Diammonium Citrate Addition to a Diet Restricted Non-Essential Amino Acids for Young Pigs

Katsumoto KAGOTA, Toshio IWASE, Toshiyuki KOJIMA,
Masayoshi NIIYAMA and Shigeo NAMIOKA

*Department of Veterinary Internal Medicine, Faculty of Veterinary Medicine,
Hokkaido University, Sapporo-shi, Hokkaido 060*

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Abstract. This feeding experiment with young pigs was conducted for 28 days to evaluate the growth effect of dietary diammonium citrate (DAC) by using three diets in which essential amino acids were supplied at the minimal requirement level for swine recommended by N.R.C. [12]. The three diets were: 1) diet restricted non-essential amino acids (basal diet); 2) basal diet plus 3.75% DAC (DAC diet); and 3) diet adjusted isonitrogenously to the DAC diet by natural feedstuffs (positive control diet). The average daily gain in the pigs given the DAC diet was greater than that in those fed the basal diet ($P < 0.05$) and in those administered the positive control diet ($P < 0.10$). The results indicate that the supplementation of DAC can improve growth when non-essential amino acids are restricted in the basal diet under conditions of adequate essential amino acids and energy supply.

It is well known that the dietary non-protein nitrogen compounds can be utilized for a protein source by ruminants. It was also shown that the addition of non-protein nitrogen to a diet composed of only purified essential amino acids (EAA) could improve the growth in rats [9, 15, 16] and in chickens [4, 10]. On the other hand, it was reported by Deguchi et al. [3] that in pigs ^{15}N labeled diammonium citrate was incorporated into the precipitates of the trichloroacetic acid of the liver and the muscles.

This experiment was conducted to evaluate the growth effect of dietary diammonium citrate (DAC) under the conditions of dietary restriction of non-essential amino acids and of an adequate supply of EAA and energy.

Materials and Methods

Twelve barrows taken from four litters of Landrace $\times$ Large white were employed. Their average body weight and age were 19.1 kg and 60 days-old,

respectively. The animals were allotted to one of the three dietary treatments, four pigs per treatment. The dietary treatments were: 1) BASAL-diet, a diet restricted NEAA with 6.4% crude protein (CP) level; 2) DAC-diet, BASAL-diet plus 3.75% DAC; 3) PC-diet, a diet adjusted isonitrogenously to DAC-diet with natural feedstuffs with 9.1% CP level for positive control. The purified EAA were used to fill the minimum requirement of EAA in all the diets. To restrict NEAA level in BASAL-diet and DAC-diet, more kinds and amounts of purified EAA were employed than those used for PC-diet. The ratio of EAA to NEAA (E/N ratio) in BASAL-diet, DAC-diet and PC-diet were calculated as 1.06, 1.06 and 0.76, respectively. The energy level was adjusted to equal in all the diets by corn starch and sucrose. Daily feeding amount was designed at a diet level of 5.0% of the body weight of the animal in DAC-diet treatment (DAC-group), 4.9% in BASAL-diet treatment (BASAL-group) and 4.9% in PC-diet treatment (PC-group). Thus, the same level of energy and EAA, recommended by N.R.C. [12], per body weight was supplied to all the three groups, and the same CP per body weight was available for DAC-group and PC-group. No antibiotic was added to the diets. The animals were fed twice daily (9:00 AM and 4:30 PM). At feeding time, one half of the daily feeding amount was mixed with a small

Table 1. Composition and nutritive values of diets

Item	International Reference No.	BASAL-diet	DAC-diet	PC-diet
Ingredients (%)				
Yellow corn	4-02-931	18.20	17.50	29.50
Dried whey	4-01-182	10.40	10.00	16.90
Wheat bran	4-05-190	5.20	5.00	8.50
Soy bean meal	5-04-604	1.60	1.50	2.60
Fish meal	5-02-025	1.60	1.50	2.60
Corn starch		58.60	56.50	36.60
L-histidine·HCl		0.03	0.04	—
L-isoleucine		0.20	0.21	0.05
L-leucine		0.02	0.05	—
L-lysine·HCl, mn 98%		0.34	0.35	0.17
DL-methionine, mn 98%		0.23	0.24	0.10
L-phenylalanine		0.09	0.10	—
L-threonine		0.16	0.17	0.01
L-tryptophan		0.05	0.05	0.01
L-valine		0.17	0.18	—
Ammonium citrate, dibasic, mn 97%		—	3.57	—
Calcium carbonate, CaCO ₃ , mn 37% Ca		0.62	0.60	0.61
Calcium phosphate, Ca ₃ (PO ₄) ₂ , mn 32% Ca, mn 18% P		1.45	1.40	1.43
Sodium chloride		0.52	0.50	0.51
Vitamin-mineral premix*		0.52	0.50	0.51
Protein and energy :				
Crude protein, analyzed, %		6.36	8.78	9.11
Digestible crude protein, %		4.88	7.35	6.80
Gross energy, kcal/kg		3549	3543	3570
Digestible energy, kcal/kg		3311	3309	3133

Remarks.

