The Effects of Dietary Lysine Deficiency on Muscle Protein Turnover in Postweanling Pigs

Yi-Ming Chang and Hen-Wei Wei*

Department of Animal Science, National Taiwan University, Taipei 106, Taiwan, ROC

ABSTRACT : The main purpose of this study is to investigate the effects of dietary lysine deficiency on protein turnover of porcine muscles. There were 18 LYD three-breed-crossing postweanling barrows from six litters cannulated with gastric tubes through the esophagus at approximate 10 kg of body weight and allocated into three treatment groups. When their body weights reached over 12 kg, one group was sacrificed for determining the initial protein masses of *m. masseter*, *m. longissimus dorsi*, *m. adductor* and *m. biceps femoris* from the right body side. The others received a diet containing 100% or 61.4% (calculated values) of the lysine requirement (NRC, 1998) multiplied by 1.103 for a period of 17 days. Daily feed provision was computed for each pig according to body weight at the same day. All pigs were infused a flooding dose of [$^2H_5$]-phenylalanine to determine the fractional protein synthesis rates (FSR) of the aforementioned muscles in the end. Their four muscles from the right body side were also dissected for measuring the fractional rates of protein accretion (FAR). As for protein degradation, fractional rates (FDR) were calculated by differences between synthesis and accretion. Results showed that the lysine deficiency resulted in, significantly ($p<0.05$), lighter body weights, smaller muscles and a slower growth rate. The protein mass, accreted by the muscles, of the deficient group was only 54% averaged of the pigs fed adequately ($p<0.05$). The FAR of these muscles in the deficient group was significantly lower ($p<0.05$) and only achieved 61.1% averaged of the control; there was no significant difference ($p>0.05$), nevertheless, in the amino-acid composition of muscles between two groups. The lysine deficiency reduced significantly ($p<0.05$) the FSR of *m. longissimus dorsi* but did not influence its FDR. *The m. biceps femoris* also presented an inhibited FSR while its FDR reduced only exhibited a very high tendency ($p = 0.055$) compared to the adequately-fed pigs. As for the *m. masseter* and *m. adductor*, both of the FSR and FDR were depressed significantly ($p<0.05$) by the lysine deficiency, and changes in the FSR were severer than those in the FDR, so that their FAR were significantly slower ($p<0.05$) in comparison with the control group. The lysine deficiency also inhibited the RNA translation activity of the muscles while the effects on RNA capacity were not significant ($p>0.05$). In conclusion, the FAR of muscle protein was changed by the current lysine deficiency through the alterations in the FSR and/or FDR. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 9 : 1326-1335)***

Key Words : Lysine Deficiency, Muscle Protein Turnover, Fractional Accretion Rate, Fractional Synthesis Rate, Fractional Degradation Rate, Postweanling Pig

INTRODUCTION

Lysine is the first limiting amino acid in feed based on corn-soybean meal for growing pigs and its deficiency will lead to retardation in growth and feed efficiency (Hamilton and Veum, 1986). The alteration in body weight, however, means a whole change in the deposition of water, lipid, protein and mineral so that Batterham et al. (1990) deemed that the accretion rate of protein would be a more suitable index than weight gain for measuring the requirement of essential amino acids.

Since protein accretion is a competitive result from a cyclical process with synthesis and degradation (Sugden and Fuller, 1991), protein turnover must be estimated overall when the effect of nutrients on protein deposition is investigated. Both Fuller et al. (1987) and Salter et al. (1990) had measured the influences of dietary lysine deficiency in the whole-body protein turnover of growing pigs and observed inhibition in protein accretion; nevertheless, these authors deduced different explanations and both of them did not investigate individual muscles or organs.

Feeding techniques will affect rigorously experimental results. In most researches, feeding *ad libitum* was adopted for measuring amino acid deficiency or requirement (NRC, 1988). Cortamira et al. (1991), however, compared intubation and feeding *ad libitum* on very young piglets receiving tryptophan deficient diets and reported that the growth retardation of the latter resulted from both the limiting amino acid and decreases in feed intake. The anorectic response was observed not only from the pigs but also from rats fed lysine deficient diets (Smriga et al., 2000). Gietzen et al. (1998) pointed out that the anterior piriform cortex of brain would detect a decrease in plasma free limiting amino acid and then release neurotransmitters to regulate ventromedial hypothalamus leading to a depression in feeding. Pair-feeding is an alternative for similarity in feed intake of all animals tested; however, it is doubtful whether the final observation from a control group could represent that of animals fed adequately.

The aim of this study is to clarify the effects only from dietary lysine deficiency on protein turnover of *m. masseter*, *m. longissimus dorsi*, *m. adductor* and *m. biceps femoris* so

* Corresponding Author: Hen-Wei Wei. Tel: +886-2-33664154, Fax: +886-2-27324070, E-mail: hwwei@ntu.edu.tw

Received September 23, 2004; Accepted March 23, 2005

Table 1. The composition of experimental diets

Ingredient	Diet [1]	
	Control	Lysine deficiency
	(%)	
Corn, dent yellow grain	15.0	15.0
Soybean meal, solvent extracted	14.0	14.0
Corn gluten meal	5.3	5.3
Feather meal	5.0	5.0
Blood meal, spray dried	2.0	2.0
Whey, dried	4.2	4.2
Soybean oil	6.24	6.24
Glucose	10.0	10.0
Corn starch	22.2	22.2
Glutamic acid	10.0	10.0
Mineral premix[2]	4.0	4.0
Vitamin premix[3]	1.0	1.0
Threonine	0.12	0.12
Methionine	0.09	0.09
Tryptophan	0.06	0.06
Lysine	0.53	-
Cellulose	0.26	0.79
Chemical composition		
ME (Mcal/kg)[4]	3,600	3,600
Moisture	8.59	8.36
Crude protein	23.5	23.4
Lysine	1.23	0.83
Threonine	0.82	0.83
Valine	0.96	0.94
Cysteine[4]	0.42	0.42
Methionine[4]	0.33	0.33
Isoleucine	0.70	0.69
Leucine	1.74	1.75
Tyrosine	0.65	0.64
Phenylalanine	0.88	0.87
Histidine	0.41	0.40
Tryptophan[4]	0.23	0.23
Arginine	6.40	6.42

[1] Multiplying the requirements (NRC, 1998) 1.103-fold.
[2] Supplying (per kilogram): calcium carbonate 9.17 g, calcium phosphate (dibasic) 16.67 g, sodium chloride 1.33 g, potassium iodate 0.25 mg, sodium sulfate (decahydrate) 3.99 g, cupric sulfate (pentahydrate) 0.93 mg, sodium selenite 0.35 mg, zinc sulfate (heptahydrate) 0.35 g.
[3] Supplying (per kilogram): retinol 2,050 IU, cholecalciferol 245 IU, α-tocopherol 22.05 IU, menadione (50%) 0.61 mg, riboflavin 1.63 mg, nicotinic acid 8.48 mg, cyanocobalamin (0.1%) 0.13 mg, folic acid (98%) 0.09 mg, d-pantothenate-hemi-calcium 13.77 mg.
[4] Calculated values.

that the intubation was chosen to avoid anorexia.

