THE UNIVERSITY OF ALBERTA

INGREDIENT INTERACTION AND RATE OF PASSAGE STUDIES WITH PIGS

by

MALEENA IMBEAH

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

ANIMAL NUTRITION

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

SPRING 1989

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K1A 0N4

ISBN 0-315-52982-2

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NAME OF AUTHOR MALEENA IMBEAH

TITLE OF THESIS INGREDIENT INTERACTION AND RATE OF PASSAGE STUDIES WITH PIGS

DEGREE FOR WHICH THESIS WAS PRESENTED DOCTOR OF PHILOSOPHY

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DEDICATION

TO MY PARENTS

Silisatopa And Ninahari Imbeah

For all the sacrifices you made

For your prayers and emotional support

For the guidance and encouragement you provided

For teaching me to trust in the Lord always

All of these together, have led me this far

God Bless You

THESIS ABSTRACT

Three experiments were conducted with ileal-cannulated pigs. The first experiment investigated the presence or absence of associative effects in the prediction of the digestible amino acid supply in barley-soybean meal (B + SBM) and barley-canola meal (B + CM) diets. Digestibilities of amino acids in B + SBM and B + CM observed experimentally and those calculated from the digestibilities determined in the single ingredients were compared. There were no differences between calculated and observed digestibilities of indispensable amino acids in B + SBM. For B + CM, observed digestibilities were higher than the calculated digestibilities. The differences (percentage units) were significant ($P<.05$) for lysine (7.3) and phenylalanine (6.4). The second part of this experiment investigated the rate of secretion of pancreatic proteolytic enzymes in pigs fed SBM or CM diets. There were no differences in the rate of secretion of trypsin or chymotrypsin.

In the second experiment, the effect of three dietary fat levels (2%, 6% and 10% canola oil) on amino acid digestibilities and the rate of passage of cornstarch-based soybean meal (SBM) and canola meal (CM) diets was investigated. Ileal digestibilities of most indispensable amino acids in the SBM diet with 10% fat were higher ($P<.05$) than those of the 2% fat diet but not different from those of the 6% diet. Ileal digestibilities of most amino acids in CM were depressed ($P<.05$) when dietary fat level was increased from 2% to 6%. A further increase in dietary fat to 10% significantly increased ($P<.05$) digestibilities of all amino acids relative to their digestibilities in the 6% fat diet,

but digestibilities did not surpass those in the 2% fat diet for most amino acids. Fecal amino acid digestibilities were mostly unaffected by dietary fat levels. The rate of passage of digesta was unaffected by dietary fat level for either SBM or CM.

The third experiment compared the marker withdrawal method and the single dose of marker, in the determination of rate of passage, using two markers. Ileal cannulated pigs fed a conventional pig grower diet were used to determine the rate of passage of feed residues following withdrawal or consumption of marker. The rate of passage of digesta following marker consumption or withdrawal were identical when chromic oxide was the marker. With dysprosium as marker, the rate of passage of digesta for the withdrawal method was higher ($P<.05$) than that for the single dose method. The rate of passage over the entire digestive tract was unaffected by method or marker.

ACKNOWLEDGEMENTS

I would like to thank Dr. R.T. Hardin, the past chairman and Dr. M.A. Price, the present chairman of the Department of Animal Science, for placing the facilities of the department at my disposal.

I am deeply grateful to my supervisor, Dr. Willem C. Sauer, for his continued support and guidance throughout my PhD. program.

I would like to give special thanks to Mr.Terry Fenton and Mrs. Myra Fenton for advice on the use of laboratory equipment.

Special gratitude is extended to Mr. Ray Weingardt for advice on statistics and computing, so generously and patiently provided to me.

I am very grateful to Mr. Mike Dugan, my colleague and friend, for being there when I needed him.

Special thanks go to my parents, Pastor Silisatopa and Mrs. Ninahari Imbeah and all my brothers and sisters for their love and prayerful support throughout my years of education.

I would like to thank John, my husband, for his patience, understanding and continued support throughout my studies.

Lastly, I wish to thank my children, Ewuraabena and Effie, as well as my nieces and nephews who served as a constant source of inspiration for me to strive on.

Financial support for these studies provided by the Natural Sciences and Engineering Research Council is gratefully acknowledged.

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1. THESIS INTRODUCTION

This chapter covers three areas and therefore, is divided into three parts. The first part covers associative effects of feeds and nutrient digestibilities. The second part deals with the effect of dietary fat levels on nutrient digestibilities, particularly protein and amino acid digestibilities. In the third part, various aspects of rate of passage and its effect on digestibility are reviewed. Associative effects and the effect of dietary fat levels on nutrient digestibilities could be taken together as the effect of ingredient interactions on nutrient digestibilities. Ingredient interactions may thus be considered as a factor which can influence nutrient digestibilities, with particular reference to amino acid digestibilities in pig diets, and thus is covered in the first section.

Associative Effects

Much work has been done on protein and amino acid digestibilities in pig feed. The effects of many factors that affect protein digestibilities, such as physical and chemical properties of the feed protein, type and amount of fiber in the diet, presence of anti-nutritional factors and heat treatment on amino acid digestibilities have been studied (Combs et al., 1967; Kakade, 1974; Liener, 1976; Restani et al., 1983 and Mitaru et al., 1984). Sauer and Ozimek (1986) postulated that differences in the rate of secretion of pancreatic proteolytic enzymes may be one of the factors responsible for differences in amino acid digestibilities between feedstuffs. The effect of ingredient interaction on nutrient digestibilities has received minimum attention. In addition, digestibility coefficients of feedstuffs are assumed to be constant. Some of the coefficients have been predicted by difference based on this assumption (Asplund and Harris, 1971, Thompson et al., 1984 and Morgan et al., 1984); thus, animal feeds are formulated based on the assumption that each ingredient makes an additive contribution to the nutritive value of the diet. This assumption may not always be valid due to possible interactions or associative effects between ingredients in a compounded feed. Interactions can alter the rates of passage (Asplund and Harris, 1971; Thompson et al., 1984 and Goetsch et al., 1987) and the overall gut environment (Mould et al., 1984a) and thereby affect nutrient digestibilities.

Associative effects have been demontrated in ruminants (Asplund and Harris, 1971 and Mould et al., 1984a) and also in horses (Thompson et al., 1984). Associative effects occur when the apparent digestibility of a nutrient in a mixture of feed ingredients is not equal to that of the sum of its digestible supply from the individual feed ingredients (Mould et al., 1984b). Asplund and Harris (1971) have shown that the digestible crude fiber content, total digestible nutrients (TDN) and biological value (BV) observed experimentally were lower ($P<.01$)than values calculated by difference for various sheep rations, indicating that the digestible nutrient supply may not always be additive. The sensitivity of BV to associative effects in ruminants is very low due to the synthesis of microbial protein. However, associative effects between feed ingredients, in which rate of passage is influenced , may affect BV in ruminants to a larger extent (Asplund and Harris, 1971).

The physical form (ground, chopped, long, pelleted, cubed or mash) and also the proportions of the feed ingredients in the diet may affect nutrient digestibility (Mould et al., 1984a). Furthermore, Thompson et al. (1984) in studies with horses observed that the addition of 20 or 40% oats to alfalfa hay reduced the crude protein digestibility of the hay, indicating a negative associative effect of oats on protein digestibility.

There is a scarcity of information on associative effects between feed ingredients in diets of monogastric animals. These associative effects could influence amino acid digestibilities and other measures of protein quality. The nutritional significance of associative effects as a factor influencing amino acid digestibilities would depend on the magnitude of the effects. Recently, some reports have shown interactions between feed ingredients which influence the fecal digestibilities of energy and protein in chickens. For example, Wallis et al. (1985) reported an interaction between wheat and other cereals whereby the digestibilities of energy and amino acids in broilers were improved ($P<.01$). Carre et al. (1987), in studies with cockerels fed peas, wheat and maize reported that the predicted protein digestibility for peas varied ($P<.05$) depending on whether they were mixed with wheat or corn. In addition, Morgan et al. (1984), in studies with pigs, observed that the digestible energy value for fish meal, calculated by difference, varied depending on its level of inclusion in a barley-based diet.

The Effect of Fat

Fat is used in the diets of farm animals as a means of increasing the energy density of the diet as well as to reduce the dustiness of the feed. Fat also facilitates the absorption of fat-soluble vitamins. In studies with chickens, fat supplementation improved the metabolisable energy (ME) content in isocaloric as well as non-isocaloric diets (Mateos and Sell, 1981; Mateos et al., 1982 and Sell et al, 1983). This improvement in the ME content was found to be associated with an increased ($P<.05$) transit time (or reduced rate of passage) over the entire digestive tract (Mateos and Sell, 1981; Mateos et al., 1982 and Sell et al., 1983). In contrast, Golian and Polin (1984) did not observe an increase in transit time associated with an improved ME content of fat-supplemented diets for young chicks. The effect of additional fat on pig performance and carcass characteristics have frequently been studied (Asplund et al., 1960; Allee and Hines, 1972 and Friend et al., 1975). Improvement of pig performance and carcass characteristics with increased dietary fat levels are normally attributed to the increased energy density. It is possible that the improved performance may not be solely due to the deposition of fat, but also to the deposition of muscle protein. Additional fat may improve the utilisation of protein by providing extra energy required for protein synthesis and deposition, leading to improved animal performance. The effect of fat on gastric emptying has also been studied extensively (Harkins et al., 1964; Hunt and Knox, 1968; Elias et al, 1968; Pirk and Skala, 1970 and Sidebottom et al., 1983). However, the effect of fat on nutrient digestibility is usually considered secondary as it is used mostly for the purpose of increasing the energy density of the diet(Lewis and Payne, 1966).

There is a scarcity of information on the effect of fat on protein and amino acid digestibilities, and what there is shows conflicting results. Marshall et al. (1959), in studies with rats fed diets containing lactoalbumin as the sole protein source and with 3 or 15% fat, observed no difference (P>.05) in protein digestibility. Lowrey et al. (1962), in studies with piglets, weaned at 3 wks and fed a corn-based diet containing SBM and fish meal as the protein sources, also observed no difference (P>.05) in protein digestibility when 10% compared to 0% fat was added to the diet. Furthermore, no differences were found in protein digestibility in studies with pigs fed corn-SBM diets that contained 0, 5, 10 or 15% fat (Greeley et al., 1964). On the other hand, Asplund et al. (1960) in studies with piglets, weaned at 3 wks and fed corn-SBM diets containing meat scraps and brewers' yeast as additional protein sources, observed an improvement (P<.05)in protein digestibility when 10% compared to 0% fat was added to the diet. Jorgensen et al. (1985) in studies with pigs, also observed an increase (P<.05) in the apparent protein digestibility (in addition to lysine, methionine and threonine) when the dietary fat content was increased from 3 to 15%. Furthermore, Tao et al. (1971) observed improvements (P<.05) in the fecal digestibilty of many of the indispensable amino acids, ranging from 8.6 to 19.7 percentage units, with broilers fed rapeseed meal diets supplemented with 3.5% fat.

It is rather difficult to consolidate the findings by the various authors to explain the underlying physiological and nutritional mechanisms for the different results. However, differences in methodology, age of animals, diet composition and different levels of fat inclusion may account for some of the contradictory findings.

Rate of Passage

Nutrient digestibilities may be affected by rate of passage. Keys and DeBarthe (1974) suggested that the digestion of starch and protein in milo and barley diets for pigs could be influenced by their respective rates of passage. Dietary fat may affect nutrient digestibilities through its effect on gastric emptying and its possible resultant effect on the rate of passage through other portions of the digestive tract, in particular in the small intestine where the majority of nutrient digestion and absorption take place. Mateos and Sell (1981) suggested that the improved ME content of poultry diets containing increased dietary fat levels was due to a reduction in the rate of passage which, in turn, would increase the time of exposure of digesta to digestive processes and absorptive surfaces of the digestive tract. Fat has been shown to delay gastric emptying (Harkins et al., 1964 and Hunt and Knox, 1968). However, the effect of fat on the rate of passage of feed residues through other sections of the digestive tract remains to be investigated. Mateos and Sell (1981), Mateos et al. (1982) and Sell et al. (1983) reported a reduction in rate of passage of feed over the entire digestive tract of laying hens fed increasing dietary fat levels. Several factors are known to affect the rate of passage in the gastro-intestinal tract which were reviewed by Warner (1981). These factors include the type of marker used, diet composition, frequency of feeding, and age of the animal. The effect of the age of animal and type of marker was clearly demonstrated by the results of Golian and Polin (1984) compared to those of Mateos and Sell (1981), Mateos et al. (1982) and Sell et al. (1983). Whereas Golian and Polin (1984) observed no reduction in rate of passage in very young chicks fed increasing dietary fat levels, using ferric oxide as marker, the Mateos team in studies with laying hens, using chromic oxide as marker, was able to measure a reduction in rate of passage.

Several factors that affect the rate of passage have been studied in pigs including the effect of feeding (1) pellets versus meal (Castle and Castle, 1957 and Seerley et al., 1962); (2) different protein sources (Maner et al., 1962); (3) different cereal grains (Keys and DeBarthe, 1974); (4) different levels of fiber (Kuan et al., 1983; Den Hartog et al.,1985; Stanogias and Pearce, 1985; Sandoval et al., 1987) and (5) different sources of fiber (Den Hartog et al., 1985). However, the effect of fat on rate of passage in the pig has not been studied. Furthermore, rate of passage studies in pigs have usually been carried out over the entire digestive tract, based on total collection of feces. There is a scarcity of information on the rate of passage through the small intestine where the majority of digestion and absorption of nutrients occur. In addition, ileal analysis as opposed to fecal analysis is the preferred method to determine amino acid digestibilities at present. therefore there is a need to relate ileal digestibilities to differences in rate of passage of digesta measured at the distal ileum rather than over the entire digestive tract.

Requirements for markers are more critical in rate of passage studies than in balance trials and digestibility studies (Uden et al., 1980). A stable equilibrium between marker and feed is essential in rate of passage studies; the association of marker with certain fractions of digesta leads to inaccurate estimates (Uden et al., 1980). One of the factors that may influence the results in rate of passage studies is the marker used, as was reviewed by Warner (1981). Many different markers have been used, e.g. Castle and Castle (1957) and Seerley et al. (1962) used stained feed particles in their studies. Many markers have been introduced in recent times and used in rate of passage studies. These include chromic oxide, polyethylene glycol, cerium, cobalt, Cr-EDTA and various dyes and oxides of metals (Maner et al., 1962; Vander Noot et al., 1967; Asplund and Harris, 1970, Keys and DeBarthe, 1974; Uden et al., 1980; Den Hartog et al., 1985). Dysprosium (Dy), a rare-earth element, has recently been added to the list of markers. Dy was first used in pigs as a digestibilty marker by Kennelly et al. (1980). Since then, Dy has been extensively used in digestibility studies with pigs at the University of Alberta but never in rate of passage studies. Measurements of rates of passage can be made with the withdrawal method or the single dose method. In the withdrawal method, the marker is fed at a constant level in the diet until steady-state conditions are established. The marker is then withdrawn and its declining concentration in digesta or feces measured (Faichney, 1975). Alternatively, the marker may be given in a single doseand its concentration in feces or digesta measured from the time of feeding for a period of several hours (Castle and Castle, 1957; Maner et al., 1962; Asplund and Harris, 1970; Keys and DeBarthe, 1974). The most widely used indicator of rate of passage is the mean retention time (MRT) which has been used to estimate the rate of passage in various animals including pigs, goats, sheep and rabbits (Castle, 1956; Castle and Castle, 1957; Seerley et al., 1962; Uden et al., 1980 and Stanogias and Pearce, 1985). This method uses the means of the times required for 5% and 95% marker excretion and assumes 100% marker excretion within the collection period. The marker excretion up to any given time can be expressed as a percentage of the total, and an excretion curve based on cummulative marker recovery can then be plotted. Thus, the 5% and 95% excretion times can be read easily and used to calculate the MRT.

