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UNIVERSITY OF ALBERTA

METHODOLOGICAL CONSIDERATIONS FOR THE DETERMINATION OF AMINO ACID DIGESTIBILITY IN PIGS

BY

MING-ZHE FAN ©

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

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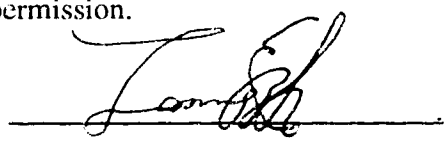

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DEDICATION

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

The main objectives of this thesis are: 1). to investigate the variability in apparent ileal digestibilities of amino acids among different samples of the same feedstuff and to identify factors responsible for the variation: 2). to investigate the effect of dietary levels of amino acids and methods of determination on apparent ileal amino acid digestibilities in feedstuffs; 3). to investigate the additivity of apparent ileal digestibilities of amino acids determined in single feedstuffs; and 4). to validate the use of the regression analysis technique for the determination of ileal endogenous amino acids and true ileal amino acid digestibilities.

The apparent ileal digestibilities of amino acids were determined in six peas, six canola meal, and six wheat samples. There was considerable variation ($P < .05$) in the digestibilities of the majority of amino acids among the six pea, canola meal, and wheat samples. Differences in the NDF content among the samples within each feedstuff were mainly responsible ($P < .05$) for the variation. Furthermore, with the exception of the wheat samples, differences in the dietary levels of most amino acids also contributed ($P < .05$), in part, to the variation in their respective digestibilities.

Effect of dietary amino acid level on the determination of apparent ileal amino acid digestibilities was investigated with six corn starch-based soybean meal diets containing six levels of amino acids. The dietary amino acid levels quadratically affected ($P < .05$) respective apparent ileal digestibilities until plateau digestibility values were reached. Differences in amino acid content in the assay diet explained, in part, the variation in corresponding apparent ileal digestibilities of amino acids, especially of the limiting amino acids, among different samples of the same feedstuff (in name).

The effect of using either the direct, difference, or regression method on the determination of apparent ileal amino acid digestibilities was investigated with barley and canola meal representing low- and high-protein feedstuffs, respectively. The use of different methods resulted in variation in the digestibilities in both types of feedstuffs.

Amino acid digestibilities in feedstuffs with a low protein content should be determined with either the difference or the regression method rather than with the direct method. Amino acid digestibilities in feedstuffs with a high protein content can be determined with either of the methods.

The additivity of apparent ileal digestibilities of amino acids in single feedstuffs was investigated with barley, wheat, canola meal, and soybean meal. This study suggested that the digestible amino acid supply in a mixture of feedstuffs can be predicted from amino acid digestibilities determined in single feedstuffs.

Results of the final study demonstrated that the endogenous amino acid levels in ileal digesta can be reliably determined by the linear relationships between dietary contents of apparent ileal digestible and total amino acids. An appropriate design of the graded dietary levels of amino acids is an important methodological consideration. Furthermore, the amount of endogenous amino acids in ileal digesta is constant at different dietary amino acid levels. Therefore, true ileal amino acid digestibilities appear to be independent of dietary amino acid levels.

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LIST OF ABBREVIATIONS NOT DEFINED IN THE TEXT

Abbreviation	Definition
ADF	Acid detergent fiber
ANOVA	Analysis of variance
BW	Body weight
℃	Degree Celsius
CP	Crude protein (%N x 6.25)
cm	Centimeter
cv	Cultivated variety
d	Day
DE	Digestible energy
DM	Dry matter
g	Gram
g	Gravity
GE	Gross energy
GLM	General linear model
h	Hour
HPLC	High performance (pressure) liquid chromatography
kg	Kilogram
i.d.	Inside diameter
mL	Microliter
min	Minute
MJ	Megajoule
N	Nitrogen
N	Normal (concentration)
NDF	Neutral detergent fiber
P	Probability
r^2	Simple coefficient of determination
r	Simple correlation coefficient
R^2	Multiple coefficient of determination
Vol	Volume
wk	Week
wt	Weight

CHAPTER I

GENERAL INTRODUCTION

The nutritive value of protein in feedstuffs for monogastric animals is determined not only by their total content in amino acids, but also by the bioavailability of the amino acids in particular, the amino acids likely to be limiting. Information concerning the bioavailability of amino acids in feedstuffs is an important determinant for accurate formulation of diets for pigs.

A. Amino Acid Digestibility and Bioavailability in Feedstuffs

Bioavailability of an amino acid in a feedstuff is defined as the proportion of the total amount of an amino acid that is not combined with compounds which interfere with its digestion, absorption or utilization by the animal consuming it, for the purpose of maintenance or growth of new tissue (ARC, 1981). As defined, amino acid bioavailability is an abstract concept and as such can not really be measured but can only estimated.

The slope-ratio assay (also referred to as growth-response assay) and the digestibility assay are the two major in vivo methods used for estimating amino acid bioavailability in feedstuffs for pigs. The different assay methods and the major processes of utilization of amino acids in feedstuffs by pigs are schematically presented in Figure I-1.

The slope-ratio assay is the most direct approach for the estimation of amino acid bioavailability in feedstuffs for pigs. It provides a combined estimation of digestibility and post-absorptive utilization of amino acids at the tissue level according to the definition of amino acid bioavailability (e.g., Austic, 1983). Results estimated with this assay are usually referred to as amino acid availability (e.g., Batterham et al., 1979). Many factors,

including the dietary balance of amino acids, dietary levels of protein and energy and sources of supply, chronology of appearance of absorbed amino acids at the tissue level, genotype, physiological stage, and other factors, can influence protein retention in the animal's body, thereby affecting results and causing large inevitable variation. Causes of high standard errors associated with amino acid availability estimates were discussed by Batterham (1992). In addition, this assay is expensive, time-consuming and provides an estimate of the availability of only one amino acid per assay (e.g., Austic, 1983; Henry, 1985). Finally, because many dietary factors affect the results, amino acid availability values determined in single feedstuffs are, in theory, not additive when used in the formulation of complete diet. It is obvious that additivity is an important consideration to determine the suitability of an assay method for the determination of the nutritive value for the purpose of diet formulation. To summarize, the slope-ratio assay seems to be an inadequate approach for the determination of the nutritive value of protein in single feedstuffs for the purpose of diet formulation.

With respect to the digestibility assay, the term amino acid digestibility should not be interchanged with amino acid availability determined by the slope-ratio assay. Amino acid digestibility is defined as the difference between the amount of amino acids in the diet and that present in ileal digesta or in feces, divided by the amount in the diet (Low, 1982; Sauer and Ozimek, 1986). As illustrated in Figure I-1, digestibility is probably the most important single determinant of amino acid utilization in feedstuffs by pigs. The amino acid digestibility assay includes both the fecal and the ileal digestibility assays, which are usually referred to as the fecal and the ileal analysis method, respectively. A large number of studies have been carried out to compare fecal and ileal analysis method for the determination of apparent ileal amino acid digestibility during the last two decades. Sufficient evidence shows that the ileal analysis method should be the method of choice for the determination of amino acid digestibilities in feedstuffs for pigs because of the

modifying action of the microflora in the large intestine on amino acids (e.g., Zebrowska, 1973; Tanksley and Knabe, 1984; Sauer and Ozimek, 1986).

Nevertheless, ileal amino acid digestibility does not always reflect amino acid bioavailability in feedstuffs, especially feedstuffs subjected to heat treatment. Special cautions should be taken for the heat-damaged feedstuffs. Some indispensable amino acids, including lysine, threonine, methionine, and tryptophan, are susceptible to heat and may be damaged by excessive heat treatment. The loss of nutritive value of amino acids in feedstuffs from excessive heat treatment is partly due to the destruction of these amino acids as shown by a decrease in their chemical content. Cug and Friedman (1989) in a study with heated casein, showed that the destruction of tryptophan reached 43%. The utilization of heat-damaged amino acids in feedstuffs by pigs can be reduced considerably in terms of amino acid digestibility and post-absorptive utilization. According to Cug and Friedman (1989), this decrease results from toxic, antinutritive derivatives that are amino acid-sugar complexes (Amadori compounds) which are formed in the presence of reducing sugars during the initial stage of the Maillard reaction. The Maillard reaction between amino acids in feedstuffs and reducing carbohydrates usually occurs between primary amino groups such as the $\varepsilon-NH_2$ groups of lysine, the $\alpha-NH_2$ of the free amino acids, and the $\alpha-NH_2$ of the terminal amino acids and the hydroxyl group (-OH) of reducing carbohydrates. Lysine, which is usually the most limiting amino acid, can be severely damaged by heat treatment; the Maillard reaction is the most widely reported reaction of this type (e.g., Hurrell and Finot, 1983). For instance, over-heating results in the formation of lysine-sugar complexes, e.g., fructoselysine, lactuloselysine, or maltuloselysine, in which the sugars are linked at the $\varepsilon-NH_2$ groups of the protein-bound lysine. Erbersdobler et al. (1989) reported that the main lysine-sugar complex, i.e. fructoselysine, can be partly released during digestive hydrolysis of protein and absorbed by passive diffusion. However, most of the absorbed fructoselysine was excreted in the urine. In this particular case, the heat-damaged amino acids are partly absorbed in a form which renders them

unavailable for protein synthesis (Figure I-1). Therefore, ileal amino acid digestibility usually overestimates amino acid bioavailability in heat-damaged feedstuffs for pigs (Batterham et al. 1990a,b; Wiseman et al. 1991). More research is required in this area to quantify the extent of the overestimation.

In principle, true ileal amino acid digestibility should be determined in feedstuffs for pigs and used in diet formulations. However, the determination of amino acid digestibility with the ileal analysis method is confounded by the presence of endogenous amino acids in digesta collected from the distal ileum (Figure I-1). Furthermore, true ileal amino acid digestibilities are usually determined from the corresponding apparent values by correcting for the endogenous amino acid contribution.

B. Variability of Apparent Ileal Amino Acid Digestibility within the Same Feedstuff

Apparent ileal amino acid digestibilities have been determined in a wide variety of feedstuffs. It is easy to recognize that there are large differences in ileal amino acid digestibility between feedstuffs. However, it comes somewhat as a surprise to note considerable variation in the ileal amino acid digestibilities among samples of the same feedstuff (in name) (Sauer et al., 1990). Some of the factors responsible for the differences in amino acid digestibilities between feedstuffs were previously discussed in detail (Sauer and Ozimek, 1986; Knabe et al., 1989). However, many factors have not been identified. The considerable variation in amino acid digestibilities among different samples within the same feedstuff reduces the reliability of the use of amino acid digestibility values in diet formulation.

Increasing production of peas in recent years has created an interest in the feed industry for their use as an alternative protein and energy source in swine diets. There is an increasing amount of information available in the literature on apparent ileal amino acid

digestibilities of peas. Gatel (1992) recently summarized the apparent ileal amino acid digestibilities in legume seeds, including peas. Compared with the other amino acids, there was much more variation in the apparent ileal digestibilities of methionine, cysteine, and tryptophan among different samples of peas. The digestibilities of methionine, cysteine, and tryptophan ranged from 58.0 to 80.7%, 44.0 to 85.0%, and 46.6 to 78.0%, respectively. Because the sulfur-containing amino acids and tryptophan are first and second limiting in protein in peas, respectively (e.g., Palisse-Roussel et al., 1984; Gatel et al., 1989), information on factors which affect the digestibilities of these amino acids is needed to optimize the use of peas in diets for pigs. There is a scarcity of information on factors affecting the variability in amino acid digestibilities in peas.

The apparent ileal amino acid digestibilities have been determined in many protein feedstuffs, especially soybean meal. Canola meal is an alternative protein supplement to soybean meal. The development and commercial use of double-zero varieties of canola (low-erucic acid and low-glucosinolate) have resulted in more extensive use of canola meal in swine diets. Apparent ileal digestibilities of amino acids have been determined only in a few studies with a limited number of canola meal samples (Sauer et al., 1982; Knabe et al., 1989). Therefore, there is a lack of information on the variability of apparent ileal amino acid digestibilities in canola meal and factors affecting the variability.

Cereal grains usually supply up to about 50% of the protein in swine diets. There is an abundance of information in the literature on apparent ileal amino acid digestibilities in cereal grains. For example, the apparent ileal digestibilities of lysine and threonine in barley ranged from 64.9 to 79.0% and from 64.4 to 76.0%, respectively; the apparent ileal digestibilities of lysine and threonine in corn ranged from 71.0 to 82.0% and from 53.8 to 78.9%, respectively; the apparent ileal digestibilities of lysine and threonine in wheat ranged from 62.3 to 81.0% and from 61.9 to 78.4%, respectively. Surprisingly, there is very little information in the literature on factors that are responsible for the wide variation in amino acid digestibilities in cereal grains, including wheat.

C. Effect of Dietary Level of Amino Acids on Variability of Apparent Ileal Amino Acid Digestibility among Different Samples of the Same Feedstuff

As was illustrated by Eggum (1973) in studies with rats, the apparent fecal CP digestibility in soybean meal-based diets increased curvilinearly with increasing dietary CP content. Similarly, it is expected that the apparent ileal amino acid digestibilities should increase curvilinearly with increasing amino acid content in the assay diet. Therefore, values for apparent ileal amino acid digestibility are only meaningful and valid under strictly standardized conditions, at least with respect to the amino acid content in the assay diet. A review of the literature reveals that, in many instances, this has not been the case. The determination and comparison of apparent ileal digestibilities of amino acids were carried out at various dietary amino acid levels as indicated by the difference in dietary CP content. For example, the CP contents in corn starch-based soybean meal diets were 21, 14, and 12% in studies by Holmes et al. (1974), Jørgensen et al. (1984), and Knabe et al. (1989), respectively. As discussed by Sauer et al. (1989), differences in CP and amino acid contents in the assay diets may explain, in part, the variation in apparent ileal amino acid digestibilities reported in the literature among different samples of the same feedstuff (in name) (e.g., Sauer and Ozimek, 1986). The effect of dietary amino acid levels on the respective apparent ileal digestibilities was previously investigated by Buraczewska and Horaczynski (1986), Furuya and Kaji (1989), and Li et al. (1993). However, the results from the aforementioned studies did not allow for the establishment of a detailed relationship between amino acid intake and apparent ileal digestibility.

