Growth and Breed Related Changes of Marbling Characteristics in Cattle[1]

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ABSTRACT

The objective of this study was to investigate the growth- and breed-related changes of marbling characteristics in cattle. Four cattle breeds with different growth impetus and muscularity were reared and slaughtered under experimental conditions. German Angus, as a typical beef cattle; Galloway, as a smaller, environmentally resistant beef cattle; Holstein-Friesian, as a dairy-type cattle; and double-muscled Belgian Blue, as an extreme type for muscle growth, were used. These 4 breeds were expected to have differences in muscle development and i.m. fat deposition. Between 5 and 15 bulls of each breed were slaughtered at 2, 4, 6, 12, or 24 mo of age.

Marbling characteristics were determined and classified in LM and semitendinosus muscle by computerized image analysis. Among breeds, differences appeared in the quantity, structure, and distribution of the marbling flecks in both muscles. The deposition of fat in the double-muscled Belgian Blue bulls remained substantially inferior to that of the other breeds, up to the age of 24 mo. Marbling in German Angus bulls particularly showed larger ($P < 0.05$) marbling fleck areas. Galloway cattle had the greatest ($P < 0.05$) number and the most regular ($P < 0.05$) distribution of the marbling flecks in young animals. Furthermore, for marbling characteristics in Holstein-Friesian animals, a great number and slightly finer structure were observed compared with the other breeds investigated. Postnatal growth-related changes of marbling in LM were characterized by as much as a 40-fold increase in the number of marbling flecks from 2 to 24 mo of age but also by up to a 4-fold enlargement in the area of the marbling flecks. The structure of marbling flecks was determined by 2 development trends. On the one hand, the marbling flecks became larger ($P < 0.05$), and the structure became coarser, which was reflected by an increasing ($P < 0.01$) proportion of long marbling flecks as well as an increasing ($P < 0.01$) maximum skeleton line length. On the other hand, continually new small, round marbling flecks appeared. This caused a decrease ($P < 0.01$) in the proportion of the 3 largest marbling fleck areas. The distribution of the marbling flecks became more regular ($P < 0.05$) with increasing proportion and number of marbling flecks.

The results suggest that hyperplasia of adipocytes plays an important role in marbling during growth of muscle in cattle.

Key words: adipogenesis, breed difference, cattle, growth, intramuscular fat, marbling

INTRODUCTION

The term marbling describes fat that is stored in adipose tissue between the muscle fiber bundles within the muscles. On the cellular level, marbling is comprised of adipocytes embedded in a connective tissue matrix in close proximity to a blood capillary network (Harper and Pethick, 2004). Marbling appears in fresh meat as white flecks or streaks of fat, as subjectively assessed by meat graders, and is related to the fat content of muscle. Different studies have dealt with growth-related changes of marbling scores or i.m. fat content. Robelin (1986) provided an excellent overview of adipose tissue development in cattle. May et al. (1992) and Van Koevering et al. (1995) found a quadratic fashion for the development of marbling scores in LM in the finishing phase before reaching a plateau. However, Bruns et al. (2004) established that marbling score and i.m. fat content increase consistently during growth. Therefore, fat in LM is not necessarily a late-developing tissue. Studies about growth- and breed-related changes relative to more detailed marbling characteristics such as quantity, structure, and distribution of marbling flecks are still lacking. However, they can provide new insights into i.m. fat deposition. Detailed marbling characteristics are provided by computerized image analysis (CIA), a technique used for objective measurement of visible fat in muscle (Hoshino, 1988; Gerrard et al., 1996; Kuchida et al., 2000). In these and other studies, images of fresh meat were used for online prediction of fat content. A newly developed technique (Albrecht et al., 1996) using stained muscle slices improves the contrast to discriminate among muscle, connective tissue, and fat. Using this technique, even small marbling flecks are visible and can be included in the measurement. The objective of the current study was to quantify, in detail and for the first time, changes in marbling from 2 to 24 mo of age in 4 breeds of cattle of different growth patterns and marbling.

MATERIALS AND METHODS

Animals

All animals were cared for and killed according to German rules and regulations for animal care. The experiment was approved by the institutional authorities and by the responsible office of the County of Mecklenburg-Vorpommern, Germany (Landesveterinär- und Lebensmitteluntersuchungsamt Mecklenburg-Vorpommern). Bulls of 4 cattle breeds with different muscle growth potential were used. The breeds represented German Angus, a typical beef cattle; Galloway, a smaller, environmentally resistant beef cattle; Holstein-Friesian, a dairy-type cattle; and double-muscled Belgian Blue, an extreme type for muscle growth.

Bulls were raised using a tethering system with individual feeding. Calves received a milk replacer diet up to 4 mo of age. After weaning, they were fed twice a day with a common diet consisting of wilted grass silage (1.8 kg of DM), corn silage (0.5 kg of DM), and concentrates (5.1 kg of DM) based on barley grain (92% OM, 15% CP, and 25% crude fiber). The proportion of concentrate in the diet was 70% (DM basis). The level of energy was 1.6- to 1.7-fold greater than the maintenance requirement of 530 kJ/kg of metabolic BW (BW0.75). This energy level ensured that the bulls could exhibit their breed-specific growth potential. The maintenance requirement for Galloway cattle was calculated as 500 kJ/kg of metabolic BW (BW0.75). Animals were restricted-fed

Table 1. Number of samples per breed and age group

	Slaughter age, mo					
Breed	**2**	**4**	**6**	**12**	**24**	**!**
German Angus	5	6	10	10	15	46
Galloway	6	8	10	10	14	48
Holstein-Friesian	10	10	10	10	12	52
Belgian Blue	6	7	8	9	14	44
!	27	31	38	39	55	190

for concentrates, depending on BW, and ad libitum for silage. The diet was adapted weekly according to the BW gain of each bull (Beyer et al., 2003). Between 5 and 15 bulls of each breed were slaughtered at 2, 4, 6, 12, and 24 mo of age (**Table 1**).