Maner et al., (1962), Mateos and Sell (1981), Mateos et al. (1982), Sell et al. (1983) and Golian and Polin (1984) estimated the rate of passage in young pigs by measuring the time of first appearance of marker in feces, referred to as the transit time. Keys and DeBarthe (1974) estimated the rate of passage of feed in pigs according to the method developed by Smith (1968). This method is based on the administration of a single dose of marker. Marker concentrations measured in feces or digesta are transformed to their natural logs. A plot of natural log vs time, starting from the point of maximum marker concentration to the last sample, gives a linear relationship which is described by the following regression equation:
$\ln Y = a + bx$; where Y is the marker concentration, x is time and b the slope or the rate constant. The rate constant provides an estimate of the rate of disappearance of marker or the rate of passage of feed residue. This method of estimating rate of passage is based on the fact that marker excretion approaches infinity and thus follows first order kinetics. The rate of passage estimated by the MRT is based on the assumption that 100% of the marker is excreted within a certain time limit (i.e. during the collection period). The excretion of marker has been demonstrated to approach infinity (Smith, 1968). Keys and DeBarthe (1974) estimated the rate of passage of feed residues in the duodenum, ileum and feces of pigs using the rate constants derived from the regression equation described by Smith (1968). Grovum and Williams

(1973) used various regression equations to determine the rate constant, transit time and half time (i.e. half life) of Cr-EDTA in sheep, based on changes in fecal marker concentration with time. In rate of passage studies in which it is not possible to collect ileal digesta or feces quantitatively, the kinetic analysis method, which is based on changing marker concentration rather than marker recovery and uses rate constant as an estimate of rate of passage, appears most appropriate. Van Soest et al. (1983) in a review, described the principal methods of expressing rate of passage. These include transit time , retention time, peak time, mean time as well as the kinetic analysis method.

Many papers have been published on amino acid digestibilities of barley-based diets supplemented with SBM or CM (Tanksley et al., 1981; Sauer et al, 1982a; 1982b; Anderson et al, 1984; Jorgensen et al, 1984; Rudolph et al, 1983; Thacker et al, 1984 and Buraczewska et al, 1985). However, there appears to be little or no information on the effect of ingredient interactions on amino acid digestibilities in pigs. In particular, interactions between SBM or CM and other components of the diet and how such interactions may affect protein and amino acid digestibilities in the pig have not been studied. Also, differences in amino acid digestibilities between different feedstuffs have generally not been interpreted in terms of differences in proteolytic enzyme secretion associated with the feedstuffs. Sauer and Ozimek (1986) postulated that differences in the rate of secretion of pancreatic proteolytic enzymes may be partly responsible for differences in apparent amino acid digestibilities between feedstuffs.

The current studies were therefore designed to investigate the effect of ingredient interactions on amino acid digestibilities in various SBM and CM diets; to determine the rate of secretion of trypsin and chymotrypsin and to relate this to amino acid digestibilities in SBM or CM diets; to study the effect of dietary fat level on amino acid digestibilities and the rate of passage of digesta in the small intestine of pigs, as well as to study two methods of measuring rates of passage in the pig.

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2. THE PREDICTION OF THE DIGESTIBLE AMINO ACID SUPPLY IN BARLEY-SOYBEAN MEAL OR BARLEY-CANOLA MEAL DIETS AND PANCREATIC ENZYME SECRETION IN PIGS[1]

INTRODUCTION

Additivity of the digestible protein and amino acid supply, determined in single ingredients, is a crucial consideration in the formulation of diets for pigs. There may be associative effects whereby the digestible supply of amino acids in a mixture of feedstuffs is not equal to the sum of the supply based on the digestibilities determined in the single ingredients. Associative effects were demonstrated in ruminants (Mould et al., 1984) and non-ruminant herbivores (horses: Thompson et al., 1984).

There is a lack of information on associative effects in pigs. Therefore, the objective of this study was to determine if there were interactions with respect to the digestible amino acid supply, determined according to the ileal analysis method (e.g. Sauer et al., 1977), between barley and soybean meal, and barley and canola meal. An additional objective was to determine the rate of secretion of protein, trypsin and chymotrypsin in pancreatic juice of pigs fed the same amount of protein from soybean meal or canola meal. Sauer and Ozimek (1986), postulated that differences in the rate of secretion of these components may be, in part, responsible for differences in apparent amino acid digestibilities between feedstuffs. Amino acid digestibilities were found to be 5-10% higher in soybean meal than in canola meal (Sauer et al., 1982).

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[1]A version of this chapter has been published. Imbeah,M., W.C.Sauer, and R.Mosenthin. 1988. J. Anim. Sci. 66: 1409.

MATERIALS AND METHODS

Experiment 1. Five barrows, average initial weight 45 kg, were obtained from the University of Alberta swine herd. The pigs were housed in stainless steel metabolism crates one week prior to the insertion of the cannulas, and fed an 18% crude protein starter diet (Sauer et al., 1983). Water was freely available from a low-pressure drinking nipple.

The barrows were fitted with a simple T-cannula, approximately 5 cm anterior to the ileo-cecal sphincter, according to a procedure adapted from Sauer et al. (1983). The cannulas were prepared according to methods described by McBride et al. (1983). The pigs were allowed a 14 d recuperation period. A detailed description of pre- and post-operative care was previously presented by Sauer (1976) and Sauer et al. (1983).

The apparent ileal digestibilities of amino acids were determined in 5 diets (table 2.1). Dextrose was included at a level of 10% to improve the palatability of the semi-purified cornstarch-based soybean meal (SBM) and canola meal (CM) diets. All diets contained 4% tallow. Vitamins and minerals were supplemented according to NRC (1979). Chromic oxide was included in the diet as the marker for the determination of the digestibilities of nutrients that were measured.

The experiment was carried out according to a 5 x 5 Latin square design. Each experimental period lasted 10 d. Ileal digesta were collected for a total of 24 h: 12 h on day 9 (0800-1000 h and alternate 2 h thereafter) and 12 h on day 10 (1000-1200 h and alternate 2h periods). The pigs were fed a 16% crude protein grower diet (Sauer et al., 1983) during the 5 d intervals between experimental periods. The animals were fed twice daily at 0800 and 2000 h, 800 g each meal.

Experiment 2. Six barrows, average initial weight 47 kg, were obtained from the University of Alberta swine herd. The pigs were housed in stainless steel metabolism crates one week prior to surgery and fed a 16% crude protein grower diet (Sauer et al., 1983). Water was freely available from a low-pressure drinking nipple.

The barrows were prepared for permanent collection of pancreatic juice. Procedures for pre-operative care, surgery, post-operative care and construction of the re-entrant cannula were carried out according to Hee et al. (1985) with modifications described by Ozimek et al. (1986). The pigs were allowed a 21 d recuperation period. A detailed description of pre- and post-operative care has been previously presented by Sauer (1976).

The rate of secretion of pancreatic juice, protein, trypsin and chymotrypsin were determined in pigs fed the SBM and CM diets (table 2.1). The ingredients that were used in this experiment originated from the same batch as in exp. 1.

The experiment was carried out according to a cross-over design. Each experimental period consisted of a 10 d adaptation period followed by a 2 d collection of pancreatic juice, from 0900 h on day 10 to 0900 h on day 12. The pigs were fed a 16% crude protein grower diet during the 7 d interval between experimental periods. The pigs were fed twice daily, at 0800 and 2000 h, 800 g each meal. The collection of pancreatic juice, sampling and return were carried out according to procedures described by Hee et al. (1985); however, the pancreatic juice was returned by aid of an adjustable peristaltic pump in the present studies. The pump was adjusted frequently so that the rate of return of pancreatic juice to the duodenum was similar to the rate of secretion.

Chemical and Statistical Analysis

Ileal digesta were frozen at -20°C immediately following collection. The samples were freeze-dried, pooled within pig and diet, ground through a .8-mm mesh screen in a Wiley mill and mixed prior to analyses. The feed samples were similarly treated.

Analyses for dry matter and crude protein were carried out according to AOAC (1980) methods. Chromic oxide was determined according to Fenton and Fenton (1979). Amino acid analyses were carried out using a Beckman 121 amino acid analyzer following hydrolysis for 24 h with 6 N HCl. The sulfur-containing amino acids and tryptophan were not determined.

Pancreatic juice was collected each hour. The volume of pancreatic juice was measured and sampled (5% by volume). The samples were frozen immediately at -30°C until analysis. Protein in pancreatic juice was determined by the method of Lowry et al. (1951). The activities of trypsin and chymotrypsin were measured according to methods described by Bergmeyer (1974).

Specific activity of pancreatic juice was expressed as units (U)/l. One unit of activity is defined as the hydrolysis of 1 μmol substrate in 1 min. Total enzyme activities were calculated as follows: specific activity(u/l) x volume(l) of pancreatic juice.

Analyses of variance to compare ileal amino acid digestibilities and pancreatic enzyme secretion were carried out according to procedures described by Steel and Torrie (1980). Where appropriate, means for significant treatment differences were compared using the Student-Newman-Keuls multiple range test. The observed amino acid digestibilities and the calculated digestibilities for barley plus soybean meal (B + SBM) and for barley plus canola meal (B + CM) were compared by means of a T-test.

RESULTS AND DISCUSSION

The protein and amino acid content of the experimental diets are presented in Table 2.2. The amino acid digestibilities were higher in SBM than CM (table 2.3). With the exception of alanine and glycine, the differences were significant ($P<.05$). Of the indispensable amino acids, the differences ranged from 10.6 (arginine) to 16.6% (valine). Of the dispensable amino acids, the differences ranged from 7.7 (glutamic acid) to 26.6% (tyrosine). Of the indispensable amino acids, the digestibility of arginine was relatively high while those of threonine and valine were relatively low, both in SBM and CM (table 2.3). The present results agree with those by Sauer et al. (1982) and results compiled by Sauer and Ozimek (1986).

The digestibilities of amino acids were higher in the B + SBM than B + CM (table 2.3). Of the indispensable amino acids, the differences were significant ($P<.05$) for arginine, histidine, phenylalanine, threonine and valine. Of the dispensable amino acids, significant differences were found for aspartic acid, glutamic acid and serine. The highest and lowest digestibility coefficients were found for arginine and valine respectively, in both diets. The present results are in general agreement with those reported by Sauer and Thacker

(1986).

The apparent ileal amino acid digestibilities in barley were lower (table 2.3) than those reported previously (e.g. Sauer et al., 1977) and those compiled by Sauer and Ozimek (1986). Sauer and Ozimek(1986), reported lysine and threonine digestibilities ranging from 64.9 to 79.0% and from 64.4 to 76.0%, respectively. However, several studies reported very low amino acid digestibility coefficients, albeit determined with the fecal analysis method. The apparent fecal lysine digestibility observed by Anderson et al. (1984) ranged from 48.1 to 61.7% in 6 barley cultivars. Anderson and Bell (1983) observed apparent fecal lysine and threonine digestibilities of 43% and 49% respectively in a sample of barley. Amino acid digestibilities, determined according to the ileal analysis method, are usually lower than those determined according to the fecal analysis method (Sauer and Ozimek, 1986). The present results, therefore, although not typical for barley, may be found in certain samples of barley. Furthermore, the barley that was used in the present studies was coarsely ground. Fineness of grinding may have a significant effect on amino acid digestibilities in cereal grains, especially when these are determined according to the ileal analysis method, as was demonstrated by Sauer et al. (1977) in wheat.

The observed and calculated digestibilities of protein and amino acids for the B + SBM and B + CM diets are presented in tables 2.4 and 2.5. The calculated digestibilities are based on the digestibilities determined in the single ingredients and the proportion of these in the complete diets. Of the indispensable amino acids, there were no differences ($P > .05$) between the observed and expected digestibilities in the B + SBM diet (table 2.4). The differences ranged from 0 (histidine) to 5.1% (valine). The observed digestibilities of most of the amino acids were slightly higher than those calculated in the B + CM diet (table 2.5). Of the indispensable amino acids, the differences were significant only for lysine and phenylalanine. Of the dispensable amino acids, there were differences ($P<.05$) for aspartic acid in the B + SBM diet and for alanine, glycine and proline in the B + CM diet between the observed and calculated digestibilities (tables 2.4 and 2.5).

The present studies indicate the presence of associative effects, albeit of a small magnitude for some of the amino acids. In general, however, these studies show that the digestible amino acid supply in a complete diet can be predicted from amino acid digestibilities determined in the single feedstuff. The digestible amino acid supply, at least, was not overestimated; the calculated digestibilities were equal to or lower than those observed. Additivity of the digestible protein and amino acid supply, determined in single feedstuffs, is a crucial consideration in the formulation of complete diets. These studies, however, do not rule out the possibility of associative effects between other feedstuffs. Investigations with a wider variety of feedstuffs may be warranted, including those with a high fiber content and/or anti-nutritional factors such as trypsin inhibitors, tannins and lectins. Anti-nutritional factors apart from affecting the digestion and/or absorption of protein in the feedstuff in which they are present, may also affect the utilization of protein in the other dietary ingredients. The digestion and absorption of endogenous protein secreted into the small intestine may be affected as well.

There were no differences ($P>.05$) in the rate of secretion of pancreatic juice, protein, chymotrypsin and trypsin between pigs fed the SBM and CM diets (table 2.6). The measurements for all parameters investigated were highly variable, which was also observed in other studies (Partridge et al., 1982; Zebrowska et al., 1983). Partridge et al. (1982) ascribe this variability to biological causes rather than to problems in analytical techniques.

The mean values for the volume of pancreatic juice secreted in 24 h were in the range of values reported for pigs that were fed semi-purified diets at a similar level of feed intake (table 2.6). The daily volume of pancreatic juice was 1.3 and 1.2 l in pigs fed cornstarch-based casein diets (Partridge et al., 1982; Zebrowska et al., 1983). Pigs fed cereal-based diets secreted twice the volume of pancreatic juice. The inclusion of fiber, depending on the type of dietary fiber, will increase the output of pancreatic juice (Maenhout et al., 1987).

