EFFECT OF METHIONINE ADDITION TO WEANLING PIG DIETS

by

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B.S., National Pingtung Institute of Agriculture, 1971

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1984

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ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to his major professor, Dr. Gary Allee, both academically and personally for his support, never ending wealth of knowledge and sound advice. The author is also sincerely appreciative of the guidance and help he received from his graduate committee, Dr. Steven Pollmann and Dr. Robert Hines.

Special appreciation to Terry Gugle, Mike Jensen, Bob LaForge and Mike Johnston for their help in his research.

Sincere gratitude is extented to Taiwan Livestock Research Institute and The National Science Council, Republic of China, for their financial support at the first year of his study in USA.

Appreciation of the understanding, patience and encouragement of the author's wife, Tsai-Chin, cannot be over emphasized.

GENERAL INTRODUCTION

The dietary requirement of a protein is determined by its ability to meet the metabolic demands for amino acids. The closer the supply of the compliment of amino acids to the needs of the pig, the lower the percentage of protein required in the diet.

The commercial availability of synthetic lysine, along with competitively priced soybean meal, has resulted in the general suggestion that crude protein content of swine diets can be reduced by 2% with the addition of synthetic lysine. However, only limited amount of work has been conducted of adding other amino acids, such as methionine, in practical starter pig diets.

Synthetic DL-methionine and its analogues are widely used in poultry diets. For swine diets, the feed company is generally adding DL-methionine in starter or grower diets. Whether this operation is necessary or economic is still in a conundrum because from the previous research, we arrive at two different conclusions. Some suggest that a improvement in growth rate will be evident by adding DL-methionine in growing swine diets; while other researchers demonstrate that adding DL-methionine results in no improvement in performance.

Recent research results with growing-finishing pigs, gilts, and sows have concluded that the present stated NRC requirements of methionine+cystine are too high and should be lowered. This may also be true with weanling pig diets. The NRC's requirements for 5 to 10 kg and 10 to 20 kg pigs were extrapolated from amino acid requirements of 20 to 35 kg pigs (Lewis, 1984). It is doubtful that the methionine requirement for weanling pigs can also be lowered using the extrapolation method.

Since almost all of the previous researchers were using natural feedstuffs for studies of methionine requirement for pigs, the results have been inconsistent and somewhat conflicting due to the variability in the methionine content of the individual feedstuffs (Trotter, 1974). Also, using the extrapolation method to predict or estimate the requirement of amino acid for weanling pigs may not be sound.

Because of the shortcomings suggested above, this study was designed to determine the methionine requirement of weanling pigs fed a semipurifed diet, approximately 21 days old and about 6 kg in average body weight.

REVIEW OF LITERATURE

Methionine Requirement

Methionine was first shown by Bell et al (1950) to be an essential amino acid for the growth of swine. He suggested that with a diet containing 10% protein, the methionine requirement for growing pigs was between 0.07 and 0.27% of the diet. No mention was made regarding the cystine content of the ration. Shelton et al (1951) reported that the methionine requirement for weanling pig (19.4 kg) was 0.6% of the ration in the absence and 0.3% methionine in the presence of adequate (0.3% or more) cystine. Curtin (1952) showed that the methionine requirement for growing-finishing pigs does not exceed 0.31%, when the ration contains 0.38% cystine and 22% protein. Becker et al (1954) calculated the methionine+cystine requirement on the basis of the minimum need for protein. One to four week old pigs (12.7 kg) required 22% protein diets containing 0.83% methionine+cystine and five to nine week old pigs required 12% protein diets conaining 0.46% methionine+cystine. They later (1955) determined that the weanling pig (12.7 kg) required 0.25% methionine in the presence of 0.17 cystine when fed a synthetic diet containing 12.6% isolated soybean protein, the combined methionine+cystine requirement being approximately 3.33% of the protein. Berry et al (1961, 1962) reported that glucose-soybean meal diets supplemented with 0.29% methionine plus threonine fed to pigs (15.9 kg) and rats (45 g) produced greater gain than when supplemented with methionine alone. Their work suggests that threonine may be the second limiting amino acid in soybean protein. Kroening et al (1965) and Berry et al

(1966) fed growing swine a methionine+cystine adequate diet based on isolated soybean protein and found the methionine+cystine requirement for two to seven week old pig was 0.5% at 12% protein, 0.6% at 18% protein and 0.7% at 25% protein.

There are indications that these requirements may have been over-estimated, since no response was often observed when diets containing less than the recommended amount were supplemented with methionine. Curtin et al (1952) fed 22% protein diet containing soybean oil meal to 11.4 kg pigs and found that there was no advantage to supplemental DL-methionine. Adding 0, 0.025, 0.05 and 0.1% of DL-methionine to a 11.8% crude protein diet for 23.4 kg barrows to provide final methionine levels of 0.22, 0.245, 0.27 and 0.32% Meade (1956) found no improvement in growth rate. Jensen et al (1965) reported that the addition 0.1% DL-methionine to a lysine supplemented diet using milo as the sole protein source had no effect on performance of 52 to 57 kg finishing pigs. In 1966, Meade used 324 growing pigs (18.5 kg) in two experiments to determine the effects of lysine and lysine+methionine supplementation in a corn-soybean diet. He found that there was no significant affect on rate of gain, feed utilization and backfat thickness. Oestemer et al (1970) working with growing swine (21 kg) used diets containing opague-2 corns and found that neither rate of gain, feed efficiency nor protein efficiency ratio (PER) was significantly improved by supplementing the basal corn diets with 0.07, 0.14, 0.21 and 0.28% DL-methionine. Stockland (1971) fed a lysine, methionine and tryptophan supplementation of corn-meat and bone meal diets to crossbred pigs (20 kg) and concluded that supplementation with 0.25% L-methionine or 0.20% L-lysine, alone or in combination, did not affect daily gain, feed efficiency or PER of pigs. Maner et al (1973) recommended that supplemental methionine in high levels of dried cassava together with plant protein sources constituting the main ingredients of composite diets, can supply a source of sulfur for cyanide detoxification. But Job (1975) and Adegbola (1977) reported the need to supplement cassava-based diets with methionine appears to be questionable. Guillermo et al (1984) reported adding 0.3% DL-methionine to diets containing 65% cassava meal for gestating and lactating gilts and growing-finishing pigs and found no advantage to methionine supplementation.

From these reports it seems that additional methionine is not necessary for growing-finishing pigs or gestating and lactating sows. This may be the reason why DL-methionine is only added to commercial pigs starter diets.