MATERIALS AND METHODS

There were 18 LYD three-breed-crossing weanling barrows (Landrace×Yorkshire×Duroc) from six litters purchased from a commercial farm, allocated into 3 treatment groups with 6 replicates and cannulated at 10 kg of body weight. Under general halothane anaesthesia after an overnight fast, a silicon gastric catheter (6 mm external diameter×4 mm internal diameter×125 cm length, Terumo Corporation, Tokyo, Japan) entered directly the pharynx through an incision in the neck for avoiding drinking hindrance and was fitted into the stomach via the oesophagus for each pig, and its end was exteriorized on the back of the neck, closed by a clip and put into an elasticated tubular bandage jacket worn by the animal. After surgery, whatever feed or water the pigs were fed by the catheter to prevent vomit and diarrhea in the first three days. Their feed intake was limited by providing proper metabolic energy to satisfy maintenance according to the following formula: 106 (body weight)$^{0.75}$/3,600 kcal/kg (NRC, 1998) for oesophagus and gaster to recover smoothly from surgery. From the forth day, water was supplied *ad libitum* but feed intake was calculated on the basis of another formula, 0.96× [(251×Body weight)-(0.99×Body weight^2)-133] kcal/3,600 kcal/kg, to provide sufficient metabolic energy for growth (NRC, 1998). When their body weights reached over 12 kg the experiment started (day 1), and one group was killed for measuring the initial protein masses of *m. masseter*, *m. longissimus dorsi*, *m. adductor* and *m. biceps femoris* from the right body side. The others received a diet (Table 1) containing 100% or 61.4% (calculated values) of the lysine requirement (NRC, 1998) multiplied by 1.103 for a period of 17 days. Because feed intake relates to dietary energy concentration and the metabolic energy density of the diets was 1.103-fold in comparison with the NRC (1998) requirement, 3,265 kcal/kg, all nutrient contents were raised by multiplying by 1.103 to provide sufficient nutrients. Daily feed provision for every pig was also computed according to its body weights as the afore-mentioned formula for growth. On day 15 two polyethylene catheters (0.86 mm internal diameter×1.52 mm external diameter×45 cm length, NT2; Portex Ltd, Hythe, Kent, UK) were implanted surgically 8 cm into both thoracic limb veins, closed by sterile needles, protected by the jacket and maintained patently by flushing once per day with 1 ml of heparinized saline (50 international units/ml) and then refilled with 1 ml of heparinized saline (500 international units/ml). At three hours after its first meal, each pig received L-[$^2H_5$]-phenylalanine enriched to 15 atom % as 150 mM solution in sterile 0.077 M NaCl by the catheter for 30 min at a rate of 6.25 ml/min on day 18 (McNurlan et al., 1994). Blood samples were taken from the other catheter at 0, 2, 7, 10, 19 and 28 min after the start of the infusion for determining the enrichment (molecule percentage excess, MPE) of plasma L-[$^2H_5$]-phenylalanine (Hunter et al., 1995). At the end of the infusion, animals were sacrified by injecting 25 ml of sodium pentobarbitone. Immediately, approximate 15 g per muscle, including *m. biceps femoris*, *m. adductor*, *m. masseter* and *m. longissimus dorsi*, from the left body side was removed and frozen in liquid nitrogen, prior to storage at -70°C until

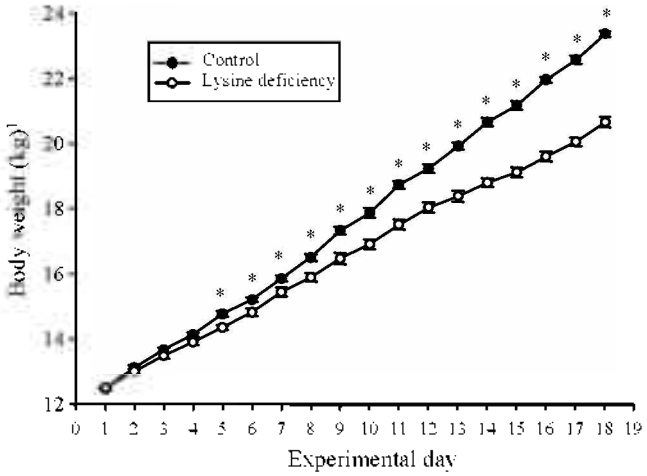

Figure 1. The effect of dietary lysine deficiency on the body weight of postweanling pigs. [1] Each value is the mean±SE of 6 postweanling pigs. * Significantly different from the lysine-deficiency group ($p<0.05$).

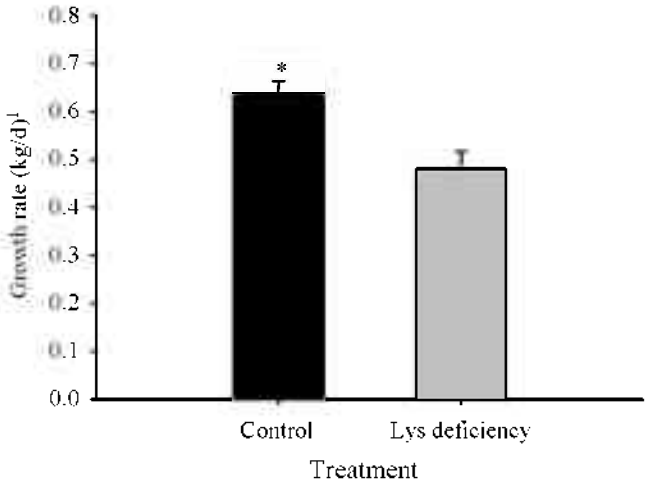

Figure 2. The effect of dietary lysine deficiency on the growth rate of postweanling pigs. [1] Each value is the mean±SE of 6 postweanling pigs. * Significantly different from the lysine-deficiency group ($p<0.05$).

analyzed to ensure the samples under complete freshness. The exact time points were recorded when every blood sample was withdrawn and also when the tissue samples were placed into liquid nitrogen. Then, the afore-mentioned muscles were also dissected and weighed from the right body side and stored at -20°C for the measurements of nitrogen contents and amino acid composition. The free lysine concentration of plasma sampled before the infusion was prepared and determined according to the description by Lee et al. (2004).

The dry matter and crude protein contents of the muscles and feed were analyzed according to the procedures of the Association of Official Analytical Chemists (AOAC, 1984). In addition, the muscle samples were hydrolyzed, similar to the method described by Williams (1994), in 6 N hydrochloric acid at 110°C for 18 h and analyzed by an amino acid analyzer (Beckman 6300) for their amino acid composition. The dietary lysine percentages were also determined to make sure that the diets were prepared properly.

