

Patterns of Nitrogen Excretion in Growing Pigs¹

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ABSTRACT : Three crossbred gilts weighing 61 ± 2 kg (mean \pm SD) and three gilts weighing 52 ± 3 kg on the day before the first treatment began (d -1) were used for each of two experiments (Exp. 1 and Exp. 2), respectively. In Exp. 1, all pigs were fed the experimental diet (CP 19%) from d -7 to the end of study (d 21) to verify that nitrogen retention is constant during the 21 -d period. In Exp. 2, pigs were fed the control diet (CP 15.5 %) from d -7 to d 8 and then the low-lysine diet from d 9 to d 16 in order to determine how rapidly dietary changes in amino acid composition results in a new equilibrium for nitrogen metabolism.

The amount of urine nitrogen loss was not different over 21 days ($p > 0.10$). Rates of nitrogen retention were not different among pigs ($p > 0.10$) nor over time ($p > 0.10$). Average nitrogen retention during the period was 1.00 g/kg BW^{0.75} per day. The apparent biological value

was 41%, which did not change over the 3-week period ($p > 0.10$). The overall efficiency of nitrogen use for nitrogen retention was 35% (Exp. 1). The amount of nitrogen loss in urine and the efficiency of nitrogen utilization for nitrogen gain reached a new equilibrium within 2 to 3 d after the diet was changed. The low-lysine diet resulted in a 20% increase of nitrogen loss in urine ($p < 0.001$) and a 9% decline in efficiency of nitrogen use for nitrogen retention ($p < 0.001$). Nitrogen retention while the pigs were fed the control diet was also higher than the retention when pigs were fed the low lysine diet ($p < 0.001$). The efficiency of nitrogen use for nitrogen retention in pigs fed the control diet was 57% (Exp. 2), which was higher ($p < 0.001$) than that from pigs fed the low-lysine diets (52%).

(Key Words: Pigs, N Retention, N Metabolism, Lysine)

INTRODUCTION

The extent to which dietary protein is utilized for protein deposition can be estimated by measuring nitrogen retention using either nitrogen balance or comparative slaughter whole-body analysis. Due to the simplicity of procedures and brevity, nitrogen balance studies have been widely used in swine researchers (Rutledge et al., 1961; Miller et al., 1969; Dunkin et al., 1986; Wray-Cahen et al., 1991; Hansen and Lewis, 1993). When estimating the efficiency of absorbed nitrogen use for nitrogen retention (i.e. apparent biological value) by nitrogen balance, separate but quantitative collections of urine and feces must be made. Females allow for a more precise separation between urine and feces. Spilled feed

that is free of hair also must be accurately measured.

Nitrogen retention (g/kg BW^{0.75} per day) declines rapidly to 45 kg and gradually in pigs weighing more than 45 kg (up to 160 kg). Therefore, a 21-d period should provide near constancy of nitrogen retention if pigs weighing more than 45 kg are used (Carr et al., 1977). If N retention is relatively constant, the number of pigs to be used can be reduced because one animal can be used for more than one treatment. Animals fitted with bladder catheters for a long time are subject to urogenital infection, but female pigs fitted with bladder catheters have been successfully used over 21 days (Fuller et al., 1979; Wang and Fuller, 1989).

The present experiments were conducted to (1) verify that N retention (g/kg BW^{0.75} per d) is sufficiently constant during a 21-day assay period (Exp. 1) and (2) to determine how rapidly dietary changes in amino acid composition result in a new equilibrium for N metabolism (Exp. 2). This validation is preliminary to conducting a series of amino acid studies using this technique.

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MATERIALS AND METHODS

Animals and housing

Three crossbred gilts (Large White × Landrace × 'multiple-breds' hybrid; PIC, USA) weighing 61 ± 2 kg (mean \pm SD) and 52 ± 3 kg on the day before the first treatment began (d -1) were used for each of two experiments (Exp. 1 and Exp. 2), respectively. Pigs were housed in metabolic cages in an environmentally controlled room with the temperature maintained at $20 \pm 1^\circ\text{C}$ and with 16:8 h light:dark cycle.

