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Effects of Palm Hydrogenated Fatty Acid Pellets on Fatty Acid Composition in the White Adipose Tissue of Japanese Black Cattle

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Abstract

This study's aim was to examine the changes in fatty acid composition of carcass adipose tissue by supplementing of palm hydrogenated fatty acid (PHFA) pellets to diets. A total of 30 castrated Japanese Black steers at about the same growth stage in the latter fattening period was used. They were fed an average of 9.0±0.7kg concentrate and 2kg of rice straw per a day, and ad libitum drinking water. The first 15 heads were partitioned into a control group and Group1, Group2, Group3, Group4 of each 3 heads of cattle. Group1 and 2 were continuously fed 600g and 300g of PHFA, relatively, per day for the last 6 months before finishing, respectively. Similarly, Group3 and Group4 were fed 600g and 300g of PHFA, relatively, per day for the last 3 months. The experiment was repeated using the other 15 head of cattle. Subcutaneous (Subcut) and rib loin eye (Rib eye) fats were used for fatty acid composition analysis. Level of unsaturated fatty acids, especially in Subcut and Rib eye in Group 3 and 4 were higher than those in the control group because of the increased C18:1 and the decreased C18:0. These results indicated that a supply of PHFA for 3 months before finishing would increase effectively USFA by possible enhancement of Δ9-desturase activity.

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Keywords : adipose tissue, palm hydrogenated fatty acid pellet, latter fattening period, fatty acid composition, Japanese Black

Introduction

Palm hydrogenated fatty acid pellets combined with hydrogen molecules is constituted from calcium and some polysaturated fatty acids. Generally, PHFA has been used as an additive to supply energy and promote development at the calf stage, increase intake in the fattening period and body weight gain, recover reproductive performance and improve milk or meat quality and quantity (Garcia-Bojalil *et al.* 1998; Sklan *et al.* 1989, 1991; Klusmeyer *et al.* 1991; Sklan & Tinsky. 1993; Espinoza *et al.* 1995; Ohtagaki *et al.* 1997). Carcasses with high degree of unsaturation in the adipose tissue tend to gain higher meat quality grades. Because PHFA is insoluble in the rumen but soluble in the abomasum, it will be possible to moderately control fatty acid composition in carcass adipose tissue without the influence on microflora in the rumen will be possible (Sukhija & Palmquist 1990; Palmquist *et al.* 1986). Jenkins & Palmquist (1984) mentioned that a diet supplemented with tallow PHFA would retain the normal the digestibility of fiber in the rumen. Ngidi et al. (1990) also reported that PHFA has not affected ruminal pH and VFA concentration and improved performance of feedlot cattle fed a high-concentrate diet although dairy gain and carcass weight have decreased. Furthermore an increase of digestibility of total fatty acids was caused by 1-4% PHFA in the diet, though it decreased when 6% PHFA was supplemented. Therefore, the chemical quality in carcass adipose tissue might

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be affected by PHFA. It is also known that part of unsaturated fatty are hydrogenated in the rumen to form saturated fatty acids such as C18:0 and C16:0. These fatty acids are altered by Δ9-desaturase into C18:1 and C16:1 in the adipose tissue and liver then stores as triacylglycerol (Kim & Ntambi. 1999; Yang *et al.* 1999). This study's aim was to examine the change of fatty acid composition in carcass adipose tissue when PHFA was supplemented in conventional diets.

Materials and Methods

Animals and management

A total of 30 Japanese Black steers in the latter fattening period was used. These were rare in accordance with the institutional guidelines of Animal Industry Research Institute, Iwate Agricultural Research Center. Age and body weight of the steers were similar at the beginning of the trial. The animals were fed an average of 9.0± 0.7kg of general concentrate and 2.0kg rice straw per a day, and *ad libitum* drinking water, respectively (Table 2). PHFA contained palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1) were mainly contained at 61.0, 38.0 and 1.0% level, respectively. In the 1st experiment, 15 heads were partitioned into 5 groups of each 3 heads, Group1, Group2, Group3, Group4 and a control group (Table 2). Group 1 and Group2 were continuously fed 600g and 300g per day of PHFA (Nihon Zenyaku Kogyo Co. Ltd., Fukushima, Japan) mixed with the conventional concentrate, relatively, for 6 months before the end of fattening (Table 2). Group3 and Group4 were similarly fed 600g and 300g per day of PHFA, respectively,

Table 1 Composition of concentrate

Ingredient	% of Diet DM
Cereals	70.0
Soybean meal	6.0
Molasses	22.0
Others	2.0
Nutrient analysis	
Crude protein	12.0 and over
Crude lipid	2.0 and over
Crude fiber	7.0 and under
TDN	73.0 and over
Calcium carbonate	0.3 and over
Crude ash content	7.0 and under
Phosphorus	0.3 and over
DCP	10.0 and over

TDN; total digestible nutrients, DCP; digestible crude protein, DM; dry matter.