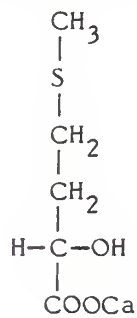
The pigs used in the previous studies were all larger and older than most pigs that are weaned today. There is only limited information available concerning the amino acid requirements of pigs weaned at approximately 21 days of age and about 5.9 to 6.4 kg. Therefore, the objective of these studies was to evaluate the sulfur amino acid requirement of the pigs weaned at approximately 21 days old, and to determine whether supplemental methionine is necessary in the practical starter diets.

Synthetic Methionine and Total Sulfur Amino Acids (TSAA)

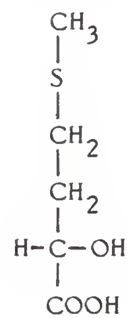
Methionine is one of the ten essential amino acids required for swine. A lack of methionine in the diet of pigs will result in reduced rate of gain and efficiency of feed utilization.

Methionine was first isolated by J.H. Muller in 1923 from casein and was characterized about five years later by Barger and Coyne (NFIA, 1984). The first source of synthetic methionine activity, DL-methionine, was manufactured in the United States by U.S. Industrial Chemicals. The market for synthetic methionine activity did not become significant until the mid-1960's. In 1979, the first synthetic source of methionine activity in liquid form was produced. This major innovation gave feed manufacturers a new option for producing more cost-efficient feeds. Liquid methionine activity combines all the nutritional advantages of conventional dry synthetic methionine sources with the easier handling and cost-saving characteristics of liquids. Today the four commonly commercial synthetic sources of methionine are: methionine hydroxy analogue calcium, methionine hydroxy analogue, DL-methionine and DL-methionine sodium. The structural formulas of each are shown below:

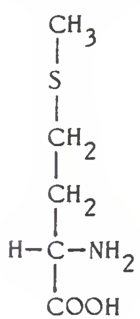
Methionine hydroxy analogue, calcium



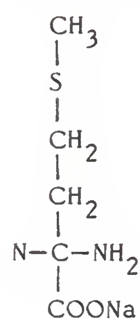
Methionine hydroxy analogue



DL-methionine



DL-methionine sodium



Methionine has a sulfur atom in its nonpolar side chain and is one of two sulfur-containing amino acids incorporated into proteins.

The cost of synthetic methionine has declined considerably since it was first used commercially; however on a per kg basis, regardless of its " nutritional worth" it still adds to the cost of the total feed. Consequently, in an effort to continue to decrease feed costs, TSAA "requirements" are continuously under scrutiny. Methionine can be converted to cystine but not vice versa. Cystine can satisfy about 50% of the total need for methionine+cystine (Shelton et al, 1951). Methionine can meet the total need for sulfur amino acids in the absence of cystine.

In the past ten years a number of experiments have suggested a lower requirement for the sulfur-containing amino acids. A report from the Netherlands (Bernede, 1980) found that daily gain and feed efficiency were maximized in 12 to 35 kg restricted-fed pigs when 18% CP diets contained 0.565% TSAA. Taylor et al (1983) indicated a TSAA requirement of 0.45% for restricted-fed growing female pigs (25-55 kg), when the lysine content is 0.95%. Reifsnyder et al (1981) reported that methionine hydroxy analogue (MHA) (free acid) was as equally effective as DL-methionine in supplying the TSAA requirement in 3 to 8 week old piglets fed liquid diets when the MHA was corrected for assay.

A review of some of the literature reveals that adding synthetic methionine in swine diets is required. On the other hand, some researchers indicated that there was no beneficial response by adding methionine to swine diets containing a lower level of TSAA than is recommended (eg. Meade 1956; Oestemer et al 1970). More recent studies done by Bernede (1980) indicated that 0.565% TSAA improves the growth rate of 12-35 kg pigs. Taylor et al (1981) indicated that 0.48% TSAA improvement in weight gain over the basal diet for growing female pigs (25 to 55 kg) was observed, when diet containing 0.95% lysine. A Soviet report (Davydenko, 1982) suggested that the TSAA requirement for 3.53 kg live weight pigs was 3.3 to 3.5% of the dietary crude protein, when fed a diet containing 20% crude protein.

All of these conclusions are slightly lower than the 1979 NRC suggested requirements for pigs. The present NRC (1979) suggested requirements of 0.76% (for 1 to 5 kg), 0.56% (for 5 to 10 kg), 0.51% (for 10 to 20 kg), 0.45% (for 20 to 35 kg), 0.40% (for 35 to 60 kg), and 0.30% (for 60 to 100 kg pigs) when fed grain-soybean meal diets ad libitium have proved adequate in most cases.

Five trials were conducted to evaluate if the addition of methionine is necessary for the early weaned pig (21 days). The first trial was conducted to evaluate amino acid addition to 17.4% CP corn-soybean diet. In the second trial, a diet containing as low a level of TSAA as possible was formulated using common grains; then, graded levels of methionine were adding to determine the pig's requirement. Another three trials were conducted using a semipurified diet to determine the methionine requirement of pigs via growth and nitrogen retention trials.

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MATERIALS AND METHODS

Experimental Animals. In these five trials, pigs were weaned at an average age of 21 days. In growth trials, pigs were housed in an enviromentally controlled nursery with woven wire floors and V-flush gutter with the pens being 1.2 x 1.5 m. Temperature was maintained at approximately 27 C. Feed was offered ad libitium in self-feeders and water was supplied by nipple waterers. Initial and final weights were recorded and daily gain, daily feed intake and feed efficiency were calculated at the conclusion of the trial. In all trials, pigs were randomly assigned to treatments. A complete randomized design was used for the growth trials. Data were subjected to analysis of variance by the GLM (SAS, 1982). Treatment means were compared the Duncan's New Multiple Range Test (Snedecor and Cochran, 1980).

In digestion trials, pigs were housed individually in metabolism cages allowing for separate collection of feces and urine. Daily feed intake was constant and the pigs were fed equal amounts twice each day. At the time of feeding, water was added to the diet to make it into a thick gruel and after feeding the troughs were filled with fresh water. A seven-day adjustment period preceded each five-day collection period. Ferric oxide was fed as a marker at the beginning and end of each five-day period.

Feces was collected daily and refrigerated. The entire five-day fecal collections were dried in a forced air oven at 50 C for seven days and then ground in a Wiley mill equipped with a 40-mesh screen.