Tissue Collection

After slaughter, chilling at 6°C for 24 h, and dressing, the LM and the semitendinosus muscle were removed from the left side of the carcass, trimmed of any external fat, and weighed. Two 2-cm thick muscle slices were removed at the 12th rib area of LM and from the thickest part of the muscle body of the semitendinosus muscle. One muscle slice was used to estimate fat content.

For CIA, muscle slices were fixed in 5% formaldehyde, cut into smaller slices (2 mm), rinsed in distilled water for a minimum of 12 h, and stained with oil red O by simply placing them in the stain solution for 6 to 8 h. The parent solution of oil red O was prepared with 0.5 g of stain in 100 mL of pure isopropanol. This was diluted with distilled water at 3:2 (3 parts oil red 0 solution to 2 parts water), to obtain a working solution, and filtered. Stained slices were rinsed overnight, differentiated for 2 to 4 h with 70% isopropanol under constant motion, and rinsed for a minimum of 12 h as described by Albrecht et al. (1996). The stained slices provided a good contrast between fat (red), connective tissue (white), and muscle (pink).

Fat Content and Marbling

The i.m. fat content of muscle samples was obtained in triplicate via the Soxhlet extraction method using petroleum ether as the solvent and was determined gravimetrically after evaporating the solvent (Association of Official Agricultural Chemists, 2000). Marbling was scored with photographic standards on a 6-point scale, where 1 = extremely low marbling and 6 = extremely high marbling. To eliminate subjective operator-to-operator differences, scoring was performed by only one experienced operator.

CIA

Images were taken with a color video camera (DXC-930P, Sony, Japan), and measurements of marbling characteristics were completed using a CIA system (Quantimet 570, Cambridge Instruments, Leica, Bensheim, Germany) as described in detail by Albrecht et al. (1996). Briefly, the area of the muscle cross-section was measured first. After that, a preprocessing procedure improved the transitions between background (muscle) and marbling flecks. After detection and interactive correction of marbling flecks, the size, position, and shape factors of each marbling fleck were measured. From this, quantitative marbling characteristics were calculated and included 1) the proportion of marbling fleck area, calculated as the ratio between the total areas of marbling flecks and the area of muscle; 2) the number of marbling flecks; and 3) size of marbling flecks calculated as the average marbling fleck area. Structure traits were 1) the proportion of the 3 largest marbling fleck areas, calculated as the ratio between the sum of the 3 largest areas and the total marbling fleck area; 2) the length of maximum skeleton line of marbling flecks; and 3) the proportion of long marbling flecks, calculated as the ratio between the sum of long-shaped areas (discriminated by a shape factor) and the total marbling fleck area. Distribution traits were 1) the distribution of marbling fleck number and 2) the distribution of marbling fleck areas. These latter 2 traits were calculated as coefficients of variation of the number or total marbling fleck area in 8 (LM) or 4 (semitendinosus muscle) parts of the muscle cross-section, respectively; therefore, lower values refer to a more even distribution.

Statistics

Data were analyzed by ANOVA using the GLM procedure of SAS Windows (Version 8, 1999; SAS Inst., Inc., Cary, NC). The factors considered were age and breed as well as their interaction. The used model was:

$$Y_{ijk} = \mu + B_i + A_j + BA_{ij} + e_{ijk},$$

where Yijk = independent variable; μ = overall mean; Bi = effect of breed i (i = 1 to 4); Aj = effect of slaughter age j (j = 1 to 5); BAij = effect of interaction; eijk = residual error.

The least squares means were compared by use of the PDIFF statement.

RESULTS

Fat Content and Marbling

Intramuscular fat content and marbling score (**Table 2**) serve as reference for data obtained by CIA. During growth, the i.m. fat content increased in German Angus, Galloway, and Holstein-Friesian (P < 0.03) breeds, whereas in double-muscled Belgian Blue bulls, the changes were not significant (P > 0.33). The increase became significant in Holstein-Friesian bulls at 12 mo of age (P < 0.01) and in German Angus and Galloway bulls at 24 mo of age (P < 0.01). Double-muscled Belgian Blue bulls had a lower (P < 0.05) i.m. fat content compared with German Angus and Holstein Friesian bulls from 12 mo of age onward and had the lowest (P < 0.01) i.m. fat content at 24 mo of age. The i.m. fat content at 24 mo of age was greater in Galloway bulls than in German Angus bulls (P = 0.004), but was similar in Holstein-Friesian bulls (P = 0.12).

Marbling scores increased (P < 0.03) during growth in all investigated breeds, except in double-muscled Belgian Blue bulls (P > 0.7). German Angus, Galloway, and Holstein-Friesian bulls received similar (P > 0.06) marbling scores, but greater (P < 0.02) scores than double-muscled Belgian Blue bulls from 12 mo of age onward.

