total length of marbling flecks in the *longissimus* muscle of cattle had been analyzed by computer image (Yang et al. 2006). In this study, the nature of marbling was quantitatively presented as area and perimeter using computer vision technique, and was related with cattle physiological age. Their correlation suggests that the physiological age of cattle could be predicted on the basis of the marbling area/perimeter.

Conclusion

Computer vision technique has been widely used to evaluate beef grades in beef industry. In this study, an online image acquisition system has been developed to acquire beef image. After image processing, marbling features including area and perimeter were determined and found to be related with WBSF and steers' month-old. Thus, in future industry, the beef tenderness and physiological age may be speculated based on the marbling features (area and perimeter), which can be determined through the online image acquisition system and image processing.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

References

- Cassar-Malek I, Picard B (2016) Expression marker-based strategy to improve beef quality. *Sci World J* 2016:2185323. <https://doi.org/10.1155/2016/2185323>
- Chen K, Qin C (2008) Segmentation of beef marbling based on vision threshold. *Comput Electron Agric* 62:223–230
- Chen K, Sun X, Qin C, Tang X (2010) Color grading of beef fat by using computer vision and support vector machine. *Comput Electron Agric* 70:27–32
- Emerson MR, Woerner DR, Belk KE, Tatum JD (2013) Effectiveness of USDA instrument-based marbling measurements for categorizing beef carcasses according to differences in longissimus muscle sensory attributes. *J Anim Sci* 91:1024–1034. <https://doi.org/10.2527/jas.2012-5514>
- Geesink GH, Koolmees PA, van Laack HL, Smulders FJ (1995) Determinants of tenderisation in beef Longissimus dorsi and Triceps brachii muscles. *Meat Sci* 41:7–17. [https://doi.org/10.1016/0309-1740\(94\)00066-G](https://doi.org/10.1016/0309-1740(94)00066-G)
- Gonzalez RC, Woods RE (2002) Digital image processing. Prentice-Hall Inc, Upper Saddle River
- Hall N, Schonfeldt HC, Pretorius B (2016) Effect of animal age and trimming practices on the physical composition of Bonsmara beef. *Food Chem* 193:160–165. <https://doi.org/10.1016/j.foodchem.2014.12.048>
- Hatem I, Tan J, Gerrard DE (2003) Determination of animal skeletal maturity by image processing. *Meat Sci* 65:999–1004. [https://doi.org/10.1016/S0309-1740\(02\)00318-2](https://doi.org/10.1016/S0309-1740(02)00318-2)
- Howard ST et al (2013) North American Beef Tenderness Survey 2011–2012: benchmarking tenderness and sample shipping procedures. *J Anim Sci* 91:5981–5988. <https://doi.org/10.2527/jas.2013-7040>
- Jackman P, Sun DW, Allen P (2009) Automatic segmentation of beef longissimus dorsi muscle and marbling by an adaptable algorithm. *Meat Sci* 83:187–194. <https://doi.org/10.1016/j.meatsci.2009.03.010>
- Jackman P, Sun DW, Allen P, Brandon K, White AM (2010) Correlation of consumer assessment of longissimus dorsi beef palatability with image colour, marbling and surface texture features. *Meat Sci* 84:564–568. <https://doi.org/10.1016/j.meatsci.2009.10.013>
- Kern SA, Pritchard RH, Blair AD, Scramlin SM, Underwood KR (2014) The influence of growth stage on carcass composition and factors associated with marbling development in beef cattle. *J Anim Sci* 92:5275–5284. <https://doi.org/10.2527/jas.2014-7891>
- Moon SS, Yang HS, Park GB, Joo ST (2006) The relationship of physiological maturity and marbling judged according to Korean grading system to meat quality traits of Hanwoo beef females. *Meat Sci* 74:516–521. <https://doi.org/10.1016/j.meatsci.2006.04.027>
- Nishimura T, Hattori A, Takahashi K (1999) Structural changes in intramuscular connective tissue during the fattening of Japanese black cattle: effect of marbling on beef tenderization. *J Anim Sci* 77:93–104
- Otsu N (1979) A threshold selection method from gray-level histogram. *IEEE Trans SMC* 15:652–655
- Pflanzer SB, de Felicio PE (2011) Moisture and fat content, marbling level and color of boneless rib cut from Nellore steers varying in maturity and fatness. *Meat Sci* 87:7–11. <https://doi.org/10.1016/j.meatsci.2010.08.009>
- Pflanzer SB, Felicio PE (2009) Effects of teeth maturity and fatness of Nellore (*Bos indicus*) steer carcasses on instrumental and sensory tenderness. *Meat Sci* 83:697–701. <https://doi.org/10.1016/j.meatsci.2009.08.011>
- Shackelford SD, Koohmaraie M, Wheeler TL (1995) Effects of slaughter age on meat tenderness and USDA carcass maturity scores of beef females. *J Anim Sci* 73:3304–3309
- Shackelford SD, Wheeler TL, Koohmaraie M (2003) On-line prediction of yield grade, longissimus muscle area, preliminary yield grade, adjusted preliminary yield grade, and marbling score using the MARC beef carcass image analysis system. *J Anim Sci* 81:150–155
- USDA (2016) United States standards for grades of carcass beef. USDA, Washington
- Wang F, Zhang Y, Li J, Guo X, Cui B, Peng Z (2016) Contribution of cross-links and proteoglycans in intramuscular connective tissue to shear force in bovine muscle with different marbling levels and maturities. *LWT Food Sci Technol* 66:413–419
- Wheeler TL, Koohmaraie M, Cundiff LV, Dikeman ME (1994) Effects of cooking and shearing methodology on variation in Warner-Bratzler shear force values in beef. *J Anim Sci* 72:2325–2330
- Wulf DM, Morgan JB, Tatum JD, Smith GC (1996) Effects of animal age, marbling score, calpastatin activity, subprimal cut, calcium injection, and degree of doneness on the palatability of steaks from limousin steers. *J Anim Sci* 74:569–576
- Xiong YL, Mullins OE, Stika JF, Chen J, Blanchard SP, Moody WG (2007) Tenderness and oxidative stability of post-mortem muscles from mature cows of various ages. *Meat Sci* 77:105–113. <https://doi.org/10.1016/j.meatsci.2007.04.012>
- Yang XJ, Albrecht E, Ender K, Zhao RQ, Wegner J (2006) Computer image analysis of intramuscular adipocytes and marbling in the longissimus muscle of cattle. *J Anim Sci* 84:3251–3258. <https://doi.org/10.2527/jas.2006-187>