The values for pancreatic secretion of protein are in the range of those reported by Zebrowska et al. (1983) and Hee et al. (1985) but higher than those by Partridge et al. (1982). Comparison of results in the different reports which would also apply to the secretion of trypsinogen and chymotrypsinogen, however, are difficult as these are confounded by differences in feed intake, feeding regimen, diet composition, pig weight and different approaches to collect pancreatic juice, which include direct cannulation of the pancreatic duct and collection of juice from a duodenal pouch. Based on the present studies and those by Partridge et al. (1982) and Zebrowska et al. (1983), it seems likely that only extreme differences between diets will elicit differences in the pancreatic secretion of protein. For example, the presence of active trypsin inhibitors (as in untoasted SBM) will increase the pancreatic protein secretion (Schumann et al., 1983). The absence of dietary protein will decrease the secretion (Corring and Saucier, 1972).

The total activities of trypsin are in general agreement with those reported by Partridge et al. (1982) and Zebrowska et al. (1983). These authors also found no effect of diet composition on the total activities of trypsin and chymotrypsin. The total activities of chymotrypsin, on the other hand, were lower than those reported by the aforementioned authors.

The lower amino acid digestibilities in CM than in SBM, therefore, do not result from differences in the rate of secretion of protein and differences in total activities of the proteolytic enzymes in pancreatic juice. A lower rate of secretion relative to SBM might have suggested a deficiency in the rate of secretion of the proteolytic enzymes. A higher rate of secretion, resulting from a negative feedback mechanism (e.g. resulting from the presence of trypsin inhibitors) could have been a reflection of the presence of compounds in CM that may form complexes with the proteolytic enzymes during digestion.

Additivity of the digestible protein and amino acid supply, determined in single ingredients, is a crucial consideration in the formulation of diets. The present studies show that the digestible amino acid supply, in diets made up of barley and canola meal and barley and soybean meal, can be predicted from amino acid digestibilities determined in the single ingredients. There was a positive associative effect for protein and some of the amino acids when barley was supplementef with canola meal. The observed digestibilities of most of the amino acids were slightly higher than those calculated in the barley and canola meal diet. Additional studies showed that differences in pancreatic protein and enzyme secretions were not responsible for the lower amino acid digestibilities in canola meal than in soybean meal.

TABLE 2.1. FORMULATION OF EXPERIMENTAL DIETS

Items	SBM[a]	B	B + SBM	CM	B+CM
Ingredients, %:					
Soybean meal	40.0		40.0		
Barley		93.3	53.7		53.6
Cornstarch	43.6			35.1	
Canola meal				50.0	40.0
Dextrose	10.0			10.0	
Tallow	4.0	4.0	4.0	4.0	4.0
Calcium carbonate	.4	1.2	1.0	.6	1.1
Dicalcium phosphate	.7	.2			
Iodized salt	.5	.5	.5	.5	.5
Vitamin premix[b]	.2	.2	.2	.2	.2
Trace-mineral premix[c]	.1	.1	.1	.1	.1
Chromic oxide	.5	.5	.5	.5	.5

[a]SBM: soybean meal; B: barley; B+SBM: barley + soybean meal; CM: canola meal; B+CM: barley + canola meal.

[b]The vitamin premix supplied the following vitamins (per kg diet): Vitamin A, 1300 IU; Vitamin D, 150 IU; Vitamin E, 11 IU; Vitamin K, 2.0 mg; Riboflavin, 2.2 mg; Niacin, 12 mg; Pantothenic acid, 11 mg; Vitamin B_{12}, 11 μg; Choline, 550 mg; Thiamine, 1.1 mg; Pyridoxine, 1.1 mg; Biotin, .1 mg and Folic acid, .6 mg.

[c]The trace-mineral mixture supplied the following minerals (per kg diet): Fe, 50 mg; Zn, 50 mg; Mn, 2 mg; Cu, 3 mg; I, .14 mg and Se, .15 mg.

TABLE 2.2. DRY MATTER, PROTEIN AND AMINO ACID CONTENT (%) OF THE EXPERIMENTAL DIETS

Items	SBM[a]	B	B+SBM	CM	B+CM
Dry matter	93.11	89.31	91.40	93.82	90.88
Crude protein[b]	19.30	10.46	27.14	20.20	22.53
Amino acids[b]					
Indispensable					
Arginine	1.34	.51	2.04	1.13	1.19
Histidine	.51	.24	.69	.48	.54
Isoleucine	.90	.37	1.17	.87	.93
Leucine	1.45	.72	1.99	1.39	1.53
Lysine	1.14	.41	1.55	1.03	1.19
Phenylalanine	.96	.51	1.46	.84	1.10
Threonine	.78	.40	1.10	.94	.98
Valine	.97	.44	1.14	1.09	.98
Dispensable					
Alanine	.84	.43	1.26	.89	.99
Aspartic acid	.79	.72	2.93	1.61	1.67
Glutamic acid	3.32	2.45	4.99	3.29	4.24
Glycine	.81	.44	1.18	.99	1.07
Proline	.96	.87	1.52	1.17	1.65
Serine	.91	.46	1.30	.85	.98
Tyrosine	.56	.24	.72	.39	.47

[a]SBM: soybean meal; B: barley; B+SBM; barley + soybean meal; CM: canola meal; B+CM: barley + canola meal.

[b]Dry matter basis.

TABLE 2.3. THE APPARENT DIGESTIBILITIES (%) OF DRY MATTER, PROTEIN AND AMINO ACIDS DETERMINED AT THE DISTAL END OF THE SMALL INTESTINE

Items	SBM	B	B + SBM	CM	B + CM	SE[a]
Dry matter	80.9b	56.6d	56.0d	65.0c	45.8e	1.3
Crude protein	81.7b	45.0e	74.3c	67.4d	64.6d	2.5
Amino acids						
Indispensable						
Arginine	91.8b	58.3d	88.2b	81.2c	77.3c	1.8
Histidine	88.2b	55.6e	81.8bc	76.3cd	69.1d	2.5
Isoleucine	85.8b	44.4d	77.5bc	70.6c	67.5c	2.8
Leucine	85.5b	52.3d	78.3bc	72.0c	69.8c	2.7
Lysine	85.6b	37.6d	80.3bc	69.8c	70.2c	3.5
Phenylalanine	83.9b	48.8d	78.9b	70.3c	69.5c	1.7
Threonine	80.1b	44.2d	73.8b	67.6c	65.3c	3.1
Valine	81.9b	29.1e	66.6c	65.3c	54.2d	3.2
Dispensable						
Alanine	81.7b	37.4d	75.8bc	71.4bc	66.7c	3.0
Aspartic acid	81.2b	46.8d	80.8b	65.5c	64.5c	2.1
Glutamic acid	87.8b	69.6e	81.4c	80.1c	75.4d	1.3
Glycine	77.1b	24.7d	69.7bc	63.3bc	61.0c	3.5
Proline	83.8b	59.3e	72.9c	65.6d	68.8c	1.7
Serline	81.8b	49.6d	77.4b	63.9c	63.4c	2.4
Tyrosine	83.4b	37.5d	74.8bc	56.8c	56.8c	3.1

[a]Standard error of the mean.

[b,c,d,e]Means in the same row followed by different letters differ at $P<.05$.

TABLE 2.4. THE OBSERVED AND CALCULATED PROTEIN AND AMINO ACID DIGESTIBILITIES[a] (%) OF BARLEY-SOYBEAN MEAL DIET

Items	Observed	Calculated
Crude protein	74.3 ± 3.3	73.2 ± 3.0
Amino acids		
Indispensable		
Arginine	88.2 ± 1.3	86.2 ± 1.7
Histidine	81.8 ± 2.1	81.8 ± 2.5
Isoleucine	77.5 ± 4.4	78.1 ± 2.3
Leucine	78.3 ± 3.5	78.7 ± 2.4
Lysine	80.4 ± 3.4	78.2 ± 3.0
Methionine	66.7 ± 8.3	67.4 ± 5.3
Phenylalanine	78.9 ± 1.6	76.2 ± 1.7
Threonine	73.8 ± 6.3	72.1 ± 3.2
Valine	66.6 ± 7.1	71.7 ± 2.4
Dispensable		
Alanine	75.8 ± 2.9	72.3 ± 3.6
Aspartic acid	80.8 ± 1.8b	76.1 ± 3.3c
Glutamic acid	81.4 ± 0.7	82.9 ± 2.9
Glycine	69.7 ± 4.5	65.5 ± 4.7
Proline	72.9 ± 2.3	75.7 ± 3.8
Serine	77.4 ± 2.3	75.3 ± 3.8
Tyrosine	74.8 ± 5.7	74.8 ± 2.6

[a]Means and standard deviation.

[b,c]Means in the same row followed by a different letter differ at $P<.05$.

TABLE 2.5. THE OBSERVED AND CALCULATED PROTEIN AND AMINO ACID DIGESTIBILITIES[a] (%) OF THE BARLEY-CANOLA MEAL DIET

Items	Observed	Calculated
Crude protein	64.0 ± 3.8b	60.6 ± 3.2c
Amino acids		
Indispensable		
Arginine	76.7 ± 3.8	75.3 ± 1.8
Histidine	72.0 ± 3.7	70.7 ± 2.6
Isoleucine	67.0 ± 3.4	63.7 ± 2.7
Leucine	67.6 ± 4.8	66.1 ± 2.8
Lysine	69.6 ± 4.3b	62.3 ± 4.0c
Phenylalanine	69.4 ± 1.5b	63.0 ± 1.9c
Threonine	64.9 ± 5.3	61.4 ± 3.2
Valine	53.9 ± 2.5	56.0 ± 2.8
Dispensable		
Alanine	65.8 ± 5.6b	61.7 ± 4.3c
Aspartic acid	63.8 ± 4.5	60.3 ± 2.5
Glutamic acid	74.9 ± 2.9	76.1 ± 1.8
Glycine	59.8 ± 5.3b	54.1 ± 3.3c
Proline	68.2 ± 2.4b	62.8 ± 1.5c
Serine	62.0 ± 5.9	59.3 ± 3.6
Tyrosine	56.9 ± 2.1b	50.0 ± 3.1c

[a]Means and standard deviation.

[b,c]Means in the same row followed by a different letter differ at $P<.05$.

TABLE 2.6. THE EFFECT OF DIET ON THE DAILY VOLUME OF PANCREATIC JUICE AND RATE OF SECRETION OF PROTEIN, CHYMOTRYPSIN AND TRYPSIN

Diet	SBM	CM	SE[a]
Volume, ml	1157.5	1042.5	152.3
Protein, g	16.4	15.4	1.5
Chymotrypsin, U x 10^{-3}	26.2	28.6	3.2
Trypsin, U x 10^{-3}	156.0	153.4	13.7

[a]Standard error of the mean.

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3. THE EFFECT OF THE DIETARY LEVEL OF FAT ON AMINO ACID DIGESTIBILITIES IN SOYBEAN MEAL AND CANOLA MEAL AND ON RATE OF PASSAGE IN GROWING PIGS

INTRODUCTION

Fat has traditionally been used in pig production to increase the energy density of the diets. The effect of dietary fat level on pig performance and carcass quality has been well documented (Asplund et al., 1960; Allee and Hines, 1972; Friend et al., 1975; Sarkar et al., 1985). Studies carried out on the effect of fat on protein digestibility have shown conflicting results. Improvements in protein digestibility were found in some (Asplund et al., 1960; Jorgensen et al., 1985) but not in other studies (Lowrey et al., 1962; Greeley et al., 1964). Furthermore, with the exception of studies by Jorgensen et al., (1985), no studies have been carried out on the effect of fat on ileal amino acid digestibilities. As was reviewed by Sauer and Ozimek (1986), the ileal rather than the fecal analysis method should be used for the determination of amino acid digestibilities. An increase in the dietary level of fat has also been shown to delay gastric emptying (Harkins et al., 1964; Hunt and Knox, 1968). However, the effect of fat on the rate of passage in other parts of the digestive tract and its nutritional implications in terms of protein and amino acid digestibilities have not been studied.

The objectives of the present studies were to determine the effect of increasing dietary fat levels on the ileal and fecal amino acid and protein digestibilities and on the rate of passage of digesta in pigs fed diets that contained SBM or CM as sole protein source.The rate of passage was determined in digesta collected from the distal ileum rather than in feces as the majority of digestion and absorption of nutrients takes place in the small intestine.

MATERIALS AND METHODS

Experiment 1. Six barrows (Yorkshire x Lacombe), with an average initial weight of 45 kg, were obtained from the University of Alberta swine herd. The pigs were housed in stainless steel metabolism crates 1 week prior to the insertion of the cannulas and fed a 16% crude protein grower diet (Sauer et al., 1983). Water was freely available from a low-pressure drinking nipple.

The barrows were fitted with a simple T-cannula, approximately 5 to 10 cm anterior to the ileo-cecal sphincter, according to procedures adapted from Sauer et al. (1983). The preparation of the cannulas followed the procedures outlined by McBride et al. (1983). However, the shape of the cannula was modified to include a larger flange and the ring was omitted (Plate 3.1). The new design of the cannula minimized the formation of intestinal adhesions which occurred frequently with the use of the original cannula. In addition, a different cap and stopper was prepared allowing for easier removal and insertion.

The pigs were returned to the metabolic crates immediately after surgery and fasted overnight. The next day they were given approximately 100 g of the grower diet, at each of two meals. The feed allowance was increased slowly until the pigs consumed 2 kg daily. This normally took 7 d to achieve. The pigs were allowed a 14-d recuperation period. A detailed description of pre- and post-operative care was previously presented by Sauer (1976) and Sauer et al. (1983).

The apparent ileal and fecal amino acid digestibilities were determined in three cornstarch-based diets that contained 15% crude protein from soybean meal (SBM) and 2, 6 or 10% canola oil (CO) (table 3.1). Dextrose was included at a level of 10% to improve the palatability of the semi-purified diets. Vitamins and minerals were supplemented to meet or exceed NRC (1979) standards. Chromic oxide was included in the diets as the marker for the determination of the digestibilities of nutrients that were measured. Solvent-extracted SBM was used.

The experiment was carried out according to a repeated 3 x 3 Latin square design. Each experimental period lasted 14 d. Feces were collected directly from the anus during each feeding on d 10 to 12. Ileal digesta were collected for a total of 24 h: 12 h on d 12 (0800 to 1000 h and alternate 2 h periods thereafter) and 12 h on d 13 (1000 to 1200 h and alternate 2 h periods thereafter). Feces and ileal digesta were pooled within pig, period and treatment. The procedures for collection of ileal digesta were adapted from Just et al. (1983). The pigs were fed 800 g twice daily, at 0800 and 2000 h.

Rate of passage studies were carried out starting on d 14 of the experimental period. The pigs received 50 ppm of dysprosium (Dy), which was administered with their morning feed (800 g at 0800 h). The Dy in the form of dysprosium chloride, was finely ground and mixed with the feed according to procedures described by Kennelly et al. (1980). Immediately after feeding, ileal digesta were collected for periods of 1 h each during the first 12 h and for periods of 2 h each during the next 12 h. A total of 18 samples was obtained for each pig on each diet over the 24 h collection period.

