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METHODOLOGY OF DETERMINATION OF ENERGY AND AMINO ACID DIGESTIBILITY VALUES IN FEEDSTUFFS FOR PIGS

BY

GUISHAN HUANG 

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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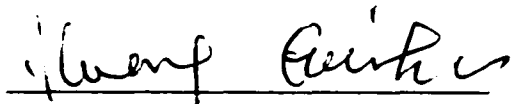

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DEDICATION

To my parents for their love and education

To my wife and my son for their love and support

To all my teachers for their education and guidance

ABSTRACT

Studies were carried out to determine: (1) application of *in vitro* enzymatic methods to predict *in vivo* fecal energy and ileal amino acid (AA) digestibility values of hulled and hulless barley for pigs, (2) effects of guanidination on apparent ileal AA digestibility values of soybean meal, rapeseed meal and peas for pigs, and (3) endogenous ileal recoveries and true ileal digestibility values of AA in the three protein sources for pigs with the homoarginine method.

Compared to hulled barley, hulless barley contained lower fiber (NDF) and higher β-glucan contents, and had higher ($P<0.05$) fecal energy digestibility and lower ($P<0.05$) ileal protein and AA digestibility values.

Two experiments indicated that correlation between *in vivo* and *in vitro* measurements of energy digestibility of barley was high ($R^2=0.90$, $P<0.05$). However, correlations between *in vivo* and *in vitro* measurements of most AA digestibility values of barley were low ($P>0.05$).

Guanidination increased protein and fiber, and decreased soluble carbohydrate and ash contents of soybean meal, rapeseed meal and peas. However, effects of guanidination on AA profile of protein in the protein sources were very small.

Unlike soybean meal and peas, apparent ileal digestibility of lysine decreased ($P<0.05$) after the guanidinated rapeseed meal was included into diet. Therefore, random and uniform of guanidination in rapeseed meal is doubted.

Guanidination increased ($P<0.05$) apparent ileal digestibility values of crude protein and most AA in peas and soybean meal, but there was little effect ($P>0.05$) on

rapeseed meal. The increase may be related to soaking the protein sources in high pH solution for few days during guanidination and/or repeated washings after guanidination.

Recoveries of endogenous ileal AA were higher ($P<0.05$) for rapeseed meal and peas than for soybean meal, there were no difference ($P>0.05$) between rapeseed meal and peas. However, true ileal digestibility values of AA were higher ($P<0.05$) in soybean meal and peas than in rapeseed meal, there were no difference ($P>0.05$) between soybean meal and peas. Therefore, output of endogenous AA for individual protein source is necessary for calculation of true ileal digestibility values of AA in order to accurately formulate diets for pigs.

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CHAPTER I

GENERAL INTRODUCTION

Energy and protein (amino acids) together account for more than 90% of the total animal feed costs. Other nutrient requirements can be met with relatively little cost. From this practical standpoint, the efficient utilization of energy and amino acids is essential for economical production of animal products. The nutritive values of energy and amino acids in feedstuffs for monogastric animals, such as pigs, are determined not only by their total contents in energy and amino acids, but also by the bioavailability of energy and amino acids, in particular the amino acids likely to be limiting to animals (Sauer et al., 2000). The availability of energy and nutrients, including amino acids, is defined as the proportion of ingested energy and nutrients that are absorbed from the digestive tract in a form available for metabolism by the animals (ARC, 1981; Sauer and Ozimek, 1986). The primary approach, normally accepted to estimate bioavailability of energy and amino acids for pigs, is the method to measure the proportion of dietary energy and amino acids that have disappeared from the gut (NRC, 1998; Sauer et al., 2000). The values determined in this manner are termed "digestibility values". In this thesis, therefore, emphasis will be placed on methodology for determination of energy and protein (amino acid) digestibility values in feedstuffs for pigs.

A. Digestive System of the Pig

The methodological improvement of feed evaluation based on energy and protein (amino acids) digestibility values, especially an *in vitro* enzymatic method for feed

evaluation, is largely decided by detailed knowledge about the digestive system of animals. It has been demonstrated very early by Aerts et al. (1977) that using chemical and enzymatic measurements to predict organic matter digestibility in ruminants and the co-efficient of determination of prediction was increased as the test method more closely approaches enzymatic digestion in the animal itself. Therefore, some knowledge of the digestive system is very important for feed evaluation, especially to determine energy and protein (amino acids) digestibility values.

The digestive process of pigs begins immediately after feed is in their mouth. Chewing feed by teeth breaks down cell walls or any other solid components of the feed into small particles, and also gives more surface area for saliva to lubricate and to soak the feed. Immediately an enzyme from saliva, α-amylase, is mixed by tongue with the feed to hydrolyze complex carbohydrates such as starch and dextrin into simpler molecules, such as maltose. The feed only remains in the mouth for a short time, then a wet round bolus is formed, and swallowed into the esophagus, which secretes mucus (from the tubuloacinar glands) lubricating the feed bolus on its passage to the stomach (Longland, 1991). Hydrochloric acid and enzymes (pepsin and rennin) are secreted into stomach to lower the pH and catalyze the digestion of protein into long chain peptides, respectively. This process facilitates the subsequent enzymatic digestion in the small intestine. Meanwhile contractions of the muscles lining the stomach physically aid to mix and break down the feed. The resulting semi-solid liquid with low pH in the stomach is called chyme. The chyme is drained through the pyloric sphincter, a valve that periodically opens to allow feed to pass into the duodenum of the small intestine. Compared to other farm animals, the small intestine of pigs is relatively long

(approximately 7 meters) and the main part of the digestive processes, and permits intensive digestion at an almost neutral pH. Other organs also are involved in digestion in the small intestine. The liver of pigs secretes bile to assist the digestion and absorption of lipids. Another important organ is the pancreas, which secretes pancreatic juice for digestion. This juice contains sodium bicarbonate, which neutralizes the acid from the stomach to raise the pH upon 7 to 8, and enzymes. The enzymes from the pancreas can be divided into three groups: amylase, like the enzyme from saliva, for carbohydrate digestion; trypsin, chymotrypsin, elastase, and carboxypeptidases A and B for protein digestion; and lipase, cholesterol esterase and phospholipase for fat digestion. Another three groups of enzymes are produced by the small intestinal mucosa: maltase, sucrase and lactase for carbohydrate digestion; aminopeptidase, di- and tri-peptidases for protein digestion; and enterokinase, which activates the inactivate precursors of enzymes from the pancreas to active enzymes. In pigs, most nutrients (amino acids, monosaccharides, fatty acids, minerals and vitamins) are absorbed in the small intestine. The undigested feed components (mainly dietary fiber, lipids, and insoluble protein) and of endogenous origin enter the large intestine, and are fermented by microflora, mainly in caecum and colon of the pig. Fermentation, microbial degradation, leads predominantly to the production of volatile fatty acids (acetic, propionic and butyric acids), gas (CO_2, H_2, NH_3 and CH_4), and heat (Noblet and Le Goff, 2001). The large intestine does not have villi, and has a small interior surface, so it is not very well suited for absorption of nutrients. In this part of the digestive system, only water, short-chain fatty acids, electrolytes and some vitamins (K and some B-vitamins) can be absorbed and contribute to the nutrient supply of the animal (Wenk, 2001). Meanwhile, the heat produced with fermentation can be used

to maintain animal body temperature. Therefore, energy (fatty acids and heat) produced by fermentation in large intestine of the pig is made available to the host animals (Ratcliffe, 1991). So fecal energy digestibility value is precisely representative of the energy bioavailability value.

All amino acids of undigested protein in the small intestine can be deaminated by microorganisms in the large intestine to yield ammonia and various amines and amides, and fatty acids (Fauconneau and Michel, 1970; Sauer et al., 2000), which also can be incorporated into amino acids synthesized *de novo*. Hence the net disappearance of an amino acid from the digestive tract (i.e. difference between the quantity of amino acids consumed and voided in faeces) does not necessarily represent absorption of these amino acids (Cho and Bayley, 1972). Net disappearances proximal to the caecum gives a minimum value for the apparent digestibility of an amino acid. Degradation in the large intestine increases and synthesis reduces estimates of AA digestibility (Holmes et al., 1974). The ammonia is absorbed and transported to the liver, where it is transformed to urea and excreted by kidney. Therefore, the ileal protein (amino acids) digestibility values are more representative of bioavailability values than fecal protein (amino acids) digestibility values.

Most digestive processes in pigs occur under anaerobic conditions. The extent of digestion depends on the quantity and composition of the diet. The size and function of the organ of the digestive tract as well as the digesta retention time in different parts of the gastrointestinal tract are also important determinants.

Considerable amounts of endogenous protein are produced namely from saliva, bile juice, pancreatic juice, intestinal juice, mucin and desquamated mucosal cells (Sauer et

al., 2000). Endogenous protein, along with dietary protein, is subjected to digestion, and their products are reabsorbed (Fuller, 1991). The majority of endogenous protein recovered at the distal ileum is composed of sloughed cells and mucin because these are partially resistant to enzymatic hydrolysis (Taverner et al., 1981; Moughan and Schuttert 1991). Digestibility values of protein and amino acids are confounded sometime by endogenous output of protein and amino acids, dependent on the feedstuffs under evaluation (Nyacoti et al., 1997_b).

It should be pointed out that digestion and absorption in live animals are complex, highly integrated, adapted, and adaptable processes, including the digestive enzyme system, gut microflora, and interactions between feed components and the digestive tract. These are dynamic processes under both neural and hormonal control and are responsive to many stimuli from the environment and dietary factors. However, it is not necessary to simulate the *in vivo* digestion exactly for the *in vitro* feed evaluation (Moughan, 1999). A major advance in feed evaluation of the *in vitro* enzymatic method for energy digestibility in pigs has particular promise for practical feed evaluation (Boisen and Eggum, 1991).

B. Development of Methodology for Determining Fecal Energy Digestibility Values

Energy is produced when organic molecules undergo oxidation. Energy is either released as heat or is trapped in high-energy bonds for subsequent use for the metabolic processes in animals (NRC, 1998). Since the original direct determinations of energy digestibility in feedstuffs for pigs by Diggs et al. (1959, 1965) and Tollett (1961), the

energy evaluation system of feedstuffs for pigs has been established. In this system, Farrell (1978), ARC (1981), and Morgan and Whittemore (1982) suggest that digestible energy is preferable in describing the energy content of feedstuffs for swine and the energy requirements of swine, because digestible energy can be easily and precisely determined and is, in principle, additive. Meanwhile, digestible energy values are available for most of the commonly used feedstuffs (NRC, 1998). Another important reason for using digestible energy is that the ratio of metabolizable energy to digestible energy is relatively constant for most practical swine diets used in North America, namely from 94 to 97 percent of digestible energy, with an average of 96 percent (Farrell, 1979; ARC, 1981). Thus, the metabolizable energy content of feedstuffs for pigs can be predicted accurately from the digestible energy content. In addition, as mentioned previously heat and volatile fatty acids produced by fermentation in the large intestine can be used by the animal, therefore fecal energy digestibility values rather than ileal digestibility values are more suitable for energy evaluation.

Conventional methods for measuring energy digestibility that involve either total collection of feces or the use of a digestibility marker are expensive and time consuming; they require relatively large amounts of feed and involve considerable expenditure for equipment and technical help. These methods, therefore, are impractical for routine and rapid feed evaluation. The mobile nylon bag technique (MNBT), described by Sauer et al. (1983), is a more rapid method. An excellent agreement was obtained between the MNBT and the conventional method for apparent energy digestibility values (Sauer et al., 1983). Beames et al. (1996) also indicated that apparent energy digestibility of feedstuffs for pigs could be predicted by dry matter digestibility determined with MNBT.

All the *in vivo* methods mentioned previously, especially those based on the use of surgically-modified animals, require special facilities which are not usually available to the feed industry. In addition, these methods are expensive and slow. Furthermore, there is increasing concern about the use of animals for routine testing of feedstuffs. For all these reasons, an *in vitro* method that could be simply used in any laboratory and give rapid and reproducible results would be very useful, especially for routine determination of the digestibility values of energy. The original *in vitro* method for the determination of digestible energy of feedstuffs involved the use of intestinal fluids of pigs (Furuya et al., 1979; Ehle et al., 1982). Lowgren et al. (1989) outlined an *in vitro* system with 3 different inocula from duodenal fluid, ileal fluid, and fecal extract. This method was successfully used to predict the digestible energy contents of feedstuffs for pigs. Using more consistent sources of purified enzymes had allowed further advances of the use of the *in vitro* method. Boisen and Fernandez (1991) developed an *in vitro* method through incubation of feedstuffs with pepsin, pancreatin and viscozyme (a microbial enzyme preparation). This method has shown promising results for prediction of fecal energy digestibility values (Boisen and Eggum, 1991).

C. Development of Methodology for Determining Ileal Digestibility Values of Protein and Amino Acids

The original methods for determining protein and amino acids digestibility values were outlined by Kuiken and Lyman (1948) by measuring the difference between the amounts of protein and amino acids ingested and excreted in feces. This method has been used extensively in studies with pigs (Dammers, 1964; Eggum, 1973). These studies, and

in particular those by Eggum (1973), opened the field of studies on the topic of amino acid digestibility. However, since undigested protein entering the large intestine of the pigs is fermented by micro-organisms, and the products of fermentation are not available to the host animal, the fecal amino acid digestibility values overestimate the amino acid availability values. For some amino acids, such as methionine and lysine (Zebrowska et al., 1978; Low, 1980; Sauer et al., 1982; Tanksley and Knabe, 1982), there are net syntheses by the microflora in the large intestine. In this case, fecal amino acid digestibility values underestimate the amino acid availability values. Therefore, Sauer and Ozimek (1986) strongly recommended using ileal amino acid digestibility instead of fecal digestibility values for feed evaluation on amino acid bioavailability. Furthermore, the ileal analysis method, as compared to the fecal analysis method, is more sensitive for detecting differences in amino acid digestibility values in feedstuffs (Sauer and Ozimek, 1986). In general, ileal digestibility values are similar to values determined by growth assays (Green and Kiener, 1989; Kovar et al., 1993; Adeola et al., 1994).

Methods for apparent ileal digestibility values of protein and amino acids

There are three methods for determination of apparent ileal protein and amino acid digestibility values, namely the direct, difference and regression methods.

For the direct method, an assay diet is formulated, in which the assay feed ingredient provides the sole dietary nutrient in question. Therefore, the nutrient digestibility in the assay feed ingredient is equal to the corresponding value in the assay diet.

The difference method involves the formulation of both a basal and an assay diet. The basal diet contains the basal feed ingredient that provides the sole assay nutrient in the

diet, whereas the assay diet consists of a mixture of the basal and the assay feed ingredients. If there is no interaction in nutrient digestibility between the basal and the assay feed ingredients, the nutrient digestibility in the assay feed ingredient can be determined by difference.

For the regression method, a basal diet and more than two assay diets with graded levels of the assay feed ingredient are formulated. The nutrient digestibility in the assay feed ingredient is extrapolated from the regression equation when the contribution level of the nutrient of the basal feed ingredient in the assay diet is equal to zero (Fan and Sauer, 1995). Like the difference method, the assumption for the regression method is that there are no interactions between the basal and the assay feed ingredients. When the direct method is used, the apparent amino acid digestibility values of feedstuffs with a low protein content are underestimated relative to feedstuffs with a high protein content because of the relatively greater contribution of endogenous amino acids (NRC, 1998). In order to minimize the effects of endogenous protein and amino acids on apparent ileal digestibility values of protein and amino acids, the difference and regression methods should be used for cereal grains with low protein contents (NRC, 1998; Sauer et al., 2000), but the all three methods are suited for protein supplements (Sauer et al., 2000).

Methods for true ileal digestibility values of protein and amino acids

It is now widely accepted that protein and amino acid digestibility values of feedstuffs for pigs is better determined based on ileal rather than fecal measurements (Low and Zebrowska, 1989). However, digesta collected at the distal ileum contain large quantities of endogenous protein. During the process of digestion in the upper digestive

tract, considerable amounts of endogenous protein enter the gut lumen (Low, 1980). The secretion and the accompanying extent of re-absorption of amino acids have a significant influence on the amount of protein recovered from the distal ileum, and thus on ileal protein and amino acid digestibility values. Several methods have been used to quantify the endogenous protein (amino acid) flow at the distal ileum of the pig. Earlier studies made use of conventional methods, including the feeding of protein-free diets, feeding diets containing protein sources with an assumed 100% digestibility, and mathematical regression techniques. More recently, the isotope dilution and the homoarginine method have been used.

When protein-free diets are fed, all protein-containing compounds in ileal digesta are assumed to be of endogenous origin. The main criticism of this method is non-physiological nature (Low, 1980) that may affect normal body protein metabolism (Millward et al., 1976) and in turn may reduce the secretion of protein compounds into the gut lumen and affect the efficiency of re-absorption (Darragh et al., 1990). The fact that animals are in a negative nitrogen balance appears to affect the endogenous losses of essential amino acids at the distal ileum of the pig (de Lange et al., 1989). As indicated by Butts et al. (1993) 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 endogenous protein output at the distal ileum. In addition, dietary constituents, such as fiber and anti-nutritional factors, may increase endogenous protein losses.

Feeding a diet containing a protein source with an assumed 100% digestibility, such as casein and crystalline amino acids, may offer a desirable alternative to using a protein-

free diet. However, the recovery of total protein at the distal ileum in pigs fed the casein and /or crystalline amino acid based diets can be lower than that 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 digestibility values of casein and synthetic amino acids are indeed 100% (Nyachoti et al., 1997a).

When regression methods are used, animals are fed graded levels of protein in the diet and the recovery of protein and amino acids at the distal ileum is related to protein and amino acid intake. By mathematical extrapolation, the recovery values of protein and amino acids at zero protein and amino acid intake can then be estimated. The regression method is believed to provide better estimates of endogenous protein losses compared to feeding protein-free diets (Fan et al., 1995). In addition, it allows for evaluation of the effects of various diets on endogenous protein losses (Souffrant, 1991; Fan et al., 1995). However, various studies have shown that estimates obtained with this method were not different from those obtained with feeding protein-free diets (e.g. Taverner et al., 1981; Furuya and Kaji, 1989; Donkoh et al., 1995). Furthermore, a basic assumption with the regression method, as well as with feeding protein-free diets, is that there is no relationship (interaction) between protein intake and endogenous protein losses. Based on various recent studies this basic assumption appears not to be valid; both methods result in an underestimation of endogenous protein losses (Nyachoti et al., 1997a).

Isotope dilution techniques using ^{15}N have been used to label either the animal's protein pool (de Lange, 1989; Huisman et al., 1992; Schulze et al., 1995) or the dietary protein (Leterme et al., 1994; Roos et al., 1994) and allow for differentiation between

endogenous and undigested dietary protein. Labeling the animal's protein pool is commonly used by continuously infusing labeled amino acids and then measuring the amount of labeled amino acids present in ileal digesta. The loss of endogenous protein may be underestimated by this method because it does not account for endogenously added mucosal cells that are synthesized from labeled 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 protein-containing compounds in the precursor pools and in endogenous gut protein are uniformly labeled when labeled amino acids are infused intravenously (de Lange et al., 1992; Lien et al., 1993). ^{15}N-labeled amino acids are very expensive and this may further limit the usefulness of this method. The ^{15}N-labeling of dietary protein poses little concerns as far as uniformity of labeling is concerned. ^{15}N supplied by dietary amino acids are quickly absorbed and incorporated into body protein thus complicating the differentiation of undigested dietary protein and endogenous protein losses (Leterme et al., 1994; Roos et al., 1994; Tamminga et al., 1995).

The homoarginine method is a relatively new technique involving transformation of dietary lysine to homoarginine through a guanidination process (a reaction with *O*-methylisourea). This method was first suggested by Hagemeister and Erbersdobler (1985) and allows for differentiation between dietary lysine (homoarginine) and endogenous lysine in digesta. Absorbed homoarginine may be hydrolyzed by arginase in the liver to lysine and urea, so there is no endogenous homoarginine entering the ileal digesta.

However, long-time and high dose usage of homoarginine may cause urea poisoning. The fact that uniform guanidination of lysine in test protein sources is not always achieved, especially in high fiber feed ingredients, may impose some limitations on this

method (Maga, 1981; Rutherfurd and Moughan, 1990; de Vrese et al., 1994). Same as the isotope dilution method, an assumption of a constant amino acid profile of endogenous protein is needed to estimate true ileal amino acid digestibility values for the homoarginine method (e. g. Nyachoti et al., 1997b).

Each method for measuring endogenous protein and amino acids, and true ileal digestibility, is subject to certain assumptions, limitations and criticisms. Also, the methods for determination of true ileal digestibility values of amino acids are very expensive, especially the isotope dilution and homoarginine techniques, and these methods are not practical for routine analyses. Furthermore, evidence at present suggests that the regulation of endogenous protein secretions, digestion and re-absorption is a highly complex matter depending on many factors. It thus seems prudent now to use apparent rather than true ileal amino acid digestibility values (Sauer and Ozimek, 1986; Knabe, 1991; Sauer et al., 2000). Meanwhile, the effects of endogenous protein and amino acid losses on apparent ileal digestibility values of protein and amino acids could be minimized if appropriate methods are chosen for determination of apparent ileal digestibility values of protein and amino acids. Then, apparent ileal amino acid digestibility values can be related to their corresponding true digestibility values.

Compared with all the *in vivo* methods, the *in vitro* methods are rapid, low cost and simple, also provide measurements that in theory reflect true ileal digestibility values of protein and amino acids. As previously reviewed by Boisen and Eggum (1991) and Moughan (1999), several *in vitro* methods simulating the digestive processes in pigs have been developed to estimate protein digestibility values. The earliest *in vitro* methods used single enzyme system such as pepsin (Sheffner et al., 1956) or trypsin (Maga et al.,

1973). At present, the *in vitro* enzymatic methods for feed evaluation (e.g. Boisen and Fernandez, 1995) have been developed to use multiple enzyme systems. Sometimes, the results correlated very well with those obtained with *in vivo* measurements. However, there is some conflicting evidence for predicting digestibility values of protein and amino acids in feedstuffs (Boisen and Fernandez, 1991), the *in vitro* method for prediction of true ileal digestibility values of amino acids may require further development before they can be applied with confidence (Moughen, 1999). There is a scarcity of information on the application of the *in vitro* method to barley evaluation for pigs.

D. Objectives (Hypotheses) of Thesis

Studies included in this thesis are to determine:

1. Application of the *in vitro* method to predict fecal energy digestibility values of hulled and hulless barleys for pigs.
2. Application of the *in vitro* method to predict ileal protein and amino acid digestibility values of hulled and hulless barleys for pigs.
3. The effects of guanidination on the chemical composition and amino acid profile of protein and on the apparent ileal amino acid digestibility values of soybean meal, rapeseed meal and peas for pigs.
4. The endogenous recoveries and true ileal digestibility value of protein and amino acids of soybean meal, rapeseed meal and peas for pigs by the homoarginine method.

