LIMITING AMINO ACIDS IN MILO FOR GROWTH IN THE PIG

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GENERAL INTRODUCTION

Milo has become one of the major feed grains in the U.S. and especially in Kansas, which ranks second only to Texas in grain sorghum production. However, it is generally accepted that milo is inadequate as the sole source of protein for swine. More specifically, it is low in several of the amino acids which are essential for growth, namely lysine, methionine and threonine.

Supplementation of lysine to fortified milo diets was recently introduced and is now being applied at the commercial level. This allows for a more efficient and economical use of protein supplement, i.e. less protein supplement is required to balance a ration in order to meet the pigs needs. This leads to the possibility of supplementing other amino acids which may be limiting in milo for growth.

There is limited published work on the amino acid adequacy of milo for growth in the pig. It has been shown that lysine is the first limiting amino acid in milo but the subsequent order has not been determined. Hence, the purpose of this study is to determine the limiting amino acids in milo for the growing pig.

REVIEW OF LITERATURE

Importance of Amino Acids

Amino acids are often referred to as the building blocks of protein. Twenty-two amino acids occur naturally in proteins and 10 of these have been classified as dietary essentials for growth in the pig (Mertz *et al*., 1952). With the identification of the essential and non-essential amino acids, came the realization that the animal's requirement for protein in the diet is in fact a requirement for amino acids (Munro, 1964). It is therefore possible to obtain performance at lower levels of dietary protein, by providing an ideal balance of acids, than was formerly recognized (Jones and Pond, 1963). The amino acids required by the animal's system are obtained from dietary protein through the digestive and absorptive processes. The majority of these amino acids are used concomitantly for protein synthesis and must be present simultaneousl Amino acid storage is virtually non-existent in the body and therefore, omission of a single essential amino acid results in cessation of protein synthesis (at the expense of dietary amino acids) when the supply of that amino acid is depleted. Thus the remaining amino acids must either be excreted or metabolized into energy, which greatly lowers their efficiency of utilization. Within one day after a single essential amino acid has been omitted from the diet, a loss of appetite and growth failure occur in young animals and a severely negative nitrogen balance is observed in adults. Omission of other essential nutrients from the diet for one day from a previously well-nourished animal has little effect on growth and intake (Rose, 1938; Geiger, 1947; Fraizer *et al.*, 1947; Munro, 1964).

Thus, diets in which the amino acids are not balanced, in relation to the requirements, contain an excess of amino acids which cannot be utilized efficiently for protein synthesis unless the deficient amino acids are supplemented (Berry *et al.*, 1962).

Limiting Amino Acids

The function of dietary protein is to serve as a source of amino acids for the body, therefore the most efficiently utilized protein will be one which provides a mixture of both essential and non-essential amino acids in the proportions needed by the body. If a given dietary protein is, by comparison with the requirements, low in any one of the essential amino acids, this will become the factor limiting the usefulness of that protein: the "limiting amino acid" (Munro, 1964).

The sequence in which the essential amino acids of a protein in a diet become limiting can be calculated from a knowledge of the amino acid composition of the protein and the requirements of the animal. The reliability of this procedure can be checked by conducting growth trials using a diet low in the dietary protein and supplementing amino acids in the order calculated to be deficient. This procedure was used to determine the limiting order of amino acids in casein for rats. The order in which the amino acids become limiting for growth as calculated theoretically, differs from that determined experimentally. Three major sources of error which could be responsible for these deviations are: (1) inaccuracies in the analysis of the amino acid composition of the protein, (2) inadequate knowledge about the availabilities of the amino acids and perhaps (3) an inaccurate estimate of the amino acid requirements (Harper, 1958).

The limiting order of amino acids for growth has been determined in Columbian floury-2 corn for the rat (Pond et al., 1971) in soybean protein (Berry et al., 1962) and corn protein (Baker et al., 1969) for growth in the pig.

Pond et al. (1953) suggested that lysine is a limiting amino acid for pigs from weaning to 40 and 55 kg in a milo-soybean meal ration (12-14% protein) and obtained the most rapid gains of pigs on these rations with the addition of 0.1% lysine. Hillier et al. (1954) obtained results which indicate that lysine is first limiting in a milo, soybean meal, alfalfa meal type ration for pigs weighing 18 to 55 kg.