Table 2. Fat content and marbling scores in LM of different cattle breeds1

		Slaughter age, mo				
Trait	**Breed**	**2**	**4**	**6**	**12**	**24**
Fat content,[3] %	German Angus	0.22^{a}	0.70^{a}	0.52^{a}	$1.34^{a,A}$	$4.24^{b,A}$
	Galloway	0.31^{a}	0.58^{a}	0.54^{a}	$1.26^{a,AB}$	$5.45^{b,B}$
	Holstein Friesian	0.18^{a}	0.21^{a}	0.72^{a}	$2.07^{b,A}$	$4.79^{c,AB}$
	Belgian Blue	0.14	0.14	0.16	0.27^{B}	0.63^{C}
Marbling[4]	German Angus	1.0^{a}	1.0^{a}	1.0^{a}	$1.6^{b,A}$	$2.8^{c,A}$
	Galloway	1.2^{ab}	1.0^{a}	1.0^{a}	$1.5^{b,A}$	$3.0^{c,A}$
	Holstein Friesian	1.0^{a}	1.0^{a}	1.0^{a}	$1.8^{b,A}$	$2.7^{c,A}$
	Belgian Blue	1	1	1	1.0^{B}	1.0^{B}

a–c: Within a trait and breed, means with different superscripts between age groups are significantly different (P < 0.05).

A–C: Within a trait and age group, means with different superscripts between breeds are significantly different (P < 0.05).

1: The sources of variation evaluated in the analysis were age group (P = 0.0001), breed (P = 0.0001), and their interactions (P = 0.0001). Values are least squares means.

3: Intramuscular fat content was obtained via the Soxhlet extraction method using petroleum ether as the solvent.

4 Marbling was scored with photographic standards using a 6-point scale, where 1 = extremely low marbling and 6 = extremely high marbling.

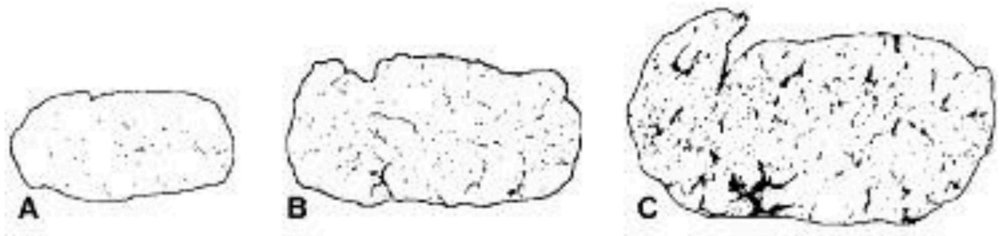

Figure 1. Binary images of 3 typical samples of longissimus muscle cross-sections, representing detected marbling flecks and borders of muscle slices. Samples were obtained from Galloway bulls at 4 (A), 12 (B), or 24 (C) mo of age. An increase in the number and size of marbling flecks during growth is clearly visible.

Image Analysis

Breed, age, and the interaction between breed and age influenced quantity, structure, and distribution of i.m. fat to various degrees (all P-values were < 0.001). The variances of the traits were explained by breed, age, and their interaction for about 80, 60, and 70% for quantity, structure, and distribution traits, respectively.

Quantity Traits.

Three samples of LM cross-sections are presented in **Figure 1** to illustrate the typical development of marbling flecks during growth. The samples were obtained at 4, 12, and 24 mo of age. In the binarized images of marbling, a definite increase in the number and size is clearly visible.

Binary images of 3 typical samples of longissimus muscle cross-sections, representing detected marbling flecks and borders of muscle slices. Samples were obtained from Galloway bulls at 4 (A), 12 (B), or 24 (C) mo of age. An increase in the number and size of marbling flecks during growth is clearly visible.

Table 3. Quantity traits of marbling in LM and semitendinosus muscle of different cattle breeds during growth[1]

		Slaughter age, mo				
Trait	**Breed**	**2**	**4**	**6**	**12**	**24**
LM						
Proportion of marbling fleck area, %	German Angus	1.17^{a}	$2.85^{ab,A}$	$1.59^{a,A}$	$3.22^{b,A}$	$6.08^{c,A}$
	Galloway	1.03^{a}	$1.75^{a,AB}$	$1.98^{a,A}$	$3.51^{b,A}$	$8.96^{c,B}$
	Holstein Friesian	0.70^{a}	$0.94^{a,BC}$	$2.65^{b,A}$	$3.92^{b,A}$	$8.08^{c,B}$
	Belgian Blue	0.08^{a}	$0.05^{a,C}$	$0.23^{a,B}$	$0.58^{a,B}$	$1.39^{b,C}$
Number of marbling flecks	German Angus	34^{a}	$107^{ab,A}$	$114^{ab,A}$	$167^{b,A}$	$427^{c,A}$
	Galloway	89^{a}	$112^{a,A}$	$136^{a,A}$	$287^{b,B}$	$615^{c,B}$
	Holstein Friesian	20^{a}	$43^{a,AB}$	$174^{b,A}$	$254^{c,B}$	$538^{d,C}$
	Belgian Blue	4^{a}	$7^{a,B}$	$13^{a,B}$	$56^{a,C}$	$170^{b,D}$
Size of marbling flecks, mm²	German Angus	1.05^{a}	$1.03^{a,A}$	$0.66^{b,A}$	$1.32^{ac,A}$	$1.59^{c,A}$
	Galloway	$0.36^{a,B}$	$0.63^{ab,B}$	$0.62^{ab,A}$	$0.82^{b,B}$	$1.31^{c,B}$
	Holstein Friesian	$0.75^{ab,AB}$	$0.69^{ab,AB}$	$0.57^{a,A}$	$0.99^{b,B}$	$1.32^{c,B}$
	Belgian Blue	$0.60^{a,B}$	$0.58^{a,B}$	$1.17^{b,B}$	$1.12^{b,AB}$	$1.14^{b,B}$
Semitendinosus						
Proportion of marbling fleck area, %	German Angus	$0.76^{a,AB}$	$1.72^{ab,A}$	$1.57^{a,A}$	$2.38^{b,A}$	$3.64^{c,A}$
	Galloway	$1.47^{ab,A}$	$1.73^{ab,A}$	$1.31^{a,A}$	$2.17^{b,A}$	$5.48^{c,B}$
	Holstein Friesian	$0.45^{a,B}$	$0.61^{a,B}$	$1.50^{b,A}$	$2.60^{c,A}$	$4.66^{d,C}$
	Belgian Blue	$0.08^{a,B}$	$0.04^{a,B}$	$0.16^{a,B}$	$0.74^{ab,B}$	$1.15^{b,D}$
Number of marbling flecks	German Angus	25^{a}	$83^{b,A}$	$55^{ab,A}$	$73^{b,AC}$	$130^{c,A}$
	Galloway	54^{a}	$62^{a,AB}$	$50^{a,AB}$	$114^{b,B}$	$242^{c,B}$
	Holstein Friesian	16^{a}	$25^{ab,BC}$	$59^{bc,A}$	$85^{c,AB}$	$166^{d,C}$
	Belgian Blue	5^{a}	$4^{a,C}$	$12^{a,B}$	$35^{ab,C}$	$70^{b,D}$
Size of marbling flecks, mm²	German Angus	0.72^{a}	0.75^{a}	1.03^{a}	$1.96^{b,AC}$	$3.05^{c,A}$
	Galloway	0.53^{a}	0.67^{a}	0.73^{a}	$0.87^{b,B}$	$2.03^{c,B}$
	Holstein Friesian	0.55^{a}	0.63^{a}	0.82^{a}	$1.53^{b,A}$	$2.46^{c,C}$
	Belgian Blue	0.45^{a}	0.43^{a}	0.57^{a}	$2.05^{b,C}$	$2.38^{b,BC}$