Table 2 Feeding scheme of the concentrate and the initial body weight of steers

Item	Control	Period and sPHFA			
		6 months		3 months	
		600 g (Group 1)	300 g (Group 2)	600 g (Group 3)	300 g (Group 4)
Number of animals: 1st	3	3	3	3	3
2nd	3	3	3	3	3
General concentrate :					
Supply ;kg/day	10.2 (0.1)	8.4 (0.1)	9.5 (0.0)	8.4 (0.0)	8.7 (0.1)
Intake ;kg/day	9.5 (0.1)	7.2 (0.2)	8.5 (0.1)	7.0 (0.1)	7.9 (0.3)
Intake/Supply×100 ;%	93.1	85.7	89.4	83.3	91.0
Age at the end of fattening ;month	26.9 (0.3)	27.2 (0.2)	26.9 (0.2)	26.9 (0.1)	26.6 (0.7)
Initial body weight at fattening ;kg	244.6 (34.5)	253.1 (35.5)	238.5 (29.2)	235.1 (23.1)	226.2 (33.6)

PHFA; palm hydrogenated fatty acid, Period; period fed PHFA, sPHFA; supply of PHFA, ( ); standard deviation.

for 3 months before the end of fattening (Table 2). The control group was fattened without supplementation of PHFA. The experiment was repeated with the other 15 head under the same conditions (Table 2). The general composition of the concentrate diet is shown in Table 1.

Tissues preparation and fatty acid composition analysis

White adipose tissues of each carcass were taken from the back subcutaneous fat (Subcut) and rib loin eye fat (Rib eye) between the 6th and 7th rib about 24 hours after slaughtering. Obtained samples were stored at - 20℃ until fatty acid composition analysis.

Extraction of total lipid from the samples (10 mg) was carried out following the method of Folch *et al.* (1957). They samples were methylated in 600 μL methanol-hydrochoric acid (Tokyo Kasei Co. Ltd., Tokyo, Japan) for 20 sec. at 50℃. The fatty acid methyl esters were extracted by normal hexane and were analyzed using a dual column gas chromatograph (GC-14, Shimazu Co., Kyoto, Japan) equipped with a glass column (DB-23, J&W Scientific; Agilent Technologies Co. Ltd., California, USA). The carrier gas was nitrogen (head pressure; 0.6 kg/cm^2, flow rate of nitrogen; 40mL/min), and the column was programmed from an initial temperature of 100℃ to a final temperature of 215℃ at the rate of 4℃/min. A standard of known composition was analyzed to verify the identity of fatty acids in the samples. The fatty acid methyl ester peak areas were quantified with an electric integrator (C-R6A, Chromatopac, Shimazu Co., Kyoto, Japan). Identification of fatty acids (myristic acid: C14:0, palmitic acid: C16:0, palmitoleic acid: C16:1, stearic acid: C18:0, oleic acid: C18:1, linoleic acid: C18:2) was made by comparing the relative retention times of fatty acid methyl ester peaks from samples with those of standards. These were calculated as normalized area percentages (%) of fatty acids.

Statistical analysis

All data were initially subjected to analysis of variance (ANOVA) to determine the effects of period fed PHFA (Period), supply of PHFA (sPHFA), part of tissue (Part), an interaction between Period and sPHFA and an interaction Part and sPHFA by using General linear model (GLM) procedure of Statistical Analysis System, SAS (SAS 1989). On the basis of the results of ANOVA, the 1st and 2nd experiment data was combined and used for analysis after that. And least squares means and standard errors for fatty acid composition ratios and indices in each group and Part were calculated, respectively, and PDIFF option was used to compare their mean differences among groups in each Part. SFA was calculated as sum of saturated fatty acid composition ratios, and USFA was done as sum of unsaturated one. Δ9-desaturase activity index (Δ9

Table 3 Analysis of variance for proportions of major fatty acids and related indices

Effect	C16:0	C16:1	C18:0	C18:1	C18:2	USFA	U/S	⊿9idx
Period	0.05	n.s.	n.s.	0.05	n.s.	0.05	n.s.	n.s.
sPHFA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Part	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Period × sPHFA	n.s.	0.05	n.s.	n.s.	n.s.	n.s.	0.05	n.s.
Part × sPHFA	0.05	0.05	n.s.	n.s.	n.s.	n.s.	0.05	n.s.