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CHAPTER II

DEVELOPING *IN VITRO* METHOD FOR DETERMINATION OF ENERGY DIGESTIBILITY IN HULLED AND HULLESS BARLEY FOR PIGS

A. Introduction

Today barley ranks fourth after wheat, rice and corn in the world cereal production (FAO, 2000). In Canada, barley production ranks second after wheat. It is grown mainly in the prairie provinces of Alberta, Saskatchewan and Manitoba. Barley is often the cheapest source of energy and the most frequently used feed grain by the feed industry of Western Canada. Growing and finishing swine generally perform satisfactorily when barley is the major source of grain. Recent advances in barley breeding have led to the development of hulless barley varieties with special traits for feed applications. Hulless barley varieties have a higher digestible energy and protein (amino acid) content than hulled barley. This will likely result in increased usage in diets for pigs. Meanwhile, developments in the commercial availability of enzyme preparations containing β-glucanase and other complex-carbohydrate digesting enzymes may also result in increased utilization of barley in swine and poultry feeding.

Many factors, such as varieties and cultivars, growing conditions, and planting locations and seasons, influence the nutritive values of barley, which make it difficult to directly take the nutritive values of barley from compilations such as those produced by NRC (1998). Consequently, diets based on average tabular values will not necessarily be balanced for the ratio of digestible energy to digestible protein and amino acids

(Moughan, 1995). Even the fine tuning that can now be achieved by classification of barley according varieties and growing condition does not overcome this problem of variation between samples. The feed industry, but also plant breeders, would benefit greatly from an evaluation system that is rapid, cheap and accurate in assessing the contents of digestible energy and digestible protein (amino acids) in individual batches of grain used for feed manufacturing. These methods need to be reproducible and cost saving. The Mobile Nylon Bag Technique (**MNBT**) (Sauer et al., 1983; Schadereit et al., 1993) was used to determine the digestible energy and protein contents in different samples of barley, including hulless barley for pigs (Beames et al., 1996). However, the use of MNBT requires surgically-modified pigs that have to be housed in metabolism crates. Recently, studies were initiated to compare results obtained with the MNBT and an *in vitro* method for determining the digestible energy and protein contents in hulled and hulless barley.

The objectives of the present studies were 1) to develop an *in vitro* method for determination of digestible energy and protein in feedstuffs with special reference to hulled and hulless barley, and 2) to establish the best equations or models for prediction of *in vivo* energy and protein digestibility values of hulled and hulless barley, based on *in vitro* digestibility measurements.

B. Experimental Procedures

Animals and Diets

Eight crossbred barrows, average initial body weight (**BW**) 40 kg, were obtained from the Swine Research Unit of the University of Alberta and housed individually in

stainless steel metabolism crates in a temperature-controlled barn (22 ± 1°C). Four days later, the barrows were fitted with a simple T-cannula at the proximal duodenum according to the procedures described by Sauer et al. (1983). The cannulas were modified according to de Lange et al. (1989). A detailed description of pre- and post-operative care was previously presented by Li et al. (1994). During the 4 d adaptation period to the crates and the 14 d recuperation period, the pigs were given a 16% crude protein (CP) diet. Water was freely available from a low-pressure drinking nipple. At the conclusion of the experiment, the barrows, average final BW 90 kg, were sacrificed, and dissected to determine whether cannulation had caused intestinal adhesions or other abnormalities.

Based on feed intake and BW, six barrows were selected and fed 6 barley diets according to a 6×6 Latin square design. The pigs were fed twice daily, equal amounts each meal at 0800 and 1800. During the first experimental period, the daily dietary allowance was provided at a rate of 5% (wt/wt) of the average BW determined at the initiation of the experiment. Thereafter, the daily allowance was increased by 100 g at each successive experimental period.

The six experimental diets (Table II-1) consisted of 95% barley. Diet A: hulled barley, c.v. Harrington I. Diet B: hulled barley, c.v. Harrington II. This source of barley c. v. Harrington was different from the source in diet A, and therefore referred to as Harrington II. Diet C: hulless barley, c.v. CDC Buck. Diet D: hulless barley, c.v. CDC Richard. Diet E: mixture of diets A and C (50% wt/wt). Diet F: mixture of diets B and D (50% wt/wt). Canola oil was included in all diets at a level of 3% to reduce the dustiness of diets. Vitamins and minerals were supplemented to meet or exceed NRC (1998)

standards. Chromic oxide was included as digestibility marker at 0.25%. The barley was finely ground through a 2-mm mesh screen prior to incorporation into the diets.

Each of the six experimental periods consisted of an 8-d adaptation to diets and a 2-d collection of feces. The feces were immediately frozen at -20°C after collection, and then freeze-dried and ground through a 1-mm mesh screen. The feces were pooled within pig and period before analyses.

The animals used in this experiment were cared for in accordance with the guidelines established by the Canadian Council on Animal Care (1993) and approved by the Faculty of Agriculture, Forestry and Home Economics Animal Care Committee of the University of Alberta.

Mobile Nylon Bag Studies

Nylon bags, with a pore size of 48 μm and approximately 25×40 mm in size, were prepared from monofilament nylon. The percentage open area in the cloth was 36%. The bags were sealed on three sides using heat provided by a sealing device. After the barley was finely ground through a 1.0-mm mesh screen, approximately 1 g barley was placed into the nylon bag, and the open end of the bag was then sealed. The bags were grouped in blocks of eight and placed in a beaker containing 500 ml of a freshly prepared pepsin solution (10 mg/ml, Fisher ChemAlert, Fair Lawn, NJ) at pH 2.0, adjusted by 1.0 M HCl or 1 M NaOH solutions. The beaker was placed in a water bath at 37°C and agitated at a rate of 90 oscillations/min for 4 h. Thereafter, the bags were removed from the beaker, washed with deionized water and frozen until required. When an animal experiment was to be initiated, the frozen bags were thawed in container placed in a water bath at 37°C for 5 min. During the feeding times on d 6 and 7 of each experimental period, 8 nylon

bags were inserted into each pig via the cannula placed in the duodenum. A total of 32 bags of each source of barley or mixture were inserted into each pig. The bags were retrieved from feces, frozen at -20°C, and freeze-dried. The residues in the bags were weighed and pooled within pig, experimental period and source of barley or mixture. Bags were discarded if they were chewed or damaged. A detailed description of the MNBT was previously provided by Sauer et al. (1983).

In Vitro Studies

The dry matter, energy and crude protein digestibility values of the barley and mixtures were determined according to the method described by Boisen (1991), but based on the results of preliminary studies, the procedure was modified as follows:

1). *In Vitro* Determination of Dry Matter and Energy Digestibility Values

One gram of barley (or mixture), finely ground through a 1.0-mm mesh screen, was weighed into a 125 ml conical flask that contained 25 ml phosphate buffer (0.1 M, pH 6.0) and 10 ml 0.2 M HCl solution. The pH was adjusted to 2 with 1 M HCl or 1 M NaOH solutions. Following, 1 ml freshly prepared pepsin (10 mg/ml, Fisher ChemAlert, Fair Lawn, NJ) and 0.5 ml chloramphenicol solutions (0.5 g/100 ml ethanol) were added. The flasks were closed with a rubber stopper and incubated in an Environ-Shaker (Lab-line Instruments, Inc., Melrose Park, Ill) at an oscillatory rate of 120 at 39°C for 6 h. Thereafter, 10 ml phosphate buffer (0.2 M, pH 6.8) and 5 ml 0.6 M NaOH solution were added and the pH was adjusted to 6.8 with 1 M HCl or 1 M NaOH solutions. The contents of the flask were mixed with 3 ml freshly prepared pancreatin solution (50 mg/ml, Sigma chemical, St. Louis, MO) and incubated in the Environ-Shaker at an oscillatory rate of 120 at 39°C for 18 h. To simulate fermentation by the microflora in the

large intestine, 20 ml of a freshly prepared cellulase solution (3 units/ml, Trichoderma Viride, Sigma-C9422) was mixed with the contents in the flask and incubated in the Environ-Shaker at an oscillatory rate of 120 at 40°C for 24 h. Then, 5 ml of 20% sulphosalicylic acid solution was added to precipitate the soluble protein for 30 min. The residue was filtered by using pre-weighed glass filter crucibles (diameter 3 cm; pore size 40-90 μm). The total residue was transferred to the crucible by rinsing with 1% sulphosalicylic acid solution, and then dried at 80°C for 18 h. The weight of the residue was determined by difference.

2). *In Vitro* Determination of Ileal Crude Protein Digestibility Values

The procedure for measurement of CP digestibility values was performed as previously described for energy digestibility with the exception of incubation with cellulase. Crude protein digestibility determined in this manner should reflect ileal CP digestibility.

Chemical Analyses

Samples of dietary ingredients, diets, feces, and residues in nylon bags and remaining after *in vitro* incubation were ground through a 0.5-mm mesh screen before analyses. Analyses for dry matter, crude fat and ash were carried out according to AOAC (1990). Gross energy and CP was determined using a Leco AC-300 Automatic Calorimeter and a Leco FP-428 Nitrogen Analyzer (Leco Corporation, St. Joseph, MT), respectively. The chromic oxide contents in diets and feces were measured according to Fenton and Fenton (1979). Neutral detergent fiber, ADF and lignin were analyzed according to principles outlined by Goering and Van Soest (1970). β-glucans in the barley and mixtures were analyzed according to principles outlined by McCleary and

Glennie-Holmes (1985) by using a Megazyme kit (Megazyme, Bray Business Park, Bray, Co. Wicklow, Ireland). The barley and mixtures were finely ground through a 0.5-mm mesh screen and approximately 0.5 g was weighed into polypropylene tubes. One mL of aqueous ethanol solution (50% v/v) was added to each tube to aid the subsequent dispersion of the sample. Then, five mL of sodium phosphate buffer (20 mM, pH 6.5) was added and the tubes were stirred on a vortex mixer. The tubes were incubated in a boiling water bath for approximately 2 min and then stirred on a vortex mixer again. After heating the tubes for a further 3 min in the boiling water bath and then cooling to 40°C, 0.2 mL of lichenase solution was added into each tube and incubated at 40°C for 1 h after capping and stirring the tubes. The contents in each tube were made up to 30 mL of volume with distilled water, mixed thoroughly and centrifuged at approximately 1,000 x g for 10 min. Three samples, 0.1 mL each, were obtained from the supernatant of each tube, and carefully transferred to 3 test tubes. To one of the three test tubes, 0.1 mL of acetate buffer (50 mM, pH 4.0) was added. To the other two test tubes, 0.1 mL β-glucanase in 50 mM acetate buffer (pH 4.0) was added. All test tubes were incubated for 15 min at 40°C. After adding 3.0 mL of glucose oxidase/peroxide reagent, the test tubes were incubated for 20 min at 40°C again. The absorbance of the solution of each sample after incubation was measured at the wavelength of 510 nm.

Statistical Analyses

The results were subjected to Analysis of Variance by using the General Linear Model procedure of SAS (1998, SAS Institute Inc., Cary, NC, USA). The main effects of diets (n=6), pigs (n=6) and periods (n=6) were included in the model. The means of diets were compared using the Student-Newman Keul's multiple range procedure and the

statistical significance level was claimed at $P < 0.05$. Correlation coefficients of determination for digestibility values with the *in vivo*, MNBT, and the *in vitro* methods were analyzed with the procedure of Regression Analysis of SAS (1998), and regressions equations were established if correlations were significant at $P < 0.05$.

C. Results and Discussion

The pigs were healthy throughout the experiment and readily consumed their daily allowances. Postmortem examinations at the conclusion of the experiment revealed no adhesions and other intestinal abnormalities.

The chemical composition of the barleys and diets are presented in Tables II-2 and II-3, respectively. The contents of β-glucans in the diets were calculated from the analyzed values in barley. The analyzed values of all other parameters measured in the diets (Table II-3) were very close to the calculated values based on the analyzed values in the barleys. The average values of all parameters measured were within the range reported by NRC (1998), Baidoo et al. (1998) and Jaikaran et al. (1998).

The digestibility values of energy and CP in barley (or diets) by the *in vivo*, MNBT and *in vitro* methods are presented in Table II-4. The *in vivo* digestibility values of energy from this experiment (76.9 to 84.7%) are within the range of energy digestibility values reported by Darroch et al. (1995) (84.1 to 87.4%), and by Beames et al. (1996) (76.7 to 88.8%). As shown in Table II-4, the dry matter digestibility values of barley mixtures E and F with the MNBT are similar to the average values calculated from the digestibility values of barleys A and C, and B and D, respectively. Not only does this

indicate that dry matter digestibility values in barley are additive, but also the reliability of the MNBT. Therefore, the advantages of the MNBT are not only cost and time saving, but are also very reliable. The energy digestibility values from this experiment are very close to previously published values (Sauer et al., 1981; van Barneveld, 1999).

Irrespective which method was used, the digestibility values of energy in hulless barley were higher ($P<0.05$) than in hulled barley; the values in the barley mixtures were intermediate. There are many factors that affect the nutrient and energy digestibility values, e.g. barley variety, fat (Shiau, 1987; Li and Sauer, 1994), fiber content, and anti-nutritional factors. Among these factors the content of fiber in barley is considered a major factor. The contents of NDF and ADF are lower in hulless than in hulled barley (Tables II-2 and II-3). Hulls normally represent the fiber component in barley. Many studies (Mitaru et al., 1984; Sauer et al., 1991) have shown that fiber is the main factor that influences the digestibility of energy. The lower dry matter and energy digestibility in hulled barley may be attributed to its higher fiber content. A higher fiber content increases the rate of passage of digesta (Stanogias and Pearce, 1985) and the losses of endogenous nitrogen (Taverner et al., 1981; Nyachoti et al., 1996; Boisen and Moughan, 1996). In addition, fiber adsorbs nutrients (carbohydrate and protein), therefore lower the digestibility (Donangelo and Eggun, 1985).

The *in vivo* CP digestibility values (71.7 to 76.6%) in the barley were lower than values reported by Sauer et al. (1974) (80.4±1.5%); Darroch et al. (1995) (84.9 to 88.6%), and Beames et al. (1996) (79.3 to 88.8%). The CP digestibility values in barley differed by approximately 8 percentage units in the aforementioned studies, which indicates that the variation in CP digestibility is larger than in energy digestibility. As

reviewed by Sauer and Ozimek (1986) and Sauer et al. (2000), the large variation of CP digestibility values in grains with a low CP content, such as barley, may be related to the methods used for determining CP digestibility values, besides other factors. The relative large contribution of endogenous CP (in relation to undigested dietary CP) and microbial fermentation of protein in the large intestine (Web, 1973; Zebrowska, 1978) may be responsible for the large variation in fecal CP digestibility among different samples of barley. It was pointed out that the content of protein in barley affects the apparent CP digestibility. The higher the CP content, the higher the apparent CP digestibility until it reaches a plateau (Fan and Sauer, 1995).

Surprisingly, unlike energy digestibility, the digestibility values of CP in hulless barley were lower than in hulled barley, although only hulless barley CDC Richard (diet D) showed a difference ($P<0.05$) from the hulled barleys. The reason for this difference may be because of the effect of β-glucans. As shown in Table II-2, barley CDC Richard (diet D) contains a higher content of β-glucans (4.1 vs 3.5%) than the other barleys. Campbell et al. (1986) reported that some hulless barleys contain higher levels of β-glucans than hulled barley. β-glucans, present in barley endosperm and aleurone cell walls, may interfere with protein digestion and amino acid absorption (Anderson et al., 1978; Low, 1980; Aman and Graham, 1987). With the supplementation of β-glucanase, there was an increase in hydrolysis of β-glucans, resulting in higher digestion of carbohydrate and protein in the gastrointestinal tract of broiler chicks (Hesselman and Aman, 1986) and young pigs (Li et al., 1996). The mechanism by which β-glucans interfere with digestion and absorption are closely related to their physico-chemical properties. β-glucans, differing from cellulose, contain approximately 30% of the

linkages in the form of β (1-3) and 70% in the form of β (1-4) linkages between glucose molecules (Fleming and Kawakami, 1977). Staudte et al. (1983) also reported a ratio of 1 to 2.5 of β (1-3) to β (1-4) linkages in β-glucans. This branched structure prevents compact folding of the molecules and increases the water-holding capacity, which results in its characteristic viscosity and gelling properties (Fadel et al., 1987; Wang et al., 1992). The degree of viscosity was also attributed to the molecular weight and concentration of β-glucans (Bengtsson et al., 1990). The viscosity and gelling properties tend to hinder intestinal motility (Holt et al., 1979), thereby decreasing the mixing of digesta with digestive enzymes and other components required for digestion and absorption (Vahouny and Cassidy, 1985; Wang et al., 1992). These properties may also delay or decrease digestion and absorption of nutrients by increasing the unstirred fluid layer, creating a physical barrier at the absorption surface on the microvilli (Johnson and Gee, 1981). Special evidence (Forrest and Wainwright, 1977), explaining why β-glucans specifically hinder CP digestion, is that 50% of the total β-glucans could be removed by solubilization in water. However, when endosperm cell walls were incubated with the proteolytic enzyme thermolysin, more than 96% of the β-glucans were extracted. Thompson and Laberge (1977) and Weltzien (1986) also pointed out that the β-glucans are firmly linked to peptides in the cell wall.

The results from this experiment suggest that the content of fiber (NDF and ADF) may be the major factor that influences energy digestibility. However, for CP digestibility, the effect of β-glucans should not be ignored, particularly when differences in fiber content between barleys are very small. This point of view was supported by a study of Beames et al. (1996), in which the difference of total dietary fiber between

hulled barley varieties ranged from 17.6 to 24.4%. The highest (83.3%) and lowest (80.7%) CP digestibility values in pigs were obtained for barley with the lowest (3.6%) and highest (4.25%) content of β-glucans, respectively. It should be pointed out that although the difference in β-glucan content between diet D (hulless barley CDC Richard) and the other diets was relatively small (0.57 to 0.78 percentage units), feces from pigs fed diet D was much more sticky than feces from pigs fed the other diets, as observed when nylon bags were retrieved from feces. If the higher viscosity of feces from diet D was not caused by the higher β-glucan content, then it was possibly caused by a different type of β-glucans in CDC Richard.

As shown in Table II-4, the *in vitro* values of energy and CP digestibility values are higher than *in vivo* and MNBT values. This results mainly from the fact that the *in vitro* method does not account for endogenous protein losses which originate from various sources including saliva, pancreatic secretions, sloughed epithelial cells, mucin (Neutra and Forstner, 1987; Boisen and Eggum, 1991; Furuya and Kaji, 1992) and bacterial protein (Souffrant, 1991). The digestibility values determined with the MNBT are higher than those determined with the *in vivo* method, possibly because some endogenous material, such as sloughed epithelial cells and bacteria which are larger than the pore size (50 μm) of the nylon bags, were shielded by the nylon bags.

The total contents of digestible dry matter, energy and CP in hulled and hulless barley are presented in Table II-5. The total contents of digestible dry matter and energy in hulless barley were higher than in hulled barley. However, the total contents of digestible CP in hulless barley were in the same range as those in hulled barley.

In order to determine the optimal conditions for *in vitro* incubation specific to barley, some preliminary studies were carried out. The results are shown in Table II-7. There were increases ($P<0.05$) in dry matter and energy digestibility values in barley as the mesh size of the screen for grinding barley decreased from 4 to 1 mm, simply because enzymes have better access to their substrate when the surface area of the ground barley is increased. However, time of incubation with pepsin did not affect ($P>0.05$) dry matter and energy digestibility. Boisen (1991) also found no difference in dry matter digestibility when the incubation time with pepsin was increased from 2 to 6 h. The digestibility values of dry matter and energy increased ($P<0.05$) when the time of incubation with pancreatin was increased from 6 to 18 h. In contrast, Boisen (1991) found no difference ($P>0.05$) in dry matter digestibility when the time of incubation with pancreatin was increased from 4 to 18 h. The explanation for this is not clear, but may relate to differences among barley cultivars. There are more than 125 cultivars of barley (van Barneveld, 1999). The digestibility values of dry matter and energy remained similar ($P>0.05$) when the incubation time with cellulase was increased from 12 to 24 h. This indicates that the activity of cellulase was sufficient for cellulose hydrolysis in 12 h. Based on these preliminary studies, the *in vitro* technique is sensitive for the determination of digestibility values in barley. These results conform in general with results from studies by Boisen (1991).

Based on the values in Table II-4, regression equations (Table II-6) were established for digestibility values of dry matter, energy and CP between the *in vivo*, MNBT and *in vitro* methods. These studies show that the *in vivo* energy and CP digestibility values in barley can be predicted ($P<0.05$) with the MNBT and *in vitro*

method. The results for predictions of the *in vivo* energy digestibility values of barley from *in vitro* and MNBT digestibility values are in agreement with other studies (Boisen, 1991; Beames et al., 1996). It should also be pointed out that the *in vivo* energy digestibility values can be predicted ($P<0.002$) by the *in vitro* values even for barley samples with small differences in fiber contents. The content of NDF differed by only 4.1 percentage units among the barley samples in this experiment. Wiseman and Cole (1983), in reviewing prediction equations of energy digestibility values based on dietary fiber content, stated that 'prediction equations are of limited application for those feedstuffs (e.g. cereal grains) where there is lack of any appreciable variability in both dependent (energy digestibility) and independent (fiber) variables.' The same conclusions were drawn in other studies (Perez et al., 1980; Just, 1982). Therefore, the *in vitro* method is superior to and supplements prediction equations based on chemical composition.

Regression equations of CP digestibility values were established between the *in vivo* method and MNBT, and between the *in vivo* and *in vitro* methods. The coefficients of determination by the *in vitro* method were not as high for the estimation of CP digestibility values (0.85 to 0.86) as for GE digestibility values (0.93 to 0.95). However, *in vitro* measurements require more repeat analyses to obtain reliable values. The prediction of the *in vivo* CP digestibility values from the *in vitro* measurements is not agreement with some other studies. Boisen (1991) initially indicated that CP digestibility values could be predicted from the *in vitro* measurements for a wide range of feedstuffs, including barley. However, results reported by Beames et al. (1996) showed that the *in vivo* CP digestibility values (true protein digestibility, apparent CP digestibility corrected for metabolic fecal nitrogen. Eggum, 1973) of barley could not accurately be predicted

from *in vitro* measurements. This result was attributed to two samples of barley (from a total of 18) of which the *in vitro* CP digestibility values were very high. In addition to barley variety and cultivars, there are many other factors that influence *in vivo* and *in vitro* digestibility values of CP. The reason for the lower correlation coefficient for CP digestibility values than for energy digestibility values may be the fact that *in vivo* CP digestibility values in low protein feedstuffs should be determined with the difference method rather than with the direct method (Fan and Sauer, 1995). Therefore, more studies are needed for prediction of *in vivo* CP digestibility values from *in vitro* measurement.

In conclusion, the energy digestibility values are higher in hulless barley than in hulled barley; the content of fiber in barley is an important factor that determines the energy digestibility. On the other hand, CP digestibility values in hulled barley were higher than in hulless barley. β-glucans are also an important factor that affect CP digestibility values of barley, especially when its fiber content is low. The *in vivo* energy digestibility values in barley can be reliably predicted with both the MNBT and the *in vitro* method. In addition, the MNBT and *in vitro* method can also be used to predict CP digestibility values. The *in vitro* method would be the method of choice as it is unlikely that it will be possible in the future to use surgically-modified animals for routine determination of digestibility values of nutrients and energy in feedstuffs for pigs.

