NUTRITIONAL SIGNIFICANCE OF ENDOGENOUS GUT NITROGEN LOSSES IN GROWING PIGS

A Thesis

Presented to

The Faculty of Graduate Studies

of

The University of Guelph

by

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In Partial Fulfilment of Requirements

for the degree of

Doctor of Philosophy

June, 1998

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ABSTRACT

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University of Guelph, 1998

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Experiments were conducted to establish the impact of diets designed to induce either low or high endogenous gut protein losses (ENL) on protein synthesis rates and energy expenditure in the visceral organs of growing pigs. First, conditions were established for converting lysine in practical feedstuffs into homoarginine (HA). This technique was then used to determine ENL in pigs fed casein-cornstarch (CC), barley (B), canola meal (CM) or a mixture of barley and canola meal (BCM) based diets. Apparent and true ileal amino acid digestibilities were also determined. Diets based on B, CM and BCM induced significantly more ENL than the CC-based diet. True ileal amino acid digestibilities in the BCM diet seemed more additive compared to apparent digestibilities.

A preliminary study was conducted to confirm that a flooding-dose of phenylalanine *per se* has no significant impact on the metabolic status of the pig. This flooding-dose technique was then used to measure protein synthesis rates in visceral organs of growing pigs fed either the CC- or the BCM-diet. The BCM diet increased daily protein synthesis rates in the colon but not in the small intestine. This finding suggests that the increase in ENL observed when feeding the BCM-based diet is due to reduced re-absorption of endogenous nitrogen rather than an increase in the

secretion of endogenous nitrogen into the gut lumen.

A further study was conducted to determine diet effect on organ mass and in vitro oxygen consumption in some visceral organs. In this study, the CC and BCM diets were evaluated as well as a BCM based diet with 30 % added alfalfa meal (BCM-ALF). The results showed that in vitro oxygen consumption per g of tissue is not affected by diet type. The BCM and BCM-ALF diets increased organ mass compared to the CC diet and because of this effect, total energy expenditure in the visceral organs of BCM and BCM-ALF pigs was higher than in the CC-fed pigs.

These findings suggest that increased ENL as a result of feeding a BCM diet compared to a CC based diet, is associated with increased energy expenditure in the hind gut. The latter is largely attributed to diet effects on organ size. It is suggested that further studies be conducted to evaluate relationships between ENL, size and energy expenditure in visceral organs when specific dietary components (e.g. feed enzymes, antinutritional factor etc.) are manipulated.

DEDICATION

To My Wife **Gertrude**
and our children **Elvis** and **Kemunto**

and

My parents
Yoventina Nyakara and **Nyachoti Ogaro.**

ACKNOWLEDGEMENTS

I would like to thank the many people with whose help I have been able to successfully complete this thesis. I thank my advisor, Dr. C. F. M. de Lange for believing in me and for his advise, guidance and encouragement throughout my studies. In particular, I thank Dr. de Lange for his reassuring words during the "trying" interludes that characterised the initial stages of my research project and for getting personally involved in the actual implementation of my experiments. I wish to express my gratitude to members of my advisory committee, Drs. Brian McBride, Steve Leeson and Henry Bayley for their invaluable contribution to my research and for their suggestions on the writing of this thesis. Special thanks to Dr. Mcbride for helping with the oxygen consumption study. I would like to thank Dr. Jim Atkinson for his interest in my progress and for dropping reading materials on my desk from time to time.

I would like to thank Linda Trouten-Radford for her technical assistance and for her willingness to help me whenever I needed her help. I thank Mr. Peter Devries and Doug Wey for their help in the animal wing and in shipping my animals and feed from Arkell to the Department, respectively. Special thanks to my colleagues in Dr. de Lange's lab: Clare Feng, Aubrey Gillis, Sonke Mohn, Erin McNeilage, Julie Blair, Kim Coudenys, Vince Gabert and Manfred Hansel for helping me with surgery, feeding and taking samples. Dr. Gabert, Thomas Volman (the visiting Dutch Student!) and Julie Blair spent a lot of time helping with my analysis in the lab and for that I thank them immensely. Thanks to Margaret

Quinton for her help in statistical analysis.

I would like to acknowledge the financial support I received from the University of Guelph which made it possible for me to start my doctoral studies. I am indebted to the Department of Animal and Poultry Science, University of Guelph, for giving me Teaching Assistantiships which helped me in paying my bills in addition to gaining valuable teaching experience. My research project was supported by research grants to Dr. de Lange from Finnfeeds International and NSERC. My experimental animals were provided by Ontario Ministry of Agriculture, Food and Rural Affairs.

I am deeply indebted to my wife Gertrude and our children Elvis and Kemunto (the little one!) for their love and support and for all the sacrifices they have had to make over the years so that I could work on my thesis. The teachings of my parents, Mr. Nyachoti Ogaro and Mrs. Yoventina N. Nyachoti helped me to deal with all the challenges I faced while working towards completion of this thesis. Thanks to my brothers and sisters for their support and encouragement throughout my studies. A big thank you to our friends in and around Guelph for their friendship and support. In particular, I wish to express my gratitude to Josephine Oreko for being there for us when Kemunto arrived!. Lastly, but certainly not least, I am grateful to God for enabling me to realise yet another goal in my life.

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CHAPTER I

General Introduction

The task of livestock feed formulation basically involves balancing dietary nutrient supply to support the desired production level. For accurate feed formulation, it is important that the availability of nutrients in feed ingredients is estimated. In practise, the availability of nutrients is often not determined mainly because it is expensive and time-consuming to do so. For this reason, nutrient digestibilities, rather than nutrient availabilities, in feed ingredients are routinely used in feed formulation (Sauer and Ozimek, 1986). In practical formulation of swine feeds, apparent ileal amino acid digestibilities are considered to give reasonable estimates of amino acid availabilities (Sauer and Ozimek, 1986).

Amino acids and nitrogen that flows past the end of the small intestine are unavailable for body protein accretion and are subsequently excreted in pig manure (Zebrowska, 1973; Just, 1981; Sauer and Ozimek, 1986). Ileal amino acid digestibilities are determined by collecting digesta from the end of the small intestine and relating amino acid intakes to amino acids recovered in ileal digesta. Digestibilities determined this way are referred to as apparent digestibilities because no differentiation is made between endogenous protein present in ileal digesta (ENL) and non-digested dietary protein. These ENL are likely to interfere with additivity of apparent ileal amino acid digestibilities in mixtures of feed ingredients and are an important contributor to maintenance amino acid and energy requirements. True ileal amino acid digestibilities are corrected for ENL by making a distinction between

undigested dietary and endogenous amino acids that are present in ileal digesta.

Many studies have been carried out in the past two decades to develop methods for quantifying ENL and on animal and dietary factors that influence ENL (de Lange et al., 1989a, b; Marty et al., 1994; Schulze et al., 1994a, b; Jansman et al., 1995). It is now known that ENL are much higher when pigs are fed diets containing practical type ingredients than previously estimated. Endogenous proteins originate from various parts of the gut, but the small intestine makes the largest contribution (Low, 1985). Since gut tissues are metabolically very active, it is conceivable that when pigs are fed diets that induce high ENL, the rates of metabolic processes such as protein synthesis and movement of Na^+ and K^+ across the cell membrane are increased. Both these processes require a large amount of energy and, therefore, any dietary treatment that will accelerate these processes will likely lead to an increase in the maintenance energy requirements of the pig. This will mean that the actual metabolic cost of feeding such diets to pigs is much higher than reflected by the amount of ENL at the terminal ileum. However, the existence of such a relationship has not been established. It is, therefore, important to avail such information so as to understand the actual metabolic cost associated with feeding diets that induce high ENL to growing pigs.

The research program reported herein was undertaken to test the hypothesis that feeding pigs diets that induce high ENL represents a significant metabolic cost to the animal in terms of energy expenditure in addition to the loss of energy contained in ENL. Experimental diets used in the current research were designed to

induce low or high ENL. In the initial stages of the project, the amounts of ENL induced when feeding these diets were quantified using the homoarginine (HA) method. As the HA method has not been extensively used in studies feeding diets based on practical feedstuffs, Experiment 1 (Chapter 3) was set up to establish appropriate conditions for using this method with the type of ingredients chosen for these studies. The amount of ENL induced by the different experimental diets was quantified at the terminal ileum using the HA method in Experiment 2 (Chapter 4). Results of the metabolic studies (gut protein synthesis and energy expenditure) are reported in Chapters 5, 6 and 7. The key findings of Chapters 3 through 7 are discussed in the General Discussion (Chapter 8) which also includes the main conclusion of these studies.

CHAPTER II

Literature Review[1]

2.1 Abstract

During the past two decades endogenous gut nitrogen losses at the distal ileum (ENL) in the growing pig have received considerable attention in swine nutrition research. Estimates of ENL are important for determining true ileal nitrogen and amino acid digestibilities in and for identifying means to improve the efficiency of nitrogen and energy utilization in growing pigs. Endogenous secretions originate from various sources including saliva, pancreatic secretions, bile, sloughed off epithelial cells, serum albumen and mucin. It has been estimated that 70 to 80 % of endogenous nitrogen secretions are digested and re-absorbed. Therefore, ENL represents only a fraction of endogenous nitrogen secreted into the gut. Increased ENL are likely associated with elevated rates of gut protein synthesis. This is bound to increase maintenance energy and amino acid requirements of pigs. Traditionally, ENL were determined by feeding protein-free diets or by the regression method. Various alternative techniques (^{15}N-isotope dilution technique, homoarginine technique, enzymatically hydrolysed casein method) are now available to estimate the ENL in pigs fed protein-containing diets. Each of these has some limitations and all

[1] A version of this chapter has been published: Nyachoti, C.M., C.F.M. de Lange, B.W. McBride, and H. Schulze, 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pis: a review. Can. J. Anim. Sci. 77:149-163.

require different assumptions. Results obtained with these alternative techniques indicate that the net ENL losses are much higher and more variable than previously estimated, and that they are affected both by animal and dietary factors. Recent estimates of ENL losses vary between 1.8 and 8.3 g kg^{-1} dry matter intake. The main factors (feed intake, body weight, content of anti-nutritional factors, fibres and [indigestible] protein) that affect ENL and approaches (plant breeding and selection, ingredient processing and use of exogenous enzymes) to reduce ENL are discussed in this review. In addition, the metabolic costs associated with ENL are estimated.

2.2 Introduction

The nutritional value of feedstuffs for pigs is largely determined by their content of available nutrients, in particular amino acids and other nutrients that supply energy. Availability of nutrients is defined as the portion of ingested nutrients that is absorbed from the digestive tract in a form that renders them available for metabolism by the animal (Sauer and Ozimek 1986). Because it is expensive and time consuming to routinely measure amino acid availabilities, apparent ileal amino acid digestibilities are usually determined and used as reasonable estimates of amino acid availabilities (Sauer and Ozimek 1986). It should be noted, however, that for certain feedstuffs (like those that have been heat treated) apparent ileal digestibilities may underestimate availabilities because amino acids may react with other constituents present in the feedstuff to form complexes that are absorbed in a form that renders them unavailable to the animal (Hurrell and Finot, 1985). In general, it is accepted

that in swine feed ingredients, ileal amino acid digestibilities provide better estimate of amino acid availabilities compared with fecal digestibilities (Sauer and Ozimek 1986; Moughan 1995). Amino acids that disappear from the hind gut are not available to the animal (Just et al. 1981). Furthermore, fecal digestibilities are confounded by the modifying and apparent equalizing effect of the microflora in the hind gut on amino acids recovered in faeces (Sauer and Ozimek 1986).

In conventional digestibility studies, it is not possible to distinguish between non-digested dietary amino acids and non-reabsorbed endogenous amino acids in ileal digesta or in faeces. The obtained digestibility values are therefore referred to as apparent digestibilities. True digestibilities can only be estimated when the recovery of endogenous amino acids in ileal digesta is determined. Recent studies (e.g. de Lange 1990; Marty et al. 1994; Schulze et al. 1994b; Jansman et al. 1995) have suggested that endogenous gut amino acid losses were previously underestimated. These studies indicate that observed differences in apparent ileal amino acid digestibilities between feed ingredients can largely be attributed to differences in the amount of ENL recovered at the distal ileum, rather than to differences in true ileal amino acid digestibilities. Given that there will be metabolic inefficiencies associated with ENL, the actual amino acid and energy losses to the animal will be larger than what is measured in ENL recovered at the end of the small intestine or in faeces.

This chapter provides a review of the techniques for estimating ENL and a discussion of the factors that affect ENL. Estimates of the metabolic cost associated with the loss of ENL and the means of reducing ENL are also discussed.

2.3 Determining apparent ileal digestibilities: methods for collecting ileal digesta from the terminal ileum in pigs

In order to determine apparent ileal digestibilities, digesta need to be sampled at the end of the small intestine. Various sampling methods have been used, including use of surgically modified (cannulated or anastomosed) animals or the slaughter of intact animals. The general principles of these procedures including their advantages and disadvantages are discussed below.

2.3.1 Cannulation techniques

Several cannulation procedures are used to facilitate collection of ileal digesta. These could be classified into three general categories, namely: re-entrant canulas, simple T-canulas, and postvalve-T-caecum canulas.

2.3.1.1 *Re-entrant canulas*

The use of the re-entrant canula technique in pigs was first suggested by Cummingham et al. (1962), but since then many variants including the ileo-ileo, ileo-caecal and ileo-colic post valve re-entrant canulas have been developed to try and overcome the problems of the initial procedure (Easter and Tanksley, 1973). The major problems with the initial re-entrant cannulation procedure are the complete transection of the small intestine which disrupts its normal functioning and the high incidence of blockage particularly with normally ground feeds (Fuller, 1991; Sauer and de Lange, 1992). The incidence of blockage is increased at high feed intakes,

increased dietary crude fibre and factors that increase digesta viscosity. Infusion of a physiological salt solution at the proximal part of the ileo-caecal re-entrant canula to make the digesta thinner so that it flows smoothly has been suggested as a possible solution to blockage problems (van Leeuwen et al., 1987), although the disadvantages are that this is more laborious and increases the need to restrain the pigs while infusing the salt solution. The ileo-colic post-valve cannulation technique, developed by Darcy-Vrillon et al. (1980), maintains the integrity of the small intestine and preserves the functional role of the ileocecal sphincter thus maintaining a more physiological state. It also allows collection of digesta as it arrives normally at the colon. However, this technique is quite laborious as well; digesta are collected continuously and need to be returned to the hindgut at regular intervals (Darcy-Vrillon and Laplace, 1990). Re-entrant cannulation has advantages in that it allows for quantitative collection and representative sampling of digesta and it does not rely on the use of digestibility markers.

2.3.1.2 *T-canulas*

The simple T-canula technique uses a T-shaped canula that is placed at the distal ileum about 5-10 cm anterior to the ileocecal valve without transecting the small intestine (Sauer and de Lange, 1992). This allows maintenance of a fairly normal physiological state since the migrating myoelectric complexes necessary for normal digesta passage are able to continue (Fuller, 1991). During collection, only a fraction of the digesta that pass by the T-cannula are collected. Therefore, this

technique relies on the use of digestibility markers and natural forces to drive digesta from the intestinal lumen through the cannula. This may pose problems regarding the limitations of using markers (e.g. the need for uniform mixing of markers in the diet and in digesta, analytical problems, uncertainties concerning the marker's absorbability) and obtaining representative samples (Udén et al., 1980; Fuller, 1991). Other concerns of using the T-cannula technique include the internal diameter of the canula, flow of digesta from diets of different composition and particle size and the amount of digesta collected to give a representative sample (Sauer and de Lange, 1992). If the T-canula is used for a long time, it may be outgrown by the animal. However, the T-canula technique is the most commonly used procedure partly because it involves less invasive surgery as compared to re-entrant canulas and it compares favourably with the use of intact animals (Livingstone and McWilliam, 1985; Moughan and Smith, 1987).

2.3.1.3 *Post-valvula T-caecum canulas*

The postvalve T-caecum canula (PVTC) technique involves removal of the entire caecum with the exception of the area surrounding the ileocecal sphincter which is prepared for placement of a T-canula. This method first suggested by van Leeuwen et al. (1988), permits quantitative collection of ileal digesta. Once the canula is opened (i.e. the plug is removed from the barrel of the cannula), the ileo-caecal sphincter moves into the barrel of the cannula allowing a free flow of digesta passing through the ileo-caecal valve to the collection tube attached to the external part of

the cannula. It also allows for determination of ileal digestibility of coarser diets and those containing high fibre levels. Furthermore, PVTC method involves a simple surgical procedure and causes less discomfort and minor negative physiological effects (van Leeuwen et al., 1988). The major concern with this method relates to the possible physiological effects of caecectomy on ileal digestibility and the fact that its superiority over the T-canula has not been established (Moughan, 1995). A modified version of this technique has been proposed by Mroz et al. (1996). Rather than removing nearly all of the caecum, these researchers suggest that a ring be placed around the ileo-caecal sphincter and that this ring is used to steer the ileo-caecal sphincter towards the caecal cannula when digesta are collected.

2.3.2 Ileo-rectal anastomosis

As a means of avoiding problems with canulas, ileo-rectal anastomosis (IRA) technique has been suggested by Fuller and Livingstone (1982). The small intestine is completely transected either at the terminal ileum or just after the ileo-caecal sphincter and then joined to the rectum such that ileal digesta is easily collected from the anus. A modified version of this technique in which a T-canula is placed in the colon to evacuate fermentation gases, has been suggested by Picard et al. (1984). Pigs with IRA are relatively easy to maintain and can be fed diets of any texture (Sauer and de Lange, 1992). However, like as with most surgical procedures, the impact of IRA on the physiology and nutrition of the animal may pose some concerns. This is particularly so with long-term protocols, as the functional role of the small intestine

is changed to make up for the missing colon. This may alter the amino acid composition of the chyme and observed digestibilities compared with that obtained when using other methods (Fuller, 1991). Anastomosed animals suffer considerable discomfort due to the continuous outpouring of digesta and this is likely unacceptable from the animal welfare standpoint.

2.3.3 Slaughter technique

As the term implies, this techniques involves slaughter of experimental animals so as to be able to collect digesta from the small intestine. The animals are killed at a certain time interval (usually 6-9 h) after feeding and digesta recovered from the last 20-40 cm of the small intestine. The major criticisms of this method concerns the fact that animals cannot be used as their own control since only one measurement can be obtained per animal and the possible difficulties of obtaining representative digesta samples. In particular, digesta can only be sampled at one instant in time when the slaughter technique is used. As a result, any (potential) diurnal variation in nutrient digestibility due to feeding schedules, and interactions between feeding schedules and diet compositions, is generally not considered when this method is used. However, in direct comparisons no differences were observed between digestibilities obtained using slaughter technique and from pigs fitted with simple T-cannulas (Moughan and Smith, 1987; Donkoh et al., 1994). The biggest advantage of this technique is that minimum disruption of the digestive function occurs since no manipulation of the digestive tract is involved and it is possible to collect digesta from

several parts of the gut. The slaughter method, unlike cannulation techniques, posses no problems with the texture of the feed thus allowing practical diets to be tested. It takes a shorter time to complete an experiment and therefore less labour input and requires no special facilities such as metabolic crates.

2.4 Endogenous Gut Nitrogen Losses

Endogenous gut nitrogen losses (ENL) are classically defined as the nitrogen found in digesta or in faeces of animals fed nitrogen-free diets (Mitchell et al., 1924). ENL mainly consists of nitrogen from enzymes, mucoproteins, desquamated cells, serum albumin, peptides, amino acids, amides and amines (Moughan et al., 1992). ENL may also contain bacteria and swallowed body hair which, strictly speaking, are not endogenous sources of nitrogen. The contribution of the latter two sources is often neglected although there is evidence suggesting that bacteria present in the ileum may affect amino acid requirements of the pig (Torrallardona et al., 1994). Dierick et al. (1986a, b) reported that ileal flora degrade substantial amounts of amino acids to form ammonia and amines and that 25-30 % of the total nitrogen in the ileum is of bacterial origin. Schulze et al. (1994b), in a study on the effect of dietary fibre level on endogenous nitrogen flow, calculated ileal bacterial nitrogen secretion to constitute more than 50 % of the total nitrogen at the distal ileum. Based on these findings and those of Rowan et al. (1992), it is apparent that bacterial contribution to endogenous nitrogen may be significant, and therefore it should be quantified and its role in whole-animal amino acid metabolism studied further.

2.4.1 *Sources of endogenous N losses*

Endogenous nitrogen enters the gastrointestinal tract at various regions. Of all these regions. The small intestine, followed by the pancreas and salivary glands, makes the largest contribution to the total amount of ENL [(Auclair (1986), cited by Souffrant (1991)]. Earlier data compiled by Low (1985; Table 2.1), although obtained under different experimental conditions, support these observations. However, as noted by Souffrant (1991) in a review of a wide range of literature, estimates of the total amount of nitrogen entering the gut varies considerably, between 16 to 33 g d^{-1} in the growing pig. Recently, Souffrant et al. (1993) calculated the endogenous nitrogen contribution from bile, pancreas, and other tissues of the gut to be 1.7, 1.9 and 7.1 g d^{-1} respectively.

2.4.2 *Recycling of endogenous N in the gut*

Endogenously secreted nitrogen is subjected to digestion, along with dietary nitrogen and their products reabsorbed (Fuller, 1991). However, the extent to which reabsorption occurs has not been clearly established; it has been estimated at 70 - 80 % before the terminal ileum (Souffrant et al., 1986, 1993; Krawielitzki et al., 1994). This indicates that only about 20-30 % of the total endogenous gut nitrogen secretions are of concern when determining ENL and true ileal amino acid digestibilities.

Free amino acids and peptides are readily reabsorbed and therefore make up only a small part of ENL at the terminal ileum (Moughan and Schuttert, 1991). The

Table 2.1. The amount (g d^{-1}) of endogenous N entering the gut calculated from various studies by Low (1985)[a]

Source	Amount	% of Total
Saliva/gastric juice	1.1	6.1
Bile	2.0	11.1
Pancreas	2.3	12.7
Small intestine	9.7	53.6
Large intestine	3.0	16.6
Total	18.1	100

[a] for a 40 kg pig consuming a barley-fishmeal or barley-soya diet at 1.7 kg d^{-1} intake level. Different surgical methods were used in the original studies.

bulk of endogenous nitrogen recovered at the distal ileum is composed of cell and mucin protein because mucin proteins are resistant to enzymatic hydrolysis (Taverner et al., 1981; Moughan and Schuttert, 1991). Consequently, proline and glycine, the main amino acids in mucin proteins, are the main contributors to endogenous nitrogen recovered at the distal ileum. Wünsche et al. (1987) [cited by Souffrant (1991)] found 65 % of endogenous nitrogen to be composed of non-essential amino acids; proline and glycine represented 25 and 12 % of the total amounts of recovered amino acids, respectively. Earlier, Sauer et al. (1977) reported slightly higher values: non-essential amino acids accounted for 78 % of total amino acids, proline and glycine represented 43 and 13 % of total amino acids, respectively.

2.4.3 *Metabolic cost of endogenous N losses*

As noted previously, ENL have recently received considerable attention because of their confounding effects on observed N and amino acid digestibilities. In addition, they contribute to maintenance amino acid and energy requirements of the pig, and possibly to the inefficiency in utilizing dietary amino acids for body protein retention (Schulze, 1994; de Lange et al., 1995; Moughan, 1995). In fact, it has been estimated that ENL are the single most important factor contributing to maintenance N requirements (de Lange et al., 1995). This also implies that the contribution of pigs to environmental pollution can be minimised by reducing ENL.

In growing animals, a fraction of amino acids that are supplied in excess of maintenance requirements is catabolized. This amino acid catabolism even applies

to amino acids that limit the rate of body protein deposition, and contributes to the inefficiency of using dietary amino acids for body protein deposition (e.g. de Lange the al., 1995; Moughan, 1995). This inefficient utilization of amino acids is referred to as inevitable amino acid catabolism (Moughan, 1995). Estimates of inevitable amino acid catabolism vary largely between .05 and .40 of available amino acid intake (de Lange et al., 1995; Moughan, 1995). Inevitable amino acid catabolism may vary with the level of amino acid intake, dietary amino acid balance and may differ between individual amino acids (de Lange et al., 1995; Moughan, 1995). If this inevitable amino acid catabolism applies to the production of endogenous gut protein and ENL, then the actual amino acid losses to the animal are in fact larger than the losses observed at the end of the small intestine (Jansman et al., 1995; Sève and Henry, 1995). In that case, part of the urinary nitrogen losses can be attributed to the inefficiencies in synthesis of gut protein and ENL. However, the enzymes required for the initial and irreversible steps of amino acid catabolism, and in particular those for the important essential amino acids, are largely present in the liver (Armentano, 1994; Simpson et al., 1998; Volman et al., 1998). In addition, at least part of the amino acids used for the synthesis of endogenous gut protein can be derived directly from intestinal lumen without passing through the liver (Hirschfield and Kern, 1969; Alpers, 1972). This suggests that inevitable amino acid catabolism only applies to a fraction of amino acids used for the synthesis of endogenous gut protein and ENL. On the other hand, inevitable amino acid catabolism may be more significant for body proteins with high turn over rates, such as those in the visceral organ. This

inevitable catabolism, or the inefficiency in the use of amino acids for endogenous gut protein synthesis and associated ENL remains to be adequately quantified (Grala et al., 1997; Sève and Leterme, 1997).

Although the gut represents a proportionally small part of the whole animal body, it is reported to account for a significant amount of the total body energy expenditure (Gill et al., 1989; Yen et al., 1989; Huntington, 1990; Yen, 1997). As a percentage of total body oxygen consumption, the gut estimated to account for 22-47 % in sheep (Kelly et al., 1993), 24.8 % in 3.5-4 months old pigs (Yen et al., 1989) and 12 % in rats (Foster and Frydman, 1978). Farrell (1988) also showed that in sheep total viscera and nervous tissue account for 58.5 % of oxygen consumption although they represent only 11 % of body weight. This phenomenon is attributable to two major energy requiring processes occurring in the gut: activities of the Na^+, K^+-ATPase enzyme and protein turnover (Lobley, 1988; Kelly and McBride, 1989; McBride and Kelly, 1990). Kelly et al. (1993) estimated Na^+ K^+ ATPase enzyme and protein synthesis activities to account for 15.2-41.4 % and 26.5-31 % as a portion of visceral oxygen consumption, respectively. Literature does not provide estimates of energy expenditure by the gut when feeds of different composition are fed to pigs. However, results of a study with beef heifers (Reynolds et al., 1991) indicated that oxygen consumption by the portal drained viscera (PDV) differed when alfalfa- or concentrate-based diets were fed. This observation was attributed to increased dry matter intake and blood flow across the PDV when alfalfa was fed.

Increased production of endogenous gut nitrogen, due to any of the dietary

factors outlined previously, will imply increased gut protein turnover, increased transport across the gut of digestion products and increased rate of blood flow through the gut tissues, all of which are important energy demanding processes (Lobley et al., 1980; Reeds et al., 1985; Black and de Lange, 1995). This in turn is bound to increase both maintenance energy and amino acid requirements of the growing pig and affect its ultimate performance (Moughan, 1989; Reynolds et al., 1991; Kelly et al., 1993). Although these relationships have not been clearly established and/or quantified, Table 2.2 does provide some evidence of its practical significance. The daily maintenance ME requirements for a 13 kg pig is about 3150 kJ d^{-1} [460 kJ $kg^{-.75}$ body weight; Agricultural Research Council (1981)]. The energetic cost of protein synthesis is estimated at 4.5 kJ g^{-1} (Webster, 1981). Furthermore, it is assumed that the rate of endogenous protein synthesis is similar to the rate of endogenous protein secretion and that the rate of endogenous secretion is 4 g g^{-1} of ENL at the end of the small intestine (75 % of the endogenous protein secreted into the gut is reabsorbed; section 2.4.2). It can then be estimated that the energy requirements for endogenous gut protein synthesis account for up to 13 % of total maintenance energy requirements or for approximately 4 % of total energy expenditure in ad libitum fed growing pigs. The values in Table 2.2, however, are an underestimation of the actual energy cost associated with ENL as it does not account for the energy required for other processes that are related to protein synthesis and for differences between the rates of endogenous protein synthesis and secretion.

