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THESIS – THÈSE

Title of Thesis – Titre de la thèse

FACTORS AFFECTING THE PROTEIN DIGESTIBILITY IN STUDIES WITH THE MOBILE NYLON BAG TECHNIQUE

Degree for which thesis was presented
Grade pour lequel cette thèse fut présentée

M Sc

Year this degree conferred
Année d'obtention de ce grade

1985

University – Université

UNIVERSITY OF ALBERTA

Name of Supervisor – Nom du directeur de thèse

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FACTORS AFFECTING THE PROTEIN DIGESTIBILITY IN STUDIES WITH THE MOBILE NYLON BAG TECHNIQUE

by

GEETHA CHERIAN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

ANIMAL NUTRITION

ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL 1985

THE UNIVERSITY OF ALBERTA

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Supervisor

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Date..... October 3 1985

To my son with love

Abstract

The mobile nylon bag technique (MNBT) was used with 5 pigs, over the weight range of 50 to 70 kg, fitted with a simple 'T' cannula in the duodenum to determine the factors that influence the protein digestibility in soybean meal (SBM), meat and bone meal (MBM) and canola meal (CM). Pre-digestion conditions such as length of time (h) of pre-digestion in a pepsin-HCl solution (0, 1.5, 2.5, 4.0), pH of the pre-digestion solution (1.0, 1.5, 2.0, 2.5) and activity (IU/l) of pepsin (188.7, 377.4) were examined. The apparent protein digestibilities of SBM, MBM and CM were also determined in conventional digestibility studies with 6 pigs over the weight range of 50 to 70 kg. The absence of pre-digestion (0 h) resulted in a lower ($P<0.05$) protein digestibility than pre-digestion for 2.5 and 4.0 h. The digestibility of protein was highest at pH 2.0. Increasing the pepsin activity of the pre-digestion solution from 188.7 to 377.4 IU/l improved the protein digestibility of SBM ($P<0.05$) but not that of MBM and CM ($P>0.05$). Another experiment was designed to examine the effect of fineness (screen size, mm) of grinding (0.5, 1.0, 1.5, 2.0), pore size (μm) of the nylon mesh (10, 48, 63, 70), the amount of feed (g) in the bag (0.5, 1.0), shape and size of the bag (2.5 x 4.0, 1.5 x 5.0), and the number of bags inserted into each pig per day (1, 4). The digestibility of protein in SBM was lower ($P<0.05$) when the sample was ground through a screen with a mesh size of 2.0 as opposed to 0.5, 1.0 or 1.5 mm. Fineness

of grinding did not effect ($P>0.05$) the protein digestibility in MBM. The digestibility of protein in all feedstuffs was lowest ($P<0.05$) when the pore size of the nylon was 10 μm. Decreasing the amount of sample from 1.0 to 0.5 g improved ($P<0.05$) the protein digestibility of SBM but not that of MBM and CM ($P>0.05$). The shape and size of the bag and the number of bags inserted per day into each pig had no effect ($P>0.05$) on protein digestibility.

The closest agreement between results obtained by MNBT and control studies occurred with a pre-digestion time of 2.5 or 4.0 h, pH of 2.0, pepsin activity of 377.4 IU/l, sample size of 0.5 g, screen size for grinding of 1.0 mm and a pore size of the nylon of 48 μm. Under these conditions, protein digestibilities determined by MNBT ranged from 89.6-91.5, 79.6- 81.4 and 76.3- 78.4 % for SBM, MBM and CM, respectively. In the same order for the feedstuffs, the apparent protein digestibilities determined in conventional studies were 93.0 ± 0.7 (SE), 79.1 ± 2.0, 78.3 ± 0.7 %, respectively. The present studies show the importance of certain factors which should be taken into consideration for further optimization of the MNBT.

KEY WORDS. MOBILE NYLON BAG TECHNIQUE, SWINE, PROTEIN DIGESTIBILITY.

Acknowledgements

I would like to thank Dr. R.T Hardin, and Dr. R.T Berg, Chairman and former Chairman of the Department of Animal Science for placing the facilities of the Department at my disposal.

I wish to express my deepest gratitude and sincere appreciation to Dr. W.C Sauer, Associate Professor of Swine Nutrition, for his invaluable assistance, guidance and constructive criticism throught this study and during the preparation of the thesis. You have made this part of my studies very enjoyable.

Thanks are also extended to Dr. L. Ozimek for his suggestions and criticism during this study.

Sincere thanks to Ray Weingardt for his patience and understanding in his assistance with the statistical analyses and computation.

Thanks to Gavin Godby and Karima Shahin for their help with textform.

The help given to me by Brenda Reminsky, and the staff of University of Alberta swine research unit is gratefully acknowledged.

The encouragement and the co-operation given to me by Joe, my husband and my parents are greatly appreciated.

Financial assistance for this research was received from the Agricultural Research council of Alberta. This is deeply appreciated.

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I. INTRODUCTION

The estimation of the nutrient requirements for livestock and the extent to which different feedstuffs supply these nutrients are essential for efficient and ecnomic feeding. The simplest evaluation of feedstuffs is on the basis of their growth effects, as in feeding trials. A further refinement is the evaluation of feedstuffs based on the digestible supply of the major nutrients.

Measuring the digestibility of nutrients in feedstuffs require large quantities of feed and a relatively long period of time for adaptation to the experimental diet. These limitations might be overcome if isolated feed samples could be artificially introduced into the digestive tract and their contents quantitatively recovered in feces. The mobile nylon bag technique (MNBT) is a promising method for the rapid determination of the protein digestibility in small samples of feedstuffs.

The basic principles of the MNBT are as follows. Pigs are surgically fitted with a 'T' cannula in the duodenum. Approximately 1 gm of a feedstuff is weighed out into a small nylon bag with approximate dimensions of 25 x 40 mm. To simulate the conditions of the stomach, the bags with feedstuffs are immersed into a beaker at 37°C for 2.5 h containing deionised water and hydrochloric acid (HCl) with a pH 2.0 to which 1.0 gm of pepsin (377.4 IU/l) has been added. Following immersion, the nylon bag is inserted into the duodenum through the duodenal cannula during the time

when the pigs are eating. These bags travel through the intestinal tract and are collected in the feces usually within 36-48 h. The bags which are usually completely enveloped by feces are taken out carefully and frozen immediately. Following freeze-drying the bags are cut open and the contents are analyzed for protein. Freeze-drying results in having a crisp and flaky nature of the undigested contents and allows for easy recovery.

The initial results that were obtained with MNBT were more variable than those obtained in conventional digestibility studies. The present studies were carried out to obtain a more detailed insight into the factors that determine the digestibility of protein as determined by aid of the MNBT. The factors that were studied included the length of time for pre-digestion, pepsin concentration, degree of fineness of grinding of the sample, sample size, pore size of the nylon, shape and size of the bag, number of bags inserted into each pig per day and the pH of the pepsin-HCl solution. The effect of these factors on protein digestibility were studied to find means to minimise the experimental variation and to obtain results as close as possible to those obtained in long term conventional digestibility trials.

II. LITERATURE REVIEW

The history of the development of methods for the determination of the nutritive value of feedstuffs for animal production goes back many years. Feeding trials were carried out in Europe during the early part of the seventeenth century. Researchers also tried to predict the nutritive value of feedstuffs by the extraction of the nutrients in feedstuffs with water, alkali, ether and alcohol.

As the science of nutrition progressed, the early methods were modified and developed in order to improve the reliability with which laboratory techniques could be used to predict the nutritive value of a feedstuff. Several modifications of in vitro methods which more precisely attempted to mimic the in vivo processes were introduced. In vivo techniques are always preferred for the evaluation of feedstuffs.

The earliest recorded studies on digestion in domestic animals included sheep and the administration of grass fitted perforated metal tubes per os. In 1756 Reaumur tried to investigate the factors that cause digestion by filling punctured metal tubes with barley for birds and grass for sheep. He concluded that digestion could not be affected by a solvent.