* Vitamin-mineral premix supplied (per kilogram of diet) : 10,000 IU vitamin A, 2,000 IU vitamin D₃, 5 mg vitamin E, 2.5 mg thiamin, 10 mg riboflavin, 10 mg nicotinic acid amide, 20 mg pantothenic acid calcium, 2.5 mg vitamin B₆, 250 mg choline chloride, 0.5 mg folic acid, 10 µg vitamin B₁₂, 100 mg Fe, 10 mg Cu, 300 mg Zn, 100 mg Mn, 1.5 mg Co and 0.5 mg I.

amount of water to reduce a loss of the diet. Fresh water was given freely after each feeding. The all animals were fed with PC-diet for 9 days (preliminary period), and fed each experimental diet, i.e., BASAL-diet, DAC-diet and PC-diet, for 28 days (experimental period). On the sixth to ninth day (preliminary stage) of the preliminary period, the 10th to 13th day (initial stage) and the 25th to 28th day (final stage) of the experimental period, nitrogen metabolism test was performed for four days at one stage. The body weight was recorded at one-week intervals, and before and after the metabolism tests.

The feces were collected in metal container under the individual cage. The feces were sprayed in the containers with 5% HCl several times a day to prevent the loss of ammonia. Daily collections of the feces were oven-dried at 70°C for 24 hours and

air-dried for 12 hours prior to analysis. The urine samples were collected in plastic bottles containing 10 ml of 25% H₂SO₄ beforehand. To determine the urinary urea nitrogen, approximately 5 ml of the daily collections were diluted 1 to 20 and frozen immediately until analysis. For nitrogen determination, the urine collected for four days was mixed, sampled (about 30 ml) and frozen until analysis. Urinary urea nitrogen was determined by diacetyl monoxime thiosemicarbazide method [2].

The nitrogen determination of the feed, feces and urine was in accordance with the Kjeldahl method as outlined in A.O.A.C. [1]. The calorie level of the diets and feces was analyzed by an automatic bomb calorimeter (Model CA-2, Shimazu Seisakusho Ltd., Kyoto, Japan).

Heparinized blood samples were collected from

the *vena cava anterior* at 8:30 AM (30 minutes before morning feeding) on the third day of the preliminary period, and on the eighth and 23rd day of the experimental period using a syringe coated with 0.1% heparin solution. A part of the heparinized blood was immediately submitted for determination of packed cell volume (microhematocrit method) and blood ammonia nitrogen (direct colorimetric method [13]). The plasma samples were then separated and used for the determination of plasma total protein (refractometric method) and blood urea nitrogen (BUN) (diacetyl monoxime thiosemicarbazide method [2]). All animals were sacrificed by bleeding on the 29th day of the experimental period, and immediately submitted to examination for necropsy findings. The carcasses were stored at 4°C overnight. On the following day, the right side of half of the carcass was examined to determine the carcass length, dressing, backfat, loin eye area, meat and fat. The data were analyzed statistically by analysis of variance methods [17].

Results

The data on one pig in DAC-group were omitted from the results because the pig showed weak appetite in the final stage by suffering injury to the hind legs due to the wired floor.

The average daily gain (Table 2) was higher in DAC-group than in BASAL-group ($P<0.05$) and PC-group ($P<0.10$). There was no significant difference in daily gain between PC-group and BASAL-group ($P<0.10$). The average feed per gain (Table 2)

was lower in PC-group than in BASAL-group ($P<0.05$). The DAC-group showed a tendency to be lower in feed per gain among the three groups, but not significant ($P<0.10$).

