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AN EVALUATION OF THREONINE AND ISOLEUCINE AS THE THIRD AND FOURTH LIMITING AMINO ACIDS IN CORN FOR GROWING SWINE[1,2,3]

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Summary

A metabolism experiment and two growth experiments were conducted to determine if threonine and isoleucine are the third and fourth limiting amino acids in corn for growing swine. In each experiment there were four dietary treatments. The basal diet contained corn fortified with minerals, vitamins, lysine and tryptophan. Threonine additions of 0 or .17% and isoleucine additions of 0 or .22% of the diet were evaluated in a 2 × 2 factorial arrangement of treatments. The addition of threonine reduced (P<.001) the daily urinary excretion of urea N and total N. There was a tendency for urea N excretion to be reduced further when isoleucine was added in combination with threonine. Addition of threonine alone improved (P<.05) feed efficiency and tended to increase weight gain and reduce feed intake. Isoleucine addition did not significantly affect any performance criteria. When threonine and isoleucine were added together, performance was poorer than when threonine was added alone. Plasma urea concentrations were reduced (P<.001) when threonine was added either alone or in combination with isoleucine. Isoleucine addition alone did not affect plasma urea concentrations. Dietary additions of either threonine or isoleucine increased the level of the same amino acid in blood plasma, but had little or no effect on the plasma concentrations of the other amino acid or of lysine or tryptophan. The results provide clear evidence that threonine is the third limiting amino acid in corn for growing swine, but do not support the hypothesis that isoleucine is fourth-limiting. Seemingly, some other amino acid or possibly isoleucine colimiting with other amino acids or nonspecific N becomes limiting after lysine, tryptophan and threonine.
(Key Words: Swine, Diet, Threonine, Isoleucine, Growth, Urinary Urea.)

Introduction

Several experiments with swine at various stages of growth have demonstrated that lysine and tryptophan are the first two limiting amino acids in corn (Clawson and Matrone, 1963; Cromwell et al., 1967; Gallo and Pond, 1968; Baker et al., 1969; Ilori and Conrad, 1977; Lewis et al., 1979). Although there is not complete agreement about which is first- or second-limiting (Baker et al., 1969), the simultaneous addition of these two amino acids to a corn diet has invariably resulted in improved pig performance.

The sequence of other limiting amino acids in corn for swine has not been determined. The calculated sequence, based on the amino acid requirements of 20- to 35-kg pigs (NRC, 1979), is: (3 and 4) isoleucine and valine—colimiting (5) threonine and (6) total sulfur amino acids. Additional experimental data on amino acid limitations are needed to permit more precise supplementation of corn-based diets for swine, particularly when computer formulations are used that take into account amino acid compositions of a variety of feedstuffs. Also, more information about the nutritional deficiencies

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TABLE 1. COMPOSITION OF BASAL DIET[a]

Item	%
Ingredient	
Corn, ground (IFN 4-02-931)	95.81
Dicalcium phosphate (IFN 6-01-080)	1.25
Limestone, ground (IFN 6-02-632)	.81
Salt, iodized (IFN 6-04-151)	.25
Trace mineral mix[b]	.10
Vitamin mix[c]	1.00
L-lysine·HCl[d]	.69
L-tryptophan	.09
Total	100.00
Calculated content[e]	
Crude protein	8.43
Lysine	.77
Tryptophan	.14
Threonine	.37
Isoleucine	.38

[a]The three other diets were formulated by adding .17% threonine (diet 2), .22% isoleucine (diet 3) or 17% threonine and .22% isoleucine (diet 4) at the expense of an equal weight of corn.

[b]Contributed the following in mg/kg of diet: Zn, 200; Fe, 100; Mn, 55; Cu, 10; Co, 1; I, 1.5.

[c]Contributed the following per kg of diet: vitamin A, 5,511 IU; vitamin D, 441 IU; vitamin E, 22 IU; menadione sodium bisulfite, 2.2 mg; riboflavin, 2.9 mg; d-pantothenic acid, 22 mg; niacin, 22 mg; choline chloride, 220 mg; vitamin B_{12}, 22 μg; ethoxyquin, 4.4 mg.

