Comparison of the Efficiency of Absorbed Nitrogen Use from Different Protein Sources in Diets Having Similar Amino Acid Balance[1]

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ABSTRACT : Nine crossbred female pigs fitted with the bladder catheters were used to investigate the effects of dietary protein form on the efficiency of absorbed nitrogen for nitrogen retention in growing pigs. Combinations of the main protein sources were corn-soybean meal (CSM; slow + slow absorption rate form), corn-hydrolyzed casein (CAS; slow + rapid absorption rate form) and corn-porcine plasma (CPL; slow + intermediate absorption rate form). All experimental diets were formulated to be isonitrogenous (CP 11%) and isocaloric (3.5 Mcal/kg) and synthetic amino acids were added to the diet as required to maintain an equivalent amino acid profile among diets. Fecal digestibility of nitrogen was not different among treatments ($p > 0.10$). Ingested nitrogen was absorbed with an apparent efficiency of 82% to 84%. Mean nitrogen retention in pigs fed the CSM diet was as high as for pigs fed the CPL diet (0.74 g N/kg $BW^{0.75}$ per d), which was higher than the N retention rate in pigs fed CAS diet (0.68 g/kg $BW^{0.75}$ per d; $p < 0.05$). Apparent biological values (ABV = 100 × N retention/absorbed nitrogen) were 63.3 %, 58.0% and 61.6% for CSM, CAS, and CPL groups, respectively ($p < 0.05$). There was no difference in mean energy digestibility among treatments. The efficiency of absorbed lysine utilization was significantly different among treatments ($p < 0.05$). Pigs fed the CAS diet were inferior to counterparts on the other diets in utilizing absorbed lysine. The ratios of free (and small peptide-bound) to protein-bound amino acids in CSM diet differed considerably from the CAS diet. This may affect the efficiency of amino acids utilization for nitrogen retention if hydrolyzed and intact amino acid pools reach the blood at different times.

(**Key Words**: Pigs, Nitrogen Absorption, Nitrogen Retention, Protein Sources, Amino Acids Balance)

INTRODUCTION

Requirements for dietary amino acids are greatly affected by changes in the efficiency of absorbed amino acids for protein deposition (Boyd et al., 1991). For pigs to maximize the efficiency of protein use for protein accretion, dietary amino acids should be well-balanced, but perhaps also 'simultaneously' absorbed and available to the tissue for protein synthesis.

Intact and hydrolyzed casein were used in semi-synthetic diets that yielded high efficiencies of amino acid use in growing pigs (Moughan and Smith, 1984; Wang and Fuller, 1989; Baker et al., 1993). Porcine plasma has been used recently to replace the milk product protein component for young growing pigs (Gatnau and Zimmerman, 1990; Hansen et al., 1993). The rates of digestion and absorption of amino acids in porcine plasma have not been evaluated, but are expected to be more rapid than those in corn and soybean meal. Hydrolyzed casein is expected to be more rapidly absorbed than either soybean meal or plasma protein. Thus, this amino acid source may likewise enhance the efficiency of nitrogen use.

This study was conducted to investigate the effects of dietary protein form on the efficiency of absorbed nitrogen for nitrogen retention in growing pigs. Combinations of the main protein sources were corn-soybean meal (CSM; slow + slow absorption rate form), corn-hydrolyzed casein (CAS; slow + rapid absorption rate form) and corn-porcine plasma (CPL; slow + intermediate absorption rate form). Synthetic amino acids were added to the diet if needed to maintain an equivalent amino acid profile among diets.

MATERIALS AND METHODS

Animals and Treatments

Nine crossbred female pigs (Large White × Landrace × 'multiple-breeds' hybrid; PIC, Franklin, KY, USA) weighing 53 ± 3 kg (mean ± SD) one day before the first

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treatment began were used for a nitrogen balance study. Pigs were randomly allotted into three groups (3 pigs/group). A group of pigs were assigned to one of three treatments each period following a 3 × 3 Latin Square (Lentner and Bishop, 1993). Pigs received the first, second and third treatment on d 1 through d 12, d 13 through d 19 and d 20 through d 26, respectively. Feces were collected once per pig on d 8 through d 12 only for the first treatment (3 pigs/treatment). Urine was collected on d 9 through d 12, d 16 through d 19, and d 23 through d 26 for the first, second and third treatment, respectively.

