# EFFECTS OF LACTIC ACID-FERMENTED FISH BYPRODUCTS ON QUALITY OF PORK MEAT

Satoshi Kawahara<sup>1</sup>, Akiko Nakajima<sup>1</sup>, Masa-aki Tsugeta<sup>2</sup>, Shuichi Nagasaki<sup>2</sup>,

Shinjiro Horinouchi<sup>3</sup>, and Masayoshi Iwakiri<sup>3</sup>

<sup>1</sup>Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan

<sup>2</sup> Yoghurt Feed Inc., Miyazaki, Japan

<sup>3</sup> Miyazaki Livestock Research Institute, Kawaminami br., Miyazaki, Japan

Abstract - We investigated the effects of a diet containing lactic acid-fermented fish byproducts (LFB) on the fatty acid composition and quality parameters of pork. Pork loin samples were collected from pigs fed a diet containing 49% LFB, and the characteristics of their meat was compared with that from animals fed conventional or 5% fishmeal diets. The LFB diet did not affect the color of lean meat, drip loss, and the melting point of subcutaneous fat. However, the LFB diet resulted in reduced lipid content and a slightly brown color of pork fat. The compositions of EPA and DHA were significantly increased by LFB feeding (P < 0.01). As a result, the n-6/n-3 ratio of pork lipid in the LFB group was improved relative to the conventional and fishmeal groups (P < 0.01). However, enrichment of n-3 polyunsaturated fatty acids (PUFA) in pork deteriorated certain sensory quality attributes, possibly due to lipid oxidation. In conclusion, LFB feeding could be a viable method of producing pork rich in n-3 PUFA, but more study is needed to improve sensory quality of pork produced with the LFB diets.

Key Words – lactic acid-fermented fish byproducts, n-3 polyunsaturated fatty acids, oxidation.

# I. INTRODUCTION

The total quantity of byproducts from fish processing in Japan is estimated to be 2.2 million tons per year. Fish byproducts are rarely recycled for animal feed because they tend to spoil quickly. We have developed a method for recycling fish byproducts by means of anaerobic lactic acid fermentation. The use of lactic acid fermentation allows the flesh of fish byproducts to be preserved without heat drying. This may provide both high nutritional quality of the final feed products and economic benefits [1].

Meanwhile. many experiments have indicated that feeding of fishery resources such as fishmeal and fish oil leads to the development of unfavorable flavors and odors in the pork. Davies [2] reported that addition to swine diets of fishmeal at levels higher than 5% imparts fishy flavor to pork. Øverland et al. [3] found high off-flavor and off-odor with 1% or more fish oil feeding to pigs. It is important to know the effect of lactic acid-fermented fish byproduct (LFB) feeding on meat flavor because consumer acceptability of meats is determined generally by their flavor.

The aims of the present study are to investigate the possible deposition of n-3 polyunsaturated fatty acids (PUFA) from LFB in meat, and to evaluate the sensory and physicochemical qualities of the resulting meat products.

# II. MATERIALS AND METHODS

Twenty-four gilts (Landrace X Large White X Duroc; 30 kg each) were assigned to 1 of the 3 dietary groups. There were 2 pigs per pen and 4 pens per treatment. The animals had free access to experimental diets and water *ad libitum*.

Conventional diet was a basal, commercial ration. Fishmeal diet was the basal ration contained 5% commercial fishmeal (CP 65%). LFB diet was a mixture of equal amounts (49% each) of the basal diet and LFB with added vitamins and minerals. The LFB used in this study contained 35% fresh byproduct of horse mackerel (*Trachurus japonicus*) provided by a marine product company. The minced ingredients and starter were well mixed, and fermented anaerobically in a polyethylene bag for 1 month at room temperature. The safety of LFB is confirmed for heavy metals (lead,

cadmium, mercury, and arsenic) by periodic self-inspection.

After about 3 months of feeding with the experimental diets, the pigs attained weights of approximately 110 kg and were slaughtered at a local commercial slaughterhouse. Loin muscles (*M. longissimus thoracis*) were separated, and a portion of each loin muscle was divided for quality measurements. The remaining portions were vacuum-packaged and stored at -60°C until analysis. The treatment and management of the animals were done according to the guidelines for the care and use of experimental animals of the Miyazaki Livestock Research Institute.

