AN ASSESSMENT OF THE BIOTIN NEEDS OF DEVELOPING GILTS AND REPRODUCING SOWS IN A MODERN PRODUCTION SYSTEM

by

Kenneth L. Bryant

Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY
in
ANIMAL SCIENCE

APPROVED:

E. T. Kornegay, Chairman

J. W. Knight

H. P. Veit

K. E. Webb, Jr.

R. F. Kelly

L. A. Swiger, Dept. Head

August, 1982
Blacksburg, Virginia

ACKNOWLEDGEMENTS

My deepest appreciation is expressed to Dr. E. T. Kornegay for his guidance, patience and help throughout this program of study and manuscript preparation. Dr. J. W. Knight's guidance, assistance and support throughout this graduate study is also gratefully appreciated.

The assistance of Dr. K. E. Webb, Jr. in setting up the biotin assay procedure and amino acid analysis is acknowledged. Sincere appreciation is expressed to Dr. H. P. Veit for his help with the histological evaluations and to Dr. R. F. Kelly for slaughtering the sows and serving on the advisory committee. I would also like to thank Dr. L. A. Swiger for his guidance and friendship during this program of study.

The statistical expertise of Dr. D. R. Notter was instrumental in the completion of this project and I am deeply indebted to him.

Appreciation is expressed to Helen Bartlett for tremendous laboratory and statistical assistance and to Susan Arthur for statistical analysis and computer assistance. I also wish to thank Cindy Eaton and Betty Gray for typing the manuscript.

Many thanks are extended to Duke Reynolds, Russ Snyder, Glenn Moreland, Dale Morse, Grady Henderson, Jerry Gibson

and Gene Ball for assistance in data collection, animal care and farm support.

I wish to thank Virginia Polytechnic Institute and State University and the Department of Animal Science for the opportunity to pursue this degree. Appreciation is expressed to Hoffmann La Roche, Inc. for their financial support.

For the love, support and sacrifices of my mother, Virginia Bryant, which made the pursuit of this degree possible, I am forever indebted.

Finally, I wish to dedicate this dissertation to my wife, Charlotte and three children - Joshua, Ashlee and Andrew. Without their love, patience, and willingness to make sacrifices this program of study would not have been completed.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Biotin is an essential water-soluble, sulfur-containing vitamin that is widely distributed in nature, but generally present in relatively small concentrations. It plays an important role in the metabolism of carbohydrates, fats and proteins. Biotin is required for maintenance of the skin, hair, foot pad, reproductive tract, nervous system and thyroid gland.

Thirty-six years have passed since biotin was shown to be an essential nutrient for swine; however, the biotin requirement of the various classes of swine is still unknown. Until the mid-1970's, it was assumed that the biotin present in feedstuffs coupled with the biotin synthesized by the gastrointestinal microflora was adequate to meet the needs of swine. During this time, several reports, primarily field cases, suggested that a spontaneous biotin deficiency in swine may occur under normal production conditions. Positive responses were obtained from biotin supplementation in a majority of these studies. Along with these recent outbreaks of an apparent biotin deficiency, there has been increased pressure to supplement swine diets with biotin. This pressure has been reinforced by the inclusion of a suggested biotin requirement (.1 mg/kg diet)

for all classes of swine in the 1979 National Research Council guidelines. This suggested requirement was extrapolated from poultry data because of a paucity of acceptable swine data.

Biotin supplementation is expensive, costing approximately one dollar per ton of diet. If swine performance could be improved through biotin supplementation, swine producers could save millions of dollars annually. However, if no benefit is derived from biotin supplementation, feed costs would be needlessly increased.

Characteristics of biotin deficiency symptoms include reduced growth rate, impaired feed conversion, alopecia, dermatitis, stiff-legged gait, hind leg spasms and foot lesions. Foot lesions include erosion of the soft heel with resultant cracking of the toe sole. Lesions also appear in the hard horn and claw wall. In 1978, alopecia, dermatitis and foot lesions were observed in the swine research and teaching herd at Virginia Tech.

Many factors can influence the occurrence of a biotin deficiency in swine or alter the level of biotin required in the diet to maximize performance. The main factors are as follows:

1) Wide variation in the biotin content within and among feed ingredients.

2) Limited bioavailability of biotin in many feedstuffs.
3) Deactivation or loss of biotin in feeds due to processing, storage, or contact with rancid fats or oils.
4) Presence of biotin antimetabolites or antagonists which interfere with biotin utilization.
5) Reduction in biotin biosynthesis in the gastrointestinal tract by various ration components, especially antibiotics.
6) Intestinal diseases or disorders which impair absorption of biotin.
7) Increased incidence of confinement rearing of swine which reduces the opportunity for coprophagy.
8) Decreased use of biotin-rich feedstuff (e.g, alfalfa, distillers solubles) as ration components in least-cost feed formulation.
9) Intense selection for faster growing swine on less feed per unit of gain and reduced daily feed intakes for breeding swine.

Poor reproductive efficiency, and thus a high culling rate is an increasingly important problem. Lameness, a complex syndrome, is a contributing factor. Biotin may be implicated in lameness through its' role in maintenance of optimal hoof integrity. Recent research reports also

suggest an improvement in reproductive performance following biotin supplementation.

Until more basic and well controlled research studies are conducted, any recognition or analysis of biotin deficiencies and requirements in swine will be based largely on speculation. Therefore, it is imperative that we reassess the role of biotin in swine nutrition.

In 1978, a study was initiated with the overall objective to assess the need for biotin supplementation to diets for developing gilts and sows housed in total confinement. The specific objectives were as follows:

1) To study the influence of biotin supplementation in diets for developing gilts from weaning to 100 kg body weight on feedlot performance, hair loss and soundness scores and the development of toe lesions;
2) to study the effects of biotin supplementation to corn- and wheat-based diets for gilts and sows on reproductive performance and various biochemical criteria;
3) to study the effects of biotin supplementation to corn- and wheat-based diets for gilts and sows on hair and skin characteristics, soundness scores and development of toe lesions; and
4) to study the influence of toe size, toe location (front vs back and inside vs outside) and

supplemental biotin on the development of toe lesions and soundness in swine throughout their production cycle.

CHAPTER II

REVIEW OF LITERATURE

The discovery and recognition of biotin as a member of the water-soluble vitamin B-complex were achieved from the mid-twenties until the early forties of this century. Lucas (1924) separated a yeast growth factor called bios into two components, bios I and bios II, while Lash Miller (1924) obtained three fractions. Miller (1933) showed that bios II contained at least two different constituents. Kogl (1935) isolated the first pure biotin as one of the factors of bios II.

Boas (1927) observed that rats fed dried egg white developed specific pathological symptoms. She found a protective factor against the egg white injury present in liver and other sources. Gyorgy (1931) studied this protective factor in liver and named it vitamin H, while Lease and Parsons (1934) called it protective factor against egg white injury. Studies of the physical, chemical and biological properties of these factors established that biotin, vitamin H and the egg white injury factor were identical (Gyorgy et al., 1940a; Gyorgy and Poling, 1940; du Vigneaud et al., 1940).

Chemical Structure, Properties and Distribution of Biotin

Numerous chemical tests (du Vigneaud et al., 1941, 1942; Hofmann et al., 1941; Melville et al., 1942) led to the determination that biotin is a monocarboxylic acid containing a cyclic urea structure with sulfur in a thioether linkage. Further work (du Vigneaud et al., 1942) revealed that biotin consisted of a five-membered urea ring fused to a five-member cyclic thioether containing a valeric acid side chain (figure 1). Confirmation of this structure was presented by x-ray crystallographic analysis (Traub, 1956).

Merck Laboratories (Harris et al., 1943) was first to chemically synthesize biotin. Commercially, biotin is now being synthesized by a method developed in the laboratories of Hoffmann-La Roche, Inc. (Gyorgy, 1954).

The free acid, d-biotin, is soluble in dilute alkali and hot water (.03 g/dl), and is practically insoluble in organic solvents. The vitamin in a dry state has a high degree of thermostability and neutral solutions are stable at 100 C. Biotin is stable to moderate acid concentrations but inactivated by strong acid or alkali solutions (Bauernfeind, 1969).

Several compounds bearing a structural similarity to biotin have been isolated from natural sources or chemically

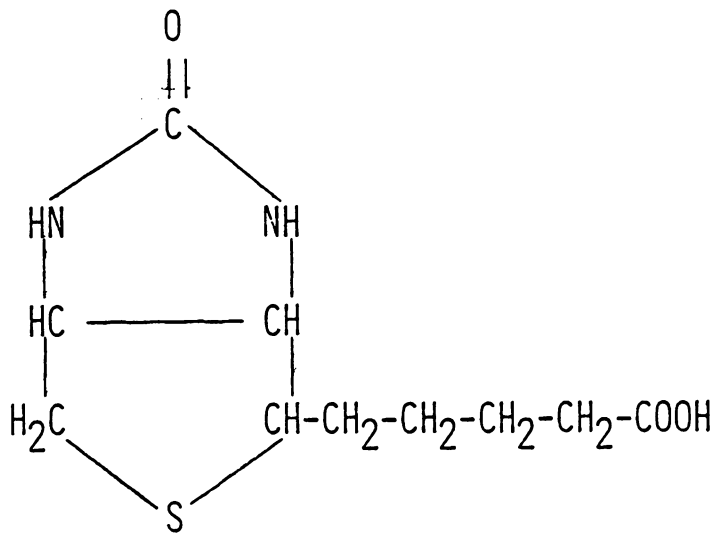


Figure 1. Structure of Biotin.

synthesized and their biological activity tested with animals and microogranisms (Bauernfeind, 1969). D-biotin is the biologically active isomer while other isomeric forms have practically no biological potency. Dethiobiotin and biotin sulfoxide are active for some microbial species but inactive in animals. Oxybiotin and biotinol can replace biotin in the diets of rats and chicks, while a bound form of biotin, biocytin, is also biologically active.

Biotin is present in nearly all plant and animal foods but in relatively small quantities. Hardinge and Crooks (1961) compiled the results of numerous studies which reported the biotin concentration in foods. Among animal tissues, liver and kidney are the major sources of biotin. Egg yolk, from which biotin was isolated, is an excellent source of biotin. Legumes and cereal concentrates are good sources of biotin while bewer's yeast (100 ug/100 g) and royal jelly (410 ug/100 g) contain abundant quanties of biotin.

Role of Biotin in Metabolism

Biotin is an essential factor in the maintenance of normal metabolism. It is directly involved in the vitally important metabolic processes of glucose and fat synthesis and can also have notable effects on other pathways by

influencing many metabolic intermediates. Therefore, biotin is necessary for utilization of nutrients, growth of body tissues and reproduction. An inadequate supply of biotin can produce serious consequences and, in livestock, result in tremendous economic loss (Whitehead, 1981).

Digestion, Storage and Excretion of Biotin

Biotin is released from natural foodstuffs by acid hydrolysis in the stomach. Few studies have been reported on the mechanism or site of absorption of biotin. It has been demonstrated in the rat that absorption occurs in the first half of the small intestine (Spencer and Brody, 1964) and the biotin molecule diffuses intact through the intestinal wall. Some destruction or loss of biotin appears to occur during digestion and absorption since the biotin requirement by subcutaneous injection is only 20% that for oral administration (Rosenburg, 1945). Sorrell et al. (1971) administrated biotin to humans orally or via a catheter into the large intestine and absorption was evaluated by measurement of biotin levels in the blood. Good absorption was obtained when biotin was given orally resulting in a three-fold increase in blood levels of biotin after 4 hours. Absorption of biotin by the large intestine was poor; nevertheless, some biotin absorption did occur.

Following absorption, biotin is bound to plasma proteins (beta- and gammaglobulins primarily) and transported to the various target tissues (Frank et al., 1970). Biotin is stored in the liver and, to a lesser degree, in the kidney (Gyorgy, 1954).

The catabolism of biotin in animal tissues is not clear. Data from Fraenkel-Conrat and Fraenkel-Conrat (1952) suggest that biotin undergoes some metabolic change but does not involve rupture of the ureido ring. Studies by Baxter and Quastel (1953) indicate that the valeric acid side chain is broken down in two-carbon fragments in a manner analogous to fatty acid oxidation. Whether the bicyclic ring system is broken down in animal tissues is not known. This catabolic process has been elucidated in microbial systems (McCormick and Wright, 1971).

Biotin absorbed in excess of requirements and storage capacity, together with biotin metabolites are excreted in the urine (Bonjour, 1977). Unabsorbed biotin or biotin synthesized by the microflora (see section on biotin biosynthesis) are excreted in the feces. Urinary excretions are usually less than intakes; however, when biotin intakes were very low (10% of normal), urinary biotin excretion in humans was greater than intake (Gardner et al., 1945). Due to biosynthesis of biotin by intestinal microflora, excretion of biotin in feces is two- to three-times higher than intake (Nielsen et al., 1942).

Biotin Dependent Enzymes

Lynen et al. (1959) first demonstrated that biotin played a role in carboxylation reactions. It is now established that biotin functions as the prosthetic group of carboxylase enzymes. Carboxylases are widely distributed in nature and catalyze energy dependent fixation of carbon dioxide to various substrates. Table 1 contains the biotin-dependent carboxylases, reactions catalyzed and their metabolic roles as presented by Achuta Murthy and Mistry (1972). Three of these enzymes, acetyl-CoA carboxylase, pyruvate carboxylase and propionyl-CoA carboxylase, are of tremendous importance in animal nutritional biochemistry.

Pyruvate carboxylase. Pyruvate carboxylase (PC) catalyzes the formation of oxalacetate from pyruvate. Utter and Keech (1960) first demonstrated its presence in animal tissue using chicken liver. Subsequent work revealed that PC is present in kidney, adipose tissue, brain, intestinal mucosa and mammary glands (see review by Achuta Murthy and Mistry, 1977).

Pyruvate carboxylase is localized in the mitochondrial matrix (Ballard et al., 1970) and the active molecule is a tetramer with a molecular weight of about 600,000 (Scrutton and Utter, 1965). The active holoenzyme contains four moles of biotin and four gram atoms of bound magnesium per mole. Acetyl-CoA is an allosteric modulator of the enzyme (Utter and Scrutton, 1969).

TABLE 1. BIOTIN-DEPENDENT CARBOXYLASES[a]

Enzyme	Reaction catalyzed (substrate——>product)	Biochemical role
Acetyl-CoA carboxylase	Acetyl-CoA——>malonyl-CoA	Fatty acid synthesis
Pyruvate carboxylase	Pyruvate——>oxaloacetate	Gluconeogenesis lipogenses and generation of 4-carbon intermediates
Propionyl-CoA carboxylase	Propionyl-CoA——>methymalonyl-CoA	Propionate metabolism in animals and microorganisms
B-methylcrotonyl-CoA carboxylase	B-methycrotonyl-CoA——>B-methyl-glutaconyl-CoA	Catabolism of leucine
Geranyl-CoA carboxylase	Geranyl-CoA——>carboxylated geranyl-CoA	Bacterial degradation of isoprenoid compounds

[a]Adapted from Achuta Murthy and Mistry, (1972).

Oxaloacetate is an intermediate in the biosynthesis of phosphoenol pyruvate and, ultimately, glucose. Therefore, PC is essential for gluconeogenesis from pyruvate. Liver and kidney are the tissues with greatest gluconeogenic capacity and they also contain the greatest PC activity (Ballard et al., 1970).

The production of oxaloacetate from pyruvate also affects lipogenesis. Acetyl-CoA generated within the mitochondrion must combine with oxaloacetate to form citrate in order to pass into the cytoplasm. Once in the cytoplasm, citrate is cleaved releasing acety-CoA which can then now enter the lipogenic pathway (Hanson et al., 1971).

Acetyl-CoA carboxylase. Acetyl-CoA carboxylase (ACC) is an ATP-dependent enzyme that catalyzes carboxylation of acety-CoA to form malonyl-CoA. This reaction is well established to be the starting point in the pathway of fatty acid biosynthesis. This was the first biotin dependent enzyme discovered (Wakil, 1958) and it has been isolated from liver, adipose tissue, mammary gland, blood, brain and intestinal mucosa in numerous animal species (see review by Achuta Murthy and Mistry, 1977).

Acety-CoA carboxylase is located in the cytosol of the cell and occurs in both an inactive monomeric form and an active polymeric form. The monomer (MW = 410,000) contains one binding site for the bicarbonate ion, one site for

acetyl-CoA and one for citrate. The presence of citrate shifts the equilibrium from inactive monomer to active polymer. It is hypothisized that conformational changes occur when citrate is bound which alter the enzyme at its' active site (Lane et al., 1974).

Malonyl-CoA, palmityl-CoA and other long chain fatty acyl-CoA derivatives inhibit ACC via a feedback mechanism (Gregolin et al., 1966). Indirect evidence suggests that in vivo fatty acid synthesis may be regulated by metabolite control of ACC (Achuta Murthy and Mistry, 1977). Enzymatic activity decreases during starvation and diabetes and increases following refeeding and insulin administration. The RNA-dependent protein synthesis inhibitor, actinomycin D, prevents this increase, suggesting de novo synthesis of the enzyme during refeeding (Allman et al., 1965).

Propionyl-CoA carboxylase. Propionyl-CoA is converted to methylmalonyl-CoA by the biotin-dependent enzyme propionyl-CoA carboxylase (PCC). The methylmalonyl-CoA can then be converted to oxaloacetate which enters the citric acid cycle or is used for glucose production. This enzyme is essential for metabolism of propionate produced in animal tissues by oxidation of odd numbered fatty acids or degradation of branched chain amino acids (Achuta Murthy and Mistry, 1977). Ruminants depend heavily on PCC to produce energy from propionic acid produced in the rumen (Kaziro and Ochoa, 1964).

Flavin et al. (1957) first identified PCC from pig heart. It is also present in the liver of numerous species, ruminal mucosa and testis of rabbit and cod fish (Achuta Murthy and Mistry, 1977). Achuta Murthy and Mistry (1972) indicated the enzyme was present in the mitochondria.

Mechanism of Action of Biotin-dependent Enzymes

Utilizing isolated, highly purified enzymes the exact mechanism of action of biotin-dependent enzymes has been elucidated. Numerous reviews have appeared on this subject (Mistry and Dakshinamurti, 1964; Lynen, 1967; Knappe, 1970; Achuta Murthy and Mistry, 1977). All known biotin dependent carboxylases utilize bicarbonate as the carboxyl donor and catalyze the same type reaction. Biotin's role is to accept the CO_2 moiety, then transfer it to the appropriate substrate.

The overall reactions catalyzed by biotin enzymes involves two successive half-reactions according to the following scheme:

a) Biotin-enzyme + HCO_2 + ATP $\longrightarrow$ CO_2-biotin-enzyme + ADP + Pi

b) CO_2-biotin-enzyme + substrate $\longrightarrow$ CO_2-substrate + biotin-enzyme.

The first partial reaction (a), driven by ATP, incorporates the CO_2 moiety into the biotin prosthetic

group. This reaction is common to all carboxylases and was validated by means of isotope exchange studies (Lynen et al., 1967; Kaziro and Ochoa, 1961). During the formation of the CO_2-biotin-enzyme intermediate, CO_2 becomes attached to the nitrogen atom of the bicyclic ring of biotin (Knappe et al., 1961).

During the second partial reaction (b), the CO_2 - biotin-enzyme intermediate transfers the CO_2 moiety to the specific substrate. This reaction takes place in the absence of ATP, ADP or Pi indicating its' independence from the first partial reaction.

The overall carboxylation reaction is postulated to proceed via a ping-pong mechanism. The biotin prosthetic group becomes carboxylated at one site on the enzyme and then is translocated to another neighboring site where CO transfer to the acceptor substrate takes place. The biotinyl group, attached to a lysine residue of the enzyme, is visualized as a mobile carboxyl carrier which flips back and forth between the two sites that bind the substrates of the first and second partial reactions, respectively (Moss and Lane, 1971).

Biochemical Effects of Biotin Deficiency

Direct effects. To study the biochemical effects of biotin deficiency numerous workers have produced a biotin

deficiency in animals using a diet rich in egg white (see section on biotin antagonist).

The initial effects of a biotin deficiency are decreases in the activities of the biotin-dependent enzymes. Since key carboxylation reactions in critical metabolic pathways are catalized by biotin-dependent enzymes, biotin deficiency would adversely affect these pathways.

Kosow and Lane (1961) reported a dramatic reduction in hepatic PCC activity in deficient animals consuming a diet containing 30% egg white. Enzyme activity was reduced early in the deficiency and after 39 d on the egg white diet, only 15% of the enzyme activity remained compared with control animals. Rats fed a diet containing 20% egg white for 45 d showed a 10- to 20-fold decrease in liver PC activity (Deodhar and Mistry, 1969). Reductions in ACC activity with biotin deficiency have also been reported (Dakshinamurti and Desjardins, 1968; Jacobs et al., 1970); however, the changes were not as drastic as for pyruvate and propionyl-CoA carboxylase.

Detailed studies at the University of Illinois suggest that individual enzymes, as well as the tissues which contain them, respond differently to a biotin deficiency. Using rats and chicks fed diets containing 20% egg white, PC and PCC activities were vastly reduced in both species (Arinze and Mistry, 1971). After only 7 to 8 d on test

diets, the hepatic activities of these two enzymes in the deficient animals were less than 50% that of the control animals. Hepatic ACC activity was reduced only 35% to 40% in the severely deficient rats (7 to 8 wk) or chicks (4 wk). However, in rat adipose tissue ACC activity was reduced to 75% of the controls. In another study (Chiang and Mistry, 1974) responses similar to those for liver were observed for rat brain, kidney and heart. In all cases liver was the tissue most adversely affected. The authors hypothesized that tissue differences may be due to the turnover rates of the various enzymes among themselves, as well as, in different tissues. Administration of a single dose of biotin (200 ug) to deficient rats resulted in rapid restoration of biotin enzyme activities (Chiang and Mistry, 1974). The most rapid increases occured during the first 4-h period and after 12 h, the enzymes showed activities equal to the control animals. Tissue differences were also noted in response time with kidney responding the quickest followed by liver, brain and heart.

The administration of actinomycin D and/or cycloheximide (inhibitors of RNA-dependent protein synthesis) to deficient rats prior to biotin injection did not prevent increased activity in hepatic PC (Deodhar and Mistry, 1969) and adipose tissue ACC (Desjardins and Dakshinamurti, 1971). These studies led to the conclusion

that in biotin deficiency the carboxylase proteins were present but catalytically inactive (apocarboxylases) without the biotin prosthetic group. When biotin is provided, an activation process, catalyzed by a synthetase enzyme, rapidly incorporates biotin into the apocarboxylase converting it to the active holocarboxylase (Achuta Murthy and Mistry, 1972).

Pyruvate carboxylase is a key enzyme which functions in gluconeogenesis from pyruvate, lactate, glycerol and gluconeogenic amino acids. When it is coupled with phosphoenol pyruvate carboxykinase the irreversible reaction catalyzed by pyruvate kinase is by-passed. Therefore,in biotin deficiency the decrease in PC activity impairs the animal's ability for endogenous glucose synthesis which leads to an accumulation of pyruvate and lactate in the cell (Lehninger, 1976). This prevents maintenance of blood glucose for energy under gluconeogenic conditions such as decreased carbohydrate intake, consumption of high protein or high fat diets and fatty acid oxidation.

Pyruvate carboxylase is present in adipose tissue yet this tissue does not undergo gluconeogensis. In adipose tissue, PC plays an important function in lipogensis (Ballard and Hanson, 1967; Hanson et al., 1971). Acetyl-CoA is the metabolite which starts fatty acid synthesis in the cell cytosol; however, acetyl-CoA is generated within the

mitochondria and cannot cross the mitochondrial membrane. To overcome this barrier, Acetyl-CoA combines with oxalacetate to form citrate which can move into the cytosol via the citrate shuttle. Citrate cleavage enzyme breaks down citrate in the cytosol, again generating oxalacetate and acetyl-CoA. Acetyl-CoA can now enter the lipogenic pathway. Pyruvate carboxylase is essential to replenish the oxalacetate in the mitochondria so it can continue to function in the citric acid cycle or maintain citrate formation for lipogensis (Hanson et al., 1971).

The reaction catalyzed by ACC is well established to be the starting point in the biosynthesis of fatty acids. Therefore, decreased activity of ACC results in a reduced rate of lipogenesis and abnormalities in fatty acid synthesis Whithead, 1981).

Indirect effects. Other metabolic reactions are impaired in biotin-deficient animals; however, the mechanism of biotin involvement in these reactions is not clear (Achuta Murthy and Mistry, 1977). Since avidin does not inhibit these reactions in vitro, Mistry and Dakshinamuriti (1964) concluded that the effects are of an indirect nature rather than due to the lack of biotin alone.

In biotin-deficient animals, protein synthesis is reduced and this is associated with an inhibition of RNA synthesis (Dakshinamurti and Litvak, 1970). Mistry et al. (1962) reported an impairment in glucose utilization and

reduction in liver glycogen in biotin-deficient rats. Ascorbic acid synthesis is reduced with biotin insufficiency and ascorbic acid content of liver and excretion in urine is markedly decreased (Dakshinamurti and Mistry, 1962). Biotin deficiency has also been shown to decrease malic enzyme activity, reduce synthesis of dicarboxylic acids, purines and citrulline (Mistry and Dakshinamurti, 1964).

Biochemical Tests of Biotin Status

At the present time there is no readily applicable test to determine the biotin status of an animal. The most specific criteria for identifying and quantifying a biotin deficiency are biochemical (Whitehead, 1981). There has been considerable progress in developing such methods for several species during the past 8 yr.

Tissue Biotin Levels

Biotin levels in blood were reported to be related to biotin intakes for chickens (Frigg et al., 1973), pigs (Glatti et al., 1975) and humans (Berger, 1950). Frigg et al. (1973) established blood levels that were consistent with good biotin status in young chicks; however, the relationship did not hold for older birds. Glatti et al. (1975) demonstrated that pigs consuming a commercial diet

containing biotin produced normal feedlot performance and plasma biotin levels over 40 ng/dl. Pigs consuming biotin-deficient diets had plasma biotin levels of 20 to 30 ng/dl. Tagwerker (1974) suggested these data be used to indicate biotin status in swine with plasma values above 40 ng/dl being adequate for young pigs. Brooks et al. (1977) used plasma biotin to diagnose biotin deficiency in sow herds affected with foot lesions and suggested that 50 ng/dl is the minimum plasma biotin level for good biotin status. Whitehead et al. (1980) reported that plasma biotin levels gave a good indication of biotin intake for 4- to 12-wk-old pigs. Tagwerker (1974) and Brooks (1982) indicated that large variability in plasma biotin level between individuals and the rapidity with which it increases after feeding limits its usefulness to the evaluation of groups or herds of swine. Bryant et al. (1982) reported no diurnal variation in plasma biotin levels when growing-finishing gilts were fed twice daily; however, significant gilt differences were observed.

Biotin concentration in liver has been used as an indicator of biotin status in chickens (Frigg and Brubacker, 1976). It has been especially useful in post-mortem studies (Hood et al., 1976) on fatty liver and kidney syndrome (see section on biotin in avian nutrition).

Biotin-Enzyme Activity

Hepatic activities of PC and ACC are related to biotin status and are reduced 20 and 60%, respectively, in biotin-deficient birds (Arinze and Mistry, 1971). Atwal et al. (1971) found PC activity in chick liver was proportional to biotin intake over a wide range of dietary biotin levels. Unfortunately, hepatic PC activities change rapidly with age and can be affected by dietary protein or fat levels (Whitehead et al., 1978).

Measurements of liver enzyme activities usually necessitates death of the animal, therefore, enzyme activity in the blood has been evaluated as a criteria of biotin status. Presence of ACC in human, pig and chicken blood has been demonstrated (Glatzle and Frigg, 1975); however, it does not show a large response to changes in dietary biotin intake.

Bannister and Whitehead (1976) obtained high PC activity in blood of young chicks and the level of activity increased with increasing dietary biotin levels. Whitehead and Bannister (1978) reported a positive sigmoidal relationship between blood PC activity and supplemental biotin level in young chickens and turkeys. Enzyme activity decreased between 2 and 4 wk of age but remained constant thereafter. In this study, alterations in the fat or protein content of the diet did not affect chick blood PC

activity. The activity of blood PC can be preserved in a glycerol-based medium extending its usefulness to field samples (Glatzle et al., 1979a). Blood PC activity changes significantly with age (Whitehead, 1981) and does not give a satisfactory test of biotin status in adult birds.

Blood PC activity has been measured in swine red blood cells (Whitehead et al., 1980); however, due to the lack of nucleated red blood cells in swine, the activity was very low. When young pigs were fed diets containing varying levels of dietary biotin, blood PC acitivity showed a maximum response to dietary biotin at 6 wk of age. By 12 wk of age there was no response to supplemental levels greater than 100 ug/kg diet. The authors questioned the use of blood PC activity as a criterion of biotin status in swine due to its low activity and rapid changes with age.

Glatzle et al. (1979a,b) reported results of biotin-dependent enzyme activation studies with chick liver and blood. Since the reduction in enzyme activity arising from a biotin deficiency is due to a lack of biotin rather than apoenzyme (see section on biotin-dependent enzymes), they hypothesized that the extent of specific enzyme activity enhanced by incubation in vitro with biotin could be used to evaluate biotin status. Activation coefficients of hepatic PC and ACC gave a good indication of biotin status. Additionally, in vitro stimulation of blood PC activity was

demonstrated. When compared to biotin levels in feed, plasma and liver, the PC activation assay appeared to be a useful tool for assessing the biotin status of chicks.

Despite low PC activity in mammalian blood, activation coefficients have been measured in rats and pigs (Bitsch et al., 1977 and Glatzle, 1979 as cited by Whitehead, 1981). Enzyme activation results with pigs (Glatzle, 1979 as cited by Whitehead, 1981) showed a close relationship with both dietary and plasma biotin levels.

Tissue Fatty Acid Patterns

Changes in tissue fatty acid patterns have been observed as an indirect effect of biotin deficiency. In deficient chicks, the proportion of palmitoleic acid is increased while stearic acid decreases (Balnave, 1966). Whitehead et al. (1976) did not observe these changes in birds fed biotin deficient diets containing high levels of fat or protein. Edwards (1974) suggested the palmitoleic acid:stearic acid ratio in liver, adipose tissue and toe lipids was useful in evaluating the biotin status of birds. The lipid content of adipose tissue has been reported to be substantially reduced in biotin-deficient pigs (Tagwerker, 1974).

Biotin Deficiency Symptoms in Animals

Due to the wide distribution of biotin in feedstuffs and intestinal biosynthesis of biotin a severe biotin deficiency rarely occurs under normal conditions. To study the clinical manifestation of biotin deficiency, experimental deficiencies have been produced in many mammalian and avian species by dietary additions of egg white (avidin) or sulfa drugs.

In view of biotin's broad range of metabolic functions biotin deficiency has a profound effect on the animal and a wide range of clinical sumptoms has been described in different species (Balnave, 1977).

Swine

Cunha et al. (1946) produced the first experimental biotin deficiency in growing pigs using a diet containing 30% raw egg white powder. Deficient pigs developed alopecia, spasticity of the hind legs, cracks in the feet and a dermatosis of the skin characterized by dryness, roughness and a brownish exudate. Rate of gain and feed efficiency were reduced by 45 and 50%, respectively. Intramuscular biotin injections of 100 ug/pig/d prevented the deficiency. These deficiency symptoms were also induced by inclusion of sulfathalidine in a basal diet (Lindley and Cunha, 1946). Suckling pigs consuming a biotin-deficient

purified diet also developed the above symptoms along with diarrhea, inflammation of the mucous membranes of the mouth, ulceration of the skin and a brown exudate in the eyes (Lehrer et al., 1952).

Glattli et al. (1975) produced a biotin deficiency in 10 to 31 d old specific pathogen free pigs and characterized the progression of deficiency symptoms. After 2 to 3 wk on the deficient diet the pig's hair coats became rough and hair loss began on the back and side of the body, and in extreme deficiency, the animals became almost hairless. The pig's skin became dry, rough, scaly and less elastic and in severe cases skin cracks and pustules developed. Encrustations and a brownish waxy exudate appeared on the skin, especially at the angles of the eye and mouth which lead to fissures. A white film formed on the upper surface of the tongue and lesions appeared in the mucous membrane of the mouth and the esophagus. Histologically, the dermal lesions were characterized by hyperkeratosis and parakeratosis. There was (1) moderate, nonpurulent subepithelial infiltration of keratin and disorientation of the stratum basale, (2) vacuolation and nuclear pyknosis of cells in the stratum spinosum and (3) an absence of the stratum granulosum. Keratinization was irregular and there was a loosening of the entire epidermis.

Five weeks after pigs were fed biotin-deficient diets, foot lesions appeared in the form of small erosions of the epidermis and cracks in the skin of the soft sole and coronet (Glattli et al., 1975). The lesions became more severe until there were deep transverse fissures across the soft sole. The hard horn became soft and rubbery losing its resistance to abrasion. This resulted in cracks forming in the hard horn of the sole and claw wall. As a result of these lesions, animals became lame and showed signs of spasticity in the hind legs (Cunha et al., 1946; Glattli et al., 1975).

Avian Species

McElroy and Jukes (1940) found that chickens fed raw egg white exhibited perosis, poor growth and a scaly dermatitis. Rough, calloused foot pads containing deep fissures and some hemorrhaging were also noted (Jukes and Bird, 1942).

Whitehead (1977) presented a description of the progressive development of biotin deficiency in chicks. The speed with which the lesions develop depends on the severity of the deficiency. In severely deficient chicks dryness and flakiness of the feet began at 14 d of age followed by slight encrustations and superficial fissures on the foot pads at 18 d of age. Encrustations occurred at the corners

of the beak at 20 d of age, and increased in severity with fissures developing on the top of the beak by 27 d. Dermatitis also developed around the eyelids between 21 to 28 d and eventually the eyelids stuck together. Perosis or bone deformities followed by slipping of the tendon occurred but is variable. Robblee and Clandinin (1970) described similar deficiency symptoms in turkey poults along with dry brittle feathers, loss of primary and secondary feathers, and diarrhea.

