

## Fatty Acid Profiles of Various Muscles and Adipose Tissues from Fattening Horses in Comparison with Beef Cattle and Pigs

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**ABSTRACT :** The present studies were designed to provide new information on fatty acid profiles of various muscles and adipose tissues of fattening horses in comparison with beef cattle and pigs. In the first study, the lipids were extracted respectively from subcutaneous, intermuscular adipose tissues, longissimus dorsi and biceps femoris muscles of fattening Breton horses (n = 8) with an average body weight of 1,124 kg. In the second study, the lipids were extracted from subcutaneous, intermuscular adipose tissues and longissimus dorsi muscle of fattening horses (n = 13), Japanese Black beef cattle (n = 5), Holstein steers (n = 5) and fattening pigs (n = 5). The fatty acids in the lipid samples were determined by gas chromatography after methylation by a combined base/acid methylation method. It was found that the lipids from horse subcutaneous and intermuscular adipose tissues contained more (p<0.05) polyunsaturated fatty acids (PUFA) which were mainly composed of linoleic acid (C18:2) and linolenic acid (C18:3) than those in the muscles. The weight percent of conjugated linoleic acids (CLA cis 9, trans 11) in lipids from biceps femoris muscle was 0.22%, which was higher (p<0.05) than that from the other depots. The horse lipids were higher (p<0.05) in PUFA but lower (p<0.05) in SFA and MUFA in comparison with those of the cattle and pigs. The percentage of C18:2 or C18:3 fatty acid in the horse lipids were respectively 2-8 fold or 5-18 fold higher (p<0.05) than those of the cattle and pigs. The percentages of CLA (cis 9, trans 11) in the horse lipids (0.14-0.16%) were very close to those of the pigs (0.18-0.19%) but much lower (p<0.05) than those of the Japanese Black beef cattle (0.55-0.94%) and Holstein steers (0.46-0.71%). The results indicated that the fatty acid profiles of lipids from different muscle and adipose tissues of fattening horses differed significantly. In comparison with that of the beef cattle and pigs, the horse lipids contained more C18:2 and C18:3 but less CLA. (*Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 11 : 1655-1661*)

**Key Words :** Horse, Cattle, Pig, Muscle, Adipose Tissue, Fatty Acids, Conjugated Linoleic Acid

### INTRODUCTION

Fatty acid composition of animal muscle or adipose tissues is affected by the factors such as species, breed, sex, anatomical location, diet and feeding regimen (Rule et al., 1995; Pitchford et al., 2002; Wang et al., 2003). Changes in the fatty acid composition could affect meat quality and nutritional value. Relatively low concentrations of saturated fatty acids in the diet are considered good for human health while some polyunsaturated fatty acids are regarded as essential dietary lipids in humans. Furthermore, some polyunsaturated fatty acids found in horse lipids were reported to have functional effects such as lowering blood cholesterol and reducing atherogenesis (Fukushima et al., 1997). However, most of the studies on the fatty acid compositions and the effects of above factors have been carried out on beef cattle, lambs, pigs and chicken (Rule et al., 1995). Although horsemeat consumption in humans is relatively small compared to that of beef and pork annually total world horsemeat production is more than half million tons (Kim, 2001).

Early studies provided information on the main fatty acids profiles of adipose tissues from ponies (Robb et al.,

1972), foals (Catalano and Quaranteilli, 1976), raw horse muscle (Badiani et al., 1997) and cured horsemeat product (Paleari et al., 2002). These showed that horsemeat was relatively high in linoleic acid (C18:2) and linolenic acid (C18:3). However, little information is available on the fatty acid profiles in various tissues from fattening horses under the same feeding conditions.

Conjugated linoleic acids (CLA) can be produced during bihydrogenation of dietary lipids in rumen by microbes (Song and Kennelly, 2003). They can also be formed in body tissues through  $\Delta^9$ -desaturation of trans 11 octadecenoic acid (Grinari et al., 2000). They have been reported to have potential health benefits (Khanal, 2004) such as anticarcinogenic, and able to reduce atherogenesis, diabetes and obesity in animal models and human cell lines. Little information was available on the CLA level in horse tissues while there were some reports on that of beef cattle and other ruminants (Fritsche et al., 1999). The horse, like ruminant animals consumes feed relatively high in fiber, but dose not ferment fiber in the fore-gut, but in the large intestine. Thus unlike ruminants it dose not break down dietary lipid or form products like CLA prior to absorption of lipid in the small intestine.

The present studies were carried out to provide new information on horse fatty acids profiles, including CLA, in comparison with those of beef cattle and pigs.

