Distribution of Fatty Acids in the *Longissimus* Muscle of Holstein Steers during the Fattening Period

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Abstract In order to study the distribution of and the changes in composition of fatty acids in the *longissimus* muscle, seven Holstein steers were fattened for 100-day (3 steers) and 200-day (4 steers) periods after being grazed. Samples of each *longissimus* muscle of the 4th, 6th, 8th, 10th and 12th thoracic vertebrae sections and of the 1st, 3rd and 5th lumbar vertebrae sections were taken for determining fatty acid composition of total lipids. There were no statistical differences in the fatty acid composition of the *longissimus* muscle sections between the 100-day and 200-day fattened steers. However, C18:0 was observed to decrease slightly and C18:1 to increase slightly during the second 100-day fattening period. The portions of *longissimus* muscle from the 4th, 6th, 8th and 10th thoracic vertebrae sections were found to have more C16:0 and C18:0 and less C16:1, C18:1 and total unsaturated fatty acid than the portions from the 12th thoracic vertebrae and from the 1st, 3rd and 5th lumbar vertebrae sections. This difference in fatty acid distribution may be related to differences in growth rate within the *longissimus* muscle.

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A number of studies have been conducted regarding the relationships in fatty acids composition within various muscle during growth periods¹⁻⁸). During the growth and fattening of cattle the amounts of intramuscular lipids increase markedly⁷). This increase is predominately in neutral lipids, especially the triglyceride portions, while the relative amounts of phospholipids remain nearly constant^{8,8,9}).

Physiological, biochemical and histological properties of individual muscles are known to vary greatly. These characteristics depend on the fatty acid composition of the muscle. The difference in fatty acid composition in muscle effects the taste of the meat¹⁰). The fatty acid composition has been found to differentiate by age^{3,4}), season⁹), sex^{2,6}), diet⁴⁻⁶) and by the differences between various muscles⁹⁻¹²) and within the muscle itself¹³).

In our previous report¹⁵), the differences in the fat content of *longissimus* muscle sections were found to be related to differences in their growth rate. Distribution of fatty acids in *longissimus* muscle and changes in fatty acids during the fattening period were examined in the study reported by this paper.

Materials and Methods

The seven Holstein steers used in this experiment are described in our previous report^{1b}. These animals were fattened for 100-day (3 steers) and for 200-day (4 steers) periods after being grazed and then slaughtered. Each *longissimus* muscle sample, after being taken from the carcasses, was cut into thoracic and lumbar vertebrae sections. The 4th, 6th, 8th, 10th and 12th thoracic vertebrae sections and the 1st, 3rd and 5th lumbar vertebrae sections were used for the determination of fatty acid composition after dehydration. Total lipid extraction was performed by the general method, utilizing chloroform and methanol solvents (v/v 2:1). After extraction, excess solvent was evaporated off under a stream of nitrogen. Esterification of fatty acids was carried out as follows. Extracted fat was methylated by HCl-anhydrous methanol in screw cap tubes and heated in boiling water for 3 hours.

The methylated fatty acids were then extracted by hexane and washed in a 2% KHCO₃ solution. The methyl esters were recovered by extraction of the residue remaining after evaporation of the solvents.

The methylated samples were injected into a Shimadzu GC-6A gas chromatograph equipped with a flame ionization detector and a chromatopac. A 250 cm glass column was packed with 5% diethylene glycol succinate on a 80/100 mesh chromosorb W support system. The column temperature was 190°C, both detector and injection temperatures were 230°C. A nitrogen flow rate of 60 ml/min was maintained. Individual fatty acid peaks were identified by comparison of retention times with those of known mixtures of standard fatty acids run under the same operating conditions.

Although as many as 17 peaks were present in the chromatograms, only 11 fatty acids were selected to test the experimental variables. They were myristic (C 14:0), myristoleic (C 14:1), pentadecanoic (C 15:0), palmitic (C 16:0), palmitoleic (C 16:1), margaric (C 17:0), heptadecanoic (C 17:1), stearic (C 18:0), oleic (C 18:1), linoleic (C 18:2) and linolenic (C 18:3) acids. Relative fatty acid values were calculated by the chromatopac equipment. All fatty acids were expressed as percentages of the total eleven fatty acids evaluated.