Table II-1. Formulation (%) of the experimental diets (as-fed basis)

Ingredients	Diets					
	A	B	C	D	E	F
Hulled barley (Harrington I) [a]	94.78	-	-	-	47.43	-
Hulled barley (Harrington II)	-	94.78	-	-	-	47.43
Hulless barley (CDC Buck)	-	-	94.78	-	47.43	-
Hulless barley (CDC Richard)	-	-	-	94.78	-	47.43
Canola oil	3.00	3.00	3.00	3.00	3.00	3.00
Biophos[b]	0.52	0.52	0.27	0.27	0.31	0.31
Calcium carbonate	0.92	0.92	1.17	1.17	1.04	1.04
Trace-mineralized salt[c]	0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride[d]	0.03	0.03	0.03	0.03	0.03	0.03
Vitamin-mineral Premix[e]	0.20	0.20	0.20	0.20	0.20	0.20
Chromic oxide[f]	0.25	0.25	0.25	0.25	0.25	0.25

[a] All barley provided by Unifeed, Vermillion, AB.

[b] Contained 18% P and 24% Ca; supplied by Continental Lime Ltd., Exshaw, AB.

[c] Provided the following (per kilogram of diet): NaCl, 2.9 g; ZnO, 12.0 mg; $FeCO_3$, 4.8 mg; MnO, 3.6 mg; CuO, 1.0 mg; $Ca(IO_3)_2$, 0.2 mg; CaO, 0.1 mg. Supplied by Windsor Salt Co., Toronto, ON.

[d] Contained choline chloride 60%. Provided by Champion Feed Service Ltd., AB.

[e] Provided the following (per kilogram of diet): vitamin A, 10,000 IU; vitamin D_3, 1,000 IU; vitamin E, 80 IU; vitamin K_3, 2.0 mg; vitamin B_{12}, 0.03 mg; riboflavin, 12 mg; niacin, 40 mg; d-pantothenic acid, 25 mg; choline, 1,000 mg; d-biotin, 0.25 mg; folic acid, 1.6 mg; thiamin, 3.0 mg; ethoxyquin, 5.0 mg; pyridoxine, 2.25 mg; Fe, 150 mg; Zn, 150 mg; Cu, 125 mg; I, .21 mg; Se, 0.3 mg. Supplied by Hoffmann-LaRoche Ltd., 2455 Meadowpine Blvd., Mississauga, ON.

[f] Fisher Scientific, Fair Lawn, NJ.

Table II-2. Chemical and amino acid composition (%) of the barleys (as-fed basis)

Item	Barley[1]					
	A	B	C	D	E	F
Dry matter	89.1	89.6	88.9	88.7	88.6	88.5
Crude protein	12.4	11.4	12.3	12.3	12.4	11.8
Gross energy (kcal/g)	4.09	4.06	4.12	4.11	4.08	4.09
Crude fat	1.7	1.7	1.8	1.3	1.7	1.5
Neutral detergent fiber	15.1	14.9	11.0	12.1	12.8	13.1
Acid detergent fiber	4.5	3.9	2.0	2.7	3.2	3.3
Lignin	0.7	0.8	0.6	0.6	0.7	0.6
β-glucans	3.51	3.49	3.41	4.10	3.49	3.84
Ash	2.2	2.0	1.9	1.9	2.0	2.0
Amino acids						
Indispensable						
Arginine	0.57	0.53	0.57	0.56	0.56	0.55
Histidine	0.28	0.26	0.29	0.27	0.28	0.27
Isoleucine	0.43	0.40	0.41	0.43	0.42	0.43
Leucine	0.91	0.82	0.84	0.85	0.87	0.85
Lysine	0.45	0.42	0.44	0.41	0.44	0.43
Phenylalanine	0.67	0.63	0.64	0.63	0.65	0.63
Threonine	0.43	0.39	0.44	0.42	0.43	0.42
Valine	0.60	0.55	0.57	0.59	0.58	0.58
Dispensable						
Alanine	0.49	0.45	0.49	0.47	0.49	0.47
Aspartic acid	0.70	0.65	0.73	0.73	0.72	0.70
Glutamic acid	3.18	2.88	3.11	3.32	3.16	3.09
Glycine	0.49	0.45	0.52	0.47	0.50	0.46
Proline	1.39	1.27	1.31	1.41	1.37	1.37
Serine	0.58	0.52	0.57	0.58	0.57	0.55
Tyrosine	0.33	0.29	0.32	0.32	0.33	0.31

[1] A: Hulled barley Harrington I; B: Hulled barley Harrington II; C: Hulless barley CDC Buck; D: Hulless barley CDC Richard; E: Mixture of Harrington I and CDC Buck (50%/50%); F: Mixture of Harrington II and CDC Richard (50%/50%).

Table II-3. Chemical and amino acid compositions (%) of the experimental diets (as-fed basis)

Item	Diets[1]					
	A	B	C	D	E	F
Dry matter	88.0	88.1	87.8	87.7	88.0	87.9
Crude protein	11.9	10.6	11.6	12.0	11.6	10.9
Gross energy, kcal/g	4.05	4.05	4.06	4.05	4.05	4.05
Crude fat	4.6	4.6	4.7	4.3	4.6	4.5
Neutral detergent fiber	14.1	14.0	10.4	11.7	12.0	12.1
Acid detergent fiber	4.0	3.8	1.7	2.5	2.7	3.2
Lignin	0.5	0.5	0.3	0.4	0.4	0.4
β-glucans	3.33	3.12	3.24	3.90	3.32	3.65
Ash	3.9	4.0	3.9	3.9	3.9	3.9
Amino acids						
Indispensable						
Arginine	0.56	0.51	0.56	0.54	0.55	0.52
Histidine	0.26	0.24	0.27	0.26	0.26	0.25
Isoleucine	0.41	0.36	0.38	0.42	0.39	0.38
Leucine	0.85	0.77	0.80	0.81	0.82	0.78
Lysine	0.42	0.40	0.42	0.39	0.43	0.39
Phenylalanine	0.62	0.58	0.60	0.59	0.61	0.58
Threonine	0.41	0.38	0.43	0.41	0.42	0.39
Valine	0.56	0.51	0.54	0.56	0.55	0.52
Dispensable						
Alanine	0.46	0.44	0.48	0.46	0.47	0.44
Aspartic acid	0.68	0.64	0.70	0.70	0.69	0.66
Glutamic acid	3.00	2.75	3.01	3.18	3.01	2.93
Glycine	0.46	0.44	0.50	0.45	0.47	0.44
Proline	1.37	1.25	1.29	1.40	1.35	1.33
Serine	0.52	0.49	0.56	0.56	0.54	0.53
Tyrosine	0.32	0.28	0.31	0.31	0.32	0.30

[1] Refer to Table II-1 for abbreviations.

Table II-4. Dry matter, energy and crude protein digestibility values (%) of barley with the *in vivo*, mobile nylon bag and *in vitro* methods

Item	Method	Barley or diets[1]						
		A	B	C	D	E	F	SEM[2]
Dry matter	*In vivo* (n=6)	81.2^{d}	80.6^{d}	88.0^{a}	84.8^{b}	84.4^{b}	82.8^{c}	0.96
	MNBT[3] (n=6)	82.3^{d}	81.5^{e}	90.3^{a}	86.1^{b}	85.7^{b}	84.0^{c}	0.32
	In vitro (n=16)	86.2^{c}	85.3^{c}	91.2^{a}	89.6^{b}	88.1$^{b\,c}$	87.6^{c}	1.02
Energy	*In vivo* (n=6)	77.6^{c}	76.9^{c}	84.7^{a}	81.4^{b}	80.9^{b}	79.3^{b}	1.05
	MNBT (n=6)	78.9^{c}	78.0^{c}	87.1^{a}	82.7^{b}	82.2^{b}	80.2^{b}	1.12
	In vitro (n=16)	81.3^{c}	80.2^{c}	89.0^{a}	85.6^{b}	82.9$^{b\,c}$	81.8^{c}	1.08
Crude protein	*In vivo* (n=6)	76.6^{a}	75.0^{a}	74.1^{ab}	71.7^{b}	75.3^{ab}	72.9^{ab}	1.07
	MNBT (n=6)	85.0^{a}	80.5^{b}	79.2^{c}	76.9^{c}	81.2^{b}	78.4^{c}	1.06
	In vitro (n=16)	90.2^{a}	88.7^{b}	85.2^{c}	84.5^{c}	87.9^{b}	85.4^{c}	1.28

[1] Refer to Table II-1 for abbreviations.

[2] Standard error of the means.

[3] Mobile nylon bag technique.

[a, b, c, d] Means in the same row with different letters differ ($P < 0.05$).

Table II-5. Digestible contents of dry matter, energy and crude protein in hulled and hulless barley (as fed basis)

Items	Barley [1]					
	A	B	C	D	E	F
Dry matter (%)	72.3	72.2	78.2	75.2	74.8	73.3
Energy (kcal/g)	3.17	3.12	3.49	3.35	3.30	3.24
Crude protein (%)	9.5	8.6	9.1	8.8	9.3	8.6

[1] Refer to Table II-2 for abbreviations.

Table II-6. Relationships of digestibility values of dry matter, energy and crude protein among the *in vivo*, mobile nylon bag and *in vitro* methods

Item	Regression equation [a]	R^2	$S_{y.x}$ [b]	P[c]	P[d]
Dry matter	$Y_{in\ vivo}=0.854X_{MNBT}+11.0$	0.99	0.03	0.0001[f]	0.0063
	$Y_{in\ vivo}=1.226X_{in\ vitro}-24.3$	0.96	0.37	0.0006[f]	0.0934
	$Y_{MNBT}=1.426X_{in\ vitro}-40.5$	0.95	0.64	0.0010[f]	0.0499
Energy	$Y_{in\ vivo}=0.863X_{MNBT}+9.7$	0.99	0.09	0.0001[f]	0.0433
	$Y_{in\ vivo}=0.839X_{in\ vitro}+10.1$	0.93	0.72	0.0020[f]	0.3551
	$Y_{MNBT}=0.977X_{in\ vitro}-0.005$	0.95	0.71	0.0010[f]	0.9996
Crude protein	$Y_{in\ vivo}=0.600X_{MNBT}+26.2$	0.91	0.35	0.0032[f]	0.0262
	$Y_{in\ vivo}=0.714X_{in\ vitro}+12.2$	0.85	0.57	0.0086[f]	0.3990
	$Y_{MNBT}=1.143X_{in\ vitro}-19.2$	0.86	1.33	0.0072[f]	0.3844

[a] Y=dependent variable: dry matter, energy or crude protein digestibility (%) values determined by different methods as subscribed;

X=independent variable: dry matter, energy or crude protein digestibility (%) values determined by different methods as subscribed.

[b] Standard error of estimate of the regression equation.

[c] The probability of significance for the slope of the regression equation.

[d] The probability of significance for the intercept of the regression equation.

[f] The linear regression equation is significant ($P < 0.05$, n=6).

Table II-7. *In vitro* dry matter and energy digestibility values of hulless barley with different particle size and time of incubation with enzymes

Factor	Level		Dry matter	Energy
Mesh size of screen (mm)	1.0		87.7[a]	82.4[a]
	2.0		83.9[b]	79.1[b]
	4.0		19.8[c]	14.1[c]
		SEM	0.78	0.92
Pepsin incubation (hours)	3.0		87.9	84.8
	4.0		87.9	83.4
	6.0		86.9	83.7
		SEM	0.66	0.68
Pancreatin incubation (hours)	6.0		87.9[b]	83.4[b]
	12.0		88.6[b]	85.1[b]
	18.0		92.6[a]	87.9[a]
		SEM	0.96	1.05
Cellulase incubation (hours)	12.0		87.3	84.3
	18.0		88.6	86.0
	24.0		87.9	83.4
		SEM	0.92	1.10

[a. b. c] Means in the same column within the same treatment with different letters differ (n=12, $P < 0.05$).

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CHAPTER III

DEVELOPING *IN VITRO* METHOD FOR DETERMINATION OF PROTEIN AND AMINO ACID DIGESTIBILITY VALUES OF BARLEY FOR PIGS

A. Introduction

Barley is one of major cereal grains produced in Canada (FAO, 2000). With more new varieties of hulless barley being cultivated for their higher digestible energy and protein content compared to hulled barley, it is expected that there will be an increased usage of barley in swine diets. In addition to digestible energy, it is important to obtain information on the digestible amino acid (AA) content in both hulled and hulless barley. The ileal, rather than the fecal, analysis method should be used to determine AA digestibility values in feedstuffs for pigs because of the modifying action of the microflora in the large intestine (Sauer and Ozimek, 1986). For the determination of ileal AA digestibility values, there are three *in vivo* methods, namely, the direct, difference and regression methods. Studies by Fan and Sauer (1995) and reviewed in detail by Sauer et al. (2000) indicated that only the difference and regression methods are suitable for the measurement of apparent ileal digestibility values of AA in low protein feedstuffs, such as barley. The direct method underestimates the digestibility values of AA in cereal grains because of the relative large contribution of endogenous AA in digesta collected from the distal ileum. Of the two other methods, the difference method is more time and cost saving. The difference method involves the formulations of both a basal diet and assay diet. The basal diet contains the basal feedstuff, such as soybean meal, which has a high protein content and provides the sole source of dietary AA. The assay diet consists

of a mixture of the basal and assay feedstuffs, for example soybean meal and barley. If there is no interaction between the basal and assay feedstuffs, then the digestibility values of AA in the assay feedstuff can be measured by difference.

The *in vitro* enzymatic method saves much more time and cost than any of the *in vivo* methods. However, the reliability of the *in vitro* method to predict the *in vivo* digestibility values needs to be verified. The values obtained with the *in vitro* method should be validated with values obtained with the *in vivo* methods.

The objectives of this experiment were: 1) to measure the ileal digestibility values of energy, crude protein (**CP**) and AA in hulled and hulless barley with the *in vivo* and the *in vitro* methods; 2) to determine the correlation coefficients of digestibility values of energy, CP and AA between the *in vivo* and the *in vitro* methods, and 3) to establish regression equations for prediction of *in vivo* digestibility values from the *in vitro* values.

B. Experimental Procedures

Animals and Diets

Eight barrows (PIC), average initial body weight (**BW**) 35 kg, were obtained from the Swine Unit of the University of Alberta and housed individually in stainless steel metabolism crates in a temperature-controlled (20 to 22°C) barn. After a 10-d adaptation period to the metabolism crates, the pigs were fitted with a simple T-cannula at the distal ileum following procedures described by Sauer et al. (1983). The cannulas were made following procedures outlined by Sauer et al. (1983) with modifications described by de Lange et al. (1989). A detailed description of pre- and postoperative care was previously

provided by Li et al. (1994). During the 10-d adaptation period to the crates and 14-d recuperation period from surgery, the barrows were fed an 18% CP diet. Water was freely available from a low-pressure drinking nipple. At the conclusion of the experiment, the barrows, average BW 90 kg, were sacrificed, and dissected to determine whether cannulation had caused adhesions or other intestinal abnormalities.

The animals used in this experiment were cared for in accordance with the guidelines established by CCAC (1993) and approved by the Faculty of Agriculture, Forestry and Home Economics Animal Care Committee of the University of Alberta.

After recuperation from surgery, seven barrows were selected, based on feed intake and BW, and fed the experimental diets according to a 7×7 Latin square design. The pigs were fed twice daily equal amounts at 0800 and 2000. During the first experimental period, the daily dietary allowance was provided at a rate of 5% of the average BW of all pigs that was determined at the start of the first experimental period. Thereafter, the daily dietary allowance was increased by 100 g at each successive experimental period.

The seven experimental diets were formulated (Table III-1) to contain 18% CP. The soybean meal diet (Diet SBM), as basal diet, was corn starch-based. Soybean meal provided the sole source of protein in this diet. The other diets (Diets A to F), assay diets, contained both soybean meal and barley. Diet A contained hulled barley, c.v. Harrington I. Diet B: hulled barley, c.v. Harrington II. Diet C: hulless barley, c.v. CDC Buck I. Diet D: hulless barley, CDC Buck II. Denotations I and II are given to indicate two different sources of Harrington and CDC Buck. Diet E: mixture of Harrington I and CDC Buck I (50%/50%, wt/wt). Diet F: mixture of Harrington II and CDC Buck II (50%/50%, wt/wt).

Canola oil was included at 3% (wt/wt) to reduce dustiness of the diets. Vitamins and minerals were supplemented to meet or exceed NRC (1998) standards. Chromic oxide (0.3%) was included in the diets as the digestibility marker. Barley was finely ground through a 2-mm mesh screen prior to incorporation into the diets.

Each experimental period lasted 9 days. Feces were collected for 48 h from 0800 on d 5 to 0800 on d 7. Ileal digesta were collected from 0800 to 2000 on d 7 and from 2000 on d 8 to 0800 on d 9. Digesta were collected into a plastic tube (length: 20 cm; width: 3 cm) that contained 10 mL 2.5 M formic acid to minimize bacterial fermentation. The bags were immediately frozen at -20°C when digesta filled approximately one third to two third of the bags. The digesta were freeze-dried and pooled within pig and experimental period before analyses.

The same barley samples and mixtures were used to determine the digestibility values of energy, CP and AA with the *in vitro* enzymatic methods.

In vitro enzymatic measurement

The energy, CP and AA *in vitro* digestibility values of the barley and mixtures were determined according to the method outlined by Boisen (1991), but with modifications described as following.

1). *In vitro* determination of energy digestibility values

One gram of barley (or mixture), finely ground through 1.0-mm mesh screen, was weighed into a 125 mL conical flask containing 25 mL of phosphate buffer (0.1 M, pH 6.0) and 10 mL 0.2 M HCl solution. The pH was adjusted to 2 with 1 M HCl or 1 M NaOH solutions. One mL of freshly prepared pepsin (10 mg/mL, Fisher ChemAlert, Fair Lawn, NJ) and 0.5 mL of a chloramphenicol solution (0.5 g/100 mL ethanol) were added.

The flasks were closed with a rubber stopper and incubated in an Environ-Shaker (Lab-line Instruments, Inc., Melrose Park, Ill) at an oscillatory rate of 120 at 39°C for 6 h. Thereafter, 10 mL of a phosphate buffer (0.2 M, pH 6.8) and 5 mL of 0.6 M NaOH solution were added and the pH was adjusted to 6.8. The contents of the flask were mixed with 3 mL of a freshly prepared pancreatin solution (50 mg/mL, Sigma Chemical, St. Louis, MO) and incubated in the Environ-Shaker at an oscillatory rate of 120 at 39°C for 18 h. To simulate fermentation by the microflora in the large intestine, 20 mL of a freshly prepared cellulase solution (3 units/mL, Trichoderma Viride, Sigma-C9422) was added into the flask and incubated in the Environ-Shaker at an oscillatory rate of 120 at 40°C for 24 h. Thereafter, 5 mL of 20% sulphosalicylic acid solution was added to precipitate the soluble protein for a period of 30 min; the residue was filtered by using pre-weighed glass filter crucibles (diameter 3 cm, pore size 40-90 μm). The total residue was transferred to the crucible by rinsing with 1% sulphosalicylic acid solution and then dried at 80°C for 18 h. The weight of the residue was obtained by difference. The dry matter (DM) and energy contents of the residues were measured.

2). *In vitro* determination of CP and AA digestibility values

The procedure for measurement of CP and AA digestibility values was performed as previously described for energy digestibility with the exception of incubation with cellulase. Crude protein and AA digestibility values determined in this manner should reflect true ileal CP and AA digestibility values, respectively.

Chemical and Statistical Analyses

Samples of ingredients, diets, feces, digesta, and residue material remaining after *in vitro* incubation were ground through a 0.5-mm mesh screen before analyses. Chemical analyses for DM, crude fat and ash were carried out according to AOAC (1990). Gross energy and CP were determined using the Leco AC-300 Automatic Calorimeter and the Leco FP-428 Nitrogen Analyzer (Leco Corporation, St. Joseph, MT), respectively. The chromic oxide contents in diets, digesta and feces were measured according to Fenton and Fenton (1979). Neutral detergent fiber and ADF were analyzed according to principles outlined by Goering and Van Soest (1970).

β-glucans in the barley and mixtures were analyzed according to the principles outlined by McCleary and Glennie-Holmes (1985) using the Megazyme kit (Megazyme, Bray Business Park, Bray, Co. Wicklow, Ireland). Samples were finely ground through a 0.5-mm mesh screen and approximately 0.5 g was weighed into polypropylene tubes. One mL of aqueous ethanol solution (50% v/v) was added to each tube to aid the subsequent dispersion of sample. Then 5.0 mL of sodium phosphate buffer (20 mM, pH 6.5) was added and the tubes were stirred on a vortex mixer. The tubes were incubated in a boiling water bath for approximately 2 min and then stirred on the vortex mixer again. After the tubes were heated for a further three min in the boiling water bath and then cooled to 40°C, a 0.2 mL of lichenase solution was added into each tube and incubated at 40°C for 1 h after capping and stirring the tubes. The contents in each tube were made up to 30.0 mL of volume by distilled water, mixed thoroughly and centrifuged at approximately 1,000 x g for 10 min. Three samples, 0.1 mL each, were obtained from the supernatant of each tube, and transferred to three test tubes. To one of the test three tubes,

0.1 mL of acetate buffer (50 mM, pH 4.0) was added. To the other two test tubes, 0.1 mL β-glucanase in 50 mM acetate buffer (pH 4.0) was added. All test tubes were incubated for 15 min at 40°C. After adding 3.0 mL of glucose oxidase/peroxide reagent, the test tubes were incubated for 20 min at 40°C again. The absorbance of the solution of each sample was measured at a wavelength of 510 nm.

For AA analyses, approximately 100 mg of sample was weighed into a screw-capped test tube and mixed with 3 mL of 6 N HCl solution. Then, the test tubes were purged with nitrogen and hydrolyzed in an oven at 110°C for 24 h. The hydrolyzed samples were mixed with the internal standard, DL-amino-n-butyric acid, and centrifuged at 1,100 × g for 15 min at 4°C. The supernatant of the sample was analyzed according to principles outlined by Jones and Gilligan (1983) using a Varian 5000 high performance liquid chromatography system with a reverse-phase column and a Varian Fluorichrom detector (Varian Canada Inc., Mississauga, ON). The amino acids were derivatized with an *o*-phthaldialdehyde reagent solution. The mobile phase consisted of two solvents with a flow rate of 1.1 mL/min. Solvent A contained 0.1 M sodium acetate (pH 7.2), methanol, and tetrahydrofuran in a ratio of 90 to 5; Solvent B was pure methanol. Peaks were recorded and integrated using the Ezchrom Chromatography Data System (version 2.12; Shimadzu Scientific Instruments Inc., Columbia, MD). Methionine, cysteine, proline and tryptophan were not determined.

The apparent fecal and ileal digestibility values of DM, energy, CP and AA in the experimental diets were determine using Equation 1:

$$D_D(\%) = [1-(I_D \times A_F)/(A_D \times I_F)] \times 100 \qquad (1)$$

Where D_D is the apparent digestibility of a nutrient or energy in the diet (%), I_D is the marker concentration in the diet (%), A_F is a nutrient or energy concentration in ileal digesta or feces (%), A_D is a nutrient or energy concentration in the diet (%), I_F is the marker concentration in ileal digesta or feces (%).

