THE SULFUR-AMINO ACID REQUIREMENT FOR REPRODUCING GILTS

by

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INTRODUCTION

Many changes have occurred in recent years in swine nutritionists' recommendations regarding the care and management of the breeding herd. Foremost among these has been the trend towards feed and protein restriction during the gestation period. Research in this area is now directed towards amino acid requirements since it is known that swine require amino acids and not protein *per se*. Also, as the price of synthetic amino acids decreases they may become competitive with the present plant and animal protein supplements.

This clearly illustrates the need for defining amino acid requirements of the swine species. Considerable work has been conducted determining the needs of the baby pig and the growing-finishing pig but the amino acid requirements of the mature gilt have only recently received attention, mainly through the work of Rippel *et al.* (1965a, b, c) with the pregnant gilt and Baker *et al.* (1966a, b, c) who demonstrated the very low amino acid levels needed to maintain the mature non-gravid gilt.

The lack of information on these requirements is borne out by the fact that the National Academy of Science, National Research Council (NRC), in 1964 did not mention any amino acid requirements for pregnant sows. In 1968, NRC listed amino acid requirements for bred sows and gilts based on reports by Rippel *et al.* (1965a, c) with an additional 15 percent safety factor.

The majority of gestation research on amino acid requirements has been conducted the last three weeks before parturition when fetal demands for growth and development are greatest (Rippel et al. 1965, a, b, c). It is likely that the total gestation requirement for amino acids is lower than the levels needed during this period and that the gilt can reproduce satisfactorily on lower protein intakes due to the phenomenon of pregnancy anabolism. The ability of the gilt to retain and store nitrogen consumed over and above the requirement for fetal development and the products of conception allows excess intake in early gestation to be stored by the body. The gilt could then draw upon these stores late in gestation if dietary protein or the intake of an essential amino acid is limiting.

Earlier work at Iowa State University (Holden et al., 1968) indicated that a balanced eight percent crude protein diet would support satisfactory reproductive performance over a long term experiment (four parities). Lucas et al. (1969) measured the amino acid composition of this diet and the levels of plasma-free amino acids of the sows consuming the diets at the 100th day of gestation and observed that methionine and cystine were the first-limiting amino acids in the eight percent protein diet. Since this basal diet was considered adequate for reproductive performance, with methionine and cystine considered to be the first limiting, an experiment was designed using this diet to estimate the dietary requirement for methionine and cystine. Since little is known of the changes in requirements with advancing pregnancy, estimates were to begin with a prebreeding phase and extend through each trimester of gestation.

REVIEW OF LITERATURE

Protein and Amino Acid Requirements for Swine Reproduction

Evvard et al. (1914) demonstrated that pregnant sows require protein in addition to that found in corn. The daily addition of 136 g of blood albumin to a corn diet (1270 g) increased litter size, birthweight and sow gestation weight gains. The offspring were more vigorous and in a fleshier condition. These results were probably due to the high levels of lysine and tryptophan in the high protein blood product relative to corn.

Historically, as the dietary protein quality has improved, pregnant swine have generally shown no reproductive performance response to different levels of protein. Davidson (1930) fed 204 or 327 g of protein daily and observed no effect on the number of ova shed. Similarly Robertson et al. (1951) observed no effect on pasture but noticed an increased number of embryos 25 days postbreeding with a higher protein diet. Boaz (1962) fed three levels of protein, 10.8, 15.3 or 19.8 percent, to gilts in drylot for three parities and although he reported some decrease in breeding efficiency on the low protein diet there were no differences in litter size or birthweight. Clawson et al. (1963) and Frobish et al. (1966) fed corn-soybean meal diets furnishing daily protein intakes ranging from 136 to 545 g. They observed that reduced protein intake did not significantly affect the number of pigs farrowed, birthweight or baby pig gain from birth to weaning.

Rippel et al. (1965d) observed gilts fed 1.8 kg of a 16 percent protein corn-soybean meal diet gained more weight during gestation than those consuming a 5 percent protein diet from the 50th day of gestation. However, there were no significant differences in litter size or weight. Holden et al. (1968) fed four levels of dietary protein, 8, 12, 16 or 20 percent, to sows through four consecutive reproductive cycles including lactation and the post-weaning period. The 20 percent protein diet consisted of 2.44 parts corn to 1.0 part soybean meal and was diluted with cornstarch and dextrose to obtain the lower protein levels. They observed a highly significant increase in gestation weight gains with increasing protein intake but no differences in the number of pigs born per litter, birthweight or number weaned.

McGillivary et al. (1964) found it necessary to feed a protein-free diet four to six estrous cycles before the number of viable embryos were reduced at 25 days postcoitum. Pond et al. (1968) fed an essentially protein-free diet (0.5 percent protein) throughout prebreeding and gestation and one gilt farrowed nine live pigs and weaned seven. Two other gilts on the protein-free diet failed to conceive and two more died during pregnancy of perforated gastric ulcers. Normal fetuses were observed in both dead gilts. A similar experiment (Pond et al., 1968) involved six gilts fed a protein-free diet beginning 24 to 28 days postmating until parturition compared to a 12 percent protein control diet. All gilts farrowed and no statistical differences were seen in number farrowed (8.6 vs. 9.6) or birthweight (1.18 vs. 1.23 kg). These reports

illustrate the sow's tremendous capacity to draw upon her body protein stores for intrauterine needs during gestation.

Rippel et al. (1965b) fed protein levels from 0 to 15 percent crude protein based on a constant ratio of 4.4 parts corn to 1.0 part soybean meal and diluted with corn starch. Using maximum nitrogen retention as the criterion, they determined the protein requirement to be 12.5 percent of the diet. Miller et al. (1969) attempted a similar experiment except that they varied the ratio of corn to soybean meal to obtain diets from 6 to 18 percent crude protein. Nitrogen retention appeared to plateau near 15 percent dietary protein or 285 g per day. Diets below this protein level were limiting in lysine and possibly tryptophan. It is likely that both these experiments overestimate the protein requirement for the entire gestation period as the trials were initiated near the 95th day of pregnancy when fetal growth is most rapid.

The preceding review indicates that a balanced corn-soybean meal diet will support satisfactory performance in sows and that the sow can support developing fetuses on a very limited protein intake. This indicates either the diets are adequate in all essential amino acids or that the sow becomes more efficient in the retention of dietary nutrients because of her pregnant condition. Johnson and Wright (1948) self-fed pregnant or non-pregnant sows on pasture a corn diet plus a hand-fed protein supplement. The bred sows consumed 5.0 percent more feed, made 13.4 percent more daily gains and consumed 6.4 percent less feed per pound gain than the open sows. After 60 days on trial they were marketed and carcass measurements were taken. Non-pregnant sows excelled

in dressing percentage, carcass firmness and belly grade but there were no differences in backfat or yield of the belly.

Rombauts (1963) observed that pregnant gilts retained 6 to 19 percent more nitrogen than non-pregnant gilts even when an allowance was made for the products of conception. Elsley et al. (1966) observed gilts consuming 2.2 kg feed per day gained more than twice the weight of non-pregnant gilts in 110 days and retained significantly more nitrogen per day (16.7 g vs. 12.4 g). Pregnant sows also had more muscle plus intercellular fat than the controls (62.52 vs. 60.07 percent) and less subcutaneous fat plus skin (22.97 vs. 25.57 percent).

Only recently has the amino acid composition of the diet come under scrutiny possibly due to the observation that gilts could perform adequately consuming as little as 90 g of protein per day. With the knowledge that swine require ten essential amino acids in the diet for growth, reproduction studies were undertaken to determine the level of each amino acid needed and how this requirement may be best met. Rippel et al. (1965a) demonstrated that the addition of lysine to a corn protein diet markedly improved nitrogen retention in pregnant gilts and that the addition of tryptophan to the corn-lysine diet caused a further increase in nitrogen retention (7.00, 9.36, 10.43 g per day). Hayward and Hafner (1941) and Mitchell and Block (1946) observed that methionine was the first limiting amino acid in soybean meal for the chick and rat and Berry et al. (1962) similarly observed methionine to be first limiting and threonine or lysine the second limiting amino acid for pigs. Long (1966),

using levels of plasma amino acids and growth rate, indicated that threonine was the second limiting amino acid for young pigs.

Lucas et al. (1969) measured plasma-free amino acids from sows consuming diets composed of 2.44 parts corn to 1.0 part soybean meal and diluted with cornstarch and dextrose to attain diets containing 8, 12, 16, and 20 percent protein. Plasma levels of methionine and cystine remained very low through the 16 percent protein diet (0.22 percent methionine + cystine) and then increased. This indicated that the sulfur-amino acids were first limiting in their corn-soybean meal diet.

Using purified diets, containing L-amino acids and casein, Rippel et al. (1965a, c) determined the level of each essential amino acid needed to support maximum nitrogen retention near the end of pregnancy. Glutamic acid was added as a nonspecific nitrogen source to bring the dietary nitrogen level to 1.92 percent or 12 percent protein. They observed the following dietary percentages of amino acids would maximize nitrogen retention on a daily intake of 1.8 kg of feed: arginine, 0.38, histidine, 0.17; isoleucine, 0.37; leucine, 0.56; lysine, 0.42; methionine, 0.19; cystine, 0.09; phenylalanine, 0.30; tyrosine, 0.33; threonine, 0.34; tryptophan, 0.07; and valine, 0.46. Cystine could provide 32 percent of the total sulfur-amino acid requirement and tyrosine could meet about half of the phenylalanine requirement.

It should be remembered that the above levels are those needed to maximize nitrogen retention near the end of pregnancy and are probably in excess of the amounts needed to support satisfactory reproductive

performance. The amount of dietary protein needed in early gestation could be considerably less due to the small amount of fetal tissue present during early pregnancy. Elsley et al. (1968) killed sows serially on the 26th, 37th and 45th days of gestation and every five days thereafter. Average fetal weight increased curvilinearly until approximately the 67th day when the fetuses weighed 161 g. Fetal weight gain then increased linearly to the 111th day of gestation and a mean fetal weight of 1132 g. Dry matter content of the fetuses increased from about 3 to 18 percent during the period of linear growth.

Holden et al. (1968) observed satisfactory sow performance through four parities on a low protein diet containing 0.13 percent sulfur-amino acids and 0.28 percent threonine. An indication of the reduced amino acid requirement of the adult gilt was demonstrated by Baker et al. (1966a, b, c). They fed purified diets devoid of leucine, arginine and histidine to adult non-gravid gilts and observed a positive nitrogen balance in excess of one gram per day, indicating these amino acids were not required for maintenance. The necessary intake of the other essential amino acids to maintain nitrogen equilibrium was only 15 to 30 percent of the recommendations of Rippel et al. (1965a,c) for maximum nitrogen retention during pregnancy. Therefore it is reasonable to assume that the amino acid requirement for pregnancy should lie between these two extremes.

The development of opaque-2 corn may allow further simplification of swine diets. Opaque-2 corn has 50 to 100 percent more lysine than normal corn and a similar increase in tryptophan (Jensen, 1968). Hesby et al.

(1968) observed no difference in number farrowed or birthweight of live pigs when opaque-2 corn (11.2 percent protein) was fed in comparison with a 15 percent or an 11.2 percent protein corn-soybean meal diet or a 9.8 percent protein all-corn diet. Gravid gilts retained more nitrogen on opaque-2 corn than on normal corn or on the 11.2 percent protein corn-soybean meal diet, indicating that the high lysine opaque-2 corn more nearly meets the amino acid requirements at this stage of the life cycle.

Rippel (1967) summarized the protein and amino acid requirements of the gravid gilt and stated that the diet of the sow has little influence on the pig at birth or its subsequent development with the exception of a few well-recognized nutrient deficiencies. Within limits, the sow has the ability to draw upon her body tissues to adequately buffer the offspring, before and after birth, against nutritional deficiencies of most feed constituents.