Experimental diets

All experimental diets were formulated to be isonitrogenous (CP 11%) and isocaloric (3.5 Mcal/kg, table 1). The pattern of amino acids approximated the ideal amino acid pattern of Wang and Fuller (1989, 1990). The amino acid contents of the experimental diets were calculated using analytical values of ingredients (table 2). Combinations of corn-soybean meal (CSM), corn-hydrolyzed casein (CAS) and corn-porcine plasma (CPL) were the dietary treatments. Diets were supplemented with synthetic amino acids as necessary. Contents of dietary protein provided by soybean meal, hydrolyzed

Table 1. Composition of experimental diet[a]

Item	Diet		
	CSM	CAS	CPL
Ingredient (%)			
Corn	68.55	68.60	68.60
Soybean meal (48.5%)	10.00	–	–
Hydolyzed casein	–	5.80	–
Porcine plasma	–	–	6.90
Corn oil	3.00	3.80	3.80
Dicalcium phosphate	2.50	2.60	2.60
Limestone	0.80	0.85	0.85
Antibiotics[b]	0.50	0.50	0.50
Salt	0.40	0.40	0.40
Vitamin-trace mineral premix[c]	0.25	0.25	0.25
L-Lysine · HCl	0.22	–	–
DL-Methionine	0.13	0.11	0.07
L-Threonine	0.06	–	–
L-Tryptophan	0.02	0.02	–
L-Glycine	0.51	0.29	0.30
L-Glutamic acid	0.21	0.71	0.70
Starch	12.85	16.07	15.00

Item	Diet		
	CSM	CAS	CPL
Chemical Composition			
DE (Mcal/kg)[d]	3.42	3.49	3.51
Crude protein (%)[e]	11.20	11.10	11.19
Calcium (%)[e]	0.79	0.79	0.89
Phosphorus (%)[e]	0.73	0.67	0.80
Amino acids (%)[f,g]			
Lysine	0.67	0.67	0.67
Met. + Cys.	0.41	0.41	0.43
Threonine	0.43	0.45	0.50
Tryptophan	0.13	0.13	0.15
Isoleucine	0.42	0.50	0.40
Valine	0.50	0.64	0.61
Leucine	1.11	1.27	1.25
Phe. + Tyr.	0.87	0.86	1.04
Histidine	0.28	0.30	0.32
IAA : CP (g/100 g)[h]	43	52	54
IBIAA : CP (g/100 g)[i]	37	37	37

[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 B_{12}, 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] Determined by a digestion study. [e] Analyzed values (AOAC, 1980).

[f] Calculated using the analytical values of ingredients (See Table 2).

[g] Approximates the ideal protein pattern (Wang and Fuller, 1989, 1990) which had relative to lysine 100, methionine + cystine 61 (methionine 31), threonine 64, tryptophan 19.5, isoleucine 60, valine 75, leucine 100, phenylanine + tyrosine 100, and histidine 32.

[h] Calculated as 100 × (sum of indispensable amino acids/CP). IAA, indispensable amino acid.

[i] Calculated as 100 × (total ideally balanced indispensable amino acids/CP). IBIAA, the ideally balance indispensable amino acids in which the proportion of indispensible amino acids in excesses relative to an ideal protein pattern is not included. For example, leucine in the CSM diet is in excess (165% of lysine). Therefore, a proportion of leucine (65% of lysine) in excess is not included in IBIAA for the CSM diet.

casein and porcine plasma were approximately 4.8% (45 % of dietary CP). Pigs were fed 6 times daily (07:00, 10:00, 13:00, 16:00, 19:00 and 22:00 h) to a total of 80 g per kg $BW^{0.75}$ and given free access to water.

Table 2. Chemical composition of ingredients

	Corn	Soybean meal	Hydrolyzed casein	Porcine plasma
Crude protein (N×6.25)[a]	7.59	48.09	82.60	69.47
Digestible energy (kcal/kg)	3,530[b]	3,680[b]	2,850[c]	3,278[d]
Calcium (%)[e]	0.05	0.36	–	0.12
Phosphorus (%)[e]	0.24	0.66	0.82	0.67
Amino acids (%)[e]				
Lysine	0.295	2.946	8.004	6.386
Methionine	0.140	0.439	2.165	0.536
Cystine	0.111	0.684	–	2.140
Threonine	0.309	1.624	4.049	4.211
Tryptophan	0.062	0.662	1.110	1.587
Isoleucine	0.312	2.066	4.944	2.713
Valine	0.421	2.153	5.977	4.680
Leucine	1.120	3.380	8.570	7.018
Phenylalanine	0.444	2.226	4.386	4.416
Tyrosine	0.280	1.941	1.943	3.536
Histidine	0.245	1.119	2.339	2.267

[a] Analyzed by Kjeldahl method. [b] Obtained from the NRC (1988).