By using soybean meal as the basal feed ingredient, the apparent ileal and fecal digestibility values of CP, AA and energy in barley were calculated by difference using Equation 2:

$$D_A = [D_D - (D_B \times S_B)]/S_A \qquad (2)$$

Where D_A is apparent digestibility of a nutrient or energy in the assay feed ingredient (%), D_D is the apparent digestibility of a nutrient or energy in the assay diet (%), obtained from Equation 1, D_B is the apparent digestibility of a nutrient or energy in the basal feed ingredient (%), S_B is the contribution level (%) of a nutrient or energy in the basal feed ingredient to the assay diet, S_A is the contribution level (%) of a nutrient or energy in the assay feed ingredient to the assay diet (%).

Results were subjected to Analyses of Variance using the General Linear Model procedure of SAS (release 6.12, the SAS Institute Inc.). The main effects of diets (n=7), pigs (n=7) and periods (n=7) were included in the model. The means of diets were compared using the Student-Newman Keul's multiple range test procedure and the significance level was claimed at $P < 0.05$. Correlation coefficients of fecal DM and energy and ileal CP and AA digestibility values between the *in vivo* and *in vitro* methods were analyzed with using the Regression Analysis procedure of SAS. Regressions equations were established and accepted if correlations were significant at $P < 0.05$.

C. Results and Discussion

The pigs remained healthy throughout the experiment and readily consumed their daily allowances. Postmortem examinations at the conclusion of the experiment revealed no adhesions and other intestinal abnormalities.

The chemical and amino acid composition of the dietary ingredients and diets are presented in Tables III-2 and III-3, respectively. The contents of β-glucans in the experimental diets were calculated from the analyzed values in barley and soybean meal. The values of all the other parameters analyzed in the experimental diets (Table III-3) were very close to the calculated values based on the analyzed values in the dietary ingredients (Table III-2). The mean values of the contents of the parameters measured in barley and soybean meal were within the range of values reported by Fan and Sauer (1995), Baidoo et al. (1998), Jaikaran et al. (1998), and NRC (1998). As expected, the contents of NDF and ADF were lower in hulless barley than in hulled barley. But the contents of β-glucans were higher in hulless barley than in hulled barley, which was expected based on previous results reported by Campbell et al. (1986) and Baidoo et al. (1998). The CP and AA contents in the hulless barleys were higher than in the hulled barley, which is in agreement with results reported by Jaikaran et al. (1998).

The apparent ileal digestibility values of DM, organic matter (OM), CP and AA in the experimental diets are presented in Table III-4. The apparent ileal digestibility values of CP and AA in the soybean meal diet are within the range of values reported by Sauer et al. (1991), Mosenthin et al. (1994), and NRC (1998). For example, the AA digestibility values in the soybean meal diet were within 2 percentage unit differences

from values reported by NRC (1998), except for isoleucine and leucine which were 3 to 4 percentage units higher. As expected, the apparent ileal digestibility values of DM, OM, CP and most of the AA in the soybean meal diet were higher ($P < 0.05$) than in the barley-containing diets. The digestibility values of CP in the diets containing hulled barley were higher ($P < 0.05$) than in the diets with hulless barley. The apparent ileal digestibility values of AA were also higher ($P < 0.05$) in the diets with hulled barley than in the diets with hulless barley, except for histidine and tyrosine ($P > 0.05$). The values from the diets containing the barley mixtures were intermediate.

The apparent ileal digestibility values of CP and AA in barley determined by the difference method are presented in Table III-5. These values are very close to values reported by Fan and Sauer (1995) and Li et al. (1996) who also used the difference method, but higher than values reported by Baidoo et al. (1998) who used the direct method. For example, the digestibility values of lysine (first-limiting AA in barley) and threonine (second-limiting AA) ranged from 58.2 to 69.7%, and from 53.7 to 59.6%, respectively. In the same order for these AA, the values reported by Fan and Sauer (1995) were 62.4 and 61.3%, respectively. Baidoo et al. (1998) reported values ranging from 45.6 to 56.6% for lysine and from 48.2 to 58.1% for threonine. As was pointed out by Fan and Sauer (1995) the direct method is not valid for determining CP and AA digestibility values in feedstuffs that are low in protein, such as barley.

As shown in Table III-5, the apparent ileal digestibility values of CP and indispensable AA, with the exception of histidine, were higher ($P < 0.05$) in hulled barley than in hulless barley; the values from the barley mixtures were intermediate. These studies show that the ileal digestibility values of CP and indispensable AA (also

dispensable AA) decreased as the β-glucan content in barley increased. As shown in Table III-3, the β-glucan contents in the diets with hulled barley (2.77 to 2.82%) are lower than in the diets with hulless barley (3.35 to 3.47%). There is no other information available in the literature on the effect of β-glucans on ileal CP and AA digestibility values in barley when these are measured with the difference method. The interpretation of results from these studies is further supported by studies by Li et al. (1996) and Baidoo et al. (1998). In the aforementioned studies, supplementation of β-glucanase to diets that contained hulless barley improved ($P < 0.05$) the CP and AA digestibility values, indicating that β-glucans have a negative effect on CP and AA digestibility values. The mechanism by which β-glucans interfere with digestion of protein and absorption of AA are closely related to their physico-chemical properties. β-glucans contain approximately 30% of the linkages in the form of β (1-3) and 70% in the form of β (1-4) between glucose molecules (Fleming and Kawakami, 1977; Aman and Graham. 1987). Staudte et al. (1983) reported a ratio of 1 to 2.5 of β (1-3) to β (1-4) linkages in β-glucans. This branched structure prevents compact folding of the molecules and increases the water-holding capacity, which results in its characteristic viscosity and gelling properties (Fadel et al., 1987, Wang et al., 1992). Bengtsson et al. (1990) reported that the degree of viscosity was attributed to the molecular weight and concentration of β-glucans. The viscosity and gelling properties tend to hinder motility of digesta in the small intestine (Holt et al., 1979), thereby decreasing the mixing of digesta with digestive enzymes and other necessary components required for digestion and absorption (Vahouny and Cassidy, 1985; Wang et al., 1992). These properties may also delay or decrease digestion and absorption of nutrients by increasing the thickness of the unstirred fluid layer, creating a

physical barrier at the absorption surface on the microvilli (Johnson and Gee, 1981). Special evidence (Forrest and Wainwright, 1977), explaining why β-glucans hinder specific digestion of CP, is that 50% of the total β-glucans could be removed by solubilization in water, however when endosperm cell walls were incubated with the proteolytic enzyme thermolysin, more than 96% of the β-glucans were extracted (Anderson et al., 1978). Thompson and Laberge (1977) and Weltzien (1986) also pointed out that β-glucans are firmly linked to peptide sequences in the cell wall.

On the other hand, based on the higher content of NDF in hulled than in hulless barley (Table III-2), it is not unreasonable to expect lower ileal CP and AA digestibility values in hulled barley than in hulless barley. It is widely accepted that fiber interferes with CP digestion and AA absorption. For example, negative correlations between the dietary content of NDF and the ileal digestibility values of CP and AA were reported for wheat (Taverner et al., 1981; Fan et al., 2001), canola meal (Fan et al., 1996), and peas (Gdala et al., 1992). However, Fan et al. (2001) found no effect of NDF (16.8 to 23.8%) on apparent ileal digestibility values of CP and AA in barley fed to growing pigs. Sauer et al. (1991) reported that the inclusion of 10% Alphafloc or barley straw in a corn starch-based soybean meal diet did not affect ileal CP and most of AA digestibility values. Li et al. (1994), also in studies with pigs, reported that the inclusion of 13.3% Alphafloc in a corn starch-based soybean meal diet did not affect ileal digestibility values of CP and most AA. From the previous studies, one can conclude that up to a certain level of fiber inclusion into the diet that there is no effect on CP and AA digestibility values. Therefore, as discussed previously (Chapter II), when differences in fiber content are relative small between diets, β-glucans are the major factor that affect ileal CP and AA

digestibility values. It should be pointed out that differences (0.53 to 0.70 percentage units) in β-glucan contents between the diets containing hulless (3.35 to 3.47%) and hulled barley (2.77 to 2.82%) were relatively small. The question remains if these small differences in β-glucan content are responsible for the differences in the apparent ileal digestibility values of CP and AA. However, it was observed that feces of pigs fed diets containing hulless barley was very sticky (indicating higher viscosity) compared to feces from pigs fed diets containing hulled barley during feces collection and retrieval of nylon bags (Chapter II). If the difference in fecal viscosity of pigs fed hulless and hulled barley diets was not caused by the difference in β-glucan content, then it is likely caused by difference in the compositions (structure) of β-glucans.

There is no information in the literature on interactions between fiber and β-glucans in diets on the digestibility values of CP in pigs. However, the results from this study and those reported previously (Chapter II) indicate that β-glucans are a determinant factor affecting CP and AA digestibility values in different barleys, especially when the fiber contents are very close. The content of fiber ranged from 8.57 to 11.9% for NDF and from 1.89 to 4.21% for ADF in this study.

As mentioned previously, the CP digestibility values were lower in hulless (62.1 and 64.5%) than in hulled barley (67.3 and 70.0%). However, taking into account the higher total CP content in the hulless (12.4 and 12.7%) than in the hulled barley (9.7 and 11.4%), the total digestible CP content is higher in hulless (7.70 to 8.19%) than in hulled barley (6.53 to 7.98%) (Table III-6). It can be stated in general that the digestible energy content is higher in hulless barley and that the digestible CP (and AA) content is also higher, or at least equal to, the content in hulled barley

The digestibility values of DM, CP and AA in barley determined with the *in vitro* method are presented in Table III-7. The digestibility values of CP and indispensable AA are close to the values reported by Boisen (1991) and Beames et al. (1996). Even though the differences in digestibility values of CP and AA between hulless and hulled barleys were not significant ($P > 0.05$), with the exception of arginine ($P < 0.05$), there was a trend towards higher digestibility values of CP and AA in hulled barley. This trend corresponds to the difference in β-glucan content between hulless and hulled barley. Beames et al. (1996) also reported that there were very small differences in digestibility values of CP, less than 2 percentage units, between hulled (c.v. Harrington) and hulless barley (c.v. CDC Richard).

The regression equations of CP and AA digestibility values between the *in vivo* and the *in vitro* methods are presented in Table III-8. The correlation coefficients of the digestibility values of DM and CP between the *in vivo* and the *in vitro* methods are significant ($P < 0.05$). This relationship was also reported by Boisen (1991) in *in vitro* studies with barley. The relationships of digestibility values of AA between the *in vivo* and *in vitro* methods are only significant ($P < 0.05$) for histidine, isoleucine, leucine, phenylalanine, valine and tyrosine. The R^2 values ranged between 0.67 and 0.85. Based on the results of this experiment, the ileal digestibility values of CP and a few of the AA can be predicted by *in vitro* measurements. However, since the digestibility values of CP and AA with the *in vitro* method corresponde to true ileal digestibility values of CP and AA, these values cannot be directly compared to *in vivo* digestibility values which represent apparent values.

The fecal digestibility values of DM, OM, energy and CP in the experimental diets with the *in vivo* method are presented in Table III-9. The fecal digestibility values of DM, OM, energy and CP in the soybean meal diet were close to the values reported by Sauer et al. (1991) and Mosenthin et al. (1994), and were higher ($P < 0.05$), as expected, than the values from the barley-containing diets. The fecal digestibility values of DM, OM and energy in the hulless barley diets were higher ($P < 0.05$) than the values in the hulled barley diets; the values in diets containing the barley mixtures were intermediate and different ($P < 0.05$) from both the diets containing hulled and hulless barleys. These differences correspond to the fiber contents in the diets. The content and composition of fiber in barley is considered a major factor that influences the digestibility values of energy (Mitaru et al., 1984; Sauer et al., 1991; Beames et al., 1996). As shown in Table III-3, the NDF and ADF contents in the diets with hulled barley (11.68 and 11.90%, and 3.99 and 4.21, respectively) are higher than those in the diets with hulless barley (8.57 and 8.82%, and 1.89 and 2.26%, respectively). The fiber components (e.g. NDF and ADF) are mainly present in the hulls of barley. The lower DM and energy digestibility in hulled barley may be attributed to its higher fiber content. An increase in fiber content results in an increase in rate of passage of digesta (Stanogias and Pearce, 1985), more adsorption of nutrients to fiber, and an increase in endogenous nitrogen losses (Just, 1982). In addition, carbohydrate and protein associated with fiber are of low digestibility (Donangelo and Eggun, 1985).

As expected, the fecal digestibility of CP is highest in the soybean meal diet (92.4%). The CP digestibility values in the barley-containing diets ranged from 86.3 to 87.6%, and were not different ($P > 0.05$). This is in contrast to the ileal CP digestibility

values, which were lower ($P < 0.05$) in the hulless barley diets than in the hulled barley diets. There is considerable microbial activity in the large intestine of the pig (Zebrowska, 1978), resulting in fermentation of β-glucans. A study by Li et al. (1996) with young pigs fed a hulless barley-soybean meal diet showed that β-glucans entering the large intestine were nearly completely (99.3%) fermented, likely also protein associated with β-glucans. In addition, a certain proportion of fiber is also fermented by the microflora in the large intestine. Therefore, both fiber and β-glucans in the hulled and hulless barleys were not significant factors that influenced the CP digestibility values measured in feces, resulting in no differences ($P > 0.05$) between these sources of barley.

In a previous experiment (Chapter II), a higher β-glucan content (3.90 vs 3.12 to 3.33%) resulted in a lower ($P < 0.05$) fecal CP digestibility. In this study, there were no differences ($P > 0.05$) in fecal CP digestibility values among the barley-containing diets. A possible reason is the higher CP content of the diets (17.6 to 18.0) in this study compared to the diets used in the previous study (10.6 to 12%) in which no soybean meal was supplemented. Also, the differences in β-glucan contents were smaller in the present study (3.35 to 3.47% vs 2.77 to 2.82%) than in the previous study. Possible effects of β-glucans may have been masked by the high CP digestibility of soybean meal.

Dry matter and energy digestibility values of barley with the difference and the *in vitro* methods are shown in Table III-10. The DM and energy digestibility values of barley from this experiment are in agreement with the range of values reported in the previous experiment (Chapter II) and other studies (Sauer et al., 1974 and 1977; Darroch et al., 1995; Beames et al., 1996). Irrespective which method was used, the digestibility values of DM and energy in hulless barley were higher ($P < 0.05$) than the values in

hulled barley; the values in the barley mixtures were intermediate. As mentioned previously, the content of fiber is the major factor that causes the differences in the digestibility values of DM and energy.

Based on the values in Table III-10, regression equations of digestibility values of DM and energy were established ($P < 0.05$) between the *in vivo* and *in vitro* methods (Table III-11). The results showed that there were strong ($P < 0.05$) correlations of DM and energy digestibility values between both methods; fecal digestibility values of DM and energy in barley can be accurately predicted from values obtained with the *in vitro* method. These results are in agreement with results from the previous study (Chapter II) and other studies (Wiseman and Cole, 1983; Boisen, 1991; Beames et al., 1996). Based on results from this study, the *in vivo* energy digestibility values can be predicted ($P < 0.002$) by the *in vitro* values for barleys with small differences in fiber contents. The NDF content among the barley samples differed by only about 5 percentage units.

In conclusion, the ileal digestibility values of CP and most AA were higher ($P < 0.05$) in hulled than in hulless barley. The higher content of β-glucans in hulless barley is likely responsible for the lower CP and AA digestibility values. However, the higher fecal digestibility values of energy in hulless barley than in hulled barley are likely due to the lower fiber content in hulless barley. The fecal energy digestibility values can be predicted with confidence from *in vitro* measurements. However, the *in vitro* method can only be used to predict the ileal digestibility values of CP and only a few of the AA. Overall, the *in vitro* method should be the method of choice as it is unlikely that it will be possible in the future to use surgically-modified animals for routine measurement of nutrient and energy digestibility values in feedstuffs for pigs.

Table III-1. Formulation (%) of the experimental diets (as-fed basis)

Ingredient	Diets						
	SBM	A	B	C	D	E	F
Hulled barley (Harrington I)	-	71.37	-	-	-	37.16	-
Hulled barley (Harrington II)	-	-	74.02	-	-	-	37.70
Hulless barley (CDC I)	-	-	-	77.49	-	37.16	-
Hulless barley (CDC II)	-	-	-	-	76.80	-	37.70
Soybean meal	39.39	23.30	20.60	17.18	17.88	20.36	19.26
Cornstarch	42.01	-	-	-	-	-	-
Dextrose	10.00						
Cellulose[1]	3.00	-	-	-	-	-	-
Canola oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Biophos[2]	1.38	0.51	0.58	0.24	0.23	0.37	0.39
Limestone	0.41	1.01	0.99	1.28	1.28	1.14	1.14
Iodized salt[3]	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride[4]	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Mineral-vitamin premix[5]	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Chromic oxide[6]	0.30	0.30	0.30	0.30	0.30	0.30	0.30

[1] Solkafloc supplied by James River Corp., Berlin, NH.
[2] Contained 18% P and 24% Ca; supplied by Continental Lime Ltd., Exshaw, AB.
[3] Provided the following (per kilogram of diet): NaCl, 2.9 g; ZnO, 12.0 mg; $FeCO_3$, 4.8 mg; MnO, 3.6 mg; CuO, 1.0 mg; $Ca(IO_3)_2$, 0.2 mg; CaO, 0.1 mg. Supplied by Windsor Salt Co., Toronto, ON.
[4] Choline chloride 60%, Champion Feed Service Ltd., Westlock, AB.
[5] Provided the following (per kilogram of diet): vitamin A, 10,000 IU; vitamin D_3, 1,000 IU; vitamin E, 80 IU; vitamin K_3, 2.0 mg; vitamin B_{12}, 0.03 mg; riboflavin, 12 mg; niacin, 40 mg; d-pantothenic acid, 25 mg; choline, 1,000 mg; d-biotin, 0.25 mg; folic acid, 1.6 mg; thiamin, 3.0 mg; ethoxyquin, 5.0 mg; pyridoxine, 2.25 mg; Fe, 150 mg; Zn, 150 mg; Cu, 125 mg; I, .21 mg; Se, 0.3 mg. Supplied by Hoffmann-LaRoche Ltd., 2455 Meadowpine Blvd., Mississauga, ON.
[6] Fisher Scientific, Fair Lawn, NJ.

Table III-2. Chemical and amino acid compositions (%) of the ingredients (as-fed basis)

Items	Ingredients[1]						
	SBM	A	B	C	D	E	F
Dry matter	88.6	88.7	88.4	87.9	87.9	88.7	88.1
Crude protein	46.1	9.7	11.4	12.7	12.4	11.2	11.8
Gross energy (kcal/g)	4.21	3.96	3.98	4.19	4.10	4.08	4.03
Crude fat	1.14	1.98	1.92	2.17	1.90	2.16	2.04
Neutral detergent fiber	8.36	13.94	13.96	9.02	9.55	11.50	11.8
Acid detergent fiber	4.26	4.38	4.21	1.98	1.47	3.20	2.88
Ash	6.27	2.04	1.84	1.94	1.75	1.99	1.80
ß-glucans	0.11	3.84	3.78	4.46	4.33	4.16	4.04
Amino acids							
Indispensable							
Arginine	2.96	0.50	0.50	0.52	0.52	0.51	0.51
Histidine	1.29	0.26	0.28	0.30	0.25	0.28	0.26
Isoleucine	2.35	0.43	0.48	0.50	0.51	0.46	0.48
Leucine	3.51	0.74	0.84	0.88	0.89	0.80	0.86
Lysine	2.88	0.38	0.41	0.47	0.48	0.43	0.44
Phenylalanine	2.21	0.51	0.62	0.63	0.61	0.57	0.61
Threonine	1.74	0.36	0.37	0.42	0.42	0.38	0.39
Valine	2.29	0.56	0.60	0.64	0.64	0.62	0.62
Dispensable							
Alanine	1.92	0.42	0.46	0.51	0.49	0.47	0.48
Aspartic acid	4.97	0.57	0.65	0.73	0.73	0.69	0.71
Glutamic acid	8.00	2.18	2.67	2.94	2.91	2.71	2.83
Glycine	2.26	0.48	0.51	0.53	0.56	0.50	0.53
Serine	1.87	0.34	0.41	0.45	0.45	0.41	0.42
Tyrosine	1.06	0.23	0.27	0.28	0.30	0.26	0.28

[1]. SBM: soybean meal; A: hulled barley (Harrington I); B: hulled barley (Harrington II); C: hulless barley (CDC I); D: hulless barley (CDC II): E: mixture of 50%/50% of A and C; F: mixture of 50%/50% of B and D.

Table III-3. Chemical and amino acid composition (%) of the experimental diets[1]

Items	Diets[2]						
	SBM	A	B	C	D	E	F
Dry matter	89.9	89.4	89.4	89.2	89.5	89.4	89.4
Crude protein	17.4	17.6	18.0	17.9	17.6	17.2	17.6
Gross energy (kcal/g)	3.95	4.11	4.05	4.06	4.05	4.05	4.06
Crude fat	3.44	4.84	4.63	4.99	4.84	4.90	4.76
Neutral detergent fiber	6.30	11.90	11.68	8.57	8.82	10.30	10.47
Acid detergent fiber	1.68	4.21	3.99	2.26	1.89	3.24	2.95
Ash	4.56	5.00	4.92	4.81	4.61	5.00	4.78
ß-glucans	0.04	2.77	2.82	3.47	3.35	3.11	3.08
Amino acids							
Indispensable							
Arginine	0.96	0.91	0.82	0.89	0.97	0.93	0.92
Histidine	0.47	0.41	0.40	0.39	0.45	0.40	0.42
Isoleucine	0.91	0.81	0.76	0.76	0.78	0.81	0.79
Leucine	1.41	1.39	1.28	1.27	1.31	1.34	1.32
Lysine	1.09	0.99	0.95	0.88	0.89	0.90	0.90
Phenylalanine	0.87	0.91	0.89	0.89	0.91	0.91	0.90
Threonine	0.75	0.59	0.61	0.56	0.60	0.58	0.58
Valine	0.73	0.89	0.82	0.91	0.96	0.94	0.92
Dispensable							
Alanine	0.75	0.75	0.69	0.75	0.78	0.78	0.77
Aspartic acid	2.05	1.63	1.44	1.48	1.64	1.57	1.57
Glutamic acid	3.27	3.66	3.55	3.88	4.11	3.83	3.90
Glycine	0.77	0.76	0.75	0.75	0.77	0.77	0.76
Serine	0.68	0.63	0.57	0.64	0.71	0.65	0.65
Tyrosine	0.40	0.38	0.36	0.44	0.46	0.41	0.46

[1] As-fed basis.

[2] Refer to Table III-1 for abbreviations of feedstuffs.