Effect of Protein Intake on Plasma Amino Acid Levels

It is well-known that the amino acid composition of dietary protein is not of the same ratio as that which is required by animals. Some amino acids are much in excess, while others appear to be limiting or not readily available. Puchal et al. (1962) observed that after feeding five different protein sources to baby pigs the plasma concentration of individual essential amino acids depended to a certain degree upon the amount present in the diet and the nature of the protein.

Hill and Olsen (1963a, b) and Dean and Scott (1966) fed amino acid mixtures void in single essential amino acids and observed corresponding decreases of these amino acids in the plasma of chicks. When chicks were fed a protein-free diet for 24 hours the concentrations of all measured amino acids decreased. Zimmerman and Scott (1965) noted that the first limiting amino acid remained very low in the blood irrespective of the severity of the deficiency. Conversely, amino acids present in the diet in excess of the requirement cause a rapid accumulation of that amino acid in the blood.

McLaughlan (1964) observed similar decreases of a plasma amino acid in rats when the supply of that amino acid in the diet was inadequate. Using a plasma amino acid score (fed level of an amino acid divided by fasted level of that amino acid expressed as a percentage) for all amino acids, he observed an exaggerated decrease in the score of the most limiting amino acid. Clark (1963) observed young pigs fed soybean meal had low levels of methionine in the plasma and reduced growth rates. Additions of methionine to the diet improved the growth rate and increased the amount of plasma methionine.

Mitchell et al. (1968) correlated nitrogen retention and plasma-free amino acids to determine the amino acid requirements of the young growing pig. Using broken-line graphs they observed that the dietary amino acid level at which plasma concentration of the test amino acid started to increase closely approximated the requirement for that amino acid as determined by nitrogen retention. Although only four amino acids

were tested they postulated that other essential amino acids would respond similarly.

Lucas et al. (1969) fed equal quality protein diets previously described and observed a highly significant linear increase of most essential amino acids in the plasma one hour after feeding as the protein content of the diets increased. Histidine increased quadratically and tryptophan was not measured. Plasma-free cystine and methionine increased when the 16 and 20 percent protein diets were fed indicating that these two amino acids were first-limiting at the lower protein intakes.

The data presented regarding the response of plasma-free amino acids to protein intake suggests that this criteria can be used to estimate the requirement for an amino acid. The adequacy and the quality of a protein for a monogastric species can also be estimated by plasma-free amino acid measurements.

Methionine-Cystine Relationships

Although animals usually require the L-isomer of amino acids, it appears that in the case of methionine both the D and L-forms are equally available. Rose (1957) noted that the D-isomer was as effective for man as was the L-isomer as determined by nitrogen balance. Wretland and Rose (1950) and Stekol (1935) observed that the D-isomer was equally available for growing rats and dogs, respectively. The Agricultural Research Council (ARC, 1967) assumed that the D-isomer is used as efficiently as the L-isomer for pigs.

Before the essential amino acids were defined, Weichselbaum et al. (1932) observed that a large part of the cystine requirement could be replaced by methionine and thought possibly methionine was converted to cystine. After methionine was found to be an essential amino acid, its requirement for the growing pig was studied. In a review, ARC (1967) estimated the methionine plus cystine requirement for the following weight pigs: 4.5 kg pig requires 0.87 percent of the dry matter intake as methionine; 9 kg, 0.78 percent; 20 kg, 0.7 percent; and 50 kg, 0.6-0.7 percent.

The methionine requirement decreases rapidly after mature size is obtained. Rippel et al. (1965a) observed pregnant gilts require only 0.28 percent methionine plus cystine in the diet and Holden et al. (1968) observed satisfactory reproductive performance on a diet containing 0.13 percent sulfur-amino acids. Baker et al. (1966c) found adult non-gravid gilts require only 0.059 percent sulfur-amino acids for maintenance. All the above gilts were fed 1.8 kg per day.

Considerable variation also exists in the replacement value of cystine for methionine. Becker et al. (1955) used growth rate of young pigs as the criteria and showed that cystine could meet 40 percent of the total sulfur-amino acid requirement, while Rippel et al. (1965a) using nitrogen balance indicated a 32 percent substitution value for bred gilts. Cystine can replace 94% of the methionine requirement for the maintenance of nitrogen equilibrium in the adult non-gravid gilt (Baker et al., 1966c).

This is due to the fact that most of the sow's maintenance needs are for the replacement of hair, hooves and tissue which contain a much higher ratio of cystine to methionine than growing muscle or fetal tissue.

EXPERIMENTAL

General Objectives

These experiments were conducted to determine the sulfur-amino acid needs of gilts during the prebreeding and gestating stages of the life cycle. Nitrogen balance and the levels of plasma-free amino acids were the primary criteria for estimating the requirements as well as litter size, birthweight, number weaned and gain from birth to weaning.

General Experimental Methods

The data reported herein are on file in the Swine Nutrition section of the Animal Science Department, Iowa State University of Science and Technology, Ames, Iowa, and identified as Swine Experiments 6808 and 6914.

Gilts used in this experiment were of Yorkshire X Landrace or of Poland China X Yorkshire X Landrace breeding. They were housed in open-front pens with concrete floors. Water was available in the pens and woodshavings were supplied as bedding in cold weather. They were fed 1.82 kg of feed each morning in individual feeding stalls and remained in them until they had consumed their ration.

Four nitrogen balance trials were conducted with each gilt during the experiment. After a two week adaptation to the diets, upon which they remained throughout the experiment, the first balance trial was conducted. Following this they were handmated to Poland China boars and successive balance trials were conducted beginning approximately 30,

68 and 106 days later. Weekly weights were recorded throughout the experiment.

The experiments were designed as split-plots with dietary treatments as the main plot and the four sampling periods, prebreeding, first trimester, second trimester and third trimester, as the subplots. Gilts in the first replicate were randomly allotted across the treatments and subsequent replicates were allotted to maintain a uniform initial weight for all treatments. Analyses of variance were conducted by the method of least squares (Harvey, 1960). Treatment effects were tested by the Rep X Treat interaction and stages by Rep X Stage or Remainder.

Nitrogen balance trials were of five days duration and conducted in raised farrowing crates equipped with collars to restrain the gilts. The front portion of the floor was plywood and the back of expanded metal. Feces were allowed to fall on and through the expanded metal onto a plastic sheet. At the end of the trial the feces were dried in an oven at 55 degrees Centigrade, allowed to come to air dryness and weighed. They were then ground in a Wiley mill and a sample was stored in a plastic bag and frozen until analyzed for nitrogen content.

Urine was collected by inserting a Foley catheter, size 24 or 26, into the bladder via the urethral opening. The catheter was attached to a length of Tygon tubing and allowed to drain into a bottle containing 45 ml of ten percent hydrochloric acid. The urine was measured daily and a 50 ml aliquot was frozen for nitrogen analysis.

Two blood samples were withdrawn from the anterior vena cava of each gilt at the end of each balance trial. The first sample was taken

following approximately a 24 hour fast and the second withdrawn one hour after feeding. A 10 ml glass syringe equipped with a 14 or 16 gauge needle, four and one-half inches long, was used to withdraw the blood. The dead space of the syringe was filled with a 10 percent solution of sodium citrate before withdrawing the blood and the blood was then transferred to a 15 ml glass centrifuge tube containing 0.1 ml of the sodium citrate solution (Hewitt, 1932). The samples were centrifuged at 600 g for 15 minutes to separate the plasma from the red blood cells. The plasma was drawn off and frozen.

The basal diet used in these experiments is shown in Table 1. It contained a ratio of 2.44 parts of corn to 1.0 part soybean meal and the methionine and cystine content was calculated to be 0.10 and 0.05 percent, respectively. Vitamins and minerals were added to meet the levels recommended by the NRC (1968). The composition of the vitamin and mineral premixes and the chemical analyses of the basal diet, corn and soybean meal used in these experiments is shown in Tables 2 through 4.

Experiment 6808

This experiment was conducted to determine whether the addition of cystine to the basal diet would enhance the nitrogen utilization of pregnant gilts. Two isonitrogenous diets shown in Table 5 were used, one containing 0.15 percent added L-cystine and the other containing 0.18 percent added L-glutamic acid (NRC grade). The trial lasted from July, 1968, to February, 1969. Three replicates, each containing two gilts, were allotted to the treatments. Plasma levels of tryptophan were not analyzed.

Experiment 6914

Four dietary levels of sulfur-amino acids were fed to six replicates of gilts to determine the level of these amino acids required for gestation. The four gilts in a replicate were fed either the basal diet containing approximately 0.15 percent calculated sulfur-amino acids or one of the diets containing added D,L-methionine. The levels of sulfur-amino acids in the diets were 0.15, 0.23, 0.36 or 0.55 percent, increasing linearly on a logarithmic scale. Glutamic acid (NRC grade) was added to keep the diets isonitrogenous (Table 6).

The experiment was started in July, 1969, and finished in April, 1970. Two gilts failed to conceive and were omitted from the experiment. Two other gilts aborted during the third trimester of gestation and available data from them are included.

Analytical Methods

Four ml of plasma was combined with four ml of 20 percent sulfosalycylic acid containing 0.333 micromoles per ml of norvaline, the internal standard. Following centrifugation at 12,350 g for 15 minutes, six ml of the deproteinized plasma were removed and the sample was neutralized to pH 7 with 5N NaOH and allowed to stand at room temperature four hours to convert cysteine to cystine (Stein and Moore, 1954). The sample was then titrated to pH 1.5 with 5N HCl, and diluted to 10 ml with deionized water and frozen until analysis for amino acid content.

One ml of this solution was analyzed by ion-exchange chromatography as described by Stein and Moore (1954). A Technicon Autoanalyzer system

employing a gradient buffer elution system with modifications as recommended by Hamilton (1962) was used. Quantitative determination of the amino acids was obtained using a standard amino acid solution and comparing the relative area of the peaks as well as the recovery of the internal standard.

Plasma tryptophan, which is lost on column chromatography, was determined by an adaptation of the procedure of Hess and Udenfriend (1959) to a Technicon Autoanalyzer system and a Turner fluorometer. The procedure is described in Appendix B.

Samples of the basal diet, corn and soybean meal were taken each time the basal diet was prepared. They were ground in a Wiley mill and frozen in a plastic bag until further analysis. Nitrogen content was determined according to the procedure of Ferrari (1960) using a Technicon Autoanalyzer continuous digestion system. Fecal and urine samples were also analyzed for nitrogen by this procedure.

Individual samples of the basal diet, corn and soybean meal were pooled for amino acid analysis and were hydrolyzed according to Kohler and Palter (1967) with modifications. Samples were hydrolyzed for 24, 48 or 72 hours, with or without previous oxidation by performic acid. After lyophilization the samples were washed into a beaker, titrated to pH 1.5 and diluted to a final concentration of 0.20 to 0.25 mg protein per ml. One ml of the sample was analyzed by column chromatography. Tryptophan levels of the diet or ingredients were not determined.

RESULTS AND DISCUSSION

Experiment 6808 - Effect of Added Cystine or Glutamic Acid

Three gilts were started on each treatment and all farrowed although one of the gilts on glutamic acid lost her pigs shortly after birth. The remaining gilts on both treatments averaged 10 live pigs farrowed with similar birthweights. Data are not presented on litter performance since no significant differences were present with the number of animals used.

Summaries and analyses of variance of plasma-free amino acids, nitrogen balance and gestation weight gain are presented in Tables 7 through 12 and Figures 1 through 3. The amino acids measured in the plasma of the gilts were not affected by the dietary treatments imposed. The addition of cystine to the basal diet failed to improve the protein quality of the diet by alleviating a possible cystine or methionine deficiency nor did the cystine addition cause an apparent imbalance of amino acids.