Spallanzani (1782) studied the digestion and rate of passage of bread and meat contained in linen bags which he swallowed. The bags were excreted in feces in less than 24

h; however, the contents had completely disappeared. Spallanzani repeated the work of Reaumur with ruminants but placed the food in large tubes. He gave six tubes to a sheep and killed the animal after a 37 h fast. Then he retrieved the tubes with their contents in the abomasum

In ruminants, the rumen bag technique has been used for many years to provide estimates of the rate and extent of disappearance of feed constituents from the the rumen (Quin *et al*.1938; Rodriguez 1968; Mehrez and Orskov 1977). Quin *et al*. (1938) used bags prepared from nylon. On the other hand, Ervin and Elliston (1959), Johnson (1966), Rodriguez (1968) and Mehrez and Orskov (1977) used bags prepared from dacron.

The rumen bag technique has been used to explore many features of the degradation process that occur within the rumen (Kempton 1980). Not only is it a powerful tool for indexing the rate at which feedstuffs degrade in the rumen, it is also a a useful tool to obtain a better understanding of the processes involved in rumen fermentation.

Several investigators have attempted to standardize the in situ fermentation time, composition of the basal diet, length of time for rinsing the bag, bag size and bag porosity (Balch and Johnson 1950; Lusk *et al*. 1962; Mehrez and Orskov 1977). Rodriguez (1968) studied the effect of time of fermentation (24 vs 48 vs 72 vs 96 h) in the rumen. Three sets of bags measuring 12 x 5 cm (long), 9.5 x 5 cm (short) and 15 cm in diameter (round) were used in the studies. A large amount of variation was reported between

bags and animals. As the fermentation time increased, the digestibility of dry matter increased, reaching a maximum at 72 h, after which it diminished. The least amount of variation was found when the time of incubation was 72 h. Van Keuren and Heinemann (1962) also studied the effect of fermentation time. They obtained similar results as Rodriguez (1968). However, with alfalfa the digestibility of dry matter increased linearly up to 96 h of fermentation. A significant increase in the disappearance with each succeeding 12 h of fermentation, independant of the diet consumed by the host animal, was reported by Bullis *et al.* (1967).

Mehrez *et al.* (1977) studied the effect of bag size. Bags measuring 5 x 8, 17 x 9 and 25 x15 cm were prepared. Four bags each, containing 5 g rolled barley (4.3 g dry matter), were prepared and incubated in the rumen of sheep for 24 h on 2 successive days. Bags measuring 5 x 8 cm, containing 4.3 g dry matter, are comparable to those used in studies by Rodriguez (1968) and Van Keuren and Heinemann (1962). Increasing the bag size up to 17 x 9 cm resulted in an increase in the proportion of dry matter disappearance from 37.5 to 85.0 %. In addition, the experimental variation between bags was reduced. The standard deviation for any four bags that were incubated together did not exceed 2.1 %. However, there was more variation between days and animals. A further increase in the bag size to 25 x 15 cm had no effect on dry matter disappearance or variation. Therefore,

it appeared that a bag size of 8 x 5 cm was too small to allow for complete mixing and removal of the end products of digestion. These results agree with those by Bullis *et al.* (1967) and Tomlin *et al.* (1967) who reported that the digestibility of dry matter diminished as the weight of the sample was increased in bags of constant size.

The effect of different pore sizes of the bag containing SBM or Barley on dry matter and nitrogen disappearance was studied by Weakly *et al.* (1983) with bags prepared from dacron of 52 and nylon of 20 μm. The extent of disappearance after 24 h was higher in dacron than in nylon bags. A reduction in substrate disappearances as a result of a decrease in bag pore size was also observed by Uden *et al.* (1974) who found a higher digestibility of guinea grass from nylon bags with a pore size of 53 as compared to 35 μm.

In ruminants since the initial utilization of the in situ technique by Quin *et al.* (1938), the rumen bag method has gained acceptance as a means for measuring fibre and dry matter digestibility and also for measuring the nitrogen disappearance from feedstuffs. Furthermore, in ruminants the factors influencing this technique have been identified and quantitated and can be controlled.

In monogastrics, digestion of the major dietary components starts in the stomach and is essentially complete in the small intestine (Keyes and De Barthe 1974). Using stained diets in fistulated pigs, these researchers measured the time taken between ingestion and maximum dye passage at

the duodenal and ileal fistula and the anus with various cereal diets. Owing to the large amount of variation these researchers did not find significant differences between the diets. They found maximum dye passage times of 2.5 - 5 h at the ileal fistula and 38 - 45 h in feces.

The factors regulating the passage of food from the stomach into the small intestine ("gastric emptying") have been studied only to a limited extent in pigs. The stimulus for the initial emptying is related to the volume, the continued emptying thereafter is influenced by nervous and hormonal inhibitory effects. Several of the gastro-intestinal hormones, notably secretin, gastric inhibitory peptide and vasoactive intestinal polypeptide will inhibit while motilin stimulates gastric contraction. However, it is not yet known which of these make a significant contribution to the control of gastric emptying under physiological conditions. Cutting the nervous connections between the stomach and the duodenum reduces the inhibitory effects of food in the duodenum, so the nervous system also contributes to the control, but the extent of its contribution is not known.

The inhibitory stimuli, whether nervous or hormonal, are initiated by the presence of various substances in the duodenum. Some substances such as fatty acids have a very marked while others such as glucose or mineral salts have a very small effect. Hunt and Knox (1975) showed that the rate of emptying is determined by the nutrient density (kcal/ml)

of a meal. The higher the nutrient density, the slower the rate of emptying, the effect being the same whether the nutrient is carbohydrate, protein or fat.

The actual process of emptying is affected by the contraction which passes down the pyloric region of the stomach into the duodenum (Neimeier 1940) and it is the motility of the pyloric region which directly influences the emptying after a meal (Auffray *et al*. 1967). Of the factors influencing motility, the pH in the duodenum has a relatively minor effect (Auffray *et al*. 1967; Laplace 1974). On the other hand, an inhibitory effect on gastric emptying is produced by food present in the first eight meters of small intestine in the pig whereas in many other species only food present in the duodenum has an effect (Auffray 1975).

The passage of stomach contents into the small intestine was measured in pigs fitted with re-entrant cannulas in the duodenum. Following a meal, passage of stomach contents continued throughout 24 h with intermittent density, the largest passage occuring 3 h after feeding (Kvasnitskii 1951). Kvasnitskii reported considerable individual variation and also variation in the rate of emptying depending on the quantity of food supplied. At 10, 16 or even 24 h after a large meal, much more remained in the stomach than after a small meal. Furthermore, the rate of stomach emptying was faster during the day than night, reflecting the activity of the animal. Thus, the two major

factors stimulating gastric emptying are the fullness of the stomach and the activity of the animal.

Auffray *et al.* (1967), in studies with pigs fitted with re-entrant cannulas in the duodenum, recorded the quantities of digesta that passed every 15 minutes. 40 % of the stomach contents passed into the duodenum during the first 15 minutes after a meal. The amount that emptied was approximately proportional to that consumed. Then followed a period when emptying was inhibited, followed by intermittent emptying over the remaining 7 h period that was studied. Measurements of portal blood sugar levels after feeding carbohydrate meals have indicated rhythmical intermittent emptying into the absorbing region of the proximal intestine (Aumaitre *et al.* 1973). Horszczaruk (1962), in studies with rubber beads, deduced that food could spend up to a maximum of 33 h in the stomach of the pig. This is in agreement with Neimeier (1940) and Kvasnitskii (1951) who showed that the stomach of the mature pig did not completely empty, even after a fast of 24 h or more.

In monogastrics, the digestion of protein starts in the stomach as a result of the action of pepsin. These enzymes are secreted as inactive precursors and are activated by the hydrolytic removal of a peptide from the N-terminal end of the molecule. The precursors of pepsin (pepsinogens) are secreted by the mucosa of the fundal and pyloric region of the stomach.

Pepsinogens are hydrolysed to pepsins in acid conditions, slowly at pH 4 and rapidly at pH 2 (Ryle 1960). At pH 4, the intramolecular activation, due to acidity alone, is very slow so that the activation predominantly results from pepsin. Below pH 3, the intramolecular activation is rapid and predominant (Al-janabi *et al.* 1972). The intramolecular activation involves a split between amino acid residues 44 and 45 (Sanny *et al.* 1975), while autocatalytic activation is due to hydrolysis of a glutamyl-isoleucyl bond with a release of a 41 amino acid peptide which then can act as an inhibitor of pepsin but is hydrolysed when pepsin is present in excess. This is probably a protective device which inhibits premature activation of the enzyme within the secretory gland, but permits rapid activation after secretion.