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## MATERIALS AND METHODS

### Animals and sampling

In the first study 2-year old castrated Breton horses (n = 8) with an average initial body weight of 780 kg were fed with a fattening diet for 12 months. The final average body weight was 1,124 kg and the carcass dress was 66.3%. The diet was composed of: 20% of commercial concentrate (formulated for beef cattle) which consisted of ground corn (56%), wheat bran (24%), soybean meal (12%), required vitamins and minerals (8%); barley, 32%; beer by-products, 10%; wheat bran, 24.8%; 13% hay and 0.2% mineral supplementation. The main nutrients composition in the diet was: 11.6% of crude protein, 3.57% of crude fat, 33.9% of neutral detergent fiber and 11.1% of acid detergent fiber. Tissue samples (Group 1) were taken from the fattening horses at slaughter. They consisted of subcutaneous fat, intermuscular fat, longissimus dorsi muscle and biceps femoris muscle (provided by Sasaki Chikusan Company in Obihiro, Hokkaido, Japan). In the second study the tissue samples (Group 2) were taken from subcutaneous, intermuscular adipose tissues and longissimus dorsi muscle of the fattening horses (n = 13), Japanese Black beef cattle (n = 5), Holstein steers (n = 5) and pigs (n = 5). The fattening horses were from Kumamoto (n = 5) and Hokkaido (n = 8), Japan. The diet for horses was the same as those in Group 1. The Japanese Black beef cattle, Holstein steers provided by Sasaki Chikusan Company (Obihiro, Hokkaido, Japan) were kept in houses treated under similar feeding conditions during fattening period. They were provided with concentrates plus roughages *ad libitum* until they reached slaughter body weight around 750 kg. The diets for beef cattle were composed of the same commercial concentrate used in fattening horses which consisted of ground corn (56%), wheat bran (24%), soybean meal (12%) and required vitamins and minerals (8%), supplemented with hay. The crossbreed pigs (Large white×Landrace×Duroc; Sasaki Chikusan Company, Obihiro, Hokkaido, Japan) were fed *ad libitum* during the fattening period until they reached the slaughter body weight around 100 kg. The diet for fattening pigs was a commercial feed for finishing pigs that composed of 45% corn; 25% rye, soybean meal 27%, wheat bran 1%, and 2% of the essential amino acids, vitamins and minerals supplementations.

### Extraction of lipids

In both groups, total lipids of the samples were extracted by a method using hexane and isopropanol for determination of fatty acids (Jiang et al., 1996). The meat or fat samples were cut to small pieces and put into 50 ml glass tube (1-3 or 0.5 g, respectively) containing 14 ml isopropanol and 10.5 ml hexane. After homogenized 5 ml

water was added to the tube and shaken vigorously. The tube was then centrifuged at 2,000 g for 5 min at 5°C. The upper layer (hexane) was transferred into a 50 ml glass tube. After washed the bottom layer with hexane twice 7 ml of 0.47 M Na<sub>2</sub>SO<sub>4</sub> was added and mixed completely. After centrifuged at 2,000 g for 5 min at 5°C the upper layer (hexane) was transferred into a glass tube and hexane was evaporated off under nitrogen.

### Methylation and determination of fatty acids

A combined base/acid methylation method (Kramer et al., 1997) with modifications was used. The lipids samples (20 mg) were placed in methylation tubes with 75 µl of Heneicosanoic acid (C21:0) methyl ester as an internal standard (5 mg/ml; Nu-Chek Prep. Inc., MN, USA). Then 2 ml of sodium methoxide (0.5 mol/L in methanol) was added to each tube and placed in a 50°C water bath for 10 min after flushed with nitrogen and mixed completely. After that 1 ml boron trifluoride (14% in methanol) was added and reheated in a 50°C water bath for another 10 min. Then 5 ml water and hexane were added and mixed completely. The upper layer (hexane) was taken into a GC vial flushed with nitrogen and stored at -30°C until fatty acid profiles were determined by gas chromatography. The fatty acids profiles were analyzed by a Shimadzu gas chromatography (GC-14B; Kyoto, Japan) with a capillary column Rtx<sup>®</sup>-2330, 30 m×0.32 mm ID×0.2 µm df (Restek, USA). Initial GC temperature was 100°C and then increased to 175°C at a rate of 25°C per min. After held for 5 min the temperature was continually increased to 225°C at a rate of 5°C per min and held for 10 min. Helium was used as the carrier gas. The fatty acids were identified by comparison to retention times of known fatty acid methyl ester standard. The concentration of identified fatty acid was then calculated based on the ratio of the fatty acid to the internal standard.

### Statistic analysis

Analysis of variance (ANOVA) was used for testing the differences on lipids concentration, detected fatty acids concentration and weight percentage of detected fatty acids from different depots (Group 1), different species or breeds of animals (Group 2). Tukey test was applied to compare the difference between the means (SPSS, 1999). The significance was set as p<0.05.

## RESULTS

### Fatty acid profiles of horses muscle and adipose tissues (Group 1)

The fatty acid profiles in lipids from the fattening horse subcutaneous and intermuscular adipose tissues or from longissimus dorsi and biceps femoris muscles are shown in

**Table 1.** Comparison on fatty acid profiles of lipids from the fattening horse subcutaneous adipose tissue, intermuscular adipose tissue, longissimus dorsi muscle and biceps femoris muscle\*