Plasma glutamic acid decreased linearly ($P \leq 0.01$) as pregnancy progressed and proline increased ($P \leq 0.05$) with an apparent quadratic trend. Apparently more of the added glutamic acid was being used as a source of non-essential amino nitrogen as fetal demands increased late in gestation. A portion of the glutamic acid or non-essential amino nitrogen may have been converted to proline and possibly hydroxyproline, which appears in large amounts in collagenous material and hair, for the development of these fetal tissues as well as maintenance of the gilts'

hair, hooves and skin. Plasma leucine was also affected by stages, with the lowest level occurring near the end of the third trimester. This would suggest that leucine may have been becoming limiting in the diet as gestation progressed.

No treatment differences were observed for nitrogen retention, again suggesting that cystine added to the basal diet did not improve the nitrogen utilization. Thus it was assumed that the basal diet was adequate in cystine and could be used to estimate the methionine and total sulfur-amino acid requirement of gestating gilts. There was a significant stage effect ($P \leq 0.01$) with retention greater during prebreeding and the third trimester, suggesting increased protein anabolism at these times.

Sow weight gains during gestation were not significantly affected by treatments but did increase slowly as gestation time increased. This is a common observation of gestation performance and has been reported by numerous researchers.

Lucas et al. (1969) showed that the sulfur-amino acids were the first limiting amino acids in this basal diet. Data presented here indicates that the addition of cystine to the diet does not improve the sulfur-amino acid content to the extent that another amino acid becomes first limiting. Therefore, the diet shown in Table 1, with methionine the first limiting amino acid, could be used to estimate the methionine requirement for gestation. Since cystine did not improve the quality of the diet, the addition of cystine was considered unnecessary.

Experiment 6914 - Effect of Added Methionine

Twenty-four gilts, six on each treatment, were originally started in this experiment. Two gilts on the first treatment failed to conceive and were removed from the experiment. Two additional gilts aborted during the third trimester, one on the second treatment and the other on the fourth treatment. Summaries and statistical analyses of all parameters measured are shown in Tables 13 through 23.

Levels of plasma-free essential amino acids and the analyses of variance are shown in Tables 13 and 14. Plasma methionine was not affected by stages, although cystine (Table 15) responded cubically with peaks at the first and third trimesters. Plasma methionine ($P \leq 0.005$) and cystine ($P \leq 0.01$) increased linearly with added methionine and methionine also responded quadratically ($P \leq 0.05$) (Figure 4). Plasma methionine increased 0.16 mg per 100 ml from treatment I to treatment II and another 0.45 mg per 100 ml to treatment III, causing the quadratic effect. The last treatment increased plasma methionine an additional 1.09 mg per 100 ml. Since Mitchell et al. (1968) and Zimmerman and Scott (1965) observed an increase in the plasma level of an amino acid when its requirement is met, it appears that the necessary intake of methionine plus cystine for these gilts is probably adequate in the first diet and does not exceed the level found in treatment II, or 0.23 percent sulfur-amino acids. With the exception of the first trimester, plasma cystine showed a similar but less dramatic increase after the second dietary treatment.

Significant linear decreases were observed for arginine ($P \leq 0.005$), isoleucine and lysine ($P \leq 0.05$) and valine ($P \leq 0.01$) with increasing methionine in the diet and leucine decreased with both quadratic and linear responses significant ($P \leq 0.05$). Plasma isoleucine and valine levels, shown in Figure 4, are representative of the decreases most essential amino acids followed as the dietary level of methionine increased. As the level of sulfur-amino acids becomes adequate it appears that the dietary intake of these amino acids may be marginal. Arginine decreased 0.99 mg per 100 ml or nearly 27 percent over the four dietary treatments, and lysine decreased 21 percent, valine 19 percent after the second treatment and isoleucine and leucine decreased 11.5 and 6.5 percent, respectively. This data indicates that arginine may be the first limiting amino acid in the diet when methionine is adequate and either lysine or valine the second limiting. Since the dietary intake of arginine is well above the estimate of Rippel et al. (1965c) it seems doubtful it is the next limiting. Lysine and valine intake may be limiting however.

Histidine, leucine, lysine, threonine and valine decreased linearly ($P \leq 0.01$) and histidine decreased quadratically ($P \leq 0.005$) as pregnancy advanced. This results from a more rapid turnover due to increasing requirements for the development of the products of conception as well as the maintenance of a larger body size. Glutamic acid and proline, as well as cystine which was discussed earlier, were significantly affected by stages (Tables 15 and 16). Glutamic acid decreased quadratically ($P \leq 0.05$) as gestation progressed and proline increased linearly

($P \leq 0.005$). This response is similar to that observed in experiment 6808 and again probably reflects the metabolic demands on available non-essential amino nitrogen, including its conversion to proline and hydroxyproline.

Compared to the plasma levels reported by Lucas et al. (1969) our observed level of plasma methionine is similar and cystine about two to three times greater than they observed on the eight percent protein diet. Plasma histidine, threonine and valine are 20 to 40 percent less. The mature sows used by Lucas et al. (1969) could be expected to require relatively more cystine than gilts because a larger share of their dietary intake is used for replacement of hair and skin rather than for growth.

Amino acid ratios were determined by dividing the fed level of each plasma amino acid by the fasted level sampled one hour previous to the collection of the fed sample. The means, expressed as percentages, and analyses of variance are shown in Tables 17 and 18. The methionine ratio, which increased linearly with added methionine was the only essential amino acid ratio significantly affected by dietary treatment. Although the postfeeding level of arginine decreased with added methionine the ratio decreased only slightly from 140 to 119 percent and at no time was it among the lowest ratios observed.

Although no statistical comparisons were made among amino acid ratios some observations can be made. Treatment and stage means were greater than 100 percent indicating that none of the essential amino acids were severely limiting. Tryptophan, leucine and isoleucine had the

lowest ratios most often which suggests that these three were among the first limiting when methionine is adequate.

Treatment means and the analyses of variance for nitrogen balance and gestation weight gains are presented in Tables 19, 20 and 21. Since the treatment response was not significant it appears that retention plateaus from the first diet on. Retention does increase slightly, however, from 9.38 to 9.98 g per day on the third treatment and then decreases to 9.68 g per day (Figure 5). Since there was no significant response to treatment, the level of sulfur-amino acids in the basal diet maximizes nitrogen retention and meets the gilts' requirement for methionine and cystine. However, the slight increase from treatment I to treatment II followed by a leveling off in nitrogen retention further suggests that the requirement does not exceed the level found in the second dietary treatment when the gilts are consuming 1.82 kg per day.

Values observed for nitrogen retention are in accordance with observations of other researchers. Rippel et al. (1965b) fed pregnant gilts diets containing 4.4 parts corn to 1.0 part soybean meal over a range of 0 to 15 percent crude protein. Gilts consuming the 9 percent protein diet retained 10.29 g per day. Miller et al. (1969) fed diets composed of different corn : soybean meal ratios and observed a similar retention on their 9 percent protein diet for gilts but only 6.40 g per day for sows. Elsley et al. (1966) fed 2.2 kg daily of a 14 percent crude protein diet and observed a linear increase in retention from 10 to 20 g per day from breeding to parturition.

As in experiment 6808 gestation weight gain was not affected by dietary treatments and there was a linear ($P \leq 0.005$) increase in gestation weight as pregnancy progressed. Litter data (Tables 22 and 23) were not affected by the dietary treatments imposed.

It was suspected that the methionine requirement would increase as pregnancy advanced, but this was not observed. Since growing gilts were used as the experimental animals they apparently maintained a relatively constant dietary need for methionine throughout the experiment. If mature sows had been used, a requirement less than that for gilts could be expected, especially in the post-weaning and prebreeding stages and early gestation.

The preceding data and discussion indicates that additional methionine added to the basal diet did not enhance nitrogen retention or improve litter size and weight. There was a linear increase in plasma methionine with increasing methionine in the diet suggesting that the level of sulfur-amino acids in the basal diet (0.15 percent) is adequate for gestating gilts and the requirement does not exceed 0.23 percent for the entire gestation period. This is further supported by the observations of Holden et al. (1968) that the reproductive performance of sows fed a similar 8 percent crude protein diet was equal to sows consuming 12, 16 or 20 percent protein diets of equal quality.

Rippel et al. (1965a, b, c) and Elsley et al. (1966) observed nitrogen retentions of 15 to 20 g per day during the final trimester of gestation. Elsley noted in a symposia (Pig Industry Development

Authority (P.I.D.A.), (1967) that the percent protein in the fetus remained near 70 percent on a dry matter basis and that different protein intakes failed to elicit a response in percent fetal nitrogen on the 70th day of pregnancy or postpartum.

Using various estimates of nitrogen retention by different tissues, a hypothetical estimate of the nitrogen requirement for gestation can be calculated. Elsley et al. (1966), P.I.D.A. (1967) observed pregnant gilts gained 53 kg during gestation or 30 kg more than non-pregnant gilts on a similar regime. The uterus and contents weighed 24 kg and contained 300 g of nitrogen, including 200 g of fetal nitrogen. Mammary tissue weighed 2.5 kg and contained 100 g of nitrogen. Thus of the 30 kg gained over non-pregnant gilts, 26.5 kg can be ascribed to products of conception and mammary tissue, a slight increase in blood volume and the remainder to pregnancy anabolism. It is assumed that the pregnant gilts' growth, independent of pregnancy, was equal to the 23 kg gained by the non-pregnant gilts (containing three percent nitrogen). Following is a summary of the estimated nitrogen requirements based on this data:

Item	Gestation nitrogen requirements, g[1]	
	Total	Per day
Growth[2]	690	6.0
Uterus + contents	300	2.6
Mammary tissue	100	0.9
Maintenance[3]	114	1.0
Pregnancy anabolism	707	6.2
Total	1911	16.7

[1]Elsley et al. (1966), P.I.D.A. (1967).

[2]23 kg times 3 percent nitrogen.

[3]Baker et al. (1966a).

Elsley et al. (1966) observed an average retention of 16.7 g nitrogen for the entire gestation. This leaves a minimum of 6.2 g per day for pregnancy anabolism or a total storage of 4.4 kg of protein. Based on these figures, a maximum of 10.5 g retained nitrogen per day should have been adequate for the maintenance of the sow and growth and development of the fetuses.

Feed and protein intake were restricted more in experiment 6914 (1.8 vs. 2.2 kg and 8 vs. 14 percent), than in the experiment conducted by Elsley et al. (1966) and allowed average gestation gain of **only** 30 kg. The average parturition weight loss was near 12 kg, which is about half that observed by Lodge et al. (1966) whose gilts were fed 2.7 kg daily of 14 percent protein diet and gained about 50 kg during gestation.

If the gilt can effectively store nitrogen throughout pregnancy she should be able to reproduce satisfactorily on about 9 of nitrogen retained per day (uterus plus contents, 2.6 g; mammary tissue, 0.9 g; maintenance 1.0 g; growth and/or pregnancy anabolism, 4.5 g). This would require an intake of 24 g of nitrogen per day with an 85 percent digestibility and a biological value of 45 percent, which is similar to the diet used. The amount of nitrogen partitioned as growth of the gilt may vary with the level of intake and may or may not include pregnancy anabolism as part of it.

In summary it appears that the gilt should be able to reproduce satisfactorily on 24 g of nitrogen intake per day or about 150 g of crude protein. The eight percent basal diet fed in this experiment supplied 146 g of relatively high quality protein per day. Based on

data obtained in this experiment as well as data of Holden *et al.* (1968), it appears that this diet meets the protein requirement of the pregnant gilts as well as the requirement for all the essential amino acids.