Microscopic examination of skin in biotin-deficient birds revealed parakeratosis, hyperkeratosis, epidermal hyperplasia, papillary growth and acanthosis (Whitehead et al., 1974; Frigg and Torhorst, 1980). In most severely affected areas, there was complete erosion of the epidermis and formation of a crust of erythrocytes, inflammatory cells, necrotic debris and secondary bacterial foci. There was a marked decrease in the amount of stainable lipid in the stratum corneum, which could contribute to the breakup of the keratin layer (Forbes and Davis, 1974).

Other signs of biotin deficiency in chickens and turkeys include decreased hatchability (Couch et al., 1948a), embryonic deformities (Cravens et al., 1942) and fatty liver and kidney syndrome (Payne et al., 1974). Egg production in commercial layers is usually not affected (Scott, 1981); however, after 13 wk on a biotin-deficient

diet egg production dropped abruptly in breeding turkeys (Ferguson et al., 1961).

Other Species

Clinical manifestations of biotin deficiency in humans include anorexia, nausea, vomiting, glossitis, pallor, depression, lassitude, substernal pain, scaly dermatitis and desquamation of the lips (Baugh et al., 1968). Symptoms cleared or improved markedly after 2 to 5 d of parenteral therapy providing 200 ug of biotin daily.

In rats and mice, biotin deficiency is characterized by an eczematous dermatitis, achromatrichia and alopecia (Rauch 1952). Spastic gait and a kangaroo-like posture was observed in biotin-deficient rats (Boas, 1927). Kennedy and Palmer (1946) reported that biotin was necessary for successful pregnancy and lactation in the rat.

Biosynthesis of Biotin by Intestinal Microflora

Assessment of biotin supplies to the animal are complicated by the presence of microorganisms in the gastrointestinal tract that synthesize biotin (Lehrer et al., 1952). Sites of most active microbial synthesis are the ceacum and large intestines (McGregor et al., 1947) where absorption is questionable (Michelsen, 1956).

However, some monogastric animals (rat, guinea pig and mouse) can absorb sufficient microbial biotin to prevent severe signs of biotin deficiency when fed a low biotin diet (Reid, 1954; Wilson et al., 1949).

The nature of carbohydrates present in the diet (Couch et al., 1949), type of microflora in the digestive tract (Brady et al., 1965) and presence of sulfa drugs (Nielson et al., 1942) have been reported to alter the amount of biotin excreted in the feces.

More than 30 microorganisms have been reported to synthesize biotin (Gyorgy and Lander, 1968) and include numerous species of Lactobacillus, Aspergillus, Pseudomonas, Mycobacterium, as well as Escherichia coli.

The sequence of biochemical reactions leading to biotin biosynthesis have been studied in detail. Two conflicting pathways for biotin biogenesis have been proposed and McCormick and Wright (1971) suggest that both pathways could be operating among the many different organisms capable of synthesizing biotin. The pathway currently favored (Eisenberg, 1973) contains the following sequence of reactions: (1) formation of pimelic acid from acetyl-CoA; (2) synthesis of dethiobiotin from pimelic acid via pelargonic acid derivatives; and (3) conversion of dethiobiotin to biotin. Metabolic control of this pathway is through negative feedback inhibition by biotin on the dethiobiotin synthetase enzyme.

Biotin Antagonists

Avidin

Avidin, a basic glycoprotein component of egg white was the earliest reported antagonist to biotin and proved to be the cause of the egg white injury (Gyorgy and Poling, 1940) in rats. It has the unique property of strongly binding free and enzyme-bound biotin.

One molecule of avidin has four biotin binding sites and possesses a tetrameric structure which dissociates in the presence of guanidine hydrochloride into four identical subunits (Green, 1964). Results from numerous studies (Fraenkel-Conrat et al., 1952; Green, 1962, 1963) indicate that the active site of avidin has tryptophan residues which possess a weak but specific affinity for ureido groups. During binding with biotin, the weak complex formed is presumably stabilized by interactions between tryptophan and the hydropholic side chains of the protein and biotin (Green, 1963).

The avidin-biotin complex is stable over a wide range of pH and temperature and is not degraded by enzymes within the gastrointestinal tract (Eakin et al., 1940).

Streptavidin

Streptavidin, a crystalline protein isolated from fermentation filtrates of streptomyces bacteria has been reported to have biotin-binding ability similar to avidin (Chaiet and Wolf, 1964). The protein binds four molecules like avidin but electrophoretic and chemical analysis indicates that streptavidin is different from avidin. Unlike avidin, streptavidin does not bind dethiobiotin or d-biotin-d-sulfoxide indicating a requirement for an intact sulfur ring in addition to the urea ring for activity (Lichstein and Birmbaum, 1965). Streptomyces bacteria can be found in soil, litter, rotting manure and oxidized feed (Anonymous, 1972).

Other Antagonists

Alpha-dehydrobiotin, a derivative of biotin isolated from streptomyces bacteria, was reported to be a potent biotin antimetabolite (Hanka et al., 1966). Homobiotin, norbiotin, lysolecithin and biotin sulfane are also regarded as antagonists to biotin utilization (Gyorgy and Lander, 1968).

Biotin Assay Procedures

The estimation of biotin in natural food products and body tissues is difficult because biotin is present in very low concentrations, with assay sensitivities from .1 to 1 ng required. Many biotin assay procedures have been reported and include: microbiological procedures, animal assays, radiochemical and various chemical tests.

Microbiological Procedures

Of the procedures devised for the estimation of biotin, microbiological procedures are the most extensively employed. Numerous microorganisms require biotin as a growth factor and a number of methods utilizing one or more of these organisms have been reported (see review by Gyorgy, 1967). To be used as a test organism for biotin assays, a microbe must be unable to produce its' own supply of biotin, thereby depending upon its' medium to supply biotin. Microbiological assays using yeast (Lampen et al., 1942), Lactobacillus casei (Shull et al., 1942) and Lactobacillus arabinosus (Wright and Skeggs, 1944) have been the most widely utilized.

The procedure developed by Wright and Skeggs (1944) which employs Lactobacillus arabinosus 17-5 (ATC #8014) as the test organism, is extensively used and recommended by the Association of Vitamin Chemists (1966). The culture

(organism) is carried in agar stabs and inocula prepared as in other standard microbiological assays. The hydrolyzed sample is added at graded levels to the culture medium. A set of standard tubes containing varying amounts of synthetic biotin (up to .5 ug per tube) are also prepared. After sterilization and inoculation, growth response is measured by titration of the acid produced after 72-h inoculation at 35 to 37 C or turbidity reading after 24 h. Results for the unknown samples are then compared to the standard curve.

Gyorgy and Lander (1968) discussed two limitations of microbiological assays for biotin determination. First, most organisms used for assay are unable to use bound forms of biotin (Lampen et al., 1942). Therefore, samples must first be hydrolyzed to release the bound biotin. Care must be taken to ensure that hydrolysis is complete but that no distruction of biotin occurs. A second limitation is the tendency for many microbes to respond (grow) non-specifically to compounds with biotin-like activity. These two limitations sometime render results of microbiological assays difficult to interpret; however, the methodology is technically simple and demands considerably less time and expense than animal assays.

Animal Assays

Bliss and Gyorgy (1967) have described animal assays for biotin in detail. They offer the advantages of being highly specific and measure the vitamin's bioavailability, in contrast to microbiological assays which measure the amount of biotin freed in chemical hydrolysis. This is an important difference since incomplete availability of biotin to animals (see section on biotin content and bioavailability) has been reported (Wagstaff et al., 1961; Frigg, 1976). The primary limitation of animal assays are their high cost and time requirements. Rats and chicks have been used as test animals for the bioassay of biotin.

Diets free of biotin and high in avidin are fed for a specific period of time to deplete the animals of biotin (Bliss and Gyorgy (1967). Traditionally, the curative effect of biotin from the test sample on the animal's growth rate is used for the bioassay because this parameter is easily assessed quantitatively. The assay is subjective to many sources of potential variation which includes: sex, litter differences, length of depletion and/or test period and the measurement of growth (Bliss and Gyorgy, 1967).

Other Methods

Radiochemical assays for biotin which use C^{14}-biotin have been reported (Dakshinamurti et al., 1974; Hood, 1975).

The assay is based on the competition between a known quantity of radioactive biotin and an unknown quantity of non-radioactive biotin for the binding sites on avidin. Following precipitation of the avidin-biotin complex the amount of excess C^{14}-biotin remaining in the supernatant will depend on the dilution of C^{14}-biotin by the non-radioactive biotin (Hood, 1975). The assay is sensitive to one nanogram and yields results which are comparable to those obtained from a microbiological assay. At present, the sensitivity of the method is limited by the specific activity of the commercially available isotope (Hood, 1975).

Viswanathan et al. (1970) developed a rapid and precise gas chromatographic method for estimating d-biotin content of premixes and injectable preparations. Biotin is converted to its silyl ester prior to being chromatographed. Biotin has also been assayed using spectrophotometric procedures (Plinton et al., 1969; Green, 1970).

Biotin Content and Bioavailability in Feedstuffs

The main source of biotin to the animal is in feedstuffs consumed. The amount of biotin in the diet available to the animal depends upon the quantity of biotin in the feedstuff, the biological availability of this biotin, the amount and type of diet consumed.

The biotin content of raw materials varies from as low as 20 ug/kg in some grains and meat meals to well over 1000 ug/kg in some samples of sunflower meal and distillers grains (Scheiner and DeRitter, 1975). Table 2 presents the biotin content and bioavailability of various feedstuffs as compiled by Scheiner and De Ritter (1975). Yeast and other sources of vegetable protein such as peanuts are rich in biotin; however, grains contain low levels of biotin.

The wide variability in the biotin content among samples of a given feedstuff is striking and the causes are numerous. These variations can result from differences in plant varieties or animal strains, harvesting season and yield, processing, length and type of storage, method of extraction and biotin assay used (Tagwerker, 1974).

Patrick et al. (1942) postulated that practical diets for poultry can be deficient in biotin due to the natural biotin in feedstuffs not being fully available. Comparing chick assays to microbiological assays, Wagstaff et al. (1961) reported that approximately one-half of the microbiologically assayed vitamin in feedstuffs is unavailable to poultry. Scott (1968) noted that biotin exists in both a free and bound form and that a high amount of the bound form is unavailable to poultry. Further evidence (Anderson and Warnick, 1970) suggested that wheat, barley, milo, fish meals, and meat and bone meal provided

TABLE 2. BIOTIN CONTENT AND BIOAVAILABILITY OF VARIOUS FEEDSTUFFS

Feedstuff	Biotin levels, ug/kg[a] Range	Avg	Biotin availability, %
Alfalfa meal, dehydrated	330- 690	490	100[a]
Barley	90- 130	110	20-50[bd]
Corn, yellow	60- 150	110	100[b]
Fish meal	420- 550	460	30[c]
Meat and bone meal, 50%	130- 310	190	70[c]
Milo	180- 280	240	20-60[b]
Oats	110- 270	190	32[b]
Soybean meal, solvent extracted	320- 460	400	100[c]
Wheat	110- 130	110	20-52[bd]
Yeast, brewers' dried	870-1520	1180	---

[a]Scheiner and DeRitter, (1975).

[b]Frigg, (1976).

[c]Anderson and Warnick, (1970).

[d]Baker, (1978).

chicks with less biotin than was found in these feedstuffs by microbiological assay.

Frigg (1976) studied the bioavailability of biotin occuring in cereals by a chick growth assay. The microbiologically determined biotin content of corn was low (45 ug/kg), however, this biotin was completely available to the chicks. In milo, barley and oats, biotin availability was reduced to 20%, 22% and 30%, respectively. No growth response was obtained with wheat, suggesting an even lower bioavailability.

Baker (1978) reported bioavailability values of 122%, 61% and 45% for corn, sorghum, barley and wheat, respectively. Similar bioavailabilities were reported by Anderson et al. (1978) and estimates of available biotin were .108 ug/kg for corn, .82 for barley, .92 for sorghum and .43 for wheat.

Table 3 presents the free, total and percent free biotin for various feedstuffs. The percent free biotin in a feedstuff does not appear to influence the bioavailability (table 2) of the feedstuff. Only 23% of the biotin in corn is in a free form, however, all the biotin present is available to chickens (Frigg, 1976; Baker, 1978). In contrast, 36% of biotin in wheat is free while less than 50% of the total biotin is available.

TABLE 3. FREE AND TOTAL BIOTIN IN VARIOUS FEEDSTUFFS[a]

Feedstuff	Free biotin ug/kg	Total biotin ug/kg	Free biotin %
Alfalfa	520	650	80
Corn	18	80	23
Herring meal	50	450	11
Meat and bone meal	30	200	15
Milo	75	230	33
Soybean meal	100	440	23
Wheat	35	98	36

[a]Scheiner and DeRitter, (1975).

Biotin in Avian Nutrition

Because biotin functions in so many important metabolic reactions, a deficiency of this vitamin has profound effects upon the economy of poultry production. In addition to gross deficiency symptoms (see section on biotin deficiency), biotin deficiency may reduce growth and feed conversion in broilers and egg production and hatchability in hens. Biotin is particularly important in turkey nutrition as evidenced by the fact that turkeys have the highest biotin requirement of any animal species studied (Scott, 1981).

Growth and Performance

McElroy and Jukes (1940) found that chickens fed raw egg white exhibited perosis, scaly dermatitis and poor growth. Using a purified diet containing all known nutrients except biotin, Jukes and Bird (1942) demonstrated that the injection of 2 ug of crystalline biotin daily prevented perosis and improved growth. Hegsted et al. (1942) confirmed biotin's role in chick growth and set the chick's biotin requirement at 70 to 100 ug/kg of diet.

These findings appeared to have little practical application until symptoms typical of biotin deficiency were diagnosed in commercial turkey flocks (Richardson and

Wilgus, 1967; Johnson, 1967). Turkeys from 8 to 20 wk of age exhibited dermatosis of the feet, increased leg deformities, weak stiff-legged gait and dry brittle feathers. Enlarged foot pads containing fissures were also noted and in severe cases the turkeys were unable to walk. In most cases treatment with biotin alleviated the symptoms. Jensen and Martinson (1969) and Robblee and Clandinin (1970) confirmed that biotin deficiency in turkey poults resulted in severe foot dermatitis. Harms et al. (1977) reported that foot pad dermatitis was significantly increased in broilers grown on wet litter and the severity and incidence of these foot lesions were reduced in birds receiving 220 ug/kg supplemental biotin in corn-soybean meal diets.

Anderson and Warnick (1970) conducted studies to determine the types of poultry diets which may require biotin supplementation. All grains were poor sources of biotin while wheat and barley were the poorest of all the grains. A practical ration containing 260 ug biotin/kg by microbiological assays still required additional biotin to produce maximum growth in broilers.

Blair (1977) recommended that broiler starter diets contain at least 150 ug/kg biotin and finisher diets 100 ug/kg to maximize growth and performance. The National Research Council (1977) recommends a biotin requirement of 150 ug/kg and 100 ug/kg for chicks 0 to 8 wk and 8 to 18 wk of age, respectively.

Wilgus (1969) studied the dietary biotin requirements of starting and growing turkeys as influenced by various production conditions. The biotin requirement of poults during the first month of life varied from 100 to 320 ug/kg diet and was influenced by carry over from the egg, type of ration, sex, diet nutrient density and interrelationships with environment. Biotin supplementation at 100 ug/kg diet in poult rations during the first 8 to 12 wk of life was recommended. Dobson (1970) reported the total biotin requirement of male broad-breasted turkey poults to be 275 to 325 ug/kg diet. The female turkey's requirement was estimated to be approximately 50 ug/kg lower than the males. The National Research Council (1977) set the biotin requirement for 0 to 8 wk-old turkey poults at 200 ug/kg and at 100 ug/kg from 8 wk to market. The total amount of biotin in the diets must be higher if feedstuffs low in biotin bioavailability such as wheat or barley are used (Scott, 1981).

Fatty Liver and Kidney Syndrome

Fatty liver and kidney syndrome (FLKS) or flip-over disease is characterized by sudden death of apparently healthy chicks having a fast growth rate (Hood, 1980). Post-mortem examination of affected birds reveals a pale, enlarged liver and kidney containing large amounts of lipid.

Fatty infiltration of the skeletal muscles, central nervous system and cardiac muscle may also be present.

Under commercial conditions, mortality from FLKS has been extremely variable with losses of 30% reported (Hemsley, 1965). Payne et al. (1974) and Pearson et al. (1976) established that a marginal deficiency of dietary biotin was centrally involved in FLKS and increasing biotin in the diet eliminated FLKS in commercial flocks. Diets high in carbohydrate, low in protein, fat and biotin have also been associated with FLKS (Butler, 1976). Hood (1980) observed that stress, such as excessive noise or sudden changes in temperature, are usually required to trigger the disorder.

When birds are stressed they catabolize glycogen and glucose. However, due to failure of gluconeogenesis, biotin-deficient birds become hypoglycemic and death results from irreversible damage to the central nervous system (Hood, 1980). The failure in gluconeogenesis is reflected in an accumulation of blood lactate. Hood et al. (1976) reported that in a marginal biotin deficiency the concentration of biotin in liver has opposite effects on the activities of hepatic PC and ACC. When liver biotin is below 80 ug/g or following a 18-h fast the activity of PC is decreased while ACC is increased. Under these conditions PC activity is insufficient to metabolize pyruvate and lactate

via gluconeogenesis. However, ACC maintains fatty acid synthesis.

Hulan et al. (1980) tested the addition of various vitamins and reported that the addition of biotin significantly reduced total mortality and mortality due to FLKS in broiler chicks. Biotin supplementation of parent flock diets reduced susceptibility of progeny to FLKS (Whitehead and Blair, 1976).

Egg Production, Hatchability and Chick Viability

In contrast to growing birds, feeding biotin-deficient diets to adult birds produces few direct effects on the birds themselves. However, the bird's reproductive capability is markedly affected by biotin. Biotin is essential for normal embryonic development, hatchability and viability of the hatching chick.

Pullets and breeding hens fed virtually biotin-devoid diets for 10 to 11 wk maintained normal egg production, egg size, weight gain and showed no signs of dermatitis or increased mortality (Cravens et al., 1942; Brewer and Edwards, 1972). Turkey hens continue to lay eggs when fed low biotin diets, however, egg size, level of production and body weight maintenance can be affected by low biotin intakes (Ferguson et al., 1961; Atkinson, 1972). Consumption of biotin-deficient diets for 13 wk by breeding

turkeys was required to significantly reduce egg production (Ferguson et al., 1961), suggesting significant biotin body stores. Twenty-three week were required to produce mild signs of foot and mouth dermatitis. Improvements in egg production from biotin supplementation to practical diets have been reported for commercial layers (Scott, 1981) and turkeys (Jensen and Martinson, 1969).

Hatchability of fertile eggs was reduced in chickens and turkeys consuming biotin-deficient diets and fell to zero in 3 to 5 wk if dietary biotin levels was sufficiently low (Cravens et al., 1942; Couch et al., 1948a; Brewer and Edwards, 1972). Biotin supplementation restored hatchability in 2 to 3 wk and increased the amount of biotin in the egg (Cravens et al., 1942; Couch e t al., 1948b). When hatchability was zero, yolk biotin content dropped to 50 ng/g and Brewer and Edwards (1972) found a yolk biotin level of 200 ng/g was required for normal hatchability. Bradley et al. (1976) increased hatchability of fertile eggs from 85% to 93% with biotin supplementation to a milo-soybean meal diet for layer breeder hens.

Perosis, syndostyly, ataxia, bone distortions and stiffness of the hock joint are birth deformities observed in embryos or hatched chicks from biotin-deficient hens (Caskey et al., 1944; Cravens et al., 1944; Couch et al., 1949). Injection of biotin into the eggs from severely

deficient hens at the start of incubation resulted in normal hatchability and no signs of abnormalities. When biotin injection was delayed until 120 h after the start of incubation, hatchability approached zero. Injection of biotin after a 96-hr incubation period produced a high incidence of perosis, suggesting that biotin is involved at an early stage in hock joint development (Couch et al., 1949).

Although biotin-deficient laying hens do not lay smaller eggs, chicks that hatch are smaller from eggs laid by biotin-deficient hens compared with those hatched from hens receiving adequate biotin (Brewer and Edwards, 1972). Chicks from biotin-deficient hens also had reduced growth rates and increased mortality, even when fed diets adequate in biotin.

Brewer and Edwards (1972) reported 100 ug of total biotin/kg diet was required for maximum hatchability and chick livability, while the National Research Council (1979) set the biotin requirement of breeding chickens at 150 ug/kg diets.

Biotin in Swine Nutrition

The essentiality of biotin as a nutrient for swine was established from early laboratory studies by Cunha et al.

(1946), Lindley and Cunha (1946) and Lehrer et al. (1952). In these studies biotin deficiency was produced in young pigs by feeding a semi-purified diet containing no biotin or diets containing avidin as egg whites. The primary deficiency symptoms included alopecia, dermatosis of the skin, cracks in feet and dramatic reductions in daily gain and feed efficiency. The deficiency syndrome was prevented by intramuscular injections of 100 ug biotin/d (Cunha et al., 1946) or dietary biotin supplementation at 200 ug/kg diet (Lindley and Cunha, 1946). A series of experiments (Buhlmann, 1973; Pohlenz, 1974; Glattli et al., 1975) confirmed these earlier observations and provided detailed information on the type and sequence of deficiency symptoms (see section on swine biotin deficiency).

Severe biotin deficiency symptoms rarely occur under practical swine production conditions. It has been assumed that biotin present in ingredients fed to swine coupled with biotin synthesized by intestinal microflora were adequate to meet the pig's needs. However, numerous reports have appeared since the mid-1960's concerning the need to supplement swine diets with biotin.

Supplemental Biotin and Feedlot Performance

The role of biotin in swine weight gain and feed efficiency was established by Cunha et al. (1946). Biotin-

deficient pigs exhibited a 45% reduction in daily gain and required 50% more feed/kg of gain. Lindley and Cunha (1946) confirmed this effect of biotin on feedlot performance utilizing a purified diet containing sulfathalidine. However, during a 5-wk trial, no beneficial effect on growth or efficiency of feed utilization was obtained when biotin was added at 200 ug/kg to a purified diet containing no antibiotics. Lehrer et al. (1952) obtained clinical biotin deficiency symptoms in baby pigs fed a synthetic milk diet free of biotin, but reported no depression in growth rate.

Adams et al. (1967) fed 192 early-weaned specific pathogen-free pigs a basal corn-milo-soybean meal diet calculated to contain 286 ug biotin/kg diet. The addition of 110 ug supplemental biotin/kg diet increased daily gain and feed efficiency 15% and 17%, respectively. Clinical biotin deficiency signs were not observed in the pigs, however, clinical signs were seen in approximately 10% of their dams.

Three experiments utilizing 270 early-weaned pigs were conducted by Peo et al. (1970) to determine the effect of biotin supplementation on feedlot performance. Supplementation with 440 or 880 ug biotin/kg diet increased daily gains approximately 10 percent. The addition of 880 ug/kg did not improve gains above that obtained with the 440 ug/kg diet. Feed efficiency was similar for all treatment groups.

To the contrary, numerous reports (Meade, 1971; Hanke and Meade, 1971; Washam et al., 1975; Easter et al., 1978; Simmins and Brooks, 1980) reported no improvement in feedlot performance when corn-soybean meal diets were supplemented with biotin. Biotin supplementation varied from 55 to 720 ug/kg diet in these reports and included starter and grower-finisher pigs. In 1979, the National Research Council suggested a biotin requirement for swine for the first time. They set the requirement at 100 ug/kg diet for swine weighing 1 to 100 kg body weight.

Biotin and Reproductive Performance

Biotin deficiency has not been experimentally produced in sows; however, the role of biotin in reproductive function was established in rats (Kennedy and Palmer, 1946) and chickens (Cravens et al., 1942). Cunha et al. (1968) was the first to suggest an improvement in reproductive performance from biotin supplementation. In a field trial, sows were indexed based on reproductive performance and sows receiving supplemental biotin produced the highest indexes. The level of biotin supplemented and biotin content of the basal diet was not given.

Brooks et al. (1977) investigated the effect of biotin supplementation for breeding sows in a herd where the sows exhibited symptoms resembling those of experimentally

induced biotin deficiency. Forty-five second and third parity sows housed in concrete-floored buildings were used. Biotin was supplemented at 250 ug/kg during gestion and 150 ug/kg during lactation to basal diets containing 76 ug available biotin/kg diet. Sows on the biotin supplemented diets farrowed more total pigs/litter than unsupplemented sows, but the difference was not significant. Second parity biotin supplemented sows farrowed significantly more (1.64) live pigs per litter than control sows. The weaning to remating interval was reduced from 15.3 d in the unsupplemented sows to 6.2 d in supplemented sows. The percentage of sows exhibiting estrus within 7 d of weaning was increased from 56% to 89% with biotin supplementation.

Corn-soybean meal (Easter et al., 1979) and barley-soybean meal diet (Newman and Elliott, 1980) supplemented with 200 ug biotin/kg failed to produce significant responses in reproductive performance when compared to unsupplemented diets. Both studies involved gilts and the supplementation period lasted through one parity. Easter et al. (1979) obtained an 8% and 15% improvement in number of pigs born alive and weaned, respectively, with biotin supplementation.

Sows receiving an estimated 145 to 220 ug of biologically available biotin daily during pregnancy and 300 to 450 ug daily during lactation exhibited deficiency

symptoms and reduced reproductive performance (Halama, 1979). Increasing daily biotin intakes to 330 to 500 ug during pregnancy and 780 to 1170 ug during lactation reduced the weaning to mating interval 2 to 3 d and increased conception rate from 76% to 85%. This field trial did not contain a control group of females for comparison. The daily intake of available biotin was subsequently fixed at 445 ug from 10 to 105 d of gestation, 550 ug from 105 d to term, 1100 to 1650 ug during lactation and 825 ug from weaning to d 10 of gestation (Halana, 1979).

Two trials using 237 gilts and primiparous sows were conducted to study the effects of biotin supplementation (200 and 300 ug/kg in trials 1 and 2, respectively) of barley-wheat-soybean meal diets on reproductive performance (Grandhi and Strain, 1980). The basal diets were calculated to contain 93 ug/kg available biotin during gestation and 104 ug/kg during lactation. All farrowing, lactation and post-weaning estrus responses were similar for control and biotin supplemented females.

Penny et al. (1981) divided a herd of 116 sows with persistent foot-lameness problems into two groups on the basis of parity, liveweight and number and severity of foot lesions. One group served as controls and the other group received biotin supplementation (1160 ug/day in gestation and 2320 ug/day in lactation) for 1 yr. The control diet

contained 203 ug/kg total biotin and 56 ug/kg biologically available biotin. Compared to controls, sows receiving biotin farrowed significantly more live pigs in parities two and four with a trend for more live pigs in parity three. There was no consistent biotin effect on the weaning to remating interval.

The National Research Council-recomended (1979) biotin requirement is 100 ug/kg diet for gestating sows and herd boars. For lactating gilts and sows daily biotin intakes of 400 to 550 ug/diet are recommended.

Effects of Biotin Supplementation on Skin and Foot Lesions

The discovery of infection and necrosis of the foot sole by Mocsy (1940) was the first reference of foot lesions in swine. Foot lesions were described by Osborne (1950) and Osborne and Ensor (1955) with the condition observed termed "foot rot". The average yearly frequency of foot rot on 25 farms was 20% and five farms had frequencies above 50 %. In these two reports, foot rot was not closely related to lameness. Penny et al. (1963) characterized numerous types of foot lesions and reported foot lesions to be common and often very severe.

Differences in claw size within animals was first reported by Nordby (1939) and confirmed by Penny et al. (1963). Fritschen (1976) reported lateral (outer) claws

were significantly longer than medial (inner) claws. He also observed that lateral claws developed more lesions than medial claws, strongly suggesting that unequal claw size is a factor in foot lesion development and distribution.

The type of flooring material swine are housed on has an impact on the development of foot lesions (Fritschen, 1979; Jensen, 1979; Newton et al., 1980). Additionally, the percent slotted area per pen and slat widths have been associated with the occurrence of foot lesions (Fritschen, 1979). Kovacs and Beer (1979) hypothized that abrasiveness, resistance to wear and slipperiness are the mechanical properties of floors which relate to foot lesions in swine.

Biotin has been incriminated as a factor precipitating foot lesions and lameness in swine of various ages (Cunha et al., 1946; Lindley and Cunha, 1946; Lehrer et al., 1952; Cunha, 1968). Cunha (1968) cited numerous field trials where biotin supplementation of swine diets reduced the occurrence of foot and skin lesions. In Yugoslavia, lame sows suffering from severe lesions of the soft-sole were treated with biotin (Tagwerker, 1974). The sows responded to biotin treatment with the most effective treatment being a combination of supplemental biotin in the diet (500 ug/kg) and twice weekly injections (5 ug/injection). Money and Laughton (1981) reported that painful foot horn lesions of 30 breeding sows responded to biotin supplementation (333

ug/kg) and the herd lameness problems were reduced. The basal herd diet contained only 40 ug available biotin/kg diet.

Brooks et al. (1977) found that biotin supplementation of sows in a university herd with a lameness problem resulted in a 28% reduction in hoof lesions after 6 mo compared with unsupplemented sows. Initially all sows had dry flaking skin and a poor hair coat. During the course of the trial, there was a general improvement in the skin condition of both groups of sows and a noticeable regrowth of hair coats. Unsupplemented diets were calculated to contain 155 ug total biotin/kg and 76 ug biologically available biotin/kg diet.

Comben (1978) reviewed numerous field reports from biotin supplementation in the United Kingdom. Most reports did not include a control group, however, all studies reported biotin supplementation reduced foot lesions and lameness. To bring about rapid improvements (1 to 2 mo) in sow herds with established foot lesions and lameness, biotin supplementations up to 2222 ug/kg diet were required.

Bane et al. (1980) studied the effect of biotin supplementation on foot lesion development in gilts individually housed in concrete-floored stalls. The addition of 220 ug biotin/kg diet had no effect on foot lesion incidence. During a two-parity sow study, biotin

supplementation did not influence the incidence of foot lesions but slightly reduced the severity of foot lesions present (Grandhi and Strain, 1980). In this study, a barley-wheat-soybean meal diet was supplemented with 200 to 300 ug biotin/kg diet.

Penny et al. (1980) obtained little response to biotin supplementation (1160 and 2320 ug/sow/day during gestation and lactation, respectively) as a treatment for established foot lesions. However, young gilts entering the herd and fed biotin supplemented diets developed fewer and less severe foot lesions. They also suggested that biotin could be useful during the growing stage before the development of foot lesions. They hypothesized that biotin could harden the hoof horn in some way, enabling it to withstand future vigours of the environment.

Brooks and Simmins (1981) fed gilts either a basal cereal-fishmeal diet containing 31/ug available biotin/kg or the basal diet plus 350 ug biotin/kg diet. Diets were initated at 35 kg body weight and continued through four parities. Supplemented females had fewer claws affected and less hoof lesions/sow at 170 d of age and after weaning each of their four litters. The treatment differences were significant following first weaning. In a second trial, compression strength of hoof horn measured on an Instron Testing Instrument increased with dietary biotin levels over the range 0 to 720 ug/kg diet.

Halama (1979) studied the effects of supplemental biotin in diets of sows containing numerous skin lesions. Before biotin supplementation, the skin disorders observed were petechial haemorrhages, dry brownish eczema, hair loss and staring hair coat. These skin disorders quickly subsided following biotin supplementation to the entire herd. Halana suggested that skin defects are a more sensitive indicator of biotin deficiency in adult pigs than claw defects and lameness.

Summary

Biotin functions in key metabolic pathways and a biotin deficiency can have profound effects on the economy of livestock production. Animals receive biotin from feedstuffs consumed and biotin synthesized by microorganisms in the gastrointestinal tract. Recent reports suggest that reduced bioavailability of biotin in some feedstuffs, changes in dietary ingredient processing or diet formulation and livestock management modifications have increased the need for biotin in animal diets. The need for biotin in poultry diets has been clearly established, however, there is a paucity of data in swine. Numerous reports on biotin in swine nutrition have been published in the last 5 yr. Many of these studies were field trials and failed to

utilize adequate control treatments for comparison. With these points in mind a series of studies were undertaken to examine the requirements of biotin for swine housed in total confinement. The studies were designed to specifically examine the role of biotin on the feedlot performance of developing gilts, reproductive performance of females through four parities and the occurrence of foot and skin lesions.

CHAPTER III
EFFECTS OF SUPPLEMENTAL BIOTIN TO CORN-SOYBEAN MEAL DIETS FOR DEVELOPING GILTS

Introduction

Several decades have passed since a biotin deficiency was first produced and described in weanling pigs (Cunha et al., 1946); however, the biotin requirement of the various classes of swine is still unknown. Until recently, it was believed supplemental biotin was not needed in normal swine diets because of the wide distribution of biotin in feedstuffs (Scheiner and DeRitter, 1975) and the synthesis of biotin by the animal's intestinal microflora (Lehrer et al., 1952).

Biotin deficiency symptoms include decreased feedlot performance, low plasma biotin levels, alopecia, dry scaly skin, transverse grooves on the tongue, erosion of the soft toe heel and extensive cracking of the toe heel and horn (Cunha et al., 1946; Glattli et al., 1975). During the past 14 yr similar claw defects and skin lesions have been reported in swine of numerous countries (Cunha et al., 1968; Tagwerker, 1974; Brooks et al., 1977). Biotin treatments in these studies were reported to give beneficial results.

The significance of foot lesions to swine lameness has been well documented (Osborne, 1950, 1955; Penny et al.,

1963, 1965). Biotin has been implicated as a factor in lameness due to its role in maintaining normal skin and foot pads. However, the role of biotin in the development of toe lesions and subsequent lameness has not been clearly delineated.

The objective of this study was to determine the influence of biotin supplementation to corn-soybean meal diets for developing gilts from weaning to 92 kg body weight on feedlot performance, plasma biotin concentrations, soundness, hair quality and development of toe lesions.

