

## PROMOTION OF INTRAMUSCULAR FAT ACCUMULATION IN PORCINE MUSCLE BY NUTRITIONAL REGULATION

Masaya Katsumata

NARO Institute of Livestock and Grassland Science, Tsukuba, Japan  
Department of Veterinary Medicine, Azabu University, Sagami-hara Japan  
e-mail: m-katsumata@azabu-u.ac.jp

### ABSTRACT

*Pork with marbling has been paid attention as good quality pork and researchers are required to develop methods to produce pork with reasonably high amount of intramuscular fat (IMF). The aim of this review paper is to describe studies on promotion of IMF accumulation in porcine muscle by nutritional regulation. The focus is on the effects of amino acid nutrition because regulating amino acid nutrition is so far the most effective and successful methods to promote IMF accumulation in the pig.*

*We found that dietary low lysine induced a series of changes relevant to energy metabolism and adipogenesis in porcine muscle: up-regulation of glucose transporter protein 4 and peroxisome proliferator-activated receptor  $\gamma$  expression and enhancement in proportion of oxidative fiber. These changes induced by dietary low level of lysine allowed us to hypothesize that it would promote IMF accumulation. Indeed, IMF content of longissimus dorsi (l. dorsi) muscle of finishing pigs subjected to dietary low lysine for two months until reaching the market weight was twice higher than that of pigs given a control diet. Further, we found a dose-dependent response of IMF accumulation in l. dorsi muscle to dietary levels of lysine; pigs given a lower lysine diet (0.40 or 0.48% total lysine in diet) had greater contents of IMF in l. dorsi muscle than those given a higher lysine diet (0.56 or 0.64% total lysine in diet) and the response was linear. Based on these results, we conducted substantive field studies in commercial farms and pork with marbling produced by dietary low level of lysine is already supplied to consumers.*

*We conducted another study to examine whether shortage of other indispensable amino acid would promote IMF accumulation in porcine muscle. Our choice was threonine because it is usually the second limiting amino acid in pig diet. Although we confirmed dietary low lysine promoted IMF accumulation in this study again, dietary low threonine did not affect IMF accumulation. Thus, the magnitudes of the effects of shortage of amino acids on IMF accumulation are different among amino acids.*

*In this review paper, I'll introduce results of other research group investigating effects of dietary lysine/protein ratio on IMF accumulation in porcine muscle; IMF accumulation is promoted when pigs are given diets with low lysine/protein ratio even though dietary levels of lysine meet the requirements.*

**Keywords:** Intramuscular Fat, Nutrition, Lysine

## INTRODUCTION

The production of pork with marbling is particularly important in East Asian countries because of the eating habits. For example, the consumption of fresh pork is two-fold higher than the consumption of processed pork in Japan. For this reason, we recently pay attention to the nutritional regulation of intramuscular fat (IMF) deposition in porcine muscle.

There are two approaches for the production of pork with a high concentration of IMF: breeding and nutritional regulations. The first approach already yielded two porcine lines accumulating high amounts of IMF in the *longissimus dorsi* muscle, the synthetic “TOKYO-X” line and the “Shimofuri-Red” Duroc line, both developed in Japan (Hyodo 1996; Takasaki *et al.*, 2005; Suzuki *et al.*, 2005). High IMF loin chops derived from these lines consistently receive good reviews in the market. Nonetheless, nutritional regulation can be advantageous compared with specialized breeding because ones do not need to rear pigs having specific genetic backgrounds. A number of studies relevant to the promotion of IMF deposition via nutritional regulation were conducted over the past twenty years. A typical strategy was to reduce the amount of protein in the diets (Castell *et al.*, 1994; Goerl *et al.*, 1995). Another strategy has been to add a single amino acid to the diet. For example, the addition of leucine (Hyun *et al.*, 2003, 2006) or arginine (Tan *et al.*, 2009) increased the IMF content in porcine muscle.