SUMMARY

Two experiments were conducted to determine the sulfur-amino acid requirement of gestating gilts as well as changes in the requirement as gestation progressed. The basal diet, with methionine the first limiting amino acid, was prepared from 2.44 parts corn and 1.0 part soybean meal and diluted with cornstarch and dextrose to formulate an eight percent crude protein diet. All animals received 1.82 kg of diet per day and remained on their respective diets throughout the experiment. The dietary intake of sulfur-amino acids was considered to be adequate when the plasma level of methionine increased linearly with added dietary methionine or when nitrogen retention plateaued. These criteria were measured prebreeding and near the end of each trimester.

In the first experiment 0.15 percent L-cystine or 0.18 percent L-glutamic acid was added to the basal diet and fed to three replicates of two gilts each. There was no increase in plasma methionine nor an improvement in nitrogen retention as a result of additional cystine over different stages of the experiment. These results indicate that methionine remained the first limiting amino acid in the basal diet when cystine was adequate.

The second experiment consisted of four levels of sulfur-amino acids, 0.15 (the level in the basal diet), 0.23, 0.36 or 0.55 percent, with increases from added D,L-methionine. Glutamic acid was added to maintain isonitrogenous diets. Six replicates were used in this study.

Nitrogen retention did not increase significantly as the methionine intake increased which suggests that the level in the basal diet was adequate. Plasma methionine increased slightly from the first to second dietary levels and then increased rapidly ($P \leq 0.005$) with additional methionine. Plasma cystine began to increase slowly ($P \leq 0.01$) after the second dietary treatment. There were no significant differences in litter size or birthweight.

Based on these observations it appears that the total methionine plus cystine requirement is adequate in the basal diet, or 0.15 percent, when gilts are consuming 1.82 kg of feed per day. The plasma amino acid response suggests a similar requirement and that it does not exceed 0.23 percent total sulfur-amino acids. The requirement remained uniform over all four stages suggesting that the gilts' growth needs offset the small fetal demands in early pregnancy.

Based upon this research, it is proposed that satisfactory reproductive performance is possible with 9 g daily nitrogen retention through the pregnancy period, which is approximately two-thirds the level previously proposed by other researchers.

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APPENDIX A

Table 1. Experiments 6808 and 6914: composition of basal diet

Ingredient	Level
Ground yellow corn	27.15
Solvent soybean meal	11.15
Starch	28.65
Dextrose	28.65
Calcium carbonate (38% Ca)	0.50
Dicalcium phosphate (26% Ca, 18% P)	2.30
Sodium chloride (iodized)	0.50
Trace mineral mix[a]	0.10
Vitamin premix 6808[b]	1.00
Total	100.00

[a]Composition of trace mineral mix is shown in Table 2.

[b]Composition of vitamin premix is shown in Table 3.

Table 2. Experiments 6808 and 6914: composition of trace mineral mix (35 C 73)

Element[a]	Percent in premix	Level in feed when added at 0.10% mg/kg
Zinc	10.0	50.0
Iron	10.0	50.0
Manganese	10.0	50.0
Copper	1.0	5.0
Cobalt	0.1	0.5
Iodine	0.3	1.5

[a]Ingredients: zinc sulfate, ferrous sulfate, manganese sulfate, iron oxide (color), copper oxide, cobalt carbonate, calcium iodate and calcium carbonate.

Table 3. Experiments 6808 and 6914: composition of vitamin premix 6808

Ingredient	Grams	Daily intake when added at 1% of the diet
Vitamin A (250,000 I.U./g)	1.0	10,000 I.U.
Vitamin D (142,000 I.U./g)	0.1	596 I.U.
Vitamin premix 1231	22.7	
Riboflavin (17.62 mg/g)		16 mg
Calcium pantothenate (35.24 mg/g)		32 mg
Niacin (79.30 mg/g)		72 mg
Choline (88.1 mg/g)		80 mg
Vitamin B_{12} premix (44 mcg Vitamin B_{12}/g)	22.7	40 mcg
Choline chloride (70%)	25.7	1216 mg
Starch	381.8	
Total	454.0 g	

Table 4. Experiments 6808 and 6914: Dry matter, crude protein and amino acid composition of the basal diet, corn and soybean meal, percent of diet

Item	Basal diet	Corn	Soybean meal
Dry matter	90.99	90.27	91.54
Crude protein	7.96	8.80	46.33
Total amino acids	7.60	8.56	46.62[a]
Alanine	0.40	0.62	2.10
Arginine	0.42	0.31	3.34
Aspartic acid	0.64	0.52	4.80
Cystine	0.06	0.03	0.27
Glutamic acid	1.52	1.79	7.67
Glycine	0.30	0.31	2.02
Histidine	0.15	0.17	1.13
Isoleucine	0.46	0.42	3.00
Leucine	0.70	0.98	3.56
Lysine	0.40	0.28	3.21
Methionine	0.12	0.19	0.72
Phenylalanine	0.37	0.41	2.43
Proline	0.53	0.82	3.25
Serine	0.32	0.44	2.15
Taurine	0.25	0.23	0.55
Threonine	0.22	0.27	1.65
Tryptophan[b]	0.10	0.09	0.60
Tyrosine	0.26	0.26	1.69
Valine	0.38	0.42	2.48

[a]Total amino acids exceeds total crude protein because of differences in recovery and sampling.

[b]Tryptophan values are calculated.

Table 5. Experiment 6808: experimental diets

Diet	Grams per 100 kg basal diet		
	L-Cystine	L-Glutamic acid	Added nitrogen
I	150	0	17.49
II	0	180	17.14

Table 6. Experiment 6914: Experimental diets[a]

Diet	Grams per 100 kg basal diet			
	Added D,L-Methionine	Total sulfur-amino acids	Added L-Glutamic acid	Total added nitrogen
I	0	150	395	37.60
II	80	230	316	37.59
III	210	360	188	37.62
IV	400	550	0	37.56

[a]Calculated level of methionine equals 0.10 percent and cystine equals 0.05 percent in the basal diet.

Table 7. Experiment 6808: Treatment means of levels of essential plasma-free amino acids taken one hour after feeding, mg per 100 ml

Treatment[a]	Amino acid									
	Arginine		Histidine		Isoleucine		Leucine		Lysine	
	Cys	Glu	Cys	Glu	Cys	Glu	Cys	Glu	Cys	Glu
Stage										
Prebreeding	3.19	3.12	1.50	1.55	2.58	2.69	3.54	3.43	3.69	4.80
1st Trimester	2.48	3.02	1.84	1.60	2.31	2.41	3.36	2.89	4.51	4.37
2nd Trimester	3.12	3.23	2.06	2.13	2.83	2.67	3.63	3.67	4.24	4.58
3rd Trimester	2.80	2.33	1.79	1.47	3.12	2.48	3.02	2.51	4.56	3.15

	Amino acid							
	Methionine		Phenylalanine		Threonine		Valine	
	Cys	Glu	Cys	Glu	Cys	Glu	Cys	Glu
Stage								
Prebreeding	0.52	0.68	1.63	2.08	2.23	1.64	3.35	3.31
1st Trimester	0.60	0.66	1.72	1.59	3.02	1.21	4.03	3.22
2nd Trimester	0.75	0.72	1.85	1.70	1.64	2.31	3.99	3.73
3rd Trimester	0.75	0.54	2.05	1.72	1.59	1.59	3.55	2.74

[a]Cys = basal diet + 0.15 percent L-cystine; Glu = basal diet + 0.18 percent L-glutamic acid.

Table 8. Experiment 6808: Analyses of variance of levels of essential plasma-free amino acids

Source	d.f.	Mean squares				
		Arginine	Histidine	Isoleucine	Leucine	Lysine
Replicate	2	1.136	0.059	0.429	0.138	0.540
Treatment	1	0.004	0.077	0.128	0.411	0.003
Stage	3	0.549	0.364	0.237	0.926**	0.435
Rep X Treat	2	1.486	0.217	0.473	0.698	0.270
Rep X Stage	6	0.192	0.181	0.115	0.070	1.293
Treat X Stage	3	0.270	0.060	0.183	0.111	1.675
Remainder	6	1.100	0.058	0.392	0.245	0.920
Total	23					

		Mean squares			
		Methionine	Phenylalanine	Threonine	Valine
Replicate	2	0.143	0.130	3.606	0.200
Treatment	1	0.000	0.007	1.128	1.391
Stage	3	0.019	0.065	0.297	0.604
Rep X Treat	2	0.006	0.154	0.552	0.523
Rep X Stage	6	0.039	0.196	1.928	0.129
Treat X Stage	3	0.036	0.169	1.667	0.233
Remainder	6	0.020	0.229	0.937	0.253
Total	23				

**$P \leq 0.01$.

Table 9. Experiment 6808: Treatment means of levels of four non-essential plasma-free amino acids taken one hour after feeding, mg per 100 ml

Treatment	Amino acid							
	Cystine		Glutamic acid		Proline		Tyrosine	
	Cys	Glu	Cys	Glu	Cys	Glu	Cys	Glu
Stage								
Prebreed.	0.09	0.15	8.06	10.37	3.71	3.17	1.53	1.78
1st Tri.	0.10	0.25	4.30	5.62	3.86	3.22	1.67	1.59
2nd Tri.	0.30	0.56	8.22	7.54	4.64	4.03	1.87	1.83
3rd Tri.	1.06	0.28	3.34	2.41	3.42	2.96	1.60	1.37

Table 10. Experiment 6808: Analyses of variance of levels of plasma-free cystine, glutamic acid, proline and tyrosine

Source	d.f.	Mean squares			
		Cystine	Glutamic acid	Proline	Tyrosine
Replicate	2	0.364	6.802	2.160	0.290
Treatment	1	0.040	1.504	3.700	0.003
Stage	3	0.383	49.020***	2.553*	0.134
Linear	1		39.030**	0.060	
Quadratic	1		0.422	2.168	
Rep X Treat	2	0.087	10.136	2.116*	0.034
Rep X Stage	6	0.130	2.748	0.503	0.113
Treat X Stage	3	0.339	33.684**	0.225	0.064
Remainder	6	0.098	2.281	0.195	0.049
Total	23				

*P ≤ 0.05.