Materials and Methods

In three separate trials, 80 crossbred gilts/trial (avg 28.6 d of age and 6.7 kg body weight), were blocked based on initial weight and ancestry and allotted to two supplemental biotin groups (0 and 220 ug d-biotin/kg diet) in a randomized complete block design. There were four pens/group and 10 gilts/pen in all trials. Feed and water were provided ad libitum. Gilts were weighed and feed intake was recorded biweekly until gilts averaged 92 kg body weight. Trials 1, 2 and 3 lasted 140, 154 and 154 d, respectively.

The basal diets, which were formulated to meet the 1979 NRC-recommendation for crude protein, Ca, P and other

nutrients for each growth phase, is presented in table 4. Rovimix[1] (220 mg d-biotin/kg) was used as the biotin source for the supplemented (SB) diet. Biotin concentration of feed samples was determined using a modification of the microbiological procedures of Wright and Skeggs (1944) and Frigg and Brubacker (1976). Samples were hydrolyzed with acid (2N H_2SO_4) and papain prior to the assay which utilized Lactobacillus plantarum (ATCC 8014) as the test organism (see appendix A). At the beginning of each trial, gilts were weaned, moved to an environmentally-controlled nursery containing expanded metal floors and placed on experimental diets. When gilts weighed approximately 50 kg, they were moved to a partially-slotted, concrete floored finishing house and remained there until completion of the trial.

At three time periods (1 = initially; 2 = 50 kg body wt; and 3 = end of trial) all feet of each gilt were evaluated for toe lesions. To minimize stress to the gilts and improve the data collection procedure, a turning device was utilized which has been described by Calabotta et al. (1982). Toe lesions were noted as to type (based on location) and severity using the classification system adapted (figure 2) from Brooks et al. (1977). Based on visual observation, each lesion was given a severity score of 1 to 5 with 1 indicating a very small lesion and 5 a very

---

[1]Hoffmann-La Roche, Inc., Nutley, NJ.

TABLE 4. COMPOSITION AND CHEMICAL ANALYSIS OF BASAL DIETS[a]

Item	Feeding period, kg				
	4 to 7	7 to 10	10 to 20	20 to 45	45 to 100
Ingredient, %					
Ground corn (IFN 4-02-931)	54.61	59.39	73.66	79.15	84.78
Soybean meal (IFN 5-04-612)	32.71	27.93	23.70	18.62	13.47
Dry whole whey (IFN 4-01-182)	10.00	10.00	———	———	———
Defl. phosphate (IFN 6-01-780)	1.03	1.03	1.09	.90	.44
Limestone (IFN 6-02-532)	.75	.58	.64	.64	.81
Salt (IFN 6-14-013)	.30	.30	.30	.30	.30
Trace mineral premix[b]	.10	.10	.08	.06	.05
Vitamin-selenium premix[c]	.25	.25	.25	.20	.15
Antibiotics[d]	.25	.25	.25	.13	.13
Chemical analysis (as fed basis):					
Protein, %	22.4	20.1	17.8	16.0	14.1
Calcium, %	.79	.75	.69	.68	.54
Phosphorus, % (total)	.65	.58	.53	.51	.40
Biotin, ug/kg[e]					
NB	187	141	128	120	103
SB	399	373	316	312	306

[a]Biotin added at .10% to provide 220 ug biotin/kg diet.
[b]Contained (%): 20 Zn, 10 Fe, 5.5 Mn, 1.1 Cu and .15 I.
[c]Supplied (per kilogram of premix): 1.76 g riboflavin, 8.8 g pantothenic acid, 8.8 g niacin, 8.8 mg vitamin B_{12}, 176 g choline chloride, 1,760,000 IU vitamin A, 176,000 IU vitamin D_3, 4400 IU vitamin E, 440 mg menadione dimethylprimidinol bisulfite (MPB) and 40 mg Se.
[d]4.5-20 kg: Chlortetracycline-sulfamethazine-penicillin; 20-100 kg: Virginiamycin.
[e]Determined by microbiological assay. NB and SB are 0 and 220 ug supplemental biotin/kg diets, respectively.

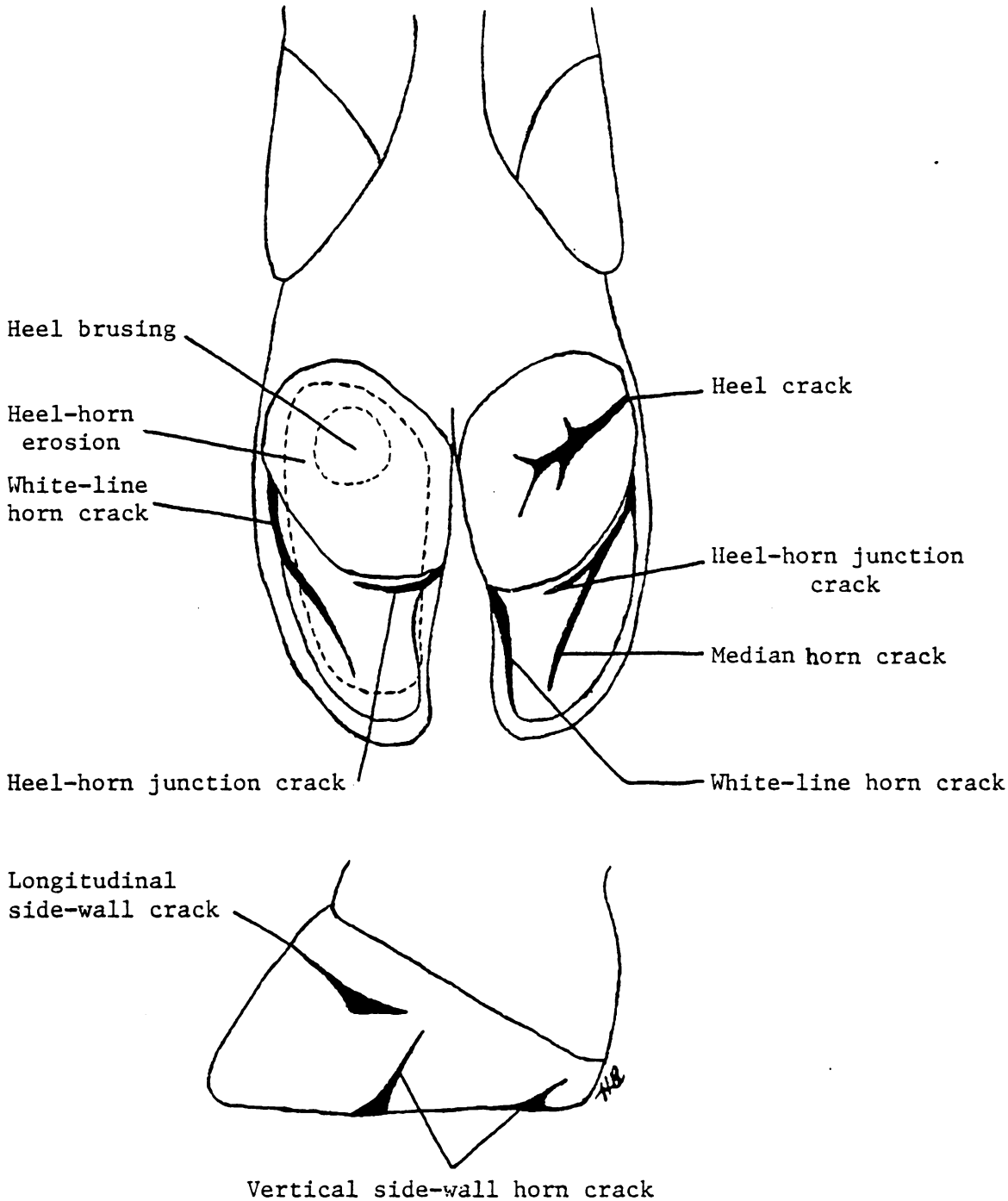


Figure 2. Toe lesion classification system adapted from Brooks et al., (1977).

large severe lesion. The total number of toe lesions/gilt and the total severity score/gilt of all toe lesions was obtained by adding the data for all eight toes of each gilt. Data for heel-horn junction cracks, white-line horn cracks and median horn cracks were combined and analyzed as horn cracks.

A committee of three, working individually, subjectively evaluated each gilt for hair loss and soundness. Hair loss scores ranged from 1 to 5 with 1 indicating a gilt with no apparent hair loss and 5 representing a gilt with extensive hair loss (alopecia). Soundness scores ranged from 1 to 15 according to the following guidelines:

1 = structurally correct, sound and free moving.

5 = slight structural abnormalities, slight limp and restriction in movement.

10 = numerous structural abnormalities, slight limp and restricted movement.

15 = excessive lameness, unable to walk.

Hair scores were determined initially and at the end of each trial, while soundness scores were determined only upon completion of each trial.

At times 1, 2 and 3 plasma samples were recovered from blood samples taken via anterior vena cava puncture. Plasma samples were composited (2 ml aliquots) by pens and composite samples assayed for plasma biotin (PB)

concentrations. Samples were hydrolyzed with papain prior to assay using the same procedure employed for the diets (see appendix A).

Statistical Analysis

Feedlot performance, PB and soundness and hair scores were analyzed as a randomized complete block design using analysis of variance procedures (Barr et al., 1979). The main effects of supplemental biotin level, replication, and trial were tested along with their two-way interactions. Each pen was considered as the experimental unit. Toe lesions within gilts were analyzed as a split-split-plot design using Harvey's (1977) mixed-model least-squares analysis procedure. Table 28 indicates the mean square used for each F statistic. When variables containing gilt means (i.e., total number of toe lesions/gilt) were tested, a split-plot design was used and the model reduced accordingly (table 29).

Results and Discussion

Performance, Soundness Score and Hair Score

Feedlot performance and soundness and hair scores of gilts fed two levels of supplemental biotin are presented in table 5. There was no trial x supplemental biotin

TABLE 5. FEEDLOT PERFORMANCE; SOUNDNESS AND HAIR OF GILTS FED TWO LEVELS OF BIOTIN. TRIALS 1 THROUGH 3 COMBINED[a]

Item	Supplemental biotin, ug/kg 0	220	S.E.[b]
No. of gilts	119	118	
Initial age, d[c]	28.5	28.6	.5
Initial weight, kg[c]	6.7	6.7	.2
Final weight, kg[c]	92.7	92.3	.9
Daily gain, kg[c]	.57	.57	.01
Daily feed, kg[c]	1.56	1.54	.02
Feed/gain	2.73	2.69	.03
Committee scores			
Hair loss[d]			
Initial	1.0	1.0	.00
Final	1.1	1.1	.02
Soundness[ce]	8.3	8.2	.17

[a]Values are least-squares means adjusted for initial weight. Pen means (10 pigs/pen) represent the experimental unit.
[b]Standard error of the mean.
[c]Trial effect ($P<.05$).
[d]Based on visual observation by three individual members on a scale of 1 to 5 with 1 indicating no hair loss and 5 excessive hair loss.
[e]Based on visual observation by three individual members on a scale of 1 to 15 with 1 indicating a sound free moving gilt and 15 a unsound, lame gilt unable to walk.

interaction (P>.10), therefore, the three trials were combined. Two gilts on SB diets and one gilt on nonsupplemental (NB) diets were removed from trial 1 due to extremely poor performance.

Daily gain, daily feed and feed/gain were not affected (P>.10) by supplemental biotin. Therefore, the NB diets fed in this study appear to contain adequate biotin (103 to 187 ug/kg diet) to promote optimal feedlot performance. These results are in agreement with numerous reports in which the level of supplemental biotin ranged from 55 to 720 ug/kg diet (Meade, 1971; Washam et al., 1975; Easter et al., 1978; Simmins and Brooks, 1980). To the contrary, Adams et al. (1967) reported an improvement in daily gain and feed efficiency when a basal corn-milo-soybean diet (286 ug biotin/kg) was supplemented with 110 ug biotin/kg diet. Peo et al. (1970) also suggested an improvement (10%) in daily gain of early-weaned pigs from supplemental biotin (440 or 880 ug/kg).

Hair coats were good in all trials across both levels of biotin and only an occasional gilt appeared to lose hair. Supplemental biotin did not influence (P>.10) hair scores. Hair scores were slightly increased (poorer) at the end of each trial compared with initial scores. It has been clearly demonstrated that severe biotin deficiency results in dry scaly skin and excessive hair loss in swine (Cunha et

al., 1946; Glattli et al., 1975). However, the level of dietary biotin necessary to maintain normal skin and hair quality has not been determined. Cunha (1968) suggested hair and skin quality could be improved by biotin supplementation under some production conditions. However, under the conditions of this study, our results do not support that hypothesis.

Soundness scores were not influenced (P>.10) by supplemental biotin. Unsoundness (lameness) was present, but was evenly distributed between both levels of biotin. There was a trial effect (P<.05) for soundness scores with gilts in trial 1 having more unsoundness problems than gilts in trials 2 and 3.

Plasma Biotin

Initially, PB averaged 39.0 ng/dl for all gilts (table 6). At time 2 (50 kg), PB was higher (P<.001) for gilts consuming SB diets and remained higher (P<.001) at time 3 (final). However, the magnitude of the difference between NB and SB gilts was slightly reduced at time 3. Trial, time, supplemental biotin x time and trial x time effects were present (P<.001).

This is the first reported data on the effects of dietary biotin supplementation on PB in growing-finishing swine and clearly indicates that biotin supplementation of

TABLE 6. PLASMA BIOTIN CONCENTRATIONS (NG/DL) IN GILTS FED TWO LEVELS OF BIOTIN[abc]

Time	Trial	Supplemental biotin, ug/kg 0	220
1 (initial)	1	41.2	40.5
	2	27.7	30.0
	3	51.2	43.7
	Mean	40.0	38.0
2 (50 kg)	1	50.6	130.2
	2	54.9	98.1
	3	92.6	167.7
	Mean	66.0	129.6
3 (final)	1	59.1	90.5
	2	48.1	91.2
	3	69.6	104.5
	Mean	58.9	95.4

[a]Least-squares means with 8 composite samples (10 gilts/composite) per mean in each trial. Standard error of the mean = 6.0 ng/dl.
[b]Trial, time and supplemental biotin effect (P<.001).
[c]Biotin x time and trial x time effect (P<.001).

swine diets will elevate swine PB. Glattli et al. (1975) studied PB in 10 to 31 d-old piglets consuming a biotin-deficient diet, a biotin-deficient diet plus biotin injections or a commercial diet for 6 wk. The biotin-deficient animals produced PB concentrations of 20 to 30 ng/dl, while piglets consuming the commercial diet had PB concentrations over 40 ng/dl. Biotin injections increased PB, but 2 wk were required for a substantial increase. Tagwerker (1974) suggested that a PB concentration over 40 ng/dl indicates an adequate biotin intake for growing-finishing swine. Based on Tagwerker's estimate, at time 1 the gilts used in this study (especially in trial 2) were in a marginal biotin status since initial PB averaged 39 ng/dl for all gilts.

Toe Lesions

All types of toe lesions shown in figure 2 were observed in this study. Toe lesions were present initially but their frequency was low and severity minor. All types of lesions increased in number and severity over the three time periods. The effects of time on toe lesions will be discussed in more detail in chapter 6.

The total number of toe lesions and total severity of those toe lesions for gilts fed two levels of biotin are shown in table 7. Biotin supplementation reduced ($P<.005$)

TABLE 7. LEAST-SQUARES MEANS FOR TOTAL NUMBER AND SEVERITY OF TOE LESIONS IN GILTS FED TWO LEVELS OF BIOTIN

Item	Trial	Supplemental biotin, ug/kg 0	220	S.E.[a]
No. of gilts		119	118	
Total no. lesions per gilt[bcd]	1	4.1	3.9	.2
	2	4.3	4.2	.2
	3	7.5	6.3	.2
	Mean	5.3	4.8	.1
Total lesion severity score per gilt[def]	1	5.8	5.4	.5
	2	8.4	7.9	.5
	3	15.6	13.8	.5
	Mean	9.9	9.1	.3

[a]Average standard error of the mean.
[b]Biotin x trial interaction (P<.03).
[c]Biotin effect (P<.005).
[d]Trial and time effect (P<.001).
[e]Sum of all severity scores/gilt. Each lesion present was scored from 1 to 5 with 1 indicating a small lesion and 5 a large, severe lesion.
[f]Biotin effect (P<.03).

the total number of toe lesions/gilt with a mean of 5.3 and 4.8 for gilts consuming NB and SB diets, respectively. This reduction in toe lesions in gilts receiving supplemental biotin was greatest in trial 3 and the biotin x trial interaction was significant (P>.03). Similar results were seen in the supplemental biotin response on total toe lesion severity score/gilt. The mean total toe lesion severity score/gilt was reduced (P<.03) from 9.9 in NB gilts to 9.1 in SB gilts. The biotin response for toe lesion severity was also greatest in trial 3; however, the biotin x trial interaction was not different (P>.10). These data suggest the response of the toe lesions to supplemental biotin is greater when the prevalence of toe lesions is high.

The frequency of gilts with one or more toe lesions for the five types of lesions is presented in table 8. Heel-horn erosion and heel cracks were the most frequent toe lesions observed across levels of biotin. Less than 10% of all gilts exhibited the other types of toe lesions. Biotin supplementation reduced the frequency of gilts with all four types of toe lesions; however, only the reduction in frequency of gilts with side-wall cracks was significant (P<.01). The magnitude of this reduction in side-wall cracks from biotin supplementation was consistent for all three trials. The biotin x trial interaction for the frequency of gilts with heel-horn erosion and heel cracks

TABLE 8. LEAST-SQUARES MEANS FOR FREQUENCY OF GILTS WITH TOE LESIONS WHEN FED TWO LEVELS OF BIOTIN

Item[ab]	Trial	Supplemental biotin, ug/kg 0	220	S.E.[c]
		%		
Heel-horn erosion[de]	1	67.4	69.3	2.4
	2	59.2	61.7	2.4
	3	94.2	88.3	2.4
	Mean	73.6	73.1	1.4
Heel crack[de]	1	57.4	60.7	3.8
	2	31.7	30.8	3.8
	3	51.7	39.2	3.8
	Mean	46.9	43.6	2.2
Heel bruising[e]	1	21.3	14.0	2.1
	2	1.7	4.2	2.1
	3	1.7	.8	2.1
	Mean	8.2	6.4	1.2
Horn crack	1	3.3	2.6	1.7
	2	6.7	3.3	1.7
	3	2.5	3.3	1.7
	Mean	4.2	3.1	1.0
Side-wall crack[f]	1	10.9	4.3	1.9
	2	7.5	3.3	1.9
	3	2.5	.8	1.9
	Mean	7.0	2.8	1.1

[a]Observations/treatment means were 117 and 114 in trial 1 and 120 and 120 in trials 2 and 3, respectively, for 0 and 220 ug/kg.
[b]See figure 2 for lesion classification.
[c]Standard error of the mean.
[d]Biotin x trial interaction (P<.10).
[e]Trial and time effect (P<.001).
[f]Biotin effect (P<.01).

was significant (P<.10). During trials 1 and 2 there was no response to supplemental biotin; however, in trial 3 the frequency of gilts with heel-horn erosion and heel cracks was reduced in SB gilts. A large number of gilts suffered from heel bruising in trial 1 and biotin appeared to be benificial; however, in trials 2 and 3, frequency of heel bruising was low.

The frequency of toes with lesions in gilts fed two levels of biotin is presented in table 9. There was a trend (P<.10) for a reduction in the frequency of toes with heel-horn erosion in gilts fed SB diets. As was the case with lesions/gilt, the greatest effect was obtained in trial 3. Biotin supplementation reduced (P<.05) the frequency of toes with heel cracks and side-wall cracks. For heel cracks, the biotin x trial interaction was significant (P<.01), with gilts fed SB diets having a much lower frequency (13.4 vs 21.8%) of heel cracks in trial 3.

Toe severity scores for heel-horn erosion and heel cracks are shown in table 10. The incidence of all other types of toe lesions was too low to permit proper statistical comparison of the severity of those toe lesions present. In general, the severity of all toe lesions was mild with only an occasional pig suffering from a severe heel, horn or side wall crack. Supplemental biotin did not influence (P>.10) the toe severity score for heel-horn

TABLE 9. LEAST-SQUARES MEANS FOR FREQUENCY OF TOES WITH LESIONS IN GILTS FED TWO LEVELS OF BIOTIN

Item[a]	Trial	Supplemental biotin, ug/kg 0	220	S.E.[b]
		%		
Heel-horn erosion[cd]	1	25.1	24.2	1.2
	2	39.4	39.3	1.2
	3	61.6	56.3	1.2
	Mean	42.0	40.0	.93
Heel crack[def]	1	17.6	18.2	1.1
	2	7.5	7.2	1.0
	3	21.8	13.4	1.0
	Mean	15.6	12.9	.98
Horn crack	1	.51	.40	.25
	2	1.25	.70	.25
	3	.30	.40	.25
	Mean	.70	.52	.19
Side-wall crack[de]	1	1.56	.65	.28
	2	1.14	.61	.27
	3	.33	.10	.27
	Mean	1.02	.46	.17

[a]See figure 2 for lesion classification.
[b]Standard error of the mean.
[c]Biotin effect (P<.10).
[d]Trial effect (P<.001).
[e]Biotin effect (P<.05).
[f]Biotin x trial interaction (P<.01).

TABLE 10. LEAST-SQUARES MEANS FOR HEEL-HORN EROSION AND HEEL CRACK SEVERITY SCORES IN GILTS FED TWO LEVELS OF BIOTIN[ab]

Item	Trial	Supplemental biotin, ug/kg 0	220	S.E.[c]
Heel-horn erosion[d]	1	1.13(266)	.96(248)	.10
	2	1.61(418)	1.52(419)	.09
	3	2.06(674)	2.14(616)	.09
	Mean	1.60	1.57	.22
Heel crack	1	1.53(164)	1.53(167)	.17
	2	1.39(72)	1.34(68)	.18
	3	1.61(212)	1.49(128)	.17
	Mean	1.51	1.46	.19

[a]Number of observations/mean in parenthesis.
[b]Scores ranged from 1 to 5 for each lesion present with 1 indicating a small lesion and 5 a very large lesion. See figure 2 for lesion classification.
[c]Average standard error of the mean.
[d]Trial effect (P<.001).

erosion or heel cracks. These results suggest that once heel-horn erosion or heel cracks develop, supplemental biotin is of little value in reducing the severity of those toe lesions.

The high incidence of toe lesions in this study, across both levels of biotin, agrees with the slaughterhouse study of Penny et al. (1963). They found foot lesions present in 65% of market hogs examined and noted the frequency to be quite variable among farms and among pigs from each farm. The large difference in toe lesions among the three trials reported here further indicate the wide variation in the development of toe lesions in growing-finishing swine. The frequent occurrence of toe lesions on the SB diets supports the hypothesis that toe lesions in swine are caused by a number of factors other than biotin. Field studies by Penny et al. (1965) suggested rough abrasive concrete floors as a precipating factor in swine foot lesions. Wright et al. (1972) described the production of foot lesions in swine up to 90 kg when housed on a rough concrete surface. There was a greater incidence of moderate and severe lesions in swine housed on the rough surface compared to a smooth one.

Abrasiveness, resistance to wear and slipperiness are three mechanical properties of floors related to foot and limb disorders (Kovacs and Beer, 1979). Type of flooring material, percentage of pen containing slats, width of slats

and slat size are all reported to influence development of foot lesions in swine (Wright et al., 1972; Smith and Robertson, 1971; Fritschen, 1976). Toe size (Penny et al., 1963) and body conformational features (Bereskin, 1979) have also been implicated in swine foot lesions. Toe sizes were obtained in this study but were not different for SB and NB gilts. The roles of toe size in toe lesion development will be reported in chapter 6.

In this study, the toe lesions observed were generally mild to moderate in severity and did not influence feedlot performance. Daily gain was positively ($P<.01$) correlated with the frequency of gilts with heel cracks; however, the correlation coefficient (.12) was small (table 11). Wright et al. (1972) and Fritschen (1976) were unable to demonstrate a reduction in growth rate or feed efficiency from moderate foot lesions. Radnai and Radnai (1976) reported that .71 kg more feed was required/unit gain in pigs suffering from foot lesions. Osborne and Ensor (1955) noted that severe toe lesions (i.e., side-wall cracks) were vulnerable to bacterial infection leading to lameness and reduced animal performance. It was hypothized that a reduction in toe lesions from supplemental biotin could lead to decreased lameness as evidenced by an improved (lower) soundness score. Under the conditions of this study, toe lesions were not correlated ($P>.10$) with soundness scores.

In agreement are the results of Osborne and Ensor (1955). Penny et al. (1963) considered foot lesions to be a common cause of lameness in pigs and noted the condition could occur at a relatively early age.

The significant reduction in number and frequency of toe lesions in gilts receiving biotin supplementation obtained in this study supports biotin's role in maintaining hoof integrity. Clearly, there are many other factors which relate to development of toe lesions in swine and the exact mechanism by which biotin protects the hoof from damage is not known. A softening of the hoof has been reported in biotin-deficient swine (Brooks et al., 1977; Comben, 1978; Whitehead, 1980) which could increase the hoof's susceptibility to wear, lesions and traumatic injury (Brooks and Simmins, 1981). Horn compression strength as measured on an Instrom Testing Machine was reported to increase with the level of dietary biotin in growing-finishing gilts at 85 kg liveweight (Brooks and Simmins, 1981). Penny et al. (1980) found biotin to be more effective in reducing foot lesions in the breeding herd when supplementation started prior to first breeding. A recent report (Brooks and Simmins, 1981) supports this finding. Gilts in the present study were continued on supplemental biotin diets (Chapter 5 and 6) to critically test this hypothesis.

Correlation coefficients among various types of toe lesions are shown in table 11. Although many of the correlation coefficients were small, numerous significant correlations were observed. As expected, the number of toe lesions/ gilt was highly correlated ($P<.01$) with toe lesion severity score/gilt. Heel-horn erosion was correlated ($P<.01$) with heel bruising, heel cracks and side-wall cracks. Side-wall cracks were the only toe lesion significantly correlated with horn cracks. These correlations support the general observation in this study that heel-horn erosion lesions develop early in the animals life and are followed by heel cracks, side-wall cracks and horn cracks.

In 1979, the National Research Council established a biotin requirement of 100 ug for all classes of swine. The results of the present study suggest that 100 ug biotin/ kg diet is adequate for normal feedlot performance in growing-finishing swine. However, under some production conditions, higher dietary levels may be required to maintain optimum hoof integrity.

TABLE 11. CORRELATION COEFFICIENTS FOR DAILY GAIN, SOUNDNESS SCORE AND TOE LESIONS

Item	Sound-ness score	Number of toe lesions/gilt	Severity of toe lesions/gilt	Frequency/gilt				
				Heel-horn erosion	Heel bruising	Heel crack	Horn crack	Side-wall crack
Daily gain	.02	.04	.02	.01	.08	.12**	-.03	.05
Soundness score	1.0	-.01	.03	-.01	-.03	-.04	.02	.06
Number of toe lesions/gilt		1.0	.92**	.19**	.02	.14**	.03	.08*
Severity of toe lesions/gilt			1.0	.15**	.01	.08*	.04	.07†
Frequency/gilt								
Heel-horn erosion				1.0	.22**	.32**	-.01	.11**
Heel bruising					1.0	.20**	.04	.17**
Heel crack						1.0	.01	.18**
Horn crack							1.0	.18**

*Significant (P<.05).
**Significant (P<.01).
†Significant (P<.10).

Summary

Three trials, utilizing a total of 240 crossbred gilts, were conducted to study the influence of 0 (NB) or 220 (SB) ug supplemental biotin/kg on feedlot performance, plasma biotin and toe lesion development in growing-finishing swine. Corn-soybean meal diets were fed to gilts from weaning to 92 kg body weight. Gilts were housed on expanded-metal floors to 50 kg body weight and partially-slotted concrete floors until completion of the trials. Feedlot performance, hair and structural soundness scores were not different (P>.10) between NB and SB gilts. Plasma biotin (PB) levels averaged 39 ng/dl initially and were elevated (P<.01) when supplemental biotin was included in the basal diet. Numerous toe lesions were observed across both levels of biotin. However, gilts consuming SB diets had fewer (P<.01) toe lesions and lower (P<.01) toe lesion severity scores compared with gilts fed NB diets. Heel-horn erosion and heel cracks were the most frequent type of toe lesions observed. Fewer gilts (P<.01) developed side-wall toe cracks when fed SB diets. There was a trial x supplemental biotin interaction (P<.10) with the frequency of gilts developing heel-horn erosion and heel cracks lower in trial 3 compared with trials 1 and 2. Supplemental biotin reduced the frequency of toes containing heel-horn

erosion ($P<.10$), heel cracks ($P<.05$) and side-wall toe cracks ($P<.05$). Severity of toe lesions was not affected ($P>.10$) by level of supplemental biotin. All toe lesions, except horn cracks, were positively correlated ($P<.01$) with each other and heel cracks were positively correlated ($P<.01$) with daily gain. These results suggest that biotin levels in corn-soybean meal diets are adequate for feedlot performance, but higher levels may be required to maintain optimum hoof integrity.

Chapter IV
INFLUENCE OF SUPPLEMENTAL BIOTIN ON REPRODUCTIVE PERFORMANCE AND VARIOUS BIOCHEMICAL CRITERA IN GILTS AND SOWS FED TWO TYPES OF GRAIN

Introduction

Interest concerning the role of biotin in swine nutrition was rekindled in the mid-1970's with the observation by Cunha (1971) that, in field trials, biotin supplementation resulted in an improvement in reproductive performance. During the last 5 yr, numerous reports have suggested a beneficial effect of supplemental biotin on litter size, conception rate and the weaning to rebreeding interval in sows (Halama, 1979; Brooks and Simmins, 1981; Penny et al., 1981; Brooks, 1982). However, other reports (Easter et al., 1979; Grandhi and Strain, 1980; Hamilton et al., 1982) failed to show an improvement from biotin supplementation. The supplemental levels reported ranged from 100 to 2,000 ug/kg diet and included basal diets containing feedstuffs of varying composition.

The biotin present in feedstuffs is not totally available to non-ruminants (Wagstaff et al., 1961; Scott, 1968; Anderson and Warnick, 1970). Frigg (1976) and Baker (1978), using chick growth assays, reported lower biotin bioavailability values for milo, barley, sorghum, oats and wheat as compared to corn. Similar bioavailabilities were reported by Anderson et al. (1978) and estimates of

available biotin were 108 ug/kg for corn, 82 for barley, 92 for sorghum and 43 for wheat.

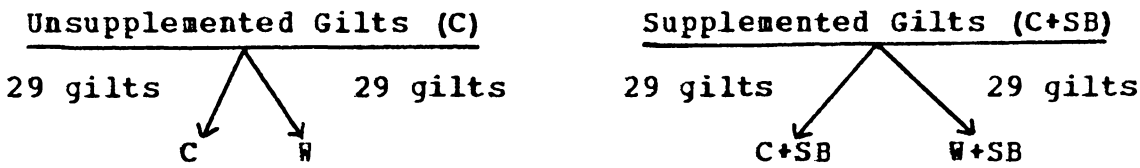
The effects of biotin supplementation on swine body stores, placental biotin transfer and milk biotin levels are not known. Answers to these questions and their relationship on swine reproductive performance are necessary to ascertain the need for biotin in sow diets.

The objective of this research was to study the influence of biotin supplementation on reproductive performance and various biochemical criteria in gilts and sows housed in total confinement for four parities and fed corn- or wheat-based diets.

Materials and Methods

Gilts from the three growing-finishing trials reported in Chapter III were utilized in this study. Following completion of trials, 116 crossbred gilts (36, 40 and 40 gilts from trials 1, 2 and 3, respectively) weighing approximately 100 kg were selected based on routine (eg. underlines, growth rate soundness, etc.) gilt selection criteria for the reproductive study. Gilts from each level of biotin during the growing-finishing study were randomly assigned (based on weight and ancestory) to either a corn-soybean meal (C) or a wheat-soybean meal (W) diet as

outlined below. Supplemental biotin (SB) was added at a level of 440 ug/kg diet to both C and W diets.



Diet compositions and analyses are presented in table 12. The four diets were initially formulated to be isonitrogenous and isocaloric, however, chemical analysis indicated the wheat diets were higher in crude protein (2.2 percentage units) than were the corn diets. Each gilt received 2.3 kg of feed daily from selection (100 kg) to 21 d following the initial breeding period. Through the remaining part of the first gestation period 1.8 kg were fed daily. During all subsequent breeding and gestation periods, 1.8 kg were fed from March 1 until November 30 and 2.3 kg were fed from December 1 until February 28 of each year. During lactation, 10% ground (coarse) oats were added to each diet. Following farrowing, feed intake was increased gradually to a maximum daily intake of 1.36 kg plus .45 kg for each pig being nursed at 7 d post-farrowing.

During breeding and gestation, females (gilts and sows) were housed in total confinement facilities containing partially-slotted concrete and solid concrete floors. On d 109 $\pm$ 1 of gestation, females were washed, disinfected and randomly assigned to one of three farrowing rooms containing

TABLE 12. DIET INGREDIENT AND CHEMICAL COMPOSITION[a]

	Diets	
	Corn	Wheat
Ingredient, %		
Yellow corn (IFN 4-02-931)	81.56	---
Soft winter wheat (IFN 4-05-284)	---	84.53
Soybean meal (IFN 5-04-604)	15.51	12.61
Limestone (IFN 6-02-532)	.68	.75
Defl. phosphate (IFN 6-01-780)	1.50	1.36
Salt (IFN 6-14-013)	.40	.40
Trace mineral premix[b]	.10	.10
Vitamin-selenium premix[c]	.25	.25
Total	100.00	100.00
Chemical analysis (as fed basis):		
Crude protein, %	14.0	16.2
Calcium, %	.92	.87
Phosphorus, % (total)	.58	.63
Gross energy, kcal/kg	3880	3844
Biotin, ug/kg[d]	105	131

[a]Biotin premix (220 mg/kg) added to obtain supplemented diets containing 440 ug biotin/kg diet.

