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I am submitting herewith a thesis written by Jeffery Michael Merrick entitled "Effects of Excess Dietary Methionine Consumption on Performance, Hematology and Plasma Free Amino Acid Concentrations in Young Poultry and Swine." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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EFFECTS OF EXCESS DIETARY METHIONINE CONSUMPTION ON PERFORMANCE, HEMATOLOGY AND PLASMA FREE AMINO ACID CONCENTRATIONS IN YOUNG POULTRY AND SWINE

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ABSTRACT

Three experiments were conducted utilizing 62 crossbred pigs, 30 pigs in experiment 1 and 16 pigs each in experiments 2 and 3. Two other experiments were conducted utilizing 168 Hubbard X Hubbard chicks; 60 chicks in experiment 4 and 108 chicks, 54 per age group, in experiment 5. The objective of these experiments was to evaluate the effects of incremental additions of D,L-methionine to the diet of chicks and weanling pigs. Pigs were allotted by weight within litter replicate and chicks were allotted within weight groups to one of four dietary treatments in experiments 1 and 5. The diets contained methionine and cystine at recommended levels (N.R.C., 1977 and 1979) for control diets and methionine at 2.5, 4.0 or 5.5 X N.R.C. requirements in treatment diets of experiments 2, 3 and 4. Diets containing the N.R.C. requirement of methionine with treatment diets having 1.5 or 3.0 X N.R.C. levels were used in experiments 1 and 5. Bodyweight, feed consumption, hematocrit, hemoglobin and plasma glucose were determined at one-week intervals in each experiment. In all but experiment 1, plasma free amino acid concentrations were also determined.

Results from the first swine experiment indicated that feed consumption was significantly reduced in pigs fed methionine at 3 X the N.R.C. requirement, however, gain per feed (gain:feed) was significantly increased in pigs consuming that level of methionine during week 2. Data from weanling pig experiments 2 and 3 indicated that gain and gain:feed were increased by the addition of 2.5 times the control dietary level of methionine but that a precipitous decrease resulted from higher

methionine supplementation during the first week of each trial. Gain and gain:feed were affected to a smaller degree in weeks 2 and 3 as compared to week 1. Hematological parameters were not affected by methionine additions in any of the weanling pig experiments.

Plasma methionine, threonine and cystine were increased in both experiments 2 and 3 in pigs fed the two highest levels of dietary methionine. Plasma glycine was depressed throughout the experiment in pigs fed 4.0 or 5.5 X the methionine requirement in experiment 2 and was depressed during week 1 only, in experiment 3. Plasma serine increased in pigs consuming the highest level of methionine in experiment 2 but did not exhibit a consistent pattern in experiment 3. Plasma cystathionine levels decreased in pigs fed 4.0 or 5.5 X the N.R.C. requirement of methionine in both pig experiments.

Weanling pigs appear to adapt to high methionine consumption evidenced by resumption of gain and gain:feed while consuming elevated levels of methionine. Also, by decreasing the plasma free methionine concentration over a short period of time while dietary methionine supplementation remains high, indicated an adaptive mechanism was functioning.

Results from the chick experiments indicated that excess methionine consumption depressed gain, feed intake and gain:feed in both young (8-day-posthatched) and older (5-week-posthatched) birds. Hematocrit and hemoglobin values were reduced in young chicks fed increasing amounts of methionine. Hematocrit levels were significantly decreased in older chicks but hemoglobin values were unaffected in chicks consuming high levels of dietary methionine. Plasma glucose was

increased in young chicks consuming the highest amount of methionine but older chicks' plasma glucose was not significantly affected.

Plasma methionine was elevated in young and old chicks consuming supplemental methionine. Plasma threonine was depressed in young chicks fed 3.0 X the N.R.C. requirement. In experiment 4, plasma glycine, serine, cystine and cystathionine were not affected by treatment. In experiment 5, plasma glycine was increased in young chicks fed 5.0 X the N.R.C. level but was decreased in older chicks consuming methionine at 3.0 or 5.0 X the requirement. Plasma serine and cystine were elevated in young chicks (experiment 5) fed the two highest levels of methionine but, in 5-week-old chicks, plasma serine and cystine were not altered by methionine supplementation of 5.0 X the N.R.C. requirement. Plasma cystathionine was not affected in either chick experiment or in either age group.

TABLE OF CONTENTS

CHAPTER		PAGE
I.	INTRODUCTION	1
II.	LITERATURE REVIEW	3
	General Considerations	3
	Specific Physical Abnormalities	5
	Methionine Pathways	9
	Biochemical Effects	10
III.	MATERIALS AND METHODS	33
	Experimental Animals and Treatments	33
	Data and Sample Collection	39
	Processing and Analysis of Samples	39
	Statistical Analysis of Data	40
IV.	RESULTS	42
	Experiment 1	42
	Experiment 2	42
	Experiment 3	52
	Experiment 4	61
	Experiment 5	64
V.	DISCUSSION	69
	Performance and Hematology	69
	Amino Acid Analysis	71
VI.	SUMMARY	76
LITERATURE CITED		79
VITA		83

LIST OF TABLES

TABLE		PAGE
1.	Percentage Composition of the Basal Diet (Experiment 1) . .	34
2.	Percentage Composition of Basal Diets for Experiments 2 and 3	36
3.	Percentage Composition of Basal Diets for Experiments 4 and 5	38
4.	Effects of Supplemental Dietary Methionine on Performance and Hematology (Experiment 1)	43
5.	Effects of Supplemental Dietary Methionine on Performance and Hematology (Experiment 2)	44
6.	Effects of Supplemental Dietary Methionine on Plasma Free Amino Acid Concentration (Experiment 2)	47
7.	Effects of Supplemental Dietary Methionine on Performance and Hematology (Experiment 3)	53
8.	Effects of Supplemental Dietary Methionine on Plasma Free Amino Acid Concentration (Experiment 3)	56
9.	Effects of Supplemental Dietary Methionine on Performance and Hematology (Experiment 4)	62
10.	Effects of Supplemental Dietary Methionine on Plasma Free Amino Acid Concentration (Experiment 4)	63
11.	Effect of Supplemental Dietary Methionine on Performance and Hematology (Experiment 5)	65
12.	Effects of Supplemental Dietary Methionine on Plasma Free Amino Acid Concentration (Experiment 5)	67

LIST OF FIGURES

FIGURE		PAGE
1.	Methionine Metabolism via the Phospholipid Choline Synthesis Pathway	11
2.	Methionine Metabolism via the Glycine Methyltransferase Pathway with the Scheme of Homocysteine Interconversion . .	12
3.	Alternate Pathway of Methionine Metabolism (Toxic Pathway)	13
4.	Effect of Supplemental Dietary Methionine on Gain by Period (Period = 7 Days; Experiment 2)	45
5.	Effect of Supplemental Dietary Methionine on Plasma Methionine by Period (Period = 7 Days; Experiment 2) . . .	49
6.	Effect of Supplemental Dietary Methionine on Plasma Threonine by Period (Period = 7 Days; Experiment 2)	50
7.	Effect of Supplemental Dietary Methionine on Plasma Glycine by Period (Period = 7 Days; Experiment 2)	51
8.	Effect of Supplemental Dietary Methionine on Gain by Period (Period = 7 Days; Experiment 3)	54
9.	Effect of Supplemental Dietary Methionine on Plasma Methionine by Period (Period = 7 Days; Experiment 3) . . .	58
10.	Effect of Supplemental Dietary Methionine on Plasma Glycine by Period (Period = 7 Days; Experiment 3)	59
11.	Effect of Supplemental Dietary Methionine on Plasma Threonine by Period (Period = 7 Days; Experiment 3)	60

CHAPTER I

INTRODUCTION

Indispensable amino acids are of major importance to the monogastric nutritionist. To maintain proper levels of these amino acids in the diet, it has been suggested that rations be balanced for specific amino acids rather than by the popular method of using crude protein. The production of certain crystalline amino acids has caused additional interest in balancing rations for amino acid content. As production techniques improve, as crystalline amino acids become increasingly economical and as greater emphasis is placed on balancing rations based on concentrations of the indispensable amino acids, the effects of excess supplementation of these amino acids must be considered.

Methionine has been shown to be the most toxic indispensable amino acid when consumed at levels exceeding its physiological requirement (Sauberlich, 1961). Studies utilizing rats have shown that growth, feed consumption and feed efficiency (gain:feed) were impaired and that tissue damage occurred when high levels of methionine (2 X the N.R.C. requirement) were consumed (Stekol and Szaran, 1962). Similar results have been observed in studies utilizing chicks (Katz and Baker, 1975a). In both rat and chick studies, methionine has been shown to affect amino acid concentrations in the blood (Klavins, 1965; Katz and Baker, 1975a). Plasma threonine, serine and glycine levels were depressed while plasma methionine increased with dietary methionine supplementation.

Considering that previous research has dealt mainly with members of the avian and rodent families and that specific indispensable crystalline amino acids may, in the future, be available for use in swine rations, this study's objective was to investigate the effects of excess methionine supplementation in weanling pigs. Also, experimentation utilizing chicks was employed to biologically test the methionine used in swine diets and to determine the response of chicks of different ages to excess dietary methionine consumption.

CHAPTER II

LITERATURE REVIEW

I. GENERAL CONSIDERATIONS

Literature concerning methionine was reviewed for this thesis. In studies designed to compare the relative toxicity of ingested amino acids, methionine has been found consistently to be the most toxic amino acid (Sauberlich, 1961). It has also been established that the isomeric form of methionine affects the degree of its toxicity. L-methionine is more toxic than its D-enantiomorph even though the D-isomer is converted to the L-isomer (Wretlind and Rose, 1950). Other conditions which must be considered when examining the adverse effects of excess methionine are age of the animal, quantity of protein in the diet, physiological state of the animal, and total food intake.

The age of the animal is important when studying the detrimental effects of disproportionate amounts of methionine. Young animals are more susceptible than older individuals due primarily to three factors (Harper et al., 1970). The first factor is that the quantities of an amino acid are often expressed as a percent of the diet.

A mature animal's intake per unit of body weight or per unit of surface area declines as the animal ages, therefore, the intake of each component of the diet (methionine) declines per unit body weight or size thus reducing the possibility of toxic effects.

The second factor is that requirements for amino acids decrease as a percent of the diet in the older animal, so that a diet that does

not meet the proper amino acid standard in a young animal will be more suitable for a more mature animal. Thirdly, the activities of several enzymes of amino acid catabolism increase as the animal ages, thus the animal has the ability to handle larger loads of dietary amino acids. Protein levels in the diet also influence the severity of effects from excess methionine. Increased protein in the ration may exert protective effects by providing additional quantities of the growth-limiting amino acids and thus stimulate growth. Furthermore, increased protein in the diet may be a defense mechanism against detrimental effects from excess methionine by providing additional quantities of amino acids for which the one in excess is antagonistic to or is needed for the catabolism of the excess amino acid. It should also be noted that the degree to which an amino acid is disproportionate with respect to the quantitites of other amino acids may be of greater importance than the absolute amount of an amino acid added to the diet.

The third factor which influences the tolerance of animals to surpluses of amino acids is the quantity of B-complex vitamins, more specifically, pyridoxine. This B-complex vitamin is an essential part of the coenzyme for many reactions of amino acid catabolism. The pyridoxine requirement increases as protein content in the diet increases, therefore, when excessive levels of an amino acid are consumed without a corresponding increase in the pyridoxine level, the catabolism of the excess amino acid may be hampered (Debey et al., 1952). The severity of adverse effects caused by excess methionine depends on two final factors. First, the physiological state or health of an animal may dictate the magnitude of the adverse response. Animals

not at their optimum physiological condition may be affected to a greater degree than healthy animals. Secondly, total food intake may contribute to the animal's response. As food intake increases, the excess amino acid intake increases, thus the probability of an adverse reaction in increased.

II. SPECIFIC PHYSICAL ABNORMALITIES

General factors which affect the intensity of the adverse effects of excess methionine consumption have been cited. However, excess methionine does cause specific abnormalities in several species of animals that have been studied. Animals studied include the human, avian (chickens and turkeys) and rodent (guinea pig, rabbit and rat). Studies done with human subjects differ from the other animal studies mainly due to the mode of administration of the methionine. Symptoms were observed after injections of methionine rather than after oral dosing or feeding of methionine as was the case with poultry and rodent studies. Human subjects receiving supplemental methionine did exhibit marked responses upon injection of thirty grams of methionine. In a study using two subjects, both individuals experienced untoward effects when methionine was infused. One subject developed nausea and vomiting while the other subject encountered a severe reaction which began soon after the infusion was completed. This reaction was characterized in the first few hours by nausea, vomiting, increased sweating, chill followed by fever, moderate hypotension, tachycardia, and intermittent disorientation. After eighteen hours, there was a mild azotemia and biochemical evidence of moderately severe hepatic

dysfunction. Electrolyte and glucose therapy were employed to correct this situation and a period of nine days was needed to return all affected body functions back to normal (Floyd et al., 1966). Excess methionine supplementation in poultry diets also created undesirable results. Experimentation utilizing nine day old chicks demonstrated a reduction in both rate and efficiency of gain when 1.25% excess methionine was present in the diet. This same level of excess methionine induced anorexia, but the reduction in gain was greater than what would be expected from the corresponding reduction in feed consumption (Katz and Baker, 1975a).

The other parameters studied in the chick were the hematological profile (hemoglobin and hematocrit), blood glucose level and condition of the spleen. One study determined that young chick's hemoglobin and hematocrit levels decreased as the amount of excess methionine increased in the diet (Harter and Baker, 1978a). In this same study, a correlation between increased spleen iron deposition and decreased blood hemoglobin was reported. It appears that excess methionine accelerates red cell destruction, thus releasing the iron bound in hemoglobin. These damaged red blood cells are filtered out via the spleen and iron accumulates creating a state known as splenic hemosiderosis (Mengel and Klavins, 1967). Blood glucose level was another factor affected by excess levels of dietary methionine. Hypoglycemia was not uncommon in a methionine-induced toxicity and a reduction in plasma glucose did indeed occur when 1.25% methionine was fed to chicks (Katz and Baker, 1975a). The process by which a state of

hypoglycemia is created in animals fed excess methionine is not yet clearly understood.

Another family of animals that has been used in methionine toxicity work is the rodent. Studies done with rats revealed that, as in the poultry experiments, a reduction in weight gain was approximately inversely proportional to the amount of methionine consumed (Stekol and Szaran, 1962).

It was also determined in experimentation with rats that the spleen was affected. Spleens examined from rats fed excessive levels of methionine were normal in shape and consistency but abnormally dark, reddish to black in color and enlarged (Stekol and Szaran, 1962). In later studies, it was discovered that excess levels of methionine fed to rats resulted in increased red blood cell accumulation and iron deposition in the spleen (Benevenga et al., 1976). This would explain the abnormalities observed in the spleens of excess methionine fed rats. Along with these findings, early investigations of the hematology of rats fed high levels of methionine explored the possibility that methionine resulted in definite anemia after one week. However, although accelerated erythrocyte destruction was indicated in high methionine diets (Mengel and Klavins, 1967), erythroid hyperplasia may occur and thus a dramatic decline in hematocrit and hemoglobin levels was not observed in the majority of studies utilizing rats (Cohen and Berg, 1956). Several similarities exist when comparing the anomalies produced in poultry and rodent methionine toxicity studies. Despite these similarities, an area of demarcation surfaces in that rats have the ability to adapt to high level methionine consumption whereas chickens

and turkeys do not. Rats gradually adapt to excess methionine diets presumably by altering their metabolic response to the elevated amino acid. This alteration may be brought about by the rat's ability to change levels of key hepatic enzymes. Adaptation to excess methionine may come about by slowing down the metabolism of the ingested excess amino acid, channeling that which is metabolized into pyruvate rather than taurine and thus provided energy for growth, and increased the urinary excretion of the excess amino acid. It should be noted that growth depression occurs before the adaptation mechanism begins (Daniel and Waisman, 1969). In a related study, the long-term adaptation of weanling rats to a high dietary level of methionine was examined. Several metabolic factors were considered and it was discovered that elevation in circulating corticosteroids was responsible for the stimulation of catabolic activity which directly affected plasma levels of the free amino acid, methionine (Girard-Globa et al., 1972). Through comparison of the effects of excess methionine on humans, poultry and rodents, marked similarities as well as differences have been demonstrated. There are also obvious differences in relation to excess methionine within a classification of animals such as rodents. The effects of high level feeding of methionine to rats have previously been cited. Rats do appear to be able to cope with excess methionine to some degree. Another member of the rodent family, the guinea pig, seems to be much more sensitive to high levels of methionine than the rat. Guinea pigs react dramatically when orally dosed with excess methionine. A study utilizing guinea pigs reported that after administration of oral doses of methionine, these animals developed

early fatty liver formation, hypoglycemia and aminoacidemia. Later, these same animals developed hypothermia, profound hypoglycemia and death within sixty hours. In striking contrast, rats, treated in the same manner as the guinea pigs, did not develop hypoglycemia or any of the problems observed in the guinea pig. Rats actually thrived on the treatment which was proven fatal to the guinea pig (Hardwick et al., 1970). The adverse effects of excess dietary methionine are produced via methionine's affect on metabolic or biochemical functions. A basic grasp of methionine catabolism is helpful in understanding the possible origin of the detrimental effects caused by excess methionine intake.