Experiment 2. With the exception of the formulation of the experimental diets, the studies described under experiment 1 were repeated under similar conditions with another six barrows of similar initial weight and of the same breed. The apparent ileal and fecal amino acid digestibilities were determined in three cornstarch-based diets that contained 15% protein from canola meal (CM) (solvent-extracted) and 2, 6 or 10% CO (table 3.1).

Chemical and Statistical Analysis

Ileal digesta and feces, which were collected for the digestibility studies, were frozen immediately following collection. The samples were freeze-dried, pooled within pig and diet, ground through a .5-mm mesh screen in a Wiley mill and mixed prior to analyses. The feed samples were ground similarly.

Analyses for dry matter, crude protein, crude fiber and fat were carried out according to AOAC (1980) methods. Chromic oxide was determined according to Fenton and Fenton (1979). Amino acid analyses were carried out according to HPLC procedures described by Jones and Gilligan (1983) using a Varian 5000 Liquid chromatograph. This method is based on pre-column derivative formation between O-phthaldialdehyde and primary amino acids in the hydrolysate. The samples were hydrolysed for 24 h with 6N HCL prior to HPLC procedures. The content of the sulfur-containing amino acids, and tryptophan and proline were not determined. Analyses were carried out in duplicate.

The digesta samples, collected for the rate of passage studies, were freeze-dried, ground through a .5-mm mesh screen in a Wiley mill and analysed in duplicate for dry matter (AOAC, 1980) and Dy which was measured by Instrument Neutron Activation analysis using the Slowpoke Reactor at the University of Alberta according to Kennelly et al. (1980).

The digestibility results for the SBM experiment were analysed according to a completely randomized design following procedures described by Steel and Torrie (1980). Animal losses during the experiment (i.e. three out of six had died by period 3), resulting in the need to replace animals after the third period, led to analysis of variance according to a completely randomized design instead of a Latin square design. Analysis of results according to a completely randomized design as opposed to a Latin square design should not effect the interpretation. Previous studies have shown no effect (P>.05) of weight of pigs, over the range of 40 to 70 kg, on apparent protein digestibility (Just Nielsen, 1968; Fernandez and Jorgensen, 1986). The digestibility results of the CM experiment were analysed according to a repeated 3 x 3 Latin square design. Where appropriate, treatment means were compared using the Student-Newman-Keuls multiple range test. In addition, regression analyses were carried out to determine the relationship between amino acid digestibilities and dietary fat levels.

Regressions of natural log with time were carried out to determine the equations for the rate of passage. The rate constants within the SBM and CM diets were compared by analysis of variance according to Steel and Torrie (1980). An orthogonal contrast was carried out to compare the rate of passage of the SBM and CM diets.

RESULTS AND DISCUSSION

The mean apparent ileal and fecal digestibilities of protein and amino acids for the SBM and CM diets are presented in Table 3.3. Irrespective of whether these were determined in ileal digesta or feces, the protein and amino acid digestibilities were higher in the SBM diets. Based on the ileal analysis method, the digestibility of threonine was lowest and arginine highest among the indipensable amino acids in both the SBM and CM diets. Based on the fecal analysis method, the digestibility of threonine was lowest in SBM and isoleucine lowest in CM. The fecal digestibilities of arginine were highest in both diets. Of the indispensable amino acids, threonine disappeared to the largest extent in the large intestine. Of the dispensable amino acids, the disappearance was most pronounced for glycine. These results are in general agreement with those previously reported by Sauer et al. (1982) and Imbeah et al. (1988). The factors responsible for the lower amino acid digestibilities in CM than in SBM include the higher fiber and tannin content in CM as was previously reviewed by Sauer et al. (1982) and Sauer and Thacker (1980).

The apparent ileal amino acid digestibilities for the individual SBM and CM diets are presented in Table 3.4. For SBM diets, the ileal digestibilities of dry matter and crude protein were not affected ($P>.05$) by the dietary level of fat. However, the inclusion of additional fat, from 2 to 10%, increased ($P<.05$) the digestibilities of most of the amino acids. Of the indispensable amino acids, the improvements ranged from 1.5 (arginine) to 4.9 (threonine) percentage units. There were no differences ($P>.05$) in the amino acid digestibilities between the SBM diets that contained 2 and 6% CO. The digestibilities of some of the indispensable amino acids (arginine, threonine and valine) were higher (P<.05) in the SBM diet with 10 than 6% CO. Of the indispensable amino acids, the digestibilities of arginine, phenylalanine, threonine and valine showed a quadratic response (P<.05) to changes in dietary fat while histidine and lysine showed a linear response (P<.05) (table 3.5).

For CM diets there was a decrease (P<.05) in the apparent ileal digestibilities for dry matter, protein and most of the amino acids when the dietary level of fat was increased from 2 to 6% (table 3.4). Of the indispensable amino acids, the decrease ranged from 2.2 (arginine) to 4.3 (threonine) percentage units. An increase in the dietary fat level from 6 to 10% improved (P<.05) the digestibilities to values similar or higher than those obtained with the 2% fat CM diet. A curvilinear relationship could be established between the ileal amino acid digestibilities and dietary fat levels in CM (table 3.5). Multiple regression analysis showed that both the linear as well as the non-linear components of this relationship were significant (P<.05) and therefore, both are important in determining the digestibilities of amino acids in CM diets with varying dietary fat levels.

The ileal rather than the fecal analysis method should be used for the determination of amino acid digestibilities (Sauer and Ozimek, 1986). Studies by Zebrowska (1973) showed that both intact or enzymatically hydrolyzed casein infused into the distal part of the ileum of pigs fed a protein-free diets was digested and absorbed; however, the absorbed material was rapidly and almost completely excreted in urine. When casein was given orally, the level of free amino acids in portal blood was high, and the level of urea was low. Other reports also clearly show that protein or amino acids infused into the large intestine make little or no contribution to the protein status of the pig (Sauer, 1976; Gargallo and Zimmerman, 1981; Wunsche et al., 1982).

The present studies show an effect of dietary fat level on the ileal amino acid digestibilities in SBM, of a linear or quadratic nature, and in CM, usually of a curvilinear nature. However, the responses in amino acid digestibilities to different levels of inclusion of CO were small. Depending on the level of dietary fat, the differences ($P<.05$) among the indispensable amino acids ranged from 1.5 to 4.9 percentage units in SBM and from 3.5 to 6.5 percentage units in CM. The highest amino acid digestibilities were observed when the diets included 10% CO. These results tend to be in agreement with studies by Jorgensen et al. (1985) who observed increases ($P<.05$) in the ileal digestibilities of lysine, methionine and threonine when the fat level was increased from 3 to 15% in diets for growing pigs.

The apparent fecal digestibilities of dry matter, protein and amino acids are presented in Table 3.6. The level of dietary fat did not affect ($P>.05$) the digestibilities in SBM. The curvilinear trend observed for the apparent ileal amino acid digestibilities in the CM diets with varying dietary fat levels was also observed for the fecal digestibilities but was not significant ($P>.05$) for most amino acids. There was a decrease ($P<.05$) in the apparent fecal digestibilities of dry matter, protein and one of the indispensable (histidine) and dispensable (glycine) amino acids when the dietary level was increased from 2 to 6% in the CM diets. Increasing the dietary fat level from 6 to 10% improved (P<.05) the digestibilities of dry matter, protein, phenylalanine, aspartic acid and glutamic acid above their digestibilities in the 6% fat diet but did not exceed those in the 10% fat diet .

These studies show no effect of dietary fat level on the fecal amino acid digestibilities in SBM and a very small effect in CM. Depending on the level of fat inclusion, the differences (P<.05) among the digestibilities of protein and indispensable amino acids ranged from 1.4 to 3.7 percentage units.

Conflicting reports on the effect of fat on protein digestibility, the results of which are summarized in Table 3.7, appear in the literature. Marshall et al. (1959) in studies with rats, fed diets containing lactoalbumin as the sole protein source and 3 or 15% fat, observed no differences (P>.05) in protein digestibility. Lowrey et al. (1962), in studies with piglets weaned at 3 wk, fed corn-based SBM and fishmeal diets also observed no differences (P>.05) in protein digestibility when 10% compared to 0% fat was added to the diets. Furthermore, no differences were found in protein digestibility in studies with pigs fed corn-SBM diets that contained 0, 5, 10 or 15% fat (Greeley et al., 1964). On the other hand, Asplund et al. (1960), in studies with piglets weaned at 3 wk, fed corn-based SBM, meat scraps and brewers' yeast observed an improvement (P<.05) in protein digestibility when 10% fat was added to the diet. Jorgensen et al. (1985) in studies with growing pigs also observed an increase (P<.05) in the apparent protein digestibility (in addition to lysine, methionine and threonine) when the dietary fat content was increased from 3 to 15%. Furthermore, Tao et al. (1971) observed improvements ($P<.05$) in the fecal digestibilities of many of the indispensable amino acids, ranging from 8.6 to 19.7 percentage units, with broilers fed rapeseed meal diets supplemented with 3.5% fat compared to no fat supplement.

It is rather difficult to consolidate the findings by the various authors and to explain the underlying physiological and nutritional mechanisms for the different results. However, differences in methodology, age of pigs, diet composition, levels of fat and differences in fat source may account for some of the contradictory findings.

Measurements of rate of passage can be made with the withdrawal method or single dose method. In the withdrawal method, the marker is fed at a constant level in the diet until steady-state conditions are established. The marker is then withdrawn and its declining concentration in digesta or feces measured (Faichney, 1975). With the single dose method, the marker is administered in a single meal and its concentration measured in digesta or feces from the time of feeding (Castle and Castle, 1957; Maner et al., 1962; Keys and DeBarthe, 1974). The most widely used method for expressing rate of passage is the mean retention time between 5 and 95% marker excretion. This method requires total collection of digesta or feces and assumes 100% marker excretion within a fixed time period (Castle and Castle, 1957). An alternative method to determine the rate of passage expresses the natural log of marker concentration from the sample with maximum concentration to the last sample according to the following equation: $\ln Y = a + bX$ (Y = marker concentration; X = time after ingestion or withdrawal of marker; b: rate of marker disappearance or rate constant), (Smith, 1968; Keys and DeBarthe, 1974). The latter method, which has the advantage that it can be used with samples rather than a total collection during a particular time period, combined with the single dose method, was used to determine the rate of passage of the SBM and CM diets in the present studies. The use of pigs, fitted with a simple T-cannula at the distal ileum which only allowed the collection of samples of digesta, was therefore possible.

The equations obtained for the six diets are presented in Table 3.8. The level of dietary fat did not influence (P>.05) the rate of passage of either the SBM or CM diets. However, the rate of passage of the SBM diets was lower (P<.05) than that of the CM diets. The times required for the first appearance of marker at the distal ileum (transit time) were 3.8, 3.2 and 3.0 h for the SBM diets and 3.0, 3.4 and 3.4 h for the CM diets with 2, 6 and 10% CO, respectively.

The present studies show that the CM diets passed through the small intestine faster than the SBM diets. These results are in agreement with studies by Shires et al. (1987) who observed significant differences (P<.05) between the mean retention times of corn-SBM (388.0 min) and corn-CM (309.8min) diets fed to chickens . These authors measured retention times over the entire digestive tract. The higher fiber content of the CM than the SBM diets (4.7 to 4.9 vs 2.3 to 2.6) (table 3.2) was possibly responsible for the faster rate of passage. Ehle et al. (1982) and Kuan et al. (1983), in studies with pigs, observed a reduction in the transit time with increasing crude fiber levels in the diet. Therefore, the faster rate of passage of the CM than SBM diets may be a contributing factor to the lower amino acid digestibilities in CM, based on the assumption that the time for digestion of protein and/or absorption of amino acids is a limiting factor.

The dietary inclusion of fat has been shown to delay gastric emptying (Harkins et al., 1964; Hunt and Knox, 1967; Elias et al., 1968; Pirk and Skala, 1970; Sidebottom et al., 1983). However, this delay in stomach emptying was not reflected by a delay in rate of passage of the diets when this was measured at the distal ileum.

A biphasal marker excretion pattern was observed, illustrated for 3 pigs receiving the SBM and CM diets that contained 2% CO (Figures 3.1 and 3.2). This pattern was usually more defined for pigs receiving the SBM diets and was apparent in 19 out of the 30 observations. Van Soest et al. (1983) in a review reported on a biphasal excretion pattern in feces in pigs which they attributed to pulsative cecal emptying. Van Soest et al. (1983) point out that these types of mixed excretion patterns are difficult to interpret. No systematic mathematical analyses has been developed for these patterns. The biphasal pattern that was observed in these studies in digesta collected from the distal ileum may result from finer feed particles leaving the stomach at a faster rate than the coarser particles. Potkins et al. (1985) observed that finely ground barley passed out of the stomach faster than coarsely ground barley. The rate constants were 0.189 and 0.113, respectively. As these studies show, the differential rate of exit of finer and coarser particles seems to carry through to the distal ileum providing a biphasal excretion pattern.

In conclusion, the present studies show that the level of dietary fat may affect the ileal amino acid digestibilities. However, the differences in amino acid digestibilities were of a small magnitude. The differences were even smaller when the fecal amino acid digestibilities were considered. Furthermore, the dietary fat level did not affect the rate of passage, measured in digesta collected from the distal ileum in pigs fed either the SBM or CM diets. However, the rate of passage of the CM diets was faster than that of the SBM diets.

TABLE 3.1. COMPOSITION (%) OF EXPERIMENTAL DIETS

Item	Diets					
	Soybean meal			Canola meal		
	2% CO[a]	6% CO	10% CO	2% CO	6% CO	10% CO
Ingrdients						
Soybean meal	32.0	32.0	32.0			
Canola meal				40.0	40.0	40.0
Cornstarch	45.25	41.25	37.25	38.25	34.25	30.25
Canola oil	2.0	6.0	10.0	2.0	6.0	10.0
Dextrose	10.0	10.0	10.0	10.0	10.0	10.0
Beet pulp	5.0	5.0	5.0	5.0	5.0	5.0
Alphafloc[b]	3.0	3.0	3.0	3.0	3.0	3.0
Calcium carbonate	.4	.4	.4	.45	.45	.45
Dicalcium phosphate	1.05	1.05	1.05			
Iodized salt	.5	.5	.5	.5	.5	.5
Vitamin premix[c]	.2	.2	.2	.2	.2	.2
Trace-mineral premix[d]	.1	.1	.1	.1	.1	.1
Chromic oxide	.5	.5	.5	.5	.5	.5

[a] CO: canola oil

[b] Brown Company, Berlin, NH, 03570

[c] The vitamin premix supplied the following vitamins (per kg diet): vitamin A, 1,300 IU; vitamin D, 150 IU; vitamin E, 11 IU; vitamin K, 2.0 mg; riboflavin, 2.2 mg; niacin, 12 mg; pantothenic acid, 11 mg; vitamin B_{12}, 11 ug; choline, 550 mg; thiamine, 1.1 mg; pyridoxine, 1.1 mg; biotin, .1 mg and folic acid, .6 mg.

[d] The trace-mineral mixture supplied the following minerals (per kg diet): Fe, 50 mg; Zn, 50 mg; Mn, 2 mg; Cu, 3 mg; I, .14 mg and Se, .15 mg.

