

Computer image analysis of intramuscular adipocytes and marbling in the longissimus muscle of cattle



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Introduction

The objective of the present study was to investigate the i.m. adipocyte and marbling traits, detected by computer image analysis, and the relationships among i.m. adipocyte size, marbling traits, and i.m. fat content. The study will contribute to a better understanding of fat deposition in muscle on a cellular level.

Materials and Methods

Animals:

Fifty one cattle of F2 generation of German Holstein and Charolais crossbreeds were used.

Samples:

After slaughter, chilling at 6°C for 24 h, and carcass dressing, LM samples were removed from the left side of the carcass at the 12th rib area. The i.m. fat content was obtained in triplicate via the Soxhlet extraction method.

Computer image analysis (CIA):

Muscle slices, 2 cm thick, were fixed in 5% formaldehyde, cut into smaller slices (2 mm), and stained with oil red O, as described in detail by Albrecht et al. (1996). Marbling traits were recorded according to Faucitano et al. (2005).

Adipocyte area determination:

Pieces of the muscle slice, containing a single marbling fleck, were cut with a cryostat microtome (CM 3050 S, Leica, Germany) and stained with eosin. The microscopic pictures of adipocytes were analyzed by AMBA software (IBSB, Germany).

Statistical analysis:

Data were analyzed using the GLM procedure of SPSS. Relationships among traits were analyzed as Pearson's correlation coefficients by SPSS. Statistical significance was defined as $P < 0.05$.

Conclusions

The i.m. fat is preferentially deposited in the ventral area of LM. Many marbling flecks seem to grow from the ventral part of the muscle cross section and spread out to dorsal direction in a treelike shape. Although the i.m. adipocyte size has an important effect on the traits of marbling flecks, hyperplasia plays a greater role in i.m. fat deposition than hypertrophy.

References

- Albrecht, E., J. Wegner, and K. Ender. 1996. *Fleischwirtschaft* 76:1145-1148.
 Faucitano, L., P. Huff, F. Teuscher, C. Garipey, and J. Wegner. 2005. *Meat Sci.* 69:537-543.

Results

Intramuscular fat is stored in islets of cells (Fig. 1) macroscopically visible as marbling flecks (Fig. 2).



Figure 1: Microscopic image of a marbling fleck, stained with eosin

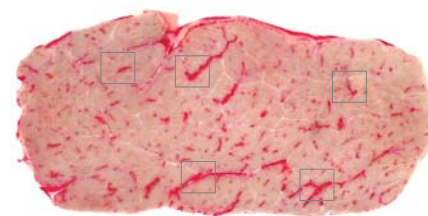


Figure 2: Longissimus muscle cross section with red stained marbling flecks

Muscle and fat traits for F2 cattle (Tab. 1) are in-between the traits of German Holstein and Charolais breeds (unpublished data) which were used to generate the F2 population.

Table 1: Longissimus muscle and fat traits of F2 bulls at 18 mo of age

Trait	Mean	Min	Max
LM cross section area, cm ²	101.9	70.5	130.8
Intramuscular fat content, %	2.5	0.8	5.4
Intramuscular adipocyte area, μm ²	4335	2358	6141
Number of marbling flecks	247	104	493
Proportion of marbling fleck areas, %	6.3	2.0	12.0
Proportion of three largest marbling fleck areas, %	34.6	13.7	70.2
Total length of marbling flecks, cm	51.9	17.7	98.7

Table 2: Correlation coefficients among i.m. adipocytes and marbling traits in F2 bulls at 18 mo of age

	i.m. fat content	Intramuscular adipocyte area
Intramuscular adipocyte area	0.71	
Number of marbling flecks	0.58	0.44
Proportion of marbling fleck areas	0.70	0.62
Proportion of three largest marbling fleck areas	0.11	0.09
Total length of marbling flecks	0.64	0.55

The i.m. adipocyte area was moderately correlated with the i.m. fat content, number of marbling flecks, proportion of marbling fleck area, and total length of marbling flecks (Tab. 2). Although the large marbling flecks always considerably influence the marbling score, the proportion of the three largest marbling flecks was not correlated with the i.m. fat content.

Adipocytes in different marbling fleck groups (different location in the muscle or different size) were measured (Tab. 3). The ventral marbling flecks had a 5-fold larger fleck area, 4-fold more adipocytes, and also larger adipocytes. Small marbling flecks contained smaller adipocytes than middle, and middle marbling flecks contained smaller adipocytes than large marbling flecks. Compared with the small marbling flecks, there were a 48-fold larger fleck area and 26-fold more adipocytes in the large marbling flecks, but only 1.5-fold larger adipocyte area.

Table 3. Adipocyte traits in different marbling fleck groups of F2 bulls at 18 mo of age

	Marbling fleck group	Area of marbling flecks, mm ²	Intramuscular adipocyte area, μm ²	Apparent adipocyte number in marbling flecks ¹	P-value for adipocyte area
Part	Dorsal	6.27	3,967	1,428	< 0.001
	Ventral	31.87	4,823	6,364	
Size of marbling flecks ²	Small	1.12	3,398	341	< 0.001
	Middle	7.33	4,273	1,783	
	Large	44.91	5,096	8,774	

¹The apparent adipocyte number in marbling flecks was calculated from the area of marbling flecks and the adipocyte area.

²For the different size, marbling flecks were divided into three groups (small < 2.5 mm², middle 2.5-15 mm², large > 15 mm²). Each group contained at least 100 marbling flecks.