[c] Metabolizable energy value for dried casein (2,740 kcal/kg) obtained from Allen (1992). Digestible energy calculated by the equation, DE = ME/.96 (NRC, 1988).

[d] Obtained from Americal Protein Corporation, Ames, IA. [e] Analytical values.

Housing and sample collection

Pigs were housed in metabolic cages in an environmentally controlled room with the temperature maintained at 20±1 ℃ and with 16:8 h light: dark cycle. The Foley bladder catheters were introduced into the urinary bladder of pigs on d 7. 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 catheter was introduced into the bladder through urethra which could be located by palpation using an index finger. Feces were collected every 2 hours from 07:00 to 24:00 h and stored at −20 ℃ until the completion of collection. All feces that were collected from each pig were thawed and homogenized using a food mixer. Sub-samples were stored at −20 ℃ for future analysis. Total urine was collected into 20-liter plastic jugs containing 400 ml of 10% HCl (v/v) to prevent N loss and microbial growth ($pH < 3$). Aliquots of daily urine output were stored at −20 ℃. Four-day samples were combined and stored at −20 ℃ again for the future analysis. All feed spilled from the feeder was collected from each pig each day. It was dried, combined at the end of each period and analyzed for N to adjust nitrogen intake. N retention was measured as the difference between nitrogen intake and the amount of N loss in the urine and feces. Apparent biological value (ABV) of nitrogen use for N retention was calculated by the equation, ABV (%) = (nitrogen retention/ absorbed nitrogen) × 100, where N absorbed = N intake − fecal N. Overall efficiency of nitrogen use was expressed as 100 × (N retention/N intake).

Chemical analysis

Feed, urine and feces were assayed in triplicate for nitrogen by Kjeldahl analysis (AOAC, 1980). Energy content of diets and feces were obtained using an oxygen bomb calorimeters (Parr Adiabatic Calorimeter, Parr Instrument Co., Mdire, Ⅱ). To prevent N loss during the drying process, 4 to 5 g of wet feces were used for nitrogen analysis (Van Soest and Robertson, 1985) and 5 ml of urine was used for N analysis. Amino acid contents of corn, soybean meal, hydrolyzed casein and porcine plasma were determined by the methods described in Lee et al. 1998.

Statistical analysis

The data were analyzed by a two-way analysis of variance (Minitab, 1991). The model was $Y = \mu + P + D + \varepsilon$, where P = pig and D = diet. Time (or period) effect was removed from the model due to insignificance ($P = 0.53$). Three observations from two pigs were missing after the completion of feces collection, so 24 observations of urine output (8 data/treatment) and 9 values of feces (3 observations/treatment) were used. Differences of treatment means were identified using Fisher's least significant difference procedure (Ott, 1988) when F-value was significant ($p < 0.10$).

RESULTS

Nitrogen and energy metabolism in growing pigs are presented in Table 3. Average body weights during the sampling periods were 63.9 kg, 64.4 kg and 64.1 kg for corn-soybean meal (CSM), corn-hydrolyzed casein (CAS), and corn-porcine plasma (CPL) treated pigs, respectively. Fecal digestibility of nitrogen was not different among treatments ($p > 0.10$). Ingested nitrogen was absorbed with an efficiency of 82% to 84%. Amount of nitrogen loss in the urine was highest in CAS treated pigs and followed by CPL and CSM treated pigs. Means of urinary nitrogen loss differed among treatments ($p < 0.05$). Mean of nitrogen retention in pigs fed CSM diet was as high as in pigs fed CPL diet (0.74 g/kg $BW^{0.75}$ per d), which was higher than the N retention rate in pigs fed CAS diet (0.68 g/kg $BW^{0.75}$ per d; $p < 0.05$). Apparent biological values (ABV = 100 × N retention/absorbed nitrogen) were 63.3 %, 58.0% and 61.6% for CSM, CAS, and CPL groups, respectively ($p < 0.05$). Overall efficiency of nitrogen use for nitrogen retention (100 × N retained/N intake) in pigs fed either CSM or CPL diet was better than in CAS treated pigs ($p < 0.05$).