**P ≤ 0.01.

***P ≤ 0.005.

Table 11. Experiment 6808: Effect of added cystine or glutamic acid on nitrogen balance and sow weight gain during gestation

	Nitrogen balance, g/day		Sow weight gain, kg	
Treatment	Cys	Glu	Cys	Glu
Stage				
Prebreeding	14.00	11.82	143.6	141.1
1st Trimester	10.57	10.89	154.7	145.9
2nd Trimester	12.59	10.09	162.5	151.2
3rd Trimester	11.90	12.88	166.4	152.8

Table 12. Experiment 6808: Analyses of variance of nitrogen retention and sow gestation weight gain

		Mean squares	
Source	d.f.	Nitrogen balance	Gestation weight gain
Replicate	2	18.93	114.7
Treatment	1	1.39	528.0
Stage	3	5.17**	363.1
Linear	1	1.54	511.0
Quadratic	1	4.74**	0.2
Rep X Treat	2	1.09	99.8
Rep X Stage	6	0.35	177.2
Treat X Stage	3	17.75	158.8
Remainder	6	4.38	81.4
Total	23		

**$P \leq 0.01$.

Table 13. Experiment 6914: Least square means of essential plasma-free amino acids taken 1 hour after feeding, mg/100 ml

Amino acid	Stage	Treatment I	II	III	IV	Stage, av.
Arginine	Prebreed.	3.03	3.13	3.17	2.61	2.99
	1st Tri.	3.99	3.62	3.00	3.36	3.49
	2nd Tri.	3.94	3.43	2.99	2.23	3.15
	3rd Tri.	3.73	3.42	2.86	2.53	3.14
	Treat. Av.	3.67	3.40	3.00	2.68	
Histidine	Prebreed.	1.55	1.44	1.51	1.54	1.51
	1st Tri.	1.36	1.29	1.27	1.31	1.31
	2nd Tri.	1.32	1.40	1.20	1.17	1.27
	3rd Tri.	1.36	1.19	1.45	1.40	1.35
	Treat. Av.	1.40	1.33	1.36	1.35	
Isoleucine	Prebreed.	1.81	2.15	1.75	1.50	1.80
	1st Tri.	2.02	2.14	1.95	2.12	2.06
	2nd Tri.	1.87	2.14	1.86	1.43	1.82
	3rd Tri.	1.91	1.82	1.88	1.60	1.80
	Treat. Av.	1.90	2.06	1.86	1.66	
Leucine	Prebreed.	2.44	2.89	2.48	2.44	2.56
	1st Tri.	2.65	2.84	2.52	2.58	2.65
	2nd Tri.	2.24	2.64	2.25	1.98	2.28
	3rd Tri.	1.99	2.00	2.13	1.70	1.96
	Treat. Av.	2.33	2.59	2.35	2.18	
Lysine	Prebreed.	4.20	3.23	3.90	3.54	3.72
	1st Tri.	3.60	4.10	3.48	3.04	3.55
	2nd Tri.	3.55	3.46	3.17	2.27	3.11
	3rd Tri.	3.01	2.95	3.32	2.44	2.95
	Treat. Av.	3.59	3.43	3.47	2.82	
Methionine	Prebreed.	0.75	0.91	1.45	2.49	1.40
	1st Tri.	0.77	0.84	1.18	2.08	1.22
	2nd Tri.	0.71	0.94	1.16	2.97	1.44
	3rd Tri.	0.72	0.92	1.61	2.22	1.37
	Treat. Av.	0.74	0.90	1.35	2.44	

Table 13. (Continued)

Amino acid	Stage	Treatment I	II	III	IV	Stage, av.
Phenylalanine	Prebreed.	1.47	1.46	1.43	1.42	1.45
	1st Tri.	1.40	1.54	1.36	1.43	1.43
	2nd Tri.	1.38	1.46	1.38	1.19	1.35
	3rd Tri.	1.64	1.50	1.56	1.54	1.56
	Treat. Av.	1.48	1.49	1.43	1.39	
Threonine	Prebreed.	1.37	1.52	1.59	1.40	1.47
	1st Tri.	1.36	1.47	1.42	1.18	1.36
	2nd Tri.	1.23	1.28	1.46	1.13	1.28
	3rd Tri.	1.19	1.15	1.20	1.02	1.14
	Treat. Av.	1.29	1.35	1.42	1.18	
Tryptophan	Prebreed.	1.00	0.98	0.96	0.88	0.95
	1st Tri.	0.93	0.78	0.69	1.00	0.85
	2nd Tri.	1.06	0.80	0.78	0.73	0.86
	3rd Tri.	0.93	1.00	0.84	0.77	0.89
	Treat. Av.	0.98	0.89	0.84	0.84	
Valine	Prebreed.	2.89	3.22	2.82	2.57	2.87
	1st Tri.	2.75	3.04	2.74	2.44	2.74
	2nd Tri.	2.46	2.61	2.62	2.04	2.43
	3rd Tri.	2.36	2.23	2.24	1.90	2.18
	Treat. Av.	2.62	2.78	2.61	2.24	

Table 14. Experiment 6914: Analyses of variance of fed levels of essential plasma-free amino acids

Source	d.f.	Mean squares				
		Arginine	Histidine	Isoleucine	Leucine	Lysine
Replicate	5	2.265	0.278*	0.228	0.379	1.739
Treatment	3	3.571*	0.013	0.612	0.688*	2.474*
Linear	1	10.658***	0.003	1.082*	0.774*	5.171*
Quadratic	1	0.010	0.021	0.602	0.847*	1.403
Cubic	1	0.044	0.017	0.150	0.384	0.846
Stage	3	0.967	0.231***	0.318*	1.969***	2.769*
Linear	1	0.016	0.290**	0.052	4.778***	7.994**
Quadratic	1	1.416	0.401***	0.395	0.860*	0.003
Cubic	1	1.470	0.002	0.507	0.269	0.310
Rep X Treat	13	0.782	0.080*	0.192*	0.153	0.657
Treat X Stage	9	0.524	0.047	0.147	0.101	0.693
Remainder	52	0.810	0.036	0.114	0.202	0.771
Total	85					

Source	d.f.	Mean squares				
		Methionine	Phenylalanine	Threonine	Tryptophan	Valine
Replicate	5	1.726	0.116	0.190	0.118	0.255
Treatment	3	12.023***	0.042	0.235	0.063	1.161**
Linear	1	31.466***	0.102	0.108	0.150	2.096**
Quadratic	1	4.445*	0.015	0.492	0.038	1.369*
Cubic	1	0.158	0.008	0.104	0.000	0.019
Stage	3	0.204	0.148	0.387*	0.044	1.955***
Linear	1	0.021	0.058	1.150**	0.043	5.726***
Quadratic	1	0.061	0.252	0.004	0.076	0.076
Cubic	1	0.053	0.133	0.008	0.131	0.064
Rep X Treat	13	0.731	0.043	0.137	0.058	0.171
Treat X Stage	9	0.309	0.028	0.021	0.062	0.066
Remainder	52	0.670	0.055	0.124	0.054	0.232
Total	85					

*$P \leq 0.05$.

**$P \leq 0.01$.

***$P \leq 0.005$.

Table 15. Experiment 6914: Least square means of some non-essential plasma-free amino acids taken 1 hour after feeding, mg/100 ml

Amino acid	Stage	Treatment I	II	III	IV	Stage av.
Citrulline	Prebreed.	1.08	1.32	1.47	1.25	1.28
	1st Tri.	1.54	1.15	1.43	1.46	1.40
	2nd Tri.	1.30	1.38	1.47	1.36	1.38
	3rd Tri.	1.62	1.34	1.51	1.51	1.50
	Treat. Av.	1.39	1.30	1.47	1.40	
Cystine	Prebreed.	0.35	0.33	0.45	0.53	0.41
	1st Tri.	0.52	0.70	0.57	0.94	0.68
	2nd Tri.	0.35	0.34	0.59	0.53	0.45
	3rd Tri.	0.36	0.42	0.80	0.76	0.58
	Treat. Av.	0.40	0.45	0.60	0.69	
Glutamic acid	Prebreed.	2.85	2.88	2.91	2.74	2.84
	1st Tri.	2.79	2.84	3.39	3.00	3.01
	2nd Tri.	2.84	3.06	3.04	2.65	2.90
	3rd Tri.	2.25	2.52	2.65	2.32	2.44
	Treat. Av.	2.68	2.83	3.00	2.68	
Ornithine	Prebreed.	1.48	1.56	1.66	1.78	1.62
	1st Tri.	1.71	1.60	1.54	1.55	1.60
	2nd Tri.	1.53	1.55	1.37	1.24	1.42
	3rd Tri.	1.55	1.40	1.36	1.14	1.36
	Treat. Av.	1.57	1.53	1.48	1.42	
Proline	Prebreed.	3.21	3.56	4.13	3.50	3.60
	1st Tri.	4.02	3.25	4.01	3.77	3.76
	2nd Tri.	4.25	3.81	3.76	4.01	3.96
	3rd Tri.	5.24	3.83	4.71	4.78	4.64
	Treat. Av.	4.18	3.61	4.15	4.01	
Tyrosine	Prebreed.	1.64	1.54	1.65	1.51	1.58
	1st Tri.	1.50	1.50	1.43	1.46	1.47
	2nd Tri.	1.48	2.26	1.31	1.32	1.59
	3rd Tri.	1.52	1.38	1.67	1.43	1.50
	Treat. Av.	1.54	1.67	1.52	1.43	

Table 16. Experiment 6914: Analyses of variance of fed levels of some non-essential plasma-free amino acids

Source	d.f.	Mean squares					
		Citrulline	Cystine	Glutamic acid	Ornithine	Proline	Tyrosine
Replicate	5	0.029	1.726	3.280**	0.116	2.391*	0.190
Treatment	3	0.110	0.357*	0.505	0.074	1.452	0.220
Linear	1	0.050	1.036**	0.002	0.220	0.112	0.301
Quadratic	1	0.000	0.004	1.216	0.002	0.631	0.209
Cubic	1	0.278	0.032	0.297	0.008	3.614*	0.152
Stage	3	0.153	0.322**	1.226*	0.338	4.124***	0.076
Linear	1	0.383*	0.077	1.651	0.923**	10.788***	0.014
Quadratic	1	0.000	0.077	2.021*	0.007	1.368	0.003
Cubic	1	0.077	0.778***	0.007	0.083	0.217	0.212
Rep X Treat	13	0.205***	0.097	0.616	0.274*	0.606	0.301
Treat X Stage	9	0.085	0.069	0.110	0.098	0.653	0.359
Remainder	52	0.057	0.070	0.428	0.124	0.686	0.291
Total	85						

*P ≤ 0.05.

**P ≤ 0.01.

***P ≤ 0.005.

Table 17. Experiment 6914: Least square means of ratios of fed/fasted plasma levels of essential amino acids, percent

Amino acid	Stage	Treatment I	II	III	IV	Stage av.
Arginine	Prebreed.	113	119	130	101	116
	1st Tri.	154	127	120	134	134
	2nd Tri.	140	133	136	108	129
	3rd Tri.	152	131	124	133	135
	Treat. av.	140	128	127	119	
Histidine	Prebreed.	120	108	121	113	116
	1st Tri.	126	108	128	110	118
	2nd Tri.	138	112	116	104	118
	3rd Tri.	118	109	113	113	113
	Treat. av.	126	109	120	110	
Isoleucine	Prebreed.	102	115	122	95	109
	1st Tri.	114	105	101	113	108
	2nd Tri.	120	119	158	100	124
	3rd Tri.	110	99	107	101	104
	Treat. av.	111	109	122	102	
Leucine	Prebreed.	101	109	112	99	105
	1st Tri.	112	105	106	112	109
	2nd Tri.	115	109	125	99	112
	3rd Tri.	110	96	108	104	104
	Treat. av.	109	105	113	103	
Lysine	Prebreed.	118	108	138	115	120
	1st Tri.	151	124	123	136	134
	2nd Tri.	157	136	141	114	137
	3rd Tri.	152	128	126	118	131
	Treat. av.	145	124	132	121	
Methionine	Prebreed.	108	150	196	218	168
	1st Tri.	113	146	198	269	182
	2nd Tri.	128	150	202	283	191
	3rd Tri.	106	135	213	239	174
	Treat. av.	114	145	202	252	

Table 17. (Continued)

Amino acid	Stage	Treatment I	II	III	IV	Stage av.
Phenylalanine	Prebreed.	95	112	120	105	108
	1st Tri.	116	108	103	124	113
	2nd Tri.	125	114	138	104	120
	3rd Tri.	118	103	108	107	109
	Treat. av.	114	109	117	110	
Threonine	Prebreed.	110	129	127	104	118
	1st Tri.	142	124	118	111	123
	2nd Tri.	113	118	123	118	118
	3rd Tri.	114	106	110	118	112
	Treat. av.	120	120	120	113	
Tryptophan	Prebreed.	108	108	101	102	105
	1st Tri.	112	110	104	119	111
	2nd Tri.	112	97	121	107	109
	3rd Tri.	96	108	103	93	100
	Treat. av.	107	106	108	105	
Valine	Prebreed.	100	113	119	101	108
	1st Tri.	117	109	115	110	113
	2nd Tri.	124	111	132	105	118
	3rd Tri.	113	107	110	102	108
	Treat. av.	114	110	119	105	
Cystine	Prebreed.	133	142	171	109	139
	1st Tri.	135	105	112	101	113
	2nd Tri.	118	122	141	117	125
	3rd Tri.	147	86	126	94	113
	Treat. av.	133	114	138	105	
Tyrosine	Prebreed.	101	117	122	109	112
	1st Tri.	123	116	113	120	118
	2nd Tri.	132	169	141	107	137
	3rd Tri.	122	102	110	115	112
	Treat. av.	119	126	121	113	