Table 2.2. Estimated energy cost for synthesising endogenous gut proteins in a 13 kg pig fed a corn starch-based semi-synthetic diet supplemented with graded levels of trypsin inhibitors[a]

Total synthesis (g d^{-1})	Energy cost of synthesis (kJ d^{-1})	% of maintenance energy requirement[b]
38.6	174	5.5
65.2	293	9.3
91.9	414	13.1

[a] calculated using endogenous protein loss data derived from Schulze 1994 and assuming 4 g of endogenous protein synthesis g^{-1} of endogenous protein recovered at the small intestine.

[b] animals were kept in individual metabolism crates (1.2-M x 1.2-M) and a room temperature and relative humidity were 23-26 °C and 50-70 % respectively.

Availing this information will enhance our understanding of the true consequences of ENL to the growing pig and will result in an identification of means to improve the energetic efficiency of meat production (McBride and Kelly, 1990).

2.4.4 *Methods for estimating endogenous N losses*

Several methods have been used to quantify endogenous nitrogen flow at the distal ileum of the pig. Earlier studies made use of conventional methods. These include feeding protein-free diets, feeding diets containing protein sources with an assumed 100 % digestibility, and mathematical regression techniques. More recently, isotope dilution, homoarginine and peptide alimentation ultrafiltration techniques have been used.

2.4.4.1 *Conventional methods: feeding protein-free diets, feeding diets containing proteins with an assumed 100 % digestibility, and mathematical regression methods*

When protein-free diets are fed, all nitrogen-containing compounds found in the ileal digesta are assumed to be of endogenous origin. The main criticism of this method is its non-physiological nature (Low, 1980) that may affect the normal body protein metabolism (Millward et al., 1976) and in turn may reduce secretion of nitrogenous compounds into the gut lumen and affect the efficiency of re-absorption (Darragh et al., 1990). The fact that animals are in negative protein balance appears to have minor effects on endogenous loss of essential amino acids at the distal ileum of the pig (de Lange et al. 1989b). In this study, only the recovery of endogenous

proline, glycine and total N were affected (reduced) when amino acids rather than saline (control) were administered intravenously to animals fed a protein-free diet. As indicated by Butts et al. (1993a) and Donkoh et al. (1995), a protein-free diet may lack the stimulatory effect on endogenous gut protein secretions. This may lead to an underestimation of ENL at distal ileum and in feces. In addition, dietary constituents, such as fibres and other antinutritional factors, that are associated with dietary proteins, may enhance ENL.

Feeding a diet containing a protein source with an assumed 100 % digestibility may offer a desirable alternative to using a protein-free diet. Leibholz (1982) found similar values for ileal ENL when either a casein-containing diet or a protein-free diet was fed to growing pigs (3.4 vs 3.0 g kg^{-1} DMI, respectively). Fuller and Cadenhead (1991), on the other hand, observed lower ileal ENL in pigs fed a protein-free diet with added casein and crystalline amino acids as compared to pigs fed just the protein-free diet (4.3 vs 5.8 g d^{-1}, respectively). The latter observation is consistent with those made by de Lange et al. (1989b) and suggests that pigs that are in a positive protein balance will have slightly lower ileal ENL than pigs that are in a negative protein balance. This also implies that the recovery of total N at the distal ileum in pigs fed casein and/or crystalline amino acid based diets can be lower than in pigs fed protein-free diets even if the true ileal digestibility of casein and crystalline amino acids is less than 100 %. The validity of this method should thus be questioned until it is proven that the true digestibilities of casein and synthetic amino acid are indeed 100 %.

When regression methods are used, animals are fed graded levels of protein in the diet and the recovery of N and amino acids at the distal ileum is related to N and amino acid intake. Via mathematical extrapolation the recovery of N and amino acid nitrogen at 0 N and amino acid intake can be estimated. The regression method is believed to provide better estimates of ENL as compared to feeding a protein-free diet (Fan et al., 1995). In addition, it allows for evaluation of effects of dietary proteins of various quality on ENL (Souffrant, 1991; Fan et al., 1995). However, various studies have shown that its estimates are not different from those obtained with protein-free diets (e.g. Taverner et al., 1981; Furuya and Kaji, 1989; Donkoh et al., 1995). Furthermore, a basic assumption with the regression, as well as with feeding protein-free diets, is that there is no relationship between protein intake and ENL. Based on various recent studies, this basic assumption appears not to be valid; both methods results in an underestimation of ENL (see Sections 2.4.4.2 to 2.4.4.5). Estimates of ENL as observed with both of these methods should therefore be referred to as the minimum ENL and endogenous amino acid losses.

For feed formulation purposes, an estimation of these minimum ENL and minimum endogenous gut amino acid losses may be meaningful. Apparent ileal amino acid and N digestibilities that are corrected for the minimum gut losses (referred to as standardized ileal amino acid digestibilities and sometimes, incorrectly, as true ileal amino acid digestibilities) are no longer related to the amino acid or N level in the test diet. These corrected digestibilities are likely to be more additive between feed ingredients than apparent digestibilities (Mariscal-Landín, 1992; Fan et al., 1995).

2.4.4.2 *Isotope dilution technique*

Isotope dilution techniques using ^{15}N have been used to label either the animal nitrogen pool (de Lange et al., 1990; Huisman et al., 1992; Schulze et al., 1994) or the dietary nitrogen (Leterme et al., 1994b; Roos et al., 1994) and allow a differentiation between endogenous N and non-digested dietary N. Labelling the animal's nitrogen pool is commonly used. This basically involves a continuous infusion of labelled amino acids and then measuring the amount of labelled amino acids present in the ileal digesta relative to that in the precursor pool for gut protein synthesis. The deproteinized fraction of blood plasma is generally considered to be the precursor pool. This technique is frequently criticised because of the difficulties of achieving two of its basic requirements; namely attainment of a steady state and choosing the right precursor pool (Moughan et al., 1992). The amount of endogenous nitrogen loss may be underestimated by this method because it does not account for endogenously added mucosal cells that are synthesized from labelled luminal dietary amino acids and then secreted (de Lange et al., 1992; Roos et al., 1994). Another point of critique on this technique is that not all N containing compounds in the precursor pool(s) and in endogenous gut N are uniformly labelled when labelled amino acids are infused intravenously (de Lange et al., 1992; Lien et al., 1993). The contribution of the various N-containing compounds (individual amino acids, urea) to total N differs substantially between the precursor pool(s) and endogenous gut proteins. In addition, these relative contributions can vary with factors such as the dietary amino acid balance and amino acid supply relative to requirements. It is,

therefore, very unlikely that the ^{15}N isotope dilution technique gives reliable estimates of ENL when various diets are fed; it will only give reliable estimates if all sources of endogenous gut N, including N in the various precursor pools, are uniformly labelled (de Lange et al., 1990, 1992; Moughan et al., 1992). ^{15}N-labelled substances are often very expensive and this may limit the usefulness of this method. The ^{15}N-labelling of dietary protein poses little concerns as far as uniformity of labelling is concerned; when ^{15}N derived from fertilizer is incorporated into plant proteins, the label is (uniformly) distributed over all amino acids (e.g. Leterme et al., 1994a). However, ^{15}N supplied by dietary amino acids can be quickly be absorbed and incorporated into body protein thus complicating the differentiation of non-digested nitrogen and ENL (Leterme et al., 1994a; Roos et al., 1994; Tamminga et al., 1995).

2.4.4.3 *Homoarginine technique*

This is a relatively new technique involving transformation of dietary lysine to homoarginine (HA) through a guanidination process (a reaction with O-methylisourea). This technique was first suggested by Hagemeister and Erbersdobler (1985) and allows a differentiation between dietary lysine (converted to HA) and endogenous gut lysine. Absorbed HA may be hydrolysed by arginase, which is present in the liver, to lysine and urea. Prerequisites for an effective use of this method include: 1) uniform, or almost complete, guanidination of lysine in test protein, 2) guanidination does not affect protein digestibility, 3) HA is not hydrolysed to urea and lysine in the gut, 4) HA is absorbed and metabolized like amino acids, 5) HA

does not re-enter the digestive tract, i.e. it is not used for protein synthesis, and 6) the presence of HA in the plasma or in the gut, *per se*, does not affect endogenous nitrogen loss (Drescher et al., 1994; Marty et al., 1994).

The suitability of this method for direct estimation of endogenous lysine flow and true lysine digestibilities has been demonstrated in a number of studies (Rutherfurd and Moughan, 1990; Schmitz et al., 1991; Marty et al., 1994). However, since homoarginine is not used for protein synthesis, this technique may only be used for a short time to avoid lysine deficiency. Tews et al. (1986) in a study with rats have shown that addition of an amino acid analogue (e.g. homoarginine) to a diet that is limiting in a natural amino acid (e.g. lysine) can induce an amino acid imbalance. However, if HA is rapidly hydrolysed by arginase in the liver to lysine and urea and HA serves as an effective source of lysine to the animal, then this concern is unjustified. The fact that optimum guanidination conditions may vary for individual protein and that near complete guanidination is not always achieved may impose some limitations on this method (Maga, 1981; Rutherfurd and Moughan, 1990; de Vrese et al., 1994). So far this method has largely been used with diets having just one, purified protein source. A key requirement, however, for any method is that it has to be applied to a wide variety of feedstuffs. It needs to be determined whether this method can be equally employed to measure endogenous nitrogen flow when feeding practical diet containing more than one protein source, and in particular where plant proteins are being evaluated. With this method, the true digestibility of amino acids other than lysine are not directly determined. Instead they are estimated

based on an assumed relationship between lysine and other amino acids in endogenous nitrogen (de Lange et al., 1990; Marty et al., 1994; Boisen and Moughan, 1996).

2.4.4.4 *Peptide alimentation ultrafiltration method*

In this method (also referred to as enzymatically hydrolysed casein, EHC), first suggested by Moughan et al. (1990), animals are fed a diet in which the sole nitrogen source is enzymatically hydrolysed casein. Hydrolysed casein contains approximately 56 % free amino acids and 41 % di- and tri-peptides (Leterme et al., 1994b). It is assumed that all endogenous nitrogen is present in fractions with molecular weight greater that 10, 000 Da. Therefore, by separating low (<5,000 Da) and high (>10,000 Da) molecular weight nitrogen compounds in the ileal digesta a distinction is made between any unabsorbed exogenous nitrogen and nitrogen of endogenous origin. In order to achieve this separation, protein precipitating agents like perchloric acid and trichloroacetic acid have been tested but found to be unsuitable (Darragh et al., 1990; Moughan et al., 1990). As an alternative, Moughan et al. (1990) suggested separation of protein in ileal digesta immediately after collection by centrifugation and ultrafiltration in which low molecular fractions (amino acids and peptides) are removed in the supernatant. Any free amino acids and small peptides of endogenous origin present in ileal digesta are discarded as well. However, according to Moughan and Schuttert (1991) free and peptide-bound are only a small proportion (11 %) of ENL. This suggests that any endogenous nitrogen lost in the supernatant is likely to

result in slight underestimation of ENL. However, the exact proportion of these components need to be clearly determined to enhance the reliability of this method. Butts et al. (1992) and Marty et al. (1994) reported values of 21 and 9.2 %, respectively, suggesting the presence of considerable variation in the contribution of free and peptide-bound amino acids to ENL.

Loss of material during sample preparation (centrifugation and ultrafiltration) may also lead to lower estimates with this method (Butts et al., 1993a). A major restriction of the peptide alimentation ultrafiltration method is the fact it cannot be used to estimate endogenous protein secretion where there is a direct association between protein and dietary factors that are known to influence endogenous secretion and ENL (e.g. in diets containing practical ingredients and/or high levels of ANFs). Donkoh et al. (1995) indicated that this method could be applied to feeds containing animal protein sources such as meat and bone meal.

2.4.4.5 *Comparison of techniques for measuring endogenous N losses*

No study has evaluated the above five techniques all together probably because of the considerable amount of resources this will require. Moreover, most studies comparing some of these techniques have used rats as models for pigs. In Table 2.3, some values on digestibility and endogenous flow of protein and amino acids obtained with different methods within studies are summarised. It is evident from these data that observed ENL and endogenous amino acid losses differ for the various methods. Feeding protein-free diets and the regression method give similar

Table 2.3. A comparison of amounts (mg kg^{-1}) of endogenous nitrogen and lysine estimated using different techniques and within studies

	Endogenous losses		
Method	N	Lys	Reference
^{15}N-isotope dil	3728	-	Roos et al. (1994)
Homoarginine	4712	-	
Protein-free diet	3668	354	Mariscal-Landin et al. (1995)
Regression	2856	382	
EHC	1866	275	Donkoh et al. (1995)
Protein-free diet	1103	172	
Regression	1019	168	
Protein-free diet	1500	252	Butts et al. (1993a)
EHC	3700	448	
^{15}N-isotope dil	3640	-	Schulze et al. (1995a)
EHC	3120	-	

values. The study of Roos et al. (1994) indicates that the homoarginine technique results in higher ENL in comparison to the ^{15}N isotope technique in which the dietary protein, rather than the animal protein, was labelled. This observation is attributed to the fact that with the ^{15}N method some of the label is absorbed, incorporated into fast turning over gut proteins, and re-secreted into the gut. The EHC method gives higher estimates of ENL as compared to both the protein-free diets and the regression method. It appears that the EHC method induces high ENL compared to feeding a diet with assumed 100% protein digestibility. This raises the question whether feeding small peptides is more representative than feeding intact proteins to determine the effects of specific dietary components (e.g. fibres, ANFs etc) on ENL. In a direct comparison, the results obtained with the EHC method were similar to those obtained with the ^{15}N-isotope dilution technique (Schulze et al., 1995a).

The results of studies summarised in Table 2.3 and other studies (e.g. de Lange et al., 1990; Makkink and Heinz, 1991; Souffrant, 1991; Huisman et al., 1992; Leterme et al., 1994b; Marty et al., 1994; Jansman et al., 1995; Schulze et al., 1995) suggest that conventional methods result in an underestimation of ENL in pigs fed protein containing diets. Moreover, results obtained with the ^{15}N-isotope dilution technique (where the animal protein, rather than the diet protein, is labelled), the HA technique and the EHC method agree reasonably well when comparable diets are fed. This is in spite of the fact that these techniques are distinctly different and require different assumptions for estimating ENL. Finally, these results show that observed ENL are highly variable. This would imply that there should be

opportunities to reduce ENL and to improve the overall efficiency of using dietary energy and amino acids for meat production (see Section 2.4.3)

2.4.5 *Dietary causes of endogenous N secretions*

Secretion and/or re-absorption of endogenous nitrogen is influenced by many factors, including body weight, diet protein quality and content, diet fibre content, dry matter intake and the presence of anti-nutritive factors (ANFs). Table 2.4 gives some selected values reported on the amount of ileal endogenous amino acids and nitrogen flow as affected by some of these factors.

2.4.5.1 *Dry matter intake and body weight*

Literature contains contradicting evidence on how dry matter intake (DMI) influences the recovery of endogenous amino acids and nitrogen at the terminal ileum. For instance, Butts et al. (1993b) measured significant increases from 14.52 to 32.59 and 2.77 to 5.9 g d^{-1} for ileal amino acids and nitrogen when DMI was increased from 0.9 to 2.17 kg d^{-1}, respectively. On the contrary, Furuya and Kaji (1992) observed no such effects when DMI was increased by almost a similar magnitude. Part of these differences can be attributed to differences in methodology and/or diet composition; Butts et al. (1993b) used the EHC method while Furuya and Kaji (1992) fed protein-free diets (see Sections 2.4.4.1, 2.4.4.4 and 2.4.4.5). It is suggested that the results from Butts et al. (1993b) are more reliable as these were obtained under more physiological conditions.

Table 2.4. The effect of various factors on ileal endogenous amino acids (only those measured in all studies) and nitrogen flow

FACTOR	AA	N	Units	BWT (kg)	CT[a]	METHOD[b]	REF[c]
DMI kg/d							
0.9	13.44	2.77	g d^{-1}	50	T-C	EHC	1
2.17	30.52	5.90					
0.8	14.31	3.13	g d^{-1}	49	T-C	PF	2
1.2	14.30	3.34					
1.16	13.68	3.01					
Fibre g/kg							
Cellulose							
30	5.62	1.36	g kg^{-1}DMI	64	IRA	PF	3
120	5.79	1.48					
Cellulose							
60	4.52	1.14	g kg^{-1}DMI	22	IRA	PF	4
90	6.08	1.58					
Pectin							
0	15.26	3.17	g kg^{-1}DMI	60	T-C	PF	5
40	18.80	3.84					
Trypsin inhibitors							
9.5	-	3.54	g kg^{-1}DMI	36	T-C	HA	6[d]
14.9	-	5.82					
0.21	-	1.55	g d^{-1}	13	PVTC	^{15}N	7[e]
2.49	-	2.61					
5.77	-	3.68					
CP (+/-)[f]	6.93	-	g kg^{-1}DMI	15	ST	EHC	8
-	13.55	-					
+							
CP quality			g d^{-1}				
skim milk	-	0.79		8	PVTC	^{15}N	9
soyabean meal	-	1.42					

[a] Technique used to collect ileal digesta: T-C = simple T-canula; IRA = ileorectal anastomosis; PVTC = post valve T-canula; ST = slaughter technique.

[b] method used to estimate endogenous losses: EHC = enzymatically hydrolysed casein; PF = protein-free diet; HA = homoarginine labelling; ^{15}N = ^{15}N-isotope dilution.

[c] Refernces: 1, Butts et al. (1993b); 2, Furuya and Kaji (1992); 3, Leterme et al. (1992); 4, Green et al. (1987); 5, de Lange et al. (1989a); 6, Marty et al. (1994); 7, Schulze (1994); 8, Butts et al. (1993a); 9, Makking and Heinx (1991).

[d] Trypsin inhibitors expressed as trypsin inhibitor units mg^{-1} of crude protein.

[e] Trypsin inhibitors expressed as mg of Trypsin inhibitor activity g^{-1} of air-dry material;

[f] With (+) or without (-) protein in the diet

Apparently the effect of DMI on ENL is closely related to the animal's body weight. In a recent study by Mariscal-Landín et al. (1995) ENL was significantly higher in 48-kg pigs consuming about 60 g DM kg^{-1} metabolic body weight ($kg^{-.75}$) as compared to 39-kg pigs consuming approximately 70 g DM kg^{-1} metabolic body weight ($kg^{-.75}$): 2.25 vs 1.83 g N kg^{-1} DMI. According to Furuya and Kaji (1992) DMI has no effect on ENL in 49- or 92-kg pigs fed at low intake levels. ENL was, however, significantly increased from 3.6 g to 7.3 g d^{-1} when the feed allowance for 80-kg pigs was increased from 1 to 3 kg d^{-1} (Fuller and Cadenhead, 1991). These data imply that at low feed intake levels, ENL is related more to animal size than DMI *per se*; at high feed intake levels ENL is more closely related to DMI. In the studies reported by Fuller and Cadenhead (1991), Furuya and Kaji (1992), and Mariscal-Landín et al. (1995), ENL was estimated by feeding protein-free diets or by using the regression method. Therefore, some care should be taken in the interpretation of these results. A final point is that more information is required to determine whether expressing ENL on the basis of variables other than feed intake (e.g. per unit of intake of indigestible materials, water-holding capacity of fibre, etc.), better explains variation in the recovery of ENL at the distal ileum in pigs fed different diets. However, it appears that the response to the intake of dry matter (and possibly other dietary factors) depends on the level of protein in the diet [e.g. Butts et al. (1993b) vs Furuya and Kaji (1992); Table 2.4].

2.4.5.2 *Anti-nutritive factors*

ANFs that are naturally present in feedstuffs have been reported to increase ileal endogenous nitrogen flow in pigs. Of the common ANFs, trypsin inhibitors (Barth et al., 1993; Marty et al., 1994; Schulze, 1994), lectins (Schulze, 1994) and tannins (Cousins et al., 1981; Mitaru et al., 1984; Jansman et al., 1995) have been shown to elevate the amount of endogenous amino acids and nitrogen passing the terminal ileum substantially. Tannins are known to interact with both dietary and endogenous nitrogen in the digestive tract and cause decreased true amino acid digestibilities as well (Jansman, 1993). ANFs increase the amount of ENL either by enhancing endogenous secretions and/or by decreasing degradation and re-absorption of endogenous nitrogen. It has not been clearly established as to which of these two is more important. Results of Barth et al. (1993) and Huisman et al. (1992) suggest that ANF such as trypsin inhibitors may be enhancing endogenous secretions rather than impairing digestion of dietary protein and amino acids. Consumption of 3000 mg of trypsin inhibitor in a casein-based diet caused a 7.8-fold rise in the recovery of endogenous protein secretion compared with exogenous protein (Barth et al., 1993). It is clear that the effect of ANFs on ENL and true N and amino acid digestibilities can be substantial, particularly at low dietary protein level and are often larger than the effects of dry matter intake and body weight (Tamminga et al., 1995).

2.4.5.3 *Dietary fibre content and quality*

Although dietary fibre refers to a wide range of chemical components with

unique physical properties and physiological effects (Graham et al., 1991), many studies have demonstrated that it influences ENL in the gut of monogastric animals. De Lange et al. (1989a) and Taverner et al. (1981) have both measured increased recovery of endogenous amino acids at the terminal ileum of pigs fed protein-free diets and increasing amounts of specific dietary fibres. In the study by de Lange et al. (1989a), the effect of purified pectin on ENL was much larger than that of purified cellulose in pigs fed protein-free diets. This indicates that different fibres have different effects on ENL. However, it is poorly understood as to how fibrous components of the diet act to enhance ENL. Secretions of pancreatic juices (Partridge et al., 1982), bile (Portman et al., 1985), mucous (Satchithanandam et al., 1990; Mosenthin et al., 1994) are reported to be increased with increasing intakes of various dietary fibres such as pectin and cellulose. Although fibre is reported to increase endogenous secretion either by sloughing of the epithelial cells due to its physical nature (Sauer et al., 1976; Shah et al., 1982) or by adsorbing peptides and amino acids and digestive enzymes (Schneeman, 1978, 1982; Bergner et al., 1981) and therefore reducing their digestion and absorption, it is still unclear from the literature which of the two is more important. Moreover, dietary fibre may have a direct effect on the size of visceral organs and may thus affect N (and energy) metabolism as well (see Section 2.4.3). For instance, Taverner et al. (1981) could not observe any signs of epithelial cell loss due to abrasion when feeding high dietary cellulose levels. Recent studies on the significance of fibre viscosity in nutrient digestion, absorption and ENL in rats (Ikegemi et al., 1990; Larsen et al., 1993) indicate that recovery of

nitrogen and amino acid at the terminal ileum is higher when feeding fibres of high viscosity (e.g. carbomethylcellulose and sodium alginate) compared with low viscosity fibres (e.g. cellulose and calcium alginate). The observation of de Lange et al. (1989a) may be explained by differences in the viscosity of pectin and cellulose fibres. Based on their results, Ikegemi et al. (1990) suggested that increased viscosity reduces diffusion of both substrates and enzymes thus hindering their effective interaction at the mucosal level. Consequently, the animal responds by increasing endogenous secretions (e.g. increased mucin and saliva production to reduce viscosity) and hence enhanced ENL. Although these studies do give some indication of the importance of the chemical properties of fibre, the results may be inconclusive since viscosity was only determined in the fibre and not in the digesta. Furthermore, whether purified forms of fibre added to semi-synthetic diet are a true representation of the properties of inherent fibre in feedstuffs is open to criticism (e.g. Schulze et al., 1994b; 1995b).

2.4.5.4 *Dietary protein quality and quantity*

It has been amply demonstrated that the amount of endogenous nitrogen secreted into the gut lumen increases substantially with increasing dietary protein levels (e.g. Snook and Meyer, 1964; Ikegemi et al., 1975; Percival and Schneeman, 1979; Moughan and Rutherford, 1990; Bergner et al., 1994). Both protein and peptides are potent stimulants of endogenous secretion in the gut (Schneeman, 1982; Darragh et al., 1990; Butts et al., 1993a) and the quantities of secretion vary with protein quality, i.e. the true protein digestibility. In general the presence of exogenous

protein in the gut appears to slow down the breakdown of endogenous protein (Snook and Meyer, 1964) although this alone may not explain the observed increase in ileal endogenous protein content. It is possible that dietary proteins both stimulate endogenous protein secretion and reduce degradation and absorption of endogenous protein. According to Percival and Schneeman (1979) and Ikegemi et al. (1975), substantial quantities of digestive enzymes may accumulate in the gut due to a low rate of breakdown rather than stimulation of secretion when poor quality protein is fed. This in turn will increase ENL.

From the foregoing sections, it is clear that direct comparisons of the effects of various factors may be inappropriate due to differences in methodology, animal size and level of specific factors included in the diet. However, it is generally evident (Table 2.4) that ileal endogenous amino acids and nitrogen flow are substantially elevated by various dietary factors. Understanding what factors in different feedstuffs used in pig feeds favour large endogenous nitrogen secretions and how they can be manipulated to reduce ENL is certainly important in studying amino acid and energy utilization in pigs and other farm animals. It should be noted that a minimum amount of endogenous secretions are required for normal digestion of dietary nutrients and that there will be a limit to the extent that ENL can be reduced.

2.5 Approaches to Minimise Endogenous N Losses

2.5.1 *Plant breeding strategies*

Plant breeding and selection offer a means of eliminating factors from

feedstuffs that are responsible for increased ENL (and/or poor nutrient utilization), such as ANFs and specific fibres. Examples include the development of soybean varieties that are low in specific anti-trypsins (Hywowitz, 1986) and of faba bean varieties that are low in condensed tannins (Jansman, 1993). However, these strategies often take a long a time before desired results are obtained and in some cases elimination of factors that favour high amounts of ENL results in other problems. For instance, elimination of tannin in sorghum predisposes the crop to more pest damage while in the field thus lowering yields (Bullard et al., 1980). Plant breeding therefore may not give immediate solutions to ENL particularly in areas where ingredients that cause high amount of ENL are the only alternative.