Pepsin has two pH optima, one near pH 2.0, the other at pH 3.5 (Taylor 1959). Pepsin from the pyloric region has a slightly lower pH optimum than pepsin from the fundal region. This is consistant with the observation that the pH in the pyloric region is usually lower than that in the fundal region. The extent to which the activity decreases with increasing pH above 3.5 depends on the substrate (Taylor 1959). Pepsins from pigs have not been found to show any activity above pH 6.0 (Taylor 1959). Kratzer and Porter (1962) showed that the ratio of activity of pig pepsin at the two maxima was different for different proteins and that the peptide mixtures produced from proteins were not the

same at the two maxima. It is not known whether this is due to the efffect of pH on the enzyme or on the substrate.

After a meal, the pH of the contents in the fundal and pyloric regions of the pig's stomach falls rapidly from 4.0-5.0 to around 2.0 (Lawrence 1972) so that, at first a slow, and then a more rapid hydrolysis of protein will occur. Some proteins, such as egg albumen, are only digested rapidly at a lower pH. No significant proteolysis will occur if the pH does not fall below 4.0. If the pH does not approach 2.0, hydrolysis of some proteins will be limited.

The gastric pH depends on many factors including time interval after feeding, age of the pig and also the particular position where it is measured (Mollgaard 1946). Kvasnitskii (1951) showed that the acidity in the stomach varied considerably from one part to another. Kvasnitskii fitted a triple lumen sampling tube into the stomach of pigs with a fundal fistula which enabled him to sample the contents at different time intervals after feeding. The acidity was highest in the lowest layer. Slivitski (1975) used the same technique with 8-10 months old pigs that were fed starch and obtained pH values of about 1.0 in the basal layer. The uppermost layer had a pH value of about 3.5 at 2 h, falling to about 2.0 at 12 to 14 h after feeding. Thus the pH of the contents of the stomach is related to the time after feeding, the nature of the diet and the position at which it is measured. It will therefore also depend on the degree to which the contents of the stomach are mixed. This

was demonstrated by Maxwell *et al*. (1970) who found that the pH of the contents of the stomach did not vary greatly from one region to another when pigs were fed a finely ground corn diet. Lawrence (1970, 1972) showed that the pH rose immediately after feeding to levels depending on both the diet and the feeding regime, but usually in the range from 4.0 to 5.0. It then fell over the next 2 to 8 h to pH 1.0 to 2.0, usually to just below pH 2. The rate at which the drop occurred depended both on the composition and the physical nature of the diet. Laplace (1974) who measured the pH in the antrum adjacent to the pylorus also found that the pH rose immediately after feeding followed by a drop . He found no differences with different feeding regimes.

Petry and Handlos (1978) introduced the nylon bag technique for studying the digestibilities of nutrients in feedstuffs for pigs. Feedstuffs were orally administered in small nylon bags. However their technique did not yield results that were compatable with conventional digestibility trials. Protein digestibilities were consistantly overestimated, ranging from 0.7 to 16.9 percentage units, depending on the feedstuff, probably resulting from prolonged retention of the bag in the stomach.

Sauer *et al*. (1983) initiated research on 'the Modified nylon bag technique' but hereafter referred to as the "Mobile nylon bag technique" (MNBT) that allows for the rapid measurement of the apparent protein digestibilities in feedstuffs for pigs. This technique was renamed to

distinguish it more clearly from nylon bag studies in ruminants in which the nylon bags with feed samples remain suspended in the rumen

The basic principles of the MNBT are as follows. Pigs are fitted with a 'T' cannula in the duodenum. Feed is ground through a 0.8 mm screen and 1.0 gm of feed is enclosed in a small bag with dimensions of 25 x 40 mm. To simulate the conditions of the stomach, the bags with feedstuffs are immersed into a beaker at 37 °C for 2.5 h containing deionised water and HCl with a pH of 2.0 to which 1g of pepsin (with an activity of 377.4 IU/l) has been added. Following immersion, the nylon bags are inserted into the duodenum through the duodenal cannula. The bags travel through the intestinal tract and are collected as they pass in the feces usually 36-48 h later. The contents in the nylon bag that are left represent the indigestible residue of the feedstuff, allowing for the determination of the protein content. The reason for inserting a duodenal cannula are two-fold. First of all, it is not very easy to "shoot" the nylon bags into the stomach (if given orally these will be chewed up). Secondly, the pyloric sphincter prevents large particles, eg. the nylon bags with contents to pass into the duodenum. After collection of the bags in feces, they are immediately frozen, and lyophilized prior to protein analysis. Apparent protein digestibilities of SBM and MBM were 88.1 and 80.9 compared with 90.1 and 79.1 % respectively determined by long term conventional

digestibility studies. Based on the previous results the MNBT offers to be a promising approach for the rapid determination of protein digestibilities in small quantities of feedstuffs.

The results obtained with MNBT were more variable than those obtained with conventional digestibility studies. The present studies were carried out obtain a more detailed insight into the factors that determine the digestibility of protein as they are determined by aid of MNBT. Pre-digestion factors such as length of time for pre-digestion, pepsin activity and pH of the pre-digestion solution and other factors such as fineness of grinding, sample size, pore size of the nylon, shape and size of the bag and the number of bags inserted per day were studied. The effect of these factors on protein digestibility were studied to find means to minimise the variation and to obtain results as close as possible to those obtained in long term conventional digestibility trials.

III. CONVENTIONAL DIGESTIBILITY STUDIES

A. Abstract

The studies were carried out with six Yorkshire x Lacombe barrows over the weight range of 50 to 70 kg, fitted with a simple 'T' cannula 5 - 10 cm from the ileo cecal junction. The pigs were used in a replicated 3 x 3 Latin Square design to determine the ileal and fecal digestibilities of protein in cornstarch-based diets formulated to contain 16% crude protein by supplementation with soybean meal (SBM), canola meal (CM) and meat and bone meal (MBM). Chromic oxide was used as digestibility marker. The digestibilities of protein determined by the fecal analysis method were 93.0, 79.4, 79.1 % for SBM, MBM and CM, respectively. In the same order for the feedstuffs, the digestibilities of protein determined by the ileal analysis method were 85.8, 73.1, and 69.5 %, respectively.

B. Introduction

Conventional digestibility studies were carried out to determine the apparent digestibility of protein in selected protein supplements. A typical protein of plant (soybean meal) and animal origin (meat and bone meal) were selected for these studies. In addition, canola meal was selected because of its increased use as a protein supplement in swine diets in Canada. The objectives of these studies were to obtain reference (control) values for follow - up studies

with the mobile nylon bag technique (Sauer *et al.* 1983).

C. Materials and Methods

Six (Yorkshire x Lacombe) barrows with average initial weight of 50 kg were fitted with a simple 'T' cannula in the distal part of the ileum according to procedures adapted from Sauer *et al.* (1983) and used in studies to allow for the measurement of protein digestibility, as determined by both the ileal and fecal analysis method (Sauer *et al.* 1977). After surgery, the pigs were returned to the metabolic crates with a heat lamp placed directly overhead for few days. The pigs were allowed to recuperate for 3 weeks during which time they were fed the grower finisher diet (Table IV.1).

The barrows were housed individually in 0.5 x 1.0 m stainless steel metabolism crates (continuous light, air temperature 23 ° C) that allowed for collection of ileal digesta and feces. They were fed cornstarch - based diets (Table III.1) formulated to contain 16 % crude protein (% N x 6.25) by supplementation with SBM, MBM and CM. Dextrose was included in the diet at a level of 10 % to possibly improve palatability. Vitamins and minerals were included according to NRC (1979). Chromic oxide (0.5%) was added as a marker for the determination of the digestibilities of the nutrients that were measured. The pigs were fed 1 kg of the diet twice daily at 0800 and 2000 h. Water was supplied ad libitum.

