EFFECT OF SUPPLEMENTAL FAT IN PRESTARTER DIETS ON PERFORMANCE AND APPARENT ABSORPTION OF FATTY ACIDS IN THE EARLY-WEANED PIG

By

TIMOTHY MARTIN FAKLER

Bachelor of Science
Oral Roberts University
Tulsa, Oklahoma
1983

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1987

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 1992

OKLAHOMA STATE UNIVERSITY

EFFECT OF SUPPLEMENTAL FAT IN PRESTARTER DIETS ON PERFORMANCE AND APPARENT ABSORPTION OF FATTY ACIDS IN THE EARLY-WEANED PIG

Thesis Approved:

Charles V. Maxwell
Thesis Adviser

Robert Teeter

George V. Odell

Thomas C. Collins
Dean of the Graduate College

ACKNOWLEDGMENTS

The author gratefully acknowledges the guidance and support provided by Dr. Charles V. Maxwell, his major professor, during the course of these investigations and the preparation of this dissertation. Thanks are also extended to Drs. Archie C. Clutter, George V. Odell and Robert S. Teeter, for serving as committee members and for their time spent in guiding the research and in providing valuable suggestions for the completion of this dissertation.

Appreciation is expressed to Dr. David L. Weeks, Dr. Christopher M. Adams, Dr. Selwyn Barron, Dr. Kwang Sohn, Troy Miller, Mary Ellen Beyl, Michelle Eells, Kathy Swenson, Paula Dubeski, Mark J. Gatschet, Thayne Vickstrom, Lloyd Sumner, Paul West, Greg Garrison, Stan Seigle, Kim Brock, Tim Cash, the chemistry "gang" and the swine barn crew for helping to complete this research.

A special thanks goes to the many international students who have broadened horizons and enriched the quality of the educational experience of the author.

Special gratitude goes to my mother, who set an example for me to follow by living through and conquering some of life's most difficult challenges.

This dissertation is dedicated to my wife Valerie and our expected baby. Their help and sacrifices have made this goal a reality.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF PLATES

LIST OF NOMENCLATURE

ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
AP-820	Plasma protein, American Protein Corporation
DGE	Tridodecyl Glyceryl Ether
FAME	Fatty Acid Methyl Ester
FE	Feed Efficiency
FWHM	Full Width Half Maximum
GC	Gas Chromatography
IgG	Immunoglobulin G
KV	Kilovolts
MCT	Medium Chain Triglycerides
TGE	Tritetradecyl Glyceryl Ether
VLDL	Very Low Density Lipoproteins
$C_{14:0}$	Tetradecanoic (Myristic) acid methyl ester
$C_{16:0}$	Hexadecanoic (Palmitic) acid methyl ester
$C_{16:1}$	9-Hexadecenoic (Palmitoleic) acid methyl ester
$C_{17:0}$	Heptadecanoic acid methyl ester
$C_{18:0}$	Octadecanoic (Stearic) acid methyl ester
$C_{18:1}$	9-Octadecenoic (Oleic) acid methyl ester
$C_{18:2}$	9,12-Octadecadienoic (Linoleic) acid methyl ester
$C_{18:3}$	9,12,15-Octadecatrienoic (Linolenic) acid methyl ester
$C_{22:0}$	Eicosanoic (Arachidic) acid methyl ester

$C_{22:4}$ 5,8,11,14-Eicosatetraenoic (Arachidonic) acid methyl ester

CHAPTER I

LIPID DIGESTION AND ABSORPTION

Brief Historical Review

Dietary lipid is a major source of energy obtained from feedstuffs. The small intestine is charged with the task of absorbing and processing not only the dietary lipid, but also the lipid contribution from bile and other endogenous sources. During the last century, many advances have been made in understanding the intricate processes of lipid digestion, absorption and metabolism. The purpose of this chapter is to provide a current description of lipid processing in the small intestine and to review how lipids are utilized in the early weaned pig.

Intestinal digestion and absorption of fat has been extensively reviewed (Friedman and Nylund, 1980; Carey, Small and Bliss, 1983; Borgström and Patton, 1991; and Caspary, 1992). In addition, Small (1991) reviewed the effects of stereospecific structure of triglycerides on absorption and processing. Readers desiring historical perspectives or additional details are directed to these reviews.

Some of the more significant landmarks in the digestion and absorption of fat are as follows. Bernard (1849) showed

that the lymphatics just distal to the pancreatic duct in the small intestine became milky after a fatty meal, suggesting the importance of pancreatic juice for normal fat absorption in the rabbit. In addition, Bernard suggested that fats were broken down by a substance in the pancreatic secretion to give fatty acids and glycerol. Dastre (1890) transposed the bile duct in the dog distally to the pancreatic duct and concluded that both bile and pancreatic juice were necessary to obtain milky lymph after a fatty meal. Rachford (1891) showed that bile stimulated the activity of pancreatic lipase. The ability of bile to solubilize fatty acids was reported by Moore and Rockwood (1897).

Mattson and Beck (1956) demonstrated that pancreatic lipase was specific for the primary esters of triglycerides and thus hydrolysis of triglycerides was incomplete and ended with the 2-monoglycerides. The partial hydrolysis mechanism resolved the dispute between those who adhered to the particle absorption theory and those who held the opinion that complete hydrolysis of triglycerides occurred prior to absorption by enterocytes.

Borgström (1955) suggested that micellular solubilization of fatty acids and monoglycerides was an important function of bile salts in fat absorption. Hofmann and Borgström (1964) later isolated and identified a micellular phase from human intestinal contents and formulated what became known as the micellular hypothesis of

fat absorption.

The structure of the mixed bile salt-phospholipid micelle was first proposed by Small et al. (1969). The micelles were shown to be in the form of a disk of bilayer phospholipids (primarily phosphatidyl choline) surrounded by a concentric monolayer of bile salt dimers. Wilson et al. (1971) reported that the rate limiting step in fatty acid uptake from a micellular solution by epithelial cells was the transversing of the unstirred water layer adjacent to the intestinal cells.

Lipid Digestion and Absorption

The absorption of fat occurs in several steps. Initially, lipids in food are solubilized as micellular dispersions within the upper gastrointestinal tract. Emulsification of lipids begins within the stomach. Partial hydrolysis of triglycerides occurs in the stomach by the action of salivary lipase (bile salts are not a requirement for activity). Triglycerides are preferentially hydrolyzed at carbon number three of glycerol by salivary lipase. In the proximal duodenum, gastric acid triggers the release of secretin, which in turn stimulates pancreatic water and bicarbonate secretion. Bicarbonate secretion causes an increase in the pH of the digesta, which is important for optimal lipase activity. In addition, the increase in pH causes the amphiphilic fatty acids to aggregate at the surface of fat droplets thus achieving emulsification of

fat.

The principle constituents of bile are bile salts and biliary lipids (particularly phospholipids). Phospholipids fuse with the emulsified fat droplets in the duodenum and form mixed micelles. Due in part to the detergent properties of fatty acids, phospholipids and bile salts, the surface area of the fat droplet is greatly enlarged. The formation of the large surface area is important for the next step of fat digestion: hydrolysis. Biliary and pancreatic secretion are triggered by the release of cholecystokinin-pancreozymin. Hydrolysis is accomplished by the pancreatic lipase/colipase system, phospholipase A_2, cholesterolase and other enzymes released from the pancreas. Hydrolysis of triglycerides to fatty acids and 2-monoglycerides is rapid and efficient. The mixed micelles that are formed contain bile acids, fatty acids, monoglycerides, cholesterol, phospholipids and fat-soluble vitamins.

The next stage of lipid digestion involves absorption of lipids through mucosal cells and eventual appearance into the blood. The absorption of fat begins within the duodenum and is essentially complete by the distal ileum. Lipid molecules are absorbed within the upper small intestine while the bile salts are reabsorbed mainly in the terminal ileum. The passage of lipid molecules from mixed micelles into mucosal cells is determined by the rate of penetration through the unstirred water layer and transport across the

mucosal cell membrane. Permeation of fatty acids through the microvillus membrane was originally thought to occur by passive diffusion (Johnson and Borgström, 1964). However, it has been shown that while protonated free fatty acids are passively absorbed, an active transport mechanism driven by a trans-membranous Na^+-gradient is the process by which fatty acid anions transverse the microvillus membrane (Stremmel, 1987).

After transport across the mucosal membrane, long chain fatty acids are taken up by a cytosolic protein that binds fatty acids. This cytosolic protein directs the fatty acids to the smooth endoplasmic reticulum, which is the site of triglyceride resynthesis. The transport of resynthesized lipids is facilitated by the synthesis of chylomicrons and very-low-density lipoproteins (VLDL). Chylomicrons and VLDL are formed by coating the hydrophobic lipids (mainly triacylglycerol and cholesterol esters) with amphiphililic compounds (phosphatidylcholine and other phospholipids) and various proteins referred to as apoproteins. The chylomicrons and VLDL formed within the Golgi apparatus are subsequently released into the intercellular space by reverse pinocytosis. The chylomicrons and VLDL enter the lymphatic system and via the thoracic duct are shunted into the jugular vein.

Determining nutrient absorption involves accurate quantification of the ingested nutrient and the unabsorbed residual nutrient. Absorption may be estimated by measuring

the quantity of the nutrient of interest in the feed ingested, and then estimating the total excretion of the nutrient (total fecal collection followed by quantification of the nutrient in the feces). It has been suggested (Hoving et al., 1977) that the validity of the nonabsorbable marker technique is based on the condition that after the oral administration of the marker and feedstuffs, intraluminal changes in the ratio of marker to test material (in this case fat) are caused exclusively by absorption. This is however, a very simplified approach, with several underlying assumptions including 1) a procedure is available for accurate determination of marker and nutrient, 2) transport of marker and nutrient within the digestive system is equivalent, 3) endogenous losses are negligible and 4) modification of the nutrient or marker does not occur within the gastrointestinal system.

Edin (1926) reported that chromium sesquioxide (chromic oxide, Cr_2O_3) was a nutritionally inert, nonabsorbable marker, and that it was suitable for use in studying the progress of absorption by comparing the ratios of nutrient to marker in feed and feces.

Carlson and Bayley (1968) however, suggested that Cr_2O_3 was inappropriate for use in lipid digestion because Cr_2O_3 separated from the lipid phase of the digesta in the stomach. Earlier, Borgström (1960) suggested that cholesterol, which is poorly absorbed and is lipid soluble, could be used to study fat absorption. However, the poor

recovery of cholesterol limits its usefulness. Sie et al. (1967) reported the use of silicon oil for studying fat absorption, but the quantitative determination is rather complicated and time consuming. Spencer et al. (1968) reported use of glycerol trioctadecenyl ether as a model for triglyceride absorption and found the triether to be very poorly absorbed.

Carlson and Bayley (1972) reported the synthesis of tridodecylglyceryl ether (DGE) and described using DGE as an indicator of fat absorption. Total recovery of DGE was comparable to that of Cr_2O_3 (97.7 and 97.4%, respectively) when these markers were included in diets given to rats. In the study, the authors measured the content of fatty acids in different regions of the gastrointestinal tract calculated using either Cr_2O_3 or DGE. Total fatty acid content in the diet was 10%, yet when calculated using Cr_2O_3 as the external marker, the stomach contents contained 18.8% fatty acids. The authors concluded that this apparent increase in fatty acid content in the stomach was actually due to Cr_2O_3 passing from the stomach faster than did the fat in the diet. The DGE and fat left the stomach at approximately the same rate. The authors concluded that DGE was a suitable indicator of fat absorption from different regions of the gastrointestinal tract. Small (1970) showed that nonpolar compounds such as DGE remained in the oil phase during the formation of micellar solutions.

Fat Utilization in the Young Pig

Early weaned pig diets are commonly supplemented with fat in order to increase the caloric density of the diet and thus more effectively replace sow's milk (30-40% fat). However, results from studies conducted in order to evaluate the effects of fat supplemented diets for early weaned pigs are conflicting. Kennington et al. (1958) found that in weanling pigs fed a practical swine growing ration, adding fat increased growth rate. When the nutrient to calorie ratio was kept constant, Aherne et al.(1982) and Allee et al.(1971) found that the addition of fat to a starter diet improved average daily gain (ADG). Several studies have found that the fat added to the diets of baby pigs did not significantly improve weight gains (Smith and Lucas, 1956; Peo et al., 1957; Sewell and Miller, 1965; Ewan, 1970 and Leibbrandt et al., 1975). In contrast, some studies concluded that addition of fat to the diet of young pigs decreased rate of gain (Frobish et al., 1969; Eusebio et al., 1965).

In addition, fat supplementation either resulted in a significant improvement in feed efficiency (Crampton and Ness, 1954; Kennington et al., 1958; Lowrey et al., 1958; Sewell and Miller, 1965; Allee et al., 1971; Allee and Hines, 1972) or no significant effect on feed efficiency relative to the control diet (Smith and Lucas, 1956; 1960; Crampton et al., 1960; Manners and McCrae, 1963; Frobish et

al., 1969, 1970, 1971) or a significant reduction in feed efficiency during the first two weeks post-weaning (Peo et al., 1957). Addition of fat resulted in either no significant effect on feed intake (Crampton and Ness, 1954; Crampton et al., 1960; Allee et al., 1971; Leibbrandt et al., 1975) or a reduction in feed intake (Kennington et al., 1958; Sewell and Miller, 1965).