After homogenized, the muscle samples were subjected to determining the concentrations of both RNA (Munro and Fleck, 1969) and protein (Lowry et al., 1951) and the enrichments of free and bound L-[$^2H_5$]-phenylalanine. The methodology for determining the MPE of L-[$^2H_5$]-phenylalanine by Gas Chromatography Mass Spectrometers (GC-MS) under electron impact ionization and selective ion recording (EI-SIR) was modified from the methods described by Calder & Smith (1988), Calder et al. (1992) and McNurlan et al. (1994). The MPE of free L-[$^2H_5$]-phenylalanine in both plasma and muscles were assessed by monitoring the pair of ions of the *t*-butyldimethylsily (*t*-BDMS) derivatives at 336 and 341 of mass-to-charge ratio (*m/z*),on a HP 5973 mass spectrometer (Hewlett Packard, USA) combining with a GC (HP 6890) and computed as described by Campbell (1974). As for the bound pool samples, because their MPE values are typically 1,000-fold lower than those of the free pool ones, the methodology of enzymatic decarboxylation to phenylethylamine before derivatization and a more precise GC (8,000 TOP, Thermo Quest Italia, Milan, Italy)-MS (Voyager quadrupole mass spectrometer, Thermo Quest Finnegan, Manchester, UK) were applied to determine another pair of ions of the *t*-BDMS derivatives at 180 (M+2-57) and 183 (M+5-57) of mass-to-charge ratio (*m/z*).

The formulas used for absolute or fractional rates of muscle protein (Garlick et al., 1973; Millward et al., 1975; McNurlan et al., 1979; Garlick et al., 1989), RNA translation activity and RNA capacity (McNurlan et al., 1981) are expressed as following:

Absolute accretion rate (AAR, g/d)
= (protein mass in ending-initiation)/number of days
Fractional accretion rate (FAR, %/d)
= AAR/(protein mass in ending+initiation)/2
Fractional synthesis rate (FSR, %/d)
= (MPE of the bound pool×100)/(MPE of the plasma and muscle free pool×*dt*)
Absolute synthesis rate (ASR, g/d)
= FSR×muscle protein mass
Fractional degradation rate (FDR, %/d)
= FSR-FAR
Absolute degradation rate (ADR, g/d)
= ASR-ADR
RNA capacity (mg RNA/g tissue protein)
= RNA content/ protein content
RNA translation activity (g protein synthesized/d/g RNA)
= (FSR×1,000)/RNA capacity

Table 2. The effects of dietary lysine deficiency on the muscle weight of postweanling pigs

	Initial	Control	Lysine deficiency
Absolute	---------- (g)[1] ----------		
M. masseter	15.83±0.53[c]	28.57±0.39[a]	21.67±0.43[b]
M. longissimus dorsi	178.50±2.13[c]	377.27±1.19[a]	303.20±0.51[b]
M. adductor	39.70±0.98[c]	71.43±0.72[a]	56.53±0.88[b]
M. biceps femoris	163.91±1.01[c]	315.80±1.27[a]	252.53±0.83[b]
Relative (to body)	---------- (%)[1] ----------		
M. masseter	0.13±0.05[a]	0.12±0.02[a]	0.10±0.02[b]
M. longissimus dorsi	1.43±0.19[b]	1.62±0.07[a]	1.47±0.03[b]
M. adductor	0.32±0.09[a]	0.31±0.04[a]	0.27±0.06[b]
M. biceps femoris	1.31±0.09[a]	1.35±0.08[a]	1.22±0.06[b]

[1] Each value is the mean±SE of 6 postweanling pigs.
[a, b, c] Means within the same row without bearing the same superscripts differ significantly ($p<0.05$).

Table 3. The effect of dietary lysine deficiency on the muscle protein mass of postweanling pigs

	Initial	Control	Lysine deficiency
	---------- (g)[1] ----------		
M. masseter	2.62±0.22[c]	3.96±0.20[a]	3.42±0.15[b]
M. longissimus dorsi	33.22±0.44[c]	60.94±0.81[a]	46.34±0.98[b]
M. adductor	7.19±0.44[c]	11.96±0.54[a]	9.82±0.41[b]
M. biceps femoris	29.22±0.48[c]	48.69±0.84[a]	39.70±0.80[b]

[1] Each value is the mean±SE of 6 postweanling pigs.
[a, b, c] Means within the same row without bearing the same superscripts differ significantly ($p<0.05$).

All data were subjected to paired t test or analysis of variance by a General Linear Models (GLM) procedures (SAS Institute, 1990). Means would be further subjected to Ducan's new multiple range test if a significant difference existed (Steel and Torrie, 1980) after analysis of variance. Probability of 5% was applied to evaluate the significant differences between means of treatments. Standard error was used instead of standard deviation to present reliability of mean.

RESULTS

The dietary lysine deficiency resulted in growth retardation (Figures 1 and 2). The initial body weights of all pigs were similar, around 12.5±0.03 kg, while significant differences existed between the adequately-fed and deficient groups from day 5 until the end. The final body weight of the pigs receiving the lysine deficient diet (20.6±0.16 kg) was only 88% compared to their counterparts (23.3±0.08 kg) and their growth rate also decreased by 25% (0.64±0.03 vs. 0.48±0.04 kg/d). A significant difference also existed in the free lysine concentration of plasma between the pigs fed adequately (11.86±0.50 μmole/dl) and deficient in lysine (2.74±0.51 μmole/dl). All of these implied that the preparation of the deficient diet was valid. On the other hand, the infusion amount of feed for the control group during the experiment was significantly more than that of the deficient group (19.1±0.14 vs. 18.0±0.18 kg) due to the differences in their body weights.

The results of muscle weights were showed in Table 2. The pigs under the deficient treatment had significantly lighter muscles than those fed adequately, whatever absolute or relative (to body) weights, and their absolute weight of muscles was decreased by a range from 19.6 to 24.2%. There were no significant differences between the initial and control groups in the relative weight of the muscles apart from the *m. longissimus dorsi*. On the other hand, the pigs receiving the lysine-deficient diet presented, significantly, smaller relative weights in the *m. masseter*, *m. adductor* and *m. biceps femoris* compared to the initial group while their relative weights of the *m. longissimus dorsi* were similar.

The dietary lysine deficiency reduced muscle protein mass (Table 3) and inhibited both accretion rates (absolute

Table 4. The effect of dietary lysine deficiency on the absolute accretion rate (AAR) and fractional accretion rate (FAR) of muscle protein of postweanling pigs

	AAR		FAR	
	Control	Lysine deficiency	Control	Lysine deficiency
	---------- (g/day)[1] ----------		---------- (%/day)[1] ----------	
M. masseter	0.08±0.01	0.05±0.01*	2.42±0.19[x]	1.59±0.50[x]*
M. longissimus dorsi	1.63±0.22	0.77±0.27*	3.45±0.20[y]	1.90±0.38[x]*
M. adductor	0.28±0.01	0.15±0.01*	2.93±0.23[z]	1.85±0.51[x]*
M. biceps femoris	1.15±0.19	0.62±0.21*	2.92±0.14[z]	1.77±0.23[x]*

[1] Each value is the mean±SE of 6 postweanling pigs.
* Significantly different from the control group ($p<0.05$).
[x, y, z] Means within a column with no common superscripts are significantly different ($p<0.05$).