Urine was collected in an 8-liter container to which 20 ml of concentrated HCl was added. Each daily collection was diluted to a constant volume (2 liters) and a 100 ml aliquot taken. Accumulated aliquots were refrigerated at 1 C until analyzed. Representative feed, fecal and urine sample were analyzed in duplicate for each sample for nitrogen as outlined by A.O.A.C.(1975).

Composition of the basal diets are shown in Table 1, 2 and 3. All essential amino acids except methionine exceeded the NRC (1979) recommended levels.

Trial 1. A growth trial was conducted using 80 crossbred (Yorkshire x Duroc) pigs averaging 6.4 kg. They were allotted to 20 pens representing four replications of five dietary treatments. They were housed four to a pen in an enviromentally controlled woven wire floor nursery. The experiment, lasting 5 weeks, had the following dietary treatments:

A. Corn-soy fortified basal diet
B. Basal + 0.1% DL-methionine
C. Basal + 0.1% L-threonine
D. Basal + 0.05% L-tryptophan
E. Basal + 0.1% DL-methionine
0.1% L-threonine
0.05% L-tryptophan.

Trial 2. A second growth trial was conducted to evaluate performance responses of graded levels of DL-methionine supplemental to a sorghum diet containing a low level of TSAA. The trial consisted of 72 crossbred pigs (Yorkshire x Duroc) averaging 6.2 kg. They were allotted to 12 pens representing four replicates of three diets. They were housed six to a pen in a temperature controlled nursery. The experiment, lasting 5 weeks, had the following treatments:

A. Sorghum-soy fortified basal diet

B. Basal + 0.1% DL-methionine

C. Basal + 0.2% DL-methionine.

Trial 3. A growth trial was conducted using 60 crossbred (Yorkshire x Duroc) pigs averaging 5.4 kg. These were allotted to 20 pens randomly representing five replications of four dietary treatments. They were housed three to a pen in an enviromentally controlled woven wire floor nursery. The experiment, lasting 28 days, included the following dietary treatments:

A. Basal diet

B. Basal + 0.1% DL-methionine

C. Basal + 0.2% DL-methionine

D. Basal + 0.3% DL-methionine.

In this trial, all facilities and procedures were the same as in the trials previously discussed. The only difference was that, at two days before the end of trial, blood samples were taken. Before taking blood samples, pigs were fasted for 6 hours by moving the feeder out of the pen. The feeder was then put back in the pen and the pigs feed for one hour, making sure that every pig had eaten enough feed. The feeder was moved out of the pen again and the pigs fasted for another 6 hours until bleeding. The blood was collected via anterior vena cava puncture with vacutainer tube. Each containing blood sample was gently shaken and immediately stored in ice. These were centrifuged within one hour after sampling. The serum was decanted into new tubes and refrigerated until they were analyzed for blood urea nitrogen (BUN) content determination (Appendex 1).

Trial 4. A digestion trial was conducted using 12 barrows (Yorkshire x Duroc) weighing an average of 6.8 kg. Three groups of four littermates were used in a randomized complete block design and allotted to four treatments as in Trial 3. Treatments within groups were randomly assigned and fed for one period (12 days), after which treatments were reallotted for another period so as to provide a second replicate. Daily feed intake was 250 g for the first period and 280 g for the second period.

Trial 5. Another digestion trial was conducted using 12 Duroc x Yorkshire barrows weighing an average of 7.1 kg. Three groups of four littermates were used in a randomized complete block design and allotted randomly to the four treatments. Similar procedures were used as discussed in Trial 4. The only difference was that a cross-over design was used. Daily feed intake was the same as in Trial 4.

TABLE 1. COMPOSITION OF BASAL DIETS FOR TRIAL 1

Ingredient	%
Yellow corn, ground (IFN 14-02-931)	54.00
Soybean meal (44%) (IFN 5-04-612)	22.50
Dried whey (IFN 4-01-182)	20.00
Dicalcium phosphate (IFN 6-01-080)	1.40
Ground limestone (IFN 6-01-069)	0.80
Salt (IFN 6-04-151)	0.20
Trace mineral premix[a]	0.10
Vitamin premix[b]	0.50
Antibiotic[c]	0.25
L-lysine HCl	0.35
Calculated analysis:	
Crude protein (%)	17.37
Lysine (%)	1.25
Tryptophan (%)	0.21
Threonine (%)	0.80
Methionine+cystine (%)	0.64
Metabolizable energy (kcal/kg)	3187

[a] Containing 5.5% Mn, 10% Fe, 1.1% Cu, 20% Zn, 0.15% I and 0.1% Co.

[b] Each kg of premix contained the following: vitamin A 880,000 IU; vitamin D_3 66,000 IU; vitamin E 4,400 IU; riboflavin 990 mg; d-pantothenic acid 2,640 mg; choline 88 gm; niacin 5,500 mg; vitamin B_{12} 4.84 mg; menadione dymethylpyrimidinol bisulfite 550 mg; ethoxyquin 6270 mg.

[c] Contributed the following per kg of diet: chlortetracycline 110 mg; sulfamethazine 110 mg; penicillin 55 mg.

TABLE 2. COMPOSITION OF BASAL DIETS FOR TRIAL 2

Ingredient	%
Ground sorghum grain (IFN 4-04-383)	65.20
Dried skim milk (IFN 5-01-175)	15.00
Soy protein concentrate 67.2%[a]	5.00
Soybean meal 44% (IFN 5-04-612)	7.50
Dried fat 7-40	3.50
Dicalcium phosphate (IFN 6-01-080)	1.60
Ground limestone (IFN 6-01-069)	0.80
Salt (IFN 6-04-151)	0.30
Trace mineral premix[b]	0.10
Vitamin premix[c]	0.50
Antibiotic[d]	0.25
L-lysine HCl	0.30
Calculated analysis:	
Crude protein (%)	17.51
Lysine (%)	1.17
Threonine (%)	0.69
Tryptophan (%)	0.22
Methionine+cystine (%)	0.59
Metabolizable energy (kcal/kg)	3187

[a] PROCON CMR® Staley, Decatur, IL 62525.

[b] Containing 5.5% Mn, 10% Fe, 1.1% Cu, 20% Zn, 0.15% I and 0.1% Co.

[c] Each kg of premix contained the following: vitamin A 880,000 IU; vitamin D_3 66,000 IU; vitamin E 4,400 IU; riboflavin 990 mg; d-pantothenic acid 2,640 mg; choline 88 gm; niacin 5,500 mg; vitamin B_{12} 4.84 mg; menadione dymethylpyrimidinol bisulfite 550 mg; ethoxyquin 6270 mg.

[d] Contributed the following per kg of diet: chlortetracycline 110 mg; sulfamethazine 110 mg; penicillin 55 mg.