Investigators have determined that the source of supplemental fat influences the ability of the young pig to utilize fat in starter diets (Cera et al., 1988; Lawrence and Maxwell, 1983; Sewell and Miller, 1965). The apparent digestibility of butterfat and coconut oil was greater than that of tallow or soybean oil (Lloyd and Crampton, 1952; Crampton et al., 1960). No difference was observed between the digestibilities of butter, soybean oil or coconut oil (Frobish et al., 1970) or between those of tallow and coconut oil (Hamilton and McDonald, 1969). Eusebio et al. (1965) found that weight gain of pigs fed lard was significantly less than that of pigs fed tallow, soybean oil or the control with no added fat. Crampton et al. (1960) studied the effects of common edible fat sources and found that ADG of pigs fed diets supplemented (20%) with butterfat, soybean oil, cottonseed oil, corn oil, linseed oil, hydrated soybean oil, hydrated cottonseed oil, hydrated coconut oil, lard, tallow and hydrated fish oil were significantly greater than pigs fed diets supplemented with rapeseed oil, coconut oil and fish oil, but no difference

was observed in feed intake due to fat source. In an earlier study, Hamilton and McDonald (1969) reported that dietary fat source did not affect ADG, feed/gain ratio or apparent digestibility.

Cera et al. (1989) found that of the commercially available plant and animal lipid sources, coconut oil addition to a corn-soy-whey starter diet gave more consistent growth and feed performance responses during the initial two weeks postweaning in pigs weaned at 21 d. Coconut oil contains a large proportion (60%) of medium chain triglycerides (MCT) which are highly digestible in early weaned pigs. Cera et al. (1990) reported that when coconut oil, soybean oil, MCT and roasted soybeans were supplemented into a corn-soy-whey starter diet, the apparent fat digestibility was highest in the MCT supplemented diet (88.0% compared to 79.8, 76.0 and 62.4 for the coconut oil, soybean oil and roasted soybean diets, respectively) and plateaued by wk 2 postweaning. Effects of MCT addition to a more complex starter diet for early weaned pigs containing little or no soybean meal have not been determined.

One possible reason for the conflicting data is that in these previous studies, pigs were fed a simple corn-soy based starter diet. Owsley et al. (1986) concluded that the young pig requires at least 6 to 9 d after weaning at 28 d to adjust to a typical corn-soy starter diet. Cera et al. (1988) demonstrated that apparent digestibility of fat in a corn-soy based diet increased each week postweaning and

appeared to reach a plateau by wk 3 postweaning. Early weaned pigs fed a diet containing soybean meal had decreased growth performance, lowered villus height and increased serum anti-soy IgG titters (Li et al., 1991).

As reported above, numerous studies have been conducted to evaluate the performance of early-weaned pigs fed fat-supplemented diets, but in these studies only performance data and fecal digestibilities were measured (Aherne et al., 1982; Allee et al., 1971; Braude and Newport, 1973; Cera et al., 1988; Cera et al., 1989; Eusebio et al., 1965; Lawrence and Maxwell, 1983). In a recent study utilizing 40-45 kg pigs, Ozimek et al. (1984) reported that in order to obtain an accurate measurement of the pig's ability to digest fat and fatty acids, digestibilities should be determined at the distal ileum, and not in the feces.

In summary, since the effects of adding fat to prestarter and starter diets have yielded conflicting results, more information is needed to fully understand fat utilization in the early-weaned pig.

Plasma Protein in Prestarter Diets

Although the young pig can utilize a simple corn-soybean diet when weaned as early as 18 days of age, performance is markedly improved by incorporating alternate sources of protein into the diet. Feeding early weaned pigs a diet containing soybean meal resulted in decreased growth performance, lowered villus height, increased serum anti-soy

IgG titers and decreased absorptive capacity (Li et al., 1991). Protein sources which have the potential of replacing the more expensive milk proteins or the soy proteins in early weaned pig diets should be readily accepted by the swine industry.

One such readily available protein source is spray dried plasma protein, a by-product of pork slaughter houses. Porcine spray dried plasma protein is produced by adding an anticoagulant to whole blood at the slaughter plant, and after cooling, the cells are separated from the plasma by centrifugation. Plasma consists of an aqueous solution of proteins, electrolytes and small organic molecules. Plasma will contain about 7 g of plasma proteins per 100 mL of solution. The plasma is stored at 25°F until it is spray dried. The cooled plasma is heated to 90°F for 25 min and spray dried for one second at 405°F. To further remove moisture, the product is kept at 200°F for 1 to 2 min. This process results in a free-flowing, light colored product that is easily mixed into early weaned pig rations.

The extent to which protein source affects utilization of other dietary ingredients has not been fully delineated.

Literature Cited

Aherne, F. X., V. Danielsen and H. E. Nielsen. 1982. Fat utilization by three-week weaned pigs. Acta. Agric. Scand. 32:151.

Allee, G. L. and R. H. Hines. 1972. Influence of fat level and calorie:protein ratio on performance of young pigs. J. Anim. Sci. 35:210(Abstr.).

Allee, G. L., D. H. Baker and G. A. Leveille. 1971. Fat utilization and lipogenesis in the young pig. J. Nutr. 101:1415.

Asplund, J. M., R. H. Grummer and P. H. Phillips. 1960. Stabilized white grease and corn oil in the diet of baby pigs. J. Anim. Sci. 19:709.

Bernard, C. 1849. Recherches sur les usages du sac pancreatique dans la digestion. Acad. Sci. 28:249.

Borgström, B. and J. S. Patton. 1991. Luminal events in gastrointestinal lipid digestion. In: B. B. Rauner (Ed.) Handbook of Physiology. The Gastrointestinal System. pp 475. American Physiological Society, Bethesda, MD.

Braude, R., H. D. Keal and M. J. Newport. 1976. Artificial rearing of pigs 5. The effect of different proportions of beef tallow or soya-bean oil and dried skim milk in the diet on growth, feed utilization, apparent digestibility and carcass composition. Br. J. Nutr. 35:253.

Braude, R. and M. J. Newport. 1973. Artificial rearing of pigs 4. The replacement of butterfat in a whole-milk diet by either beef tallow, coconut oil or soya-bean oil. Br. J. Nutr. 29:447.

Cary, M. C., D. M. Small and C. M. Bliss. 1983 Lipid digestion and absorption. Ann. Rev. Physiol. 45:651.

Caspary, W. F. 1992. Physiology and pathophysiology of intestinal absorption. Am. J. Clin. Nutr. 55:299S.

Cera, K. D., D. C. Mahan and G. A. Reinhart. 1988. Weekly digestibilities of diets supplemented with corn oil, lard or tallow by weanling swine. J. Anim. Sci. 66:1430.

Cera, K. R., D. C. Mahan and G. A. Reinhart. 1989. Apparent fat digestibilities and performance responses of postweaning swine fed diets supplemented with coconut

oil, corn oil or tallow. J. Anim. Sci. 67:2040.

Cera, K. R., D. C. Mahan and G. A. Reinhart. 1990. Evaluation of various extracted vegetable oils, roasted soybeans, medium-chain triglyceride and an animal-vegetable fat blend for postweaning swine. J. Anim. Sci. 68:2756.

Crampton, E. W. and O. M. Ness. 1954. A meal mixture suitable as the entire ration to be self-fed dry to pigs weaned at ten days of age. J. Anim. Sci. 13:357.

Crampton, E. W., R. K. Shaw, V. G. Mackay and D. C. Schad. 1960. Comparative feeding value of common edible fats as measured by the growth of prematurely weaned pups, guinea pigs and swine. J. Nutr. 70:81.

Dastre, A. 1890. Recherches sur la bile. Arch. Physiol. Ser. 5, 2:315.

Eusebio, J. A., V. W. Hayes, V. C. Speer and J. T. McCall. 1965. Utilization of fat by young pigs. J. Anim. Sci. 24:1001.

Ewan, R. C. 1970. Effect of protein quality on fat utilization by baby pigs. J. Anim. Sci. 31:1020(Abstr.)

Friedman, H. I. and B. Nylund. 1980. Intestinal fat digestion, absorption, and transport. A review. Am. J. Clin. Nutr. 33:1108.

Frobish, L. T., V. W. Hayes, V. C. Speer and R. C. Ewan. 1969. Effect of diet form and emulsifying agents on fat utilization by young pigs. J. Anim. Sci. 29:320.

Frobish, L. T., V. W. Hayes, V. C. Speer and R. C. Ewan. 1970. Effect of fat source and level on utilization of fat by young pigs. J. Anim. Sci. 30:197.

Frobish, L. T., V. W. Hayes, V. C. Speer and R. C. Ewan. 1971. Effect of fat source on pancreatic lipase activity and specificity and performance of baby pigs. J. Anim. Sci. 33:385.

Hamilton, R.M.G. and B. E. McDonald. 1969. Effect of dietary fat source on the apparent digestibility of fat and the composition of fecal lipids of the young pig. J. Nutr. 97:33.

Johnson, J. M. and B. Borgström. 1964. The intestinal absorption and metabolism of micellar solution on lipids. Biochim. Biophys. Acta 84:412.

Kennington, M. H., T. W. Perry and W. M. Beeson. 1958. Effect of adding animal fat to swine rations. J. Anim. Sci. 17:1166(Abstr.).

Lawrence, N. J. and C. V. Maxwell. 1983. Effect of dietary fat source and level on the performance of neonatal and early weaned pigs. J. Anim. Sci. 57:936.

Leibbrandt V. D., V. W. Hays, R. C. Ewan and V. C. Speer. 1975. Effect of fat on performance of baby and growing pigs. J. Anim. Sci. 40:1081.

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, R. D. Klem and R. D. Goodband. 1991. Interrelationship between hypersensitivity to soybean proteins and growth performance in early-weaned pigs. J. Anim. Sci. 69:4062.

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, J. D. Hancock, G. L. Allee, R. D. Goodband and R. D. Klem. 1990. Transient hypersensitivity to soybean meal in the early-weaned pig. J. Anim. Sci. 68:1790.

Lloyd, L. E. and E. W. Crampton. 1952. The relation between certain characteristics of fats and oils and their apparent digestibility by young pigs, young guinea pigs and pups. J. Anim. Sci. 16:377.

Lowrey, R. S., W. G. Pond and J. H. Maner. 1958. The effect of the calorie-protein ratio on digestibility, feed efficiency and weight gain in growing swine. J. Anim. Sci. 17:1165(Abstr.).

Manners, M. J. and M. R. McCrea. 1963. Protein requirement of baby pigs 2. Requirement on high-fat diets and effect of replacing carbohydrate by fat. Br. J. Nutr. 17:357.

Moore, B. and D. P. Rockwood. 1897. On the mode of absorption of fats. J. Physiol. London. 21:58.

Owsley, W. F., D. E. Orr and L. F. Tribble. 1986. Effects of nitrogen and energy source on nutrient digestibility in the young pig. J. Anim. Sci. 63:492.

Ozimek, L., W. C. Sauer, W. Kozikowski and H. Jorgensen. 1984. The ileal and fecal digestibility of fat and fatty acids in pigs fed semi-purified diets containing beef tallow or rapeseed oil. 1984 Feeders Day Report, Univ. Alberta, Edmonton, 14.

Peo, E. R., G. C. Ashton, V. C. Speer and D. V. Catron. 1957. Protein and fat requirements of baby pigs. J. Anim. Sci. 16:885.

Rachford, B. K. 1891. The influence of bile on the fat splitting properties of pancreatic juice. J. Physiol. London 12:72.

Sewell, R. F and I. L. Miller. 1965. Utilization of various dietary fats by baby pigs. J. Anim. Sci. 24:973.

Small, D. M. 1970. Fedn. Proc. Fedn. Am. Soc. Exp. Biol. 29:1320.

Small, D. M. 1991. The effects of glyceride structure on absorption and metabolism. Ann. Rev. Nutr. 11:413.

Smith, H. and I.A.M. Lucas. 1956. The early weaning of pigs. I. The effect upon growth of variations in the protein, fat, sucrose, antibiotic vitamin and mineral contents of diets for pigs of 8-25 lb. live weight and a comparison of wet and dry feeding. J. Agric. Sci. 48:220.

Walker, W. R., G. L. Morgan and C. V. Maxwell. 1986. Ileal cannulation in baby pigs with a simple T-cannula. J. Anim. Sci. 62:407.

Wolfe, R. G., C. V. Maxwell and E. C. Nelson. 1978. Effect of age and dietary fat level on fatty acid oxidation in the neonatal pig. J. Nutr. 108:1621.

CHAPTER II

EFFECT OF REPLACING SOYBEAN MEAL WITH PLASMA PROTEIN AND SOURCE OF FAT IN PRESTARTER DIETS ON EFFICIENCY OF GAIN AND FEED UTILIZATION IN EARLY-WEANED PIGS

Abstract

A study utilizing 384 Yorkshire and Hampshire and Yorkshire x Hampshire crossbred early-weaned pigs was conducted to determine the effects fat source and protein source on performance. In experiment one, 108 pigs were fed a complex prestarter diet that included either soybean meal or porcine plasma protein and 8% added fat (coconut oil, soybean oil or tallow) for two weeks; weekly gain and feed intake measurements were recorded. Pigs fed a complex prestarter diet containing plasma proteins had superior ($P<.01$) performance when compared to pigs fed a complex prestarter diet containing soybean meal. During week 1, pigs fed diets containing soybean oil or tallow gained weight faster ($P<.01$) than pigs fed the diet containing coconut oil. Experiment two was conducted to determine if treatment responses observed in experiment one would be similar in pigs reared in more conventional nursery

facilities. Pigs housed in individual pens (experiment one) performed comparably to pigs housed in a more conventional nursery with six pigs per pen (experiment two). In experiment three, 144 early-weaned pigs were used to compare the effects of adding medium chain triglycerides or soybean oil to complex prestarter diets. Experimental diets were fed for two weeks (Period 1), followed by a common starter diet for three weeks (Period 2). In experiment three, pigs fed the diet containing plasma proteins had superior ($P<.01$) performance when compared to pigs fed a diet with soybean meal. Replacing soybean oil with medium chain triglycerides (included at 8% of the diet) in prestarter diets resulted in reduced average daily gain in weeks 1 and 2.