Table III.1. Composition of diets

Diets	Soybean Meal	Canola Meal	Meat and Bone Meal
Ingredient (%):			
Soybean meal (47.5% C.P.)	33.7		
Canola meal (36.0% C.P.)		44.5	
Meat and Bone meal (51.7% C.P.)			30.9
Cornstarch	44.8	38.2	50.8
Dextrose	10.0	10.0	10.0
Alphafloc	6.2	2.5	6.6
Canola oil	2.7	2.2	
Calcium carbonate	0.4	0.4	
Calcium phosphate	1.5	1.5	
Sodium tripoly phosphate			1.0
Trace-mineralized salt	0.5	0.5	0.5
Vitamine-mineral premix[1]	0.2	0.2	0.2
Chromic oxide	+	+	+

[1]The premix provided the following per kilogram of diet. Vitamins: 1,300 IU vitamin A; 150 IU vitamin D; 11 IU vitamin E; 2 mg vitamin K; 2.2 mg riboflavin; 12 mg pantothenic; 11 μg vitamin B_{12}; 50 mg iron; 50 mg zinc; 20 mg manganese; 3 mg copper; .15 mg selenium.

The experiments were conducted in the form of a 3 x 3 Latin Square design. Each experimental period lasted 12 days. Feces were collected during 3 days from 0800 h on day 8 to 0800 h on day 11 of each experimental period. Ileal digesta were collected on days 11 (0800 - 1000 h, 1200 - 1400 h, 1600 - 1800 h) and on day 12 (1000 - 1200 h, 1400 - 1600 h, 1800 - 2000 h) according to procedures outlined by (Just *et al.* 1983).

Analytical and Statistical Procedures

Digesta and feces were frozen immediately upon collection. At the conclusion of the experiment, feed, feces and digesta were freeze dried, ground in a Wiley mill through a 0.8 mm mesh screen and thoroughly mixed before samples were taken for analyses. Analysis for dry matter and nitrogen in feed, feces and digesta were performed according to AOAC (1980) methods. Chromic oxide levels were determined according to the method of Fenton and Fenton (1979). The results were analysed by two way analysis of variance using Anova and SPSS (SPSS user's Guide).

D. Results and Discussion

The apparent ileal and fecal digestibilities of protein sources are shown in Table III.2. The digestibilities of protein measured in digesta collected from the end of the small intestine were 85.8, 73.1 and 69.5 % for SBM, MBM and CM, respectively. In the same order for the protein supplements, the apparent fecal protein digestibilities were

Table III.2. Apparent ileal and fecal digestibilities of protein Soybean Meal, Meat and Bone Meal and Canola Meal

Items	Soybean Meal	Canola Meal	Meat and Bone Meal
Ileal	85.8 ± 3.0	73.1 ± 2.4	69.5 ± 0.8
Fecal	93.0 ± 0.7	79.1 ± 2.0	79.4 ± 1.7
Difference	7.2	6.0	9.9

93.0, 79.1 and 79.4 %, respectively. The present results are in agreement with results compiled by Sauer and Ozimek (1985). These researchers reported ileal digestibility values in the range of 79.3 - 85.3, 59.2 - 66.0 and 68.2 - 70.3 % for SBM, MBM and CM, respectively. In the present studies the difference between the ileal and fecal digestibilities were 7.2, 6.3 and 9.9 % for SBM, MBM and CM, respectively. The digestibilities as measured by the fecal analysis method were higher than those determined by the ileal analysis method. This was found to be in agreement with previous studies (Sauer *et al.* 1977).

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IV. EFFECT OF PRE-DIGESTION FACTORS ON PROTEIN DIGESTIBILITY BY THE MOBILE NYLON BAG TECHNIQUE

A. Abstract

The mobile nylon bag technique (MNBT) was used with 5 pigs, over the weight range of 50 to 70 kg, fitted with a simple 'T' cannula in the duodenum to determine the factors that influence the protein digestibility in soybean meal (SBM), meat and bone meal (MBM) and canola meal (CM). Pre-digestion conditions such as length of time (h) of pre-digestion in a pepsin-HCl solution (0, 1.5, 2.5, 4.0), pH of the pre-digestion solution (1.0, 1.5, 2.0, 2.5) and activity (IU/l) of pepsin (188.7, 377.4) were examined. The absence of pre-digestion (0 h) resulted in a lower ($P<0.05$) protein digestibility than pre-digestion for 2.5 and 4.0 h. The digestibility of protein was highest at pH 2.0. Increasing the pepsin activity of the pre-digestion solution from 188.7 to 377.4 IU/l improved the protein digestibility of SBM ($P<0.05$) but not that of MBM and CM ($P>0.05$). The closest agreement between results obtained by MNBT and control studies occurred with a pre-digestion time of 2.5 h, at a pH of 2.0 and pepsin activity of 377.4 IU/l. Under these conditions, protein digestibilities determined by MNBT ranged from 89.6 - 91.5, 79.6 - 81.4 and 76.3 - 78.4 % in SBM, MBM and CM, respectively. In the same order for the feedstuffs, the apparent protein digestibilities determined in conventional studies were 93.0 ± 0.7 (SE), 79.1 ± 2.0 and

78.3 ± 0.7 %, respectively.

B. Introduction

Protein digestion and absorption in the pig may be divided into three phases namely the gastric, pancreatic and intestinal phase. In the MNBT, the gastric phase is simulated in vitro by incubation of samples with pepsin-HCl. The other two phases take place in vivo under normal physiological conditions of digestion. The gastric phase of protein digestion is first initiated in the stomach by pepsin, which is derived from pepsinogen, by the hydrolytic removal of a peptide from the N-terminal end. Pepsin itself activates pepsinogen so that the process becomes autocatalytic. There is a scarcity of information on protein digestion in the stomach. Research suggests that only a small proportion of the protein is hydrolysed there (Zebrowska 1980). The extent of gastric digestion of protein is determined by the physical state of the ingested protein, the length of time it stays in the stomach, the activity of pepsin, the pH of the stomach contents and hormonal as well as non - hormonal factors. To obtain a better understanding of the factors that affect protein digestion, as determined with MNBT, in vitro pre-digestion factors simulating conditions in the stomach were studied.

A series of three experiments were performed with pigs to determine the influence of pre-digestion factors such as length of time for pre-digestion, pH of the pre-digestion

solution and activity of pepsin on MNBT protein measurements.

In order to study these factors, a typical protein of plant (soybean meal) and animal origin (meat and bone meal) were selected. In addition, canola meal was selected as a feedstuff in these studies due to its increased use in Canada as a protein supplement in swine diets.

C. Materials and Methods

Animals

Five Yorkshire x Lacombe barrows, with average initial weight of 45 kg were obtained from the University of Alberta swine herd. Pigs were housed individually in 0.5 x 1.0 m stainless steel metabolism crates (continuous light, air temperature 23 °C) one week prior to surgery and fed a 16% crude protein grower diet (Table IV.1) ad libitum. Water was supplied ad libitum from a low pressure drinking nipple.

Following surgery the pigs were returned to the metabolic crates with a heat lamp placed directly overhead for a few days. The pigs were not fed on the day following surgery and the next day onwards they were fed approximately 100 g of the grower diet twice daily. The feed allowance was increased slowly until the pigs started consuming about 900 g of diet twice daily. Water was supplied ad libitum. The pigs were allowed to recuperate for a minimum period of three weeks. Once the trial started, the pigs were fed 900 g of the grower diet (Table IV.1) twice daily, at 0800 and

Table IV.1. Composition and partial chemical composition of the grower finisher diet

Ingredient (% as fed):	
Wheat	31.5
Barley	50.0
Soybean meal	15.0
Calcium phosphate	1.0
Calcium carbonate	1.0
Iodized salt	0.5
Vitamine-mineral premix[1]	1.0
Chemical analyses:	
Dry Matter (%)	89.0
Gross Energy (MJ/kg)	15.9
Ether Extract (%)	1.4
Crude Protein	16.7
Ash(%)	5.3

[1]The premix provided the following per kilogram of diet: (1) minerals - 120 mg zinc; 48 mg manganese; 100 mg iron; 10 mg copper; 1 mg selenium;
(2) vitamins: 7500 IU vitamin A ;700 IU vitamin D_3;45 IU vitamin E; 12 mg riboflavin; 40 mg niacin; 25 mg pantotheninc acid; 28μ g B_{12}.

1600 h, throughout the experiment. The grower diet was formulated to meet the National Acadamy of Sciences National Research Council recommended levels of nutrients (NRC 1979).