In all groups, the nitrogen retention (Table 3) increased in the initial stage ($P<0.01$), and decreased during the final stage ($P<0.01$). In the initial stage, the retention was higher in BASAL-group than DAC-group ($P<0.01$) and PC-group ($P<0.05$). In the initial stage, the retention was higher in BASAL-group than in DAC-group ($P<0.10$) and PC-group ($P<0.05$). The DCP efficiency (gain per gram of the digestive crude protein) (Table 3) in all groups increased in the experimental period as compared to the preliminary period ($P<0.01$). During the initial and final stage of the experimental period, the DCP efficiency was higher in BASAL-group than in PC-group ($P<0.01$). The DCP efficiency was higher in BASAL-group than in DAC-group during the initial stage ($P<0.10$) and in the final stage ($P<0.01$). The DCP efficiency became higher in DAC-group as compared to PC-group in the final stage ($P<0.05$), while there was no significant difference between DAC-group and PC-group in the initial stage ($P<0.10$).

There was no significant difference in

Table 2. Average daily gain, feed intake and feed per gain in young pigs fed diet with or without diammonium citrate supplementation

Item	Dietary treatments ^a		
	BASAL-group	DAC-group	PC-group
Average daily gain, kg ^c	0.426±0.018 ^b	0.508±0.019	0.455±0.013
Average daily feed intake, kg	1.34	1.44	1.34
Average feed per gain ^d	3.14 ±0.04	2.88 ±0.09	2.95 ±0.06

Remarks.

^a Four pigs per treatment for BASAL-group and PC-group, and three pigs for DAC-group.

Average initial body weight was 22.5 kg. Twenty-eight days on experimental diet.

^b Mean ± standard error.

^c Means of BASAL-group vs. DAC-group different, $P<0.05$; and DAC-group vs. PC-group different, $P<0.10$.

^d Means of BASAL-group vs. PC-group different, $P<0.05$.

Table 3. Average nitrogen intake, nitrogen retention and gain per digestive crude protein in young pigs fed diets with or without diammonium citrate supplementation

Item	Dietary treatments		
	BASAL-group	DAC-group	PC-group
Average N intake, g/day			
Preliminary stage ^a	16.92±1.40 ^b	16.90±1.57	16.03±0.53
Initial stage	13.07±0.68	19.54±0.98	18.36±0.53
Final stage	15.11±0.50	22.64±0.73	21.90±0.51
Average N retention/N intake, %			
Preliminary stage ^c	47.8 ±2.9	45.5 ±2.1	45.4 ±1.5
Initial stage ^d	65.9 ±1.6	50.0 ±3.5	54.6 ±3.6
Final stage ^e	50.3 ±2.6	35.8 ±6.2	39.3 ±3.3
Average gain/DCP/day			
Preliminary stage ^f	1.49±0.62	2.68±0.16	1.62±0.14
Initial stage ^g	5.72±0.49	3.76±0.75	3.66±0.12
Final stage ^h	5.77±0.15	4.18±0.14	3.48±0.21

Remarks.

^a The values of each stage were measured for four days: preliminary stage is shown from the 6th to 9th day of the preliminary period; the initial stage from the 10th to 13th day; and the final stage from the 25th to 28th day of the experimental period.

^b Mean ± standard error.

^c Time effect of the preliminary stage vs. initial stage, and the initial stage vs. final stage, different, $P < 0.10$.

^d Means of BASAL-group vs. DAC-group different, $P < 0.01$; and BASAL-group vs. PC-group different, $P < 0.05$.

^e Means of BASAL-group vs. PC-group different, $P < 0.05$; and BASAL-group vs. DAC-group different, $P < 0.10$.

^f Time effect of the preliminary stage vs. initial and final stage, different, $P < 0.01$.

^g Means of BASAL-group vs. PC-group, different, $P < 0.01$; and BASAL-group vs. DAC-group, different, $P < 0.01$.

^h Means of BASAL-group vs. DAC-group and BASAL-group vs. PC-group different, $P < 0.01$; and DAC-group vs. PC-group different, $P < 0.05$.

Table 4. Carcass characteristics in young pigs fed with diets with or without diammonium citrate supplementation

Item	Dietary treatments ^a		
	BASAL-group	DAC-group	PC-group
Dressing, %	72.0±1.2 ^b	72.5±1.1	71.0±0.8
Length, cm	64.3±1.9	62.8±2.6	63.1±2.7
Backfat, cm ^c	1.7±0.1	1.8±0	1.9±0.1
Loin eye area, cm ²	9.4±0.5	9.3±0.7	8.7±0.6
Meat, %	55.5±1.4	53.5±0.9	55.2±1.4
Fat, %	25.0±1.4	26.5±0.5	24.6±1.3

Remarks.

^a Four pigs per treatment for BASAL-group and PC-group, and three pigs for DAC-group. Average initial body weight was 22.5 kg. Twenty-eight days on experimental diet.

^b Mean ± standard error.

^c Average of three measurements at shoulder, back and loin.