In contrast, our present strategy involves the reduction of lysine levels in the diet. We previously reported that a low dietary lysine level up-regulated glucose transporter protein 4 (GLUT4) and peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) mRNA expression (Katsumata *et al.* 2001; Katsumata *et al.* 2008). Further, dietary low lysine enhanced proportion of oxidative fiber in muscle (Katsumata *et al.* 2008). Given that the oxidative capacity of muscle affects IMF content (Gerbens 2004; Goto *et al.*, 1994; Hocquette *et al.* 2003), we went on to show that the shortage of a single amino acid, lysine, enhanced IMF deposition in the pig (Katsumata *et al.*, 2005, 2012). The aim of this review paper is to describe studies relevant to the promotion of IMF deposition in porcine muscle by nutritional regulation.

### Effect of low food intake on GLUT4 mRNA expression

The idea was to improve quality of pork by altering energy metabolism of porcine muscle; the initial focus was on glucose transporter proteins because the rate limiting step of energy metabolism in the cell was thought to be transport of glucose through transmembrane via its transporter proteins. We elucidate effects of reduced food intake on gene expression of glucose transporter protein 1 (GLUT1) and GLUT4 in muscle of growing pigs because these two proteins were major glucose transporter proteins functioning in mammalian muscle. We found that expression of GLUT1 and GLUT4 mRNA in *l. dorsi* and *rhomboideus* muscles was up-regulated by low food intake (Katsumata *et al.* 1999). The experiment revealed that low food intake up-regulated GLUT1 and GLUT4 mRNA expression in skeletal muscle of growing pigs. However, knowledge about effect of each nutrient on expression of glucose transporter proteins was limited. Therefore, we decided to focus on the effect of specific nutrient.

### Effect of dietary low lysine on GLUT4 mRNA expression

Our interest was in effects of dietary lysine levels because we had already found that dietary low lysine reduced concentrations of IGF I in plasma in growing pigs (Katsumata *et al.* 2002). In order to elucidate effects of dietary lysine levels on GLUT4 expression in skeletal muscle of growing pigs, we prepared two diets: a control diet of which lysine concentration was 1.15% and a low lysine diet of which lysine concentration was 0.70% (Katsumata *et al.* 2001). The diets were iso-protein and iso-energetic. Concentrations of all the essential amino acids other than lysine of the diets were equivalent and met the requirements. The trial started when the pigs were 6 weeks old and the pigs were given those diets for 3 weeks. GLUT4 mRNA expression in *l. dorsi* and *rhomboideus* muscles was higher in the low lysine group (Fig. 1) than that in the control group. Further, GLUT4 protein levels in *rhomboideus* muscle were also higher in the low lysine group than those in the control group. We conducted a similar experiment to elucidate the effects of dietary low threonine and observed that GLUT4 mRNA expression was higher in *l. dorsi* muscle of pigs fed a low threonine diet than those fed a diet meeting requirement of threonine (Katsumata *et al.* 2004). Thus, it was found that not only low food intake but also low intake of a single amino acid up-regulates GLUT4 expression in porcine skeletal muscle.