	Adipose tissue		Muscle		SEM	p-value
	Subcutaneous	Intermuscular	Longissimus dorsi	Biceps femoris		
Lipids (g/100 g tissue)						
Total	70.28 <sup>b</sup>	84.39 <sup>c</sup>	12.73 <sup>a</sup>	7.71 <sup>a</sup>	1.84	0.001
Detected FA (g/100 g lipids)						
Total	82.23	84.86	82.87	80.95	1.02	0.087
Composition of FA (% total detected FA)						
C10:0	0.05 <sup>a</sup>	0.07 <sup>b</sup>	0.09 <sup>b</sup>	0.08 <sup>b</sup>	0.00	0.001
C12:0	0.19 <sup>a</sup>	0.21 <sup>ab</sup>	0.24 <sup>b</sup>	0.21 <sup>ab</sup>	0.01	0.065
C14:0	3.26 <sup>a</sup>	4.06 <sup>b</sup>	4.18 <sup>b</sup>	3.98 <sup>b</sup>	0.16	0.003
C14:1	0.39 <sup>a</sup>	0.46 <sup>a</sup>	0.78 <sup>b</sup>	0.74 <sup>b</sup>	0.05	0.001
C15:0	0.29	0.37	0.35	0.33	0.02	0.091
C16:0	24.46 <sup>a</sup>	26.98 <sup>b</sup>	26.41 <sup>ab</sup>	26.07 <sup>ab</sup>	0.53	0.020
C16:1	7.56 <sup>a</sup>	7.55 <sup>a</sup>	11.09 <sup>b</sup>	11.04 <sup>b</sup>	0.51	0.001
C17:0	0.34 <sup>ab</sup>	0.41 <sup>b</sup>	0.32 <sup>ab</sup>	0.31 <sup>a</sup>	0.02	0.021
C17:1	0.67	0.72	0.78	0.71	0.04	0.337
C18:0	2.71 <sup>ab</sup>	3.06 <sup>b</sup>	2.52 <sup>a</sup>	2.81 <sup>ab</sup>	0.12	0.036
C18:1	35.49 <sup>ab</sup>	33.47 <sup>a</sup>	37.13 <sup>b</sup>	36.38 <sup>ab</sup>	0.83	0.023
C18:2	20.51 <sup>b</sup>	19.47 <sup>b</sup>	13.24 <sup>a</sup>	14.14 <sup>a</sup>	0.88	0.001
C18:3	2.50 <sup>b</sup>	1.89 <sup>ab</sup>	1.53 <sup>a</sup>	1.31 <sup>a</sup>	0.19	0.002
C20:0	0.09	0.08	0.08	0.09	0.01	0.274
C20:1	0.57 <sup>c</sup>	0.49 <sup>bc</sup>	0.38 <sup>a</sup>	0.42 <sup>ab</sup>	0.02	0.001
C20:2	0.45 <sup>b</sup>	0.39 <sup>b</sup>	0.26 <sup>a</sup>	0.30 <sup>a</sup>	0.02	0.001
C20:3	0.07 <sup>ab</sup>	0.05 <sup>a</sup>	0.09 <sup>b</sup>	0.14 <sup>c</sup>	0.01	0.001
C20:4	0.18 <sup>ab</sup>	0.10 <sup>a</sup>	0.33 <sup>b</sup>	0.67 <sup>c</sup>	0.04	0.001
C24:1	0.04 <sup>ab</sup>	0.03 <sup>a</sup>	0.05 <sup>bc</sup>	0.07 <sup>c</sup>	0.01	0.002
CLA c9, t11	0.16 <sup>ab</sup>	0.14 <sup>a</sup>	0.15 <sup>a</sup>	0.22 <sup>b</sup>	0.01	0.007
SFA	31.40 <sup>a</sup>	35.24 <sup>b</sup>	34.19 <sup>b</sup>	33.89 <sup>b</sup>	0.61	0.002
MUFA	44.73 <sup>a</sup>	42.72 <sup>a</sup>	50.21 <sup>b</sup>	49.35 <sup>b</sup>	1.07	0.001
PUFA	23.87 <sup>b</sup>	22.04 <sup>b</sup>	15.60 <sup>a</sup>	16.76 <sup>a</sup>	1.03	0.001

\* Group 1. FA: Fatty acids; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

<sup>ac</sup> Means within the row without the same letter differ significantly ( $p < 0.05$ ),  $n = 7$  in the group of subcutaneous adipose tissue or biceps femoris muscle,  $n = 8$  in the groups of intermuscular adipose tissue or longissimus dorsi muscle.

Table 1. Total lipid concentration was 7.71 and 12.73% in longissimus dorsi and biceps femoris muscles, respectively, while that of subcutaneous and intermuscular adipose tissues was 70.28 and 84.39% respectively. The weight percentage of the total saturated fatty acids (SFA, 31.4%) of the horse subcutaneous adipose tissue was lower ( $p < 0.05$ ) than that of the other depots. The percentage of monounsaturated fatty acids (MUFA) in the horse muscles was higher ( $p < 0.05$ ) than that in the subcutaneous and intermuscular adipose tissues. By contrast, the percentage of polyunsaturated fatty acids (PUFA) in the subcutaneous and intermuscular adipose tissues (23.87 and 22.04% respectively) was higher ( $p < 0.05$ ) than that in the longissimus dorsi and biceps femoris muscles (15.60% and 16.76%, respectively).