Pond et al. (1958) studied the amino acid adequacy of milo for growth of rats. Using a low protein, fortified milo basal diet, he studied the limiting order of amino acids in milo. By adding various levels of lysine, threonine, isoleucine, methionine, tryptophan and valine to the basal diet and observing weekly gain, it was determined that lysine was first limiting and threonine second. No conclusions were drawn as to the remaining order.

Jensen et al. (1965) evaluated milo as the sole source of protein for the finishing pig. The response obtained from adding either 0.20 or 0.25% lysine to the 8.0% crude protein milo diet suggested lysine was first limiting. Addition of 0.1% DL-methionine had no effect, suggesting it is not second limiting. This agrees with the results of Pond et al. (1958).

Amino Acid Requirements

The level of dietary protein required for maximum growth of the young animal is that amount which supplies the required quantities of biologically available essential and non-essential amino acids (Jones, 1963). The requirement for an individual amino acid depends upon the amount needed for protein synthesis, the amount needed for synthesis of other body constituents and the amount that is degraded (Aguilar et al., 1972).

No information was available on amino acid requirements of swine prior to 1948 (Mertz et al., 1952). The exact quantitative levels of each amino acid required by swine is still not known, but reliable data has been published in the literature.

Lysine. Mertz et al. (1949) first demonstrated lysine to be essential to the nutrition of swine when he reported a diet containing 2.0% DL-lysine·HCl as capable of supporting growth in the weanling pig. Becker et al. (1951) reported the lysine requirement of a 5-9 week old pig on a 12% protein diet to be 0.72% of the diet or 6.0% of the dietary protein. Hutchinson et al. (1957), using a yellow corn diet (11.69% protein) reported that pigs fed a diet with 0.52% lysine made significantly ($P<.01$) faster gains and had better feed efficiency than pigs on lower levels of lysine. McWard et al. (1959) found that a 14 kg weanling pig, on a 12.8% protein diet, required 0.71% lysine in the diet or 5.5% of the protein. At 21.7% protein, the lysine need was 0.95% of the diet or 4.38% of the dietary protein. Becker et al. (1966) set the requirement at 0.79, 0.74 and 0.68% lysine for 18, 16 and 14% dietary protein levels, respectively for a 14 kg pig. N.R.C. (1968) defines the lysine requirement of the weanling pig (20-40 kg) as 0.70% of the diet based on an 18% protein diet.

Methionine. Bell et al. (1950) initially recognized methionine to be essential for swine. Shelton et al. (1951) fed purified diets (21% protein) and set the DL-methionine requirement at 0.6% in the absence of cystine or 0.3% in the presence of adequate cystine (0.3% or more). The requirement for 2-7 week old pigs was found to be 0.5, 0.6 and 0.7% at 12, 18 and 25% protein levels, respectively by Kroening et al. (1965). Becker et al. (1955) stated that 0.25% DL-methionine in the presence of 0.17% cystine supported satisfactory rate and efficiency of gain. Becker et al. (1966) recommended 0.53, 0.50 and 0.45% DL-methionine for 18, 16 and 14% protein diets and stated that cystine can replace 40% of the methionine need. Baker et al. (1969) reported cystine could provide at least 56% of the requirement for the total dietary sulfur amino acids. N.R.C. (1968) defines the DL-methionine requirement as 0.50% of the diet (18% protein) and states that cystin can replace 40% of the need for methionine.

Threonine. Shelton et al. (1950) demonstrated that threonine must be present for growth in swine. Kroening et al. (1965)

reported a 2-7 week old pig required 0.63% threonine while Becker (1951) suggested a requirement of 0.61% for the 5-9 week old pig. Mitchell et al. (1968) reported the 10 kg pig as requiring 0.60% threonine as determined by nitrogen balance. Mertz et al. (1952) determined the requirement of the weanling pig to be no more than 0.4% of the diet while Evans (1963) reported it to be 0.45%. Becker et al. (1966) suggested the needs of a 14 kg pig to be 0.49, 0.45 and 0.42% for 18, 16 and 14% protein diets respectively. N.R.C. (1968) defines the threonine requirement for the weanling pig as 0.45%.