D. Effect of Method on the Determination of Apparent Ileal Amino Acid Digestibility in Feedstuffs

Nutrient and energy digestibilities in feedstuffs can be determined with the direct, difference, and regression method (Giger and Sauvant, 1983). Apparent ileal digestibilities of amino acids in most low-protein feedstuffs including cereal grains and protein supplements are usually determined with the direct method (e.g., Lin et al., 1987; Knabe et al., 1989). The difference method is often used to determine amino acid digestibilities in feedstuffs that are of poor palatability (e.g., Kreienbring et al., 1988; Knabe et al., 1989). To our knowledge, the regression method has never been used for the determination of apparent ileal digestibilities of amino acids in feedstuffs for pigs. The likelihood exists that inappropriate use of methods for determination of amino acid digestibilities may have contributed, in part, to the variation in digestibilities within the same feedstuff that have been reported in the literature (e.g., Sauer and Ozimek, 1986). Furthermore, valid apparent ileal digestibilities are also necessary to determine the corresponding true digestibilities whenever the amount of endogenous amino acids in ileal digesta can be accurately quantified in future research. There is a scarcity of information in the literature on the evaluation of different methods to determine amino acid digestibility in feedstuffs.

E. Additivity of Apparent Ileal Amino Acid Digestibility Determined in Single Feedstuffs

Additivity of apparent ileal amino acid digestibilities, determined in single feedstuffs, is a crucial consideration in the formulation of diets for pigs. In diet formulation, it is usually assumed that the supply of digestible amino acids in a mixture of feedstuffs is equal to the total of the supply based on the digestible supply determined in the single feedstuffs.

There may be associative effects between different feedstuffs. However, there is a scarcity of information on the additivity of the apparent ileal digestible amino acid supply. Furthermore, the information that is available is contradictory. Imbeah et al. (1988) reported that the digestible amino acid supply in a barley and soybean meal or canola meal diet can be predicted from amino acid digestibilities determined in the single feedstuffs. On the other hand, Furuya and Kaji (1991) found significant differences between the observed and calculated digestibilities of most amino acids in a barley and soybean meal diet but not in a corn and soybean meal diet. Therefore, further experiments should be carried out to address this discrepancy.

F. Methodological Considerations for Estimating True Ileal Amino Acid Digestibility in Feedstuffs by Regression Analysis

True ileal amino acid digestibilities, which are independent of endogenous contributions of amino acids, should be determined in all feedstuffs for pigs. The key issue for the determination of true ileal amino acid digestibilities is to quantify the levels of endogenous amino acids in digesta collected from the distal ileum.

The feeding of protein-free diets is the classical technique to determine the amount of endogenous amino acids (e.g., Carlson and Bayley, 1970). While the feeding of protein-free diets remains a useful technique to study the effect of dietary factors other than protein on the recoveries of amino acids in ileal digesta (e.g., De Lange et al., 1989), it is questionable whether results obtained with this technique are valid.

Using the ^{15}N-isotope dilution technique, a differentiation can be made between non-digested dietary and endogenous protein (e.g., Souffrant et al., 1981; De Lange et al., 1990; Lien et al., 1993). Nevertheless, this technique can usually be used to estimate total endogenous protein, not the amount of each of the amino acids. A constant amino acid

profile of endogenous protein is assumed to estimate true ileal amino acid digestibility (e.g., De Lange et al., 1990).

Alternatively, linear relationships between apparent ileal digestible and dietary amino acid contents can be used to estimate the levels of endogenous amino acids under normal dietary conditions in pigs (Furuya and Kaji, 1986; 1989). However, this technique has been questioned, as it is not clear yet whether dietary levels of amino acids can influence the amount of endogenous amino acids and whether the linear relationships are affected by differences in ranges of graded dietary amino acid levels (De Lange et al., 1989; Souffrant, 1991). Furthermore, the relationships between dietary levels of amino acids and true ileal amino acid digestibilities have not been established.

As these questions are directly related to methodology for estimating the amount of endogenous amino acids in ileal digesta by the regression analysis technique, further studies need to be conducted to validate this technique for the determination of true ileal amino acid digestibility in feedstuffs for pigs.

G. Objectives of Research

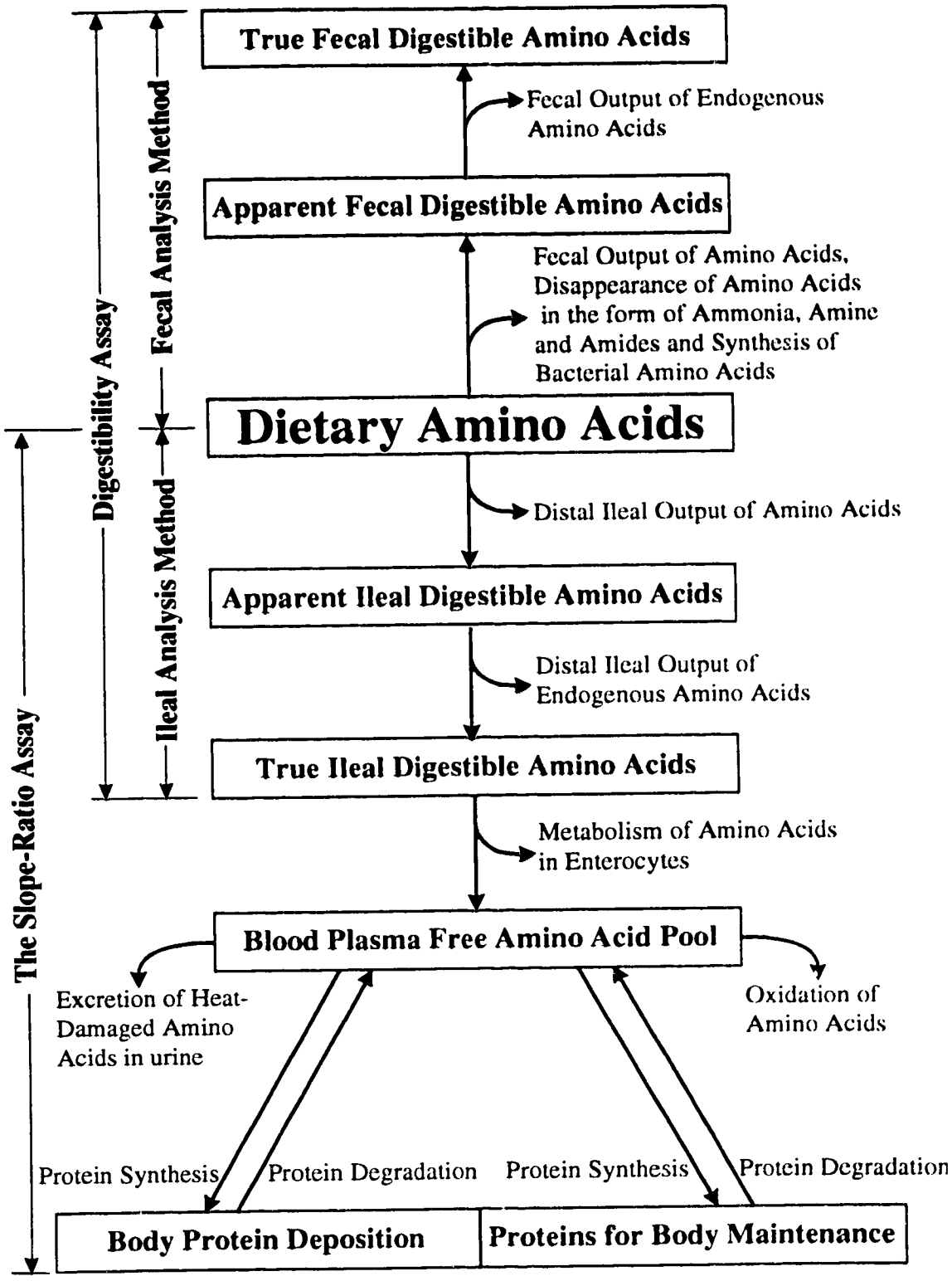
Based on the issues addressed in this introduction, the objectives of the studies carried out for this thesis were as follows:

1. to investigate variability in apparent ileal amino acid digestibilities among different samples of peas, canola meal, and wheat and to identify factors responsible for this variation.

2. to investigate effect of dietary level of amino acids on the determination of apparent ileal amino acid digestibilities.

3. to investigate the effect of different methods on the determination of apparent ileal amino acid digestibilities in different types of feedstuffs.
4. to investigate the additivity of apparent ileal amino acid digestibilities determined in single feedstuffs.
5. to investigate methodological aspects of the use of the regression technique for the determination of true ileal amino acid digestibilities in feedstuffs.

Figure I-1. Schematic representation of the utilization of amino acids in feedstuffs by pigs and different assay methods


True Fecal Digestible Amino Acids
Fecal Output of Endogenous Amino Acids
Apparent Fecal Digestible Amino Acids
Fecal Output of Amino Acids, Disappearance of Amino Acids in the form of Ammonia, Amine and Amides and Synthesis of Bacterial Amino Acids
Dietary Amino Acids
Distal Ileal Output of Amino Acids
Apparent Ileal Digestible Amino Acids
Distal Ileal Output of Endogenous Amino Acids
True Ileal Digestible Amino Acids
Metabolism of Amino Acids in Enterocytes
Blood Plasma Free Amino Acid Pool
Excretion of Heat-Damaged Amino Acids in urine
Oxidation of Amino Acids
Protein Synthesis
Protein Degradation
Protein Synthesis
Protein Degradation
Body Protein Deposition
Proteins for Body Maintenance
Digestibility Assay
Fecal Analysis Method
Ileal Analysis Method
The Slope-Ratio Assay

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CHAPTER II

VARIABILITY OF APPARENT ILEAL AMINO ACID DIGESTIBILITY IN DIFFERENT SAMPLES OF PEAS FOR PIGS

A. Introduction

Although peas are grown primarily for human consumption, they also have considerable potential as a feedstuff for pigs. Increasing production of peas in recent years has created an interest in the feed industry for their use as an alternative protein and energy source in swine diets. Two subspecies of peas are grown in Canada: *Pisum sativum ssp. arvense* which has dark-coloured flowers and is primarily used as forage and *Pisum sativum ssp. hortense* which has white flowers and whose seeds are used mainly for consumption by humans and pigs. The average CP content in pea cultivars is 25% (wt/wt, as-fed), ranging from 20 to 30% (Grosjean and Gatel, 1986). The content of lysine in peas, as a percentage of CP, is relatively high (6.6%, wt/wt as-fed); the content of the sulphur-containing amino acids, as in many other grain legumes, is relatively low (2.1%, wt/wt as-fed) (NRC, 1988). There is a large variation in CP and amino acid contents among pea cultivars (Savage and Deo, 1989). This variation is affected by both genetic and environmental factors (Reddy et al., 1979). Moreover, the nutritive value of protein in peas is determined not only by its total chemical content in amino acids, but also by their digestibilities.

As has been pointed out frequently during the last decade, the ileal rather than fecal analysis method should be used to determine amino acid digestibility (e.g., Tanksley and Knabe, 1984; Sauer and Ozimek, 1986). In recent years, several studies have been carried

out to determine the apparent ileal amino acid digestibilities in peas (Leterme et al., 1990b; Gdala et al., 1992; Fan et al., 1994). Relatively large differences were observed in amino acid digestibilities between different samples of peas in the aforementioned studies.

The objectives of this study were to investigate the variability in apparent ileal amino acid digestibilities in six pea samples and to identify factors responsible for this variation.

B. Experimental Procedures

Animal Trial Procedures

Six barrows (Lacombe x Yorkshire), average initial BW 32.5 kg, were surgically fitted with a simple T-cannula at the distal ileum according to procedures adapted from Sauer et al. (1983). The cannulas were modified according to De Lange et al. (1989). After surgery, the barrows were housed individually in stainless steel metabolic crates in a temperature-controlled barn (20 to 22°C). During a 14-d recovery period, the barrows were fed a 16% CP grower diet (Sauer et al., 1983). A detailed description of pre- and post-operative care was previously presented by Sauer (1976) and Sauer et al. (1983). After recovery, the barrows were fed one of the six pea diets according to a 6 x 6 Latin square design. The barrows were fed 800 g twice daily, at 0800 and 2000 h. Water was freely available from a low-pressure drinking nipple. At the conclusion of the experiment, the barrows, average final BW 65.4 kg, were electrically stunned before being killed, bled out, and dissected to determine whether cannulation had caused intestinal abnormalities.

The pea samples selected for these studies were from six cultivars, i.e. Progreta, Radley, Century, Tara, Express, and Trapper. The peas were ground through a 4.8-mm mesh screen prior to diet incorporation. The diets were formulated to contain 16.5% CP (% N x 6.25, as-fed basis) with the specific pea cultivar sample as the sole protein source (Table II-1). All diets included 10% dextrose to improve the palatability. Canola oil was

included at a level of 3.0% to reduce dustiness of the diets. Vitamins and minerals were supplemented according to NRC (1988) standards. Chromic oxide (.3%) was included in the diets as a digestibility marker.

Each experimental period comprised 7 d. Ileal digesta were collected for a total of 24 h: from 0800 to 1000 h on d 6 and every other 2 h thereafter until 0800 h on d 7 and from 1000 to 1200 h on d 7 and every other 2 h thereafter until 0800 h on d 8. Ileal digesta were collected in soft plastic tubing (length, 15 cm; i.d., 2.5 cm), which was attached to the barrel of the cannula with Velcro tape. The tubing contained about 10 mL of a solution of formic acid (10%, vol/vol) to minimize further microbial activity. The tubing was removed and replaced as soon as it was partially filled with digesta. Digesta were immediately frozen at -20°C after collection.

The experimental proposal, surgical procedures, and procedures for care and treatment of the barrows were reviewed and approved by the Faculty of Agriculture and Forestry Animal Care Committee of the University of Alberta. The barrows used in this experiment were cared for in accordance with the guidelines established by CCAC (1980).

Chemical Analyses

After the conclusion of the animal trials, the digesta samples were freeze-dried, pooled within the barrow and the period for the same dietary treatment, ground through a .8-mm mesh screen, and mixed before analyses. The samples of peas and diets were ground similarly.

Analyses for DM, CP, and ether extract were carried out according to AOAC (1984) methods. Analysis for NDF was carried out according to principles outlined by Goering and Van Soest (1970). Chromic oxide content was determined according to the procedure of Fenton and Fenton (1979).