The most abundant fatty acid in the lipids from these depots was oleic acid (C18:1, 33.47 to 37.13%) while stearic acid (18:0) was relatively low (2.52 to 3.06%). The percentage of C18:1 in the muscles was higher than that in

the adipose tissues, however, a significant difference ( $p < 0.05$ ) was found only between the longissimus dorsi muscle and the intermuscular adipose tissue. By contrast, the percentage of C18:0 was higher ( $p < 0.05$ ) in the intermuscular adipose tissue than that in the longissimus dorsi muscle. The percentages of palmitic acid (C16:0) and myristic acid (C14:0) of the subcutaneous adipose tissue were lower ( $p < 0.05$ ) than those of the other depots. The percentages of myristoleic acid (C14:1) and palmitoleic acid (C16:1) of the subcutaneous and intermuscular adipose tissues were lower ( $p < 0.05$ ) than those of the longissimus dorsi and biceps femoris muscles. The percentages of fatty acids with carbons of 20 or more generally were very low. Eicosenoic acid (C20:1) and eicosadienoic acid (C20:2) in the adipose tissues were higher while those of dihomo- $\gamma$ -linoleic acid (C20:3), arachidonic acid (C20:4) and nervonic acid (C24:1) were lower than those in the muscle.

Linoleic acid (C18:2) was the third abundant fatty acid among the detected fatty acids. Significant difference

**Table 2.** Comparison of fatty acid profiles in lipids from subcutaneous adipose tissues of the fattening horses, beef cattle or pigs\*

	Horse	Beef cattle		Pig	SEM	p-value
		Japanese Black	Holstein steer			
Lipids (g/100 g tissue)						
Total	73.91	78.67	78.31	76.63	2.00	0.397
Detected FA (g/100 g lipids)						
Total	82.89 <sup>c</sup>	73.91 <sup>a</sup>	74.89 <sup>ab</sup>	78.40 <sup>b</sup>	0.80	0.001
Composition of FA (% total detected FA)						
C14:0	3.27 <sup>c</sup>	2.05 <sup>ab</sup>	2.70 <sup>bc</sup>	1.28 <sup>a</sup>	0.16	0.001
C14:1	0.40 <sup>a</sup>	1.34 <sup>b</sup>	1.50 <sup>b</sup>	0.02 <sup>a</sup>	0.10	0.001
C16:0	24.25 <sup>b</sup>	19.83 <sup>a</sup>	23.36 <sup>b</sup>	23.93 <sup>b</sup>	0.56	0.001
C16:1	7.85 <sup>c</sup>	4.85 <sup>b</sup>	5.56 <sup>b</sup>	1.73 <sup>a</sup>	0.42	0.001
C18:0	2.57 <sup>a</sup>	8.02 <sup>b</sup>	10.43 <sup>b</sup>	17.08 <sup>c</sup>	0.86	0.001
C18:1	35.19 <sup>a</sup>	56.66 <sup>d</sup>	49.31 <sup>c</sup>	42.10 <sup>b</sup>	1.01	0.001
C18:2	21.08 <sup>c</sup>	2.90 <sup>a</sup>	2.51 <sup>a</sup>	9.89 <sup>b</sup>	0.38	0.001
C18:3	2.52 <sup>b</sup>	0.20 <sup>a</sup>	0.14 <sup>a</sup>	0.55 <sup>a</sup>	0.06	0.001
C20:2	0.45 <sup>b</sup>	0.04 <sup>a</sup>	0.03 <sup>a</sup>	0.42 <sup>b</sup>	0.02	0.001
C20:4	0.16 <sup>b</sup>	0.04 <sup>a</sup>	0.04 <sup>a</sup>	0.13 <sup>b</sup>	0.01	0.001
CLA c9, t11	0.16 <sup>a</sup>	0.94 <sup>b</sup>	0.71 <sup>b</sup>	0.18 <sup>a</sup>	0.09	0.001
CLA t10, c12	0.00 <sup>a</sup>	0.05 <sup>b</sup>	0.05 <sup>b</sup>	0.03 <sup>b</sup>	0.01	0.001
SFA	30.95 <sup>a</sup>	31.08 <sup>a</sup>	38.39 <sup>b</sup>	43.46 <sup>c</sup>	1.31	0.001
MUFA	44.62 <sup>a</sup>	64.67 <sup>c</sup>	58.08 <sup>b</sup>	45.28 <sup>a</sup>	1.17	0.001
PUFA	24.43 <sup>c</sup>	4.25 <sup>a</sup>	3.53 <sup>a</sup>	11.26 <sup>b</sup>	0.73	0.001

\* Group 2. FA: Fatty acids; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

<sup>a-c</sup> Means within the row without the same letter differ significantly ( $p < 0.05$ ).

n = 13 in the group of horses. n = 5 in the groups of Japanese Black beef cattle, Holstein steers or pigs.

( $p < 0.05$ ) was found between those in the adipose tissues (20.51 and 19.47%) and the muscles (13.24 and 14.14%). Similarly, the percentage of linolenic acid (C18:3) in the horse subcutaneous and intermuscular adipose tissues (2.5% and 1.89% respectively) was also higher than those in the muscles (1.53 and 1.31%). The main CLA in the horse lipids was cis 9, trans 11 (c9, t11), that in horse biceps femoris muscle was 0.22%, higher ( $p < 0.05$ ) than those in the other depots (0.14-0.16%), while that of CLA t10, c12 was less than 0.01% (data are not shown).

### Comparison of fatty acid profiles of horses, cattle and pigs (Group 2)

Comparison of fatty acid profiles in lipids from the animal subcutaneous, intermuscular adipose tissues, and longissimus dorsi muscle are shown in Tables 2, 3 and 4, respectively. The total lipid concentrations of the subcutaneous and intermuscular adipose tissues were very similar between the species and breeds, while that of longissimus dorsi muscle from Japanese Black beef cattle (31.55%) was much higher ( $p < 0.05$ ) than those from Holstein steers (5.90%), pigs (6.67%) and horses (8.8%).