Part; part of tissue, Period × sPHFA; interaction between Period and sPHFA, Part × sPHFA; interaction between Part and sPHFA, n.s.; non-significance, C16:0; palmitic acid, C16:1; palmitoleic acid, C18:0; stearic acid, C18:1; oleic acid, C18:2; linoleic acid, USFA; unsaturated fatty acid, U/S; ratio of USFA to SFA, ⊿9idx; ⊿9-desaturase activity index; ratio of C18:1 to sum of C18:0 and C18:1, See Table 2 for other footnotes.

idx) was calculated as ratio of C18:1 to sum of 18:0 and C18:1 using formula described by Malau-Aduli *et al.* (1997, 1998). U/S was shown as the ratio of USFA to SFA.

Results and Discussion

Animals at the about same age in the latter fattening period were used their intake of the was kept relatively constant (Table 2). To examine the effects that influenced on fatty acid com-

Table 4 Least squares means (standard errors) of fatty acid composition and related in the adipose tissues

	Part	Group 1	Group 2	Group 3	Group 4	Control
C14:0 (%)	Subcut	2.6(0.6)	1.7(0.7)	0.7(0.7)	1.1(0.7)	2.1(0.8)
C16:0 (%)	Subcut	26.3(1.7)	21.4(1.9)	23.1(1.9)	21.7(1.9)	27.2(2.3)
C16:1 (%)	Subcut	9.8(1.5)	12.9(1.6)	9.5(1.6)	9.6(1.6)	8.9(2.0)
C18:0 (%)	Subcut	3.6(2.1)	6.8(2.3)	4.3(2.3)	2.7(2.3)	8.8(2.8)
C18:1 (%)	Subcut	46.4(3.2)^{b}	43.3(3.5)^{b}	52.1(3.5)^{ab}	56.2(3.5)^{a}	43.6(4.2)^{b}
C18:2 (%)	Subcut	10.9(2.0)	13.6(2.2)	9.6(2.2)	8.7(2.2)	9.1(2.7)
SFA (%)	Subcut	32.6(3.1)^{ab}	30.0(3.4)^{ab}	28.6(3.4)^{ab}	25.3(3.4)^{b}	38.2(4.1)^{a}
USFA (%)	Subcut	67.3(3.1)^{ab}	69.9(3.4)^{ab}	71.3(3.4)^{ab}	74.6(3.4)^{a}	61.7(4.1)^{b}
U/S	Subcut	2.2(0.4)^{ab}	2.9(0.4)^{ab}	2.6(0.4)^{ab}	3.2(0.4)^{a}	1.7(0.5)^{b}
⊿9idx	Subcut	0.9(0.03)	0.8(0.03)	0.9(0.03)	0.9(0.03)	0.8(0.04)
C14:0 (%)	Rib eye	2.5(0.6)	1.0(0.7)	0.5(0.9)	2.0(0.8)	2.5(0.8)
C16:0 (%)	Rib eye	25.8(1.7)^{a}	20.8(2.3)^{ab}	16.9(2.3)^{b}	23.2(2.1)^{ab}	25.3(2.3)^{a}
C16:1 (%)	Rib eye	9.6(1.5)^{b}	11.9(2.0)^{ab}	15.0(2.0)^{a}	7.1(1.8)^{b}	9.9(2.0)^{ab}
C18:0 (%)	Rib eye	4.3(2.1)	6.2(2.8)	2.9(2.8)	5.4(2.5)	4.3(2.8)
C18:1 (%)	Rib eye	48.1(3.2)	50.1(4.3)	48.5(4.3)	53.0(3.8)	47.2(4.2)
C18:2 (%)	Rib eye	9.4(2.0)	9.7(2.7)	15.9(2.7)	9.1(2.4)	10.6(2.7)
SFA (%)	Rib eye	32.7(3.1)^{a}	28.1(4.2)^{ab}	20.4(4.2)^{b}	30.7(3.7)^{ab}	32.2(4.1)^{ab}
USFA (%)	Rib eye	67.2(3.1)^{b}	71.8(4.2)^{ab}	79.5(4.2)^{a}	69.2(3.7)^{ab}	67.7(4.1)^{ab}
U/S	Rib eye	2.1(0.4)^{b}	3.0(0.5)^{ab}	4.4(0.5)^{a}	2.3(0.5)^{b}	2.2(0.5)^{b}
⊿9idx	Rib eye	0.8(0.03)	0.8(0.04)	0.9(0.04)	0.9(0.04)	0.9(0.04)

Subcut; Subcutaneous fat, Rib eye; Rib loin eye fat, $^{a, b}$; Difference among means with different super scripts in the same row was significant at 5 % level., See Table 2 and 3 for other footnotes.