TABLE 3.2. PROXIMATE AND AMINO ACID COMPOSITION (%) OF EXPERIMENTAL DIETS

Item	Diets					
	Soybean meal			Canola meal		
	2% CO[a]	6% CO	10% CO	2% CO	6% CO	10% CO
Proximate						
Dry matter	91.7	91.2	91.8	91.5	91.7	92.5
Crude protein	15.7	14.6	15.5	14.6	14.3	15.6
Crude fat	2.0	5.9	10.0	2.3	6.0	10.0
Crude fiber	2.3	2.5	2.6	4.9	4.7	4.9
Amino acids						
Indispensable						
Arginine	1.07	1.01	1.07	.94	.92	1.04
Histidine	.38	.38	.38	.41	.39	.44
Isoleucine	.74	.67	.72	.68	.66	.72
Leucine	1.22	1.14	1.21	1.19	1.15	1.26
Lysine	1.02	.96	1.04	.98	.95	1.06
Phenylalanine	.78	.73	.77	.66	.64	.70
Threonine	.64	.59	.66	.77	.76	.83
Valine	.73	.68	.72	.82	.81	.88
Dispensable						
Alanine	.76	.68	.75	.80	.78	.88
Aspartic acid	1.84	1.75	1.83	1.27	1.23	1.36
Glutamic acid	2.99	2.79	2.98	2.98	2.93	3.24
Glycine	.72	.70	.73	.91	.88	1.00
Serine	.87	.88	.88	.76	.76	.85
Tyrosine	.49	.45	.50	.51	.49	.55
Amino acid N recovery[b]	91.9	92.6	92.9	95.0	95.0	97.1

[a] CO: canola oil

[b] Percentage of amino acid nitrogen in total nitrogen

TABLE 3.3. THE MEAN APPARENT ILEAL AND FECAL DIGESTIBILITIES[a] (%) OF DRY MATTER, PROTEIN AND AMINO ACIDS IN THE SOYBEAN MEAL AND CANOLA MEAL DIETS.

Item	Soybean meal		Canola meal	
	Ileum	Feces	Ileum	Feces
Dry matter	76.4 ± .65	92.9 ± .21	65.6 ± 1.09	82.7 ± .36
Protein	81.0 ± .85	90.9 ± .48	66.6 ± 1.44	80.0 ± .81
Amino acids				
Indispensable				
Arginine	91.5 ± 0.57	95.2 ± 1.03	82.8 ± 0.95	89.5 ± 1.75
Histidine	87.0 ± 0.47	94.7 ± 0.61	81.7 ± 0.80	87.2 ± 1.03
Isoleucine	85.6 ± 0.62	90.3 ± 0.76	71.4 ± 1.04	79.8 ± 1.28
Leucine	85.6 ± 0.57	91.4 ± 0.57	73.6 ± 0.96	84.3 ± 0.95
Lysine	85.5 ± 0.50	91.6 ± 0.59	72.8 ± 0.85	82.9 ± 0.99
Phenylalanine	86.4 ± 0.58	91.9 ± 0.41	73.8 ± 0.97	82.4 ± 0.68
Threonine	73.6 ± 1.01	87.8 ± 0.89	62.1 ± 1.70	82.4 ± 1.51
Valine	82.2 ± 0.68	89.5 ± 0.73	69.0 ± 1.14	82.7 ± 1.23
Dispensable				
Alanine	80.3 ± 0.78	88.4 ± 0.90	70.9 ± 1.31	82.3 ± 1.51
Aspartic acid	83.0 ± 1.05	92.4 ± 0.53	66.4 ± 1.76	80.8 ± 0.90
Glutamic acid	87.8 ± 0.82	94.9 ± 0.30	81.4 ± 1.38	91.4 ± 0.51
Glycine	74.2 ± 1.71	89.5 ± 0.35	63.1 ± 2.88	85.9 ± 0.95
Serine	83.4 ± 0.80	93.1 ± 0.85	69.1 ± 1.34	85.1 ± 1.44
Tyrosine	83.4 ± 0.90	88.2 ± 1.31	69.6 ± 1.52	82.1 ± 2.21

[a] Means and standard error of the digestibility coefficients of all the diets with SBM or CM.

TABLE 3.4. EFFECT OF LEVEL OF FAT ON APPARENT ILEAL DIGESTIBILITIES (%) OF DRY MATTER, PROTEIN AND AMINO ACIDS

	Diets							
	Soybean meal				Canola meal			
Item	2% CO[a]	6% CO	10% CO	SE[b]	2% CO	6% CO	10% CO	SE[b]
Dry matter	76.0	77.0	75.6	.73	65.7[c]	62.9[d]	66.3[c]	.57
Crude protein	80.1	80.6	79.8	.51	68.1[d]	63.6[e]	70.1[c]	.42
Amino acids								
Indispensable								
Arginine	90.2[d]	90.4[d]	91.7[c]	.32	84.5[c]	82.3[d]	85.8[c]	.44
Histidine	85.9[d]	87.4[cd]	87.9[c]	.41	80.0[d]	79.8[d]	83.4[c]	.58
Isoleucine	83.7	87.2	85.2	.54	74.1[c]	70.2[d]	75.8[c]	.62
Leucine	83.2	83.9	85.5	.53	76.8[c]	73.5[d]	78.7[c]	.55
Lysine	83.6[d]	84.7[cd]	85.9[c]	.44	74.9[e]	71.0[d]	76.9[c]	.43
Phenylalanine	84.2[c]	84.8[cd]	86.4[c]	.48	76.6[cd]	74.2[d]	78.5[c]	.53
Threonine	69.7[d]	71.8[d]	74.6[c]	.77	64.4[c]	60.1[d]	66.6[c]	.67
Valine	79.6[d]	80.2[d]	82.7[c]	.56	71.6[c]	68.0[d]	73.6[c]	.65
Dispensable								
Alanine	77.7[d]	78.2[d]	80.8[c]	.59	73.9[c]	70.7[d]	75.9[c]	.56
Aspartic acid	80.5	81.2	82.7	.70	69.5[cd]	65.7[d]	71.5[c]	1.05
Glutamic acid	85.6	86.5	88.3	.68	83.3[c]	81.1[d]	84.4[c]	.58
Glycine	69.3	74.0	73.6	1.44	66.1[c]	61.4[d]	68.5[c]	.81
Serine	80.9[d]	81.3[d]	83.7[c]	.53	70.1[d]	70.6[d]	73.6[c]	.70
Tyrosine	80.4[d]	80.3[d]	85.3[c]	1.03	71.6[d]	69.2[e]	75.4[c]	.50

[a] CO: canola oil

[b] Standard error of the mean

[c,d,e] Means in the same row followed by a different superscript, within soybean meal or canola meal, differ at P<.05.

TABLE 3.5. RELATIONSHIP BETWEEN THE APPARENT ILEAL DIGESTIBILITIES OF THE INDISPENSABLE AMINO ACIDS (Y) AND DIETARY FAT LEVEL (X).

Item	Soybean meal			Canola meal			
	Regression equation	r^2	Probability	Regression equation	r^2	Probability X	Probability X^2
Amino acids							
Arginine	$Y=89.9 + 0.2 X^2$	.76	.0113	$Y=92.4 - 10.5 X + 2.8X^2$	.92	.0036	.0025
Histidine[a]	$Y=85.1 + 1.0 X$	.80	.0116	$Y=83.8 - 6.0 X + 2.0X^2$	.89	.0982	.0407
Isoleucine[b]				$Y=87.5 - 19.0 X + 4.7X^2$	.87	.0026	.0019
Leucine[b]				$Y=88.7 - 15.8 X + 4.1X^2$	.92	.0011	.0008
Lysine[a]	$Y=82.4 + 1.2 X$	.83	.0052	$Y=88.4 - 18.0 X + 4.7X^2$	.96	.0001	.0001
Phenylalanine	$Y=83.9 + 0.3 X^2$	.80	.0118	$Y=85.5 - 18.9 X + 3.2X^2$	.86	.0073	.0049
Threonine	$Y=69.2 + 0.6 X^2$	.88	.0019	$Y=77.0 - 17.0 X + 4.5X^2$	.87	.0064	.0044
Valine	$Y=79.0 + 0.4 X^2$	.85	.0040	$Y=84.4 - 17.0 X + 4.5X^2$	.88	.0030	.0021

[a] Histidine and lysine digestibilities in SBM diets show a linear response to fat, while the other amino acids show a non linear response.

[b] Dietary fat level had no effect on the ileal digestibilities of isoleucine and leucine in SBM.

TABLE 3.6. EFFECT OF LEVEL OF FAT ON APPARENT FECAL DIGESTIBILITIES (%) OF DRY MATTER, PROTEIN AND AMINO ACIDS

Item	Diets							
	Soybean meal				Canola meal			
	2% CO[a]	6% CO	10% CO	SE[b]	2% CO	6% CO	10% CO	SE[b]
Dry matter	92.0[d]	93.4[c]	92.2[d]	.21	83.7[c]	82.3[d]	83.9[c]	.23
Crude protein	90.5	91.3	90.8	.62	80.4[c]	78.6[d]	81.4[c]	.42
Amino acids								
Indispensable								
Arginine	94.9	95.7	95.9	.36	89.9	90.0	92.4	1.63
Histidine	94.6	94.6	93.7	.88	90.2[c]	89.2[d]	90.6[c]	.22
Isoleucine	89.2	90.7	91.3	.65	77.9	79.0	82.9	.97
Leucine	90.2	92.0	92.3	.55	84.1[cd]	81.8[d]	85.5[c]	.59
Lysine	90.6	92.4	92.3	.66	81.5	81.8	85.1	.74
Phenylalanine	90.9	92.4	92.6	.49	81.3[d]	81.3[d]	84.5[c]	.44
Threonine	86.3	90.0	88.8	.88	81.0	78.6	80.9	1.09
Valine	88.5	90.1	90.8	.70	82.6	79.8	83.5	.80
Dispensable								
Alanine	87.3	88.8	89.7	.85	82.3	78.9	82.9	1.03
Aspartic acid	91.9	93.1	93.2	.49	79.7[d]	79.7[d]	82.9[c]	.60
Glutamic acid	94.4	95.2	95.4	.37	91.2[cd]	90.3[d]	91.8[c]	.29
Glycine	88.6	90.8	90.4	.60	85.9[c]	82.7[d]	86.4[c]	.59
Serine	92.3	93.3	93.7	.49	84.9	82.7	85.6	1.18
Tyrosine	86.7	89.3	90.2	1.12	80.6	76.8	81.7	1.43

[a] CO: canola oil

[b] Standard error of the mean

[c,d] Means in the same row followed by a different superscript, within soybean meal or canola meal, differ at P<.05.

TABLE 3.7. SUMMARY OF RESULTS IN THE LITERATURE ON THE EFFECT OF DIETARY FAT LEVEL ON PROTEIN DIGESTIBILITY.

Authors	Animal specie and age/wt.	Major Dietary Ingredients	Fat Source and level	Effect of fat on protein digestibility
Asplund et. al., 1960	Piglets 3-4 wks	SBM, Meat scraps, Brewers' yeast, corn	Grease 10%	Improvement (P<0.001) in fecal protein digestibility by 7% units.
Tao et al., 1971	Broiler chicks 1.3-1.8kg	Cornstarch Dextrose Rapeseed meal	Vegetable oil 3.5%	Improvement (P<0.05) in fecal digestibilities of amino acids ranging from 8.6 (arginine) to 19.7 (valine) percentage units.
Jorgensen et al., 1985	Pigs, 80kg		3 vs 15%	Improved (P<0.01) ileal and fecal digestibilities of protein, methionine by 2 percentage units.
Marshall et al., 1959	Rats 200 or 400 d old	Lactoalbumin, cornstarch, or sucrose	Corn oil Crisco, lard 3% vs 15%	No effect
Lowrey et al., 1962	Pigs 3 wk or growing pigs 112lb	Corn, SBM + fishmeal Corn, SBM + meat scraps	Tallow 10%	No effect
Greeley et al., 1964	Pigs 60lb	Corn cornstarch SBM	Corn oil, lard tallow 0%, 5%, 10%, 15%	No effect

TABLE 3.8. TRANSIT TIME, PEAK TIME AND MEAN TREATMENT a AND b VALUES FROM LINEAR REGRESSION EQUATION LN Y = a + b X OBTAINED FROM RATE OF PASSAGE STUDY.

Item	Transit time (h)	Peak time (h)	a	b	Standard error of the mean	r^2
Diets						
Soybean meal						
2% CO	3.8 ± .5	6.6 ± .6	13.48	-.25[a]	.016	.84
6% CO	3.2 ± .2	7.4 ± .6	13.81	-.28[a]	.020	.85
10% CO	3.0 ± .0	7.6 ± .9	13.48	-.25[a]	.020	.80
Canola meal						
2% CO	3.0 ± .0	8.0 ± .5	13.99	-.32[b]	.022	.88
6% CO	3.4 ± .2	8.2 ± .7	13.60	-.29[b]	.021	.84
10% CO	3.4 ± .2	8.0 ± .5	14.05	-.32[b]	.025	.84

[a,b] Means in the same column followed by a different superscript differ at $P < .05$

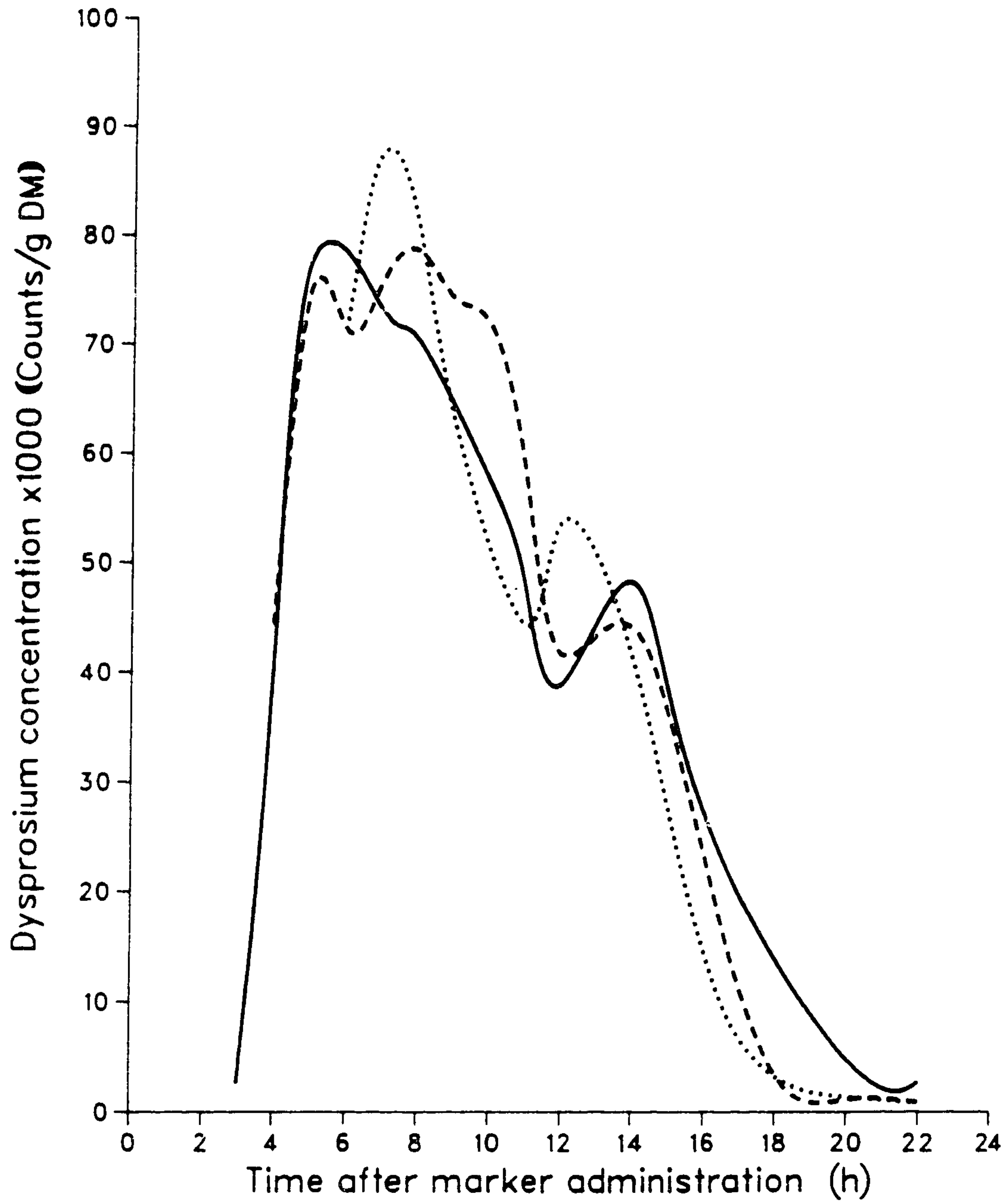

Figure 3.1 The marker excretion pattern in ileal digesta of three pigs fed the soybean meal diet with 2% canola oil.