Table III-4. Apparent ileal digestibility values (%) of dry matter, organic matter, crude protein and amino acids in the experimental diets

Items	Diets[1]							SEM
	SBM	A	B	C	D	E	F	
Dry matter	73.6^{a}	59.2^{b}	59.7^{b}	61.3^{b}	61.5^{b}	59.2^{b}	59.3^{b}	1.25
Organic matter	77.2^{a}	61.7^{b}	62.2^{b}	63.7^{b}	64.0^{b}	61.8^{b}	61.9^{b}	1.10
Crude protein	80.6^{a}	75.4^{b}	75.6^{b}	71.7^{c}	70.7^{c}	72.7^{c}	71.2^{c}	0.81
Amino acids								
Indispensable								
Arginine	90.9^{a}	85.7^{b}	84.8^{bc}	81.2^{d}	80.8^{d}	83.0^{cd}	82.6^{cd}	0.89
Histidine	89.8^{a}	84.1^{b}	82.8^{b}	81.4^{b}	82.0^{b}	83.3^{b}	82.3^{b}	1.02
Isoleucine	88.9^{a}	84.4^{b}	82.4^{bc}	75.9^{d}	76.0^{d}	78.0^{cd}	78.9^{cd}	1.42
Leucine	87.1^{a}	82.2^{b}	80.5^{bc}	76.3^{d}	76.1^{d}	77.8^{cd}	77.4^{d}	1.08
Lysine	87.4^{a}	82.3^{b}	79.0^{c}	75.0^{d}	75.7^{cd}	77.7^{c}	76.2^{cd}	0.98
Phenylalanine	87.4^{a}	83.1^{b}	81.4^{bc}	78.5^{c}	78.2^{c}	79.4^{bc}	79.0^{c}	1.12
Threonine	82.9^{a}	73.0^{b}	72.8^{bc}	67.7^{d}	69.0^{cd}	70.1^{bcd}	70.2^{d}	1.10
Valine	83.2^{a}	81.2^{ab}	78.3^{bc}	74.7^{c}	74.9^{c}	76.7^{bc}	76.2^{c}	1.57
Dispensable								
Alanine	82.5^{a}	74.1^{b}	71.6^{bc}	68.6^{c}	70.1^{c}	71.3^{bc}	70.2^{c}	0.98
Aspartic acid	83.6^{a}	78.8^{b}	76.2^{bc}	74.0^{c}	74.7^{c}	75.6^{bc}	75.3^{c}	1.02
Glutamic acid	84.4^{a}	82.8^{ab}	82.7^{ab}	79.3^{c}	80.9^{bc}	82.9^{ab}	82.2^{abc}	0.91
Glycine	74.3^{a}	68.7^{b}	68.1^{b}	61.5^{cd}	58.8^{d}	64.3^{bc}	62.7^{cd}	1.62
Serine	81.2^{a}	74.5^{b}	70.5^{c}	72.3^{bc}	74.8^{b}	73.7^{b}	72.5^{bc}	1.01
Tyrosine	86.1^{a}	79.3^{b}	77.6^{b}	78.4^{b}	79.4^{b}	78.6^{b}	79.5^{b}	0.90

[1] Refer to Table III-1 for abbreviations of feedstuffs.

[a, b, c] Means in the same row with different superscript letters differ ($P < 0.05$, n=7).

Table III-5. Apparent ileal digestibility values (%) of dry matter, crude protein and amino acids in barley with the difference method

Items	Feedstuffs[1]						
	A	B	C	D	E	F	SEM
Dry matter	54.5	55.8	58.6	58.7	55.0	55.6	1.12
Crude protein	67.3^{a}	70.0^{a}	64.5^{b}	62.1^{b}	66.7^{ab}	64.8^{b}	0.92
Amino acids							
Indispensable							
Arginine	75.7^{a}	74.7^{a}	69.0^{b}	67.4^{b}	69.5^{b}	70.3^{b}	1.02
Histidine	74.9	73.8	73.4	72.6	73.8	72.5	1.01
Isoleucine	76.4^{a}	73.5^{a}	62.4^{c}	62.2^{c}	63.3^{bc}	66.4^{b}	1.32
Leucine	74.6^{a}	72.8^{ab}	66.7^{c}	66.0^{c}	70.4^{bc}	67.3^{c}	0.98
Lysine	69.7^{a}	62.6^{b}	58.2^{c}	59.4^{c}	62.0^{b}	60.5^{bc}	0.92
Phenylalanine	77.0^{a}	75.4^{a}	71.6^{b}	70.4^{b}	73.8^{ab}	71.2^{b}	1.05
Threonine	57.4^{a}	59.6^{ab}	53.7^{c}	55.6^{c}	55.8^{bc}	57.4^{ab}	1.01
Valine	78.5^{a}	73.1^{a}	68.0^{b}	68.0^{b}	69.1^{b}	69.6^{ab}	1.32
Dispensable							
Alanine	61.6^{a}	58.9^{b}	57.0^{b}	58.8^{b}	57.7^{b}	58.6^{b}	0.96
Aspartic acid	65.1^{a}	60.5^{b}	59.5^{b}	60.6^{b}	60.9^{b}	60.5^{b}	1.02
Glutamic acid	82.7^{a}	79.3^{b}	77.2^{bc}	79.7^{b}	82.2^{ab}	79.8^{b}	1.05
Glycine	60.1^{a}	60.5^{a}	49.4^{b}	44.2^{c}	50.8^{b}	50.1^{b}	1.59
Serine	62.5^{b}	56.9^{c}	64.1^{b}	68.6^{a}	63.4^{b}	62.6^{b}	0.95
Tyrosine	69.1	68.3	71.9	73.9	70.9	73.1	1.22

[1] Refer to Table III-2 for abbreviations of feedstuffs.

[a, b, c] Means in the same row with different superscript letters differ ($P < 0.05$, n=7).

Table III-6. Digestible contents (%) of dry matter, energy, crude protein and amino acids in barleys

Items	Barley[1]					
	A	B	C	D	E	F
Dry matter, fecal	70.9	71.7	75.9	77.0	73.0	73.0
Dry matter, ileal	48.3	49.3	51.5	51.6	48.8	49.0
Energy, kcal/g, fecal	3.16	3.21	3.59	3.56	3.35	3.33
Crude protein, ileal	6.53	7.98	8.19	7.70	7.47	7.65
Amino acids, ileal						
Indispensable						
Arginine	0.38	0.37	0.36	0.35	0.35	0.36
Histidine	0.19	0.21	0.22	0.18	0.21	0.19
Isoleucine	0.33	0.35	0.31	0.32	0.29	0.32
Leucine	0.55	0.61	0.59	0.59	0.56	0.58
Lysine	0.26	0.26	0.27	0.29	0.27	0.27
Phenylalanine	0.39	0.47	0.45	0.43	0.42	0.43
Threonine	0.21	0.22	0.23	0.23	0.21	0.22
Valine	0.44	0.44	0.44	0.44	0.43	0.43
Dispensable						
Alanine	0.26	0.27	0.29	0.29	0.27	0.28
Aspartic acid	0.37	0.39	0.43	0.44	0.42	0.43
Glutamic acid	1.80	2.12	2.27	2.32	2.23	2.26
Glycine	0.29	0.31	0.26	0.25	0.25	0.27
Serine	0.21	0.23	0.29	0.31	0.26	0.26
Tyrosine	0.16	0.18	0.20	0.22	0.18	0.20

[1]Refer to Table III-2 for abbreviations of feedstuffs.

Table III-7. Digestibility values (%) of dry matter, crude protein and amino acids in barley with the *in vitro* method

Items	Barley[1]						SEM
	A	B	C	D	E	F	
Dry matter	81.8[c]	82.1[c]	86.2[a]	86.0[a]	83.6[b]	83.3[b]	0.26
Crude protein	90.5	91.2	90.0	90.0	89.9	90.1	0.40
Amino acids							
Indispensable							
Arginine	89.9[a]	89.6[a]	86.0[b]	85.1[b]	89.3[ab]	86.3[ab]	1.36
Histidine	93.2[ab]	93.9[a]	92.0[bc]	90.8[c]	92.7[abc]	91.7[bc]	0.57
Isoleucine	91.7[a]	91.1[a]	89.7[ab]	90.0[ab]	89.8[ab]	89.4[b]	0.45
Leucine	91.9	91.5	90.6	90.6	90.3	90.6	0.54
Lysine	90.0	90.2	89.6	90.0	90.5	90.0	0.58
Phenylalanine	91.8[a]	91.7[a]	90.5[ab]	90.1[b]	90.2[b]	90.8[ab]	0.42
Threonine	91.0[abc]	92.1[a]	89.6[bc]	88.4[c]	91.8[ab]	89.8[bc]	0.89
Valine	91.2	91.0	89.9	89.8	90.4	89.8	0.47
Dispensable							
Alanine	85.4	85.7	85.3	84.3	84.8	84.6	0.99
Aspartic acid	87.0	87.4	85.4	86.8	87.2	86.4	0.81
Glutamic acid	95.5	95.3	94.8	94.7	95.0	95.1	0.37
Glycine	77.7	77.4	75.3	72.4	73.7	75.3	2.15
Serine	92.4	92.2	90.5	91.0	91.1	90.7	1.07
Tyrosine	91.2	91.7	90.3	90.7	91.2	90.4	1.06

[1] Refer to Table III-2 for abbreviations of feedstuffs.

[a, b, c] Means in the same row with different superscript letters differ ($P < 0.05$, n=6).

Table III-8. Relationship of dry matter, crude protein and amino acid digestibility values between the *in vivo* and *in vitro* methods

Items	Equation[a]	R^2	P value[b]
Dry matter	$Y_{in\ vivo}=0.88010X_{in\ vitro}-17.42$	0.85	0.0095[c]
Crude protein	$Y_{in\ vivo}=6.04594X_{in\ vitro}-481.06$	0.84	0.0490[c]
Amino acids			
Indispensable			
Arginine	$Y_{in\ vivo}=1.26291X_{in\ vitro}-39.66$	0.65	0.0532
Histidine	$Y_{in\ vivo}=0.60181X_{in\ vitro}+17.83$	0.75	0.0246[c]
Isoleucine	$Y_{in\ vivo}=6.03321X_{in\ vitro}-477.29$	0.81	0.0149[c]
Leucine	$Y_{in\ vivo}=4.66257X_{in\ vitro}-354.31$	0.69	0.0399[c]
Lysine	$Y_{in\ vivo}=4.12293X_{in\ vitro}-309.20$	0.08	0.5761
Phenylalanine	$Y_{in\ vivo}=2.88065X_{in\ vitro}-188.50$	0.68	0.0433[c]
Threonine	$Y_{in\ vivo}=0.81978X_{in\ vitro}-17.57$	0.34	0.2237
Valine	$Y_{in\ vivo}=5.63598X_{in\ vitro}-438.14$	0.75	0.0260[c]
Dispensable			
Alanine	$Y_{in\ vivo}=0.69823X_{in\ vitro}-0.61$	0.05	0.6661
Aspartic acid	$Y_{in\ vivo}=1.09634X_{in\ vitro}-33.87$	0.17	0.4183
Glutamic acid	$Y_{in\ vivo}=4.26451X_{in\ vitro}-325.20$	0.35	0.2188
Glycine	$Y_{in\ vivo}=2.91156X_{in\ vitro}-166.71$	0.86	0.0730
Serine	$Y_{in\ vivo}=-2.49722X_{in\ vitro}+291.04$	0.23	0.2716
Tyrosine	$Y_{in\ vivo}=-3.36019X_{in\ vitro}+376.69$	0.67	0.0474[c]

[a] Y=dependent variable: dry matter, crude protein or amino acid digestibility (%) values by the difference method as subscribed;

X=independent variable: dry matter, crude protein or amino acid digestibility (%) values by the *in vitro* method as subscribed.

[b] The probability of significance for the slope of the regression equation.

[c] The linear regression equation is significant ($P < 0.05$, n=6)

Table III-9. Fecal digestibility values (%) of dry matter, organic matter, energy and crude protein in the experimental diets

	Diets[1]							
Items	SBM	A	B	C	D	E	F	SEM
Dry matter	93.4[a]	83.2[f]	83.8[e]	87.7[c]	88.7[b]	84.9[d]	85.2[d]	0.23
Organic matter	95.9[a]	87.4[f]	88.0[e]	91.3[c]	92.1[b]	89.2[d]	89.3[d]	0.18
Energy	94.3[a]	83.4[e]	83.7[e]	87.3[c]	88.2[b]	84.8[d]	85.0[d]	0.25
Crude protein	92.4[a]	86.3[b]	87.4[b]	87.0[b]	87.2[b]	86.6[b]	87.6[b]	0.39

[1] Refer to Table III-1 for abbreviations of feedstuffs.

[a, b, c, d, e, f] Means in the same row with different superscript letters differ ($P < 0.05$, n=7).

Table III-10. Digestibility values (%) of dry matter and energy in barley with the difference method and the *in vitro* method

Method	Items	Barley samples[1]						SEM
		A	B	C	D	E	F	
Difference method	Dry matter	79.9^{e}	81.1^{d}	86.4^{b}	87.6^{a}	82.3^{c}	82.9^{c}	0.22
	Energy	79.8^{d}	80.7^{d}	85.7^{b}	86.8^{a}	82.2^{c}	82.6^{c}	0.23
In vitro method	Dry matter	86.7^{c}	87.3^{c}	92.1^{a}	92.8^{a}	90.1^{b}	90.3^{b}	0.25
	Energy	83.5^{e}	85.0^{d}	88.3^{ab}	89.0^{a}	86.8^{c}	87.4^{bc}	0.46

[1] Refer to Table III-2 for abbreviations of feedstuffs.

$^{a, b, c, d, e}$ Means in the same row with different superscript letters differ ($P < 0.05$, n=7).

Table III-11. Relationships of dry matter and energy digestibility values in barley between the *in vivo* and the *in vitro* methods

	Equations[a]	R^2	P value[b]
Dry matter digestibility	$Y_{in\ vivo}=1.16711X_{in\ vitro}-21.53740$	0.91	0.0032
Energy digestibility	$Y_{in\ vivo}=1.25008X_{in\ vitro}-25.37339$	0.88	0.0055

[a] Y=dependent variable: dry matter or energy digestibility (%) values by the difference method;

X=independent variable: dry matter or energy digestibility (%) values by the *in vitro* method.

[b] The probability of significance for the slope of the regression equation ($P < 0.05$, n=7).

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CHAPTER IV

INFLUENCE OF GUANIDINATION ON APPARENT ILEAL AMINO ACID DIGESTIBILITY VALUES OF SOME PROTEIN SOURCES FOR PIGS

A. Introduction

Since the homoarginine method was proposed by Hagemeister and Erbersdobler (1985) for distinguishing between endogenous and dietary sources of amino acid (AA) in ileal digesta, several workers have used this method to determine the recoveries of endogenous ileal lysine and other AA in rats (Moughan and Rutherfurd, 1990), poultry (Siriwan et al., 1994; Angkanaporn et al., 1997), and pigs (Marty et al., 1994; Nyachoti et al., 1997; Caine et al., 1998). This method involves transformation of dietary lysine to homoarginine by a guanidination reaction with *O*-methylisourea. Because no endogenous homoarginine enters the digestive tract, the ileal digestibility value of homoarginine is assumed to be similar to the true ileal digestibility value of lysine. This technique is based on the assumption that the guanidination has no effect on AA profile of the protein and on protein susceptibility to proteolysis during digestion (Bryden et al., 1996). However, this assumption has not been experimentally validated in previous studies with pigs. Concerns associated with soaking of feedstuffs in high pH solution for a few days during the guanidination and then washings with distilled water three times after the guanidination are often raised and these largely relate to possible changes in chemical and AA composition of the feedstuffs under investigation (Caine et al., 1998).

The objectives of the present study were to determine the effects of guanidination on chemical compositions, AA profile of protein and apparent ileal AA digestibility values of soybean meal, rapeseed meal and peas for pigs.

B. Experimental Procedures

Animals and Management

Eight crossbred barrows, average initial body weight (**BW**) 18 kg, were obtained from the Swine Research Unit of the Hohenheim University, and housed individually in stainless steel metabolism crates in a temperature-controlled (20 to 22°C) barn. After a 7 d adaptation period, the barrows were surgically fitted with a simple T-cannula at the distal ileum following the procedures described by Sauer et al. (1983). The pigs were returned to their respective metabolism crates after surgery and fasted for 24 h. Then the barrows were provided with 25 g of a starter diet four times daily at 6 h interval. The dietary allowance was gradually increased until all the barrows consumed the diet at a rate of 5% of the average BW which occurred within 7 d. During the recuperation period, the temperature in crates was maintained at 30 to 32°C by adjusting the infrared heating lamp. The surgical procedures and pre- and post-operative care were performed as described by Sauer et al. (1983). During the 7 d adaptation and 14 d recuperation periods, the barrows were fed an 18% crude protein (**CP**) diet. Water was freely available from a low-pressure drinking nipple. At the conclusion of the experiment, the barrows with an average final BW of 31 kg, were sacrificed, and dissected to determine whether cannulation had caused adhesions or other intestinal abnormalities.

The experiment was carried out according to a repeated 3 × 3 Latin square design. Based on feed intake and BW, Six barrows were selected and assigned randomly into three groups. Each of the three experimental periods comprised 10 d. Three normal diets containing normal soybean meal, rapeseed meal or peas were fed from d 1 to 7 of each experimental period. Digesta were collected from 0800 to 2000 on d 6 and from 2000 of d 7 to 0800 of d 8. On d 9, three guanidinated diets with 50% of the protein of the normal diets replaced by respective guanidinated feedstuffs were used. Ileal digesta were collected continuously for 24 h starting immediately after the guanidinated diets were offered. A diet containing casein enzymatic hydrolysate was fed on d 8 and 10 to separate the digesta originating from the normal and guanidinated diets.

The barrows were fed twice daily, equal amounts each meal, at 0800 and 2000. The daily dietary allowance was provided at a rate of 4% of the individual BW of each barrow at the beginning of each experimental period.

Digesta were collected into plastic bags (approximately 20 cm in length × 3 cm in width) that contained 10 mL 2.5 M formic acid to minimize microbial fermentation, and frozen at -20°C immediately after collection. The digesta were freeze-dried and pooled within barrow and period before chemical and AA analyses.

The animals and experimental procedures used in this experiment followed the Standards and Guidelines of the German Law for Animal Care (Regierungspraesidium Stuttgart, Germany).

Preparation of Experimental Diets

Seven corn starch-based diets (Table IV-1) were formulated to contain 18% CP according to NRC (1998) standards. Three normal diets, without homoarginine,

contained normal soybean meal, rapeseed meal or peas as sole protein source. Another three guanidinated diets were prepared by replacing 50% of the protein source in each normal diet with the respective guanidinated feedstuff. Additionally a diet was prepared with casein enzymatic hydrolysate as sole protein source. All dietary ingredients were ground through a 1.0 mm mesh screen prior to guanidination and/or incorporation into the diets. For all seven diets, soybean oil was included at a level of 3% to reduce the dustiness of the diets. Dextrose was used to improve the palatability. Vitamins, minerals, methionine, and tryptophan were supplemented to meet or exceed NRC (1998) standards. Titanium oxide was included in six of the experimental diets as a digestibility marker. Chromic oxide was used in the guanidinated diets in expense of half the titanium oxide in the normal diets to differentiate the digesta originating from the guanidinated diets by the green color of chromic oxide during digesta collection.

Guanidination was performed by using procedures described by Schmitz et al. (1991) for soybean meal, and by Nyachoti et al. (1997) for rapeseed meal and peas. Feedstuffs, each batch containing 200 g protein, were mixed with 1 L of 0.5 M *O*-methylisourea (**MIU**) solution. Then the feedstuffs with MIU solution was incubated at pH 10.3 for soybean meal, and 10.5 for rapeseed meal and peas at 4°C for 6 d. The MIU solution was prepared by reacting *O*-methylisourea sulfate (Sigma Chemical, St. Louis, MO) and barium hydroxide (Sigma Chemical, St. Louis, MO) with stirring for 30 min, followed by centrifugation for 10 min at 4,000 × g to precipitate and separate the barium sulfate. The feed material of the incubation was thoroughly stirred to ensure uniform conditions for guanidination, and the pH during guanidination was monitored daily and adjusted accordingly through the incubation period. At the end of incubation, the

guanidination reaction was stopped by lowering the pH to the iso-electric point of each protein source (soybean meal: 4.5; rapeseed meal and peas: 4.6) by adding 1 M HCl solution. Then, the guanidinated feed materials were centrifuged at 4,000 × g at 4°C for 10 min. The supernatant was discarded to remove MIU. After three further cycles of washing with distilled water to re-suspend the precipitate and centrifugation, the precipitated feed material was freeze-dried and ground prior to incorporation into the guanidinated diets.

Chemical and Statistical Analyses

Samples of dietary ingredients, diets, and digesta were ground through a 0.5-mm mesh screen before analyses. Dry matter, crude fat, and ash contents were determined according to AOAC (1990). Gross energy and CP contents were analyzed using a Bomb Calorimeter and Automated Nitrogen Analyzer (Leco Corporation, St. Joseph, MO), respectively. Analyses for the contents of NDF and ADF were carried out according to principles outlined by Goering and van Soest (1970). Titanium oxide was measured by using Atomic Absorption Spectroscopy as described by Brandt and Allam (1987).

Amino acids including homoarginine were determined by using an Eppendorff Biotronik LC 3000 Amino Acid Analyzer (Eppendorff, Hamburg, Germany) following hydrolysis with 6 N HCl solution in sealed and evacuated tube at 113°C for 24 h (Naumann and Bassler, 1988). Methionine and cysteine (only in feedstuffs) were determined as methionine sulfone and cysteic acid after oxidation with 98% performic acid overnight according to AOAC (1990). The oxidized samples were then hydrolyzed and analyzed in the same manner as the other AA. Tryptophan was not determined.

The results were subjected to Analysis of Variance by using the General Linear Model of SAS program (SAS Institute Inc., Cary, NC). The main effects of diets (n=6), pigs (n=6) and periods (n=3) were included in the model. The means of diets were compared using the Student-Newman Keul's multiple range test procedure and the statistical significance level was claimed at $P < 0.05$.

C. Results and Discussion

The pigs were healthy throughout the experiment and readily consumed the guanidinated diets at the same rate of intake as the respective normal diets. Postmortem examinations at the conclusion of the experiment revealed no adhesions and other intestinal abnormalities.

Dry matter recovery rates (wt/wt) of soybean meal, rapeseed meal and peas after guanidination were 77.1, 84.3 and 95.7%, respectively. The recovery rate of soybean meal was very close to the result reported by Caine et al. (1998) for autoclaved Nutrisoy, for which the recovery rate was 76%. Except for the results in this study, there are no data available on the recovery rates of rapeseed meal and peas after guanidination.

The chemical compositions of soybean meal, rapeseed meal and peas before and after guanidination are presented in Table IV-2. The contents of dry matter, CP, crude fat, NDF, and ADF in soybean meal, rapeseed meal and peas before guanidination are close to the contents reported by NRC (1998).