Isoleucine. Shelton et al. (1950) demonstrated isoleucine to be essential for the growing pig. Maximum response in growth and feed efficiency were obtained with a diet (22% protein) that contained 0.70% isoleucine when fed to young swine (Bringegar et al., 1950). Becker et al. (1963) determined a 5 kg pig required 0.76% isoleucine when fed a 22% protein diet. Oestemer et al. (1973) predicted a lower need for 5.8 kg pigs based on ADG, G/F and PER, respectively, to be 0.52, 0.48 and 0.45%. The need of weanling pigs was found to be 0.65% and 0.46% for 26.7 and 13.35% protein diets, respectively (Becker et al., 1957). This agrees closely with Evans (1963), suggesting a requirement of 0.60% isoleucine. Bravo et al. (1970) reported a lower need for 20 kg pigs of 0.27-0.32% isoleucine when ADG, G/F and plasma-free isoleucine were used as a response criteria. Becker et al. (1966) determined the requirement for a 14 kg pig to be 0.48, 0.52 and 0.57% for 14, 16 and 18% dietary protein, respectively. N.R.C. (1968) defines the requirement for a 20-40 kg pig to be 0.50% of the diet.

Tryptophan. Tryptophan was proved to be indispensible for growing pigs by Beeson et al. (1949) who suggested a level of 0.40% DL-tryptophan as adequate to meet the normal requirement in pigs weighing 23 to 45 kg. A ration containing 0.2% DL-tryptophan supplied sufficient tryptophan for optimum growth and efficiency of gain in pigs. Maximum rates of gain were observed with this diet and higher levels of DL-tryptophan did not result in an increase growth response (Shelton et al., 1951). Becker et al. (1955) established that the pig requires no more than 0.115% tryptophan in a diet containing 15.3% protein. Evans (1963) and Baker et al. (1971) have determined similar requirements of 0.15 and 0.121% tryptophan, respectively, as did Becker et al. (1966) with requirements of 0.11, 0.12 and 0.13% for 14, 16 and 18% protein levels, respectively. N.R.C. (1968) defines the tryptophan requirement as 0.13% for a 20-40 kg pig.

Thompson et al. (1952) showed that D-tryptophan may be used to some extent by the pig when he reported that averages of gain tended to favor baby pigs fed 0.10% DL-tryptophan in the diet as compared to 0.05% L-tryptophan. Baker et al. (1971) found that the young pig can utilize D-tryptophan approximately 60% as well as L-tryptophan.

Amino Acids in Milo

It has been demonstrated that milo is comparable to corn in producing gains (Peo and Hudman, 1958). It has also been shown that milo is inadequate as the sole source of protein (amino acids) for swine (Jensen, 1965; Allee and Hines, 1971). It does contain all of the 10 essential amino acids but not in the quantities to support maximum growth. With the expanded use of irrigation, fertilizer, and hybrid seeds in farming practices, the amino acid content of sorghum grain may vary more than previously (Waggle et al., 1966). The lysine content of milo is very low and the amounts of methionine and threonine are also below the level required by the pig. In R-109 milo, lysine is 38% adequate (content in milo expressed as a percent of the pigs requirement), methionine, 48% and threonine, 73%. Isoleucine and tryptophan are often marginally deficient. As indicated previously, much work has been published on supplementing feed grains with lysine to improve its feeding value. Also, that supplementing milo with amino acids other than lysine will have no effect upon performance since lysine is first limiting. Some work has been published on supplementing methionine to milo rations (Jensen et al., 1965; Pond et al., 1958) but very limited work is published on additions of other amino acids to deficient diets.

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INTRODUCTION

Milo has been shown to be inadequate as the sole source of protein for growth of swine (Jensen, Becker and Harman, 1965; Allee and Hines, 1971). It is deficient or marginally-so in several amino acids for the growing pig, including lysine, methionine, threonine, isoleucine and tryptophan. Lysine is the first limiting amino acid in milo for swine (Pond, Hillier and MacVicar, 1953; Hillier, MacVicar and Pond, 1954).