Results and Discussion

1. Changes in fatty acid composition in the *longissimus* muscle during fattening.

Significant differences of fatty acid composition were not found in the *longissimus* muscle between the 100-day and 200-day fattened steers (Tables 1 and 2). Sum of the major fatty acids (C 16:0, C 16:1, C 18:0 and C 18:1) was observed to be approximately 92% in total fatty acids, showing the same trend as the results reported by many workers^{1,5,9,10,12}. In the four fatty acids, however, C 18:0 was observed to decrease slightly and C 18:1 to increase slightly during the second fattening period. It is well-known that the fatty acid composition of muscle is influenced by age^{3,4}, nutrition⁴⁻⁶, exercise¹¹, sex^{2,6}, season⁹ and is different among various muscles⁹⁻¹² and the location of the sample portion¹³.

Distribution Fatty Acids in *Longissimus* Muscle

Table 1. The means of fatty acid contents in *longissimus* muscle sections of cattle finished on 100-day fattening periods after grazing (n=3)

	Thoracic vertebrae					Lumbar vertebrae			All sections	
	4 th	6 th	8 th	10 th	12 th	1 st	3 rd	5 th	Mean	S D
C14:0	2.4	2.3	2.2	2.2	2.2	2.1	2.2	2.2	2.2 ± 0.1	
C14:1	0.6	0.6	0.6	0.6	0.7	0.7	0.8	0.8	0.7 ± 0.1	
C15:0	0.8	0.6	0.9	1.1	0.8	0.9	0.8	0.9	0.9 ± 0.1	
C16:0	28.2	27.7	27.7	27.7	26.5	26.9	27.1	26.1	27.2 ± 0.7	
C16:1	3.9	3.7	3.7	4.0	4.5	4.5	4.4	4.9	4.2 ± 0.4	
C17:0	0.7	0.8	0.7	0.7	0.9	0.7	0.6	1.0	0.8 ± 0.1	
C17:1	0.5	0.6	0.6	0.8	0.7	0.7	0.6	1.0	0.7 ± 0.2	
C18:0	19.2	20.4	20.1	18.6	17.7	17.2	16.9	16.1	18.3 ± 1.5	
C18:1	41.0	40.7	40.6	40.8	42.5	43.0	42.8	43.2	41.8 ± 1.1	
C18:2	2.7	2.5	2.8	3.4	2.9	3.1	3.2	3.3	3.0 ± 0.3	
C18:3	0.2	0.2	0.2	0.3	0.6	0.2	0.5	0.5	0.3 ± 0.2	
Total saturated	51.1	51.7	51.5	50.2	48.1	47.8	47.6	46.2	49.3 ± 2.0	
Total unsaturated	48.9	48.3	48.5	49.8	51.9	52.2	52.4	53.8	50.7 ± 2.0	

Fatty acid contents are expressed as a percent of the total.

Table 2. The means of fatty acid contents in *longissimus* muscle sections of cattle finished on 200-day fattening periods after grazing (n=4)

	Thoracic vertebrae					Lumbar vertebrae			All sections	
	4 th	6 th	8 th	10 th	12 th	1 st	3 rd	5 th	Mean	S D
C14:0	2.6	2.6	2.6	2.7	2.9	2.6	2.6	2.6	2.6 ± 0.1	
C14:1	0.9	0.8	0.9	0.8	1.0	1.1	1.1	1.0	1.0 ± 0.1	
C15:0	0.4	0.3	0.5	0.5	0.5	0.5	0.5	0.5	0.5 ± 0.1	
C16:0	27.9	27.9	28.0	28.6	28.1	27.5	27.0	26.9	27.7 ± 0.5	
C16:1	4.1	4.1	4.1	4.1	4.7	4.9	4.9	4.9	4.5 ± 0.4	
C17:0	1.1	1.1	1.0	0.8	0.8	0.9	1.0	0.9	1.0 ± 0.1	
C17:1	0.7	0.7	0.7	0.6	0.8	0.7	0.7	0.8	0.7 ± 0.1	
C18:0	17.8	18.4	18.1	17.9	16.0	15.3	15.4	15.3	16.8 ± 1.3	
C18:1	42.3	41.6	42.0	41.9	43.4	44.3	44.7	45.0	43.2 ± 1.3	
C18:2	2.0	2.3	2.0	2.0	1.9	2.3	2.0	2.2	2.1 ± 0.2	
C18:3	0.4	0.4	0.4	0.5	0.0	0.2	0.7	0.1	0.3 ± 0.2	
Total saturated	49.7	50.3	50.2	50.4	48.3	46.7	46.5	46.0	48.5 ± 1.8	
Total unsaturated	50.3	49.7	49.8	49.6	51.7	53.3	53.5	54.0	51.5 ± 1.8	

Fatty acid contents are expressed as a percent of the total.