Total lipids were extracted with chloroform and methanol [4]. Fatty acid methyl esters (FAME) were prepared with HCl/methanol [5]. The resulting FAME was analyzed on a gas chromatograph (GC–2010; Shimadzu, Japan). The conditions used for gas chromatography were the same as previously described [5].

The concentration of  $\alpha$ -tocopherol was determined using a high-performance liquid chromatograph (LC-10AT; Shimadzu) equipped with a fluorescence detector (RF-530; Shimadzu). The sample-preparation method and HPLC conditions were the same as the previous paper [5].

The surface color (CIE L\*, a\*, b\*) of lean meat and fat around the lean portions was measured in triplicate on a freshly cut surface after a 30-min bloom time by using a Minolta ChromaMeter (Minolta CR-200, Japan).

Drip loss was measured in duplicate by the method of Barton-Gade *et al.* [6], and the results are expressed as a percentage of the original weight. The drip loss was measured on non-illuminated chops after 48 h at 4°C.

The melting point of subcutaneous fat was measured according to the method described by the Japan Oil Chemists' Society [7].

Thiobarbituric acid-reactive substances (TBARS) value of pork after refrigerated storage (at 10°C for 7 days) was determined by use of a steam distillation method described [5].

Sensory evaluation was conducted using 24 panelists. Before the sensory evaluation, each panelist twice underwent a brief training program in evaluating meats according to the guidelines [8]. The panelists first evaluated the appearance of the raw meat and then the texture,

odor, and taste of cooked meat. Meat samples (3-cm cube) were boiled in 1% saline at 95°C for 10 min, and immediately chopped into 1-cm cubes. The cooked samples were randomly served to the panelists to reduce the effect of the order of presentation.

The data were analyzed with analysis of variance (ANOVA). When significant effects were observed from the F test, we carried out a post-hoc test (Tukey's test) to compare the means.

## III. RESULTS AND DISCUSSION

The feed intake of pigs was higher in the LFB group than in the other groups (Table 1). However, the daily gain of pigs raised with the LFB diet was lower than those with the other diets. The amount of dry matter in the LFB diet was lower than those in the other diets. Thus, the poor growth performance could be caused partially by a decreased feed efficiency of the LFB diet containing higher level of water.

Table 1 Effects of LFB feed on growth performance, color, drip loss, and intermuscular fat content of pork loin

Daramatara	Dietary group			
Farameters	Conventional	Fishmeal	LFB	
Growth performance				
Dairy gain (g / day)	950 (80) <sup>a</sup>	874 (80) <sup>ab</sup>	784 (77) <sup>b</sup>	
Feed intake (kg)	$192(21)^{a}$	$192(10)^{a}$	286 (27) <sup>b</sup>	
Lean meat				
Lightness, L*	51.4 (3.8)	54.6 (1.3)	53.3 (1.1)	
Redness, a*	12.0 (0.5)	11.2 (0.7)	12.9 (0.6)	
Yellowness, b*	10.3 (0.2)	9.1 (0.3)	9.2 (0.2)	
Fat				
Lightness, L*	76.1 (0.1) <sup>a</sup>	75.3 (0.9) <sup>ab</sup>	73.2 (1.0) <sup>b</sup>	
Redness, a*	$4.9(0.3)^{a}$	$5.3(0.5)^{a}$	$6.8(0.4)^{b}$	
Yellowness, b*	$7.8(0.5)^{a}$	$6.8(0.3)^{a}$	$10.9(1.1)^{b}$	
Drip loss, %	5.78 (1.46)	5.17 (1.78)	5.31 (1.25)	
Fat content, %	7.42 (3.26) <sup>a</sup>	4.75 $(1.15)^{ab}$	3.40 (1.01) <sup>b</sup>	
Melting point (°C)	30.6 (1.0)	30.5 (0.9)	30.6 (0.7)	

Data were presented as means (n=8). Values in parentheses were standard deviations. <sup>a,b,c</sup> Within a row, means without a common superscripts differ statistically (P < 0.01).

There were no effects of diet on lean meat color and drip loss. The color of fat located around the lean meat portions was significantly influenced by LFB feeding (P < 0.05). While

the difference in color was not visually apparent, the fat around the lean meat portions in the LFB group was slightly brown.