[b]Contained (%): 20 Zn, 10 Fe, 5.5 Mn, 1.1 Cu and .15 I.

[c]Supplied (per kilogram of premix): 1.76 g riboflavin, 8.8 g pantothenic acid, 8.8 g niacin, 8.8 mg vitamin B_{12}, 176 g choline chloride, 1,760,00 IU vitamin A, 176,000 IU vitamin D_3, 4400 IU vitamin E, 440 mg menadione dimethylprimidinol bisulfite (MPB) and 40 mg Se.

[d]Determined by microbiological assay. Corn + biotin and wheat + biotin diets contained 470 and 490 ug total biotin/kg diet, respectively.

partially-slotted farrowing crates which were cleaned and disinfected prior to each farrowing. Females received vaccinations for Leptospirosis and Brucellosis every 6 mo and Atrophic Rhinitis vaccines (Bordetella bronchiseptica) 2 and 6 wk prior to farrowing. All females were dewormed for internal parasites and sprayed for external parasites every 2 to 3 mo.

Following selection and assignment to dietary treatments at approximately 100 kg and 180 d of age, all gilts were checked for estrus once daily using intact boars. Gilts were not bred until they had exhibited at least one normal estrous cycle. During specified breeding periods, estrus checks were conducted twice daily at approximately 12-h intervals. Females were bred 12, 24 and 36 h following initial detection of estrus by artificial insemination. Boars were collected daily and fresh semen was used for at least one of the three breedings.

All females were weighed at d 109 $\pm$ 1 of gestation and at weaning during each parity and weight loss during lactation was calculated. Farm personnel were present during parturition and provided routine assistance when needed. At birth, piglet naval cords were tied with twine, clipped and dipped in a 5% tincture of iodine solution. At 8 to 16 h of age, piglets were weighed, tails docked and needle teeth clipped. Each pig was injected with 200 mg of

iron as iron dextran and male pigs were castrated at 3 to 5 d of age. Pigs were vaccinated against Atrophic Rhinitis (Bordetella bronchiseptica vaccine) at 7 d of age and 3 to 5 d prior to weaning at 24 ± 3 d of age. The total number of pigs/litter was determined from the number of live, stillborn and mummified fetuses at parturition. At 21 d post-farrowing, number of pigs/litter, pig body weight, total litter weight and survival rate of pigs born alive were determined for each litter.

Plasma was recovered from blood samples (20 ml) taken via anterior vena cava puncture in each female at selection, then at d 109 ± 1 of gestation and at weaning during each parity. From the last 30 litters farrowed, plasma samples were obtained from two male and two female pigs selected at random. Samples were taken via vena cava puncture prior to nursing and at 14 d of age. Replacement pigs were selected at random for those pigs dying prior to d 14 of age. The four pig samples were composited by litters (1 ml aliquot/pig) within sampling times and assayed for plasma biotin (PB) concentration. During parities 3 and 4, one pig was sacrificed immediately after birth from 41 litters and the liver removed and frozen at -20 C until assayed for biotin content. Fifty-three sows (parities 3 and 4) were given 3 ml oxytocin intramuscularly and a milk sample obtained by manual expression of all milk from one side of

the udder. Biotin determinations were made on fat-free milk samples (see appendix A). Five to seven d following weaning at parity 4, sows were slaughtered and liver samples obtained for determination of biotin and pyruvate carboxylase activity. Pyruvate carboxylase activity was determined on fresh liver tissue according to the procedure of Deodhar and Mistry (1969) and activity expressed as muMoles ^{14}Co fixed/min/mg protein. Protein concentrations were determined on homogenized liver samples by the procedure of Lowry et al. (1951). All biotin determinations were performed using the microbiological procedure outlined in appendix A.

Females were removed from the experiment if they (1) failed to conceive following two successive breeding periods, (2) failed to cycle by 400 d of age, (3) failed to return to estrus within 60 d following weaning, (4) encountered farrowing problems which would significantly affect the sow's performance during the next parity and were felt unrelated to dietary treatments and/or (5) developed unsoundness which prevented them from rising and walking to obtain food and water. Table 13 summarizes the number and percentage of females removed from the study by reason.

TABLE 13. NUMBER AND PERCENTAGE OF FEMALES REMOVED FROM THE EXPERIMENT BY REASON

Reason	Number of females	Percent of total females
Completed four parities	40	34.5
Anestrus		
Never cycled[a]	8	6.9
Postpuberty[b]	4	3.5
Postpartum[c]	15	12.93
Failure to conceive	17	14.7
Unsoundness[d]	12	10.3
Died	4	3.4
Rectal prolapse	3	2.6
Farrowing problems	2	1.7
Failed to complete four parities before termination of study	8	6.9
Miscellaneous	3	2.6
Total	116	100.0

[a]Defined as failure to exhibit one normal estrus cycle before 400 d of age.
[b]Defined as failure to exhibit 2 or more normal estrus cycles, consecutively.
[c]Defined as failure to return to estrus with 40 d postweaning.
[d]Defined as unable to rise, walk and obtain food and water.

Statistical Analysis

Conception rates and biochemical criteria were analyzed as a 3 x 2 x 2 factorial design using analysis of variance procedures. Animal group, level of biotin and type of grain were main effects. Breeding period means were the experimental unit for conception rate, while females and/or pigs were the experimental unit for the biochemical data. Reproductive data were analyzed as a split-plot design with Harvey's (1977) mixed-model least-squares analysis procedure. In addition to the three main effects, parity, housing (at farrowing) and various two-way interactions between main effects, parity and housing were tested. Sources of variation, degrees of freedom and denominator for the F statistic are presented in appendix table 30.

Results and Discussion

Only 40 females (34.5%) of the 116 gilts that started the study completed four parities (table 13). Eight gilts failed to complete four parities before the study was terminated, however, if the study had been continued a portion of these eight gilts would certainly have reached four parities. Anestrus behavior (failure to exhibit estrus as defined in materials and methods section) was high with 27 females (23.3%) culled for this reason. Eight of these

27 anestrus females failed to reach puberty while the remaining 19 became anestrus following puberty (4) or weaning (15). Examination of the ovaries from 23 females culled for anestrus and so classified revealed small ovaries containing no active corpora lutea. Ovaries from four females contained corpora lutea and corpora albicantia indicating cyclic activity but lack of estrus expression (silent heat). Anestrus activity was evenly distributed across dietary treatments. Seventeen females (14.7%) were culled for failure to conceive following two successive breedings with 12 and 5 females coming from unsupplemented (NB) and biotin supplemented (SB) diets, respectively. Twelve females (5 from NB and 7 from SB) were culled for unsoundness.

Biotin x type of grain interactions were infrequent and the data were summarized by main effects of supplemental biotin and type of grain. A total of 245 litters were produced in the study. Table 14 presents a summary of the number of litters produced at each parity by type of grain and level of biotin.

Breeding Performance

Age at first estrus was greater ($P<.07$) for wheat-fed (W) gilts compared with corn-fed (C) gilts (table 15). There was a biotin x grain interaction with C + SB gilts

TABLE 14. NUMBER OF LITTERS PRODUCED AT EACH PARITY SUMMARIZED BY TYPE OF GRAIN AND LEVEL OF BIOTIN

Item	Grain		Supplemental biotin, ug/kg	
	Corn	Wheat	0	440
No. gilts, initial	58	58	58	58
No. females farrowing:				
One litter	42	50	47	45
Two litters	33	33	31	35
Three litters	24	25	23	26
Four litters	20	18	17	21
Total litters farrowed	119	126	118	127

TABLE 15. LEAST-SQUARES MEANS FOR THE REPRODUCTIVE PERFORMANCE OF GILTS AND SOWS FED TWO LEVELS OF BIOTIN AND TWO TYPES OF GRAIN.
MAIN EFFECTS

Item	Grain		Supplemental biotin/ug/kg		S.E.[a]
	Corn	Wheat	0	440	
No. of litters	119	126	118	127	
Breeding performance					
Age first estrus, d[gh]	269	281	278	272	3.2
Conception rate, %[ij]	80.2	79.4	75.3	83.9	2.4
Weaning to estrus interval, d[k]	13.0	11.7	14.5	10.2	1.1
Farrowing performance					
Total pigs born/litter[b]	11.8	10.6	11.1	11.3	.24
Live pigs born/litter[b]	10.7	9.7	10.1	10.4	.22
Pig birth wt, kg	1.38	1.31	1.35	1.34	.02
Total litter birth wt, kg[c]	14.7	12.7	13.5	13.9	.33
Lactation performance to 21 d					
No. of pigs/litter	8.9	8.5	8.5	8.9	.20
Pig body wt, kg	5.14	5.09	5.10	5.12	.07
Total litter wt, kg	45.4	42.6	42.9	45.1	.96
Pig survival rate, %[d]	83.3	88.8	85.6	87.0	1.3
Sow wt loss, kg[e]	26.5	24.1	26.3	24.3	1.0
Daily feed intake/sow, kg[ef]	4.2	4.1	4.2	4.2	.05

[a]Standard error of the mean.
[b]Grain effect (P<.05).
[c]Grain effect (P<.01).
[d]No. of pigs alive at 21 d ÷ no. of pigs born alive x 100.
[e]From day 109±1 of gestation to 21 d post-farrowing.
[f]Maximum daily feed intake was 1.36 kg plus .45 kg/pig nursing.
[g]Grain effect (P<.07).
[h]Supplemental biotin x grain effect (P<.05).
[i]No. of females pregnant ÷ no. of females mated x 100.
[j]Supplemental biotin effect (P<.07).
[k]Supplemental biotin effect (P<.05).

exhibiting estrus at the earliest age (260 d) and W + SB gilts exhibiting estrus at the greatest age (284 d). The mean age at first expressed estrus (puberty) was 272 d. These results are much higher than those reported by Mavrogenis and Robison (1976); however, the incidence of gilts with delayed puberty has been reported to range from 10 to 50% under modern production systems (Christenson and Ford, 1979; Rampacek et al., 1981). There was a significant group effect ($P<.001$) with gilts in group 3 averaging 358 d to puberty as compared with 231 and 236 d for group 1 and 2, respectively. Some undiagnosed condition is believed responsible for this dramatic effect on group 3 and based on the symptoms a viral infection is hypothesized.

The weaning to estrus interval was extremely variable (range, 5 to 60 d) and, as noted earlier, 19 females became anestrus following puberty. Of these 19 anestrus females, 12 became anestrus following weaning. The number of anestrus sows was high. However, a recent survey indicated that the incidence of post-weaning estrus in sows varied from 4% to 40% among herds (Crabo, 1982). Only the data from females returning to estrus within 40 d following weaning were used to test for statistical differences. The weaning to estrus interval was reduced ($P<.05$) from 14.5 to 10.2 d when females received SB diets compared with NB diets. However, the percentage of gilts returning to estrus

within 7 d was similar (49% vs 45%) for NB and SB females, respectively. More SB females (32% vs 12%) returned to estrus from 7 to 14 d compared with NB females with this difference primarily responsible for the overall biotin effect. Brooks et al. (1977) and Halama (1979) have also reported a reduction in the weaning to rebreeding interval. To the contrary, Penny et al. (1981) and Grandhi and Strain (1980) noted no response in the weaning to remating interval with biotin supplementation.

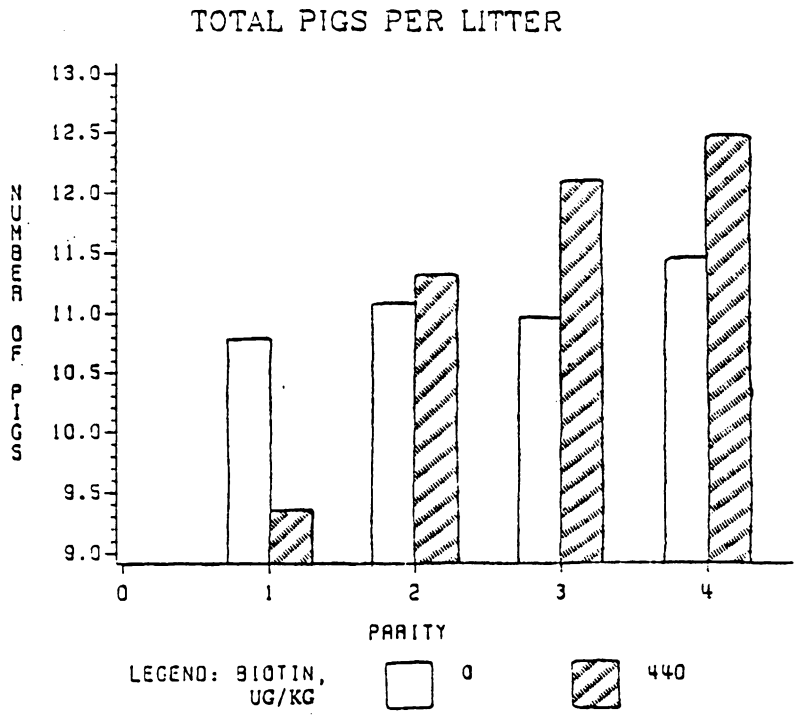
Females consuming SB diets had a 9% higher ($P<.05$) conception rate compared to NB females. Halama (1979) also reported a 9% improvement in conception rate when biotin was supplemented to diets for sows showing visual signs of hair loss and foot lesions. Grandhi and Strain (1980) failed to obtain a response in conception rate with biotin supplementation.

Farrowing Performance

Females consuming C diets farrowed a greater ($P<.03$) number of total pigs/litter and a greater ($P<.05$) number of live pigs/litter (table 15) compared to females receiving the W diets. The average litter birth weight was 2 kg greater ($P<.01$) for C females as compared with W females. The reason for this reduction in farrowing performance when W diets were fed is unknown. Wheat diets were isocaloric

(based on ME) with C diets, and contained 2% higher crude protein; therefore, eliminating a lack of dietary crude protein or energy as a factor.

Based on the combined data for four parities, biotin supplementation did not improve (P>.10) any farrowing responses tested. However, there was a biotin x parity interaction (figure 3) for average number of total pigs/litter (P<.08) and average number of live pigs/litter (P<.10). Females consuming SB diets farrowed fewer total pigs (1.5 pigs) and live pigs (1.2 pigs) per litter during the first parity compared with NB females. At parity 2, the response in total pigs/litter was similar for NB and SB females but number of live pigs/litter was .7 pigs higher for SB females compared with NB females. During parities 3 and 4 the improvements in litter size for SB females were almost identical with increases of 1.0 and .8 pigs/litter for average number of pig and average number of live pigs, respectively. Studies from Europe and Mexico, which used basal diets of varying composition have reported improvements in litter size ranging from 4 to 14 percent (Brooks et al., 1977; Halana, 1979; Penny et al., 1981; Michel and Mastachi, 1981). Two of these studies (Brooks et al., 1977; Penny et al., 1981) also noted greater responses in litter size in sows as compared to gilts. No improvements in farrowing performance have been reported for



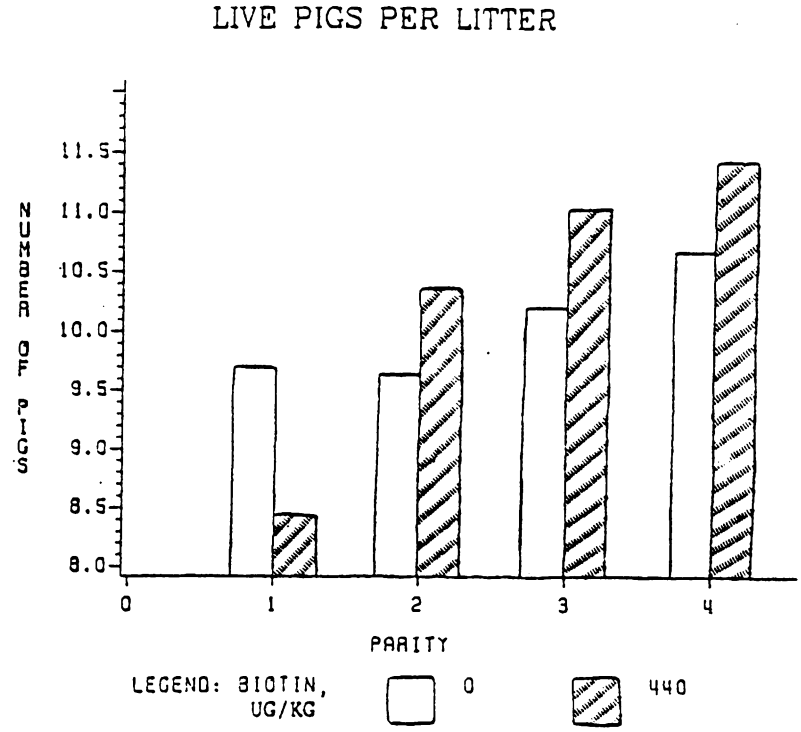


Figure 3. Biotin x parity interaction for total number of pigs and live pigs per litter.

females consuming corn-soybean meal diets (Easter et al., 1979; Hamilton et al., 1982) and barley-wheat-soybean meal diets (Grandhi and Strain, 1980).

Lactation Performance

There were no significant (P>.10) responses in lactation performance for level of biotin or type of grain fed. All responses favored the females fed SB diets, compared with the NB diets and the combined lactation responses resulted in SB females having 2.2 kg heavier litters at 21 d of lactation. Other reports on the lactation performance of sows consuming diets containing supplemental biotin have reported slight to no response (Brooks et al., 1977; Easter et al., 1979; Hamilton et al., 1982).

The significant advantages in litter size and litter weight obtained at farrowing for C females were not present (P>.10) at 21 d of lactation. However, all responses at 21 d of lactation, except pig survival rate, favored the C females compared with W females.

Biochemical Criteria

Other than a reduction (P<.06) in baby pig PB at 14 d of age, the type of grain fed did not influence (P>.10) sow and pig biotin body stores or sow hepatic pyruvate

TABLE 16. LEAST-SQUARES MEANS FOR VARIOUS BIOCHEMICAL CRITERIA AS INFLUENCED BY BIOTIN SUPPLEMENTATION AND TYPE OF GRAIN FED[a]

Item	Grain				Supplemental biotin, ug/kg				S.E.[b]
	Corn		Wheat		0		440		
Plasma biotin, ng/dl[c]									
Initial	101	(53)	99	(57)	60	(55)	141	(55)	4.3
Gestation, day 109[d]	102	(95)	91	(109)	51	(97)	132	(107)	2.9
Weaning[d]	111	(82)	108	(99)	69	(88)	150	(93)	3.1
Milk biotin, ug/l[ce]	44	(25)	47	(28)	24	(23)	68	(30)	2.8
Liver biotin, ug/gDM[c]	1.10	(38)	1.20	(37)	.96	(39)	1.34	(36)	.06
Liver pyruvate carboxylase activity[f]	10.6	(32)	10.1	(26)	10.2	(30)	10.5	(28)	12.31
Pig									
Plasma biotin, ng/dl[c]									
Day 0	841	(14)	883	(14)	425	(11)	1299	(17)	111
Day 14[g]	229	(14)	146	(15)	62	(11)	313	(18)	22
Liver biotin, ug/gDM	.77	(21)	.75	(20)	.79	(17)	.73	(24)	.04

[a]No. of observations/mean in parenthesis.
[b]Standard error of the mean.
[c]Biotin effect (P<.001).
[d]Mean for 4 parities.
[e]Grain x biotin effect (P<.03).
[f]Expressed as muM$^{14}CO_2$ converted/min/mg protein.
[g]Grain effect (P<.06).

carboxylase activity (table 16). Biotin supplementation elevated (P<.001) sow and pig PB levels and biotin content of sow milk and liver. Pig liver biotin content (at birth) and sow hepatic pyruvate carboxylase activity were not influenced (P>.10) by level of biotin. The initial PB levels shown in table 16 reflect the elevation in PB from biotin supplementation during the growing-finishing period reported in chapter 3. Plasma biotin remained higher (P<.001) at d 109 of gestation and at weaning during each parity for females consuming SB diets and support the results in chapter 3 that biotin-supplementation of swine diets increases PB levels. Plasma biotin levels were lower (P<.05) at d 109 of gestation as compared with PB levels at weaning. This higher PB at weaning may reflect the elevated daily feed intake during lactation. Females consuming SB diets had a three-fold increase in milk biotin concentration and the magnitude of the difference between NB and SB females was similar to the difference observed for PB. There was a positive correlation (P<.01) between sow milk biotin level and pig PB with elevations in milk biotin resulting in higher pig PB at 14 d of age (table 17). Plasma biotin was extremely high in newborn pigs but there was a seven-fold decrease in pig PB at 14 d of age for NB and SB females, respectively. Sow PB at weaning was negatively correlated (P<.01) with pig PB at birth (table 17).

TABLE 17. CORRELATION COEFFICIENTS FOR BIOCHEMICAL CRITERIA[a]

Item	Biotin content						Sow pyruvate carboxylase activity
	Sow plasma			Pig plasma		Pig liver	
	Gestation	Weaning	Sow milk	Birth	Day 14	birth	
Biotin content							
Sow plasma							
Gestation	1.0	.14	.01	-.22	.14	-.32	-.24
Weaning		1.0	.09	-.71**	.07	-.46	-.05
Sow milk			1.0	.09	.65*	-.16	.32
Pig plasma							
Birth				1.0	-.13	-.01	-.22
Day 14					1.0	-.06	.43
Pig liver, birth						1.0	.72**
Sow pyruvate carboxylase activity							1.0

[a] 28 observations/comparison.
*(P<.05).
**(P<.01).

Correlation coefficients for female PB and reproductive performance are presented in table 18. As expected, the various reproductive criteria were highly correlated (P<.01). Female PB at d 109 of gestation was postively correlated with number of pigs alive/litter at 21 d (P<.10) and total litter weight at 21 d of age (P<.05). Even though the correlation coefficients were small, this relationship suggests that biotin is important during lactation to maximize the number and weight of pigs weaned. A positive correlation (P<.01) was obtained for PB levels in females at d 109 of gestion and weaning (table 18) but was not present in the data set used for comparisons of various biochemical criteria (table 17).

The results of this study indicate that sow and pig body stores of biotin (except for pig liver at birth) can be increased by supplementing breeding herd diets with biotin at 440 ug/kg diet. The porcine placenta appears capable of transporting large quantities of biotin to the fetus and the amount transported is related to level of biotin intake. Barrett and Everson (1951) revealed a rapidly changing need for B vitamins as pregnancy progressed in rats, and Lewis and Everson (1952) found biotin concentrations in rat fetal and maternal tissues to rise dramatically during the last trimester of pregnancy. Frank et al. (1970) reported that vitamins cross the human placental barrier at different

TABLE 18. CORRELATION COEFFICIENTS FOR SOW PLASMA BIOTIN LEVELS AND REPRODUCTIVE PERFORMANCE[a]

Item	Pigs born alive	Litter birth wt	Pigs alive at 21 d	Litter wt at 21 d	Sow plasma biotin	
					Gestation	Weaning
Pigs born alive	1.0	.83**	.86**	.63**	.07	-.01
Litter birth wt		1.0	.80**	.79**	.06	-.03
Pigs alive at 21 d			1.0	.82**	.15+	.01
Litter wt at 21 d				1.0	.20*	.02
Sow plasma biotin						
Gestation					1.0	.24**
Weaning						1.0

[a] 210 observations used for each comparison.
*(P<.05).
**(P<.01).

rates; rapid transfer rates were observed for biotin. Kaminetzky et al. (1974) observed much higher biotin blood levels in human neonates at birth compared to maternal biotin blood levels and hypothesized that the placental transfer mechanism accumulates biotin in fetal circulation against a concentration gradient.

Under the conditions of this study, swine reproductive performance, especially at farrowing, was slightly reduced when wheat was fed as the grain source compared with corn. The reason for this decrease in reproductive performance with wheat is unclear. Data on the utilization of nutrients in wheat are sparse. Frape et al. (1969) observed no differences in nutrient digestibility between samples of hard and soft wheat offals. To the contrary, Ivan and Farrell (1976) found that protein of hard wheat was of better quality than that of soft wheat based on ileal recovery of amino acids and the advantage was largely due to a greater absorption of lysine from hard wheat. Nelson and Kirby (1982) reported the digestibility of the amino acids in wheat, except for glycine, to range from 94 to 99%, which are similar to those of corn.

Significant reductions in the bioavailability of biotin in milo, barley, oats and wheat have been reported for the chicken (Frigg, 1976; Baker, 1978; Anderson et al., 1978). It is hypothesized that this reduction in available biotin

in some feedstuffs also occurs in swine and when diets containing these ingredients are fed, the need for supplemental biotin may be increased (Brooks et al., 1977). In the present study, type of grain fed did not influence the females response to supplemental biotin and thus, our results do not support the above hypothesis for wheat.

The National Research Council-recommended (1979) biotin requirement for gilts and sows is 100 ug/kg diet. Although not conclusive, results from the present study indicate this level may be insufficient for optimal reproductive performance in breeding swine housed in total confinement. The mechanism by which biotin might increase conception rate and reduce the weaning to estrus interval are unclear. Penny et al. (1981) suggested biotin's role in numerous enzyme reactions, ultimately involved in energy production and utilization, could be a factor in biotin's improvement of litter size for second through fourth parity sows. This hypothesis could also be involved in the conception rate and post-weaning improvements observed in this study. The present study is the first report on biotin supplementation which presents the combined reproductive responses of conception rate, farrowing performance and the weaning to estrus interval. The results suggest the need for further study of biotin's role in swine reproduction.

Summary

Data from 116 females and 245 litters were used to study the influence of 0 (NB) or 440 (SB) ug of supplemental biotin to corn- (C) or wheat- (W) based diets for gilts and sows housed in total confinement. Reproductive performance through four parities and various sow and pig biochemical criteria were evaluated. Females fed W diets were older ($P<.07$) at first estrus, farrowed litters that were lighter ($P<.01$) at birth and that contained fewer ($P<.05$) total and live pigs compared with C females. Pigs nursing W females had lower ($P<.06$) plasma biotin (PB) levels at 14 d of age. Biotin supplementation did not influence ($P>.10$) farrowing and lactation performance, however, litters from SB females were 2.2 kg heavier at 21 d of age. Conception rate was increased ($P<.07$) by 9% and the weaning to estrus interval was reduced ($P<.05$) from 14.5 to 10.2 d with SB. There was a supplemental biotin x parity interaction ($P<.10$) for total and live pigs/litter with SB females farrowing smaller litters at parity 1 but larger litters at parities 3 and 4. Biotin supplementation increased ($P<.001$) the biotin content of sow plasma, milk and liver while sow liver pyruvate carboxylase activity was not altered ($P>.10$). Pigs farrowed by SB females had three- and five-fold higher ($P<.001$) levels of PB at birth and 14 d of age, respectively; however

liver biotin levels at birth were not different for pigs from NB and SB females. These results suggest that reproductive performance, especially at farrowing, is reduced when wheat-based diets are fed. Although not conclusive, the significant improvements in conception rate and weaning to remating indicate that biotin supplementation to diets for gilts and sows housed in total confinement may be required to maximize reproductive performance.

Chapter V
INFLUENCE OF SUPPLEMENTAL BIOTIN ON TOE LESIONS, SOUNDNESS SCORES AND HAIR AND SKIN CHARACTERISTICS IN GILTS AND SOWS FED TWO TYPES OF GRAIN

Introduction

Foot lesions have been closely associated with lameness in swine (Penny et al., 1963; Smith and Robertson, 1971; Fritschen, 1976). The continued trend to confinement housing of the breeding herd has increased the incidence of foot lesions,inflated culling rates due to lameness and increased economic losses due to decreased breeding herd efficiency (Jensen, 1979).

The role of nutrition in the development of foot lesions and subsequent lameness has not been clearly established. However, a deficiency of biotin has been shown to produce foot lesions in swine (Cunha et al., 1946; Glattli et al., 1975). The report by Brooks et al. (1977) of a 28% reduction in foot lesions in a breeding herd after 6 mo of dietary biotin supplementation has stimulated interest on the biotin needs of breeding swine. Recent field reports have also demonstrated benefical effects from supplemental biotin in breeding herds with foot lesions and lameness problems (Comben, 1978; Halama, 1979; Money and Laughton, 1981). Penny et al. (1980) reported that biotin supplementation to be of little value in alleviating

established foot lesions in sows but helpful if the supplementation began during in gilt development. Halama (1979) also observed an improvement in hair coat and a reduction in hair loss following biotin supplementation.

The present study was conducted to more clearly evaluate the influence of supplemental biotin on foot lesions and hair characteristics in gilts and sows housed in total confinement and fed two types of grain.

Materials and Methods

Females from the reproductive study (chapter 4) were used in this study. The females received either 0 (NB) or 440 (SB) ug/kg of supplemental biotin to a corn- (C) or wheat-based (W) diet. Allottment, housing, experimental diets, feeding levels and management of the females were presented in chapter 4. Initially and 7 $\pm$ 3 d following weaning at each of four parities, each female was evaluated for toe lesions, hair loss and soundness. The mean age (d) and weight (kg) was 240, 117; 521, 156; 732, 162; 916, 172 and 1090, 180 for the five time points, respectively. All feet on each female were examined for toe lesions as outlined in chapter 3 (figure 2). Median horn cracks were combined with white-line horn cracks and analyzed as white-line horn cracks. Therefore the six types of toe lesion

tested were (1) heel-horn erosion, (2) heel bruising, (3) heel crack, (4) heel-horn junction crack, (5) white-line horn crack and (6) side-wall horn crack. The frequency of toe lesions was evaluated on a female basis, as well as, a toe within female basis. The frequency of toe lesions was calculated as the percent of females or toes containing one or more lesions/type of lesion. The total number of toe lesions/female and the total severity score/female for all six types of toe lesions was obtained by adding the values for all eight toes of each female. Three individuals scored each female for hair loss and soundness using the scoring system presented in chapter 3. The three individual scores were combined and the mean scores for hair loss and soundness were used for statistical comparison.

At the start of the sow study (approximately 100 kg), an ellipitical-shaped brand was placed on the lower ham of each gilt using freeze-branding techniques. This brand served as a reference point to ensure taking hair counts from the same location. An elliptical-shaped grid containing two 1-cm^2 sections was placed over each brand and hair shafts counted within each 1-cm^2 section. The four values (two/ham) were combined to obtain the average number of hairs/cm^2 skin for each female.

Females were removed from the study after farrowing four litters, failure to cycle, failure to conceive or

development of unsoundness which prevented them from rising and walking to obtain feed or water (see materials and methods, chapter 4). When females were removed from the study, three skin samples were taken for histological evaluation. One skin sample was taken at the point of the left shoulder. The other two samples were taken from the right ham (one inside and one outside of the elliptical brand). All samples were placed in 10% neutral buffered-formalin until slides were prepared. Skin sections, 5 to 6 microns thick, were cut, stained with hematoxylin and eosin solutions, mounted on slides and examined with light microscopy. The presence of and degree of abnormalities of the epidermis, dermis and adnexa were noted.

Statistical Analysis

Data were analyzed as a split-plot (means/female) or split-split-plot (means/toe) design using Harvey's (1977) mixed model least-squares analysis procedure. Whole plot effects included level of biotin, type of grain and replicate (animal group). Female within supplemental biotin, type of grain and replicate mean square was used to test whole plot effects and all two-way interactions of whole plot effects. Time and all two-way interactions of time and whole plot effects were tested with the residual error mean square. The time effect was also partitioned

into linear, quadratic and cubic orthogonal components. To test for differences between means/toe, the same whole plot effects and sub-plot effects and interactions were employed while sub-sub-plot effects of foot (front vs back) and toe (inside vs outside) plus their two-way interactions with whole and sub-plot effects were tested. Appendix table 31 indicates the mean square error term used for calculating each F statistic for the split-split-plot design. T-tests were used to test for differences in main effects at each time. The biotin response was determined by subtracting the response obtained for 440 ug supplemental females from the 0 ug biotin response and dividing this difference by the effect of 0 ug biotin and multiplying by 100.

Results

Toe Lesions

All types of toe lesions (chapter 3, figure 2) were observed across both levels of supplemental biotin and each type of grain. Toe lesions varied in severity from minor to very severe. Twelve females (5 from NB and 7 from SB diets) were culled due to lameness (chapter 4, table 13); however, only 6 of these females (4 NB and 2 SB) were culled due to severe foot lesions. Four of the females culled suffered from a severely abscessed, necrotic foot termed "foot rot"

(Osborne, 1955) or "bush foot" (Penny et al., 1965). Numerous other females (across all dietary treatments) developed minor cases of bush foot that did not necessitate culling from the breeding herd. In general, the number of females with bush foot and the severity of bush foot was less for females consuming SB diets.

Biotin x type of grain interactions ($P>.10$) were not observed. Various replicate (animal group) x biotin and replicate x type of grain interactions ($P<.05$) were obtained. However, these interactions did not show a significant pattern of occurrence or biological significance. Type of grain fed did not influence ($P>.10$) any response criteria tested and clearly indicates that feeding of wheat compared with corn does not influence the development of toe lesions, hair loss and soundness scores. For reference, the least-squares means by type of grain fed for the various response criteria are presented in tables 32-36 but will not be discussed in this section.

The frequency of females with toe lesions (one or more/type) when fed two levels of biotin are summarized in table 19 by type of toe lesion. All the females developed heel-horn erosion and support the findings in chapter 3 and of Penny et al. (1965) that erosion of the toe is a normal and frequent occurrence in swine housed in confinement. Heel bruising was 15% greater for SB females compared with

TABLE 19. LEAST-SQUARES MEAN FOR THE FREQUENCY OF FEMALES WITH TOE LESIONS WHEN FED TWO LEVELS OF BIOTIN[a]

Item[b]	Supplemental biotin, ug/kg 0	440	S.E.[c]	Biotin response, %[d]
No. of observations	171	174		
Heel-horn erosion	100.0	100.0	.00	0
Heel bruising	12.8	14.8	2.3	-16
Heel crack[e]	96.8	84.7	2.2	+13
Heel-horn junction crack[e]	40.4	23.9	2.9	+41
White-line horn crack	38.7	30.7	2.8	+21
Side-wall horn crack[e]	36.3	20.9	2.6	+42

[a]Incidence values expressed as the percentage of females with one or more lesions.
[b]See figure 2 for lesion classification.
[c]Standard error of the mean.
[d]Biotin response = $\frac{0 \text{ ug} - 440 \text{ ug}}{0 \text{ ug}} \times 100$.
[e]Biotin effect ($P<.01$).