Animals were fed individually for 7 days in metabolism cages and then fitted with Foley bladder catheters (14Fr., 15 cc; Rüsck Manufacturing Ltd, Germany). Procedures described by Fuller et al. (1979) were modified for catheterization as follows. The exterior area of pigs and the operator's hands were washed with water and cleaned with a solution of Betadine (The Perdue Fredrick Co., USA). The lubricated tip of the catheter was introduced into the bladder through urethra which could be located by palpation using an index finger. To minimize bladder infection, 2 ml of Tribisson (80 mg trimethoprim and 400 mg sulphadiazine/ml; Coopers Animal Health Inc., USA) was introduced via intramuscular injection daily for 3 d after the animals were fitted with catheters. Daily feed intake and body temperature were carefully monitored to validate the health of the pigs over the experimental period. The protocol was reviewed and approved by the Cornell University Animal Care and Use Committee.

Diets and treatments

The diets used in Exp. 1 and 2 are shown in table 1 and table 2, respectively. Amino acid profiles of the diet used in Exp. 1 and the control diet in Exp. 2 approximated the ideal protein amino acid pattern of Wang and Fuller (1990). In Exp. 1, all pigs were fed the experimental diet (CP 19%, table 1) from d -7 to the end of study (d 21) to validate that nitrogen retention is constant during the 21-d period. Day 1 was defined as the first day when animals received the experimental treatment. In Exp. 2, pigs were fed the control diet (CP 15.5%, table 2) from d -7 to d 8 and then the low-lysine diet from d 9 to d 16 in order to determine how rapidly dietary changes in amino acid composition results in a new equilibrium for nitrogen metabolism. The low-lysine diet in Exp. 2 differed from the control diet in that supplemental synthetic lysine was replaced by corn starch to create diet which would result in a markedly lower N

retention. In Exp. 1 pigs were fed 4 times (08:00, 12:00, 16:00, and 20:00 h) daily at 100 g/kg BW^{0.75} which is 3 times the maintenance energy requirement (NRC, 1988). In Exp. 2 pigs were fed 6 times (07:00, 10:00, 13:00, 16:00, 19:00, and 22:00 h) daily at 80 g/kg BW^{0.75} (2.5 times the maintenance energy requirement). All pigs were given free access to water. The protein contents (N × 6.25) of the experimental diets were estimated by Kjeldahl analysis (AOAC, 1980).

Table 1. Composition of experimental diet (Exp. 1)

Item	% of diet ^a
Ingredient	
Corn	66.80
Soybean meal (48.5% protein)	26.90
Corn oil	2.00
Limestone	1.80
Dicalcium phosphate	0.81
Salt	0.55
Antibiotics ^b	0.50
Vitamin-trace mineral premix ^c	0.25
L-Lysine · HCl	0.21
DL-Methionine	0.10
L-Threonine	0.08
Chemical Composition^d	
Digestible energy (Mcal/kg)	3.5
Crude protein (%) ^e	19.00
Lysine (%)	1.17
Methionine + cystine (%)	0.75
Threonine (%)	0.78
Tryptophan (%)	0.24
Calcium (%)	0.94
Phosphorus (%)	0.51
Available phosphorus (%)	0.24

^a Presented on an as-fed basis.

^b Provided the following antibiotics per kilogram of complete diet: Chlortetracycline · HCl, 110 mg; Sulfamethazine, 110 mg; Penicillin, 55 mg.

^c Provided the following nutrients per kilogram of complete diet: vitamin A, 5,510 IU; vitamin D, 1,320 IU; vitamin E, 20 IU; vitamin K, 2.2 mg; pantothenic acid, 17.6 mg; riboflavin, 4.4 mg; niacin, 35.2 mg; choline 95.6 mg; vitamin B₁₂, 25.5 µg; Mg, 270 mg; Zn, 80 mg; Fe, 80 mg; Mn, 40 mg; Cu, 10 mg; I, 1 mg; Se, 0.3 mg.

^d Calculated values (NRC, 1988) unless otherwise indicated.

^e Analyzed by Kjeldahl N assay method.