Milo and corn are generally considered to be of comparable nutritive value (Hillier *et al.*, 1954), but corn has been the subject of more amino acid studies. Baker *et al.* (1969) determined tryptophan and lysine to be first and second-limiting in corn protein for the growing pig. Gallo and Pond (1968) observed the same results on all-corn diets for the finishing pig. Baker *et al.* (1969) also showed that methionine was not third-limiting as did Oestemer *et al.* (1970) when no response was obtained from supplementa methionine. Lysine was found to be first-limiting and tryptophan second-limiting for both early weaned pigs fed all-corn diets (Gallo, Pond and Logomarsino, 1968) and young rats fed Columbian *floury*-2 corn (Pond, Maner and Linares, 1971).

Additional research has determined the limiting order in milo for growth in the rat (Pond, Hillier and Benton, 1958), proving lysine to be first-limiting and suggesting threonine second-limiting. However, it is difficult to find published data on the sequence in which amino acids other than lysine become limiting for growth in pigs fed all-milo diets. The increased occurrence of amino acid supplementation makes it important to learn the qualitative and quantitative amino acid limitations of milo protein.

EXPERIMENTAL PROCEDURE

General. In the growth trials, pigs were housed in 1.2 x 1.8 m wooden cages with expanded metal floors in a building where temperature was maintained at approximately 21° C. Feed and water were supplied *ad libitum*. Initial and final weights were recorded and daily gain and feed efficiency were determined at the conclusion of the trials. Trials varied from 21 to 28 days in length. A randomized complete block design was used for all growth trials and the data was treated statistically using analysis of variance and Duncans New Multiple Range test (Snedecor and Cochran, 1971).

In the nitrogen retention studies the pigs were housed individually in metal metabolism cages allowing for separate collection of feces and urine. Daily feed intake was constant and fed in two equal portions at approximately 8:00 a.m. and 5:00 p.m. Fresh water was supplied each day. A five day pre-test period preceded a five day collection period. A ferric oxide marker was fed at the beginning and end of each 5-day period. Feces were collected daily and stored in a refrigerator. The entire 5-day fecal collection was dried in a forced-air oven at 50° C for 7 days, allowed to come to air-dry weight, weighed and ground in a Wiley mill equiped with a 40-mesh screen. Urine was collected in an 8-liter container to which 20 ml. of concentrated HCl had been added. Each daily collection was diluted to a constant volume and a 100 ml. aliquot taken. Accumulated aliquots were stored in a refrigerator at 1°C until analyzed. Representative feed, fecal and urine samples were analyzed in duplicate for nitrogen as outlined by A.O.A.C. (1970).

Composition of the fortified milo basal diet is shown in Table I. Amino acid composition of the basal milo diet is given in Table II. Crystalline amino acids were added to the basal diet to equal the amino acid content of a 16% protein milo-soybean meal diet in trials 1 and 2. In trials 3, 4 and 5, amino acids were supplemented to meet the N.R.C. (1968) requirements. Amino acids were added in the order calculated to be limiting and re-arranged in subsequent trials. Only the L-isomer of amino acids was added except in the case of DL-methionine. Lysine was added in the form of L-lysine•HCl, feed grade (78% L-lysine).

Trial 1. Thirty Hampshire and Yorkshire pigs weighing 17 kg were allotted based on weight, breed and sex to the following five treatments (Table III): (1) basal milo diet, (2) as 1 plus 0.56% lysine, (3) as 2 plus 0.22% DL-methionine, (4) as 3 plus 0.33% threonine and (5) a 16% C.P. milo-soybean meal positive control. The trial consisted of three pigs per replicate and lasted 28 days.

Trial 2. A nitrogen metabolism study was conducted using four littermate Yorkshire gilts weighing an average of 18 kg. The gilts were randomly assigned to treatments 1, 2, 3 and 4 (Table IV) as in trial 1. A Latin Square design was used and daily intake was held constant at 1135 g throughout the trial.

TABLE I. COMPOSITION OF BASAL DIET[a]

Ingredient	Percent
Milo [b]	95.86
Dicalcium phosphate	1.92
Ground limestone	0.42
Salt	0.50
Vitamin premix [c]	1.00
Trace mineral premix [d]	0.05
Antibiotic premix [e]	0.25

[a] Diet was ground.

[b] Crude protein in trials 1, 2 and 3 = 8.7%; in trials 4 and 5 = 9.1%.