Pork with high levels of PUFA in its fat is generally disfavored because of its poor appearance, which is attributable to a low melting point of fat. In this study, the melting points of subcutaneous fats did not differ among the groups fed the different diets. Interestingly, the fat content of pork loin was significantly lower in the LFB group than in the conventional group (P < 0.05), although the crude fat content in the feeds was higher in the LFB diet than in the other diets (Table 1).

The levels of EPA and DHA in pork loin from the LFB group were significantly higher than those of the other groups (P < 0.01; Table 2). The total percentage of the two kinds of fatty acids in the LFB group with respect to total fatty acids was in excess of 3.5%, whereas the corresponding percentage in the other groups was below 0.3%.

Table 2 Effect on fatty acid composition (% of total fatty acids) and vitamin E content ( $\mu$ g/100 g) of pork from pigs fed LFB

Daramatara	Dietary group			
Parameters	Conventional	Fishmeal	LFB	
C14:0	$1.3 (0.1)^{a}$	$1.5 (0.1)^{a}$	1.7 (0.2) <sup>b</sup>	
C16:0	24.6 (0.6)	25.0 (1.0)	25.0 (0.4)	
C18:0	$12.2 (0.6)^{a}$	12.5 (0.6) <sup>a</sup>	13.9 (0.9) <sup>b</sup>	
C18:1 n-9	$40.9(0.6)^{a}$	$40.1(1.3)^{a}$	32.3 (2.6) <sup>b</sup>	
C18:2 n-6	$9.1(1.1)^{a}$	$9.3(1.8)^{a}$	11.8 (1.6) <sup>b</sup>	
C18:3 n-3	$0.3 (0.1)^{a}$	$0.3 (0.1)^{a}$	$0.5 (0.1)^{b}$	
C20:4 n-6	$0.8 (0.4)^{a}$	$0.9 (0.4)^{ab}$	$1.4 (0.6)^{b}$	
C20:5 n-3	$0.0 (0.0)^{a}$	$0.1 (0.0)^{a}$	$1.5(0.6)^{b}$	
C22:6 n-3	$0.0 (0.0)^{a}$	$0.2 (0.1)^{a}$	$1.9 (0.4)^{b}$	
Total SFA	38.6 (0.9) <sup>a</sup>	39.4 (1.7) <sup>a</sup>	41.4 (1.0) <sup>b</sup>	
Total MUFA	50.8 (0.6) <sup>a</sup>	$49.2(1.5)^{a}$	40.8 (2.6) <sup>b</sup>	
Total PUFA	$10.6 (1.1)^{a}$	$11.4(2.4)^{a}$	17.8 (2.8) <sup>b</sup>	
PUFA/SFA	$0.28 (0.04)^{a}$	$0.29 (0.07)^{a}$	$0.43 (0.07)^{b}$	
n-6/n-3	30.5 (11.2) <sup>a</sup>	14.4 (0.6) <sup>b</sup>	$3.5(0.6)^{c}$	
Vitamin E	$318(81)^{a}$	330 (28) <sup>b</sup>	$204(81)^{c}$	

Data were presented as means (n=8). Values in parentheses were standard deviations. <sup>a,b,c</sup> Within a row, means without a common superscripts differ statistically (P < 0.01).

The total PUFA composition dramatically increased in the LFB group relative to the other groups. However, the P/S ratio of the pork fat remained at approximately 0.4. On the other hand, the LFB feeding considerably decreased the n-6/n-3 ratio of pork fat to a value of 3.5. The recommended n-6/n-3 ratio of a human diet

is below 4.0 [9]. This result suggests that feeding of LFB to growing/finishing pigs is a possible means for improving the nutritional value of pork with respect to lipids.

Vitamin E levels in the LFB group dropped by 35% relative to the other groups. The vitamin E levels in the diets were of 38.9 mg/kg DM for the conventional diet, 39.0 mg/kg DM for the fishmeal diet, and 34.1 mg/kg DM for the LFB diet, respectively.