TABLE 3. COMPOSITION OF BASAL DIET FOR TRIALS 3, 4 and 5

Ingredient	%
Soy protein concentrate 67.2%[a]	20.00
Corn starch	70.50
Dried fat 7-60	5.00
Dicalcium phosphate (IFN 6-01-080)	1.40
Ground limestone (IFN 6-01-069)	0.80
Salt (IFN 6-04-151)	0.50
Trace mineral premix[b]	0.10
Vitamin premix[c]	1.00
L-lysine HCl	0.40
L-threonine	0.10
Isoleucine	0.15
DL-tryptophan	0.04
Antibiotic[d]	0.25
Calculated analysis:	
Crude protein (%)	13.50
Lysine (%)	1.24
Threonine (%)	0.67
Tryptophan (%)	0.18
Isoleucine (%)	0.76
Methionine+cystine (%)	0.36
Metabolizable energy (kcal/kg)	3488

[a] PROCON CMR® Staley, Decatur, IL 62525.

[b] Containing 5.5% Mn, 10% Fe, 1.1% Cu, 20% Zn, 0.15% I and 0.1% Co.

[c] Each kg of premix contained the following: vitamin A 880,000 IU; vitamin D_3 6,000 IU; vitamin E 4,400 IU; riboflavin 990 mg; d-pantothenic acid 2,640 mg; choline 88 gm; niacin 5,500 mg; vitamin B_{12} 4.84 mg; menadione dymethypyrimidinol bisulfite 550 mg; ethoxyquin 6270 mg.

[d] Contributed the following per kg of diet: chlortetracycline 110 mg; sulfamethazine 110 mg; penicillin 55 mg.

RESULTS

Trial 1. The influence on performance of adding different synthetic amino acids on the performance of pigs weaned at approximately 21 days of age is shown in Table 4. There were no significant differences in average daily gain or daily feed intake between pigs fed the basal diets and those with the supplemented amino acids diets. However, pigs fed the diet with 0.1% DL-methionine added tended to consume slightly less feed than pigs fed the basal diet or diets with other synthetic amino acids.

All pigs performed poorly during the first week of the trial due to the stress involved in weaning. Three pigs died (one in treatment A, the control diet; one in treatment B and one in treatment D) within the first three days of the experiment. Death did not appear to be related to treatment.

Trial 2. Performance of pigs fed diets containing various level of added DL-methionine is shown in Table 5. There were no significant differences in average daily gain and feed consumption among treatments. No pigs died in this trial.

Trial 3. The growth performance of weaned pigs fed semi-purified diets containing graded levels of DL-methionine is shown in Table 6. There was a significant difference in growth rate and daily feed consumption between the basal and methionine supplemented diets. However, there was no difference within the methionine supplemented diets. The addition of 0.1% DL-methionine resulted in a faster growth rate (P<.05) than the basal diet. Pigs fed the basal diet consumed less feed (P<.05) than those fed the diet with added methionine.

Feed efficiency improved linearly (P<.05) with added methionine. The basal diet had the poorest feed efficiency (P<.05). The growth rate and feed efficiency of the semi-purified diets in this trial was poorer than the practical diets in Trial 1 and 2. As in previous growth trials, there was no problem with scouring.

BUN content decreased linearly with added supplementation, but the 0.1% added level was significantly different than the other two. Minimum urea nitrogen in blood was obtained in pigs feed about 0.56% methionine+cystine. The BUN level of pigs from all diets of this trial was lower than average because of the low protein content of the semipurified diets.

Trial 4. The results of the digestion trial for diets containing graded levels of DL-methionine are shown in Table 7. The apparent N digestibility was similar for all diets. The nitrogen retention (grams per day) of the basal diet was significantly (P<.05) lower than the supplemented diets; the results were with a plateau at 0.2% added DL-methionine. Nitrogen retention expressed as a percentage of intake also plateaued with 0.2% added DL-methionine.

Trial 5. The second digestion trial for diets containing graded levels of DL-methionine is shown in Table 8. As in the previous digestion trial, nitrogen digestibility was not different between treatments. Nitrogen retention (grams per day) of pigs fed the diet with 0.2% DL-methionine was higher (P<.05) than that of pigs fed the basal diet. No response was observed from adding more than 0.2% DL-methionine. Similar results were observed when nitrogen retention was expressed as a percentage of intake.

TABLE 4. PERFORMANCE OF WEANED PIGS FED DIETS SUPPLEMENTED WITH VARIOUS ESSENTIAL AMINO ACIDS (Trial 1)[ab]

Item	Diets					SE
	Basal	Basal + .1%DL-met	Basal + .1%L-thr	Basal + .05%L-trp	Basal + .1%DL-met + .1% L-thr + .05%L-trp	
Initial weight (kg)	6.39	6.31	6.24	6.33	6.30	.12
Final weight (kg)	20.21	19.57	19.89	19.31	20.34	.54
Average daily gain (kg)	.39[b]	.38[b]	.39[b]	.37[b]	.40[b]	.03
Daily feed intake (kg)	.67[b]	.60[b]	.66[b]	.65[b]	.68[b]	.05
Feed / gain	1.69[b]	1.60[b]	1.69[b]	1.76[b]	1.70[b]	.10

[a] Each value is the mean of 16 pigs (4 pigs per pen and 4 replications per treatment).

[b] Means within a row different superscripts differs significantly ($P<.05$).

TABLE 5. PERFORMANCE OF WEANED PIGS FED SORGHUM BASED DIETS WITH ADDED LEVELS OF DL-METHIONINE (Trial 2)[ab]

Item	Diets			SE
	Basal	Basal + .1%DL-met	Basal + .2%DL-met	
Initial weight (kg)	6.04	6.54	6.05	.52
Final weight (kg)	16.60	16.88	16.24	.84
Average daily gain (kg)	.30[b]	.30[b]	.29[b]	.03
Feed intake(kg/day)	.48[b]	.48[b]	.47[b]	.04
Feed / gain	1.57[b]	1.62[b]	1.60[b]	.02

[a] Each value is the mean of 24 pigs (6 pigs per pen and 4 replications per treatment).

[b] Means within a row with different superscripts differ significantly ($P<.05$).