III. METHIONINE PATHWAYS

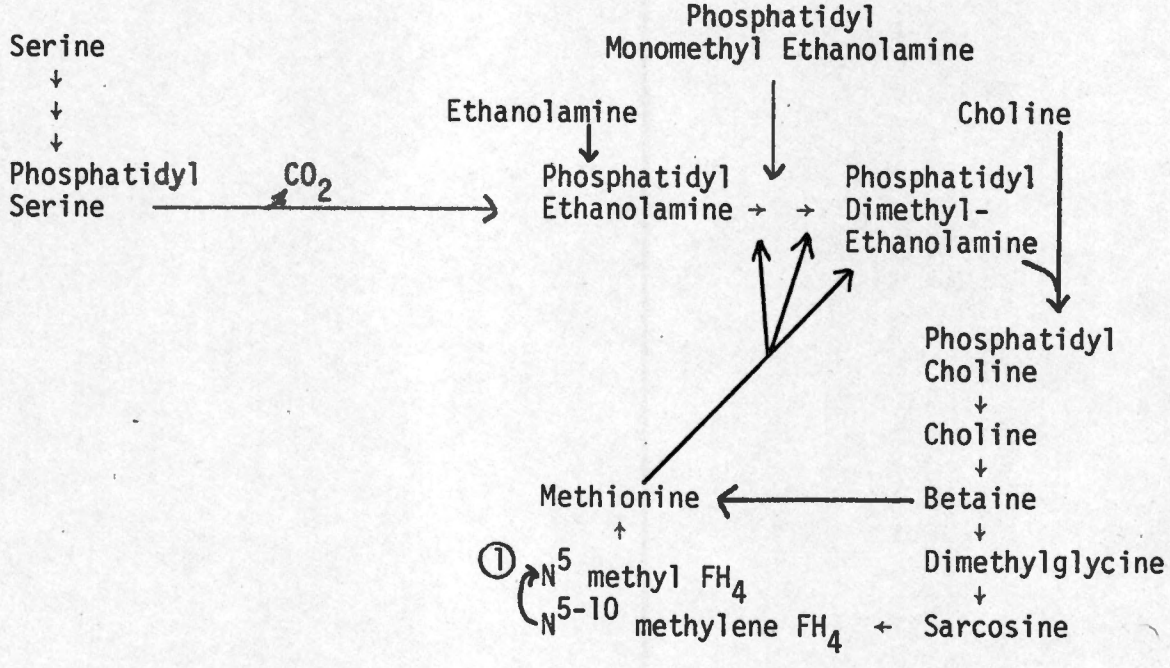
Adverse effects observed following consumption of excessive levels of methionine are due to the aberrant metabolism of the methyl group (Benevenga, 1974). At least three pathways are involved in the conversion of the methyl group to CO_2. One involves the phospholipid choline synthesis pathway and is apparently saturated at low concentrations of methionine. Another involves the formation of sarcosine (N-methylglycine) and may account for a portion of the metabolism of methionine at concentrations over that which can be handled by the choline synthesis pathway. The pathway involving sarcosine formation is known as the glycine methyltransferase pathway.

A third pathway, possibly the pathway of toxicity, is competitively inhibited by S-methyl-L-cysteine and appears to account for a majority of the metabolism of methionine at high concentrations. This pathway is termed the S-methyl-L-cysteine-like pathway. It is an

intermediate or end product of the degradation of methionine via this pathway that is postulated to be the toxic product. The following schematic diagrams of methionine metabolism illustrate portions of these three pathways (Figures 1, 2, and 3).

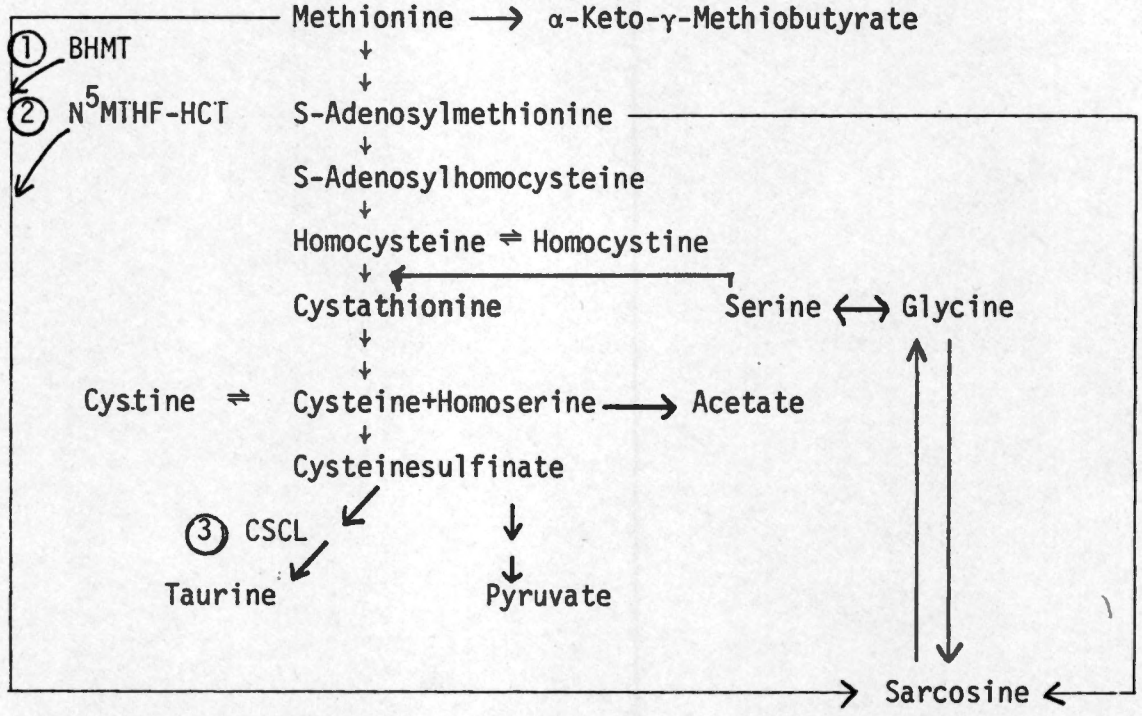
IV. BIOCHEMICAL EFFECTS

The biochemical information contained within the previously cited metabolic pathways was utilized by researchers in their investigations of the adverse effects of methionine. One early report, using rats fed a low protein diet containing 5% D,L-methionine, suggested that detrimental occurrences appeared to be related to the dietary needs and the relative abundance of methionine in tissues (Sauberlich, 1961). Methionine, which is normally found in the least amount in tissues of the rat, was toxic when fed in excess. Major general metabolic implications were derived from this study. It was noted that alterations in the balance or ratio of the free amino acids in the plasma, as a result of feeding excessive levels of methionine, may have been responsible for some of the adverse effects. Elevated levels of methionine in the plasma reduced feed consumption and thus reduced rate of gain. Consequences of changing plasma amino acid balance was the possible interference of transporting amino acids into cells while hindering enzymatic ability and equilibrium for tissue protein synthesis. High plasma free amino acids may also interfere in the reabsorption of filtered essential or limiting amino acids by the kidney, thereby creating a deficiency of some essential amino acids. Furthermore, the rate of detoxification, metabolism and removal of excess methionine or



① N^5-methyl tetrahydrofolate.

Figure 1. Methionine Metabolism via the Phospholipid Choline Synthesis Pathway. (Benevenga, 1974)



① Betaine-homocysteine methyltransferase.

② N^5-methyl tetrahydrofolate-homocysteine methyltransferase.

③ Cysteinesulfinate carboxylase.

Figure 2. Methionine Metabolism via the Glycine Methyltransferase Pathway with the Scheme of Homocysteine Interconversion. (Benevenga, 1974; Harter and Baker, 1978b)

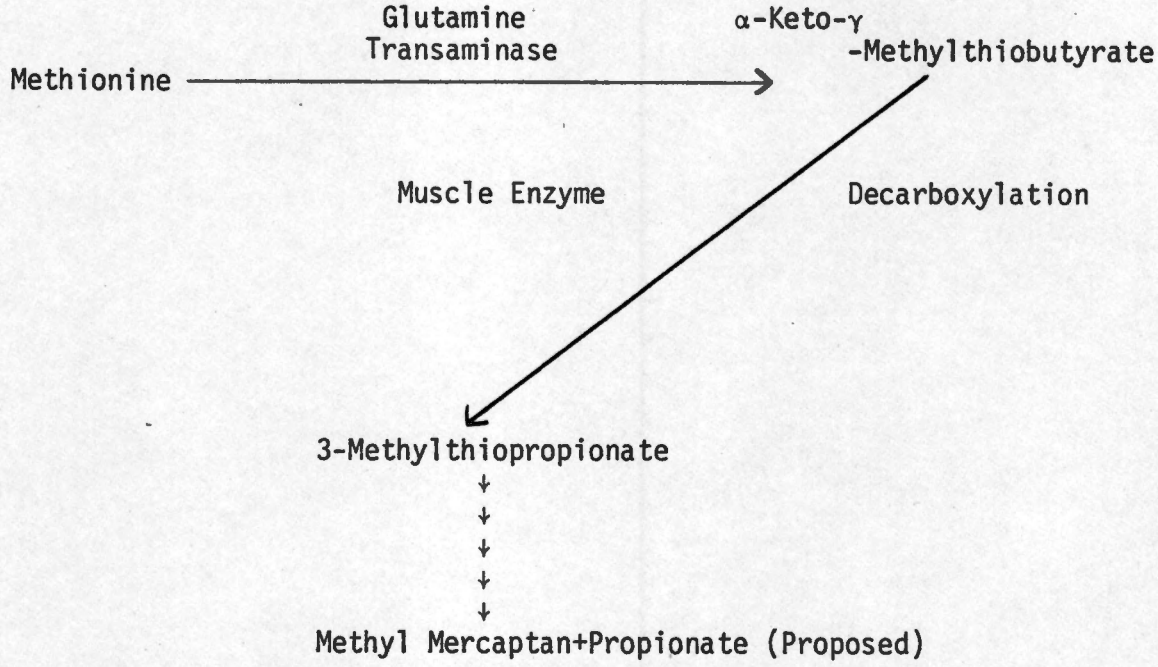


Figure 3. Alternate Pathway of Methionine Metabolism (Toxic Pathway). (Adapted from reports by Mitchell and Benevenga, 1978; Harter and Baker, 1978a; Steele et al., 1979.)

its breakdown products in the plasma may have been a contributing factor to the adverse effects observed with high level methionine intake. Also, limitations in the availability of compounds necessary in the removal of detoxification of methionine may have accounted for toxic effects. To substantiate these hypotheses, Sauberlich (1961) supplemented the high methionine diet with glycine (possibly limiting when methionine is present in excessive levels). It was discovered that additional dietary glycine did increase food intake when compared to high methionine diets without supplemental glycine.

In a later study, using rats fed 4% D,L-methionine in a low protein diet, other metabolic alterations were observed (Klain et al., 1963). Notable increases in the activity of specific liver enzymes such as arginase, tryptophan pyrrolase, glutamic-oxaloacetic and pyruvic transaminase were reported. Decreased levels of nicotinamide adenine dinucleotide (NAD) and slightly elevated levels of total and neutral fat were observed. Other abnormalities which developed due to ingestion of excess methionine were increased levels of some amino acids in the urine and severe disorders in nitrogen metabolism. As in the previous study, supplemental glycine counteracted some of the maladies created by high methionine consumption.

Further work utilizing rats fed 4.5% D,L-methionine was conducted to examine the effects of methionine on free amino acids in serum (Klavins, 1965). In this study, three experimental groups of rats were used. The first group was fed excess methionine ad libitum while the second group was fed the basal diet in an amount similar to that being consumed by group one (Pair feeding). Group three was fed the

basal diet in amounts to cause the same rate of gain as group one (Weight control feeding). Results from this study indicated that excess methionine caused significant alterations in serum amino acid composition. Increased levels of methionine, α-aminobutyric acid, lysine and glutathione in the serum were noted. Excess dietary methionine was antagonistic towards serine, glycine, threonine, valine and leucine. The effect methionine exerts on serum amino acid concentrations was more evident in those amino acids, with the exception of lysine, which are normally in serum at higher concentrations than methionine. The elevated serum glutathione levels were attributed to possible release of this compound from red blood cells due to methionine-induced hemolysis. It was also determined in this study that excess methionine caused increased requirements of glycine and/or arginine. The methionine-induced hemolysis of red blood cells was established well before the Klavins study of 1965. The earlier study discovered the occurrence of erythroid hyperplasia and increased red cell turnover in rats fed high methionine diets (Cohen and Berg, 1956). However, that early work did not explain the mechanism of the methionine-induced anemia. A project designed to ascertain the mechanism was accomplished (Mengel and Klavins, 1967). Their study utilized rats fed diets in amounts to produce isocaloric intake. Three diets which varied in supplemental methionine (.15 molal, .30 molal, 0 methionine) were used. They reported that feeding methionine resulted in definite anemia after one week of the experiment. Anemia was slightly higher in the rats fed the highest level of methionine. It was also noted that a decrease in hematocrit, an increase in reticulocyte count and an increase in fecal

urobilinogen excretion occurred. Further investigation revealed that the half-life of red blood cells in methionine-fed rats was markedly reduced. Accelerated red blood cell destruction was also indicated in rats consuming high methionine diets. These authors theorized that since there was an absence of methemoglobin formation or presence of Heinz bodies, and that an increase in glucose-6-phosphate dehydrogenase activity with reduced glutathione content occurred, it was unlikely that hemolysis reflected an overload of the hexose monophosphate shunt. Also, normal autohemolysis and osmotic fragility of erythrocytes would not support a gross defect in glycolysis in the red blood cell. In the opinion of these researchers, hemolysis was the cause of anemia, but the mechanism inducing this anemia was not elucidated. In another report, amino acid levels and enzyme activity in tissues of rats force fed diets differing in methionine content was examined (Sanchez and Swendseid, 1969). Animals were force fed one of three diets for a period of three days. The control group received a complete amino acid diet. Experimental groups received either a diet similar to the complete amino acid diet minus methionine and cystine or a diet similar to the complete amino acid diet plus 2% or 4% L-methionine as a supplement. Results from this study indicated that excess methionine caused alterations in the concentration of a number of amino acids, particularly in muscle tissue. Increases were detected in methionine, histidine, alanine and α-aminobutyric acid levels in samples of tissue, plasma and liver. Conversely, glycine was decreased in all tissues. Other notable results were the increased activity of threonine dehydratase and the enhanced activity of amino acid catabolizing

enzymes in animals consuming 4% supplemental methionine. Also, methionine was shown to lower ornithine and arginine levels as a result of alterations in the urea cycle enzymes. One final conclusion drawn from this report was that with excess methionine feeding, increased blood corticosterone levels developed.

The biochemical occurrences and the adaptation of weanling rats to diets containing excess methionine were considered in the following study (Daniel and Waisman, 1969). A 24% protein laboratory feed was ground and fed as the control diet. The high methionine diet consisted of the 24% chow diet with 3% supplemental L-methionine. Some rats were fed ad libitum while others were pair-fed as a group. Biochemical alterations resulting from high methionine feeding were decreased cystathionine synthetase and cysteine-sulfinate carboxylase activities. Increases in serine dehydratase tryptophan pyrrolase, tyrosine transaminase and glutamic acid dehydrogenase activities were discovered during the most toxic stages of methionine feeding. Other results caused by high methionine consumption were a fifteen-fold increase in hepatic free methionine, an increase in liver taurine levels, and a decrease in liver serine levels. These findings occurred rapidly following excess methionine intake, but after five to seven days, growth resumed and adaptation was evident even though toxic levels of methionine were not reduced. These researchers postulated that rats adapt to excess methionine by three methods. First, adaptation occurs by slowing down the metabolism of the ingested excess amino acid. Secondly, rats adapt by channeling that which is metabolized into pyruvate rather than taurine and thus provided a source of energy for

growth. Finally, increased urinary excretion of the excess amino acid aids in the adaptive process. Another observation presented was that growth depression occurs before the adaptation mechanism begins.