The TBARS values at day 0 did not differ among the dietary groups. After 7 days of storage at 10°C, the TBARS values of the LFB group were much higher than those of the other groups (P < 0.01), indicating that significant lipid oxidation occurred in the LFB meat during storage (Figure 1). Pork from the LFB group contained more PUFAs such as EPA and DHA than the pork from the other 2 groups. Thus, the pork fat from the LFB group would be rapidly oxidized during cold storage for 7 days. Additionally, the vitamin E level in the LFB pork was decreased by 65% relative to meats from the other groups (Table 2). Vitamin E is the major lipid-soluble antioxidant in animal tissue, and acts post-mortem to delay the oxidative deterioration of meat.



Figure 1. Effect of dietary LFB on TBARS values of pork loin after storage at 10°C for 7 days.

The results of the sensory evaluation are shown in Figure 2. The appearance (including its smell) of raw pork did not differ among the different groups. The panelists did not prefer the texture of cooked pork of both the LFB and fishmeal groups, especially the latter. The panelists noted that the LFB pork was "dried" and the fishmeal pork was "firm." The panelists expressed dissatisfaction with the LFB pork in terms of smell and taste. The panelists further pointed out that they detected a fishy odor or a rancid flavor (P < 0.05). Many experiments have indicated that enrichment of n-3 PUFA by means of fishmeal or fish oil feeding leads to the development of unfavorable flavors and odors in the pork [3]. Further, the intensity of this undesirable flavor is positively correlated with the levels of lipid oxidation [10]. Thus, it is appears that deteriorations in palatability of the LFB pork are partly caused by lipid oxidation.



Figure 2. Sensory evaluation scores of loin meats. Y-axis: 2.0 = favorable; -2.0 = unfavorable. Means with different letters are significantly different at P < 0.01.

## IV. CONCLUSION

The present study indicates that LFB may be used as an efficient source of n-3 PUFA such as EPA and DHA for growing and finishing pigs. The LFB feeding consequently improved some nutritional value such as the n-6/n-3 ratio. Thus, addition of LFB to feeds might provide a viable strategy for producing n-3 PUFA-rich pork. Additionally, feeds containing LFBs may represent a good model of recycling of byproducts into food/feed resources. However, this study has revealed that n-3 PUFA-enriched meat has unfavorable sensory effects, which appear to be due to oxidation of pork fat. Further studies into supplementation of feeds with antioxidants such as vitamin E in order to minimize lipid oxidation and deterioration of meat flavor are currently in progress.

### ACKNOWLEDGEMENTS

This study was supported by a program of technological development for recycling from the Miyazaki Prefectural Industrial Support Foundation.

### REFERENCES

- Martin, A. M. (1996). Lactic acid fermentationaided biomass conversion. Renewable Energy, 9: 942-945.
- Davies, W. L. (1939). Fishiness as a flavour and a taint. Flavours, 2:18-21.
- Øverland, M., Taugbøl, O., Haug, A., Sundstøl, E. (1996). Effect of fish oil on growth performance, carcass characteristics, sensory parameters, and fatty acid composition in pigs. Acta Agriculturae Scandinavica Section A – Animal Science, 46: 11-17.
- Folch, J., Lees, M., Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226: 497-509.
- Kawahara, S., Takenoyama, S., Takuma, K., Muguruma, M., Yamauchi, K. (2009). Effects of dietary supplementation with conjugated linoleic acid on fatty acid composition and lipid oxidation in chicken breast meat. Animal Science Journal, 80: 468–474.
- Barton-Gade, P. A., Demeyer, D., Honikel, K. O., Joseph, R. L., Poulanne, E., Severini, M., Smulders, F. J. M., Thornberg, E. (1993). Reference methods for water holding capacity in meat & meat products: procedures recommended by an OECD working group. In Proceedings of the 39<sup>th</sup> International Congress of Meat Science and Technology (S4 A02.WP), Calgary, Canada.
- Japan Oil Chemists' Society. (2003). Standard Methods for the Analysis of Fats, Oil and Related Materials, 3.2.2.2-1996, pp. 1-2. Tokyo.
- American Meat Science Association (AMSA). (1978). Guidelines for Cookery and Sensory Evaluation of Meat. AMSA, National Livestock and Meat Board, Chicago, IL.
- Wood, J. D., Enser, M. (1997) Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. British Journal of Nutrition, 78: S49-S60.
- 10. Pearson, A. M., Love, J. D., Shortland, F. B. (1977). "Warmed-over" flavor in meat, poultry, and fish. Advances in Food Research, 23: 1-74.