Table 2. Composition of experimental diet (Exp. 2)

Item	% of diet ^a	
	Control	Low lysine
Ingredient		
Corn	74.89	74.89
Canola meal	10.10	10.10
Soybean meal (48.5% protein)	9.62	9.62
Corn oil	2.00	2.00
Limestone	0.86	0.86
Dicalcium phosphate	0.40	0.40
Salt	0.40	0.40
Antibiotics ^b	1.00	1.00
Vitamin-trace mineral premix ^c	0.25	0.25
L-Lysine · HCl	0.31	—
DL-Methionine	0.08	0.08
L-Threonine	0.06	0.06
L-tryptophan	0.03	0.03
Corn starch	—	0.31
Chemical Composition^d		
Digestible energy (kcal/kg)	3,450	3,450
Crude protein (%) ^e	15.46	15.30
Lysine (%)	0.98	0.74
Methionine + cystine (%)	0.66	0.66
Threonine (%)	0.68	0.68
Tryptophan (%)	0.21	0.21
Calcium (%)	0.60	0.60
Phosphorus (%)	0.47	0.47
Available phosphorus (%)	0.20	0.20

^a Presented on an as-fed basis.

^b Provided the following antibiotics per kilogram of complete diet: Chlortetracycline · HCl, 440 mg; Sulfamethazine, 440 mg; Penicillin, 220 mg.

^c Provided the following nutrients per kilogram of complete diet: vitamin A, 5,510 IU; vitamin D, 1,320 IU; vitamin E, 20 IU; vitamin K, 2.2 mg; pantothenic acid, 17.6 mg; riboflavin, 4.4 mg; niacin, 35.2 mg; choline 95.6 mg; vitamin B₁₂, 25.5 µg; Mg, 270 mg; Zn, 80 mg; Fe, 80 mg; Mn, 40 mg; Cu, 10 mg; I, 1 mg; Se, 0.3 mg.

^d Calculated values (NRC, 1988) unless otherwise indicated.

^e Analyzed by Kjeldahl N assay method.

Urine collection and analysis for nitrogen

Catheters were introduced into the urinary bladder on d-3 of each experiment. Urine collection started on d 4. In Exp. 1, urine was collected daily for the last 4 days of each week (d 4 to d 7, d 11 to d 14, and d 18 to d 21). In Exp. 2, urine was collected daily from d 4 to d 16 for two pigs and from d 4 to d 25 for one pig that exhibited a high fever (> 40.5°C). Urine was collected daily in 20-liter closed plastic jugs containing 400 ml of 10% (v/v) HCl to prevent loss of NH₃ and the growth of microbes (pH < 3). Sub-samples of the urine were transferred to 250 ml plastic bottles and stored at -20°C until N was determined by Kjeldahl analysis. Daily urine samples were used for N assay.

Statistical analysis

Data obtained from Exp. 1 were analyzed by two-way analysis of variance (pig and time, no interaction). The average 4-day urine N output during each period was used as one observation. This provided one datum per pig each week. Data are presented as means. For data obtained from pigs in Exp. 2, five-day means of urine N loss and N Eff (efficiency of N use calculated as N retention/N intake) for pigs fed the control diet were compared to the same variables for the pigs fed the low-lysine diet.

RESULTS

Experiment 1

Nitrogen metabolism over the 3-week study period in growing pigs is shown in table 3. The amount of urine nitrogen loss was not different over 21 days ($p > 0.10$). The rates of nitrogen retention were not different among pigs (figure 1; $p > 0.10$) nor over time (table 3 and figure 1; $p > 0.10$) when nitrogen digestibility was

Table 3. N metabolism over a 3-week period in growing pigs (Exp. 1)^a

Week ^b	N intake ^c	Urinary N ^c	N retention ^c	ABV (%) ^d	N Eff (%) ^e
1	2.90	1.44	1.00	41.0	34.5
2	2.87	1.40	1.01	42.1	35.3
3	2.85	1.41	0.98	41.1	34.5
SEM ^f	0.03	0.04	0.04	1.7	1.4

^a Data present treatment means. ^b Urine collected for the last 4 days of each week.