2.5.2 *Processing techniques*

Various techniques such as high pressure combined with heat treatment, physical or chemical dehulling are frequently used as a means of improving the feeding value of several feed ingredients. Heat treatment has been used in various forms to inactivate trypsin inhibitors, an important ANF in soybeans. Studies, such as reported by Marty et al. (1994), have shown that ingredient processing can reduce ENL in pigs. However, heat treatments need to be conducted under closely controlled conditions to avoid reducing the availability of amino acids, particularly lysine (Van der Poel et al., 1990). Physical dehulling (removal of seed coat) can be effective when the factors responsible for poor nutrient utilization (fibre, ANFs) are located in the seed coat. Dehulling of tannin- and trypsin inhibitors-containing

feedstuffs (Chibber et al., 1978; Jansman, 1993; Grala et al., 1998) has been show to improve nutrient utilization by monogastric animals. Feeding pre-germinated feedstuffs with high levels of ANFs reduces endogenous gut protein loss (Table 2.5; Schulze et al., 1994a). These researchers found a significant reduction in ileal crude protein loss and total amino acids passing the terminal ileum from 51.9 to 27.4 and 48.6 to 21.4 g kg DMI^{-1}, respectively, as a result of germination. Chemical dehulling acts by reacting with the ANF (particularly tannins) present in the seed coat. Chemical dehulling requires that the right amounts are used to avoid damaging some nutrients and not to disrupt the acid base balance of the animal (Schutte and Smith, 1991). Dehulling equipment is often expensive and other useful nutrients may be lost along with the hull thus posing some concerns with such techniques. Similarly, as noted by Huisman et al. (1990) in their review on antinutritional factors in pig production, one needs to be careful with different processing methods since they can influence the nutritional quality through interactions between nutrients and factors that favour increased ENL.

2.5.3 *Use of synthetic enzymes*

There are several types of enzyme preparations used to inactivate ANFs, ordegrade fibres, that are present in feedstuffs to enhance nutrient utilization. Although some studies with both poultry (Bedford and Classen, 1991; Bedford, 1993) and pigs (Inborr et al., 1991, 1993; Danielsen, 1994; Li et al., 1994) have found positive effects of enzyme supplementation on animal performance, others (Graham

Table 2.5. Ileal digestibilities (%) of protein and amino acids and the amount of endogenous protein and total amino acids passing the terminal ileum in growing pigs fed casein-based diets supplemented with raw, germinated or porcine pancreatin-treated beans[a]

Parameter	Untreated raw beans	Germinated beans	Pancreatin treated beans
Apparent CP digestibilty	74.3	86.8	75.3
Apparent total amino acids digestibility	75.5	88.6	78.2
True CP digestibility	88.1	93.2	93.4
Ileal CP loss (g kg^{-1} DMI)	51.9	27.4	51.1
Total AA passing terminal ileum (g kg^{-1} DMI)	48.6	21.4	42.2

[a] After Schulze et al. 1994a.

et al., 1986; Thacker et al., 1988; McClean and McCraken, 1992) did not observe any improvements in animal performance. This may be partly explained by variations in activity of the enzymes used, their stability during feed manufacturing and passage through the gastrointestinal tract, the rate of passage and viscosity of digesta in the gut, and the characteristics of their substrates (Inborr and Bedford, 1994; Fthenaskis and Kyriazakis 1994; van der Meulen et al., 1994). For those studies reporting positive effects, there is no clear-cut understanding of how exogenous enzyme supplementation causes improved animal performance. However, it appears that these enzyme preparations help in breaking or disrupting the non-structural polysaccharides of the endosperm cell wall thus releasing entrapped nutrients for digestion and improve nutrient utilization by reducing digesta viscosity (Chesson, 1993). Inborr et al. (1993), in an experiment with early-weaned pigs, found reduced endogenous enzyme secretion with diets supplemented with exogenous enzymes. Jensen et al. (1994) similarly measured reduced protein content in pancreatic juice from starter pigs fed enzyme-supplemented diets. Results of these studies indicate that addition of exogenous enzymes to animal feeds have a sparing effect on endogenous enzyme secretion and likely a reduction in ENL. Whether exogenous enzymes improve animal performance just by reducing digesta viscosity is controversial, and as reported by Inborr et al. (1991), this may not wholly explain the improved animal performance. Exogenous enzymes may be facilitating fibre breakdown which in turn will reduce ENL from the gut and therefore allow better protein utilization. This aspect is, however, yet to be studied.

2.6 Conclusions

In this review it has been attempted to demonstrate the significance of ENL at the distal ileum in pig nutrition. Most studies cited in this review focused on methodologies to estimate ENL, and on the effects of dietary components on ENL and true N and amino acid digestibilities. Few studies have been conducted to determine the actual metabolic cost (including changes in maintenance energy and amino acid requirements) associated with changes in ENL. It is concluded that the conventional methods (feeding protein-free diets, feeding diets with an assumed 100% digestibility, regression methods) underestimate ENL in pigs fed protein-containing diets. Alternative techniques (such as the ^{15}N-isotope dilution technique, the homoarginine technique, and the enzymatically hydrolyzed casein method) all have some limitations for routinely measuring ENL. The HA technique will likely become a method of choice for determining ENL in a wide range of feedstuffs, provided that a representative fraction or all of lysine in these feedstuffs is converted to homoarginine. Based on an increasing number of observations, obtained with these alternative techniques, ENL appear to be highly variable and affected by factors such as body weight, dry matter intake, and the contents of anti-nutritive factors, fibres and (digestible) protein in the diet. A reduction in ENL will improve the efficiency of using dietary amino acids and energy for pork production. Therefore, the specific components in pig feed ingredients that cause these losses should be identified. In addition, means to inactivate or reduce the levels of these components in pig feed ingredients (breeding and selection, ingredient processing, synthetic enzymes) should

be evaluated. Continued research is required to further develop methodologies to accurately quantify ENL under varying conditions and to quantify the total metabolic cost associated with ENL.

CHAPTER III

Guanidination of Lysine in Casein, Barley, and Canola Meal.

3.1 Abstract

In order to estimate true ileal amino acid digestibilities in feedstuffs for pigs, it is important that a distinction is made between the undigested dietary and endogenous amino acids present in the ileal digesta. Dietary guanidinated proteins can be used to differentiate between undigested dietary and endogenous lysine in intestinal digesta provided that a representative part of dietary lysine is converted into homoarginine (HA) during guanidination. This is likely achieved at high lysine conversion rates. The effect of methylisourea (MIU) concentration and incubation period on the extent of lysine guanidination in casein, barley and canola meal was studied. Conversion rates were higher in casein than in barley and canola meal and were affected by both MIU concentration and reaction period. Treatment with 0.4 M MIU for 4 or 6 d gave guanidination rates of 72.5 % and 78.5 % for barley and 72.3 % and 75.2 % for canola meal, respectively. After 6 d, treatment with 0.5 M MIU solution gave conversion rates of 88.0 % and 84.6 % for barley and canola meal, respectively, and, therefore, these conditions are recommended for guanidination of lysine in barley and canola meal.

3.2 Introduction

There is increasing evidence suggesting that pig feeds should be formulated

based on true rather than apparent ileal amino acid digestibilities (Moughan, 1995; Nyachoti et al., 1997b). In order to estimate true ileal amino acid digestibilities in feedstuffs for pigs, a distinction must be made between the undigested dietary (ie. exogenous) and endogenous amino acids present in the ileal digesta. As a means to differentiate endogenous from exogenous lysine, a method in which dietary lysine is chemically converted to homoarginine (HA) in a guanidination reaction with methylisourea (MIU) has been suggested (Hagemeister and Erbersdobler, 1985). When using this method, it is important that a representative (random) proportion of dietary lysine is converted into HA; a requirement that is likely achieved with high guanidination rates (Rutherfurd and Moughan, 1990; Siriwan et al., 1994). The extent of guanidination is known to vary with incubation conditions and protein source thus suggesting differences in optimal guanidination conditions (Maga, 1981; Rutherfurd and Moughan, 1990). For instance, Siriwan et al. (1994) found a 66.1 % conversion in casein while others report values ranging from 89-99.6 % (Schmitz et al., 1991; Barth et al., 1993; Imbeah et al., 1996). Values for isolated soy protein range from 68-83 % (Rutherfurd and Moughan, 1990; Siriwan et al., 1994) while in soybean meal and full fat soybeans, more conventional feedstuffs, about 63.1-79.8 % of the lysine was transformed into HA (Marty et al., 1994; Siriwan et al., 1994; Imbeah et al., 1996). Furthermore, this technique was developed using purified proteins and has had limited application in studies involving practical feedstuffs. Consequently, for the HA method to be routinely used for determining true digestibilities, it is essential that it is evaluated with a wide range of feedstuffs (particularly plant proteins sources)

commonly used in pig diets to establish optimal guanidination conditions.

The objective of the present study was to determine the extent of conversion of lysine to HA in barley, canola meal and casein and to establish how this is influenced by changing some of the reaction conditions.

3.3 Materials and Methods

O-methylisourea (O-MIU) (Pfalzer & Bayer, Connecticut, OH), barium hydroxide, hydrochloric acid (reagent grade), and sodium hydroxide (Sigma Chemicals, St. Louis, MO) were used in this study. Methylisourea (MIU) solution was prepared by reacting O-MIU with barium hydroxide followed by centrifugation at 4,000 x g to remove the precipitated barium sulphate. Casein (89 % CP, Prairie Micro-Tech Inc., Kitchener, ON), barley (12 % CP) and canola meal (36 % CP) were used in this study. Barley and canola meal were ground to pass through 1 mm screen before use.

This study was conducted in two consecutive assays. In Assay 1, the procedure described by Schmitz et al. (1991) was used to determine the guanidination rates in casein, barley and canola meal. Briefly, duplicate samples of each ingredient containing 200 g of crude protein were soaked in 1 L of double distilled water and then mixed thoroughly with 1 L of 0.4 M MIU solution. The pH of the mixture was adjusted to 10.5 using 1 M NaOH before incubating the mixture in a fridge set at 4 °C (to minimize isomerization of amino acids) for 4 d. Based on the results of Assay 1, Assay 2 was conducted to investigate the effects of increasing both the MIU

concentration and the time of incubation on the extent of lysine conversion to HA. Duplicate samples of each ingredient were mixed with either 0.5 or 0.6 M MIU and incubated for either 4 or 6 d. Another set of samples was reacted with 0.4 M MIU and incubated for 6 d only. In both assays, the pH was monitored twice daily and adjusted accordingly and the material thoroughly stirred to ensure uniform conditions in the mixture during incubation. At the end of incubation, the guanidination reaction was stopped by lowering the pH to the isoelectric point of each ingredient (casein, 4.5; barley, 5.6; canola meal, 4.6) using 1 M HCl (Sosulski and Bakal, 1969; Preaux and Lontie, 1975). Samples were then centrifuged at 4,000 x g and 4 °C to recover the guanidinated protein. Unreacted MIU was removed by re-suspending the precipitate in water adjusted to the isoelectric pH of each protein and centrifuged again. The latter was repeated thrice, each time discarding the supernatant. The clean samples were then freeze-dried, finely ground (barley and canola meal) and analyzed in duplicate for HA and lysine by high performance liquid chromatography following hydrolysis with 6 *N* HCl in sealed, evacuated tubes at 110 °C for 24 h (Bidlingmeyer et al., 1987). The percentage conversion of lysine to HA was calculated as:

$$\% \text{ conversion} = \frac{MC_{HA}}{MC_{HA} + MC_{LYS}} \times 100$$

where MC_{HA} and MC_{LYS} are the molar concentrations of HA and lysine, respectively. Data were statistically analyzed using the GLM procedures of SAS (1985).

3.4 Results and Discussion

Both barley and canola meal formed a thick gruel once mixed with MIU and the pH adjusted to 10.5 thus making it more difficult to maintain a homogenous reaction mixture as compared to the casein sample. During washing and centrifugation to remove unreacted MIU, barley formed a very hard pellet that required a considerable amount of time to pulverize between washings. However, a simple procedure for large-scale guanidination of casein and soybean meal, a practical ingredient, recently described by Imbeah et al. (1996) may help overcome such difficulties.

The rates of conversion (%) of lysine to HA in casein, barley and canola meal are presented in Table 3.1. The extent of guanidination in casein (89.2 %) obtained in the present study fall within the range of lysine conversion of 68-99.6 % reported in the literature (Schmitz et al., 1991; Barth et al., 1993; Siriwan et al., 1994) and was higher ($P < .05$) compared to conversion rates observed in barley and canola meal. Conversion rates in barley and canola meal in Assay 1 were 72.5 and 72.3 %, respectively (Table 3.1). Published data on barley and canola meal are not available. However, guanidination levels obtained in the present study for barley are substantially higher than reported for other cereals like corn (57.3 %), sorghum (61.2 %) and wheat (62.6 %) (Siriwan et al., 1994). In canola meal, guanidination was within the range of values (63.1-79.8 %) reported for soybean meal, a similarly processed feedstuff (Marty et al., 1994; Siriwan et al., 1994; Imbeah et al., 1996). Higher conversion rates could be expected for canola meal than barley since the

Table 3.1. Effect of incubation period and methylisourea concentration on the rate (%) of lysine conversion into homoarginine in casein, barley and canola meal proteins[1]

Incubation period	Protein source	Methylisourea concentration (M)		
		0.4	0.5	0.6
4 days	Casein	88.3 ± 0.7^{by}	95.3 ± 0.7^{a}	95.1 ± 0.6^{a}
	Barley	72.5 ± 0.4^{by}	85.7 ± 3.1^{a}	87.0 ± 1.0^{a}
	Canola meal	72.3 ± 0.9^{cy}	81.3 ± 0.9^{by}	86.6 ± 0.5^{ay}
6 days	Casein	93.4 ± 1.2^{ax}	95.6 ± 1.8^{b}	95.0 ± 1.2^{b}
	Barley	78.5 ± 0.4^{ax}	88.0 ± 2.2^{b}	MV[2]
	Canola meal	75.2 ± 0.5^{ax}	84.6 ± 0.4^{bx}	82.5 ± 0.6^{bx}

[1] values are mean ± standard deviation of two individually treated samples

[2] missing value

[abc] means for each protein source within a row having a common superscript are not significantly different ($P > 0.05$)

[xy] means for each protein source between days and within a column having a common superscript are not significantly different ($P > 0.05$)

former is a processed product in which protein is likely more accessible when exposed to MIU than in barley.

Assay 2 showed that the extent of lysine guanidination in all three ingredients is affected by MIU concentration and period of incubation (Table 3.1). There was an increase in the extent of guanidination as the MIU concentration was increased from 0.4 to 0.5 M in all three proteins. A further increase in MIU concentration to 0.6 had inconsistent effects on the extent of guanidination in different proteins thus indicating the presence of interactions between the protein type and guanidination conditions (Table 3.1). Under the conditions of the present study, increasing the MIU concentration beyond 0.5 M had no further guanidination in casein and barley after 4 or 6 d but a significant improvement ($P < .05$) was observed in canola meal after 4 d. This observation agrees with data reported by Maga (1981) but contradicts recent findings by Imbeah et al. (1996) suggesting that increasing MIU concentration from 0.4 to 0.6 M results in equal levels of guanidination. Lengthening the incubation period from 4 to 6 d resulted in significant ($P < .05$) increases in the extent of guanidination in all proteins when reacted with 0.4 M solution and only in canola meal was the effect of incubation period substantial ($P < .05$) when reacted with 0.5 or 0.6 M MIU solutions.

The whole purpose of converting dietary lysine to HA is to use it as a means of distinguishing exogenous from endogenous nitrogen present in ileal digesta. In order to reliably apply this technique for this purpose, it is important that a representative (random) proportion of dietary lysine is converted into HA (other

assumptions are discussed in Chapter II, section 2.4.4.3). This is unlikely achieved at low levels of guanidination as shown by Siriwan et al. (1994). It was not determined whether or not a representative proportion of lysine was guanidinated in the protein sources used in the current study. However, given the high levels of guanidination obtained in barley and canola meal when treated with 0.5 M MIU for 6 d, it is likely that conversion of a representative proportion of lysine was indeed achieved. It has been shown that the degree of randomness in lysine conversion into HA increases with guanidination rates (Rutherfurd and Moughan, 1990; Siriwan et al., 1994).

The current study and others (Maga 1981; de Vrese et al., 1995; Imbeah et al., 1996) show that the extent of guanidination of lysine varies with the protein source and the guanidination conditions used and that the extent of lysine conversion is higher in animal proteins than in plant proteins. It is not clear why low guanidination rates are obtained with vegetable proteins, but a close linkages between protein and other vegetable polymers may limit effective interaction between MIU and lysine residues thus leading to lower conversion rates in plant proteins. Also this may indicate the relative chemical unavailability of lysine in plant proteins compared to animal proteins (Nair et al., 1978; Rutherfurd and Moughan, 1990).

3.5 Implications

This study has shown that the extent of guanidination varies with the protein type and the guanidination conditions used and that this can be improved by identifying optimal guanidination conditions for different protein sources. However,

care must be taken to ensure that such manipulations do not interfere with the digestibility of treated material. It is suggested that for barley and canola meal, a 0.5 M MIU solution and 6-d incubation period should be used.

CHAPTER IV

Estimating Endogenous Amino Acid Flows at the Terminal Ileum and True ileal Amino Acid Digestibilities in Feedstuffs for Growing Pigs Using the Homoarginine Method[2]

4.1 Abstract

True ileal lysine digestibilities were determined using the homoarginine method (HAM) in casein-, barley-, canola meal- and barley-canola meal-based diets fed to growing pigs. Four Yorkshire barrows (25 to 49 kg BW) equipped with ileal T-cannulas were fed one of the diets according to a 4 x 4 latin square design. All diets, except for the barley diet, were formulated to be similar in DE:CP ratio. Ileal digesta was collected continuously for 24 h on d 7 and 9 of each experimental period for determining apparent and true ileal digestibilities, respectively. True ileal lysine digestibility and endogenous flows were determined by feeding diets in which 50 % of the protein containing ingredients was guanidinated; the conversion of lysine to homoarginine (HA) allows for direct determination of true lysine digestibilities. The apparent ileal CP and amino acid digestibilities were higher ($P < .05$) in the casein diet compared to the barley, canola meal and barley-canola meal diets. The CP digestibility was higher ($P < .05$) in the barley-canola meal diet than in barley and canola meal diets. Endogenous lysine losses were influenced by diet ($P < .05$) and

[2] A version of this chapter has been published: Nyachoti, C.M., C.F.M. de Lange, and H. Schulze, 1997. Estimating endogenous amino acid flows at the terminal ileum and true ileal amino acid digestibilities in feedstuffs for growing pigs using the homoarginine method. J. Anim. Sci. 75: 3206-3213.

ranged between 586 to 1429 mg/kg DM intake. True ileal lysine digestibilities in barley, canola meal and barley-canola meal diets were similar ($P > .05$) and lower ($P < .05$) than in the casein diet. The true ileal amino acid digestibilities were estimated for other amino acids. Unlike apparent digestibilities, true digestibilities seemed additive in the barley-canola meal mixture.

4.2 Introduction

For accurate formulation of pig feeds in terms of amino acid supply, it is essential that amino acid availabilities in individual ingredients are determined and that these be additive in mixtures of ingredients. Apparent ileal amino acid digestibilities provide a reasonable estimate of amino acid availabilities in most pig feed ingredients (Sauer and Ozimek, 1986; Moughan, 1995). Recent studies suggest that endogenous gut N losses are higher than previously estimated and that differences in apparent digestibilities between feedstuffs are attributed to differences in endogenous gut N losses rather than differences in true digestibilities (Marty et al., 1994; Schulze et al., 1994b).

To determine true ileal amino acid digestibilities, the undigested dietary amino acids present in the ileal digesta should be differentiated from endogenous amino acids. The homoarginine (HA) method, which involves the transformation of dietary lysine to its amino acid analogue, homoarginine, in a guanidination reaction with methylisourea, is a promising method for determining true lysine digestibilities in pig feed ingredients (Hagemeister and Erbersdobler, 1985; Rutherfurd and Moughan,

1990; Marty et al., 1994). However, the HA method has not been tested with most commonly used pig feed ingredients.

The objectives of the study described herein were to determine endogenous amino acid flow at the terminal ileum using the HA method and to estimate true ileal amino acid digestibilities in casein-, barley-, canola meal- or barley-canola meal-based diets fed to growing pigs. The barley-canola meal diet was included to allow assessment of additivity of apparent and true ileal amino acid digestibilities.

4.3 Materials and Methods

4.3.1 *Animals and Housing*

Four Yorkshire growing barrows with an average initial BW of 25 kg were obtained from the University of Guelph Arkell Swine Research farm for use in the present study. Pigs were housed in large .9 x 1.5-m adjustable metabolism cages with smooth, transparent plastic sides and tender foot floors in a temperature-controlled (20 to 22°C) room. After a 7-d adaption period, pigs were surgically fitted with a simple T-cannula (20 mm i.d.; Central Hydraulics Mfg. Co. Ltd, Edmonton, Alberta) at the terminal ileum following the procedures described by Sauer et al. (1983). The design of the cannulas was modified according to de Lange et al. (1989a). After surgery the pigs were immediately returned to the metabolism cages and allowed a 14-d recovery period. During this period they were fed twice daily increasing amounts of a corn and soybean meal based grower diet and had unlimited access to water.

The use of animals in the present study was reviewed and approved by the

Animal Care Committee of the University of Guelph and the pigs were cared for according to the guidelines of the Canadian Council on Animal Care. After the study the pigs were killed to determine if cannulation had caused any intestinal abnormalities.

4.3.2 *Preparation of Experimental Diets*

Four diets were formulated to contain a similar DE:CP ratio (except the barley-based diet) (Table 4.1). Diet 1 was a semi-synthetic diet based on casein and cornstarch that, based on previously reported data (Butts et al., 1993a), was assumed to be highly digestible and was used to estimate the minimum endogenous N losses. Diets 2, 3 and 4 contained barley, canola meal and a combination of barley and canola meal as protein sources, respectively. The barley and canola meal were ground in a hammer mill through a 2-mm screen before diet preparation. Cornstarch was used to supply energy in diet 3, and sucrose was included in diets 1, 3, and 4 to improve palatability. Vitamins and minerals were supplemented to meet or exceed NRC (1988) recommendations. Chromic oxide (.5%) was included in each diet as an indigestible marker for determining nutrient digestibilities.

For the estimation of true ileal amino acid digestibilities and endogenous amino acid flows, samples of the four protein sources were guanidinated before diet preparation. The guanidination of casein was achieved by using the procedure described by Schmitz et al. (1991) and that of barley and canola meal was accomplished by increasing the methylisourea (MIU) concentration to 0.5 *M* and the

Table 4.1. Ingredient composition (%), calculated DE and DE:CP of the experimental diets (as fed basis)

Ingredient	Diet 1[a]	Diet 2	Diet 3	Diet 4
Barley	-	92.75	-	61.15
Canola meal	-	-	42.1	22.0
Casein	20.82	-	-	-
Corn starch	61.28	-	41.53	-
Sucrose	10.0	-	10.0	10.0
Limestone	.6	.85	.85	1.2
Dicalcium phosphate	2.4	1.5	.62	.75
Fat (A/V blend)	3.5	3.5	3.5	3.5
Iodized salt	.5	.5	.5	.5
Vitamin premix[b]	.3	.3	.3	.3
Mineral premix[c]	.1	.1	.1	.1
Chromic oxide	.5	.5	.5	.5
Calculated DE, kcal/kg	3978	3217	3439	3253
DE:CP ratio	215	302	215	212

[a] Diet 1, casein corn starch diet; Diet 2, barley diet; Diet 3, canola meal diet; Diet 4, barley-canola meal diet.

[b] Vitamin premix supplied the following per kg of finished feed: Vit A, 12000 IU; Vit D, 1200 IU; Vit E, 48; Vit K, 3 mg; choline, 600 mg; pantothenic acid, 18 mg; Riboflavin, 6 mg; Folic acid, 2.4 mg; Niacin, 30 mg; Thiamin, 1.8 mg; Vit B_6, 1.8 mg; Biotin, 0.24 mg; Vit B_{12}, 0.03 mg.

[c] Mineral premix supplied the following per kg of finished feed: Cu, 15 mg; Zn, 100 mg; Fe, 100 mg; Mn, 20 mg; I, 0.3 mg; Se, 0.3 mg.

incubation period to 6 d as established in Chapter 3. Briefly, material calculated to contain 200 g of crude protein were soaked in 1 L of double distilled water and then thoroughly mixed with a litre of MIU solution (0.4 *M* for casein and 0.5 *M* for barley and canola meal). The pH of the mixture was adjusted to 10.5 using 1 *M* NaOH before incubating the mixture in a fridge set at 4 °C for 4 d (for casein) or 6 d (for barley and canola meal). The MIU solution was prepared by reacting O-methylisourea (Pfalzer and Bayer, Connecticut, OH) with barium hydroxide (Sigma Chemical, St. Luois, MO) followed by centrifugation at 4,000 x g to remove the precipitated barium sulphate. The pH was monitored twice daily and adjusted accordingly, and the material thoroughly stirred to ensure uniform conditions in the mixture during incubation. At the end of incubation, the guanidination reaction was stopped by lowering the pH to the isoelectric point of each protein (casein, 4.5; barley, 5.6; canola meal, 4.6) using 1 *M* HCl. Samples were then centrifuged at 4,000 x g and 4 °C to recover the guanidinated protein. Unreacted MIU was removed by resuspending the precipitate in water adjusted to the isoelectric pH of each protein and centrifuged again. The later was repeated thrice, each time discarding the supernatant. The clean material was freeze-dried before it was used in diet preparation. Lysine conversion to HA in casein, barley and canola meal was 94.1 %, 86.6% and 80.1%, respectively. The HA diets were prepared by replacing 50 % of the protein source in each diet (Table 4.1) with guanidinated samples. Dysprosium chloride (Sigma) was used in place of chromic oxide as an indigestible marker and included at a level of 100 ppm (Marty et al., 1994) so as to have a marker unique for

the HA diets.

4.3.3 *General Conduct of Study*

The experiment was designed and conducted according to a 4 x 4 Latin square. After recovering from surgery, the pigs were offered one of the four experimental diets twice daily at 0800 and 1800 as a wet mash. Feed refusals and spillage were recorded and used to determine actual DMI. Pigs were fed at 2.6 times maintenance energy requirement (ARC, 1981) based on their BW at the beginning of each experimental period. Because there were differences in calculated diet DE contents, feed allowance varied among diets. At the end of each feeding, drinking water was placed in the feed troughs so that it was always available.

The experimental periods lasted 9 d each. Pigs were allowed to acclimatize to their respective experimental diets for 6 d. On d 7, a 24-h continuous ileal digesta collection was conducted for determination of apparent digestibilities in the regular non-guanidinated diets. On d 9, a meal of the diets with guanidinated protein was fed only at 0800 followed by a 24-h continuous digesta collection for determining endogenous amino acid losses and true amino acid digestibilities. Digesta were collected through soft transparent plastic tubes (4 cm i.d.; Alpine Plastics Ltd, Edmonton, Alberta) which were attached to the barrel of the ileal cannulas as described by de Lange et al. (1989a). The lower section of the tube was clamped shut, filled with 10 ml of 10 % formic acid to minimize bacterial activity, and kept immersed in a plastic container filled with ice water. Every 1 to 2 h, the collected

digesta were removed and immediately frozen at -20°C until further analysis.

4.3.4 *Sample Preparation and Analyses*

Digesta were pooled per pig and collection day. Digesta samples were freeze-dried, and along with diet samples, ground in a Wiley mill through a .5-mm screen and thoroughly mixed before analyses. Nitrogen and DM content in diet and digesta were determined according to AOAC (1990). Chromic oxide was determined using the method of Fenton and Fenton (1979); dysprosium chloride was determined by flame atomic absorption spectrophotometry (Everson, 1975). Amino acid and homoarginine content in feed and digesta were determined using a Beckman System Gold amino acid analyzer [Beckman Instruments (Canada) Ltd., Mississauga, ON] following hydrolysis with 6 *N* HCl in sealed, evacuated tubes at 110 °C for 24 h (Mason et al., 1980). The sulphur containing amino acids and tryptophan were not determined. All analyses were performed in duplicate.