For amino acid analyses, with the exception of the sulfur-containing amino acids and tryptophan, the samples were hydrolyzed with 6 *N* HCl at 110°C for 24 h, derivatized as

ninhydrin-positive compounds, and detected colorimetrically according to HPLC-procedures adapted from Mason et al. (1980) using an automatic amino acid analyzer (Beckman model 6300, Beckman Inst., Inc., Palo Alto, CA.). Methionine and cysteine contents were determined as methionine sulfone and cysteic acid after oxidation with performic acid; the oxidation process was carried out according to AOAC (1984). The oxidized samples were then hydrolyzed and analyzed in the same manner as the samples that were not oxidized. Tryptophan analysis was carried out according to procedure described by Jones et al. (1981).

The trypsin inhibitor activity (**TIA**) was expressed in trypsin inhibited unit (TIU). TIA in the pea samples was assayed as described by Kakade et al. (1974).

Calculations and Statistical Analyses

The apparent ileal digestibilities of CP and amino acids in the experimental diets (and also in the peas) were determined with the direct method using equation [1].

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

D_D: apparent digestibility of a nutrient in the diet (percentage); I_D: marker concentration in the diet (percentage); A_F: nutrient concentration in ileal digesta (percentage); I_F: marker concentration in ileal digesta (percentage); A_D: nutrient concentration in the diet (percentage).

The digestibility values were first subjected to ANOVA for a 6 x 6 Latin square design. Sources of variation were diets (t = 6), periods (p = 6), and barrows (n = 6). Periods and barrows were the controlled factors in the 6 x 6 Latin square design. Where appropriate, treatment means were compared using the Student-Newman Keuls' multiple range test. To establish linear relationships between the content of CP and amino acids in peas, data obtained from another six pea samples (Fan et al., 1994) were also included in the linear regression analyses. The ANOVA, the multiple comparisons, and the linear regression analyses were carried out using the GLM Procedures of SAS (1990) according

to the principles described by Steel and Torrie (1980). Pearson partial correlation analyses were conducted to determine the relationships between the apparent ileal digestibilities of CP and amino acids and the TIA values and NDF content in the pea samples and the dietary levels of CP and amino acids using the GLM Procedure of SAS (1990). Variation contributed by barrows and periods was removed by creating five dummy variables for barrows and periods, respectively and obtaining partial correlations when the dummy variables were forced into the analyses (Draper and Smith, 1981).

C. Results and Discussion

The pigs remained healthy and consumed their meal allowances throughout the experiment. Postmortem examinations, carried out at the conclusion of the experiment, revealed no intestinal adhesions.

The chemical composition of the diets and the pea samples are presented in Tables II-2 and 3, respectively. The analyzed values of CP and amino acids in the pea diets were close to the calculated values based on the analyzed values in the different pea samples. The contents of ether extract, NDF, and TIA values in the pea diets were calculated from the analyzed values in the pea samples.

The content of CP was highest in the sample of cv Progreta and lowest in the sample of cv Trapper. The rate of protein synthesis and accumulation of seed storage proteins can vary widely in peas depending on genetic and environmental factors (Pandy and Gritton, 1975; Savage and Deo, 1989). The storage proteins of peas, which make up 65 to 80% of the extractable proteins of peas, are globulins and can be divided into two major fractions: legumins and vicilins (Savage and Deo, 1989). In addition, Murray (1979) pointed out that albumins contributed significantly to the total protein in peas and suggested that they might, therefore, also be considered storage proteins. The albumins make up 20 to 35% of the extractable proteins of peas (Schroeder, 1982). In this context, it should be mentioned

that the amino acid content and composition in peas are dependent on the content and relative proportions of legumins, vicilins, and albumins, as these have different amino acid profiles. For example, albumins are rich in the sulphur-containing amino acids and tryptophan while vicilins are very poor in these amino acids (Huet et al., 1987).

The content of each of the amino acids increased as the CP content in the pea samples increased (Table II-3). Using additional results from six pea samples from a previous study (Fan et al., 1994), linear relationships were established between the content of CP and each of the amino acids in peas (Table II-4). With the exception of cysteine, leucine, lysine, threonine, and tryptophan, linear relationships were obtained between the content of CP and each of the remaining amino acids. Holt and Sosulski (1979) reported positive correlations between the N content and nine amino acids in 16 samples of peas. Furthermore, Huet et al. (1987), in studies with 33 pea samples, showed linear relationships between the CP content and all the individual amino acids. They ascribed these linear relationships to the fact that the amino acid composition of additional proteins, accumulated in pea seeds higher in protein than the lowest protein seeds, remains constant. They also noted that these additional proteins were particularly rich in amino acids with a high N content, including arginine. In agreement with these studies, the higher content of CP in the sample of cv Progreta, compared with the other cultivar samples, resulted primarily from a greater proportion of arginine.

The apparent ileal digestibilities of CP and amino acids in the pea samples are shown in Table II-5. The digestibility of CP in the pea samples ranged ($P < .05$) from 74.9 (cv Century) to 79.7% (cv Express). With the exception of arginine, cysteine, histidine, proline, and methionine, the digestibilities of the remaining amino acids were different ($P < .05$) among the pea samples. Of the indispensable amino acids, the differences in digestibilities ranged from 3.0 (arginine) to 17.3 (tryptophan) percentage units. Of the dispensable amino acids, the differences in digestibilities ranged from 5.3 (aspartic acid) to 10.3 (glycine) percentage units.

The NDF content in the pea samples ranged from 14.6 to 18.2% (Table II-3). The apparent ileal digestibilities of CP and amino acids, with the exception of arginine, cysteine, proline, and tryptophan, were negatively correlated ($P < .05$) with the NDF content in the pea samples (Table II-6). These results suggest that differences in NDF content were partly responsible for the variability in the digestibilities of CP and most of the amino acids among the pea samples. Gdala et al. (1992) also reported a negative correlation between NDF content and amino acid digestibilities in peas. The mechanism(s) by which differences in NDF content contribute to the variability in amino acid digestibilities is not clear. It is possible that NDF is associated with protein in peas making it resistant to hydrolysis by the proteolytic enzymes.

The TIA values in the pea samples ranged from 3.63 to 10.96 TIU/mg DM and were highest in the samples of cv Progreta and cv Radley (Table II-3). The TIA values were in the range of those reported by Leterme et al. (1990a), who also reported high TIA values in the samples of cv Progreta and cv Radley. With the exception of the apparent ileal digestibility of tryptophan which was negatively correlated ($P < .05$), there were no correlations ($P > .05$) between TIA values and the digestibilities of the other amino acids in the pea samples (Table II-6). Previous studies by Fan et al. (1994), in which the digestibility of tryptophan was not measured, also showed no correlation between TIA values and amino acid digestibilities.

With the exception of arginine, cysteine, and proline, the apparent ileal digestibilities of CP and the remaining amino acids were positively correlated ($P < .05$) with their respective dietary levels of CP and amino acids (Table II-6). The study in Chapter V showed that apparent ileal digestibilities of amino acids were quadratically affected by their respective dietary levels. Therefore, differences in the dietary levels of amino acids were also, in part, responsible for the variability in amino acid digestibilities.

It is of interest to consider the apparent ileal digestibility of tryptophan which is one of the limiting amino acids in protein from peas (e.g., Eggum and Beames, 1983). Among

the pea samples, tryptophan showed the largest variation in apparent ileal digestibility, which, as was previously discussed, resulted, in part, from differences in TIA values. However, a large proportion of the variation likely resulted from differences in the content of tryptophan in peas *per se* and in the pea diets. As was discussed in Chapter V, a relatively small difference in tryptophan content between diets, as its content is low, may elicit a relatively large difference in its apparent ileal digestibility.

Within each pea sample, compared with the other amino acids, the apparent ileal digestibilities of arginine, glutamic acid, and lysine were usually high, whereas those of cysteine, glycine, proline, and tryptophan were relatively low (Table II-5), which was also observed by Leterme et al. (1990b), Gdala et al. (1992), and Fan et al. (1994). The relatively high digestibilities of arginine and lysine in this study suggest that enzyme specificity is an important determinant of apparent amino acid absorption in the small intestine. Low (1980) suggested that arginine and lysine would be expected to appear first after enzymatic hydrolysis based on the known specificity of the proteases and peptidases involved. The relatively low ileal digestibilities of glycine and proline, may, in part, result from their relatively high concentrations in endogenous secretions. Studies by Holmes et al. (1974), Sauer et al. (1977), and Taverner et al. (1981) showed a relatively high abundance of glycine and proline in digesta collected from the distal ileum of growing pigs fed a protein-free diet. Glycine, a major constituent base of the bile salt conjugates, accounts for more than 90% of the total of the amino acids secreted in porcine bile juice (Souffrant, 1991). The bile salt conjugates are degraded in the distal ileum by intestinal bacteria; 90% of the bile salts is re-absorbed via active transport and enters the enterohepatic circulation. However, deconjugated glycine escapes re-absorption and enters the large intestine (Newsholme and Leech, 1984; Shiau, 1987). Furthermore, the small intestinal secretions, which include mucins, supply the largest proportion of N to the endogenous N secretions in the small intestine (Auclair, 1986). As was shown by Neutra and Forstner (1987), "native" mucin which represents over 95% of mucin glycoprotein is

very rich in serine and proline. As was reviewed by Huisman and Jansman (1991), the Bowman-Birk protease inhibitor family, to which the trypsin inhibitors in peas belong, contains a very high content of cysteine. Up to 50% of the total amount of cysteine in peas may be located in the trypsin inhibitors (Begbie and Pusztai, 1989). Irreversible binding between the trypsin inhibitors and trypsin and chymotrypsin, which are also rich in cysteine, further increases the endogenous loss of cysteine. Additionally, the content of cysteine in peas is low, which also contributes to its low apparent ileal digestibility. Lastly, the low digestibility of tryptophan can also be attributed, in part, to its low content in peas and, as was discussed previously, the trypsin inhibitor has an adverse effect on its digestibility.

In conclusion, there were differences in the apparent ileal digestibilities of the majority of amino acids among the pea samples. Differences in NDF content were, in part, responsible for this variation. On the other hand, differences in the dietary levels of most amino acids were also, in part, responsible for the variation. Furthermore, the relatively low digestibilities of cysteine, methionine, and tryptophan further accentuate the limitation of these amino acids in protein from peas.

Table II-1. Formulation (%) of the pea diets

	Diets[a]					
Items	1	2	3	4	5	6
Peas	68.2	74.7	68.9	78.6	72.0	78.4
Corn starch[b]	15.9	9.4	15.2	5.6	12.1	5.7
Dextrose[c]	10.0	10.0	10.0	10.0	10.0	10.0
Canola oil	3.0	3.0	3.0	3.0	3.0	3.0
Calcium carbonate	.6	.6	.6	.6	.6	.6
Dicalcium phosphate	1.2	1.2	1.2	1.2	1.2	1.2
Trace-mineralized salt[d]	.5	.5	.5	.5	.5	.5
Vitamin premix[e]	.2	.2	.2	.2	.2	.2
Mineral premix[f]	.1	.1	.1	.1	.1	.1
Chromic oxide[g]	.3	.3	.3	.3	.3	.3

[a]Diet 1, cv Progreta; diet 2, cv Radley; diet 3, cv Century; diet 4, cv Tara; diet 5, cv Express; diet 6, cv Trapper.

[b]St. Lawrence Starch Company, Mississauge, ON.

[c]Corn Products, Englewood Cliffs, NJ.

[d]Supplied by Windsor Salt, Toronto, Canada. Composition (percentage): NaCl, 96.5; ZnO, .40; $FeCO_3$, .16; MnO, .12; CuO, .033; $Ca(IO_3)_2$, .007; CaO, .004.

[e]The vitamin premix supplied the following vitamins (mg/kg diet): retinyl palmitate, 5.2; cholecalciferol, .38; all-rac-α-tocopherol acetate, 44.0; menadione, 3.0; riboflavin, 2.2; niacin, 12.0; d-pantothenic acid, 11.0; vitamin B_{12}, .012; choline, 550; thiamine, 1.1; pyridoxine, 1.1; d-biotin .1; folic acid, .6; corn starch was used as carrier.

[f]The trace-mineral premix supplied the following minerals (mg/kg diet): Fe, 50; Zn, 50; Mn, 2; Cu, 3; I, .14; Se, .15; corn starch was used as carrier.

[g]Fisher Scientific, Fair Lawn, NJ.

Table II-2. The chemical composition[a] (%) of the pea diets

	Diets[b]					
Items	1	2	3	4	5	6
DM	92.7	92.0	92.9	92.3	92.5	93.0
Ether extract	3.8	3.9	3.6	3.9	3.6	3.9
NDF	9.5	11.8	12.5	11.8	10.7	13.6
CP	18.9	19.2	18.1	19.2	18.7	18.0
Amino acids						
Indispensable						
Arginine	2.03	1.99	1.95	1.94	1.85	1.74
Histidine	.45	.46	.42	.47	.45	.42
Isoleucine	.74	.79	.70	.81	.77	.75
Leucine	1.34	1.40	1.25	1.42	1.36	1.30
Lysine	1.30	1.37	1.18	1.38	1.29	1.25
Methionine	.20	.22	.20	.22	.20	.19
Phenylalanine	.82	.88	.68	.82	.83	.68
Threonine	.80	.85	.74	.83	.77	.63
Tryptophan	.13	.15	.14	.16	.16	.15
Valine	.87	.90	.81	.91	.87	.85
Dispensable						
Alanine	.73	.77	.67	.75	.71	.70
Aspartic acid	2.07	2.13	1.93	2.12	2.03	1.86
Cysteine	.27	.29	.26	.26	.25	.27
Glutamic acid	3.15	3.22	2.96	3.30	3.06	2.83
Glycine	.75	.79	.72	.80	.76	.73
Proline	.70	.73	.67	.74	.70	.68
Serine	.89	.92	.83	.93	.84	.79
Tyrosine	.47	.50	.43	.42	.42	.35
TIA, TIU/mg DM	7.35	6.51	2.65	4.39	2.61	4.42

[a]DM basis.

[b]Refer to Table II-1.

