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Effects of Dietary Protein and Fat Levels on Growth Performance and Meat Quality in Finishing Pigs while Maintaining Sufficient Lysine

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Abstract

We conducted a study to evaluate the effects of dietary protein and fat levels, while maintaining appropriate levels of lysine and protein for nutrition, on growth performance, carcass characteristics, and meat quality in finishing pigs. Crossbred pigs (n = 32) were assigned to a 2 × 2 factorial arrangement of four dietary treatments: two protein levels (20% vs. 23%) and two fat levels (approximately, 2.5% vs. 6.2%). The examined diets were filled lysine content for finishing pigs. Each diet had similar digestible energy content. Average daily gain was not affected by dietary protein levels. No significant differences were observed in carcass characteristics. Intramuscular fat content in the longissimus dorsi muscle increased with higher dietary protein and the moisture and shear force were decreased. Most measurements of meat quality except for fat quality were not significantly affected by dietary fat. Our study indicates that intramuscular fat and marbling increase with increased dietary protein (assuming the diet contains sufficient lysine according to the feed standard), without having an adverse effect on the growth performance of finishing pigs and that intramuscular fat was affected little by dietary fat level.

Introduction

To maximize profits from pig farming it is necessary to produce high quality pork at low cost. In pork, a greater content of Intramuscular Fat (IMF) results in improved palatability [1]. Several studies have demonstrated that the IMF content of pork can be increased either by selective breeding or by dietary control [2-7]. Although low protein or low lysine methods have the desired effect of IMF and are easy to implement, the methods have not gained popularity because they result in decreases in feed intake, daily gain and lean meat quantity [2,4].

In Japan, many brands of well-marbled pork with a high IMF content have been produced using the dietary method, by feeding the pigs on bread crumbs [3]. This method, in which the breadcrumbs have low lysine and high protein content, has been successful in increasing the IMF in finishing pigs at lower cost and without adverse effects on feed intake and growth performance [5,6]. We reported that dietary decreasing lysine/protein ratio as 0.046 to 0.035 with sufficient fixed crude protein as 16% and adequate (not too much) lysine contents as 0.58% and 0.75% increased IMF without adverse effect on the growth performance in finishing pigs [7]. The question remains whether the dietary protein higher than 16% such as 20% and 23% could increase IMF. Moreover, the effect of dietary higher protein on pork quality is indispensable information for marbled pork production by by-product feed that sometimes contains high protein.

On the other hand, dietary fat is another possible mechanism for elevation of IMF in pork [6-8]. However, it is not clear whether the increase in IMF is caused by an increase in dietary fat or calories. It is important to determine how dietary protein and fat levels of by-product feed affect IMF in pork. Therefore, we aimed to evaluate the effects of dietary protein and fat levels with high protein and adequate (not too much) lysine levels on the growth performance, carcass characteristics, and meat quality of finishing pigs.

Materials and Methods

Dietary design and animal management

A total of 32 cross bred pigs (Landrace×Large White sow×Duroc sire, average body weight 54.5 ± 5.8 kg) were used for this experiment. The pigs were assigned to four groups of eight pigs (four barrows and four gilts per group) each to account for differences in initial body weight and sex.

As the experiment was carried out on a pig farm near a city, food industry by-products were used to make up the pigs' diet in the interests of practicality. The experiment pigs' diets were contained

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boiled, expired Chinese noodles from supermarkets and broken or misshapen chocolate from factories.

Four dietary treatments were prepared by computer software 'Ecofeed_ver2.xls' [9], with two levels of protein (15.5% and 18.5%) and two fat content levels (2.5% in Lower-Fat and 6.0% in Higher-Fat) on a dry matter basis. These diets contained sufficient lysine and crude protein for finishing pigs, in line with the Japanese Feeding Standard [10]. Data on the digestible energy in boiled Chinese noodles were taken from the standard table of food composition [11], as in our previous report [7]. The calculated digestible energy of the Lower-Fat and Higher-Fat treatments was 3.9 Mcal/kg and 4.1 Mcal/kg, respectively, and kept similar between diets by substituting fat with sugar (Table 1). Analytical composition of experiment diets composed two levels of protein (20% vs 23%) and two levels of fat (Lower Fat at 2.43% and 2.66% vs Higher Fat at 5.25% and 7.25%), and contained lysine at 0.61% to 0.78% (Table 2). The dietary lysine/protein ratio was 0.037 for20% protein and Lower- fat, 0.030 for 20% protein and Higher- fat, 0.032 for 23% protein and Lower- fat, 0.034 for 23% protein and Higher- fat. The each formulated feed was packed in plastic bag and sealed in flexible container until use.