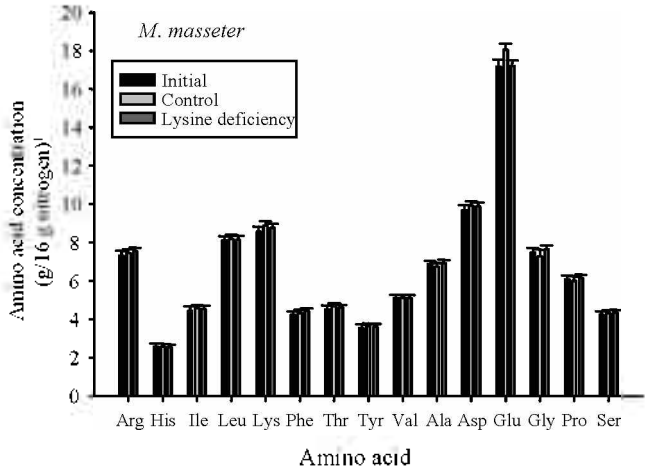

Figure 3. The effect of dietary lysine deficiency on the amino acid pattern of *m. masseter*. [1] Each value is the mean±SE of 6 postweanling pigs.

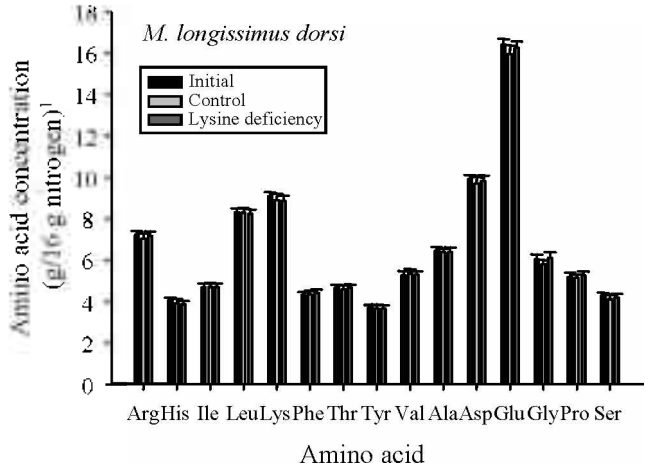

Figure 4. The effect of dietary lysine deficiency on the amino acid pattern of *m. longissimus dorsi*. [1] Each value is the mean±SE of 6 postweanling pigs.

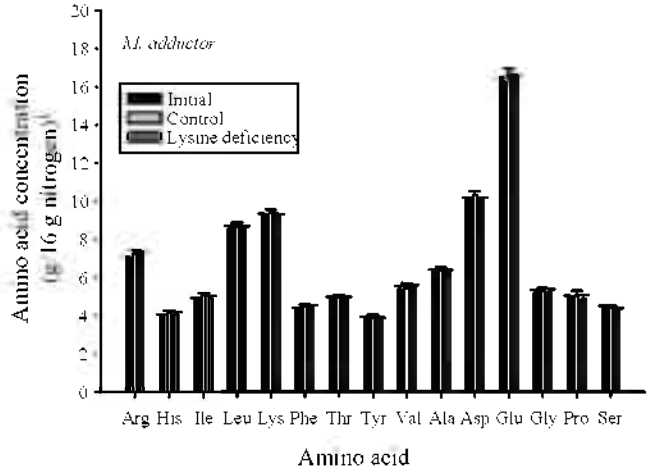

Figure 5. The effect of dietary lysine deficiency on the amino acid pattern of *m. adductor*. [1] Each value is the mean±SE of 6 postweanling pigs.

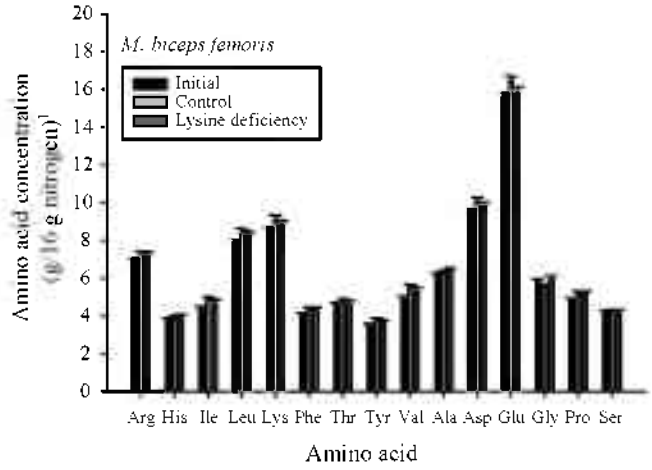

Figure 6. The effect of dietary lysine deficiency on the amino acid pattern of *m. biceps femoris*. [1] Each value is the mean±SE of 6 postweanling pigs.

and fractional) of muscle protein (Table 4). The protein mass accreted. AAR and FAR of the muscles were decreased by 46, 45.7 and 38.9%, averaged respectively, due to the lysine deficiency. Significant differences existed in the FARs of the muscles dissected from the adequately-fed pigs but were not observed in the deficient group. The *m. masseter* and *m. longissimus dorsi* showed the smallest and largest FARs, respectively, and both were significantly different from the *m. adductor* and *m. biceps femori* whose FARs were similar. The amino acid composition of the muscles was presented in Figure 3, 4, 5 and 6 and no significant differences existed between these groups, no matter which muscle.

The change in the enrichment of plasma free phenylalanine over time was shown in Figure 7 and both groups reached their plateaus around the 7th min after the beginning of infusion. The flooding infusion was successful because the ratio of free phenylalanine enrichment of the individual muscle to the final plasma sample in both groups was approximate to 1.00 (Table 5) and this implies that the free pool of those muscle samples was still flooding when frozen in liquid nitrogen and at sequential -70°C. The MPE was significantly higher in the lysine-deficient pigs (12.72 ±0.07%) than in the adequately-fed group (11.66±0.01%) after the 7th min and this is attributed to more free phenylalanine to dilute the MPE of L-[$^2H_5$]-phenylalanine because of the larger body size of the control pigs.

The dietary lysine deficiency inhibited the muscle FSR by 31% averaged while only the *m. masseter* and *m. adductor* presented significant decreases in the FDR reduced by 30%, approximately, due to the deficient treatment (Table 6). Although the FDR of *m. biceps femoris* was also restrained, the difference compared to the adequately-fed pigs only showed a very high tendency ($p =$

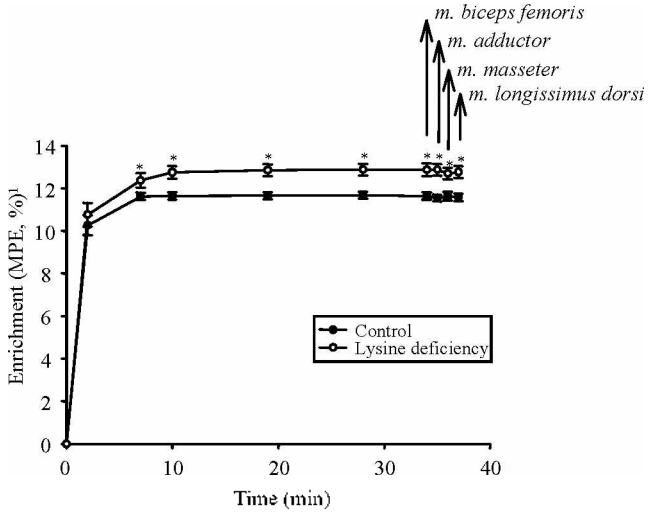

Figure 7. The curves of plasma and muscle free phenylalanine enrichment over time. [1] Each value is the mean±SE of 6 postweanling pigs. * Significantly different from the control group ($p<0.05$).