Interest in the effects of excess methionine consumption in various animals compared to effects cited in studies utilizing rats has resulted in a number of experiments. One such comparative study was accomplished using guinea pigs and rats (Hardwick et al., 1970). Animals were gavage fed either water or L-methionine in addition to being fed a fox diet ad libitum. Some of the animals receiving excess methionine were given injections of adenine intraperitoneally in a single dose. This was carried out to test the assumption that biochemical changes induced by methionine intoxication were mediated through an hepatic adenosine triphosphate (ATP) insufficiency. Findings from this report demonstrated that species differences may occur. Methionine administered to guinea pigs was lethal in most instances. Hepatic lipid was always present in the terminal state along with depression of hepatic glycogen, severe hypoglycemia and generalized aminoacidemia. Hepatic ATP concentrations were about 30% of that of control animals. It was discovered that adenine prevented the development of depressed blood sugar as well as the onset of decreased hepatic ATP concentration. Although excess methionine intake adversely affected guinea pigs in this particular study, rats were found to be less sensitive to L-methionine at the levels used. Various biochemical observations were noted in this study. The fall of hepatic ATP was found to be concurrent with abrupt rises in hepatic S-adenosyl methionine and hepatic total adenosylthionium products (S-adenosyl-methionine and S-adenosylhomocysteine). The rise in

S-adenosylhomocysteine did slowly decline one hour after dosing. Other occurrences reported were that the rise in adenosylthionium products was about the same magnitude as the total fall in hepatic ATP concentration and the fall in hepatic and pancreatic ATP concentrations were concurrent to rises in renal and splenic ATP concentrations. Also mentioned was a tendency toward increased heart, psoas muscle, and lung ATP concentrations in methionine-fed animals.

Another avenue of research has been the possibility of a metabolite in the transmethylation pathway (normal methionine metabolism pathway) being the causative factor in methionine toxicity. A study comparing methionine and homocystine was undertaken to investigate the occurrence and alleviation of these toxicities (Benevenga and Harper, 1967). Results from this study indicated that rats fed a diet containing 10% casein and 3% L-methionine were adversely affected to a greater degree than rats fed a similar diet with isosulfurous amounts of homocystine. Portions of the toxicity state (growth depression) of methionine and homocystine were alleviated, but not prevented, by glycine and serine supplementation. Glycine appeared to be more effective than serine in reducing methionine toxicity but serine was more effective in alleviating homocystine toxicity. Several amino acids were tested for their ability to alleviate methionine and homocystine toxicity, but only glycine and serine were found to favorably reverse these toxicities.

Using results compiled from several reports concerned with methionine toxicity, a summary of that data was published (Harper et al., 1970). One aspect of methionine toxicity discussed was a theory

proposing that the adverse effects of excess methionine came about as a result of the labile methyl group of methionine. In a previously cited study (Benevenga and Harper, 1967), homocystine was found to adversely affect animals in a fashion similar to excess methionine.

If homocystine was the toxic product, the labile methyl theory would have proven untenable. However, excess consumption of homocystine did not produce iron accumulation in the spleen as has been shown to be the case in methionine toxicity. Thus, the labile methyl group theory was not discounted. In an attempt to reinforce this theory, methyl donors such as choline, betaine, dimethylthetin and methionine sulfoxide were fed and were reported to have essentially the same affect as methionine. The alleviation of methionine toxicity was also discussed. Noted were the beneficial affects of additions of both arginine and glycine. Glycine and arginine are precursors of guanidino-acetic acid, which accepts the methyl group of methionine to form creatine. This was thought to be a mechanism by which methionine catabolism could have been increased. This has been proven to be unlikely for three reasons: (1) glycine alone was as effective as a combination of the two; (2) glycine and arginine additions did not enhance creatinine or creatine excretion; and (3) guanidionacetic acid has been proven to be toxic. One final postulation was that methyl acceptors (nicotinic acid) do not seem to alleviate the symptoms of methionine toxicity. As information on the biochemical aspects of methionine toxicity were discovered, other studies were conducted to add to the existing data. The long-term adaptation of weanling rats to high dietary levels of methionine and serine was investigated

(Girard-Globa et al., 1972). In this study, five week old rats were fed an 18% casein diet with either 2% D,L-methionine, 4% excess serine, or 4% excess serine plus 2% D,L-methionine. Some rats in the control group were fed restricted levels of the basal diet and other control rats were pair-fed with excess methionine fed rats. Rats did adapt to high methionine as evidenced by decreasing levels of free methionine in the plasma. Adding serine to excess methionine diets lowered plasma methionine but did not bring the methionine levels back to normal. Methionine sulfoxide was elevated by excess methionine feeding but additional serine brought it back to within the normal range. Other amino acids affected by high methionine intake were α-aminobutyric acid and taurine. Both were elevated initially in excess methionine-fed rats. Additional serine affected taurine differently over time. Early in the experiment, taurine was elevated in rats fed a diet with 2% supplemental methionine. Serine was apparently the limiting factor in taurine synthesis. At a later time, additional serine in the diet lowered taurine levels relative to those noted in methionine-fed rats. Taurine may no longer have been the degradation product of cystathionine formed from methionine and serine interaction. Cysteine, glycine and threonine were also affected by excess methionine consumption. Cysteine was elevated by an oral load of methionine. Glycine levels were decreased in rats fed excess methionine and excess serine. It was also postulated that plasma threonine was decreased by methionine due to stimulated activity of serine dehydratase brought about by excess methionine consumption.

Studies aimed at defining the mechanism by which methionine exerts its toxic effects gave still more insight into the methionine toxicity investigations. Researchers devised a biochemical comparison in an effort to explain the alternate pathway (toxic pathway) of methionine toxicity. The use of a compound that resembled methionine in its toxic activity (S-methyl-L-cysteine) was studied (Benevenga, 1974). S-methyl-L-cysteine is not found in vivo, but it has been helpful as a means for deducing alternate methionine metabolism. In this endeavor, S-methyl-L-cysteine and methionine were suggested to compete at a transport site or at some early site of catabolism. Noting that S-methyl-L-cysteine is not activated to S-adenosylmethionine as methionine may be and that the S-methyl-L-cysteine pathway may result in production of the methionine metabolite responsible for toxicity, it seemed plausible that these compounds may indeed competitively inhibit one another in an alternate pathway leading to a toxic end product. A subsequent study utilized the hypotheses from the previously cited work to determine the growth depression and tissue reaction to the consumption of excess dietary methionine and S-methyl-L-cysteine (Benevenga et al., 1976). Results from this study indicated similarities in the effects of high methionine and S-methyl-L-cysteine (SMC) intake in rats.

Consumption of the SMC diet caused an 85% depression in growth and a 65% reduction in food intake. Greater reductions in these parameters were observed when excess methionine was supplemented in the diet. It was also clear from this study that consumption of excessive levels of methionine or SMC resulted in an increase in red blood cells

in splenic sinusoids and increased deposition of iron in the spleen. Other findings presented were that the methyl groups of methionine and SMC were rapidly converted to CO_2 and that SMC and methionine may have related or identical toxic metabolites. The ability of serine to partially correct methionine aberrations was not noted in an experiment where serine was added to SMC diets. The theory that adverse effects of excess consumption of methionine is due to the metabolism of the methyl group was reiterated. Another study investigating the metabolic mechanism of methionine toxicity examined the role of transamination of methionine (Mitchell and Benevenga, 1978). The product of methionine transamination in the rat was identified as α-keto-γ- methiolbutyrate. This transamination of methionine may be an alternate pathway for methionine oxidation which does not involve its activation to S-adenosyl-L-methionine. This seems to be a valid hypothesis since L-methionine toxicity has not been explained by way of known pathways of methionine. These researchers stated that the transamination of the D-isomer or hydroxy analog of methionine makes them available for further utilization. Furthermore, a keto acid can be transaminated with glutamine to yield methionine. Proper enzymes must be present if transamination of methionine is to occur. The appropriate enzymes were evaluated in their study. Glutamine transaminase was active toward both methionine and α-keto-γ-methiolbutyrate. Leucine transaminases were also shown to transaminate methionine with α-keto-glutarate at rates identical to those obtained with leucine. It was of importance to note that a keto acid can be transaminated to produce methionine because α-keto butyrate is known to be a product of methionine metabolism via

the trans-sulfuration pathway. The α-keto-γ-methiolbutyrate compound was determined to be the most effective keto acid co-substrate for the transamination of methionine, while α-keto butyrate and homoserine (homoserine is rapidly converted to α-keto-γ-butyrate) were shown to be inhibitors of methionine oxidation. Also mentioned was the use of amino-oxyacetic acid, a potent inhibitor of pyridoxal phosphate dependent aminotransferases and a competitor with the amino acid substrate, as a compound to establish evidence that a link exists between transamination and oxidation of methionine.

Even though the majority of research cited thus far has pertained to studies utilizing rats, several studies have examined the toxic manifestations exhibited when members of the avian specie consumed excessive amounts of methionine. Nutritional and metabolic implications of methionine toxicity in the chick were investigated (Katz and Baker, 1975a). Consumption of excess methionine at a level of 1.25% caused a reduction in plasma glucose, impaired threonine utilization and was antagonistic toward glycine. Glycine, serine, arginine, threonine and adenine were tested for their ability to alleviate toxic symptoms. It was found that the reduction of both rate and efficiency of gain and depressed blood glucose were improved when the methionine imbalanced diet was supplemented with glycine, serine, or threonine, but neither arginine or adenine aided in reversal of these parameters. Although adenine supplementation did not improve growth or feed efficiency, it was effective in reversing plasma glucose depression. Metabolic observations were numerous. The oxidation of threonine to CO_2 was increased in response to excess methionine feeding. This occurrence

may have made threonine growth limiting. Plasma threonine levels may be lowered by the increase in serine-threonine dehydratase which occurs during excess methionine consumption or by a reduction in the utilization of dietary threonine. The beneficial response of adding threonine to the excess methionine diet was probably due to its furnishing glycine via the threonine aldoase pathway, after which threonine becomes limiting. Glycine was found to be even more effective than threonine in alleviating methionine toxicity. This may be the case due to glycine's involvement in methionine metabolism both as a methyl acceptor and as a source of serine to convert homocysteine to cystathionine. In the chick, homocysteine accumulation in plasma and tissues may explain the bulk of the effects attributable to excess methionine consumption. These researchers stated that glycine may become the first limiting amino acid in methionine toxicity. This may implicate the cystathionine synthetase catalyzed reaction leading to cystathionine formation from homocysteine as a possible rate-limiting reaction in methionine catabolism. Another study involving methionine toxicity in the chick attempted to further define the metabolic processes occurring during consumption of excess methionine (Katz and Baker, 1975b). It was discovered that methionine, calcium hydroxy analog (OH-M(ca)) and ethionine lowered concentrations of hepatic ATP. They also noted that homocysteine and homocystine were as toxic as methionine. Using this information, these researchers indicated that the theory of the methyl group of methionine causing toxicity was questionable. Metabolism of the methyl group of methionine precedes the formation of homocysteine and thus could not be involved in the

toxicity resulting from consumption of high levels of homocysteine. To further substantiate the toxic effects of methionine intake in the avian species, poults and chicks were fed 2% D,L-methionine to determine whether high levels of methionine affected them in a comparable fashion (Hafez et al., 1978). Results from this study revealed that excess methionine did cause growth depression in poults. Equimolar amounts of homocystine also depressed growth but to a lesser degree than excess methionine. The addition of betaine, a methyl donor, improved the condition of birds fed homocystine but not those consuming methionine. It was also observed that poults receiving excess methionine or homocystine developed a cervical paralysis similar to that caused by a folic acid deficiency. For this reason, folic acid was added to the experimental diets. The addition of folic acid did not improve growth or cervical paralysis or perosis in the poults. From this study, it was concluded that the severe perosis observed in homocystine fed groups was probably a result of a deficiency of methyl groups necessary for choline synthesis which is required for the prevention of perosis. These researchers also discounted the theory that methionine toxicity is due to its methyl group. They suggested that the homocysteine moiety is involved in methionine toxicity.

As research data from various experiments examining methionine toxicity was analyzed, some theories on the mechanism of methionine toxicity became questionable. The theory of depleting hepatic ATP in adenylating methionine to S-adenosylmethionine and the theory of depleting methyl acceptors in the conversion of S-adenosylmethionine to S-adenosyl homocysteine have been proven unplausible. A third theory

investigating the metabolism of the labile methyl group of methionine via an alternate pathway, not requiring S-adenosylmethionine formation, has been the focus of recent experimentation. A study consisting of six separate experiments was conducted to establish if this third theory was acceptable (Harter and Baker, 1978a). The six experiments conducted were designed to: (1) determine if excess methionine or 3-methylthiopropionate (alternate pathway metabolite) caused decreased gain and increased spleen iron; (2) determine the effect of excess methionine or homocysteine on the plasma amino acid concentration of methionine, homocystine, cystathionine and cystine (metabolites in the trans-sulfuration catabolic pathway); (3) relate spleen iron deposition with decreased blood hemoglobin induced by growth reducing intakes of methionine; (4) determine if choline, betaine, methyl acetate (all three are methyl donors), methyl mercaptoacetate or methyl mercapton (both supply thiomethyl groups) would cause spleen iron deposition; (5) determine if supplemental glycine and threonine would alleviate spleen iron accumulation as well as improve gain and efficiency; and finally (6) determine if supplemental glycine or threonine would affect spleen iron deposition caused by 3-methylthiopropionate. All trials were done utilizing chicks as the experimental animal. Experimental findings revealed that excess methionine and 3-methylthiopropionate caused slower gains, reduced efficiency and increased spleen iron. Consumption of 3-methylthiopropionate elicited a greater adverse response in each case. Growth rate and efficiency were also reduced when D,L-homocysteine was fed. Plasma methionine increased when excess methionine was consumed. Homocysteine also increased plasma levels

slightly. Homocysteine's effect on plasma methionine may have been a result of the transmethylation of homocysteine back to methionine. Levels of homocysteine, cystathionine and cystine in the plasma were not increased when excess methionine was fed. However, homocysteine feeding did increase plasma homocystine without affecting plasma methionine, cystathionine or cystine levels. As noted in previous studies, hemoglobin levels decreased while spleen iron accumulation increased when methionine was given in excessive amounts. Other results demonstrated that the methyl donors tested did not affect gain, efficiency, hemoglobin or spleen iron. It was discovered that methyl mercaptoacetate and methyl mercaptan reduced growth rate and feed efficiency but did not increase spleen iron or depress hemoglobin. The supplementation of excess methionine diets with glycine and threonine improved both weight gain and feed efficiency. Glycine and threonine supplementation also reduced spleen iron accumulation caused by excess methionine. This type of supplementation may have restored metabolic efficiency which resulted in improved conditions. Homocysteine diets were also supplemented with glycine and threonine. Supplementation improved gain and restored feed efficiency but did not eliminate the anorexia caused by homocysteine consumption. The effect of additional glycine and threonine in 3-methylthiopropionate diets was examined. Glycine and threonine additions did improve weight gain but not back to the level of birds fed the basal diet. Also, glycine and threonine supplementation to diets with 3-methylthiopropionate did not decrease spleen iron accumulation. The assumption that homocysteine's transmethylation back to methionine was the homocysteine toxic pathway

seemed untenable to these researchers. Differences in lesions associated with methionine and homocysteine toxicity were noteworthy. Spleens of animals fed excess homocysteine appeared normal while spleens of methionine fed birds were dark and small. Hemolytic anemia, which is an adverse effect of excess methionine consumption, did not occur when excess homocysteine was fed. It was also determined that excess homocysteine consumption did not increase the oxidation of threonine to carbon dioxide as was noted when excess methionine was fed. The thought that methionine toxicity was caused by a build-up, in plasma or tissues, of normal catabolic pathway intermediates (homocysteine, cystathionine) was also dismissed. Findings from this study demonstrated that methionine metabolism via an alternate pathway, which forms toxic metabolites, may possibly have been the source of methionine toxicity. Consumption of 3-methylthiopropionate, an endproduct of the oxidative decarboxylation of methionine's keto analog, produced effects similar to those observed when excess methionine was fed. The feeding of 3-methylthiopropionate decreased weight gain, feed efficiency, hemoglobin levels and increased spleen iron to an equal or greater extent than the intake of excess methionine. Another consideration investigated was the metabolism of the labile methyl group of methionine in the alternate pathway resulting in a toxic product. These researchers found this not to be the case in chickens. Methyl donors (betaine, choline) did not reduce or affect gain, feed efficiency, hemoglobin or splenic hemosiderosis. One other mechanism studied was the conversion of 3-methylthiopropionate to methyl mercaptan or methylthioacetate (toxic endproducts). Again, these researchers determined that

methyl mercaptan or methylthioacetate were not part of the mechanism responsible for the toxicity. Feeding these proposed toxic end-products did not result in spleen iron accumulation while 3-methylthio-propionate feeding did produce that effect. This may suggest that 3-methylthiopropionate is the end product of the alternate pathway. Finally, factors influencing saturation of excess methionine on normal pathway activity was discussed. Excess methionine may saturate the activity of normal catabolic pathway enzymes, however, this does not seem to be the case. Conversely, pathway substrate depletion, namely substrates involved in the conversion of homocysteine to cystathionine, may have reasonable application. Methionine increases serine-threonine dehydratase which in turn may reduce plasma serine and threonine levels. Decreased serine will adversely affect the conversion of homocysteine to cystathionine while a reduction in plasma threonine may cause a secondary threonine deficiency. Glycine and threonine supplementation to toxic diets have been shown to reduce the growth depression caused by excess methionine intake, but they do not improve the adverse conditions resulting from alternate pathway metabolites (3-methylthio-propionate). Glycine and threonine, both of which can be metabolized to serine, may facilitate increased catabolism of homocysteine to cystathionine which may then result in more methionine catabolism via the normal trans-sulfuration pathway and less by the proposed alternate pathway. The role of an alternate pathway for methionine catabolism and the metabolites of that pathway have been the subject of a recent study. The effects of dietary 3-methylthiopropionate on metabolism, growth and hematopoiesis in the rat were examined to

determine if consumption of excessive amounts of 3-methylthiopropionate (MTP) resulted in a toxicity similar to that of excess methionine (Steele et al., 1979). A 10% casein diet with either supplemental methionine, methionine plus glycine, methionine plus serine, MTP, MTP plus glycine, or MTP plus serine were fed ad libitum to young male rats. Growth and food intake patterns of rats fed MTP diets were found to be similar to those observed with rats fed high levels of methionine. Also, MTP fed rats' spleens were darkened and enlarged as was the case with spleens from rats fed excessive levels of methionine. Glycine or serine supplementation to MTP diets resulted in no differences in growth or food intake when compared to control groups. Supplemental glycine or serine improved bodyweight but not as dramatically as when added to high methionine diets. Splenic hemosiderosis was increased in MTP-fed rats while splenic hematopoiesis was decreased in these same animals. Hematocrit and hemoglobin levels of MTP-fed rats were normal. The finding that glycine and serine alleviation of methionine toxicity occurs by enhanced degradation via the trans-sulfuration pathway was analogous with findings from the previous study. Glycine alleviates methionine toxicity by accepting the methyl group from S-adenosylmethionine to form sarcosine and serine reduces the toxic effects by reacting with homocysteine to form cystathionine. Glycine or serine supplementation did not eliminate the adverse effects due to excess dietary MTP, but this seems reasonable since MTP does not result from trans-sulfuration pathway metabolism. A final point discussed was the metabolic adaptation of rats to excess methionine. Metabolic adaptation did occur as evidenced by increased oxidation of methionine methyl groups in rats

fed excess dietary methionine and the diminishing of methionine's detrimental effects as time advanced.