Each treatment group of eight pigs was fed in each pen, thus four pens were used for the experiment. The pigs were cared for according to Japanese Basic Rule for Keeping and Managing Farm Animals [12]. All animals had free access to drinking water and feed. The average daily gain was calculated based on initial and final body weights.

Table 1: Composition of examined feed for growing-finishing pigs.

Protein levels	20% Protein		23% Protein	
Fat levels	Lower Fat	Higher Fat	Lower Fat	Higher Fat
	Ingredients‡			
Boiled Chinese noodles§	81.53	81.84	81.47	81.88
Wheat bran	9.64	8.39	8.76	5.63
Soybean meal	1.15	1.79	4.54	5.52
Chocolate¶		4.32		4.47
Defatted rice bran	1.02	1.7		
Bread crumbs and stale bread, Dry	3.77		2.63	
Vegetables†1	1.02	1.02	1.02	1.02
Corn gluten meal, 60%CP			0.55	0.74
Sugar	0.92		0.2	
Lysine hydrochloride	0.11	0.1		
Dicalcium phosphate	0.43	0.43	0.43	0.43
Calcium carbonate	0.31	0.31	0.31	0.21
Vitamin mineral premix‡1	0.1	0.1	0.1	0.1

‡wet form, fresh as fed basis.

§ wet form, boiled. DM=35.0%. Lysine content was 0.128%

¶chocolates which were mixed with black and white.

†1 fresh leaves of lettuce and the cabbage which which were discarded in the supermarket.

‡1 Vitamin mineral premix: Provided per kilogram diet; 2,500 IU Vitamin A , 3,500IU Vitamin D, 0.5mg α-tocopherol, 0.1mg thiamin nitrate, 600mg riboflavin, 0.05mg pyridoxine hydrochloride, 0.001mg cyanocobalamin, 0.12mg nicotinic acid amide, 0.2mg menadione, 0.5mg D-(+)-Pantothenic acid calcium salt, 10mg Choline chloride, 0.1mg folacin, 0.602mg $MnCO_3$,2,170 $ZnCO_3$, 4.2mg $FeSO_4$, 2.7mg $CuSO_4$, 0.25mg $CoSO_4$, 0.006µg Calcium iodate, 0.002µg d-Biotin, 10µg Lysine hydrochloride.

Carcass composition

Table 2: Analytical composition of examined feed as dry matter basis for growing-finishing pigs.

Protein levels	20% Protein		23% Protein	
Fat levels	Lower Fat	Higher Fat	Lower Fat	Higher Fat
Crude protein, %	19.95	20.17	23.11	23
Crude fat, %	2.66	5.25	2.43	7.25
Crude ash	4.52	4.14	4.87	4.5
Ca, %	0.8	0.66	0.75	0.73
P, %	0.64	0.61	0.71	0.65
Amino acids, %				
Ile	0.61	0.64	0.75	0.8
Leu	1.14	1.16	1.41	1.5
Lys	0.74	0.61	0.75	0.78
Met	0.45	0.47	0.56	0.45
Cys	0.37	0.36	0.41	0.35
Phe	0.82	0.86	0.95	0.93
Tyr	0.53	0.58	0.66	0.65
Thr	0.56	0.58	0.71	0.7
Trp	0.19	0.19	0.22	0.18
Val	0.77	0.77	0.95	1.03
Arg	0.74	0.75	0.92	0.9
His	0.4	0.39	0.46	0.45
Ala	0.72	0.77	0.92	0.98
Asp	0.98	1.05	1.41	1.4
Glu	4.52	4.36	4.94	4.93
Gly	0.74	0.75	0.88	0.85
Pro	1.49	1.46	1.58	1.63
Ser	0.82	0.83	1	0.98
Fatty acids, %				
C14:0	1.54	1.39	2.19	2.17
C14:1	0.56	0.4	1.16	1.12
C16:0	28.03	23.82	22.35	24.24
C16:1	1.67	1.75	3.71	2.9
C18:0	10.34	17.43	11.89	17.62
C18:1	31.55	36.55	40.63	34.78
C18:2	25.99	18.44	17.05	16.69
C18:3	0.32	0.22	1.02	0.48
Saturated	39.91	42.64	36.43	44.03
Monounsaturated	33.78	38.7	45.5	38.8
Polyunsaturated	26.31	18.66	18.07	17.17