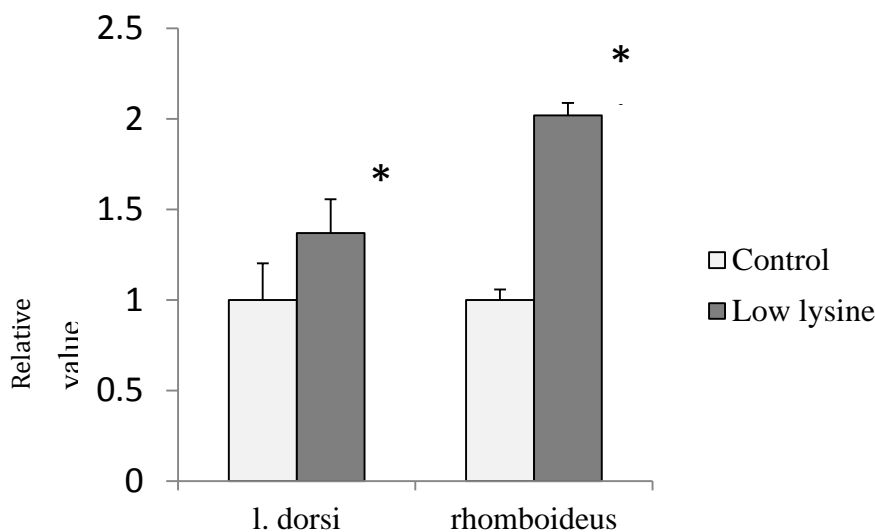


Fig. 1. GLUT4 mRNA expression (relative value/50µg RNA) in l. dorsi and *rhomboideus* muscles (n=8). Least square means and standard errors. \*, P<0.05, \*\*, P<0.01 Means of the control diet groups are expressed as 1.

### Effect of dietary low lysine on oxidative capacity of muscle

As already mentioned, the rate limiting step of energy metabolism in the cell was thought to be transport of glucose through transmembrane via GLUT. Therefore, it may be reasonable to consider that post-GLUT4 energy metabolism is also affected by dietary low lysine. We determined activities of two enzymes relevant to energy metabolism in l. dorsi and *rhomboideus* muscles and found that dietary low lysine enhanced the activities of citrate synthase in the both muscles whereas it did not affect those of hexokinase (Katsumata *et al.* 2003). We inferred that oxidative capacity of porcine skeletal muscle would be enhanced by dietary low lysine because citrate synthase is an indicator of oxidative capacity of the cell.

Contractile and metabolic properties of muscle are dependent on muscle fiber type and biochemical property of each muscle fiber. Biochemical properties of muscle fibers are classified according to energy substrates on which they mainly depend: some of them are muscle fibers mainly dependent on oxidation of substrates such as fatty acids and others are mainly dependent on breakdown of glycogen. The former is classified into oxidative fiber while the latter is classified into glycolytic fiber. Considering the histochemical characteristics of muscle, we hypothesized that feeding low lysine diet to growing pigs would shift biochemical property of muscle from glycolytic to oxidative. Therefore, in order to test this hypothesis, we elucidated effects of a low lysine diet on activities of NADH-dehydrogenase, an enzyme of the complex I of mitochondrial respiratory chain, in l. dorsi and *rhomboideus* muscles of growing pigs by enzyme histochemistry. We found that proportions of oxidative fiber were higher in the low lysine group in the both muscles (Fig. 2; Katsumata *et al.* 2008). These observations agreed with our hypothesis. There is a certain relation between triacylglyceride content in muscle fiber and muscle fiber type; triacylglyceride content of oxidative fibers is higher than that of more glycolytic fibers (Gerbens 2004). In the case of farm animals, it was reported that oxidative fibers were associated with higher amount of IMF in cattle (Goto *et al.* 1994; Hocquette *et al.* 2003). Moreover, we also observed that levels of PPAR $\gamma$  mRNA in l. dorsi and *rhomboideus* muscles were up-regulated by dietary low lysine (Katsumata *et al.* 2008). PPAR $\gamma$  is a nuclear hormone receptor widely accepted as a master regulator of adipogenesis (Tontonoz *et al.* 1994a,b). We hypothesized that feeding a low lysine diet to finishing pigs might promote accumulation of IMF in porcine muscle.



Fig. 2. Proportion of oxidative fibers in l. dorsi and *rhomboideus* muscles (n=5). Least square means and standard errors. \*\*; P<0.01

### Dietary low lysine promotes IMF accumulation in finishing pigs

We prepared two diets: control diet of which lysine concentration met the requirement of finishing pigs and a low lysine diet (0.40% lysine in the diet) of which lysine concentration was approximately 30% lower than the requirements. The pigs were given those diets for two months until their body weight reached the market weight (110kg). The diets were iso-protein and iso-energetic and the concentrations of all the essential amino acids other than lysine were met the requirements. As we expected, the IMF content of l. dorsi muscle of the pigs fed the low lysine diet was twice higher than those of the control pigs: 3.5% for the control group vs. 6.7% for the low lysine group (Table 1). Feed efficiency was lower in the low lysine group than that in the control group (Table 1). Further, the low lysine group took five days longer to reach the market weight (Table 1) than that in the control group. These hindered performances had been expected because the level of lysine in the low lysine diet did not meet the requirement. If the responses of the performances and the concentrations of IMF in l. dorsi muscle to dietary lysine levels are dose-dependent, we might be able to find out a dietary lysine level low enough to produce pork with marbling while the performances of pigs are acceptable. For the next step, we examined whether or not effects of dietary lysine levels on concentrations of IMF in l. dorsi muscle of pigs would be dose-dependent. The details of the experiment described in this paragraph are reported elsewhere (Katsumata *et al.* 2005).