4.3.5 *Calculations and Statistical Analysis*

Apparent ileal amino acid and protein digestibilities (%) were calculated using the observations made on d 7 and chromic oxide as the indigestible marker. The following formula was used:

$$AD = 100 - (100 * ([AA]_{digesta} * [Cr]_{diet})/([AA]_{diet} * [Cr]_{digesta})) \quad [1]$$

where $[AA]_{diet}$ and $[AA]_{digesta}$ are the concentrations (mg kg^{-1} DM) of nitrogen and amino acid in the diet and digesta, respectively and $[Cr]_{diet}$ and $[Cr]_{digesta}$ are the

concentrations (mg/kg DM) of chromic oxide in the diet and digesta, respectively. The total flow (mg/kg DMI) of nitrogen, amino acids and homoarginine at the terminal ileum was calculated as:

$$AA_{flow} = [AA]_{digesta} * ([Marker]_{diet}/[Marker]_{digesta}) \quad [2]$$

where AA_{flow} is the flow of an amino acid at the terminal ileum and $[Marker]_{diet}$ and $[Marker]_{digesta}$ are the concentrations of the appropriate indigestible marker (chromic oxide for d 7 and dysprosium chloride for d 9 observations) in the diet and digesta, respectively. The true digestibility (%) of lysine (TD_{lys}) was assumed to be equal to the apparent digestibility of HA and was calculated as:

$$TD_{lys} = ([HA]_{diet} - HA_{flow}) * 100/[HA]_{diet} \quad [3]$$

where $[HA]_{diet}$ and HA_{flow} are the homoarginine concentration in the diet (mg/kg DM) and the flow of homoarginine at the terminal ileum, respectively (Moughan and Rutherfurd, 1990; Marty et al., 1994). The flow of endogenous lysine (LYS_{eflow}) was calculated as the total flow of lysine at the distal ileum (LYS_{flow}, determined with equation 2 and chromic oxide as the indigestible marker) minus the flow of unabsorbed dietary lysine using the equation:

$$LYS_{eflow} = LYS_{flow} - ([LYS]_{diet} * (1 - TD_{lys} * 0.01)) \quad [4]$$

where $[LYS]_{diet}$ is the concentration of lysine in the diet. The endogenous flow of amino acids other than lysine (AA_{eflow}) was calculated from the observed flow of endogenous lysine and the amounts of other amino acids relative to lysine as reported by Boisen and Moughan (1996) except for proline and glycine for which ratios from de Lange et al. (1989b) were used. True digestibilities (%) of amino acids other than

lysine were then calculated as follows:

$$TD_{AA} = ([AA]_{diet} - (AA_{flow} - AA_{endo}))*100/[AA]_{diet} \quad [5]$$

Endogenous flow of N was determined by multiplying N flow at the distal ileum with the ratio of lysine in endogenous nitrogen and the results employed in deriving true N digestibilities using equation 5.

Data were subjected to analysis of variance using the GLM procedures of SAS (1985). The experimental design used was a 4 x 4 latin square and effects of period (df = 3), animal (df = 3), and diet (df = 3) were included in the statistical model. When a significant *F*-value ($P < .05$) was indicated by the analysis of variance, means of diet treatments were compared using Duncan's multiple-range test (Steel and Torrie, 1980). The observed amino acid digestibility values in the barley-canola meal diet were compared to those calculated from digestibilities in the pure ingredients by means of a *t*-test so as to assess additivity of apparent and true digestibilities.

4.4 Results and Discussion

All four pigs appeared healthy, readily consumed their daily feed allowance and grew normally throughout the study. At the end of the experiment their BW averaged 48.9 ± 2.8 kg while their DMI during the experiment were 980, 1311, 1098, and 1229 g/d for the casein, barley, canola meal, and barley-canola meal diets, respectively. The amount offered for each diet differed due to their differences in calculated energy content (Table 4.1) and this led to the significant ($P < .05$)

differences in DMI. A postmortem examination indicated that cannulation did not result in any intestinal abnormalities or adhesions and that cannulas were inserted at the proper location.

The DM, CP and amino acid content of casein, barley, canola meal, and the experimental diets are shown in Table 4.2. Amino acid analysis of ingredients are within the range of published values (NRC, 1988; Fan et al., 1995; Degussa, 1996) although levels in canola meal are somewhat lower than NRC (1988). For most amino acids and CP, the analysed contents in the diets were very similar to calculated values based on analysed amounts in the individual ingredients and their respective inclusion levels in the diets.

The apparent ileal digestibilities of DM, CP and amino acid in the experimental diets (Table 4.3) were within the range of reported values for these feedstuffs and diets (Sauer and Ozimek, 1986; de Lange et al., 1990; Darcy-Vrillon et al., 1991; Heartland Lysine, 1995). As expected, DM, CP, and amino acid digestibilities were highest ($P < .05$) in the casein diet. Apparent ileal DM digestibility was lowest ($P < .05$) in the barley-canola meal diet. Apparent ileal CP digestibilities were similar in the barley and canola meal diets; both were lower ($P < .05$) than that in the barley-canola meal diet. Of the essential amino acids, only apparent ileal arginine and lysine digestibilities differed ($P < .05$) among the barley, canola meal and barley-canola meal diets. The apparent ileal lysine digestibility value (53.6 %) obtained in the present study for barley agrees closely with results of others (Furuya and Kaji, 1991; Fan et al., 1995). However, values that are substantially

Table 4.2. Dry matter, protein and amino acid content (%) in ingredients and diets (as fed basis)

Item	Ingredient			Diet[a]			
	Casein	Barley	Canola	1	2	3	4
Dry matter	93.00	86.7	88.6	90.6	90.9	87.8	88.1
Crude protein	89.02	11.55	34.95	18.6	12.0	14.5	15.0
Amino acids:							
Indispensable							
Arginine	3.28	.50	1.67	.67	.55	.80	.63
Histidine	2.57	.25	.78	.64	.21	.34	.36
Isoleucine	4.72	.34	.93	.93	.32	.40	.41
Leucine	8.84	.72	1.98	1.85	.66	.82	.90
Lysine	7.34	.35	1.47	1.54	.31	.57	.55
Phenylalanine	4.64	.51	1.13	.99	.47	.49	.55
Threonine	4.01	.31	1.32	.83	.47	.54	.55
Valine	5.89	.45	1.17	1.23	.45	.53	.51
Dispensable							
Alanine	2.88	.41	1.23	.69	.38	.64	.68
Aspartic acid	6.65	.58	2.12	1.43	.54	1.24	1.35
Glutamic acid	18.55	2.62	5.20	3.76	2.36	3.09	2.68
Glycine	1.75	.40	1.49	.37	.36	.51	.64
Proline	9.64	1.10	2.57	2.13	1.04	1.82	1.35
Serine	5.19	.47	1.30	1.19	.45	.75	.64
Tyrosine	4.06	.31	.86	1.12	.33	.36	.39

[a] Diet 1, casein corn starch diet; Diet 2, barley diet; Diet 3, canola meal diet; Diet 4, barley-canola meal diet.

Table 4.3. Apparent dry matter, crude protein and amino acid digestibilities (%) in pig fed casein, barley, canola meal or barley-canola meal based diets

Item	Diet 1[a]	Diet 2	Diet 3	Diet 4	SEM
Dry matter	92.6[b]	65.0[c]	66.3[c]	60.8[d]	1.1
Crude protein	89.0[b]	64.1[d]	64.7[d]	68.2[c]	1.4
Amino acids:					
Indispensable					
Arginine	92.5[b]	68.1[d]	81.9[c]	76.5[cd]	2.7
Histidine	94.7[b]	68.8[c]	74.8[c]	71.3[c]	2.4
Isoleucine	92.9[b]	67.9[c]	73.2[c]	78.6[c]	2.9
Leucine	93.9[b]	65.3[c]	65.1[c]	71.7[c]	2.6
Lysine	95.4[b]	53.5[d]	62.6[cd]	63.6[c]	2.8
Phenylalanine	94.4[b]	77.7[c]	71.7[c]	76.9[c]	2.0
Threonine	89.0[b]	63.7[c]	62.4[c]	67.7[c]	5.3
Valine	92.9[b]	67.6[c]	69.5[c]	69.2[c]	4.2
Dispensable					
Alanine	86.1[b]	51.3[d]	71.6[c]	67.1[c]	2.5
Aspartic acid	90.1[b]	52.0[d]	71.7[c]	74.1[c]	2.3
Glutamic acid	93.2[b]	78.5[c]	83.9[c]	77.3[c]	2.3
Glycine	68.3[b]	45.8[c]	50.7[bc]	69.8[b]	4.4
Proline	94.2[b]	79.0[c]	74.4[c]	73.7[c]	2.2
Serine	88.2[b]	55.2[d]	72.0[c]	69.6[c]	4.6
Tyrosine	96.3[b]	73.0[c]	67.5[c]	74.5[c]	2.9

[a] Diet 1, casein corn starch diet; Diet 2, barley diet; Diet 3, canola meal diet; Diet 4, barley-canola meal diet.

[bcd] Means in the same row with different superscripts differ ($P < .05$)

SEM, pooled standard error of the mean.

higher or lower than those observed here have been reported (Imbeah et al., 1988; de Lange et al., 1990; Heartland Lysine, 1995). The value observed for canola meal was somewhat lower that previously reported (Imbeah et al., 1988; de Lange et al., 1990; Fan et al., 1995; Heartland Lysine, 1995), and the value for the barley-canola meal diet agreed closely with that reported by Fan et al. (1995). The apparent ileal digestibility of threonine was 63.7 %, 62.4 % and 67.7 % for the barley, canola meal and barley-canola meal diets, respectively. These values agrees closely with previously reported data (de Lange et al., 1990; Fan et al, 1995). Differences in apparent ileal digestibilities of dispensable amino acids were significant ($P < .05$) only for alanine, aspartic acid, glycine, and serine among the diets containing plant protein. Of these, glutamic acid had the highest apparent ileal digestibility for barley, canola meal, and barley-canola meal diets and glycine had the lowest digestibility value except for the barley-canola meal diet.

The flow of lysine at distal ileum is presented in Table 4.4. There were significant differences ($P < .05$) in the total and endogenous flows of lysine at the distal ileum of pigs fed the four experimental diets. Endogenous lysine flow was lowest ($P < .05$) in the casein diet compared to the other diets. The canola meal diet had the highest flow, and amounts for the barley and barley-canola meal diets were intermediate (Table 4.4). The endogenous lysine flow observed for the casein diet was close to that reported by Butts et al. (1993a) who used the enzymatically hydrolysed casein method, and these can be considered as the minimum gut losses. Estimates of endogenous lysine flow for the barley and canola meal diets were 1101 and 1429

Table 4.4. Total, endogenous, and exogenous lysine flows at the distal ileum of pigs fed casein, barley, canola meal or barley-canola meal based diets

	Diet				
Item	Diet 1[a]	Diet 2	Diet 3	Diet 4	SEM
Total flow:					
mg/kg DMI	743.6[d]	1521.5[c]	2396.6[b]	2149.6[b]	99.3
mg/d	726.3[c]	2006.4[b]	2661.0[b]	2655.3[b]	188.1
Endogenous flow:					
mg/kg DMI	585.6[d]	1100.8[c]	1428.8[b]	1296.9[bc]	112.3
mg/d	570.1[c]	1458.4[b]	1607.5[b]	1615.1[b]	188.6

[a] Diet 1, casein corn starch diet; Diet 2, barley diet; Diet 3, canola meal diet; Diet 4, barley-canola meal diet.

[bcd] Means in the same row with different superscripts differ ($P < .05$)

SEM, pooled standard error of the mean.

mg/kg DMI, respectively, which are in close agreement with previous estimates obtained with the ^{15}N-isotope dilution technique (de Lange et al., 1990; 1111 and 1223 mg/kg DMI, respectively). The amount observed for the canola meal diet was very similar to the amount determined by Marty et al. (1994; 1329 mg/kg DMI) in 50-kg pigs fed soybean meal-based diets using the HA method. The calculated quantities of endogenous N recovered for the casein, barley, canola meal and barley-canola meal diets were 1.16, 2.19, 2.77, and 2.73 g/kg DMI, all of which fall within the wide range of values previously reported for endogenous N losses in growing-finishing pigs (Nyachoti et al., 1997a).

The current data indicate that the HA method gives reasonable estimates of endogenous lysine losses, even though assumptions have to be made when this method is used. These assumptions have been discussed elsewhere (Boisen and Moughan, 1996; Nyachoti et al., 1997a) and addressed in a number of recent studies (Moughan and Rutherfurd, 1990; Schmitz et al., 1991; Marty et al., 1994). The results clearly demonstrates that conventional methods of estimating endogenous N losses (N-free, purified diets) underestimates the actual amount of endogenous N losses when practical diets are fed. Others have made similar observations (e.g. Darragh et al., 1990; de Lange et al., 1990; Butts et al., 1993a).

The recovery of endogenous gut protein at the distal ileum of growing-finishing pigs represents a balance between secretion and reabsorption (Souffrant, 1991; Nyachoti et al., 1997a). Secretion and(or) reabsorption of endogenous gut protein is influenced by such factors as the BW, dietary fibre content, DMI and the presence

of anti-nutritive factors in the diet. The role of these factors in inducing endogenous gut protein losses has been reviewed in detail elsewhere (Boisen and Moughan, 1996; Nyachoti et al., 1997a). The amount of DMI and dietary crude fibre differed among diet treatments in the present study, and this may account for part of the differences observed in endogenous gut protein losses. It is apparent that other factors also affect endogenous losses.

A limitation of the HAM is that only endogenous lysine flow is determined. Flow of other amino acids can be calculated if assumptions are made about the amino acid composition of endogenous gut protein. Boisen and Moughan (1996), in a recent review, concluded that the amino acid composition of endogenous gut protein is relatively constant. However, they reported substantial differences in amino acid composition of endogenous gut protein between individual studies. Part of these differences may be attributed to the variable proline and glycine content in endogenous N which appears to be related to the overall N-balance in the pig (de Lange et al., 1989b). Even though it was assumed in the calculations of true ileal amino acid digestibilities that the amino acid composition of endogenous gut protein is constant, it is suggested that the effect of diet on the amino acid composition of endogenous gut protein be further evaluated (e.g. Souffrant, 1991; Boisen and Moughan, 1996).

The true ileal N and amino acid digestibilities are presented in Table 4.5. There were no significant differences ($P > .05$) in true ileal N digestibilities among the four diets although the casein diet had a numerically higher value compared to

Table 4.5. True ileal amino acid digestibilities (%) in pigs fed casein, barley, canola meal or barley-canola meal based diets

Item	Diet 1[a]	Diet 2	Diet 3	Diet 4	SEM
Nitrogen	99.3	90.8	89.8	88.5	5.2
Amino acids:					
Indispensable					
Arginine	100.6[b]	86.6[c]	98.6[b]	92.9[bc]	3.0
Histidine	99.2[b]	93.5[bc]	93.4[bc]	85.6[c]	3.0
Isoleucine	97.9	95.1	99.7	100.2	3.9
Leucine	98.0[b]	86.4[c]	85.4[c]	87.2[c]	2.7
Lysine	99.0[b]	87.1[c]	84.6[c]	85.8[c]	1.2
Phenylalanine	100.0	99.8	97.6	96.1	2.4
Threonine	99.0	97.0	97.8	96.6	5.1
Valine	98.3	89.1	97.4	96.4	4.5
Dispensable					
Alanine	94.4[b]	78.5[d]	91.0[bc]	82.0[cd]	2.8
Aspartic acid	99.3	97.2	95.4	92.4	2.8
Glutamic acid	98.3[b]	93.2[bc]	97.6[b]	90.2[c]	2.0
Glycine	91.4	89.2	88.1	94.7	4.3
Proline	97.2[b]	90.0[c]	82.1[d]	82.0[d]	2.4
Serine	95.6	90.8	98.0	94.9	4.8
Tyrosine	99.6	94.0	90.7	91.8	3.9

[a] Diet 1, casein corn starch diet; Diet 2, barley diet; Diet 3, canola meal diet; Diet 4, barley-canola meal diet.

[bcde] Means in the same row with different superscripts differ ($P < .05$)

SEM, pooled standard error of the mean.

the other three diets. The casein diet had the highest ($P < .05$) true ileal lysine digestibility, but this did not differ between the other three diets. True ileal digestibilities for amino acids other than lysine differed ($P < .05$) for arginine, histidine and leucine only among the indispensable amino acids while only for alanine, glutamic acid and proline were differences ($P < .05$) observed among the dispensable amino acids. In general, the true ileal N and amino acid digestibilities obtained for both barley and canola meal were in close agreement with the values obtained by de Lange et al. (1990) using the ^{15}N isotope dilution technique. These observations suggest that differences in the flow of endogenous gut protein losses contribute to differences in apparent amino acid digestibilities between feedstuffs (Marty et al., 1994; Schulze et al., 1994b).

The observed and calculated apparent ileal digestibilities in barley-canola meal diet were only significantly different ($P < .05$) for aspartic acid and glycine among all the amino acids (Table 4.6). However, observed and calculated true ileal digestibilities did not differ ($P > .05$; Table 4.6) for any of the amino acids. The numerical differences between observed and calculated digestibilities were smaller for true digestibilities than for apparent digestibilities of all amino acids. It seems that at least for some amino acids apparent digestibilities determined in pure ingredients are not additive when included in a mixture of ingredients (i.e. in a complete diet). Similar observations have been made previously (Imbeah et al., 1988). This indicates that it is more appropriate to use true ileal amino acid digestibilities in feed formulation since the digestible amino acid supply in complete a diet can be predicted from true

Table 4.6. Observed and calculated apparent and true ileal amino acid digestibilities in a barley-canola meal based diet fed growing pigs

	Apparent Digestibilities		True digestibilities	
Amino acid	Observed	Calculated	Observed	Calculated
Indispensable				
Arginine	76.5±6.4	75.6±0.7	92.9±4.2	93.1±2.0
Histidine	71.3±4.8	72.0±2.0	85.6±3.8	93.4±3.7
Isoleucine	78.6±4.7	70.6±1.4	100.1±4.1	97.4±3.8
Leucine	71.8±3.3	65.2±2.8	87.2±3.6	85.9±2.5
Lysine	63.6±6.5	59.0±1.7	85.6±1.6	85.8±1.2
Phenylalanine	76.9±4.6	75.0±1.2	96.1±2.0	98.8±3.4
Threonine	67.7±10.4	62.9±3.4	96.6±8.1	97.5±2.5
Valine	72.1±6.9	65.7±1.2	96.4±5.6	93.1±2.4
Dispensable				
Alanine	67.1±5.6	61.8±1.2	82.0±3.5	85.0±3.2
Aspartic acid	74.1±4.6[a]	63.1±0.5[b]	92.4±2.0	96.4±3.2
Glutamic acid	77.3±4.0	80.7±1.6	90.2±2.2	95.0±1.6
Glycine	69.8±3.1[a]	48.6±3.1[b]	94.7±4.0	88.6±4.6
Proline	73.7±3.5	68.4±8.8	81.9±3.5	86.4±1.9
Serine	69.6±7.9	63.6±3.3	95.0±5.3	94.4±4.8
Tyrosine	74.5±4.4	70.2±2.7	91.8±4.7	92.3±4.9

[ab] Means in the same row for either apparent or true digestibility with different superscripts differ ($P < .05$)

digestibilities determined in pure ingredients. This is more so in corn-based diets (Fan et al., 1995).

4.5 Implications

The homoarginine HA method is a relatively simple and inexpensive means for determining endogenous gut lysine losses and true lysine digestibilities in pigs fed practical type feeds. When the HA method is used to estimate the true ileal digestibilities of amino acids, other than lysine, assumptions have to be made about the amino acid composition of endogenous gut protein losses (ENL). True rather than apparent ileal amino acid digestibilities should be used when formulating swine feeds, but this will require further development of methods for routine estimation of ENL. Improvements in protein utilization should be sought via reducing ENL and improving true ileal amino acid digestibilities.

CHAPTER V

The Application of the Phenylalanine "flooding dose" Procedure to Measure Protein Synthesis in pigs

5.1 Abstract

The metabolic effect of infusing a large dose of phenylalanine into growing pigs was investigated in a study with five 22 kg Yorkshire barrows. Pigs were infused with a saline control or one of the two phenylalanine solutions (75 or 150 mmol L^{-1}) at a rate of 10 mL kg^{-1} body weight over a 12 min period. Blood samples (5 ml) were taken 10 min prior to the start of infusion, at the end of infusion and thereafter at regular intervals for 2 h. After determining packed cell volume (PCV), plasma was recovered for measuring concentrations of glucose, insulin and plasma free amino acids. Packed cell volume and glucose levels were not affected by infusing a large dose of phenylalanine ($P > .05$). Insulin concentration was similar ($P > .05$) for the two phenylalanine solutions at the end of infusion and significantly higher ($P < .05$) compared to the control. Plasma free amino acid levels were determined only in the pre- and end of infusion samples and in samples taken 10 and 20 min after the end of infusion. At the end of infusion, phenylalanine concentration was higher ($P < .05$) for the phenylalanine treatments and among all other amino acids only plasma tyrosine levels was increased ($P < .05$). Plasma amino acids levels were similar among treatments at other time periods. The results show that a flooding-dose of phenylalanine may not significantly alter the metabolic status (as shown by the

changes in levels of the key indicators of metabolism measured) of the pig and therefore its protein metabolism. These data supports the use of the flooding-dose method using labelled amino acids to estimate fractional rates of protein synthesis in tissues with high protein turnover rates in pigs.

5.2 Introduction

In conventional tissue protein synthesis studies, procedures involving continuous infusion of radiolabelled amino acids have been used. Typically, these procedures require a 6 to 8 h infusion period which renders them unsuitable for studying protein synthesis rates in tissues with high turnover rates such as visceral organs (Lobley et al., 1980). The limitations of these methods are further aggravated by the fact that the specific radioactivity (SRA) in the amino acids in the precursor pool for protein synthesis can not be determined with certainty. To minimise these limitations, Garlick et al. (1980) proposed a method that involves administration of a large ("flooding") dose of an amino acid and killing the animals a short time later (usually 10 min). The use of a flooding dose raises the SRA in the various free amino acid pools to similar levels thus eliminating the uncertainties regarding the precursor pool (Garlick et al., 1980). Furthermore, only a short incorporation time of labelled amino acid into tissue protein is allowed thus reducing the extent of recycling of label in tissues with high turnover rates. Despite these advantages, the flooding dose technique has been used mainly in studies with rodents (McNurlan et al., 1979; Garlick et al., 1980) and only in a few studies with domestic farm animals (Attaix et

al., 1986; Sève et al., 1986; Southorn et al., 1992).

One of the concerns of using the flooding dose technique is that infusing a large amount of an amino acid may cause perturbation in the metabolic status (eg hormonal or substrate levels) of the animal which in turn may alter rates of protein synthesis. Indeed Lobley et al. (1990) and Southorn et al. (1992) have reported such metabolic effects in sheep infused with large doses of different amino acids. In pigs, this technique has only been used in a study with piglets in which no attempt was made to establish the metabolic consequences of infusing large amounts of phenylalanine (Sève et al., 1986). It is, therefore, not known how administering a large amount of a given amino acid to a pig affects its metabolic status (i.e. the ability to utilize nutrients and to maintain normal physiological levels of various metabolites).

The present study was undertaken to investigate the metabolic impact of infusing a large dose of phenylalanine into growing pigs. This was achieved by examining changes in plasma insulin, glucose and amino acid levels and packed cell volume as indicators of metabolic homeostasis.

5.3 Materials and Methods

5.3.1 *Animals, Housing and Diets*

The use of animals in this study was reviewed and approved by the Animal Care Committee of the University of Guelph and animals were cared for according to the guidelines of the Canadian Council on Animal Care. Five growing barrows

(Yorkshire, from the University of Guelph Swine herd) with an average initial body weight of 18 kg were used in the present study. They were housed in individual metabolic crates with smooth, transparent side walls and tender foot floors in a temperature controlled room (20-22 °C). Pigs were fed a casein corn starch diet (same as Diet 1, Table 4.1) mixed with water in a 1:1 ratio in two equal amounts (at 08.00 and 20.00 h) Intake was restricted to 2.6 times maintenance energy requirement (ARC, 1981). Drinking water was provided ad libitum from a low pressure nipple. During the last 3 d prior to the start of infusion, the feeding schedule was changed to 3-hourly feeding so as to maintain steady state conditions (Lobley et al., 1992).

5.3.2 *Study Procedures*

After a 7 d adjustment period to the diet and new environment, pigs were surgically fitted with 2 silicon catheters (1.6 mm i.d., 2.41 mm o.d., Dow Corning, Midland, MG) in the right and left external jugular veins under halothane general anaesthesia for infusion and sampling of blood according to de Lange et al. (1990). Following a 7 d recovery period, each pig was subjected in a random order to 3 treatment infusions consisting of a 150 mmol L^{-1} solution of saline (control), 75 mmol L^{-1} (LPHE) or 150 mmol L^{-1} (HPHE) solutions of L-phenylalanine on 3 consecutive days. All solutions were filter-sterilised through 0.22 μ filters (Millipore, Bedford, MA) before use. Each solution was infused for a 12 min period starting 1.5 h after feeding at a rate of 10 ml/kg body weight from a Watson Marlow variable speed

peristaltic pump[3] (Watson-Marlow, Inc., Wilmington, MA). Blood samples (5 ml) were drawn at the beginning (baseline) and end of infusion period and at 10 min intervals thereafter for 1 h and at 90 and 120 min after the end of infusion. Samples were analyzed for packed cell volume and plasma levels of insulin, glucose and amino acids.

5.3.3 *Analytical Procedures*

Packed cell volume was determined by centrifuging blood in haematocrit tubes to tightly pack the red blood cells (Cunningham, 1992). Plasma was recovered by centrifuging at 1500 x g for 15 min and stored at -20 °C until required for analysis. Plasma insulin and glucose levels were determined by a radioimmunoassay (Coat-A-count, Diagnostic Products, Los Angeles, CA.) and the method of Trinder (1969) (Sigma Diagnostic Procedure No. 315), respectively. Plasma free amino acid concentrations before infusion, at the end of infusion (time 0) and at 10 and 20 min after the end of infusion were determined by the method of Bidlingmeyer et al. (1984). Briefly, 200 μL of plasma samples were deproteinized with 1 mL of 0.5 % trifluoroacetic acid in methanol after adding of 40 μL of 2.5 μmol/ml norleucine as an internal standard. The samples were then centrifuged at 5000 rpm for 5 min and the supernatant recovered for determining plasma free amino acids. After freeze drying the supernatant, 50 μL of a mixture of triethylamine:methanol:water (1:1:3)

[3] model 504S/RL IP55

was added and the samples freeze dried again for 1-2 h. Samples were then derivatized by adding 20 μL of a derivatising reagent (1:1:1:7 mixture of triethylamine : water : phenylisothiocyanate : methanol) and allowed to stand for 35 min before stopping the reaction by freezing the samples in liquid N. After freeze drying, samples were reconstituted in a sample diluent (95 % phosphate buffer plus 5 % acetonitrile), centrifuged and loaded on the HPLC for amino acid separation using a 3.9 mm x 30 cm Pico.Tag reverse-phase column (Millipore/Waters, Mississauga, ON) maintained at 48 °C.