Pigs were slaughtered at a commercial abattoir in Osaka. Carcass weight, back fat thickness, and dressing percentage were measured. Back fat thickness was measured at the thinnest section between the 9th and 13th ribs by Japan Meat Grading Association. After the carcasses were chilled for approximately two days at 1°C, the loin (20 cm thickness) with subcutaneous back fat was removed from the 5-6th ribs to the caudal end on the right side of each carcass. The cuts were vacuum-packed in plastic bags and stored at −20 °C for analysis.

Meat quality

Meat and fat color were measured using the Japanese pork color standards based on a 6-point scale for meat (1=extremely pale pink, 6=extremely dark red) and a 4-point scale for fat (1=very white, 4=very colored) [13], respectively. The color of the meat was measured according to the 1976 CIELAB system using a colorimeter of CM–S100w (Konica Minolta Holdings Inc., Tokyo, Japan). Marbling and seam fat score (1=little fat, 5= much fat) were assessed according to the Pork Standards of National Pork Producers Council [14,15].

The longissimus dorsi muscle (LM) from each loin sample was minced for chemical analysis. Moisture, crude protein, ether extracts and ash content were measured using the following methods, respectively: oven drying at 135°C for 2 h, Kjeldahl digestion, Soxhletextraction and muffle heating (600°C for 3 hours). Water holding capacity of the LM was measured by the beads methods with high-speed centrifuge [16].

A core of LM 5 cm in diameter was taken, the raw weight measured, and a thermossensor inserted into the meat. The sample was packed with glass beads in a plastic bag and heated in boiled water at 100 °C until the internal temperature reached 71°C. After cooling, the meat was weighed and the cooking loss was calculated from the weight difference. The cooked sample was stored at 4°C for 24 h. Then, the Warner-Bratzler (W-B) shear force was measured on a cylindrical core 1.3 cm in diameter taken from the meat by Instron Universal Testing machine (model 3344, Instron Inc., Glenview, Illinois, USA).

The melting point and refractive index of the inner layer of backfat from the LM were measured using a capillary tube [17] and a reflectometer (Atago, Tokyo, Japan) [18], respectively. Lipids from the LM and back fat were extracted with chloroform and methanol solution and assessed for fatty acid composition. After methyl esterification with boron trifluoride methanol, the fatty acid composition was determined by using a gas chromatograph (Shimadzu Inc., Kyoto, Japan) equipped with a capillary column (DW–WAX122–7062, 0.25 mm × 60 m). The temperatures of the column and detecting device were 160°C and 270°C, respectively. Eight fatty acids (14:0, 14:1, 16:0, 16:1, 18:0, 18:1, 18:2, and 18:3) were identified from the relative retention time of each standard material, and the total of these 8 ingredients was expressed as 100%.

Statistical analysis

Two-way ANOVA and Tukey-Kramer test were adopted to determine the statistical significance of differences in dietary protein level, fat level, and the interaction between these variables by using JMP®7. To investigate the relationships between IMF or marbling score and other meat characteristics in 30 pigs, simple regression analysis was performed. Two sets of data for carcass traits and meat quality from two pigs were not obtained as a result of an accident during transportation. Therefore, the number of data for carcass traits and meat quality were 20% protein (Lower-Fat, n = 7, and Higher-Fat, n = 7) and 23% protein (Lower-Fat, n = 8, and Higher-Fat, n = 8).