Key Words: Early Weaning, Plasma Protein, Fat Source, Medium Chain Triglycerides

Introduction

Maintenance of growth during the early post-weaning period would logically require a high energy diet to replace sow's milk (30-40% fat). Early-weaned pig diets are commonly supplemented with fat in order to increase the caloric density of the diet and thus more fully meet the energy requirements of the young pig. Many studies have been conducted to evaluate the effects of fat supplementation in diets for early-weaned pigs. Factors that influence the magnitude of response to added fat include the source of fat (Cera et al., 1988; Sewell and

Miller, 1965), inclusion rate (Aherne, et al, 1982), the form of carbohydrate (Aherne, et al. 1982) and energy:protein ratio (Aherne, et al. 1982; Allee et al., 1971). Other factors (such as the level of performance) may also exert an influence on the utilization of added fat in diets fed to early-weaned pigs.

Most fat sources used in swine diets are composed of long-chain fatty acids (triglycerides) derived from plants or animals. Fats of vegetable origin are generally more digestible than fats derived from animals (Cera et al., 1989a). Apparent digestibility of fat increases with time postweaning (Cera et al., 1988). Digestibility of triglycerides containing medium chain fatty acids is higher than triglycerides containing long chain fatty acids (Cera et al., 1989b). Medium chain triglycerides are a mixture of C6:0, C8:0, C10:0 and C12:0 medium chain fatty acids esterified to glycerol. Medium chain fatty acids (products of hydrolysis of medium chain triglycerides) are absorbed at a faster rate from the intestinal lumen than long chain fatty acids (Friedman and Nylund, 1980). Dietary fats containing medium chain triglycerides may be utilized more effectively than fats composed of long-chain fatty acids. De Rodas et al. (1990) observed an increase in performance with increasing levels of medium chain triglycerides in the diet (up to 6% maximum).

Many researchers have observed conflicting data when studying the utilization of fat in the young pig (Kennington

et al., 1958; Leibbrandt et al., 1975 and Aherne et al., 1982). One possible reason for the conflicting data is the age of pigs at weaning in these studies varied from 17 to 35 days of age. In addition, in previous studies, pigs were fed a simple corn-soybean based starter diet and exhibited very modest to poor performance.

Although the young pig can utilize a simple corn-soybean diet when weaned as early as 18 days of age, performance is markedly improved by incorporating alternate sources of protein into the diet. Li et al., (1991) reported that feeding early-weaned pigs a diet containing soybean meal resulted in decreased growth performance, lowered villus height, increased serum anti-soybean IgG titers and decreased absorptive capacity. Protein sources which have the potential of replacing soybean proteins in early-weaned pig diets should be readily accepted by the swine industry. One such readily available protein source is spray dried plasma protein, a by-product of pork slaughter houses. Sohn et al. (1991) reported that early-weaned pigs consuming diets containing plasma protein had improved gain and feed efficiency when compared to pigs fed a complex prestarter diet containing 10% dried skim milk.

The extent to which protein source affects utilization of other dietary ingredients has not been fully delineated. Therefore, this study was conducted in order to determine the effects of fat source and protein type on performance in early-weaned pigs fed a complex prestarter diet.

Materials and Methods

Pigs were housed in an environmentally controlled room in elevated pens with ad libitum access to both feed and water. A temperature of 84 to 86°F was maintained during week 1 and was decreased 2°F per week for the remainder of the experiment. Diets were formulated (Table 1) to meet or exceed 1988 NRC requirements for 5-10 kg pigs (period 1) and 10-20 kg pigs (period 2) with the exception of crude protein during period 2 (19.40% and 18.21%, respectively, compared to the NRC requirement of 20.0%). Plasma protein was substituted for soybean meal on an equal lysine basis.

All data were analyzed using the GLM procedure of SAS (1988) with pen as the experimental unit. The model included the effects of protein source, fat source, litter and replication. All possible interactions were evaluated. Specific differences between means were determined using the PDIFF procedure (SAS, 1988).

Experiment One

One hundred eight Yorkshire pigs (36 in each of three trials) were group weaned at 21 to 26 d of age and allotted by sex and weight within litter to one of six dietary treatments providing a total of eighteen pigs per treatment. Pigs were housed in individual, elevated metal pens in an environmentally controlled room. For the duration of each trial (14 days), pigs were assigned to one of six dietary

treatments in a 2 X 3 factorial arrangement of treatments. Diets (Table 1) used were: 1) complex prestarter diet containing soybean meal with 8% added tallow; 2) diet one with 8% coconut oil; 3) diet one with 8% soybean oil; 4) complex prestarter diet containing plasma protein with 8% added tallow; 5) diet four with 8% coconut oil; and 6) diet four with 8% soybean oil.

Experiment Two

This experiment was conducted to determine if responses observed in experiment one would be similar in pigs reared in more conventional nursery facilities. One hundred thirty-two Yorkshire and Hampshire pigs (72 in trial one and 60 in trial two) were group weaned at 21 to 26 days of age and assigned to pens with six pigs per pen in trial one and five pigs per pen in trial two. Each pen was randomly assigned to one of the six treatments imposed in experiment one. During the trial, two pigs died and three sick pigs were removed from treatment.

Experiment Three

One hundred forty-four Yorkshire, Hampshire and Yorkshire x Hampshire crossbred pigs (72 pigs in each of two trials) were group weaned at 21 to 28 days of age and stratified by weight, litter and sex and assigned to pens with six pigs in each pen. Pens were randomly assigned to one of four treatments in a 2 x 2 factorial experiment.

Dietary treatments during the first 14 days postweaning (Period 1) consisted of: 1) diet containing plasma protein with 8% added medium chain triglycerides as the fat source; 2) Diet 1 with 8% soybean oil as the fat source; 3) diet containing soybean meal with 8% added medium chain triglycerides as the fat source and 4) Diet 3 with 8% added soybean oil as the fat source. Diets were formulated (Table 1) to contain 20% edible grade whey, 10% dried skim milk and 1.4% lysine. All pigs were fed a common starter diet (Table 1) for an additional 3 weeks (Period 2) to evaluate any carry-over effects on performance from diets fed during period 1.

Results

Experiment One

The effect of fat source and inclusion of soybean meal on average daily gain, average daily feed intake and feed efficiency is presented in Table 2. Main effect means are presented since no trial*treatment or protein source*fat source interaction was observed. During week 1, pigs fed diets containing plasma protein tended (P=.20) to grow faster than pigs fed the diets with soybean meal and pigs fed soybean oil or tallow grew faster (P<.01) than those fed coconut oil. For week 2 and for the two week period, pigs fed diets containing plasma protein grew 16 and 13% faster (P<.01) respectively than pigs fed diets with soybean meal.

For the two week period, pigs fed tallow grew more rapidly (P=.05) than those fed coconut oil. Pigs fed diets containing plasma protein consumed 18% more feed (P=.0001) during week 2 than pigs fed the diets containing soybean meal. Observed differences in feed intake due to fat source during week 1, week 2 or the entire period were not significant (P>.05). Efficiency of feed utilization was not affected (P>.05) by fat source during week 1 or for the two week period, but during week 2, pigs fed coconut oil were 9% more efficient (P=.03) in gain per unit of feed than those fed soybean oil. In addition, pigs fed the diet containing soybean meal grew more efficiently (P=.01) than pigs fed the plasma protein diet. This difference may have been influenced by the difficulty in obtaining an accurate estimate of feed intake when pigs were housed individually.

Experiment Two

Data were combined and main effects means are presented since no trial*treatment or fat source*protein source interactions were observed. Pigs consuming diets that contained plasma protein grew faster (P<.05) during all experimental periods than pigs fed soybean meal based diets (24, 18 and 21% faster during week 1, week 2 and for the overall period respectively, Table 3). Average daily gain did not differ (P>.3) due to fat source. Average daily feed intake (Table 3) during weeks 1 and 2 and for the overall period did not differ (P>.08) due to fat or protein source.

Feed efficiency (Table 3) in pigs consuming diets containing plasma protein during week 1 and for the overall two week period was greater (24 and 14% respectively, P<.05) than feed efficiency values for pigs fed the complex prestarter diets containing soybean meal. Differences in feed efficiency due to fat source were not significant (P>.25).

Experiment Three

Main effect means are presented except in instances where a fat source*protein source or an interaction (P<.1) was observed. Data from both trials were combined since there was no trial*treatment interaction. Least squares means for average daily gain are presented in Table 4. For weeks 1 and 2 and during period 1, pigs fed the complex prestarter diets containing plasma protein grew 30, 11 and 15% faster (P<.01 for week 1 and P<.05 for week 2 and period 1), respectively than pigs fed the soybean meal based diet. In contrast, pigs receiving diets containing soybean meal during period 1 grew 24% faster (P=.005) during week 3 (the first week that a common corn-soybean meal diet was fed) when compared to pigs receiving the plasma protein diets in period 1. Pigs fed diets containing the soybean oil grew 27, 21 and 24% faster (P<.05) during week 1, week 2 and period 1 respectively, than pigs consuming diets containing medium chain triglycerides. Rate of gain was similar (P>.15) among treatments during weeks 4, 5 and period 2. However, pigs receiving diets containing soybean oil during

period 1 grew 9% faster (P<.05) during the total experiment than pigs fed the medium chain triglyceride fat source.

Average daily feed intake during week 2 and period 1 was 15 and 11% lower (P<.05) respectively, in pigs fed diets with soybean meal compared to pigs fed diets containing plasma protein (Table 5). During week 3 when pigs were offered a common corn-soybean meal diet, average daily feed intake was 10% higher (P<.05) in pigs fed diets containing soybean meal during period 1. Pigs offered diets containing soybean oil during week 1, week 2 and period 1 consumed 16, 15 and 16% more (P<.05) feed respectively, than pigs receiving diets containing medium chain triglycerides.

There was a diet*treatment interaction (P<.01) during period 1 and weeks 2 and 4, therefore the subclass means are presented (Table 6). During week 2 and period 1, pigs consuming the diets containing soybean meal with added medium chain triglycerides had a lower (P<.05) average daily feed intake than pigs fed diets with soybean oil, whereas feed intake was not affected by fat source in pigs fed the plasma protein complex diet.

Feed efficiency (gain:feed) during week 1 was 19% greater (P<.05) for pigs receiving the diets with plasma protein (Table 7). In contrast, pigs consuming the diets with plasma protein during the first period had a 19% lower (P<.05) feed efficiency during week 3 when all pigs were fed a common corn-soybean meal diet. During all other periods, no significant difference (P>.05) in feed

efficiency due to treatments was observed.

Discussion

Feeding early-weaned pigs a complex prestarter diet containing porcine plasma proteins resulted in an increase in performance when compared to pigs fed a complex prestarter diet containing soybean meal. These results are consistent with previous research indicating that plasma proteins will improve performance in early-weaned pigs (Hansen, et al., 1991 and Sohn et al., 1991). The ADG data presented here agree with the results of Gatnau and Zimmerman (1991) in that the benefits of porcine plasma protein addition to starter diets are pronounced in the early post-weaning period

Pigs receiving diets containing soybean meal during period 1 grew faster (P=.005) during week 3 when compared to pigs receiving the plasma protein diets in period 1. This suggests that adaptation to soybean protein was still necessary two weeks postweaning in pigs that were fed diets devoid of soybean proteins. In addition, pigs fed the diet containing soybean meal during period 1 had higher average daily feed intake (P=.02) during week 3 than pigs fed diets containing soybean meal during period 1. This increased intake is consistent with the observed increased gain and may be due to the consumption of soybean meal during period 1, which may increase the subsequent utilization of soybean proteins during week 3.

Soybean oil was a better fat source than the medium chain triglyceride source used in experiment three. In previous studies (De Rodas and Maxwell, 1990), medium chain triglycerides improved performance in early-weaned pigs when fed up to 6% of the diet . The lack of consistency in the effect of medium chain triglycerides may be due to the higher inclusion rate (8%) of medium chain triglycerides used in this study. Cera et al. (1989) reported that pigs fed diets supplemented with coconut oil had higher weight gains than pigs fed corn oil or tallow supplemented diets each week for four weeks in pigs weaned at 21 d of age. This data is in conflict with the data presented here. However, it should be noted that the diets in Cera et al. (1989) used were formulated to contain 35.3 % SBM which may have biased the results (ADG of .1 and .3 kg for weeks one and two respectively).

Although no statistical analyses could be performed due to differing experimental units, pigs housed in individual pens (experiment one) performed comparably to pigs housed in a more conventional nursery with six pigs per pen (experiment two). This suggests that data obtained from conducting experiments using the metabolism crates in this experiment may be used as a estimate of performance in conventional nurseries.

Literature Cited

Cera, K. R., D. C. Mahan and G. A. Reinhart. 1989. Apparent fat digestibilities and performance responses of postweaning swine fed diets supplemented with coconut oil, corn oil or tallow. J. Anim. Sci. 67:2040.

De Rodas, B. and C. V. Maxwell. 1990. The effect of creep feeding on postweaning performance in early weaned pigs. Okla. Agric. Exp. Sta. Res. Rep. 278.

Friedman, H. I. and B. Nylund. 1980. Intestinal fat digestion, absorption and transport. Am. J. Clin. Nutr. 33:1108.

Hansen, J. A., J. L. Nelssen and R. D. Goodband. 1991. Evaluation of plasma proteins and meat extract as replacement protein sources for dried skim milk in starter swine diets. Kansas Swine Day Proceedings, p. 44.