Most workers reported that C18:1 increased with increasing age and fattening^{1,2,4,6,11}). While, some workers reported that it decreased with increasing age^{3,14}). So there is obviously a difference in the results obtained by different workers. As to age and season, the steers were slaughtered at different chronological ages and different seasons, that is, the 100-day fattened steers were slaughtered in the summer and the

200-day fattened steers in the autumn. LINK *et al.*³⁾ reported that differences in fatty acid composition during growth were related to the season of the year rather than to the chronological age of the animals and that the total amount of saturated fatty acids was highest during the spring and early summer. This observation is in agreement with our results. So we concluded that age and season contribute to fatty acid composition in muscle.

As to the feed, in the present trials comparisons were made using the same breed (Holstein) and fed almost the same feed, mainly concentrates, until finishing. The fatty acid composition in the muscle was similar in both groups of steers. There was almost the same fatty acid composition of the *longissimus* muscles in this study and in those reported by OTAKE *et al.*¹²⁾, in spite of the differences in the type of cattle. In making a comparison of this study and their results, it is seen that the management of the animals was almost the same from rearing to finishing since they were both managed at the Grassland Research Institute.

Furthermore, DAVID *et al.*⁶⁾ showed that the muscle of grass-fed animals contained more saturated fatty acids and less unsaturated fatty acids. From this suggestion, the feed may be a main factor influencing fatty acid composition, especially concentrates may cause an increase in the proportion of C 18:1 in muscle during fattening. However, the slight change of fatty acid composition during the second fattening period is not thereby clearly explained.

2. The distribution of fatty acids within *longissimus* muscle.

During the fattening period studied, the relative proportion of fatty acids showed only small changes among the *longissimus* muscle sections. As the averages of all seven steers for each section, the main fatty acid compositions are shown in Fig. 1. There were some tendencies of fatty acid distribution in the *longissimus* muscle. The portions of *longissimus* muscle in the 4th, 6th, 8th and 10th thoracic vertebrae sections have more C 16:0 and C 18:0 and less C 16:1, C 18:1 and total unsaturated fatty acids than the portions in the 12th thoracic and 1st, 3rd and 5th lumbar vertebrae as shown in Fig. 1.

As to *longissimus* muscle growth, the portions in the 12th thoracic and the 1st, 3rd and 5th lumbar vertebrae showed greater growth rates than the portions in the 4th, 6th and 8th thoracic vertebrae sections during the second fattening period as reported in our previous paper¹⁸⁾.

The *longissimus* muscle consists of two parts, *longissimus thoracic* and *lumbar*, which can not be divided into two clear portion. Considered from the standpoint of fatty acid distribution and muscle growth, the two portions have slightly different muscle characteristics and may have a different metabolic activity as reported by ALLEN *et al.*¹⁶⁾. Differences in fatty acids in the two portions of the muscle may be related to the muscle growth rate. So the muscle growth may be related to the fatty acid composition and the difference of fatty acids composition in muscle may be explained on the basis of a greater degree of metabolic activity in the muscle and its subsequent utilization of lipid as reported by TERREL and BRAY⁹⁾.

Distribution Fatty Acids in *Longissimus* Muscle

Fig. 1. Distribution of C16:0, C16:1, C18:0, C18:1 and total unsaturated fatty acids in the *longissimus* muscle of fattened steers (n=7). Each fatty acid contents is expressed as a percent of the total and is combined for the two groups of steers. Each vertical bar indicates standard deviation.

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肥育期間中におけるホルスタイン種去勢牛の 最長筋肉内の脂肪酸分布について

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肥育期間中における最長筋肉の脂肪酸組成の変化および分布について知るために，放牧後 100 日間（3 頭）および 200 日間（4 頭）肥育したホルスタイン種去勢牛 7 頭を供試した。各々の最長筋肉から，（第 4, 6, 8, 10 および 12 胸椎と第 1, 3 および第 5 腰椎）全脂質を抽出し，その脂肪酸組成について調べた。100 日間と 200 日間肥育した牛の筋肉内の脂肪酸組成は統計的になら相違は見られなかった。しかしながら，肥育期間中に C18:0

は若干減少し，C18:1 は若干増加の様子が観察された。第 4, 6, 8 および 10 胸椎の最長筋は第 12 胸椎と第 1, 3 および腰椎のそれと比較すると，C16:0 と C18:0 が多く，C16:1, C18:1 および全不飽和脂肪酸含量が少ないことが見いだされた。この脂肪酸組成の相違は最長筋肉内における成長速度の相違に関連があるものと推定される。

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