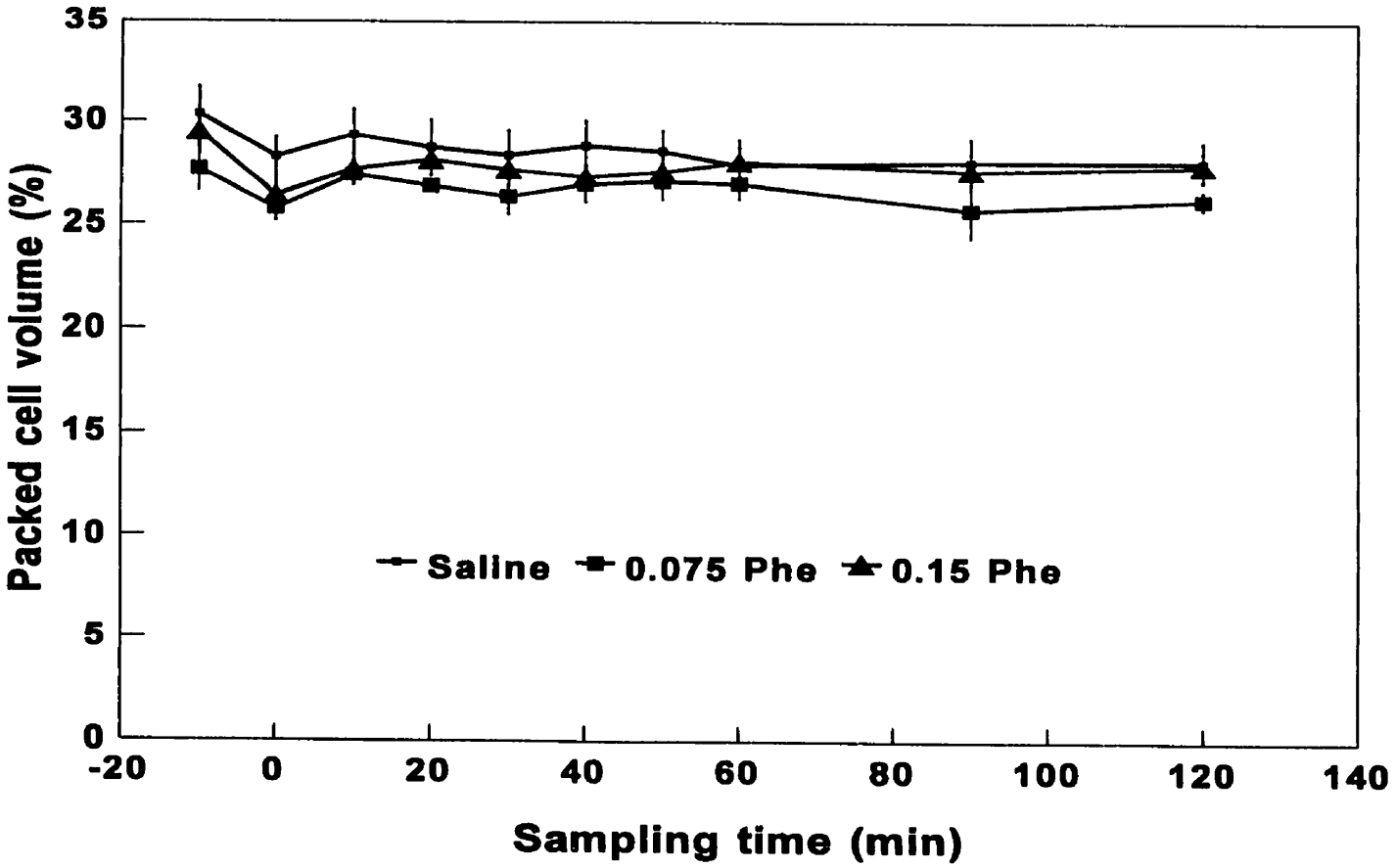
5.3.4 *Statistical analysis*

Data on blood parameters were analyzed by the GLM procedures of SAS (1985) with treatments and animals as sources of variation. Insulin, glucose and amino acid concentrations were compared at every sampling point using repeated measure analysis and polynomial contrast for control vs phenylalanine solutions and between the phenylalanine solutions. The mean of the phenylalanine treatments at each time and over time 10 min to 120 min were compared with control values. When a significant *F*-value ($P < .05$) was obtained means were separated using Scheffe multiple-range test (Steel and Torrie, 1980).

5.4 Results and Discussion

Packed cell volume was not influenced ($P > .05$) by the infusion of saline or phenylalanine solutions and it remained stable throughout the two h study period for

Figure 5.1. Packed cell volume (%) in growing pigs fed a casein-cornstarch diet and infused with saline or a flooding dose of phenylalanine (75 or 150 mmol L^{-1}) (mean ± SE).



all three treatments (Figure 5.1). A similar observation has been made in sheep given a flooding dose of phenylalanine (Southorn et al., 1992). These results indicate that a flooding dose of phenylalanine does not interfere with the amount of red blood cells per volume of blood and therefore general blood homeostasis or relative plasma volume. This implies that a flooding dose of phenylalanine as used in the present study is not a huge insult to the animal.

Plasma insulin concentrations increased by 35-51 pmol L^{-1} following infusion of phenylalanine solutions (Table 5.1). The LPHE solution elicited a higher but non-significant ($P > .05$) increase in plasma insulin levels compared to the HPHE solution (Table 5.1). Plasma insulin levels did not change when physiological saline was infused; it averaged 85.5 pmol L^{-1} over the 2 h study period. This was very similar to the baseline value of 84.4 pmol L^{-1} observed here and 89 pmol L^{-1} reported by Le Floc'h et al. (1995). Compared to the saline treatment, the impact of phenylalanine infusion was significant ($P < .05$) only at the end of infusion (0 min) and at 120 min after infusion, although insulin concentrations in phenylalanine infused pigs were numerically higher at most sampling times (Table 5.1). The observed difference in insulin levels between the control and phenylalanine treatments at 120 min is unusual and difficult to explain, although the fact that this may have been a direct result of phenylalanine infusion cannot be ruled out. This is more so considering that changes in plasma glucose and amino acid concentrations at this point were similar in all treatments (Table 5.2; Figure 5.2). Plasma insulin levels were similar ($P > .05$) between the two phenylalanine treatments at 120 min after infusion.

Table 5.1. Plasma insulin concentration (pmol L^{-1}) in growing pigs fed a casein corn starch diet and infused with either saline, 0.075 M or 0.15 M phenylalanine solutions

	Treatment			
Sampling time	Saline	0.075 M Phe	0.15 M Phe	SEM
Baseline	82.2	83.0	88.1	7.0
0[1]	81.6[b]	134.2[a]	122.9[ab]	14.8
10	93.3	78.0	96.9	9.2
20	83.7	144.2	110.6	19.1
30	88.5	118.4	118.6	20.6
40	87.0	110.3	90.7	15.4
50	74.1	106.5	86.4	15.9
60	94.3	113.6	113.5	21.8
90	94.3	82.3	109.9	14.0
120	76.0[b]	109.3[a]	116.3[a]	9.1

[1] time 0 denotes the end of the 12 min infusion period

[ab] means within a row bearing different superscript letters differ ($P < .05$)

SEM, pooled standard error of the means

Table 5.2. Plasma glucose concentration (mmol L^{-1}) in growing pigs fed a casein corn starch diet and infused with either saline, 0.075 M or 0.15 M phenylalanine solutions

	Treatment			
Sampling time	Saline	0.075 M Phe	0.15 M Phe	SEM
Baseline	5.90	6.26	6.53	.36
0[1]	5.94	6.14	6.23	.34
10	5.68	6.01	5.91	.32
20	5.61	6.23	6.13	.28
30	5.84	6.44	5.99	.32
40	5.79	6.28	5.84	.39
50	5.84	6.27	5.67	.33
60	5.69	6.24	5.67	.39
90	5.84	6.06	6.01	.44
120	5.87	6.50	6.35	.40

[1] time 0 denotes the end of the 12 min infusion period.

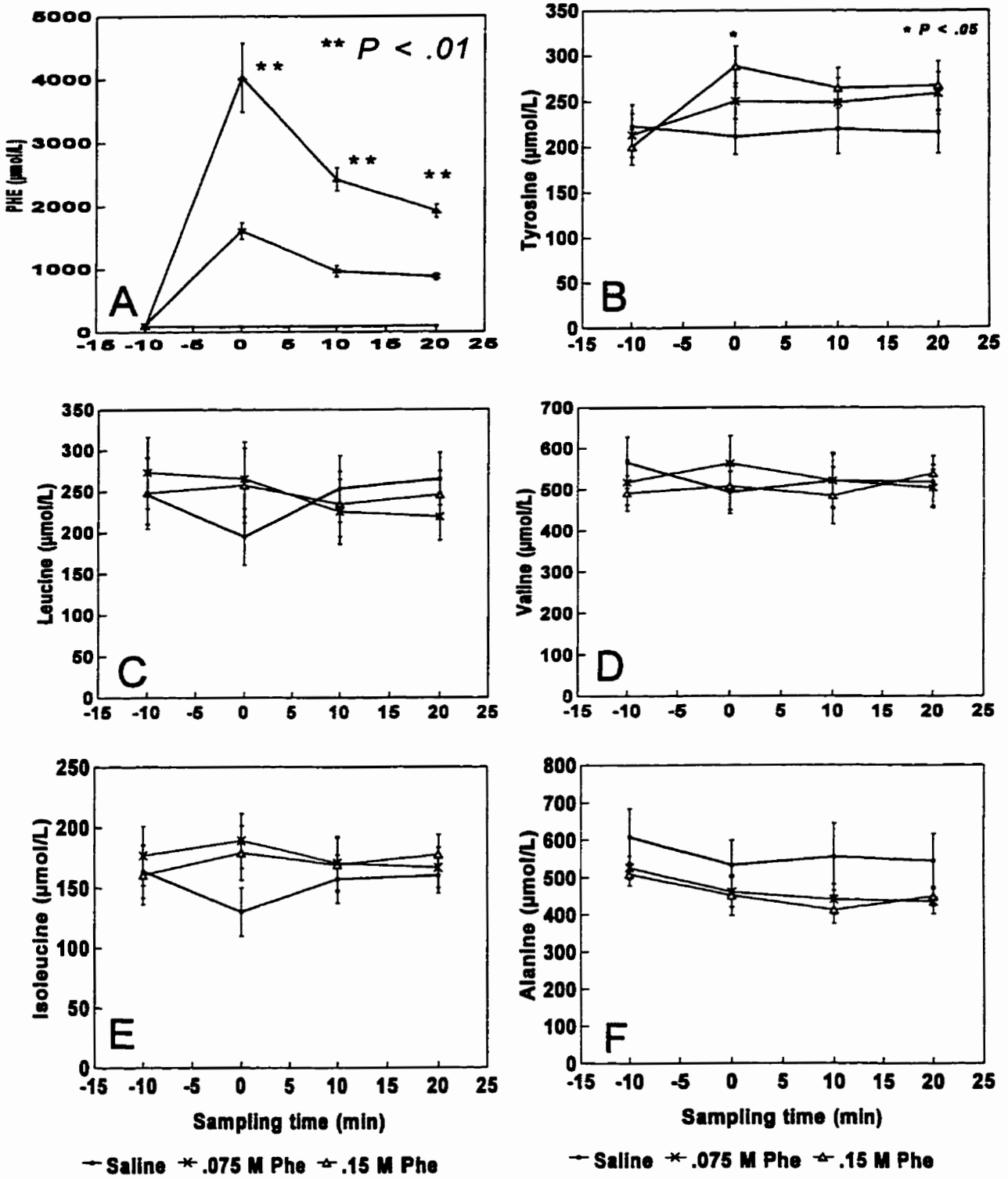
The effects of infusing a flooding dose of an amino acid into animals on plasma insulin concentration has been reported in a number of previous studies (Lobley et al., 1992; Southorn et al., 1992). In these studies, as in the current study, infusing a large amount of phenylalanine cause perturbations in the plasma insulin concentration. The magnitude of change in plasma insulin levels depends, among other factors, on the rate of infusion; it is more pronounced if the treatment is administered over a short time as opposed to a longer infusion period (Lobley et al., 1992; Southorn et al., 1992). In the current study the phenylalanine flooding dose was given over a 12 min period (same as the long period used by Southorn et al., 1992) which may have been responsible for the mild perturbation observed. It should be noted that the plasma insulin levels were likely already elevated in all animals since they were frequently consuming a highly digestible high starch diet; a situation that may have lessened the response to phenylalanine infusion. Whether actually increased plasma insulin concentration has a direct influence on the rate of protein synthesis in the growing pig is unclear. However, the surge in insulin levels seen here, as in most previous studies, lasted only for a short time (usually < 10 min) and was unlikely to cause major changes in rates of protein synthesis. Indeed, Garlick et al. (1994) could not find evidence in the literature that the transient insulin spike due to infusion of a flooding dose of an amino acid influences protein synthesis in rats and humans. In a similar literature review, Rennie et al. (1994) also asserted that the observed stimulation of protein synthesis in muscle following a flooding dose of leucine cannot be ascribed to a stimulation of hormones such as insulin. Earlier, in

a study with rats, Garlick et al. (1983) found that although plasma insulin level was significantly increased by a factor of 4 above baseline levels, this did not change the rate of protein synthesis. In the current study plasma insulin levels were increased by a maximum factor of 1.6 above control levels; an effect that is unlikely to have influenced rates of protein synthesis in these animals. It is also important to note that where insulin has been shown to affect protein synthesis, the translational efficiency (i.e. g protein synthesis d^{-1} g RNA^{-1}) may have been suboptimal because rats used in that study were starved (Reeds, 1988; Lobley, 1993). It is important to note that the effect of insulin on protein synthesis in gut tissues has not been determined. Also the differences in fractional rates of protein synthesis determined with either the flooding-dose or continuous infusion techniques are mainly due to methodological differences as opposed to effects on the hormonal changes (Rennie et al., 1994).

Plasma glucose concentrations were not affected ($P > .05$) by any of the three treatments and concentrations remained stable throughout the study (Table 5.2) and averaged 6.03 mmol L^{-1} over the 2 h experimental period. Plasma glucose levels are reported to range from 5.48 to 5.64 mmol L^{-1} in growing pigs (Antinmo et al., 1978; Le Floc'h et al., 1995) which is close to levels observed in the present study. That infusing a large phenylalanine dose has no significant impact on plasma glucose levels has been demonstrated in sheep as well (Southorn et al., 1992). This observation does illustrates that the changes in insulin levels are indeed very small.

Trends in plasma free amino acid concentrations are presented in Figure 5.2. Infusing a large amount of physiological saline did not alter ($P > .05$) concentrations

Figure 5.2. Concentrations of selected plasma free amino acids in growing pigs fed a casein-cornstarch diet and infused with saline or a flooding dose of phenylalanine solutions (75 or 150 mmol L^{-1}).



of most plasma free amino acids. In response to phenylalanine infusion, plasma free phenylalanine was increased by an average of 10 and 32 times baseline values, respectively, for LPHE and HPHE solutions. The plasma free phenylalanine concentrations differed ($P < .05$) among the three treatments at 0, 10 and 20 min after the end of infusion (Figure 5.2a). Of all other amino acids, only tyrosine concentration was increased ($P < .05$) above control levels by infusing the HPHE solution. This was expected as phenylalanine is converted to tyrosine (Figure 5.2b). However, this impact was only evident at the end of infusion. There was no distinct effect ($P > .05$) of phenylalanine infusion on the concentrations of all other amino acids in plasma. The baseline levels of phenylalanine and tyrosine were similar during all three experimental days thus indicating that the impact of phenylalanine infusion had subsided within 24 h.

The general lack of effect of a flooding dose of phenylalanine on plasma concentration of other amino acids contradicts previous reports with other species showing that a flooding dose of an amino acid does cause perturbation in concentrations of other plasma free amino acids (Lobley et al., 1990; Southorn et al., 1992). It could be expected that if a flooding dose of phenylalanine causes changes in plasma amino acid levels, then large neutral amino acids could be affected most as they share the same transport system with phenylalanine which will have a competitive advantage when present in large amounts (Christensen, 1990). Based on the present data, a flooding dose of phenylalanine administered over a 12 min period into growing pigs does not seem to cause significant changes in plasma amino acid

levels; which again suggest that the insulin effect in these animals was minor. It has been suggested that a flooding dose of an amino acid in itself may interfere with the process of protein synthesis by changing the amino acid composition of the precursor pools and therefore the availability of amino acids for protein synthesis (Rennie et al., 1994). However, the current data seem to indicate that this may not be of any major concern in growing pigs since only a minor insulin effect was observed and the plasma free amino acid concentrations remained essentially unaltered.

5.5 Implications

The current results show that a flooding dose of phenylalanine given to growing pigs does not cause significant changes in their metabolic status as shown by changes in plasma insulin, glucose and amino acid concentrations. Giving a large-dose of phenylalanine in itself may, therefore, not influence fractional rates of protein synthesis in the gut. It was concluded that the phenylalanine flooding-dose method is an appropriate technique for measuring protein synthesis rates in tissues with high protein turnover rates in growing pigs.

CHAPTER VI

Effect of diet type on gut protein synthesis in growing pigs as measured with the phenylalanine flooding dose technique.

6.1 Abstract

The effect of diet on protein synthesis rates (PSR) in visceral organs (liver, pancreas, duodenum, jejunum, ileum, colon, and caecum) and skeletal muscle of growing pigs was assessed using a flooding dose of L-[ring 2, 6-^{3}H] phenylalanine (PHE). Twelve Yorkshire barrows with an average initial body weight (BW) of 18 kg and with catheters in the right and left jugular veins were fed either a casein-cornstarch (CC) or barley-canola meal (BCM) based diets formulated to a similar DE:CP ratio. Pigs were fed at 2.6 x maintenance energy requirements twice daily for 3 weeks and then every 3 h for 3 d before measurement of PSR. Each pig was infused with 10 mL/kg BW of a 150 mM PHE solution containing 230 MBq/L PHE for 12 min and then killed 20 min later. Blood samples (5 ml) were taken for determining plasma insulin and glucose levels and specific radioactivity (SRA) in plasma free PHE. CC pigs had higher ($P < .05$) plasma glucose (5.8 ± .2 vs 5.2 ± .3 mmol/L) and insulin (80.6 ± 9.3 vs 55.7 ± 5.8 pmol/L) levels than BCM pigs. In all pigs, the SRA of plasma free PHE rose to a plateau value within 3 min after the start of the infusion and did not change significantly ($P > .05$) throughout the experiment. Fractional PSR (Ks, %/d) values based on SRA in plasma or intracellular free PHE were similar ($P > .05$) in all tissues except pancreas ($P < .05$). Diet

affected Ks in liver ($P < .01$) and colon ($P < .05$) but not in the other tissues. Based on the SRA in plasma, liver Ks were 85.4 ± 11.0 vs 60.5 ± 5.2 in CC and BCM pigs, respectively; these values were 82.3 ± 4.7 vs 98.2 ± 5.8 in the colon. Average Ks for other organs were: pancreas, 132.1; duodenum, 93.9; jejunum, 81.9; ileum, 89.3; caecum, 91.2; muscle, 28.3. The absolute amount of protein synthesis (g/d) was higher ($P < .05$) in the liver and pancreas of the CC pigs compared to BCM pigs. No dietary effect was observed in all other organs ($P > .05$). The present results seem to suggest that feeding diets that induce high endogenous protein losses does not affect PSR in the visceral organs and skeletal muscle of growing pigs.

6.2 Introduction

There is a continuous secretion of large amounts of endogenous protein into the gut lumen resulting from the processes of digestion and maintenance of the animal's organs and tissues (Tamminga et al., 1995). While a substantial portion of endogenously secreted protein is reabsorbed, about 25 % passes the terminal ileum thus becoming unavailable to the pig (Souffrant, 1991). Results of several recent studies (Butts et al., 1993a; Marty et al., 1994; Schulze et al., 1994a, b) have shown that these endogenous gut protein losses are much higher than previously estimated using the conventional methods of feeding a protein-free diet or mathematical regression to zero protein intake. It has further been shown that these losses are higher when feeding diets containing certain feedstuffs and/or anti-nutritional factors (de Lange et al., 1989a; Schulze et al., 1994; Jansman et al., 1995).

Studies on endogenous protein secretion in pigs have concentrated on its flow at the distal ileum under various experimental conditions but not its possible influence on protein synthesis rates (PSR) in gut tissues (Nyachoti et al., 1997a). Because of the significant implications of gut PSR on amino acid and energy requirements of the animal (Moughan, 1995; Nyachoti et al., 1997a), it is important to understand how PSR in visceral organs is influenced by diets that stimulate high endogenous gut nitrogen losses.

Visceral organs have high protein turnover rates as compared to other body tissues (Lobley et al., 1980). In order to study the relationship between endogenous gut protein loss and PSR in visceral organs, a method is thus required for estimating PSR of proteins with high turnover rates. Conventional methods, based on a continuous infusion of labelled amino acids, underestimate PSR because they do not reflect accurately the PSR of proteins that turn over rapidly or that are exported from the tissues. Furthermore, conventional methods present problems with selecting the amino acid precursor pool. A procedure involving injection of a flooding-dose of isotope-labelled amino acids (McNurlan et al., 1989; Garlick et al., 1980) has been used to estimate PSR in individual rat tissues within 10 min, thus allowing measurements to be made in tissues such as the liver and small intestines which have high protein turn over rates. Use of the flooding-dose method to measure PSR in large animals is not feasible without changes largely because of costs. Southorn et al. (1992) have demonstrated the use of this method to estimate intestinal and liver PSR in sheep by decreasing the specific radioactivity to one tenth of the level used by

Garlick et al. (1980) and increasing the incorporation time from 10 to 20, 40 or 60 min. The 20 min interval values were higher and most likely representative of rates of rapidly turning over proteins. Other than the study by Sève et al. (1986) with baby pigs, the flooding-dose technique is yet to be used in older pigs. Applying this adapted method to estimate PSR in the visceral organs of the pig should provide an insight into the significance of endogenous protein losses in pig nutrition.

The current study aimed at using the phenylalanine flooding-dose technique to estimate PSR in the visceral organs of growing pigs fed two different diets formulated to induce either low or high endogenous gut protein losses.

6.3 Materials and Methods

6.3.1 *Animals, Housing and Diets.*

Growing Yorkshire barrows with an average initial body weight (BW) of 18 kg were obtained from Arkell Swine Research unit at University of Guelph for use in the present study. They were housed in individual metabolic crates with smooth, transparent side walls and tender-foot floors in a temperature controlled room (20-22 °C) and allowed to adapt to their new environment and diets for a minimum of 2 week before undergoing surgery and a further 1 week between surgery and the measurement of PSR.

Two diets based on either casein-cornstarch (CC) or barley-canola meal (BCM) (same as Diet 1 and 4 used in Chapter IV, Table 4.1) were used in this experiment. The diets were formulated to meet or exceed NRC (1988) requirements

for vitamins and minerals and to contain a similar digestible energy to protein ratio. Pigs were given their daily feed allowance in two equal amounts (at 08.00 and 20.00 h) and intake was restricted to 2.6 times maintenance energy requirement (ARC, 1981). During the last 3 d prior to the start of infusion, the feeding schedule was changed to 3-hourly feeding so as to maintain steady state conditions (Lobley et al., 1992).

6.3.2 *General Conduct of Study*

Surgical procedures, preparation of infusate and infusion procedures used in the current study were exactly the same as described in Chapter V of this thesis. Six animals were assigned at random to one of the two diet treatments. Starting 1.5 h after feeding, each pig was infused with a solution of unlabelled phenylalanine (150 mmol/L) in water containing 230 MBq/L L-[ring 2, 6-^{3}H] phenylalanine (American Radiolabelled Chemicals Inc., St. Luois, MO) for a 12 min period at a rate of 10 mL/kg BW to give a dose of about 2.3 MBq/kg BW. This dose level was almost twice that used by Southorn et al. (1992) and one eighth that used in the original study by Garlick et al. (1980). Southorn et al. (1992) found significant differences in phenylalanine specific radioactivity (SRA) in plasma and intracellular free pools and only a 57 % flooding level in the liver. Increasing the amount of radioactivity infused in this study was done so as to minimize such differences and increase the level of flooding in all tissues studied. After 20 min, timed from the end of infusion, pigs were killed by a lethal injection of sodium pentobarbitone via the infusion catheter. This

method of killing pigs was used so as to minimize sloughing of the mucosal tissue following death (Moughan and Smith, 1987). The start of infusion was sequentially delayed to allow a 3 h interval between animals. Blood samples (5 mL) were drawn 10 min before the start of infusion, at 3 min intervals during infusion, and at 5 min intervals after infusion until slaughter. Time 0 indicates the start of the infusion period. A sample of the liver, pancreas, duodenum (taken as the first 1 m of the small intestine (SI)), jejunum, ileum (taken as the last 1 m of the SI), colon and caecum were quickly excised immediately after death and chilled with ice-cold irrigation saline to minimize postmortem metabolism (Southorn et al., 1992). Each sample was then blotted with an absorbent paper, weighed (except for muscle) and then wrapped in an aluminum foil before rapid freezing in liquid nitrogen. Measurements of PSR in muscle were done to allow comparisons with other studies. The time from dissecting to chilling of each sample was recorded accurately and considered in the calculation of protein synthesis. Sampled organs were weighed to allow calculation of total protein synthesis. The whole sampling procedure was accomplished in less than 5 min following death. The experimental protocol was approved by the Animal Care Committee at the University of Guelph and pigs were cared for according to the guidelines of the Canadian Council on Animal Care.

6.3.3 *Sample Preparation and Analyses*

Blood samples were centrifuged at 1500 x g for 15 min and the recovered plasma subdivided into three batches before being stored at -20 °C until required for

analysis. Plasma insulin concentration was determined using a radioimmunoassay (Coat-A-count, Diagnostic Products, Los Angeles, CA.) while plasma glucose concentration was measured using the method of Trinder (1969) (Sigma Diagnostic Procedure No. 315). Plasma free phenylalanine concentration was determined using procedures similar to Bidlingmeyer et al. (1984). Briefly, 200 μL of plasma samples were deproteinized with 1 mL of 0.5 % trifluoroacetic acid in methanol and the precipitated protein removed by centrifugation at 13,000 rpm for 5 min. After freeze drying the supernatant, 300 µL of a redrying mixture (1:1:3 triethylamine : methanol : water) was added and the mixture freeze dried again for about 2 h. To the dried samples, 100 µL of a derivatising reagent (1:1:1:7 water : triethylamine : phenylisothiocyanate (PITC): methanol) was added and the reaction allowed to proceed for 35 min. At completion, the reaction was stopped by freezing the samples in liquid N followed by freeze drying. After drying, samples were reconstituted in 300 µL of a 95 % phosphate buffer in methanol, centrifuged at 13,000 rpm for 5 min to remove particulate matter and the supernatant kept frozen at - 20°C until required for analysis by high performance liquid chromatography (HPLC) (usually within two weeks).

Tissue samples (1 g) were homogenised in 5 mL of 2% (w/v) perchloric acid with an Ultra-Turax T25 tissue disrupter (Janke & Kunkel, IKA Labortechnik, Staufen, Germany) and then centrifuged at 1500 x g for 15 min. The supernatant was recovered and kept frozen at - 20°C until required for further processing. The precipitate was washed twice with 8 mL of 2 % perchloric acid, resuspended in 8 mL

of 1 M sodium hydroxide and then left to stand in a water bath set at 37 °C for 1.5 h to solubilize the proteins. The solubilized protein was recovered by adding 4 mL of 20 % cold perchloric acid and letting the mixture stand on ice for 20 min. Precipitated protein was recovered by centrifugation at 2000 x g for 15 min followed by 2 washings with 8 mL of 2 % perchloric acid.