a–d: Within the same muscle, trait, and breed, means with different superscripts between age groups are significantly different ($P < 0.05$).

A–D: Within the same muscle, trait, and age group, means with different superscripts between breeds are significantly different ($P < 0.05$).

1: The sources of variation included in the analysis were age group ($P = 0.0001$), breed ($P = 0.0001$), and their interactions ($P = 0.0001$). Values are least squares means.

As shown in **Table 3**, the proportion of marbling fleck area and the number and size of marbling flecks increased ($P < 0.05$) in the muscle cross-sections of both muscles. This development is different among breeds. In Holstein-Friesian bulls, an intensive incorporation of fat begins at 6 mo of age ($P < 0.01$), and in German Angus and Galloway bulls, this incorporation begins at 12 mo of age ($P < 0.05$) in both muscles. In double-muscled Belgian Blue bulls, the proportion and number of marbling flecks were enlarged ($P < 0.01$) only at 24 mo of age. Double-muscled Belgian Blue bulls, in general, had a very low proportion and number of marbling flecks during growth. Galloway bulls showed the greatest ($P < 0.02$) number of marbling flecks at 24 mo of age compared with the other investigated breeds. Comparing the muscles, LM had a 1.5-fold greater proportion of marbling fleck area and a 2.5- to 4-fold greater number of marbling flecks than semitendinosus muscle.During growth from 2 to 24 mo of age, the proportion of marbling fleck area in LM increased 5-, 9-, 12-, and 17-fold, and in semitendinosus muscle, the proportion of marbling fleck area increased 5-, 4-, 10-, and 14-fold, in German Angus, Galloway, Holstein-Friesian, and double-muscled Belgian Blue bulls, respectively. The number of marbling flecks in LM increased 13-, 7-, 27-, and 43-fold, and in semitendinosus muscle, the number of marbling flecks increased 5-, 4-, 10-, and 14-fold, in German Angus, Galloway, Holstein-Friesian, and double-muscled Belgian Blue bulls, respectively.

Although the proportion of marbling fleck area and the number of marbling flecks intensely increased during growth, the size of marbling flecks (**Table 4)** increased only about 2-fold. The marbling flecks became larger ($P < 0.01$) in double-muscled Belgian Blue bulls at 6 mo of age in LM and at 12 mo of age in semitendinosus muscle; the size remained constant until 24 mo. In the other breeds, the size of marbling flecks was greater ($P < 0.05$) at 12 or 24 mo of age, respectively. Comparing the breeds at 24 mo of age, German Angus bulls had the largest ($P < 0.05$) marbling flecks in both muscles. A tendency for larger marbling fleck areas in LM can be observed in this breed at all investigated ages.

Structure Traits of Marbling Flecks.

The proportion of the 3 largest marbling fleck areas (**Table 4**) is great if the marbling is dominated by large marbling flecks. The proportion of the 3 largest marbling fleck areas in the LM decreased ($P < 0.01$) during growth, except in Galloway bulls ($P > 0.09$). Double-muscled Belgian Blue bulls showed greater ($P < 0.04$) values up to 6 mo of age compared with the other breeds. In semitendinosus muscle, the proportion of the 3 largest marbling fleck areas decreased ($P < 0.01$) in double-muscled Belgian Blue bulls. In the other breeds, the values tended to drop first, followed by an increase ($P < 0.05$) up to 24 mo.