Table 1. Effects of dietary low lysine on concentrations of IMF in l. dorsi muscle, live weight gain, feed efficiency, and age reaching to the market weight

	Control (n=5)	Low lysine (n=6)	
IMF in l. dorsi muscle (%)	3.5 ± 0.7	6.7 ± 0.7	**
Live weigh gain (g/d)	784 ± 39	715 ± 36	
Feed efficiency	0.32 ± 0.01	0.30 ± 0.01	†
Age reaching to the market weight (d)	173 ± 1	178 ± 1	**

Least square means and standard errors. † P<0.10, \*\* P<0.01

### Dose-dependent response of IMF contents in l. dorsi muscle to dietary lysine levels

In order to elucidate response of accumulation of IMF in l. dorsi muscle to different levels of dietary lysine, we prepared test diets of 4 levels of lysine (0.40, 0.48, 0.56, and 0.64 %) diet. Barrows and gilts aged approximately 100 days (average body weight was 58 kg) were used. The diets were again iso-protein and iso-energetic, and contained equal amounts of indispensable amino acids apart from lysine in the recommended amounts. When the pigs were reached to the market weight (110 kg), l. dorsi muscle samples were taken. The effects of dietary lysine levels on live weight gain and feed efficiency were significant (Table 2); the lower the lysine content was, the lower the weight gain and the feed efficiency were. These responses were linearly dose-dependent. Concentrations of IMF in l. dorsi muscle were affected by dietary lysine levels; pigs given a lower lysine diet (0.40 or 0.48 % lysine) had higher concentrations of IMF in l. dorsi muscle than those given a higher lysine diet (0.56 or 0.64 % diet) (Table 2). The response was linearly dose-dependent and there was a tendency of quadratic effect (Table 2). Dose-dependent effects of dietary protein levels on concentrations of IMF in l. dorsi muscle were already reported (Castel *et al.* 1994; Goerl *et al.* 1995). However, to our knowledge, this was the first evidence demonstrating that accumulation of IMF in pig muscle dose dependently responded to levels of a single amino acid in diet. Concentrations of IMF in l. dorsi muscle could be regulated by adjusting dietary lysine levels when they are below the requirements. As responses of growth performances were also dose-dependent, we might be able to find out a dietary lysine level producing both reasonable high concentration of IMF and reasonable growth performance. The details of this experiment were reported elsewhere (Katsumata *et al.* 2012).

Table 2. Effects of dietary lysine levels on concentrations of IMF in l. dorsi muscle (n=10)

	0.40%	0.48%	0.56%	0.64%	SEM	L <sup>a</sup>	Q <sup>b</sup>
IMF in l. dorsi muscle (%)	6.9	4.9	4.3	3.6	0.6	**	†
Live weight gain (g/d)	804	857	943	935	34	**	
Feed efficiency	0.27	0.29	0.32	0.32	0.01	**	

Least square means and SEM. <sup>a</sup>Linear response to dietary lysine levels. <sup>b</sup>Quadratic response to dietary lysine levels. † P<0.10, \*\* P<0.01