^c g/kg BW^{0.75} per day.

^d Apparent biological value of N = $100 \times (\text{N retention}/\text{N absorbed})$.

Nitrogen digestibility was assumed to be 84% (Wray-Cahen et al., 1991).

^e Overall efficiency of N use for N retention = $100 \times (\text{N retention}/\text{N intake})$.

^f Standard error of mean ($n = 3$).

assumed to be 84% (Wray-Cahen et al., 1991). Average nitrogen retention during the period was estimated to be 1.00 g/kg BW^{0.75} per d (table 3). The apparent biological value was estimated to be on the order of 41%, which did not change over the 3-week period ($p > 0.10$, figure 2). The overall efficiency of nitrogen use for nitrogen retention was 35%.

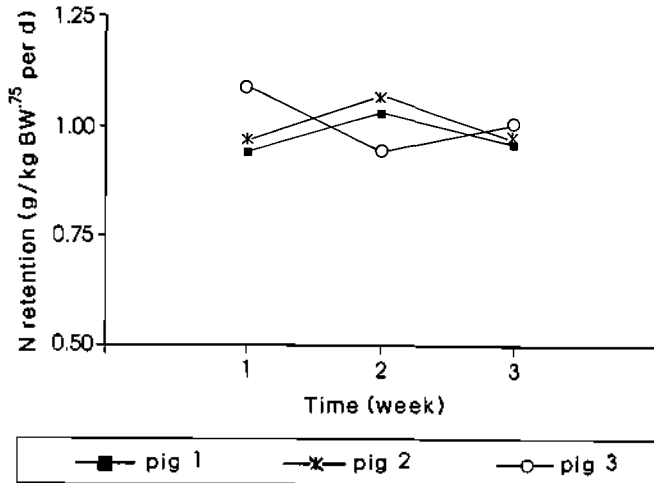


Figure 1. Change in nitrogen retention over a 21-d period. Nitrogen retention was measured as the difference between N intake and N loss in the urine and feces. Apparent fecal N digestibility was assumed to be 84% (Wray-Cahen et al., 1991). Urine was collected for the last 4 days of each week. Mean of N retention was not different among pigs ($p > 0.10$) nor over a 3-week period ($p > 0.10$). SEM = 0.04, $n = 3$.

Experiment 2

The daily variation of urinary nitrogen excretion and the overall efficiency of nitrogen use for nitrogen retention (N Eff; N retention/N intake) of two pigs (#1 and #2) used in Exp. 2 are illustrated in figure 3. The amount of nitrogen loss in urine and the efficiency of nitrogen utilization for nitrogen gain reached a new equilibrium within approximately 2 to 3 d after the diet was changed. One pig (#3) required 12 d, however, due to sickness, high fever and refusal of feed for two days (d 9 to 10, figure 4). The low-lysine diet resulted in a 20% increase of nitrogen loss in urine ($p < 0.001$) and a 9% decline in efficiency of nitrogen use for nitrogen retention ($p < 0.001$, table 4). Nitrogen retention while the pigs were fed the control diet was also higher than the retention when pigs were fed the low lysine diet ($p < 0.001$). The efficiency of nitrogen use for nitrogen retention in pigs fed the control diet was 57%, which was

higher ($p < 0.001$) than that from pigs fed the low-lysine diets (52%).