Table 3. Nitrogen and energy metabolism in growing pigs

Criterion	CSM	CAS	CPL	SEM (n = 7)[b]
Body weight (kg)	63.9	64.4	64.1	2.0
N intake (g/kg $BW^{.75}$ per d)	1.42	1.40	1.42	0.01
Fecal N (g/kg $BW^{.75}$ per d)[a]	0.25	0.23	0.23	0.01
Absorbed N (g/kg $BW^{.75}$ per d)[a]	1.17	1.17	1.19	0.01
% of N intake	82.3	83.6	83.8	1.1
Urinary N (g/kg $BW^{.75}$ per d)	0.43^{x}	0.49^{y}	0.46^{xy}	0.01
N retention (g/kg $BW^{.75}$ per d)	0.74^{x}	0.68^{y}	0.74^{x}	0.01
% of N absorbed	63.3^{x}	58.0^{y}	61.6^{x}	1.0
% of N intake	52.1^{x}	48.5^{y}	51.8^{x}	0.8
Gross energy intake (GE; mcal/d)[a]	7.09	7.11	7.11	0.07
Energy digested (mcal/d)[a]	6.14	6.21	6.30	0.06
% of GE[a]	86.6	87.3	88.6	0.88

[a] n = 3. [b] Standard error of mean.

[x,y,z] Means in the same row with different superscript letters differ ($p < 0.05$).

Pigs consumed an average of 7.1 Mcal gross energy per day during the sampling periods. Digestibility of energy for CSM, CAS and CPL diets was 86.6%, 87.3% and 88.6 %, respectively. There was no difference in mean energy digestibility among treatments. Average digestible energy intake for all pigs was 6.2 Mcal/d which is equivalent to 2.5 times the maintenance energy requirement (NRC, 1988).

The efficiency of absorbed lysine utilization (figure 1) for predicted protein accretion (N retention × 6.25 / absorbed lysine) was significantly different among treatments ($p < 0.05$). Pigs fed CAS diet was inferior to other pigs in utilizing absorbed lysine. One gram of absorbed lysine resulted in 10.1 g, 8.7 g and 10.5 g of protein accretion (N retention × 6.25) in CSM, CAS, and CPL treated pigs, respectively. The ratios of free (and small peptide-bound) to protein-bound amino acids in CSM diet and CAS diet differed considerably, which might affect the efficiency of amino acids utilization for nitrogen retention (figure 2).

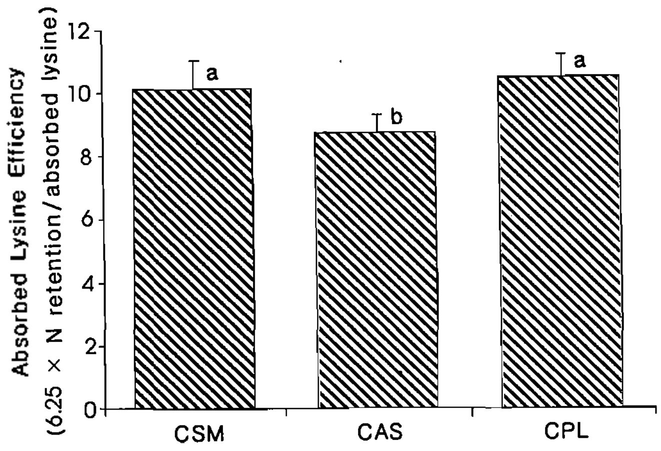

Figure 1. Efficiency of absorbed lysine use for apparent protein accretion (6.25 × N retention/absorbed lysine) in diets containing different proteins. Vertical lines represent SD of treatment means. Digestibility (availability) of lysine in corn, soybean meal, hydrolyzed casein and porcine plasma was assumed to be 76.5% (Mean value of 8 literatures), 84.5% (Mean value of 15 literatures), 100% (Kies et al., 1986; Wang and Fuller, 1989) and 84% (obtained from American Protein Corporation, Ames, IA), respectively. Different letters on columns indicate that treatment means significantly differ ($p < 0.05$).