Presently, the mechanism by which methionine toxicity exerts its adverse conditions has not been totally substantiated. However, the current hypotheses involve the theory of an alternate (toxic) pathway initiating the toxicity. More specifically, it appears that methionine toxicity is due to the aberrant metabolism of the methylthiol (CH_3-SH) group (Benevenga, 1979) or methionine's conversion to 3-methylthio-propionate with concomitant production of hydrogen sulfide and methyl mercaptan (Baker, 1979). Furthermore, the alternate pathway may become dominant when a cystine deficiency occurs or an excess amount of methionine is consumed.

CHAPTER III

MATERIALS AND METHODS

I. EXPERIMENTAL ANIMALS AND TREATMENTS

A series of 5 experiments was conducted. The first three experiments utilized 62 cross bred pigs with the remaining experiments utilizing 60 chicks in experiment 4 and 108 chicks in experiment 5. Chicks utilized in the experiments were of the Hubbard X Hubbard strain. Pigs in all experiments were allotted within litter blocks and by weight. The pigs in experiment 1 were housed in 2.1 x 8.5 meter pens in the growing-finishing facilities at The University of Tennessee Blount Swine Unit. A self-feeder with two feeding spaces was provided in each pen and water was available via nipple waterers. The floor of each pen was solid concrete, sloped toward a gutter outside the pens. Areas housing animals were scraped daily. Pigs utilized in the first experiment were 5 to 6 weeks of age and averaged 13.0 to 13.9 kilograms (kg) at the beginning of the trial. Three rations were fed during this experiment with two replications (6 pigs per replication) per ration. The composition of basal and experimental diets are presented in Table 1. Diets differ only in the amount of D,L-methionine and cornstarch (additional D,L-methionine replaced equivalent amounts of cornstarch in diets two and three). The basal diet (diet 1) contained the National Research Council (NRC) requirements of sulfur amino acids (SAA) for 20 to 35 kilogram pigs. Diets 2 and 3 contained

Table 1. Percentage Composition of the Basal Diet (Experiment 1)[a]

Ingredient	International Reference Number	Percent
Cornstarch	4-02-889	To 100
Soybean Meal (48% C.P.)	5-04-612	32.0
Soybean Oil	4-07-983	2.0
Dicalcium/Monocalcium Phosphate	6-01-080	2.9
Limestone	6-02-632	0.6
Salt		0.5
Vitamin-Trace Mineral Premix[b]		0.6
Antibiotic[c]		0.1
D,L-Methionine[d]		Variable

[a]The basal diet contained no supplemental D,L-methionine. Diets two and three contained 0.64 and 1.40% D,L-methionine, respectively. The basal diet contained .45% methionine and cystine.

[b]Provided the following per kilogram of diet: vitamin A, 5,291 USP units; vitamin D-3, 1,323 IC units; vitamin E, 6.6 Int units; riboflavin, 5.3 mg; niacin, 26.5 mg; d-pantothenic acid, 13.2 mg; vitamin B-12, 19.8 mg; choline chloride, 265 mg; zinc, 150 mg; iron, 150 mg; manganese, 110 mg; copper, 20 mg; cobalt, 2.7 mg; iodine, 1.1 mg; selenium, .13 mg.

[c]Tylan 10 (tylosin phosphate) 22 ppm.

[d]Degussa, Federal Republic of Germany, D,L-methionine (purity of 99%).

levels of D,L-methionine at 1.5 and 3.0 times the N.R.C. requirement. Feed was offered ad libitum.

Pigs in experiments 2 and 3 were individually housed in expanded metal pens measuring 76 centimeters (cm) in length X 60 cm in width X 57 cm in height. Animals were pair-fed a constant amount of their diets based on the consumption of their litter mate on the highest methionine diet twice daily. Pigs were hand-watered after each feeding and water was also added to the diets to form a more palatable gruel feed. The floor beneath each pen was cleaned daily. A period of 5 days was used to allow animals time to acclimate to the pens and feeding routine. In the event of scours, Biosol-M (neomycin sulfate and methscopolamine bromide) was administered either through the water or by direct dosing until scouring ceased. The 16 pigs utilized in experiment 2 were 6 to 8 weeks of age and had treatment group averages of 13.1 to 14.0 kilograms bodyweight at the initiation of the experiment. The 16 pigs utilized in experiment 3 were 3 to 4 weeks of age and averaged 7.9 to 9.3 kilograms bodyweight depending on the treatment group. Four rations were fed in experiment 2 and experiment 3. Treatments were replicated 4 times in each experiment with the pig receiving the highest level of methionine determining the amount of feed the other 3 animals in its replicate block would be fed. The composition of the experimental diets are presented in Table 2. As in experiment 1, diets differed only in the amount of D,L-methionine and cornstarch (supplemental D,L-methionine replaced an equivalent amount of cornstarch in diets 2, 3 and 4). In both experiments 2 and 3, diet 1 contained the sulfur amino acids at levels recommended by the N.R.C.

Table 2. Percentage Composition of Basal Diets for Experiments 2 and 3

Ingredient	International Reference Number	Percent Experiment 2[a]	Percent Experiment 3[b]
Cornstarch	4-02-889	To 100	To 100
Soybean Meal (48% C.P.)	5-04-612	38.6	37.4
Soybean Oil	4-07-983	2.0	2.0
Dicalcium/Monocalcium Phosphate	6-01-080	1.9	1.9
Limestone	6-02-632	1.1	1.1
Salt		0.5	0.5
Vitamin-Trace Mineral Premix[c]		0.6	0.6
Antibiotic[d]		0.2	0.2
D,L-Methionine[e]		Variable	Variable
Dextrose	4-02-125	5.0	5.0
Fiber[f]		1.5	1.5

[a]Methionine was calculated to be .55, 1.37, 2.20 and 3.02% in diets 1, 2, 3 and 4, respectively, in experiment 2.

[b]Methionine was calculated to be .53, 1.33, 2.13 and 2.93% in diets 1, 2, 3 and 4, respectively, in experiment 3.

[c]Provided the following per kilogram of diet: vitamin A, 5,291 USP units; vitamin D-3, 1,323 IC units; vitamin E, 6.6 Int units; riboflavin, 5.3 mg; niacin, 26.5 mg; d-pantothenic acid, 13.2 mg; vitamin B-12, 19.8 mg; manganese, 110 mg; copper, 20 mg; cobalt, 2.7 mg; iodine, 1.1 mg; selenium, .13 mg; choline chloride, 265 mg; zinc, 150 mg; iron, 150 mg.

[d]Tylan 10 (tylosin phosphate) 22 ppm.

[e]Degussa, Federal Republic of Germany, D,L-methionine (purity of 99%).

[f]Alpha-cell (wood cellulose) was used in experiment 2. Silica was used in experiment 3.

with diets 2, 3 and 4 containing the sulfur amino acids at levels 2.5, 4.0 and 5.5 times the N.R.C. requirements.

Experiment 4 was conducted utilizing 60, 8-day-old chicks. Male chicks of the Hubbard X Hubbard strain were allotted by weight and then experimental groups were selected to have the same initial mean weight and a similar weight distribution. Chicks were placed on their respective experimental diets. Each experimental diet was fed to triplicate groups of 5 birds per replicate. The composition of experimental diets are presented in Table 3. Experimental diets differ in sulfur amino acid content only. Amounts of cornstarch were subtracted as equivalent amounts of D,L-methionine were added. The basal diet (diet 1) contained sulfur amino acids at levels recommended by the N.R.C. Diets 2, 3, and 4 had 2.5, 4.0 and 5.5 times, respectively, the N.R.C. requirement. Chicks were housed in wire-bottomed, electrically heated chick batteries in continuous light, kept at 27 to 30°C. Feed and water were supplied ad libitum. Experiment 5 utilized 54, 8-day-old chicks and 54, 5-week-old chicks. Both age groups of birds were sorted and allotted similarly to the selection procedure used in experiment 4. After selection, each group of chicks was randomly allotted to the proper pen and offered the appropriate experimental diet. Each experimental diet was fed to triplicate groups of 6 birds per replicate. The composition of the experimental diets are presented in Table 3. All diets are similar except for differences in sulfur amino acid content. D,L-methionine was added at the expense of cornstarch. The basal diet (diet 1) for chicks in age group 1 contained sulfur amino acids at levels recommended by the N.R.C. Diets 2 and 3 had, respectively, 3 and

Table 3. Percentage Composition of Basal Diets for Experiments 4 and 5

Ingredient	International Reference Number	Experiment 4[a]	Experiment 5 Age Group 1[b]	Experiment 5 Age Group 2[c]
Cornstarch	4-02-889	To 100	To 100	To 100
Soybean Meal	5-04-612	45.00	45.00	41.00
Dextrose	4-02-125	25.00	25.00	25.00
Soybean Oil	4-07-983	2.50	2.50	2.50
Cellulose		1.00	1.00	1.00
Dicalcium Phosphate	6-01-080	2.20	2.20	2.20
Limestone	6-02-632	1.00	1.00	1.00
Salt		0.40	0.40	0.40
Vitamin Premix[d]		0.60	0.60	0.60
Manganous Sulfate		0.05	0.05	0.05
D,L-Methionine[e]		Variable	Variable	Variable

[a]The basal diet (diet 1) contained .3% supplemental methionine which brought the SAA content of diet 1 up to .93%. Diets 2, 3 and 4 contained 1.40, 2.82, and 4.23% supplemental D,L-methionine, respectively. The SAA content of diets 2, 3 and 4 were calculated to be 2.33, 3.72 and 5.12%, respectively.

[b]The basal diet (diet 1) for experiment 5 fed to chicks of age group 1 contained .28% supplemental D,L-methionine (total SAA = .93%). Diets 2 and 3 for age group 1 birds contained 1.86 and 3.72% D,L-methionine, respectively. Diet 2 contained 2.77% total SAA and diet 3 contained 4.61% total SAA.

[c]The basal diet (diet 1) for experiment 5 fed to birds in age group 2 contained .13% supplemental D,L-methionine (total SAA = .72%). Diets 2 and 3 for age group 2 contained 1.44 and 2.88% D,L-methionine, respectively. Diet 2 contained 2.15% total SAA and diet 3 contained 3.57% total SAA.

[d]Provided per kilogram diet: cholecalciferol, 750 ICU, vitamin A, 4.270 Int units; riboflavin, 4.73 mg; vitamin B-12, 0.011 mg; choline chloride, 468 mg; niacin, 42 mg; Ca-pantothenate, 7.33 mg.

[e]Degussa, Federal Republic of Germany, D,L-methionine (purity of 99%).

5 times the N.R.C. requirement for SAA. The basal diet (diet 1) for 5-week-old chicks (age group 2) met the N.R.C. requirement for SAA, while diets 2 and 3 had 3 and 5 times, respectively, the level of SAA as the basal diet. Chicks utilized in experiment 5 had similar accommodations as those in experiment 4. Five-week-old birds were not housed in batteries with electric heat capacity. Feed and water were supplied ad libitum.

II. DATA AND SAMPLE COLLECTION

Pigs in experiment 1 were weighed and bled and feed consumption was measured on a weekly basis for 4 weeks. Pigs from experiments 2 and 3 were also weighed and bled (after overnight fasting) weekly for 3 weeks. Feed pans used in experiments 2 and 3 were scraped following each feeding and uneaten feed was dried and weighed to more accurately determine feed consumption. Blood samples were taken via anterior vena cava puncture. Chicks utilized in experiments 4 and 5 were weighed after a 1-week trial. Feed consumption was determined and blood samples were taken at the termination of each experiment. Blood samples were taken via heart puncture. All birds, except the 5-week-old chicks, were killed by cervical dislocation and the spleens removed.

III. PROCESSING AND ANALYSIS OF SAMPLES

Blood hemoglobin was determined by the method of Crosby et al. (1954) and hematocrit levels determined by the microhematocrit method of McGovern et al. (1955) on heparinized whole blood collected at each bleeding. After hemoglobin and hematocrit determination, whole blood

samples were centrifuged at 5,000 rpm for ten minutes. Plasma was removed after centrifugation and prepared for plasma glucose and amino acid analysis. Sample preparation for amino acid analysis consisted of treating 2 milliliters of plasma with 50 mg of 5'-sulfo-salicylic acid. Samples were then placed in ice for ten minutes after which they were centrifuged and the supernatant collected. Proper dilutions were determined for the physiological samples. Samples were analyzed by an automatic amino acid analyzer utilizing a 5 buffer-lithium citrate system with norleucine as an internal standard for analysis of plasma as described by Dionex (1980). Plasma glucose levels were determined by the method described by Sigma (1980).

IV. STATISTICAL ANALYSIS OF DATA

The data were analyzed by the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) (Barr et al., 1979). The GLM procedure is a multivariate regression analysis, determining differences by significant F tests. One degree of freedom orthogonal polynomial comparisons were used to establish the presence of linear, quadratic or cubic responses determined by the independent variable's influence on the dependent variables. The independent variables used in the analysis of variance were sex, dietary level of methionine (treatment), pen within treatment and sex X treatment interaction for experiment 1; block, period, dietary methionine level, and period X treatment interaction for experiments 2 and 3; dietary methionine level for experiment 4; and age, dietary methionine level and age X treatment interaction for experiment 5. The dependent variables were gain, feed

consumption, gain:feed, hematocrit and hemoglobin for all 5 experiments. Experiments 2 through 5 also had dependent variables plasma glucose level, and plasma threonine, serine, glycine, cystine, methionine, and cystathionine levels.

CHAPTER IV

RESULTS

I. EXPERIMENT 1

Performance and hematological data are presented in Table 4. Gain and feed intake were not significantly affected during week 1 or 2. However, feed consumption was significantly reduced in pigs fed methionine level 3, during weeks 3 ($P < .05$) and 4 ($P < .01$) while gain remained unaffected. Gain:feed was significantly increased, during week 2, due to methionine supplementation of 3 times the N.R.C. requirement. Hematocrit and hemoglobin values were not affected by feeding elevated levels of methionine.