Results

Growth performance and carcass traits

There were no significant effects of dietary protein, fat levels, or their interaction on final body weight and the duration of fattening period in finishing pigs (Table 3). Daily gain was slightly greater with the Higher-fat diets. There were also no significant effects of dietary protein or fat level on carcass weight, back fat thickness or dressing percentage.

Meat characteristics

The color score of the pork (according to the pork color standard, PCS) was significantly lower with increased dietary protein contents, but was not affected by dietary fat level (Table 4). Increased dietary protein level resulted in a higher marbling score (P = 0.03), but dietary fat level did not affect marbling. The seam fat score was tended to be

Table 3: Effects of dietary protein and fat levels at adequate lysine content on growth and carcass characteristics for growing-finishing pigs.

	Protein level		Fat level		P-value			
	20%	23%	Lower	Higher	Pooled SEM	Protein	Fat	Protein × Fat
Growth performance								
Initial body weight, kg	54.4	54.6	54.3	54.7	1.03	0.9	0.9	1
Final body weight, kg	118.5	117.3	117.2	118.6	0.38	0.1	0.1	0.66
Fatting period, days	58.75	57.5	59.63	56.63	1.4	0.7	0.3	0.67
Daily gain, kg/day	1.1	1.1	1.07	1.13	0.02	1	0	0.55
Carcass characteristics								
Carcass weight, kg	78.5	77.4	77.5	78.3	0.42	0.2	0.4	0.77
Backfat thickness, cm	3.3	3.3	3.3	3.3	0.12	0.9	0.8	0.7
Dressing percentage	66.2	65.9	66	66	0.25	0.6	1	0.77

20% Protein + Lower Fat n=7, 20% Protein+ Higher Fat n=7, 23% Protein + Lower Fat n=8, 23% Protein + Higher Fat n=8.

* indicate significant difference (P < 0.05).

Table 4: Effects of dietary protein and fat levels at adequate lysine content on meat quality of logissimusdorsi muscle.

	Protein level		Fat level		P-value			
	20%	23%	Lower	Higher	Pooled SEM	Protein	Fat	Protein × Fat
Pork color standard	2.7	2.3	2.6	2.3	0.1	0.02	0.18	0.93
Marbling score	3.4	4.3	3.8	3.9	0.2	0.03	0.86	0.85
Seam fat score	1.6	2	1.7	2	0.1	0.06	0.08	0.62
Muscle, L*	58.2	60.5	60	58.9	0.91	0.24	0.56	0.57
a*	2.3	2.9	2.2	3	0.23	0.16	0.1	0.51
b*	16.8	17.4	16.8	17.4	0.2	0.09	0.09	0.58
pH	5.7	5.71	5.69	5.72	0.01	0.2	0.09	0.27
Water holding capacity, %	66.63	63.29	65.93	63.77	0.73	0.01	0.09	0.06
Cooking loss, %	20.6	18.51	20.55	18.42	0.65	0.1	0.1	0.5
Shear force, N	19.93	14.65	17.84	16.38	1.05	< 0.01	0.43	0.07
Intramuscular fat, % §	7.91	10.65	9.86	8.88	0.63	0.03	0.41	0.56
Moisture, %	69.9	67.9	68.7	68.9	0.52	0.06	0.79	0.46
Crude protein, %	21.1	20	20.3	20.8	0.17	< 0.01	0.12	0.72
Crude ash, %	1.1	0.99	1.01	1.07	0.02	< 0.01	0.08	0.68

20% Protein + Lower Fat n= 7, 20% Protein+ Higher Fat n=7, 23% Protein + Lower Fat n=8, 23% Protein + Higher Fat n=8.

*indicate significant difference (P< 0.05). **indicate significant difference (P< 0.01).