Table 18. Experiment 6914: Analyses of variance of plasma ratios of essential amino acids

Source	d.f.	Mean squares					
		Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine
Replicate	5	0.103	0.051	0.146	0.052	0.212	0.270
Treatment	3	0.124	0.116	0.160	0.043	0.192	7.218**
Linear	1	0.322	0.078	0.026	0.007	0.294	21.387***
Quadratic	1	0.006	0.014	0.195	0.018	0.029	0.146
Cubic	1	0.044	0.256	0.259	0.105	0.254	0.120
Stage	3	0.164	0.010	0.163	0.026	0.126	0.208
Linear	1	0.296	0.004	0.003	0.000	0.158	0.085
Quadratic	1	0.080	0.025	0.193	0.067	0.218	0.490
Cubic	1	0.116	0.000	0.293	0.011	0.000	0.050
Rep X Treat	13	0.162	0.067	0.150	0.050	0.172	0.910**
Treat X Stage	9	0.075	0.020	0.104	0.025	0.075	0.130
Remainder	52	0.095	0.034	0.088	0.034	0.088	0.343
Total	85						

**P ≤ 0.01.

***P ≤ 0.005.

Table 18. (Continued)

Source	d.f.	Mean squares					
		Phenylalanine	Threonine	Tryptophan	Valine	Cystine	Tyrosine
Replicate	5	0.047	0.134	0.067	0.056	0.307	0.068
Treatment	3	0.029	0.025	0.003	0.082	0.521	0.071
Linear	1	0.000	0.045	0.002	0.031	0.296	0.090
Quadratic	1	0.007	0.021	0.002	0.072	0.143	0.117
Cubic	1	0.078	0.008	0.004	0.143	1.125	0.007
Stage	3	0.066	0.046	0.044	0.043	0.303	0.297
Linear	1	0.016	0.050	0.011	0.003	0.430	0.059
Quadratic	1	0.132	0.077	0.119	0.103	0.112	0.482
Cubic	1	0.049	0.010	0.000	0.023	0.369	0.350
Rep X Treat	13	0.068	0.104	0.043	0.061	0.351	0.208
Treat X Stage	9	0.074	0.054	0.032	0.021	0.138	0.130
Remainder	52	0.045	0.083	0.022	0.037	0.146	0.119
Total	85						

Table 19. Experiment 6914: Average nitrogen retention data, g per day

Stage	Treatment	Nitrogen intake	Fecal nitrogen	Urine nitrogen	Nitrogen retention
Prebreeding	I	23.80	2.36	12.45	8.99
	II	24.35	3.72	10.30	10.32
	III	24.35	2.21	11.36	10.78
	IV	23.57	2.93	9.57	11.07
1st Tri.	I	23.93	4.10	9.61	10.22
	II	23.66	4.26	9.65	9.75
	III	23.93	4.23	9.80	9.90
	IV	23.93	4.47	9.86	9.60
2nd Tri.	I	23.84	5.13	10.11	8.60
	II	23.99	5.23	9.36	9.40
	III	23.99	4.48	9.33	10.18
	IV	23.99	4.75	9.78	9.46
3rd Tri.	I	24.30	6.38	9.94	7.98
	II	24.06	5.50	9.56	9.00
	III	24.17	5.62	9.48	9.07
	IV	24.06	6.08	8.81	9.17

Table 20. Experiment 6914: Least square treatment means for nitrogen balance and gestation weight gain

Nitrogen balance, gm/day Stage	Treatments I	II	III	IV	Stage av.
Prebreeding	9.43	10.32	10.77	11.07	10.40
1st trimester	10.66	9.75	9.90	9.60	9.98
2nd trimester	9.04	9.40	10.18	9.46	9.52
3rd trimester	8.42	9.67	9.07	9.34	9.13
Treat. av.	9.38	9.78	9.98	9.86	
Gestation weight, kg					
Prebreeding	115.4	117.7	118.4	116.6	117.0
1st trimester	127.0	128.6	130.3	129.6	128.9
2nd trimester	139.7	139.0	139.2	143.4	140.3
3rd trimester	147.2	144.3	146.7	151.1	147.3
Treat. av.	132.4	132.4	133.6	135.2	

Table 21. Experiment 6914: Analyses of variance of nitrogen balance, urinary nitrogen and sow gestation weight

Source	D.F.	Mean squares	
		Nitrogen balance	Gestation weight
Replicate	5	39.86**	626.8
Treatment	3	1.11	36.4
Linear	1	1.98	98.4
Quadratic	1	1.33	10.1
Cubic	1	0.01	0.8
Stage	3	6.17	3623.6***
Linear	1	18.49*	10732.5***
Quadratic	1	0.00	122.1
Cubic	1	0.00	16.3
Rep X Treat	13	11.65**	255.7***
Treat X Stage	9	1.53	15.7
Remainder	52	5.35	31.8
Total	85		

*P ≤ 0.10.

**P ≤ 0.05.

***P ≤ 0.005.

Table 22. Experiment 6914: Least square treatment means for number born, number born live, birth weight, number weaned and gain from birth to weaning

Item	Treatment			
	I	II	III	IV
Number born	7.6	9.4	8.8	8.3
Born live	5.9	8.1	7.7	8.3
Birthweight, kg	1.22	1.22	1.25	1.22
Number weaned	5.0	5.0	5.7	6.6
Gain, birth to weaning, kg	1.62	1.75	1.82	1.62

Table 23. Experiment 6914: Analyses of variance for number born, number born live, birthweight, number weaned and gain from birth to weaning

Source	d.f.	Mean squares				
		Number born	Born live	Birth-weight	Number weaned	Gain, birth to weaning
Replicate	5	2.47	2.51	0.01	4.32	0.16
Treatment	3	2.48	4.68	0.00	2.59	0.05
Linear	1	0.26	8.18	0.00	6.59	0.00
Quadratic	1	5.54	2.05	0.00	1.13	0.13
Cubic	1	1.62	3.82	0.00	0.06	0.01
Remainder	11	4.11	7.89	0.02	4.99	0.19
Total	19					

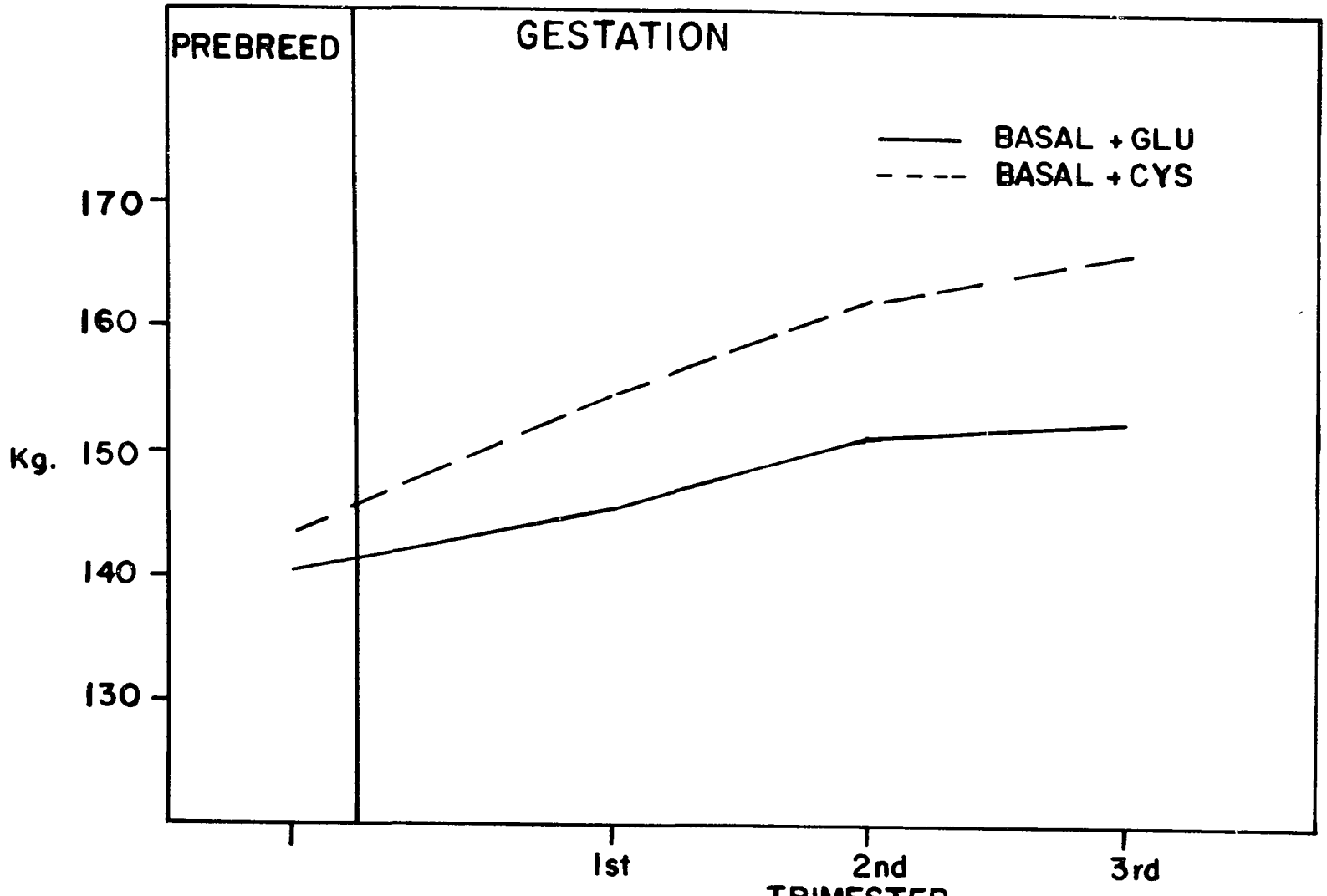


Figure 1. Experiment 6808. Effect of glutamic acid or cystine on gestation weight