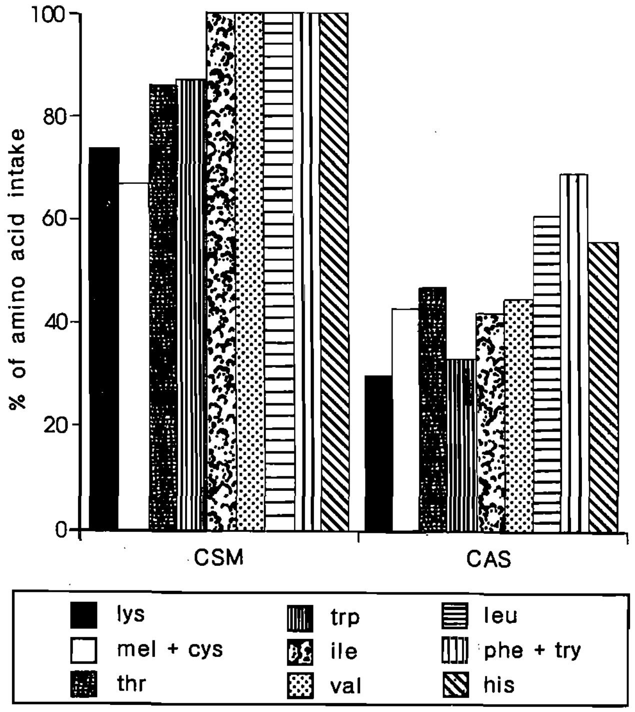

Figure 2. Fraction of protein-bound amino acids in corn-soybean (CSM) and corn-hydrolyzed casein) CAS diets. It was assumed that enzymatically hydrolyzed casein contained only small peptides and free amino acids. Each protein-bound amino acid in the diet is expressed as % of the corresponding amino acid in diets (e.g. 100 × protein-bound lysine/total lysine), respectively.

DISCUSSION

The present study was conducted to investigate the effects of different protein sources on the efficiency of absorbed nitrogen utilization for nitrogen retention in growing pigs. The protein sources selected were soybean meal, hydrolyzed casein and porcine plasma because of potentially different absorption rates of amino acids.

The efficiency of absorbed nitrogen (table 3) and lysine utilization (figure 1) for nitrogen retention in pigs fed corn-hydrolyzed casein (CAS) diet was lower than in pigs fed either corn-soybean meal (CSM) or corn-porcine plasma (CPL) diet ($p < 0.05$). This result was not in agreement with other observations, which showed that amino acids in casein (intact or hydrolyzed) are almost perfectly absorbed and utilized for N metabolism when casein and crystalline amino acids are used as sole nitrogen or amino acid sources (Moughan and Smith, 1984; Kies et al., 1986; Wang and Fuller, 1989).

A nitrogen balance study was conducted using a diet that consisted exclusively of enzymatically hydrolyzed casein and synthetic amino acids as nitrogen sources (Moughan and Smith, 1984). The efficiency of dietary nitrogen utilization was estimated to be as high as 94%. Wang and Fuller (1989) also reported that amino acids in diets containing a sole mixture of intact casein and synthetic amino acids were efficiently utilized for nitrogen retention (+ 80%).

All amino acids should be available to the tissues at the approximately same time in order to be utilized for protein synthesis. The high efficiency of amino acid use in a mixture of casein and supplemental amino acids could be attributed to a proper balance and the similar absorption rates of amino acids. It was reported that the amino acids in intact casein are absorbed as rapidly as free amino acids (Gupta et al., 1958). In addition, the absorption rates of some amino acids in enzymatically hydrolyzed casein exceeded those of free amino acids from an amino acid mixture simulating casein (Rerat et al., 1988; Matthew, 1991) because hydrolyzed casein has a high proportion of small peptides as well as free amino acids. The transport systems which are associated with amino acid absorption in brush border and basolateral membranes are usually shared by more than one amino acid (Alpers, 1987; Hopfer, 1987; Webb, 1990; Matthew, 1991). The absorption rates of amino acids which are linked to the same transport system are subject to inhibition by one another. In contrast, small peptides would inhibit free amino acid transport little if any at the brush border membrane, nor compete with other peptides (Burston and Matthews, 1990). In fact, some small peptide-bound amino acids in casein hydrolysate are absorbed more rapidly than free amino acids in the casein-simulating mixture (Matthew, 1991).