0.055) of reduction. In addition, the FAR/FSR of the *m. longissimus dorsi* was significantly greater in the control group than in the deficient one. The pigs receiving the same treatment did not present significant differences in FSRs between the muscles; however, the *m. masseter* of the control group presented a significantly higher FDR in comparison with the others. The adequately-fed pigs also presented significant differences in the FAR/FSR of the muscles but this did not happen in the deficient group. Both the *m. adductor* and *m. biceps femori* showed the significantly different FAR/FSR from those of the *m. longissimus dorsi* and *m. masseter*.

Table 5. The ratio[1] of the free phenylalanine enrichment of individual muscles to the final plasma sample

	Control	Lysine deficiency
M. masseter	1.00±0.02	0.99±0.01
M. longissimus dorsi	0.99±0.01	0.99±0.01
M. adductor	0.99±0.01	1.00±0.02
M. biceps femoris	1.00±0.01	1.00±0.02

[1] Each value is the mean±SE of 6 postweanling pigs.

The RNA capacity of the muscles was not influenced by the lysine deficiency; however, the RNA translation activity was decreased by 30% averaged (from 26.8 to 33.5%, Table 7).

DISCUSSION

The growth retardation observed due to the lysine deficiency was similar to the phenomena reported in the studies of lysine- or other amino-acid- requirements by dose-response methodology for swine (Batterham et al., 1990; Chung and Baker, 1992; Mahan et al., 1993). Although the tube-feeding technique changed the intake behavior, the growth rates of both the groups were better than that, 0.45 kg/d, for 10-20 kg of weight assigned by NRC (1988). That might result from the hybrid vigor of the three-breed-crossing barrows and/or the finely-ground feed for facilitating feeding and being digested easily due to decreases in size and then increases in surface area of feed particles.

The final body weight and growth rate did not seem to be as accurate indices in the current study because their

Table 6. The effect of dietary lysine deficiency on the fractional rates of synthesis (FSR), degradation (FDR) and FAR/FSR of the muscles of postweanling pigs

	FSR		FDR		FAR/FSR	
	Control	Lysine deficiency	Control	Lysine deficiency	Control	Lysine deficiency
	(%/day)[1]				(%)[1]	
M. masseter	6.23±0.39^{x}	4.21±0.23^{x}*	3.81±0.50^{x}	2.62±0.40^{x}*	39.6±3.1^{x}	37.8±5.6^{x}
M. longissimus dorsi	5.42±0.48^{x}	3.99±0.33^{x}*	1.97±0.54^{y}	2.09±0.61^{x}	64.7±2.6^{y}	48.8±6.2^{x}*
M. adductor	5.54±0.27^{x}	3.68±0.38^{x}*	2.61±0.38^{y}	1.83±0.61^{x}*	53.3±3.3^{z}	50.6±6.6^{x}
M. biceps femoris	5.65±0.27^{x}	3.91±0.31^{x}*	2.73±0.33^{y}	2.15±0.42^{x}	52.0±1.9^{z}	45.9±3.7^{x}

[1] Each value is the mean±SE of 6 postweanling pigs.

* Significantly different from the control group ($p<0.05$).

^{x,y,z} Means within a column with no common superscripts are significantly different ($p<0.05$).

Table 7. The effect of dietary lysine deficiency on the RNA capacity and translation activity of the muscles of postweanling pigs

	RNA capacity		RNA activity	
	Control	Lysine deficiency	Control	Lysine deficiency
	(mg RNA/g tissue protein)[1]		(g protein synthesized/day/g RNA)[1]	
M. masseter	9.58±0.26	9.24±0.31	6.50±0.34	4.56±0.04*
M. longissimus dorsi	7.70±0.37	7.67±0.26	7.08±0.53	5.18±0.23*
M. adductor	7.86±0.26	7.44±0.39	7.07±0.31	4.91±0.15*
M. biceps femoris	8.60±0.30	8.55±0.20	6.65±0.44	4.42±0.30*

[1] Each value is the mean±SE of 6 postweanling pigs.

* Significantly different from the control group ($p<0.05$).

extents of decrease. 12% and 25%. were less sensitive than that. 38.7%. of FAR averaged for the muscles when the dietary lysine content measured was decreased by 32.5%. Since body protein deposition is the single largest factor determining dietary amino-acid requirements (De Lange et al., 2001). the deficiency in dietary lysine more easily reflected to the FAR of the muscles. In addition, the limiting amino acid restricted incorporation of other amino acids into protein so that their fate was converted to provide extra energy. Because most of the variation in chemical body composition can be attributed to the contents of body lipid considering that close associations exist among body protein, water and ash except fat (De Lange et al., 2001), the disproportional decrements in the final body weight and growth rate to the dietary lysine deficiency probably resulted from an increase in body lipid content. as demonstrated by Hogberg and Zimmerman (1979).

Despite inhibition in the relative weights and FAR of the muscles due to the lysine deficiency. the amino-acid compositions of the four muscles were not changed. This implies that balance of deposited individual muscle proteins was still consistent although deposition was lessened. Changes in the amino acid compositions of muscles probably would happen if pigs received diets deficient in lysine more severely considering the fact that the reactions of three types of muscle protein. including myofibrillar. sarcoplasmic and stroma proteins. to severe alterations in nutritional status. such as full-fed vs. fasting. were different (Reeds et al.. 1993) and the amino acid compositions of muscle proteins including myosin. actin and collagen varied greatly (Wei. 1999).