Kennington, M. H., T. W. Perry and W. M. Beeson. 1958. Effect of adding animal fat to swine rations. J. Anim. Sci. 17:1166.

Leibbrandt, V. D., V. W. Hays, R. C. Ewan and V. C. Speer. 1975. Effect of fat on performance of baby and growing pigs. J. Anim. Sci. 40:1081.

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, R. Klemm and R. D. Goodband. 1991. Interrelationship between hypersensitivity to soybean proteins and growth performance in early-weaned pigs. J. Anim. Sci. 69:4062.

SAS. 1988. SAS/STAT User's Guide (6th Ed.). SAS Inst. Inc., Cary, NC.

Sohn, K. S., C. V. Maxwell and D. S. Buchanan. 1991. Spray dried plasma protein as a protein source for early weaned pigs. Okla. Agric. Exp. Sta. Res. Rep. 342.

Table 1. Composition[a] of diets (Exp. 1, 2 and 3)

Ingredient	Period 1		Period 2
	Plasma protein	SBM	
Dried skim milk	10.0	10.0	--
Whey	20.0	20.0	--
Corn	46.2	38.4	66.65
AP-820[b]	6.8	--	--
SBM, 44%	--	15.0	28.5
Fat source[c]	8.0	8.0	--
Lysine, HCl	.25	.25	.15
Fishmeal, Menhaden	5.0	5.0	--
Ethoxyquin	.025	.02	--
FOA[d] 390	1.0	1.0	1.0
Flavor, Berry	.1	.1	--
$CuSO_4$	.1	.1	.075
DL-Methionine	--	.2	--
Vitamin premix[e]	.74	.74	.375
Calcium carbonate	--	--	.90
Dical	1.75	1.35	--
Salt	--	--	.40
Calculated composition of diet:			
ME (Mcal/lb)	1.62	1.62	1.45
CP (%)	18.21	19.40	18.32
Lys (%)	1.40	1.40	1.11
Trp (%)	.25	.25	.24
Thr (%)	.91	.86	.72
Met + Cys (%)	.72	.70	.61
Ca (%)	1.07	.99	.88
P (%)	.85	.85	.74

[a] As fed basis.
[b] Plasma protein source, American Protein Corporation, Ames, Iowa.
[c] Coconut oil, soybean oil, tallow or medium chain triglycerides (MCT).
[d] Provided 10 g Furazolidone, 5 g Oxytetracycline, 4.5 g Arsanilic acid per lb of diet.
[e] Supplied 4,160 IU vitamin A, 416 IU vitamin D, 18 IU vitamin E, 20 mg pantothenic acid, 28 mg niacin, 4.0 mg riboflavin, 7.3 mg menadione sodium bisulfate, .02 mg vitamin B_{12}, 1.3 mg biotin, 2.7 mg pyridoxine, .9 mg folic acid, 3.9 mg thiamin, 267 mg choline, .1 mg selenium, .03 g manganese, .1 g zinc, .1 g iron, .1 g copper, .2 g magnesium, .43 g potassium and .2 mg iodine per lb of feed.

Table 2. Effect of protein and fat source on performance (Exp. 1)[a]

Item	Protein source		Fat source		
	AP-820	SBM	Coconut	Soy oil	Tallow
Average daily gain (lb)					
Week 1	.49	.45	.40[b]	.51[c]	.51[c]
Week 2	.96[b]	.83[c]	.88	.89	.92
Period	.71[b]	.63[c]	.62[d]	.69[de]	.70[e]
Average daily feed intake (lb)					
Week 1	.89	.86	.84	.88	.91
Week 2	1.39[b]	1.09[c]	1.19	1.30	1.24
Period	1.25	1.11	1.07	1.23	1.24
Average daily feed efficiency (gain:feed)					
Week 1	.61	.58	.54	.65	.60
Week 2	.75[b]	.82[c]	.82[d]	.76[e]	.79[de]
Period	.68	.69	.66	.70	.69

[a] Least squares means.
[b,c] Means within row and within main effect not bearing a common superscript differ ($P<.01$).
[d,e] Means within row and within main effect not bearing a common superscript differ ($P<.05$).

Table 3. Effect of protein and fat source on performance (Exp. 2)[a]

Item	Protein source		Fat source		
	AP-820	SBM	Coconut	Soy oil	Tallow
Average daily gain (lb)					
Week 1	.52[b]	.42[c]	.46	.49	.45
Week 2	.87[b]	.74[c]	.83	.82	.76
Period	.70[b]	.58[c]	.64	.66	.61
Average daily feed intake (lb)					
Week 1	.53	.52	.55	.52	.52
Week 2	1.16	1.04	1.08	1.18	1.04
Period	.85	.78	.81	.85	.78
Average daily feed efficiency (gain:feed)					
Week 1	.99[b]	.80[c]	.84	.94	.89
Week 2	.76	.71	.77	.72	.72
Period	.84[b]	.74[c]	.79	.79	.78

[a] Least squares means.
[b,c] Means within row and within main effect not bearing a common superscript differ ($P<.05$).

Table 4. Effect of protein and fat source on average daily gain (Exp. 3)[a]

	Protein source		Fat source	
Item (lb)	AP-820	SBM	MCT	Soy oil
Week 1	.39[b]	.30[c]	.30[d]	.38[e]
Week 2	.84[d]	.76[e]	.72[b]	.87[c]
Period 1	.61[b]	.53[c]	.51[b]	.63[c]
Week 3	.71[b]	.88[c]	.76	.82
Week 4	1.01	.97	.97	1.00
Week 5	1.03	1.07	1.05	1.04
Period 2	.92	.97	.93	.96
Total	.80	.80	.76[d]	.83[e]

[a] Main effect least squares means, experiment three.

[b,c] Means within main effect in the same row not bearing a common superscript differ (P<.01).

[d,e] Means within main effect in the same row not bearing a common superscript differ (P<.05).

Table 5. Effect of protein and fat source on average daily feed intake (Exp. 3)[a]

	Protein source		Fat source	
Item (lb)	AP-820	SBM	MCT	Soy oil
Week 1	.41	.38	.36[c]	.43[b]
Week 2	1.00[b]	.85[c]	.87[b]	.99[c]
Period 1	.70[d]	.62[e]	.61[d]	.71[e]
Week 3	1.35[b]	1.48[c]	1.38	1.46
Week 4	1.85	1.85	1.85	1.85
Week 5	1.98	1.98	1.99	1.98
Period 2	1.73	1.77	1.74	1.76
Total	1.15	1.11	1.13	1.13

[a] Main effect least squares means, experiment three.

[b,c] Means within main effect in the same row not bearing a common superscript differ ($P<.05$).

[d,e] Means within main effect in the same row not bearing a common superscript differ ($P<.01$).

Table 6. Effect of protein and fat source on average daily feed intake (Exp. 3)[a]

	Plasma protein		SBM	
Item (lb)	MCT	Soy oil	MCT	Soy oil
Week 2[b]	1.00[c]	1.00[c]	.73[d]	.97[c]
Period 1[b]	.69[c]	.72[c]	.54[d]	.69[c]
Week 4[b]	1.78	1.92	1.92	1.76

[a] Least squares means, experiment three.
[b] Diet*fat interaction (P<.1), therefore simple effects are presented.
[c,d] Means within rows not bearing a common superscript differ (P<.05).

Table 7. Effect of protein and fat source on feed efficiency (Gain:Feed)[a]

	Protein source		Fat source	
Item	AP-820	SBM	MCT	Soy oil
Week 1	.94[b]	.79[c]	.83	.90
Week 2	.85	.90	.86	.89
Period 1	.88	.87	.86	.89
Week 3	.51[b]	.58[c]	.54	.55
Week 4	.55	.53	.53	.54
Week 5	.52	.54	.53	.53
Period 2	.53	.55	.53	.55
Total	.60	.62	.59	.63

[a] Main effect least squares means.
[b,c] Means within main effect in the same row not bearing a common superscript differ (P<.05).

CHAPTER III

SYNTHESIS AND SPECTRAL CHARACTERIZATION OF 1,2,3-TRITETRADECYL GLYCERYL ETHER: A HYDROPHOBIC, EXTERNAL MARKER FOR USE IN DIGESTIBILITY STUDIES

Abstract

A simplified procedure is described for the synthesis of 1,2,3-tritetradecyl glyceryl ether (TGE). The ether is prepared via a three step alkylation of glycerol in the presence of NaOH (alkaline conditions) using bromotetradecane as the alkylating agent. This compound has the potential to be used as a nonabsorbable, lipid soluble marker in determining fatty acid digestibilities or any other lipid soluble fraction in the diet. TGE is lipid soluble and therefore should be more closely associated with the lipid fraction of the diet. Cr_2O_3 has been shown to travel through the digestive tract at a different rate than lipid components. TGE and fatty acid methyl esters can be analyzed in one step via gas chromatography in less than 30 min under optimum conditions. Cr_2O_3 requires a separate analysis thus necessitating use of more time and resources and introducing an additional source of error in the analytical procedure. Very small quantities (.05%) of TGE

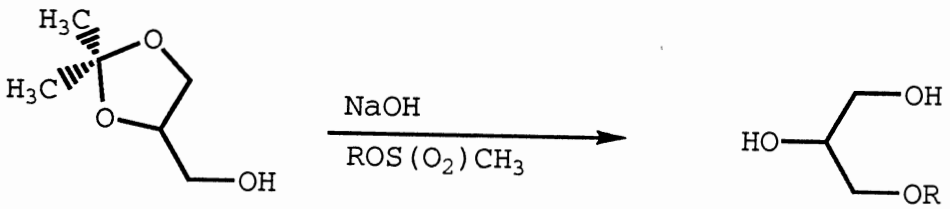
are sufficient for use as a marker, due to the sensitivity of the quantification system.

Key Words: External Lipid Soluble Marker, Absorption, GC

Introduction

Determining lipid absorption involves accurate quantification of the ingested and unabsorbed residual lipid. Absorption can be estimated by measuring the quantity of lipid ingested, and then estimating the total excretion of lipid from total fecal collection. Total collection of feces is not practical. Researchers have utilized nonabsorbable markers as an alternative to total fecal collection. It has recently been suggested (Owens and Hanson, 1992) that the characteristics of an ideal marker include 1) it must not be absorbed; 2) it must not affect or be affected by the digestive tract; 3) it must flow parallel with or be physically similar to or intimately associated with the material it is to mark; and 4) a specific and sensitive method to quantify the marker must be available.

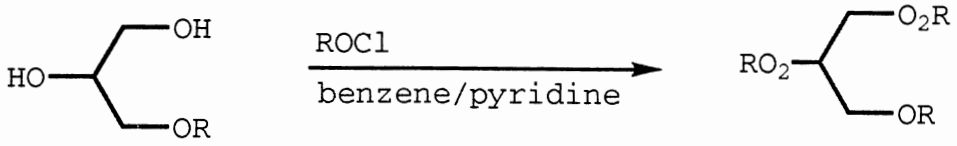
Baumann and Mangold (1964) used methanesulfonates of saturated and unsaturated long-chain alcohols to synthesize alkyl glyceryl-(1) ethers (3-alkoxy-1,2-propanediols, **1**) by



1

reaction with 1,2-O-isopropylidene-glycerol.

The alkyl glyceryl ethers were allowed to react with acid chlorides in benzene-pyridine to prepare alkoxydiglycerides **2** (Baumann and Mangold, 1966).