As shown in Table IV-2, guanidination caused larger change in chemical composition of soybean meal and rapeseed meals compared to peas. Contents of CP in

soybean meal, rapeseed meal and peas increased 19.0, 11.6 and 2.8 percentage units after guanidination, respectively. Caine et al. (1998) also reported that protein content increased 20% after guanidination of Nutrisoy. The contents of NDF increased 6.0, 6.4 and 2.2 percentage units in the same order of feedstuffs. Guanidination also increased the concentration of ADF in rapeseed meal, namely by 5.2 percentage units, but not in the other feedstuffs. However, the content of ash decreased 3.9, 4.1 and 1.8 percentage units in the same order of feedstuffs, respectively. The dry matter losses of the feedstuffs after guanidination possibly consisted of both soluble carbohydrates, this can be extrapolated from the change in composition of the feedstuffs, and minerals, as shown in Table IV-2. These losses may have resulted mainly from the multiple washings to remove *O*-methylisourea after guanidination, thereby also remove some soluble materials, like soluble carbohydrate. This point of view is in agreement with that from Caine et al. (1998). Another reason for the increase in CP content is because of two nitrogenous radicals more in homoarginine than in lysine after guanidination:

Lysine ($_2$HN-[CH$_2$]$_4$-CH[NH$_2$]-COOH) to homoarginine ($_2$HN-C[=NH]-NH-[CH$_2$]$_4$-CH[NH$_2$]-COOH).

Based on the combination of the difference in dry matter recovery rates and the changes in chemical compositions of the three feedstuffs after guanidination, it seems that the lower the recovery of dry matter after guanidination, the larger the change in chemical compositions in the feedstuff, especially with respect to CP. Therefore, the chemical composition of the guanidinated feedstuff should be analyzed if its recovery rate of dry matter is low. Furthermore, if the change in chemical composition caused by guanidination could affect protein and AA digestibility values, the guanidinated feedstuff should be used cautiously to replace its normal feedstuff. It is better in this case to replace

at a lower rate in order to keep the chemical composition of the guanidinated diet to be similar to the normal diet. Otherwise the guanidinated diet would not represent its normal diet. However, it also should be ensured that the homoarginine level in the guanidinated diets is high enough to be accurately measured. That means the diets should contain a certain minimum level of homoarginine for analysis. As shown in Table IV-4, replacing 50% of the protein in normal diets with protein from its guanidinated counterpart feedstuff did not result in a large difference in the chemical compositions of the diets in this study. This rate of replacement was same as used by Nyachoti et al. (1997).

The effects of guanidination on AA profile of protein in soybean meal, rapeseed meal and peas are shown in Table IV-3. The change of each AA concentration in protein after guanidination ranged within 2 percentage units in soybean meal and rapeseed meal, and 3 percentage units in peas except for lysine, which was converted to homoarginine. Therefore, the effects of guanidination on AA profile of protein in the feedstuffs are small, at least relative small compared to the effects on chemical composition of the feedstuffs. These results suggest that the effect of guanidination on the basic structure of protein was not remarkable. The total AA contents (no tryptophan) in CP of soybean meal, rapeseed meal and peas after guanidination decreased 3.61, 2.61 and 8.85 percentage units, respectively. This indicated that non-AA nitrogen increased in CP after guanidination, especially in peas. The reasons may be that the methylisourea was not removed entirely by washings (Caine et al., 1998), or the methylisourea reacted with some residues of non-lysine molecules (Lingnert, 1990).

The apparent ileal digestibility values of CP and AA in the normal diets and the guanidinated diets are presented in Table IV-5. The values from the normal diets

containing normal soybean meal, rapeseed meal and peas were within the range of values previously reported by Jansman et al. (1988), de Lange et al. (1989), and NRC (1998). For example, the apparent ileal lysine and threonine digestibility values of soybean meal, rapeseed meal, and peas reported by NRC (1998) were 85 and 78%, 74 and 69%, and 84 and 73%, respectively. In this study, the corresponding values for lysine and threonine digestibilities in soybean meal, rapeseed meal and peas were 84.9 and 79.2%, 72.4 and 62.6%, and 82.0 and 69.3%, respectively. Comparisons with the literature were made for lysine and threonine for reasons that these AA are often limiting in feedstuffs for pigs. The apparent ileal digestibility values of CP and AA were highest ($P < 0.05$) in soybean meal and lowest ($P < 0.05$) in rapeseed meal. The digestibility values of CP and AA in peas were intermediate. The differences in digestibility values among these feedstuffs may be, in part, related to differences in the content of fiber and anti-nutritional factors. The NDF contents in soybean meal, rapeseed meal, and peas were 12.0, 23.6 and 12.3%, respectively (Tables IV-2). Negative correlations between NDF content and apparent ileal CP and AA digestibility values were reported in some feedstuffs, including canola meal (Fan et al., 1996), peas (Gdala et al., 1992; Fan and Sauer, 1999) and wheat (Taverner and Farrell, 1981). A higher NDF content, such as in rapeseed meal including high contents of pectin and lignin, may cause an increase in the rate of passage of digesta (Stanogias and Pearce, 1985), blocking enzyme-substrate interactions (Johnson and Gee, 1981; Anderson et al., 1990), and promoting an increased loss of endogenous CP and AA in ileal digesta (Taverner et al., 1981; Furuya and Kaji, 1992; Li et al., 1994). Peas contain variable amounts of trypsin inhibitors and tannins. Rapeseed meal also contains high level of tannins. As was reviewed by Huisman and Jansman (1991), the Bowman-

Birk protease inhibitor family, to which trypsin inhibitor in peas belongs, irreversible bind to trypsin (and also chymotrypsin) thereby decreasing protein digestion and increasing endogenous protein and AA losses (Barth et al. 1993). The digestion of both exogenous and endogenous proteins is affected. Dietary tannins are able to decrease CP and AA digestibility through several mechanisms, including formation of hydrogen bonds and hydrophobic interactions of their hydroxyl groups with the carbonyl groups of dietary proteins, decreasing the activities of gastric pepsin, pancreatic trypsin and chymotrypsin and intestinal brush border peptidases, changing the morphology of the gastrointestinal mucosa, decreasing transmembrane nutrient uptake, and increasing endogenous protein secretions (Jansman et al., 1988). This would partially explain the fact that CP and AA digestibility are usually lower in rapeseed meal and peas than in soybean meal (Sauer and Ozimek, 1986).

There were no differences in apparent ileal lysine digestibility values between normal diet and the guanidinated diet for both soybean meal and peas. This indicated that the guanidination of soybean meal and peas were uniform. Unlike soybean meal and peas, however, the apparent ileal lysine digestibility value for rapeseed meal was 10.9 percentage units lower ($P < 0.05$) in the guanidinated diet (61.5%) than in the normal diet (72.4%). Combining the lower conversion rate (wt/wt, calculated from the data in Table IV-3) of lysine to homoarginine in rapeseed meal (77.8) than in soybean meal (79.0%) and peas (89.7), it can be assumed that the guanidination in rapeseed meal was not as random and uniform as in soybean meal and peas. Firstly, the lower levels of conversion rate may reflect the higher inaccessibility of some lysine residues in the protein closely associated with other polymeric materials and secondly, the lower lysine digestibility in

the guanidinated-rapeseed meal diet than in the normal rapeseed meal diet indicated that the higher digestible lysine must be converted to homoarginine and the higher indigestible lysine must be blocked from guanidination and stay as lysine. In other words, if the lysine in feedstuffs was converted to homoarginine randomly and uniformly by guanidination, the apparent ileal digestibility value of lysine in normal feedstuff should be same as, or very close to the value in its guanidinated feedstuff. The non-uniform guanidination of rapeseed meal could be explained, at least part, by the high content of fiber (NDF and ADF in Tables IV-2 and IV-4) in rapeseed meal. Fiber could cement and anchor a part of the AA in the fiber matrix, and fiber is hard structure matrix that could stiffen cell walls thus preventing the chemical reaction of AA with other chemicals and enzymes (Bach Knudsen, 2001). Therefore, if the conversion rate of lysine to homoarginine is very low, it is essential to examine whether or not the conversion of lysine residues to homoarginine was random because if guanidination is not uniform, the endogenous AA losses by homoarginine method could be underestimated. This would result in overestimation of true ileal AA digestibility values. The uniformity of guanidination could be studied by determining *in vivo* digestibility values of AA, like the present experiment, or by following the ratio of homoarginine to other AA during sequential digestion of the guanidinated protein *in vitro* using enzymes that simulated digestion *in vivo* (Siriwan et al., 1994). However, a low level of conversion rate by guanidination doesn't necessarily indicate that the homoarginine method is unsuitable for rapeseed meal, because this issue could be overcome by optimizing the conditions of guanidination, for example increasing *O*-methylisourea concentration and/or prolong the guanidination time. More study is needed to find optimal guanidination conditions and

verify whether the homoarginine method is suitable for determining true ileal digestibility of rapeseed meal. No other AA ileal digestibility values in the normal rapeseed meal diet were lower than in the guanidinated-rapeseed meal diet. This indicated that the decrease of lysine ileal digestibility value after guanidination is not caused by endogenous lysine flow in distal ileum, because the most prominent AA in endogenous protein are proline, glycine, threonine, serine, aspartic acid and glutamic acid (Sauer et al. 1977; de Lange et al., 1989; Boisen and Moughan, 1996). However, it should be aware that optimal conditions of guanidination vary for individual proteins within each feedstuff, and near complete guanidination is not always achieved, which may impose some limitation on the homoarginine method (Maga, 1981; Rutherd and Moughan, 1990; de Vrese et al., 1994).

As shown in Table IV-5, there were no significant ($P > 0.05$) differences in the apparent ileal digestibility values of CP and AA between the normal rapeseed meal diet and the guanidinated-rapeseed meal diet, except for lysine, arginine, leucine and phenylalanine, the values of the latter three AA were higher ($P < 0.05$) by approximately 3 percentage units in the guanidinated-rapeseed meal diet than its normal diet. However, the apparent ileal digestibility values of CP and most AA in the guanidinated-peas diet were significantly ($P < 0.05$) higher by approximately 4 to 8 percentage units compared to the normal pea diet; especially the digestibility value for proline in the guanidinated-pea diet was 12 percentage units higher ($P < 0.05$) than in the normal pea diet. On the other hand, values for lysine and glycine were not ($P > 0.05$) different between normal pea diet and the guanidinated-pea diet. The apparent ileal digestibility values of CP and most AA were about 2 to 6 percentage units higher ($P < 0.05$) in the guanidinated-soybean meal diet than in the normal soybean meal diet. However, no differences in

digestibility values were observed for arginine, isoleucine, lysine, aspartic acid, glycine and proline ($P > 0.05$) between the two diets. There was a scarcity of references about the effect of guanidination on CP and AA digestibility of feedstuffs for pigs. A study (Ravindran et al., 1998) on broilers reported that the apparent ileal digestibility values of overall AA in the guanidinated-soybean meal diet was slightly (2 percentage units) but not significantly ($P > 0.05$) higher than in the normal soybean meal diet. Any factor that disrupts the interactions between protein molecules and their conformational structure during guanidination could lead to changes in their physico-chemical and functional properties. The increases in apparent ileal digestibility values of CP and AA in the guanidinated feedstuffs may possibly results from the following reasons: First, multiple washings with distilled water after guanidination could remove some soluble carbohydrates, like water soluble tannins in peas and rapeseed meal. Secondly soaking feedstuffs in a solution with pH 10.3 or 10.5 for few days during guanidination would fully expand the fiber matrix; the AA and other molecules trapped in the fiber matrix would dissolve, and the water and/or ions would bind with fiber (Bach Knudsen 2001). Thirdly, soaking in alkaline solution and then acidic situation (pH 4.5 or 4.6) would denature or inactivate some anti-nutritional factors (Anderson and Wolf, 1995), such as trypsin inhibitors and tannins. Meanwhile this may also explain why the digestibility of proline increased by 12 percentage units from the normal pea diet to the guanidinated-pea diet. Studies (Sauer, 1976) and reviews (de Lange et al., 1989; Boisen and Moughan, 1996) indicated that the content of proline in endogenous protein recovered from the distal ileum was very high. Mehansho et al. (1987) reported that rats and mice adapted to dietary tannins by an induced synthesis of proline-rich salivary protein. This protein

showed a strong affinity for tannins, and may serve as a defense mechanism against the anti-nutritional effects of tannins. A study by Barth et al. (1993) indicated that trypsin inhibitor(s) reduced the digestion of dietary protein and increased endogenous protein losses; the effects of trypsin inhibitors on endogenous protein losses were larger than on dietary protein digestion. Meanwhile, soaking and drying could change the non-covalent forces (electrostatic, hydrogen bonding, and hydrophobic interactions) of AA side chains and the covalent disulfide links between thiol groups of cysteine residues, consequently altering the chemical and biological functions of a protein. Therefore, it is possible that guanidination indirectly cause the increases in the apparent ileal digestibility values of CP and AA in peas and soybean meal.

In summary, the contents of CP and NDF in soybean meal, rapeseed meal and peas increased after guanidination, but ash and soluble carbohydrate contents decreased. The effects of guanidination on chemical composition are different in the individual feedstuffs, with larger effects in soybean meal and rapeseed meal than in peas. The effects guanidination on the AA profile of protein in the feedstuffs are relatively small. Apparent ileal digestibility values of CP and AA in soybean meal are higher than in rapeseed meal; the values in peas are intermediate. The uniformity and randomness of guanidination in rapeseed meal may be questioned on the basis of decrease in apparent ileal lysine digestibility values from the normal to guanidinated rapeseed meals. Guanidinaion increased the apparent ileal CP and most AA digestibility values of peas and soybean meal, but there was little effect on the values of rapeseed meal.

Table IV-1. Formulation (%) of experimental diets (as-fed basis)

Ingredients	Diets[1]						
	SBM	RSM	PS	G-SBM	G-RSM	G-PS	CEH
Soybean meal	43.70	-	-	17.00	-	-	-
Guanidinated soybean meal	-	-	-	17.00	-	-	-
Rapeseed meal	-	54.70	-	-	22.60	-	-
Guanidinated rapeseed meal	-	-	-	-	22.60	-	-
Peas	-	-	88.70	-	-	40.70	-
Guanidinated peas	-	-	-	-	-	40.70	-
Casein enzymatic hydrolysate	-	-	-	-	-	-	20.10
Cornstarch	34.55	24.90	-	44.02	34.2	-	58.30
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Dextrose	14.00	14.00	3.66	14.00	14.00	10.90	14.00
Monocalcium phosphate	0.92	-	0.29	1.20	-	0.45	1.48
Calcium carbonate	0.93	0.50	1.32	0.88	0.70	1.26	0.64
Mineral-vitamin premix[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Antibiotics	0.20	0.20	0.20	0.20	0.20	0.20	0.20
DL-methionine	-	-	0.10	-	-	0.10	-
Tryptophan	-	-	0.03	-	-	0.03	-
Chromic oxide	-	-	-	0.20	0.20	0.20	-
Titanium oxide	0.40	0.40	0.40	0.20	0.20	0.20	-

[1] SBM: soybean meal; RSM: rapeseed meal; PS: peas; G-SBM: guanidinated SBM; G-RSM: guanidinated RSM; G-PS: guanidinated PS; CEH: casein enzymatic hydrolysate.

[2] Mineral-vitamin premix provided the following vitamins and minerals per kg of diet: vitamin A, 12,000 IU; vitamin D_3, 1,200 IU; vitamin E, 40 IU; vitamin K_3, 0.6 mg; vitamin B_1, 0.7 mg; vitamin B_2, 2.0 mg; vitamin B_6, 1.4 mg; vitamin B_{12}, 9.0 μg; niacin, 11mg; D-panthothenic acid, 6.0 mg; folacin, 0.3 mg; Zn, 100 mg; Mn, 4 mg; Fe, 100 mg; Cu, 20 mg; Se, 0.26 mg.

Table IV-2. Chemical compositions (%) of normal (N) and guanidinated (G) soybean meal, rapeseed meal and peas (as-fed basis)

	Soybean meal		Rapeseed meal		Peas	
Item	N	G	N	G	N	G
Dry matter	88.6	91.9	87.7	91.1	92.5	94.3
Crude protein	44.7	63.7	33.9	45.5	20.7	23.5
Gross energy (MJ/kg)	17.7	20.2	18.2	20.6	17.0	17.5
Crude fat	2.0	1.9	4.8	5.0	1.0	1.0
Neutral detergent fibre	12.0	18.0	23.6	30.0	12.3	14.5
Acid detergent fibre	7.8	7.3	16.1	21.3	7.7	7.6
Starch	1.3	1.2	0.4	0.7	46.1	47.5
Ash	6.2	2.3	6.9	2.8	2.8	1.0

Table IV-3. Amino acid profile (%) of protein in the normal (N) and guanidinated (G) soybean meal, rapeseed meal and peas (crude protein basis)

	Soybean meal			Rapeseed meal			Peas		
Amino acid	N	G	D[1]	N	G	D	N	G	D
Indispensable									
Arginine	6.90	6.50	0.40	5.60	5.60	0	6.80	6.70	0.10
Histidine	2.60	2.46	0.14	2.60	2.50	0.10	2.45	2.15	0.30
Isoleucine	4.14	4.10	0.04	3.80	3.60	0.20	4.00	3.70	0.30
Leucine	7.50	7.40	0.10	6.94	6.90	0.04	7.34	7.10	0.24
Lysine	6.25	1.46	4.79	5.50	1.40	4.10	7.55	0.86	6.69
Methionine	1.30	1.20	0.10	2.05	1.80	0.25	0.94	0.76	0.18
Phenylalanine	4.84	4.80	0.04	3.90	3.80	0.10	4.90	4.50	0.40
Threonine	4.00	3.80	0.20	4.50	4.36	0.14	3.94	3.40	0.54
Valine	4.25	4.30	-0.05	4.70	4.74	-0.04	4.40	4.00	0.40
Dispensable									
Alanine	4.06	3.94	0.12	4.10	4.00	0.10	4.20	3.70	0.50
Aspartic acid	11.40	10.80	0.60	7.30	6.74	0.56	11.60	10.20	1.40
Cysteine	1.40	1.16	0.24	2.10	2.00	0.10	1.50	1.00	0.50
Glutamic acid	18.40	17.30	1.10	16.80	15.50	1.30	17.50	14.75	2.75
Glycine	4.40	4.00	0.40	5.00	4.80	0.20	4.40	3.65	0.75
Proline	5.10	4.56	0.54	5.60	5.40	0.20	3.75	3.40	0.35
Serine	5.35	5.10	0.25	4.36	4.30	0.06	5.20	4.60	0.60
Tyrosine	3.30	3.20	0.10	2.70	2.60	0.10	3.00	2.70	0.30
Homoarginine	-	5.50	-	-	4.90	-	-	7.45	
Total	95.19	91.58	3.61	87.55	84.94	2.61	93.47	84.62	8.85

[1] Difference in amino acid concentrations in protein between the normal and guanidinated feedstuffs.

Table IV-4. Chemical and amino acid compositions (%) of the experimental diets (as-fed basis)

Items	Diets[1]					
	SBM	G-SBM	RSM	G-RSM	PS	G-PS
Dry matter	89.2	89.7	89.9	90.1	91.5	90.8
Crude protein	18.0	18.3	18.0	18.0	17.6	17.7
Gross energy (MJ/kg)	15.99	16.20	17.08	17.23	16.75	16.71
Crude fat	3.5	3.5	5.8	5.4	3.8	3.8
Neutral detergent fiber	8.66	9.48	17.98	17.63	12.26	12.59
Acid detergent fiber	6.34	4.89	12.44	15.08	7.65	7.23
Ash	7.35	5.58	6.88	5.23	6.60	5.33
Amino acids						
Indispensable						
Arginine	2.87	4.14	1.93	2.55	1.55	1.64
Histidine	1.08	1.57	0.90	1.14	0.49	0.53
Isoleucine	1.72	2.61	1.31	1.64	0.80	0.91
Leucine	3.12	4.71	2.39	3.14	1.46	1.74
Lysine	2.60	0.93	1.89	0.64	1.50	0.21
Phenylalanine	2.01	3.06	1.34	1.73	0.98	1.10
Threonine	1.66	2.42	1.55	1.98	0.79	0.83
Valine	1.77	2.74	1.62	2.16	0.88	0.98
Dispensable						
Alanine	1.69	2.51	1.41	1.82	0.84	0.91
Aspartic acid	4.74	6.88	2.51	3.07	2.31	2.50
Glutamic acid	7.66	11.01	5.79	7.06	2.49	3.61
Glycine	1.83	2.55	1.72	2.19	0.88	0.89
Proline	2.12	2.90	1.93	2.46	0.75	0.83
Serine	2.23	3.25	1.50	1.96	1.04	1.13
Tyrosine	1.37	2.04	0.93	1.18	0.60	0.66

[1] Refer to Table IV-1 for abbreviations of diets.

Table IV-5. Apparent ileal digestibility values (%) of amino acids in diets for pigs

Item	Diets[1]						SEM
	SBM	G-SBM	RSM	G-RSM	PS	G-PS	
Dry matter	73.08^{b}	83.14^{a}	61.02^{c}	62.16^{c}	65.60^{c}	74.51^{b}	1.59
Crude protein	80.57^{b}	86.42^{a}	63.85^{d}	66.32^{d}	73.16^{c}	81.06^{b}	1.82
Amino acid							
Indispensable							
Arginine	89.57^{a}	91.78^{a}	76.56^{d}	79.62^{c}	85.71^{b}	89.51^{a}	1.63
Histidine	87.68^{b}	91.09^{a}	77.72^{d}	78.12^{d}	80.89^{c}	85.96^{b}	1.11
Isoleucine	87.38^{a}	90.05^{a}	70.55^{d}	70.72^{d}	78.89^{c}	83.86^{b}	1.33
Leucine	84.97^{b}	90.04^{a}	70.97^{d}	75.27^{c}	77.11^{c}	84.61^{b}	1.24
Lysine	84.88^{a}	84.01^{a}	72.42^{b}	61.54^{c}	81.99^{a}	80.70^{a}	1.80
Phenylalanine	86.42^{b}	90.37^{a}	70.87^{e}	74.04^{d}	81.19^{c}	84.99^{b}	1.18
Threonine	79.21^{b}	84.01^{a}	62.63^{d}	63.07^{d}	69.58^{c}	76.47^{b}	1.75
Valine	82.63^{b}	87.23^{a}	66.33^{d}	67.76^{d}	72.87^{c}	80.50^{b}	1.53
Dispensable							
Alanine	80.13^{b}	86.44^{a}	68.84^{d}	70.74^{cd}	73.71^{c}	79.55^{b}	1.52
Aspartic acid	85.35^{a}	88.30^{a}	63.75^{c}	65.10^{c}	78.72^{b}	85.03^{a}	1.68
Glutamic acid	86.88^{b}	90.93^{a}	78.22^{d}	80.05^{cd}	81.96^{c}	87.63^{b}	1.45
Glycine	70.95^{ab}	77.78^{a}	58.78^{c}	55.76^{c}	64.77^{b}	71.27^{ab}	3.01
Proline	67.89^{a}	76.11^{a}	49.75^{b}	46.29^{b}	52.33^{b}	64.22^{a}	7.05
Serine	83.27^{b}	87.73^{a}	64.93^{d}	66.35^{d}	74.08^{c}	81.54^{b}	1.47
Tyrosine	85.60^{b}	89.68^{a}	68.59^{d}	70.38^{d}	77.65^{c}	84.07^{b}	1.36

[1] Refer to Table IV-1 for abbreviations of diets.

$^{a, b, c, d, e}$ Means in the same row with different superscript letters differ ($P < 0.05$, n=6).