The percentages of SFA, MUFA and PUFA of the fattening horses, beef cattle and pigs differed significantly ( $p < 0.01$ ). Compared to the beef cattle and pigs, horses had lower SFA and MUFA but higher ( $p < 0.05$ ) PUFA. Compared to beef cattle, the pigs had higher ( $p < 0.05$ ) PUFA

in subcutaneous and intermuscular lipids. Within the beef cattle, the Japanese Black had lower ( $p < 0.05$ ) percentage of SFA in all the depots but higher ( $p < 0.05$ ) percentage of MUFA in subcutaneous and intermuscular adipose tissues.

The sequences of the concentration of the top four abundant fatty acids, from high to low, were C18:1, C16:0, C18:2 and C16:1 for the fattening horses, C18:1, C16:0, C18:0 and C16:1 for the beef cattle, and C18:1, C16:0, C18:0 and C18:2 for the pigs. The values of C18:0 and C18:1 in the fattening horses muscles and adipose tissues were lower ( $p < 0.05$ ) than those of beef cattle and pigs. Those of C14:0 and C16:1 in the fattening horses muscles and adipose tissues were higher ( $p < 0.05$ ) than those of the other animal species. The concentration of C18:2 was 21.08, 20.35 and 12.79% for the horse subcutaneous, intermuscular adipose tissues and longissimus dorsi muscle, respectively. It was 4-8 folds higher ( $p < 0.05$ ) than that of the beef cattle and 2 more folds higher ( $p < 0.05$ ) than that of the pigs. Similarly, the proportion of C18:3 was 2.52, 2.15 and 1.47% for the horse subcutaneous, intermuscular adipose tissues and longissimus dorsi muscle, respectively. It was 8-18 fold higher ( $p < 0.05$ ) than that of the beef cattle and 5-6 folds higher ( $p < 0.05$ ) than that of the pigs. CLA c9, t11 was the main CLA in lipids from all these animal species. The percentage of CLA c9, t11 in lipids from the fattening horses (0.14-0.16%), which was comparable to that of the pigs (0.18-0.19%), was much lower ( $p < 0.05$ )

**Table 3.** Comparison of fatty acid profiles in lipids from intermuscular adipose tissues of the fattening horses, beef cattle or pigs\*

	Horse	Beef cattle		Pig	SEM	p-value
		Japanese Black	Holstein steer			
Lipids (g/100 g tissue)						
Total	82.61	79.54	81.37	76.96	1.73	0.202
Detected FA (g/100 g lipids)						
Total	84.09 <sup>b</sup>	83.82 <sup>b</sup>	78.50 <sup>a</sup>	81.08 <sup>ab</sup>	0.97	0.001
Composition of FA (% total detected FA)						
C14:0	3.85 <sup>c</sup>	2.01 <sup>b</sup>	2.36 <sup>b</sup>	1.33 <sup>a</sup>	0.14	0.001
C14:1	0.42 <sup>b</sup>	0.74 <sup>c</sup>	0.55 <sup>b</sup>	0.02 <sup>d</sup>	0.03	0.001
C16:0	26.54 <sup>b</sup>	20.59 <sup>a</sup>	21.98 <sup>a</sup>	24.33 <sup>b</sup>	0.54	0.001
C16:1	7.51 <sup>b</sup>	3.14 <sup>a</sup>	2.90 <sup>a</sup>	2.24 <sup>a</sup>	0.26	0.001
C18:0	3.07 <sup>a</sup>	13.05 <sup>b</sup>	17.46 <sup>c</sup>	15.89 <sup>c</sup>	0.68	0.001
C18:1	33.21 <sup>a</sup>	53.57 <sup>c</sup>	47.15 <sup>b</sup>	44.23 <sup>b</sup>	1.08	0.001
C18:2	20.35 <sup>c</sup>	2.78 <sup>a</sup>	2.81 <sup>a</sup>	8.20 <sup>b</sup>	0.48	0.001
C18:3	2.15 <sup>b</sup>	0.19 <sup>a</sup>	0.16 <sup>a</sup>	0.46 <sup>a</sup>	0.07	0.001
C20:2	0.43 <sup>b</sup>	0.04 <sup>a</sup>	0.03 <sup>a</sup>	0.37 <sup>b</sup>	0.02	0.001
C20:4	0.11 <sup>b</sup>	0.04 <sup>a</sup>	0.05 <sup>a</sup>	0.21 <sup>c</sup>	0.01	0.001
CLA c9, t11	0.14 <sup>a</sup>	0.70 <sup>b</sup>	0.60 <sup>b</sup>	0.19 <sup>a</sup>	0.05	0.001
CLA t10, c12	0.00 <sup>a</sup>	0.03 <sup>b</sup>	0.05 <sup>c</sup>	0.03 <sup>b</sup>	0.00	0.001
SFA	34.47 <sup>a</sup>	37.09 <sup>a</sup>	44.13 <sup>b</sup>	42.58 <sup>b</sup>	1.09	0.001
MUFA	42.30 <sup>a</sup>	59.05 <sup>c</sup>	52.07 <sup>b</sup>	47.91 <sup>b</sup>	1.25	0.001
PUFA	23.23 <sup>c</sup>	3.86 <sup>a</sup>	3.80 <sup>a</sup>	9.52 <sup>b</sup>	0.58	0.001