The phenylalanine content in the precipitated protein pellet was determined following hydrolysis in 10 mL of 6 *N* hydrochloric acid in sealed, nitrogen flushed tubes at 110 °C for 24 h. The hydrolysed samples were left to cool for 30 min and then thoroughly mixed before transferred into 125 mL Erlenmeyer flasks. Each tube was rinsed twice with deionized water and the washing added to their respective hydrolysates. The samples were then diluted to approximately 50 mL using deionized water and mixed thoroughly by swirling before filtering about 4 mL of each sample through 0.22 μ filters with low protein binding ability (Millipore Corp. Mississauga, ON). One mL of the filtered hydrolysate samples and all of the supernatant samples were cleaned through a Dowex cation exchange resin to remove salts and other contaminants that interfere with derivatization of amino acids with PITC (Sève et al., 1986; Southorn et al., 1992). Briefly, 2 mL of Dowex resin in 3 cc syringes were washed with 10 mL of deionized water and then the samples loaded followed with another washing with 10 mL of water. Amino acids were eluted with 6 mL of ammonium hydroxide which was subsequently evaporated to dryness. The dry hydrolysate and supernatant samples were processed in the same manner as the plasma samples for HPLC analysis. Plasma and supernatant samples were analyzed

in duplicate while hydrolysates were analyzed in triplicate. Thirty-five μL of each sample were injected for amino acid separation using a 3.9 mm x 30 cm Pico.Tag reverse phase column (Millipore/Waters, Mississauga, ON) maintained at 48 °C. The run time was shortened from 90 to 30 min and the gradients modified to allow a clear separation of the phenylalanine peak. The phenylalanine peak was collected over a time window starting and ending at least 1 min before and after the elution time of phenylalanine using a Waters Fraction Collector (Millipore/Waters, Milford, MA, USA). The level of radioactivity in the collected fractions was determined by liquid scintillation counting on a liquid scintillation system[4] (Beckman Instruments, Fullerton, CA, USA) after adding 10 mL of Biodegradable Counting Scintillant (Amersham Canada Ltd., Oakville, ON). With each set of samples (~30 samples per run), phenylalanine standards were analysed in duplicate. A standard curve relating peak areas to the amount of phenylalanine injected was derived by injecting 5, 10, 15, 25, and 35 μL of a standard solution containing 2.5 μmol phenylalanine per mL. The amount of phenylalanine in the collected fractions from the samples was then determined from the standard curve and related to the amount of radioactivity present. Tissue protein content was measured according to Smith et al. (1985) by the calorimetric reaction with bicinchoninic acid[5].

[4] Model LS 600

[5] Sigma Chemicals, St. Louis, MO

6.3.4 *Calculations and Statistical Analysis*

The level of flooding achieved in different tissues was calculated by expressing the phenylalanine SRA in the intracellular free pool of each tissue as a percentage of phenylalanine SRA in plasma. Flooding level was also assessed by expressing the phenylalanine SRA in plasma as a percentage of phenylalanine SRA in the infusate. Visceral organ weights were also expressed relative to empty body weight (EBW). For calculating EBW, gut fill in the CC- and BCM-fed pigs was assumed to be 3% and 5%, respectively (Mohn and de Lange, unpublished observation; Jorgensen et al. 1996). Fractional protein synthesis rates (Ks) were calculated according to the method of Garlick et al. (1983) using the following equation:

$$Ks = \frac{SRA_h \times 100}{SRA_f \times t}$$

where K_s is expressed as a percentage of the tissue protein pool synthesized per day; SRA_h is the specific radioactivity of bound phenylalanine in the protein hydrolysate from the tissue, SRA_f is the phenylalanine specific radioactivity in the precursor pool used for calculation and t is the incorporation time in d of ^{3}H-phenylalanine into protein. Fractional protein synthesis rates were calculated in four different ways; assuming t to be the time from the start or end of infusion to chilling of tissue samples, and the SRA in the precursor pool for protein synthesis to be similar to the SRA in either the plasma free or intracellular free pool. The phenylalanine SRA in plasma was averaged over the various sampling times from 3 min after the start of infusion to the slaughter time. The absolute PSR in grams of protein per d were

calculated by multiplying the Ks with the total protein content present in each tissue on the d of the experiment. They were also expressed as grams of protein per d per kg $BW^{0.75}$ to adjust for differences in final BW and to allow comparison with data from other studies.

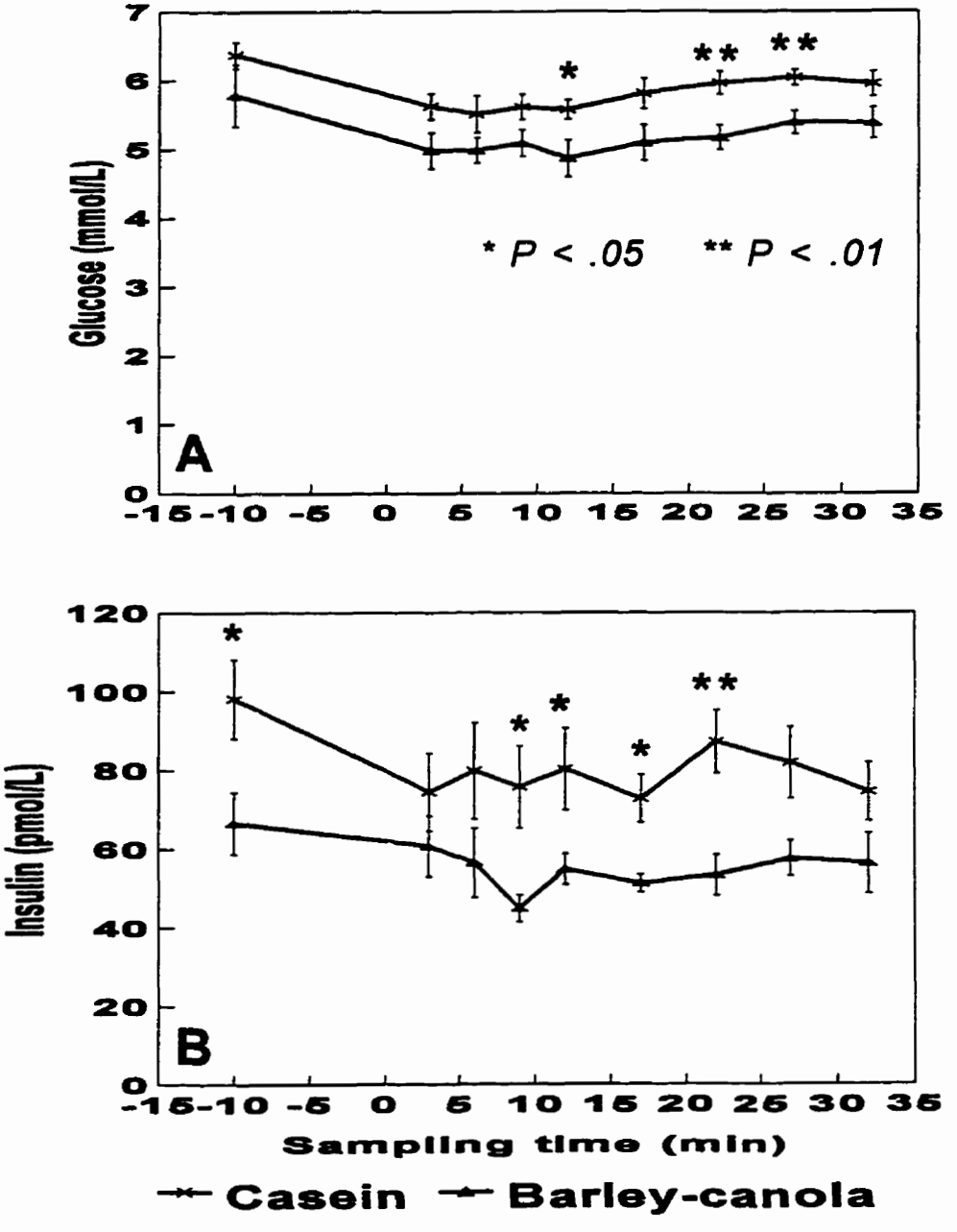
Data were analyzed by the GLM procedures of SAS (1985). Plasma insulin, glucose and phenylalanine levels were compared at all sampling times using repeated measure analysis of variance. Treatment means for protein synthesis and level of specific radioactivity in the plasma or intracellular precursor pool were compared by Student's t test (Steel and Torrie, 1980). Means were declared significantly different at a probability level of $P < .05$.

6.4 Results

All pigs readily consumed their experimental diets. They all quickly recovered from surgery and consumed all their daily feed allowances on the day of surgery. They all remained healthy and grew normally throughout the experiment; averaging (mean ± SD) 27.1 ± 0.8 and 23.7 ± 0.5 kg BW for the CC and BCM fed pigs, respectively, at the conclusion of the trial.

Figure 6.1A shows the plasma glucose concentration during and after the infusion of the flooding-dose of ^{3}H-phenylalanine. Averaged over the entire experimental period, the diet effect on plasma glucose concentration was significant ($P < .05$); at most sampling times it was higher in the CC fed pigs than in the BCM fed pigs. Within each diet there was no change ($P > .10$) in plasma glucose

Figure 6.1. Plasma glucose (A) and insulin (B) concentration in growing pigs fed either a casein-cornstarch- or barley-canola meal-based diet and infused with a flooding dose of 150 mmol L^{-1} of ^{3}H-phenylalanine solution at a rate of 10 mL/kg body weight for 12 min starting at time 0 (mean ± SE).



concentration during and after infusion and no significant diet and time interaction was observed ($P > .10$). Over the entire study period, it averaged 5.78 ± 0.18 and 5.19 ± 0.25 mmol L^{-1} of plasma for the CC and BCM fed pigs, respectively. The trend for plasma insulin concentration is presented in Figure 6.1B. There were significant ($P < .01$) differences in plasma insulin concentration between the two dietary treatments; the CC fed pigs had higher plasma insulin levels at most sampling times than those consuming the BCM diet. Within each diet treatment, there was a slight but significant drop ($P < .05$) in insulin concentration from baseline values (i.e. those obtained 10 min prior to the start of the infusion) within 3 min after the start of the infusion but this remained relatively stable for the rest of the study. There was no significant diet and time interaction in plasma insulin levels ($P > .10$).

The plasma free phenylalanine concentration rose rapidly during the infusion period reaching a maximum concentration of about 2960 μmol L^{-1} at the end of infusion in the casein fed pigs and 3090 μmol L^{-1} at 6 min after infusion in the BCM fed pigs. It then dropped quickly before levelling off at about 1586 and 1787 μmol L^{-1} of plasma, respectively for the casein and BCM fed pigs (Figure 6.2). The pattern was very similar for the two dietary treatments. Plasma phenylalanine concentrations were higher ($P < .05$) in the BCM fed pigs at some sampling times than in the CC fed pigs. The SRA of phenylalanine in plasma rose to a plateau value within 3 min of starting the infusion and did not change ($P > .10$) until the end of the study (Figure 6.3). There were no differences ($P > .10$) in plasma phenylalanine SRA between the casein and BCM fed pigs at any sampling time. The level of flooding achieved in the

Figure 6.2. Concentration of plasma free phenylalanine in growing pigs fed either a casein-cornstarch- or barley-canola meal-based diet and infused with a flooding dose of 150 mmol L^{-1} of ^{3}H-phenylalanine solution at a rate of 10 mL/kg body weight for 12 min starting at time 0 (mean ± SE).

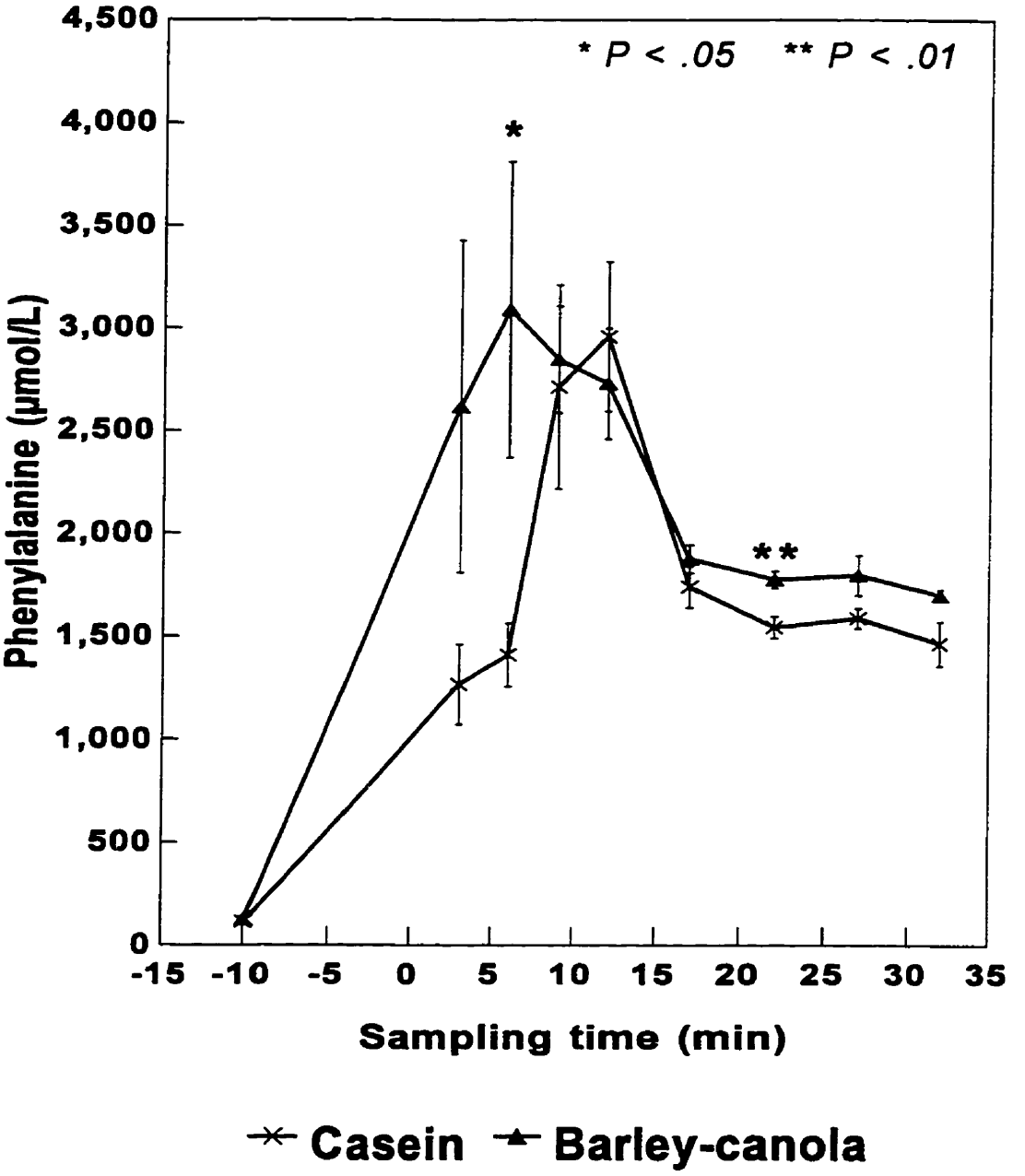


Figure 6.3. Plasma free phenylalanine specific radioactivity in growing pigs fed either a casein-cornstarch- or barley-canola meal-based diet and infused with a flooding dose of ^{3}H-phenylalanine (150 mmol L^{-1}, 1.5 Bq/nmol) solution at a rate of 10 mL/kg body weight for 12 min starting at time 0 (mean ± SE).

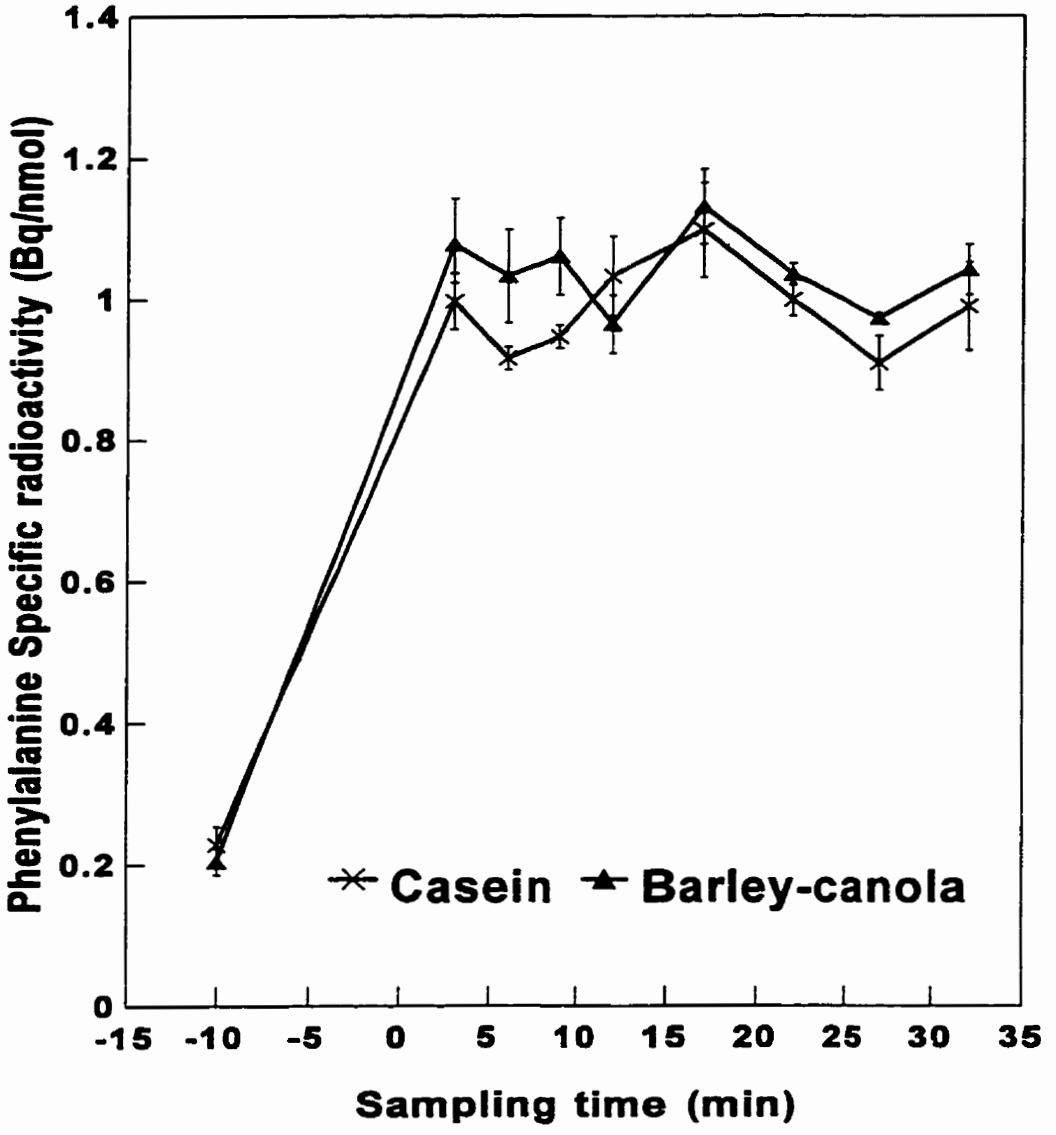


Table 6.1. Level of flooding (%) achieved in the visceral organs and skeletal muscle of growing pigs fed either a casein-cornstarch or barley-canola meal-based diet and infused with a flooding-dose of ^{3}H-phenylalanine (150 mmol L^{-1}, 1.5 Bq/nmol) solution at 10 mL/kg BW for 12 min (mean ± SE).

	Flooding[1]	
Tissue	Casein	Barley-canola
Liver	83.1 ± 1.9^{ab}	83.6 ± 1.9^{a}
Pancreas	67.8 ± 2.9^{c}	67.7 ± 5.2^{b}
Duodenum	75.8 ± 1.8^{bc}	80.7 ± 3.3^{a}
Jejunum	76.6 ± 3.3^{abc}	76.8 ± 4.1^{ab}
Ileum	85.5 ± 3.1^{a}	79.8 ± 3.1^{ab}
Colon	75.7 ± 2.9^{bc}	71.7 ± 2.9^{ab}
Caecum	79.8 ± 3.1^{ab}	80.2 ± 4.7^{ab}
Muscle	82.1 ± 3.2^{ab}	78.1 ± 3.0^{ab}

[1] determined by expressing the SRA of phenylalanine in the intracellular free pool as a percentage of the SRA in plasma.

^{abc} means ± SE within a column bearing dissimilar superscript letter differ ($P < .05$)

intracellular free pool in the studied organs is presented in Table 6.1. The levels of flooding in all tissues were similar ($P > .05$) for the two dietary treatments. In both treatments, the lowest ($P < .05$) flooding level (~67 %) was observed in the pancreas. The flooding levels calculated by expressing mean phenylalanine SRA in plasma as a percentage of phenylalanine SRA in the infusate were (mean±SE) 72.4 ± 1.3 % and 74.9 ± 1.4 %, for the casein and BCM diets, respectively. These values did not differ ($P > .10$).

The wet weights of all the organs studied except for muscle are presented in Table 6.2. The CC-fed pigs had significantly heavier ($P < .05$) livers than those consuming the BCM diet. Barley-canola meal fed pigs tended to have heavier ($P < .10$) duodena and colons compared to the CC-fed pigs whose jejunum tended to be heavier ($P < .10$) compared to the BCM-fed pigs. Ileum, pancreas and caecum were not significantly different between the 2 diets ($P > .10$). Table 6.2 also presents the weights of the visceral organs relative to EBW. The relative weights of duodenum and colon were significantly higher ($P < .05$) in BCM-fed pigs than in CC-fed pigs. The relative weight of the caecum tended to be heavier ($P < .08$) in BCM-fed pigs than in CC-fed pigs (Table 6.2).

Table 6.3 presents the Ks based on 20 or 32 min of incorporation time. Among the organs studied, dietary treatment affected Ks only in the liver ($P < .01$) and colon ($P < .05$). Within the two treatments, the pancreas and muscle had the highest and lowest Ks, respectively, compared to other tissues. The Ks in the

Table 6.2. Wet weights of visceral organs of growing pigs fed either a casein-cornstarch or barley-canola meal-based diet.

Tissue	Weight (g)		Weight (g/kg empty BW)	
	Casein	Barley-canola	Casein	Barley-canola
Liver	775.4 ± 30.9[a]	675.6 ± 15.2[b]	29.6 ± 1.1	30.0 ± 0.5
Pancreas	44.9 ± 6.0	36.0 ± 5.1	1.7 ± 0.3	1.6 ± 0.2
Duodenum	54.1 ± 5.0	65.1 ± 2.7	2.1 ± 0.2[b]	3.0 ± 0.1[a]
Jejunum	606.9 ± 24.9	546.9 ± 13.6	23.2 ± 1.0	24.3 ± 0.7
Ileum	66.7 ± 3.5	67.4 ± 7.0	2.5 ± 0.1	2.9 ± 0.3
Colon	248.4 ± 18.2	302.4 ± 22.0	9.5 ± 0.7[b]	13.5 ± 1.1[a]
Caecum	55.7 ± 5.9	60.6 ± 5.7	2.1 ± 0.2	2.7 ± 0.2

[ab] means ± SE in a row for weights (g) or relative weights bearing different superscript letter differ ($P < .05$).

Table 6.3. Fractional rates (%/d ±SE) of protein synthesis in visceral organs and muscle of growing pigs fed either a casein-cornstarch- or barley-canola meal-based diet and determined assuming the SRA in either the intracellular (I) or plasma (P) free phenylalanine pool is the same as that in precursor pool for protein synthesis

Tissue		20 min incorporation time		32 min incorporation time	
		Casein	Barley-canola	Casein	Barley-canola
Liver	I	101.6 ± 8.8[a]	67.5 ± 5.1[b]	65.2 ± 5.7[a]	42.8 ± 3.2[b]
	P	85.4 ± 7.4[a]	60.5 ± 5.2[b]	54.8 ± 4.8[a]	38.4 ± 3.3[b]
Pancreas	I	185.5 ± 11.0[x]	162.3 ± 10.8[x]	119.8 ± 7.5[x]	106.6 ± 7.4[x]
	P	141.2 ± 11.6[y]	122.9 ± 14.5[y]	93.2 ± 7.9[y]	80.7 ± 9.7[y]
Duodenum	I	121.9 ± 14.4	111.6 ± 9.2	78.7 ± 9.0	71.4 ± 5.7
	P	94.8 ± 9.9	93.0 ± 8.3	61.9 ± 6.3	59.4 ± 5.1
Jejunum	I	109.6 ± 13.9	98.2 ± 9.7	71.4 ± 9.0	64.4 ± 6.7
	P	86.5 ± 9.4	77.3 ± 7.5	56.9 ± 6.1	50.7 ± 5.0
Ileum	I	105.1 ± 12.4	104.0 ± 7.9	69.2 ± 7.9	67.7 ± 5.2
	P	91.0 ± 8.8	87.5 ± 5.1	60.5 ± 5.5	56.9 ± 3.4
Colon	I	110.1 ± 5.3[b]	137.5 ± 7.3[a]	72.9 ± 3.4[b]	90.3 ± 5.0[a]
	P	82.3 ± 4.7	98.2 ± 5.8	71.9 ± 16.9	64.5 ± 3.9
Caecum	I	101.3 ± 10.7	123.1 ± 16.8	66.5 ± 6.9	80.5 ± 10.4
	P	83.6 ± 10.6	98.5 ± 12.9	55.5 ± 6.6	65.2 ± 8.6
Muscle	I	34.9 ± 4.7	33.4 ± 3.0	23.3 ± 3.1	22.1 ± 1.9
	P	29.1 ± 4.6	27.2 ± 2.3	19.4 ± 3.0	17.9 ± 1.6

[ab] means ± SE in a row within incorporation time bearing different superscript letter differ ($P < .05$).
[xy] means ± SE within a column and within the same tissue bearing different superscript letter differ ($P < .05$).

pancreas calculated using phenylalanine SRA in the intracellular free pool were higher ($P < .05$) than those calculated using plasma free phenylalanine SRA for both treatments. In all other tissues intracellular free pool based values were only numerically higher than those derived based on the plasma free pool. As expected, fractional rates of protein synthesis were lower after 32 min as compared to 20 min of incorporation (Table 6.3). However, the trends were exactly the same as observed at the 20 min incorporation period.

The percentage of protein in the various organs ranged from 6.7 ± 0.7 % and 6.1 ± 0.5 % in the colon to 11.3 ± 1.4 % and 10.3 ± 1.7 % in the liver of the CC BCM fed pigs, respectively. The total amount (g) of protein present in each organ on the day the protein synthesis was measured was not different ($P > .05$) between the two dietary treatments (Table 6.4). However, there was a trend towards a higher protein mass in the liver ($P < .07$) of casein-fed pigs compared to their BCM-fed counterparts. The absolute protein synthesis rates in g per day and in g per kg $BW^{0.75}$ are presented in Tables 6.5 and 6.6, respectively. The amounts of protein synthesised in g per day were higher ($P < .05$) in the liver and pancreas in the casein-fed pigs than in the BCM-fed pigs. No differences were observed in all other visceral tissues although synthesis rates in the casein fed pigs were numerically higher in most tissues compared to the BCM-fed pigs (Table 6.5). However, a trend towards a higher protein content in jejunum of casein-fed pigs than BCM-fed pigs was observed when calculated assuming plasma as precursor pool after 20 min of incorporation. When expressed as grams of protein per day per kg $BW^{0.75}$, protein synthesis rates were

Table 6.4. Total protein mass and protein mass per kg $BW^{0.75}$ present in visceral organs of growing pigs fed either a casein-cornstarch or barley-canola meal-based diet on the day that protein synthesis rates were measured in the visceral organs (mean ± SE).