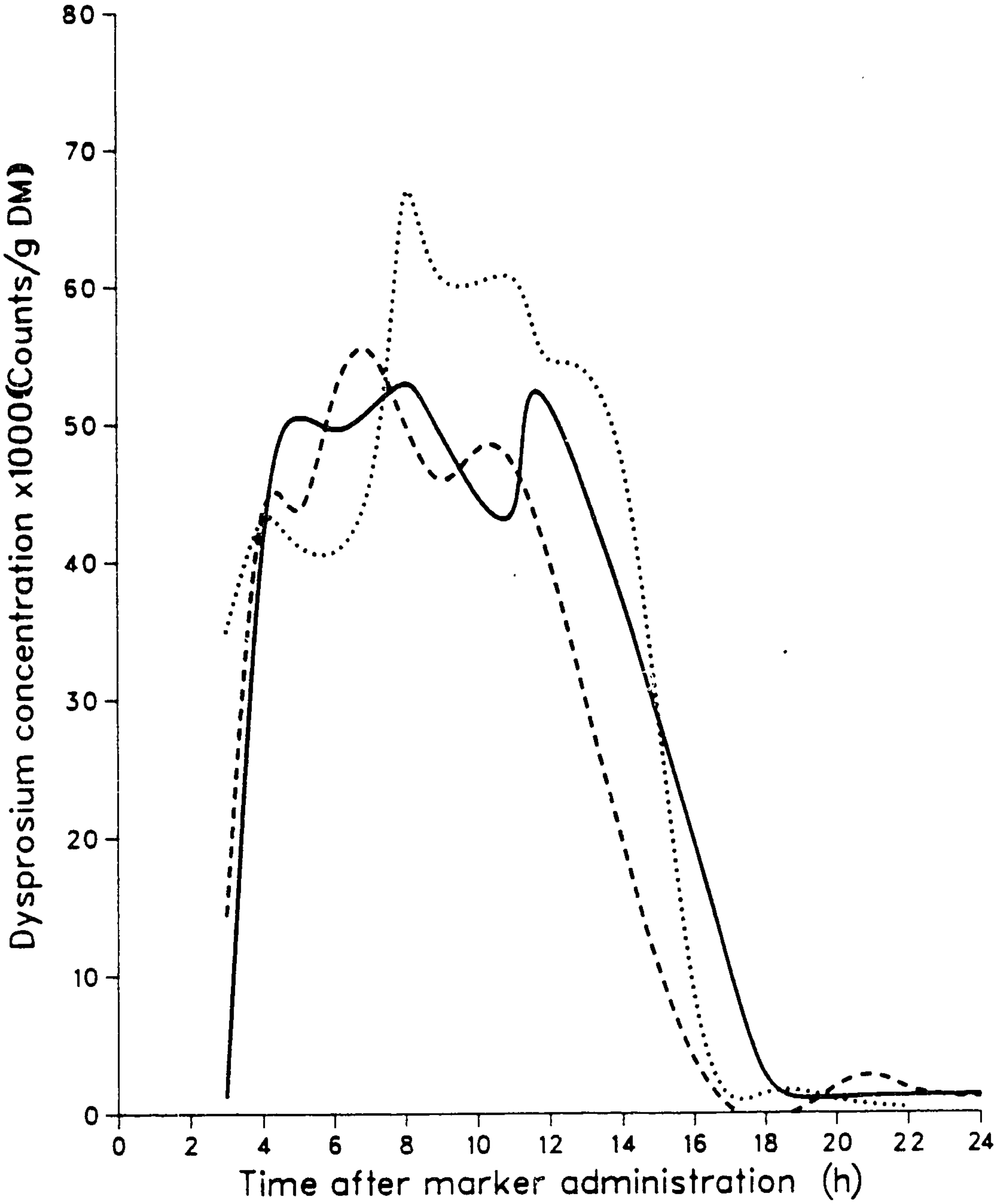

Figure 3.2 The marker excretion pattern in ileal digesta of three pigs fed the canola meal diet with 2% canola oil.

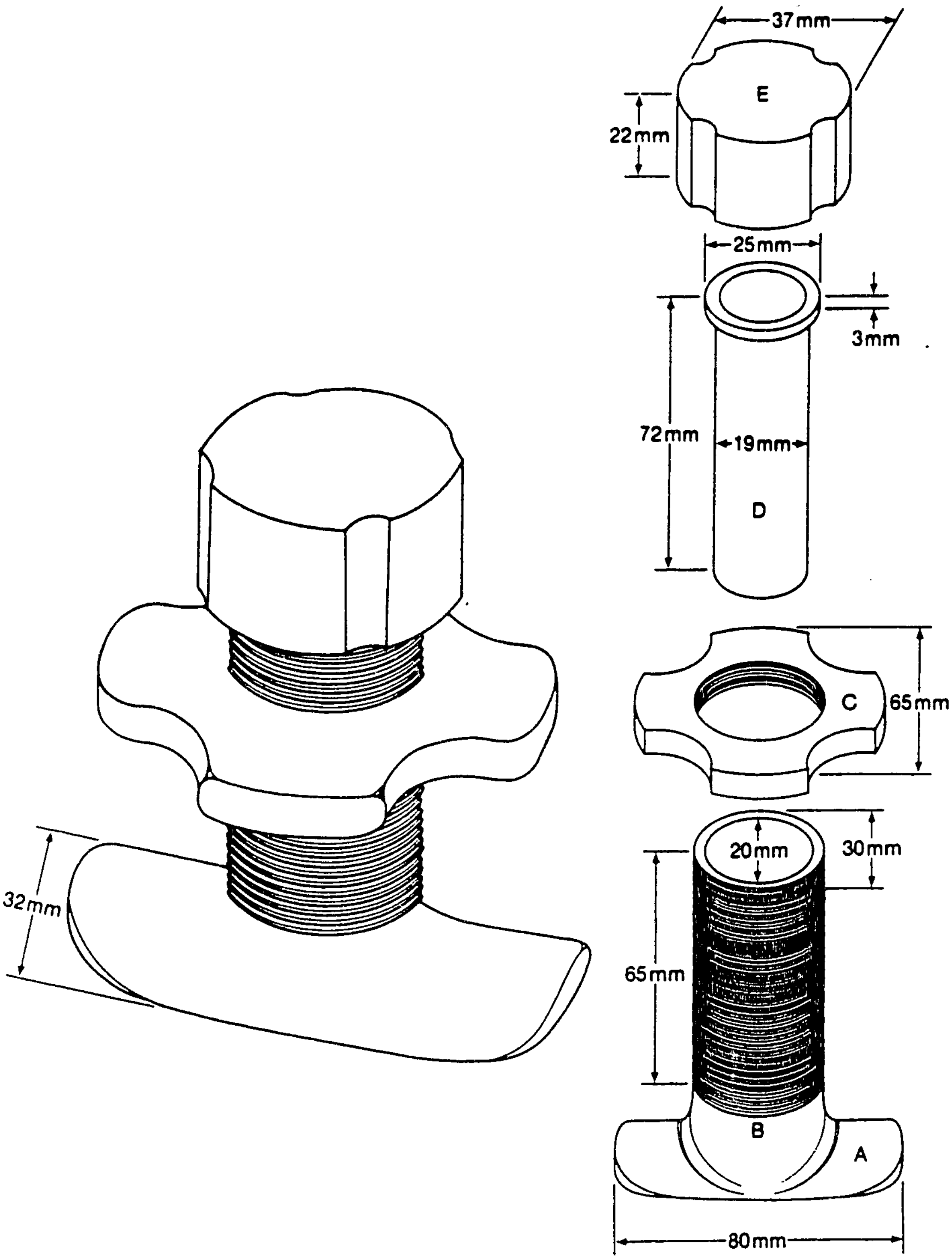

Plate 3.1 Parts and dimensions of the simple T-cannula.
A: Flange; B: barrel; C: collar; D: stopper; E: cap.

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4. COMPARISON OF THE SINGLE DOSE AND WITHDRAWAL METHODS FOR MEASURING THE RATE OF PASSAGE OF MARKER IN ILEAL DIGESTA AND FECES IN GROWING PIGS

INTRODUCTION

Rate of passage studies with pigs have traditionally been performed on the entire gastro-intestinal tract by total collection of feces or by sacrifice of the animals and collection of digesta at various points along the digestive tract (Castle and Castle, 1956; Maner et al., 1962; Seerley et al., 1962; Ehle et al., 1982; Pond et al. 1986). Various markers, including chromic oxide and rare-earth elements have been used to determine the rate of passage. Measurements of rate of passage can be made with the withdrawal or the single dose method. In the withdrawal method, the marker is administered at a constant level in the diet until steady-state conditions are reached, then withdrawn (Faichney, 1975). With the single dose method, the marker is administered with a single meal. (Castle and Castle, 1957; Seerley et al., 1962; Grovum and Williams, 1973; Ehle et al., 1982).

The objective of the present studies was to compare the single dose and withdrawal methods for measuring rate of passage using two markers, chromic oxide and dysprosium (Dy). The rate of passage was measured in digesta collected from the distal ileum of pigs fitted with a simple T-cannula, and in feces. The rate of marker disappearance, or rate constant, was used as the indicator of rate of passage.

MATERIALS AND METHODS

Six barrows (Yorkshire x Lacombe), with an average initial weight of 50 kg, were obtained from the University of Alberta swine herd. The pigs were housed in stainless steel metabolism crates 1 wk prior to the insertion of the cannulas, and fed a 16% crude protein grower diet (Table 4.1). Water was freely available from a low-pressure drinking nipple.

The barrows were fitted with a simple T-cannula, according to procedures adapted from Sauer et al. (1983). The cannulas were prepared from polyvinylchloride plastisol according to procedures described by Sauer (1976). The pigs were returned to the metabolic crates immediately after surgery and fasted for the remainder of that day. The next day, they were given approximately 100 g of an 18% crude protein starter diet (Sauer et al., 1983), twice daily. The feed allowance was increased slowly until the pigs consumed 2 kg daily. This level of food consumption was reached 7 d after sugery. The pigs were allowed a 14 d recuperation period. A detailed description of pre- and post-operative care was previously presented by Sauer (1976) and Sauer et al. (1983).

Experiment 1. Studies were carried out to compare the rate of passage, as determined with the withdrawal and single dose methods, in digesta collected from the distal ileum. The pigs were divided into two groups of three and fed twice daily, at 0800 and 2000 h, 1 kg each meal. The first group received the grower diet with chromic oxide (.5%)and Dy (25 ppm). These markers were withdrawn at the 0800 h feeding on day 11 of the experimental period. The second group received the grower diet without markers for 10 days. On d 11, at 0800 h, they were given a single dose of chromic oxide (.5%) and Dy (25ppm), mixed in the grower diet. Ileal digesta were collected every h from both groups for 36 h following withdrawal or administration of the markers. The treatments were then crossed-over for the second experimental period.

Experiment 2. Following the conclusion of Experiment 1, the same pigs were used to compare the rate of passage, as determined with the withdrawal and single dose methods, in feces. The experiment was carried out under the same experimental conditions and design described under Exp.1. Feces were collected during each 6 h period for a total of 5 d following withdrawal or administration of the markers at the 0800 h meal on d 11 of the experimental period.

Chemical and Statistical Analysis

Ileal digesta and feces were frozen immediately following collection. Prior to analyses, the samples were freeze-dried and ground through a .5-mm mesh screen in a Wiley mill. The feed samples were ground similarly. The dry matter content in the samples was determined according to AOAC (1980) methods. Chromic oxide was determined according to Fenton and Fenton (1979). Dy was measured by Instrument Neutron Activation Analysis using the Slowpoke Reactor at the University of Alberta according to procedures outlined by Kennelly et al. (1980).

The concentrations of markers in samples of digesta and feces were transformed to their natural logs, from the sample with maximum marker concentrations to the last sample that was obtained for each pig. Simple regression analysis was performed to establish the relationship between the natural log of marker concentration with time for both methods and both markers in ileal digesta and feces. The slopes of the regression lines, which represent the rate of marker disappearance, were compared for both methods and each marker with the students T-test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The changes in the concentrations of Dy and chromic oxide in digesta collected from the distal ileum, following the withdrawal of the markers, are presented in Fig.4.1. Both curves show an increase in marker concentration during the first 3 to 4 h, then a gradual decline. The lower concentrations of the markers that were observed in the samples collected immediately after feeding may result from incomplete mixing of digesta with the markers and digesta without the markers.

The changes in the concentrations of the markers in digesta collected from the distal ileum following the administration of the markers with a single meal are presented in Fig.4.2. There was an increase in the concentration during the first 3 to 6 h after feeding, then a decline and once more an increase at 10 to 12 h followed by a gradual decline. This biphasal excretion pattern was also observed in a previous study (Imbeah and Sauer, 1989) and may result from finer particles leaving the stomach at a faster rate than the coarser particles (Potkins et al., 1985). The biphasal excretion pattern was observed for each pig.