Table II-3. The chemical composition[a] (%) of the pea samples

	Peas[b]					
Items	P1	P2	P3	P4	P5	P6
DM	91.2	92.3	92.2	91.4	92.5	93.2
Ether extract	.8	.9	.5	.9	.4	1.0
NDF	14.6	15.8	18.2	15.2	14.8	17.3
CP	28.3	25.5	26.9	24.8	26.2	22.4
Amino acids						
Indispensable						
Arginine	2.90	2.50	2.65	2.36	2.36	1.98
Histidine	.67	.61	.60	.63	.61	.51
Isoleucine	1.11	1.06	1.01	1.03	1.08	.92
Leucine	2.00	1.85	1.78	1.78	1.83	1.59
Lysine	1.97	1.84	1.75	1.78	1.75	1.52
Methionine	.30	.29	.27	.28	.26	.21
Phenylalanine	1.25	1.20	1.14	1.06	1.11	.75
Threonine	1.18	1.12	1.05	1.05	1.04	.57
Tryptophan	.21	.22	.23	.21	.23	.20
Valine	1.29	1.20	1.15	1.15	1.18	1.03
Dispensable						
Alanine	1.12	1.06	.99	.97	.99	.87
Aspartic acid	3.08	2.84	2.77	2.70	2.68	2.16
Cysteine	.39	.39	.38	.35	.35	.32
Glutamic acid	4.74	4.33	4.25	4.23	4.14	3.52
Glycine	1.14	1.06	1.04	1.03	1.06	.90
Proline	1.03	.96	.93	.96	.95	.80
Serine	1.33	1.23	1.18	1.18	1.09	.87
Tyrosine	.83	.82	.74	.56	.65	.41
TIA, TIU/mg DM	10.96	8.68	3.87	5.64	3.63	5.63

[a]DM basis.

[b]P1, cv Progreta; P2, cv Radley; P3, cv Century; P4, cv Tara; P5, cv Express; P6, cv Trapper.

Table II-4. The linear relationships between the content of CP and the content of each of the amino acids in the pea samples

Items	Regression equations[a] [b]	r^2	S_{yx}	Probability[c]	
Indispensable					
Arginine[d]	Y = 17.19 + 3.75X	.71	1.30	.0001	.0006
Histidine[d]	Y = 2.56 + 39.01X	.66	1.42	.6365	.0014
Isoleucine[d]	Y = 4.92 + 19.00X	.50	1.72	.4684	.0103
Leucine	Y = .90 + 14.05X	.78	1.13	.8302	.7841
Lysine	Y = 16.87 + 4.39X	.21	2.16	.0098	.1350
Methionine[d]	Y = 60.30 + 9.82X	.40	1.88	.1372	.0273
Phenylalanine[d]	Y = 11.23 + 13.41X	.56	1.61	.0190	.0052
Threonine	Y = 19.17 + 6.95X	.29	2.04	.0001	.0693
Tryptophan	Y = 89.41 + 6.29X	.29	1.90	.7001	.2708
Valine[d]	Y = -3.86 + 24.90X	.82	1.04	.4014	.0001
Dispensable					
Alanine[d]	Y = - .71 + 25.81X	.72	1.28	.8931	.0005
Aspartic acid[d]	Y = 6.98 + 6.65X	.78	1.14	.0485	.0001
Cysteine	Y = 51.83 + 6.01X	.27	2.08	.5674	.0842
Glutamic acid[d]	Y = 3.40 + 2.87X	.86	.92	.2632	.0001
Glycine[d]	Y = 9.73 + 16.17X	.43	1.84	.1224	.0210
Proline[d]	Y = 23.12 + 3.97X	.76	1.11	.5545	.0242
Serine[d]	Y = 17.36 + 7.91X	.35	1.95	.0006	.0414
Tyrosine[d]	Y = 21.13 + 7.92X	.37	1.93	.0001	.0360

[a]Y = The content of CP in the pea samples (percentage, DM basis).

[b]X = the content of an amino acid in the pea samples (percentage, DM basis).

[c]The probabilities of significance for the intercepts and the slopes of the regression equations.

[d]The linear regression equations are significant ($P > .05$, n = 12).

Table II-5. The apparent ileal digestibilities (%) of DM, CP, and amino acids in the pea diets

	Diets[a]						
Items	1	2	3	4	5	6	SEM[b]
DM	69.5^{cd}	70.8^{c}	65.0^{d}	66.1^{d}	68.6^{cd}	64.8^{d}	1.18
CP	76.1^{de}	77.9^{cd}	74.9^{e}	77.9^{cd}	79.7^{c}	75.8^{de}	.57
Amino acids							
Indispensable							
Arginine	89.3	91.3	89.1	89.5	91.0	88.3	.77
Histidine	79.7	82.1	77.1	82.4	81.9	80.8	1.30
Isoleucine	75.9^{de}	79.5^{c}	73.1^{e}	80.0^{c}	81.5^{c}	78.4^{cd}	.97
Leucine	77.1^{de}	79.8^{cd}	74.1^{e}	80.8^{cd}	81.9^{c}	78.8^{cd}	1.04
Lysine	81.8^{d}	84.1^{cd}	78.7^{e}	84.9^{c}	85.2^{c}	83.3^{cd}	.79
Methionine	71.6	73.2	69.4	74.8	75.4	69.6	1.46
Phenylalanine	77.4^{d}	81.3^{c}	72.5^{e}	80.8^{c}	83.3^{c}	75.6^{d}	.97
Threonine	72.8^{cde}	74.5^{cde}	66.5^{e}	75.3^{cd}	76.5^{c}	67.7^{de}	2.02
Tryptophan	53.1^{f}	64.1^{d}	59.8^{e}	65.9^{d}	70.4^{c}	62.3^{de}	1.05
Valine	72.4^{cd}	74.7^{c}	68.3^{d}	75.8^{c}	77.1^{c}	74.1^{c}	1.59
Dispensable							
Alanine	69.9^{c}	73.7^{c}	64.6^{d}	72.4^{c}	74.0^{c}	71.1^{c}	1.18
Aspartic acid	78.3^{cd}	81.0^{c}	75.8^{d}	80.6^{c}	81.1^{c}	77.5^{cd}	.86
Cysteine	56.2	62.7	53.8	59.2	62.4	61.2	2.55
Glutamic acid	83.0^{cd}	86.0^{c}	81.1^{d}	85.7^{c}	85.4^{c}	83.4^{cd}	.88
Glycine	62.5^{cd}	69.0^{c}	58.7^{d}	67.5^{c}	68.1^{c}	65.3^{cd}	2.10
Proline	63.0	68.2	64.5	69.6	71.7	69.5	2.93
Serine	73.3^{cd}	76.3^{c}	70.3^{d}	76.7^{c}	77.0^{c}	73.0^{cd}	1.34
Tyrosine	74.5^{cd}	77.5^{c}	70.2^{de}	75.9^{c}	77.4^{c}	68.0^{c}	1.60

[a]Refer to Table II-1.

[b]Standard error of mean (n = 6).

c, d, e, f Means in the same row with different superscript letters differ ($P < .05$).

Table II-6. Correlation coefficients between apparent ileal digestibilities of CP and amino acids and NDF content and TIA in the pea samples and the dietary levels of CP and amino acids

	NDF[a] (n = 36)		TIA[b] (n = 36)		Dietary levels[c] (n = 36)	
	r	*p*	*r*	*p*	*r*	*p*
CP	-.59	.0015	-.13	.5167	.51	.0077
Amino acids						
Indispensable						
Arginine	-.31	.1264	.03	.8830	.19	.3475
Histidine	-.39	.0479	.06	.7798	.44	.0245
Isoleucine	-.51	.0078	-.10	.6145	.72	.0001
Leucine	-.52	.0062	-.08	.7087	.63	.0005
Lysine	-.56	.0030	.01	.9911	.66	.0003
Methionine	-.52	.0062	-.05	.8189	.39	.0471
Phenylalanine	-.72	.0001	.06	.7833	.77	.0001
Threonine	-.65	.0004	.14	.5025	.52	.0070
Tryptophan	-.13	.5429	-.63	.0005	.90	.0001
Valine	-.48	.0134	-.02	.9168	.54	.0044
Dispensable						
Alanine	-.58	.0020	.13	.5228	.60	.0011
Aspartic acid	-.60	.0012	.09	.6709	.59	.0015
Cysteine	-.21	.2992	-.03	.9019	.15	.4779
Glutamic acid	-.48	.0132	.07	.7400	.46	.0170
Glycine	-.39	.0491	.03	.8742	.53	.0051
Proline	-.09	.6545	-.27	.1879	.16	.4269
Serine	-.53	.0055	.03	.8784	.39	.0476
Tyrosine	-.63	.0006	.19	.3602	.53	.0051

[a]NDF content in the pea samples (percentage, DM basis).

[b]TIA in the pea samples (TIU/mg DM).

[c]Dietary levels of CP and amino acids (percentage, DM basis).

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CHAFTER III

VARIABILITY OF APPARENT ILEAL AMINO ACID DIGESTIBILITY IN DIFFERENT SAMPLES OF CANOLA MEAL FOR PIGS

A. Introduction

Canola meal is an alternative protein supplement to soybean meal. The development and commercial use of the double-zero varieties of canola (low-erucic acid and low-glucosinolate) has resulted in more extensive use of canola meal in swine diets. There are large differences in the contents of CP and amino acids among canola meal samples obtained from different processing plants (Bell and Keith, 1991). For instance, as summarized by Bell and Keith (1991), the contents of CP, methionine, threonine, and tryptophan ranged from 38.0 to 43.5%, .73 to .96%, 1.73 to 2.11%, and .45 to .57%, respectively. Although most of the variation in chemical composition among the canola meal samples was associated with the different processing plants, it is unlikely that these plants *per se* were responsible for the differences (Bell and Keith, 1991). Furthermore, the nutritive value of protein in canola meal is not only determined by the total content of amino acids, but also by their digestibilities. Therefore, variability in the total content of amino acids does not reflect the real differences in the nutritive value of protein among different canola meal samples.

Presently, it is generally accepted that the ileal rather than fecal analysis method should be used to determine amino acid digestibility in feedstuffs for pigs because it measures digestibility prior to microbial degradation and synthesis of amino acids in the large intestine (Zebrowska, 1973; Tanksley and Knabe, 1984; Sauer and Ozimek, 1986).

There is a scarcity of information on the variability of apparent ileal amino acid digestibilities among samples of canola meal. Apparent ileal amino acid digestibilities have been determined only in a few studies with a limited number of canola meal samples (Sauer et al., 1982; Knabe et al., 1989).

The objectives of this study were to investigate the variability in apparent ileal digestibilities of amino acids among six canola meal samples and secondly to identify factors responsible for this variation. Samples of canola meal, obtained from different processing plants in Western Canada, were selected for this study.

B. Experimental Procedures

Animal Trial Procedures

Six barrows (Lacombe x Yorkshire), average initial BW 32.5 kg, were surgically fitted with a simple T-cannula at the distal ileum according to procedures adapted from Sauer et al. (1983). The cannulas were modified according to De Lange et al. (1989). After surgery, the barrows were housed individually in stainless steel metabolic crates in a temperature-controlled barn (20 to 22°C). During a 14-d recovery period, the barrows were fed a 16% CP grower diet (Sauer et al., 1983). A detailed description of pre- and post-operative care was previously presented by Sauer (1976) and Sauer et al. (1983). After recovery, the barrows were fed one of six canola meal diets according to a 6 x 6 Latin square design. The barrows were fed 800 g twice daily, at 0800 and 2000 h. Water was freely available from a low-pressure drinking nipple. At the conclusion of the experiment, the barrows, average final BW 65.4 kg, were electrically stunned before being killed, bled out, and dissected to determine whether cannulation had caused intestinal abnormalities.

Six canola meal samples were obtained from different processing plants and are referred to as Russel, Canbra, Neepawa, Altona, Alberta Terminal, and Fort Saskatchewan

canola meal, respectively. Corn starch-based diets were formulated to contain 16.5% CP (%N x 6.25, as-fed basis) with the specific canola meal as the sole protein source (Table III-1). All diets included 10% dextrose to improve palatability. Canola oil was included at a level of 3.0% to reduce dustiness of the diets. Vitamins and minerals were supplemented according to NRC (1988) standards. Chromic oxide (.3%) was included in the diets as the digestibility marker.

Each experimental period comprised 7 d. Ileal digesta were collected for a total of 24 h: from 0800 to 1000 h on d 6 and every other 2 h thereafter until 0800 h on d 7 and from 1000 to 1200 h on d 7 and every other 2 h thereafter until 0800 h on d 8. Ileal digesta were collected in soft plastic tubing (length, 15 cm; i.d., 2.5 cm), which was attached to the barrel of the cannula with Velcro tape. The tubing contained about 10 mL of a solution of formic acid (10%, vol/vol) to minimize further microbial activity. The tubing was removed and replaced as soon as it was partially filled with digesta. Digesta were immediately frozen at -20°C after collection.

The experimental proposal, surgical procedures, and procedures for care and treatment of the barrows were reviewed and approved by the Faculty of Agriculture and Forestry Animal Care Committee of the University of Alberta. The barrows used in this experiment were cared for in accordance with the guidelines established by CCAC (1980).

Chemical Analyses

After the conclusion of the animal trial, the digesta samples were freeze-dried, pooled within barrow and period for the same dietary treatment, ground through a .8-mm mesh screen, and mixed before analyses. Samples of ingredients and diets were ground similarly.

Analyses for DM, CP, and ether extract were carried out according to AOAC (1984) methods. Analysis for NDF was carried out according to principles outlined by Goering

and Van Soest (1970). Chromic oxide content was determined according to the procedure of Fenton and Fenton (1979).

For amino acid analyses, with the exception of the sulfur-containing amino acids and tryptophan, the samples were hydrolyzed with 6 *N* HCl at 110°C for 24 h, derivatized as ninhydrin-positive compounds, and detected colorimetrically according to HPLC-procedures adapted from Mason et al. (1980) using an automatic amino acid analyzer (Beckman model 6300, Beckman Inst., Inc., Palo Alto, CA.). Methionine and cysteine contents were determined as methionine sulfone and cysteic acid after oxidation with performic acid; the oxidation process was carried out according to AOAC (1984). The oxidized samples were then hydrolyzed and analyzed in the same manner as the samples that were not oxidized. Tryptophan analysis was carried out according to the procedure described by Jones et al. (1981).

The content of condensed tannins in the canola meal samples was measured as catechin equivalents using the vanillin-sulfuric acid method of Kuhla and Ebmeier (1981).

Calculations and Statistical Analyses

The apparent ileal digestibilities of DM, CP, and amino acids in the experimental diets (also in the canola meal samples) were determined using equation [1].