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CHAPTER V

ESTIMATING ENDOGENOUS ILEAL RECOVERY AND TRUE ILEAL DIGESTIBILITY VALUES OF AMINO ACIDS OF PROTEIN SOURCES FOR PIGS WITH THE HOMOARGININE METHOD

A. Introduction

The nutritive value of protein sources of feedstuffs for pigs is largely determined by their contents of available crude protein (**CP**) and amino acids (**AA**). In general, it is accepted that ileal AA digestibility values provide better estimates of AA availability values than fecal digestibility values in protein sources for swine (Sauer and Ozimek, 1986; Moughan, 1995). However, apparent ileal AA digestibility values in feedstuffs do not take into the endogenous AA outputs. The endogenous AA output is not constant and varies from feedstuff to feedstuff (Souffrant, 1991). True ileal digestibility values are independent of endogenous outputs, and eventually are necessary for animal nutritionists to accurately formulate diets to truly meet AA requirements of pigs (NRC, 1998). There are several methods for determination of recovery values of endogenous ileal CP and AA, such as the conventional methods of the feeding of protein-free diet and the regression technique, and the more recent ^{15}N-isotope dilution and homoarginine methods.

Various studies have shown that estimates obtained with the regression technique are not different from those obtained when protein-free diets are fed (e. g. Taverner et al., 1981; Furuya and Kaji, 1989; Donkoh et al., 1995). And both methods underestimate the endogenous CP and AA outputs (Boisen and Moughan, 1996; Nyachoti et al., 1997_a).

The ^{15}N-isotope dilution technique is very expensive and requires special instrumentation. This method may also underestimate the endogenous CP and AA outputs because of possible recycling of isotope-labeled AA in the digestive tract.

The homoarginine method is a technique that distinguishes between endogenous and dietary AA in ileal digesta so that the true ileal AA digestibility values of feedstuffs can be determined (Angkanaporn et al., 1997; Caine et al., 1998). This method involves transformation of dietary lysine to homoarginine through a guanidination process. Then the absorbed homoarginine is hydrolyzed by arginase into lysine and urea in the liver. Since no endogenous homoarginine enters the digestive tract, the ileal digestibility of homoarginine is assumed to be similar to the true ileal digestibility of lysine. Therefore, endogenous output and/or true ileal digestibility values of the other AA can be calculated based on the ratio of lysine to the other AA in endogenous protein.

Soybean meal, rapeseed meal and peas were chosen in this study based on the following considerations. Soybean meal, to which other feedstuffs are often compared in feed evaluation, is the major protein supplement used for non-ruminant animals in most areas of the world where intensive animal and poultry productions are performed. Soybean meal is very palatable and has low fiber content. Therefore, the digestibility values of AA in soybean meal are relatively high and stable. Rapeseed meal is a dietary ingredient that is used more and more in swine diets, especially in Canada. The production of canola, a new kind of rapeseed, has increased considerably during the last decade in western-Canada. Peas have low content of anti-nutritive factors and high contents of both protein and energy (Fan and Sauer, 1999), and are used more and more as dietary ingredients in swine diets in western-Canada. (Castell et al., 1996).

The objective of this study was to determine endogenous ileal recoveries and true ileal digestibility values of CP and AA in soybean meal, rapeseed meal, and peas for pigs with the homoarginine method.

B. Experimental Procedures

Animal and Management

A detail description of the experimental procedures, including formulation and preparation of the experimental diets was presented previously (Chapter IV). Eight crossbred barrows, average initial body weight (**BW**) 18 kg, were obtained from the Swine Research Unit of the Hohenheim University, and housed individually in stainless steel metabolism crates in a temperature-controlled (20 to 22°C) barn. After a 7 d adaptation period, the pigs were surgically fitted with a simple T-cannula at the distal ileum following the procedures described by Sauer et al. (1983). The pigs were returned to their respective metabolism crates after surgery and fasted for 24 h. Then the pigs were provided with 25 g of an 18% CP diet four times daily at 6 h interval. The dietary allowance was gradually increased until all the pigs consumed the diet at a rate of 5% of the average BW that occurred within 7 d. During the recuperation period, the temperature in crates was maintained at 30 to 32°C by adjusting the infrared heating lamp. The pre- and post-operative cares were previously provided by Sauer et al. (1983). Water was freely available from a low-pressure drinking nipple. At the conclusion of the experiment, the barrows with average final BW 31kg were sacrificed, and dissected to determine whether cannulation had caused adhesions or other intestinal abnormalities.

Based on feed intake and BW, six pigs were selected and assigned randomly into three groups. The pigs were fed one of the three guanidinated diets in three experimental periods according to a repeated 3 × 3 Latin square design. Each experimental period lasted 10 d. For pigs to adapt the experimental diets, from d 1 to 7 (inclusive) the barrows were fed diets containing normal feedstuffs: soybean meal, rapeseed meal or peas. On d 8 and 10 of each experimental period, all pigs were fed a diet containing casein enzymatic hydrolysate to separate digesta from the normal feedstuff diets and the respective guanidinated diets (containing homoarginine) that were offered on d 9 of each experimental period. Ileal digesta were collected continuously for 24 h starting immediately after the guanidinated were offered.

Digesta were collected into plastic bags (approximately 20 cm × 3 cm) that contained 10 mL 2.5 M formic acid to minimize microbial fermentation, and frozen at -20°C immediately after collection. The digesta were freeze-dried and pooled within pig and period before chemical and AA analyses.

The animals and experimental procedures used in this experiment followed the Standards and Guidelines of the German Law for Animal Care (Regierungspraesidium Stuttgart, Germany).

Preparation of Experimental Diets

Seven corn starch-based diets (Table IV-1 in Chapter IV) were formulated to contain 18% CP according to NRC (1998) standards. Three normal diets contained normal soybean meal, rapeseed meal or peas as sole protein source. Three guanidinated diets were prepared by replacing 50% of the protein source in each normal diet with the corresponding guanidinated feedstuffs. Another diet contained casein enzymatic

hydrolysate as sole protein source. All dietary ingredients were ground through a 1.0 mm mesh screen prior to guanidination and/or incorporation into the diets. For all the seven diets, soybean oil was included at a level of 3% to reduce the dustiness of the diets. Dextrose was used to improve the palatability. Vitamins, minerals, methionine, and tryptophan were supplemented to meet or exceed NRC (1998) standards. Titanium oxide was included in the guanidinated diets as digestibility marker. Chromic oxide was used in the guanidinated diets to differentiate the digesta originating from the guanidinated diets by the green color of chromic oxide during digesta collection.

Guanidinations were performed by using procedures described by Schmitz et al. (1991) for soybean meal, and by Nyachoti et al. (1997_b) for rapeseed meal and peas. Feedstuffs, each batch containing 200 g protein, were mixed with 1 L of 0.5 M *O*-methylisourea (**MIU**) solution. Then incubation of the feedstuffs with MIU solution was performed under pH 10.3 for soybean meal, and 10.5 for rapeseed meal and peas at 4°C for 6 d. The MIU solution was freshly prepared by reacting *O*-methylisourea sulfate (Sigma Chemical, St. Louis, MO) and barium hydroxide (Sigma Chemical, St. Louis, MO) with stirring for 30 min, followed by centrifugation for 10 min at 4,000 × g to precipitate and separate the barium sulfate. The feed material of the incubation was thoroughly stirred every day to ensure uniform conditions for guanidination. The pH for guanidination was monitored daily and adjusted accordingly during 6 d incubation. At the end of incubation, the guanidination reaction was stopped by lowering the pH to iso-electric point of each protein source (soybean meal: 4.5; rapeseed meal and peas: 4.6) by adding 1 M HCl solution. Then, the guanidinated feed materials were centrifuged at 4,000 × g at 4°C for 10 min. The supernatant was discarded to remove MIU. After three

times of washing with distilled water to re-suspend the precipitate and then to centrifuge, the precipitated feed material was freeze-dried prior to incorporation into the diets as guanidinated feedstuffs.

Chemical and Statistical Analyses

Samples of dietary ingredients, diets, and digesta were ground through a 0.5-mm mesh screen before analyses. Dry matter, crude fat, and ash contents were determined according to AOAC (1990). Gross energy and CP contents were analyzed using a Bomb Calorimeter and Automated Nitrogen Analyzer (Leco Corporation, St. Joseph, MO, USA), respectively. Analyses for the contents of NDF and ADF were carried out according to principles outlined by Goering and van Soest (1970). Titanium oxide was measured by using Atomic Absorption Spectroscopy as described by Brandt and Allam (1987).

Amino acids including homoarginine were determined by using an Eppendorff Biotronik LC 3000 Amino Acid Analyzer (Eppendorff, Hamburg, Germany) following hydrolysis with 6 N HCl solution in sealed and evacuated tube at 113°C for 24 h (Naumann and Bassler, 1988). Methionine and cysteine (only in feedstuffs) were determined as methionine sulfone and cysteic acid after oxidation with 98% performic acid overnight according to AOAC (1990). The oxidized samples were then hydrolyzed and analyzed in the same manner as the other AA. Tryptophan was not determined.

The total flow of CP or each AA ($\mathbf{AA_{flow}}$, g/kg dry matter intake (**DMI**)) in ileal digesta was calculated using the CP or AA concentration in ileal digesta ($\mathbf{AA_{digesta}}$) and titanium oxide (**Ti**) concentrations in the diet and digesta according to the following equation:

$$AA_{flow}=AA_{digesta}\times Ti_{diet}/ Ti_{digesta} \quad [1]$$

The true ileal lysine digestibility values (**TD_{lys}, %**) were assumed equal to the ileal homoarginine digestibility value, calculated as follow:

$$TD_{lys} =100 \times (HA_{diet} - HA_{flow})/ HA_{diet} \quad [2]$$

where HA_{diet} and HA_{flow} are homoarginine concentration in diet and total flow in ileal digetsta, respectively.

The endogenous recovery of lysine (**Lys_{enflow}**, g/kg DMI) in ileal digesta was calculated from the following equation:

$$Lys_{enflow} = Lys_{flow} - Lys_{diet} \times (1 - TD_{lys}/100) \quad [3]$$

where Lys_{flow} is the total flow of lysine from equation [1], Lys_{diet} is lysine concentration in diets, TD_{lys} is the true ileal lysine digestibility from equation [2]. The endogenous flow of AA other than lysine was calculated from the observed flow of endogenous lysine and the amounts of other AA relative to lysine as reported by Boisen and Moughan (1996), except for proline and glycine, for which the average ratios of the values from Boisen and Moughan (1996) and de Lange et al. (1989_b) were used.

The exogenous (dietary) recovery of each amino acid (**AA_{exflow}**, g/kg DMI) in ileal digesta was calculated as follows:

$$AA_{exflow}= AA_{flow}- AA_{enflow} \quad [4]$$

The true ileal AA other than lysine digestibility (**TD_{AA}, %**) values were calculated using the following equation:

$$TD_{AA} = 100 \times [AA_{diet}- AA_{exflow}]/AA_{diet} \quad [5]$$

The results were subjected to Analysis of Variance by using the General Linear Model of SAS (SAS Institute Inc., Cary, NC, USA). The main effects of diets (n=3), pigs

(n=6) and periods (n=3) were included in the model. The means of diets were compared using the Student-Newman Keul's multiple range test procedure and the statistical significance level was claimed at $P < 0.05$.

C. Results and Discussion

The pigs were healthy throughout the experiment and readily consumed the guanidinated diets at the same rate of intake as the normal diets with soybean meal, rapeseed meal or peas. Postmortem examinations at the conclusion of the experiment revealed no adhesions and other intestinal abnormalities.

The chemical and AA compositions of the experimental diets with normal or guanidinated soybean meal, rapeseed meal, or peas are presented in Table V-1 (dry matter basis). Among the three guanidinated diets the contents of energy, CP, and ash were very closes each other. The concentrations of histidine in the guanidinated-pea diet and phenylalanine in the guanidinated-rapeseed meal diet were relatively lower. But all the contents of indispensable AA in the diets were higher than the requirements of NRC (1998) standards. The contents of NDF, ADF and fat were higher in the guanidinated-rapeseed meal diet than in the guanidinated-soybean meal diet, the contents in the guanidinated-peas were intermediate.

The recoveries of endogenous CP and AA in ileal digesta from the diets containing guanidinated soybean meal, rapeseed meal and peas are shown in Table V-2. The endogenous CP recoveries for soybean meal, rapeseed meal and peas were 27.99, 46.46 and 40.18 g/kg DMI, respectively. These values were close to the values reported

by de Lange et al. (1990), Boisen and Moughan (1996), and Nyachoti et al. (1997_b). The values reported by de Lange et al. (1990) were 25.5 and 30.5 g/kg DMI for soybean meal and rapeseed meal, respectively, with the ^{15}N-isotope dilution technique. Huisman et al. (1992) reported 34 g/kg DMI for peas with ^{15}N-isotope dilution studies. The relative higher recoveries of endogenous CP in this study, especially rapeseed meal, possibly were because of using the different methods. The amount of endogenous nitrogen loss may be underestimated by ^{15}N-isotope dilution technique because it does not account for endogenously added mucosal cells that are synthesized from labeled luminal dietary AA and then secreted (de Lange et al., 1992; Roos et al., 1994). Homoarginine method overestimated endogenous CP recovery from rapeseed meal diet if guanidination was not complete uniform in rapeseed meal (Chapter IV). Limited information is available on comparison of amount endogenous protein recovery of same feedstuff by different methods. However, the recoveries of endogenous CP and AA were usually higher for rapeseed meal and peas than for soybean meal. Boisen and Moughan (1996) summarized the endogenous ileal protein loss into three levels, a basal loss after ingestion of experimental protein-free diets is 10-15 g/kg DMI; a normal loss when protein-containing diets are given is about 20 g/kg DMI, like soybean meal diet; an extra losses specially induced by the presence of dietary fiber and/or antinutritional factors are typically in the range of 20 to 40 g/kg DMI, like the rapeseed meal and pea diets in this study.

As shown in Table V-2, the endogenous CP and AA recoveries in ileal digesta were higher ($P < 0.05$) in rapeseed meal and peas than in soybean meal, no difference ($P > 0.05$) between rapeseed meal and peas. There are many factors that determine the amounts of endogenous CP and AA recoveries. Among these factors, the type and level

of fiber and anti-nutritional factors in feedstuffs are very important determinants (Sauer and Ozimek, 1986; Boisen and Moughan, 1996). The levels of NDF and ADF were higher in the two diets containing guanidinated rapeseed meal and peas than in the diet containing guanidinated soybean meal, namely 19.57 and 16.74, 13.87 and 7.96, and 10.57 and 5.45, respectively. The NDF comprises cellulose, hemicellulose, and lignin. Lignin is especially higher in rapeseed meal than soybean meal and peas (Bach Knudsen, 2001). Meanwhile, the water-soluble fiber, pectin and ß-glycoside-linked oligosaccharides are higher in peas (Savage and Deo, 1989). Firstly, dietary fiber, like NDF, directly stimulates the secretion of digestive tract, including pancreatic protein and juice (Langlois et al., 1987), bile (Portman et al., 1985), mucus (Low, 1989), and sloughed epithelial cells (Shah et al., 1982). Secondly, in more general terms, fiber adsorbs AA and peptides on it and inhibits absorption of AA and peptides, including the digested endogenous sources (Bergner et al., 1981; Mitaru et al., 1984). Thirdly, by forming an unstirred water jelly (layer) because of its high water-holding capacity, fiber creates a physical barrier to block enzyme-substrate interactions and AA absorption of endogenous protein (Johnson and Gee, 1981; Chesson, 1990, Graham and Aman, 1991). Fiber in pea endosperm, characterized by a very high water-holding capacity (10-12 g water/kg DM), caused a dramatic increase in endogenous ileal protein and AA excretion (Leterme et al., 1996). Many studies reported that fiber increase loss of endogenous CP and AA in ileal digesta (Sauer et al., 1977; Taverner et al., 1981; de Lange et al., 1989; Furuya and Kaji, 1992; Leterme et al., 1992).

The level and type of anti-nutritional factors in feedstuffs may also affect the recoveries of endogenous CP and AA. There are two major types of trypsin inhibitors in

feedstuffs: the Kunitz, originally isolated from soybean, and Bowman and Birk, which are more common in other legume seeds, like peas (Castell et al., 1996). As was reviewed by Huisman and Jansman (1991), the Bowman-Birk protease inhibitors could irreversibly bind to trypsin and chymotrypsin in the intestine. The inactivation of trypsin and chymotrypsin stimulate further secretion of the two enzymes with pancreatic juice by a hormone (Cholecystokinin) action, thereby increasing endogenous protein and AA losses and decreasing protein digestion (Barth et al., 1993). Another kind of anti-nutritional factors is tannins in peas and rapeseed meal. It is a kind of polyphenolic substances. Dietary tannins are able to increase endogenous CP and AA by several mechanisms, including easy formation of hydrogen bonds and hydrophobic interaction of their hydroxyl groups with the carbonyl groups of protein, such as secreted enzymes, dietary protein and protein of gastrointestinal mucosa. Most of the complexes with tannins are water-insoluble, then decreasing the activity of enzymes, changing the morphological structure of the mucosa, decreasing trans-membrane AA and peptide uptake, and therefore increasing endogenous protein secretions (Jansman, 1993). Very interestingly, some mammals (e.g. rats, mule deer and moose) have developed mechanisms for adaptation to dietary tannin by induced synthesis of proline-rich salivary proteins (Mehansho et al., 1987; Hagerman et al., 1992; Hagerman and Robbins, 1993), these protein show a strong affinity for tannins and may serve as a defense mechanism against the antinutritional effects of tannins. To what extent this mechanism is operative in other species of animals, like pigs, is not known. The combined effects of fiber and anti-nutritional factors, trypsin inhibitors (peas) and tannins (peas and rapeseed meal) would

partially explain the higher endogenous CP and AA in rapeseed meal and peas than in soybean meal, in which there was lower fiber and anti-nutritional factors.

The endogenous recoveries of lysine in this study were within the range of values reported by de Lange et al. (1990), Boisen and Moughan (1996), and Nyachoti et al. (1997_b). For example, the recovery of endogenous ileal lysine from the guanidinated-rapeseed meal diet were 1.40 in this study and 1.43 (g/kg DMI) in the study about canola meal by Nyachoti et al. (1997_b). Boisen and Moughan (1996) and de Lange et al. ($1989_{a,b}$) indicated that the AA composition of endogenous protein is relatively constant for different diets and methods of determination, with exception of proline and glycine (de Lange et al., 1989_b). When AA were administered intravenously at a level meeting AA requirements for growing pigs, the proportion of proline ($P < 0.05$) and glycine ($P > 0.05$) decreased in the endogenous ileal CP than protein-free diets or saline administering intravenously (de Lange et al., 1989_b). Boisen and Moughan (1996) also reported that the endogenous recovery of proline was higher from protein-free method than from enzyme hydrolyzed casein method. It seems that the proportions of proline and glycine in endogenous ileal CP maybe decrease with the increase of dietary AA level available to pigs. In this study, the dietary allowance of CP and AA belong to a restricted level, at 4% BW of each pig in each experimental period. So for calculation of endogenous ileal proline and glycine recoveries, the average ratio of the two values from Boisen and Moughan (1996) and de Lange (1989_b) was used. Otherwise the true ileal digestibility values of proline and glycine would be higher than 100% or unreasonable too low.

As shown in Table V-2, the major AA in endogenous CP was glutamic acid, aspartic acid, proline, glycine, threonine and serine for all the diets. Endogenous CP and

AA originate from many sources such as saliva, digestive enzymes from stomach, pancreas and intestine, bile, sloughed epithelial cells, and mucin. Pancreatic juice, the main source of the proteolytic enzymes, has a high content of the branched-chain AA, glycine, aspartate and glutamate (Corring and Jung, 1972; Gabert et al., 1996). Glycine is also a major constituent base of the bile salt conjugates, and accounts for 90% of the total AA secreted in porcine bile juice (Souffrant, 1991). The bile salt conjugates are degraded in the small intestine; 90% of the bile salts are re-absorbed before the distal ileum. However, the deconjugated glycine, which escapes re-absorption and enters the large intestine (Newsholme and Leech, 1984; Shiau, 1987). Furthermore, the small intestinal secretion, mucin that is resistant to enzymatic hydrolysis, is very rich in threonine, proline and serine (Neutra and Forstner, 1987; Lien et al., 1997). As de Lange demonstrated (1989_b) that when pigs fed a protein-free diet, endogenous ileal proline output was higher than when pigs were parenterally administered with AA. It means that animals with negative AA balance in body will mobilize body protein, especially muscle protein, to supply AA for vital metabolic functions. Alanine and, especially, glutamine account for more than 50% of total α-amino-acid nitrogen related from muscle tissue (Rodwell, 1985). The tissue of the intestinal tract takes up large quantities of glutamate plus ammonia, citrulline and proline (Rodwell, 1985; Rogers and Phang, 1985). It should be pointed out that more than 65% of endogenous CP is composed of dispensable AA (Souffrant, 1991), only threonine in indispensable AA is relative higher in endogenous CP, the indispensable AA make more significance to animal nutrition (Sauer et al., 1977).

The true ileal digestibility values of CP and AA in soybean meal, rapeseed meal and peas are presented in Table V-3. The values of this study were close to the values

reported by Marty et al. (1994) for soybean meal and Nyachoti et al. (1997_b) for rapeseed meal. For example, the true ileal digestibility values of lysine in soybean meal and rapeseed meal were 96.0 and 83.3, respectively, in this study (Table V-3). In the same order of the two feedstuffs, the reported values were 97.7 (Marty et al. 1994) and 84.6% (Nyachoti et al., 1997_b), respectively, in pigs by the homoarginine method. No data in references is available for peas by the homoarginine method.

The true ileal digestibility values of CP and AA were higher ($P < 0.05$) in the diets containing soybean meal and peas than in the diet with rapeseed meal, but no difference ($P > 0.05$) between soybean meal diet and pea diet (Table V-3). These relationships were corresponding to the fiber contents in the three diets, especially to lignin contents. As summarized by Bach Knudsen (2001), lignin contents in non-starch polysaccharides of soybean meal, rapeseed meal and peas were 7.4, 60.9 and 6.7%, respectively. Following this ratios of lignin in polysaccharides, the lignin contents in the three diets with soybean meal, rapeseed meal and peas were approximately 0.78, 11.92 and 0.93% (DM basis). Therefore, it seems that lignin, not cellulose, was a determinant factor on true ileal digestibility values of CP and AA. Many reports indicated that the effects of cellulose on ileal digestibility values of CP and AA were doubted (Sauer et al., 1991; Li et al., 1994; Fan and Sauer, 2001). As a major component of the cell wall, lignin is very branched networks built up by phenylpropane units, partly linked to non-cellulosic polysaccharides, and serves principally two main functions. It cements and anchors the cellulose microfibrils and other matrix polysaccharides and, as the lignin-polysaccharide complex is hard, and stiffen the wall thus preventing biochemical degradation and physical damage of nutrients in the cell wall (Liyama et al., 1994). So

even though NDF content was 3 percentage units higher in the guanidinated-pea diet than in the guanidinated-soybean meal diet, the true ileal digestibility values of CP and AA were no difference between the two diets, because lignin contents were very close in the two diets. NRC (1998) reported the differences in true ileal digestibility values of indispensable AA between soybean meal and peas were within 3 percentage units.