### Effects of dietary threonine levels on IMF accumulation in muscle of pigs

As described above, shortage of a single amino acid lysine in diets enhanced concentrations of IMF in l. dorsi muscle. From these results, we hypothesized that shortages of other indispensable amino acids would enhance concentrations of IMF in pig muscle. We decided to focus on threonine because it is usually the second limiting amino acid in pig diets. Twelve barrows and four gilts (10 weeks old and 36 kg) were assigned to one of four diets: lysine control (LC), low lysine (LL), threonine control (TC), and low threonine (LT). The LC and LL were positive control groups of this experiment. The pigs were fed these diets for 8 weeks. Both the LL and LT diets reduced body weight gain and feed efficiency of the pigs (data not shown). Although the LL diet certainly enhanced the IMF contents both in l. dorsi and *rhomboideus* muscles, the LT diet did not affect them (Table 3). Thus, we failed to confirm our hypothesis that in addition to dietary low lysine, dietary low threonine enhances IMF contents in pig muscle. This calls for a new hypothesis that the magnitudes of the effects of shortage of amino acids on IMF accumulation are different among amino acids.

In this experiment, we obtained an interesting result; concentrations of serum insulin of the LL and LT groups tended to be lower than those of the control groups. On the other hand, the concentrations of serum glucose were not affected by dietary amino acid levels. These results suggested that the response of peripheral tissues to insulin might be larger in the dietary low dietary amino acid groups. Up-regulation of GLUT4 expression induced by dietary low lysine and threonine might be involved in this response. The details of this experiment are reported elsewhere (Kobayashi *et al.* 2012).

Table 3. Effects of dietary lysine and threonine levels on concentrations of IMF in *l. dorsi* and *rhomboideus* muscles (%)

	Lysine control	Low lysine	Threonine control	Low threonine	SEM
<i>L. dorsi</i>	1.3 <sup>b</sup>	3.9 <sup>a</sup>	1.9 <sup>b</sup>	1.9 <sup>b</sup>	0.3
<i>Rhomboideus</i>	2.6 <sup>c</sup>	4.4 <sup>a</sup>	3.2 <sup>bc</sup>	3.6 <sup>ab</sup>	0.3

Least square means and SEM. a, b, and c P<0.05

### Control of IMF accumulation by manipulating dietary lysine/protein ratio

This review paper has been describing the results from our own studies on promotion of IMF accumulation by dietary low lysine. Although IMF accumulation is promoted by dietary low lysine, it hinders growth performance as well; dietary low lysine reduces body weight gain and feed efficiency. We need to overcome this disadvantage through further investigations. A research group of Miyazaki University, Japan, has been also studying nutritional regulation in order to promote IMF accumulation in porcine muscle. Their tactic is to promote IMF accumulation in porcine muscle by manipulating dietary lysine/protein ratio. Using by-product-feeds mainly consisting of bread crumbs allowed them to manipulate dietary lysine/protein ratio. Ingredients and chemical composition of diets are shown in Table 4. They used a commercial diet for finishing pigs as the control diet. While the lysine/protein ratio of the control diet was 0.039, those of the test diets 1, 2, 3, and 4 were 0.034, 0.026, 0.043, and 0.033, respectively. Further, the concentrations of lysine of the test diets 1 and 3 were higher than the lysine requirement of finishing pigs. The pigs were given those diets from 70 to 105 kg body weight. Fig. 3 shows concentrations of IMF of *l. dorsi* muscle of the pigs. The concentration of IMF of the control group was 4.0%. The concentrations of IMF of the test groups were higher than that of the control group: 8.1% for the test diet 1 group, 8.7% for the test diet 2 group, 6.2% for the test diet 3 group, and 9.6% for the test diet 4 group, respectively. Significant differences between the concentration of IMF of the control group and those of the test diet 1, 2, and 4 groups were detected. Although the difference between the concentration of IMF of the control group and that of the test diet 3 group was not significant, 6.2% seems to be high enough from a practical point of view. In their another study, the concentration of IMF of *l. dorsi* muscle was as high as 9.5% when the pigs were given a diet lysine/protein ratio of which was 0.035 (the concentrations of lysine and crude protein were 0.58% and 16.2%, respectively). The daily live weight gain of those pigs was 990 g and high enough as that of finishing pigs. These results indicate that concentrations of IMF in *l. dorsi* muscle are enhanced without hindering growth performance of pigs by manipulating dietary lysine/protein ratio. The details of these studies are reported elsewhere (Takahashi *et al.* 2013; Maeda *et al.* 2014).