§Ether extract was considered as IMF content.

a little higher with increased dietary protein (P = 0.06) and higher fat levels (P = 0.08). There was no interaction detected between the effects of dietary protein and fat on the marbling or seam fat score.

The colorimeter measurements, a* and b* tended to be slightly higher with increased dietary protein and higher fat (P = 0.09–0.16). Cooking loss tended to be slightly lower with increased dietary protein and higher fat (P = 0.10). The W-B shear force and water holding capacity were significantly lower with a higher dietary protein (P<0.01).

Average IMF content was 1.35 times higher in the 23% protein groups (10.65%) than in the 20% protein groups (7.91%). The range in IMF content was 4.61–17.01% in the 23% protein groups and 3.02–10.66% in the 20% protein groups. Mean IMF content of 20% protein Lower-fat group, 20% protein Higher-fat group, 23% protein Lower-fat group and 23% protein Higher-fat group were8.76%, 7.04%, 10.82% and 10.48%, respectively.

IMF content was strongly positively correlated with marbling score (r = 0.84, P< 0.01) and strongly negatively correlated with moisture (r = –0.97, P< 0.01) (Table 5). Both IMF content and marbling score were positively correlated with seam fat score, a* and b* (P< 0.05) and negatively correlated with moisture, crude protein, crude ash and W-B shear force (P< 0.05).

Fat characteristics

With higher dietary protein, the contents of C14:0, C18:2and polyunsaturated fatty acid were slightly lower in the LM, while C16:0 content was higher (P< 0.05) (Table 6). The C18:0content of the LM tended to be lower with higher dietary fat (P = 0.09).

The melting point of the inner layer back fat was not affected by dietary protein level, but was slightly lower (by 1.6°C) with the Higher-fat diets. The refractive index of the back fat was slightly higher with Higher-fat diet, and there was a significant interaction between dietary protein and fat level in determining the refractive index. The contents of saturated fatty acid and C18:0 were slightly lower, and the contents of C14:0, C14:1, C18:3 and monounsaturated fatty acid were slightly higher, with the Higher-fat diets.

Table 5: Simple regression coefficients between intramuscular fat content or marbling score and other characteristics in longissimus dorsi muscle.

	vs Intramuscular fat†	vs Marbling score
Marbling Score	0.84**	
Pork color standard	-0.37*	-0.15
Seam fat score	0.51**	0.49**
Muscle, L*	0.56**	0.32
a*	0.37*	0.54**
b*	0.53**	0.55**
pH	-0.16	-0.15
Moisture	-0.97**	-0.86**
Crude protein	-0.62**	-0.41*
Crude ash	-0.71	-0.62**
Water holding capacity, %	-0.15	-0.06
Cooking loss	-0.24	-0.47**
Shear force	-0.53**	-0.45*

† Intramuscular fat was considered as ether extract. n=30.

20% Protein + Lower Fat n=7, 20% Protein+ Higher Fat n=7, 23% Protein + Lower Fat n=8, 23% Protein + Higher Fat n=8.

* indicate significant correlation (P< 0.05). ** indicate significant correlation (P<0.01).

Table 6: Effects of dietary protein and fat levels at adequate lysine content on fat quality.