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

D_D: apparent digestibility of a nutrient in the diet (percentage); I_D: marker concentration in the diet (percentage); A_F: nutrient concentration in ileal digesta (percentage); I_F: marker concentration in ileal digesta (percentage); A_D: nutrient concentration in the diet (percentage).

The digestibility values were first subjected to ANOVA for a 6 x 6 Latin square design. Sources of variation were diets (t = 6), periods (p = 6), and barrows (n = 6). Periods and barrows were the controlled factors in the 6 x 6 Latin square design. Where appropriate, treatment means were compared using the Student-Newman Keuls' multiple

range test. The ANOVA and the multiple comparisons were carried out using the GLM Procedures of SAS (1990) according to the principles described by Steel and Torrie (1980). Pearson partial correlation analyses were conducted to determine the relationships between the apparent ileal digestibilities of CP and amino acids and the contents of NDF and tannins in the canola meal samples and the dietary levels of CP and amino acids using the GLM Procedure of SAS (1990). Variation contributed by barrows and periods was removed by creating five dummy variables for barrows and periods, respectively and obtaining partial correlations when the dummy variables were forced into the analyses (Draper and Smith, 1981).

C. Results and Discussion

The pigs seemingly remained healthy and consumed their meal allowances throughout the experiment. Postmortem examinations, carried out at the conclusion of the experiment, revealed no intestinal adhesions.

The chemical composition of the diets and the canola meal samples are presented in Tables III-2 and 3, respectively. The directly determined dietary contents of CP and amino acids were close to the calculated values based on the analyzed contents in the canola meal samples. The dietary contents of tannins and NDF were calculated from the values determined in the corresponding canola meal samples.

The contents of ether extract, NDF, CP, and amino acids were within the range of values summarized by Bell and Keith (1991) and Bell (1993a). The CP content ranged from 35.3 to 40.6% among the canola meal samples. Changes in amino acid contents paralleled the changes in CP content among the samples, which was also observed by Bell and Keith (1991).

The samples of canola meal were obtained from processing plants in different regions of Western-Canada. The cultivars could not be identified as canola seeds are

usually blended before processing. According to Bell and Keith (1991), most of the variation in chemical composition among samples of canola meal is associated with the regions, rather than the processing plants *per se*, from which the samples were obtained. This variation results sometimes from the specific cultivars grown in that region and environmental conditions. Species and cultivar differences in the CP and amino acid contents of *B. napus* and *B. campestris* were reported by Finlayson et al. (1969) who analyzed eight cultivars of these two species. Norton (1989) reported that the composition of the storage proteins (globulins) in canola is genetically controlled. However, the content of globulins in canola may vary due to environmental factors including, soil conditions, fertilizer application, and weather conditions. Based on the previous discussion, a combination of genetic and environmental factors was likely responsible for the differences in CP and amino acid contents among the canola meal samples.

The apparent ileal digestibilities of CP and amino acids in the canola meal samples are presented in Table 4. With the exception of proline, there were differences ($P < .05$) in the digestibilities of CP and all amino acids among the canola meal samples. The digestibility of CP ranged from 62.4 to 70.3%. Of the indispensable amino acids, the differences in digestibilities ranged from 4.5 (histidine) to 8.4 (lysine) percentage units. Of the dispensable amino acids, the differences in digestibilities ranged from 4.7 (glutamic acid) to 10.2 (tyrosine) percentage units. Furthermore, the digestibilities of amino acids in the canola meal samples reported in this study were much more variable than those reported in the literature. For example, as summarized by Sauer and Ozimek (1986), the digestibilities of lysine and threonine ranged from 73.5 to 76.3% and 65.6 to 67.2%, respectively, whereas these ranged from 68.3 to 76.7% and 59.7 to 66.5%, respectively in this study.

With the exception of a lower tannin content of one canola meal sample (CM2, .14%), the content of tannins in the remaining canola meal samples were similar, ranging from .21 to .24% (Table III-3). However, no relationships ($P > .05$) were observed

between the apparent ileal digestibilities of CP and amino acids and the tannin content in the canola meal samples.

The NDF content in the canola meal samples ranged from 19.3 to 24.9% (Table III-3). The apparent ileal digestibilities of amino acids, with the exception of arginine, were negatively correlated ($P < .05$) with the NDF content in the canola meal samples (Table III-5). These results indicate that differences in NDF content were, in part, responsible for the variability in the digestibilities of amino acids among the samples. The NDF fraction comprises cell wall material, including cellulose, hemicellulose, and lignin (Goering and Van Soest, 1970). Cellulose may contribute up to 24.1% of the polysaccharide fraction in rapeseed meal. (Siddiqui and Wood, 1971). As was reviewed by Naczk and Shahidi (1990), cellulose is the major constituent in hulls, together with hemicellulose, lignin, and pectic substances, which act as cementing material. Pectin, which is water-soluble, is not included in the NDF fraction. However, one can postulate that there is a positive correlation between the NDF and pectin content. However, it should be kept in mind that pectic substances are also present in cotyledons (Naczk and Shadidi, 1990), therefore not all pectic substances are correlated with the NDF content.

The dietary inclusion of cellulose and pectins may affect the apparent ileal amino acid digestibilities. Cellulose is a water-insoluble fiber source. As was reviewed by Li et al. (1994), the dietary inclusion of water-insoluble fiber may decrease the apparent ileal digestibilities of amino acids by increasing the recovery of endogenous amino acids at the distal ileum. However, they also pointed out that increases in the recoveries of endogenous amino acids become quantitatively important only when a certain dietary level of inclusion of water-insoluble fiber (threshold level) is exceeded. As was reviewed by Li et al. (1994), the dietary inclusion of water-soluble fiber decreases the apparent ileal digestibilities of amino acids. Studies by Anderson et al. (1990) showed that the gelatinization and viscosity properties of water-soluble fiber decreased the digestion and absorption of nutrients by reducing the mixing of intestinal contents, blocking enzyme-substrate

interaction, and by forming an unstirred water layer, thereby creating a physical barrier to nutrient absorption. Apart from the aforementioned mechanisms, fiber components, contained in the protein-containing feedstuffs including these in canola meal, can decrease amino acid digestibilities by physically hindering the access of proteolytic enzymes to proteins and by directly binding to proteins. To summarize, it is likely that differences in cellulose and pectin contents among the canola meal samples are, in part, responsible for the variability in the digestibilities of amino acids. Furthermore, variability may also, in part, be related to differences in the content of hulls among the canola meal samples. Differences in the contents of hulls between canola meal samples of different cultivar has been reported (e.g., Bell and Shires, 1982). Hulls represent about 30% by weight of canola meal (Bell, 1993b). The hull fraction contains up to 22.5% CP (Bell, 1993b) which is of very low digestibility (Cichon and Sauer, 1980; Bell and Shires, 1982).

The apparent ileal digestibilities of amino acids, with the exception of aspartic acid, glycine, proline, serine, threonine, tryptophan, and valine, were positively correlated ($P < .05$) with their dietary levels (Table III-5). The study in Chapter V with soybean meal diets showed quadratic relationships between the dietary amino acid contents and their respective digestibilities. Therefore, differences in the dietary levels of these amino acids were also, in part, responsible for the variability in digestibilities among the canola meal samples.

Compared to soybean meal (e.g., Sauer and Ozimek, 1986), the apparent ileal digestibilities of amino acids in canola meal were lower. For instance, the digestibilities of lysine and threonine ranged from 68.3 to 75.2% and 59.7 to 66.5%, respectively in canola meal samples in this study (Table III-4) and from 80.1 to 90.7% and 70.7 to 82.2%, respectively in soybean meal samples (Sauer and Ozimek, 1986). As was discussed by Sauer et al. (1982), the lower digestibilities in canola meal may be attributed to its higher fiber content, as a result of a larger proportion of hulls, and protein associated with hulls which is of low digestibility.

Within each canola meal sample, compared with the other amino acids, the apparent ileal digestibilities of arginine and glutamic acid were relatively high while those of glycine, threonine, and tryptophan were relatively low (Table III-4), which was also observed by Sauer et al. (1982) and Knabe et al. (1989). The relatively high digestibility of arginine may, in part, result from enzyme specificity, an important determinant factor in protein digestion, which was discussed in detail by Low (1980). Glutamic acid, distributed mainly in storage protein, is usually the most abundant amino acid in cereal, legume, and oil seeds. Most of the glutamic acid in canola meal is contained in globulins, the storage protein located in the protein bodies of the cotyledons in canola seed. Storage protein is of relatively high digestibility, which explains the high digestibility of glutamic acid. On the other hand, the relatively low digestibilities of glycine and threonine, observed quite often in the other feedstuffs, in part, result from their relatively high concentrations in endogenous secretions. Studies by Holmes et al. (1974) and Sauer et al. (1977) showed relatively high contents of threonine and glycine in digesta collected from the distal ileum of growing pigs fed a protein-free diet. Furthermore, the relatively low digestibility of tryptophan in the canola meal samples may largely result from the fact that a large proportion of tryptophan in canola meal is located in the hull fraction which is high in fiber and of low digestibility. Expressed as a percentage of CP, hulls and dehulled canola meal contained 2.5 and 1.3% tryptophan, respectively (Bell, 1993b).

In conclusion, there was considerable variation in the apparent ileal digestibilities of amino acids among the canola meal samples. Differences in NDF content were mainly responsible for the variation. In addition, differences in the dietary levels of most amino acids were also, in part, responsible for the variation.

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

	Diets[a]					
Items	1	2	3	4	5	6
Canola meal	42.8	45.6	43.7	40.9	50.3	45.3
Corn starch[b]	42.3	39.6	41.4	44.2	34.9	39.8
Dextrose[c]	10.0	10.0	10.0	10.0	10.0	10.0
Canola oil	3.0	3.0	3.0	3.0	3.0	3.0
Calcium carbonate	.8	.8	.8	.8	.8	.8
Trace-mineralized salt[d]	.5	.5	.5	.5	.5	.5
Vitamin premix[e]	.2	.2	.2	.2	.2	.2
Mineral premix[f]	.1	.1	.1	.1	.1	.1
Chromic oxide[g]	.3	.3	.3	.3	.3	.3

[a]Diet 1, canola meal from Russel, CPS LTD; diet 2, canola meal from Canbra; diet 3, canola meal from Neepawa; diet 4, canola meal from Altona; diet 5, canola meal from Alberta Terminal Canola Crushers LTD; diet 6, canola meal from Fort Saskatchewan.

[b]St. Lawrence Starch Company, Mississauge, ON.

[c]Corn Products, Englewood Cliffs, NJ.

[d]Supplied by Windsor Salt, Toronto, Canada. Composition (percentage): NaCl, 96.5; ZnO, .40; $FeCO_3$, .16; MnO, .12; CuO, .033; $Ca(IO_3)_2$, .007; CaO, .004.

[e]The vitamin premix supplied the following vitamins (mg/kg diet): retinyl palmitate, 5.2; cholecalciferol, .38; all-rac-α-tocopherol acetate, 44.0; menadione, 3.0; riboflavin, 2.2; niacin, 12.0; d-pantothenic acid, 11.0; vitamin B_{12}, .012; choline, 550; thiamine, 1.1; pyridoxine, 1.1; d-biotin .1; folic acid, .6; corn starch was used as carrier.

[f]The trace-mineral premix supplied the following minerals (mg/kg diet): Fe, 50; Zn, 50; Mn, 2; Cu, 3; I, .14; Se, .15; corn starch was used as carrier.

[g]Fisher Scientific, Fair Lawn, NJ.

Table III-2. The chemical composition[a] (%) of the experimental diets

	Diets[b]					
Items	1	2	3	4	5	6
DM	95.4	94.7	94.6	95.7	94.4	94.2
Ether extract	4.7	4.7	4.9	4.5	4.9	5.0
NDF	10.2	11.1	8.4	8.7	12.8	10.2
CP	18.1	18.0	18.5	17.6	18.3	17.8
Amino acids						
Indispensable						
Arginine	1.24	1.22	1.33	1.25	1.25	1.25
Histidine	.48	.49	.53	.49	.50	.49
Isoleucine	.68	.69	.74	.68	.71	.69
Leucine	1.28	1.31	1.39	1.27	1.33	1.30
Lysine	1.04	1.01	1.11	1.01	1.08	1.06
Methionine	.37	.38	.40	.37	.38	.39
Phenylalanine	.69	.71	.76	.69	.72	.71
Threonine	.79	.83	.85	.77	.85	.83
Tryptophan	.22	.23	.23	.22	.22	.21
Valine	.90	.94	.98	.89	.95	.92
Dispensable						
Alanine	.83	.85	.89	.81	.85	.84
Aspartic acid	1.45	1.50	1.54	1.43	1.45	1.47
Cysteine	.49	.46	.53	.49	.48	.47
Glutamic acid	3.42	3.34	3.65	3.44	3.31	3.30
Glycine	.92	.96	1.00	.91	.98	.94
Proline	1.17	1.18	1.25	1.16	1.16	1.17
Serine	.80	.82	.85	.78	.83	.82
Tyrosine	.42	.41	.49	.40	.45	.46
Tannins	.10	.06	.10	.10	.11	.10

[a]DM basis.

[b]Refer to Table III-1.

Table III-3. The chemical composition[a] (%) of the canola meal samples

	Canola meal samples[b]					
Items	CM1	CM2	CM3	CM4	CM5	CM6
DM	95.8	92.8	94.2	94.9	96.5	95.6
Ether extract	3.8	3.6	4.3	3.3	3.3	3.9
NDF	23.7	24.9	19.3	21.4	24.7	22.3
CP	40.6	39.4	40.0	41.7	35.3	38.8
Amino acids						
Indispensable						
Arginine	2.62	2.46	2.71	2.77	2.30	2.50
Histidine	1.10	1.06	1.13	1.14	.98	1.04
Isoleucine	1.51	1.45	1.57	1.58	1.37	1.46
Leucine	2.87	2.82	2.96	2.98	2.55	2.76
Lysine	2.33	2.18	2.38	2.39	2.13	2.29
Methionine	.84	.83	.86	.84	.77	.80
Phenylalanine	1.58	1.56	1.64	1.64	1.41	1.53
Threonine	1.76	1.79	1.82	1.79	1.66	1.74
Tryptophan	.52	.54	.54	.56	.45	.47
Valine	2.00	1.98	2.07	2.03	1.80	1.92
Dispensable						
Alanine	1.85	1.84	1.89	1.91	1.67	1.78
Aspartic acid	3.14	3.23	3.28	3.31	2.87	3.06
Cysteine	1.08	.99	1.11	1.14	.91	1.00
Glutamic acid	7.67	7.23	7.76	8.08	6.35	7.12
Glycine	2.08	2.07	2.12	2.14	1.86	2.01
Proline	2.65	2.59	2.67	2.73	2.30	2.48
Serine	1.75	1.77	1.82	1.83	1.62	1.73
Tyrosine	1.11	1.09	1.14	1.13	1.04	1.09
Tannins	.23	.14	.22	.24	.21	.21

[a]DM basis.