The length of the maximum skeleton line (**Table 4**) increased ($P < 0.01$) markedly from 6- to 41-fold in both muscles in all breeds during growth, except in double-muscled Belgian Blue bulls (P > 0.14). Comparing the breeds, the length of the maximum skeleton line was smallest in double-muscled Belgian Blue bulls (P < 0.01) in both muscles at 24 mo of age. German Angus and Holstein-Friesian bulls did not differ (P > 0.25) in this trait. Galloway had the longest (P < 0.01) maximum skeleton lines at 24 mo of age in LM. In the semitendinosus muscle, there were no differences among German Angus, Galloway, and Holstein-Friesian bulls (P > 0.06) in this trait.

The proportion of the area of long marbling flecks in LM (**Table 4**) increased (P < 0.01) in Holstein-Friesian, Galloway, and double-muscled Belgian Blue bulls during growth. However, German Angus bulls showed no changes (P > 0.13) in this trait in LM. This trend of marbling flecks becoming longer with age was clearer in semitendinosus muscle. In all 4 breeds, the proportion of the area of long marbling flecks increased (P < 0.05) during growth. At 24 mo of age, up to 77% of the marbling fleck area was included in long marbling flecks, but only about 20% of the marbling flecks had a long shape. Comparing the breeds, double-muscled Belgian Blue bulls had the lowest (P < 0.05) proportion of long marbling flecks.

Distribution of Marbling Flecks.

A distinction was made between the distribution of fat localizations (distribution of marbling fleck number) and distribution considering the area and localization of marbling flecks (distribution of marbling fleck areas) in the muscle cross-section. The more regular the distribution, the lower were the values. During growth, the distribution of marbling flecks became more regular in all breeds. The values for the distribution of marbling fleck number (**Table 5**) decreased (P < 0.04) in the LM, approximating to an ideal distribution represented by the value 0. Differences among the breeds were greatest at 2 and 4 mo of age, than diminished during growth. At 24 mo of age, there were no differences among breeds. Galloway bulls had the lowest (P < 0.02) values for the distribution of marbling fleck number at 2 mo of age, whereas double-muscled Belgian Blue bulls had the greatest (P < 0.01) values at 2, 4, and 6 mo of age.

The values for the distribution of marbling fleck areas decreased (P < 0.01) during growth as well. The data indicate that the most regular distribution of fat in Galloway bulls already existed in young animals. In double-muscled Belgian Blue bulls, the distribution of marbling fleck areas was not as regular as in the other breeds and reflected in greater (P < 0.05) values, except at 12 mo of age.

Regarding the distribution of marbling flecks, there was no such clear improvement with age in semitendinosus muscle. During growth, the values for the distribution of marbling fleck number decreased (P < 0.05) in all 4 breeds. German Angus and Galloway bulls exhibited lower (P < 0.04) values for the distribution of marbling fleck number at 2 and 4 mo of age. The decrease in the distribution of marbling fleck areas was only significant in Holstein-Friesian bulls and in double-muscled Belgian Blue bulls (P < 0.01). Differences between the breeds coincided with the pattern that showed in LM. The values for the distribution of the marbling fleck areas were greater (P < 0.05) in double-muscled Belgian Blue bulls at almost all ages.

Development of i.m. Fat from an Anatomical Point of View.

In all investigated breeds and ages, >50% of the marbling flecks was located in the ventral or cranial half (i.e., bone oriented) of the LM

Table 4. Structure traits of marbling in LM and semitendinosus muscle of different cattle breeds during growth[1]

		Slaughter age, mo				
Trait	**Breed[2]**	**2**	**4**	**6**	**12**	**24**
LM						
Proportion of 3 largest marbling fleck areas, %	German Angus	$53.4^{a,AC}$	$32.9^{bc,AB}$	$33.7^{b,A}$	$36.7^{b,A}$	21.5^{c}
	Galloway	22.2^{B}	29.5^{A}	26.0^{A}	20.6^{B}	19.6
	Holstein Friesian	$55.6^{a,A}$	$42.3^{b,B}$	$26.5^{c,A}$	$29.2^{c,AB}$	18.7^{c}
	Belgian Blue	$71.2^{a,C}$	$70.3^{a,C}$	$74.2^{a,B}$	$28.7^{b,AB}$	28.7^{b}
Length of maximum skeleton line, mm	German Angus	1.74^{a}	2.77^{ab}	2.16^{a}	$6.01^{b,A}$	$9.75^{c,A}$
	Galloway	0.63^{a}	1.93^{a}	2.00^{a}	$3.22^{a,AB}$	$13.67^{b,B}$
	Holstein Friesian	0.76^{a}	1.24^{a}	2.20^{ab}	$4.78^{bc,AB}$	$7.99^{c,A}$
	Belgian Blue	0.28	0.25	1.04	1.40^{B}	3.01^{C}
Proportion of long marbling flecks, %	German Angus	64.7^{A}	58.0^{A}	60.2	68.3^{A}	69.0^{A}
	Galloway	$32.6^{a,BC}$	$52.0^{b,A}$	57.5^{bc}	$57.2^{bc,AB}$	$68.3^{c,A}$
	Holstein Friesian	$42.1^{a,B}$	$46.6^{ab,A}$	58.4^{bc}	$66.6^{c,A}$	$65.4^{c,AB}$
	Belgian Blue	$26.2^{a,C}$	$12.2^{b,B}$	64.0^{c}	$50.5^{c,B}$	$56.8^{c,B}$
Semitendinosus						
Proportion of 3 largest marbling fleck areas, %	German Angus	$40.0^{abc,A}$	$29.7^{a,A}$	$36.6^{ab,A}$	$47.8^{bc,A}$	$48.2^{c,A}$
	Galloway	$36.3^{ab,A}$	$37.3^{ab,A}$	$28.5^{a,A}$	$27.8^{a,B}$	$39.6^{b,AB}$
	Holstein Friesian	$46.2^{a,A}$	$36.6^{ab,A}$	$29.4^{b,A}$	$40.8^{a,A}$	$42.4^{a,AB}$
	Belgian Blue	$87.8^{a,B}$	$84.4^{ab,B}$	$71.3^{b,B}$	$39.8^{c,A}$	$33.4^{c,B}$
Length of maximum skeleton line, mm	German Angus	$0.90^{a,A}$	1.95^{a}	1.92^{a}	5.39^{a}	$14.13^{b,A}$
	Galloway	$1.08^{a,A}$	1.87^{a}	0.87^{a}	2.34^{a}	$17.66^{b,A}$
	Holstein Friesian	$0.40^{a,B}$	0.63^{ab}	1.26^{ab}	5.09^{b}	$16.37^{c,A}$
	Belgian Blue	0.21^{B}	0.15	0.37	1.72	2.89^{B}
Proportion of long marbling flecks, %	German Angus	$51.4^{a,A}$	$63.2^{ab,A}$	$62.6^{a,A}$	$76.2^{bc,A}$	$77.2^{c,A}$
	Galloway	$53.5^{a,A}$	$67.1^{ab,A}$	$55.6^{a,A}$	$54.6^{a,B}$	$69.0^{b,A}$
	Holstein Friesian	$26.5^{a,B}$	$42.1^{b,B}$	$52.4^{b,A}$	$75.9^{c,A}$	$74.6^{c,A}$
	Belgian Blue	$28.3^{a,B}$	$2.6^{b,C}$	$22.8^{a,B}$	$56.9^{c,B}$	$58.8^{c,B}$