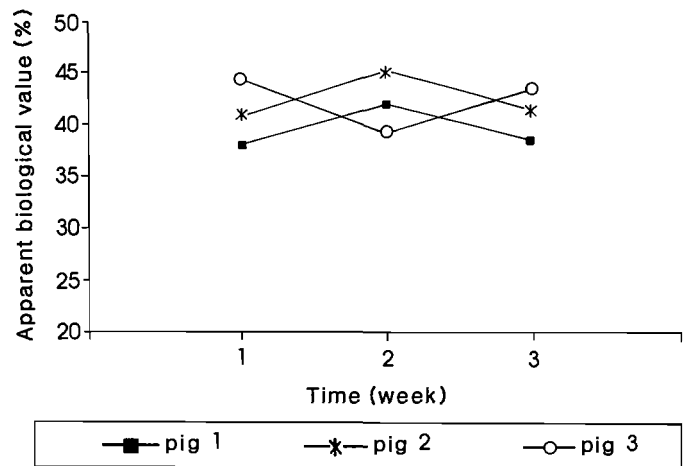


Figure 2. Change in apparent biological value (ABV) over a 3 week period in growing pigs. Digestibility of N was assumed to be 84%. ABV (%) was calculated as (N retention/absorbed N) \times 100. Mean of ABV was not different among pigs ($p > 0.10$) nor over a 3-week period ($p > 0.10$). SEM = 1.7, $n = 3$.

DISCUSSION

The present experiments were conducted to determine if the rate of nitrogen retention is sufficiently constant over a 21-d period and to determine how rapidly dietary changes in amino acids composition result in a new equilibrium for nitrogen metabolism. This validation was prerequisite to conducting a series of nutritional studies using this technique.

In a report by Carr et al. (1977), the rate of nitrogen retention declined by approximately 0.006 g per d with an increase of 1 kg BW in growing pigs weighing more than 45 kg. Since the mean body weight among periods in Exp. 1 increased by as much as 11 kg (64.0 kg for wk 1, 69.6 kg for wk 2 and 75.1 kg for wk 3), the decrease of nitrogen balance during the study period was expected to be only 0.067 g/kg BW^{0.75} per d based on the equation of Carr et al. (1977). The results from Exp. 1 (table 3, figure 1 and 2) confirmed that nitrogen retention and efficiency of absorbed nitrogen use for N retention (ABV) was relatively constant over the 21-d period for pigs fed the same diet.

Nitrogen retention varies with body weight. In young growing pigs weighing 40 kg, the maximum N retention was achieved to be 1.2 to 1.3 g/kg BW^{0.75} per d (Wang and Fuller, 1990). Using the equation of Carr et al. (1977),