[b]CM1, canola meal from Russel, CPS LTD; CM2, canola meal from Canbra; CM3, canola meal from Neepawa; CM4, canola meal from Altona; CM5, canola meal from Alberta Terminal Canola Crushers LTD; CM6, canola meal from Fort Saskatchewan.

Table III-4. The apparent ileal digestibilities (%) of DM, CP, and amino acids in the experimental diets

Item	Diets[a]						SEM[b]
	1	2	3	4	5	6	
DM	69.5[cd]	66.4[d]	71.9[c]	70.8[c]	61.8[e]	66.8[d]	.71
CP	66.7[cd]	61.4[f]	71.3[c]	67.7[de]	64.0[ef]	64.8[def]	.81
Amino acids							
Indispensable							
Arginine	81.6[cd]	79.4[d]	85.2[cd]	81.6[cd]	82.9[cd]	84.4[c]	1.14
Histidine	77.7[d]	76.1[d]	81.1[c]	78.9[cd]	77.8[d]	78.1[cd]	.77
Isoleucine	78.5[cd]	66.2[e]	74.8[c]	71.1[cd]	68.8[de]	71.1[cd]	1.1
Leucine	78.8[cd]	69.8[d]	77.1[c]	78.3[cd]	71.9[d]	74.2[cd]	1.14
Lysine	78.5[cd]	68.3[e]	74.7[c]	74.4[cd]	71.3[c]	78.2[cd]	1.11
Methionine	79.8[cde]	77.3[e]	84.4[c]	79.3[cde]	78.8[de]	81.1[cd]	.84
Phenylalanine	78.2[d]	69.1[d]	78.6[c]	78.3[d]	78.9[d]	78.2[d]	1.1
Threonine	61.2[cd]	55.7[d]	66.8[c]	61.8[cd]	61.8[cd]	68.8[c]	1.44
Tryptophan	64.4[d]	61.8[d]	67.8[c]	66.7[cd]	61.7[d]	64.4[d]	.91
Valine	67.8[d]	66.3[d]	71.3[c]	68.8[d]	66.8[d]	69.3[cd]	1.14
Dispensable							
Alanine	71.8[cd]	66.8[d]	75.3[c]	71.1[c]	69.2[d]	71.6[cd]	1.11
Aspartic acid	65.4[cde]	61.7[e]	69.4[c]	66.7[cd]	63.7[de]	67.8[cd]	1.14
Cysteine	71.3[c]	67.7[d]	75.4[c]	74.6[cd]	68.3[d]	71.6[c]	.97
Glutamic acid	80.3[cd]	75.3[d]	83.1[c]	81.1[cd]	80.6[cd]	81.8[cd]	.97
Glycine	62.3[d]	60.1[d]	68.3[c]	66.1[cd]	61.9[d]	64.2[cd]	1.14
Proline	66.2	66.1	69.8	71.1	66.6	71.1	1.01
Serine	69.6[de]	63.6[e]	69.3[c]	66.8[cde]	64.6[de]	68.4[cd]	1.14
Tyrosine	66.6[de]	62.3[e]	71.3[c]	66.6[de]	67.3[de]	69.2[cd]	1.41

[a]Refer to Table III-1.

[b]Standard error of mean (n = 6).

c, d, e, f[Means in the same row followed by different superscript letters differ ($P < .05$).

Table III-5. Correlation coefficients between apparent ileal digestibilities of CP and amino acids and the NDF content in the canola meal samples and the dietary levels of CP and amino acids

	NDF (n = 36)[a]		Dietary levels[b] (n = 36)	
Items	*r*	*p*	*r*	*p*
CP	-.76	.0001	.23	.2767
Amino acids				
Indispensable				
Arginine	-.30	.1376	.53	.0007
Histidine	-.68	.0001	.51	.0079
Isoleucine	-.74	.0001	.41	.0383
Leucine	-.68	.0001	.41	.0402
Lysine	-.72	.0001	.49	.0104
Methionine	-.65	.0003	.52	.0069
Phenylalanine	-.71	.0001	.47	.0145
Threonine	-.61	.0011	.21	.3135
Tryptophan	-.72	.0001	.31	.1245
Valine	-.73	.0001	.26	.2073
Dispensable				
Alanine	-.67	.0002	.43	.0328
Aspartic acid	-.72	.0001	.22	.2934
Cysteine	-.87	.0001	.74	.0001
Glutamic acid	-.63	.0006	.64	.0006
Glycine	-.76	.0001	.17	.4003
Proline	-.47	.0166	.11	.5972
Serine	-.70	.0001	.24	.2463
Tyrosine	-.62	.0007	.71	.0001

[a]NDF content in the canola meal samples (percentage, DM basis).

[b]Dietary levels of CP and amino acids (percentage, DM basis).

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CHAPTER IV

VARIABILITY OF APPARENT ILEAL AMINO ACID DIGESTIBILITY IN DIFFERENT SAMPLES OF WHEAT FOR PIGS

A. Introduction

Wheat is a major ingredient in swine diets in Western Canada. This cereal grain, together with barley, usually supplies approximately one half of the protein in diets for growing and finishing pigs. There is a considerable variation in the CP and amino acid content in wheat samples; the CP content can range from 6 to 18% (Simmonds, 1989). Furthermore, the nutritive value of protein in wheat is not only determined by its total content in amino acids, but also by their digestibilities, especially of the limiting amino acids.

The ileal analysis is preferred to the fecal analysis method for the determination of amino acid digestibilities in feedstuffs for pigs because of the modifying action of the microflora in the large intestine on amino acids, which usually results in overestimation of amino acid digestibility with the fecal analysis method (e.g., Zebrowska, 1973; Tanksley and Knabe, 1984). The apparent ileal digestibilities of amino acids have been determined in many samples of wheat; considerable variation in the digestibilities have been reported (e.g., Sauer and Ozimek, 1986).

Apart from differences in methodological approaches, there is a scarcity of information on the effect of inherent factors in wheat on amino acid digestibilities. Accurate information on factors affecting amino acid digestibilities is of importance for economical

formulation and/or amino acid supplementation to wheat-based diets and for the plant breeder to improve protein quality.

The objectives of this study were to investigate the variability in apparent ileal digestibilities of amino acids in six wheat samples and to identify factors responsible for this variation. Samples of commonly used wheat cultivars were selected for these studies.

B. Experimental Procedures

Animal Trial Procedures

Six barrows (Lacombe x Yorkshire), average initial BW 35.3 kg, were surgically fitted with a simple T-cannula approximately 5 cm anterior to the ileo-cecal sphincter according to procedures adapted from Sauer et al. (1983). The cannulas were modified according to De Lange et al. (1989). After surgery, the barrows were housed individually in stainless steel metabolic crates in a temperature-controlled barn (20 to 22°C). During a 14-d recovery period, the barrows were fed a 16% CP grower diet (Sauer et al., 1983). A detailed description of pre- and post-operative care was previously presented by Sauer (1976) and Sauer et al. (1983). After recovery, the barrows were fed one of six experimental diets according to a 6 x 6 Latin square design. They were fed 800 g twice daily, at 0800 and 2000. Water was freely available from a low-pressure drinking nipple. At the conclusion of the experiment, the barrows, average final BW 61.2 kg, were electrically stunned before killing, bled out, and dissected to determine whether cannulation had caused intestinal abnormalities.

The six wheat samples selected for these studies were obtained from four different cultivars and three different locations, namely, cv Katepwa from Alberta Wheat Pool, cv Katepwa from Alberta United Grain Growers, cv Katepwa from Saskatchewan Wheat Pool, cv Neepawa from Saskatchewan Wheat Pool, cv Kyle from Saskatchewan Wheat Pool, and cv Columbus from Saskatchewan Wheat Pool. The wheat samples supplied the

sole dietary protein (Table IV-1). Vitamins and minerals were supplemented according to NRC (1988) standards. Chromic oxide (.3%) was included in the diets as the digestibility marker. All the wheat samples were ground through a 4.8-mm mesh screen prior to diet incorporation.

Each experimental period comprised 7 d. Ileal digesta were collected for a total of 24 h: from 0800 to 1000 h on d 6 and every other 2 h thereafter until 0800 h on d 7 and from 1000 to 1200 h on d 7 and every other 2 h thereafter until 0800 h on d 8. Ileal digesta were collected in soft plastic tubing (length, 15 cm; i.d., 2.5 cm), which was attached to the barrel of the cannula with Velcro tape. The tubing contained 10 mL of a solution of formic acid (10%, vol/vol) to minimize further microbial activity. The tubing was removed and replaced as soon as it was partially filled with digesta. Digesta were immediately frozen at -20°C following collection.

The experimental proposal, surgical procedures, and procedures for care and treatment of the barrows were reviewed and approved by the Faculty of Agriculture and Forestry Animal Care Committee of the University of Alberta. The barrows used in this experiment were cared for in accordance with the guidelines established by CCAC (1980).

Chemical Analyses

After the conclusion of the animal trial, the digesta samples were freeze-dried, pooled within barrow and period for the same dietary treatment, ground through a .8-mm mesh screen, and mixed before analyses. The samples of wheat and diets were ground similarly.

Analyses for DM, CP, and ether extract were carried out according to AOAC (1984) methods. Analysis for NDF was carried out according to principles outlined by Goering and Van Soest (1970). Chromic oxide content was determined according to the procedure of Fenton and Fenton (1979).

For amino acid analyses, with the exception of the sulfur-containing amino acids and tryptophan, the samples were hydrolyzed with 6 *N* HCl at 110°C for 24 h, derivatized as

ninhydrin-positive compounds, and detected colorimetrically according to HPLC-procedures adapted from Mason et al. (1980) using an automatic amino acid analyzer (Beckman model 6300, Beckman Inst., Inc., Palo Alto, CA.). Methionine and cysteine contents were determined as methionine sulfone and cysteic acid after oxidation with performic acid; the oxidation process was carried out according to AOAC (1984). The oxidized samples were then hydrolyzed and analyzed in the same manner as the samples that were not oxidized. Tryptophan analysis was carried out according to the procedure described by Jones et al. (1981).

Calculations and Statistical Analyses

The apparent ileal digestibilities of CP and amino acids in the experimental diets (and also in the wheat samples) were determined with the direct method using equation [1].

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

D_D: apparent digestibility of a nutrient in the diet (percentage); I_D: marker concentration in the diet (percentage); A_F: nutrient concentration in ileal digesta (percentage); I_F: marker concentration in ileal digesta (percentage); A_D: nutrient concentration in the diet (percentage).

Three different pigs went off feed during the experiment, one during the first, third, and fourth experimental period, respectively, which resulted in three missing values. The digestibility values were first subjected to least square analyses of variance (**LSAV**) for a 6 x 6 Latin square design. Periods and barrows were the controlled factors in the 6 x 6 Latin square design. Where appropriate, least square means (**LSM**) for treatments were compared using the probability of difference (**PDIFF**) Procedure in GLM (SAS, 1990). The LSAV and PDIFF were carried out using the GLM Procedures of SAS (1990) according to the principles described by Steel and Torrie (1980). Pearson partial correlation analyses were conducted to determine the relationships between the apparent ileal digestibilities of CP and amino acids and the content of NDF in the wheat samples and

the dietary levels of CP and amino acids using the GLM Procedure of SAS (1990). Variation contributed by barrows and periods was removed by creating five dummy variables for barrows and periods, respectively and obtaining partial correlations when the dummy variables were forced into the analyses (Draper and Smith, 1981).

C. Results and Discussion

The pigs, with the exception of the instances mentioned previously, remained healthy and consumed their meal allowances throughout the experiment. Postmortem examinations, carried out at the conclusion of the experiment, revealed no intestinal adhesions.

The chemical composition of the diets and the wheat samples are shown in Tables IV-2 and 3, respectively. The analyzed values of CP and amino acids in the diets were close to the calculated values based on the analyzed values in the different wheat samples. The dietary NDF contents were calculated from the values determined in the wheat samples. The contents of CP, lysine, and threonine measured in the wheat samples in this study ranged from 17.0 to 19.9%, .43 to .50%, and .49 to .58%, respectively. These values, and also those of the other amino acids, were considerably higher than those reported by Sauer et al. (1981) and the average values compiled by NRC (1988) for wheat.

Like in many other plant seeds, the rate of protein synthesis and accumulation in wheat is genetically controlled and affected by many environmental factors (e.g., Byers et al., 1978; Simmonds, 1989). Based on the original classification proposed by Osborne (1909), wheat proteins can be distinguished into albumins, globulins, prolamins (gliadins), and glutenins depending on their solubility in water, saline solution, 70 to 80% aqueous ethanol, and diluted acid or alkali solutions, respectively. As was reviewed by Simmonds (1989), the storage proteins of wheat, which make up about 72% of the total protein, are primarily prolamins and glutenins which are located in the endosperm; the remaining

proteins are metabolically active cytoplasmic proteins, i.e. albumins and globulins. These proteins comprise enzymes, membrane, ribosomal proteins, and other regulatory and some structural (cell wall) proteins and are distributed in the aleurone layer, the pericarp, and embryo tissues and account for 16, 4, and 8% of the total protein of wheat, respectively. In this context, it should be mentioned that the amino acid content and composition in wheat is dependent on the content and relative proportions of these four proteins, as there are large differences in amino acid composition between these proteins (Lásztity, 1983; Simmonds, 1989). For example, the contents of lysine and threonine (g/100 g protein) are much higher in albumins and globulins than in glutenins and prolamins and vice versa for the contents of glutamic acid and proline (e.g., Simmonds, 1989).