TABLE 6. GROWTH PERFORMANCE AND BLOOD UREA NITROGEN (BUN) CONTENT OF WEANED PIG FED SEMIPURIFIED DIETS WITH VARIOUS LEVELS OF DL-METHIONINE (TRIAL 3)[abcd]

Item	Diet				SE
	Basal	Basal + .1%DL-met	Basal + .2%DL-met	Basal + .3%DL-met	
Initial weight (kg)	5.43	5.41	5.43	5.41	.02
Final weight (kg)	8.24	10.45	11.22	10.90	.25
Average daily gain (kg)	$.10^{b}$	$.18^{c}$	$.21^{c}$	$.20^{c}$	.02
Daily feed intake (kg)	$.25^{b}$	$.33^{c}$	$.37^{c}$	$.35^{c}$	.03
Feed / gain	2.55^{b}	1.84^{c}	1.80^{c}	1.78^{c}	.09
BUN (mg/100 ml)	6.31^{b}	3.38^{c}	1.14^{d}	1.12^{d}	.79

[a] Each value is the mean of 15 pigs (3 pigs per pen and 5 replication per treatment).

[bcd] Means within a row with different superscripts differ significantly ($P<.05$).

TABLE 7. NITROGEN DIGESTIBILITY AND RETENTION OF WEANED PIGS FED DIETS WITH VARIOUS LEVELS OF DL-METHIONINE (TRIAL 4)[abcde]

Item	Diets				SE
	Basal	Basal + .1%DL-met	Basal + .2%DL-met	Basal + .3%DL-met	
N digestibility (%)	90.65^{a}	90.25^{a}	91.16^{a}	90.48^{a}	.93
Daily nitrogen (g/day)					
Intake	6.24^{a}	6.41^{a}	6.54^{a}	6.37^{a}	.25
Excreted in urine	1.91^{a}	1.17^{b}	$.70^{c}$	$.82^{c}$	.10
Excreted in feces	$.59^{a}$	$.62^{a}$	$.58^{a}$	$.61^{a}$	.03
Retention	3.74^{a}	4.62^{b}	5.26^{c}	4.94^{bc}	.17
Percentage of N retention to N intake (%)	60.36^{a}	72.28^{b}	80.57^{c}	77.58^{d}	.86

[abcd] Means within a row with different superscripts differ significantly ($P<.05$).

[e] Average initial weight of pigs are: 6.77 kg for A, 6.95 kg for B, 6.86 kg for C and 6.55 kg for D.

TABLE 8. NITROGEN DIGESTIBILITY AND RETENTION OF WEANED PIGS FED ADDED LEVELS OF DL-METHIONINE (TRIAL 5)[abcde]

Item	Diets				SE
	Basal	Basal + .1%DL-met	Basal + .2%DL-met	Basal + .3%DL-met	
N digestibility (%)	88.01[a]	88.06[a]	88.26[a]	89.37[a]	.88
Daily nitrogen (g/day)					
Intake	7.04[a]	7.44[b]	7.10[c]	6.88[d]	.03
Excreted in urine	2.35[a]	1.55[ab]	.98[b]	1.59[ab]	.44
Excreted in feces	.84[ab]	.88[a]	.83[ab]	.73[b]	.05
Retention	3.85[a]	5.01[ab]	5.29[b]	4.56[ab]	.40
Percentage of N retention to N intake (%)	54.59[a]	67.14[ab]	74.52[b]	66.80[ab]	6.21

[abcd] Means within a row with different superscripts differ significantly ($P<.05$).

[e] Average initial weight of pigs are: 7.00 kg for A, 7.14 kg for B, 7.14 for C and 7.05 for D.

DISCUSSION

In trial 1 and 2, the addition of 0.1% or 0.2% DL-methionine to a basal corn-soy diet or to a sorghum-soy diet showed no improvement in the growth rate of pigs weaned at 21 days. This indicates that supplementation of DL-methionine is not required to obtain maximum growth. Establishing confident requirement levels is economically important to the swine industry. The results of trial 1 suggest that a 17% protein corn-soy-whey diet meets the amino acid requirement of pigs weaned at 3 weeks of age and supplementation with synthetic amino acids (except lysine) is not necessary. Stockland et al (1971) suggested that lysine, methionine and tryptophan supplementation of corn-meat and bone meal diet did not affect daily gain, feed efficiency or protein efficiency ratio (PER) of pigs whether added singularly or in combination. However, both results do not agree with the work done by Russell et al (1983). They concluded that adding 0.1% methionine to a lysine-fortified basal diet improved growth rate, but not feed efficiency; and that supplementation of 0.03% tryptophan to a lysine methionine threonine fortified basal diet had the optimal growth rate and feed efficiency of growing pig (18 to 34 kg). This difference was probably due to Russell using a low protein (12% CP) diet; whereas, we used a level of 17% CP.

In trial 2, it was determined that a commonly used sorghum based diet had sufficient TSAA for weaned pigs. Additional DL-methionine showed no benefit but did increase the cost of the diets. Robinson (1951) concluded that synthetic methionine was of no benefit when added to rations of yellow corn, soybean oil meal, ground alfalfa and minerals for growing-finishing pigs. Curtin (1952) suggested that there is no practical need for methionine supplementation in swine rations containing good quality solvent extracted soybean oil meal as the only high protein feed.

In trial 3, the pigs fed the semipurified diet with supplemental DL-methionine had significant improvements in average daily gain, feed intake, and feed efficiency. These data show that adding 0.2% methionine, to give a TSAA of 0.56%, resulted in maximum weight gain of weaned pigs. No significant improvement in growth rate was seen at the 0.3% supplemental methionine level. Pigs in trial 3 gained less than those in trials 1 and 2 possibly because the semipurified diets used in the methionine study caused reduced food intake. Becker et al (1955) and Shelton et al (1951) concluded that a total of 0.25% methionine and 0.17% cystine supported satisfactory rate and efficiency of gain. Oestemer et al (1970) working with Opaque-2 corn diets suggested that the methionine+cystine requirement of growing pigs is somewhat less than 0.42 to 0.50% of the diet.

The BUN concentrations decreased as the added DL-methionine level increased to 0.2% at which point it leveled off (Figure 1). This reduction in BUN presumably reflected more efficient nitrogen utilization and less urea synthesis by the pigs that received the higher methionine levels. These results are similar to a Canadian report (Keith et al, 1972), in which they used the serum amino acids technique of Knipfel et al (1972) to determine the serum methionine concentration at the end of each experimental period and found the methionine requirement is 0.46% of the diet. The BUN level of pigs of this trial seem lower than average which may be due to the low protein content of the semipurified diets. Munchow and Bergner (1968) found that the blood urea content increased with the protein content in the diet.

The growth trial indicated that 0.1% supplementation is optimum, but that 0.2% produced the most desirable BUN content.