II. EXPERIMENT 2

Data concerning performance and hematology are presented in Table 5 and Figure 4. During week 1, pigs consuming methionine at 4.0 or 5.5 times the N.R.C. levels gained less and had depressed gain:feed values compared to pigs fed methionine at N.R.C. or 2.5 times N.R.C. levels (cubic effect of methionine on gain; ($P < .05$); quadratic effect of methionine on gain:feed, ($P < .01$)). After week 1, no reductions due to supplemental methionine were observed for gain or gain:feed (quadratic effect of period on gain and gain:feed ($P < .01$)). The interaction between period (week) and methionine supplementation level was significant for gain and gain:feed (linear effect of period X cubic effect of

Table 4. Effects of Supplemental Dietary Methionine on Performance and Hematology (Experiment 1)

Item	Methionine Level (X N.R.C.)[a] 1	1.5	3	PSEM
Gain (grams)				
Week 1	2810	2905	2540	588.72
Week 2	2747	3223	3255	800.00
Week 3	2858	3271	2794	915.82
Week 4	2715	2858	2556	754.49
Feed Intake (grams)[b]				
Week 1	3493	4128	3763	636.67
Week 2	5382[d,e]	5922	5287	857.97
Week 3	5922	6668[d]	4953[e]	1094.31
Week 4	6128[d]	6176[d]	5160[e]	58.93
Feed Efficiency[c] (gain:feed)				
Week 1	.81	.70	.73	.15
Week 2	.52[f]	.55[f]	.63[g]	.10
Week 3	.49	.49	.57	.12
Week 4	.44	.47	.50	1.87
Hematocrit (%)				
Week 1	33.39	32.43	34.87	1.81
Week 2	33.24	33.04	33.81	1.24
Week 3	33.52	34.11	35.25	1.70
Week 4	34.50	37.80	35.25	1.91
Hemoglobin (grams/dl)				
Week 1	11.49	11.03	11.47	.51
Week 2	11.11	11.03	11.00	.36
Week 3	10.91	11.24	11.61	.57
Week 4	11.78	12.42	11.90	.66

[a]Methionine level 1 = .45% total SAA. Methionine levels 1.5 and 3 contained .66% and 1.40% total SAA, respectively.

[b]Methionine effect significant ($P < .05$) during week 3. Methionine effect significant ($P < .01$) during week 4.

[c]Methionine effect significant during week 2 ($P < .05$).

[d]Values in the same row with different superscripts are significantly different.

[e]Values in the same row with different superscripts are significantly different.

[f]Values in the same row with different superscripts are significantly different.

[g]Values in the same row with different superscripts are significantly different.

Table 5. Effects of Supplemental Dietary Methionine on Performance and Hematology (Experiment 2)[a]

Item	Methionine Level (X N.R.C.)[b]				
	1	2.5	4.0	5.5	PSEM
Gain (grams)[c]					
Week 1	3527	3765	3098	2177	286.53
Week 2	3720	4525	3958	3663	286.53
Week 3	3379	3788	3844	3425	286.53
Feed Efficiency[d] (Gain:Feed)					
Week 1	.58	.65	.55	.40	.047
Week 2	.56	.68	.61	.57	.047
Week 3	.48	.55	.56	.50	.047
Hematocrit (%)					
Week 1	38.40	37.60	38.60	41.88	1.81
Week 2	37.73	35.95	38.93	38.95	1.81
Week 3	39.10	38.53	39.45	39.23	1.81
Hemoglobin (grams/dl)					
Week 1	12.51	12.43	12.51	13.44	.68
Week 2	13.02	12.35	12.90	13.01	.68
Week 3	13.83	13.37	13.63	13.53	.68
Plasma Glucose (mg/dl)					
Week 1					
Week 2	60.23	60.22	63.35	63.64	6.45
Week 3	56.25	50.64	59.06	59.06	6.45

[a]Experiment 2 animals were pair-fed. Feed consumption not a dependent variable.

[b]Methionine levels 1, 2.5, 4.0, and 5.5 correspond to total SAA concentrations of .55%, 1.37%, 2.20% and 3.02%, respectively.

[c]Methionine effect significant ($P < .01$). Period effect significant ($P < .01$). Cubic effect of methionine significant ($P < .05$). Quadratic effect of period significant ($P < .05$). Linear effect of period X cubic effect of methionine significant ($P < .058$).

[d]Methionine effect significant ($P < .01$). Quadratic effect of period significant at ($P < .05$). Quadratic effect of methionine significant ($P < .01$). Linear effect of period X linear effects of methionine significant ($P < .05$).

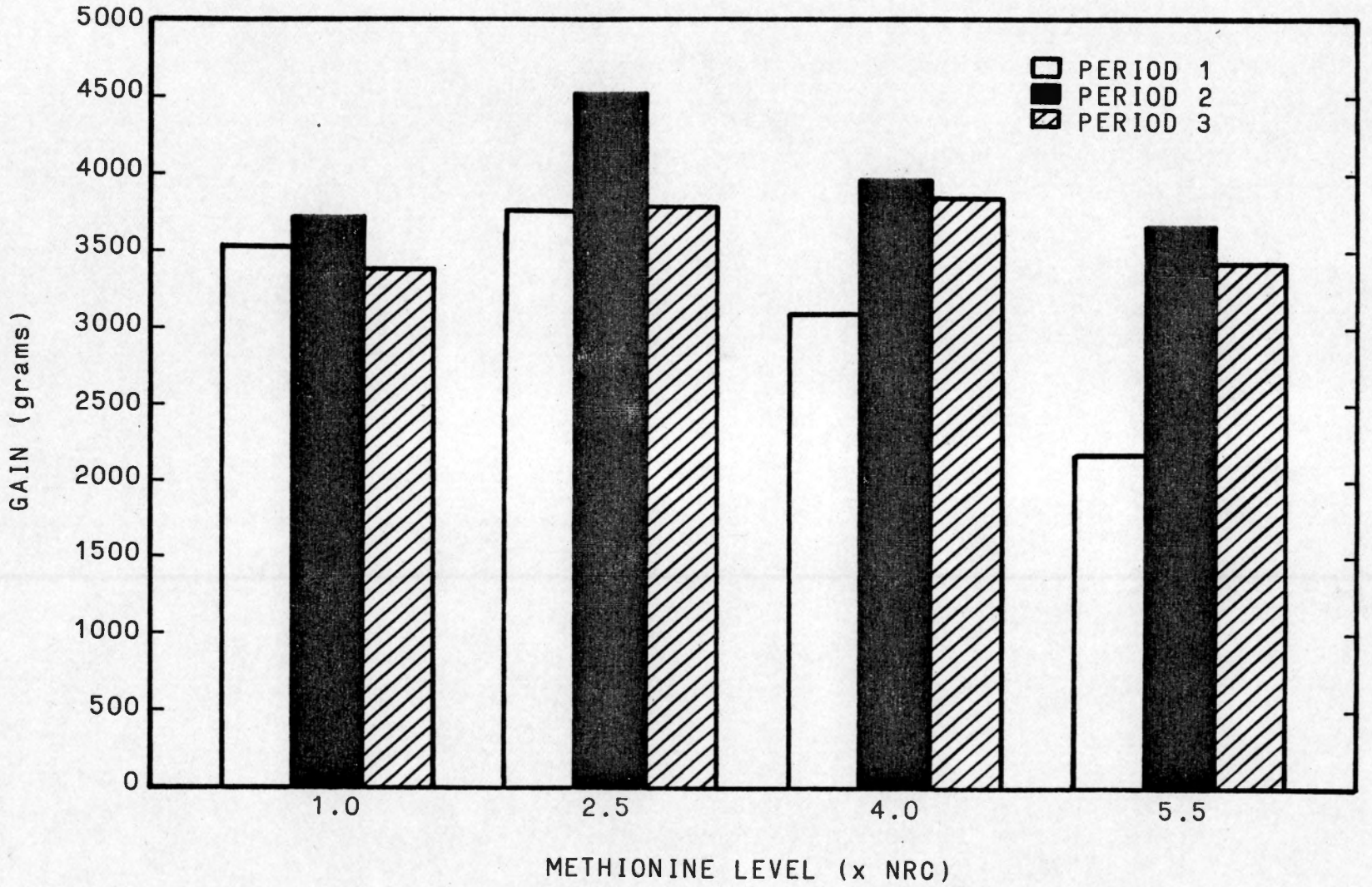


Figure 4. Effect of Supplemental Dietary Methionine on Gain by Period (Period = 7 Days; Experiment 2).

methionine on gain ($P < .058$); linear effect of period X linear effect of methionine ($P < .05$)).

Supplementation with methionine at 2.5 times the requirement caused significantly increased growth and gain:feed when compared to pigs fed methionine at the N.R.C. level. As observed in experiment 1, hematocrit, hemoglobin and plasma glucose levels were not affected by supplemental methionine consumption. Results of amino acid analysis are presented in Table 6 and Figures 5, 6, and 7. Pigs fed supplemental methionine at 4.0 and 5.5 times the N.R.C. requirement had increased plasma free amino acid concentrations of methionine, threonine, and cystine throughout the study. Cubic effect of methionine supplementation on methionine, threonine and cystine was significant at ($P < .01$) compared to pigs consuming methionine at the N.R.C. or 2.5 times the N.R.C. level. With the exception of week 1 at 5.5 times the N.R.C. level for methionine, plasma glycine was reduced, in comparison to N.R.C. or 2.5 times N.R.C. groups, in pigs fed the higher two amounts of methionine (cubic effect of methionine significant at $P < .05$). Plasma serine concentration was increased in pigs fed 5.5 times N.R.C. requirements for methionine during weeks 1, 2 and 3 (cubic effect of methionine significant at $P < .05$).

Generally, no significant differences were observed in plasma serine concentration in the pigs fed methionine at 1, 2.5 or 4.0 times the N.R.C. requirement during the course of experiment 2. Reductions in plasma cystathionine were noted in pigs consuming 4.0 or 5.5 times the N.R.C. level during weeks 1 and 2 when compared to the diets containing lower levels of methionine (cubic effect of methionine

Table 6. Effects of Supplemental Dietary Methionine on Plasma Free Amino Acid Concentration (Experiment 2)[a]

Plasma Free Amino Acid (nmoles/ml)	Methionine Level (X N.R.C.)[b]				
	1	2.5	4.0	5.5	PSEM
Methionine[c]					
Week 1	107	325	1214	1631	166.63
Week 2	28	76	1161	1458	166.63
Week 3	35	55	717	1967	166.63
Threonine[d]					
Week 1	302	280	645	806	85.98
Week 2	236	154	574	785	85.98
Week 3	221	175	565	849	85.98
Glycine[e]					
Week 1	1185	1064	690	979	129.55
Week 2	1340	969	938	887	129.55
Week 3	1187	951	907	866	129.55
Serine[f]					
Week 1	317	279	305	418	49.2
Week 2	238	189	229	316	49.2
Week 3	229	201	316	391	49.2
Cystine[g]					
Week 1	47	124	233	353	41.95
Week 2	48	49	335	259	41.95
Week 3	95	101	355	280	41.95
Cystathionine[h]					
Week 1	197	181	156	156	13.30
Week 2	191	175	146	146	13.30
Week 3	148	151	121	129	13.30

[a]Mean of four samples per methionine level.

[b]Methionine levels 1, 2.5, 4.0 and 5.5 correspond to total SAA amounts .55, 1.37, 2.20 and 3.02%, respectively.

[c]Methionine effect significant ($P < .01$). Period effect significant ($P < .05$). Cubic effect of methionine significant ($P < .01$). Linear effect of period significant ($P < .01$).

[d]Methionine effect significant ($P < .01$). Cubic effect of methionine significant ($P < .01$).

[e]Methionine effect significant ($P < .01$). Cubic effect of methionine significant ($P < .05$).

Table 6 (continued)

[f]Methionine effect significant ($P < .01$). Period effect significant ($P < .058$). Cubic effect of methionine significant ($P < .05$). Quadratic effect of period significant ($P < .05$).

[g]Methionine effect significant ($P < .01$). Cubic effect of methionine significant ($P < .01$).

[h]Methionine and period effect significant ($P < .01$). Cubic effect of methionine significant ($P < .01$). Linear effect of period significant ($P < .01$).

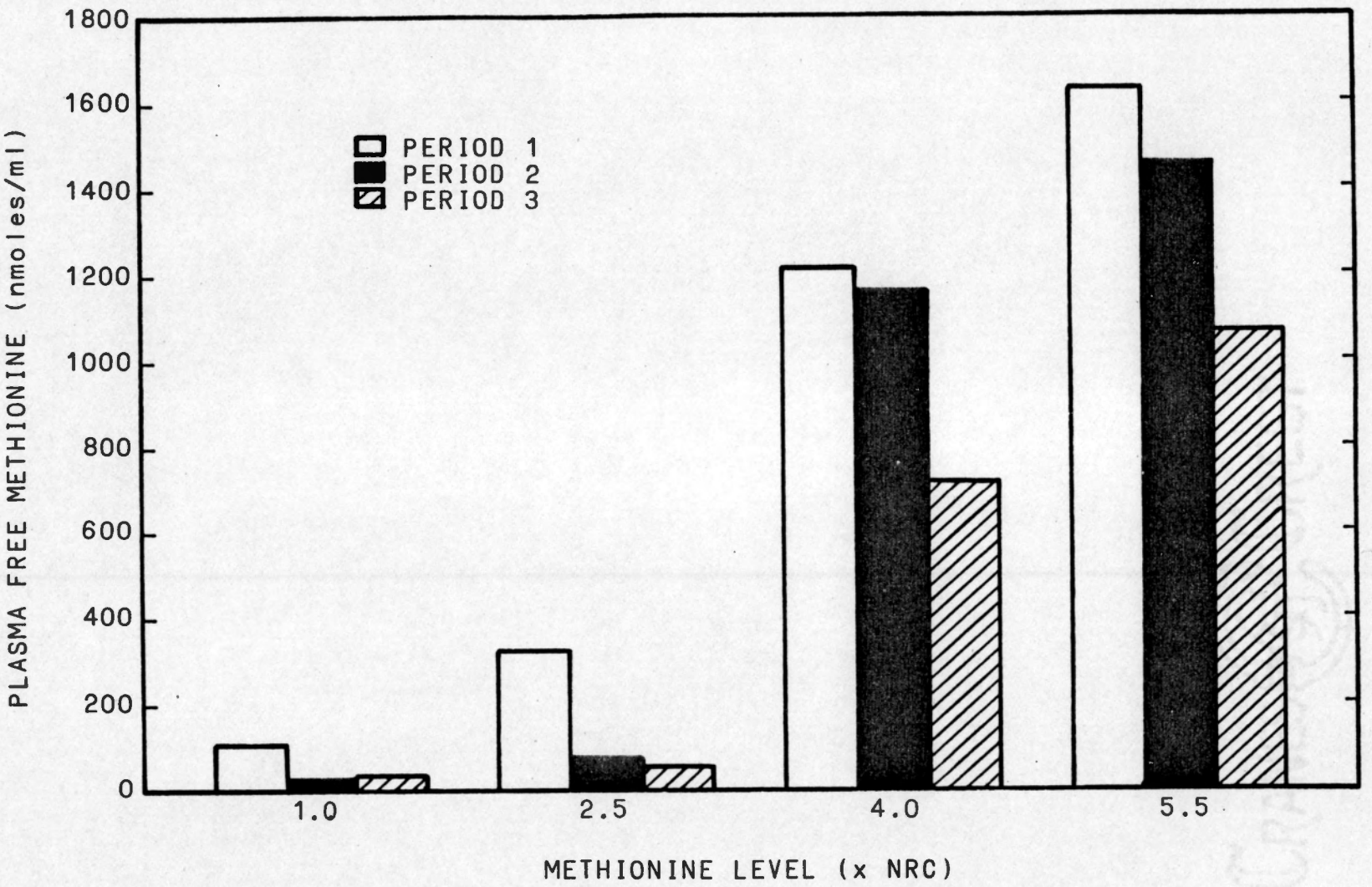


Figure 5. Effect of Supplemental Dietary Methionine on Plasma Methionine by Period (Period = 7 Days; Experiment 2).