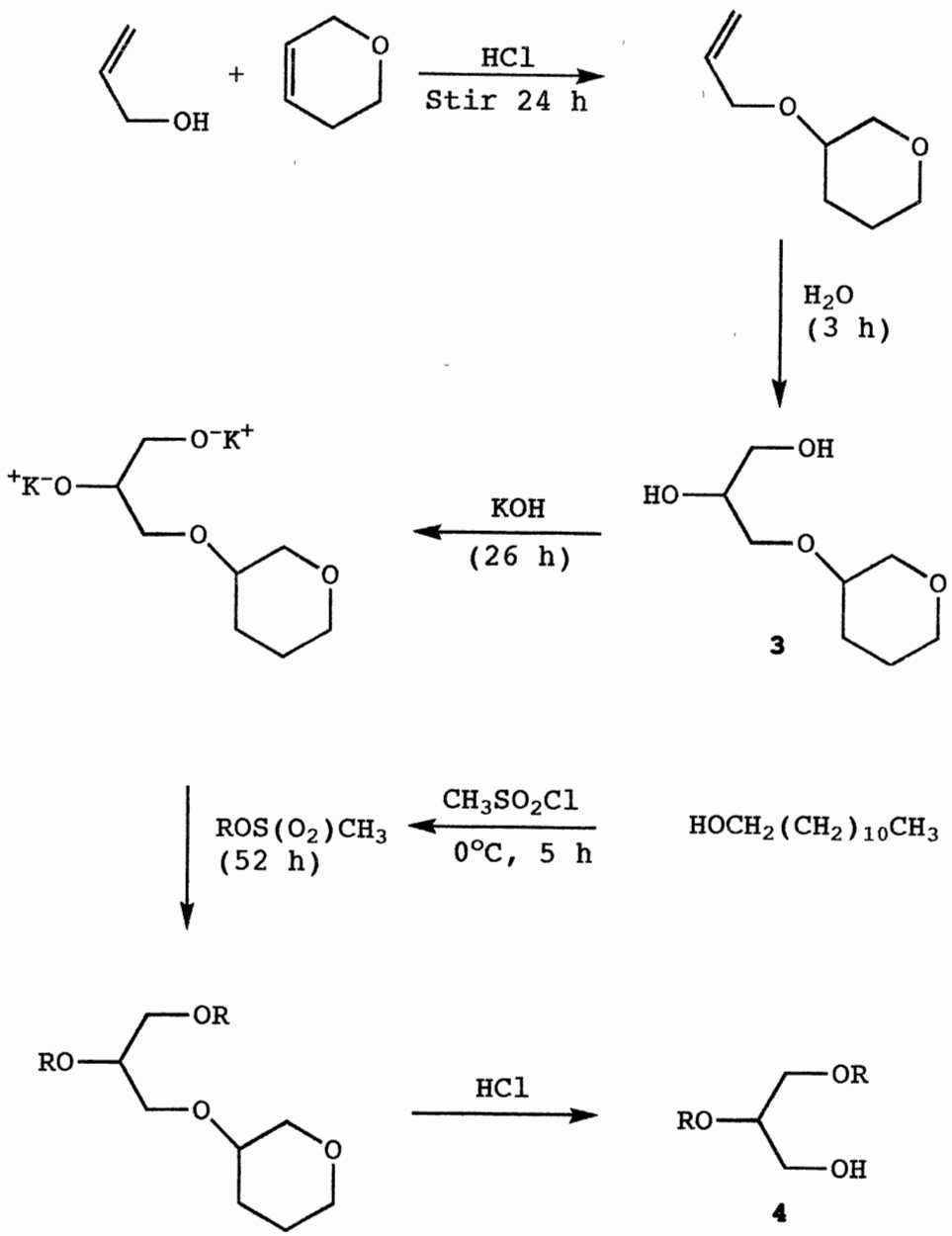


2

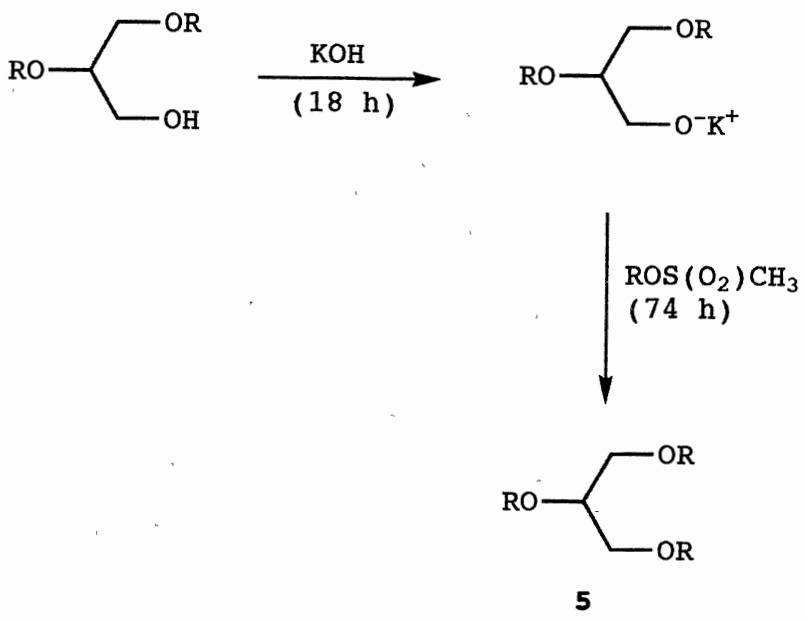
Paltauf and Spener (1968) reported the synthesis of trialkyl glyceryl ethers starting with 3-tetrahydropyranyl glycerol ether (**3**) and alkyl methylsulfonates. The ether **3** was prepared following the procedure of Barry and Craig (1955) and the methylsulfonates by the method of Baumann and Mangold (1964). The tetrahydropyranyl moiety serves as a protecting group for the hydroxyl group on the third carbon of glycerol and it is easily removed by acid cleavage after alkylation of the other two hydroxyl groups. The 1,2-dialkyl glyceryl ether (**4**) was made by allowing the 3-tetrahydropyranyl glycerol ether (**3**) to react with two equivalents of an alkyl methlysulfonate in the presence of KOH. Acid hydrolysis of the tetrahydropyranyl moiety gave the ether **4**. After isolation and purification of the diether, the third equivalent of alkyl methylsulfonate was added to afford the 1,2,3-trialkyl ether **5**.

One possible reason for the limited use of this marker in the past 20 years is the arduous synthetic procedure

utilized by Paltauf and Spener (1968) to obtain the 1,2,3-tridodecyl glyceryl ether.



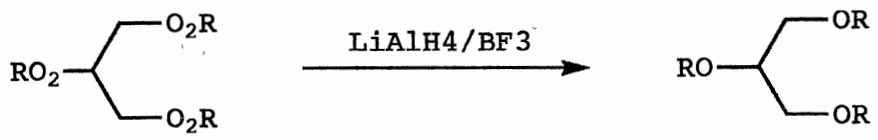
Spener et al. (1968) later used trioctadecenyl glyceryl ether to study the intestinal absorption of fats and found the triether to be very poorly absorbed from the gastrointestinal tract of rats.



5

Attempts have been made to simplify synthesis of glycerol triethers (Mangold, 1979) but limited success has been achieved. Go and Branen (1975) reported the reduction of triglycerides to their corresponding ethers **6** derivatives using a combination of $LiAlH_4$ and BF_3 with yields of less than three percent.

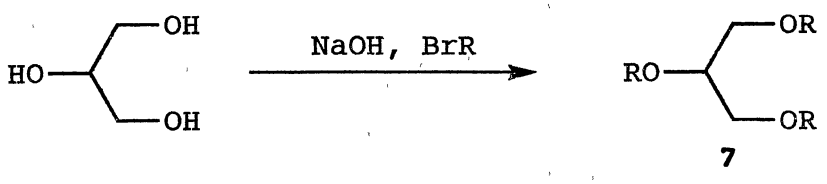
It has been reported that attempts to obtain trialkyl ethers via the Williamson ether synthetic route were



6

unsuccessful (Carlson, 1970 and Mangold, 1979). Müller

(1977), however, reported the direct alkylation of glycerol to trialkyl glycerol ethers (7). The authors reported yields of 22-83%. The major drawback of this procedure was the long reaction times at very reduced pressures (0.1-1.0



7

mm Hg). Most nutrition labs are not equipped to run reactions at .1 mm Hg.

Carlson and Bayley (1972) reported the synthesis of tridodecyl glyceryl ether and described using the triether as an indicator of fat absorption. Total recovery of the triether was comparable to that of Cr_2O_3 when these markers were included in diets given to rats. In addition, the Cr_2O_3 passed from the stomach faster than did the fat in the diet, while the triether and fat left the stomach together. The authors concluded that the triether was a suitable indicator of fat absorption from different regions of the gastrointestinal tract.

Evaluation of triethers as fecal flow markers has also been compared to ß-sitosterol. St. Clair et al. (1976) fed diets containing ß-sitosterol, 1-hexadecyl-2,3-didodecyl glyceryl triether and chromic oxide to African green monkeys. For all animals studied, excretion of ß-sitosterol

and triether paralleled one another.

Hoving et al. (1977a) suggested the use of radio labeled triethers for use as markers for triglycerides during gastric emptying. In an additional study, Hoving et al. (1977b) reported the simultaneous use of ^{131}I-triolein and ^{75}Se-triether in a single oral dose to estimate fat absorption. The results in normal rats showed that this dual isotope technique gave a valid estimate of fat absorption by analysis of only a single stool sample.

Therefore, this research was initiated to find a more facile procedure for the synthesis of trialkyl glyceryl ethers. A simplified synthetic procedure combined with an accurate and sensitive quantification system should allow for broader use of this lipid-soluble, nonabsorbable marker, especially within animal agriculture.

Materials and Methods

Chemical reagents were purified before use when deemed necessary. The ^{1}H and ^{13}C NMR spectral data were obtained on a Varian XL-400 NMR spectrometer operating at 399.9 MHz for ^{1}H NMR and at 100.6 MHz for ^{13}C NMR. All NMR data were recorded in δ or ppm values downfield from tetramethyl silane (TMS) with $DCCl_3$ as the solvent. Liquid Secondary Ion Mass Spectrometry (LSIMS) analyses were performed on a VG Analytical (Manchester, Eng.) ZAB2 SE high resolution spectrometer tuned to a resolution of 10,000 (FWHM definition) and operated at 8 KV accelerating voltage with a

Cs ion gun potential of 35 KV. Data were acquired over the mass range of 50-800 using a scan time of 15 sec. The matrix used was 3-nitrobenzyl alcohol. GC/MS data were obtained using a VG Analytical TS 250 mass spectrometer tuned to a resolution of 500 (FWHM definition) and operated at 70 eV electron energy and 4 KV accelerating potential. Data were acquired at a rate of 2 s/scan from 50-500 m/Z. Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and were uncorrected. Liquid chromatography was accomplished using a Chromatotrom Model 7924T (Harrison Research Inc., 840 Moana Court, Palo Alto, CA 94306) with silica gel as the adsorbent.

Procedure for Using the Chromatotron

The plates used for the centrifugal chromatography were prepared by shaking 115 g (0-5°C) of silica gel (PF type 60, EM Science) and 200 mL (0-5°C) of distilled water in a 500-mL Erlenmeyer flask for 60 sec. The slurry was then poured onto the previously washed glass plate (120 mm radius) beginning with the edge and moving inward with a spiral pattern. The plate was allowed to harden (stand for 30 min.) and then placed on a rotor and turned at room temperature for 24 h. The plate was scraped with the equipment provided with the Chromatotron to give a 4 mm thick plate with an inner radius of 40 mm and an outer radius of 113 mm.

For separations, a sample was dissolved in a minimum amount of hexane and slowly added to a plate that was previously saturated with hexane. The separation was accomplished by using a gradient elution series with hexane as the nonpolar solvent and diethyl ether as the polar solvent. A chromophore was not present, therefore 10 mL fractions were collected in test tubes. The separation was monitored by thin-layer chromatography using silica gel as the solid phase and 10% ether in hexane as the solvent. Plates were developed by spraying the plates with a solution of potasium dichromate followed by heating on a hot plate set to 150^{o}C.

Gas-Liquid Chromatography

Analyses were performed utilizing a Hewlett-Packard 5890A gas-liquid chromatograph fitted with a splitless injector, flame ionization detector and an HP 3396 Series II integrator. A DB-WAX capillary column (J&W Scientific, 30 m length, .25 mm inner diameter and .25 μm film thickness) was used for all analyses. Parameters were optimized by comparing the number of theoretical plates and the baseline resolution obtained from the separation of a standard mixture of FAME (The order of elution of was $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{19:0}$, $C_{18:3}$, $C_{22:0}$, $C_{22:4}$ and TGE; See Plate IV). The ditetradecyl ether by-product eluted before the triether, with total run times of less than 30 min. Peaks were identified by comparing mass

spectral data from purified compounds obtained from the synthetic procedure to follow and the mass spectra obtained from GC-MS analyses.

Synthesis of 1,2,3-Tritetradecyl Glyceryl Ether

Into a 500 mL round bottom flask equipped with a nitrogen inlet, a dean-stark trap, a pressure-equalizing addition funnel (with a rubber septum sealing the 14/20 ground glass joint) and a magnetic stirrer was placed 10.0 g (.25 mol) of powdered NaOH, 18.42 g of glycerol (.20 mol) and 200 mL of toluene. Stirring was initiated and the mixture was heated and allowed to reflux for 2 h. After allowing the mixture to cool to room temperature, freshly distilled bromotetradecane (74.87 g, .27 mol) was added and the resulting slurry was heated to reflux. After heating for 24 h, the solution was again cooled, a second equivalent of NaOH (10.0 g, .25 mol) and bromotetradecane (74.87 g, .27 mol) were added and heating was resumed. Following an additional 24 h of heating, the resulting slurry was cooled and the third equivalents of NaOH (10.0 g, .25 mol) and bromotetradecane (74.87 g, .27 mol) were added and heating to reflux was resumed. Heating was terminated after 48 h, but the reaction was stirred for an additional 2 h.

The resulting slurry was transferred to a 1000 mL separatory funnel and the reaction flask was rinsed with H_2O (3 X 100 mL) and diethyl ether (3 X 100 mL). The mixture was extracted with ether (3 X 200 mL) and the combined

extracts were dried ($MgSO_4$) overnight. The solvent was removed (rotary evaporator) and the resulting light colored oil was fractionally distilled to give 100 g (70%) of crude 1,2,3-tritetradecylglyceryl ether. Purified 35 was obtained by following the general procedure described for the Chromatotron. The entire separation was completed in 1 h. The solvent was removed (rotary evaporator), and the resulting oil solidified upon cooling to give purified ether **2** as a white powder: mp 34.0-34.5°C; Mass spectrum - see Plate I; NMR data - see Plates II and III.

Results and Discussion

Tritetradecyl glyceryl ether was prepared in good yield via a simplified three step addition of bromotetradecane to glycerol in the presence of NaOH. Isolation of the triether from the reaction mixture was relatively facile. The semi-purified triether that was obtained after fractional distillation was contaminated with small amounts of the ditetradecyl ether. This compound did not interfere with the gas chromatographic analysis of the triether nor fatty acid methyl esters (up to $C_{22:0}$) and thus the semi-purified ether may be used in digestibility studies.

Identification of **3** was accomplished by obtaining and interpreting the appropriate spectral data. The ^1H NMR shown in Spectrum 11 gave chemical shift values consistent with the expected triether structure. The ^{13}C NMR spectra (Spectrum 12) gave only 14 spectral lines, with the four

downfield carbon signals [77.90 C(2), 71.66 C(1,3), 70.85 C(1',3') and 70.62 C(2')] in excellent agreement with expected chemical shift values. The lack of resolution of the side-chain aliphatic carbons is consistent with the spectra of the starting bromotetradecane (Spectrum 3) and the ditetradecyl ether by-product (Spectrum 6) and is due to coalescing of the methylene carbons on the aliphatic side chains. The exact mass obtained (calc.: 681.7125; found: 681.7135 $[M+H]^+$) was within acceptable limits (10 PPM) for current analytical publication.