The reason why the efficiency of absorbed nitrogen and lysine use for nitrogen retention in pigs fed CAS diets was lower than in pigs fed CSM diets may be related to the absorption rates of amino acids in the diets (figure 2). Amino acids in corn and soybean meal are absorbed at much slower rates, but perhaps more synchronously, than amino acids in casein or supplemental amino acids (Gupta et al., 1958; Rolls et al., 1972; Bach Knudsen and Jorgensen, 1980; Hara and Kiriyama, 1991). The proportion of protein-bound sulfur amino acids (SAA) in the CSM diet was about 70% of total SAA. Therefore, at least 70% of indispensable amino acids in the CSM diet would be almost simultaneously absorbed, provided to the tissues, and utilized for protein synthesis. On the other hand, amino acids in the CAS diet would be absorbed at differential rates because amino acids were provided as half protein-bound form and half free form (or small peptide form). This may have resulted in decreased efficiency of amino acid utilization for protein synthesis.

We are not aware of published information on the quantity of protein-bound amino acids in porcine plasma, so we did not illustrate the protein-bound amino acid profiles of the CPL diet in figure 2. Since the content of amino acids derived from corn in the CAS diet was identical to that in the CPL diet and since porcine plasma may contain free amino acids and small peptides as well as intact proteins (e.g. globulins, albumins etc.), proportion of total protein-bound amino acids in the CPL diet may be intermediate compared to those in the CSM and CAS diets. Free amino acids or small peptides in porcine plasma may be absorbed as rapidly as amino acids in a supplemental amino acid mixture or amino acids and peptides in hydrolyzed casein whereas protein-bound amino acids would be absorbed slowly since they should be hydrolyzed prior to being absorbed. Hence, overall absorption rates of amino acids in porcine plasma would be intermediate as compared to those in corn, soybean meal and hydrolyzed casein. The efficiency of absorbed nitrogen and lysine use for nitrogen retention in pigs fed the CPL diet was similar to that in pigs fed the CSM diet, but higher than in pigs fed the CAS diet.

Studies have shown that pigs fed diets supplemented with a large amount of free amino acids once or twice a day are inferior in growth performance to pigs fed the same diets more frequently (Dent et al., 1970; Batterham and O'Neill, 1978; Partridge et al., 1985). In the present study, pigs were fed 6 times a day to minimize the effect of feeding frequency on efficiency of nitrogen utilization, but pigs fed the CAS diet exhibited lower efficiency of nitrogen and lysine use than pigs fed other diets. We attribute this effect to rapid absorption rates of amino acids and small peptides in hydrolyzed casein in contrast to corn and the resulting imbalance in profile of amino acids that were absorbed.

Recently, Hara and Kiriyama (1991) reported that portal concentrations of amino acids in rats fed diets containing a mixture of casein and oligo-L-methionine increased as soon as 20 to 40 minutes after a feeding. Both rates and extent of absorption of amino acids in rats fed the diet containing a mixture of oligo-L-methionine and casein were faster and greater than in rats fed the diet antaining a mixture of oligo-L-methionine and soy protein.

The ratio of free (and small peptides) to protein-bound amino acids in the CAS diet varies considerably among individual amino acids. For example, 30% of lysine in the CAS diet is free or peptide-bound whereas 60 to 70% of aromatic amino acids, leucine and histidine were free or peptide-bound (figure 2). Hence, there may be an imbalance among amino acids available for protein synthesis relative to need when rapidly and slowly absorbed amino acids, respectively, reach the tissue. We expect that absorbed amino acids in relative excess at a given time are unable to be utilized efficiently for protein synthesis and will be eventually catabolized. This might result in a decrease in the efficiency of absorbed nitrogen use in pigs fed the CAS diets.

Amino acids in porcine plasma were complementary to those in corn. Hence profile of amino acids in CPL diet well matched the ideal protein amino acid pattern (Wang and Fuller, 1989, 1990) used in this study when plasma (supplemented with a small amount of methionine) replaced soybean meal and supplemental amino acids.

Results from this study indicate that the efficiency of absorbed nitrogen use for nitrogen retention is affected by dietary protein sources even if amino acid profiles are similar. Due to a possible differential absorption rate (slow + rapid forms) of amino acids in the CAS diet, the efficiency of absorbed nitrogen use for nitrogen gain by pigs fed the CAS diet was inferior to the efficiency by pigs fed the CSM diet in which the absorption rates of amino acids were expected to be similar (slow + slow forms). We assumed that the absorption rates of amino acids bound to protein (e.g. amino acids in corn-soybean meal) are much slower than those of free amino acids or small peptides (e.g. amino acids in hydrolyzed casein). To validate this, the absorption rates of amino acids in blood (e.g. portal absorption) would have to be measured by uptake techniques.

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