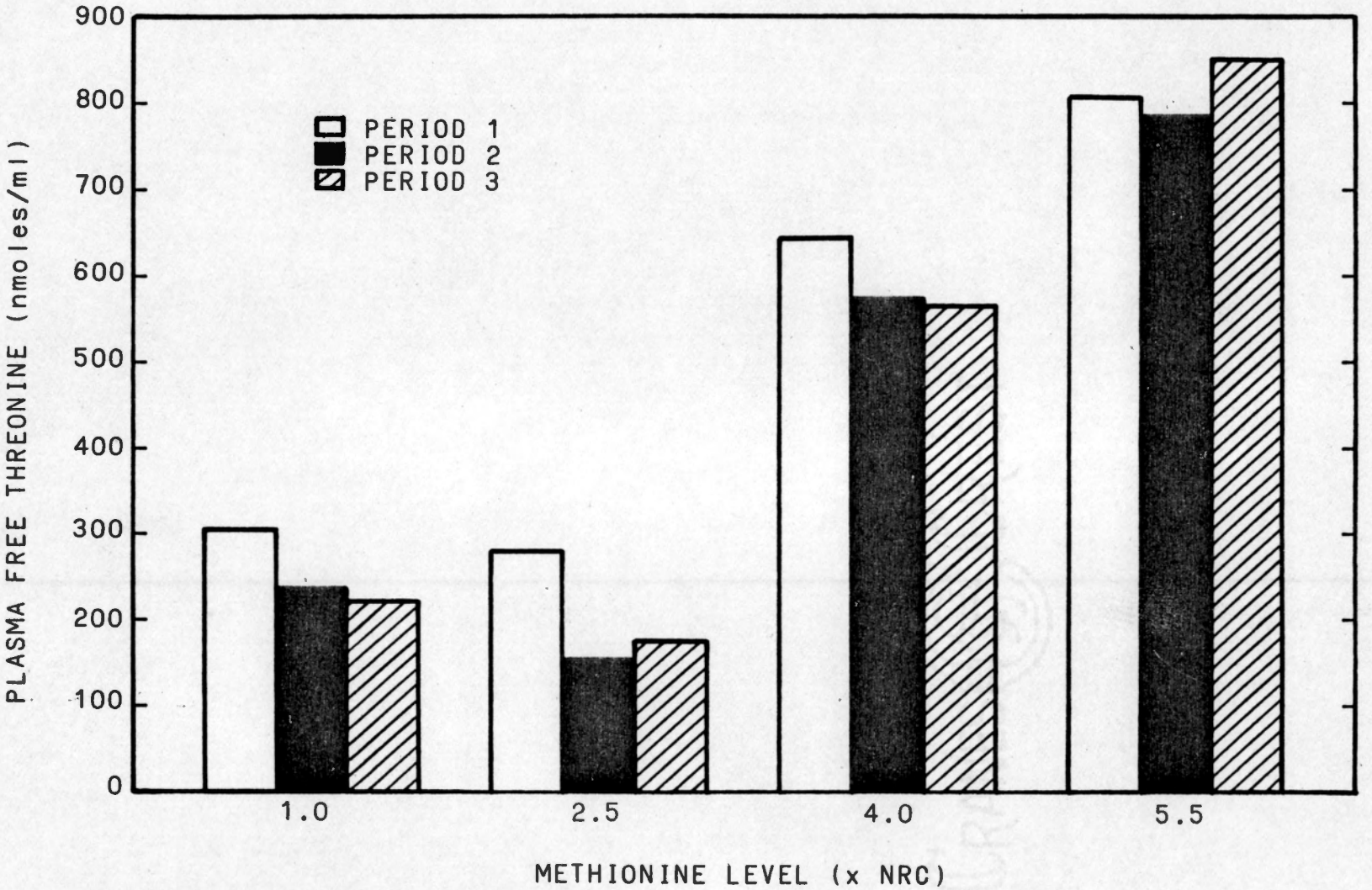


Figure 6. Effect of Supplemental Dietary Methionine on Plasma Threonine by Period (Period = 7 Days; Experiment 2).

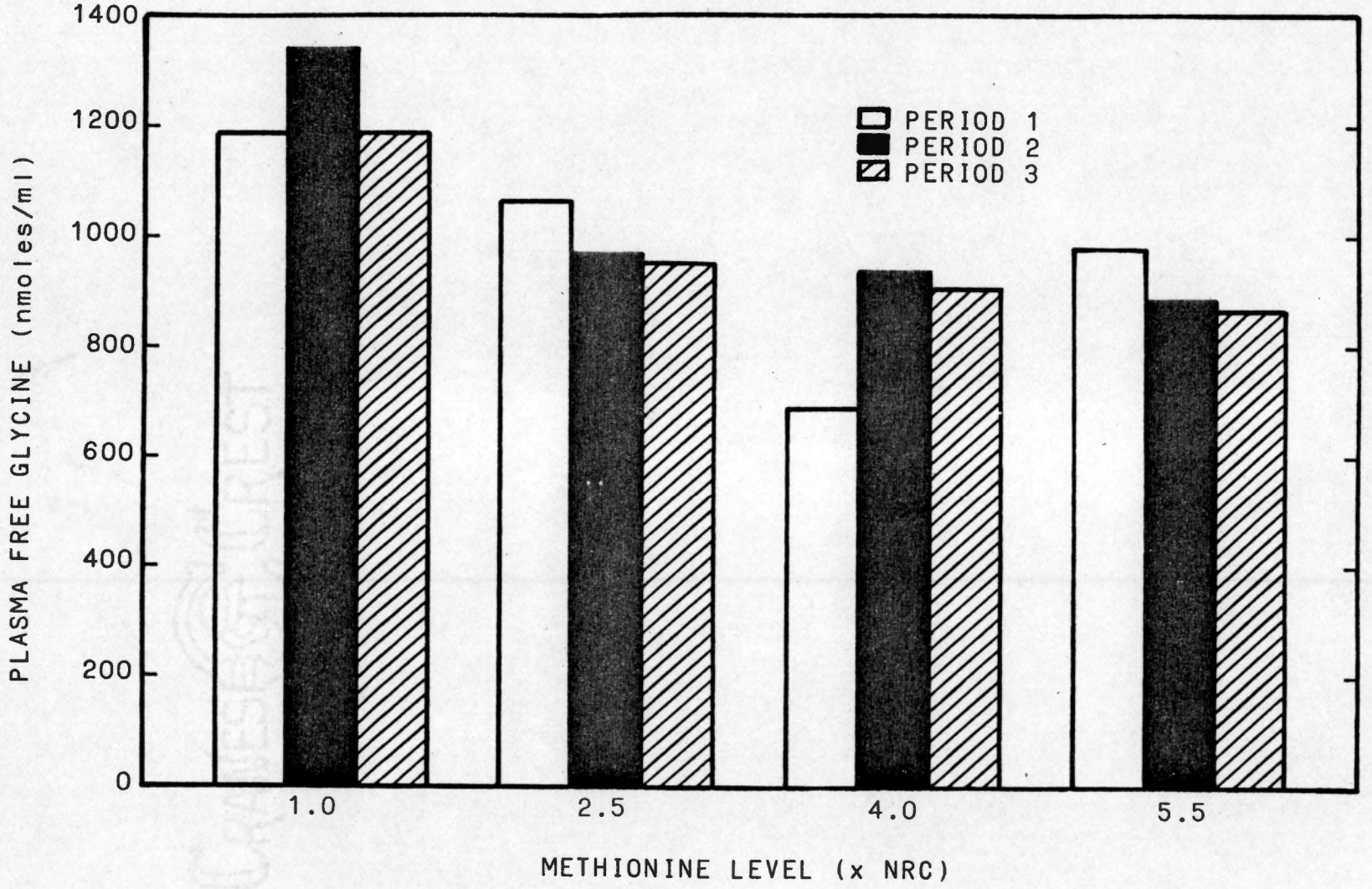


Figure 7. Effect of Supplemental Dietary Methionine on Plasma Glycine by Period (Period = 7 Days; Experiment 2).

significant at $P < .01$). At the end of the third week, no differences were observed in plasma cystathionine in pigs fed any level of supplemental methionine. Methionine supplementation was not the only source of variation in experiment 2. Period or weeks in which the samples were obtained also affected the plasma free amino acid profile. In groups fed the higher two levels of methionine, marked reductions were noted between plasma methionine concentration during week 1 and week 3 (linear effect of period significant at $P < .01$). Plasma serine concentration in pigs fed the highest level of methionine was reduced from week 1 to week 2 but increased from week 2 to week 3 (quadratic effect of period significant at $P < .05$). Plasma cystathionine concentrations in all treatment groups declined significantly from week 1 to week 3 (linear effect of period significant at $P < .01$).

III. EXPERIMENT 3

Performance and hematology results are presented in Table 7 and Figure 8. Pigs fed 5.5 times the N.R.C. requirement for methionine gained less and had reduced gain:feed during week 1 (methionine effect significant at $P < .05$). Groups consuming 2.5 times the N.R.C. requirement had increased gain and gain:feed values compared to group 1 (methionine at N.R.C. level) during week 1. Again, as in the previous two experiments, no changes in hematocrit or hemoglobin levels were observed throughout the trial. However, plasma glucose quadratically responded to methionine supplementation during the first week. After week 1, little or no differences in gain, gain:feed or plasma glucose were found (quadratic effect of period on gain significant at $P < .01$);

Table 7. Effects of Supplemental Dietary Methionine on Performance and Hematology (Experiment 3)[a]

	Methionine Level (X N.R.C.)[b]				
Item	1	2.5	4.0	5.5	PSEM
Gain (grams)[c]					
Week 1	1452	1939	1622	1100	203.12
Week 2	1962	2087	1826	1701	203.12
Week 3	3549	4026	3493	3618	203.12
Feed Efficiency[d] (Gain:Feed)					
Week 1	.48	.64	.54	.37	.064
Week 2	.59	.64	.56	.52	.064
Week 3	.70	.78	.69	.75	.064
Hematocrit (%)					
Week 1	34.22	33.75	31.73	32.85	.97
Week 2	35.60	36.83	34.75	35.38	.97
Week 3	36.40	35.45	36.53	34.80	.97
Hemoglobin (grams/dl)					
Week 1	11.33	12.28	10.79	11.55	.58
Week 2	13.24	12.80	11.70	12.36	.58
Week 3	11.23	11.63	13.09	11.43	.58
Plasma Glucose[e] (mg/dl)					
Week 1	70.41	57.25	57.61	62.56	4.56
Week 2	66.76	60.65	61.51	62.51	4.56
Week 3	58.20	53.04	55.08	57.84	4.56

[a]Experiment 3 animals were pair-fed. Feed consumption not a dependent variable.

[b]Methionine levels 1, 2.5, 4.0 and 5.5 correspond to total SAA amounts of .535, 1.326, 2.127 and 2.929%, respectively.

[c]Methionine effect significant ($P < .05$). Period effect significant ($P < .01$). Cubic effect of methionine significant ($P < .05$). Quadratic effect of period significant ($P < .01$).

[d]Period effect significant ($P < .01$). Cubic effect of methionine significant ($P < .058$). Linear effect of period significant ($P < .01$).

[e]Quadratic effect of methionine significant ($P < .05$).

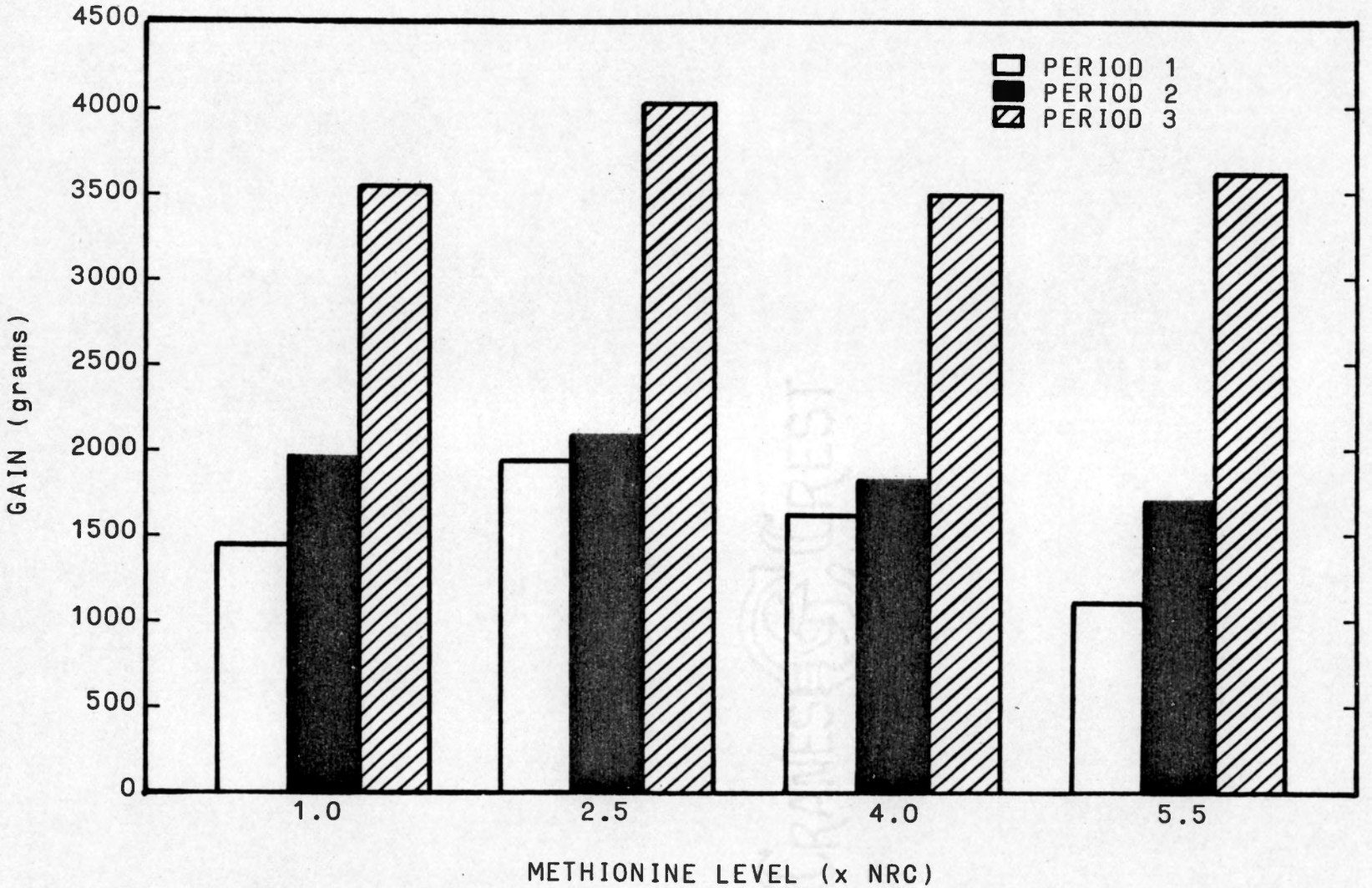


Figure 8. Effect of Supplemental Dietary Methionine on Gain by Period (Period = 7 Days; Experiment 3).

linear effect of period on gain:feed significant at $P < .01$) at any level of methionine supplementation. Data from amino acid analyses are presented in Table 8 and Figures 9, 10, and 11. Pigs consuming methionine at 4.0 and 5.5 times the N.R.C. requirement had increased plasma methionine, threonine and cystine concentrations (cubic effect of methionine supplementation on plasma methionine, threonine and cystine significant at $P < .01$). This trend was consistent during each week of the experiment. Each addition of supplemental methionine to the diet caused decreased levels of plasma glycine during week 1 (cubic effect of methionine supplementation significant at $P < .055$). Plasma glycine concentrations were not affected during weeks 2 and 3. Plasma serine concentrations did not exhibit a consistent pattern at each weekly bleeding. Pigs fed 2.5 times the N.R.C. methionine requirement did have depressed levels of plasma serine compared to levels in the N.R.C. requirement fed pigs during weeks 1 and 2 (cubic effect of methionine significant, $P < .05$). However, pigs receiving the highest two levels of methionine did not have significantly reduced plasma serine concentrations during weeks 1 and 2. During week 3, plasma serine concentration was significantly increased in pigs fed 5.5 times the N.R.C. level versus plasma serine values of pigs fed methionine at the N.R.C. or 2.5 times the N.R.C. requirement. Feeding the highest two levels of methionine resulted in decreased concentrations of plasma cystathionine throughout the three-week trial (cubic effect of methionine significant, $P < .01$).

Table 8. Effects of Supplemental Dietary Methionine on Plasma Free Amino Acid Concentration (Experiment 3)[a]

Plasma Free Amino Acid (nmoles/ml)	Methionine Level (X N.R.C.)[b] 1	2.5	4.0	5.5	PSEM
Methionine[c]					
Week 1	31	190	994	1226	186.51
Week 2	18	178	855	2035	186.51
Week 3	53	212	950	1360	186.51
Threonine[d]					
Week 1	343	260	590	626	83.79
Week 2	343	220	582	753	83.79
Week 3	306	303	654	753	83.79
Glycine[e]					
Week 1	1553	1142	903	695	193.30
Week 2	1507	1230	1163	1147	193.30
Week 3	1349	1156	983	1038	193.30
Serine[f]					
Week 1	360	209	284	281	55.15
Week 2	306	178	290	262	55.15
Week 3	331	301	411	464	55.15
Cystine[g]					
Week 1	79	106	466	689	79.06
Week 2	109	141	360	542	79.06
Week 3	95	79	366	414	79.06
Cystathionine[h]					
Week 1	161	173	134	128	17.68
Week 2	159	138	96	89	17.68
Week 3	184	180	138	123	17.68

[a]Mean of four samples per methionine level.

[b]Methionine levels 1, 2.5, 4.0 and 5.5 correspond to total SAA amounts of .535, 1.326, 2.127 and 2.929%, respectively.

[c]Methionine effect significant ($P < .01$). Cubic effect of methionine significant ($P < .01$). Cubic effect of methionine X quadratic effect of period significant ($P < .05$).

[d]Methionine effect significant ($P < .01$). Cubic effect of methionine significant ($P < .01$).

[e]Methionine effect significant ($P < .05$). Cubic effect of methionine significant ($P < .055$).

Table 8 (continued)

[f]Period effect significant ($P < .05$). Cubic effect of methionine and quadratic effect of period significant ($P < .05$)..

[g]Methionine effect significant ($P < .01$). Cubic effect of methionine significant ($P < .01$).

[h]Methionine effect significant ($P < .01$). Period effect significant ($P < .05$). Cubic effect of methionine and quadratic effect of period significant ($P < .01$).