Surgical Procedures

Pigs were starved for 36 h and anaesthetised with a halothane oxygen mixture (5 % halothane, 1.75 % strength dose) through a face mask. No pre medication was used. Aseptic technique and standard surgical procedures were as follows: the pig was placed in left lateral recumbency, the right thoracic and abdominal wall was clipped with an electric shaver, scrubbed with betadine iodine solution, rinsed with 70 % ethanol and draped. A 15 cm incision was made caudal and parallel to the last rib through the skin and the external abdominal oblique muscle. The remaining two muscle layers were split by blunt dissection and the peritoneum was cut parallel to the incision. The right side of the rib cage was elevated and the proximal duodenum was retracted with intestinal forceps. Saline moistened Telfa pads (Kendall Canada, Toronto Ontario) were placed over the incision edges beneath the exposed section of the doudenum. The duodenum is the first part of the small intestine and is closely attached to the body wall by a short mesentry, the mesoduodenum. Ducts from the pancreas enter the first part of the duodenum. The duodenum leaves the pylorus of the stomach and passes caudal on the right side towards the pelvic inlet. The duodenum then crosses to the left side behind the root of the great mesentery and thus forward to

join the jejunum. The beginning of the duodenum was identified by gentle palpation and held between the index and thumb finger. A simple purse string suture, using a 3-0 chromic catgut was put in oval shape. A 2 cm incision was made with a scalpel in between the purse string suture. This suture was loosened. The base of the cannula was introduced through the incision into the intestinal lumen and the suture tightened. Manipulation of the intestine was made easier by pouring sterile saline (at room temperature) into the peritoneal cavity during the beginning of the surgery, causing smooth muscle contraction and increased tone of the gut. The smooth contact surface provided by the Telfa pads further reduced tissue damage. Surgical gloves with a textured finish should be avoided. Hemostasis was maintained by ligating major blood vessels. One additional purse string suture was secured around the cannula base. A circle of skin approximately 15 mm diameter was excised, the underlying tissue was split by blunt dissection and the peritoneum was cut. The intercostal opening was stretched sufficiently to pass the cannula guide. The cannula was exteriorized between the 11th or 12th intercostal space which is normally close to the duodenal cannulation site. The stab incision exteriorizing the cannula thus provided a tight seal and avoided necrosis. Before closing the abdominal cavity 200 mg oxytetracycline hydrochloride and 250 ml of sterile saline were introduced in to the peritoneal cavity.

Preparation of the Nylon Bags

Unless otherwise indicated, bags measuring 2.5 x 4.0 cm were prepared from monofilament nylon with a pore size of 48 µm (Thompson & Co Ltd, 235, Montepellar BLVD, St Laurent, Montreal P.Q) by aid of a seal master (Packing Aids Corporation, 469 Bryant Street, San Francisco, California 94102, U.S.A) by hot sealing. This method of hot sealing allowed for a faster production of the bags as compared to the conventional sewing method which was previously described by Sauer *et al.* (1983). In addition, the bags were more secure.

Pre - digestion Procedure

Unless otherwise indicated, 10 bags at a time with 1.0 g of a sample were incubated for 2.5 h at 37 °C in a beaker that contained 500 ml of a pepsin-HCl solution with a pH of 2.0. The activity of pepsin in this solution was 377.4 IU/l (Purified pepsin powder, Fisher scientific company, Chemical Manufacturing division, Fairlawn, N.J, U.S.A). The beaker was placed in a shaker (Lab-Line orbit Environ shaker, Lab line Instruments, INC, Melrose Park, IL, U.S.A) at 90 oscillations / minute for 2.5 h at 37 °C and agitated. Thereafter, the bags were removed, washed with deionised water and frozen until used

Insertion and Collection of bags.

Prior to insertion the frozen bags were taken out of the freezer and thawed in a water bath at 37 °C for 5 minutes. Two bags were inserted in the morning and two in

the evening into the duodenum of each of 5 pigs while the pigs were eating. The bags were collected during daily searches between 0800 - 0900 and 1500 - 1600 h. The bags, which were often completely enveloped by feces, were carefully isolated and immediately frozen. Only bags recovered within 72 h were used in the studies. Some of the bags were collected after 72 h. These included bags that were lost and washed during the cleaning of the metabolic crates. Washing results in the removal of soluble nitrogen containing compounds from the bag. Occasionally, bags were found that had been chewed. These bags were also discarded.

Experiments.

A series of three experiments were conducted.

Experiment 1. The effect of the pH of the pre - digestion solution on MNBT protein digestibility measurements was studied. Pre - digestion solutions with a pH of 1.0, 1.5, 2.0 and 2.5 were prepared. As in the other experiments, 4 bags for each treatment, 2 during the morning and 2 during the evening meal, were inserted into each of 5 pigs.

Experiment 2. The effect of pepsin activity on MNBT protein measurements was studied. Pre - digestion solutions with a pepsin activity of 188.7 and 377.4 IU/l were prepared.

Experiment 3. The effect of time of pre - digestion on MNBT protein digestibility measurements was studied. Pre - digestion periods of 0, 1.5, 2.5 and 4.0 h were selected.

Chemical Analysis.

Analyses were initiated immediately after the completion of the experiment. Following freeze-drying the bags were cut open carefully and the contents were analyzed for protein by aid of the micro - Kjeldahl method (Fawcett 1979). Freeze-drying resulted in a crispy and flaky nature of the contents allowing for easy recovery.

Statistical Analysis.

The data were analyzed by Analysis of Variance Mehlenbacher (1978). Where appropriate treatment means were tested for significance ($P<0.05$) using Student-Newman-Kuels' multiple range test (Steel and Torrie 1980).

D. Results and Discussion

The fistula was virtually leak proof and required little maintenance and were functional until the pigs were slaughtered. Protein digestibility values obtained with the nylon bag technique are referred to as 'apparent'(Sauer *et al.* 1983).

The effect of pH of the pre - digestion solution on protein digestibility is shown in Table IV.2. The apparent protein digestibilities were highest at pH 2.0 ($P<0.05$) for all the feedstuffs tested and closest to those obtained in the conventional studies. The protein digestibilities were lower at pH 1.0 and 2.5 than at pH 2.0. These differences were significant ($P<0.05$) for MBM and CM. The present results likely reflect an optimum pH of 2.0 for the pepsin

Table IV.2. The effect of the pH of the pre-digestion solution on protein digestibility, [1] % (Exp. 1)

Feedstuffs		*Soybean meal*	*Meat and bone meal*	*Canola meal*
pH:	1.0	88.1 ± 0.5[3] (11)a	75.7 ± 0.6 (10)b	71.1 ± 1.3 (20)b
	1.5	85.7 ± 0.4 (11)b	78.5 ± 0.6 (11)a	74.7 ± 0.4 (20)a
	2.0	89.9 ± 0.6 (12)a	81.4 ± 1.0 (15)a	76.7 ± 0.5 (18)a
	2.5	88.3 ± 0.5 (10)a	75.7 ± 0.6 (9)b	71.2 ± 0.8 (18)b
Control[2]		93.0 ± 0.7	79.1 ± 2.0	78.3 ± 1.8

1- Means ± standard errors.
2- Control values determined in conventional digestibility studies (part 3).
3- Values in parenthesis indicate the number of nylon-bag determinations.
a, b Means in the same column not followed by the same letter are significantlly different at $P < 0.05$.

that was used in the present studies. The pH of the contents in the stomach is not constant. After a meal, the pH rapidly drops from 4.0 - 5.0 to 2.0 (Lawrence 1970). Therefore, at first a slow, and then a more rapid hydrolysis of protein will occur. The fall in pH will depend on the buffering capacity of the diet, which in particular depends on the level of protein, and also on the feeding regimen (Maxwell *et al.* 1970). No significant changes in protein digestion will occur if the pH does not fall below 4.0. Furthermore, if the pH does not approach 2.0, hydrolysis of some proteins will be limited. The latter will account for the lower protein digestibility at pH 2.5 than at pH 2.0 (Table IV.2).