The changes in marker concentration can be described by the equation $\ln Y = a + b X$ (Ln Y: natural log of marker concentration; X: time (h) after withdrawal or administration of the marker; b = rate of marker disappearance or rate constant). The regression equations based on the withdrawal and single dose methods with chromic oxide or Dy as marker, are presented in Table 4.2. Similar regression equations were obtained with both the withdrawal and single dose methods when chromic oxide was used. The r^2 values were .91 and .87 for the withdrawal and single dose methods, respectively. The times required for the first appearance of chromic oxide at the distal ileum (transit time) and maximum marker concentration (peak time) were 1.7 $\pm$ .45 and 6.7 $\pm$ .5 h, respectively.

Based on Dy, the rates of passage measured in ileal digesta with the withdrawal and single dose methods, were different ($P<.05$), Table 4.2. The rate of passage was faster when measured with the withdrawal rather than single dose method. It is not clear which equation represents the actual rate of passage at the distal ileum. Dy is chemically less inert than chromic oxide and is known to form precipitates and radiocolloids with hydroxides and phosphates (Uden et al., 1980). In addition, Dy, like other rare-earth markers, may migrate from originally marked to previously unmarked particles, thus affecting estimates of rate of passage. The migration increases with decreasing pH (Crooker et al., 1982). Dy is a particulate marker and the strength of the bond is stronger at a higher pH and weaker at a lower pH (Hartnel and Satter, 1979). Thus, the bond may dissociate under acidic conditions, e.g. in the stomach, and affect marker concentration. Dy can also form complexes with organic compounds even under existing pH conditions in the digestive tract (Teeter et al., 1984). Despite these limitations, the use of Dy may provide a dependable estimate when marker recoveries rather than concentrations are used to determine rate of passage (Young et al., 1976). The aforementioned factors that influence the concentration of Dy in digesta may have been effective to different extents with the single dose and withdrawal methods. The r^2 for both methods, however, were identical i.e. .90 and .89 for the withdrawal and the single dose methods, respectively. The transit and peak time of Dy in digesta collected from the distal ileum were 1.0 $\pm$ 0.0 and 6.0 $\pm$ 0.6, respectively and similar to those obtained for chromic oxide. As was the case with chromic oxide, a biphasal excretion pattern was observed for Dy.

The excretion patterns of the markers in feces were similar to those observed in digesta (Fig.4.3 and 4.4). Van Soest et al. (1983) in a review, also reported a biphasal excretion pattern in pigs which they attributed to pulsative cecal emptying. The cecum of the pig is small compared to the large intestine; therefore one would not expect cecal emptying to influence the excretion pattern in the large intestine. Rather, the biphasal excretion pattern observed in feces is more likely related to the excretion pattern in digesta. Potkins et al. (1985) reported a faster gastric emptying rate for finer compared to coarse particles. Since feed contains both fine and coarse particles, their differential rate of passage from the stomach is likely to carry through over the entire digestive tract, giving rise to the biphasal pattern of marker excretion in both digesta and feces.

In feces, there were no differences (P>.05) between the regression equations determined with the withdrawal and single dose methods when chromic oxide or Dy was used as marker (Table 4.2).

The rate constants observed in digesta collected from the distal ileum were higher than in feces (Table 4.2), indicating a faster rate of passage of digesta through the small rather than large intestine.

Keys and DeBarthe (1974), in studies with growing pigs, fed a diet containing 70% barley as well as 9% solka floc, and obtained the following equation with the single dose method and chromic oxide as marker in ileal digesta: Ln Y = 11.69 - 0.38 X compared to Ln Y = 3.67 - 0.16 X in the present studies in which a diet containing 56.6% barley and 25% wheat was fed. The differences between the two equations may be due, in part, to differences in diet composition. The diet used by Keys and DeBarthe (1974) was higher in fiber content, hence the higher rate constant. Based on chromic oxide excretion in feces the equation Ln Y = 10.33 - 0.063 X was obtained by Keys and DeBarthe (1974) compared to Ln Y = 4.01 - 0.056 X in these studies. Whereas the rate constants determined in ileal digesta in studies by Keys and DeBarthe (1974) were approximately twice the value of those obtained in the present studies, the rate constants determined in feces were nearly the same (.063 vs .056).

In summary, these studies show that the rate of passage, measured in ileal digesta or feces, were similar when these were determined with the withdrawal or single dose method when chromic oxide was used as marker. However, with Dy the two methods provided different rate constants determined in digesta collected from the distal ileum. This may result from the partial reactivity of Dy. Similar rate constants were observed in feces for the two methods with Dy. The correlation coefficients for both methods and both markers were relatively high ranging from .87 to .91 when rate of passage was measured at the distal ileum and from .80 to .90 when the rate of passage was measured in feces. Therefore, either method or marker can be used to identify differences in rate of passage between different diets, although the use of chromic oxide provided more consistent results.

TABLE 4.1. COMPOSITION OF CROWER DIET

Ingredients	%
Wheat	25.0
Barley	56.6
Soybean meal	14.5
Iodized salt	.4
Calcium carbonate	1.5
Dicalcium phosphate	1.0
Vitamin and mineral premix[a]	1.0

[a] Supplied per kg of diet: 7500 I.U. vitamin A; 700 I.U. vitamin D_3; 45 I.U. vitamin E; 12 mg riboflavin; 40 mg niacin; 25 mg pantothenic acid; 28 ug vitamin B_{12}; 120 mg zinc, 48 mg manganese; 100 mg iron; 10 mg copper; .1 mg selenium.

TABLE 4.2. MEAN TREATMENT a AND b VALUES FROM LINEAR REGRESSION EQUATION LN Y = a + b X OBTAINED FROM RATE OF PASSAGE STUDY

	a	b	Standard error of b mean	r^2	Significance of b mean
Ileal digesta					
Chromic oxide					
Withdrawal	3.67	-.161	.005	.91	N.S.
single dose	3.66	-.161	.006	.87	
Dysprosium					
Withdrawal	10.36	-.145	.004	.90	P < .05
single dose	10.11	-.131	.005	.89	
Feces					
Chromic oxide					
Withdrawal	4.22	-.056	.003	.87	N.S.
single dose	4.01	-.056	.004	.80	
Dysprosium					
Withdrawal	10.65	-.042	.002	.90	N.S.
single dose	10.56	-.044	.003	.83	

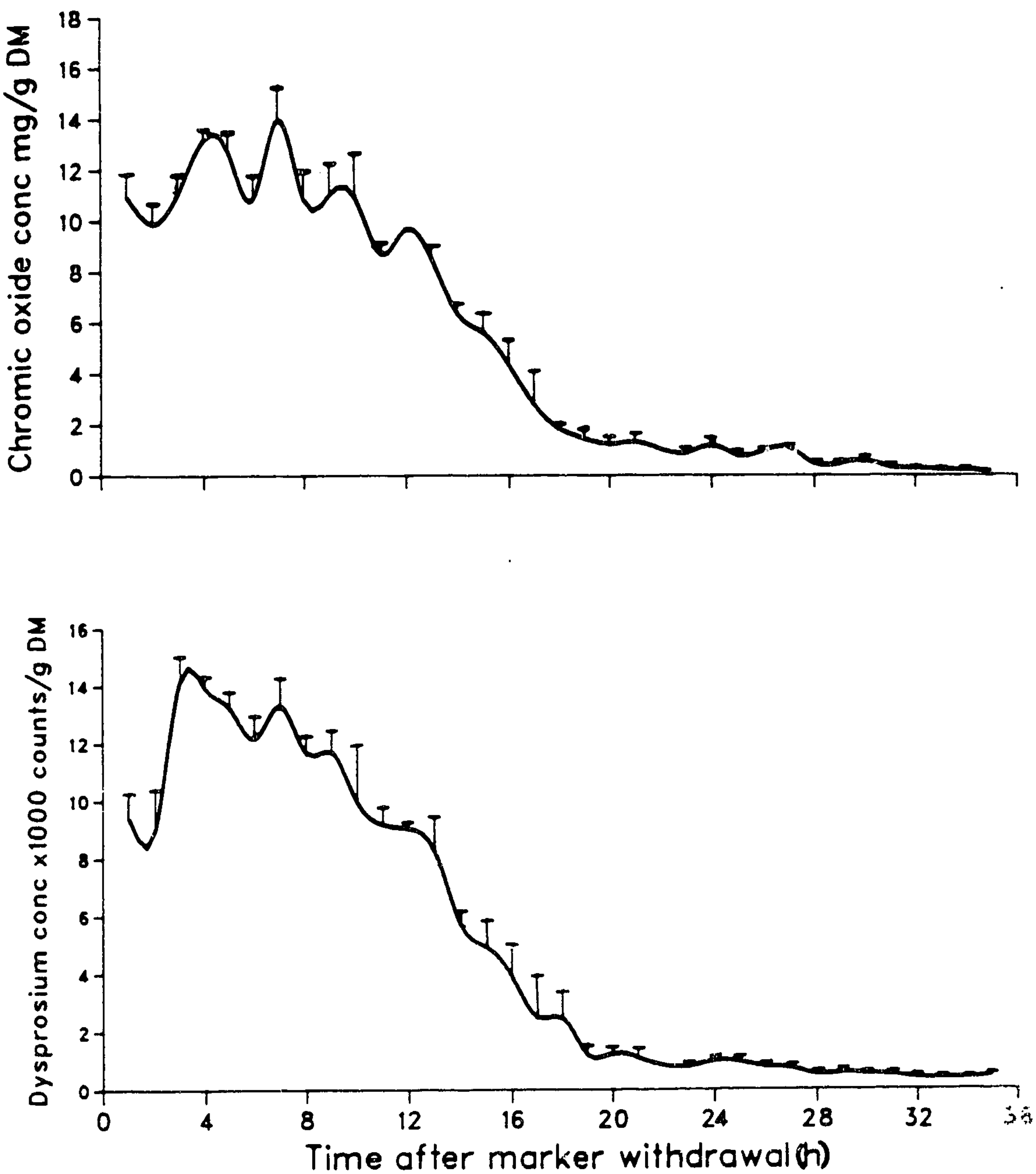

Figure 4.1 Changes in the concentrations of chromic oxide (top) and dysprosium (bottom) in digesta collected from the distal ileum with time after marker withdrawal.

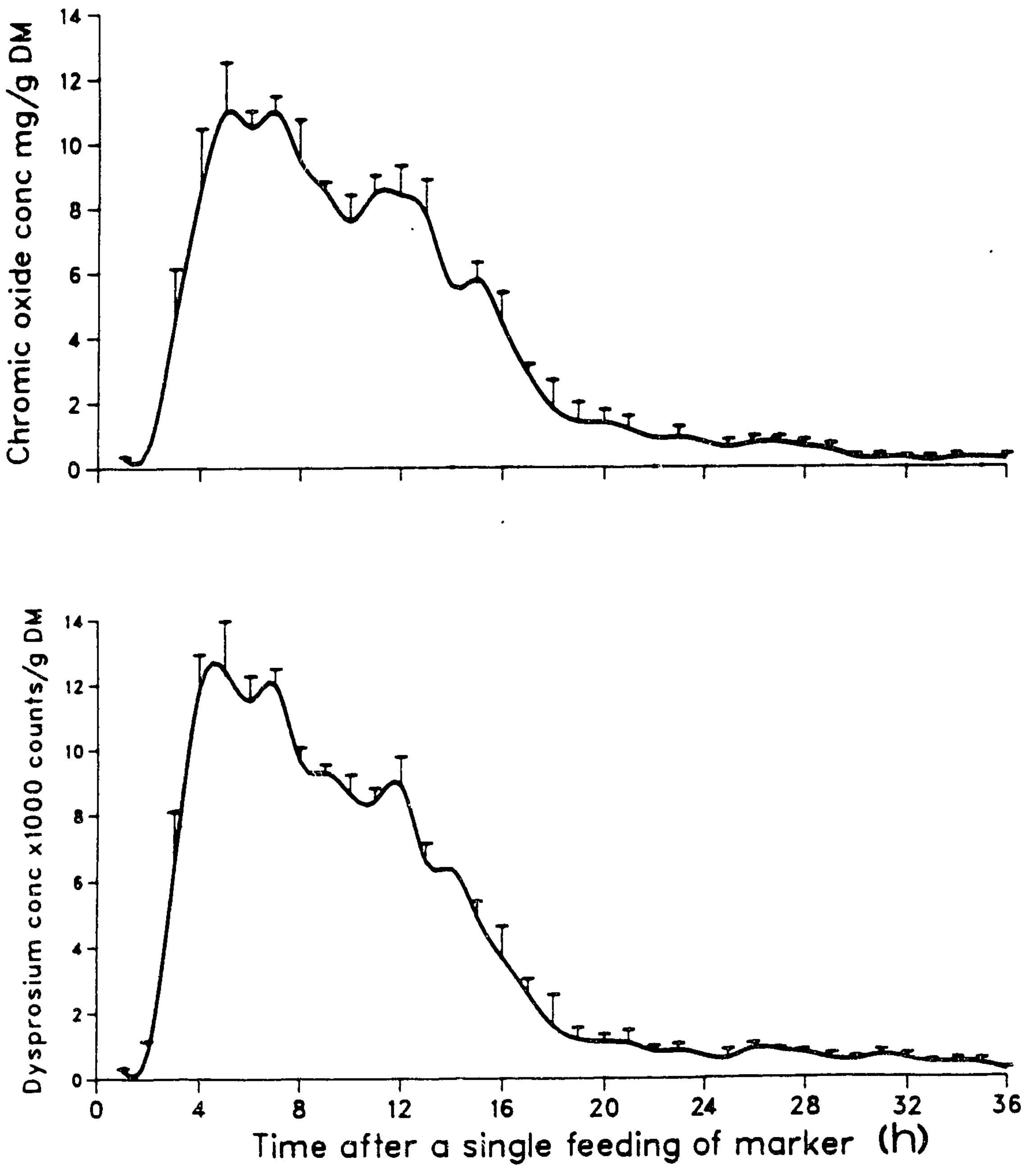

Figure 4.2 Changes in the concentrations of chromic oxide (top) and dysprosium (bottom) in digesta collected from the distal ileum with time after marker administration.