	Protein level		Fat level		P-value			
	20%	23%	Lower	Higher	Pooled SEM	Protein	Fat	Protein × Fat
Longissimus muscle								
Fatty acids, %								
C14:0	1.9	1.59	1.66	1.82	0.06	< 0.01	0.11	0.65
C14:1	0.52	0.25	0.36	0.4	0.09	0.14	0.82	0.42
C16:0	27.54	28.46	28.27	27.79	0.2	0.02	0.2	0.49
C16:1	3.62	3.33	3.38	3.54	0.11	0.21	0.48	0.81
C18:0	14.53	14.94	15.17	14.32	0.25	0.41	0.09	0.83
C18:1	47.29	47.67	47.16	47.82	0.44	0.68	0.48	0.9
C18:2	4.31	3.63	3.79	4.11	0.16	0.04	0.31	0.67
C18:3	0.29	0.13	0.21	0.2	0.05	0.13	0.93	0.35
SFA	43.97	44.99	45.1	43.93	0.39	0.19	0.13	0.68
MUFA	51.43	51.25	50.9	51.76	0.4	0.83	0.3	0.91
PUFA	4.6	3.76	4	4.31	0.2	0.04	0.42	0.56
Subctaneous inner fat								
Pork fat color standard	1.9	1.9	1.9	1.9	0.1	0.96	1	0.51
Refractive index, nD	1.4546	1.4546	1.4549	1.4551	0.00004	0.66	0.03	< 0.01
Melting point, ℃	38.4	39.1	39.6	38	0.49	0.48	0.09	0.23
Fatty acids, %								
C14:0	1.76	1.96	1.68	2.04	0.07	0.12	< 0.01	0.18
C14:1	0.35	0.47	0.32	0.51	0.03	0.07	< 0.01	0.3
C16:0	27.54	27.19	27.8	26.91	0.32	0.59	0.18	0.58
C16:1	2.18	1.99	2.08	2.09	0.11	0.42	0.96	0.19
C18:0	19.05	19.04	19.66	18.43	0.23	0.99	< 0.01	0.98
C18:1	40.54	40.79	40.06	41.28	0.31	0.68	0.06	0.66
C18:2	8.39	8.33	8.22	8.49	0.11	0.79	0.22	0.61
C18:3	0.19	0.23	0.18	0.25	0.01	0.07	< 0.01	0.8
SFA	48.35	48.19	49.14	47.38	0.36	0.81	0.02	0.76
MUFA	43.07	43.25	42.46	43.88	0.34	0.79	0.04	0.88
PUFA	8.58	8.56	8.4	8.74	0.11	0.94	0.14	0.64

20% Protein + Lower Fat n=7, 20% Protein+ Higher Fat n=7, 23% Protein + Lower Fat n=8, 23% Protein + Higher Fat n=8.

* indicate significant correlation (P< 0.05). ** indicate significant correlation (P< 0.01).

SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.

Discussion

In the present study, two sexes of pigs, barrows and gilts, were adopted, because the study carried out under the practical condition in pig farm in Wakayama prefecture, to provide the effective method on famer and pig industries. The barrows and gilts were kept in same pen that was practical in many pig farms. The main effect of sex were significantly observed in only seam fat score (P = 0.01), and no significant interaction were observed in Sex×Protein, Sex×Fat and Sex×Protein×Fat. Thus, we discussed the effects of Protein, Fat and Protein×Fat, below.

Effect on growth performance

In this study, growth performance in terms of feed intake, duration of the fattening period, daily gain and feed conversion were similar to the values obtained with routine diets at our research station. Average daily gain for each dietary group was more than 1 kg, as expected according to the daily weight gain in the Japanese feeding standard [10]. Daily gain was not affected by dietary protein level, but was slightly greater on the Higher-fat diets. This was as expected, because these diets contained 0.2 Mcal/kg more digestible energy. An increase in dietary energy by means of oil supplementation also results in an increase in daily gain in pigs [19].

In the present study, the growth performance was normal, as the result of a sufficient intake of crude protein, lysine, and energy. According to the Japanese daily nutrient requirements for pigs [10], ensuring a daily gain of 1.00 kg/d requires a nutritional fill percentage of171% in 23% protein and 148% in 20% protein diets for crude protein, and 103% in Lower-fat and 112% in higher-fat diets for digestible energy. Our dietary treatments therefore fulfilled the daily nutrient requirements for crude protein, energy and lysine to ensure proper growth performance. This suggests that increasing dietary protein with sufficient (not too much) lysine is a practical method for increasing IMF content, as discussed below.

Effect on carcass traits

The different dietary protein and fat levels in the diets did not significantly affect carcass weight, back fat thickness or dressing percentage. Although the average ether extract of the Higher-fat diets was 2.4 times higher than that of the Lower-fat diets, the effect of dietary fat level on these aspects of the carcass was not significant, because all four diets differed indigestible energy at only 0.2 Mcal/kg. Thus, digestible energy difference at 0.2 Mcal/kg could affect daily gain, but not carcass traits.