[d]Contributed .54% L-lysine.

[e]Calculated from feed ingredients composition table (NRC, 1979).

of corn for swine is needed to help plant breeders improve the protein quality of corn, either by traditional selection or by genetic engineering.

In experiments with rats, Lewis et al. (1982) found that threonine and isoleucine were the third and fourth limiting amino acids in corn. Therefore, a metabolism experiment and two growth experiments were conducted to test the hypothesis that threonine and isoleucine are third- and fourth-limiting in corn for growing swine.

Experimental Procedure

Diets. Four diets were fed in each of the three experiments. Diet 1 (the basal diet), which is presented in table 1, contained corn supplemented with minerals, vitamins, lysine and tryptophan. This basal diet was calculated to provide adequate amounts of all nutrients

(NRC, 1979) except protein and certain amino acids (isoleucine, methionine + cystine, threonine, valine). Diets 2 and 3 consisted of the basal diet supplemented with threonine and isoleucine, respectively. Diet 4 was the basal diet supplemented with both threonine and isoleucine. Thus, the four diets comprised a 2 × 2 factorial arrangement of treatments. The additions of threonine and isoleucine (.17 and .22%, respectively) were calculated to raise the total dietary contents of these amino acids to 120% of the NRC (1979) requirement. In all experiments, animals were allowed ad libitum access to feed and water, and every animal was observed daily.

Exp. 1. Thirty-six crossbred gilts, with an average initial weight of 26 kg, were assigned to three groups on the basis of their age and weight. The experimental regimen was initiated on all animals within a group at the same time. The gilts were fitted with Foley bladder catheters (size 14)[7] using a procedure similar to that described by Fuller et al. (1979). Animals were anesthetized during catheter insertion. Anesthesia was induced with sodium thiamylal (1 ml of

a 4% solution per 5 kg body weight administered iv) and was maintained by closed-circuit inhalation of halothane. Catheters were treated with sulfa-urea cream to reduce the possibility of infection, and urine collections were started 1 to 2 d after catheter insertion. During the experimental periods, the gilts were kept in individual (.91 × .40 m) metabolism stalls with wire-mesh floors. The room temperature was 24 C and there was continuous fluorescent light. The experiment was conducted in two consecutive periods of 7 d for each group. During the first period, all pigs received the basal diet. Total daily urine collections were made from d 4 to 7. At the end of the first period, all gilts for which complete collections had been made were allotted at random within their age and weight groups to the four diets. As in the first period, total daily urine collections were made from d 4 to 7. Urine was allowed to flow through the catheters into collection vessels to which 50 ml of a 10% (v/v) solution of technical grade concentrated HCl were added. After measuring the total daily urine volume for each pig, an aliquot was removed and stored at −20 C until analyzed. Urine samples were analyzed for ammonia N, creatinine N, urea N and total N, and the daily excretion of each of these components was calculated.

Exp. 2. Twenty-four pigs (12 barrows and 12 gilts) with an average initial weight of 24 kg were assigned at random within sex to the four dietary treatments. They were housed individually in 1.6 × 1.8 m pens with one-third slatted floors. Room temperature was 30 C for the first 7 d and was then progressively lowered to 24 C. There was continuous fluorescent light. Pigs were weighed and feed consumption was measured weekly throughout a 28-d growth period. In addition, the pigs were bled weekly. Blood from the anterior vena cava was collected in heparinized tubes, centrifuged and the plasma frozen (−20 C) until analyzed for urea.

Exp. 3. Twenty-four pigs (12 barrows and 12 gilts) with an average initial weight of 24 kg were allotted to dietary treatments in the same manner as described in Exp. 2. Housing and data collection were also similar to Exp. 2, except that the experiment was continued for

45 d rather than 28 d. Blood samples were collected on d 14, 28 and 40. All plasma samples were analyzed for urea. Samples collected on d 40 were also analyzed for lysine, tryptophan, threonine and isoleucine.