The effect of pepsin activity on protein digestibility was examined in experiment 2 (Table IV.3). As the pepsin activity was increased, the protein digestibility of SBM and CM, but not that of MBM, increased. The results obtained at the higher pepsin activity were closest to those obtained in the conventional studies. The increase in protein digestibility for some of the feedstuffs with increasing pepsin concentration indicates differences in the requirement of pepsin for protein hydrolysis. The rate of digestion may vary from one protein to another. Nehring and Schroder (1965) compared the digestion by pepsin of protein in lucerne, rapeseed meal, barley and lupin with meat meal. Amino acids and smaller peptides were released in the greatest quantity from rapeseed meal. The amino acids preferably released either as free amino acids or as small

Table IV.3. Effect of the activity of pepsin on protein digestibility,[1] % (Exp.2).

Feedstuffs		*Soybean meal*	*Meat and bone meal*	*Canola meal*
Pepsin activity, IU/l	188.7	88.8 ± 2.0 (8)b	82.6 ± 0 7 (17)	76.7 ± .55 (18)
	377.4	91.5 ± 1.5 (10)a	81.0 ± 1.0 (17)	78.4 ± .86 (20)

1- Means ± standard error.
2- Values in parenthesis indicate the number of nylon bag determinations.
a, b Means in the same column not followed by the same letter are significantlly different at P< 0.05.

peptides also varied from one protein to another.

Gastric secretion has been studied in pigs by means of isolating the stomach, by aid of stomach pouches and also in the intact animal (Kvasnitskii 1951; Maxwell *et al.* 1970; Lawrence 1972). However, none of the studies allowed for estimation of the total secretion (and the activity) of pepsin from the stomach. Low (1982) and Zebrowska *et al.* (1983) studied the total pepsin activity in the duodenum, as an indication of pepsin activity in the stomach. Low (1982) reported mean total pepsin activities (24 h) in duodenal digesta of pigs fed 3 diets to be 7764400, 6078400 and 5801600 units/24 h, respectively. One unit of pepsin activity has been defined as the increase in absorbance of 0.001/minute at 280 nm due to the relese of TCA soluble hydrolysis products. However, Zebrowska *et al.* (1983) showed a total pepsin activity of 760449 and 1466571, in pigs fed casein and barley - soybean meal diets. Feed intake was similar in both studies. The estimates that were cited differ by a factor of 5 to 10. Low (1982) used hemoglobin, whereas Zebrowska *et al.* (1983) used casein as a substrate for the determination of pepsin activity. However, considering that the activities of trypsin and chymotrypsin are about 288 times higher when these are determined with casein as substrate as compared to hemoglobin (a similar trend can be assumed for pepsin activity), the difference becomes even larger. Therefore, a comparison of results obtained by Low (1982) and Zebrowska *et al.* (1983) is

valueless. The previous discussion shows that more research is needed to determine the total pepsin activity in the stomach under in vivo conditions, and the extent to which the in vivo activity relates to the activity of pepsin used in MNBT. In the present studies the pepsin activity of 377.4 IU/l was measured using the procedures described in the method of enzymatic analysis by Bergmeyer (1963).

The effect of time of pre - digestion on protein digestibility were studied in experiment 3 (Table IV.4). The absence of pre - digestion (0 h) resulted in a lower protein digestibility ($P<0.05$) than pre - digestion for 2.5 or 4.0 h for all the feedstuffs that were tested. In addition, the protein digestibility in CM was lower at 1.5 than at 2.5 or 4.0 h of pre - digestion. The closest agreement between results obtained by MNBT and conventional studies occurred with a pre-digestion period of 2.5 and 4.0 h. Under these conditions, the protein digestibilities determined by MNBT were 90.7, 80.6 and 76.3 % for SBM, MBM and CM respectively. These results are in agreement with those obtained by Sauer *et al.* (1983) when samples were pre-digested for 2.5 h in pepsin-HCl solution. For SBM there seemed to be a trend towards a higher protein digestibility when the sample was incubated for 4.0 h, however this was not found to be significant ($P>0.05$). The present results show the relative importance of digestion of protein by pepsin. Although the digestion of protein in the stomach is not essential there will be a decrease in protein digestibility when absent

Table IV.4. Effect of the time of pre-digestion on the protein digestibility,[1] % (Exp. 3)

Feedstuffs		*Soybean meal*	*Meat and bone meal*	*Canola meal*
Time, h	0	79.2 ± 0.4 (12)[2]b	56.5 ± 0.4 (14)b	65.2 ± 2.0 (17)b
	1.5	92.0 ± 0.4 (11)a	79.6 ± 0.5 (11)a	73.2 ± 1.0 (19)b
	2.5	90.9 ± 0.3 (16)a	80.6 ± 0.3 (16)a	76.3 ± 0.6 (18)a
	4.0	94.1 ± 0.4 (11)a	80.1 ± 0.4 (17)a	77.8 ± 0.8 (18)a

1- Means ± standard error.
2- Values in parenthesis indicate the number of nylon bag determinations.
a, b Means in the same column not followed by the same letter are significantlly different at P< 0.05.

($P>0.05$).

The extent of gastric digestion of protein which takes place under in vivo conditions is determined by the physical state of the ingested protein and the length of the time it stays in the stomach. Horzczaruk (1962) remarked that food could spend up to 33 h in the stomach of the pig. This is in agreement with the findings of Neimeier (1940) and Kvasnitskii (1951) showing that the stomach of the mature pig did not fully empty even after 24 hours or more. Keyes and De Barthe (1974), using stained diets in fistulated pigs, measured the time taken between ingestion and maximum dye passage at the duodenal fistula, and they observed maximum dye passage times of 2.5 - 5 h for the duodenal fistula. Under normal conditions the duration of food in the stomach may depend on the type of food, whether liquid or solid (the liquid phase tends to empty faster), the nature of the food, whether fibrous or not, and also on the volume of the food.

However difficult it is to apply information obtained from nylon bag studies in ruminants, which relate to fermentation, to MNBT studies in pigs, some analogies may be drawn. Rodriguez (1968) studied the effect of fermentation period in the rumen. With a short incubation period of 24 h, these researchers reported larger variation between bags and animals. As the fermentation time was increased, the digestibility of dry matter increased reaching a maximum at 72 h. Their results were similar to those by Van Keuren and

Heineman (1962). However, in the present study with different pre - digestion periods there was no decrease in variation between bags and animals.

The present studies present a detailed insight into the factors that should be taken into consideration for the maximum optimization of the MNBT. Protein digestibility values obtained at a pH of 2.0, pepsin activity of 377.4 IU/l and samples pre - digested for 2.5 to 4.0 h gave values similar to those obtained in long term conventional digestibility studies.

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V. EFFECT OF OTHER FACTORS ON PROTEIN DIGESTIBILITY BY THE MOBILE NYLON BAG TECHNIQUE

A. Abstract

The mobile nylon bag technique (MNBT) was used with 5 pigs, over the weight range of 50 to 70 kg, fitted with a simple 'T' cannula in the duodenum to determine the factors that influence the protein digestibility in soybean meal (SBM), meat and bone meal (MBM) and canola meal (CM). Fineness (screen size, mm) of grinding (0.5, 1.0, 1.5, 2.0), pore size (μm) of the nylon mesh (10, 48, 63, 70), number of bags inserted into each pig per day (1,4), shape and size of the bag (2.5 x 4.0 vs 1.5 x 5.0 cm) and the amount of the feed (g)in the bag (0.5, 1.0) were examined. The digestibility of protein in SBM was lower ($P<0.05$) when the sample was ground through a screen with a mesh size of 2.0 as opposed to 0.5, 1.0 or 1.5 mm. Fineness of grinding did not effect ($P>0.05$) the protein digestibility in MBM. The digestibility of protein in all the feedstuffs was lowest ($P<0.05$) when the pore size of the nylon was 10 μm. Decreasing the amount of sample from 1.0 to 0.5 g improved ($P<0.05$) the protein digestibility of SBM but not that of MBM and CM ($P>0.05$). The shape and size of the bag and the number of bags inserted per day into each pig had no effect ($P>0.05$) on protein digestibility. The protein digestibilities obtained with a fineness of 1.0 mm, pore size of 48 μm and a sample size of 0.5 g were closest to

those obtained in conventional digestibility studies. Under these conditions the protein digestibilities determined by MNBT ranged from 89.6 - 91.5, 79.6 - 81.4 and 76.3 - 78.4 % for SBM, MBM and CM, respectively. In the same order for the feedstuffs, the apparent protein digestibilities determined in conventional studies were 93.0 ± 0.7 (SE), 79.1 ± 2.0 and 78.3 ± 0.7 %, respectively.