The content of each of the amino acids usually increased correspondingly as the CP content increased among the wheat samples (Table IV-3). Wyatt (1993), in studies with 48 Canadian hard red spring and 47 American wheat samples, observed strong positive linear relationships between the content of CP and amino acids and concluded that the amino acid content in wheat can be reliably predicted from its CP content. In this study, as the CP content increased 2.9 percentage units from the lowest to the highest protein wheat sample, the contents of all amino acids also increased correspondingly. However, expressed as a percentage of the total CP content in wheat, the contents of the limiting amino acids, lysine and threonine, decreased. These results are in agreement with Simmonds (1989) who reported that high-protein wheat samples contain a greater proportion of their protein as storage protein, especially prolamins which are deficient in lysine and threonine.

The apparent ileal digestibilities of CP and amino acids in the wheat samples are presented in Table IV-4. There were differences ($P < .05$) in the ileal digestibilities of CP and all the amino acids among the wheat samples. The digestibility of CP ranged from 74.7 to 80.5%. Of the indispensable amino acids, the differences in digestibilities ranged from 4.7 (isoleucine, phenylalanine, and tyrosine) to 11.3 (lysine) percentage units. Of the

dispensable amino acids, the differences in digestibilities ranged from 2.6 (glutamic acid) to 12.0 (glycine) percentage units.

The NDF content in the wheat samples ranged from 11.7 to 17.6% (Table IV-3). With the exception of arginine, glutamic acid, proline, and tyrosine, the apparent ileal digestibilities of CP, and amino acids were negatively correlated ($P < .05$) with the NDF content in the wheat samples. These results suggest that differences in NDF content among the wheat samples were, in part, responsible for the variation in the digestibilities of CP and most amino acids. A negative relationship between protein digestibility and crude fiber content in cereal grains was also reported in studies with pigs and rats (Eggum and Beames, 1983). As was reviewed by Simmonds (1989), an average of 20% of the total wheat protein is deposited in the aleurone layer, the pericarp, and the seed coat. These fractions make up wheat bran after milling. The protein contained in bran is of relatively low digestibility and can be classified into two types (Saunders and Kohler, 1972). The first type of protein actually resides inside the thick cellulosic cell walls in the aleurone layer; the second type of proteins is tightly bound to the cellulosic matrix of the aleurone cells. Therefore, differences in the NDF content among the wheat samples may reflect their differences in the thickness of the aleurone cell wall and the amount of protein contained within the aleurone cell and/or bound to the cellulosic matrix. This, in turn, explains how differences in the NDF content among the wheat samples are responsible for the variation in digestibilities.

The study in Chapter V showed that the apparent ileal digestibilities of amino acids are quadratically affected by their respective dietary levels. Although there were differences in the dietary levels of amino acids among the wheat diets (Table IV-2), the apparent digestibilities of the amino acids were not correlated ($P > .05$) with their respective dietary levels.

The apparent ileal digestibilities of amino acids in the wheat samples in this study fall within the range of values summarized by Sauer and Ozimek (1986). However, much more

variation in the digestibilities of amino acids among different samples of wheat has been reported in the literature. For instance, as was summarized by Sauer and Ozimek (1986), the digestibilities of lysine and threonine ranged from 62.3 to 81.0% and 61.9 to 78.4%, respectively, whereas these ranged from 59.0 to 70.3% and 64.2 to 73.0%, respectively in this study. Other factors including fineness of grinding and methodological approaches, which were previously discussed by Sauer and Ozimek (1986) and in Chapter V were likely responsible for the additional variation in amino acid digestibilities among the wheat samples reported in the literature.

In agreement with studies by Ivan and Farrell (1976), Sauer et al. (1981), and Green et al. (1987), among the amino acids in wheat, the apparent ileal digestibilities of glycine, lysine, and threonine were low while those of glutamic acid, phenylalanine, and proline were relatively high (Table IV-4). The low digestibilities of lysine and threonine and high digestibilities of glutamic acid and proline likely result from differences in the distribution of these amino acids among the protein fractions in wheat. As was discussed previously, the contents of lysine and threonine are very low in glutenins and prolamins (protein in endosperm) and high in albumins and globulins (protein in the aleurone layer) and vice versa for the contents of glutamic acid and proline. Proteins "locked-up" inside the aleurone cell walls are of low digestibility, whereas the storage proteins in endosperm are of high digestibility. As was shown in studies with rats, the true protein digestibilities in wheat bran (derived primarily from the aleurone layer and pericarp) and white flour (primarily from endosperm) ranged from 63 to 73% and from 93 to 96%, respectively (Simmonds, 1989). Furthermore, the low apparent ileal digestibility of threonine may also, in part, result from its relatively high concentration in endogenous protein recovered from the distal ileum (e.g., Holmes et al., 1974; Sauer et al., 1977). In addition, the low digestibility of glycine can also be attributed to its high content in endogenous protein, as was discussed in detail in Chapter VII.

In conclusion, measured with the ileal analysis method, there were differences in the apparent digestibilities of all amino acids among the wheat samples. Differences in NDF content among the wheat samples were, in part, responsible for the variation. Furthermore, the relatively low digestibilities of lysine and threonine further accentuate the limitation of these amino acids in protein from wheat.

Table IV-1. Formulation (%) of the experimental diets

Ingredients	%
Wheat[a]	97.0
Calcium carbonate	1.1
Dicalcium phosphate	.8
Trace-mineralized salt[b]	.5
Vitamin premix[c]	.2
Mineral premix[d]	.1
Chromic oxide[e]	.3

[a]The six wheat diets contained the following wheat samples: diet 1, cv Katepwa from Alberta Wheat Pool; diet 2, cv Katepwa from Alberta United Grain Growers; diet 3, cv Katepwa from Saskatchewan Wheat Pool; diet 4, cv Neepawa from Saskatchewan Wheat Pool; diet 5, cv Kyle from Saskatchewan Wheat Pool; diet 6, cv Columbus from Saskatchewan Wheat Pool.

[b]Supplied by Windsor Salt, Toronto, Canada. Composition (percentage): NaCl, 96.5; ZnO, .40; $FeCO_3$, .16; MnO, .12; CuO, .033; $Ca(IO_3)_2$, .007; CaO, .004.

[c]The vitamin premix supplied the following vitamins (mg/kg diet): retinyl palmitate, 5.2; cholecalciferol, .38; all-rac-α-tocopherol acetate, 44.0; menadione, 3.0; riboflavin, 2.2; niacin, 12.0; d-pantothenic acid, 11.0; vitamin B_{12}, .012; choline, 550; thiamine, 1.1; pyridoxine, 1.1; d-biotin .1; folic acid, .6; corn starch was used as carrier.

[d]The trace-mineral premix supplied the following minerals (mg/kg diet): Fe, 50; Zn, 50; Mn, 2; Cu, 3; I, .14; Se, .15; corn starch was used as carrier.

[e]Fisher Scientific, Fair Lawn, NJ.

Table IV-2. The chemical composition[a] (%) of the wheat diets

	Wheat diets[b]					
	1	2	3	4	5	6
DM	90.9	90.4	90.0	90.4	90.5	90.5
Ether extract	1.0	1.2	1.1	1.4	1.2	1.3
NDF	11.5	14.4	13.2	12.9	11.4	17.2
CP	18.2	17.4	16.6	17.1	19.4	18.5
Amino acids						
Indispensable						
Arginine	.95	.88	.86	.95	1.00	.98
Histidine	.40	.37	.34	.39	.43	.43
Isoleucine	.59	.54	.51	.56	.65	.59
Leucine	1.22	1.13	1.07	1.13	1.31	1.21
Lysine	.47	.45	.43	.50	.46	.46
Methionine	.31	.29	.29	.29	.32	.30
Phenylalanine	.86	.77	.74	.75	.87	.82
Threonine	.54	.51	.48	.52	.54	.53
Tryptophan	.20	.21	.22	.21	.20	.21
Valine	.75	.69	.66	.71	.78	.73
Dispensable						
Alanine	.61	.57	.56	.59	.65	.62
Aspartic acid	.91	.86	.86	.93	.98	.94
Cysteine	.43	.40	.39	.43	.44	.43
Glutamic acid	6.24	5.66	5.45	5.51	6.56	6.37
Glycine	.72	.66	.65	.69	.69	.73
Proline	2.06	1.86	1.85	1.84	2.09	2.11
Serine	.86	.80	.76	.80	.89	.88
Tyrosine	.36	.33	.30	.28	.41	.31

[a]DM basis.

[b]Refer to Table IV-1.

Table IV-3. The chemical composition[a] (%) of the wheat samples

	Wheat samples[b]					
Items	W1	W2	W3	W4	W5	W6
DM	89.3	89.9	90.4	89.9	90.5	91.2
Ether extract	1.0	1.3	1.2	1.6	1.3	1.4
NDF	12.2	14.9	13.5	13.4	11.7	17.6
CP	19.2	18.3	17.0	17.7	19.9	19.1
Amino acids						
Indispensable						
Arginine	1.00	.90	.86	.98	1.02	1.00
Histidine	.45	.40	.31	.40	.39	.43
Isoleucine	.64	.57	.51	.57	.65	.58
Leucine	1.30	1.18	1.07	1.17	1.33	1.22
Lysine	.50	.48	.43	.49	.46	.48
Methionine	.32	.29	.30	.30	.33	.32
Phenylalanine	.88	.76	.71	.72	.87	.78
Threonine	.58	.53	.49	.53	.55	.54
Tryptophan	.20	.21	.22	.23	.20	.21
Valine	.81	.72	.66	.75	.80	.73
Dispensable						
Alanine	.64	.58	.56	.60	.66	.64
Aspartic acid	.93	.86	.86	.98	1.02	1.00
Cysteine	.46	.41	.40	.43	.44	.44
Glutamic acid	6.47	5.80	5.57	5.57	6.74	6.51
Glycine	.76	.69	.65	.70	.71	.75
Proline	2.13	1.89	1.87	1.82	2.12	2.13
Serine	.91	.83	.76	.81	.91	.89
Tyrosine	.35	.29	.24	.22	.43	.24

[a]DM basis.

[b]W1, cv Katepwa from Alberta Wheat Pool; W2, cv Katepwa from Alberta United Grain Growers; W3, cv Katepwa from Saskatchewan Wheat Pool; W4, cv Neepawa from Saskatchewan Wheat Pool; W5, cv Kyle from Saskatchewan Wheat Pool; W6, cv Columbus from Saskatchewan Wheat Pool.

Table IV-4. The apparent ileal digestibilitiies (%) of DM, CP, and amino acids in the wheat diets

	Diets[a]						
Items	1	2	3	4	5	6	LSM[b]
No. of observations	6	4	6	6	5	6	
DM	74.9[c]	67.7[ef]	69.4[e]	72.6[d]	75.1[c]	66.1[f]	.67
CP	80.5[c]	78.0[cd]	76.9[de]	79.4[cd]	80.1[cd]	74.7[e]	1.11
Amino acids							
Indispensable							
Arginine	82.0[c]	80.2[c]	82.6[c]	81.8[c]	81.4[c]	77.8[d]	.83
Histidine	83.8[c]	79.8[c]	82.9[c]	80.5[c]	81.6[c]	74.0[d]	1.49
Isoleucine	83.0[c]	80.1[de]	81.6[cd]	81.7[cd]	80.5[de]	78.3[e]	.81
Leucine	84.8[c]	81.4[e]	83.4[cd]	83.4[cd]	82.1[de]	79.9[e]	.79
Lysine	70.3[c]	64.0[de]	67.5[cd]	65.0[d]	69.4[cd]	59.0[e]	1.68
Methionine	82.4[c]	78.6[de]	81.0[d]	81.1[d]	81.2[d]	76.0[e]	.84
Phenylalanine	87.5[c]	83.5[d]	86.4[c]	86.6[c]	85.3[cd]	82.8[d]	.71
Threonine	73.0[c]	68.7[e]	69.9[c]	69.8[cd]	71.8[c]	64.1[d]	1.51
Tryptophan	79.5[c]	75.4[e]	76.7[de]	77.6[cde]	79.0[cd]	71.9[f]	.89
Valine	79.5[c]	75.1[de]	77.0[cd]	76.8[cd]	76.9[cd]	71.6[e]	1.01
Dispensable							
Alanine	74.8[c]	70.8[cd]	72.0[cd]	70.7[d]	71.1[cd]	65.7[e]	1.05
Aspartic acid	73.9[c]	70.6[cd]	71.7[cd]	69.2[d]	71.7[cd]	64.8[e]	1.01
Cysteine	79.4[c]	76.0[c]	76.5[c]	76.9[c]	79.8[c]	71.3[d]	1.41
Glutamic acid	90.4[c]	88.9[de]	91.7[cd]	90.5[c]	89.3[e]	88.9[e]	.41
Glycine	74.2[c]	67.8[de]	67.6[de]	68.0[d]	69.7[cd]	62.3[e]	1.69
Proline	87.2[c]	85.6[ef]	81.3[de]	84.7[cd]	81.1[cd]	81.4[d]	1.08
Serine	85.8[c]	80.6[e]	82.3[a]	81.8[c]	81.4[c]	78.2[c]	.96
Tyrosine	77.0[c]	73.2[c]	76.2[c]	77.9[c]	74.2[c]	73.7[c]	1.68

[a]Refer to Table IV-3.

[b]LSM (n = 6).

c, d, e, f[Means in the same row followed by different superscript letters differ ($P < .05$).

Table IV-5. Correlation coefficients between apparent ileal digestibilities of CP and amino acids and NDF content in the wheat samples and the dietary levels of CP and amino acids

	NDF[a] (n = 33)		Dietary levels[b] (n = 33)	
	r	*p*	*r*	*p*
CP	-.66	.0006	.11	.6143
Amino acids				
Indispensable				
Arginine	-.36	.0884	.16	.4556
Histidine	-.72	.0001	.11	.4226
Isoleucine	-.59	.0032	.16	.4707
Leucine	-.62	.0015	.14	.5140
Lysine	-.77	.0001	.05	.8276
Methionine	-.79	.0001	.24	.2691
Phenylalanine	-.67	.0005	.03	.8853
Threonine	-.70	.0002	.09	.6880
Tryptophan	-.85	.0001	.31	.1013
Valine	-.71	.0002	.10	.6476
Dispensable				
Alanine	-.68	.0003	.16	.4556
Aspartic acid	-.71	.0002	.33	.1212
Cysteine	-.78	.0001	.08	.7125
Glutamic acid	-.30	.1602	.13	.4433
Glycine	-.69	.0003	.05	.8130
Proline	-.29	.1775	.09	.6772
Serine	-.65	.0009	.23	.2823
Tyrosine	-.27	.2122	.17	.4333

[a]NDF content in the wheat sample (percentage, DM basis).