Analytical Methods. Urea N in urine and urea in plasma were determined by the automated (AutoAnalyzer[8]) procedure of Marsh et al. (1965). Ammonia N and creatinine N in urine were also analyzed by automated procedures, based on the method described by Streeter et al. (1970) for ammonia and on the method described by Chasson et al. (1961) for creatinine. Total N in urine was determined by a standard Kjeldahl procedure similar to that described by the AOAC (1980). For the analysis of lysine, threonine and isoleucine, plasma was first deproteinized by adding solid sulfosalicylic acid according to the method of Perry and Hansen (1969). The supernatant fluid was analyzed for amino acids using an automated ion-exchange separation (Blackburn, 1978), followed by fluorometric detection (Benson and Hare, 1975). Plasma tryptophan was determined by the automated method of Lewis et al. (1976) after deproteinization with trichloroacetic acid.

Statistical Methods. Data were analyzed using analysis of variance techniques appropriate for factorial plans (Steel and Torrie, 1980). The general linear model procedure of the Statistical Analysis System (SAS, 1979) was used for computation. In Exp. 1 differences in excretion of urinary components were adjusted for differences in feed intake using intake as a covariate.

Results and Discussion

Brown and Cline (1974) and Fuller et al. (1979) demonstrated that the addition of an amino acid to a basal diet first-limiting in that amino acid results in a decrease in urinary excretion of urea by growing pigs. The urea excretion becomes relatively stable within 3 d after a change of diet. Exp. 1 was designed to utilize this response to test whether threonine or isoleucine were limiting in a corn + lysine + tryptophan diet.

Of the 36 gilts that were originally assigned to Exp. 1, 21 completed the two collection periods. The other gilts were lost from the experiment for a variety of reasons. Two pigs died during the insertion of the catheter, apparently as a reaction to the anesthesia. Another gilt was

removed from the experiment because of a rectal prolapse. The other gilts were lost from the experiment when catheters pulled out of their urethras. Initially, attempts were made to reinsert catheters, but irritation of the urinary tract made it difficult to reinsert and maintain the catheters. When catheters were removed, all gilts returned to normal in 2 to 3 d. Only data from those gilts that completed both collection periods were utilized for analysis. The number and average initial weight of gilts within each group completing the experiment is identified in table 2.

The feed intakes and excretion of major nitrogenous compounds in the urine of the pigs in Exp. 1 are also presented in table 2. During period 1, when all pigs were given the basal diet, there were no significant differences in feed intake or N excretion among the four groups. Pigs ate the basal diet readily, even though they were confined in metabolism crates. The average feed intake during period 1 was 2,181 g/d; this compares well with an expected intake of 1,500 g/d suggested by the NRC (1979) for pigs weighing 20 to 35 kg. The urinary excretions of creatinine N, urea N and total N were similar to those listed in a table of standard values for swine (Altman and Dittmer, 1974). The daily excretion of urea N (5,154 mg/d) was much greater than the 2,880 mg/d reported by Brown and Cline (1974) for 25-kg pigs fed a corn diet supplemented with lysine and tryptophan. However, Brown and Cline (1974) did not report the feed intakes of the pigs in their experiment. They stated that feed intake was equalized, which implies that it was less than ad libitum, and probably less than the intakes in this experiment. Considering the average values for all pigs during this period, ammonia N, creatinine N and urea N accounted for 23, 5 and 77% of the total N, respectively. Thus, the sum of these three compounds apparently accounted for slightly more than the total N excreted (105% of the determined total N).