B. Introduction

Measuring the digestibility of nutrients for domestic animals requires large quantities of feed and a long period of adaptation to the experimental diets. These limitations would be overcome if the feed samples could be artificially introduced into the digestive tract and the undigested contents recovered quantitatively in feces. The increasing number of feedstuffs that are used and the many new varieties of the various cereal grains that are introduced have made it necessary for feed manufacturers and nutritionists to search for rapid and less expensive techniques. The MNBT could be a very useful solution for such screening purposes, because of its "in vivo" nature and because this method is rapid and inexpensive.

In general, the results obtained with MNBT were more variable than those obtained in conventional digestibility studies (Sauer *et al.* 1983). Therefore, studies were conducted to determine the reasons for the larger experimental variation and to find means to reduce the

variation. A series of experiments were performed with 5 pigs to determine some of the factors other than those that were previously described (Part IV) that may influence the protein digestibility as determined with MNBT. Factors such as the fineness of grinding, pore size of the nylon, the amount of feed in the bag, the shape and size of the bag and also the number of bags that are inserted into each pig per day were examined. The same feedstuffs namely, SBM, MBM and CM were used in the present studies.

C. Materials and Methods

Animals

Five Yorkshire x Lacombe barrows with average initial weight of 45 kg, were obtained from the University of Alberta swine herd. Pigs were housed individually in 0.5 x 1.0 m stainless steel metabolism crates (continuous light, air temperature 23 °C) one week prior to surgery and fed a 16 % crude protein grower diet (Table IV.1) ad libitum. Water was supplied ad libitum from a low pressure drinking nipple.

Following surgery the pigs were returned to the metabolic crates with a heat lamp placed directly overhead for a few days. The pigs were not fed on the day following surgery and the next day onwards they were fed approximately 100 g of the grower diet twice daily. The feed allowance was increased slowly until the pigs were consuming about 900 g of diet twice daily. Water was supplied ad libitum. The pigs

were allowed to recuperate for a minimum period of 3 weeks. Once the trial started, the pigs were fed 900 g of the grower diet (Table IV.1) twice daily, at 0800 and 1600 h, throughout experiment. The grower diet was formulated to meet the National Acadamy of Sciences National Research Council recommended levels of nutrients (NRC 1979).

Surgical Procedures

As described in Part IV.

Preparation of the Nylon Bags

Unless otherwise indicated, bags measuring 2.5 x 4.0 cm were prepared from monofilament nylon with a pore size of 48 μm (Thompson & Co Ltd, 235 Montepellar BLVD, St Laurent, Montreal P.Q) by aid of a seal master (Packing Aids Corporation, 469 Bryant Street, San Francisco, California 94102, U.S.A) by hot sealing. This method of hot sealing allowed for a faster production of the bags as compared to the conventional sewing method which was previously described by (Sauer *et al*. 1983). In addition, the bags were more secure.

Pre - digestion Procedure

Unless otherwise indicated, 10 bags at a time with 1.0 g of a sample were incubated for 2.5 h at 37 °C in a beaker that contained 500 ml of a pepsin-HCl solution with a pH of 2.0. The activity of pepsin in this solution was 377.4 IU/l (Purified pepsin powder, Fisher scientific company, Chemical Manufacturing division, Fairlawn, N.J, U.S.A). The beaker was placed in a shaker (Lab-Line orbit Environ shaker, Lab

line Instruments, INC, Melrose Park, IL, U.S.A) at 90 oscillations / minute for 2.5 h at 37 °C and agitated. Thereafter, the bags were removed, and washed with deionised water and frozen until used.

Insertion and Collection of bags.

Prior to insertion the frozen bags were taken out of the freezer and thawed in a water bath at 37 °C for 5 minutes. Two bags were inserted in the morning and two in the evening into each of 5 pigs while the pigs were eating. The bags were collected during daily searches between 0800 - 0900 and 1500 - 1600 h. The bags, which were often completely enveloped by feces, were carefully isolated and immediately frozen. Only bags recovered within 72 h were used in the studies. Some of the bags were collected after 72 h. These included bags that were lost and washed during the cleaning of the metabolic crates. Washing results in the removal of soluble nitrogen containing compounds from the bag. Occasionally, bags were found that had been chewed. These bags were also discarded.

Experiments.

A series of five experiments were conducted.

Experiment 1. The MNBT was used to determine the fineness of grinding of the feedstuff on protein digestibility. Mesh sizes varying from 0.5, 1.0, 1.5 and 2.0 mm were used. As in the previous experiment (Part IV), 4 bags of each treatment, two during the morning and two during the evening meal, were inserted into each of 5 pigs.

Experiment 2. The effect of pore size of the nylon on protein digestibility was studied. Nylon mesh with pore sizes of 10, 48, 63, and 70 μm were selected.

Experiment 3. The effect of the amount of feed in the bag on protein digestibility was measured. Bags containing either 0.5 or 1.0 g were prepared.

Experiment 4. The effect of the size and shape of the nylon bag on protein digestibility was examined. Bags measuring 1.5 x 5.0 as compared to 2.5 x 4.0 cm were used.

Experiment 5. The effect of the total number of bags inserted into each pig per day on protein digestibility was examined. One as opposed to 4 bags were inserted into each pig per day.

Chemical Analysis.

Analyses were initiated immediately after the completion of experiment. Following freeze-drying the bags were cut open carefully and the contents were analyzed for protein using the micro - Kjeldahl method (Fawcett 1979). Freeze-drying results in a crisp and flaky nature of the undigested contents and allows for easy recovery.

Statistical Analysis.

The data were analyzed by analysis of variance Mehlenbacher (1978). Where appropriate, treatment means were tested for significance ($P<0.05$) using Student-Newman-Kuels' multiple range test (Steel and Torrie 1980).

D. Results and Discussion

The effect of fineness of grinding on protein digestibility is shown in table V.1. Decreasing the screen size from 2.0 to 0.5 mm increased the protein digestibility ($P<0.05$) SBM from 88.2 to 91.3 %. The digestibility of protein in MBM did not increase significantly, but tended to be lower when the mesh size of the screens were 1.5 and 2.0 mm. The present results show the effect of grinding, which to a certain extent simulates the mechanical work of mastication and the stomach, to be dependant on the physical and/or chemical properties of the particular feedstuff that was tested. Superior results have been obtained by fine grinding especially for feeds that are high in fibre content (Crampton and Bell 1946). This might have attributed to the higher digestibility at 0.5 than at 2.0 mm mesh size for SBM as compared to MBM. The improvement in digestibility may also be due to the greater surface area that some feedstuffs present upon fine grinding. In addition, differential effects on pH changes in the stomach resulting from barley that was fine or coarsely ground was noted by Lawrence (1970). The coarser particles tended to give more acid conditions and digesta in the stomach were much less fluid than when the finer particles were fed. Rate of passage was also affected. The coarser particles passed more quickly than the finer particles resulting in a lower digestibility.

The effect of the pore size of the nylon on apparent protein digestibility is shown in Table V.2. Protein

Table V.1. Effect of fineness of grinding on protein digestibility,[1] % (Expt. 1)

Feedstuffs	Soybean Meal	Meat and Bone Meal
Screen size, mm		
0.5	91.3 ± 0.6 (17)[2]a	79.8 ± 1.4 (18)a
1.0	90.8 ± 0.3 (20)a	79.9 ± 0.2 (19)a
1.5	90.0 ± 0.9 (18)a	78.7 ± 1.0 (17)a
2.0	88.2 ± 1.8 (18)b	78.6 ± 0.9 (19)a

1- Means ± standard errors.
2- Values in parenthesis indicate the number of nylon bag determinations.
a, b Means in the same column not followed by the same letter are significantlly different at $P < 0.05$.