[c] Amount per kilogram: 880,000 USP Units of vitamin A, 66,000 USP Units of vitamin D_3, 990 mg of Riboflavin, 2,640 mg of d-Pantothenic acid, 66,000 mg of Choline, 5,500 mg of Niacin, 4,400 I.U. vitamin E, 4.84 mg of vitamin B_{12} and 12.54 g preservative (BHT).

[d] Containing 0.1% cobalt, 1.0% copper, 0.3% iodine, 10% iron, 10% manganese and 10% zinc.

[e] Supplied as ASP-250.

TABLE II. AMINO ACID COMPOSITION OF FORTIFIED MILO BASAL DIET

	% in	
Amino Acids	Trials 1, 2 & 3	Trials 4 & 5
Lysine	.230	.299
Methionine	.093	.096
-Cystine	.140	.144
Threonine	.315	.343
Isoleucine	.380	.366
Leucine	1.217	1.271
Tryptophan	.163	.115
Arginine	.378	.453
Valine	.458	.455
Histidine	.214	.262
Phenylalanine	.470	.490
-Tyrosine	.345	.388

<u>Trial 3</u>. Forty-two Duroc, Hampshire and Yorkshire pigs weighing an average of 22 kg were allotted based on weight, breed and sex to the following seven treatments (Table V): (1) basal milo (2) as 1 plus 0.47% lysine, (3) as 2 plus 0.13% threonine, (4) as 3 plus 0.27% methionine, (5) as 4 plus 0.12% isoleucine, (6) as 5 plus 1.70% glycine and 1.70% glutamic acid and (7) 16% C.P. milo-soybean meal control. Diets 6 and 7 were isonitrogenous. The trial was replicated with a total of 6 pigs per treatment and lasted 21 days.

<u>Trial 4</u>. Thirty Duroc and Yorkshire pigs weighing an average of 17 kg were allotted based on weight and breed to the following five treatments (Table VI): (1) Basal plus 0.40% lysine, (2) as 1 plus 0.11% threonine, (3) as 1 plus 0.26% methionine, (4) as 1 plus 0.11% threonine plus 0.26% methionine and (5) 16% C.P. milo-soybean meal control. The trial consisted of three pigs per replicate and lasted 22 days.

<u>Trial 5</u>. A nitrogen retention study was conducted using twelve Duroc and Yorkshire barrows weighing an average of 16 kg. Three groups of four littermates were used in a randomized complete block design. Within each group treatments 1, 2, 3 and 4 (Table VII) as in trial 4, were randomly assigned and fed for one period (10 days) after which treatments were re-allotted for another period so as to provide a second replicate. Daily intake was 900 g for the first period and 1000 g for the second.

RESULTS

Trial 1. Results of this growth trial are presented in Table III. Supplementation of the basal diet with lysine (0.56%) resulted in a marked ($P<.05$) improvement in the daily gain and feed/gain. The addition of methionine (0.22%) in the presence of lysine resulted in a slight improvement in daily gain and feed efficiency although these responses were not statistically significant. With the addition of threonine (0.33%) in the presence of lysine and methionine, a significant ($P<.05$) response was obtained in both daily gain and feed/gain. As expected, the milo-soybean meal diet produced the fastest ($P<.05$) and most efficient gains.

Table 2. The results of the nitrogen metabolism study are presented in Table IV. The addition of lysine (0.56%) to the basal diet significantly ($P<.05$) increased nitrogen retention. Addition of methionine (0.22%) in the presence of lysine increased nitrogen retention by 0.78 g per day over that observed for lysine alone. However, this difference was not statistically significant. The addition of threonine (0.33%) in the presence of lysine and methionine, resulted in greater ($P<.05$) nitrogen retention than that observed on the basal diet supplemented with lysine or lysine and methionine.