	Casein	Barley-canola	Casein	Barley-canola
Tissue	(g)		(g/kg^{75})	
Liver	88.0 ± 7.2	70.2 ± 4.9	7.42 ± 0.59	6.53 ± 0.45
Pancreas	3.9 ± 0.5	2.8 ± 0.5	0.33 ± 0.04	0.26 ± 0.05
Duodenum	4.3 ± 0.5	5.0 ± 0.4	0.36 ± 0.04	0.46 ± 0.03
Jejunum	57.9 ± 3.8	50.3 ± 4.5	4.87 ± 0.28	4.67 ± 0.39
Ileum	5.6 ± 0.2	5.6 ± 0.7	0.47 ± 0.02	0.52 ± 0.07
Colon	19.9 ± 1.7	19.1 ± 0.8	1.68 ± 0.15	1.78 ± 0.08
Caecum	3.7 ± 0.5	3.7 ± 0.5	0.31 ± 0.03	0.35 ± 0.04

similar ($P > .05$) in all tissues irrespective of diet treatment (Table 6.6).

6.5 Discussion

One of the major concerns regarding the use of the flooding-dose procedure for measuring PSR is that the administration of a large dose of an amino acid to an animal may by itself influence the rate of protein synthesis (Rennie et al., 1994). Plasma glucose and insulin concentrations were determined in the present study to assess the impact of infusing a flooding-dose of phenylalanine on these indicators of metabolic status in the pig and to confirm our earlier observations (Chapter V). Within each dietary treatment, the glucose concentration in plasma did not change significantly over the entire experimental period, although concentrations were different between the two treatments (Figure 6.1B). During the experiment, the levels of plasma glucose averaged 5.78 ± 0.18 and 5.19 ± 0.25 mmol L^{-1} for the CC-fed and BCM-fed pigs, respectively. These values are in close agreement with the range of values (5.48 to 6.03 mmol L^{-1}) reported for plasma glucose concentration in growing pigs (Antinmo et al., 1978; Le Floc'h et al., 1995; Chapter V). The present results also agrees with our earlier finding indicating no significant change in plasma glucose concentration following a flooding dose of phenylalanine. In a study with sheep, Southorn et al. (1992), also found no change in plasma glucose concentration over a period of 60 min when a flooding dose of phenylalanine was administered at the same rate as used in the current study.

Repeated measure analysis of variance showed that for each treatment,

Table 6.5. Absolute protein synthesis rates (g/d) in visceral organs of growing pigs fed either a casein-cornstarch- or barley-canola meal-based diet and determined assuming that the SRA in either the intracellular (I) or plasma (P) free phenylalanine pool is the same as that in the precursor pool for protein synthesis

Tissue		20 min incorporation time		32 min incorporation time	
		Casein	Barley-canola	Casein	Barley-canola
Liver	I	87.2 ± 6.2[a]	61.4 ± 8.6[b]	56.0 ± 4.1[a]	39.4 ± 5.6[b]
	P	57.8 ± 1.9[a]	43.5 ± 6.1[b]	36.7 ± 1.2[a]	27.6 ± 3.9[b]
Pancreas	I	7.0 ± 0.9[a]	4.3 ± 0.7[b]	4.6 ± 0.6[a]	2.8 ± 0.4[b]
	P	5.4 ± 0.7	3.4 ± 0.7	3.6 ± 0.5	2.2 ± 0.5
Duodenum	I	5.4 ± 1.0	4.8 ± 0.7	3.5 ± 0.7	3.1 ± 0.5
	P	4.6 ± 0.5	4.7 ± 0.6	2.9 ± 0.3	3.0 ± 0.4
Jejunum	I	62.8 ± 8.1	44.5 ± 7.6	41.0 ± 5.4	29.2 ± 4.9
	P	56.0 ± 4.9	39.8 ± 6.4	36.7 ± 3.3	26.1 ± 4.3
Ileum	I	6.0 ± 0.9	5.2 ± 1.0	3.9 ± 0.6	3.5 ± 0.6
	P	5.8 ± 0.5	4.9 ± 0.7	3.8 ± 0.3	3.2 ± 0.5
Colon	I	28.2 ± 7.1	26.2 ± 1.7	18.6 ± 4.6	17.2 ± 1.2
	P	22.4 ± 6.6	18.6 ± 0.8	14.9 ± 4.3	12.2 ± 0.6
Caecum	I	4.1 ± 0.7	4.5 ± 0.7	2.7 ± 0.4	3.0 ± 0.4
	P	3.4 ± 0.6	3.7 ± 0.6	2.3 ± 0.4	2.4 ± 0.4

[ab] means ± SE in a row within incorporation time bearing different superscript letter differ ($P < .05$).

Table 6.6. Absolute protein synthesis rates (g/d/kg^{75}) in visceral organs of growing pigs fed either a casein-cornstarch- or barley-canola meal-based diet and determined assuming that the SRA in either the intracellular (I) or plasma (P) free phenylalanine pool is the same as that in the precursor pool for protein synthesis[1]

Tissue		20 min incorporation time		32 min incorporation time	
		Casein	Barley-canola	Casein	Barley-canola
Liver	I	7.34 ± 0.46	5.72 ± 0.82	4.71 ± 0.30	3.67 ± 0.53
	P	4.87 ± 0.11	4.05 ± 0.59	3.09 ± 0.07	2.57 ± 0.37
Pancreas	I	0.56 ± 0.08	0.41 ± 0.06	0.39 ± 0.05	0.27 ± 0.04
	P	0.45 ± 0.05	0.32 ± 0.07	0.30 ± 0.04	0.21 ± 0.04
Duodenum	I	0.45 ± 0.09	0.45 ± 0.07	0.29 ± 0.06	0.29 ± 0.04
	P	0.39 ± 0.04	0.44 ± 0.06	0.25 ± 0.03	0.28 ± 0.04
Jejunum	I	5.25 ± 0.59	4.14 ± 0.71	3.43 ± 0.39	2.72 ± 0.46
	P	4.72 ± 0.38	3.71 ± 0.60	3.09 ± 0.25	2.43 ± 0.40
Ileum	I	0.50 ± 0.07	0.49 ± 0.09	0.33 ± 0.47	0.32 ± 0.06
	P	0.49 ± 0.05	0.46 ± 0.07	0.32 ± 0.03	0.30 ± 0.05
Colon	I	2.37 ± 0.59	2.45 ± 0.18	1.56 ± 0.38	1.61 ± 0.12
	P	1.87 ± 0.54	1.73 ± 0.09	1.25 ± 0.36	1.14 ± 0.06
Caecum	I	0.28 ± 0.07	0.42 ± 0.06	0.19 ± 0.05	0.27 ± 0.04
	P	0.23 ± 0.06	0.34 ± 0.05	0.16 ± 0.04	0.22 ± 0.03

[1] values are mean ± SE

circulating plasma insulin concentration dropped significantly within 3 min after the start of the infusion and then remained relatively unchanged until the pigs were killed. The higher level of insulin (and glucose) in the CC-fed pigs compared to the BCM-fed pigs could be expected as the CC diet was much more digestible compared to the BCM diet (Nyachoti et al., 1997b). The overall plasma insulin concentration in the CC-fed pigs was 80.6 ± 9.2 pmol L^{-1}, a value that is similar to the concentration determined in pigs at similar BW and fed the same diets (Chapter V; 84.4 pmol L^{-1}) and agrees closely with concentration (89 pmol L^{-1}) observed by Le Floc'h et al. (1995). The absence of an increase in circulating insulin levels in the current study contradicts earlier observations in the rat suggesting that injecting a flooding-dose of phenylalanine instantly increases the concentration of circulating insulin followed by a very rapid fall (McNurlan et al., 1982). However, the spike in plasma insulin concentration seems to be related to the length of time over which the dose is applied. Indeed, studies with sheep have shown a significant increase in plasma insulin concentration when a flooding dose of phenylalanine was administered over a shorter infusion period as opposed to a longer period (Lobley et al., 1992; Southorn et al., 1992). The fact that we infused phenylalanine over a relatively long period (12 min) as opposed to giving a bolus injection (usually lasting 10-15 sec) may explain why plasma insulin levels remained relatively unchanged after infusing a flooding-dose of phenylalanine.

Both the glucose and insulin data indicate that the flooding dose procedure used here did not have significant impact on the measured indicators of metabolic

status of the pigs during the study. This observation is important considering the expressed fear that a flooding-dose of phenylalanine results in a surge in circulating insulin levels and that this may influence the process of protein turnover and, therefore, confound results derived using this procedure (Rennie et al., 1994). Whether insulin really has an influence on protein synthesis in pig tissues has not been demonstrated. In an extensive literature review, Garlick et al. (1994) could not find any evidence that a transient rise in plasma insulin concentration when using a flooding dose procedure has any influence on protein synthesis. In cases where a flooding dose of phenylalanine has been shown to significantly increase the circulating insulin concentration, the rate of protein synthesis in skeletal muscle of the rat was affected only at insulin levels 6.5 times baseline values (Garlick et al., 1983).

The purpose of administering a flooding-dose of labelled phenylalanine is to rapidly bring the SRA of phenylalanine in the various amino acid pools to the same level so as to eliminate the uncertainty regarding the precursor pool for protein synthesis (McNurlan et al., 1979; Garlick et al., 1980; Davis et al., 1989). Specific radioactivity of phenylalanine in plasma rose to the plateau value within 3 min of starting the infusion and this value did not change significantly over the course of the experiment for both treatments. This suggests that flooding was indeed achieved in the present study (Figure 6.3). The ratio between plasma free phenylalanine SRA to SRA in the infusate is one way of assessing the level of flooding achieved with the flooding-dose procedure. In the present study these levels were (mean ± SE) 72.4 ± 1.3 % and 74.9 ± 1.4 % in the CC- and BCM-fed pigs, respectively. These values are

higher than the value of 52 % observed in sheep (Southorn et al., 1992) but are between the values of 65 % and 80 % reported by Garlick et al. (1980) and Attaix et al. (1986), respectively. The amount of radioactivity in our infusate was 230 MBq/L, 110 MBq/L higher than the amount used by Southorn et al. (1992) and may explain part of the observed differences. Another way of assessing the success of flooding is to determine whether flooding is achieved in individual tissues. This is done by dividing the SRA of phenylalanine in the intracellular free pool by phenylalanine SRA in the plasma free pool. Theoretically, this ratio should approach unity. However, it will never be unity because of the dilution of partly labelled plasma amino acids with unlabelled amino acids derived from protein degradation in the intracellular free amino acid pool (Davis et al., 1989). Furthermore, the level of flooding in various tissues will be a reflection of the rate with which amino acids are absorbed from the blood plasma.

The level of flooding achieved in the current study in the various tissues ranged from 67.8 ± 2.9 % and 67.7 ± 5.2 % in the pancreas of the CC- and BCM-fed pigs, respectively to 83.8 ± 3.3 % in the ileum of the CC-fed pigs to 83.6 ± 1.9 % in the liver of the BCM-fed pigs (Table 6.1). The flooding levels observed in the liver (83.1 ± 1.9 % and 83.6 ± 1.9 % for CC- and BCM-fed pigs, respectively) and jejunum 75.1 ± 3.8 % and 76.8 ± 4.1 % for the CC- and BCM-pigs, respectively) agrees closely with findings of earlier studies with the flooding dose procedure in rats which showed a flooding level of 75 % in jejunal mucosa (McNurlan et al., 1979) and 85-90 % in the liver (McNurlan et al., 1979; Garlick et al., 1980). In sheep, Southorn

et al. (1992) observed a substantially lower flooding level (57 to 67 %) in the liver compared to the current results; an observation they attributed to possible difference in hepatic structure among species or contamination with highly labelled blood. Flooding level in the duodenum was lower in the current study compared with the 92 % level observed in sheep (Southorn et al., 1992).

The flooding level in muscle of about 80 % seen in the current study is somewhat lower than expected considering that skeletal tissues have lower protein turn over rates compared to visceral organs (Lobley, 1988). However, it has been suggested that it is more difficult to flood muscle tissues because of the relatively slow uptake of plasma amino acids into muscle cells (Davis et al., 1989). High flooding in the muscle (91 to 100 %) relative to SRA in the infusate has been observed in piglets given a bolus injection of ^{3}H-phenylalanine and killed 10 min later (Sève et al., 1986). The differences in the amount of radioactivity in infusates, mode of administration (injection vs 12 min infusion) and length of incorporation time (10 min vs 32 min) may explain part of the differences in flooding levels observed in the two studies.

Protein synthesis rates were calculated based on either the plasma free or intracellular free pool as the amino acid precursor pool for protein synthesis. These values were statistically similar in all tissues except in the pancreas. This is in agreement with observation made by Garlick et al. (1994), who suggested that measurements based on either the plasma or intracellular free amino acid pool is adequate to define the precursor SRA. However, PSR based on the SRA in intracellular free pool were numerically higher than those based on the plasma free

pool SRA. It should be noted that the intracellular free pool is used more frequently (McNurlan et al. 1979; Reeds et al., 1982; Garlick et al., 1983; Sève et al., 1986) while others have used both the intracellular and plasma free pools (Lobley et al., 1980; Davis et al., 1981; Southorn et al., 1992). Fern et al. (1974) have shown that the intracellular free pool provides a better estimate of the SRA in the aminoacyl-tRNA than does the SRA in the plasma free pool. For this reason it is suggested that PSR values derived from the intracellular free SRA provide better estimates of the actual PSR than those derived from the plasma free SRA. Since phenylalanine SRA in plasma was at a plateau 3 min after the start of infusion (see Figure 6.3), values determined assuming a 32 min incorporation time (i.e. since the start of the infusion) are likely a better reflection of the PSR in this organs.

Of all the tissues studied, significant diet effects on Ks were observed only in the liver and colon (Table 6.3). The availability of nutrients in the CC-based diet was significantly higher than in the BCM diet as shown in our previous study (Nyachoti et al., 1997b). According to Armentano et al. (1994) and Volman et al. (1998), the liver is a major site for amino acid metabolism and, therefore, the high protein turnover rates observed in the livers of CC-fed pigs was likely due to their adaptation to metabolize the relatively large amount of available nutrients in the CC diet. The absolute amount of protein synthesised per d was significantly different only in the liver and pancreas. The values observed in the liver (87.2 ± 6.2 and 61.4 ± 8.6 g/d, for the CC- and BCM-fed pigs, respectively) based on SRA in the intracellular free pool and 20 min of incorporation are comparable with the value of 60.1 g/d reported

by Garlick et al. (1976). There were no significant differences when the synthesis rates were corrected for the metabolic BW ($kg^{0.75}$) of the pigs. Based on the SRA in plasma free amino acids, the values of (mean ± SE) 4.87 ± 0.11 and 4.05 ± 0.59 observed in the liver of CC- and BCM-fed pigs after 20 min incorporation time, respectively, are comparable with a value of 3.78 ± 0.32 $g/d/kg^{0.75}$ observed in the liver of 4 week old broiler chickens (Tesseraud et al., 1996). In that study, the intracellular free SRA and 10 min incorporation time was used for calculating PSR.

Based on phenylalanine SRA in the plasma free pool, the rates of muscle protein synthesis after 32 min of incorporation for the casein (19.4 ± 3.0 %) and BCM (17.9 ± 1.6 %) fed pigs are in close agreement with results (16.4 to 18.2 %) in 10 d suckled piglets (Sève et al., 1986). However, the current results, and those of Sève et al. (1986) indicate that protein synthesis in the muscle of growing pigs is much higher than the rate of 7.6 % per d obtained in the gastrocnemius muscle of 20-30 kg pigs (Edmunds et al., 1978; Simon et al., 1978) or in the leg muscle of 70 kg pigs (Garlick et al., 1976). It is important to note, however, that both Edmunds et al. (1978) and Garlick et al. (1976) used the constant infusion of tracer amino acids; a technique that is know to underestimate the rates of protein turnover in various tissues (Lobley, 1988).

In a previous study we showed that feeding the BCM diet used in the present study induces a significantly higher amount of endogenous N flow at the distal ileum compared to feeding the CC-based diet (Nyachoti et al., 1997b). Based on this observation, we hypothesized that feeding a diet that induces a high amount of

endogenous N flow at the distal ileum will also induce a high rate of protein synthesis in the gut tissues to replace the lost protein. The Ks observed in the current study, however, does not support this hypothesis (Table 6.3). Of all the tissues studied, significant diet effects on Ks were observed only in the liver and colon (Table 6.3). Among the factors that influence the flow of endogenous N at the terminal ileum is the content and type of dietary fibre (Boisen and Moughan, 1996; Nyachoti et al., 1997a). Dietary fibre increases endogenous N not only through its abrasiveness which increases the sloughing off of the gut mucosa, but also through increasing digesta viscosity which in turn hinders adequate interaction between endogenous N and digestive enzymes (Chesson, 1993; Inborr et al., 1994). This results in inadequate recycling of endogenous N in which case most of it is excreted. Although digesta viscosity was not determined in the current study, it is suggested that the differences in endogenous N flow observed in the experiment reported in Chapter V was due to the differences in the efficiency of reabsorbing endogenous gut proteins rather than due to differences in rates of protein synthesis in the gut tissues. A similar proportion has also been suggested by Grala (1998). It is important to note, however, that in the current study we looked at the overall protein synthesis rates as opposed to synthesis rates of specific proteins. It may as well be that the synthesis rates of certain types of proteins (eg. mucoproteins) were affected to different extent as hypothesised by (Lien et al., 1997).

6.6 Implications.

Diets that induce high endogenous gut protein losses did not have a significant effect on the rates of protein synthesis in the small intestine of growing pigs. It seems, therefore, that the main consequence of feeding such diets is the effect on endogenous protein losses *per se* and therefore ways of minimizing such losses should be identified.

CHAPTER VII

Effect of Diet Composition on Organ Size and *In vitro* Oxygen Consumption by the Visceral Organs in Growing Pigs

7.1 Abstract

Organ size and *in vitro* oxygen consumption by the visceral organs were determined in growing pigs. Fifteen Yorkshire barrows (with an average final BW of 26.3 kg) were fed diets based on casein-cornstarch (CC), barley-canola meal (BCM) or barley-canola meal-alfalfa meal (BCM-ALF) in a completely randomised design for three weeks. All diets were formulated to a similar DE:CP ratio. Pigs were fed at 2.6 x maintenance energy requirements twice daily for 3 weeks and then every 3 h for 3 d before measuring oxygen consumption. Pigs were killed 1 h postfeeding and samples of the liver, jejunum, colon and caecum taken immediately after death. *In vitro* oxygen uptake was determined polarographically using a Yellow Springs Instruments (YSI) oxygen monitor. BCM and BCM-ALF fed pigs had heavier ($P < .05$) visceral organs relative to empty body weight (EBW) compared to the CC fed pigs. Per g of liver and mucosal tissue, oxygen consumption was not influenced by diet ($P > .10$). However, per g of tissue, oxygen consumption was higher ($P < .05$) in the caecum muscularis of pigs fed BCM and BCM-ALF than in the CC-fed pigs. Per kg of EBW, total oxygen uptake in the liver ($P < .05$) and colon ($P < .001$) of BCM and BCM-ALF fed pigs was higher than in CC pigs. It was concluded that feeding pigs high fibre diets, lead to heavier visceral organs relative to EBW and this has direct

implications on the efficiency of energy utilization by repartitioning nutrients from the accretion of edible carcass to the visceral organs.

7.2 Introduction

Although the visceral organs represents a proportionally small part of the whole animal body, they are reported to account for a significant amount of the total body energy expenditure (Gill et al., 1989; Yen et al., 1989; Huntington, 1990). For instance, Yen et al. (1989) estimated the contribution of gut tissues and the liver to whole animal oxygen consumption to be about 25 % and 20 %, respectively, in the growing pig. Two major processes, namely the Na^+, K^+ pump and protein turnover are known to be responsible for the high energy expenditure by gut tissues and the liver (Lobley et al., 1988; Kelly and McBride, 1989; McBride and Kelly, 1990). Studies with other livestock species show that these processes are influenced by the level of nutrition and diet composition (Yen et al., 1989; Kelly et al., 1993; Finegan, 1996). Although in the experiment reported in Chapter VI diet did not have a significant effect on gut protein synthesis, this study was conducted to confirm these observations. A high fibre diet was included in the present study to ensure that any possible dietary effects are detected.

Diet composition and in particular dietary fibre content influences the mass of visceral organs. Pigs fed high fibre diets have heavier visceral organs and longer gastrointestinal tracts relative to empty body weight (EBW) compared to those fed low fibre diets (Pond et al., 1988; Jorgensen et al., 1996). Diets that are high in fibre

or other anti-nutritional factors are also known to increase the amount of endogenous gut N losses in pigs (Butts et al., 1993; Boisen and Moughan, 1996; Nyachoti et al., 1997a). Feeding diets that increase both endogenous N losses and visceral organ mass, may lead to increased metabolic activities in the gut tissues with a net effect of elevating their energy demand (Reeds et al., 1985). However, the existence of such a relationship has not been demonstrated in pigs fed different feeds. Estimates of oxygen consumption by the gut and measuring the sizes of various gut organs may provide a better understanding of the metabolic consequences of feeding growing pigs diets that induces high endogenous gut N losses and increase sizes of visceral organs.

The present study was undertaken to determine *in vitro* oxygen consumption by the visceral organs in growing pigs fed a casein-cornstarch-based control diet or two high fibre diets based on barley-canola meal with or without alfalfa meal. The effect of diet on organ weights was also assessed.

7.3 Materials and methods

7.3.1 *Animals, Housing and Diets.*

The use of animals in this study was reviewed and approved by the Animal Care Committee of the University of Guelph and animals were cared for according to the guidelines of the Canadian Council on Animal Care. Fifteen growing Yorkshire barrows with an average initial body weight (BW) of 18 kg were obtained from Arkell Swine Research unit at University of Guelph for use in the present study. They were housed in individual pens with tender foot flooring in a temperature controlled room

(20 to 22 °C) and allowed to adapt to their new environment and diets for a minimum of 3 weeks before measurement of *in vitro* oxygen consumption in selected organs.

Two diets based on either casein-cornstarch (CC) or barley-canola meal (BCM) (same as Diet 1 and 4 used in Chapter IV, Table 4.1) and a third diet based on barley-canola meal and alfalfa meal (BCM-ALF) were used in this experiment (Table 7.1). The diets were formulated to meet or exceed NRC (1988) requirements for vitamins and minerals and to contain a similar digestible energy to protein ratio. Pigs were given their daily feed allowance in two equal amounts (at 08.00 and 20.00 h) and intake was restricted to 2.6 times maintenance energy requirement (ARC, 1981). During the last 3 d prior to the start of the study, the feeding schedule was changed to 3-hourly feeding so as to maintain steady state conditions (Lobley et al., 1992).

7.3.2 *General conduct of the study*

Five pigs were randomly assigned to one of the three diets. For the measurement of *in vitro* oxygen consumption, three pigs were killed per d by an intra-cardiac injection of a lethal dose of sodium pentobarbitol on 5 consecutive days. On each d, one pig from each treatment was picked at random and killed 1 h after feeding. A time interval of 3 h was allowed between animals. Samples of liver (left lobe), jejunum (2 m from stomach), colon (40 cm from caecum), and caecum were taken immediately after death, cleaned with ice-cold Krebs-Henseleit buffer and then

Table 7.1. Ingredient composition (%), calculated DE and DE:CP of the experimental diets (as fed basis)

Ingredient	Casein	Barley-canola	BCM-ALF[a]
Alfalfa meal	-	-	30
Barley	-	60.95	44
Canola meal	-	22.5	10
Casein	21.32	-	-
Corn starch	59.08	-	-
Sucrose	10	10	10
Limestone	.6	1.2	-
Dicalcium phosphate	2.4	.75	1.4
Fat (A/V blend)	3.5	3.5	3.5
Iodized salt	.5	.5	.5
Vitamin premix[b]	.5	.5	.5
Mineral premix[c]	.1	.1	.1
Alfafloc	2	-	-
Calculated DE, kcal/kg	4002	3337	2978
DE:CP ratio	211	214	212

[a] BCM-ALF, barley-canola meal-alafalfa meal diet.

[b] Vitamin premix supplied the following per kg of finished feed: Vit A, 12000 IU; Vit D, 1200 IU; Vit E, 48; Vit K, 3 mg; choline, 600 mg; pantothenic acid, 18 mg; Riboflavin, 6 mg; Folic acid, 2.4 mg; Niacin, 30 mg; Thiamin, 1.8 mg; Vit B_6, 1.8 mg; Biotin, 0.24 mg; Vit B_{12}, 0.03 mg.

[c] Mineral premix supplied the following per kg of finished feed: Cu, 15 mg; Zn, 100 mg; Fe, 100 mg; Mn, 20 mg; I, 0.3 mg; Se, 0.3 mg.

placed in fresh ice-cold Krebs-Henseleit buffer for transport to the laboratory. Measurement of oxygen consumption started within 20 min of slaughter and was completed in about 1 h. Duplicate subsamples (15 to 30 mg fresh weight) of liver (thin slices), intact mucosa (from each gut segment) and muscularis (from caecum only) were dissected out under x10 magnification, blotted dry on Kimwipes and weighed. Samples were then placed in 4 mL of M199 medium (Sigma Chemicals, St. Louis, MO) followed by oxygen consumption measurement. *In vitro* oxygen uptake was measured polarographically using a YSI (Yellow Springs Instruments Inc., Yellow Springs, OH) Clark-style oxygen electrode. The tissues were maintained in 4 mL of M199 medium at pH 7.4 and 37 °C during measurement of oxygen uptake for 10 min (Kelly et al., 1993). Measurements were derived from the linear phase of oxygen uptake charts. The DM content of the samples used in determining oxygen uptake were estimated from values determined in similar samples (40 to 50 mg) taken from the same spot. Another larger (~ 2.5 g) section of each gut segment was dissected, blotted and then weighed before scrapping off the mucosal tissue using a microscope slide. The mucosa and muscularis tissues were weighed separately prior to determining DM contents by oven drying at 100 °C for 12 h (AOAC, 1990) and the values used in calculating the total dry weight of mucosa and muscularis, respectively, in the gut segments. The rest of the liver and segments of the gut were cleaned of digesta and weighed.

7.3.3 *Calculations and statistical analysis*

Oxygen consumption was calculated either as mL of oxygen per g of tissue dry matter per h (weight-specific oxygen consumption) or as total mL of oxygen consumed by the whole tissue per h. Weight-specific oxygen consumption determined in the caecum muscularis was assumed to be the same as that in the muscularis of the small intestine and colon for calculating total oxygen consumption. Total oxygen consumption by each organ was also calculated relative to EBW. For calculating EBW, it was assumed that gut fill in the CC-, BCM- and BCM-ALF-fed pigs was taken to be 3%, 5% and 8.2% of EBW, respectively (Mohn and de Lange, unpublished observation; Jorgensen et al., 1996). Relative organ weights (g/kg EBW) were calculated by dividing the weight of each organ by the EBW. The data were subjected to analysis of variance using the GLM procedures (SAS, 1985) with diet as the source of variation. When significant diet effects were obtained, differences between means were compared using Duncan's multiple range test (Steel and Torrie, 1980).