a–c: Within the same muscle, trait, and breed, means with different superscripts between age groups are significantly different (P < 0.05).

A–C: Within the same muscle, trait, and age group, means with different superscripts between breeds are significantly different (P < 0.05).

1: The sources of variation included in the analysis were age group (P < 0.002), breed (P = 0.001), and their interactions (P < 0.01). Values are least squares means.

Table 5. Distribution traits of marbling in LM and semitendinosus muscle of different cattle breeds during growth[1]

		Slaughter age, mo				
Trait	**Breed**	**2**	**4**	**6**	**12**	**24**
LM						
Distribution of marbling fleck number, %	German Angus	$16.3^{a,A}$	$6.7^{b,A}$	$5.6^{b,A}$	$3.4^{b,AB}$	3.2^{b}
	Galloway	$8.1^{a,B}$	$6.7^{a,A}$	$5.2^{ab,A}$	$3.5^{ab,A}$	2.6^{b}
	Holstein Friesian	$21.7^{a,A}$	$12.5^{b,B}$	$4.9^{c,A}$	$4.0^{c,AB}$	2.5^{c}
	Belgian Blue	$29.4^{a,C}$	$23.1^{b,C}$	$19.9^{b,B}$	$7.8^{c,B}$	5.4^{c}
Distribution of marbling fleck areas, %	German Angus	$26.7^{a,A}$	$15.2^{b,A}$	$10.7^{b,A}$	$10.5^{b,AB}$	$5.7^{c,A}$
	Galloway	$9.6^{ab,B}$	$11.6^{b,A}$	$8.4^{ab,A}$	$6.2^{a,A}$	$4.5^{a,A}$
	Holstein Friesian	$26.9^{a,A}$	$21.0^{b,B}$	$8.6^{c,A}$	$8.7^{c,AB}$	$5.6^{c,A}$
	Belgian Blue	$34.7^{a,C}$	$27.1^{b,C}$	$24.5^{b,B}$	$11.5^{c,B}$	$9.9^{c,B}$
Semitendinosus						
Distribution of marbling fleck number, %	German Angus	$17.1^{a,A}$	$12.0^{ab,A}$	$15.5^{a,A}$	$11.1^{ab,A}$	$10.2^{b,A}$
	Galloway	$14.8^{ab,A}$	$14.2^{ab,A}$	$15.8^{a,A}$	$9.7^{b,A}$	$8.9^{b,A}$
	Holstein Friesian	$25.3^{a,B}$	$20.5^{a,B}$	$12.6^{b,A}$	$10.8^{b,A}$	$9.3^{b,A}$
	Belgian Blue	$41.9^{a,C}$	$41.5^{a,C}$	$34.2^{b,B}$	$18.7^{c,B}$	$15.5^{c,B}$
Distribution of marbling fleck areas, %	German Angus	21.8^{A}	17.3^{A}	21.6^{A}	20.3^{A}	20.2
	Galloway	21.0^{A}	22.2^{AB}	19.7^{A}	17.1^{A}	18.2
	Holstein Friesian	$30.0^{a,B}$	$24.5^{ab,B}$	$19.3^{bc,A}$	$19.8^{bc,A}$	17.6^{c}
	Belgian Blue	$48.2^{a,C}$	$43.0^{ab,C}$	$37.8^{b,B}$	$27.2^{c,B}$	20.5^{d}

a–d: Within the same muscle, trait, and breed, means with different superscripts between age groups are significantly different (P < 0.05).

A–C: Within the same muscle, trait, and age group, means with different superscripts between breeds are significantly different (P < 0.05).