The average total N excretion during period 1 (6,660 mg/d) is equivalent to approximately 562 mg·(kg body wt$^{.75}$)$^{-1}$·d^{-1}. This value is remarkably similar to the value of 570 mg·(kg body wt$^{.75}$)$^{-1}$·d^{-1} recorded by Fuller et al. (1979) for pigs fed barley supplemented with its first two limiting amino acids, lysine and threonine. In the experiments of Fuller et al. (1979), pigs were given 120 g feed·(kg body wt$^{.75}$)$^{-1}$·d^{-1}, whereas in our experiment, they

consumed approximately 184 g·(kg body wt$^{.75}$)$^{-1}$·d^{-1}. However, because of the difference in the N content of barley and corn, the N intakes of the pigs were 2.16 and 2.48 g·(kg body wt$^{.75}$)$^{-1}$·d^{-1} for the barley and corn diets, respectively.

During period 2, when pigs were fed the four diets, feed intakes were somewhat lower. Nevertheless, the average intake of each group still compared well with the intakes suggested by the NRC (1,500 g/d for pigs weighing 20 to 35 kg and 2,000 g/d for pigs weighing 35 to 60 kg).

The daily excretions of ammonia N and creatinine N in urine were similar to those observed during period 1 and were not affected (P>.1) by dietary treatment. There were, however, significant treatment differences in the daily excretions of both urea N and total N. Addition of threonine to the corn + lysine + tryptophan diet substantially (main effect of threonine, P<.001) reduced the urinary excretion of both of these components. The data demonstrate an improved utilization of the ingested N and indicate that threonine is the third limiting amino acid in corn. The main effect of isoleucine was not significant, confirming that it was not third-limiting. Urea N excretion indicated some evidence of an interaction between the two amino acids (P=.100). The addition of isoleucine alone tended to increase urea N excretion (from 4,937 to 5,205 mg/d). However, the addition of isoleucine in combination with threonine reduced urea N excretion to 927 mg/d. This interaction suggests that isoleucine may be fourth-limiting. Although the interaction was not significant for total N excretion, the daily excretion rates tended to follow the same pattern as for urea N excretion.

The total urinary N excretions of pigs fed the diet with threonine and the diet with both threonine and isoleucine were approximately 367 and 300 mg·(kg body wt$^{.75}$)$^{-1}$·d^{-1}, respectively. These values are similar to the value of 270 mg·(kg body wt$^{.75}$)$^{-1}$·d^{-1} recorded by Fuller et al. (1979) for pigs fed barley diets supplemented with lysine, threonine and histidine. According to the estimates of Fuller et al. (1979), the irreducible minimum urinary N loss in swine is of the order of 230 mg·(kg body wt$^{.75}$)$^{-1}$·d^{-1}. The composition of this residual N is, presumably, predominantly non-urea N such as creatinine, allantoin, nucleic acids, amino N and minor contributions from vitamins. If these estimates are correct, the data

TABLE 2. FEED INTAKE (G/D) AND EXCRETION OF NITROGEN (MG/D) IN VARIOUS COMPONENTS OF URINE (EXP. 1)[a]

Diet number:	1	2	3	4		P values[b]		
No. of gilts:	5	6	5	5				
Average initial wt, kg:	25.5	25.3	26.3	26.5				
Item					CV[c], %	Thr	Ile	Thr × ile
			Period 1					
Diet	Basal	Basal	Basal	Basal				
Feed intake	2,138	2,179	2,303	2,106	10.5	.450	.657	.254
Ammonia N	1,588	1,336	1,715	1,506	34.3	.338	.528	.930
Creatinine N	351	277	381	351	29.3	.257	.249	.626
Urea N	5,079	4,760	5,569	5,209	27.7	.599	.464	.975
Total N	6,626	6,327	7,080	6,607	27.8	.646	.658	.919
			Period 2					
Diet	Basal (B)	B + thr	B + ile	B + thr + ile				
Feed intake	1,675	1,639	1,914	1,838	17.0	.675	.112	.880
Ammonia N	1,509	1,306	1,459	1,308	28.6	.326	.900	.885
Creatinine N	434	334	407	374	27.4	.173	.901	.478
Urea N	4,937	2,018	5,205	927	27.7	<.001	.342	.100
Total N	6,900	4,343	6,990	3,552	19.0	<.001	.479	.340

[a]Least-squares means. Values (except for feed intake) were adjusted for differences in intake. Analysis of variance model include threonine (thr), isoleucine (ile) and their interaction.