NB females but this difference was not significant (P>.10). The frequency of females with heel cracks, heel-horn junction cracks and side-wall horn cracks was reduced (P<.01), 13, 41 and 42%, respectively, when females were fed SB diets. Biotin supplementation also reduced the frequency (21%) of females with white-line horn cracks but the difference was not significant (P>.10). These results support the finding in the growing-finishing study (chapter 3) where biotin supplementation reduced the frequency of gilts with side-wall horn cracks and showed a trend for a reduction in the frequency of other types of toe lesions.

Females fed SB diets had fewer (P<.001) total toe lesions, heel cracks, heel-horn junction cracks, side-wall horn cracks and white-line horn cracks (P<.03) per female compared with females consuming NB diets (table 20). When the data were combined for all five time points, the percent reduction in number of toe lesions from biotin supplementation was 16, 28, 56, 37 and 61% for total toe lesions, heel cracks, heel-horn junction cracks, white-line horn cracks and side-wall horn cracks, respectively. Time x biotin interactions (P<.01) were obtained for the number of total toe lesions, heel-horn junction cracks, white-line horn cracks and side-wall horn cracks per female (figure 4). After time periods 1 and 2, total toe lesion, heel-horn junction cracks, side-wall horn cracks and white-line horn

TABLE 20. LEAST-SQUARES MEANS FOR THE NUMBER AND TOTAL SEVERITY OF TOE LESIONS IN GILTS AND SOWS FED TWO LEVELS OF BIOTIN[a]

Item[b]	Supplemental biotin, ug/kg 0	440	S.E.[c]	Biotin response, %[d]
No. of observations	171	174		
Total toe lesions				
No. lesions[ef]	14.2	12.0	.19	+16
Severity[e]	41.5	34.5	.72	+17
Heel-horn erosion				
No. lesions	7.9	7.9	.03	- .1
Severity	23.8	24.2	.31	+ 2
Heel bruising				
No. lesions	.24	.21	.03	+13
Severity	.69	.64	.10	+ 7
Heel crack				
No. lesions[e]	3.7	2.7	.12	+28
Severity[e]	9.6	6.0	.35	+38
Heel-horn junction crack				
No. lesions[ef]	.66	.29	.06	+56
Severity[e]	1.9	.8	.19	+58
White-line horn crack				
No. lesions[fg]	.86	.54	.06	+37
Severity[h]	2.2	1.3	.16	+41
Side-wall horn crack				
No. lesions[ef]	.83	.32	.06	+61
Severity[e]	3.3	1.6	.20	+52

[a]Data represents the total number of toe lesions and total toe lesion severity scores for all eight toes/female. Each lesion was given a severity score of 1 to 5 with 1 denoting a very small lesion and 5 a very large lesion.

[b]See figure 2 for lesion classification.

[c]Standard error of the mean.

[d]Biotin response = $\frac{0 \text{ ug} - 440 \text{ ug}}{0 \text{ ug}} \times 100$.

[e]Biotin effect (P<.001).

[f]Biotin x time interaction (P<.01).

[g]Biotin effect (P<.03).

[h]Biotin effect (P<.01).

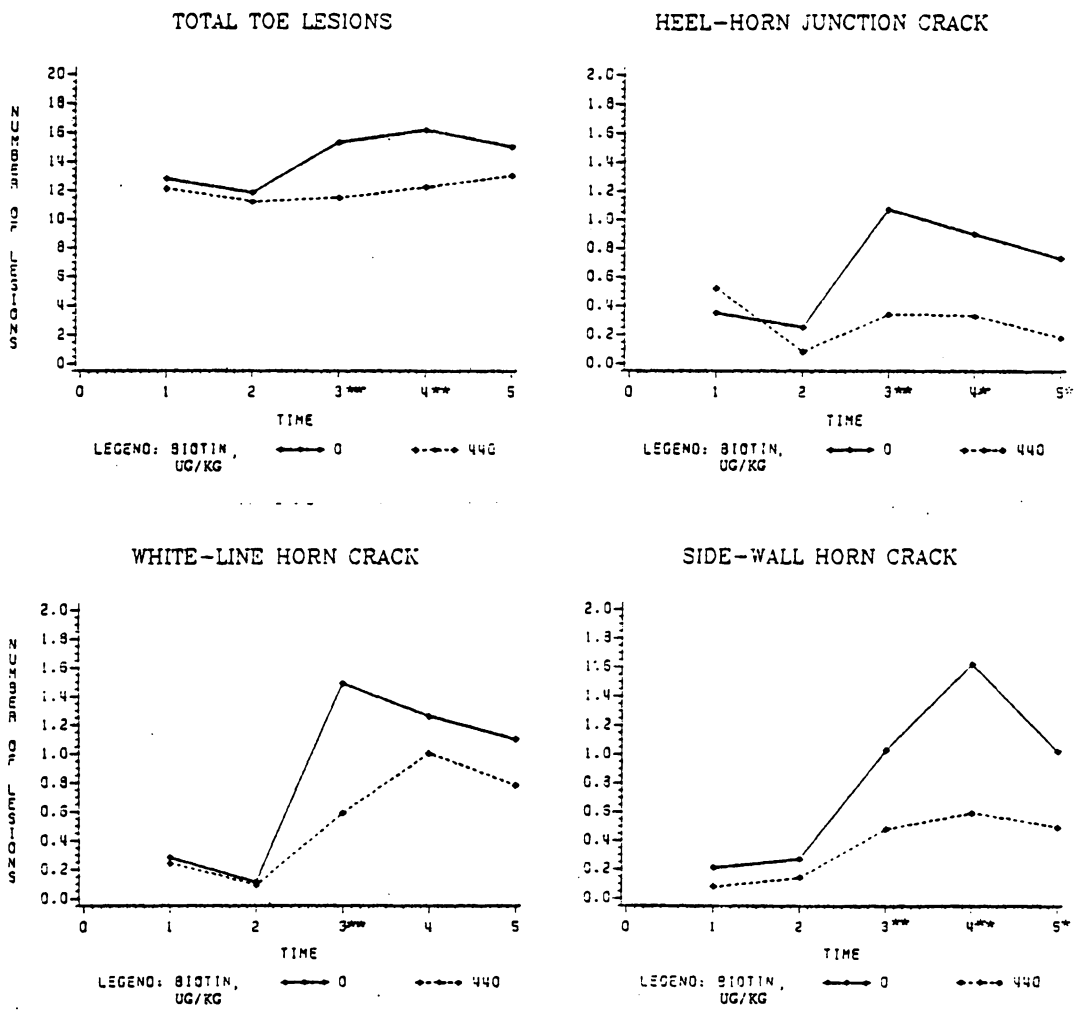


Figure 4. Biotin x time interactions for number of toe lesions per female. Time 1 is initial, while times 2 through 5 correspond to weaning at parities 1 through 4, respectively. Differences between biotin levels at each time are indicated (*P<.05; **P<.01).

cracks were less for sows fed SB diets compared with sows fed NB diets. The total severity (sum of all severity scores/ female for each type of lesion) of total lesions, heel cracks, heel-horn junction cracks, white-line horn cracks and side-wall horn cracks was also reduced ($P<.01$) when females were fed SB diets (table 20). In general, the reductions in total severity were of the same magnitude as the respones observed for number of lesions. The reductions in the number of toe lesions with biotin supplementation observed for sows in this study are much more dramatic than the results obtained during the growing-finishing study (chapter 3). This suggests that biotin is most beneficial for older sows. In a small field trial (19 sows), Money and Laughton (1981) reported a reduction from 10.3 to 2.6 active foot lesions/sow in 4 mo when a diet containing barley, dried blood, lucerne meal and bone flour (estimated to provide 68 ug biotin/d) was supplemented with biotin (360 and 319 ug/kg diet during gestation and lactation, respectively). The number of foot lesions remained high (11.8 lesions/sow) in the control sows. In a four-parity sow study, Brooks and Simmins (1981) also reported a reduction in the mean number of lesions/sow at 170 d of age and following each parity, however, the differences were not significant.

In the present study, heel-horn erosion was the most frequent toe lesion observed and occurred in approximately 88% of the toes examined (table 21) while heel bruising was rare and occurred in only 3% of all toes. Females fed SB diets had lower frequencies of toes containing heel cracks ($P<.001$), heel-horn junction cracks ($P<.001$), white-line horn cracks ($P<.03$) and side-wall horn cracks ($P<.001$) compared with NB females. The greatest reduction (61%) in frequency of toe lesions occurred for side-wall horn cracks which are closely associated with the necrotic bush-foot condition (Osborne, 1955). There was a biotin x time interaction ($P<.03$) for the frequency of toes with heel-horn junction cracks, white-line horn cracks and side-wall horn cracks (figure 5). These interactions were almost identical to those reported for the number of lesions/female (figure 4). There was a reduction in heel cracks ($P<.01$) at all five time points. These results show that biotin supplementation to sow diets will reduce the frequency of toes with heel and horn cracks and that the response is greatest with multiparous sows.

Brooks et al. (1977) found that biotin supplementation of sows in a herd with toe lesions and a lameness problem resulted in a 28% reduction in toe lesions after six mo compared with a 1% increase in toe lesions for unsupplemented sows. The major reductions occurred for

TABLE 21. LEAST-SQUARES MEANS FOR THE FREQUENCY OF TOES WITH LESIONS IN GILTS AND SOWS FED TWO LEVELS OF BIOTIN[a]

Item[b]	Supplemental biotin, ug/kg 0	440	S.E.[c]	Biotin response, %[d]
No. of observations	1368	1392		
Heel-horn erosion	87.3	87.8	.6	- .5
Heel bruising	3.0	2.6	.3	+13
Heel crack[e]	46.6	33.7	1.0	+28
Heel-horn junction crack[e]	7.6	3.5	.5	+54
White-line horn crack[f]	10.5	6.8	.5	+35
Side-wall horn crack[e]	10.3	4.0	.5	+61

[a]Frequency values expressed as the percentage of toes within a female with one or more lesions.
[b]See figure 2 for lesion classification.
[c]Standard error of the mean.
[d]Biotin response = $\frac{0 \text{ ug} - 440 \text{ ug}}{0 \text{ ug}} \times 100$.
[e]Biotin effect ($P<.001$).
[f]Biotin effect ($P<.03$).

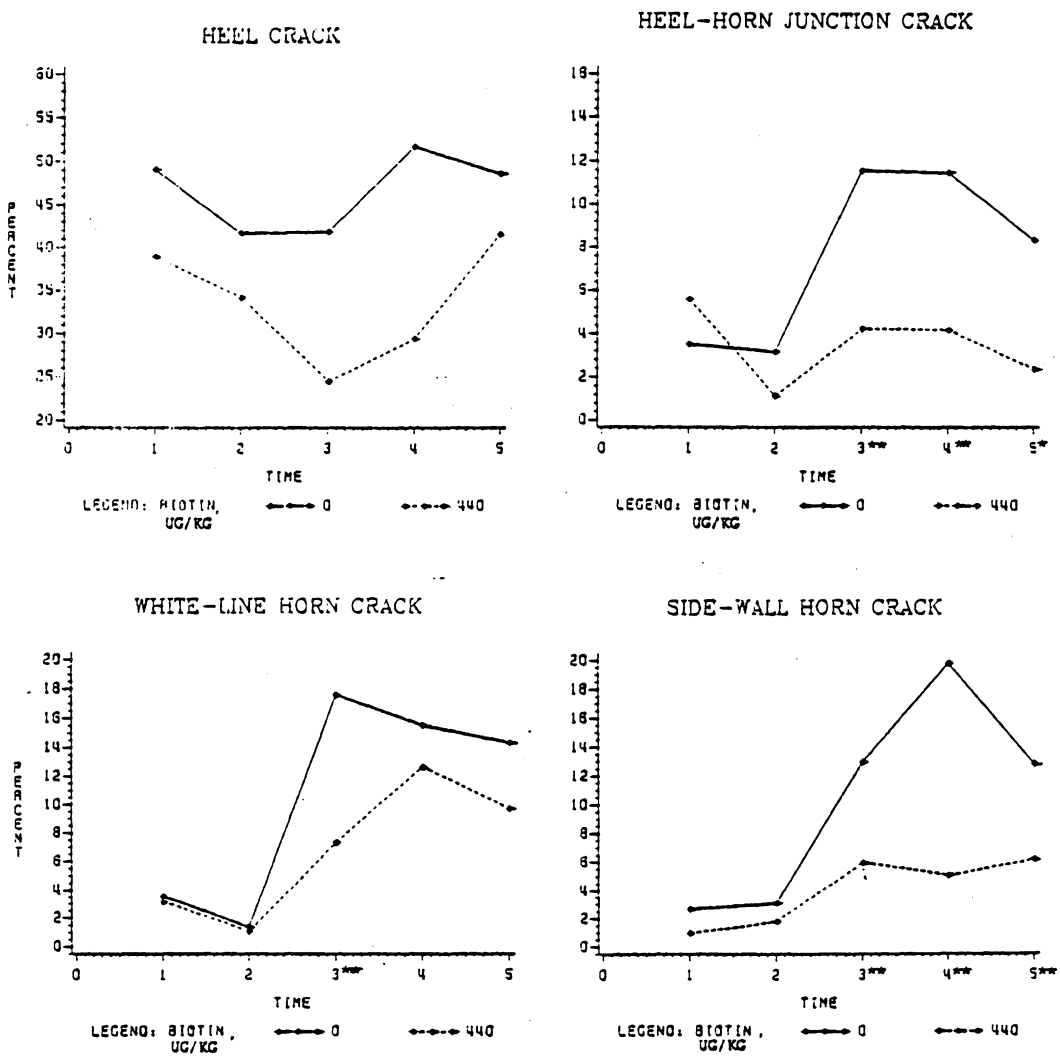


Figure 5. Biotin x time interaction for frequency (percent) of females with toe lesions. Time one is initial while times 2 through 5 correspond to weaning at parities 1 through 4, respectively. Differences between biotin levels at each time are indicated (*P<.05; **P<.01).

heel-horn junction cracks (55%) and horn cracks (42%). Penny et al. (1980) reported significant reductions in the number of lesions/toe for heel erosion of the lateral fore and hind toes and for white-line cracks, heel bruising and total number of lesions of the lateral toe. To the contrary, Bane et al. (1980) found that supplementation of a corn-soybean meal diet with 220 ug biotin/kg diet had no effect on foot lesion incidence in gestating gilts.

The mean severity of toe lesions for the five time points combined are summarized in table 22 by level of biotin. Heel-horn erosion was the most severe toe lesion while the remaining four types of toe lesions were of similiar severity. Heel crack severity scores were lower ($P<.01$) for SB females compared with NB females (2.54 vs 2.09). Although not significant ($P>.10$), there was a trend for supplemental biotin to reduce the severity of all three types of horn cracks. Penny et al. (1980) noted that the total severity score of toe lesions on the lateral hind toe was reduced when sows were fed biotin supplemented diets. Grandhi and Strain (1980) reported only a slight reduction in the severity of toe lesions following biotin supplementation to barley-wheat-soybean meal gestation-lactation diets.

TABLE 22. LEAST-SQUARES MEANS FOR TOE LESION SEVERITY SCORES IN GILTS AND SOWS FED TWO LEVELS OF BIOTIN[ab]

Item	Supplemental biotin, ug/kg 0	440	S.E.	Biotin response, %[c]
Heel-horn erosion	3.00(1355)	3.04(1382)	.02	- 1
Heel crack[d]	2.45(595)	2.09(458)	.04	+15
Heel-horn junction crack	2.77(94)	2.32(49)	.45	+16
White-line horn crack	2.48(102)	2.31(70)	.13	+ 7
Side-wall horn crack	2.81(102)	2.56(41)	.25	+ 9

[a]Severity scores ranged from 1 to 5 with 1 denoting a small lesion and 5 a very large lesion.

[b]No. of observations/mean in parenthesis.

[c]Biotin response = $\frac{0 \text{ ug} - 440 \text{ ug}}{0 \text{ ug}} \times 100$.

[d]Biotin effect ($P<.01$).

Hair Characteristics

Females fed SB diets had greater numbers of hairs/ cm^2 ($P<.001$) and lower (better) hair scores ($P<.001$) compared with females fed NB diets (table 23). The biotin x time interaction was significant ($P<.001$) for hair scores (figure 6). There was no difference in hair scores for NB and SB females at times 1, 2 and 5, while at times 3 and 4 SB females had lower scores compared with NB females. Hair scores at times 2 and 5 showed a trend for improvement with biotin supplementation. Hair scores were positively correlated ($P<.01$) with total toe lesions/ female and number of white-line horn cracks/female (table 24). There was also a trend ($P<.10$) for hair scores to be positively correlated with heel bruising, heel cracks and heel-horn junction cracks. Even though the correlation coefficients are low, the trends indicate that hair loss as reflected by hair score may be related to the development of toe lesions. These correlations suggest that the factor or factors involved in the development of toe lesions in swine also play a role in the maintenence of normal hair coats. Hair scores were not correlated ($P>.10$) with hair counts. Therefore, the mechanism of hair loss on the lower ham does not appear to be the same as hair loss on other parts of the pig's body.

TABLE 23. LEAST-SQUARES MEANS FOR HAIR COUNTS, HAIR SCORES AND SOUNDNESS SCORES FOR GILTS AND SOWS FED TWO LEVELS OF BIOTIN

Item	Supplemental biotin, ug/kg 0	440	S.E.[a]
No. of observations	171	174	
No. of hairs/cm^2[bc]	4.10	5.07	.09
Hair score[cd]	2.10	1.73	.04
Soundness score[e]	8.61	8.70	.12

[a]Standard error of the mean.
[b]Mean value for four observations (two/hair) taken from the base of the hair.
[c]Biotin effect ($P<.001$).
[d]Mean value for three committee members. Each female received a score of 1 to 5 where 1 indicated no hair loss and 5 excessive hair loss.
[e]Mean value for three committee members. Each female received a score of 1 to 15 where 1 indicated a very sound, free moving female and 15 a lame, unsound female unable to walk.

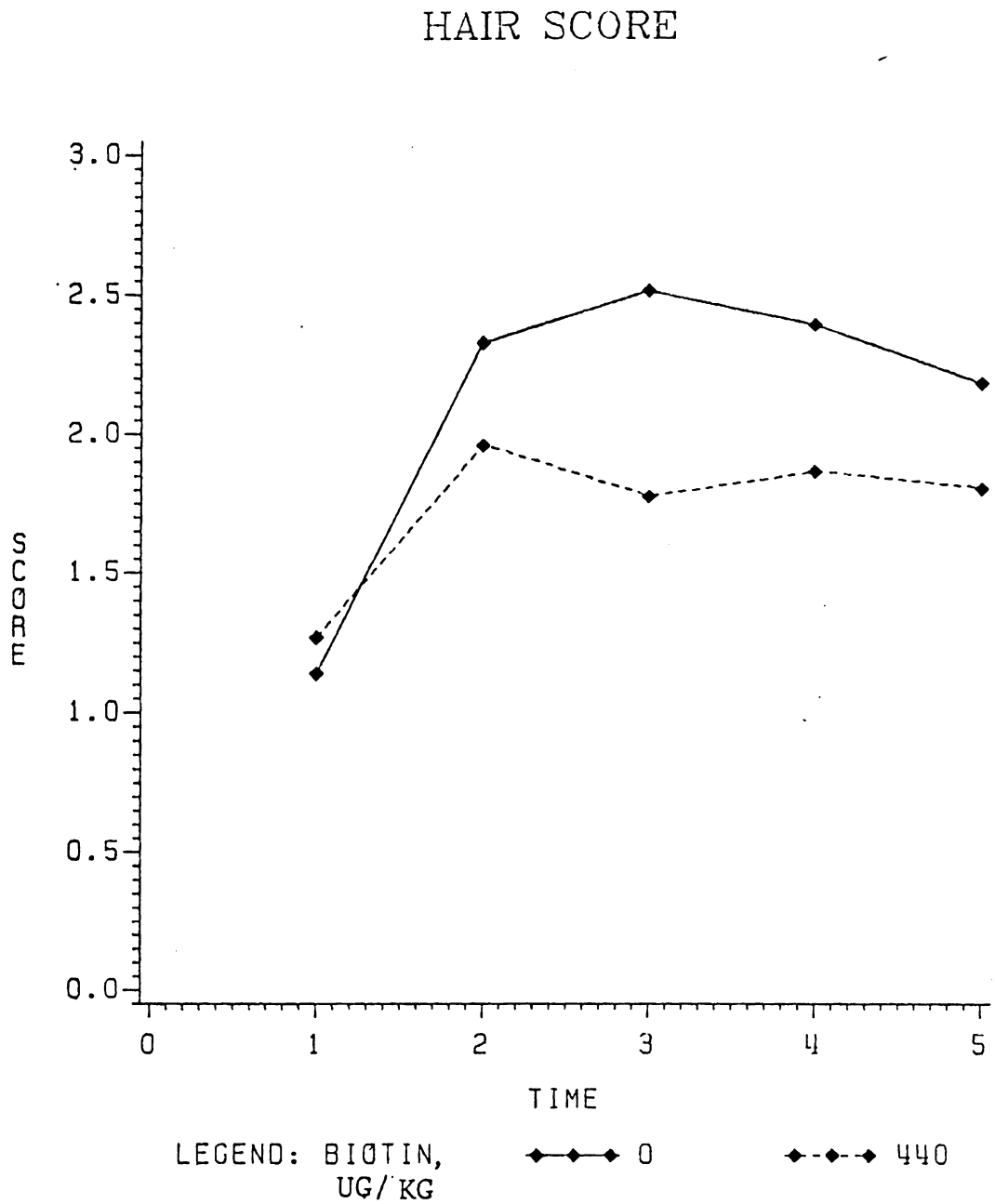


Figure 6. Biotin x time interaction for hair scores in gilts and sows.

TABLE 24. CORRELATION COEFFICIENTS FOR SOUNDNESS AND HAIR SCORES AND TOE LESIONS[a]

Item	Hair score	No. of toe lesions/female: Total lesions	Heel erosion	Heel bruising	Heel crack	White-line horn crack	Heel-horn junction crack	Side-wall horn crack
Soundness score	.17**	.12†	-.01	.04	.12*	-.03	.08	.08
Hair score	1.0	.20**	.03	.11†	.12†	.19**	.12†	.06
No. of toe lesions/female								
Total lesions		1.0	.15*	.29**	.74*	.59**	.52**	.55**
Heel erosion			1.0	-.32**	.09	.04	-.01	-.01
Heel bruising				1.0	.10	.16**	,12†	.10
Heel crack					1.0	.16**	.08	.11
White-line horn crack						1.0	.25**	.33**
Heel-horn junction crack							1.0	.28**

[a]345 observations for each comparison.
*(P<.05).
**(P<.01).
†(P<.10).

The role of biotin in maintaining good hair coats, reducing dermatitis of the skin and preventing the developing of foot lesions is well documented (Cunha et al, 1946; Buhlmann, 1973; Glattli et al., 1975). Cunha (1968) and Halama (1979) reported a reduction in skin dermatitis and hair loss following biotin supplementation to sow diets in field trials. Comben (1978) also reported many sows in field trials exhibited dry scaly skin and hair loss, however, no consistant response was obtained from biotin supplementation. A subjective skin assessment also failed to demonstrate a difference between sows receiving 0 or 1160 ug/d of supplemental biotin (Penny et al., 1981). Numerous significant correlations were observed for number of toe lesions among the 6 types of toe lesions. These findings support those obtained in chapter 3 and suggests that the development of one type of lesion could lead to the development of other types of lesions.

Soundness Scores

Soundness scores were not affected ($P>.10$) by level of supplemental biotin (table 23). A positive correlation ($P<.05$) was observed for soundness score and number of heel cracks/female, however, the correlation coefficient (.12) was low. There was also a trend ($P<.10$) for soundness score to be positively correlated with total number of toe

lesions/female. Hair scores were correlated ($P<.01$) with soundness scores such that females with high (poorer) hair scores also had high soundness scores. Although not conclusive, the data suggest that females with heel cracks and apparent hair loss have more unsoundness problems.

Histological Data

Numerous abnormal skin characteristics were observed from the skin sections evaluated. However, these abnormal skin characteristics were equally divided among levels of biotin and type of grain. The most common skin changes were irregularity of the skin surface, thinning or thickening of the keratinized layer, extension of the keratinized layer into the dermis (rete pegs) and convolution of the epidermis. Although not present on all slides, there were some skin sections which contained abnormal hair follicles. These abnormal hair follicles were unrelated to level of biotin.

Discussion

Until recently, many nutritionists have refused to consider the possibility that supplemental biotin may be necessary in swine diets. It was felt that the biotin content of feedstuff and biotin biosynthesis in the

gastrointestinal tract were adequate to meet the animal's needs. The results of the present study coupled with other recent reports (Brooks et al., 1977; Brooks and Simmins, 1981; Penny et al., 1980) strongly suggest that foot health can be improved with biotin supplementation of sow diets. Numerous changes in the feeding, housing and management of sows may predispose the development of biotin responsive foot lesions and most notable the movement to confinement housing of the breeding herd.

Animal age, length of time in the sow herd and the prevalence of toe lesions in the herd appears to be closely related to the animal's responsiveness to biotin supplementation. Controlled studies which have evaluated the effects of biotin supplementation on development of foot lesions in gilts and primiparous sows have mostly obtained no response, (Bane et al., 1980; Grandi and Strain, 1980). In the present study, the major reductions (except for heel cracks) in toe lesions did not occur until the sows had farrowed approximately two litters (600 to 700 d of age). The recent results of Brooks and Simmins (1981) also support this time and/or age-related biotin response hypothesis. The results in figures 4 and 5 also indicate the reduction in number and frequency of lesions with biotin supplementation is generally greater as the presence of toe lesions increases in the herd.

Brooks et al. (1977) hypothized that biotin supplemenmtation reduced toe lesions by hardening the hoof, which reduced wear and/or tramatic injury to the toe. In support of this hypothesis horn compression strength has been reported to increase with dietary biotin level over the range of 0 to 720 ug supplemental biotin/kg diet (Brooks and Simmins, 1981). Comben (1978) reported that sows from herds exhibiting visual signs of biotin deficiency had soft and rubbery toe horns which indented under pressure from a finger nail. Horn hardness was not evaluated in the present study, however, softening of the horn did not appear to occur.

Penny et al. (1980) indicated that established foot lesions in sows did not seem to benefit from biotin supplementation, but the feet of young biotin-supplemented gilts, with few lesions on entry to the herd were in some way improved. Brooks and Simmins (1981) also noted that toe lesions were reduced when biotin supplementation was initiated at 25 kg body weight. The results of the present study support these findings; however due to the experimental design (females remained on NB or SB diets during growth and reproductive phases) we can not conclusively say that early biotin supplementation is necessary for optimum hoof integrity.

The data show that when gilts and sows are housed in total confinement foot lesions will develop and the addition of 440 ug supplemental biotin/kg diet will reduce the incidence and number of various types of toe lesions. Additional studies are needed to determine how early in the animals life and at what level biotin should be supplemented to ensure optimal hoof and hair condition.

Summary

The influence of supplementing 0 (NB) or 440 (SB) ug d-biotin/kg to corn- or wheat-based diets on toe lesions and hair characteristics in 116 crossbred female swine was studied from selection (100 kg) until completion of four parities. Females were housed in buildings containing partially-slotted and solid concrete floors. Toe and hair evaluations were made at a mean female age of 240, 521, 732, 916 and 1090 d. Type of grain fed did not influence ($P>10$) any response criteria evaluated. Seven types of toe lesions were observed across all dietary treatments and varied in severity from minor to very severe. The frequency of females with heel cracks, heel-horn junction cracks and side-wall horn cracks was reduced ($P<.01$) when females were fed SB diets. Females fed SB diets had fewer ($P<.001$) total lesions, heel cracks, heel-horn junction cracks, side-wall

horn cracks and white-line horn cracks ($P<.03$) compared with females consuming NB diets. Reductions in the frequency of toes with heel cracks ($P<.001$), heel-horn junction cracks ($P<.001$), white-line horn cracks ($P<.03$) and side-wall horn cracks ($P<.001$) were obtained with biotin suplementation. There were biotin x time interactions ($P<.03$) for the number of lesions/female and the frequency of females with lesions. In general, biotin supplementation was more effective in reducing the number and frequency of toe lesions in multiparous sows compared with gilts and primiparous sows. Biotin supplementation increased ($P<.001$) the number of hairs/cm^2 skin and improved ($P<.001$) hair scores. Histological evaluation and soundness scores were not affected ($P>.10$) by biotin fed. Soundness scores were positively correlated with number of heel cracks/female ($P<.05$) and hair score ($P<.01$); however, the coefficients were low. These results indicate that foot health and hair coats of sows housed in confinement can be improved by supplementation of 440 ug biotin/kg diet.

CHAPTER VI.
THE INFLUENCE OF TOE SIZE, TOE LOCATION AND SUPPLEMENTAL BIOTIN ON THE DEVELOPMENT OF TOE LESIONS IN SWINE

Introduction

The frequency and type of toe lesions in swine are well documented (Osborne, 1950; Osborne and Ensor, 1955; Penny et al., 1963, 1965; Penny, 1979; Smith, 1979). Numerous factors have been shown to cause foot lesions in swine; however, the mechanism leading to the development of toe lesions in swine still remains unclear.

Type of flooring material (Wright et al., 1972; Fritschen, 1979; Jenson, 1979; Newton et al., 1980), percent slotted area/pen and slat widths (Fritschen, 1979) and incorrect slat design (Smith and Robertson, 1971) can influence the development of toe lesions. Kovacs and Beer (1979) hypothesized that abrasiveness, resistance to wear and slipperiness are the mechanical properties of floors which relate to foot lesions in swine.

Differences in claw size within swine was first reported by Nordby (1939) and confirmed by Penny et al. (1963). The lateral (outer) toes are larger than medial (inner) toes (Fritschen, 1979; Kornegay et al., 1980; Calabotta et al., 1981; Arthur et al., 1982). These authors also reported that lateral toes developed more lesions than medial toes, strongly suggesting that unequal toe size is a factor in foot lesion development and distribution.

Biotin deficiency has been implicated as a factor precipitating toe lesions and lameness in swine of various ages (Cunha et al., 1946; Lehrer et al., 1952; Cunha, 1968; Glattli et al., 1975). Results from numerous studies suggest that toe lesions can be reduced in swine housed in confinement with biotin supplementation (Cunha, 1968; Tagwerker, 1974; Brooks et al., 1977; Comben et al., 1978; Penny et al., 1980). To the contrary, Bane et al. (1980) and Grandhi and Strain (1980) reported that supplementation was not effective in reducing toe lesions in swine.

The objective of the present study was to study the influence of toe size, toe location and supplemental biotin on the development of toe lesions in swine housed in confinement from weaning through four parities.

Materials and Methods

Females described in chapters 3, 4 and 5 were used for this study. The females were fed corn-soybean meal diets containing 0 (NB) or 220 (SB) ug of supplemental biotin/kg diet from weaning to 100 kg and corn- or wheat-based diets containing 0 (NB) or 440 ug (SB) supplemental biotin/kg diet from 100 kg through 4 parities. Females remained on either NB or SB diets throughout all phases of the study. Allotment, housing, experimental diets, feeding levels,

management and number of females were described in chapters 3 and 4 (see material and methods section). The toe lesion data and hair scores obtained in chapters 3 and 5 were combined and used for this study. Detailed procedures for the collection of toe lesion data and hair scores are presented in chapter 3. The same six types of toe lesions studied in chapter 5 were tested in this study.

At the same eight time points that toe lesions and hair scores were obtained, four toe measurements were taken on one fore and one hind limb using an Omega caliper calibrated in millimeters (figure 7). An estimate of toe base area was obtained by multiplying measurement 2 by measurement 4. The mean female age (d) and weight (kg) was 40, 10; 115, 51; 178, 92; 240, 117; 521, 156; 732, 162; 916, 172; and 1090, 180 for the eight time points.

Statistical Analysis

Data were analyzed as a split-split-plot design using Harvey's (1977) mixed model least-squares analysis procedure. The data were combined by level of biotin for the corn- and wheat-based diets fed during the reproductive study. Whole plot effects included level of biotin and replicate (animal group). Female within biotin and animal group mean square was used to test whole plot effects and their two-way interaction. Time and all two-way

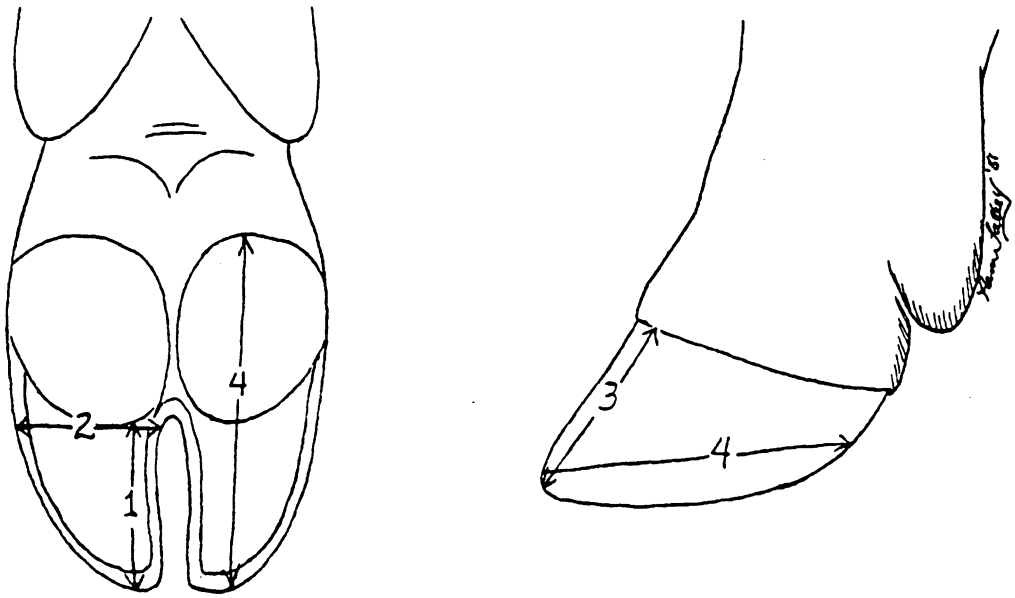


Figure 7. Toe measurements: Horn length (1), distance from distal periphery of the pad to the tip of the toe; Toe width (2), widest part of the toe along the distal perphery of the pad; Horn height (3), distance from the coronary band to the tip of the toe; and Toe length (4), distance from the proximal periphery of the pad to the tip of the toe.

interactions of whole plot effects were tested with the female x time mean square. Sub-sub-plot effects of foot (front vs back) and toe (inside vs outside) plus their two-way interactions with time and whole plot effects were tested using the residual mean square. Appendix table 37 presents the source of variation, degrees of freedom and mean square error term used to calculate each F statistic. The time effect was also partitioned into linear, quadratic, cubic and quartic orthogonal components.