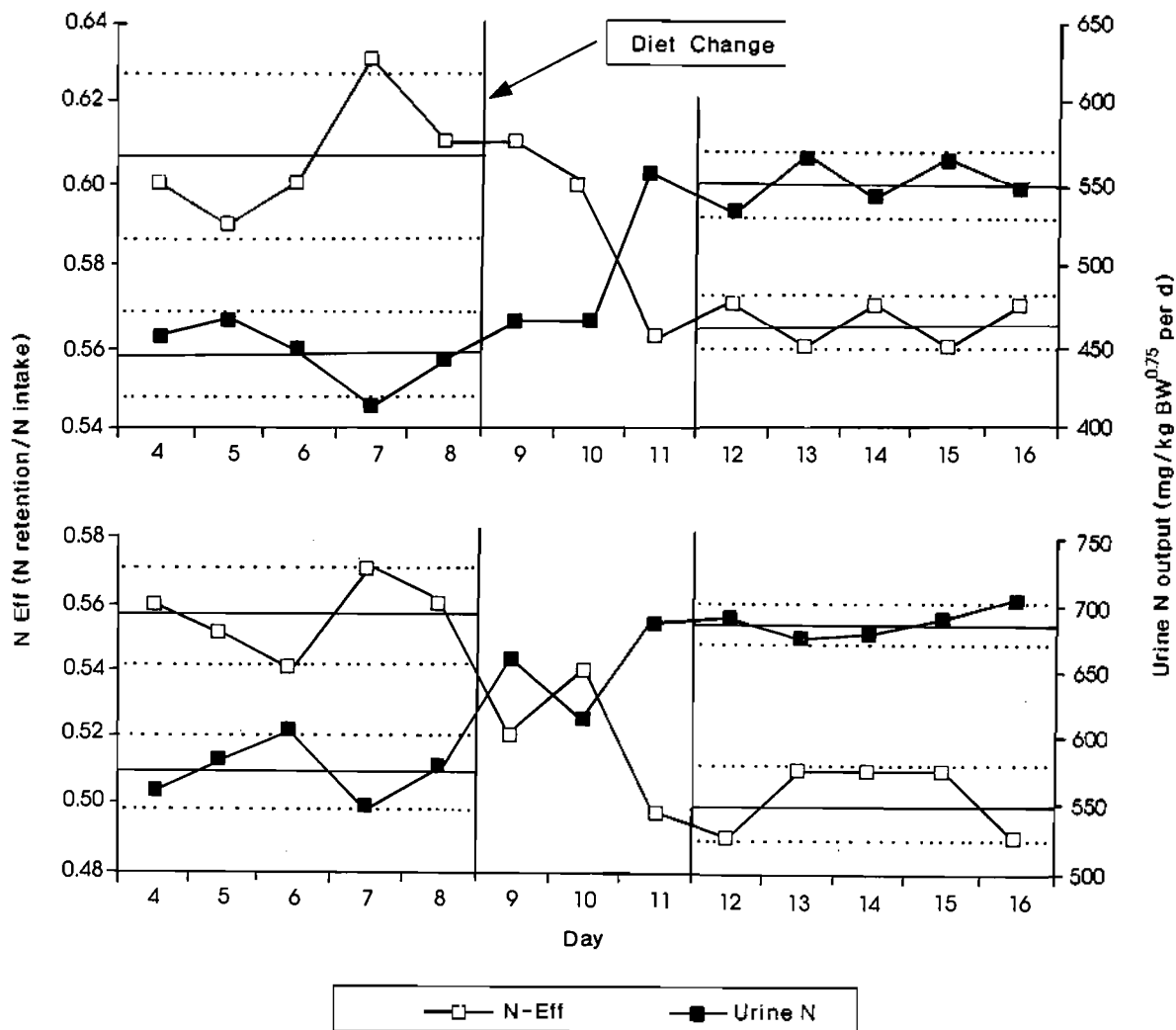


Figure 3. Time required to establish equilibrium of urine N output and N Eff (N retention/N intake) after a diet is changed. Top and bottom diagrams show N metabolism of #1 pig and #2 pig, respectively. Control diet (high-lysine) were given from d-7 to d-8 and then the low-lysine diet fed from d 9 to d 16. Solid horizontal lines represent 5 d-sample means of urine N and N Eff. Dotted horizontal lines show the 95% confidential intervals of the means. Urine N loss and N Eff reached a new equilibrium within 2 to 3 after a meal changed.

N retention for pigs of 65 kg is estimated to be 0.86 g/kg $BW^{0.75}$ per d. Hansen and Lewis (1993) have recently estimated that N retention was 0.98 g/kg $BW^{0.75}$ per d for female pigs of 65 kg. N retention was 1.0 g/kg $BW^{0.75}$ per d in Exp. 1 for 70 kg pigs and 1.10 g/kg $BW^{0.25}$ per d in Exp. 2 for 57 kg (average weight) pigs fed control diets (table 3 and 4). These results are similar to Hansen and Lewis' estimate, but higher than Carr et al.'s assessment. Discrepancy in the N retention reported by different research groups may be due to different rates of protein deposition achievable for the genotypes or environmental conditions. The pigs used by Hansen's group would be superior in their rates of growth and lean deposition to pigs used in the 1960s to 1970s when most

of studies reviewed by Carr's group were conducted. Different sexes also contribute to different N retention. Intact male pigs would have higher rates of N gain than females and castrated males. Maximum N retention for boars was estimated to be 1.2 g/kg $BW^{0.75}$ per d at 65 kg BW (Hansen and Lewis, 1993) and 0.9 g/kg $BW^{0.75}$ per d at 75 kg BW (Dunkin et al., 1986).