Back fat thickness in all groups was greater than the “good” range (1.3-2.4 cm) as defined by the Japanese pork carcass standard [13]. Indeed, the average back fat thickness (3.3 cm) was higher than normal for pig’s from our research institute. This can be explained by the high digestible energy in feed 3.9-4.1 Mcal/kg compared to the standard Japanese nutrient requirement [10]. In the present study, digestible energy in the examined feed were higher than commercial feed of finishing pigs, because one of the primary aim in this study was to evaluate the effect of dietary fat level on IMF content, hence the examined feed included high energy. Beaulieu et al., [20] reported that back fat thickness increases linearly when dietary energy is increased via supplementation of fat. This also results in an increase in dressing ratio. It has been reported that a 5% oil supplement results in increased back fat [21], but this would be because of the increase in dietary energy.

Effect on meat quality

In this study, IMF and marbling score were greater with higher dietary protein, under conditions of similar sufficient lysine for the growth of finishing pigs. Moreover, in the present study it showed that the major cause of IMF increase is dietary increase in protein with some constant lysine. The increase in dietary protein in the present studyhad no effect on growth performance. Increasing dietary protein on by-product feed may therefore be a less costly way of increasing the production of marbled pork, since it does not affect the growth of finishing pigs.

In the present study, IMF increased with increasing dietary protein as well as dietary amino acids, as arginine, leucine, threonine and glutamic acid. These results are consistent with the hypothesis that increasing dietary specified amino acids result in increasing IMF as follow. Ma et al., [22] reported that supplementing the diet with 1% arginine resulted in increased IMF content. According to Hyun et al., [23], a high-leucine diet with 0.5% lysine increased IMF. Plitzner et al., [24] reported that IMF increased as result of increasing the dietary threonine/lysine ratio from 0.60 to 0.68 and 0.81.Hu et al., [25] reported dietary supplementation with 1% arginine and 1% glutamic acid increased IMF. On the other hand, it was reported that dietary protein deficient caused increasing IMF. Castell et al., [26] reported that IMF increased with decreased dietary crude protein from 17.6% to 11.9%. Goerl et al., [27] reported that IMF increased when dietary protein decreased from 13% to 10%, while Allee et al., [28] reported that a high protein diet reduced fat synthesis in one instance but not in another. Similarly, it was reported that dietary lysine deficient resulted in increasing IMF. Hyun et al., [23] reported that a lysine-deficient diet might reduce protein synthesis, resulting in fat accumulation in the muscle. Katsumata [4] argued that this effect might be caused by an increase in the oxidative capacity of porcine muscle due to enhanced production of citrate synthase. These reports differ from our results in that increasing dietary protein, as amino acids, caused increasing IMF. One plausible explanation for these differences in contents of dietary protein to influence on IMF contents is that the two types of specified dietary amino acids may affect the IMF content; the some dietary amino acids may increase IMF and the other such as lysine may decreases IMF.

Another likely explanation is decreasing dietary lysine/protein ratio. We reported that adequate dietary protein and lysine, with decreasing dietary lysine/protein ratio resulted in increasing IMF [7]. Additionally, Takahashi et al., [5] reported that the interaction between dietary lysine and protein levels has a significant effect on IMF, and concluded that dietary lysine/protein ratio is one of the most important factors for increasing IMF. However, in the present study, dietary lysine/protein ratio did not decrease when IMF was increased. It can be presumed that increasing dietary protein lead to increasing dietary arginine and leucine, and may decrease lysine absorption or may decrease lysine/protein ratio at the absorption stage. The digestion and absorption of amino acids were affected by the presence of other amino acids. For example, there may be antagonisms between lysine and arginine and between leucine, isoleucine and valine [29]. Our results suggest other possibilities for this mechanism, specifically related to suitable lysine and higher protein. There is a possibility that some excess amino acids, which are not utilized in protein synthesis, may promote lipid synthesis in the muscle tissue, under the condition of limiting the dietary lysine that inhibits IMF accumulation. We hypothesize that some excess amino acids not utilized in protein synthesis promote lipid metabolism, although further investigations are needed to identify the effects of dietary specified amino acids on increasing IMF content of pork.