7.4 Results and Discussion

All pigs remained healthy and consumed all their feed throughout the experiment. The CC- and BCM-fed pigs had similar BW and EBW at the end of the study and both were heavier ($P < .01$) compared to those fed the BCM-ALF diet. All pigs were killed 1 h post feeding so as to allow measurements of oxygen uptake to be made approximately 2 h from death; a time interval that corresponds to the

interval used in the protein synthesis study (Chapter VI).

Barley-canola meal and BCM-ALF fed pigs had heavier ($P < .01$) colons as compared to the CC fed pigs. Wet weights of the other organs were not affected by diet ($P > .10$) (Table 7.2). In general, the BCM and BCM-ALF fed pigs had heavier ($P < .05$) visceral organs relative to EBW compared to those fed on the CC diet (Table 7.3). After correcting for DM, the effect of diet on the relative weights of visceral organs was significant only in the liver ($P < .05$) and colon ($P < .01$). However, numerically, it was larger in the small intestine, caecum and total gut of BCM-ALF-fed pigs than in the CC-fed pigs. Due to the small number of pigs per treatment and the large variability, substantial numerical effects are not statistically significant. The present data corroborates previous evidence suggesting that feeding high fibre diets causes an increase in the visceral organ mass in rats, pigs and sheep (Kelly et al., 1993; Zhao et al., 1995; Jorgensen et al., 1996). Per kg EBW, the wet weights of colon and caecum were 15.5 - 17.5 and 2.22 - 2.94 g, respectively, which agrees closely with the values (17.23 and 2.83, respectively) reported by Jorgensen et al. (1996). The small intestine weights relative to EBW were higher in the present study compared to the values observed by Jorgensen et al. (1996). High fibre diets have greatest impact on the colon; an observation that is most likely due to the distension and lengthening of this part of the gut where most of the fibrous feed components are digested (Jorgensen et al., 1996; Rérat, 1996). The heavier colons in pigs fed high fibre diets is associated with increased protein synthesis rates in this organ as seen in Chapter VI.

Table 7.2. Body weights, wet organ weights and dry weight of gastrointestinal mucosal and muscularis tissues and liver of growing pigs fed casein-cornstarch- (CC), barley-canola meal- (BCM) or barley-canola meal-alfalfa meal (BCM-ALF)-based diets.

	Diet			
Item	CC	BCM	BCM-ALF	SEM[1]
Intial body weigh (kg)	18.6	19.6	18.4	0.66
Final body weight (kg)	27.28[a]	27.2[a]	24.32[b]	0.49
Empty body weight[2] (kg)	26.46[a]	25.84[a]	22.44[b]	0.46
Wet organ weight (g)				
Liver	657.0	724.0	665.0	22.5
Small intestine	826.0	775.6	795.6	39.1
Colon[3]	268.5[b]	400.2[a]	391.4[a]	22.9
Caecum	53.4	57.4	65.6	5.58
Total GIT[4]	1175.0	1233.2	1252.6	56.8
Dry weight - Mucosal tissue (g)				
Small intestine	116.88	102.02	106.51	8.45
Colon	17.18[b]	29.96[a]	27.53[a]	1.78
Caecum	3.10	3.20	3.60	0.35
Dry weight - Muscularis (g)				
Small intestine	30.02	30.01	31.74	1.98
Colon	36.14	39.92	38.79	3.05
Caecum	8.21	8.19	8.39	0.71
Dry weight Liver (g)	131.16	152.17	141.64	7.09

[1] pooled standard error of the means.

[2] contribution of gut fill to BW: 3%, 5% and 8.2% for the casein, BCM, and BCM-ALF fed pigs, respectively;

[3] n = 4 for the casein diet

[4] GIT excludes the stomach

[ab] means in a row with dissimilar superscripts differ ($P < .05$).

Table 7.3. Visceral organ weights (g/kg empty BW[1]) in growing pigs fed casein-cornstarch- (CC), barley-canola meal- (BCM) or barley-canola meal-alfalfa meal (BCM-ALF)-based diets.

Item	Diet			SEM[2]
	CC	BCM	BCM-ALF	
Wet organ weights:				
Liver	24.8[b]	28.0[a]	29.6[a]	0.6
Small intestine	31.2	30.1	35.5	1.6
Colon	10.1[b]	15.5[a]	17.5[a]	0.9
Caecum	2.01[b]	2.22[b]	2.92[a]	0.19
Total GIT[3]	41.3[b]	47.9[ab]	56.0[a]	2.9
Dry organ weights:				
Liver	4.97[b]	5.9[ab]	6.34[a]	0.34
Small intestine	5.55	5.13	6.18	0.42
Colon	1.99[b]	2.71[a]	2.98[a]	0.19
Caecum	0.43	0.44	0.54	0.04
Total GIT[3]	7.57	8.28	9.69	0.69

[1] contribution of gut fill to BW: 3%, 5% and 8.2% for the casein, BCM, and BCM-ALF fed pigs, respectively.

[2] pooled standard error of the means.

[3] GIT excludes the stomach.

[ab] means in a row with dissimilar superscripts differ ($P < .05$).

The total amount of mucosa in the colon of BCM and BCM-ALF fed pigs was higher ($P < .01$) than in the CC fed pigs (Table 7.2). No diet effect was observed in the weight of the muscularis tissue from different segments of the gut ($P > .10$). The relative contribution of mucosa to the total dry weight of the gut segments was significantly influenced by diet in the jejunum ($P < .05$), and colon ($P < .01$) but not in the caecum ($P > .10$). The total amount of mucosa represented (mean ± SE) 0.795 ± 0.008, 0.772 ± 0.005 and 0.768 ± 0.009 of the total dry weight of the jejunum in CC, BCM and BCM-ALF, respectively. These respective values were 0.322 ± 0.306, 0.431 ± 0.013 and 0.416 ± 0.007 in the colon. In the caecum, the dry mucosa weight as a proportion of the total dry caecum weight averaged 0.285 across treatments. The dry jejunal mucosa weight observed in the present study contributed to approximately 0.775 of the total jejunal dry weight; this value agrees closely with values of 0.62 to 0.76 reported in sheep fed concentrate-based diets (Rompala and Hoagland, 1987; Finegan, 1996). The contribution of mucosal weight to the total weight in the colon of sheep (range 0.55 to 0.61) reported by Finegan (1996) is somewhat larger than seen in the present study. The trends in mucosal development seen here fits in well with what could be expected based on the differences in site of digestion and nutrient absorption when pigs are fed the type of diets used in the current experiment (Rérat, 1996). The CC diet is highly digestible (Nyachoti et al., 1997b) and therefore nutrients were likely readily available in the upper gut thus leading to more development of the mucosa in this segment of the gut to facilitate nutrient absorption. Relatively a larger proportion of the BCM and BCM-ALF diets

is digested by microbial fermentation in the hind gut and specifically in the colon which stimulated mucosal development in this segment of the gut (Pond et al., 1988; Jorgensen et al., 1996; Rérat, 1996).

Oxygen consumption per g DM of the liver, mucosa and muscularis tissue of gut segments is presented in Table 7.4. Dietary treatment did not influence weight-specific oxygen uptake in the liver and mucosal tissue of jejunum, colon and caecum ($P > .05$). Weight-specific respiration in the muscularis tissue is also shown in Table 7.4 and was different ($P < .05$) among treatments. It was higher in the BCM and BCM-ALF diets compared to the CC diet. The higher respiration rate in the muscularis tissue is likely a reflection of the amount of (undigested) material that needs to be moved through the digestive tract. The lack of diet effect on weight-specific respiration observed in the present study, is in good agreement with results of previous studies with other livestock species (Burrin et al.; 1990; Kelly et al.; 1993; Finegan, 1996). To our knowledge, similar data are not available for pigs. Weight-specific metabolic activity in sheep fed either forage or concentrate based diets for 8 weeks was reported by Finegan (1996) to be 7.56 vs 6.42 in jejunum, 4.79 vs 4.74 in caecum and 5.77 vs 3.66 mL/g/h in the colon. The data obtained in the current study agrees closely with the results seen in the sheep colon and caecum but not in jejunum. Species differences and differences in stage of development and growth rates may explain the differences in weight-specific oxygen uptake seen in the jejunum of pigs compared to the values reported for the sheep. It is likely that the demand for nutrient supply in a young fast growing pig as those used in the current

Table 7.4. Oxygen consumption by the liver and gastrointestinal tract mucosal and muscularis tissues (mL/g dry matter/h) in growing pigs fed casein-cornstarch- (CC), barley-canola meal- (BCM) or barley-canola meal-alfalfa meal (BCM-ALF)-based diets.

	Diet			
Tissue	CC	BCM	BCM-ALF	SEM[1]
Liver	3.63	3.31	3.84	0.24
Mucosal tissue				
Jejunum	12.72	12.25	12.77	0.58
Colon	5.63	7.13	6.77	0.83
Caecum	6.00	6.56	5.70	0.64
Muscularis tissue				
Caecum	1.28[b]	2.26[a]	2.49[a]	0.29

[1] pooled standard error of the means.
[ab] means in a row with dissimilar superscripts differ ($P < .05$).

study is much higher than in a mature slow growing animal as used in the sheep studies.

The total oxygen consumption by the liver, mucosa and muscularis tissues of the gut is presented in Table 7.5. Diet had significant effect on the total oxygen consumption by the mucosal tissue of the colon. It was lower ($P < .05$) in the CC fed pigs than in the BCM and BCM-ALF fed pigs, which in turn were similar ($P > .05$). However, diet had no effect ($P > .05$) on the total amount of oxygen consumed by the liver and mucosal tissue of the small intestine and caecum although values for the caecum mucosa in the BCM and BCM-ALF fed pigs were numerically higher than in the CC pigs (Table 7.5). Diet treatment had a significant ($P < .05$) impact on the total amount of oxygen consumed by the muscularis tissue of the small intestine, colon and caecum (Table 7.5). It was higher in the BCM and BCM-ALF diets than in the CC diet; an observation that was likely due to increased motor activity in the colons of pigs fed diets with low digestibilities (Fioramonti and Bueno, 1980).

Per kg of EBW, total oxygen consumption was significantly affected by diet treatment in the liver ($P < .05$) and colon ($P < .001$) and was numerically higher in the caecum and total gut of BCM and BCM-ALF fed pigs compared to CC fed pigs (Table 7.6). In general, these observations are in line with previous reports that total-gut oxygen consumption is affected more by mass changes than by weight-specific respiration (Burrin et al., 1990; Kelly et al., 1993; Finegan et al., 1996).

The present study together with results of others (Pond et al., 1988; Anugwa et al., 1988; Jorgensen et al., 1996) has provided evidence that diet type affects the

Table 7.5. Total oxygen consumption by the liver and gastrointestinal tract mucosal and muscularis tissues (mL/h) in growing pigs fed casein-cornstarch- (CC), barley-canola meal- (BCM) or barley-canola meal-alfalfa meal (BCM-ALF)-based diets.

	Diet			
Tissue	CC	BCM	BCM-ALF	SEM[1]
Liver	521.68	490.49	540.88	31.36
Mucosal tissue				
Small intestine	1454.8	1322.3	1236.8	89.1
Colon	93.01[b]	210.23[a]	185.87[a]	18.18
Caecum	17.20	22.62	19.90	2.05
Muscularis tissue				
Small intestine	38.3[b]	69.5[ab]	79.9[a]	10.3
Colon	48.64[b]	93.56[a]	94.36[a]	11.51
Caecum	10.99[b]	18.2[ab]	20.61[a]	2.85

[1] pooled standard error of the means.
[ab] means in a row with dissimilar superscripts differ ($P < .05$).

Table 7.6. Total oxygen consumption (mL/h/kg empty BW[1]) by the visceral organs in growing pigs fed casein-cornstarch- (CC), barley-canola meal- (BCM) or barley-canola meal-alfalfa meal (BCM-ALF)-based diets.

	Diet			
Tissue	CC	BCM	BCM-ALF	SEM[2]
Liver	19.66[b]	18.99[b]	24.30[a]	1.38
Small intestine	60.88	47.89	75.02	8.8
Colon	5.29[b]	11.77[a]	12.41[a]	0.96
Caecum	1.18	1.70	2.09	0.27
Total GIT[3]	61.4	67.3	72.9	4.3

[1] contribution of gut fill to BW: 3%, 5% and 8.2% for the CC, BCM, and BCM-ALF fed pigs, respectively.

[2] pooled standard error of the means.

[3] GIT excludes the stomach.

[ab] means in a row with dissimilar superscripts differ ($P < .05$).

metabolic activity of the gut tissues in the pig. This effect is mainly related to diet effects on relative organ weights. According to Koong et al. (1982, 1983), the weights of the visceral organs are positively and significantly correlated with fasting heat production of pigs. Furthermore, Yen et al. (1989) reported that in growing pigs, the hepatic-portal drained viscera including the gut accounted for a disproportionately high (25 %) amount of the whole-body oxygen consumption although they only represent a small part of the body weight. These findings plus those of the current study suggest that diet induced effects on energy expenditure in visceral organs has an impact on whole-body energy expenditure in growing pigs. Feeding diets that induce high visceral organ mass will reduce the efficiency of utilizing available energy intake for lean meat accretion. In addition diet effects on endogenous gut nitrogen losses in the pig should be considered (de Lange et al., 1989a; Schulze et al., 1994b, 1995b; Chapter IV). The BCM diet used in the present study was shown in Chapter IV of this thesis to actually increase endogenous gut nitrogen losses compared to the CC diet. These effects on whole body energy expenditure and ENL should be considered in addition to diet effects on the supply of truly digestible nutrients.

Implications

Per g of tissue, energy expenditure by the liver, small intestine, colon and caecum is not affected by diet composition. However, the total amount of energy expended by these organs is significantly influenced by diet composition mainly because of diet effects on the organ mass. It is concluded that feeding diets that will

lead to heavier visceral organs will reduce the efficiency of converting dietary energy into energy retained in lean meat.

CHAPTER VIII

General Discussion

In formulating swine diets with respect to amino acid supply, apparent ileal digestibilities are used as reasonable estimates of amino acid availability. However, apparent amino acid digestibilities underestimates their true digestibilities because apparent digestibilities are confounded with endogenous gut protein that is secreted into the gut lumen and that is not reabsorbed (Sauer and Ozimek, 1986). Furthermore, there are concerns regarding the additivity of apparent as opposed to true ileal amino acid digestibilities (de Lange 1989; Moughan, 1995; NRC, 1998). For proper feed formulation, it is important that amino acid digestibilities determined in individual feedstuffs are additive in a mixture of ingredients. Finally, protein that is not absorbed prior to the terminal ileum cannot be used for protein accretion; it is eventually excreted in urine and faeces and may thus contribute to environmental pollution (Zebrowska et al., 1978; Krawielitzki et al., 1983). For these reasons, endogenous gut protein secretions have received a lot of attention in swine nutrition research. Many of these studies have focused on methodologies for proper quantification of endogenous N losses (ENL) at the end of the small intestine and on diet and animal factors that induce high ENL (de Lange et al., 1989a, b; Butts et al., 1993a; Marty et al., 1994; Schulze et al., 1994b, 1995a, b). These studies have clearly shown that feeding pigs practical feeds causes much higher ENL than feeding purified and/or protein-free diets. As discussed in Chapter II, Section 2.4.3 and recently by Grala (1998), high ENL are likely to represent a metabolic cost to the pig which is

larger than the energy and amino acid content of ENL *per se*. The studies described in this thesis were designed to address the hypothesis that feeding diets that induce high ENL will also lead to enhanced energy expenditure in the gut tissues.

The issue regarding the method of choice for measuring ENL in pigs is not yet resolved. As discussed in Chapter II, all the methods that have been tried thus far have strengths and limitations. Based on a review of the literature (Chapter II), the homoarginine (HA) method which involves the chemical transformation of dietary lysine into its amino acid analogue, homoarginine, in a guanidination reaction with methylisourea (Hagemeister and Erbersdobler, 1985), was chosen to quantify the amount of ENL induced by the diets used in the present studies. Because the HA method has had limited evaluation with practical feedstuffs, research reported in Chapter III was devoted to determining the optimal conditions for lysine guanidination in the feedstuffs used in the current studies. The results showed that by manipulating the concentration of methylisourea and the length of reaction time, the rate of conversion of dietary lysine into HA can be increased substantially. This finding is important because it indicates that a great degree of randomness in lysine conversion can be achieved in practical feedstuffs which is a major requirement for a reliable use of this method (Rutherfurd and Moughan, 1990; Siriwan et al., 1994). It was reported in Chapter IV that the HA method can be used to quantify ENL in pigs fed practical feedstuffs such as canola meal and barley and that this method can be used in diets containing more than one protein source. The results of this experiment showed that feeding diets that contain barley or canola meal, causes much

higher ENL than reported for purified diets or protein free diets. This again confirmed results of others (de Lange et al., 1989b; Butts et al., 1993a; Marty et al., 1994). The question of additivity of amino acids in a mixture of commonly used feedstuffs was addressed in this experiment by including the barley-canola meal (BCM) diet. It was demonstrated that true ileal amino acid digestibilities are additive in a mixture of barley and canola meal. In contrast, it was observed that apparent ileal digestibilities were not additive. This suggests that true digestibilities should be used in practical swine feed formulation. These findings provide a strong reason for further refinement of methods that can be used routinely to quantify ENL so as to allow regular use of true ileal amino acid digestibilities in formulating swine diets. Such a method will also facilitate studies aimed at reducing ENL as a means of reducing the negative impact of swine production on the environment.

Results of the metabolic consequences of feeding diets that induce high ENL are reported in Chapters VI and VII which addresses the impact on protein synthesis and energy expenditure in visceral organs, respectively. It should be noted that because of the composition of the diet used to induce high ENL, it is not possible to establish a relationship between specific dietary components and ENL, protein synthesis rates or energy expenditure in visceral organs. Such relationships can only be established by using diets that differ in only one component know to enhance ENL such as specific antinutritional factors or non-starch polysaccharides. Prior to conducting these experiments, it was necessary to validate the method used to measure protein synthesis (Chapter V). Because visceral organs have high protein

turnover rates, the flooding-dose procedure which allows measurements to be made within a short time (usually 10 to 20 min), was proposed (Garlick et al., 1980). Its use in large livestock species has been validated in sheep (Southorn et al., 1992) but not in the pig. The current studies show that administering a flooding dose of phenylalanine into growing pigs *per se* does not alter the metabolic status of the animal as evidenced by the insignificant impact on the circulating levels of plasma insulin, glucose and free amino acids. Based on this finding, it was concluded that phenylalanine flooding-dose procedure is an appropriate technique for measuring protein synthesis rates in the gut tissues of growing pigs. It was expected that when pigs are fed diets that increase ENL, rates of protein synthesis in the gut tissues will be elevated to replace the lost proteins (Low, 1980). However, the findings of the protein synthesis study (Chapter VI) do not support this supposition. Instead, the current findings show that the availability of nutrients and the site of nutrient absorption are important factors influencing the process of protein synthesis in visceral organs. In particular, feeding the casein-cornstarch (CC) diet, led to higher protein synthesis rates in the liver compared to feeding the BCM diet. Amino acid supply to the liver in the CC fed pigs was much higher than feeding the BCM diet as shown in Chapter IV. The higher protein synthesis rate observed in this organ was likely an adaptive response to the increased supply of nutrients (Pond et al., 1988). Protein synthesis was higher in the colon of BCM fed pigs compared to the CC fed pigs which corresponds with the observed heavier colons in Chapter VII. This further supports the reasoning that nutrient absorption is a major stimulant for protein

synthesis. It has been shown that non-starch polysaccharides are digested mainly in the colon with the help of microbial fermentation (Pond et al., 1988; Jorgensen et al., 1996; Rérat, 1996).

The results of the protein synthesis experiment (Chapter VI) suggest that enhanced ileal ENL observed when feeding practical diets may not be related to protein synthesis in the small intestine. Increased ENL is likely related to the efficiency of reabsorption of endogenous protein secreted into the gut. Feeding practical feeds to pigs not only causes high ENL but also leads to an increase in the bulkiness and possibly the viscosity of digesta as compared to purified diets (Marty et al., 1994; Ikegemi et al., 1990; Jorgensen et al., 1996). This in turn hinders effective interaction between digestive enzymes and ENL with a net effect of reduced efficiency of recycling endogenous N (Ikegemi et al., 1990). This finding points out the need for practical solutions to manipulate the physical-chemical characteristics of digesta so as to enhance reabsorption of ENL. Of the strategies discussed in Chapter II, section 2.5.3, supplementation with exogenous enzymes and feed processing may be possible solutions to this but further developmental work will be required before these becomes useful tools in swine nutrition.

In Chapter VII, the effect of feeding diets that induce either low or high ENL on energy expenditure by the visceral organs in pigs was studied. In addition, a BCM diet with added alfalfa meal (BCM-ALF) was included in this experiment as a high fibre diet that was expected to elicit an effect on the metabolic activity in the visceral organs (Jorgensen et al., 1996). The results of this experiment showed that diet

composition has a significant effect on the weights of visceral organs and the colon in particular relative to empty body weight (EBW). Measurements of in vitro oxygen consumption by these organs showed that diet composition has no effect on the weight-specific respiration rates. This observation confirms the results of previous studies in other species (Burrin et al., 1990; Kelly et al., 1993; Finegan, 1996). When expressed relative to EBW, total oxygen consumption was influenced by diet; being higher in the BCM and BCM-ALF diets compared to the low fibre CC diet. The basic objective in commercial pork production is to efficiently produce lean meat. Feeding diets that promote organ mass is undesirable for various reasons. First, offals are of low economic value and often represent a nuisance to handle at slaughter houses. Second, and more importantly, visceral organs are metabolically active tissues that consume a disproportionately high amount of energy relative to their sizes (Koong et al., 1982, 1983; Yen et al., 1989). This implies that feeding diets that favour heavier visceral organs will divert a substantial amount of energy away from producing lean meat to supporting these organs; a phenomenon that will have serious consequences on the efficiency of utilizing energy for the production of lean meat. Since such diets are also likely to enhance ENL (as shown in Chapter IV), the actual consequences of feeding this type of diets to swine are much higher than indicated by the metabolic results. As discussed in Chapter II and by Grala (1998), ENL is likely associated with increased dietary N requirements. These effects on whole body energy expenditure and ENL should be considered in addition to diet effects on the supply of truly digestible nutrients. It should be noted that gut development due to

feeding the BCM or BCM-ALF diets is an important adaptive response that allows pigs to utilize such diets. Whether or not one should feed such diets will be determined by their cost effectiveness. The present studies have demonstrated the influence of such diets on nutrient metabolism in the gut. Therefore, we should now look for means of reducing such effects.

In conclusion, it is suggested that future studies should focus on identifying means of reducing endogenous gut protein losses and the impact of diet on organ size and the associated energy expenditure as a way of improving the efficiency of dietary protein and energy utilization for pork production. In addition, studies should be conducted to evaluate the relationships between endogenous gut protein losses, organ size and energy expenditure in visceral organs when specific dietary components (e.g. feed enzymes, antinutritional factors) are manipulated.

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IMAGE EVALUATION
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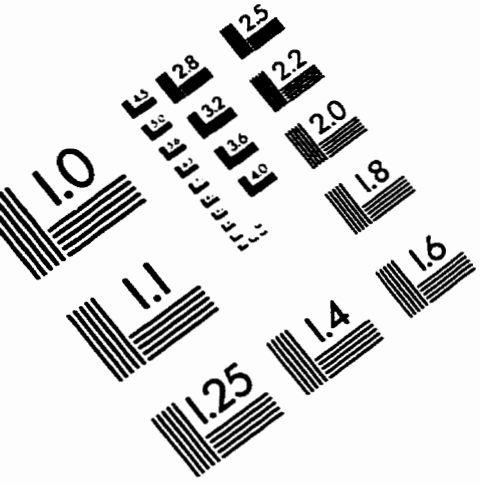

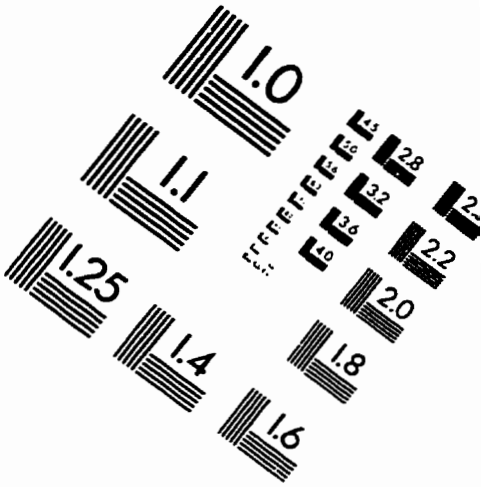

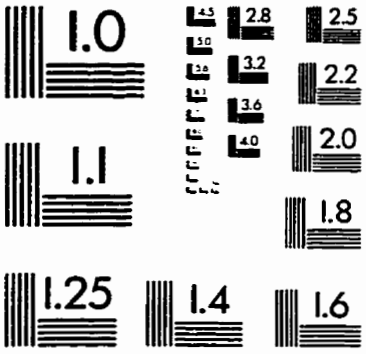

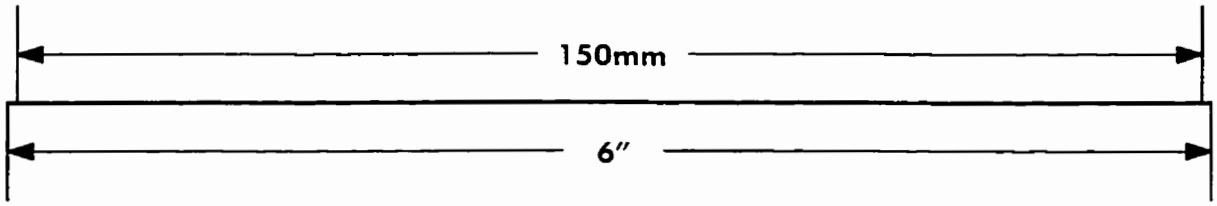

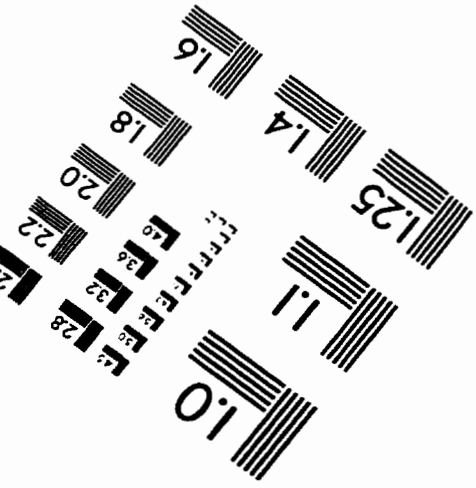

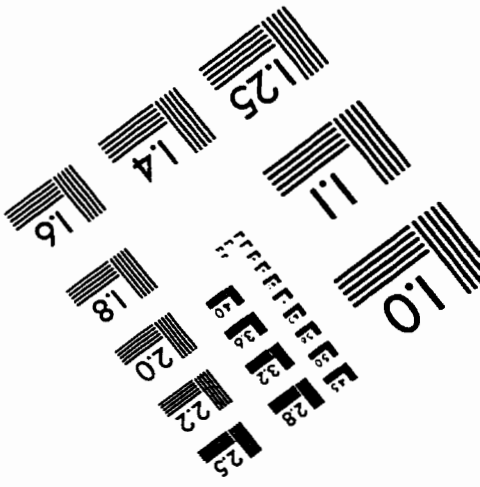

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