It was demonstrated that N retention in the growing castrates weighing 45 to 90 kg (average 67.5 kg) increased as energy intake increased up to 330 kcal DE/kg $BW^{0.75}$ per d (Campbell and Taverner, 1988) which corresponded to 3 times the maintenance energy requirement (NRC, 1988). It was also observed that nitrogen retention increased linearly as ME intake

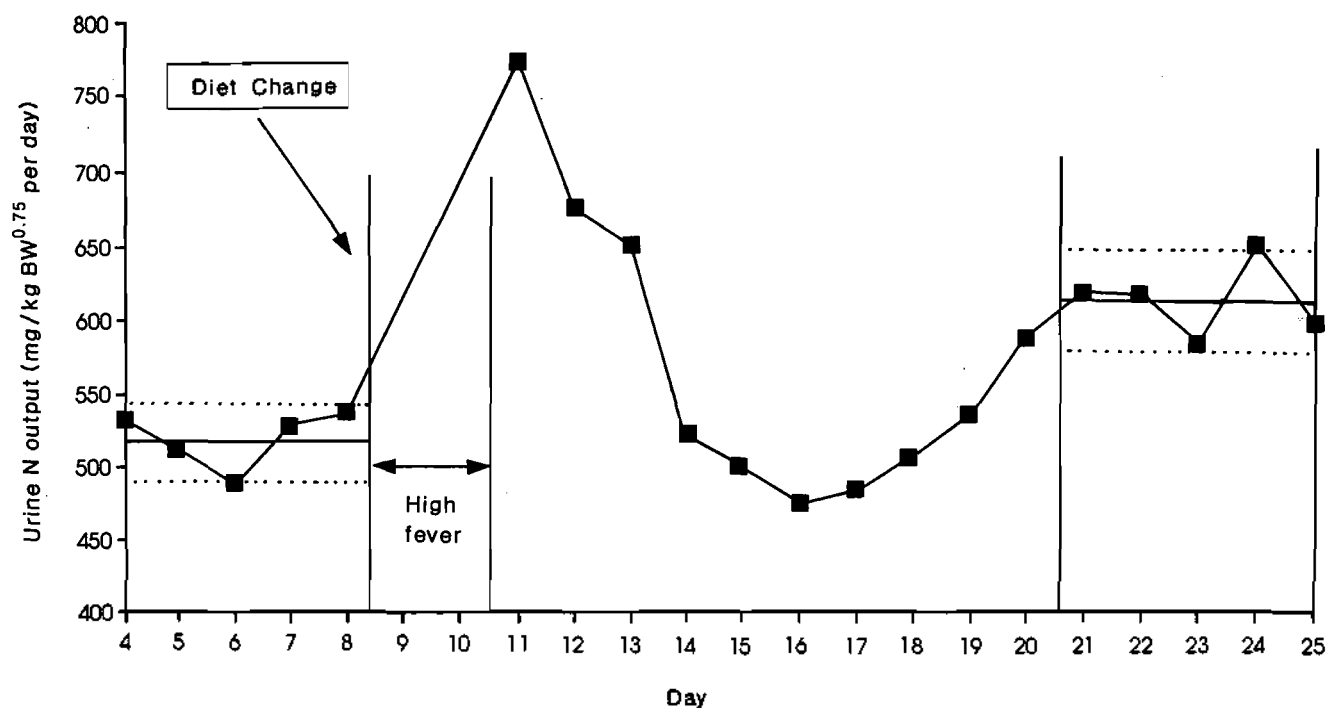


Figure 4. Time required to establish equilibrium of urine N output in the pig which had a high fever during the study period. Control diet (high-lysine) was given from d -7 to d 8 and then the low-lysine diet fed from d 9 to d 25. The pig was sick (high fever > 40.5°C) and consumed very little feed on d 9 and 10. The catheter was removed on d 9 and replaced on d 11 when the body temperature decreased to below 39.4°C. Solid horizontal lines represent 5 d-sample means of urine N. Dotted horizontal lines show the 95% confidential intervals of the means.

Table 4. Nitrogen metabolism in growing pigs (Exp. 2)^{a, b}

Criterion	Diet		SEM (n = 15) ^e	Statistical significance
	Control ^c	Low-lysine ^d		
N intake (g/kg BW ^{0.75} /per d)	1.91	1.95	0.01	p = 0.11
Urine N (g/kg BW ^{0.75} per d)	0.51	0.61	0.01	p < 0.001
N retention (g/kg BW ^{0.75} per d)	1.10	1.02	0.01	p < 0.001
N Eff (%) ^f	57.3	52.5	0.35	p < 0.001

^a Data present treatment means. ^b N digestibility was assumed to be 84% (Wray-Cahen et al., 1991).