The structure of 1,2,3-tritetradecyl glyceryl ether is chemically similar to several compounds which have been shown to be suitable for use as lipid soluble, external marker (Hoving et al., 1977; Morgan and Hofmann, 1970; Oswald et al., 1966; 1966; Spener et al., 1968; St Clair et al., 1975).

The relative ease of the GC analysis combined with the ease of synthesis and isolation of 1,2,3-tritetradecyl glyceryl ether should enhance the use of this triether as a nonabsorbable, inert, lipid soluble, external marker for use in studying lipid absorption and metabolism.

Literature Cited

Barry, P. J. and B. M. Craig. 1955. Glyceride Synthesis I. Synthesis of symmetrical diglycerides from dihydroxy acetone and allyl alcohol. Can. J. Chem. 31:716.

Baumann, W. J. and H. K. Mangold. 1964. Reactions of aliphatic methanesulfonates. I. Synthesis of long-chain glyceryl-(1) ethers. J. Org. Chem. 29:3055.

Baumann, W. J. and H. K. Mangold. 1966. Reactions of aliphatic methanesulfonates. II. Synthesis of long-chain di- and trialkyl glyceryl ethers. J. Org. Chem. 31:498.

Carlson, W. E. 1970. Tridodecyl glyceryl ether as a fat absorption indicator. Ph.D. Dissertation, U. Guelph, Canada.

Carlson, W. E. and H. S. Bayley. 1972. Preparation and use of a glyceryl triether as an indicator of fat absorption. Br. J. Nutr. 28:295.

Go, H. C. and A. L. Branen. 1975. Synthesis of long chain alkyl glyceryl ethers from triglycerides using boron trifluoride etherate and lithium aluminum hydride. J. Am. Oil Chem. Soc. 52:427.

Hoving, J., J.H.P. Wilson, J. J. Pratt and M. G. Woldring. 1977a. Reevaluation of triethers as markers for triglycerides during gastric emptying. Digest. Disease 22:449.

Hoving, J., J.H.P. Wilson, A. J. Valkema and M. G. Woldring. 1977b. Estimation of fat absorption from single fecal specimens using ^{131}I-triolein and ^{75}Se-triether. Gastroenterology 72:406.

Mangold, H. K. 1979. Synthesis and biosynthesis of alkoxylipids. Angew. Chem. Int. Ed. Engl. 18:493.

Morgan, R.G.H. and A. F. Hofmann. 1970. Use of 3H-labeled triether, a nonabsorbable oil-phase marker, to estimate fat absorption in rats with cholestyramine-induced steatorrhea. J. Lipid Res. 11:231.

Müller, V. V. 1977. Synthese und eigenschaften gesättigter trialkylglycerinäther. Fette Seifen Anstrichm. 79:305.

Oswald, E. O.; C. Piantadosi, C. E. Anderson and F. Snyder. 1966. The synthesis of ^{14}C- and ^{3}H-labeled glycerol ethers. Lipids 1:241.

Owens, F. N. and C. F. Hanson. 1992. External and internal markers for appraising site and extent of digestion in ruminants. J. Dairy Sci. 75:2605.

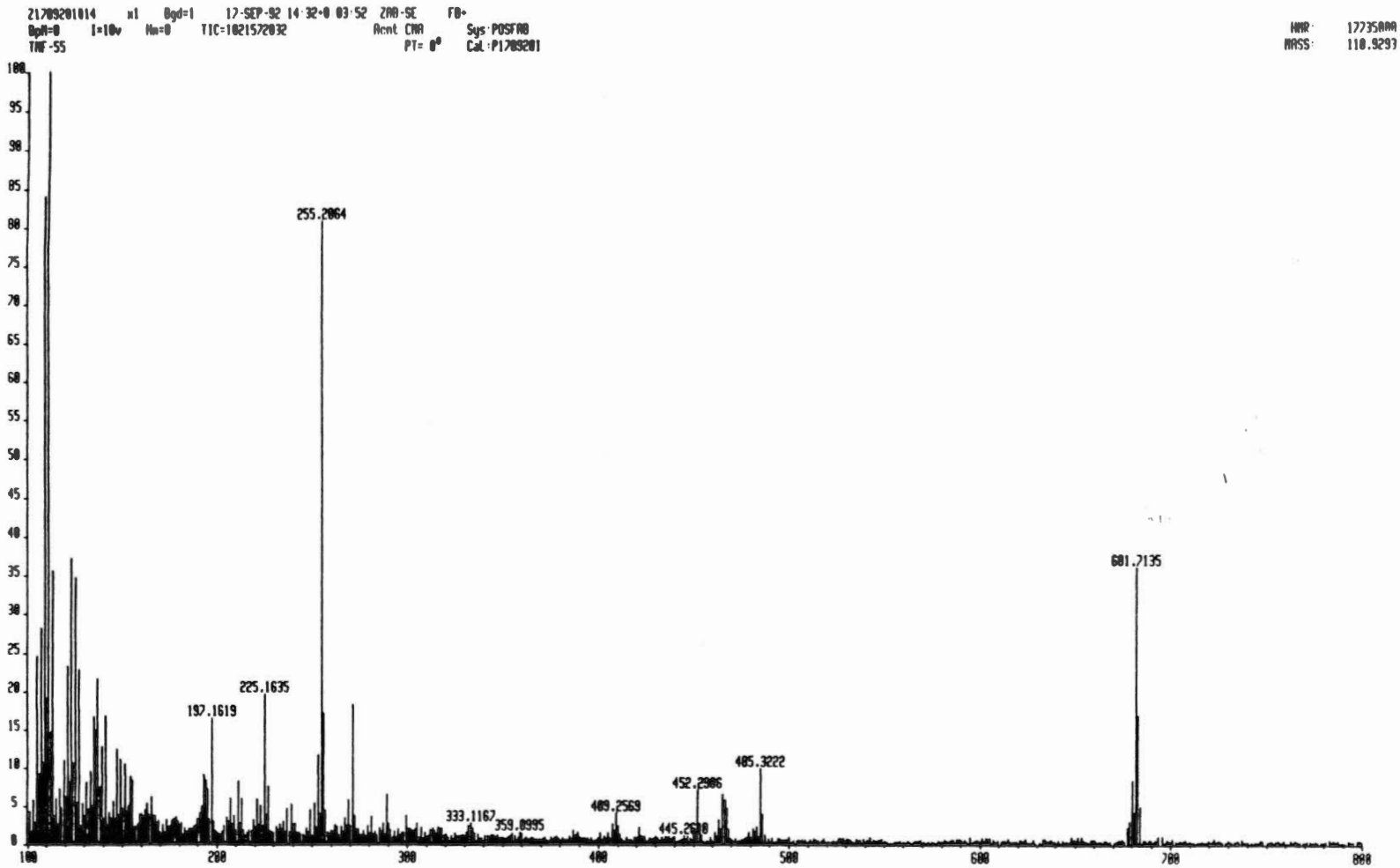
Paltauf, F. and F. Spener. 1968. An improved synthesis of 1,2-dialkyl glycerol ethers and the synthesis of 14C labeled trialkyl glycerol ethers. Chem. Phys. Lipids 2:168.

Spener, F., F. Paltauf and A. Holasek. 1968. The intestinal absorption of glycerol trioctadecenyl ether. Biochim.

Biophys. Acta 152:368.

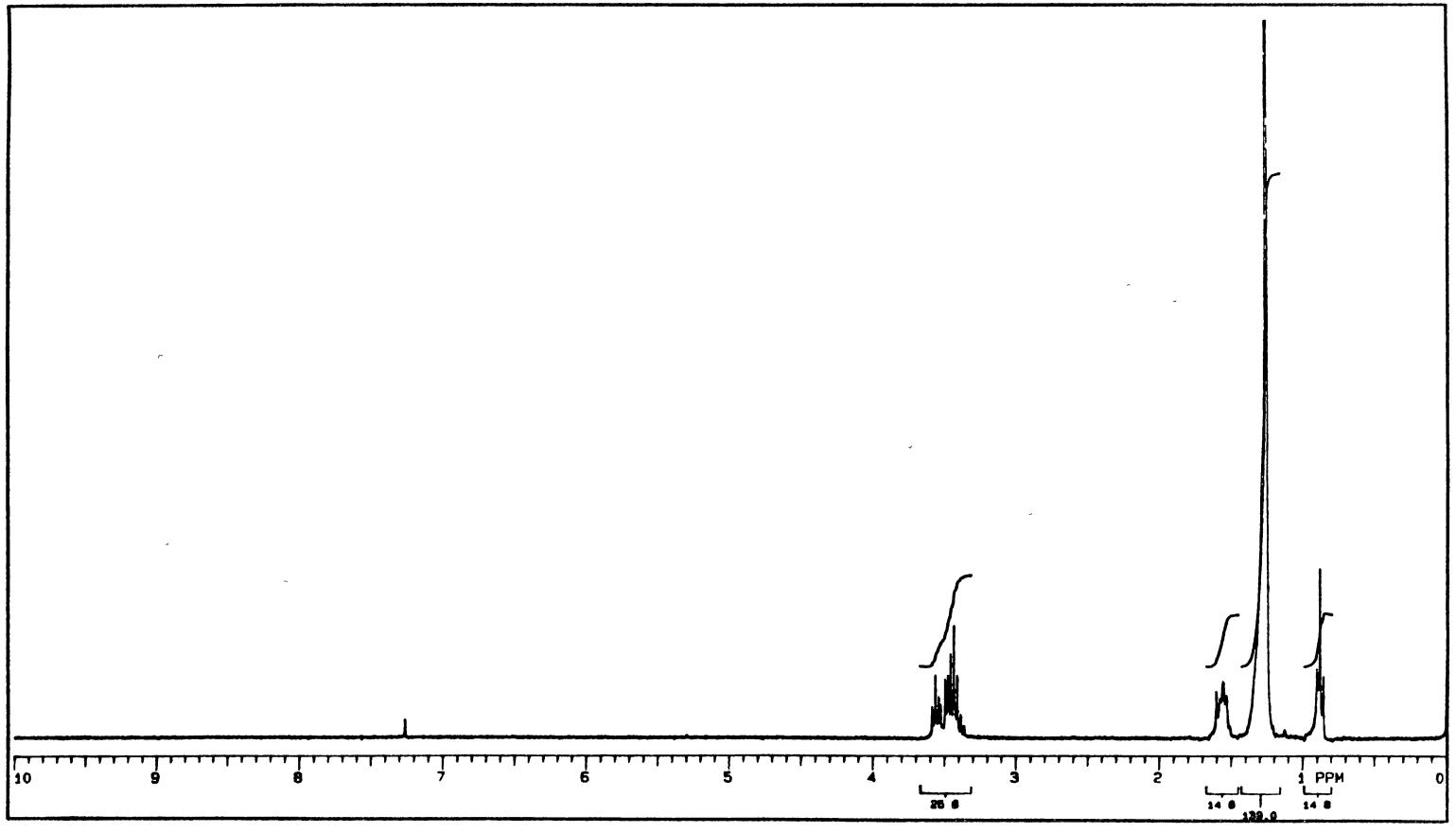
St. Clair, R. W., N.D.M. Lehner and T. E. Hamm. 1975. Evaluation of chromic oxide, glycerol triether, and B-sitosterol as fecal flow markers in two species of nonhuman primates. Lipids 10:25.

Plate I



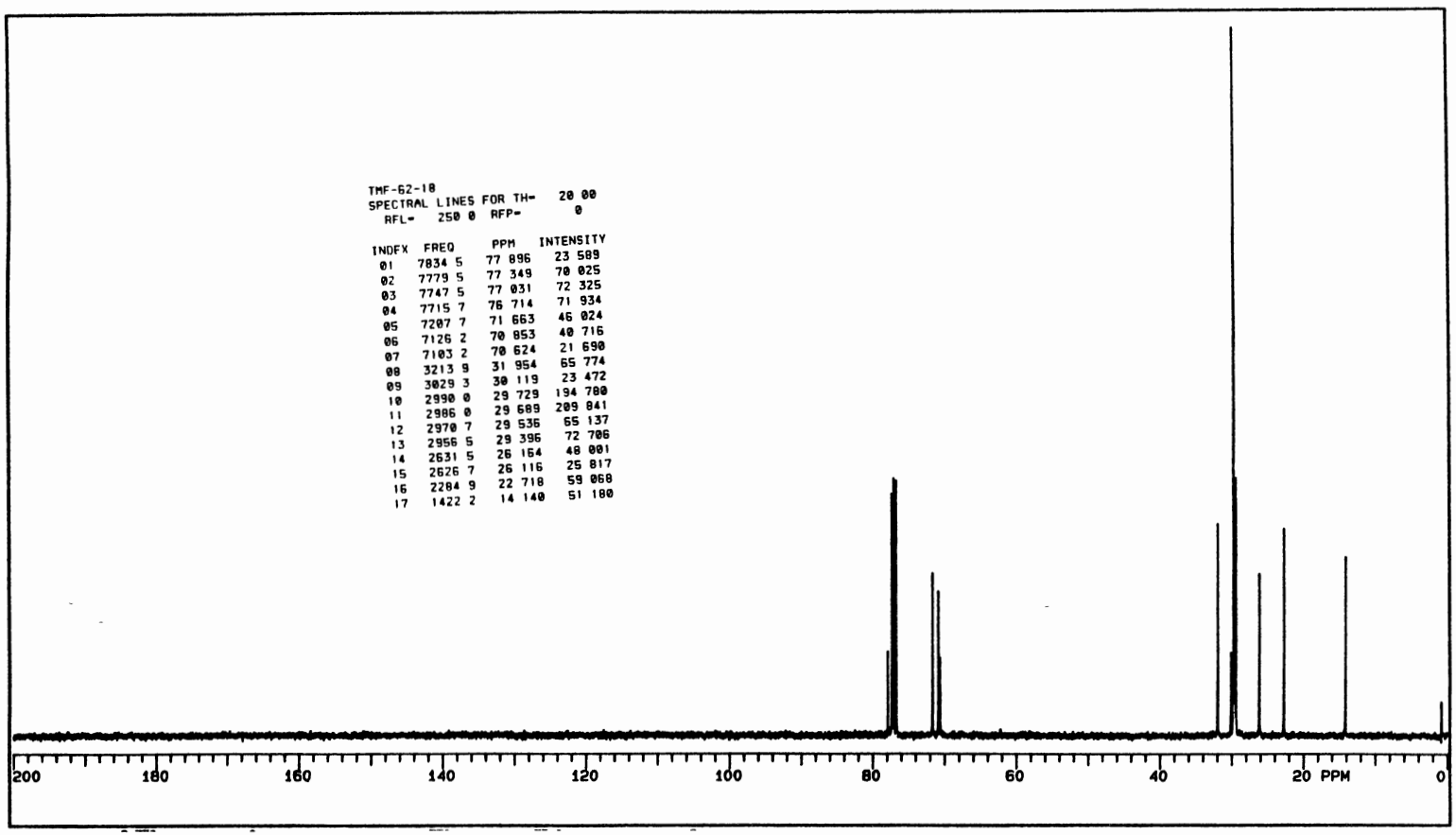
Mass Spectrum of Tritetradecyl Glyceryl Ether