There were no significant differences in apparent N digestibility, because the basal and experimental diets are both semipurified and contain the same main protein sources. For daily N retention, there was a clear difference between the supplemented diets and the basal diet. Trial 4 showed the optimum supplemental level of methionine to be 0.2%; which means 0.56% TSAA is adequate for pigs weaned at 3 weeks. The lower N retention in the basal diet resulted from a higher excretion of nitrogen in the urine. If N retention is expressed as a percentage of N intake, the maximum nitrogen retention was at 0.2% added level. These result suggest that 0.56% TSAA has the maximum N retention. The value of percentage of N retention to N intake is improved from 60 to 80% or 55 to 75%. Kroening et al (1965) reported the percent of absorbed nitrogen retained on isolated soybean protein was improved from 58 to 72% by proper methionine and threonine supplementation. However, for both trials, the results of N retention were quadratic (Figure 2). Pick (1968) supplemented an Opaque-2 corn diet containing 0.2% methionine+cystine with 0.1, 0.2 and 0.3% L-methionine and found that rats fed 0.3% TSAA gained 6.2 g per day compared to gains of 6.2 and 6.1 g for rats given 0.4% and 0.5% TSAA respectively. Bressani et al (1969) were also unable to demonstrate improvements in rats due to supplemental DL-methionine to Opaque-2 corn diet.

The reasons why the higher levels of DL-methionine were reducing performance is difficult to explain. Inhibition of growth by feeding excessive levels of methionine has been studied extensively in the rat. Brown and Allison (1948) observed that an addition of 4.8% of DL-methionine to a 12% casein diet produced weight losses in the rat. Van Pilsum and Berg (1950) reported that excessive methionine in an amino acid mixture would suppress the growth of the rat. Similar observations have been reported by Wretlind and Rose (1950) when 1.4% L-methionine was fed in a synthetic rat diet. Henson et al (1954) reported that an addition of 0.2% DL-methionine to a corn-tankage ration inhibited growth in the pig. Mitchell et al (1968) found that additions of methionine at levels above 0.28% nitrogen retention tended to decline although slower than might have been expected in view of the well-recognized toxic effects of excess methionine. Rosell (1984) indicated that 0.40% excess methionine added to the basal diet had no effect on the performance of the weanling pig.

Difficulties in interpretation are, also, influenced by shortcomings in analytical procedures for the estimation of sulfur amino acids, discrepancies between chemical and microbiological estimates, lack of knowledge on the availability of methionine and cystine and of their complementary effects and interactions with other nutrients (Braude et al, 1973).

In these studies, we did not use carcass composition as a criteria of response. Possibly in the future, more results of the chemical analysis of the whole carcass will help to resolve some of the discrepancies in N retention experiments. Braude et al 1973 concluded that the amounts of lean and fat in the carcasses were affected by DL-methionine supplementation, as were the linear measurements for mid-back fat and fat over eye muscle. However, on the contrary, Fetuga et al (1975) concluded that graded addition of DL-methionine within each protein level did not influence carcass characteristics.

Our results suggest that the total sulfur amino acid requirement of pigs weaned at 3 weeks of age does not exceed 0.56% of the diet. This is exactly the value of 1979 NRC recommendation. In agreement with our data, Becker et al (1955) concluded that 0.15% methionine plus 0.17% cystine was deficient in methionine to support normal performance of the weanling pig, but a level of 0.2% methionine and 0.17% cystine produced satisfactory rate and efficiency of gain. Pfander et al (1955) reported that no response was obtained above 0.56% of the diet, but this was the lowest level that they used. Since the commonly used practical diets meet this requirement of TSAA it is not necessary to supplement DL-methionine in diets for pigs weaned at 3-weeks of age.