Trial 3. Average daily gain (ADG) and feed/gain (F/G) data are presented in Table V. Supplementation of lysine (0.47%) to the basal milo diet produced a three-fold increase in ADG and reduced F/G by one-half. Both responses were statistically

TABLE III. PERFORMANCE OF GROWING PIGS FED FORTIFIED MILO DIETS SUPPLEMENTED WITH L-LYSINE, DL-METHIONINE AND L-THREONINE[a] (Trial 1)

Diets	Daily Gain(g)	Feed/ Gain
1. Basal	104 [c]	7.52 [c]
2. as 1 + 0.56% lysine [b]	182 [d]	5.07 [d]
3. as 2 + 0.22% methionine	245 [d]	4.34 [de]
4. as 3 + 0.33% threonine	331 [e]	3.35 [de]
5. Milo + soybean meal	627 [f]	2.26 [e]

[a] Six pigs per diet, avg initial wt., 17 kg, 28 day trial.
[b] Supplied as 0.73% L-lysine·HCl.
[cdef] Means with different superscripts in the same column are significant ($P<.05$).

TABLE IV. NITROGEN RETENTION OF PIGS FED FORTIFIED MILO DIETS SUPPLEMENTED WITH L-LYSINE, DL-METHIONINE AND L-THREONINE[a] (Trial 2)

	Daily N(g)			
Diets	Intake	Urine	Fecal	Retained
1. Basal	16.62	6.29	4.08	6.25 [c]
2. as 1 + 0.56% lysine [b]	18.18	5.19	4.39	8.60 [d]
3. as 2 + 0.22% methionine	18.52	4.70	4.44	9.38 [d]
4. as 3 + 0.33% threonine	19.05	3.71	4.02	11.32 [e]

[a] Four Yorkshire gilts weighing 18 kg, 4 X 4 Latin Square design.
[b] Supplied as 0.73% L-lysine·HCl.
[cde] Means with different superscript letters are statistically different ($P<.05$).

TABLE V. EFFECT OF ADDITIONS OF VARIOUS AMINO ACIDS TO A FORTIFIED MILO DIET ON WEIGHT GAIN AND FEED/GAIN[a] (Trial 3)

Diets	Daily Gain (g)	Feed/ Gain
1. Basal	68 [c]	11.46 [c]
2. as 1 + 0.47% lysine [b]	204 [d]	5.33 [de]
3. as 2 + 0.13% threonine	254 [de]	4.33 [ef]
4. as 3 + 0.27% methionine	213 [de]	4.56 [ef]
5. as 4 + 0.12% isoleucine	300 [e]	3.73 [fg]
6. as 5 + 1.70% glycine + 1.70% glutamic acid	114 [c]	5.92 [d]
7. Milo + SBM	636 [f]	2.43 [g]

[a] Six pigs per diet, avg. initial wt., 22 kg, 21 day trial.
[b] Supplied as 0.60% L-lysine·HCl.
[cdefg] Means with different superscripts in the same column are statistically different ($P < .05$).

significant ($P<.05$). The addition of threonine (0.13%) in the presence of lysine increased ADG by 50 g over that observed for lysine alone and decreased F/G although neither was statistically significant. Adding 0.27% methionine in the presence of lysine and threonine produced a lower ADG and higher F/G than observed by adding only lysine and threonine to the basal diet. When isoleucine (0.12%) was added in the presence of lysine, threonine and methionine, an increase in ADG was observed along with a lower F/G compared to the other basal plus amino acid diets. The inclusion of non-essential amino acids in the presence of lysine, threonine, methionine and isoleucine resulted in a marked reduction in gain and feed efficiency compared to the basal diet supplemented with the essential amino acids. The 16% C.P. milo-soybean diet produced markedly greater ($P<.05$) gains and the lowest ($P<.05$) F/G.

Trial 4. Results of this growth trial are presented in Table VI. Supplementing the basal diet with 0.40% lysine produced a daily gain of 331 g and a 3.84 F/G. Addition of threonine (0.11%) to the basal + lysine diet increased ($P<.10$) daily gain by 73 g and lowered ($P<.05$) F/G. There was a significant ($P<.10$) negative response to the addition of methionine (0.26%) to the basal + lysine diet. F/G was increased although not significantly. The addition of both threonine and methionine in the presence of lysine resulted in almost identical responses in both ADG and F/G as that observed when only threonine was added in the presence of lysine. ADG for the milo-soybean meal diet was greater ($P<.10$)

TABLE VI. PERFORMANCE OF GROWING PIGS FED FORTIFIED MILO DIETS SUPPLEMENTED WITH VARIOUS AMINO ACIDS[a] (Trial 4)