Toe lesions were analyzed as to their frequency and severity. Frequency of toe lesions was defined as the percentage of toes examined that contained one or more toe lesions. Severity scores ranged from 1 to 5 with 1 indicating a small lesion and 5 a very large lesion. Those observations (toes) that did not contain a severity score (no lesion present) were deleted prior to analysis. The small numbers of some types of toe lesions (e.g., heel-horn junction crack) and missing sub-cells necessatated reductions in the statistical model for severity of types of some toe lesions. The frequency of heel bruising was too low to permit proper statistical comparison.

Results and Discussion

Toe Measurements

Toe location influenced toe size with the foot x toe effect significant ($P<.01$). The foot x age and toe x age interactions were also significant ($P<.01$) for all toe measurements (table 25). Horn length was greatest for rear outside (RO) toes and smallest for rear inside toes (RI). As the animals increased in age heel-horn erosion (see chapter 3, figure 2) was frequently of such severity that the distal periphery of the pad was difficult to discern, thus, measurement of the horn length was difficult. Therefore, this measurement may lack precision and the results may be questionable. Toe width was greatest for front outside (FO) toes and dramatically smaller (4.2 mm) for RI toes while front inside (FI) and RO toes were similiar in width. Horn height was greatest for RO toes, intermediate for FO and RI toes and smallest for FI toes. Rear outside toes were the longest while RI toes were the shortest. Toe base area was greatest (14.7 mm) for FO toes and smallest (11.7 mm) for RI toes. The data show that inside (I) toes are smaller than outside (O) toes and the magnitude of the difference is greatest for rear toes.

The FR x age and IO x age interactions were significant for toe base area, toe length, horn height, toe width ($P<.01$) and horn length ($P<.05$). All toe measurements

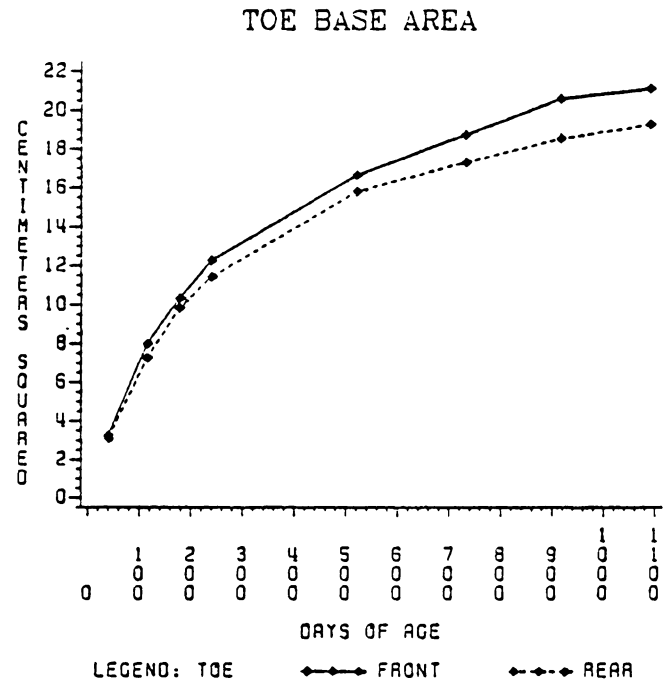
TABLE 25. LEAST-SQUARES MEANS FOR MEASUREMENTS OF INSIDE (I) AND OUTSIDE (O) TOES ON FRONT (F) AND REAR (R) FEET OF GILTS AND SOWS[a]

Item[b]	Toe				S.E.[c]
	FI	FO	RI	RO	
Horn length, mm[ef]	21.5	22.9	20.8	23.0	.09
Toe width, mm[eg]	24.9	26.8	22.6	24.8	.07
Horn height, mm[eg]	40.2	41.0	41.8	43.5	.12
Toe length, mm[eg]	49.3	51.4	48.7	52.9	.12
Toe base area, cm^2[deg]	13.0	14.7	11.7	14.0	.05

[a]Combined data from eight observations/toe.
[b]See figure 7 for explanation of measurements.
[c]Standard error of the mean.
[d]Calculated by multiplication of toe width x toe length.
[e]Foot (front vs rear) and toe (inside vs outside) effect ($P<.001$).
[f]Foot x time effect ($P<.05$).
[g]Foot x time and toe x time effect ($P<.001$).

increased linearly and quadratically (P<.001) over time in a similar manner. Therefore, only the data for toe base area will be discussed (figure 8). For reference, data for the other four toe measurements are presented in figures 13 and 14 (see Appendix C). The ratio of front toe base area to rear toe base area was 1.05:1 at 40 d of age. As the females increased in age, the difference between F toe base area and R toe base area increased and the ratio was 1.10 to 1 at 1090 d of age. Outside toes also had larger toe base areas than I toes initially (ratio=1.07:1) and the difference increased with age to a final outside to inside ratio of 1.19:1.

Total length of the I toes was observed to be 1 to 4 cm shorter than the length of O toes by Nordby (1939). Penny et al. (1963) confirmed this difference in toe size between I and O toes and noted I:O ratios of 1.11:1 and 1.13:1 for toe length and width, respectively. Arthur et al. (1982) reported differences in the I:O ratios for F and R feet with an average ratio of 1.05:1 and 1.13:1 for toe length on the F and R foot, respectively. Penny et al. (1963) suggested that differences in toe size were present at birth while Smith and Mitchell, (1977) concluded that differences could not be detected until 5 to 14 d of age. Fritschen (1976) and Newton et al., (1980) reported similar findings on claw size and reported that type of slats and amount of slatted area in the pen influenced toe size.



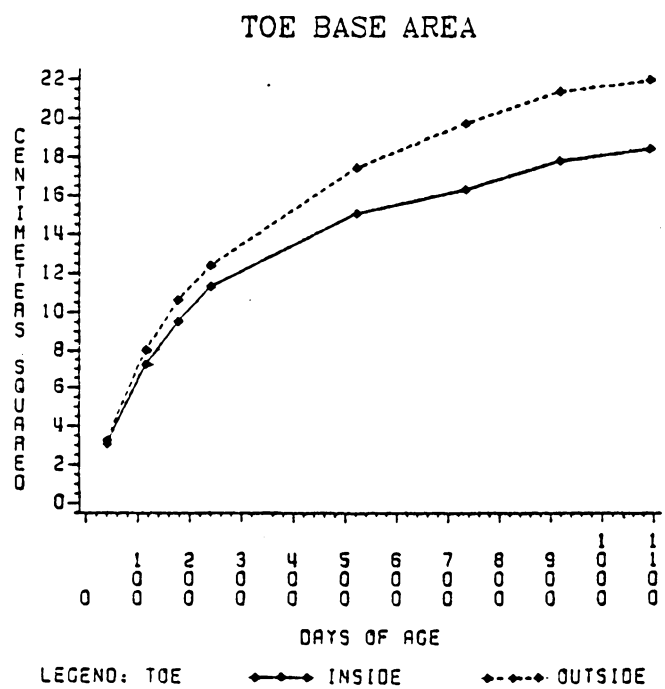


Figure 8. Effect of age on toe base area of inside or outside toes in gilts and sows.

A FB x IO x age interaction was observed (P<.01) for toe base area and and gives an indication of the relative differences in toe growth among toes. All toes grew at approximately the same rate up to 200 to 250 d of age. At that point the growth rate of the inside toes was slower compared to the outside toes with the RO growing dramatically slower. These results agree with the recent findings of Lepine (1982).

Supplemental biotin did not affect (P>.10) toe measurements. The role of nutrition on toe size is poorly understood. Recent findings suggest that dietary calcium and phosphorus and crude protein do not influence toe size (Kornegay et al., 1981; Calabotta et al., 1982; Lepine, 1982; Arthur et al., 1983; Kornegay et al., 1983).

Toe Lesions

All types of toe lesions indicated in figure 2 (chapter 3) were observed during the course of the study. Toe location influenced the frequency of toe lesions (table 26) and the FR x IO interaction was significant (P<.01) for all toe lesions. With the exception of heel-horn erosion and heel-horn junction cracks, the RO toes had the highest frequency of toe lesions. The FO toes were the second most frequently affected toes. Inside toes had very low frequencies (< 3%) of heel bruising, heel-horn junction cracks, white-line horn cracks and side-wall horn cracks.

TABLE 26. LEAST-SQUARES MEANS FOR THE FREQUENCY (PERCENT) OF TOE LESIONS ON INSIDE (I) AND OUTSIDE (O) TOES FROM FRONT (F) AND REAR (R) FEET OF GILTS AND SOWS[a]

Item[b]	Toe				S.E.[c]
	FI	FO	RI	RO	
Heel-horn erosion[de]	83.2	78.9	76.9	73.3	.91
Heel bruising[de]	.4	2.1	1.5	4.0	.32
Heel crack[de]	21.9	36.1	20.8	42.5	1.00
Heel-horn junction crack[d]	1.2	5.9	1.8	5.8	.37
White-line horn crack[d]	2.7	7.3	2.4	8.1	.36
Side-wall horn crack[de]	1.5	5.5	2.6	8.6	.37

[a]2112 observations/mean.
[b]See figure 2 for lesion classification.
[c]Standard error of the mean.
[d]Toe (inside vs outside) effect ($P<.001$).
[e]Foot (front vs rear) effect ($P<.01$).

The frequency and severity of toe lesions was low initially and increased linearly and quadratically ($P<.001$) with increased age of the females (figure 9). Heel-horn erosion was the toe lesion that increased most rapidly, with approximately 100% of the females exhibiting heel-horn erosion at 240 d of age. This finding suggests that heel-horn erosion is a common occurrence in confinement housed swine and supports the work of Penny et al. (1963). Heel cracks were the second most common toe lesion and were observed frequently across dietary treatments.

The frequency of toes with lesions was similar for I and O toes up to 178 d. After 178 d, the frequency of toes containing lesions rose more preceptiously for the O toes compared with I toes. The frequency of O toes with lesions remained high throughout the study except for heel-horn erosion at 1090 d and heel bruising at 916 and 1090 d. These results indicate that toe location plays an important role in the distribution of toe lesions with the outside toes containing more toe lesions ($P<.001$) than inside toes. The F vs R effects on toe lesion distribution are less clear (figure 10). The F toes had higher frequencies of heel-horn erosion ($P<.001$) but lower frequencies of heel bruising. The frequency of toes with heel cracks and side-wall horn cracks was greater for the R toes, however, the response was variable at each time point. The percentage of toes

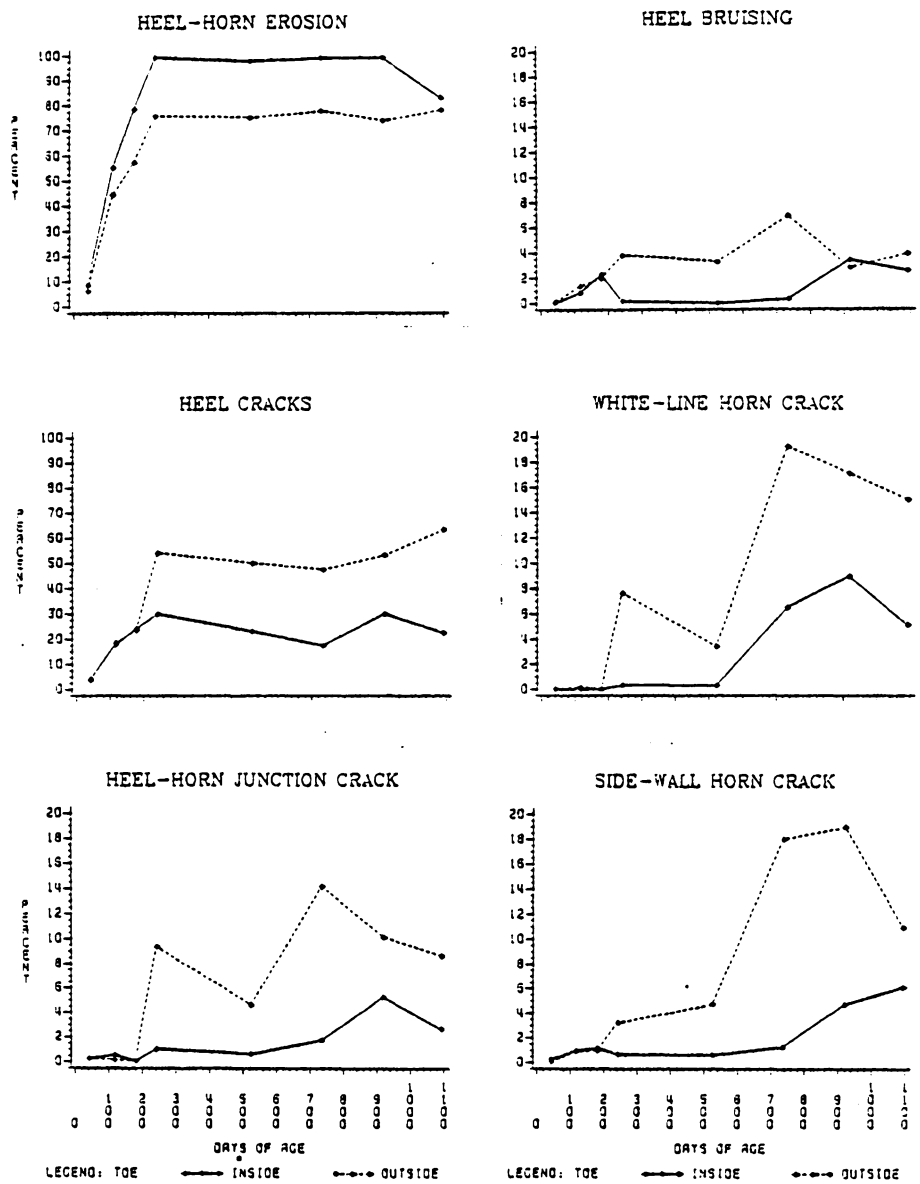


Figure 9. The effect of age on the frequency of inside or outside toes with lesions.

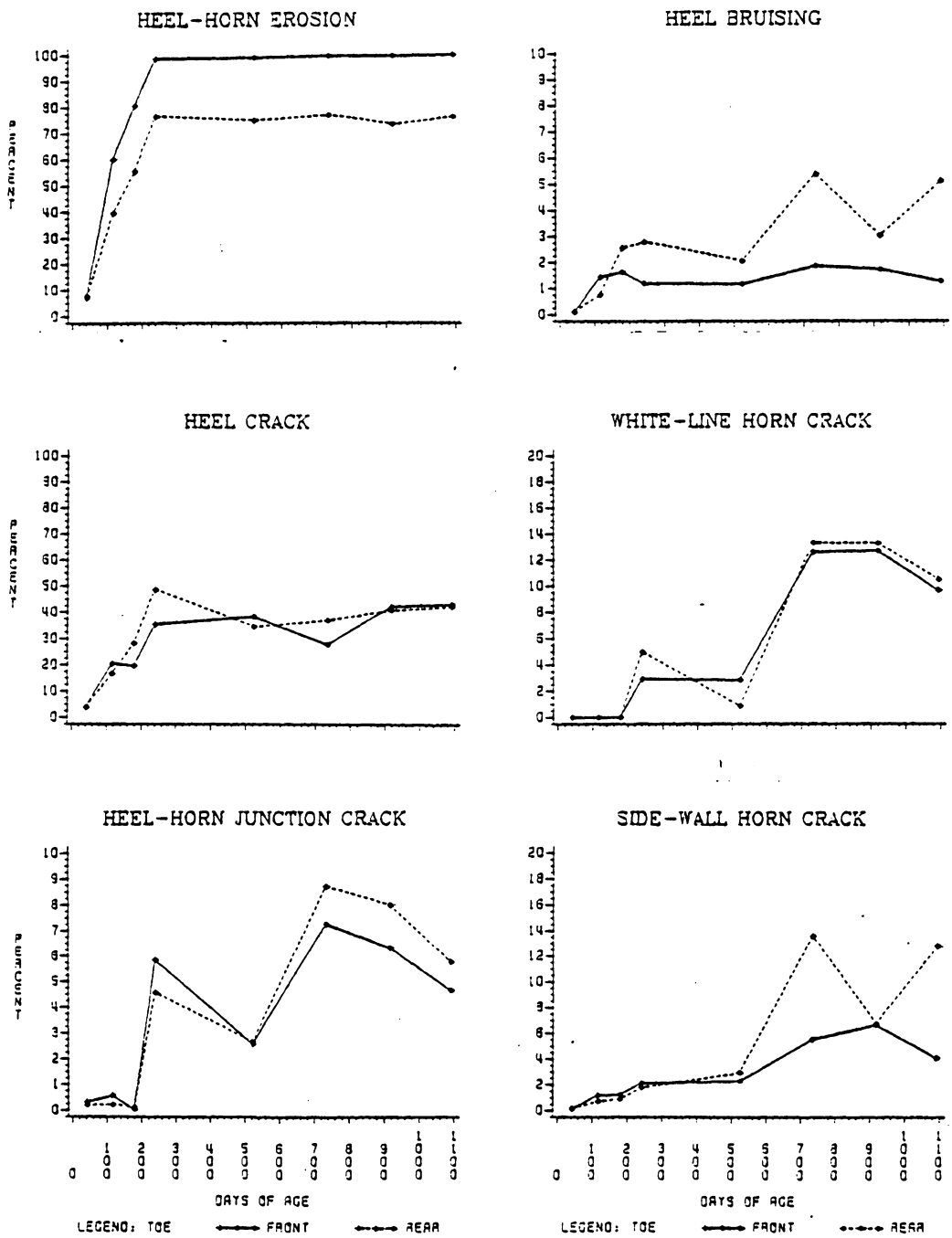


Figure 10. The effect of age on the frequency of front and rear toes with lesions.

containing white-line horn cracks or heel-horn junction cracks was not different (P>.10) for F and R toes.

The occurrence of more toe lesions on outside toes compared to inside toes is well documented (Penny et al., 1965; Fritschen, 1979; Penny, 1979; Arthur et al., 1982; Lepine, 1982). However, a definite relationship between toe size and toe lesions has not been established. Unfortunately, confounding of toe size with toe location make interpretation of results difficult.

The correlation coefficients in table 27 suggest that toe size influences the development of toe lesions; however, the magnitude of the correlation is small. As one would expect, toe base area among toes is positively correlated. Positive correlations (P<.01) among the toes for total toe lesions were also noted. The most important correlations are those between IO toe base area differences and total toe lesions/toe for F and R feet. As the IO difference in toe base area for F toes increases (e.g. inside toe gets smaller and/or outside toe get larger) the total toe lesions/toe decreases for the FI toe and increases for the FO toe. A similar and slightly greater effect is noted for the rear IO difference in toe base area. These findings indicate that differences between toe size within a foot are related to the toe lesions that develop on that foot. Penny (1979) reported significantly more lateral claws with severe

TABLE 27. CORRELATION COEFFICIENTS FOR TOE SIZE, TOTAL TOE LESIONS AND SOUNDNESS SCORE[a]

Item	Toe base area[b]				Front[c] IO diff.	Rear[d] IO diff.	Total toe lesions/toe				Soundness score
	FI	FO	RI	RO			FI	FO	RI	RO	
Toe base area											
FI	1.0	.60**	.50**	.53**	-.34**	.13**	.08*	-.04	-.01	.03	.01
FO		1.0	.45**	.61**	.55**	.24**	-.01	.11**	-.09*	.08	-.01
RI			1.0	.40**	.01	-.40**	.01	-.13**	.03	-.12**	-.01
RO				1.0	.15**	.68**	-.01	.07	-.11**	.18**	.04
Front IO difference					1.0	.15**	-.10**	.17**	-.11**	.07	-.01
Rear IO difference						1.0	-.01	.18**	-.13**	.28**	.05
Total toe lesions/toe											
FI							1.0	.33**	.37**	.26**	.07†
FO								1.0	.13**	.41**	.04
RI									1.0	.24**	-.01
RO										1.0	.02
Soundness score											1.0

[a] 580 observations/comparison.
[b] F=front foot; R=rear foot; I=inside toe; O=outside toe.
[c] FO - FI toe base area.
[d] RO - RI toe base area.
*(P<.05).
**(P<.01).
†(P<.10).

lesions in pigs with unequal-sized claws compared with pigs containing equal-sized claws. Fritschen (1976) suggested that unequal claw size resulted in unequal weight distribution which subsequently leads to toe lesions developing on outside toes. Penny (1979) hypothesized that the distribution of toe lesions are the manifestation of variations in use, wear and exposure to injury. He further concluded that lateral claws may get more lesions due to their location rather than size and the RI claw might be the toe best protected.

Supplemental Biotin Effects

Toe Lesions. Biotin supplementation did not influence ($P>.10$) the frequency of toes with heel-horn erosion or heel bruising. However, females fed SB diets had fewer ($P<.001$) heel cracks, white-line horn cracks, heel-horn junction cracks and side-wall horn cracks. A biotin x age interaction was also present ($P<.001$) for these four types of lesions (figure 11). The biotin response on frequency of toes with lesions was minor up to 240 d of age. Starting at 240 d and continuing throughout the study, biotin supplementation reduced the frequency of toes with heel cracks and side-wall horn cracks. Reductions in white-line horn cracks and heel-horn junction crack were not observed until the females reached 500 d of age. Similar reductions

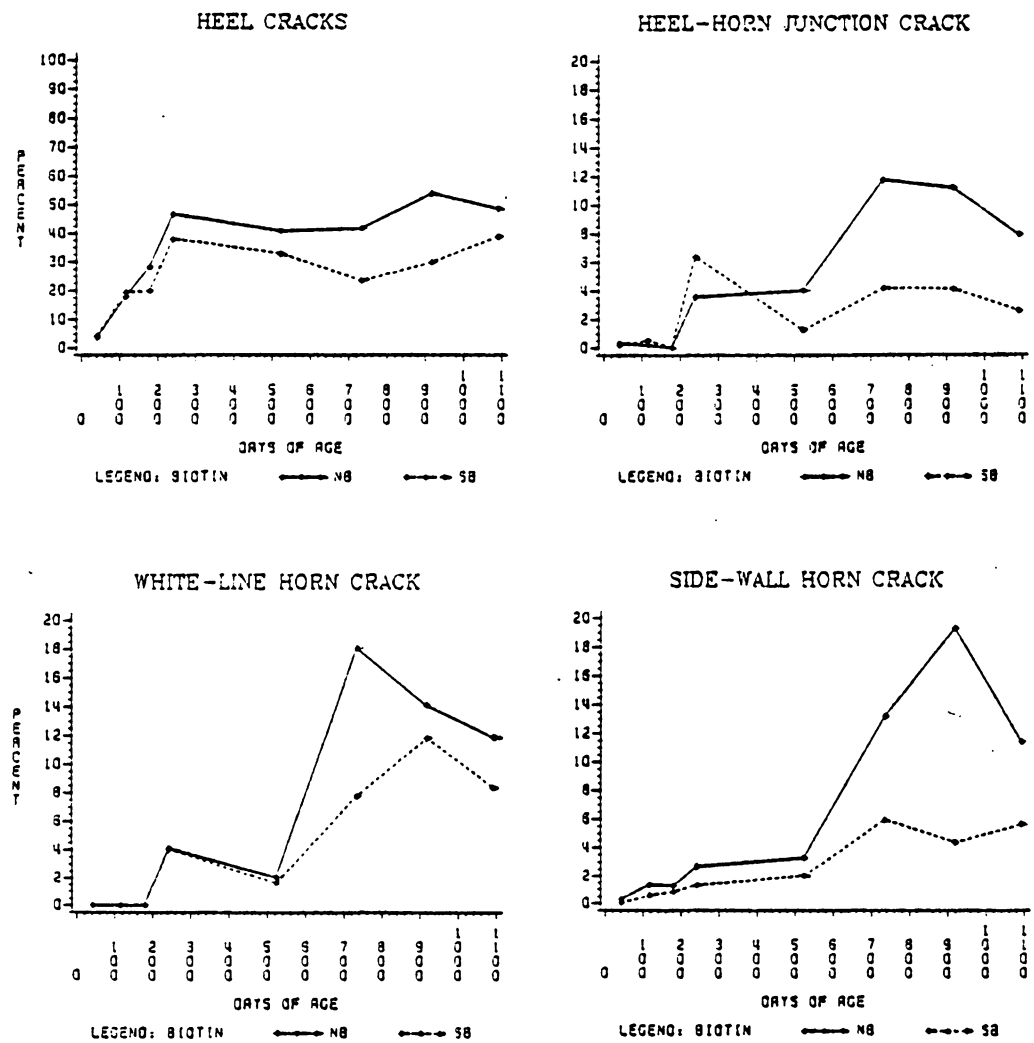


Figure 11. The effect of age on the frequency of toes with lesions in gilts and sows fed biotin supplemented (SB) or unsupplemented (NB) diets.

in toe lesions have recently been reported by Brooks and Simmins (1981) in females fed SB diets from 25 kg body weight through four parities. They also reported an increased biotin response as the animal increased in age. The reason for this age related biotin response is unclear. In chapter's 3 and 5 it was hypothesized that the biotin response on toe lesions increased as the prevalence of toe lesions increased. Animal ages of 300 to 400 d were required for the frequency of some types of lesions to increase appreciably in the study.

Hair Scores. There also was a biotin x age interaction ($P<.01$) for hair scores (figure 12). Biotin did not affect hair scores up to 240 d of age. However, from 240 d until the completion of the study hair scores were better for SB females compared to NB females. Halama (1979) reported, improvements in hair quality following biotin supplementation to a breeding herd with symptoms of hair loss. To the contrary, Penny et al., 1980 observed no difference in hair coats between NB and SB females.

The results of the present study indicate that toe size, toe location and supplemental biotin influence toe lesion development in swine. It is hypothesized that all three factors plus other environmental influences are working together to establish the level and distribution of foot lesions in swine herds.

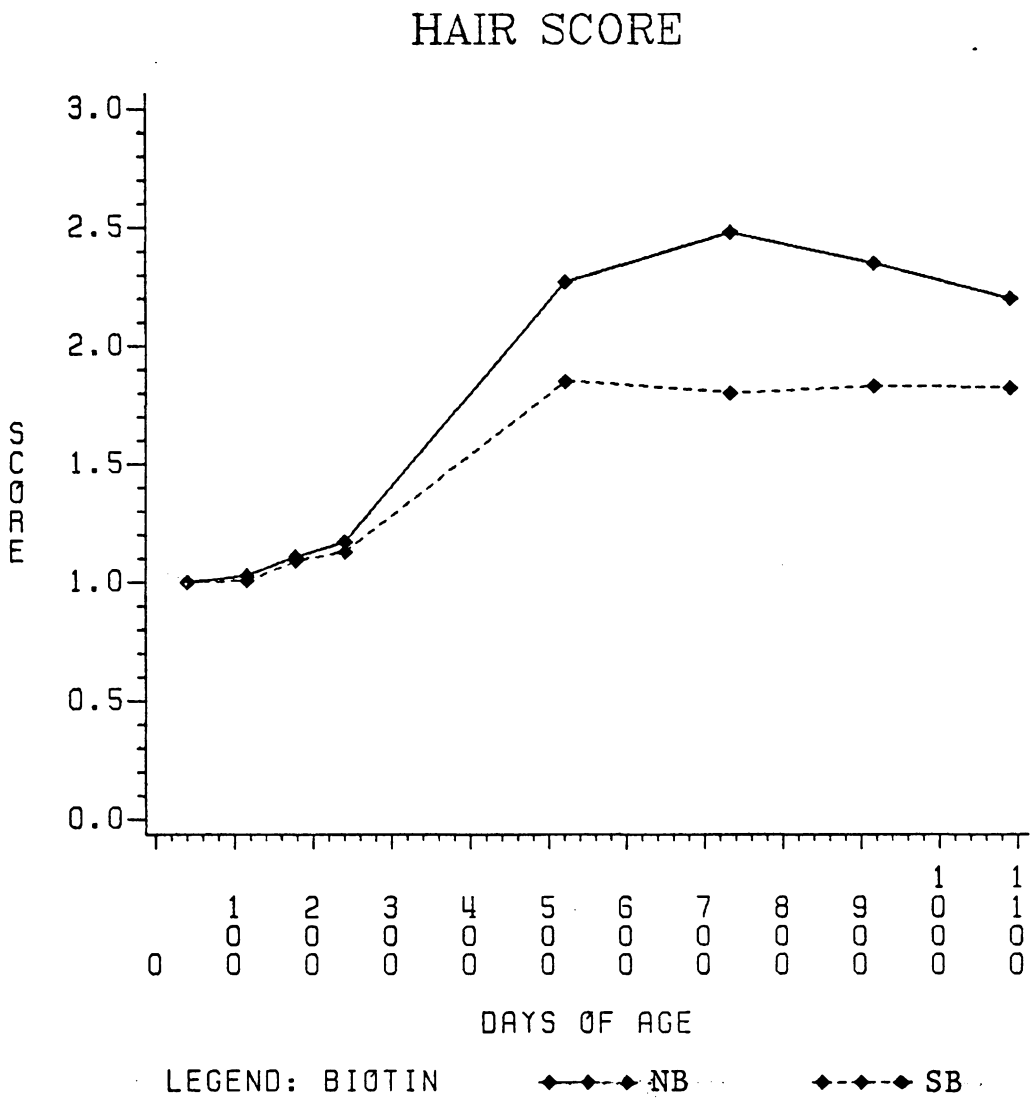


Figure 12. Effect of age on hair scores in gilts and sows fed two levels of biotin.

Summary

The influence of toe size, toe location and supplemental biotin on the development of toe lesions and hair scores in swine were evaluated from weaning through four parities. Females were fed corn-soybean meal diets containing 0 (NB) or 220 ug (SB) d-biotin/kg up to 100 kg bodyweight and corn- or wheat-based diets containing 0 (NB) or 440 (SB) ug d-biotin/kg diet until completion of the study. Toe measurements, frequency of toes with lesions and hair scores were obtained at a mean animal age of 40, 115, 178, 240, 521, 732, 916 and 1090 d of age. Front (F) toes were larger ($P<.001$) than rear (R) toes while outside (O) toes were larger ($P<.001$) than inside (I) toes. Significant FR x age and IO x age interactions ($P<.01$) were observed for toe length, horn height, toe width, toe base area and horn length ($P<.05$). All toe measurements increased linearly and quadratically ($P<.001$) over time. The FB x IO x age interaction ($P<.01$) indicated that all toes grew at a similar rate up to 200 to 250 d of age, after which, the growth rate of the inside toes (especially RI) were slower relative to the outside toes. The FR x IO interaction was significant for toe lesions ($P<.01$) with the frequency of toes with lesions highest for RO toes and lowest for the RI toes.

Correlation coefficients indicated that as the IO difference in toe base area increase the frequency of toes with lesions decreases for I toes and increases for O toes.

Biotin supplementation reduced ($P<.001$) the frequency of toes with heel cracks, white-line horn cracks, heel-horn junction cracks and side-wall horn cracks with the response primarily occurring between 500 and 1000 d of age. Hair scores were also improved with biotin supplementation.

These results indicate that toe size, toe location and supplemental biotin are related to the development and distribution of toe lesions in swine.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The results of the present study support the belief that swine diets composed of corn and soybean meal provide adequate levels of biotin to permit normal feedlot performance. However, the improvements in foot health are noteworthy. The reduction in total toe lesions and total toe lesion severity scores/gilt with the supplementation of 220 ug d-biotin/kg indicate that biotin levels higher than those present in corn-soybean meal diets may be necessary for maximum hoof integrity. Since side-wall cracks often lead to bush foot and lameness, the reduction in side-wall cracks with SB diets also appear significant. The biotin responses were mostly limited to trial 3 and make conclusions difficult in regard to the need to supplement growing-finishing swine diets with biotin. The variability in biotin reponsiveness among trials suggest there are other environmental factors which when present increase the pig's need for biotin.

The positive correlation between types of toe lesions in the growing-finishing study point out the need to prevent the development of toe lesions. The presence of heel-horn erosion and heel cracks (the most common toe lesion) often leads to the development of subsequent toe lesions.

The optimum plasma biotin level for swine is not known. However, our results indicate that levels above 40 ug/dl (suggested by Tagwerker, 1974 to be adequate) may not be sufficient to prevent development of toe lesions. More research is needed to evaluate the relationship between plasma biotin and toe lesion development.

The results of the sow study provide evidence that biotin may be more important in swine reproductive performance than previously thought. Although the improvements in reproductive performance with biotin supplementation in this study were not striking some interesting findings were observed.

Biotin supplementation resulted in improvements for conception rate and the weaning to estrus interval. The 9% improvement in conception rate would be of significant economic importance for the swine producer. Halama (1979) also reported an improvement in conception rate in a field trial, however, no control group was utilized for comparison. The reduction in the weaning to estrus interval in the present study supports the finding of Brook et al. (1977) and Halama (1979). However, they are contradictory to those reported by Grandhi and Strain (1980) and Penny et al. (1980). Additional research is needed in this area before conclusions can be reached.