FIG 1. EFFECT OF METHIONINE ADDITION ON BUN CONTENT OF WEANED PIGS

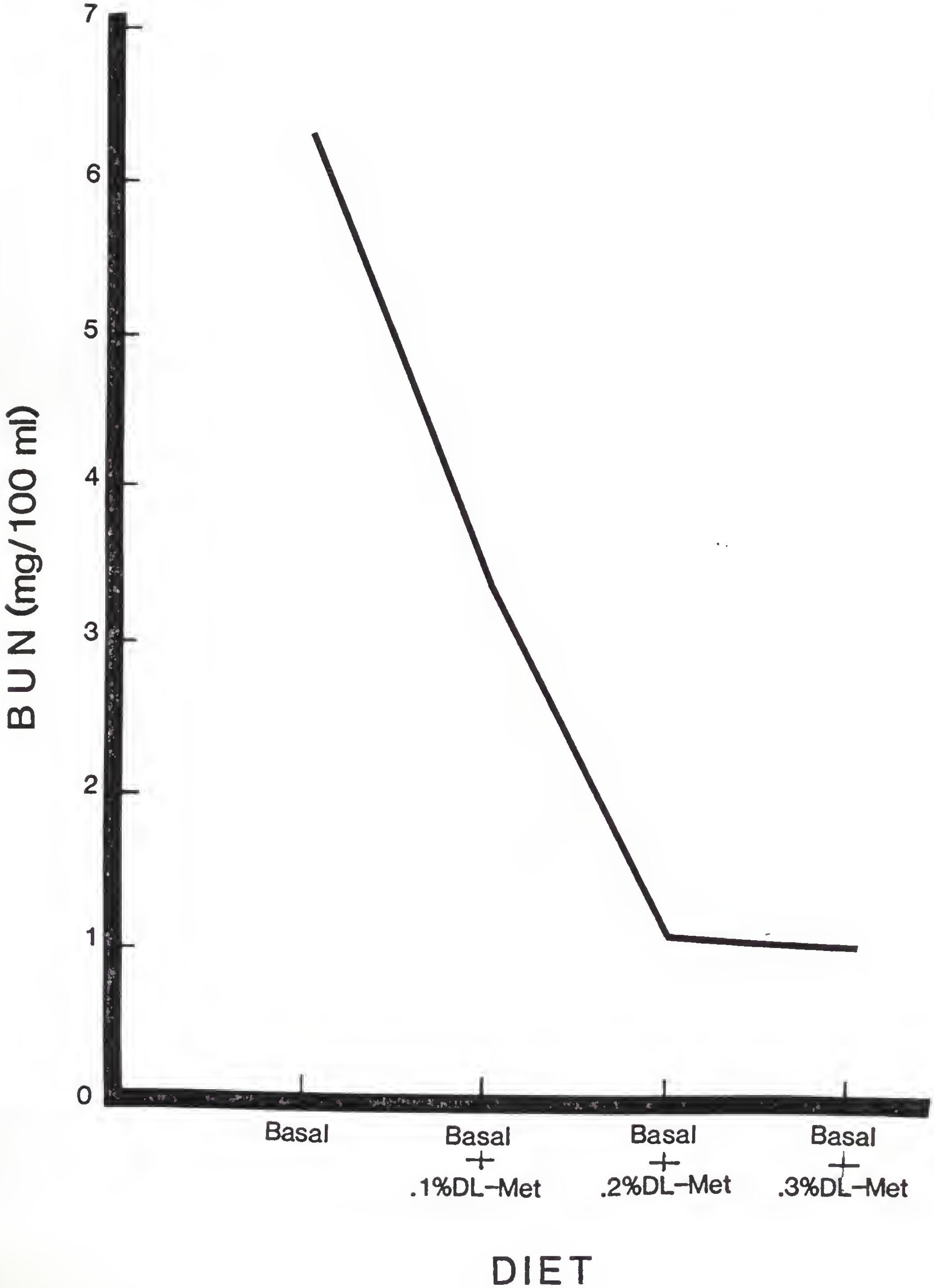
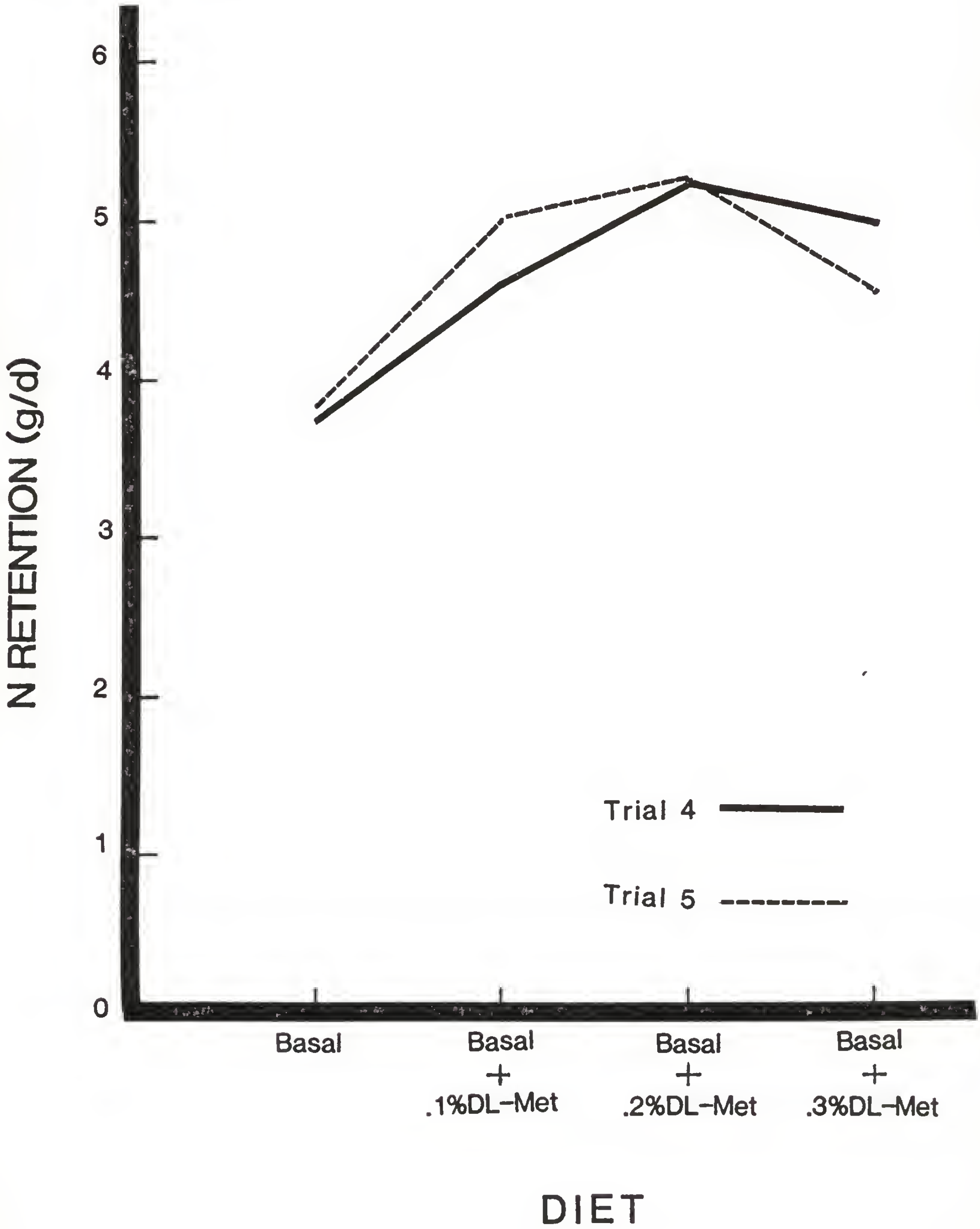


FIG 2. EFFECT OF METHIONINE ADDITION ON N RETENTION OF WEANED PIGS



SUMMARY

Three growth trials and two digestion trials utilizing 236 pigs were conducted to evaluate the effect of amino acids addition to a practical pig diet and to estimate the methionine requirement of the weanling pig using growth and nitrogen retention as criteria.

In trial 1, the addition of 0.1% DL-methionine, 0.1% L-threonine, 0.05% L-tryptophan or all three to a corn-soy fortified basal diet did not improve pig performance. In trial 2, there was no significant difference in average daily gain or feed consumption among pig fed 0, 0.1 or 0.2% added DL-methionine to sorghum-soy fortified basal diet.

A semipurified diet with added levels of DL-methionine was used to estimate the methionine requirement. In the growth trial, there was a significant difference in daily gain and feed intake between the basal and supplemented diets; but no difference within the supplemental diets (0.1%, 0.2% and 0.3% added DL-methionine). However, feed efficiency linearly improved significantly with added methionine. BUN content decreased linearly with added supplementation. In the digestion trial, the apparent N digestibility was similar for all treatments. The N retention, both in grams per day and in percentage of nitrogen intake, was significantly lower in the basal diet than that in the supplemented diets; the results were quadratic peaking at the 0.2% added methionine level.

From these results, it was concluded that the total sulfur amino acid requirement of weanling pigs does not exceed 0.56% of the diet. The addition of methionine to a 17% protein corn or sorghum-soybean meal does not improve pig performance.