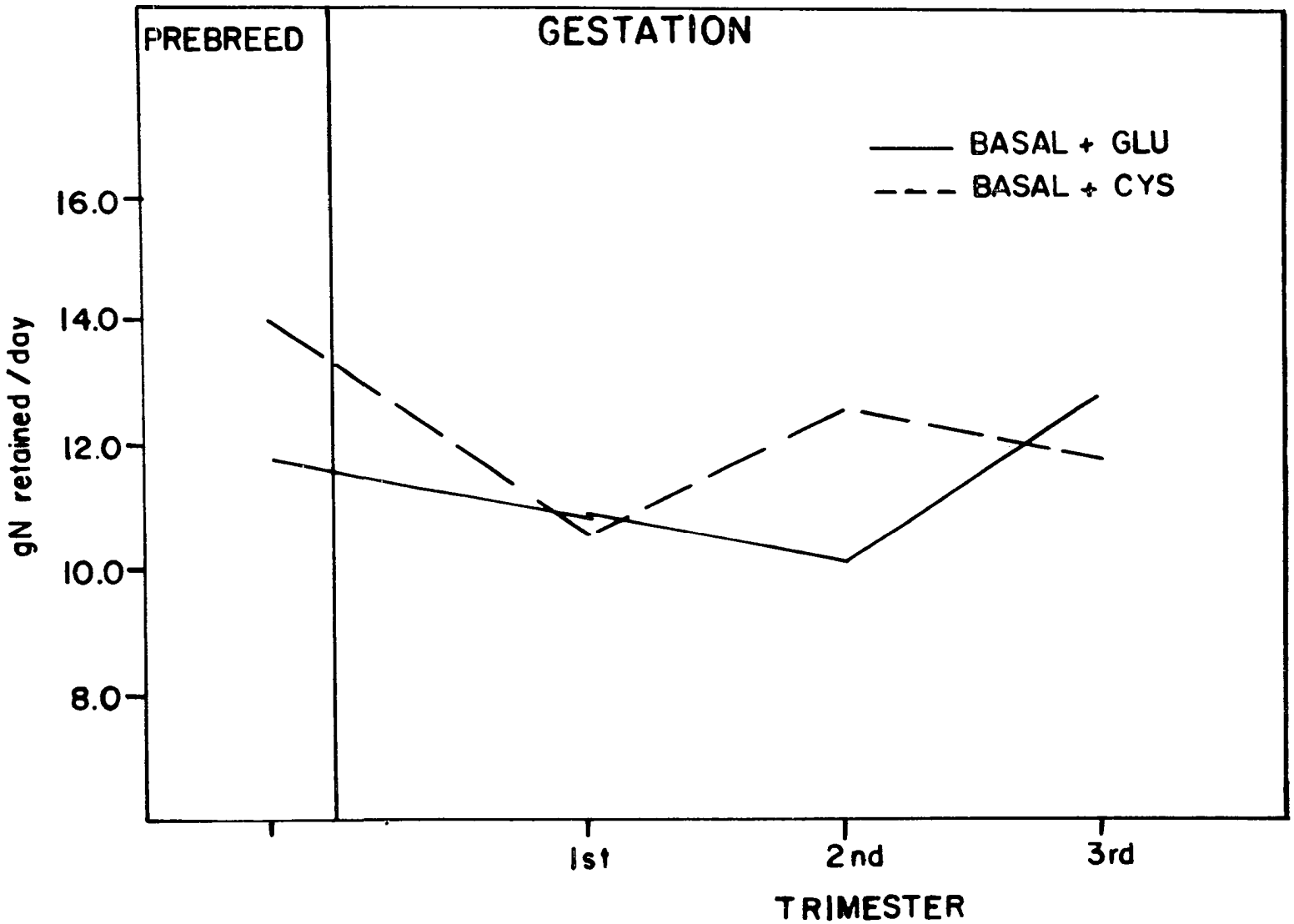


Figure 2. Experiment 6808. Effect of glutamic acid or cystine on nitrogen retention

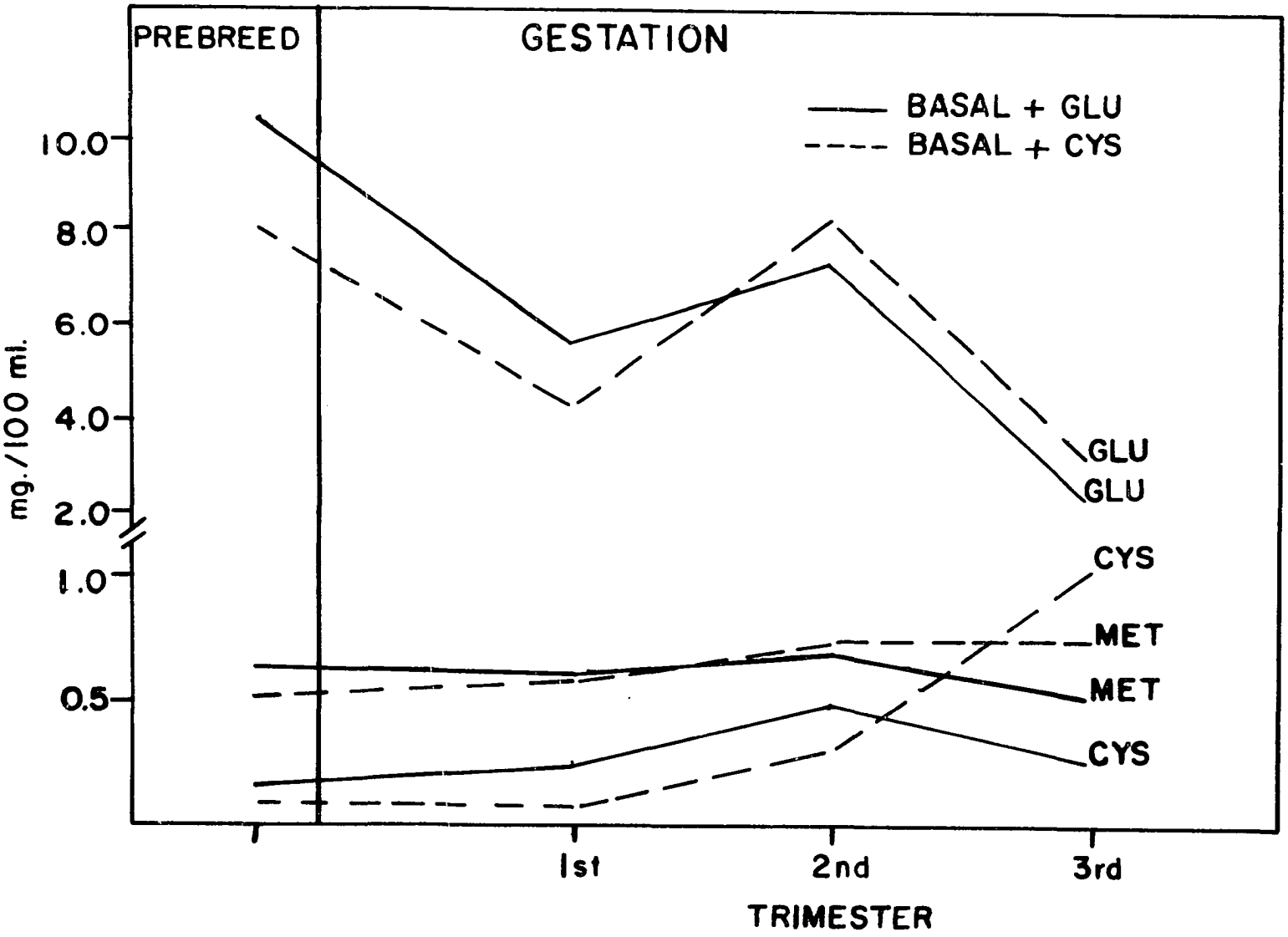


Figure 3. Experiment 6808. Effect of glutamic acid or cystine on plasma free cystine, glutamic acid and methionine

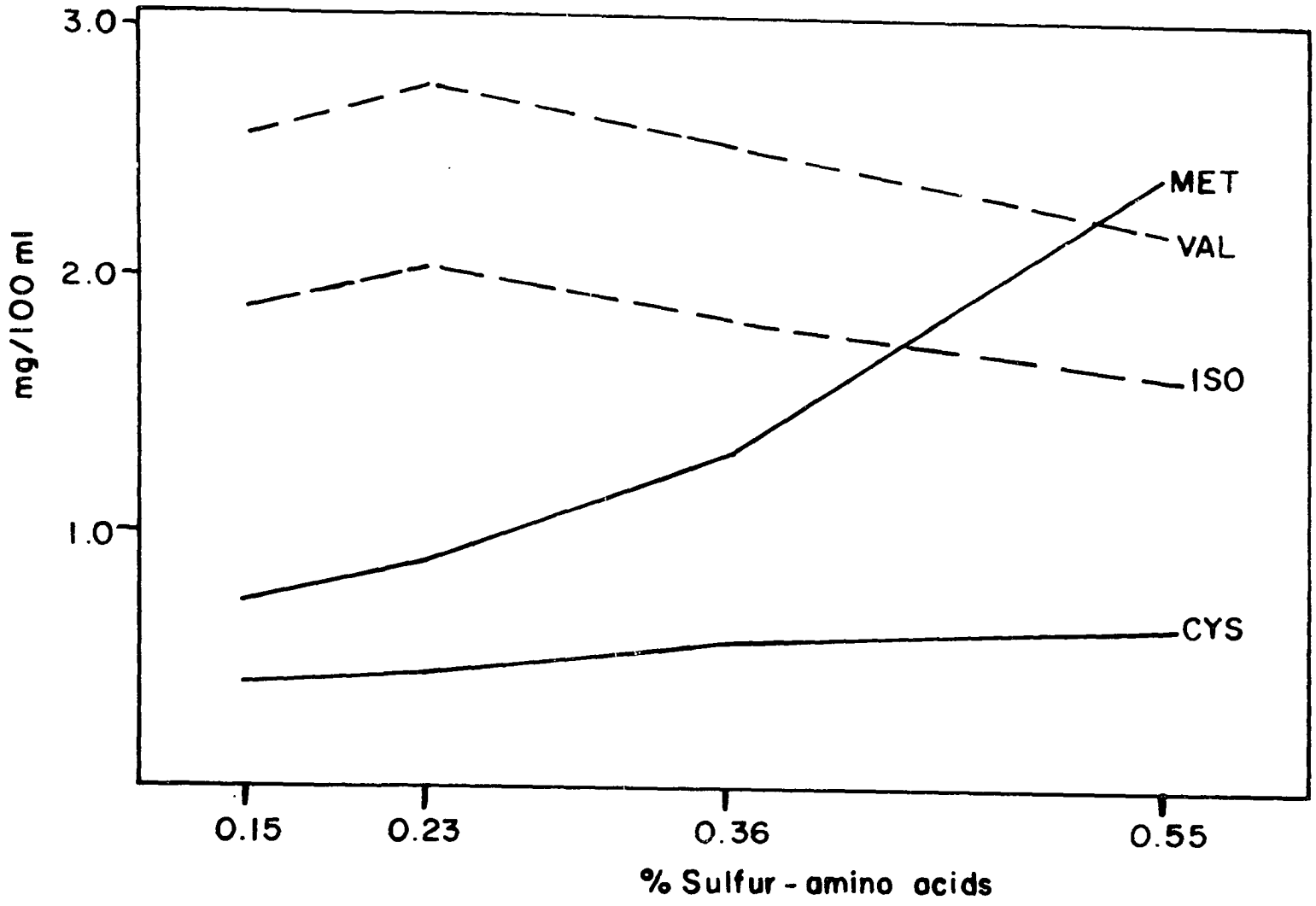


Figure 4. Experiment 6914. Effect of sulfur-amino acids on plasma-free cystine, isoleucine, methionine and valine