[b]Probability values for the F-test of the main effect of threonine, isoleucine and their interaction, respectively.

[c]Coefficient of variation.

imply that pigs make efficient use of the N they absorb from a barley diet supplemented with lysine, threonine and histidine, and from a corn diet supplemented with lysine, tryptophan, threonine and isoleucine. In support of this concept, the proportion of urinary total N contributed by urea N was much lower for pigs supplemented with threonine (46%) or threonine + isoleucine (26%) than for pigs fed the basal diet (72%).

The results of the first growth experiment are presented in table 3. There was a tendency for the addition of threonine to reduce feed intake (main effect of threonine, P=.091). This was particularly evident when threonine and isoleucine were added together. Neither the main effect of threonine nor that of isoleucine was significant for weight gain. However, there was an interaction (P=.055) between the two amino acids. Addition of threonine alone tended to improve weight gain, but the combination of threonine and isoleucine together reduced weight gain below that achieved by pigs fed the basal diet. Feed efficiency (gain/feed) was improved by threonine addition (P=.019), but again there was evidence of an interaction (P=.079). Apparently the addition of threonine improved feed efficiency, but the further addition of isoleucine tended to nullify that improvement.

Plasma urea concentrations showed the same effects regardless of bleeding period (d 7, 14, 21 and 28) and consequently the mean of the four periods is presented in table 3. Plasma urea concentrations were lower (P<.001) in pigs that received added threonine regardless of whether this was alone or in combination with isoleucine. There was no evidence of an interaction between threonine and isoleucine for plasma urea.

The data obtained in Exp. 2 are in agreement with those of Exp. 1, confirming that threonine is third-limiting in corn. They do not, however, provide support for the hypothesis that isoleucine is fourth-limiting. Because Exp. 2 was rather short (28 d), a somewhat longer test (45 d) was utilized in Exp. 3.

The performance and plasma urea concentrations of the pigs in Exp. 3 (table 4) were in most respects similar to the results of Exp. 2. Additions of threonine reduced (P=.005) feed intake, but this was mainly because of a large reduction when threonine and isoleucine were added together (interaction, P=.023). Weight gain seemed to be increased by the single addition of either threonine or isoleucine, but neither of the main effects was significant. Addition of threonine and isoleucine together resulted in the lowest weight gain of any treatment (interaction, P=.004). Threonine addition improved (P<.001) feed efficiency, but, as in Exp. 2, this improvement was reduced when isoleucine was also added (interaction, P=.004). The response in plasma urea concentration was similar to Exp. 2. Responses were similar at each bleeding period (d 14, 28 and 40) and mean values are presented in table 4. Addition of threonine dramatically reduced (P<.001) plasma urea, but there was no further reduction when threonine and isoleucine were added in

TABLE 3. PERFORMANCE AND PLASMA UREA CONCENTRATIONS OF PIGS (EXP. 2)[a]

Item	Basal (B)	B + thr	B + ile	B + thr + ile	CV[c], %	P values[b]		
						Thr	Ile	Thr × ile
Feed intake, kg/d	1.54	1.50	1.56	1.37	10.6	.091	.404	.284
Weight gain, kg/d	.45	.50	.47	.42	13.4	.925	.215	.055
Gain/feed	.29	.34	.30	.31	8.1	.019	.316	.079
Plasma urea, mg/dl[d]	15.45	5.94	16.31	5.98	32.6	<.001	.759	.782

[a]Six individually fed pigs/treatment (initial weight 24 kg; final weight 37 kg) were on test for 28 d.

[b]Probability values for the F-test of the main effect of threonine (thr), isoleucine (ile) and their interaction, respectively.

[c]Coefficient of variation.