\* Group 2. FA: Fatty acids; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

<sup>a-d</sup> Means within the row without the same letter differ significantly ( $p < 0.05$ ).

n = 13 in the group of horses. n = 5 in the groups of Japanese Black beef cattle, Holstein steers or pigs.

than that of the Japanese Black cattle (0.55-0.94%) and the Holstein steers (0.46-0.71%). The percentages of fatty acids in total lipid with 20 or more carbons were generally very low. The levels of C20:2 and C20:4 in lipids from the fattening horse subcutaneous and intermuscular adipose tissues were also close to those of the pigs, but higher ( $p < 0.05$ ) than those of the beef cattle.

## DISCUSSION

Fatty acids in the diet can be absorbed and utilized by horses as source of energy or as precursors for synthesis of triacylglycerols (TAG) or other fatty acids after being desaturated and/or elongated. The diet of horses, as with ruminants normally, is composed of a relatively large amount of hay. In the lipids of such diets the percentage of linoleic acid (C18:2) in the total fatty acids can be around 50%. The horse could effectively digest and utilize these PUFAs from the diet. The accumulation from dietary fatty acids containing 54% C18:2 and 5.8% C18:3 in the total fatty acids was the main explanation for this because these fatty acids can not be *de novo* biosynthesized (Rule et al., 1995). A previous study found that C18:2 and C18:3 in blood serum of saddle horses fed with hay-barley diet were 33.97% and 1.03% of total fatty acids, respectively (Bergero et al., 2002). There are some reports on the fatty acid composition of both cured horsemeat (Paleari et al.,

2003) and horse oil that was obtained commercially from animal tissue by rendering (Fukushima et al., 1997). Those data also showed that C18:1, C16:0, C18:2 and C18:3 were main fatty acids in the horse oil products and had similar values as those found in present study except C18:3 that was even higher (Fukushima et al., 1997). The variants in rendering processes of oil products including the additives to prevent the development of oxidation, different sources of animals and the dietary differences in C18:3 may cause the differences among these data.

The obvious differences in percentage of C18:2, C18:3 and C20:4 suggest that the adipose tissues and muscles may be different in uptake, utilization and/or storage of these fatty acids. In the adipose tissues of fattening horses the fatty acids from diet may be provided and incorporated to synthesise TAG, while in the muscles there are more requirements on providing energy and as precursors for other essential fatty acids such as C20:4 or eicosanoids. The higher percentage of C20:4 in the muscles may due to the higher concentration of phospholipids in membrane of muscles.

The differences in the profiles of fatty acids between animal species and breeds may relate to their different digestive physiological characters. Animals can use both acetate and glucose for *de novo* synthesis of lipids. For lipid biosynthesis the principle precursor in pigs is glucose while in ruminants, acetate may be even more important for this

**Table 4.** Comparison of fatty acid profiles in lipids from longissimus dorsi muscle of the fattening horses, beef cattle or pigs\*

	Horse	Beef cattle		Pig	SEM	p-value
		Japanese Black	Holstein steer			
Lipids (g/100 g tissue)						
Total	8.80 <sup>a</sup>	31.55 <sup>b</sup>	5.90 <sup>a</sup>	6.67 <sup>a</sup>	2.10	0.001
Detected FA (g/100 g lipids)						
Total	81.28 <sup>b</sup>	81.88 <sup>b</sup>	75.50 <sup>a</sup>	80.50 <sup>b</sup>	0.88	0.004
Composition of FA (% total detected FA)						
C14:0	4.01 <sup>c</sup>	2.56 <sup>b</sup>	2.21 <sup>b</sup>	1.42 <sup>a</sup>	0.13	0.001
C14:1	0.76 <sup>c</sup>	0.85 <sup>c</sup>	0.50 <sup>b</sup>	0.02 <sup>a</sup>	0.04	0.001
C16:0	26.91 <sup>b</sup>	23.89 <sup>a</sup>	22.97 <sup>a</sup>	24.48 <sup>ab</sup>	0.58	0.001
C16:1	11.67 <sup>b</sup>	3.65 <sup>a</sup>	3.04 <sup>a</sup>	3.02 <sup>a</sup>	0.31	0.001
C18:0	2.51 <sup>a</sup>	11.40 <sup>b</sup>	15.63 <sup>c</sup>	14.41 <sup>c</sup>	0.51	0.001
C18:1	36.93 <sup>a</sup>	51.47 <sup>b</sup>	47.81 <sup>b</sup>	48.92 <sup>b</sup>	0.91	0.001
C18:2	12.79 <sup>b</sup>	2.58 <sup>a</sup>	3.15 <sup>a</sup>	4.64 <sup>a</sup>	0.34	0.001
C18:3	1.47 <sup>b</sup>	0.17 <sup>a</sup>	0.15 <sup>a</sup>	0.24 <sup>a</sup>	0.05	0.001
C20:2	0.25 <sup>c</sup>	0.03 <sup>a</sup>	0.03 <sup>a</sup>	0.21 <sup>b</sup>	0.01	0.001
C20:4	0.43 <sup>b</sup>	0.07 <sup>a</sup>	0.40 <sup>b</sup>	0.36 <sup>b</sup>	0.05	0.001
CLA c9, t11	0.15 <sup>a</sup>	0.55 <sup>b</sup>	0.46 <sup>b</sup>	0.18 <sup>a</sup>	0.05	0.001
CLA t10, c12	0.00 <sup>a</sup>	0.03 <sup>b</sup>	0.05 <sup>b</sup>	0.03 <sup>b</sup>	0.01	0.001
SFA	34.37 <sup>a</sup>	39.20 <sup>b</sup>	42.83 <sup>c</sup>	41.23 <sup>bc</sup>	0.90	0.001
MUFA	50.43 <sup>a</sup>	57.30 <sup>b</sup>	52.80 <sup>b</sup>	53.03 <sup>b</sup>	1.41	0.002
PUFA	15.20 <sup>b</sup>	3.50 <sup>a</sup>	4.37 <sup>a</sup>	5.74 <sup>a</sup>	0.43	0.001