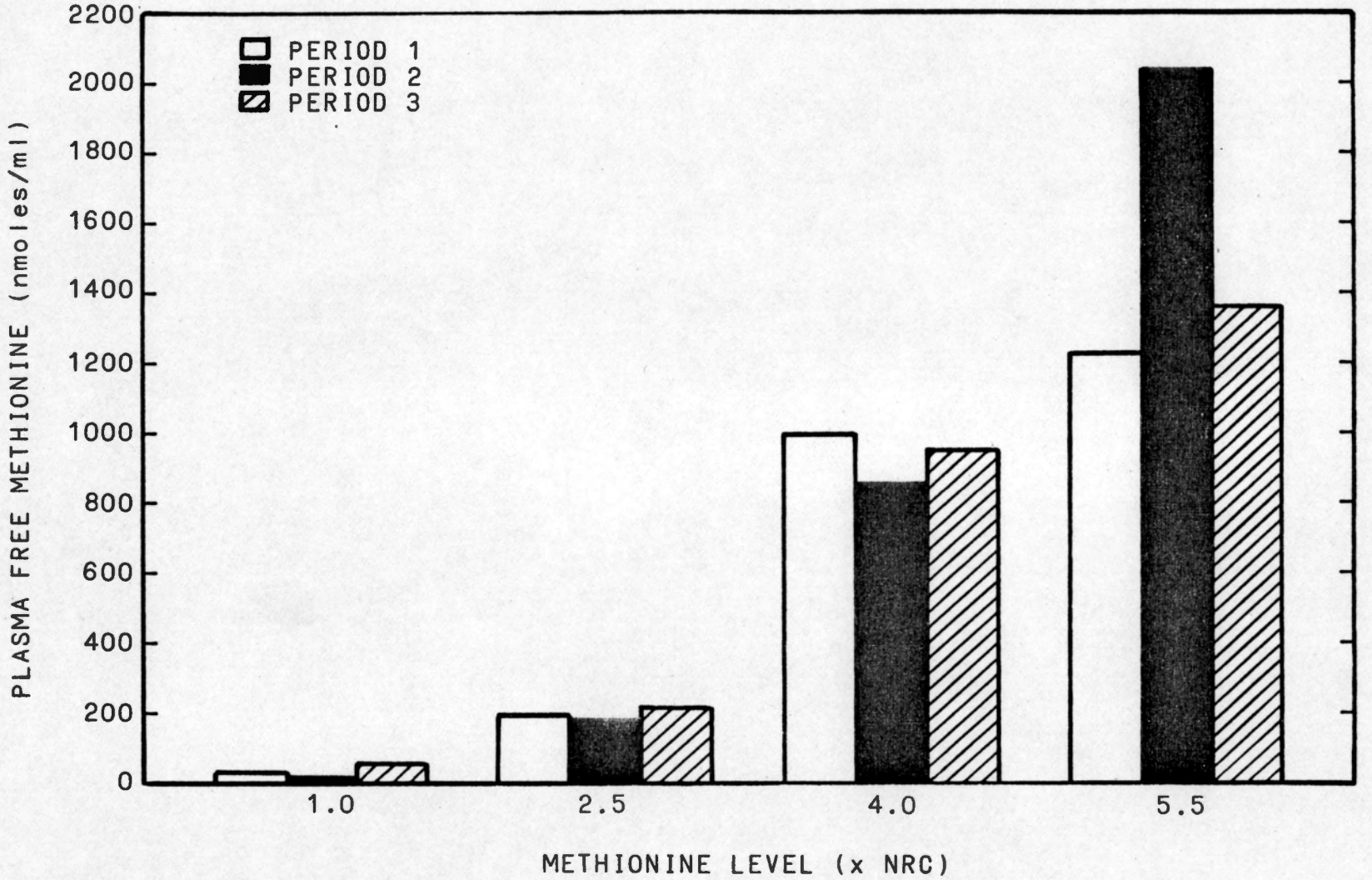


Figure 9. Effect of Supplemental Dietary Methionine on Plasma Methionine by Period (Period = 7 Days; Experiment 3).

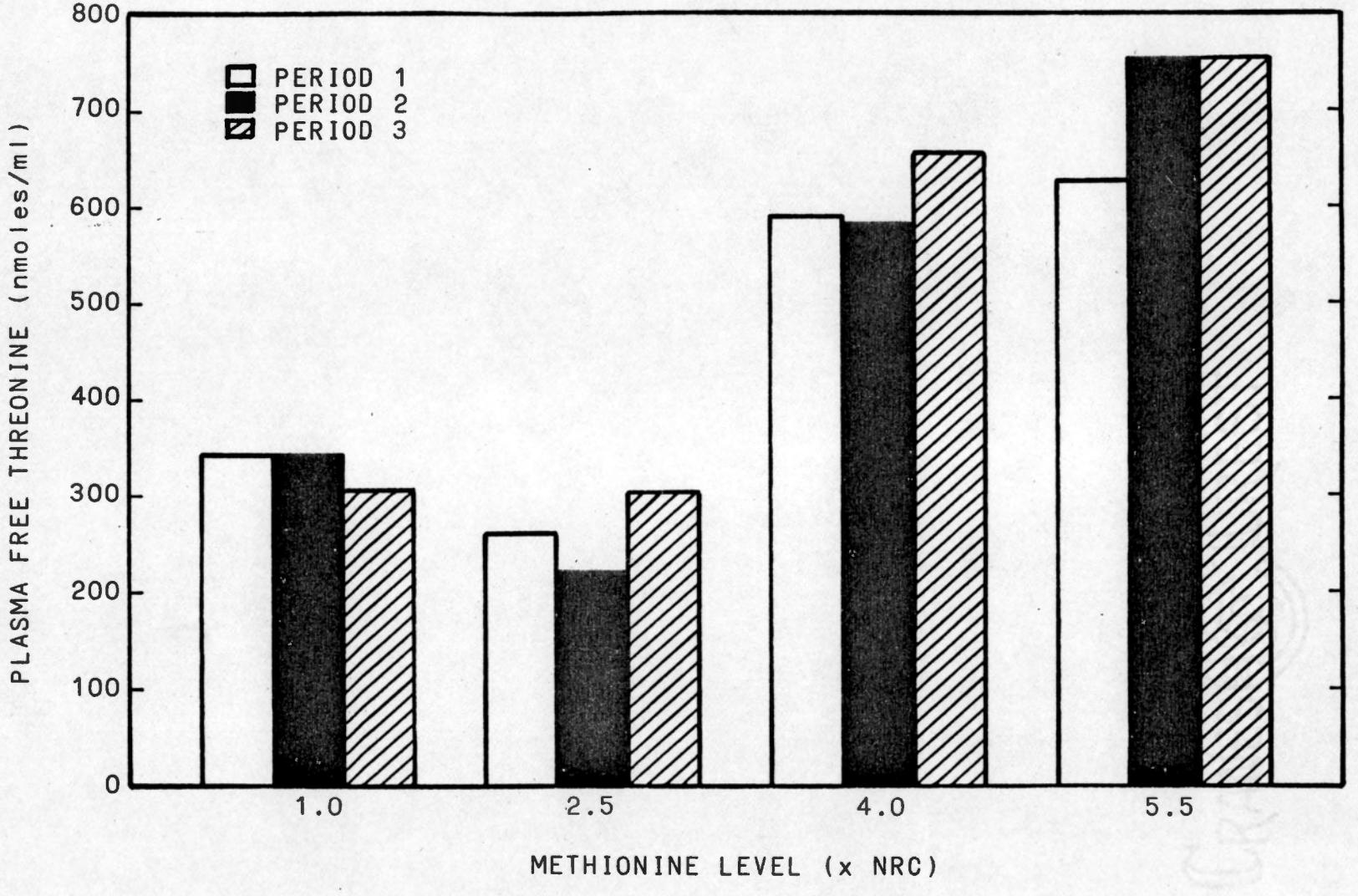


Figure 10. Effect of Supplemental Dietary Methionine on Plasma Threonine by Period (Period = 7 Days; Experiment 3).

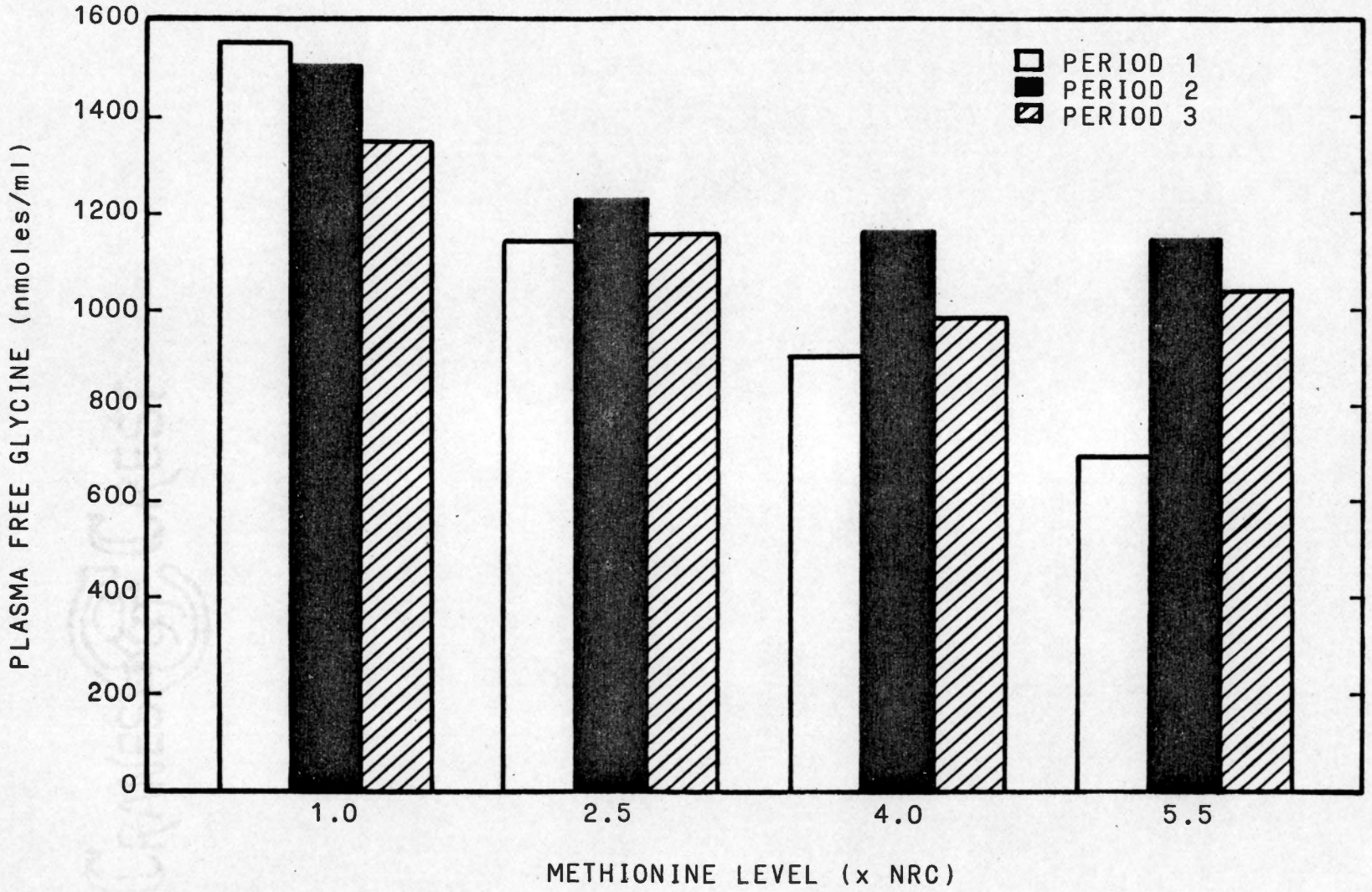


Figure 11. Effect of Supplemental Dietary Methionine on Plasma Glycine by Period (Period = 7 Days; Experiment 3).

IV. EXPERIMENT 4

Performance and hematological data are presented in Table 9. Chicks fed methionine at 4.0 and 5.5 times the N.R.C. requirement gained less, consumed less feed and had depressed gain:feed compared to chicks fed the lower levels of methionine (cubic effect of methionine supplementation on gain, feed intake and gain:feed significant, $P < .01$). Chicks receiving the highest level of methionine were adversely affected to a greater extent than those fed 2.5 or 4.0 times the N.R.C. requirement. Hematocrit and hemoglobin levels were depressed by supplemental methionine feeding. Chicks fed 2.5, 4.0 or 5.5 times the N.R.C. requirement had lower hematocrit and hemoglobin values than chicks fed methionine at the N.R.C. recommended level. Chicks consuming the two highest levels of methionine had lower hematocrit and hemoglobin values than chicks fed 2.5 times the N.R.C. level (cubic effect of methionine supplementation on hematocrit and hemoglobin significant, $P < .01$). Plasma glucose levels were not affected by methionine levels of 2.5 or 4.0 times the N.R.C. amount, but a significant increase in plasma glucose was noted from chicks fed the N.R.C. level of methionine to chicks consuming the highest methionine level (cubic effect of methionine significant, $P < .01$). Results of amino acid analyses are presented in Table 10. Plasma methionine concentration increased in chicks fed 4.0 and 5.5 times the N.R.C. requirement as opposed to plasma methionine levels in chicks consuming methionine at the N.R.C. or 2.5 times the N.R.C. level.

Plasma threonine concentration was decreased in chicks fed 2.5, 4.0 and 5.5 times the N.R.C. requirement compared to chicks fed

Table 9. Effects of Supplemental Dietary Methionine on Performance and Hematology (Experiment 4)[a]

	Methionine Level (X N.R.C.)[b]				
Item	1	2.5	4.0	5.5	PSEM
Gain[c] (grams)	154.5	142.5	61.5	16.1	10.43
Feed Intake[d] (grams)	244.3	225.0	134.0	83.8	7.34
Feed Efficiency[e] (gain:feed)	.63	.63	.46	.19	.33
Hematocrit (%)[f]	25.67	23.57	21.47	20.07	.73
Hemoglobin[g] (grams/dl)	8.67	7.40	6.44	6.27	.23
Plasma Glucose[h] (mg/dl)	157.80	171.48	187.28	204.24	19.29

[a]Mean of triplicate pooled groups of five male chicks.

[b]Methionine levels 1, 2.5, 4.0, and 5.5 correspond to total SAA amounts of .93, 2.33, 3.75 and 5.16%, respectively.

[c]Methionine effect significant ($P < .01$). Cubic effect of methionine significant ($P < .01$).

[d]Methionine effect significant and cubic effect of methionine significant ($P < .01$).

[e]Methionine effect significant and cubic effect of methionine significant ($P < .01$).

[f]Methionine effect and cubic effect of methionine significant ($P < .01$).

[g]Methionine effect and cubic effect of methionine significant ($P < .01$).

[h]Methionine effect and cubic effect of methionine significant ($P < .01$).

Table 10. Effects of Supplemental Dietary Methionine on Plasma Free Amino Acid Concentration (Experiment 4)[a]

Plasma Free Amino Acid (nmoles/ml)	Methionine Level (X N.R.C.)[b]				
	1.0	2.5	4.0	5.5	PSEM
Methionine[c]	98	447	1301	1191	343.47
Threonine[d]	1164	768	686	507	87.19
Glycine	776	795	956	869	79.10
Serine	813	788	923	851	62.02
Cystine	137	297	291	427	148.75
Cystathionine	177	175	172	138	16.03

[a]Mean of triplicate pooled groups of five male chicks.

[b]Methionine levels 1, 2.5, 4.0 and 5.5 correspond to total SAA amounts of .93, 2.33, 3.75, and 5.16%, respectively.

[c]Methionine effect significant ($P < .10$).

[d]Methionine effect significant ($P < .01$).

required amounts of methionine. Chicks consuming the highest level of methionine supplementation exhibited the greatest depression in plasma threonine. Methionine fed at all four levels did not significantly reduce or increase plasma glycine, serine, cystine or cystathionine.

V. EXPERIMENT 5

Performance and hematological data are presented in Table 11. Gain and feed intake were depressed with each additional level of methionine supplementation in both 8-day-old and 5-week-old chicks. Gain:feed in both age groups was decreased only in chicks fed 5.0 times the N.R.C. requirement for methionine. Hematocrit and hemoglobin levels were depressed in chicks consuming 3.0 or 5.0 times the N.R.C. level of methionine compared to chicks fed the N.R.C. level of methionine. However, the older birds' hematocrit values were only depressed at the highest level of methionine supplementation. Also in the older birds, the hemoglobin value actually increased from methionine level 1 to methionine level 3, but methionine level 1 and methionine level 5 were not significantly different. Plasma glucose was increased in the younger birds by increasing levels of methionine, but older birds' plasma glucose was not significantly affected. Data on amino acid analyses are presented in Table 12. Plasma methionine concentration significantly increased in young birds with each subsequent addition of methionine. In older birds, a significant increase in plasma free methionine was noted only at 5.0 times the N.R.C. requirement. Supplemental methionine at 3.0 or 5.0 times the N.R.C. requirement depressed plasma threonine levels in both age group chicks.