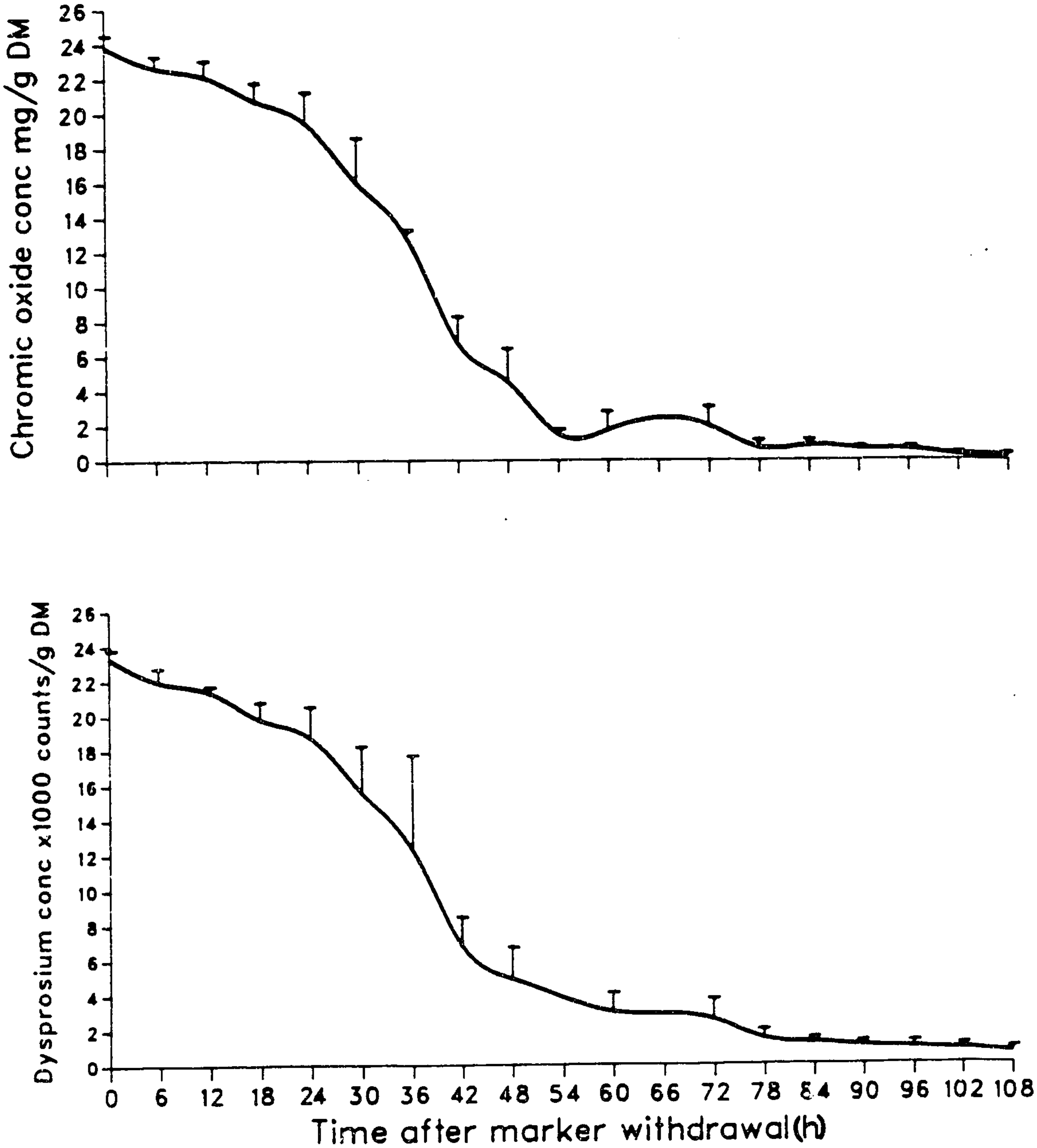

Figure 4.3 Changes in the concentrations of chromic oxide (top) and dysprosium (bottom) in feces with time after marker withdrawal.

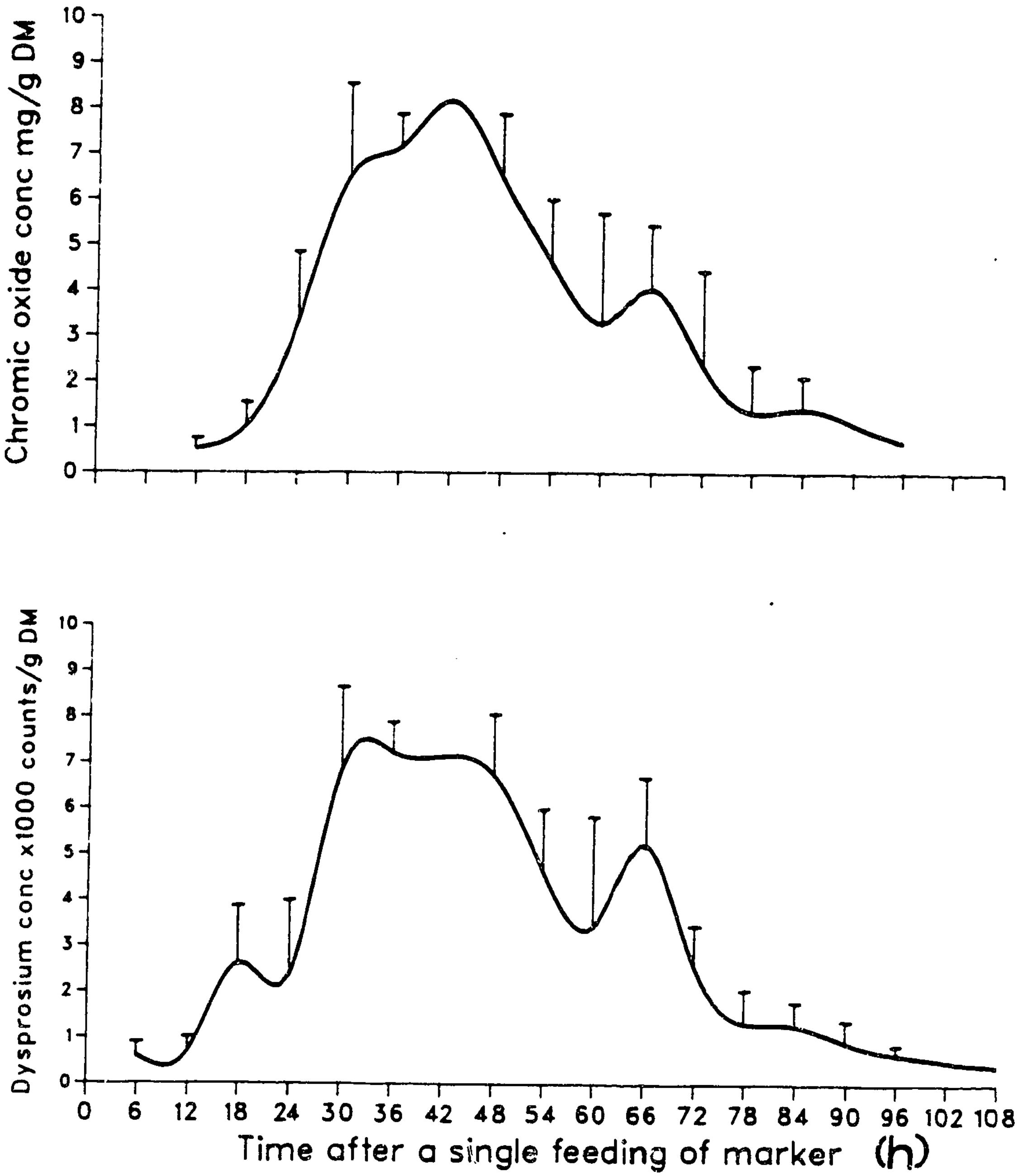

Figure 4.4 Changes in the concentrations of chromic oxide (top) and dysprosium (bottom) in feces with time after marker administration.

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5. GENERAL DISCUSSION

Interaction between ingredients within compounded feeds has not received much attention in research. There is some uncertainty about the additivity of nutritional values of ingredients in feeds. The presence of associative effects observed between canola meal and barley in this study, though of small magnitude (chapter 2), sheds some light on ingredient interactions within feeds. Whereas associative effects have been well documented for ruminants (Asplund and Harris, 1971 and Mould et al., 1984) and in horses (Thompson et al., 1984), there is a lack of information on such effects in the pig. In the current studies, positive associative effects were observed for lysine and phenylalanine as well as some dipensable amino acids. Lysine is usually the first limiting amino acid in diets for pigs, thus if an interaction between ingredients can indeed improve the digestible supply of this amino acid, then the producer could exploit such an interaction to his advantage.

Wallis et al. (1985) observed interactions between wheat and various combinations of other cereal grains (oats, maize, etc) which led to improved ($P<.05$) digestibilities of energy and amino acids for broilers. Carre et al. (1987) reported that predicted protein digestibility for peas fed to cockerels was higher ($P<.05$) when combined with maize than when combined with wheat. These observations together with the interaction between canola meal and barley from the present studies are an indication of associative effects in non-ruminants. Therefore, digestibility coefficients obtained by difference should be used with caution in the formulation of feeds for non-ruminants. For ingredient combinations which have a positive effect on nutrient digestibilities, the use of predicted digestibility coefficients in formulating rations should pose no nutritional problem. However, for ingredients whose interaction may have a negative effect on nutrient digestibilities, the use of calculated digestibility coefficients in diet formulation may give a lower than expected digestible amino acid supply and could lead to poor animal performance depending on the magnitude of such effects.

Interactions within compounded feeds may not be limited to those between the grains and protein supplements (i.e. the major components). There could be interactions between other components of the diet. Fat is normally a minor component of feeds, which is included to reduce dustiness and to increase energy density. There is a general belief that increasing dietary fat level would increase the digestibilities of protein and amino acids. This belief is probably based on the fact that high dietary fat delays gastric emptying. However, reports on the effect of fat on the digestibilities of protein and amino acids are very conflicting. With regard to the effect of dietary fat on rates of passage in the small intestine of the pig, there appears to be no information. In poultry, Mateos and Sell (1981), Mate[illegible] al.(1982) and Sell et al.(1983) have reported that increasing dietary fat for hens reduced ($P<.05$) the rate of passage of feed residues over the entire digestive tract and also improved ($P<.01$) the metabolisable energy content of the diet. Results from the present studies show that, increasing dietary fat can improve ileal amino acid digestibilities without affecting the rate of passage in the small intestine. The effect of fat on ileal amino acid digestibilities appears to show a fat level x protein source interaction. In the soybean meal diets, increasing fat level from 2 to 6% had no effect on amino acid digestibilities, but a 10% fat level improved (P<.05) the ileal digestibilities of most amino acids, relative to digestibilities on the 2% fat diet. The improvements ranged from 1.5 to 3.9 percentage units. This seems to suggest that amino acid digestibilities in soybean meal require a fairly high dietary fat increment in order for improvement to occur. For canola meal diets, increasing dietary fat level from 2% to 6%, depressed (P<.05) ileal digestibilities of most amino acids. The magnitude of the depression ranged from 2.2 to 4.7 percentage units. A further increase in dietary fat from 6% to 10% improved amino acid digestibilities relative to their digestibilities on the 6% fat diet, the improvements ranging from 3.0 to 7.1 percentage units. The response of ileal amino acid digestibilities for the soybean meal diets was non-linear for all amino acids that showed a response, except histidine and lysine which did show linear responses. The response in canola meal diets was curvilinear for all amino acids, with both linear and non-linear components being significant in determining digestibilities (table 3.5). The results from the canola meal diets show that smaller ranges of fat levels (2 - 6%; 6 - 10%) were required to give a response while a wider range (2% - 10%) may appear not to show a response due to the parabolic nature of the response curve. Thus for canola meal, though there were dramatic changes in amino acid digestibilities with changes in fat levels, there were net improvements in the digestibilities only in the case of protein, histidine and lysine when dietary fat level was increased from 2% to 10%. For the remainder of the amino acids, their digestibilities on the 2% and 10% fat diets were not significantly different (P>.05). Therefore, the conflicting reports in the literature (Marshall etal., 1959; Asplund et al., 1960; Lowrey et al., 1962; Partridge et al., 1986 and Cole and Hutchesen, 1987) on the effect of fat on protein and amino acid digestibilities may be the result of differences in the ranges of fat used, differences in diet composition, especially with regard to protein sources, and differences in the species of animals used by the various authors.

Canola meal diets are known to have relatively low apparent digestibilities of protein and amino acids (McKinnon and Bowland, 1977; Sauer et al., 1982 and Sauer and Thacker, 1986). Animal performance on canola meal diets is relatively poor compared to performance on soybean meal diets (Castell, 1977; McKinnon and Bowland, 1977 and McIntosh et al., 1986). This poor performance may be attributed to the low digestibilities of amino acids in canola meal diets. Sauer and Ozimek (1986) postulated that differences in amino acid digestibilities between feedstuffs may be related to differences in the rate of secretion of pancreatic proteolytic enzymes associated with those feedstuffs. In the present studies, there were no differences ($P>.05$) in the rate of secretion of trypsin and chymotrypsin between pigs fed SBM or CM diets (chapter 2). Therefore the lower apparent digestibilities of amino acids observed for canola meal diets were due to factors other than the rate of secretion of trypsin and chymotrypsin. Differences in fibre contents and rates of passage between SBM and CM diets may partly explain the lower amino acid digestibilities of the CM diets. In the present studies, the CM diets had higher fibre contents than the SBM diets (4.8 vs 2.5), and also a faster ($P<.05$) rate of passage (chapter 3). Fibre has been shown to increase the rate of passage of feed residues (Kuan et al.,1983), thereby reducing the time of exposure to digestive enzymes and absorptive surfaces and hence reducing protein and amino acid digestibilities. Since canola meal is a major oil meal, it would be of interest to identify positive interactions between this protein supplement and other ingredients in order to improve amino acid digestibilities and thereby improve animal performance on canola meal diets.

The final study (chapter 4) compared the rates of passage of markers via digesta and feces following marker withdrawal or a single dose of marker. A biphasal pattern of marker excretion following a single dose of marker was observed in both the fat experiment and the final study where the single dose method and the withdrawal method were compared. These two studies seem to show that marker excretion after a single dose of marker, follows a biphasal pattern in the large as well as the small intestine. Van Soest et al. (1983) explained the cause of fecal biphasal marker excretion, but there appears to be no information on biphasal marker excretion at the end of the small intestine. One could only speculate on this observation on the basis of differential rates of passage of finer and coarse particles from the stomach, which follow through to the end of the small intestine. Potkins et al. (1985) observed that finely ground barley passed out of the stomach of pigs faster ($P<.001$) than coarsely ground barley. It could be assumed that the feeds used in the present studies contain both fine and coarse particles and these can travel the digestive tract at different rates and can give rise to the biphasal pattern of marker excretion. This study also showed that when chromic oxide was the marker, the rate of passage in the small intestine was the same for the withdrawal and the single dose method. With dysprosium as the marker, the rate of passage following marker withdrawal was higher ($P<.05$) than the rate of passage after a single dose of marker. For rates of passage over the entire digestive tract, the two methods gave identical results with both markers. It appears that chromic oxide may be a better marker for studying the rates of passage along the small intestine. Dysprosium is a particulate marker according to Hartnel and Satter (1979); such markers may dissociate in acidic conditions (such as in the stomach) and may thus alter marker concentrations in digesta at different times. Therefore, Dy may not provide the stable equilibrium necessary for accurate measurements of rate of passage.

These studies have shown that associative effects or ingredient interactions do occur in pigs, even though the effects may be small. In addition, it has been shown that ileal amino acid digestibilities can be improved by increasing dietary fat, depending on the protein source and the range of fat levels used, without affecting the rate of passage of feed residues through the small intestine. Also, the comparison of the marker withdrawal method and the single dose method show that the two methods are identical for the determination of rates of passage over the entire digestive tract. However, the rates of passage in the small intestine could be different for the two methods, depending on the marker used. Chromic oxide appears to be a better marker than dysprosium for rate of passage studies. This is demonstrated by the fact that for both the single dose and the withdrawal methods, chromic oxide gave identical estimates of the rate of passage to the terminal ileum as well as passage over the entire tract.

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