[d]Mean of values from blood samples collected on d 7, 14, 21 and 28.

TABLE 4. PERFORMANCE AND PLASMA UREA AND AMINO ACID CONCENTRATIONS OF PIGS (EXP. 3)[a]

Item	Basal (B)	B + thr	B + ile	B + thr + ile	CV,[c] %	P values[b]		
						Thr	Ile	Thr × Ile
Feed intake, kg/d	1.79	1.71	2.12	1.44	16.7	.005	.811	.023
Weight gain, kg/d	.52	.61	.68	.48	19.1	.248	.749	.004
Gain/feed	.29	.36	.32	.33	6.7	<.001	.694	.004
Plasma urea, mg/dl	15.66	7.54	18.00	7.60	21.7	<.001	.280	.307
Plasma lysine, mg/dl	9.93	6.58	7.44	7.89	27.7	.132	.531	.054
Plasma tryptophan, mg/dl	1.67	1.81	1.75	1.77	20.2	.589	.877	.661
Plasma threonine, mg/dl	3.77	11.41	4.02	10.52	35.3	<.001	.781	.616
Plasma isoleucine, mg/dl	.50	.32	1.89	1.95	35.1	.730	.001	.491

[a]Six individually fed pigs/treatment (initial weight 24 kg; final weight 50 kg) were on test for 45 d. Plasma urea concentrations represent the mean of values from blood samples collected on d 14, 28 and 40. Plasma amino acid concentrations were determined from blood samples taken on d 40.

[b]Probability values for the F-test of the main effect of threonine (thr), isoleucine (ile) and their interaction, respectively.

[c]Coefficient of variation.

combination. Addition of isoleucine alone did not reduce plasma urea.

Blood plasma samples obtained on d 40 of Exp. 3 were analyzed for lysine, tryptophan, threonine and isoleucine. The results are presented in table 4. Although plasma lysine and tryptophan were not significantly altered by additions of threonine and(or) isoleucine, there was a tendency for plasma lysine levels to be reduced, particularly when threonine was added (main effect of threonine, $P=.132$). A lower plasma lysine concentration would be consistent with the premise that threonine supplementation improved the dietary amino acid pattern, and consequently improved lysine utilization. The plasma levels of threonine and isoleucine were greatly elevated by dietary additions of the respective amino acid, but there were no interactions.

The increase in weight gain due to threonine addition was 50 g/d in Exp. 2 and 90 g/d in Exp. 3. These improvements are reasonably similar to the increase (107 g/d) that would be predicted from the reduction in urinary urea observed in Exp. 1.

The results of the three experiments indicate that the determined sequence of limiting amino acids in corn for swine is different than that calculated from requirement tables. Instead of isoleucine being third-limiting (with valine also) and threonine being fifth-limiting, the data indicate that threonine is the third limiting amino acid.

The lack of improvement in growth or feed efficiency when isoleucine was added in combination with threonine was not anticipated. Lewis et al. (1982) obtained large increases in weight gain and feed efficiency in two separate experiments with growing rats when isoleucine was added as the fourth amino acid in a corn diet. Also, the reductions in urinary excretion of urea N and total N reported in Exp. 1 implicate isoleucine as being fourth-limiting. Particularly unexpected was the apparent decrease in feed intake and weight gain when threonine and isoleucine were added together. This response, which occurred in both Exp. 2 and 3, is difficult to explain. In diets in which isoleucine is not limiting, its addition would be expected to cause an amino acid imbalance. However, if this is so, the imbalance caused increased intake in the absence of threonine, and decreased intake in the presence of threonine (when presumably some other amino acid was limiting). These data imply that the manner in which growing

swine respond to an amino acid imbalance may be specific for individual amino acids.

Although it is possible that isoleucine is co-limiting with some other essential amino acids or with nonspecific N, it does not appear to be clearly fourth-limiting alone for swine. The difference in results obtained with swine and rats may represent a real difference between species, or may be related to differences in physiological ages of the animals.

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