The present results that IMF content is not affected by dietary fat levels agree with our previous study [7]. This does not mean that dietary fat is unrelated to increasing IMF when digestible energy is changed. In addition, Albin et al., [30] reported that dietary fat content affects amino acid digestion. The effect of dietary fat and amino acid digestion on IMF production requires further investigation.

In the present study, average IMF was so rich (7.04–10.82% in all groups). There are some factors affecting IMF. For example, Suzuki et al., [31] reported IMF increased by breeding and Ashihara et al., [32] reported that restriction of dietary protein and energy in suckling piglets result in increasing IMF contents.

There was a significant correlation ($r = 0.51$, $P < 0.01$) between IMF and seam fat score. Seam fat score was slightly higher in the higher protein and Higher-fat groups than in the Lower protein and Lower-fat groups. These feed effects were small on seam fat as well as on back fat thickness, compared with IMF. The effects on fat accumulation probably vary in different tissues. Doran et al., [33] reported that a reduced protein diet increased stearoyl-CoA desaturase protein expression in muscle tissue, but not in subcutaneous adipose tissue in pigs. In an in vitro study, Wang et al., [34] suggested that differences between the differentiation of lipid accumulation in intramuscular and subcutaneous preadipocytes might be related to differences in glucose utilization and lipid metabolism. Madeira et al., [35] reported that when pigs were fed a reduced-protein diet, an increase in IMF content was accompanied by increased fatty acid synthase and stearoyl-CoA desaturase mRNA levels.

Pork color score of PCS and colorimeter measurement were significantly affected by increasing dietary protein. The changes in meat color were caused principally by deposition of white fat in the LM.

We observed negative correlations between IMF and moisture, crude protein and crude ash. Thus, the increase in IMF was accompanied by a decrease in these elements of muscle, in particular, the moisture. This decrease in moisture with increasing IMF has also been reported by other researchers [1,21,23].

The shear force of the LM decreased with an increase in dietary protein and was negatively correlated with IMF, which is consistent with previous reports [1,36]. Cooking loss tended to decrease with higher dietary protein ($P = 0.01$) and higher dietary fat ($P = 0.01$), and was negatively correlated with marbling ($P< 0.01$). This decrease in cooking loss and increase in marbling score with higher dietary protein may improve the palatability of pork. Indeed, Hodgson et al., [1] reported that cooking loss was negatively correlated with marbling and the overall palatability of pork.

Effect on fat characteristics

The melting point of the inner layer of back fat was not affected by dietary protein level and was slightly lower with higher dietary fat ($P = 0.09$), although the difference was only 1.6°C. This decrease in melting point could be explained by the decrease in saturated fatty acids and increase in monounsaturated fatty acids caused by the increase in dietary fat contained in the chocolate. The increase in dietary fat resulting from chocolate supplementation did not result in soft fat in the present study [7,8]. In general, increasing dietary fat tends to result in soft fat in pork with an elevated C18:2 content [37,38]. A possible reason for this is that chocolate is made from palm and coconut oils, which contain over 60% saturated fatty acid [11], so the dietary saturated fatty acid content in the Higher-fat group may be higher than in the Lower-fat group.

Conclusion

Our results suggest that the increase in dietary protein, maintaining sufficient lysine for growth, can result in increased IMF without adversely affecting daily gain in finishing pigs. Dietary contents and balance of various amino acids are probably important for IMF of pork. Although dietary protein is expensive in commercial feed, protein is rich in many by-products. By-products such as cereals, bread, noodle etc have low lysine content. It is easy to make the feed (we call the feed with “amino acid balance method”) with high protein and suitable lysine. Indeed, pig farmers near city have already succeeded to produce the marbled pork by feeding by-products. The marbled pork is traded at higher price than the ordinary pork fed commercial feed. Use of by-products is also useful for recycled society. Further developments are expected in the near future.

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