Table V.2. The effect of the pore size of the nylon bags on protein digestibility,[1] % (Exp. 2)

Feedstuffs		*Soybean meal*	*Meat and bone meal*	*Canola meal*
pore size,um:	10	87.3 ± 0.8 (19)b	67.4 ± 1.0 (20)b	69.8 ± 0.6 (20)b
	48	91.1 ± 0.7 (17)a	79.6 ± 0.6 (18)a	76.7 ± 0.6 (10)a
	63	91.1 ± 0.2 (8)a	87.7 ± 1.5 (11)a	79.4 ± 0.9 (20)a
	70	95.3 ± 0.3 (4)a	80.8 ± 1.0 (10)a	79.8 ± 0.6 (20)a

1- Means ± standard errors.
2- Values in parenthesis indicate the number of nylon bag determinations.
a, b Means in the same column not followed by the same letter are significantlly different at $P < 0.05$.

digestibilities were lower ($P<0.05$) when the sample was enclosed in nylon with a mesh size of 10 μm as compared to 48, 63 or 70 μm. The protein digestibility coefficients obtained at 10 μm were of the same order as those obtained by the ileal analysis method in conventional studies: 87.3 vs 85.8 %, 67.4 vs 69.5 % and 69.8 vs 73.1 % for SBM, MBM and CM, respectively (Tables V.2 and Table III.2). The digestibility of protein, as determined by the ileal analysis method (Sauer *et al*. 1977), represents the protein digestibility in a feedstuff as this is not modified by the action of the microflora. In the pig, the microflora is predominantly present in the large intestine. A mesh size of 10 μm may prevent the penetration of a large number of the microbes, resulting in protein digestibilities that would be in the range of those obtained by the ileal analysis method. However, additional research should be carried out to verify these postulations. On the other hand, a decrease in substrate disappearance and / or enzyme penetration may be responsible for the lower digestibility of protein at 10 as compared to 48, 63 and 70 μm (Table V.2). Although the protein digestibility for SBM was higher at 70 as compared to 48 and 63 μm, the differences were not significant ($P>0.05$).

The effect of differences in pore size of the bag has been studied in ruminants by Weakly *et al*. (1983), who studied the disappearance of SBM and Barley from dacron (52 μm) or nylon (20 μm) bags. The disapperance of dry matter

and nitrogen was greater from dacron than from nylon bags. Similar results were obtained by Uden *et al.* (1974), who found a greater cell wall digestibility of guinea grass from nylon bags with a pore size of 53 as compared to 20 or 35 μm.

In experiment 3, nylon bags were prepared that contained 0.5 g or 1.0 g samples of SBM, MBM and CM. The apparent protein digestibility in SBM increased ($P<0.05$) as the sample size decreased from 1.0 g to 0.5 g. Sample size did not effect ($P>0.05$) the protein digestibility in MBM and CM (Table V.3). For some feedstuffs, a sample size of 1.0 g may be too large to allow for complete mixing, enzyme penetration and removal of end products of digestion from the bag. The same effect was noticed by Uden *et al.* (1974) in studies with ruminants. Increasing the sample size from 6.5 to 50 mg/cm² decreased the disappearance of guinea grass cell fractions from 54 to 38 %. The effect of sample size on dry matter disappearance was also studied by Van Keuren and Heinemann (1962). A larger sample size resulted in a lower dry matter disappearance. Furthermore, these results agree with results obtained by Bullis *et al.* (1967), who reported that the disappearance diminished as the weight of the sample was increased in bags of similar size.

The shape and size of the bags did not effect ($P>0.05$) the digestibility of protein (Table V.4). Although protein digestibility was not effected, the longitudinal bags (1.5 x 5.0 cm) moved faster through the digestive tract. The effect

Table V.3. Effect of sample size on the protein digestibility,[1] % (Exp. 3)

Feedstuffs		*Soybean meal*	*Meat and bone meal*	*Canola meal*
Sample size, g	0.5	93.3 ± 0.5 (16)[2]a	82.6 ± 0.7 (8)	76.6 ± .94 (18)
	1.0	89.6 ± 0.5 (15)b	81 2 ± 0.8 (8)	76.7 ± 56 (18)

1- Means ± standard error.
2- Values in parenthesis indicate the number of nylon bag determinations.
a, b Means in the same column not followed by the same letter are significantlly different at P< 0.05.

Table V.4. Effect of the shape of the bag on the protein digestibility, [1] % (Expt. 4)

Feedstuffs		Soybean Meal	Meat and Bone Meal
Bag size, cm	1.5 cm x 5.0	90.6 ± 0.7 [2] (11)	81.0 ± 1.1 (10)
	2.5 cm x 4.0	90.7 ± 0.3 (11)	80.9 ± 0.3 (15)

1- Means ± standard errors.
2- Values in parenthesis indicate the number of nylon bag determinations.

of size of the bag was studied in ruminants by Mehrez and Orskov (1977). Two sets of bags, measuring 5 x 8 and 17 x 9 cm, each containing 5 g of rolled barley, were incubated in the rumen of sheep for 24 h on 2 consecutive days. Increasing the bag size increased the dry matter disappearance from 37.5 to 85 %. The difference in bag size in the present studies may not have been large enough to cause a difference in protein digestibility.

There were no difference in protein digestibility if one or four bags were inserted each day (Table V.5). From these results it may be postulated that it is unlikely that the insertion of bags has any effect on the normal processes of digestion and absorption that take place in the pig.

The digestibility values obtained with a screen size of 1.0 mm, pore size of 48 μm and with a sample size of 0.5 gm gave values similar to those obtained with the conventional studies. Under these conditions, protein digestibilities determined by MNBT ranged from 89.6 - 91.5, 79.6 - 81.4, and 76.3 - 78.4 % for SBM, MBM and CM respectively. Protein digestibilities determined by the conventional methods were 93.0, 79.1 and 78.3 % for SBM, MBM and CM respectively. The present studies show the importance of certain factors which should be taken into consideration for further optimization of MNBT.

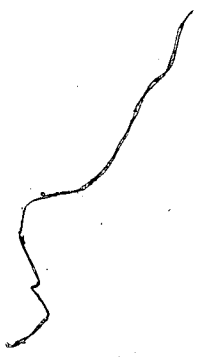

Table V.5. Effect of the number of bags inserted per day on protein digestibility, % (Exp. 5)

Feedstuffs		Soybean Meal	Meat and Bone Meal
No: of bags	1	90.4 ± 1.5 (5)a	79.7 ± 0.8 (8)a
	4	90.7 ± 0.3 (16)a	80.9 ± 0.3 (15)a

1- Means ± standard errors.
2- Values in parenthesis indicate the number of nylon bag determinations.

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General Summary and Conclusion

The present studies were carried out to obtain a more detailed insight into the factors that should be taken into consideration for the maximum optimization of the MNBT. The pre-digestion of feedstuffs in a pepsin-HCl solution seemed to have a great influence on protein digestibility. The absence of pre-digestion (0 h) resulted in a lower ($P<0.05$) digestibility than pre-digestion for 2.5 and 4.0 h. The digestibility of protein was highest at pH 2.0. Increasing the pepsin activity of the pre-digestion solution from 188.7 to 377.4 IU/l improved the protein digestibility of SBM ($P<0.05$) but not that of MBM and CM ($P>0.05$).

The digestibility of protein in SBM was lower ($P<0.05$) when the sample was ground through a screen with a mesh size of 2.0 as opposed to 0.5, 1.0 or 1.5 mm. However, fineness of grinding did not effect ($P>0.05$) the protein digestibility in MBM. The digestibility of protein in all feedstuffs was lowest ($P<0.05$) when the pore size of the nylon was 10 μm. Decreasing the amount of sample from 1.0 to 0.5 g improved ($P<0.05$) the protein digestibility of SBM but not that of MBM and CM ($P>0.05$). The shape and size of the bag and the number of bags inserted per day into each pig had no effect ($P>0.05$) on protein digestibility. The closest agreement between results obtained by MNBT and control studies occurred with a pre-digestion time of 2.5 or 4.0 h, at a pH of 2.0, pepsin activity of 377.4 IU/l, sample size of 0.5 g, pore size of the nylon of 48 μm and with a

finer degree of grinding of 1.0 mm. Under these conditions, protein digestibilities determined by MNBT ranged from 89.6 - 91.5, 79.6 - 81.4 and 76.3 - 78.4 % in SBM, MBM and CM, respectively. In the same order for the feedstuffs, the apparent protein digestibilities determined in conventional studies were 93.0, 79.1 and 78.3 %, respectively.

The present series of investigations show some of the variables that effect MNBT measurements. In general, these variables had more effect on the protein digestibility of SBM than on that of MBM and CM. In conclusion the MNBT offers to be a promising method for the determination of the digestible protein in feedstuffs for pigs.

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