The FSR of muscle would be greater in young animals than in older ones (Barrett and Gelfand. 1989), and the data from the current control pigs combined with those in some literature also presented this decreasing trend with age. Cortamira et al. (1991) reported that muscle FSR of piglets at 4 to 6 kg of weight was 10.1 %/d averaged. and this is higher than 6.0 %/d (22 kg of weight. Mulvaney et al., 1985). 5.7 %/d (23.3 kg. the current study) and 4.8 %/d (45 kg. Mulvaney et al.. 1985). In other reports. nevertheless. the FSR of porcine muscles was higher in older ones compared to the current data. for instance: 8.1 and 7.0 %/d for 30 and 35 kg. respectively (Simon et al.. 1978; Wykes et al., 1996), which might be due to different breeds, possibly. In addition. Cortamira et al. (1991) and Ponter et al. (1994) observed that the FSR was greater in the muscles containing mainly fast-twitch glycolytic fibre. characterized by high myofibrillar ATPase and low citrate synthase activities (Laborde et al., 1985; Remignon et al.. 1994). than in those mainly with slow-twitch oxidative fibre or mixed fibre in the very young piglets. This phenomenon. however. was opposite to the observations from older pigs (the current study; Mulvaney et al.. 1985), broilers (Tesseraud et al., 1996a) and rats (Lewis et al.. 1984). although the FSR of porcine slow-twitch muscles only showed a higher tendency in comparison with the other types of muscles. Cortamira et al. (1991) attributed the discrepancy to a change in the development of the muscles with age. As to degradation. the muscles with majority in slow-twitch fibre presented significantly greater FDR than those with major fast-twitch or mixed fibre did in not only the postweanling pigs (the current study) but also broilers (Tesseraud et al.. 1996a). In addition. the methodology adopted in the current study for FDR based on the difference between FSR (measured in 40 min) and FAR (measured over weeks) might be somewhat unsatisfactory (Rathmacher. 2000) because the calculation rested on the assumption that FSR was constant over the time when FAR was determined while protein synthesis would vary with age and then result in underestimate in degradation.

Despite the similarity in the FSR, the present FAR and FAR/FSR data of *m. longissimus dorsi* is much higher than those (3.45 vs. 0.7 %/d; 64.7 vs. 13.5%) reported by Mulvaney et al. (1985). Since this muscle was considered as exhibiting high impetus for postnatal growth (Davis. 1974). its FAR and FAR/FSR should be higher to present a smaller FDR calculated and then low protein turnover compared to muscles with low or intermediate impetus. A contradictory observation. however. existed in the three impetus types of porcine muscles at 22 kg of weight (Mulvaney et al., 1985). Because the authors did not show any information concerning the growth rate or the initial and final muscle protein mass of their pigs. it is very difficult to give a reasonable interpretation. The current data of FAR/FSR and relative weight to whole body supported that the growth impetus was higher in *m. longissimus dorsi*, intermediate in *m. adductor* and *m. biceps femori* and lower in *m. masseter*.

Protein accretion is a consequence from the competition between synthesis and degradation (Lobley. 1997) so that any alteration in both would result in changes in the FAR. In the current study. the pattern of protein turnover of the muscles from the deficient group could be divided into two categories: one. including *m. masseter*, *m. adductor* and *m. biceps femori*, presented decreases in both FSR and FDR; another was *m. longissimus dorsi* whose FSR was also reduced but the FDR was enhanced slightly. Due to lacking of literature concerning the protein turnover of porcine muscles under dietary lysine deficiency. information from broilers is cited for reference. Although both *m. longissimus dorsi* of swine and *m. pectoralis major* of chickens contained mainly fast-twitch fibre. the latter exhibited increases in both FSR and FDR but decline in muscle weight and ASR under lysine deficiency (Tesseraud et al.. 1996b). *i.e.* the increment was larger in the FDR than in the FSR. This possibly could be attributed to the different species because the *m. anterior latissimus* (a typical slow-

twitch fibre type) and *m. sartorius* (a typical mixed-fibre type) of the chicks also showed the same phenomenon (Tesseraud et al., 1996a) while this differed from the performance of the current porcine *m. masseter* containing majority in slow-twitch oxidative fibre and the *m. adductor* and *m. biceps femori* whose fibre belong to the mixed type. On the other hand, the dissimilarity between these two researches probably resulted from different feeding methodology. Essential amino-acid deficiencies caused low feed-intake in animals fed *ad libitum* (Gietzen et al., 1998; Smriga et al., 2000) so that consequent growth retardation would become severe due to deficiency in all nutrients. The intubation, nevertheless, could avoid this disadvantage (Cortamira et al., 1991) to maintain normal feed intake according to daily metabolic body weight apart from the limiting amino acid. Since Tesseraud et al. (1996a, b) fed their birds with a lysine deficient diet *ad libitum*, the severity of growth retardation in the muscles was inevitable. That's why their data of extents in decreases was more austere in muscle weight and protein content than in ASR and resulted in an increase in the FSR.

The ratio of FAR to FSR can be considered as an opposite index for protein turnover, *i.e.* less protein deposited from synthesis means faster protein turnover, and it is decided by both synthesis and degradation. The protein turnover of the *m. longissimus dorsi*, with the significantly higher FAR/FSR, was enhanced by the lysine deficiency while the effects on the other muscles were not significant. *i.e.* despite inhibition in the protein syntheses of all the muscles, only the degradation of the *m. longissimus dorsi* was not reduced. In addition, although significant differences existed in the FAR and FDR of the muscles with the different fibre types of the adequately- fed pigs, the current lysine deficiency blurred such differences. This did not occur, however, in broiler data (Tesseraud et al., 1996a). For synthesis, because the RNA capacity of all the muscles was not changed by the lysine deficiency, it implied that the RNA content was sufficient while the translational activity was lessened. One possible link would be that corresponding amino-acyl tRNA levels to limiting amino acids were insufficient and then resulted in a lack of polysome aggregation and increases in free ribosome subunits, monosomes and disomes (Allen et al., 1969; Pronczuk et al., 1970; Ip and Harper, 1974). Furthermore, Shenoy and Rogers (1977) demonstrated the insufficient charge level would not be the only cause. The regulation to the formation of initiation complex by eukaryotic initiation factors (eIF) in the process of translation was considered as relating to the decrease in polysome aggregation (Flaim et al., 1982, Kelly and Jefferson, 1985; Preedy and Garlick, 1986). For instance, Kimball et al. (1998) observed with myoblasts *in vitro* that deprivation of leucine or histidine resulted in a decrease in protein synthesis with an increase in eIF2 phosphorylation. In addition, translation would not be inhibited when eIF4E binding protein was in a phosphorylated state (Hara et al., 1997).

As to degradation, a possible link would be differential sensitivities of proteolyses to the lysine deficiency. There are four systems of muscle proteases including lysosomal hydrolases (cathepsins), Ca^{2+}-activated proteases (calpains), a soluble ATP-dependent proteolytic system involving a cofactor ubiquitin and a nonlysosomal process independent of ATP (Tawa et al., 1992). Van Den Hemel-Grooten et al. (1995) fell to observed alterations in the activities of calpains and cathepsin in muscles from the barrows receiving a nitrogen-free diet for two weeks. Wing and Goldberg (1993) demonstrated that increases in the activities of lysosomal and nonlysosomal ATP-dependent proteolyses in muscles from rats starved for one day while the activities of Ca^{2+}-activated proteases were without changes. The current lysine deficiency is different from those situations including the nitrogen-free one, lacking of all amino acids, and the starvation, devoid of all nutrients, so that the alterations in the activities of such four proteolysis systems may be inconsistent.

To sum up, the present lysine deficiency changed the FAR of muscle protein through the alterations in the FSR and/or FDR, and the further works will be to tap the effects of dietary lysine deficiency on the phosphorylation of the eIF2 and the eIF4E binding protein and on the alterations in the four proteolysis systems of the muscles.