\* Group 2. FA: Fatty acids; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

<sup>a-c</sup> Means within the row without the same letter differ significantly ( $p < 0.05$ ).

n=13 in the group of horses. n=5 in the groups of Japanese Black beef cattle. Holstein steers or pigs.

(He et al., 1997b, 1998). In horses the diet contains not only soluble carbohydrate that provides glucose after digestion by enzymes, but also relatively large amount of insoluble carbohydrate that can be fermented by the bacteria in the hind gut to produce volatile fatty acids (VFAs). Horses and pigs are able to digest fat and incorporate the dietary fatty acids into adipocytes efficiently. However, in beef cattle most of these fatty acids in diet are transformed to C18:1 and C18:0 by the biohydrogenation by rumen bacteria, which may be the main explanation for the higher C18:0 and C18:1 but lower C18:2 and C18:3 in beef (Scollan et al., 2001). It was also found in the second study that the Japanese black beef cattle were lower in C18:0 but higher in C18:1 compared to Holstein steers, which was consistent with those reported before (Yoshimura and Namikawa, 1985). The percentage of C18:0 in subcutaneous adipose tissues of Holstein steers decreased while that of C18:1 increased with the enlargement of the adipocytes and the body weight (He et al., 1997a, 2000). The difference in the enlargement of adipocytes between these breeds may also cause the difference in the fatty acid profiles.

The concentration of CLA in lipids from adipose tissues and muscles of beef cattle was higher than that in fattening horse and pigs, which is similar to the different levels of CLA in milk fat of these animals (Jahreis et al., 1999). It was reported that CLA in horse milk fat was only 0.1%

while that in pigs and cows milk were 0.2% and 0.7% respectively (Jahreis et al., 1999). It was reported (Fritsche et al., 1999) that CLA in lipids from pork was 0.06% (U.S. origin) or 0.12-0.15% (German origin) while that in beef was 0.29% (round, U.S. origin) or 0.65% (beef, German origin). In rumen the CLA can be produced during the biohydrogenation of C18:2 by rumen bacteria such as *Butyrivibrio fibrisolvens* (Kepler, 1966; Choi and Song, 2005). They can be also formed through  $\Delta^9$ -desaturation of trans 11 octadecenoic acid in animal tissues (Griinari et al., 2000; An et al., 2003). In the fattening horse most dietary C18:2 which is the main precursor of CLA may be digested and absorbed before reaching the caecum and is thus not available for CLA production. It is suggested that the CLA accumulated in horse meat may arise from endogenous synthesis or be derived from dietary lipids if there are some.

In conclusion, the results showed that the fatty acid profiles in lipids from different muscle and adipose tissues of fattening horses differed significantly. When compared to the lipids of beef cattle and pigs, the horse lipids contained more C18:2 and C18:3 but less CLA.