Plate II



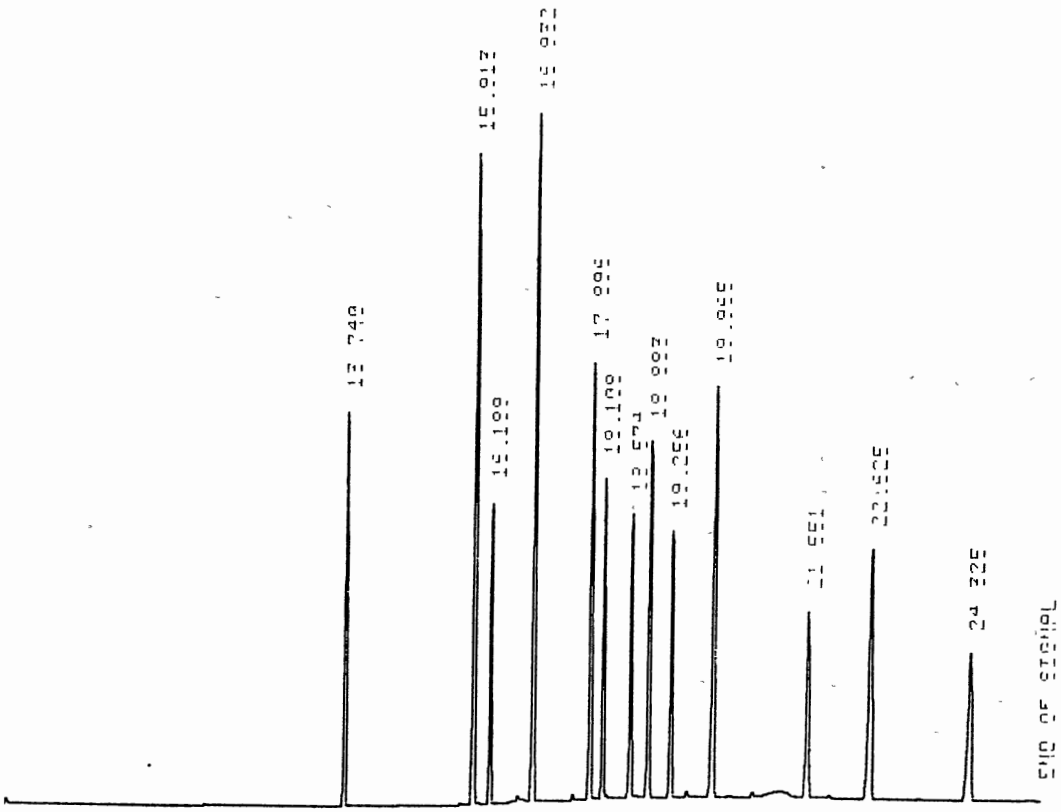
^{1}H NMR Spectrum of Tritetradecyl Glyceryl Ether

Plate III



^{13}C NMR Spectrum of Tritetradecyl Glyceryl Ether

Plate IV



Chromatogram of FAME Standards and Tritetradecyl Glyceryl Ether

CHAPTER IV

APPARENT ABSORPTION OF FATTY ACIDS FROM THE GASTROINTESTINAL TRACT OF THE EARLY-WEANED PIG: EFFECT OF FAT SOURCE, WEEK ON TRIAL AND SAMPLING SITE

Abstract

Four pigs were used in a replicated 2 x 2 Latin square experiment. Digestive contents were sampled at the distal ileum and the rectum with the intent of determining if sampling site affected the absorption estimate. At 17 d of age, pigs were removed from the sow and surgically fitted with a T-type cannula in the distal ileum. After a 4 d recovery on the sow, pigs were weaned and assigned to treatments. The two treatments used were complex pre-starter diets supplemented with either soybean oil or tallow at 8% of the diet. Diets were devoid of soybean proteins and contained porcine plasma proteins soybean in an attempt to provide the pigs with a high producing diet. 1,2,3-Tritetradecyl glyceryl ether was added to the diets as an external marker. Pigs consuming the soybean oil supplemented diet grew at a faster rate (P<.001) and

exhibited a higher (P<.003) feed efficiency than pigs fed the tallow supplemented diet. Palmitic acid was absorbed more fully (P<.02) from the soybean oil supplemented diet than from the tallow supplemented diet. Apparent absorption of stearic acid was lower (P<.03) from the tallow diet when measured at the distal ileum, but no difference (P>.9) was observed in the fecal absorption values. Calculating apparent absorption at the distal ileal gives different estimates of apparent absorption than the fecal index method. These data suggest that differences occured in specific fatty acid utilization within the early-weaned pig.

Key Words: Fatty Acid Absorption, Ileal, Fecal, Soybean Oil, Tallow

Introduction

Determining nutrient absorption involves accurate quantification of the ingested nutrient and the unabsorbed residual nutrient. The most common procedure used for determining fat and fatty acid digestibility has been the fecal index method. This method involves measuring the amount of specific fatty acid in the diet and the amount voided in the feces. However, the fecal index method fails to account for the modifying effects of the micro flora as the digesta transverses the large intestine (Carlson and Bayley, 1968). More accurate information on the digestibility of fatty acids may be obtained by measuring the quantity of fatty acids at the distal ileum (Ozimek et

al., 1984).

Carlson and Bayley (1968) suggested that Cr_2O_3 (or any other water soluble marker) was inappropriate for use in lipid digestion because the Cr_2O_3 separated from the lipid phase of the digesta in the stomach and passed from the stomach faster than dietary lipid. Spener et al. (1968) reported use of glycerol trioctadecenyl ether as a model for triglyceride absorption and found the triether to be very poorly absorbed. Carlson and Bayley (1972) synthesized tridodecyl glyceryl ether and described procedures for using the triether as an indicator of fat absorption. Recovery of the triether was comparable to that of Cr_2O_3 when these markers were included in diets given to rats.

In a recent report, Cera et al. (1989) studied fatty acid absorption in barrows weaned at 21 d of age. Fecal samples were collected for 5 d periods each week of the four week study. The authors concluded that the digestibility of the long chain fatty acids (>16C) could not be ascertained from the data due to the contribution of endogenous fecal lipids.

The present study was undertaken to determine the effects of fat source on ileal and fecal fatty acid digestibilities in early-weaned pigs fed a complex prestarter diet containing plasma protein and devoid of soybean meal. Tritetradecyl glyceryl ether (TGE) was used as the external marker. Quantification of fatty acids and TGE were accomplished by gas chromatographic analysis of

feed, feces and ileal contents.

Materials and Methods

Sample collection

To determine the ileal and fecal digestibilities of fatty acids from prestarter diets, five Yorkshire pigs (17 d old) were surgically fitted with a simple T-type cannula inserted into the distal ileum (Walker et al., 1986). Pigs were returned to the sow immediately following surgery and allowed to remain on the sow for a four day recovery period. After recovery, the pigs were weaned and moved to individual, elevated metal pens in an environmentally controlled room. Four of the pigs were utilized in a replicated 2 x 2 Latin square arrangement of treatments with main effects of fat source and week of experiment. Diets (Table 8) were supplemented with either soybean oil (soy) or tallow and with 1,2,3-tritetradecyl glyceryl ether (TGE) added as a non-absorbed inert marker (.05%). Interim gain and efficiency estimates were measured on d 7 and 14 post weaning. After feed intake and weight gain were estimated on day 7, the diets were switched, so that pigs consuming the soybean oil supplemented diet during week one received the tallow supplemented diet during week two. Ileal and fecal samples were obtained on d 5, 6, 7 (week 1) and d 12, 13 and 14 (week 2) and frozen for future analysis.

All data were analyzed using the GLM procedure of SAS (1988) with the model including the effects of Latin square, pig within Latin square, week, fat source and sampling site. All valid interactions were evaluated. For digestibility data analyses, week*fat source*Latin square was used as the mean square error term. Specific differences between means were determined using the PDIFF procedure (SAS, 1988). Individual pig (experiment one) or pen (experiments two and three) was used as the experimental unit in statistical analyses.

Fatty Acid Methyl Ester Preparation

Ileal and fecal samples were lyophilized (approximately 72 h) and samples from within the same week were combined on an equal dry weight basis. Following the procedure reported by Sukhija and Palmquist (1988), duplicate 200 mg samples of feed, lyophilized feces or lyophilized ileal samples were accurately weighed and transferred to a culture tube (15 mm by 150 mm) fitted with a Teflon lined cap. Into each tube was added 2 mL of toluene containing 2.0 mg/mL of heptadecanoic acid (internal standard) and 3.0 mL of 5% methanolic hydrochloric acid (MeOH-HCl, prepared by slowly adding 10 mL of acetyl chloride to 100 mL of anhydrous methanol). After capping, the culture tubes were vortexed and then placed in a block heater. The tightly capped tubes were heated for .5 h at 90^{o}C. The tubes were removed from

the heating block and vortexed (30 s) and returned to the heating block for an additional .5 h at 90°C. The samples were cooled to room temperature and 5.0 mL of 6% Na_2CO_3 was added (increasing the pH to neutrality is essential in order to prevent damage to the GC column). After vortexing for .5 min at medium speed, the tubes were centrifuged for 10 min in a bench top centrifuge. The upper organic phase was transferred via a Pasteur pipet to a clean test tube and dried by adding 1 g of Na_2SO_4 to each test tube. To the lower aqueous phase was added an additional 2 mL of toluene for a second extraction. After vortexing (.5 min) and centrifuging (10 min) the upper organic layer was added to the first extraction. The tubes were capped and kept in the dark until the GC analyses were completed (up to 24 h).

Gas-Liquid Chromatography

Fatty acid analyses were performed utilizing a Hewlett-Packard 5890A gas-liquid chromatograph fitted with a splitless injector, flame ionization detector and an HP 3396 Series II integrator. A DB-WAX capillary column (J & W Scientific, 30 m length, .25 mm inner diameter and .25 um film thickness) was used for all analyses. Parameters were optimized by comparing the number of theoretical plates and the baseline resolution obtained from the separation of a standard solution. The total time for each chromatographic run was 30 min. Parameters for optimum separation of fatty acid methyl esters were:

Injector temperature:	300°C
Detector temperature:	320°C
Linear velocity: (toluene at 80°C)	25 cm/s
Temperature ramp:	80°C for 1 min
	80 - 250°C @ 10°C/min
	250°C for 12 min
Carrier gas:	Helium

Each sample was used for a minimum of two GLC analyses. Samples were injected using a 5 μL syringe (Hamilton). For each analysis, the syringe was filled to contain .5 μL of air, .5 μL of toluene, .5 μL of air, .2 μL of sample and finally .5 μL of air.

A standard fatty acid methyl ester mixture was prepared by combining individual fatty acid methyl esters (Sigma Chemical Company, St. Louis, MO). Standards were analyzed by GC/MS to identify each ester and to obtain a mass spectrogram of each ester for latter use as a standard for identification of peaks from feed, fecal and ileal samples. Fatty acid methyl esters used included $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{22:0}$ and $C_{22:4}$.

Results and Discussion

It has been suggested that differences in utilization of dietary fat in the young pig may be due to differences in the fatty acid composition and the ratio of saturated to

unsaturated fatty acids (Lawrence and Maxwell, 1983). Fatty acid composition of the diets used in the experiment presented herein are reported in Table 9. The tallow supplemented diet contained greater amounts of the three saturated fatty acids that were quantified. In addition, the oleic acid content of the tallow diet was greater than the oleic acid content of the soybean oil supplemented diet. The fatty acid compositions are consistent with those reported by Braude and Newport (1973).

Performance data (Table 10) shows that the pigs were performing within expected parameters for pigs weaned at three weeks of age. Daily gain and intake during week two were significantly greater ($P<.001$) than during week one. Feed efficiency (gain:feed) was significantly lower ($P<.003$) during week two when compared to week one. Pigs consuming the soybean oil supplemented diet grew faster ($P<.001$) and exhibited higher ($P<.003$) feed efficiency values than pigs fed the tallow supplemented diets. It should be noted that the intent of this experiment was to compare digestibility data and not performance data, therefore it was sufficient for this experiment to determine that the pigs performance was comparable to earlier studies.