Overall farrowing and lactation performance was not affected by supplemental biotin, however, there appeared to be a response in litter size with biotin supplementation in multiparous sows. Number of live pigs/litter were notably higher for SB sows during parities 2, 3, and 4. The mechanism for this parity x biotin interaction is unknown, but does indicate the need for long-term sow studies when evaluating biotin. The results of the present study clearly indicate that biotin body stores in the sow, fetus and piglet can be increased with biotin supplementation. However, there were no strong relationships between any biochemical criteria and reproductive performance.

The response to biotin supplementation was not affected by the type of grain fed. This finding does not support the hypothesis (at least for sows) that the decreased bioavailablility of biotin in wheat increases the need to supplement biotin in wheat-based diets for swine. Large variability of the biotin availability among variaties of grain have been reported (Frigg, 1978) and may be a contributing factor in the lack of response obtained in this study.

In general, the reproductive performance of females consuming wheat diets was poorer than that of corn fed females with the major reductions occurring during farrowing. Wheat diets were 2% higher in crude protein and

contained levels of ME similar to the corn diets. There were slight differences in the amino acid content of the two diets (table 38). The most notable difference in amino acid content was a 32% increase in the glutamic acid content of the wheat-based diets. These differences in amino acid content could be partially responsible for the differences in performance between females fed corn or wheat-based diets.

Toe lesions are a frequent occurrence in confinement housed swine as evidenced by the numerous types of lesions noted across dietary treatments. Major reductions in the development of toe lesions were obtained with biotin supplementation in the sow study and serve to support the early findings of Cunha et al., (1946) and Glattli et al., (1975) that biotin is required to maintain normal hoof integrity. It has been hypothesized (Brooks et al., 1977) that biotin supplementation hardens the hoof making it less susceptible to injury. Soft hoofs (although not closely evaluated) did not appear to be present in this study suggesting other factors are responsible for biotin's reduction in lesion development. The development of toe lesions were not correlated with soundness scores; however, the association between foot lesions and lameness is no longer speculative.

Hair scores and number of hairs/cm^2 were improved with biotin supplementation. Although these variables lack

economic importance, they serve to strengthen the role of biotin in maintaining normal skin and hoof tissue.

Our results support the early finding of Nordby (1939) that toe sizes vary according to location and that outside toes develop more lesions than inside toes (Penny et al., 1963). Correlations between toe size and toe lesions were noted in this study, however, the coefficients were low. The findings of this study lead to the conclusion that toe location and differences in toe size are a factor in toe lesion development, but other factors such as housing, flooring, patterns of animal movement and nutrition are also interrelated with the development of toe lesions in swine.

Although not conclusive, our results indicate that biotin supplementation to swine diets must be seriously considered. The development of foot lesion in swine, especially sows, can be reduced with biotin supplementation and improvements in reproductive performance may be attainable. Therefore, those producers with herds containing excessive toe lesions and apparent hair loss may be able to justify the additional expense of biotin supplementation.

Literature Cited

Achuta Murthy, P. N. and S. P. Mistry. 1972. Synthesis of biotin-dependent carboxylases from their apoproteins and biotin. Biochem. Rev. 43:1.

Achuta Murthy, P. N. and S. P. Mistry. 1977. Biotin. Prog. Fed. Nut. Sci. 2:405.

Adams, C.R., C. E. Richardson and T. J. Cunha. 1967. Supplemental biotin and vitamin B-6 for swine. J. Anim. Sci. 26:903(Abstr.).

Allman, D. W., D. D. Hubbard and D. M. Gibson. 1965. Fatty acid synthesis during fat-free refeeding of starved rats. J. Lipid Res. 6:63.

Anderson, J. O. and R. E. Warnick. 1970. Studies of the need for supplemental biotin in chick rations. Poul. Sci. 49:569.

Anderson, P. A., D. H. Baker and S. P. Mistry. 1978. Bioassay determination of the biotin content of corn, barley, sorghum and wheat. J. Anim. Sci. 47:654.

Anonymous. 1972. Biotin in animal nutrition. Roche(Basle) Bulletin No. 1267., p. 16.

Arinze, J. C. and S. P. Mistry. 1971. Activities of some biotin enzymes and certain aspects of gluconeogenesis during biotin deficiency. Comp. Biochem. Physiol. 38B:285.

Arthur, S. A., E. T. Kornegay, H. R. Thomas, H. P. Veit, D. R. Notter and R. A. Barczewski. 1982. Restricted energy intake and elevated calcium and phosphorus intake for gilts during growth. III. Characterization of feet and limbs and soundness scores of sows during three parities. J. Anim. Sci. (in press).

Association of Vitamin Chemist. 1966. Methods of Vitamin Assay. 3RD Edition. J. Wiley; New York. pp. 245-255.

Atkinson, R. L. 1972. Interactions between biotin and feed ingredients. Texas Turkey Ind. Day Rep. Temple, Texas. pp. 9-21.

Atwal, A. S., A. R. Robblee and L. P. Milligan. 1971. Effects of dietary biotin on liver pyruvate carboxylase and 32P incorporation into nucleic acids in livers of chicks. J. Nutr. 101:1555.

Baker, D. H. 1978. Nutrient bioavailability in feedstuffs: Methodology for determining amino acid and B-vitamin availability in cereal grains and soybean meal. Ga. Nutr. Conf. pp. 1-12.

Ballard, F. J. and R. W. Hanson. 1967. The citrate cleavage pathway and lipogenesis in rat adipose tissue: replacement of oxaloacetate. J. Lipid Res. 8:73.

Ballard, F. J., R. W. Hanson and L. Reshef. 1970. Immunochemical studies with soluble and mitochondrial pyruvate carboxylase activities from rat tissues. Biochem. J. 119:735.

Balnave, D. 1966. Effect of gonadal hormones and biotin deficiency on growth and lipid composition of liver and tissue in the immature female chick. Proc. XIII World Cong., Kiev. p. 213.

Balnave, D. 1977. Clinical symptoms of biotin deficiency in animals. Amer. J. Clin. Nutr. 30:1408.

Bane, D. P., H. D. Hilley and R. J. Meade. 1980. Influence of d-biotin and housing on hoof lesion in swine. J. Anim. Sci. 50(Suppl. 1):138.

Bannister, D. W. and C. C. Whitehead. 1976. Presence of pyruvate carboxylase in the blood of the domestic fowl (Gallus domesticus) and an assay procedure. Int. J. Biochem. 7:619.

Barr, A. J., J. H. Goodnight, J. P. Sall, W. H. Blair and D. M. Chilko. 1979. SAS User's Guide. SAS Institute, Inc., Cary, NC.

Barrett, M. and G. Everson. 1951. Deposition of B vitamins in normally developing fetuses as evidence for increased vitamin needs of the rat for reproduction. I. Thiamine and riboflavin. J. Nutr. 45:493.

Bauernfeind, J. C. 1969. Biotin - A ubiquitous and versatile vitamin. Feedstuffs. 41(34):32.

Baugh, C. M., J. H. Malone and C. E. Butterworth, Jr. 1968. Human biotin deficiency: A case history of biotin deficiency induced by raw egg consumption in a cirrhotic patient. Amer. J. Clin. Nutr. 21:173.

Baxter, R. M. and J.H. Quastel. 1953. The enzymatic breakdown of d-biotin in vitro. J. Biol. Chem. 201:751.

Bereskin, B. 1979. Genetic aspects of feet and leg soundness in swine. J. Anim. Sci. 48:1322.

Berger, H. 1950. Urinary excretion of biotin in children without pathological skin conditions with special reference to the relations of seborrhoeic dermatitis and eczema. Int. Z. Vitamforsch. 22:190.

Blair, R. 1977. Biotin-considerations for the Canadian feed manufacturer. Proc. Univ. of Guelph Nutr. Conf. for Feed Manufacturers, May, 1977, pp. 70-74.

Bliss, C. I. and P. Gyorgy. 1967. Animal assays for vitamins. In: P. Gyorgy and W. N. Pearson (Ed.) The Vitimans Vol III pp. 1-20. Academic Press, New York.

Boas, M. A. 1927. The effect of desiccation upon the nutritive properties of egg-white. Biochem. J. 21:712.

Bonjour, J. P. 1977. Biotin in man's nutrition and therapy - a review. Int. J. Vit. Nutr. Res. 47:107.

Bradley, J. W., R. L. Atkinson and W. F. Krueger. 1976. Relationship of biotin to reproductive performance of layer-type hens. Poul. Sci. 55:2490.

Brady, R. N., L. F. Li, D. B. McCormick and L. D. Wright. 1965. Microbial and enzymatic degradation of biotin. Biochem. Biophys. Res. Comm. 19:777.

Brewer, L. E. and H. M. Edwards, Jr. 1972. Studies on the biotin requirement of broiler breeders. Poul. Sci. 51:619.

Brooks, P H. 1982. Biotin in pig nutrition. News and Information. 3:29.

Brooks, P. H. and P. H. Simmins. 1981. The effect of supplementing breeding pig diets with biotin on the maintenance of hoof integrity. In: Gieseche. D., G. Dirksen and M. Stangassinger (Ed). Proc. of the 4th Int. Conf. on Production Diseases in Farm Animals. Munich, W. Germany. pp. 1-4.

Brooks, P.H., D. A. Smith and V.C.R. Irwin. 1977. Biotin-supplementation of diets; the incidence of foot lesions and the reproductive performance of sows. Vet. Rec. 101:46.

Bryant, K. L., E. T. Kornegay, J. W. Knight, D. R. Notter and H. S. Bartlett. 1982. Influence of biotin injection on plasma biotin concentrations in swine. VPI&SU Livestock Res. Rep. No. 2. pp. 53-58.

Buhlmann, R. 1973. Changes in fat metabolism with biotin deficiency in swine. Ph. D. Disertation. Basle.

Butler, E. J. 1976. Fatty liver diseases in domestic fowl - A review. Avian Pathol. 5:1.

Calabotta, D. F., E. T. Kornegay, H. R. Thomas, J. W. Knight, D. R. Notter and H. P. Veit. 1982. Restricted energy intake and elevated calcium and phosphorus intake for gilts during growth. I. Feedlot performance and foot and leg measurements and scores during growth. J. Anim. Sci. 54:565.

Caskey, C. D., L. C. Norris and G. F. Heuser. 1944. A chronic congenital ataxia in chicks due to manganese deficiency in the maternal diet. Poul. Sci. 23:516.

Chaiet, L. and F. J. Wolf. 1964. The properties of streptavidin, a biotin binding protein produced by streptomycetes. Arch. Biochem. Biophys. 106:1.

Chiang, G. S. and S. P. Mistry. 1974. Activities of pyruvate carboxylase and propionyl CoA carboxylase in rat tissues during biotin deficiency and restoration of the activities after biotin administration. Proc. Soc. Exp. Biol. Med. 146:21.

Christenson, R. K. and J. J. Ford. 1979. Puberty and estrus in confinement -reared gilts. J. Anim. Sci. 49:743.

Comben, N. 1978. Biotin for breeding sows. Field experiences 1976-1978. In: Proceedings, Roche Symposium; London, October 1978. pp. 1-22.

Couch, J. R., W. W. Cravens, C. A. Elvehjem and J. G. Halpin. 1948a. Relation of biotin to congenital deformities in the chick. Anat Rec. 100:29.

Couch, J. R., W. W. Cravens, C. A. Elvehjem and J. G. Halpin. 1948b. Daily uptake of biotin by the hen's egg. Poul. Sci. 27:823.

Couch, J. R., W. W. Cravens, C. A. Elvehjem and J. G. Halpin. 1949. Studies on the function of biotin in the domestic fowl. Arch. Biochem. 21:77.

Crabo, B. G. 1982. Post-weaning anestrus in swine: Endocrine factors and prevention. National Pork Producers 1982 Research Investment Report, p. 23.

Cravens, W. W., W. H. McGibbon and E. E. Sebesta. 1944. Effect of biotin deficiency on embryonic development in the domestic fowl. Anat. Rec. 90:55.

Cravens, W. W., E. E. Sebesta, J. G. Halpin and E. B. Hart. 1942. Effect of biotin on reproduction in the domestic fowl. Proc. Soc. Exp. Biol. Med. 50:101.

Cunha, T. J. 1971. Present status on vitamin K and biotin for the pig. Feedstuffs 43:20.

Cunha, T. J., C. R. Adams and C. E. Richardson. 1968. Observations on the biotin needs of the pig. Feedstuffs. 40:22.

Cunha, T. J., D. C. Lindley and M. E. Ensminger. 1946. Biotin deficiency syndrome in pigs fed desicated egg white. J. Anim. Sci. 5:219.

Dakshinamurti, K. and P. R. Desjardins. 1968. Lipogenesis in biotin deficiency. Can. J. Biochem. 46:1261.

Dakshinamurti, K., A. D. Landman, L. Ramamurti and R. J. Constable. 1974. Isotope dilution assay for biotin. Anal. Biochem. 61:225.

Dakshinamurti, K. and S. Litvak. 1970. Biotin and protein synthesis in rat liver. J. Biol. Chem. 245:5600.

Dakshinamurti, K. and S. P. Mistry. 1962. Ascorbic acid synthesis and biotin deficiency. Arch. Biochem. Biophys. 99:254.

Deodhar, A. D. and S. P. Mistry. 1969. Gluconeogenesis in biotin deficiency: In vivo synthesis of pyruvate holocarboxylase in biotin deficient rat liver. Biochem. Biophys. Res Comm. 34:755.

Desjardins, P. R. and K. Dakshinamurti. 1971. Acetyl-CoA holocarboxylase synthesis in biotin-deficient rat adipose tissue. Arch. Biochem. Biophys. 142:292.

Dobson, D. C. 1970. Biotin requirement of turkey poults. Poul. Sci. 49:546.

Du Vigneaud, V., K. Hofmann, D. B. Melville and P. Gyorgy. 1941. The preparation of free crystalline biotin. J. Biol. Chem. 140:763.

Du Vigneaud, V., D. B. Melville, K. Folkers, D. E. Wolf and S. A. Harris. 1942. The structure of biotin: a study of desthiobiotin. J. Biol. Chem. 146:475.

Du Vigneaud, V., D. B. Melville, P. Gyorgy and C. S. Rose. 1940. Special article on the identity of Vitamin H with biotin. Science 92:62.

Eakin, R. E., E. E. Snell and R. J. Williams. 1940. A constitutent of raw egg white capable of inactivating biotin in vitro. J. Biol. Chem. 136:801.

Easter, R. A., J. R. Corley, N. W. Esch and D. L. Wende. 1979. Vitamin B-6, biotin, folacin and thiamin supplementation of corn-soybean meal diets. J. Anim. Sci. 49(Suppl. 1):239.

Easter, R. A., E. J. Michel, P. A. Anderson and J. R. Corley. 1978. Effect of vitamin B-6 and biotin additions to corn-soybean meal diets for growing -finishing swine. J. Anim. Sci. 47(Suppl. 1):300.

Edwards, H. M. 1974. Diagnosing B-vitamin deficiencies with biochemical methods. Proc. Ga. Nutr. Conf. pp. 3-13.

Eisenberg, M. A. 1973. Biotin: Biogenesis, Transport and Their Regulation. In: A. Meister (Ed.). Advances in Enzymology. Vol. 38. pp. 317-372. Interscience, New York.

Ferguson, T. M., C. H. Whiteside, C. R. Creger, M. L. Jones, R. L. Atkinson and J. R. Couch. 1961. B-vitamin deficiency in the mature turkey hen. Poul. Sci. 40:1151.

Flavin, M., H. Castro-Murdosa and S. Ochoa. 1957. Metabolism of propionic acid in animal tissues. J. Biol.Chem. 229:981.

Forbes, P. D. and R. E. Davis. 1974. Cutaneous changes in the feet of biotin-deficient poults and chicks. Fed. Amer. Soc. Exp. Biol. 33:666.

Fraenkel-Conrat, J. and H. Fraenkel-Conrat. 1952. Metabolic fate of biotin and of avidin-biotin complex upon parenteral administration. Biochem. Biophys. Acta. 8:66.

Fraenkel-Conrat, H., N. S. Snell and E. D. Ducay, 1952. Avidin. II Composition and mode of action of avidin. Arch. Biochem. Biophys. 39:97.

Frank, O., G. Walbroehl, A. Thomson, H. Kaminetzky, Z. Kubes and H. Baker. 1970. Placental transfer: Fetal retention of some vitamins. Am. J. Clin. Nutr. 23:662 (Abst.).

Frape, D. L., J. Wilkinson and L. G. Chubb. 1969. A note on the digestibility of hard and soft wheat offals in growing pigs. Anim. Prod. 11:429.

Frigg, M. 1976. Bio-availability of biotin in cereals. Poul. Sci. 55:2310.

Frigg, M. and G. Brubacker. 1976. Biotin deficiency in chicks fed a wheat-based diet. Int. J. Vit. Nutr. Res. 46:314.

Frigg, M. and J. Torhorst. 1980. Histological and cytological alterations in the skin of biotin-deficient chicks. Res. Vet. Sci. 28:17.

Frigg, M., H. Weiser and A. Bollinger. 1973. Biotin deficiency in chicks. Clinical and chemical alterations. Proc. 5th Int. Congr. World Vetr. Poult. Assoc., Munich. 2: 1286.

Fritschen, R. D. 1976. Effect of slat width and type on claw lesions. Nebraska Swine Rep. (E. C.), 79:219.

Fritschen, R. D. 1979. Housing and its effect on feet and leg problems. Proc. the Pig Vet. Soc. 5:95.

Gardner, J., H. T. Parsons, W. H. Peterson. 1945. Human biotin metabolism on various levels of biotin intake. J. Amer. Med. Assoc. 211:198.

Glattli, H. R., J. Pohlenz, K. Streiff and F. Ehrensperger. 1975. Clinical and morphological findings in experimental biotin deficiency in pigs. Zbl. Vet. Med. A. 22:102.

Glatzle, D. and M. Frigg. 1975. Acetyl-coenzyme A carboxylase activity in whole blood and its dependency on the biotin status of chickens. Biochem. Biophys. Res Comm. 66:368.

Glatzle, D., M. Frigg and F. Weber. 1979a. Acetyl-CoA carboxylase activities in blood and liver of chicks and their dependency on biotin status. Acta. Vitam. Enzym. 1-6:11.

Glatzle, D., M. Frigg and F. Weber. 1979b. Pyruvate carboxylase activities in red blood cells and liver of chicks and their dependency on biotin status. First results with activation assays. Acta. Vitam. Enzym. 1-6:19.

Grandhi, R. R. and J. H. Strain. 1980. Effect of biotin supplementation on reproductive performance and foot lesions in swine. Can. J. Anim. Sci. 60:961.

Green, N. M. 1962. Spectroscopic evidence for the participation of tryptophan residues in the binding of biotin by avidin. Biochim. Biophys. Acta. 59:244.

Green, N. M. 1963. Avidin 3. The nature of the biotin-binding site. Biochem. J. 89:599.

Green, N. M. 1964. The molecular weight of avidin. Biochem. J. 92:16c.

Green, N. M. 1970. Spectrophotometric determination of avidin and biotin. In: McCormick, D. B. and L. D. Wright (Ed.). Vol. XVIII. pp. 418-424. Academic Press, New York.

Gregolin, C., E. Ryder, A. K. Kleinschmidt, R. C. Warner and M. D. Lane. 1966. Molecular characteristics of liver acetyl CoA carboxylase. Proc. Natl. Acad. Sci., U.S. 56-148.

Gyorgy, P. 1931. Studies on the protective factor present in liver. Z. Arztl. Fortbild. 28:317.

Gyorgy, P. 1954. Biotin. In: Sebrell, W. H., Jr. and R. S. Harris. (Ed.). The Vitamins. Vol. I. pp. 560-599. Academic Press, New York.

Gyorgy, P. 1967. Biotin. In: P. Gyorgy and W. N. Pearson (Ed.). The Vitamins. Vol VIII. pp. 303-312. Academic Press, New York.

Gyorgy, P. and B. W. Lander, Jr. 1968. Biotin. In: W. H. Sebrell and R. S. Harris (Ed.). The Vitamins. Vol. II. pp. 261-359. Academic Press, New York.

Gyorgy, P., D. B. Melville, D. Burk and V. du Vigneaud. 1940. The possible identity of Vitamin H with biotin and coenzyme R. Science 91:243.

Gyorgy, P. and C. E. Poling. 1940a. Further experiments on nutritional achromotrichia in rats and mice. Proc. Soc. Exp. Biol. Med. 45:773.

Gyorgy, P. and C. E. Poling. 1940b. Further experiments on nutritional achromotrichia in rats and mice. Proc. Soc. Exp. Biol. Med. 45:773.

Halama, A. K. 1979. Biotin deficiency in breeding pigs. Wiener Tierarztliche Monatsschrift. 66:(12):370.

Hamilton, C. R., T. L. Veum and L. Vanskike. 1982. Effects of added biotin on sow and litter performance in confinement. J. Anim. Sci. 5(Suppl 1): .

Hanka, L. J., M. E. Bergy and R. B. Kelly. 1966. Naturally occurring antimetabolite antibiotic related to biotin. Science. 154(12):1667.

Hanke, H. E. and R. J. Meade. 1971. Biotin and pyridoxine additions to diets for pigs weaned at an early age. Swine Res. Rep., Univ. of Minnesota. pp. 19-20.

Hanson, R.W., M. S. Patel, L. Reshef and F. J. ballard. 1971. In: Regulation of gluconeogenesis. H. Solig and B. Willms (eds.) Verlag, Stuttgart. pp. 255-276.

Hardinge, M. G. and H. Crooks. 1961. Lesser known vitamins in foods. J. Amer. Dietet. Ass. 38:240.

Harms, R. H., B. L. Damron and C. F. Simpson. 1977. Effect of wet litter and supplemental biotin and/or whey on the production of foot pad dermatitis in broilers. Poul Sci. 56:291.

Harris, S. A., D. E. Wolf, R. Mozingo and K. Folkers. 1943. Synthetic biotin. Science. 97:447.

Harvey, W. R. 1977. Mixed Model Least-squares andMaximum Likelihood Computer Program. User's Guide for LSML76 (mimeo.).

Hegsted, D. M., R. C. Mills, G. M. Briggs, Jr., C. A. Elvehjem and E. B. Hart. 1942. Biotin in chick nutrition. J. Nutr. 23:175.

Hemsley, L. A. 1965. The fatty liver and kidney syndrome of young chickens. Vet. Rec. 77:124.

Hofmann, K., D. B. Melville and V. du Vigneaud. 1941. The effects of certain reagents on the activity of biotin. J. Biol.Chem. 141:85.

Hood, R. L. 1975. A radiochemical assay for biotin in biological materials. J. Sci. Fd. Agr. 26:1847.

Hood, R. L. 1980. The fatty liver and kidney syndrome in broiler chickens: An Austrailian experience. Feedstuffs. 52(53):13.

Hood, R. L., A. R. Johnson, A. C. Fogerty and J. A. Pearson. 1976. Fatty liver and kidney syndrome in chicks. II. Biochemical role of biotin. Austrailian J. Biol. Sci. 29:429.

Hulan, H. W., F. G. Proudfoot and K. B. McRae. 1980. Effect of vitamins on the incidence of mortality and acute death syndrome in broiler chickens. Poul. Sci. 59:927.

Ivan, M. and D. J. Farrell. 1976. Nutritional evaluation of wheat. 5. Disappearance of components in digesta of pigs prepared with two re-entrant cannulae. Anim. Prod. 23:111.

Jacobs, R., E. Kilburn and P. W. Majerus. 1970. Acetyl coenzyme A carboxylase--The effects of biotin deficiency on enzyme in rat liver and adipose tissue. J. Biol. Chem. 245:6462.

Jensen, A. H. 1979. The effects of enviornmental factors, floor design and materials on performance and on foot and limb disorders in growing and adult pigs. Proc. the Pig. Vet. Soc. 5:85.

Jensen, L. S. and R. Martinson. 1969. Requirement of turkey poults for biotin and effect of deficiency on incidence of leg weakness in developing turkeys. Poul. Sci. 48:222.

Johnson, C. W. 1967. Field evaluation of d-biotin supplementation for biotin deficient turkey poults and older turkeys. Poul. Sci. 46:1276.

Jukes, T. N. and F. H. Bird. 1942. Prevention of perosis by biotin. Proc. Soc. Exp. Biol. Med. 49:231.

Kaziro, Y. and S. Ochoa. 1961. Mechanism of the propionyl carboxylase reaction. I. Carboxylation and decarboxylation of the enzyme. J. Biol. Chem. 236:3131.

Kaziro, Y. and S. Ochoa. 1964. The metabolism of propionic acid. In: F. F. Nord. (Ed.). Advances in Enzymology. Vol. 26. pp. 283-378. Interscience, New York.

Kaminetzky, H. A., H. Baker, O. Frank and A. Langer. 1974. The effects of intravenously administered water-soluble vitamins during labor on maternal and neonatal blood vitamin levels at delivery. Am. J. Obstet. Gynecol. 120:697.

Kennedy, C. and L. S. Palmer. 1946. Biotin deficiency in relation to reproduction and lactation. Arch. Biochem. 7:9.

Knappe, J. 1970. Mechanism of biotin action. Ann. Rev. Biochem. 39:757.

Knappe, J., E. Ringelmann and F. Lynen. 1961. The role of ATP and bicarbonate in carboxylase reactions. Biochem. Z. 335:101.

Kogl, F. 1935. Isolation of two liver fractions. Chem. Ber. 68:16.

Kornegay, E. T., H. R. Thomas and J. L. Baker. 1981. Phosphorus in swine. IV. Influence of dietary calcium and phosphorus and protein levels on feedlot performance, serum minerals, bone development and soundness scores in boars. J. Anim. Sci. 52:1070.

Kornegay, E. T., H. P. Veit, J. W. Knight, D. R. Notter, H. S. Bartlett and D. F. Calabotta. 1982. Restricted energy intake and elevated calcium and phosphorus intake in boars during growth. II. Foot and leg measurements and toe and soundness scores. J. Anim. Sci. (in review).

Kosow, D. P. and M. D. Lane. 1961. Restoration of biotin-deficiencyinduced depression of propionyl carboxylase activity in vivo and in vitro. Biochem. Biophys. Res Comm. 4:92.

Kovacs, A. B. and G. Y. Beer. 1979. The mechanical properties and qualities of floors for pigs in relation to limb disorders. Proc. the Pig Vet. Soc. 5:99.

Lampen, J. O., G. P. Bahler and W. H. Peterson. 1942. The occurence of free and bound biotin. J. Nutr. 23:11.

Lane, M. D., J. Moss and S. E. Polakis. 1974. Acety coenzyme A carboxylase. Curr. Top. Cell Regal. 8:139.

Lash Miller, W. 1924. Wildier's bios. Science. 59:197.

Lease, J. J. and H. T. Parsons. 1934. Effects of dietary egg white and of a specific factor given orraly or potentally. Biochem. J. 28:2109.

Lehninger, A. L. 1976. Biochemistry. pp. 363-587. Worth Publishers, Inc. New York.

Lehrer, W. P., Jr., A. C. Wiese and P. R. Moore. 1952. Biotin deficiency in suckling pigs. J. Nutr. 47:203.

Lepine, A. J. 1982. Effect of restricted growth rate and elevated levels of minerals and vitamins on feet and leg characteristics, soundness scores and metacarpal and metatarsal characteristics of growing boars. M. S. Thesis. Virginia Polytechnic Institute and State Univ., Blacksburg.

Lewis, H. and G. Everson. 1952. Deposition of B vitamins in normally developing fetuses as evidence for increased vitamin needs of the rat for reproduction. II. Pantothenic acid and biotin. J. Nutr. 46:27.

Lichstein, H. C. and J. Birnbaum. 1965. Combinability of avidin and streptavidin with analogs of biotin. Biochem. Biophys. Res. Comm. 20:41.

Lindley, D. C. and T. J. Cunha. 1946. Nutritional significance of inositol and biotin for the pig. J. Anim. Sci. 5:47.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193:265.

Lucas, G. H. W. 1924. The fractionation of bios and comparison of bios with vitamins B and C. J. Phys. Chem. 28:1180.

Lynen, F. 1967. The role of biotin-dependent carboxylations in biosynthetic reactions. Biochem. J. 102:381.

Lynen, F., J. Knappe, E. Lorch, G. Jutling and E. Ringlemann. 1959. The role of biotin in carboxylation reactions. Angew. Chem. 71:481.

Mavrogenis, A. P. and O. W. Robinson. 1976. Factors affecting puberty in swine. J. Anim. Sci. 42:1251.

McCormick, D.B. and L.D. Wright. 1971. Biotin transport and metabolism. In: Comprehensive Biochemistry. Vol 21. pp. 81-110. Elsevier, Amsterdam.

McElroy, L. W. and T. N. Jukes. 1940. Formation of the anti-egg-white injury factor (biotin) in the rumen of the cow. Proc. Soc. Exp. Biol. Med. 45:296.

McGregor, M. A., H. T. Parsons and W. H. Peterson. 1947. Biotin balance in the albino rat. J. Nutr. 33:517.

Meade, R. J. 1971. Biotin and pyridoxine supplementation of diets for growing pigs. Swine Res. Rep., Univ. of Minnesota. pp. 45-46.

Melville, D. B., K. Hofmann and V. du Vigneaud. 1942. The structure of biotin: The formation of thiophenevaleric acid from biotin. J. Biol. Chem. 146:487.

Michel, E. and I. Mastchi. 1981. Biotin in the nutrition of pregnant sows. Sintesis Porcina 12(1):3.

Michelsen, P. 1956. Intestinal synthesis of vitamins in the nonruminant. In: Harris, R. S., G. F. Marrian and K. V. Thimann (Ed.). Vitamins and Hormones. Vol. 15. pp. 1-56. Academic Press, New York.

Miller, 1933. Cited by H. R. Rosenburg. 1944. In: Chemistry and Physiology of the Vitamins. p 470. Interscience, New York.

Mistry, S. P. and K. Dakshinamurti. 1964. Biochemistry of biotin. In: R. S. Harris, I. G. Wool and J. A. Loraine (Ed.). Vol. 22. pp. 1-56. Academic Press, New York.

Mistry, S. P., K. Dakshinamurti and V. V. Modi. 1962. Impairment of glucose utilization in biotin deficiency. Arch. Biochem. Biophys. 96:674.

Mocsy, V. 1940. Cited by Penny, R. H. C. 1979. Genetical, physiological and anatomical factors contributing to foot and limb disorders in growing and adult pigs. Proc. The Pig Vet. Soc. 4:85.

Money, D. F. L. and G. L. Laughton. 1981. Biotin responsive lameness in New Zealand pigs. New Zealand Vet. J. 28:33.

Moss, J. and M. D. Lane. 1971. The biotin-dependent enzymes. In: A. Meister. (Ed.). Advances in Enzymology. Vol. 35. pp. 321-442. Interscience, New York.

National Research Council. 1977. Nutrient Requirement of Poultry. 7th Revised Ed. National Academy of Sciences, Washington, D. C.

National Research Council. 1979. Nutrient requirements of domestic animals. II. Nutrient requirements of swine. National Academy of Science. National Research Council, Washington, D. C.

Nelson, T. S. and L. K. Kirby. 1982. Amino acid content and digestibility in feedstuffs for chickens. Feedstuffs 54(12):14.

Newman, C. W. and D. O. Elliott. 1980. Supplemental pyridoxine, biotin and choline in gestation diets of first parity sows. J. Anim. Sci. 50 (Suppl. 1):215.

Newton, G. L., C. V. Booram, O. M. Hale and B. G. Mullinix, Jr. 1980. Effect of four types of floor slats on certain feet characteristics and performance of swine. J. Anim. Sci. 50:7.

Nielsen, E., E. M. Shull and W. H. Peterson. 1942. Response of bacteria, yeast and rats to peroxide-treated biotin intestinal synthesis of biotin in the rat. J. Nutr. 24:523.

Nordby, J. E. 1939. Inequalities in the digits in swine. J. Heredity. 30:307.

Osborne, H. G. 1950. Footrot in pigs. Australian Vet. J. 26:316.

Osborne, H. G. and Ensor. 1955. Some aspects of the pathology, aetiology and therapeutics of foot-rot in pigs. New Zealand Vet. J. 3:91.

Patrick, H., R. V. Boucher, R. S. Dutcher and H. C. Knandel. 1942. The nutritional significance of biotin in chick and poult nutrition. Poul. Sci. 21:476.

Payne, C. G., P. Gilchrist, J. A. Pearson and L. A. Hemsley. 1974. Involvement of biotin in the fatty liver and kidney syndrome of broilers. Brit. Poul. Sci. 15:489.

Pearson, J. A., A. R. Johnson, R. L. Hood and A. C. Fogerty. 1976. Fatty liver and kidney syndrome in chicks. I. Effect of biotin in diet. Australian J. Biol. Sci. 29:419.

Penny, R. H. C., A. D. Osborne, A. I. Wright and T. K. Stephens. 1965. Foot-rot in pigs: Observations on the clinical disease. Vet. Rec. 77:1101.

Penny, R. H. C., R. D. A. Cameron, S. Johnson, P. J. Kenyon, H. A. Smith, A. W. P. Bell, J. P. L. Cole and J. Taylor. 1980. Foot rot of pigs: The influence of biotin supplementation on foot lesions in sows. Vet. Rec. 107:350.

Penny, R. H. C., R. D. A. Cameron, S. Johnson, P. J. Kenyon, H. A. Smith, A. W. P. Bell, J. P. L. Cole and J. Taylor. 1981. Influence of biotin supplementation on sow reproductive efficiency. Vet. Rec. 109:80.

Penny, R. H. C., A. D. Osborne and A. I. Wright. 1963. The causes and incidence of lameness in store and adult pigs. Vet. Rec. 75:1225.

Peo, E. R., Jr., G. F. Wehrbein, B. Moser, P. J. Cunningham and P. E. Vipperman, Jr. 1970. Biotin supplementation of baby pig diets. J. Anim. Sci. 31:209(Abstr.).