Table 11. Effect of Supplemental Dietary Methionine on Performance and Hematology (Experiment 5)[a]

Item	Methionine Level (X N.R.C.)[b] 1.0	3.0	5.0	PSEM
Gain (grams)[c]				
Age 1	188.1	130.8	40.24	12.69
Age 2	192.0	136.5	52.2	12.69
Food Intake[d] (grams)				
Age 1	274.1	211.9	92.4	18.93
Age 2	640.8	529.9	418.5	18.93
Feed Efficiency[e] (gain:feed)				
Age 1	.69	.62	.44	.03
Age 2	.30	.26	.13	.03
Hematocrit (%)[f]				
Age 1	27.97	22.87	24.43	.62
Age 2	27.57	26.43	25.70	.62
Hemoglobin[g] (grams/dl)				
Age 1	9.47	6.80	6.95	.54
Age 2	8.65	10.15	8.65	.54
Plasma Glucose[h] (mg/dl)				
Age 1	165.13	185.64	197.26	6.86
Age 2	167.19	171.97	175.73	6.86

[a]Mean of triplicate pooled groups of six male chicks. Age 1 = 8-day-posthatched chicks; age 2 = 5-week-posthatched chicks.

[b]Methionine levels 1, 3 and 5 correspond to total SAA amounts of .93, 2.79 and 4.65%, respectively, for age group 1 chicks and to .72, 2.16 and 3.60%, respectively, for age group 2 chicks.

[c]Methionine effect significant ($P < .01$). Quadratic effect of methionine significant ($P < .05$).

[d]Methionine and age effect significant ($P < .01$).

[e]Methionine and age effect significant ($P < .01$).

[f]Methionine effect significant ($P < .01$). Age effect significant ($P < .05$). Methionine X age interaction is significant ($P < .05$). Linear effect of methionine significant ($P < .01$).

Table 11 (continued)

[g]Age effect significant ($P < .01$). Methionine X age interaction significant ($P < .05$). Linear effect of methionine significant ($P < .01$).

[h]Methionine effect significant ($P < .05$). Linear effect of methionine significant ($P < .01$).

Table 12. Effects of Supplemental Dietary Methionine on Plasma Free Amino Acid Concentration (Experiment 5)[a]

Plasma Free Amino Acid (nmoles/ml)	Methionine Level (X N.R.C.)[b] 1.0	3.0	5.0	PSEM
Methionine[c]				
Age 1	53	968	1560	208.21
Age 2	45	89	565	208.21
Threonine[d]				
Age 1	1191	996	987	50.23
Age 2	846	381	741	50.23
Glycine[e]				
Age 1	701	644	819	41.01
Age 2	578	324	403	41.01
Serine[f]				
Age 1	779	1039	1038	90.61
Age 2	878	606	999	90.61
Cystine[g]				
Age 1	110	468	369	69.28
Age 2	81	125	185	69.28
Cystathionine[h]				
Age 1	197	175	205	17.33
Age 2	149	153	120	17.33

[a]Means of triplicate pooled groups of six male chicks. Age 1 = eight-day-posthatched chicks. Age 2 = five-week-posthatched chicks.

[b]Methionine levels 1, 3 and 5 correspond to total SAA amounts of .93, 2.79 and 4.65%, respectively, for age group 1 and .72, 2.16, and 3.60%, respectively, for age group 2.

[c]Methionine and age effect significant ($P < .01$).

[d]Methionine and age effect significant ($P < .01$). Methionine X age interaction significant ($P < .01$).

[e]Methionine and age effect significant ($P < .01$). Methionine X age interaction significant ($P < .05$).

[f]Methionine effect significant ($P < .10$). Methionine X age interaction significant ($P < .05$).

[g]Methionine effect significant ($P < .05$). Age effect significant ($P < .01$).

[h]Age effect significant ($P < .01$).

Plasma glycine in 8-day-posthatched chicks increased when methionine was fed at 5.0 times the N.R.C. requirement. Plasma glycine, in older chicks, decreased when methionine was fed at 3.0 and 5.0 times the N.R.C. value. Plasma serine in young chicks increased when methionine was fed at the two higher levels. The concentration of plasma serine in older chicks decreased when chicks were fed 3.0 times the N.R.C. requirement but it was not significantly affected by methionine at 5.0 times the N.R.C. level. Plasma cystine concentration was increased in younger birds fed the two higher methionine diets. Plasma cystine in older birds was unaffected at any level of methionine tested. In both age groups, plasma cystathionine concentrations were not significantly affected by level of methionine supplementation.

CHAPTER V

DISCUSSION

I. PERFORMANCE AND HEMATOLOGY

The detrimental effects of feeding levels of methionine above (2 times) the National Research Council's (N.R.C.) established requirement has been well documented in the chick and rat. Reports concerning these two species have noted that excessive methionine consumption depressed gain, feed intake and gain:feed (Katz and Baker, 1975a; Sauberlich, 1961). In experiment 1, gain:feed was depressed. Gain and gain:feed in experiments 2 and 3 coincided with trends observed in previous chick and rat studies. Experiments 4 and 5 (chick experiments) also followed similar gain, feed intake and gain:feed responses determined in earlier studies.

Hematological parameters in the pig experiments (experiments 1, 2 and 3) were not affected by supplemental methionine. This type of response is somewhat similar to that observed in rats (Rotruck and Boggs, 1977). Hematocrit (Hct) and hemoglobin (Hb) levels were unaffected in rats, however, rats developed hemolytic anemia. No signs of anemia (reduced Hct or Hb) were observed in pigs. Studies utilizing chicks have demonstrated excess methionine's adverse effects on hematocrit, hemoglobin and blood glucose (Katz and Baker, 1975a). These adverse effects were not observed in any of the three experiments utilizing pigs. However, 8-day-posthatched chicks used in experiments 4 and 5 did exhibit reduced hematocrit and hemoglobin values when fed

elevated levels of methionine. Contrary to earlier chick work in which blood glucose was decreased when dietary methionine was increased (Katz and Baker, 1975a), young chicks (8-day-posthatched) in experiments 4 and 5 had elevated plasma glucose levels when fed 5.5 times N.R.C. in experiment 4 or 3.0 and 5.0 times the N.R.C. requirement in experiment 5. At this time, no plausible explanation has been postulated for the increase in chick plasma glucose. The 5-week-posthatched birds did not have significantly elevated or depressed plasma glucose levels and hematocrit values were only significantly depressed when they consumed the highest level of methionine. Also, older chicks did not have reduced hemoglobin levels. It appears from comparative observations of older and younger birds that excess dietary methionine causes depressed gain, feed intake and gain:feed equally in both age groups, but older chicks are less sensitive to metabolic abnormalities associated with excess methionine consumption. Perhaps the 5-week-old birds reacted simply to an imbalance in the amino acid profile whereas the 8-day-old chicks may have encountered biochemical problems (alternate pathway metabolites) which led to alterations in the hematological parameters examined.

Although performance parameters were affected in both pig and chick experimentation, results from the pig trials demonstrated that the adaptation process observed in rats (Daniel and Waisman, 1969; Girard-Globa et al., 1972) may exist in pigs. After the first week of experiments 2 and 3, pigs exhibited improved gain, feed intake and gain:feed. By the end of the third week, pigs consuming supplemental methionine were performing as well as pigs on the diet with methionine

supplied at N.R.C. requirement levels. Pigs consuming methionine at 2.5 times the N.R.C. requirement surpassed all other treatment groups in performance. This may indicate that current N.R.C. requirements for the weanling pigs are inadequate for proper growth or that the sulfur amino acids in soybean meal are not readily available for utilization by the young pig. It was further determined from the experiments utilizing pigs, that optimal growth and gain:feed is accomplished at 2.38 times the N.R.C. requirement. This level of methionine was estimated by using the first derivative of the linear, quadratic and cubic effects and solving for X at zero.

II. AMINO ACID ANALYSIS

Methionine supplementation at 4.0 or 5.5 times the N.R.C. requirement increased plasma methionine in pigs. This agrees with findings from both rat and chick studies (Klavins, 1965; Harter and Baker, 1978a). Conversely, when pigs were fed the two highest levels of methionine, plasma threonine was significantly increased. This is contrary to other studies in which plasma threonine decreased when rats and chicks were fed excess methionine (Klavins, 1965; Katz and Baker, 1975b). The increase in plasma threonine may indicate that excess dietary methionine does not stimulate threonine dehydratase in the pig as it has been postulated to do in the chick and rat (Katz and Baker, 1975b; Sanchez and Swenseid, 1969). Furthermore, methionine may increase the absorption of threonine which could account for elevated plasma threonine in pigs fed supplemental methionine. This was not investigated however. To further substantiate the theory that threonine

or serine dehydratase is not stimulated at levels of methionine tested in the experiments using pigs, plasma serine was noted to increase in pigs from experiment 2 throughout the study. Also, after weeks 1 and 2 in experiment 3, serine was not significantly affected by 4.0 or 5.5 times the N.R.C. requirement. Plasma serine was elevated in pigs fed 5.5 times the requirement for methionine. Although not significantly different, pigs fed 2.5 times the methionine requirement (pigs with highest gain and gain:feed) had depressed plasma threonine and serine values.

In experiment 2, pigs had depressed plasma glycine during week 2 when fed 2.5, 4.0 or 5.5 times the N.R.C. requirement. During week 3, plasma glycine was depressed only on the two highest levels of methionine supplementation. Pigs utilized in experiment 3 also had depressed plasma glycine on 2.5, 4.0 and 5.5 times the methionine requirement during the first week, but thereafter, plasma glycine was not significantly different in pigs fed any level of methionine tested. This coincides with studies done with the rat and the chick in which methionine seemed to be antagonistic toward plasma glycine (Klavins, 1965; Katz and Baker, 1975b). It may be that glycine becomes first limiting in high-methionine-fed pigs. With threonine and serine increasing in the plasma of high-methionine-fed pigs, it appears the metabolism of these two amino acids to glycine may be impaired resulting in decreasing plasma glycine as methionine consumption increases. Plasma free cystathionine concentration was decreased during weeks 1 and 2 in experiment 2 by feeding pigs 4.0 or 5.5 times the N.R.C. requirement. Plasma cystathionine was depressed throughout the trial at dietary

methionine levels of 4.0 or 5.5 times the requirement. Plasma cystine was elevated in pigs fed 4.0 or 5.5 times the methionine requirement for the duration of both experiments 2 and 3. Considering methionine's effect on plasma cystathionine and plasma cystine, this may indicate that the cystathionine synthetase-catalyzed reaction leading to cystathionine formation is the possible rate limiting reaction in the metabolism of methionine at elevated levels. It appears from the results of these studies utilizing weanling pigs that the adverse effects of supplemental methionine are probably not due to alternate pathway metabolism.

Supplemental dietary methionine elevated plasma methionine in chicks as it did in pigs. Levels 3.0 times the requirement or greater increased plasma methionine in young chicks but only 5.0 times the requirement elevated 5-week-old chicks plasma methionine. Older chicks may be less sensitive to elevated dietary methionine since methionine is required to a greater degree for maintainence than for growth. Plasma threonine decreases observed in young chicks fed excess methionine in experiments 4 and 5 were in agreement with results reported by other researchers (Katz and Baker, 1975b). In experiment 5, feeding young chicks 5.0 times the N.R.C. requirement of methionine caused increased plasma glycine and although plasma glycine was not significantly increased in experiment 4, it did increase when high levels (4.0 times N.R.C.) of methionine were consumed. These increases in plasma glycine may result from threonine's catabolism to serine with concomitant production of glycine. Plasma serine was significantly elevated in young chicks (experiment 5) fed 3.0 or 5.0 times the N.R.C. requirement.

In experiment 4, plasma serine was increased when chicks were fed 4.0 or 5.5 times the methionine requirement but this increase was not significant. Increases in plasma serine might develop from its conversion to glycine since plasma glycine levels were elevated. Young chicks in experiments 4 and 5 had increased plasma free cystine concentrations but only differences in experiment 5 were significant. With elevated dietary methionine being consumed, it is reasonable to expect increases in plasma cystine since methionine can be metabolized to cystine. In both chick experiments, plasma cystathionine was not significantly altered in either age group or chick. Even though plasma cystathionine was not changed significantly, a trend toward reducing the level of plasma cystathionine in chicks fed high methionine diets existed. Possibly methionine was being metabolized through a pathway not leading to cystathionine formation (alternate pathway). In general, older chicks utilized in experiment 5 demonstrated similar responses to high methionine supplementation as the younger birds. However, some variation was evident. Plasma methionine was increased only when dietary methionine was consumed at the highest level tested. Plasma threonine decreased more at 3.0 times the N.R.C. requirement than at 5.0 times the requirement. Glycine in young chicks increased but decreased in older birds. Plasma serine decreased when supplemental methionine was fed at 3.0 times the N.R.C. requirement but was not significantly different, although elevated, when methionine was fed at 5.0 times the requirement. Plasma cystine was increased, but not significantly, with excess methionine consumption and plasma cysthathionine was not altered at any level of methionine supplementation

offered. Considering the responses of the plasma amino acids examined to excess methionine, it appears, as was earlier postulated, that older chicks are more resistant to methionine toxicity due to the greater need of methionine for maintainence in a bird approaching maturity. As the bird ages, the shift from growth requirements to maintainence requirements occurs. Thus, with methionine being in greater demand for maintainence processes, older birds are prepared to handle heavier dietary loads of methionine than are young birds.

CHAPTER VI

SUMMARY

Three experiments were conducted utilizing 62 crossbred pigs, 30 pigs in experiment 1 and 16 pigs each in experiments 2 and 3. Two other experiments were conducted utilizing 168 Hubbard X Hubbard chicks; 60 chicks in experiment 4 and 108 chicks, 54 per age group, in experiment 5. The objective of these experiments was to evaluate the effects of incremental additions of D,L-methionine to the diet of chicks and weanling pigs. Pigs were allotted by weight within litter replicate and chicks were allotted within weight groups to one of four dietary treatments in experiments 2, 3 and 4 and to one of three treatments in experiments 1 and 5. The diets contained methionine and cystine at recommended levels (N.R.C., 1977 and 1979) for control diets and methionine at 2.5, 4.0, or 5.5 X N.R.C. requirements in treatment diets of experiments 2, 3 and 4. Diets containing the N.R.C. requirements of methionine with treatment diets having 1.5 or 3.0 X N.R.C. levels were used in experiments 1 and 5. Bodyweight, feed consumption, hematocrits, hemoglobin and plasma glucose were determined at one-week intervals in each experiment. In all but experiment 1, plasma free amino acid concentrations were also determined.

Results from the first swine experiment indicated that feed consumption was significantly reduced in pigs fed methionine at 3 X the N.R.C. requirement, however, gain per feed (gain:feed) was significantly increased in pigs consuming that level of methionine during week 2.

Data from weanling pig experiments 2 and 3 indicated that gain and gain:feed were increased by the addition of 2.5 times the control dietary level of methionine but that a precipitous decrease resulted from higher methionine supplementation during the first week of each trial. Gain and gain:feed were affected to a smaller degree in weeks 2 and 3 as compared to week 1. Hematological parameters were not affected by methionine additions in any of the weanling pig experiments.

Plasma methionine, threonine and cystine were increased in both experiments 2 and 3 in pigs fed the two highest levels of dietary methionine. Plasma glycine was depressed throughout the experiment in pigs fed 4.0 or 5.5 X the methionine requirement in experiment 2 and was depressed during week 1 only, in experiment 3. Plasma serine increased in pigs consuming the highest level of methionine in experiment 2 but did not exhibit a consistent pattern in experiment 3. Plasma cystathionine levels decreased in pigs fed 4.0 or 5.5 X the N.R.C. requirements of methionine in both pig experiments.

Weanling pigs appear to adapt to high methionine consumption evidenced by resumption of gain and gain:feed while consuming elevated levels of methionine. Also, by decreasing the plasma free methionine concentration over a short period of time while dietary methionine supplementation remains high, indicated an adaptive mechanism was functioning.

Results from the chick experiments indicated that excess methionine consumption depressed gain, feed intake and gain:feed in both young (8-day-posthatched) and older (5-week-posthatched) birds. Hematocrit and hemoglobin values were reduced in young chicks fed increasing amounts of methionine. Hematocrit levels were significantly

decreased in older chicks but hemoglobin values were unaffected in chicks consuming high levels of dietary methionine. Plasma glucose was increased in young chicks consuming the highest amount of methionine but older chicks' plasma glucose was not significantly affected.

Plasma methionine was elevated in young and old chicks consuming supplemental methionine. Plasma threonine was depressed in young chicks fed 3.0 X the N.R.C. requirement. In experiment 4, plasma glycine, serine, cystine and cystathionine were not affected by treatment. In experiment 5, plasma glycine was increased in young chicks fed 5.0 X the N.R.C. level but was decreased in older chicks consuming methionine at 3.0 or 5.0 X the requirement. Plasma serine and cystine were elevated in young chicks (experiment 5) fed the two highest levels of methionine but, in 5-week-old chicks, plasma serine and cystine were not altered by methionine supplementation of 5.0 X the N.R.C. requirement. Plasma cystathionine was not affected in either chick experiment or in either age group.

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