1: The sources of variation included in the analysis were age group (P = 0.0001), breed (P = 0.0001), and their interactions (P = 0.0001). Values are least squares means.

or semi-tendinosus muscle, respectively (**Table 6**). At 2 mo of age in German Angus bulls, 87% (and in Holstein-Friesian bulls, 80%) of marbling flecks was ventrally located. During growth, the differences between the ventral and dorsal half of muscle decreased in these 2 breeds ($P < 0.01$). An indication for a ventral-to-dorsal gradient is also that the largest marbling fleck area was ventrally located in 88, 76, 88, 67% of the samples of German Angus, Galloway, Holstein-Friesian, double-muscled Belgian Blue bulls, respectively. Differences between the ventral and dorsal half were smaller in Galloway bulls, which was already evident in young animals. No reliable results were obtained for double-muscled Belgian Blue bulls because of the low number of marbling flecks, which appeared in varying positions.

The proportion of marbling fleck area in the cranial half of semitendinosus muscle (**Table 6)** decreased ($P < 0.01$) during growth in Holstein-Friesian and double-muscled Belgian Blue bulls, but remained unchanged in German Angus and Galloway bulls ($P > 0.4$). Breed and the interaction between breed and age were not significant for the variance in the proportion of marbling fleck area in the cranial half ($P = 0.33$ and 0.19, respectively). At 24 mo of age, about 70% of marbling fleck area is located in the cranial half of the semitendinosus muscle in all breeds. This gradient between cranial- and caudal-located marbling flecks in semitendinosus muscle is also reflected by the localization of the largest marbling flecks. The largest marbling flecks were cranially located in 89, 93, 96, 86% of the samples of German Angus, Galloway, Holstein-Friesian, double-muscled Belgian Blue bulls, respectively. Especially in double-muscled Belgian Blue bulls, where almost no fat was visible, single marbling flecks appeared first in the cranial half of the muscle cross-section.

Table 6. Proportion of marbling fleck area located ventral in LM and cranial in semitendinosus muscle of different cattle breeds during growth[1]

		Slaughter age, mo				
Trait	**Breed**	**2**	**4**	**6**	**12**	**24**
LM						
Proportion of marbling fleck area, %	German Angus	$86.9^{a,A}$	$61.6^{bc,AB}$	$71.9^{ab,A}$	$75.0^{ab,A}$	56.4^{c}
	Galloway	55.1^{B}	59.3^{AB}	65.8^{A}	62.5^{B}	56.5
	Holstein Friesian	$79.5^{a,A}$	$71.8^{ab,A}$	$63.2^{bc,A}$	$67.9^{abc,AB}$	57.1^{c}
	Belgian Blue	$29.4^{a,C}$	$57.7^{bd,B}$	$93.2^{c,B}$	$63.1^{ab,AB}$	51.5^{d}
Semitendinosus						
Proportion of marbling fleck area, %	German Angus	75.4^{A}	66.1	77.8	69.2	70.9
	Galloway	76.4^{A}	79.5	71.5	68.7	68.5
	Holstein Friesian	$92.5^{a,AB}$	71.6^{b}	71.8^{b}	75.3^{b}	68.3^{b}
	Belgian Blue	$99.6^{a,B}$	74.2^{bc}	61.3^{c}	83.6^{ab}	74.6^{bc}

a–d: Within the same muscle, trait, and breed, means with different superscripts between age groups are significantly different ($P < 0.05$).

A–C: Within the same muscle, trait, and age group, means with different superscripts between breeds are significantly different ($P < 0.05$).

1: The sources of variation included in the analysis were age group ($P = 0.0001$, $P = 0.325$), breed ($P = 0.004$, $P = 0.011$), and their interactions ($P = 0.0001$, $P = 0.193$) for LM and semitendinosus, respectively. Values are least squares means.

DISCUSSION

Studies about the relationship of marbling to meat quality often refer to subjective marbling score and eating quality, assessed as tenderness, juiciness, and flavor (Wheeler et al., 1994; Thompson, 2004). Whereas the influence of marbling on tenderness is rather low and variable (Dikeman, 1996), marbling has a greater influence on juiciness and flavor (Millar, 1994). Therefore, marbling is an accepted indicator for meat quality and is assessed in abattoirs by meat graders. Previously, Moody and Cassens (1968) provided a quantitative and morphological analysis of i.m. fat deposition in cattle LM. Considerable effort has been placed on developing methods to increase the objectivity and reproducibility of marbling assessment. Computerized image analysis is already established for the prediction of i.m. fat content or marbling score of beef (Jones et al., 1995; Shackelford et al., 1998; Kuchida et al., 2000). In a review of objective measurement technologies for evaluation of marbling, Ferguson (2004) stated that the total amount of fat, size, and distribution of the i.m. fat depots are relevant, and CIA is the preferred technique to obtain these data. Previously, Dufey (1989) used simple techniques to determine distribution and fineness of fat structures in muscle. Marbling traits measured by CIA show a much more differentiated picture than subjectively graded marbling in beef (Mc Donald and Chen, 1990; Albrecht et al., 1996) and pork (Faucitano et al., 2005). Gerrard et al. (1996) measured the size of marbling flecks by CIA and partitioned marbling flecks into 3 size classes. The number of small marbling flecks was greater. However, the summarized fat area from large marbling flecks was greater. Furthermore, they concluded that the marbling scoring is overestimated by the large marbling flecks. In the current study, we also observed that marbling is overestimated if larger fat streaks appear. Conversely, marbling is underestimated if only a few very small marbling flecks appear. However, the accuracy of the chemical extraction methods is also limited because the extracted fat also contains phospholipids of membranes and lipids in muscle fibers. No such limitations exist with the objective measurement of stained muscle slices. Even a small, single marbling fleck, consisting of a few adipocytes, provides a result different from zero. Therefore, the data from CIA are more evident and useful to compare breeds and changes during growth.