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APPENDIX

THE QUANTITATIVE DETERMINATION OF UREA NITROGEN BY THE DIACETYL MONOXIME METHOD

I. Reagents[1]

A. BUN acid reagent:

Ferric chloride in phosphoric and sulfuric acids. Stock No. 535-3.

B. BUN color reagent:

Diacetyl monoxime, 0.18% (w/v), and thiosemicarbazide. Stock No. 535-5.

C. Urea nitrogen standard solution:

Urea at a urea N level of 30 mg/dL (10.7 mmol/L). Stock No.535-30.

Urea at a urea N level of 150 mg/dL. Stock No. 535-150.

Benzoic acid as preservative.

D. Trichloroacetic acid solution:

Trichloroacetic Acid (TCA), 3% (w/v). Stock No. 635-3.

II. Instrument and materials required

A. Instrument: PERKIN-ELMER 552 spectrophotometer.

B. Materials: colorimeter cuvets, test tubes, pipets, centrifuge, boiling water bath and timer.

III. Procedure (with deproteinization)

A. Into a centrifuge tube or test tube, pipet:

1.8 ml of cold 3% trichloroacetic acid.

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[1] Sigma chemical company, P.O. Box 14508, St. Louis, Mo. 63178.

2.0 ml serum, plasma or whole blood.

Mix well by shaking and allow to stand approximately 5 minutes to precipitate proteins.

B. Centrifuge 5-10 minutes to obtain clear supernatant.

C. Decant and save supernatant into separate tube.

D. Label 3 or more test tubes: Blank, Standard, Test 1, Test 2, etc.

E. To each, add:

2.8 ml BUN acid reagent.

2.0 ml BUN color reagent.

Mix. It is convient to premix these reagents by combining 7 parts BUN acid reagent and 5 parts BUN color reagent. Then, 4.8 ml of this mixture is used. Premix only amount needed for daily use.

F.

To Blank, add:	To Standard, add:	To Test, add:
0.2 ml 3% TCA.	0.2 ml of a 10-fold dilution of urea nitrogen standard solution in 3% TCA.	0.2 ml of clear supernatant from step C.

Mix throughly.

G. Simultaneously using a rack, place all tubes in boiling water bath for exactly 10 minutes.

H. Quickly remove tubes and immediately place in a container of cold tap water for 3-5 minutes.

I. Transfer contents to cuvets and read Absorbance of Standard and Test vs Blank as reference at the same wavelength and in the same instrument as used in preparing calibration curve. Complete readings within 20 minutes.

J. Calculations:

Determine values for standard and test directly from calibration curve. Absorbance of standard serves to verify validity of curve. Value for standard (urea N 30 mg/100 ml) should be within a range of 28-32 mg/100 ml. If not, a new calibration curve should be prepared. If test value is greater than 75 mg/100 ml, repeat assay using one-half volume of sample and multiply result by 2.

K. Calibration:

A single calibration curve is used for both test procedures.*

1. Prepare urea nitrogen diluted standard by pipeting the solutions indicated into test tubes and mixing thoroughly. The use of 0.1% benzoic acid solution in place of water will prolong the stability of the diluted standards for at least 3 months when refrigerated.

Diluted Standard No.	Urea Nitrogen Standard Solution (150mg/100ml).	Water (ml).
1	0.5	4.5
2	1.0	4.0
3	1.5	3.5
4	2.0	3.0
5	2.5	2.5

2. Lable 5 test tubes 1-5 and a 6th tube Blank.

To each add: 3.0 ml BUN acid reagent.

2.0 ml BUN Color Reagent.

Mix. Then pipet 0.02 ml of each of the solutions as indicated in column 2 below:

Tube	Addition (0.02 ml)	Absorbance	Urea N(mg/100 ml)
1	Water	reference	0
2	Diluted Std. 1		5
3	Diluted Std. 2		10
4	Diluted Std. 3		15
5	Diluted Std. 4		30
6	Diluted Std. 5		45
7	Diluted Std. 6		60
8	Diluted Std. 7		75

Wavelength used or filter used is 528 .

3. Simultaneously place all tubes in boiling water bath for exactly 10 minutes.
4. Quickly remove tubes and place in a container of cold tap water for 3-5 minutes.
5. Transfer contents to cuvets and within 20 minutes read Absorbance vs Blank as reference at 515-540 nm. Record Absorbance in column 3.
6. Prepare urea N calibration curve using Absorbance vs corresponding urea N concentration (mg/100 ml) in column 4.

* A calibration curve should be prepared for each new lot of BUN color reagent or BUN acid reagent.

IV. Results

Values are derived directly from calibration curve. Validity of the curve is verified by simultaneous assay of a standard.

EFFECT OF METHIONINE ADDITION TO WEANLING PIG DIETS

by

TZE-CHOW ONG

B.S., National Pingtung Institute of Agriculture, 1971

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1984

ABSTRACT

Five trials were conducted involving 236 pigs weaned at 21 days of age, to evaluate the effects of amino acids additions to starter diet and to estimate the methionine requirement of the weanling pig.

All growth trials were conducted for four or five weeks. Pigs were randomly assigned to pens in an enviromentally controlled nursery with average daily gain (ADG), feed intake (FI) and feed efficiency (F/G) used as response criteria. In trial 1, a 17.4% crude protein basal diet were supplemented with 0.1% DL-methionine, 0.1% L-threonine, 0.05% L-tryptophan or all three to give five treatments. There were no differences in pig performance between treatments. In trial 2, 0.1% or 0.2% DL-methionine was added to a practical sorghum-soy fortified basal diet giving three treatments. The results showed no differences in ADG, FI or F/G among treatments.

To estimate the methionine requirement of the weanling pig a semipurified diet was used in a growth trial (Trial 3) and two nitrogen retention trials (Trials 4 & 5). A 13.5% crude protein semipurified basal diet was supplemented with 0.1%, 0.2% and 0.3% DL-methionine to provide four treatments. In the growth trial, pigs fed the diet containing 0.1% supplemental methionine gained significantly faster than pigs on the basal diet. Feed efficiency linearly improved significantly with added methionine. Plasma BUN content was decreased significantly with the addition of methionine leveling off at the 0.2% addition level. In the N digestion trials, maximum nitrogen retention was obtained when 0.2% DL-methionine was added to a 13.5% crude protein basal diet containing 0.56% TSAA.

These studies suggest that 0.56% TSAA is adequate for pigs weaned at three weeks of age. Additionally, a 17.4% protein diet supplemented with lysine is adequate in all other amino acids for pigs weaned at three weeks of age.