ACKNOWLEDGMENT

The authors wish to thank the National Science Council in Taiwan Government for financial support to this research project, NSC 90-2313-B-002-370. We are also grateful to Prof. Jih-Tay Hsu (National Taiwan University) for his guidance in surgery, to Prof. W. C. Chang and Mrs. J. Y. Tsai (National Taiwan University) for their technique assistance in measuring amino acids, to Dr. Cheng-Huang Lin (National Taiwan Normal University) for his generosity in lending the GC-MS for the free pool measurement and to Dr. Gerald Lobley and Miss Susan Anderson (Rowett Research Institute, Scotland) for their kindliness in helping the bound pool analyses.

REFERENCES

Allen, R. E., P. L. Raines and D. M. Regen. 1969. Regulatory significance of transfer RNA charging levels: I. Measurements of charging levels in livers of chow-fed rats, fasting rats, and rats fed balanced or imbalanced mixtures of amino acids. Biochim. Biophys. Acta. 190:323-336.

AOAC. 1984. Offical Methods of Analysis. 14th edn. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Barrett, E. J. and R. A. Gelfand. 1989. The *in vivo* study of cardiac

and skeletal muscle protein turnover. Diabetes Metab. Rev. 5:133-148.

Batterham, E. S., L. M. Andersen, D. R. Baigent and E. White. 1990. Utilization of ileal digestible amino acids by growing pigs: effect of dietary lysine concentration on efficiency of lysine retention. Br. J. Nutr. 64:81-94.

Calder, A. G. and A. Smith. 1988. Stable isotope ratio analysis of leucine and ketoisocaproic acid in blood plasma by gas chromatography/ mass spectrometry. Use of tertiary butyldimethylsilyl derivatives. Rapid Commun. Mass Spectrom. 2:14-16.

Calder, A. G., S. E. Anderson, I. Grant, M. A. McNurlan and P. J. Garlick. 1992. The Determination of low d_5-phenylalanine enrichment (0.002-0.09 atom percent excess), after conversion to phenylethylamine, in relation to protein turnover studies by gas chromatography/electron ionization mass spectrometry. Rapid Commun. Mass Spectrom. 6:421-424.

Campbell, I. M. 1974. Incorporation and dilution values their calculation in mass spectrally assayed stable isotope labeling experiments. Bioorg. Chem. 3:386-397.

Chung, T. K. and D. H. Baker. 1992. Efficiency of dietary methionine utilization by young pigs. J. Nutr. 122:1862-1869.

Cortamira, N. O., B. Seve, Y. Lebreton and P. Ganier. 1991. Effect of dietary tryptophan on muscle, liver and whole-body protein synthesis in weaned piglets: relationship to plasma insulin. Br. J. Nutr. 66:423-435.

Davies, A. S. 1974. A comparison of tissue development in Pietrain and Large White pigs from birth to 64 kg of live weight. Anim. Prod. 19:377-387.

De Lange, C. F. M., S. H. Birkett and P. C. H. Morel. 2001. Protein, fat and bone tissue growth in swine. In: Swine Nutrition. 2nd Ed. (Ed. A. J. Lewis and L. L. Southern). CRC Press, Florida, USA. pp. 65-81.

Flaim, K. E., W. S. Liao, D. E. Peavy, J. M. Taylor and L. S. Jefferson. 1982. The role of amino acids in the regulation of protein synthesis in perfused rat liver: II. Effects of amino acid deficiency on peptide chain initiation, polysomal aggregation, and distribution of albumin mRNA. J. Biol. Chem. 257:2939-2946.

Fuller, M. F., P. J. Reeds, A. Cadenhead, B. Seve and T. Preston. 1987. Effects of the amount and quality of dietary protein on nitrogen metabolism and protein turnover of pigs. Br. J. Nutr. 58:287-300.

Garlick, P. J., D. J. Millward and W. P. James. 1973. The diurnal response of muscle and liver protein synthesis *in vivo* in meal-fed rats. Biochem. J. 136:935-945.

Garlick, P. J., J. Wernerman, M. A. McNurlan, P. Essén, G. E. Lobley, E. Milne, A. G. Calder and E. Vinnars. 1989. Measurement of the rate of protein synthesis in muscle of postabsorptive young men by injection of a 'flooding dose' of [1-^{13}C]leucine. Clin. Sci. (Lond). 77:329-336.

Gietzen, D. W., L. F. Erecius and Q. R. Rogers. 1998. Neurochemical changes after imbalanced diets suggest a brain circuit mediating anorectic responses to amino acid deficiency in rats. J. Nutr. 128:771-781.

Hamilton, C. R. and T. L. Veum. 1986. Effect of biotin and (or) lysine additions to corn-soybean meal diets on the performance and nutrient balance of growing pigs. J. Anim. Sci. 62:155-162.

Hara, K., K. Yonezawa, M. T. Kozlowski, T. Sugimoto, K. Andrabi, Q. P. Weng, M. Kasuga, I. Nishimoto and J. Avruch. 1997. Regulation of eIF-4E BP1 phosphorylation by mTOR. J. Biol. Chem. 272:26457-26463.

Hogberg, M. G. and D. R. Zimmerman. 1979. Effects of protein nutrition in young pigs on developmental changes in the body and skeletal muscles during growth. J. Anim. Sci. 49:472-481.

Hunter, K. A., P. E. Ballmer, S. E. Anderson, J. Broom, P. J. Garlick and M. A. McNurlan. 1995. Acute stimulation of albumin synthesis rate with oral meal feeding in healthy subjects measured with [ring-$^2H_5$] phenylalanine. Clin. Sci. (Lond). 88:235-242.

Ip, C. C. and A. E. Harper. 1974. Liver polysome profiles and protein synthesis in rats fed a threonine-imbalanced diet. J. Nutr. 104:252-263.

Kelly, F. J. and L. S. Jefferson. 1985. Control of peptide-chain initiation in rat skeletal muscle: Development of methods for preparation of native ribosomal subunits and analysis of the effect of insulin on formation of 40 S initiation complexes. J. Biol. Chem. 260:6677-6683.

Kimball, S. R., R. L. Horetsky and L. S. Jefferson. 1998. Implication of eIF2B rather than eIF4E in the regulation of global protein synthesis by amino acids in L6 myoblasts. J. Biol. Chem. 273:30945-30953.

Laborde, D., A. Talmant and G. Monin. 1985. Activitiés enzymatiques métaboliques et contractiles de 30 muscles du porc: Relations avec le pH ultime atteint aprés la mort (Enzyme metabolic and contractile activity in 30 pig muscles and their relationship to ultimate postmortem pH.). Reprod. Nutr. Dévelop. 25:619-628.

Lee, D. N., Y. H. Cheng, Y. S. Chung, J. L. Shive, Y. M. Lian, H. W. Wei and C. F. Weng. 2004. Effects of dietary taurine supplementation on the growth performance, serum constituents and antibody production of broilers. Asian-Aust. J. Anim. Sci. 17:109-115.