Diets	Daily Gain(g)	Feed/ Gain
1. Basal + 0.40% lysine [b]	331 [c]	3.84 [g]
2. as 1 + 0.11% threonine	404 [d]	2.92 [h]
3. as 1 + 0.26% methionine	222 [e]	4.71 [g]
4. as 1 + 0.11% threonine + 0.26% methionine	400 [d]	2.94 [h]
5. Milo + SBM	663 [f]	2.19 [h]

[a] Six pigs per diet, avg initial wt., 17 kg, 22 day trial.
[b] Supplied as 0.51% L-lysine•HCl.
[cdef] Means with different superscripts are statistically different ($P<.10$).
[gh] Means with different superscripts are statistically different ($P<.05$).

TABLE VII. NITROGEN RETENTION OF BARROWS FED BASAL MILO DIET SUPPLEMENTED WITH L-LYSINE, L-THREONINE AND DL-METHIONINE[a] (Trial 5)

	Daily N(g)			
Diets	Intake	Urine	Fecal	Retained
1. Basal + 0.40% lysine [b]	13.45	3.35	3.99	6.11 [c]
2. as 1 + 0.11% threonine	13.56	2.44	4.17	6.95 [d]
3. as 1 + 0.26% methionine	13.68	3.31	4.14	6.23 [c]
4. as 1 + 0.11% threonine + 0.26% methionine	13.81	3.37	3.81	6.63 [cd]

[a] Six pigs per diet, initial wt., 16 kg.
[b] Supplied as 0.51% L-lysine•HCl.
[cd] Means with different superscripts are statistically different ($P<.10$).

than other treatments although the F/G was not statistically ($P<.10$) lower than that observed on the diets with added threonine.

Trial 5. The results of the nitrogen retention study are presented in Table VII. Addition of 0.40% lysine to the basal diet resulted in 6.11 g of nitrogen retained per day. Adding 0.11% threonine to the basal + lysine diet produced a significantly ($P<.10$) larger daily nitrogen retention of 6.95 g. The addition of methionine (0.26%) to the basal + lysine diet did not improve nitrogen retention. The addition of methionine in the presence of lysine and threonine did not increase nitrogen retention over that observed on the basal diet supplemented with lysine and threonine.

DISCUSSION

The studies reported herein confirm and extend previous findings by demonstrating, using growth and nitrogen retention studies, that lysine is the first-limiting amino acid in milo for the growing pig and threonine is the second-limiting amino acid in milo protein. The addition of lysine to the fortified milo basal diet resulted in a marked improvement in daily gain, feed efficiency and nitrogen retention. The marked response to the addition of lysine was expected since numerous workers have demonstrated that lysine is the most limiting amino acid for the growing pig (Pond et al., 1953; Hillier et al., 1954), the finishing pig (Jensen et al., 1965) and the growing rat (Pond et al., 1958).

These experiments demonstrate that threonine is the second-limiting amino acid in milo for the growing pig although it is calculated to be third-limiting. The addition of threonine in the presence of lysine or lysine and methionine resulted in an improvement in daily gain and feed efficiency. Similarly, threonine increased nitrogen retention when added to diets containin either lysine or lysine and methionine. The work of Pond et al. (1958) suggests that threonine is the second-limiting amino acid in milo for growth in the rat. The order in which amino acids are calculated to become limiting for growth does not always agree with results obtained using growth trials. Such was the case for the growing rat fed casein diets (Harper, 1958) and the young pig fed corn diets (Gallo et al., 1968). These authors suggested the following factors as possible reasons for contradictory results: (1) lower biological availability of amino acids in the protein, (2) inaccurate estimates of the requirements and (3) inaccuracies in the amino acid analysis of the protein.

Although methionine is calculated to be the second-limiting amino acid in milo protein, the addition of methionine to lysine fortified diets failed to increase daily gain or nitrogen retention. Jensen et al. (1965) demonstrated methionine was not second limiting for the finishing pig fed a milo diet. Pond et al. (1958) obtained no response from adding methionine in the presence of lysine and other amino acids to milo diets for the growing rat.