Table 5. Average packed cell volume, plasma total protein, blood ammonia nitrogen, plasma urea nitrogen and urinary urea nitrogen in young pigs fed diet with or without diammonium citrate supplementation

Item	Dietary treatments ^a		
	BASAL-group	DAC-group	PC-group
Packed cell volume, % ^{b, d}			
Preliminary stage	33.1±2.3 ^c	36.6±2.1	33.7±1.2
Initial stage	39.7±1.6	42.4±1.2	40.1±1.6
Final stage	39.1±0.7	40.5±1.4	38.8±1.8
Plasma total protein, g/100 ml ^{b, e}			
Preliminary stage	6.2±0.2	6.2±0.3	6.4±0.2
Initial stage	6.7±0.1	6.6±0.3	7.2±0.3
Final stage	6.7±0.3	6.4±0.4	6.8±0.3
Blood ammonia N, g/100 ml ^{b, f}			
Preliminary stage	69 ±18	50 ±18	22 ±10
Initial stage	48 ±15	62 ±7	48 ±14
Final stage	106 ±14	99 ±25	83 ±11
Plasma urea N, mg/100 ml ^{b, g}			
Preliminary stage	6.9±0.4	5.7±2.0	7.2±1.2
Initial stage ^h	0.1±0.1	3.2±0.5	2.5±0.5
Final stage ^h	1.4±0.5	6.0±0.7	4.5±0.5
Urinary urea N, g/day			
Preliminary stage	4.0±0.5	4.2±0.2	4.1±0.5
Initial stage ⁱ	1.4±0.3	6.7±1.5	4.8±1.0
Final stage ^j	1.3±0.2	8.1±0.9	5.3±0.6

Remarks.

^a Four pigs per treatment for BASAL-group and PC-group, and three pigs for DAC-group. Average initial body weight was 22.5 kg. Twenty-eight days on experimental diet.

^b Blood sample was collected on the 3rd day (preliminary stage) of the preliminary period, eight day (initial stage) and 23rd day (final stage) of the experimental period.

^c Mean ± standard error.

^d Time effect of preliminary stage vs. initial stage, and preliminary stage vs. final stage, different, $P < 0.01$.

^e Time effect different, $P < 0.10$.

^f Time effect of preliminary stage vs. final stage and initial stage vs. final stage different, $P < 0.01$.

^g Time effect of preliminary stage vs. initial stage and preliminary stage vs. final stage different, $P < 0.01$.

^h Means of BASAL-group vs. DAC-group and BASAL-group vs. PC-group different, $P < 0.01$.

ⁱ Means of BASAL-group vs. DAC-group different, $P < 0.01$; and BASAL-group vs. PC-group different, $P < 0.05$.

^j Means of BASAL-group vs. DAC-group and BASAL-group vs. PC-group different, $P < 0.01$; and DAC-group vs. PC-group different, $P < 0.05$.

carcass characteristics among the treatments (Table 4).

The levels of packed cell volume and plasma total protein (Table 5) in all groups increased when the experiment began ($P < 0.01$, $P < 0.10$, respectively). There was no

significant difference in the packed cell volume level and plasma total protein level among the groups ($P < 0.10$). The blood ammonia nitrogen level (Table 5) was higher in the final stage than in the initial stage in all groups ($P < 0.01$). No other significant

difference was found among the three stages and among the groups ($P < 0.10$). The BUN levels (Table 5) decreased in the initial stage as compared to the preliminary stage in all groups ($P < 0.01$). In both stages in the experimental period, BUN level was lower in BASAL-group than in PC-group and DAC-group ($P < 0.01$). There was no significant difference in BUN levels between DAC-group and PC-group. The levels of urinary urea nitrogen (Table 5) were lower in BASAL-group than in DAC-group at the initial and final stage ($P < 0.01$). In BASAL-group, moreover, the levels were lower than in PC-group during the initial stage ($P < 0.05$) and in the final stage ($P < 0.01$). Although there was no significant difference in the urinary urea nitrogen levels between DAC-group and PC-group in the initial stage ($P < 0.10$), the levels were higher in DAC-group than in PC-group in the final stage ($P < 0.05$).

According to the postmortem examination, no lesion caused by any infection or other abnormality on the pigs was observed.

Discussion

On the basis of clinical, clinico-pathological and necropsy findings, it was confirmed that the pigs were healthy and were not affected with ammonia intoxication.