position in adipose tissue and related indices, the results of ANOVA are shown in Table 3. Least squares means of the fatty acid composition in the adipose tissue related indices for Group1, Group2, Group3, Group4 and the control group are shown in Table 4. Statistical significance was obtained for the effect of Period and the interaction between Part and sPHFA for C16:0, also for the interaction between Period and sPHFA and between Part and sPHFA for C16:1. C16:0 of Rib eye in Group 3 contained significantly less C16:0 compared with the control group ($P<0.05$). Although the difference was not significant C16:1 of Rib eye in Group3 tended to be more than the control group. The effect of Period for C18:1 was significant ($P<0.05$). Subcut in Group 1 - 4 tended to have less C18:0 than one in the control group. C18:0 of Rib eye, especially in Group 3, tended to be less than the control group. The levels of C18:1 of Subcut in Group 1 - 4 tended to be higher than the control group and levels of C18:1 of Subcut in Group1-4 tended to be higher than the control group and that in Group3 was especially high ($P<0.05$). Level of C18:1 of Rib eye in each group was consistent with that of Subcut. C18:2 was not influenced by the examined effects. Chang *et al.* (1992) suggested that, because C18:0 was the primary fatty acid available for absorption in ruminants, increase of C18:1 and decrease of C18:0 in adipose tissues might be influenced by an adaptive response of stearoyl-CoA desaturase and Δ9-desaturase. If it is so, Δ9-desaturase of mono-unsaturation enzyme in the adipose tissue might have been activated by supplementation of PHFA and the activity of supplementation for 3 months tended to be higher for supplementing PHFA for 3 months than for 6 months although estimated Δ9idx was not influenced by the considered effects in this experiment. It would be necessary to measure the actual activity of Δ9-desaturase by using biochemical methods to compare it more precisely for these experimental conditions. The levels of USFA of Subcut and Rib eye in Group1 were higher than that in the control group. The level of USFA, especially, of Subcut in Group4 and that of Rib eye in Group3 were significantly high compared to those in the control group ($P<0.05$). Thus, the differences in U/S ratio among the groups were similar to the differences in USFA. Furthermore, Mitsuhashi *et al.* (1988a) reported that fatty acid composition of adipose tissue of cattle in the fattening period seemed to be changed by the quantity of total unsaturated fatty acids and fatty acid composition of fat in the diet. Eweedah *et al.* (1997) suggested that diets supplemented with full fat soybean or sunflower seed containing rich polyunsaturated fatty acids would increase and store USFA in carcass adipose tissue. Therefore, these results indicated that PHFA would increase and store USFA in carcass adipose tissue, possibly by actively unsaturating polysaturated fatty acids with Δ9-desaturase (Smith *et al.* 2002). To effectively, increase USFA, it is important to supply approximately 3-5% of PHFA in the diet for the last 3 months during fattening.

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黒毛和種去勢牛におけるパーム硬化脂肪酸ペレットの蓄積体脂肪の脂肪酸組成に及ぼす効果

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反芻家畜の肉質において，蓄積体脂肪の脂肪酸組成の不飽和度が高いものが好まれる．本研究では，第一胃での飽和化を避け，不飽和脂肪酸 (USFA) の効率的な蓄積が期待されるパーム硬化脂肪酸ビーズ (PHFA) の蓄積体脂肪の脂肪酸組成への効果を検討した．1回目として肥育後期の黒毛和種去勢牛15頭を3頭づつ1～4区と対照区の5区に分け，1区と2区には肥育終了の6ヶ月前から一日当たり600gと300gのPHFAを，3区と4区にはその3ヶ月前から600gと300gをそれぞれ与え，対照区は無添加とした．他の15頭を用い同様の条件で2回目を行った．各区に一日当たり平均9.0±0.7kgの後期用濃厚飼料と2.0kgの稲ワラを，そして飲用水を任意に与えた．脂肪酸組成分析のための脂肪組織を屠殺後に背皮下脂肪 (Subcut) とロース芯部 (Rib eye) から採取した．特に3区と4区におけるSubcutとRib eyeのUSFAは，対照区に比較して高い傾向がみられ，それはC18:1の増加とC18:0の減少によるものと考えられた．以上の結果から，肥育終了前6ヶ月の長期間よりも短期間の3ヶ月程度で，また飼料給与量の3－5%PHFA添加で蓄積体脂肪のUSFAは増加することが明らかになった．

キーワード：蓄積体脂肪，パーム硬化脂肪酸ペレット，肥育後期，脂肪酸組成，黒毛和種

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