Table 4. Ingredients and chemical composition of diets (%)

	Control	Test 1	Test 2	Test 3	Test 4
Ingredients (%)					
Commercial diet	100				
By-product-feed		88.9	89.02	98.52	98.64
Soybean meal		9.9	9.89		
L-lysine-HCl		0.12		0.39	0.27
Mineral mix		0.98	0.99	0.99	0.99
Vitamin mix		0.1	0.1	0.1	0.1
Chemical composition (%) <sup>1)</sup>					
Crude protein	14.5	16.3	16.3	12.9	12.9
Lys	0.57	0.55	0.43	0.55	0.43
Lys/Protein ratio	0.039	0.034	0.026	0.043	0.033

<sup>1)</sup>Calculated values as-fed basis.

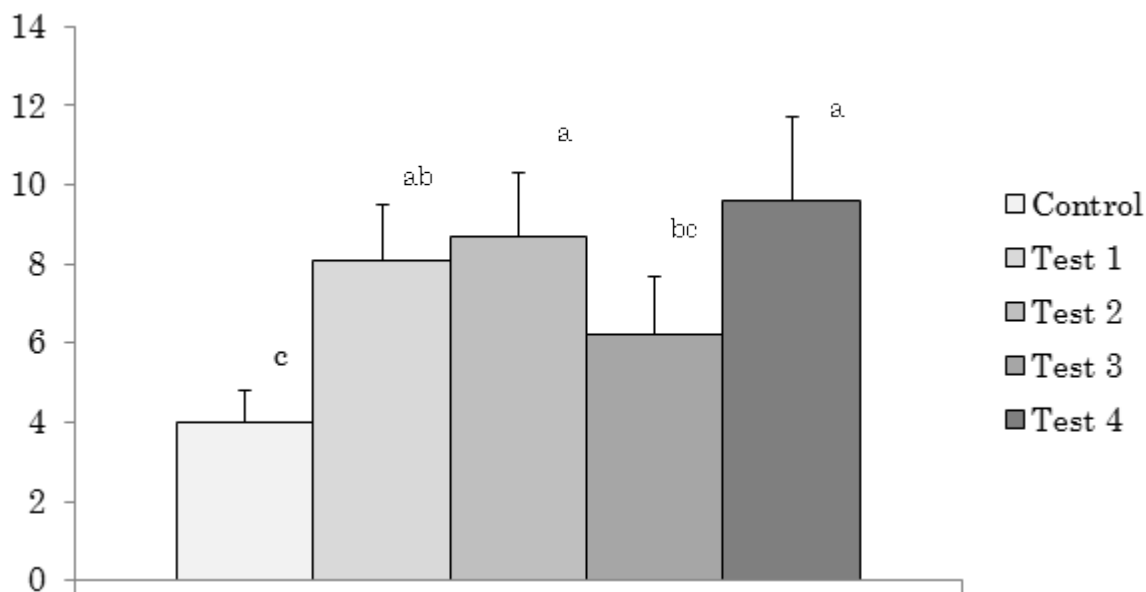


Fig. 3. Effects of by-product-feeds mainly composed of bread crumbs on concentrations of IMF in l. dorsi muscle (n=8). Means and standard deviations. a, b, c P<0.05

### CONCLUSION

Dietary low lysine promotes IMF accumulation in porcine muscle. Further, we observed a dose-dependent response of IMF contents in l. dorsi muscle to dietary lysine levels. This dose-response might allow us to find out a dietary lysine level producing both reasonable high concentrations of IMF and growth performance. Indeed, although it's still a small-scale implementation, pork with high concentration of IMF produced by dietary low lysine is already supplied to consumers in Japan. However, there still some issues remain to be solved. For instance, if producers give their pigs an extremely low concentration of lysine or subject their pigs to excessive long period of feeding a low lysine diet, aiming further enhancement in concentration of IMF, might result in unacceptable negative effects on growth performance. Thus, we need to conduct further studies to brush up on our method and appropriately instruct the producers to avoid these issues.

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