The true ileal digestible contents of CP and AA in soybean meal, rapeseed meal and peas are presented in Table V-4. The true ileal digestible contents of CP and AA in soybean meal were higher than in peas, and the contents in rapeseed meal were intermediate. The three highest contents of indispensable AA in the three feedstuffs were leucine, arginine and lysine, and the relative lower indispensable AA was histidine, threonine and isoleucine.

In summary, recoveries of endogenous CP and AA in ileal digesta differ from feedstuff to feedstuff (and diet), the recoveries were higher from rapeseed meal and peas than soybean meal, but no difference between rapeseed meal and peas. The reasons for the differences among the feedstuffs possibly were type and amounts of fibers and antinutritional factors, like trypsin inhibitors and tannins. However, the true ileal digestibility values of CP and AA were higher in soybean meal and peas than rapeseed meal, no difference between soybean meal and peas. The factors responding for the differences among the three feedstuffs may be the component of NDF, especially lignin. Based on the true ileal digestible contents of CP and AA, the values of soybean meal were higher than peas, and rapeseed meal was intermediate. Based on the results of this study, in the formulation of diets for pigs it is not appropriate to apply a constant correction for the output of endogenous AA. Therefore, accurate information on both

endogenous outputs and true ileal digestibility values of AA is necessary for accurate formulation of diets for pigs.

Table V-1. Chemical and amino acid compositions (%) of the experimental diets[1]

Items	Diets[2]					
	SBM	G-SBM	RSM	G-RSM	PS	G-PS
Dry matter	89.2	89.7	89.9	90.1	91.5	90.8
Crude protein	20.2	20.4	20.0	20.0	19.2	19.5
Gross energy (MJ/kg)	4.28	4.31	4.54	4.57	4.37	4.30
Crude fat	3.9	3.9	6.5	6.0	4.2	4.2
Neutral detergent fiber	9.71	10.57	20.00	19.57	13.40	13.87
Acid detergent fiber	7.11	5.45	13.84	16.74	8.36	7.96
Ash	8.24	6.22	7.65	5.80	7.21	5.87
Indispensable amino acids						
Arginine	1.59	1.36	1.19	1.13	1.55	1.44
Histidine	0.58	0.51	0.55	0.50	0.48	0.44
Isoleucine	0.94	0.81	0.76	0.71	0.79	0.76
Leucine	1.67	1.49	1.45	1.37	1.44	1.41
Lysine	1.39	0.70	1.17	0.64	1.46	0.77
Phenylalanine	1.11	0.97	0.82	0.79	0.96	0.95
Threonine	0.93	0.81	0.97	0.91	0.79	0.75
Valine	0.98	0.85	0.98	0.92	0.86	0.84
Dispensable amino acids						
Alanine	0.95	0.84	0.90	0.84	0.84	0.81
Aspartic acid	2.61	2.27	1.57	1.44	2.36	2.22
Glutamic acid	4.22	3.65	3.58	3.26	3.43	3.22
Glycine	0.96	0.84	1.07	0.99	0.87	0.80
Proline	1.09	0.94	1.15	1.09	0.75	0.72
Serine	1.22	1.08	0.95	0.90	1.02	0.98
Tyrosine	0.70	0.60	0.53	0.50	0.58	0.56
Homoarginine	-	0.61	-	0.51	-	0.81

[1] Dry matter basis.

[2] SBM: soybean meal; RSM: rapeseed meal; PS: peas; G-SBM: guanidinated SBM; G-RSM: guanidinated RSM; G-PS: guanidinated PS.

Table V-2. Recoveries of endogenous protein and amino acids in ileal diesta[1]

Items	Guanidinated Diets			SEM
	Soybean meal	Rapeseed meal	Peas	
Crude protein	27.99[b]	46.46[a]	40.18[a]	3.14
Indispensable amino acid				
Arginine	0.84[b]	1.39[a]	1.21[a]	0.94
Histidine	0.42[b]	0.70[a]	0.60[a]	0.05
Isoleucine	0.70[b]	1.16[a]	1.00[a]	0.08
Leucine	1.12[b]	1.86[a]	1.61[a]	0.14
Lysine	0.84[b]	1.40[a]	1.20[a]	0.09
Phenylalanine	0.84[b]	1.39[a]	1.20[a]	0.09
Threonine	1.26[b]	2.09[a]	1.81[a]	0.14
Valine	0.98[b]	1.63[a]	1.41[a]	0.11
Dispensable amino acids				
Alanine	1.15[b]	1.90[a]	1.65[a]	0.12
Aspartic acid	1.85[b]	3.07[a]	2.65[a]	0.21
Glutamic acid	2.32[b]	3.86[a]	3.34[a]	0.26
Glycine	1.75[b]	2.86[a]	2.22[a]	0.19
Proline	1.76[b]	2.87[a]	2.48[a]	0.18
Serine	1.18[b]	1.95[a]	1.69[a]	0.12
Tyrosine	0.56[b]	0.93[a]	0.80[a]	0.06

[1] g per kg dry matter intake.

[a, b, c] Means in the same row with different superscript letters differ ($P < 0.05$).

Table V-3. True ileal digestibility values (%) of amino acids in feedstuffs for pigs

Item	Guanidinated Diets			SEM
	Soybean meal	Rapeseed meal	Peas	
Crude protein	97.0[a]	89.4[b]	96.4[a]	0.85
Indispensable amino acid				
Arginine	97.9[a]	92.0[b]	97.8[a]	0.97
Histidine	97.1[a]	89.0[b]	93.8[a]	0.50
Isoleucine	98.7[a]	87.1[b]	97.1[a]	0.70
Leucine	97.5[a]	88.8[b]	96.0[a]	0.57
Lysine	96.0[a]	83.3[b]	96.4[a]	0.80
Phenylalanine	99.0[a]	91.9[b]	97.8[a]	0.60
Threonine	99.6[a]	86.3[b]	100.5[a]	0.89
Valine	98.8[a]	85.3[b]	97.2[a]	0.74
Dispensable amino acids				
Alanine	100.1[a]	93.4[b]	99.9[a]	0.65
Aspartic acid	96.4[a]	86.4[b]	96.9[a]	0.91
Glutamic acid	97.3[a]	91.9[b]	98.0[a]	0.65
Glycine	97.9[a]	88.0[b]	100.9[a]	1.20
Proline	94.5[a]	75.5[b]	92.3[a]	4.04
Serine	98.6[a]	88.0[b]	98.7[a]	0.84
Tyrosine	99.0[a]	89.0[b]	98.4[a]	0.58

[a. b. c] Means in the same row with different superscript letters differ ($P < 0.05$).

Table V-4. True ileal digestible contents (%) of protein and amino acids in the protein sources for pigs (as-fed basis)

Item	Protein sources		
	Soybean meal	Rapeseed meal	Peas
Crude protein	43.4	30.3	20.0
Indispensable amino acid			
Arginine	2.81	1.78	1.52
Histidine	1.05	0.80	0.46
Isoleucine	1.70	1.14	0.78
Leucine	3.04	2.12	1.40
Lysine	2.45	1.57	1.45
Phenylalanine	1.99	1.23	0.96
Threonine	1.65	1.34	0.79
Valine	1.75	1.38	0.86
Dispensable amino acids			
Alanine	1.69	1.32	0.84
Aspartic acid	4.57	2.24	2.24
Glutamic acid	7.45	5.32	2.44
Glycine	1.79	1.51	0.88
Proline	2.00	1.46	0.69
Serine	1.20	1.32	1.03
Tyrosine	1.36	0.83	0.59

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CHAPTER VI

GENERAL DISCUSSION AND CONCLUSIONS

Conventional *in vivo* methods for determination of energy and nutrient digestibility values, involving either total collection of feces or the use of digestibility markers, are expensive, time-consuming, require relatively large amounts of feed, and involve considerable labor expenditure. Therefore, these methods are impractical for routine and rapid feed evaluation (Cherian et al., 1988), particularly, when many samples of multiple varieties of cereals are evaluated, like barley of which there are more than 50 varieties in Canada. It is not possible to directly take the nutritive values of different varieties of barley from compilations such as those produced by NRC (1998). Consequently, diets based on average tabular values will not necessarily be balanced for the ratio of digestible energy to digestible protein and AA. No doubt, the mobile nylon bag technique (**MNBT**) offers a cheaper and more rapid method for determining digestibility values of energy in cereals (Sauer et al., 1983). However, the MNBT requires the use of surgically-modified animals. In the future it is questionable if the use of surgically-modified animals for routine evaluation of feedstuffs will be allowed. The *in vitro* enzymatic method shows promise for the future because it is simple, rapid and inexpensive for routine determination (Boisen and Fernandez, 1995). There is scarcity of information on the *in vitro* method specific to use for barley samples.

The homoarginine method for the determination of the endogenous recoveries and true ileal digestibility of CP and AA is a relative novel method. This method has many

advantages over other methods, as it is suitable to evaluate almost all feedstuffs and mixtures under normal physiological conditions. However, with this method one has to make some assumptions, namely that there is no effect of guanidination on the AA profile of the protein and on its digestibility (Bryden et al., 1996). These assumptions have not been experimentally examined with pigs, such as using soybean meal, rapeseed meal and peas for pigs.

The nutritional value of feedstuffs for pigs is largely determined by their contents of available energy and nutrients, in particular AA. In general, it is accepted that ileal AA digestibility values provide better estimates of AA availability values than fecal digestibility values in dietary ingredients for swine (Sauer and Ozimek, 1986; Moughan, 1995). However, apparent ileal AA digestibility values in feedstuffs do not take into account the endogenous AA outputs that are associated with the characteristics of these feedstuffs (Boisen and Moughan 1996), such as the level and type of fiber and antinutritional factors, for example trypsin inhibitors and tannins in peas and rapeseed meal. Therefore, the true ileal digestibility values of CP and AA, independent of endogenous AA losses, should be determined.

A. *In Vitro* Methods for Determination of Energy and Amino Acid Digestibility Values of Barley for Pigs

Two experiments were carried out to study the correlations of fecal energy and ileal CP and AA digestibility values in hulled and hulless barley between the *in vivo* (including MBNT) and *in vitro* methods in order to predict the *in vivo* values from *in*

vitro measurements. Results showed that the fecal energy digestibility values of hulless barley were higher ($P < 0.05$) than of hulled barley. The differences were suggested to be related to the higher fiber content in hulled barley compared to hulless barley. Many studies (Mitaru et al., 1984; Sauer et al., 1991) and reviews (Wenk, 2001; Noblet and Le Goff, 2001) have reported that fiber is an important factor that negatively influences energy digestibility values of feedstuffs. However, the ileal CP and AA digestibility values of hulless barley were lower ($P < 0.05$) than of hulled barley. This was attributed to the higher contents of β-glucans in hulless than in hulled barley. There is a scarcity of information on the interaction between fiber and β-glucans on ileal CP and AA digestibility values of barley in pigs, but some reports have indicated that a proportion of the β-glucans are firmly linked to peptides in the cell wall (Forrest and Wainwright, 1977). In addition, β-glucans increase the viscosity of digesta that may result in a decrease in the ileal digestibility values of protein (Bach Knudsen, 1993). However, the total ileal digestible contents of CP and AA in hulless barley are still higher than, or at least equal to, the total digestible contents in hulled barley because of the higher CP and AA contents in hulless barley. For the fecal energy digestibility values of hulless and hulled barley, the values from both the MNBT and the *in vitro* technique showed very high correlations ($P < 0.05$) with measurements from the conventional *in vivo* method. The result from two experiments confirmed that the *in vitro* method can be used to predict the fecal energy digestibility values of both hulled and hulless barley. These results are in agreement with those reported by Boisen (1991) and Beames et al. (1996). However, the *in vitro* method for prediction of ileal AA digestibility values of hulled and hulless barley may require further development before it can be applied to hulless and

hulled barley. Beames et al. (1996) and Moughan (1999) came to a similar conclusion. The possible reason is that the digestibility values of AA in barley by the *in vitro* method corresponde to true ileal digestibility values of AA, and not to apparent ileal digestibility values which are influenced by endogenous CP and AA output. It is also not possible to reproduce in a single model to simulate all the complex biochemical and physiological events that comprise the *in vivo* processes of digestion and absorption. The *in vitro* assays have many limitations. For example, the model does not include enzymes from the brush border of the epithelial cells in the small intestine, co-enzymes, and co-factors.

B. Effects of Guanidination on Apparent Ileal Digestibility Values of Amino Acids in Protein Sources for pigs

The effect of guanidination on AA profile of the protein and on the protein susceptibility to digestion is a major methodological consideration when the homoarginine method is used (Bryden et al., 1996; Ravindran et al., 1998). The results of this study indicated that guanidination changed the chemical composition of soybean meal, rapeseed meal and peas, by increasing CP and NDF contents and decreasing soluble carbohydrate and ash contents. The effects of guanidination on the chemical composition of soybean meal and rapeseed meal were larger than that of peas. However, the effect of guanidination on the AA profile of protein in the feedstuffs were relative small. Caine et al. (1998) also reported that there was an increase in CP and decrease in carbohydrate contents after guanidination. The reason was attributed to the repeated washings to remove methylisourea after guanidination.

Based on the evidence that the apparent ileal digestibility value of lysine decreased ($P < 0.05$) after the guanidinated rapeseed meal was included into the diets, it is doubtful that there was random and uniform guanidination of rapeseed meal. However, the apparent ileal digestibility values of lysine were similar ($P > 0.05$) after guanidinated soybean meal and peas were included into the diets, indicating that there were random and uniform guanidinations of soybean meal and peas. These results suggest that optimal conditions of guanidination for higher fiber feedstuffs, such as rapeseed meal, should be investigated further.

Guanidination increased ($P < 0.05$) the apparent ileal digestibility values of CP and most AA in peas and soybean meal, but it had little effect ($P > 0.05$) on that in rapeseed meal. This indicates that the protein in the feedstuffs after guanidination became more digestible and/or caused lower endogenous protein recoveries in ileal digesta. Results from Ravindran et al. (1998) also showed that the apparent ileal digestibility values of AA in guanidinated soybean meal diet for broilers was 2 percentage units higher than for a normal soybean meal diet, even though the differences were not significant ($P > 0.05$). The possible reasons for the increase in apparent ileal digestibility values of AA may be related to soaking feedstuffs for few days during guanidination and/or repeated washings after guanidination, thereby changing the chemical and nutritional composition of the feedstuffs, such as removing the water-soluble tannins from peas, decreasing the activity of trypsin inhibitors in peas and soybean meal (if any), and swelling of fiber so that more protein and AA were released for digestion and absorption.

Apparent ileal digestibility values of CP and AA in soybean meal were higher ($P > 0.05$) than in rapeseed meal; the values in peas were intermediate, and lower ($P < 0.05$) than in soybean and higher ($P < 0.05$) than in rapeseed meal. Fiber and anti-nutritional factors were possibly responsible for the differences among the feedstuffs in this study.

C. Estimating Endogenous Ileal Recovery and True Ileal Digestibility Values of Amino Acids in Protein Sources for Pigs with the Homoarginine Method

The recoveries of endogenous CP and AA in ileal digesta were higher ($P < 0.05$) from rapeseed meal and peas than from soybean meal; there were no differences ($P > 0.05$) between rapeseed meal and peas. The possible reasons for these differences among the feedstuffs were firstly type and amount of fiber, which was highest in rapeseed meal followed by peas, and secondly antinutritional factors, such as trypsin inhibitors in peas and tannins in rapeseed meal and peas. However, the true ileal digestibility values of CP and AA were higher ($P < 0.05$) in soybean meal and peas than in rapeseed meal; there were no differences ($P > 0.05$) between soybean meal and peas. The factor responsible for the differences among the feedstuffs may be NDF, especially lignin, of which the content was much higher in rapeseed meal than in peas and soybean meal.

The combined results of apparent ileal digestibility values, endogenous ileal recoveries and true ileal digestibility values of CP in soybean meal, rapeseed meal and peas are presented in Figure VI-1. The highest ileal apparent and true digestibility values and the lowest endogenous CP loss were observed on soybean meal. The lowest ileal apparent and true digestibility values and the highest endogenous CP loss were observed on rapeseed meal. Interestingly, if only based on the apparent ileal digestibility value of

CP, peas were intermediate, lower ($P<0.05$) than soybean meal and higher ($P<0.05$) than rapeseed meal. However, the endogenous loss of CP from peas was the same ($P > 0.05$) as from rapeseed meal, because of a higher content of trypsin inhibitors and tannins in peas. Removing the endogenous CP losses associated with peas, the true ileal CP digestibility of peas was similar ($P > 0.05$) to soybean meal. As discussed in Chapter IV, The reason of guanidination increasing the apparent ileal digestibility value of CP in peas is possibly because the repeated washings that removed some of water-soluble tannins, decreased the activity of trypsin inhibitor, and released protein from fiber matrix. These studies suggest that the nutritive value of peas could be improved by diminishing endogenous CP losses, for example by heat processing to denature of trypsin inhibitor and by extracting of tannins in peas.

Based on the results of this study, in the formulation of diets for pigs it is not appropriate to apply a constant correction for the output of endogenous AA. Therefore, accurate information on both endogenous outputs and true ileal digestibility values of AA is necessary for accurate formulation of diets for pigs.

D. General Conclusions

To summarize, the following conclusions can be drawn:

1. Compared to hulled barley, hulless barley contained lower fiber (NDF) and higher β-glucan contents, and consequently had higher ($P<0.05$) fecal energy digestibility and lower ($P<0.05$) ileal protein and AA digestibility values.

2. The result from two experiments confirmed that *in vitro* method could be used to predict fecal energy digestibility values of the barley. However, *in vitro* method for prediction of ileal AA digestibility values of the barley may require further development because of low correlation between *in vivo* and *in vitro* methods.
3. Guanidination increased CP and NDF contents, but decreased soluble carbohydrate and ash contents in soybean meal, rapeseed meal and peas. The effect of guanidination on AA profile of protein in the protein sources was relative small.
4. Based on evidence that apparent ileal digestibility of lysine decreased after guanidinated rapeseed meal was included into diet, the random and uniform guanidination in rapeseed meal is doubted.
5. Guanidination increased apparent ileal digestibility values of CP and most AA in peas and soybean meal, but there was little effect on the values in rapeseed meal.
6. Apparent ileal digestibility values of CP and AA in soybean meal were higher than in rapeseed meal; the values in peas were intermediate.
7. Recoveries of endogenous ileal CP and AA were higher from rapeseed meal and peas than from soybean meal; there were no difference between rapeseed meal and peas.
8. The true ileal digestibility values of CP and AA were higher in soybean meal and peas than in rapeseed meal; there were no differences between soybean meal and peas.
9. It is not appropriate to apply a constant correction for the output of endogenous AA. Therefore, accurate information on both endogenous outputs and true ileal digestibility values of AA is necessary for accurate formulation of diets for pigs.

E. Future Research

1. Determination of interactions between fiber and β-glucans on ileal CP and AA digestibility values of hulled and hulless barley.
2. Improvement of the *in vitro* method for specific determination of ileal AA digestibility values in hulled and hulless barley.
3. Determination of optimal condition of guanidination for high fiber feedstuffs such as rapeseed meal.
4. Studies on the effects of guanidination on fiber and antinutritional factors, such as trypsin inhibitors and tannins in soybean meal, rapeseed meal and peas.
5. Studies on processing to improve the nutritional quality of peas.

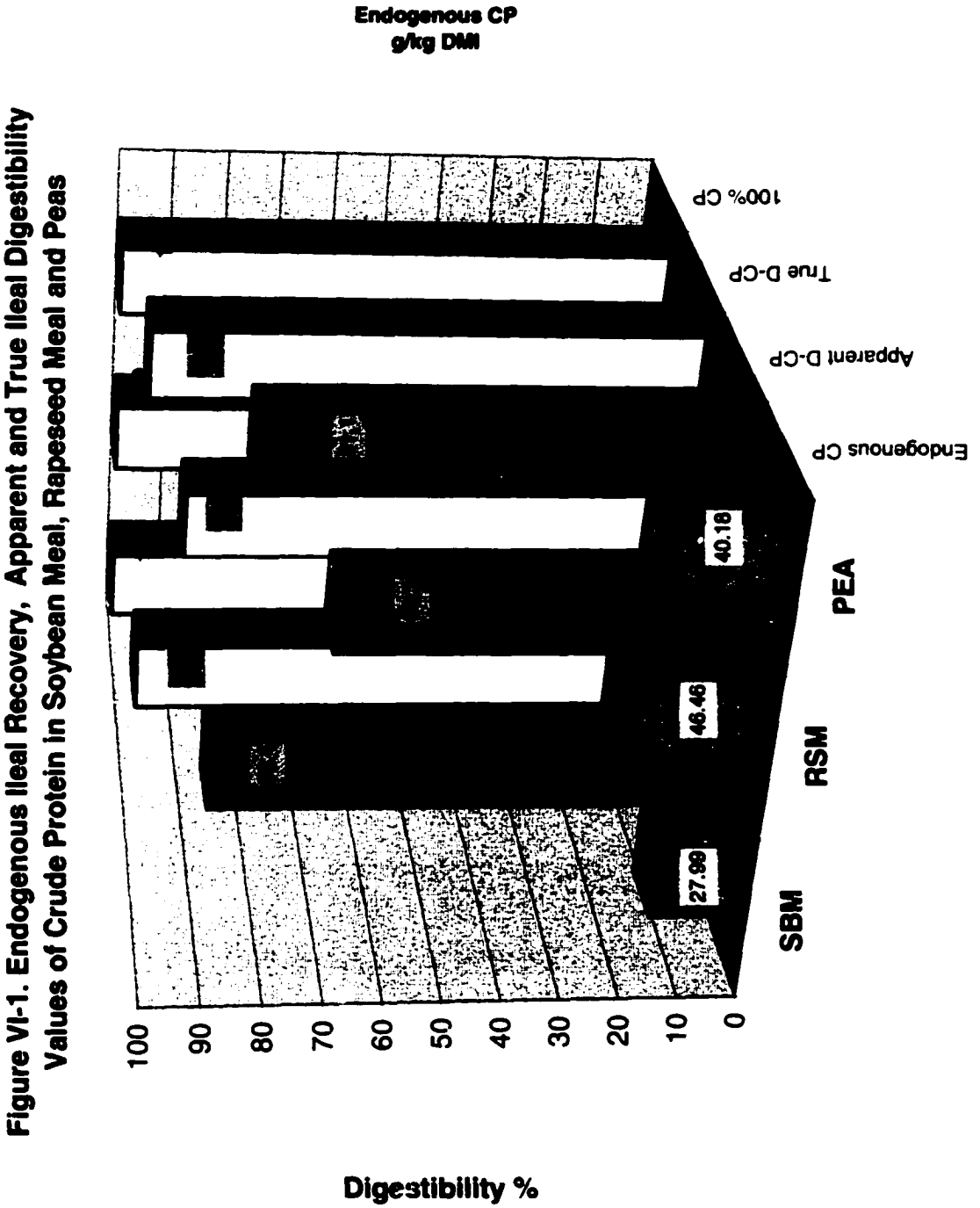

Figure VI-1. Endogenous Ileal Recovery, Apparent and True Ileal Digestibility Values of Crude Protein in Soybean Meal, Rapeseed Meal and Peas

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