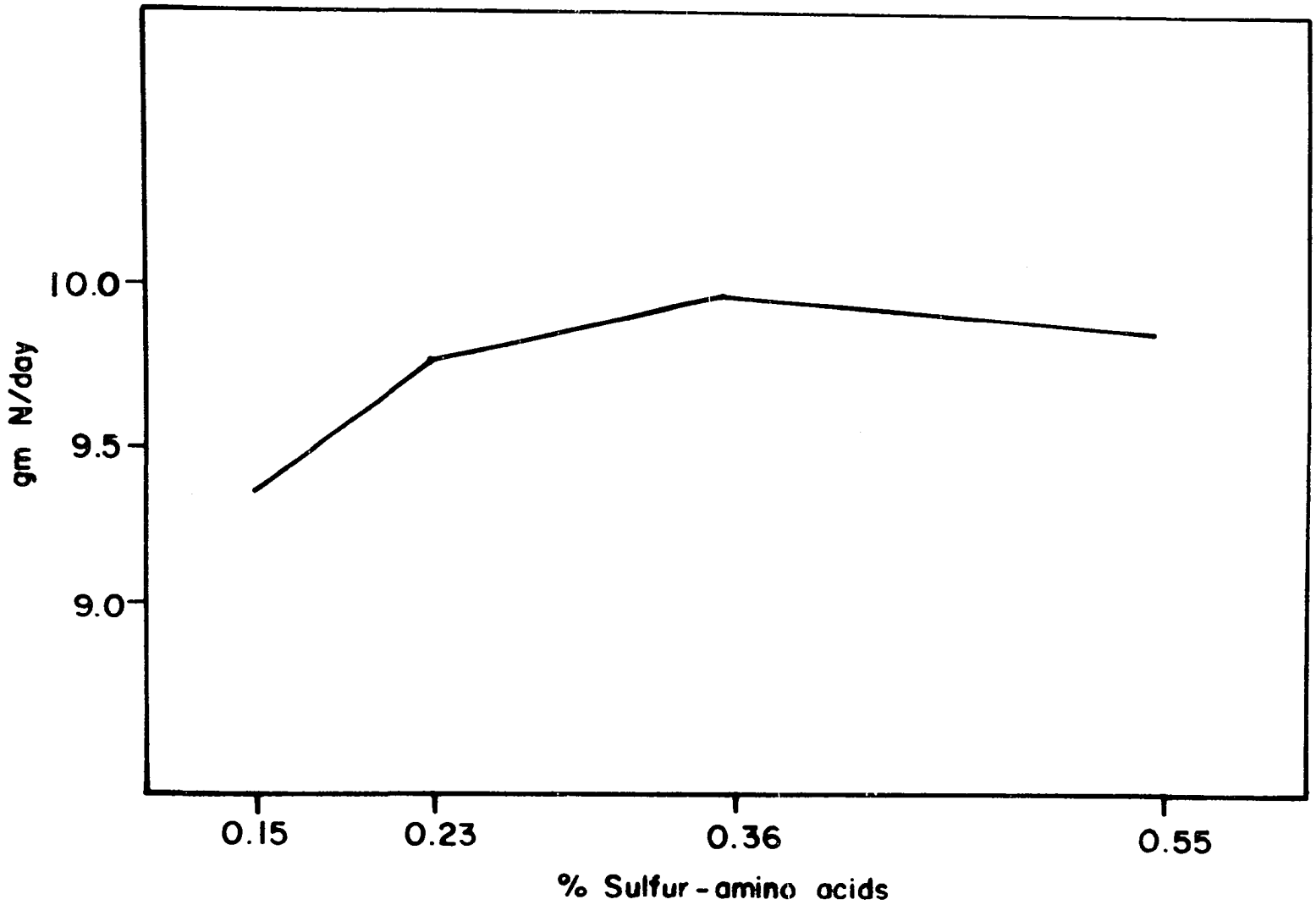


Figure 5. Experiment 6914. Effect of sulfur-amino acids on nitrogen retention

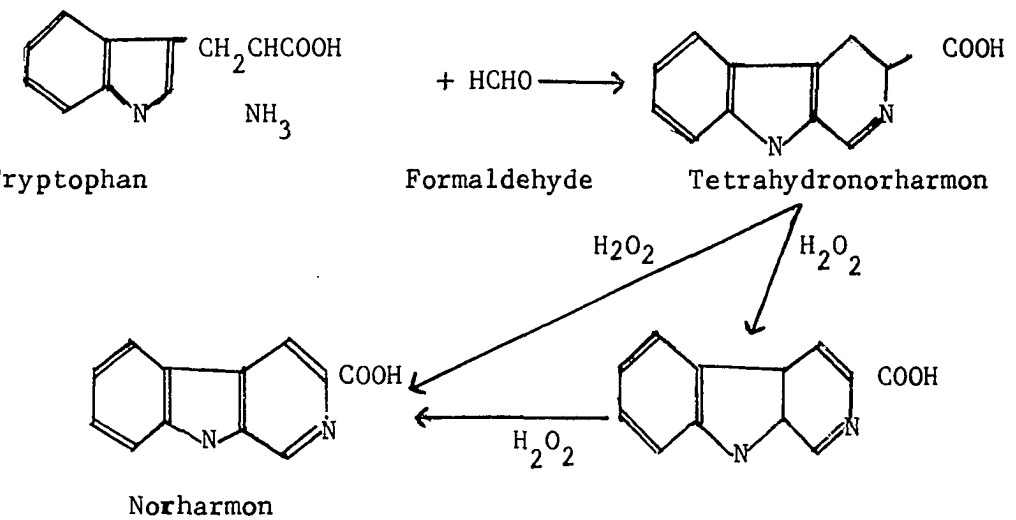
APPENDIX B

AUTOMATED FLUOROMETRIC METHOD FOR THE DETERMINATION OF TRYPTOPHAN IN PLASMA

Reference: Hess, S. M. and S. Udenfriend. 1959. A fluorometric procedure for measurement of tryptamine in tissue. Journal of Pharmacology and Experimental Therapeutics 127: 175-177.

Principle: After deproteinization with trichloroacetic acid (TCA), free tryptophan in plasma or serum is cyclized to form nonfluorescent tetrahydronorharmon. Treatment with hydrogen peroxide (H_2O_2) converts tetrahydronorharmon to fluorescent norharmon which absorbs at 300 and 360 mu and emits fluorescence at 440 mu.

Chemistry:



Equipment:

Sampler
Proportioning pump
Heating bath - 90° C
Fluorometer (Turner 111 with 7-60 activation filter and no. 3 emission filter
Recorder

Reagents:

1. Trichloroacetic acid (TCA, 8%). Dissolve 8 gm TCA in 100 ml deionized water.
2. Trichloroacetic acid (4%). Dissolve 4 gm TCA in 100 ml deionized water.
3. Formaldehyde (HCHO, 25.2%). Dilute 7 ml 36% HCHO to 10 ml with deionized water.
4. Hydrogen peroxide (H_2O_2, 10%). Dilute 4 ml 30% $H2O2$ to 10 ml with deionized water.
5. Sulfuric acid (H_2SO_4, 0.2N) Dilute 5.6 ml concentrated H_2SO_4 to 1000 ml with deionized water.

Sample preparation: Combine 1 ml plasma with 4 ml 4% TCA and centrifuge at approximately 12,350 g for 15 minutes. Decant the supernate into a culture tube for storage. This sample may be poured directly into the sample cups in the sampler without further dilution.

Standard preparation:

1. Stock solution. Dissolve 200 mg tryptophan in 100 ml deionized water. This solution will contain 2 mg tryptophan per ml.
2. Dilute stock. Dilute one ml of stock solution to 100 ml deionized water. This solution contains 0.02 mg tryptophan per ml and is used to prepare the following standards.

Table 24. Tryptophan standards

ml dilute stock	ml 8% TCA	ml H_2O	mg Try. per ml	mg Try. per 100 ml sample with 1 to 5 dilution
0.5	4.0	5.5	.001	0.5
1.0	4.0	5.0	.002	1.0
2.0	4.0	4.0	.004	2.0
3.0	4.0	3.0	.006	3.0
4.0	4.0	2.0	.008	4.0
5.0	4.0	1.0	.010	5.0

Procedure:

1. Set up the apparatus as shown in Figure 1.
2. Start the fluorometer by turning on power switch and depressing start switch for 5 seconds.
3. Switch the recorder to standby position. The chart speed should be set at 0.2 inch per minute and the range should be linear from 0 to 100 percent fluorescence.
4. The sampling rate is 30 per hour with a ratio of one part sample to two parts wash. The standards and samples are poured into two ml cups and run consecutively without alternating wash cups.
5. Place all lines in reagents and after 20 minutes adjust the recorder range.
6. Start the sampler and run one set of standards with approximately every 20 samples.
7. After completion of recording transfer all reagent lines to deionized water and turn the fluorometer and recorder off.
8. After flushing the lines with deionized water for 20 minutes stop the proportioning pump and relax the tubes.

Calculations: The results are calculated by comparing the peak heighth of the samples with the standards. A standard curve is plotted on a plastic overlay with the concentration of the standards plotted on the X-axis and the percent fluorescence plotted on the Y-axis and a smooth curve drawn through the points. The point on the curve that is intersected by the peak heighth of the sample corresponds to the level of tryptophan in mg per 100 ml or mg percent.

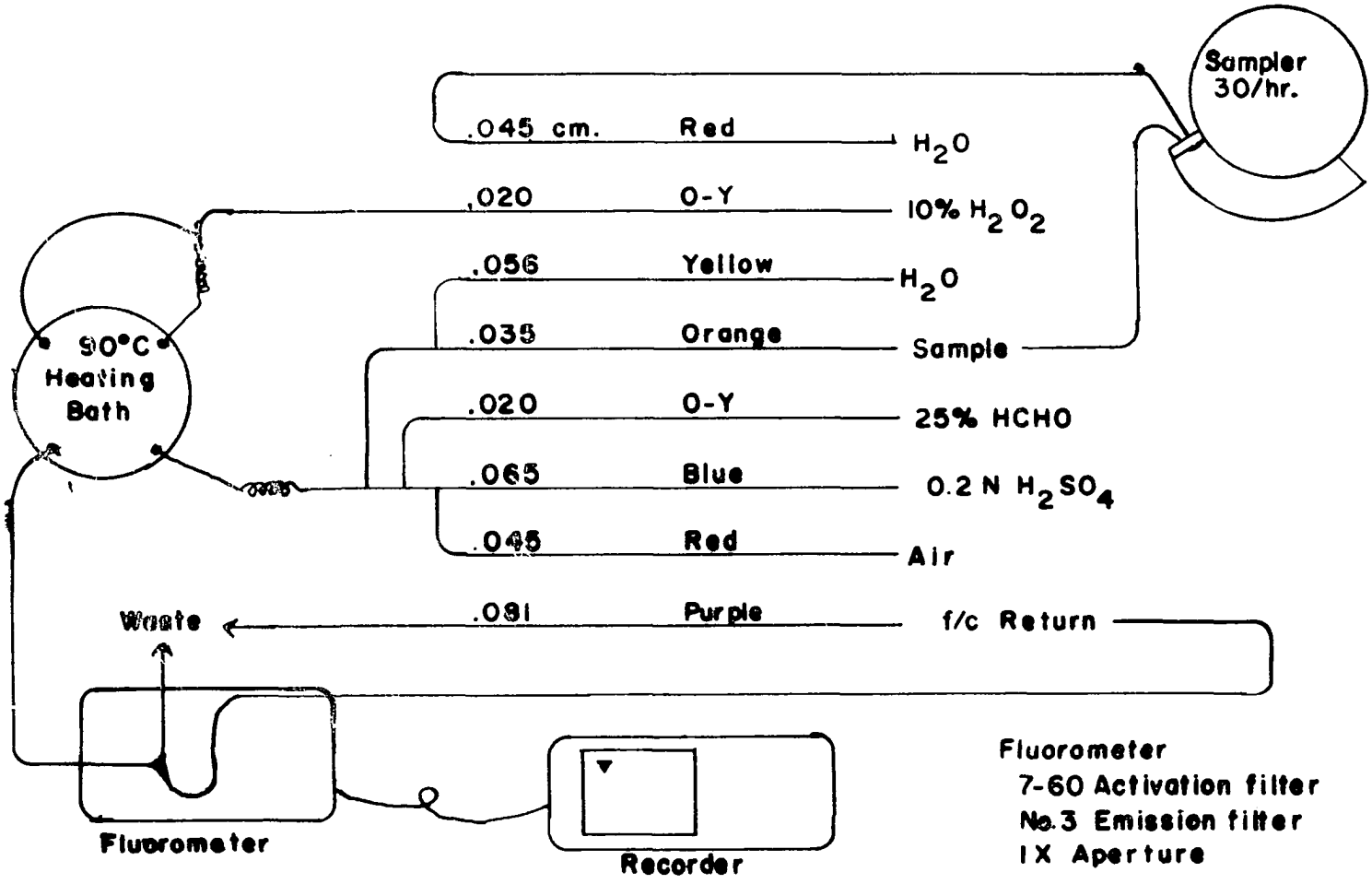


Figure 6. Flow diagram for automated determination of tryptophan