Variable results were obtained with the methionine supplementation. Nitrogen retention was slightly improved with added methionine in the presence of lysine. A slight improvement in daily gain was noticed contrasted to a marked ($P<.10$) growth depression in separate trials where methionine was added in the presence of lysine. Results of a third trial in which methionine was added in the presence of lysine and threonine were similar to results obtained from the lysine-alone diet. Feed/gain results followed an associated pattern to that of daily gain. Henson, Beeson and Perry (1954) and Becker et al. (1955) have observed gain depression from excess dietary methionine. Oestemer et al. (1970) concluded that the lack of response from supplemental methionine was suggestive of a lower methionine requirement for the growing pig than previously accepted values.

The addition of isoleucine in the presence of lysine, threonine and methionine supported greater ADG and improved F/G compared to diets not containing isoleucine. This suggests that isoleucine may be the third limiting amino acid in milo. Benton, Harper and Elvehjem (1955) showed that isoleucine would produce a marked increase in the growth of rats fed a corn diet supplemented with lysine, tryptophan, threonine and valine.

Addition of non-essential amino acids (glycine and glutamic acid) in the presence of lysine, threonine, methionine and isoleucine resulted in depressed daily gain and poor feed efficiency which can be partially explained by a low feed intake. Apparently, non-essential amino acids were sufficient in the diets and additional quantities contributed to an imbalance.

The superiority of performance by pigs fed the 16% C.P. milo-soybean meal diet indicates that additional amino acids other than the ones studied are limiting or perhaps the amino acids are not contained in the available quantities and proportions as in the milo-soybean diet.

SUMMARY

Three growth trials and two nitrogen retention trials were conducted with 118 growing pigs to determine the limiting amino acids in milo. Lysine supplementation of the basal milo diet resulted in a marked improvement in daily gain, feed efficiency and nitrogen retention. The addition of threonine increased daily gain and improved feed efficiency when added to diets containing supplemental lysine or lysine and methionine. Similarly, nitrogen retention was increased with the addition of threonine to those diets. Supplementing the basal milo diet with methionine in the presence of lysine slightly increased daily gain in one trial and produced a marked growth depression in another. Another trial in which methionine was added in the presence of lysine and threonine produced results similar to that obtained from pigs fed the lysine-alone diet. Adding isoleucine in the presence of lysine, threonine and methionine produced greater gains and feed efficiency than diets without added isoleucine. No beneficial response was obtained by supplementing non-essential amino acids

(glycine and glutamic acid) in the presence of all other amino acids studied.

These data demonstrate lysine is the first-limiting and threonine the second-limiting amino acid in milo protein for the growing pig and that methionine is not third-limiting. Additionally, these data suggest that isoleucine may be the third-limiting amino acid in milo protein for the growing pig.

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LIMITING AMINO ACIDS IN MILO FOR GROWTH IN THE PIG

by

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ABSTRACT

Three growth trials and two nitrogen retention trials were conducted with 118 growing pigs to determine the limiting amino acids in milo. Lysine supplementation of the basal milo diet resulted in a marked ($P<.05$) improvement in ADG, F/G and nitrogen retention. The addition of threonine increased ADG and improved F/G when added to diets containing supplemental lysine or lysine and methionine. Similarly, nitrogen retention was increased ($P<.10$) with the addition of threonine to those diets. Supplementing the basal milo diet with methionine in the presence of lysine slightly increased ADG in one trial and produced a marked ($P<.10$) growth depression in another. A third trial in which methionine was added in the presence of lysine and threonine produced results similar to that obtained from pigs fed the lysine-alone diet. Adding isoleucine in the presence of lysine, threonine and methionine produced greater gains and lower F/G than diets without added isoleucine. No beneficial response was obtained by supplementing non-essential amino acids (glycine and glutamic acid) in the presence of all other amino acids studied.

These data demonstrate lysine is the first-limiting and threonine the second-limiting amino acid in milo protein for the growing pig and that methionine is not third-limiting. These data also suggest that isoleucine may be the third-limiting amino acid in milo protein for the growing pig. Non-essential amino acids appear to have been in adequate quantity in the

basal diet and supplementation of such produced an imbalance. The greatly superior ($P<.05$) performance obtained from the milo-soybean meal diet indicate that other amino acids are still limiting in the milo diets or they are not present in the amount or balance as that of the control diet.