Plinton, C., F. P. Mahn, M. Hawrylyshyn, V. S. Venturella and B. Z. Senkowski. 1969. Colorimetric determination of biotin. J. Pharm. Sci. 58:875.

Pohlenz, F. 1974. Clinical and morthological results with experimental biotin deficiency in swine. F. Veterinarmed. 20:249.

Radnai, I. and I. Radnai. 1976. Cited by R. H. C. Penny. 1979. Genetical, physiological and anatomical factors contributing to foot and limb disorders in growing and adult pigs. Proc. Pig Vet. Soc. 4:85.

Rampacek, G. B., R. B. Kraeling and T. E. Kiser. 1981. Delayed puberty in gilts in total confinement. Theriogenology. 15:491.

Rauch, H. 1952. The effects of biotin deficiency on hair development and pigmentation. Physio. Zool. 25:145.

Reid, M. E. 1954. Nutritional studies with the guinea pig: B-vitamins other than panothenic acid. Proc. Soc. Exp. Biol. Med. 85:547.

Richardson, C. E. and H. S. Wilgus. 1967. Biotin--A limiting factor in turkey rations? Feedstuffs, 39(32):52.

Robblee, A. R. and R. Clandinin. 1970. The role of biotin in the nutrition of turkey poults. Poul. Sci. 49:976.

Rosenberg, H. R. 1945. Biotin. In: Chemistry and physiology of the vitamins. pp 475-476. Interscience Publishers, Inc., New York.

Scheiner, J. and E. DeRitter. 1975. Biotin content of feedstuffs. J. Agr. Food Chem. 23:1157.

Scott, M. L. 1968. Rediscovery of biotin as a factor for prevention of leg weakness in turkeys. Feedstuffs. 40(11):24.

Scott, M. L. 1981. Importance of biotin for chickens and turkeys. Feedstuffs. 53(8):59.

Scrutton, M. C. and M. F. Utter. 1965. Pyruvate carboxylase. III. Some physical and chemical properties of the highly purified enzyme. J. Biol. Chem. 240:1.

Shull, G. M., D. R. Miller and W. H. Peterson, 1942. A microbiological assay for biotin. J. Biol. Chem. 142:913.

Simmins, P. H. and P. H. Brooks. 1980. The effect of dietary biotin level on the physical characteristics of pig hoof tissue. Anim. Prod. 30:469.

Smith, W. J. and A. M. Robertson. 1971. Observations on injuries to sows confined in part slatted stalls. Vet. Rec. 89:531.

Sorrell, M. F., O. Frank., A. D. Thomson, H. Aquino and H. Baker. 1971. Absorption of vitamins from the large intestine in vivo. Nutr. Rep. Int. 3:143.

Spencer, R. P. and K. R. Brody. 1964. Biotin transport by small intestine of rat, hamster and other species. Amer. J. Phys. 206:653.

Traub, W. 1956. Crystal structure of biotin. Nature. 178:649.

Tagwerker, F. 1974. Recent research on biotin in the nutrition of pigs and chickens. Roche Information Service Bull. No. 1494.

Utter, M. F. and D. B. Keech. 1960. Formation of oxaloacetate from pyruvate and carbon dioxide. J. Biol. Chem. 235:PC17.

Utter, M. F. and M. C. Scrutton. B. L. Horecker and E. R. Stadtman (Ed.). 1969. In: Current Topics in Cellular Regulation. Academic Press, New York, Vol. I. pp. 253-296.

Viswanathan, V., F. P. Mahan, V. S. Venturella and B. Z. Senkowski. 1970. Gas-liquid chromatography of d-biotin. J. Pharm. Sci. 59:400.

Wagstaff, R. K., D. C. Dobson and J. O. Anderson. 1961. Available biotin content of barley. Poul Sci. 40:503.

Wakil, S. J. 1958. A malonic acid derivative as an intermediate in fatty acid synthesis. J. Amer. Chem. Soc. 80:6465.

Washam, R. D., J. E. Sowers and L. W. DeGoey. 1975. Effect of zinc proteinate or biotin in swine starter rations. J. Amer. Sci. 40:179(Abstr.).

Whitehead, C. C. 1977. The use of biotin in poultry nutrition. World's Poul. Sci. J., August 1977, pp. 140-154.

Whitehead, C. C. 1981. The assessment of biotin status in man and animals. Proc. Nutr. Soc. 40:165.

Whitehead, C. C. and D. W. Bannister. 1978. Blood pyruvate carboxylase (ec. 6.4.1.1) activity as a criterion of biotin status in chickens and turkeys. Brit. J. Nutr. 39:547.

Whitehead, C. C., D. W. Bannister and M. E. Clelland. 1978. Metabolic changes associated with the occurrence of fatty liver and kidney syndrome in chicks. Brit. J. Nutr. 40:221.

Whitehead, C. C., D. W. Bannister and J. P. F. D'Mello. 1980. Blood pyruvate carboxylase activity as a criterion of biotin status in young pigs. Res. Vet. Sci. 29:126.

Whitehead, C. C., D. W. Bannister, A. J. Evans, W. G. Siller and P. A. L. Wight. 1976. Biotin deficiency and fatty liver and kidney syndrome in chicks given purified diets containing different fat and protein levels. Brit. J. Nutr. 35:115.

Whitehead, C. C., D. W. Bannister, P. A. L. Wight and H. Weiser. 1974. Studies on biotin requirements and deficiency in chicks. Fifteenth World Poul. Congr., pp. 70-72.

Whitehead, C. C. and R. Blair. 1976. The involvement of further nutritional factors in the fatty liver and kidney syndrome in chicks. Res. Vet. Sci. 21:141.

Wilgus, H. S. 1969. Biotin supplementation of practical turkey feeds. Proc 24th Ann. Texas Nutr. Conf. pp., 69-83.

Wilson, J. W., E. H. Leduc and D. H. Winston. 1949. The production of biotin deficiency in the mouse. J. Nutr. 38:73.

Wright, A. I., A. D. Osborne, R. H. C. Penny and E. M. Gray. 1972. Foot-rot in pigs: Experimental production of the disease. Vet. Rec. 90:93.

Wright, L. D. and H. R. Skeggs. 1944. Determination of biotin with lactobacillus arabinosus. Proc. Soc. Exp. Biol. Med. 56:95.

APPENDIX A

BIOTIN ASSAY PROCEDURES

General

The microbiological assay for biotin determination utilized Lactobacillus plantarum[1] (ATCC 8014) as the test organism. The micro-organism was maintained in Bacto-Micro Assay Culture Agar[1] (Difco Code 0319-02) and cultivated in Bacto-Micro Inocolum Broth[1] (Difco Code 0320-02). The assay was carried out with Bacto-Biotin Assay Medium[1] (Difco Code 0419-15). A modification of the sample hydrolysis procedures of Frigg and Brubacker (1976) and the assay procedure of Wright and Skeggs (1944) was employed. The assay is a microvitamin assay sensitive to biotin concentrations of .01 ng, therefore, extreme measures were necessary to prevent contamination and obtain satisfactory repeatable results. Water purity was essential and fresh deionized-distilled water was used exclusively for all phases of the assay. All glassware was washed thoroughly with soap and water, rinsed 4 to 6 times with tap water followed by 3 to 4 rinsings with deionized water and finally 2 to 3 rinsings with deionized-distilled water. Disposable, serological pipets were used. Between assays all glassware

---

[1]Difco, Inc., Detroit, MI.

and solutions coming in contact with L. plantarum were autoclaved for 30 min at 15 psi (121 C). Unless stated otherwise all autoclaving was at 15 psi (121 C) and all incubations in a water bath at 37 C.

Solution Preparation

Stock Culture Agar. Difco Micro Assay Culture Agar was rehydrated by dissolving .47 g in 100 ml deionized-distilled water and heated to boiling for 1 to 2 min. Ten ml aliquots were placed in 16 x 150 mm culture tubes and autoclaved for 15 min.

Inoculum Broth. Difco Micro Inoculum Broth was rehydrated by dissolving 3.7 g in 100 ml deionized-distilled water, distributed in 10 ml aliquots in tubes of 13 to 16 mm diameter and sterilized in the autoclave for 15 min at 15 psi (121 C). Broth was removed from the autoclave shortly after sterilization since prolonged heating will damage broth.

Biotin Assay Medium. Difco Bacto-Biotin Assay Medium was rehydrated by suspending 75 g in 1 liter deionized-distilled water and heated to boiling for 2 to 3 min. Extreme care was taken here. Even the slightest contamination of medium at this step gave unsatisfactory results. All glassware, spatula, weigh paper and water must

be free of biotin. If possible, prepare in separate location from the standard curve.

Papain Hydrolysis Solution. On the day samples were to be hydrolyzed, a papain-citrate buffer hydrolysis solution was prepared. To prepare this solution, 1 g of papain was dissolved (mixed thoroughly) in 4.4 ml of a citric acid solution (2.1 g citric acid/dl) and 5.6 ml of a disodium phosphate solution (2.99 g Na_2HPO_4 /dl) and diluted with deionized-distilled water to 100 ml. All solutions were prepared fresh daily.

Volatile Preservative. One vol 1, 2-dichloroethane, 1 vol chlorobenzeme, and 2 vol 1-chlorobutane were mixed in a test tube immediately prior to hydrolysis.

Biotin Stock and Standard Curve Solutions. Biotin stock solution was prepared by dissolving .01 g pure d-biotin in 100 ml of deionized-distilled water. The stock solution was maintained under toluene and refrigerated when not in use. Fresh stock solutions were prepared at 2 mo intervals.

To prepare the standard curve solutions, 2 ml of the stock solution were diluted to 100 ml with deionized-distilled water (Std A). Standard A was further diluted by adding 5 ml to 95 ml deionized-distilled water (Std B).

Standard B was used to prepare the following standard solutions which were hydrolyzed and assayed along with the samples:

Std	Std B ml added	H_2O added	ng biotin/ml
0	0	100	0
.05	.5	95.5	.5
.10	1.0	99	1.0
.15	1.5	98.5	1.5
.20	2.0	98	2.0
.25	2.5	97.5	2.5
.35	3.5	96.5	3.5
.45	4.5	95.5	4.5

Hydrolysis Procedures

Feed Samples. A 2.5 g sample, which had been ground through a 0.5 mm screen, on a Wiley mill, was mixed in 25 ml 2 N H_2SO_4 and autoclaved for 30 min. The pH was adjusted to 6.8 using 5 N NaOH and the solution diluted with water to a volume of 250 ml. One ml of this solution was added to 9 ml papain solution and 5 drops of preservative. The mixture was vortexed thoroughly, and incubated for 18 hr followed by autoclaving for 10 min. The solution was filtered (Whatman No 42) and 4 to 5 ml water was used to rinse the incubation tube and filter paper (these rinsings were added to the filtrate). Prior to the assay the filtrate was diluted to a concentration of approximately .20 ng biotin/ml.

Liver Samples. Liver samples (4 to 5 g) were lypholyzed for 24 hr, ground through a .5 mm screen on a Wiley mill and

dessicated until hydrolysis. For hydrolysis, .2 g liver were suspended in 15 ml papain solution containing 7 to 8 drops preservative. Following an 18-hr incubation, samples were autoclaved for 10 min. Samples were then filtered (Whatman No 42) and 4 to 5 ml water used to rinse the incubation tubes and filter paper. Filtrates were diluted to a final biotin concentration of approximately .20 ng/ml.

Plasma Samples. One ml of plasma was placed in 4 ml papain solution and 3 drops of preservative added. The solution was vortexed, incubated 18 hr and then autoclaved for 10 min. Samples were diluted to 10 ml followed by centrifugation (10 min at 3000 x g) and filtered through Whatman No 42 paper.

Milk Samples. Milk samples (approximately 15 ml) were centrifuged (10,000 x g) under refrigeration for 15 min and the lipid pellet formed on the top of the sample removed. The fat-free samples were vortexed vigorously to resuspend the remaining milk constituents. One ml fat-free milk sample was added to 5 ml papain solution and 3 drops preservative added. This solution was vortexed, hydrolyzed and filtered in the same manner as plasma samples.

Standards. One ml of each standard solution was added to duplicate hydrolysis tubes containing 4 ml papain solution and 3 drops of volatile preservative. Tubes were vortexed, hydrolyzed and filtered in the same manner as plasma samples.

Assay Procedure

Day 1

1) L. plantarum was aseptically transfered from stab cultures to two culture tubes each containing 10 ml sterile inoculum broth and incubated at 37 C for 18 to 20 hr.

Day 2

1) Five drops of the d 1 inoculum was aseptically transfered to two culture tubes as in d one.
2) Standard curve solutions were prepared (see solution preparation section).
3) Standards and samples were hydrolyzed in duplicate (see hydrolysis procedures).

Day 3

1) Three drops of the d 2 inoculum was transfered to two 13 x 100 mm culture tubes (matched for same optical density with Spectronic 20) containing 5 ml sterile inoculum broth and incubated at 37 C for 18-20 hr.
2) Hydrolyzed samples were autoclaved, filtered and diluted (see hydrolysis procedures).

Day 4

1) One ml of each duplicate standard hydrolysate was added to triplicate assay tubes containing 5 ml assay media, followed by 4 ml water and vortexed.

2) The appropriate amount (1 to 5 ml) of each duplicate sample hydrolysate to contain approximately .2 ng of biotin was added to triplicate assay tubes containing 5 ml assay media. Water was added to a final volume of 10 ml/tube and vortexed.
3) Five ml of water was added to two tubes containing 5 ml assay media and vortexed. These tubes were used as uninoculated blanks to check for proper sterilization and biotin contamination during assay.
4) All assay tubes were autoclaved for 10 min and removed from the autoclave as soon as possible and all tubes allowed to reach room temperature.
5) The d 3 inoculum was centrifuged (10 min, 2000 x g) and supernatant aseptically removed. Cells were resuspended in 5 ml sterile saline, vortexed, centrifuged and supernatant removed. The inoculum was then carried to an optical density of .5 at 640 mu using sterile saline. This permitted the addition of approximately equal numbers of microbes/assay tube.
6) One drop of prepared inoculum was aseptically added to all assay tubes except the blanks. All tubes were vortexed then incubated for 18-22 hr at 37 C.

Day 5

1) After incubation, tubes were placed in the refrigerator for 30 min to stop additional growth of the microorganism.
2) Turbidity readings were obtained on each assay tube using a Spectronic 20 (640 mu) which was blanked (100% T) with the 0 ng standard.
3) Using the turbidimetric values of the standard tubes, a standard curve was plotted (density against ng of biotin).
4) The biotin content of the unknown samples was then calculated from this standard curve. Duplicate hydrolysate that varied by more than 10% from each other were repeated.

APPENDIX B

TABLE 28. ANALYSIS OF VARIANCE TABLE FOR SPLIT-SPLIT-PLOT STATISTICAL MODEL USED IN THE GILT STUDY[a]

Source	df	Denominator for F statistic
Biotin level (B)	1	Gilt
Replicate (R)	3	Gilt
Trial (E)	2	Gilt
B x R	3	Gilt
B x E	2	Gilt
R x E	6	Gilt
Gilt/(B-R-E)	213	Gilt x T
Time (T)	2	Gilt x T
T x B	2	Gilt x T
T x R	6	Gilt x T
T x E	4	Gilt x T
Gilt x T	456	Residual
Foot (F)	1	Residual
Toe (O)	1	Residual
F x O	1	Residual
F x T	2	Residual
O x T	2	Residual
F x B	1	Residual
O x B	1	Residual
F x R	3	Residual
O x R	3	Residual
F x E	2	Residual
O x E	2	Residual
Residual	4946	

[a]Data for frequency and severity of each type of toe lesion were analyzed using this analysis. Due to small numbers of observations and missing cells the model was reduced for some toe lesion severity variables.

TABLE 29. ANALYSIS OF VARIANCE TABLE FOR SPLIT-PLOT STATISTICAL MODEL USED IN THE GILT STUDY[a]

Source	df	Denominator for F statistic
Biotin level (B)	1	Gilt
Replicate (R)	3	Gilt
Trial (E)	2	Gilt
B x R	3	Gilt
B x E	2	Gilt
R x E	6	Gilt
Gilt/(B-R-E)	219	Residual
Time (T)	2	Residual
T x B	2	Residual
T x R	6	Residual
T x E	4	Residual
Residual	460	

[a]Data for total toe lesions, total severity and frequency of gilts with each type of toe lesion were analyzed with this analysis.

TABLE 30. ANALYSIS OF VARIANCE TABLE FOR SPLIT-PLOT STATISTICAL MODEL USED IN THE REPRODUCTIVE STUDY[a]

Source	df	Denominator for F statistic
Biotin level (B)	1	Female
Replicate (R)	2	Female
Grain (G)	1	Female
B x R	2	Female
B x G	1	Female
R x G	2	Female
Female/(B-R-G)	82	Residual
Parity (P)	3	Residual
Housing (H)	2	Residual
Season (S)	3	Residual
B x P	3	Residual
B x H	2	Residual
B x S	3	Residual
G x P	3	Residual
G x H	2	Residual
G x S	3	Residual
Residual	129	

[a]Data for age at first estrus, weaning to remating intervals, farrowing performance, and lactation performance to 21 d were analyzed using this analysis.

TABLE 31. ANALYSIS OF VARIANCE TABLE FOR SPLIT-SPLIT-PLOT STATISTICAL MODEL USED IN THE SOW STUDY[a]

Source	df	Denominator for F statistic
Biotin level (B)	1	Sow
Replicate (R)	2	Sow
Grain (G)	1	Sow
B x R	2	Sow
B x G	1	Sow
R x G	2	Sow
Sow/(B-R-G)	93	Sow x T
Time (T)	4	Sow x T
T x B	4	Sow x T
T x R	7	Sow x T
T x G	4	Sow x T
Sow x T	250	Residual
Foot (F)	1	Residual
Toe (O)	1	Residual
F x O	1	Residual
F x T	4	Residual
O x T	4	Residual
F x B	1	Residual
O x B	1	Residual
F x R	2	Residual
O x R	2	Residual
F x G	1	Residual
O x G	1	Residual
Residual	2360	

[a]Data for frequency and severity of each type of toe lesion were analyzed using this analysis. Due to small numbers of observations and missing cells the model was reduced for some toe lesion severity variables.

TABLE 32. EFFECTS OF TYPE OF GRAIN FED ON THE FREQUENCY OF FEMALES WITH TOE LESIONS

Item[b]	Grain		S.E.[c]
	Corn	Wheat	
No. of observations	165	180	
Heel-horn erosion	100.0	100.0	.00
Heel bruising	11.8	15.9	2.3
Heel crack	89.0	92.5	2.2
Heel-horn junction crack	31.7	32.6	2.9
White-line horn crack	34.1	35.2	2.8
Side-wall horn crack	29.5	27.8	2.6

[a]All values are least-squares means expressed as the percentage of females with one or more lesions. Combined data from five time points.
[b]See figure 2 for lesion classification.
[c]Standard error of the mean.

TABLE 33. LEAST-SQUARES MEANS FOR THE NUMBER AND TOTAL SEVERITY SCORE OF TOE LESIONS PER FEMALE. GRAIN EFFECTS[a]

Item[b]	Grain		S.E.[c]
	Corn	Wheat	
No. of observations	165	180	
Total toe lesions			
No. lesions	13.3	13.0	.19
Severity	38.6	37.4	.72
Heel-horn erosion			
No. lesions	7.94	7.93	.03
Severity	23.9	24.0	.31
Heel bruising			
No. lesions	.19	.25	.03
Severity	.58	.75	.10
Heel crack			
No. lesions	3.38	3.04	.12
Severity	8.4	7.3	.35
Heel-horn junction crack			
No. lesions	.46	.50	.06
Severity	1.3	1.5	.19
White-line horn crack			
No. lesions	.70	.70	.06
Severity	1.9	1.7	.16
Side-wall horn crack			
No. lesions	.60	.55	.06
Severity	2.5	2.3	.20

[a]Combined data from five time points.
[b]See figure 2 for lesion classification.
[c]Standard error of the mean.
[d]Total number of toe lesions/female.
[e]Total severity score of toe lesions/females. Value was obtained by adding the severity scores for all eight toes for each type of lesion. Severity scores ranged from 1 to 5 with 1 denoting a very small lesion and 5 a very large lesion.

TABLE 34. LEAST-SQUARES MEANS FOR THE FREQUENCY OF TOES WITH LESIONS IN GILTS AND SOWS FED TWO TYPES OF GRAIN[a]

Item[b]	Grain		S.E.[c]
	Corn	Wheat	
No. of observations	1320	1440	
Heel-horn erosion	87.6	87.6	.6
Heel bruising	2.4	3.2	.3
Heel crack	42.3	38.0	1.0
Heel-horn junction crack	5.4	5.8	.5
White-line horn crack	8.7	8.5	.5
Side-wall horn crack	7.4	6.8	.5

[a]Frequency values expressed as the percentage of toes within a female with one or more lesions. Combined data from five time points.
[b]See figure 2 for lesion classification.
[c]Standard error of the mean.

TABLE 35. LEAST-SQUARES MEANS FOR TOE LESION SEVERITY SCORES IN GILTS AND SOWS FED TWO TYPES OF GRAIN[ab]

Item	Grain		S.E.[c]
	Corn	Wheat	
Heel-horn erosion	3.02(1306)	3.02(1431)	.02
Heel crack	2.38(540)	2.16(513)	.04
Heel-horn junction crack	2.69(62)	2.40(81)	.45
White-line horn crack	2.35(80)	2.45(92)	.13
Side-wall horn crack	2.54(68)	2.74(75)	.25

[a]Severity scores ranged from 1 to 5 with 1 denoting a small lesion and 5 a very large lesion.
[b]Values are from five time points combined with total no. of observations/mean in parenthesis.
[c]Standard error of the mean.

TABLE 36. LEAST-SQUARES MEANS FOR HAIR COUNTS, HAIR SCORES AND SOUNDNESS SCORES FOR GILTS AND SOWS FED TWO TYPES OF GRAIN[a]

Item	Grain		S.E.[b]
	Corn	Wheat	
No. of observations	165	180	
No. of hairs/cm^2[c]	4.54	4.62	.09
Hair score[d]	1.92	1.91	.04
Soundness score[e]	8.48	8.74	.12

[a]Combined data from five time points.
[b]Standard error of the mean.
[c]Mean value for four observations (two/ham) taken from the base of the ham.
[d]Mean value for three committee members. Each female received a score of 1 to 5 where 1 indicated no hair loss and 5 excessive hair loss.
[e]Mean value for three committee members. Each female received a score of 1 to 15 where 1 indicated a very sound, free moving female and 15 a lame, unsound female unable to walk.

TABLE 37. ANALYSIS OF VARIANCE TABLE FOR SPLIT-SPLIT-PLOT STATISTICAL MODEL. GILT AND SOW STUDY COMBINED[a]

Source	df	Denominator for F statistic
Biotin level (B)	1	Female
Replicate (R)	2	Female
B x R	2	Female
Female/(B-R)	231	Female x T
Time (T)	7	Female x T
T x B	7	Female x T
T x R	13	Female x T
Gilt x T	792	Residual
Foot (F)	1	Residual
Toe (O)	1	Residual
F x O	1	Residual
F x T	7	Residual
O x T	7	Residual
F x B	1	Residual
O x B	1	Residual
F x R	2	Residual
O x R	2	Residual
Residual	7369	

[a]Data for toe measurements, frequency of toes with lesions and the severity of those toe lesions were analyzed with this analysis. Low numbers of observations and missing cells necessitated reducing the model for some toe lesion severity variables.

TABLE 38. AMINO ACID CONTENT OF BASAL CORN- AND WHEAT-BASED DIETS. SOW STUDY[a]

Amino acid	Basal diet	
	Corn	Wheat
Aspartic acid	1.39	1.38
Threonine	.54	.57
Serine	.66	.71
Glutamic acid	2.53	3.73
Proline	1.40	1.85
Glycine	.57	.65
Alanine	.85	.64
Valine	.56	.59
Cysteine	.26	.25
Methionine	.16	.18
Isoleucine	.51	.54
Leucine	1.44	1.15
Tyrosine	.53	.49
Phenylalanine	.68	.70
Lysine	.71	.68
Histidine	.38	.36
Arginine	.90	.93
Essential	5.87	5.72
Nonessential	8.20	9.70
Total	14.08	15.43

[a]Determined using ion exchange chromotography.

APPENDIX C

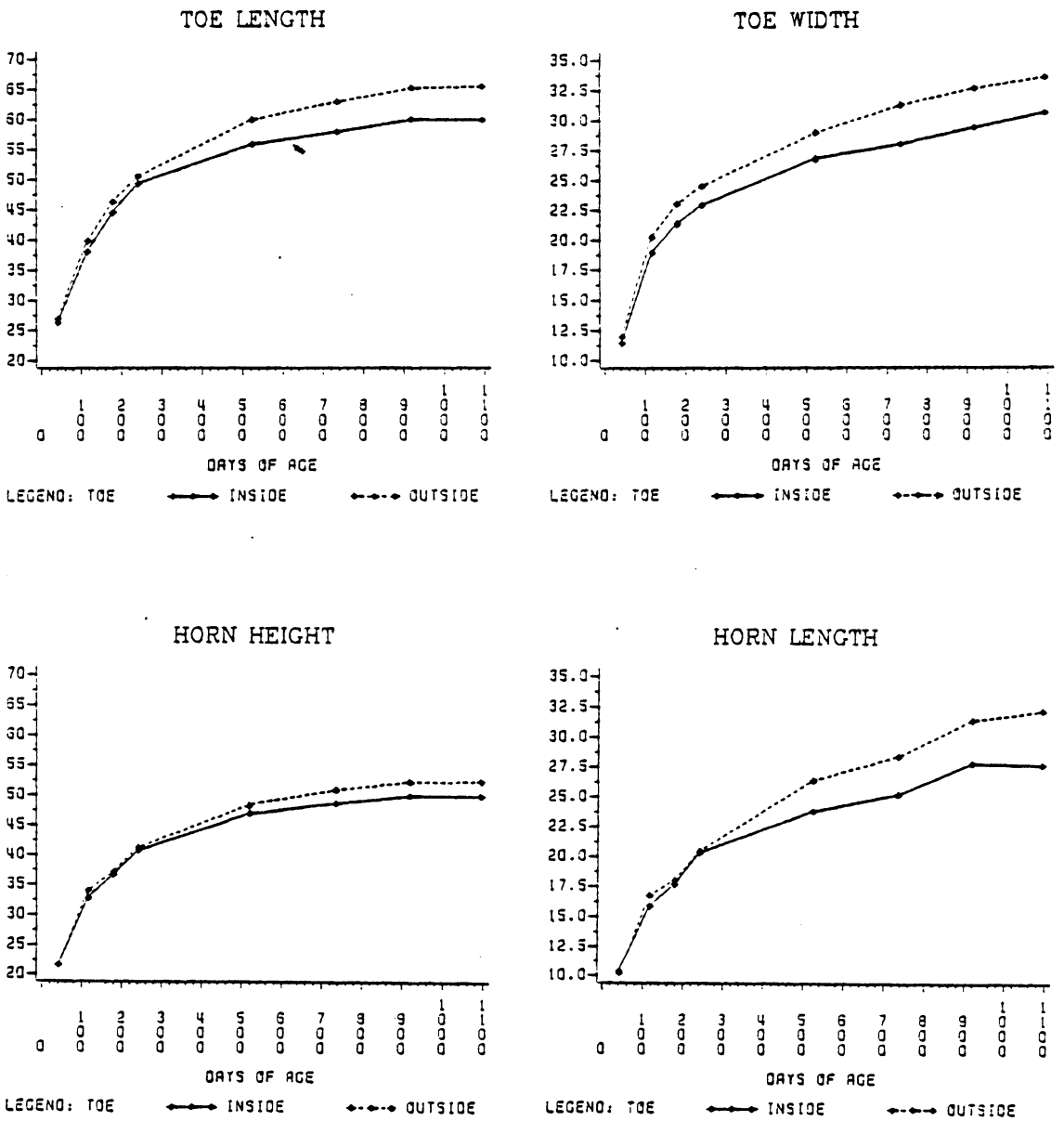


Figure 13. The effect of age on size of inside and outside toes in gilts and sows.

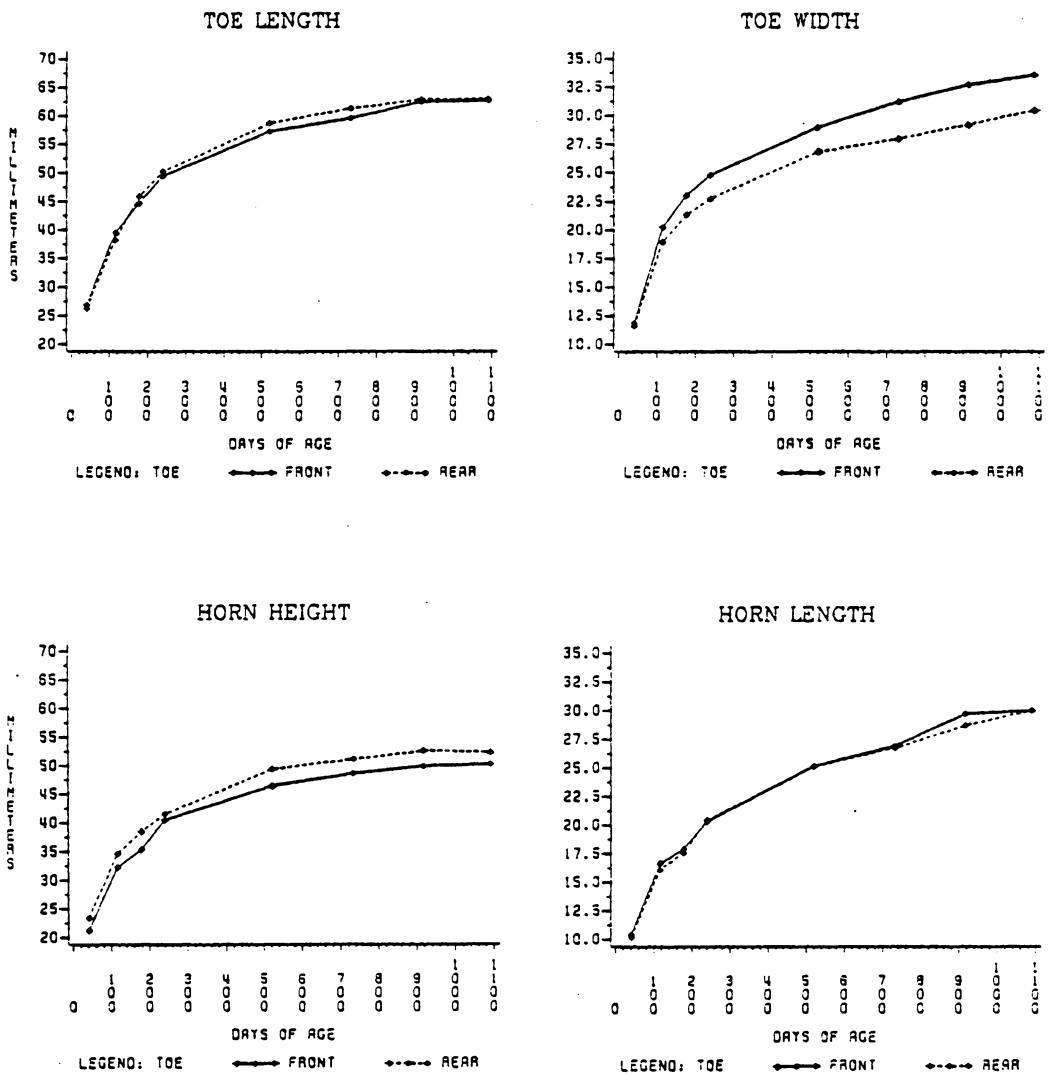


Figure 14. The effect of age on size of front and rear toes in gilts and sows.

The vita has been removed from the scanned document

AN ASSESSMENT OF THE BIOTIN NEEDS OF DEVELOPING GILTS AND REPRODUCING SOWS IN A MODERN PRODUCTION SYSTEM

by

Kenneth L. Bryant

(ABSTRACT)

To assess the biotin needs of developing gilts and sows, female swine were fed corn-soybean meal diets supplemented with 0 (NB) or 220 ug (SB) d-biotin/kg diet in three growing-finishing trials and corn (C) or wheat-based (W) diets supplemented with 0 (NB) or 440 (SB) ug d-biotin/kg in a four-parity sow study. During the growing-finishing trials, feedlot performance, hair and soundness scores were unaffected by dietary treatment, however, SB elevated ($P<.001$) plasma biotin (PB) and reduced the number and frequency of various toe lesions. Conception rate and the weaning to estrus interval were improved ($P<.05$) with SB. No significant responses were noted in farrowing and lactation performance. There was a trend for SB to improve litter size in multiparous females. Feeding SB diets elevated ($P<.001$) the biotin content of sow plasma, liver and milk, while sow hepatic pyruvate carboxylase activity

was unchanged. Fetal plasma biotin levels were high in pigs from NB females and were increased four-fold when dams received SB diets. Farrowing performance was reduced in W females; however, level of biotin x type of grain interactions were not present. The frequency (%) of females with heel cracks, heel-horn junction cracks and side-wall horn cracks was reduced ($P<.01$) when females were fed SB diets. Females fed SB diets had fewer ($P<.001$) total toe lesions, heel cracks, heel-horn junction cracks, side-wall horn cracks and white-line horn cracks ($P<.03$) compared with NB females. Level of biotin x age interactions ($P<.05$) indicated that SB was more effective in reducing toe lesions in multiparous females with most reductions occuring following the first parity. Supplemental biotin did not alter soundness scores ($P>.10$) but increased ($P<.001$) the number of hairs/cm^2 and improved ($P<.001$) hair scores. Toe location influenced toe size and lesion development with outside toes being larger and containing more lesions. Toe lesions and toe size were not correlated ($P>.10$) to soundness. The results of this study suggest that the development of toe lesions in swine can be reduced with biotin supplementation and improvements in reproductive performance may be attainable.