Studies about the development of marbling are mostly restricted to LM and to the finishing phase. May et al. (1992) and Van Koevering et al. (1995) regressed marbling scores against days on feed and showed that marbling developed in a quadratic fashion before reaching a plateau, when animals attained their genetic potential to deposit i.m. fat. Brethour (2000) showed that, in his experiments, during a 180-d feeding period, animals with high marbling when slaughtered had greater initial values and also had a faster rate of increase of marbling. In agreement, Bruns et al. (2004) concluded that fat in LM is not necessarily a late-developing tissue. Regressed as a component of growth over HCW, they established that i.m. fat increases consistently during growth. In a recent study, Harper and Pethick (2004) suggested that producers should focus on initiation of more preadipocytes in muscle of animals that have the genetic disposition to marble. During finishing, these preadipocytes differentiate and are filled with lipids to become visible marbling. Furthermore, Smith et al. (2000) stated that the postnatal growth of i.m. fat involves substantial hypertrophy of adipocytes and also a period of hyperplasia of preadipocytes. Our results on the number of marbling flecks suggest that hyperplasia plays an important role in marbling. New marbling flecks appear only by recruiting and filling of preadipocytes.

From the results, it can be concluded that there were 2 different tendencies during growth. First, the appearance of larger fat streaks, long-shaped and branched, dominates the images. Second, newly developing marbling flecks, small and round-shaped, provide a positive counterpart. Decreasing values of the mean size of marbling flecks and the proportion of the 3 largest marbling fleck areas reflect the influence of those new small marbling flecks. They consist of adipocytes located around small blood vessels. When marbling flecks grow, adipocytes develop along connective tissue between muscle fiber bundles. The shape becomes inevitably long and often branched. This is reflected by greater values for the length of maximum skeleton line and the number of their branching points.

By microscopic studies of mature Japanese cattle, Hoshino et al. (1990) established that dotted fat is intrafascicular fat, and retiform fat is interfascicular fat. In the current study, intrafascicular fat was found after 6 mo of age. Similarly interfascicular fat begins as dotted marbling flecks and changes shape to longer, branched streaks during growth.

The influence of breed on the quantity of stored lipids has been described by many researchers (Marshall, 1994; Zembayashi and Lunt, 1995; Chambaz et al., 2002). Previously, Boccard (1981) described a very low i.m. fat content in double-muscled cattle. Data of CIA in our study confirmed this finding for double-muscled Belgian Blue bulls. Whereas in German Angus, Galloway, and Holstein-Friesian bulls at 24 mo of age, the relatively great amount of fat led to fusion of single marbling flecks to larger fat streaks in both muscles, in double-muscled Belgian Blue bulls, there were no such large, branched fat streaks. Gregory et al. (1994) described an early incorporation of i.m. fat in German Angus in comparison with 8 other beef breeds. In the current study, German Angus bulls exhibited larger marbling flecks already in young animals, but the number of marbling flecks was greater in Galloway and Holstein-Friesian bulls. In summary, for Galloway bulls, the marbling of the LM is characterized by a great number of regularly distributed marbling flecks, indicating a great potential for marbling. Marbling in the Holstein-Friesian bull is characterized by a great number of marbling flecks with a fine structure, without large, branched fat streaks.

In comparison with already published data about fat content in semitendinosus muscle (Wegner et al., 2000), the results of the current study show a greater fat content and, therefore, a greater proportion of marbling fleck area in LM. According to Nishimura et al. (1999), the increase in fat content is lower in semitendinosus muscle during growth. Smaller marbling flecks are developed in LM (i.e., a greater number of small marbling flecks are developed), which leads to a lower mean size. The structure of marbling fat is finer, and the distribution of marbling flecks over the muscle cross-section is more regular. The development of fat depots is similar in both muscles, beginning in the deeper layers of the muscle, near large blood vessels, where nutrients are better provided. Hoshino et al. (1990) described a connection between the size of arterioles and the size of fat depots. In the semitendinosus muscle of the investigated breeds in the current study, often large blood vessels were surrounded by fat and located near the branching points of the large connective tissue streak. In older animals, these marbling flecks fused to one large streak. Additional marbling flecks appeared far away from large blood vessels in the center of tertiary muscle fiber bundles. However, in double-muscled Belgian Blue bulls, not all visible blood vessels were surrounded by fat, and no fat was located within muscle fiber tertiary bundles.

In this study, the gradient from deeper to outer layers of muscle decreased with age. Whereas in Galloway bulls the distribution of marbling flecks was relatively regular, in the other breeds, the distribution became more regular by new developing marbling flecks in older animals.

In conclusion, data of CIA not only reflect the subjectively assessed marbling score in an objective matter, moreover this technique fills a gap between macroscopical appearance of marbling and microscopically visible adipocytes. The presented results show, for the first time with CIA, how marbling develops in different breeds of cattle from 2 to 24 mo of age in respect to quantity, structure, and distribution of marbling flecks.

IMPLICATIONS

Intramuscular fat storage begins early in the life of cattle. The development of marbling flecks differs between breeds and muscles in amount, structure, and distribution. Computerized image analysis of stained muscle slices makes it possible to pay more attention to small, regularly distributed marbling flecks, which are not visible in fresh meat. These small marbling flecks provide a contribution to meat quality even at low intramuscular fat content. Further investigations should combine morphological studies with cellular studies to verify whether small marbling flecks are the regions of intramuscular adipogenesis. Such studies contribute to a better understanding of intramuscular fat deposition to produce good marbled meat without increasing the overall amount of fat in the animal.

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