Subclass least squares means for apparent absorption are presented for fat source*sampling site and sampling site*week interactions (Tables 11 and 12 respectively). No differences in apparent absorption due to sampling site, fat source or week of trial were observed for myristic acid.

Apparent absorption of palmitic acid was lower from tallow supplemented diets than from soybean oil supplemented diets when measured from either fecal ($P<.02$) or ileal ($P<.02$) contents. Palmitoleic acid was estimated to be better absorbed ($P<.03$) from the tallow supplemented diet than from the soybean oil supplemented diet when the apparent absorption was calculated from ileal contents, while no difference ($P>.9$) in the fecal digestibility values were observed. Apparent absorption of stearic acid was lower ($P<.03$) in tallow fed pigs when measured at the distal ileum. In contrast, no difference ($P>.3$) was observed in fecal digestibilities of stearic acid. The three unsaturated C_{18} fatty acids exhibited similar apparent absorption differences for fecal vs ileal values. While no differences ($P>.5$ for $C_{18:1}$; $P>.8$ for $C_{18:2}$; $P>.4$ for $C_{18:3}$) due to fat source were observed for ileal absorption values, pigs fed tallow supplemented diets had lower apparent fecal absorption values ($P<.03$ for $C_{18:1}$; $P<.03$ for $C_{18:2}$; $P<.02$ for $C_{18:3}$).

Ozimek et al. (1984) reported the digestibility of tallow and rapeseed oil in growing pigs (40-45 kg) that were fitted with a simple T-cannula at the end of the small intestine. The apparent digestibility of fat was greater at the distal ileum as compared to the fecal digestibility, and thus shows a net appearance of fat in the large intestine. The apparent fecal digestibilities of the saturated fatty acids ($C_{16:0}$ and $C_{18:0}$) were lower than their corresponding

ileal digestibilities. In addition, the authors found that the apparent absorption of stearic acid in pigs fed tallow supplemented diets was significantly lower when measured from fecal samples (35.9% for fecal estimate compared to 62% for ileal estimate), thus suggesting a net appearance of stearic acid in the digesta as it passes through the large intestine. Conversely, the fecal digestibilities of most of the unsaturated fatty acids ($C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:1}$ and $C_{22:1}$) were higher than their corresponding ileal digestibilities. The increase in the level of saturated fatty acids accompanied by a decrease in unsaturated fatty acids between the distal ileum and the feces was probably due to hydrogenation of the unsaturated fatty acids by the micro flora within the large intestine. The authors concluded that a more valid estimation of fat absorption could be obtained if samples were taken from the distal ileum. Later, Endres et al. (1988) reported lowered estimates of fat absorption by the fecal collection method when compared to the ileal collections in 28 d old pigs. Carlson and Bayley (1968) reported apparent reduction in the observed absorption of stearic acid and attributed the reduction to saturation of C_{18} fatty acids by the microflora within the large intestine and suggested the need for samples to be taken from distal ileum rather than the feces in order to give "a truer indication of the pig's ability to digest fat".

This phenomenon of reduced absorption of stearic acid in pigs when comparing ileal to fecal estimates was not observed for both fat sources used in this experiment. In pigs fed the tallow supplemented diet, no difference ($P>.2$) in apparent absorption between the ileal and fecal estimates was detected. In contrast, pigs fed diets supplemented with soybean oil exhibited a decrease ($P<.03$) in apparent absorption measured by fecal estimates when compared to the value measured at the distal ileum. This difference in fat source may be affected by the content of stearic acid within the diet. The increased amount of stearic acid in tallow (1.42% for tallow diet; .37% for soy diet) may have contributed to a lower ileal apparent absorption and thus no net increase in apparent absorption was observed.

When only ileal estimates of apparent absorption are considered, no differences ($P>.5$ for $C_{16:0}$; $P>.9$ for $C_{16:1}$; $P>.6$ for $C_{18:0}$; $P>.7$ for $C_{18:1}$; $P>.9$ for $C_{18:2}$; $P>.9$ for $C_{18:3}$) in apparent absorption over time were detected for any of the fatty acids measured (Table 12). However, differences in apparent absorption over time were detected for stearic ($P<.06$), oleic ($P<.05$) and linoleic ($P<.05$) acids when fecal absorption estimates were used. Cera et al. (1988) reported apparent fat digestibility and dietary fat absorption increased with age or time postweaning. In addition, the authors stated that digestibility differences amoung fatty acids were of a greater magnitude during the

initial weeks postweaning, however, no data were presented to support this conclusion.

These data show that differences occur in apparent absorption of specific fatty acid within the early weaned pig. Some of these differences may be attributed to the source of added fat in the diet. In addition, the age of the pig and thus the status of the digestive system may also contribute to differential utilization of fat. The apparent discrepancies in values for fatty acid absorption by the young pig indicate that there may be many unknown or uncontrolled factors that affect apparent absorption determinations. The most reliable estimate for apparent absorption should be obtained by taking measurements at the distal ileum (Carlson and Bayley, 1968; Ozimek and Sauer, 1984).

Literature Cited

Braude, R. and M. J. Newport. 1973. Artificial rearing of pigs 4. The replacement of butterfat in a whole-milk diet by either beef tallow, coconut oil or soya-bean oil. Br. J. Nutr. 29:447.

Carlson, W. E. and H. S. Bayley. 1968. Utilization of fat by young pigs: Fatty acid composition of ingesta in different regions of the digestive tract and apparent and corrected digestibilities of corn oil, lard and tallow. Can. J. Anim. Sci. 48:315.

Carlson, W. E. and H. S. Bayley. 1972. Preparation and use of a glyceryl triether as an indicator of fat absorption. Br. J. Nutr. 28:295.

Cera, K. D., D. C. Mahan and G. A. Reinhart. 1988. Weekly digestibilities of diets supplemented with corn oil, lard or tallow by weanling swine. J. Anim. Sci. 66:1430.

Cera, K. R., D. C. Mahan and G. A. Reinhart. 1989. Apparent fat digestibilities and performance responses of postweaning swine fed diets supplemented with coconut oil, corn oil or tallow. J. Anim. Sci. 67:2040.

Endres, B., F. X. Aherne, L. Ozimek and H. Spicer. 1988. The effects of fat supplementation on ileal versus fecal fat digestibilities, performance and body composition of weaned pigs. J. Anim. Sci. 68:225.

Lawrence, N. J. and C. V. Maxwell. 1983. Effect of dietary fat source and level on the performance of neonatal and early weaned pigs. 57:936.

Ozimek, L., W. C. Sauer, G. Ozimek and W Kozikowski. 1983. Ileal and fecal digestibility of fatty acids in pigs fed beef tallow or rapeseed oil. J. Anim. Sci. 57(Suppl. 1): 261(Abstr.).

Ozimek, L., W. C. Sauer, W. Kozikowski and H. Jorgensen. 1984. The ileal and fecal digestibility of fat and fatty acids in pigs fed semi-purified diets containing beef tallow or rapeseed oil. 1984 Feeders Day Report, Univ. Alberta, Edmonton, 14.

SAS. 1988. SAS/STAT User's Guide (6th Ed.). SAS Inst. Inc., Cary, NC.

Spener, F., F. Paltauf and A. Holasek. 1968. The intestinal absorption of glycerol trioctadecenyl ether. Biochim. Biophys. Acta. 152:368.

Sukhija, P. S. and D. L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36:1202.

Walker, W. R., G. L. Morgan and C. V. Maxwell. 1986. Ileal cannulation in baby pigs with a simple T-cannula. J. Anim. Sci. 62:407.

Table 8. Composition[a] of diets

Ingredient	% in ration
Dried skim milk	10.0
Whey	20.0
Corn	46.2
AP-820[b]	6.8
SBM, 44%	--
Fat source[c]	8.0
Lysine, HCl	.25
Fishmeal, Menhaden	5.0
Ethoxyquin	.025
FOA[d] 390	1.0
Flavor, Berry	.1
$CuSO_4$	.1
DL-Methionine	--
Vitamin premix[e]	.74
Calcium carbonate	--
Dical	1.75
Salt	--
Calculated composition of diet:	
ME (Mcal/lb)	1.62
CP (%)	18.21
Lys (%)	1.40
Trp (%)	.25
Thr (%)	.91
Met + Cys (%)	.72
Ca (%)	1.07
P (%)	.85

[a] As fed basis.
[b] Plasma protein source, American Protein Corporation, Ames, Iowa.
[c] Soybean oil (soy) or tallow.
[d] Provided 10 g Furazolidone, 5 g Oxytetracycline, 4.5 g Arsanilic acid per lb of diet.
[e] Supplied 4,160 IU vitamin A, 416 IU vitamin D, 18 IU vitamin E, 20 mg pantothenic acid, 28 mg niacin, 4.0 mg riboflavin, 7.3 mg menadione sodium bisulfate, .02 mg vitamin B_{12}, 1.3 mg biotin, 2.7 mg pyridoxine, .9 mg folic acid, 3.9 mg thiamin, 267 mg choline, .1 mg selenium, .03 g manganese, .1 g zinc, .1 g iron, .1 g copper, .2 g magnesium, .43 g potassium and .2 mg iodine per lb of feed.

Table 9. Fatty acid composition of diets[a]

	Fat source	
Fatty acid	Soybean oil	Tallow
$C_{14:0}$	.07	.27
$C_{16:0}$	.97	1.91
$C_{16:1}$	.05	.23
$C_{18:0}$	.37	1.42
$C_{18:1}$	1.68	3.40
$C_{18:2}$	3.85	.30
$C_{18:3}$	.58	.03

[a] Percent of diet on dry matter basis.

Table 10. Effect of week and fat source on performance criteria[a]

Item	Week		Fat source		SE
	One	Two	Soy	Tallow	
ADG[b] (g)	325.6[b]	460.2[c]	416.5[b]	369.3[c]	.43
ADFI[b] (g)	339.8[b]	775.1[c]	541.0	573.9	8.73
FE (Gain:Feed)	0.96	0.60[e]	0.82	0.73[g]	.01

[a] Least squares means.
[b,c] Means within main effect in the same row not bearing a common superscript differ (P<.001).
[d,e] Means within the same row not bearing a common superscript differ (P<.005).
[f,g] Means within the same row not bearing a common superscript differ (P<.05).

Table 11. Effect of sampling site and fat source on apparent absorption of fatty acids[a]

	Ileal		Fecal		
Fatty Acid	Soy	Tallow	Soy	Tallow	SE
$C_{14:0}$	94.1	92.5	92.4	92.6	1.23
$C_{16:0}$	93.8[b]	80.4[c]	91.8[b]	79.7[c]	2.37
$C_{16:1}$[d]	92.5[b]	96.2[c]	97.9[c]	98.0[c]	.85
$C_{18:0}$[e]	88.6[b]	69.0[c]	71.2[c]	77.8[bc]	4.28
$C_{18:1}$	97.2[b]	95.7[bc]	98.4[b]	91.1[c]	1.63
$C_{18:2}$[d]	97.8[b]	96.8[b]	99.3[b]	79.2[c]	4.29
$C_{18:3}$[e]	97.3[b]	100.0[b]	99.6[b]	87.5[c]	2.41

[a] Least squares means.
[b,c] Means within the same row not bearing a common superscript differ (P<.05).
[d] Sample site*fat source interaction (P<.1).
[e] Sample site*fat source interaction (P<.03).

Table 12. Effect of sampling site and week on apparent absorption of fatty acids[a]

	Ileal		Fecal		
Fatty Acid	Week 1	Week 2	Week 1	Week 2	SE
$C_{14:0}$	93.7	92.8	93.3	91.8	1.23
$C_{16:0}$	88.1	86.1	85.6	85.8	2.37
$C_{16:1}$	94.4^{b}	94.3^{b}	98.9^{c}	96.9^{bc}	.85
$C_{18:0}$	80.4	77.1	82.1^{b}	66.9^{c}	4.28
$C_{18:1}$[d]	96.8^{bc}	96.2^{bc}	91.7^{b}	97.8^{c}	1.63
$C_{18:2}$	97.7^{b}	97.0^{b}	81.3^{c}	97.2^{b}	4.29
$C_{18:3}$	98.9	98.4	92.1	95.0	2.41

[a] Least squares means.
[b,c] Means within the same row not bearing a common superscript differ ($P<.05$).
[d] Week*fat source interaction ($P<.1$).

VITA

Timothy M. Fakler

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECT OF SUPPLEMENTAL FAT IN PRESTARTER DIETS ON PERFORMANCE AND APPARENT ABSORPTION OF FATTY ACIDS IN THE EARLY-WEANED PIG

Major Field: Animal Nutrition

Biographical:

Personal Data: Born in Hays, Kansas, September 15, 1961, the son of Frederick F. and Teresa E. Fakler.

Education: Graduated from Albert Lea Senior High School, Albert Lea, Minnesota, in June, 1979; earned Bachelor of Science Degree from Oral Roberts University in May, 1983; earned Master of Science degree from Oklahoma State University in December, 1987; completed requirements for the Doctor of Philosophy degree at the Oklahoma State University in December, 1992.

Professional Experience: Worked in the capacity of a Graduate Teaching Assistantship, Department of Chemistry, Oklahoma State University, August. 1983 to July, 1986.; worked in the capacity of a Graduate Research and Teaching Assistantship, Department of Animal Science, Oklahoma State University, Spring 1988 to December , 1992.

Professional Organizations: The American Society of Animal Science, The American Association for the Advancement of Science, The American Chemical Society, Alpha Epsilon Delta (Pre-med Honor Society), Phi Lamda Epsilon (Chemistry Honor Society).