It was reported in small laboratory animals that dietary ammonia nitrogen was incorporated into tissue amino acids and utilized for protein synthesis [4, 5, 9, 16]. The ammonia nitrogen transfer into the tissue protein in pigs was also observed by Deguchi et al. [3] who investigated the incorporation of the isotopic nitrogen into the trichloroacetic acid precipitates of the liver and the muscle obtained from the baby pigs given ^{15}N labeled diammonium citrate. Some studies on the growth of pigs fed ammonium salts, however, failed in demon-

stration of growth effect of dietary ammonium salts [6, 8, 11, 14, 18]. On the other hand, it was suggested in rats [7], chickens [10] and pigs [11, 19], that non-essential amino acids could be a limiting factor for growth, and that supply of non-essential amino acids improved growth when the dietary non-essential amino acids were remarkably reduced or absent.

The BASAL-diet was designed to contain the essential amino acids and energy levels recommended by N.R.C. [12]. Namely, BASAL-diet had a E/N ratio of 1.06 which was clearly higher than that of Zimmerman's low protein diet [19], of which non-essential amino acids was a limiting factor for growth.

The animals fed with the BASAL-diet showed lower blood urea concentration and decreased urea excretion into urine with a elevated nitrogen retention. But the growth in BASAL-group was retarded compared with other animals. These data suggested that the retardation in growth of BASAL-group was caused by the shortage of non-essential amino acids, in spite of their nitrogen metabolism operated effectively under the nitrogen restricted condition of their diet. Compared with BASAL-group, the animals fed with DAC-diet were well grown with higher daily gain, lower feed per gain, in spite of lower nitrogen retention. And the growth curve of DAC-group was relatively similar to the animals fed with the positive control diet, i.e., PC-diet, composed of the natural feedstuffs in higher crude protein level than BASAL-diet. The DAC-group differed from BASAL-group only in the dietary condition of addition of diammonium citrate. The difference of growth between BASAL-group and DAC-group, therefore, was considered to be resulted from the addition of diammonium citrate. Released citrate from DAC in the

intestine could not have caused the growth increase energetically, since the analyzed levels of gross energy and digestible energy of DAC-diet were nearly equal to those of BASAL-diet. Rose et al. [16] reported that there was no effect of the citrate on the growth of rats when trisodium citrate was administered orally. The growth effect of DAC was, therefore, due to its ammonium portion.

From the reasons that mentioned above, it was considered that the improved growth of the pigs fed DAC-diet was resulted from the substitutional effect of ammonium portion of diammonium citrate for non-essential amino acids.

In this experiment, however, urinary urea excretion was increased in DAC-group at the final stage, which was larger than that of PC-group fed the natural feedstuffs. This fact may indicate that the ingested amount of ammonia nitrogen might be too much for the expected growth level which was equal to that of PC-group, when diammonium citrate was administered with abundant of the purified essential amino acids.

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要 約

非必須アミノ酸制限食に対するクエン酸2アンモニウム添加が子豚の発育に及ぼす効果：籠田勝基・岩瀬俊男・小島敏之・新山雅美・波岡茂郎（北海道大学獣医学部家畜内科学教室）——非タンパク態窒素化合物の豚発育に及ぼす効果を知るために、クエン酸2アンモニウム（DAC）を用いて豚の飼養試験を実施した。給与飼料は厳しい低タンパクの条件のもとで、必須アミノ酸はNRC標準の要求量を満たすように添加し、非必須アミノ酸が窒素の制限因子となるように設計された。すなわち、1) 粗タンパク（CP）含量 6.4%，非必須アミノ酸制限（基礎飼料区）。2) 基礎飼料+DAC 3.6% 添加（DAC区）。3) DAC区とCPを等しくしたDAC無添加区（Positive Control, PC区）である。消化エネルギーは各区とも 3.3 kcal/g とした。平均体重 22.5 kg の secondary SPF 豚12頭を4群に分け、それぞれ単飼ケージに収容し、1日2回の制限給餌で28日間飼養した。日増体重および飼料要求率の測定とともに窒素代謝試験と Ht, TP, BUN および血中アンモニアを測定した。DAC区の平均日増体重は 508 g で基礎飼料区の 426 g および PC 区の 455 g より有意に高い値を示した ($p<0.05$ と $p<0.10$)。飼料要求率は基礎飼料区 3.14, DAC区 2.86 および PC 区 2.94 で基礎飼料区が他の区より高い傾向を示した。Ht, TP および血中アンモニアは何れの区でも正常範囲内にあり、臨床所見からもアンモニア中毒は認められなかった。以上の成績から、非必須アミノ酸を制限因子とした飼養条件下では DAC が豚の発育に利用されることが明らかとなった。