^c Urine collected from d 4 to d 8 was used for estimates of N loss in urine. Daily samples were used for Kjeldahl N assay.

^d The pig which was sick on d 9 and d 10 reached the re-equilibrium in urinary N loss 12 d after the diet changed. For this pig fed low-lysine diet, urine N output was estimated from samples obtained from d 21 to d 25. For the other two pigs, urine collected from d 9 to d 13 was used for N analysis. Daily samples were used for Kjeldahl N assay.

^e Standard error of mean. ^f Efficiency of nitrogen utilization for N retention; 100 × (N retention/N intake).

increased up to 260 kcal/kg BW^{0.75} per d (270 kcal DE) in intact male pigs of 75 kg BW (Dunkin et al., 1986) and 280 kcal/kg BW^{0.75} per d (290 kcal DE) in female pigs of 60 kg BW (Fuller et al., 1976). Therefore, 2.5 to 3 times maintenance energy requirement was expected to support the maximum N retention for female and castrate pigs over 50 kg. Pigs were provided with 353 kcal DE/

kg BW^{0.75} per d in Exp. 1 and 275 kcal DE/kg BW^{0.75} per d in Exp. 2, which successfully supported N retention in both experiments.

The overall efficiency of N use for absorbed N retention (N retained/N absorbed) was 41.4% in Exp. 1, which was much lower than that observed from other N balance studies (Brown et al., 1973; Hansen and Lewis,

1993). This probably was a result of the high nitrogen intake of high protein content (19%) of the diet relative to need in Exp. 1. The results of Exp. 2 which involved a lower dietary protein level (15.5%) are in good agreement with other results on N Eff and re-emphasize the need to determine N efficiency in the linear portion of the response curve (i.e. protein levels below the requirement).

Removal of all free synthetic lysine from the control diet in Exp. 2 resulted in a marked decrease in N Eff and N retention ($p < 0.001$). However, the extent to which N retention and N Eff declined was much less than the degree of dietary lysine deletion (25%, table 2). According to Wang and Fuller's assumption (1989), when the first limiting amino acid is deleted from the diet, the degree of decline in N retention would correspond to the degree of deletion. If this were applied to Exp. 2, the extent to which N retention declined with a removal of free lysine in Exp. 2 should have been close to 25% since lysine was the co-first limiting amino acid with some other indispensable amino acids (e.g. methionine + cystine, threonine). However, the decline in N retention was only 7%. The reason for this is not clear. Of course, if all amino acids in control diets exceeded the requirement, 25% deletion of lysine would not decrease the N retention as much as 25%. Therefore, it is questionable whether lysine as well other indispensable amino acids in the control diet were used with maximum efficiency for N retention. If indispensable amino acids including lysine were in excess relative to dispensable amino acids at a given N intake, a fraction of indispensable amino acids would be catabolized without being efficiently utilized or converted into non specific N. In this case, the degree of decline in N retention would be less than that of lysine deletion. More research is needed to determine the optimum ratio of indispensable to dispensable amino acids.

All pigs fitted with catheters during N balance studies are subject to tissue irritation. To minimize the duration of catheterization, it is important to determine how rapidly dietary changes by nutrient alteration results in a new equilibrium for N retention of N excretion into urine and feces. As shown in figure 3, N output in urine reached a new equilibrium within 2 to 3 d after a removal of free lysine in two pigs. Our results are in agreement with Fuller et al. (1979) who observed that the rate of daily urea excretion was re-equilibrated within 2 to 3 d after additions of limiting amino acids.

One of the pigs used in Exp. 2 became sick and consumed very little feed on d 9 to 10 (data not shown)

when the diet changed. The rate of N excretion in urine subsequently increased for 3 d (on d 11 to d 14) and then rapidly decreased and remained very low for 5 to 6 d (one d 15 to d 20) before it increased and reached the expected equilibrium on d 21 to 25 (figure 4). The extremely low rate of urine N excretion after sickness probably can be attributed to the compensatory growth which may occur after a temporary shortage of nutrient intake.

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