Lewis, S. E. M., F. J. Kelly and D. F. Goldspink. 1984. Pre- and post- natal growth and protein turnover in smooth muscle, heart and slow- and fast- twitch skeletal muscles of the rat. Biochem. J. 217:517-526.

Lobley, G. E. 1997. Nutritional and hormonal control of peripheral tissue metabolism in farm species. Livestock Prod. Sci. 56:91-114.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

Mahan, D. C., R. A. Eater, G. L. Cromwell, E. R. Miller and T. L. Veum. 1993. Effect of dietary lysine levels formulated by altering the ratio of corn:soybean meal with or without dried whey and L-lysine.HCl in diets for weanling pigs. NCR-42 Committee on Swine Nutrition. J. Anim. Sci. 71:1848-52.

McNurlan, M. A., A. M. Tomkins and P. J. Garlick. 1979. The effect of starvation on the rate of protein synthesis in rat liver and small intestine. Biochem. J. 178:373-379.

McNurlan, M. A. and P. J. Garlick. 1981. Protein synthesis in liver and small intestine in protein deprivation and diabetes. Am. J. Physiol. 241:E238-E245.

McNurlan, M. A., P. Essen, A. Thorell and A. G. Calder. 1994. Response of protein synthesis in human skeletal muscle to insulin: an investigation with L-[$^2H_5$]phenylalanine. Am. J. Physiol. 267:E102-E108.

Millward, D. V., P. J. Garlick, R. J. C. Stewart, D. O. Nnanyelugo and J. C. Waterlow. 1975. Skeletal-muscle growth and protein turnover. Biochem. J. 150:235-243.

Mulvaney, D. R., R. A. Merkel and W. G. Bergen. 1985. Skeletal muscle protein turnover in young male pigs. J. Nutr. 115:1057-1064.

Munro, H. N. and A. Fleck. 1969. Analysis of tissue and body fluids for nitrogenous constituents. In: Mammalian Protein Metabolism (Ed. H. N. Munro). Academic Press, New York, USA. pp. 424-525.

National Research Council. 1988. Nutrient Requirements of Swine, 9th Ed., National Academy Press, Washington, DC.

National Research Council. 1998. Nutrient Requirements of Swine 10th Ed. National Academy Press, Washington, DC.

Ponter, A. A., N. O. Cortamira, B. Seve, D. N. Salter and L. M. Morgan. 1994. The effects of energy source and tryptophan on the rate of protein synthesis and on hormones of the entero-insular axis in the piglet. Br. J. Nutr. 71:661-674.

Preedy, V. R. and P. J. Garlick. 1986. The response of muscle protein synthesis to nutrient intake in postabsorptive rats: the role of insulin and amino acids. Biosci. Rep. 6:177-183.

Pronczuk, A. W., Q. R. Rogers and H. N. Munro. 1970. Liver polysome patterns of rats fed amino acid imbalanced diets. J. Nutr. 100:1249-1258.

Rathmacher, J. A. 2000. Measurement and significance of protein turnover. In: Farm Animal Metabolism and Nutrition. (Ed. J. P. F. D'Mello). CAB International, Oxon, UK. pp. 25-47.

Reeds, P. J., D. G. Burrin, T. A. Davis, M. A. Fiorotto, H. J. Mersmann and W. G. Pond. 1993. Growth regulation with particular reference to the pig. In: Growth of the Pig. (Ed. G. R. Hollos). CAB International, Oxon, UK. pp. 1-32.

Remignon, H., L. Lefaucheur, J. C. Blum and F. H. Ricard. 1994. Effects of divergent selection for body weight on three skeletal muscles characteristics in the chicken. Br. Poult. Sci. 35:65-76.

Salter, D. N., A. I. Montgomery, A. Hudson, D. B. Quelch and R. J. Elliott. 1990. Lysine requirements and whole-body protein turnover in growing pigs. Br. J. Nutr. 63:503-513.

SAS Institute Inc. 1990. SAS/STAT User's Guide: Version 6. 4th edn. SAS Institute, Inc., Cary, North Carolina.

Shenoy, S. T. and Q. R. Rogers. 1977. Effect of starvation on the charging levels of transfer ribonucleic acid and total acceptor capacity in rat liver. Biochim. Biophys. Acta. 476:218-227.

Simon, O., R. Münchmeyer and H. Bergner. 1978. Estimation of rate of protein synthesis by constant infusion of labeled amino acids in pigs. Br. J. Nutr. 40:243-251.

Smriga, M., H. Murakami, M. Mori and K. Torii. 2000. Effects of L-lysine deficient diet on the hypothalamic interstitial norepinephrine and diet-induced thermogenesis in rats *in vivo*. Biofactors 12:137-142.

Steel, R. D. G. and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd edn. McGraw-Hill Book Company, New York, New York.

Sugden, P. H. and S. J. Fuller. 1991. Regulation of protein turnover in skeletal and cardiac muscle. Biochem. J. 273:21-37.

Tawa, N. E., Jr., I. C. Kettelhut and A. L. Goldberg. 1992. Dietary protein deficiency reduces lysosomal and nonlysosomal ATP-dependent proteolysis in muscle. Am. J. Physiol. 263:E326-E334.

Tesseraud, S., N. Maaa, R. Peresson and A. M. Chagneau. 1996a. Relative responses of protein turnover in three different skeletal muscles to dietary lysine deficiency in chicks. Br. Poult. Sci. 37:641-650.

Tesseraud, S., R. Peresson, J. Lopes and A. M. Chagneau. 1996b. Dietary lysine deficiency greatly affects muscle and liver protein turnover in growing chickens. Br. J. Nutr. 75:853-856.

Van den Hemel-Grootn, H. N. A., M. Koohmaraie, J. T. Yen, J. R. Arbona, J. A. Rathmacher, S. L. Nissen, M. L. Fiorotto, G. J. Garssen and M. W. A. Verstegen. 1995. Comparison between 3-methylhistidine production and proteinase activity as measures of skeletal muscle breakdown in protein-deficient growing barrows. J. Anim. Sci. 73:2272-2281.

Wei, H. W. 1999. Effect of Long-term Amino-acid Deficiency on the Amino-acid Composition of the Body. Ph.D. Thesis, University of Aberdeen, Aberdeen, Scotland, UK.

Willian, A. P. 1994. Recent developments in amino acid analysis. In: Amino acids in farm animal nutrition (Ed. J. P. F. D'Mello). CAB International, Oxon, UK. pp. 11-36.

Wing, S. S. and A. L. Goldberg. 1993. Glucocorticoids activate the ATP-ubiquitin-dependent proteolytic system in skeletal muscle during fasting. Am. J. Physiol. 264:E668-E676.

Wykes, L. J., M. Fiorotto, D. G. Burrin, M. D. Rosario, M. E. Frazer, W. G. Pond and F. Jahoor. 1996. Chronic low protein intake reduces tissue protein synthesis in a pig model of protein malnutrition. J. Nutr. 126:1481-1488.