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## REFERENCES

- An, B. K., C. W. Kang, Y. Izumi, Y. Kobayashi and K. Tanaka. 2003. Effect of dietary fat sources on occurrences of conjugated linoleic acid and trans fatty acids in rumen contents. *Asian-Aust. J. Anima. Sci.* 16:222-226.
- Badiani, A., N. Nanni, P. P. Gatta, B. Tolomelli and M. Manfredini. 1997. Nutrient profile of horse meat. *J. Food Compos. Anal.* 10:254-269.
- Bergero, D., N. Miraglia, M. Polidori, M. Ziino and D. Gagliardi. 2002. Blood serum and skin fatty acid levels in horses and the use of dietary polyunsaturated fatty acids. *Anim. Res.* 51:157-163.
- Catalano, A. L. and A. Quarantelli. 1976. Carcass characteristics and chemical composition of the meat from milk-fed foals. *Clin. Vet.* 102:489-506.
- Choi, S. H. and M. K. Song. 2005. Effect of C18-polyunsaturated fatty acids on their direct incorporation into the rumen bacterial lipids and CLA production *in vitro*. *Asian-Aust. J. Anim. Sci.* 18:512-515.
- Fritsche, J., R. Rickert and H. Steinhart. 1999. Formation, contents and estimation of daily intake of conjugated linoleic isomers and trans-fatty acids in foods. In: *Advances in conjugated linoleic acid research, Volume I* (Ed. M. P. Yurawec, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza and G. J. Nelson). AOCS Press, Champaign, Illinois. pp. 378-396.
- Fukushima, M., Y. Takayama, T. Habaguchi and M. Nakano. 1997. Comparative hypocholesterolemic effects of capybara (*hydrochoerus hydrochaeris dabbeneri*) oil, horse oil, and sardine oil in cholesterol-fed rats. *Lipids* 32:391-395.
- Grinari, J. M., B. A. Corl, S. H. Lacy, P. Y. Chouinard, K. V. V. Nummela and D. E. Bauman. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by  $\Delta^2$ -desaturase. *J. Nutr.* 130:2285-2291.
- He, M. L., S.-G. Roh, H. Oka, S. Hidaka, N. Matsunaga and H. Hidari. 1997a. The relationship between fatty acid composition and the size of adipocytes from subcutaneous adipose tissue of Holstein steers during the fattening period. *Anim. Sci. Tech.* 68:838-842.
- He, M. L., S. Hidaka, N. Matsunaga, S.-G. Roh and H. Hidari. 1997b. Effect of different experimental conditions on lipogenesis and substrate oxidation in isolated adipocytes from fattening Holstein steers. *Reprod. Nutr. Dev.* 37:529-539.
- He, M. L., S.-G. Roh, E. Shibasaki, S. Hidaka, N. Matsunaga and H. Hidari. 1998. The relationship between lipogenic activity and the size of adipocytes from subcutaneous adipose tissue of Holstein steers during the fattening period. *Anim. Sci. Tech.* 69:424-432.
- He, M. L., S. Hidaka, N. Matsunaga and H. Hidari. 2000. Comparison of fatty acid composition among isolated bovine adipocytes with different sizes. *J. Anim. Physiol. a. Anim. Nutr.* 83:215-223.
- Jahreis, G., J. Fritsche and J. Kraft. 1999. Species-dependent, seasonal, and dietary variation of conjugated linoleic acid in milk. In: *Advances in conjugated linoleic acid research, Volume I* (Ed. M. P. Yurawec, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza and G. J. Nelson). AOCS Press, Champaign, Illinois. pp. 215-225.
- Jiang, J., L. Bjoerck, R. Fonden and M. Emanuelson. 1996. Occurrence of conjugated cis-9, trans-11-octadecadienoic acid in bovine milk: effects of feed and dietary regimen. *J. Dairy Sci.* 79:438-445.
- Kepler, C. R., K. P. Hirons, J. J. McNeil and S. B. Tove. 1966. Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*. *J. Biol. Chem.* 241:1350-1354.
- Khanal, R. C. 2004. Potential health benefits of conjugated linoleic acid (CLA): A review. *Asian-Aust. J. Anim. Sci.* 17:1315-1328.
- Kim, Y.-S. 2001. Meat production. In: *Meat science and application* (Ed. Y. H. Hui, W.-K. Nip, R. W. Rogers and O. A. Young). Marcel Dekker, Inc., New York, Basel. pp. 563-579.
- Kramer, J. K. G., V. Fellner, M. E. R. Dugan, F. D. Sauer, M. M. Mossoba and M. P. Yurawecz. 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total *trans* fatty acids. *Lipids* 32:1219-1228.
- Paleari, M. A., V. M. Moretti, G. Beretta, T. Mentasti and C. Bersani. 2003. Cured products from different animal species. *Meat Sci.* 63:485-489.
- Pitchford, W. S., M. P. B. Deland, B. D. Siebert, A. E. O. Malau-Aduli and C. D. K. Bottema. 2002. Genetic variation in fatness and fatty acid composition of crossbred cattle. *J. Anim. Sci.* 80:2825-2832.
- Robb, J., R. B. Harper, H. F. Hintz, J. T. Reid, J. E. Lowe, H. F. Schryver and M. S. S. Rhee. 1972. Chemical composition and energy value of the body, fatty acid composition of adipose tissue, and liver and kidney size in the horse. *Anim. Prod.* 14:25-34.
- Rule, D. C., S. B. Smith and J. R. Romans. 1995. Fatty acid composition of muscle and adipose tissue of meat animals. In: *The biology of fat in meat animals-Current advances* (Ed. S. B. Smith and D. R. Smith). The American Society of Animal Science, Champaign, Illinois. pp. 144-165.
- Scollan, N. D., M. S. Dhanoa, N. J. Choi, W. J. Maeng and M. Enser. 2001. Biohydrogenation and digestion of long chain fatty acids in steers fed on different sources of lipid. *J. Agric. Sci.* 136:345-355.
- Song, M. K. and J. J. Kennelly. 2003. Biosynthesis of conjugated linoleic acid and its incorporation into ruminant's products. *Asian-Aust. J. Anim. Sci.* 16:306-314.
- SPSS, 1999. SPSS 10.0 for Windows. SPSS®Inc.
- Wang, J. H., S. H. Choi and M. K. Song. 2003. Effects of concentrate to roughage ratio on the formation of cis 9, trans 11-CLA and trans 11-octadecenoic acid in rumen fluid and plasma of sheep when fed high oleic or high linoleic acid oils. *Asian-Aust. J. Anim. Sci.* 16:1604-1609.
- Yoshimura, T. and K. Namikawa. 1985. Influence of breed, sex and anatomical location on lipid and fatty acid composition of bovine intermuscular fat. *Jpn. J. Zootech. Sci.* 56:122-129.