[b]Dietary levels of CP and amino acids (percentage, DM basis).

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CHAPTER V

DETERMINATION OF APPARENT ILEAL AMINO ACID DIGESTIBILITY IN PIGS: EFFECT OF DIETARY AMINO ACID LEVEL[1]

A. Introduction

The ileal rather than the fecal analysis method should be used to determine amino acid digestibility in pigs because of the modifying action of the microflora in the large intestine (Zebrowska, 1973). The apparent ileal amino acid digestibilities have been determined in a wide variety of feedstuffs (e.g., Tanksley and Knabe et al., 1984). However, there is a large amount of variation in the apparent ileal amino acid digestibilities among different samples of the same feedstuff, especially in cereal grains (Sauer and Ozimek, 1986). This variability may result, in part, from differences in methodological approaches (Sauer et al., 1989).

In theory, the apparent ileal amino acid digestibilities are dependent on the amino acid contents in the assay diet. As was illustrated by Eggum (1973) in studies with rats, the apparent fecal CP digestibility in soybean meal (**SBM**) increased curvilinearly with increasing dietary CP content. Similarly, it is expected that the apparent ileal amino acid digestibilities will increase curvilinearly with increasing amino acid contents in the assay diet. Therefore, values for apparent ileal amino acid digestibility are only meaningful and valid under strictly standardized conditions, at least with respect to the amino acid content in the assay diet. A review of the literature reveals that, in many instances, this has not been

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the case. The determination and comparison of apparent ileal digestibilities of amino acids were carried out at various dietary amino acid levels as indicated by the differences in dietary CP content. For example, the CP contents in corn starch-based SBM diets were 21, 14, and 12% in studies by Holmes et al. (1974), Jørgensen et al. (1984), and Knabe et al. (1989), respectively. Differences in CP and amino acid contents in the assay diets may explain, in part, the variation in apparent ileal amino acid digestibilities reported in the literature (e.g., Sauer and Ozimek, 1986) among different samples of the same feedstuff (in name). The effect of dietary amino acid levels on apparent ileal digestibilities was previously investigated by Buraczewska and Horaczynski (1986), Furuya and Kaji (1989), and Li et al. (1993). However, the results from the aforementioned studies did not allow for the establishment of a detailed relationship between amino acid intake and apparent ileal digestibility.

The objective of the present study was to investigate the effect of dietary amino acid content on the determination of apparent ileal amino acid digestibility. Six corn starch-based SBM "model" diets, formulated to contain six different levels of CP, were used in these studies.

B. Experimental Procedures

Animals and Diets

Six barrows (Lacombe x Yorkshire), average initial BW 35 kg, were surgically fitted with a simple T-cannula at the distal ileum according to procedures adapted from Sauer et al. (1983). The cannulas were modified according to De Lange et al. (1989). After surgery, the animals were individually housed in stainless steel metabolic crates in a temperature-controlled barn (20 to 22°C). During a 14-d recovery period, the barrows were

fed a 16% CP grower diet (Sauer et al., 1983). A detailed description of pre- and post operative care was previously presented by Sauer (1976).

After recovery, the barrows were fed one of the six experimental diets (Table V-1) according to a 6 x 6 Latin square design. They were fed twice daily, equal amounts of each meal, at 0800 and 2000 h. The dietary allowance was 1,600 g/d during Period 1 and increased by 100 g/d for each following period. Water was freely available from a low-pressure drinking nipple. The barrows were electrically stunned before killing and bled out at the conclusion of the experiment and dissected to determine whether cannulation had caused intestinal abnormalities. The average BW of the barrows at the conclusion of the experiment was 78.6 kg.

The six corn starch-based diets were formulated to contain six levels of CP from 48% SBM that was solvent-extracted (Table V-1). Canola oil was included at a level of 3.5% to increase the DE content similar to the level recommended by NRC (1988) and to reduce the dustiness of the diets; dextrose was included at a level of 10% to improve the palatability of the diets. Vitamins and minerals were supplemented according to NRC (1988) standards. Chromic oxide (.4%) was included in the diets as the marker for the determination of the digestibilities of the nutrients that were measured.

Each experimental period lasted 8 d. Ileal digesta were collected for a total of 24 h: from 0800 to 1000 h on d 7 and every other 2 h thereafter until 0800 h on d 8 and from 1000 to 1200 h on d 8 and every other 2 h thereafter until 0800 h on d 9. Ileal digesta were collected in soft plastic tubing (length, 10 cm; i.d., 1.5 cm), which was attached to the barrel of the cannula with Velcro tape. The tubing contained 10 mL of a solution of formic acid (10%, vol/vol) to minimize further bacterial activity. The tubing was removed and replaced as soon as it was filled with digesta. Digesta were immediately frozen at -20°C.

The experimental proposal, surgical procedures, and procedures for care and treatment of the barrows were reviewed and approved by the Faculty of Agriculture and Forestry Animal Care Committee at the University of Alberta. The animals used in this

experiment were cared for in accordance with the guidelines established by CCAC (1980).

Chemical Analyses

After the conclusion of the experiment, the digesta were freeze-dried, pooled within the barrow and the period for the same diet, ground in a Wiley mill through a 1-mm mesh screen, and mixed before analysis. The samples of the diets and SBM were ground similarly. All analyses were performed in duplicate.

Analyses for DM, CP, and ether extract were carried out according to AOAC (1984) methods. Analyses for ADF, NDF, and lignin were carried out according to principles outlined by Goering and Van Soest (1970). Chromic oxide content was determined according to the procedure of Fenton and Fenton (1979).

For amino acid analysis, except for methionine and cysteine, approximately .1 g of sample was weighed into screw-capped test tubes and mixed with 3 mL of 6 *N* HCl. The tubes were flushed with nitrogen and then heated 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 1,110 x *g* for 15 min at room temperature. The samples were analyzed according to the method of Jones and Gilligan (1983) using a Varian 5000 HPLC system (Varian Associates, Sunnyvale, CA.) with a reversed-phase column previously described by Dugan et al. (1989). The amino acids in the sample were derivatized with an *o*-phthaldialdehyde reagent solution and detected spectrofluorometrically. Methionine and cysteine contents were determined as methionine sulfone and cysteic acid, respectively, after oxidation with performic acid; the oxidation procedure was carried out according to AOAC (1984). The oxidized samples were dried according to the procedure described by Dugan et al. (1992), then hydrolyzed and analyzed in the same manner as the samples that were not oxidized.

Calculations and Statistical Analyses

The apparent ileal digestibilities of DM, CP, and amino acids in the experimental diets were calculated according to equation [1].

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

D_D: apparent ileal amino acid digestibilitiy in the assay diet (percentage); I_D: marker concentration in the assay diet (percentage); A_F: amino acid concentration in ileal digesta (percentage); A_D: amino acid concentration in the assay diet (percentage); I_F: marker concentration in ileal digesta (percentage).

The digestibility values were first subjected to ANOVA for a 6 x 6 Latin square design. The interval between the treatment levels of CP (and amino acids) were designed to be equal by increasing equal amounts of SBM (8.4%) in the diets at the expense of corn starch. The treatment effect was partitioned and tested according to the equally spaced orthogonal polynomials based on principles described by Steel and Torrie (1980). The ANOVA and the orthogonal polynomial analyses were carried out using the GLM Procedures of SAS (1990).

The dietary CP and amino acid levels were the treatment factor and periods and barrows the controlled factors in the 6 x 6 Latin square design. Inspection of the apparent ileal CP and amino acid digestibilities showed that not only were the ileal CP and amino acid digestibilities quadratically affected by their corresponding dietary contents but they also approached plateau values. Therefore, the relationships between the apparent ileal digestibilities of CP and amino acids and their respective dietary CP and amino acid contents were fitted with the segmented quadratic with plateau model (SAS, 1990) according to equations [2] and [3].

$$Y = a + bX + cX^2 \qquad \text{if } X < X_0 \quad [2]$$

$$Y = P \qquad \text{if } X > X_0 \quad [3]$$

For values of X less than X_0, the equation relating Y and X is quadratic; for values of X greater than X_0 the equation is constant, i.e. reaching a plateau. The two sections of the

curve must meet at X_0 on the X-coordinate and the curve must be continuous and smooth as expressed by equations [4] and [5].

$$X_0 = -b / (2c) \quad [4]$$

$$P = (a - b^2) / 4c \quad [5]$$

Y: apparent ileal CP or amino acid digestibility (percentage); a: the intercept of the quadratic equation; b: the slope of the linear effect; X: CP or amino acid content (percentage, DM basis); c: the slope of the quadratic effect; X_0: the breakpoints on the X-coordinate between the quadratic and the plateau sections (percentage, DM basis); P: the plateau apparent ileal digestibilities of CP and amino acids (percentage).

The determination of the quadratic relationships between the apparent ileal CP and amino acid digestibilities and the dietary CP and amino acid contents and the plateau digestibility values were quantitatively analyzed as a segmented quadratic with plateau model using the non-linear procedure (**NLIN**) of SAS (1990). The plateau digestibility values and their corresponding lower endpoints of 95% confidence intervals were obtained from the NLIN procedure of SAS (1990).

C. Results and Discussion

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

The chemical composition of SBM and the diets are presented in Table V-2. The contents of fiber components in the diets were calculated from the analyzed values in SBM. With the exception of tyrosine (**TYR**) in the 16% CP diet for which the difference was 6.8%, the analyzed values of CP and amino acids in all other diets were within 5% of the calculated values based on the analyzed values in SBM.

The apparent ileal digestibilities of DM, CP, and amino acids in the diets are presented in Table V-3. There was a linear decrease ($P < .01$) in the apparent ileal digestibility of DM as the dietary CP content was increased, which was a direct result from replacing corn starch with SBM, indicating that SBM had a lower apparent ileal DM digestibility than corn starch. There were quadratic increases ($P < .01$) in the apparent ileal digestibilities of CP and all amino acids when the dietary CP content was increased from 4 to 24.5%. For CP, the increase was 26.7 percentage units. Of the indispensable amino acids, the increases ranged from 11.8 (phenylalanine) to 30.9 (threonine) percentage units. Of the dispensable amino acids, the increases ranged from 7.1 (glutamic acid) to 47.7 (glycine) percentage units. The increases in apparent ileal digestibilities of amino acids with increasing CP content were greatest at the lower CP levels; the increases become negligible at the higher CP levels because endogenous CP accounts for a smaller proportion of CP in ileal digesta. Differences in the apparent ileal digestibilities of CP and amino acids in relation to their dietary content were also observed by Furuya and Kaji (1989).

The quadratic relationships between apparent ileal digestibilities and dietary contents, the breakpoints (X_0) on the X-coordinate and their 95% confidence intervals, and the plateau apparent ileal digestibility values and their 95% confidence intervals are presented in Table V-4. The quadratic with plateau relationships between apparent ileal digestibilities and dietary contents are graphically illustrated in Figure V-1 for lysine (**LYS**), methionine (**MET**), and threonine (**THR**). Initially, the apparent ileal amino acid digestibilities increased sharply; thereafter the increases became smaller and reached their plateau values after which there were no further increases and the digestibility values became independent of the dietary amino acid levels. With the exception of glutamic acid (**GI** similar pattern was observed for CP and other amino acids. These quadratic with plateau relationships between dietary amino acid contents and the apparent ileal digestibilities were not observed in the studies by Furuya and Kaji (1989) and Li et al. (1993). This likely resulted from the limited number of dietary treatments (three or four). In addition, the

dietary treatments were not evenly designed from low to high amino acid content as in the present study.

Of the amino acids, the apparent ileal digestibility of GLU tended to decrease (Table V-3) as its dietary content was increased from 3.72 to 4.63% (Table V-2), which is graphically illustrated in Figure V-1. Studies by Li et al. (1993) with early-weaned pigs fed corn starch-based SBM diets also showed trends toward decreases in the apparent ileal digestibilities of CP and the majority of the amino acids as the dietary CP content was increased from 19.5 to 25.5% (DM basis). As was discussed by these authors, these decreases, with an increase in dietary CP content, may result from decreases in the efficiency of protein digestion and (or) amino acid and peptide absorption. As the uptake of free amino acids and small peptides into the enterocytes is an active process involving a multiplicity of transport systems (e.g., Hoper, 1987; Webb, 1990), it is possible that the total supply of amino acids and (or) small peptides from the high protein diets may have exceeded the maximum capacity of efficiency of transport. Furthermore, it should be pointed out that the GLU content was the highest among all amino acids in SBM.

The lower endpoints of 95% confidence intervals of the plateau apparent ileal digestibilities of CP and amino acids are presented in Table V-5. The lower endpoints of 95% confidence intervals of the plateau apparent ileal digestibility values are defined to be the initial plateau digestibilities. The dietary CP and amino acid contents, corresponding to the initial plateau digestibility values, are referred to as the dietary threshold levels and are illustrated for LYS, MET, and THR in Figure V-1. In order to determine the dietary threshold levels for CP and amino acids, the initial plateau apparent ileal digestibility values of CP and amino acids were inserted into the corresponding quadratic regression equations in Table V-4. The dietary threshold levels for CP and amino acids, estimated by solving the root square of the quadratic equations corresponding to the initial plateau digestibility values, are presented in Table V-5.

The dietary threshold levels of amino acids derived from the present study are not warranted to be absolutely constant under other experimental conditions. Dietary factors that influence the amount of endogenous amino acids recovered at the distal ileum, including sources and levels of fiber (e.g., Sauer et al., 1977; Taverner et al., 1981) and antinutritional factors (e.g., Begbie and Pusztai, 1989), can alter the ratio of exogenous : endogenous amino acids, thereby affecting the threshold levels. However, the dietary threshold levels of amino acids in SBM determined in this study remain to be a valuable reference. Furthermore, the plateau values of apparent ileal digestibilities of the amino acids in SBM are only characteristic to this protein supplement. Amino acids in other protein-containing feedstuffs, depending on their dietary contents and the level of inclusion in the assay diets, will have different plateau values for their respective apparent ileal digestibilities.

The dietary CP contents corresponding to the threshold levels of the individual amino acids are presented in Table V-5. As is illustrated in Figure V-2 for CP, MET, THR, and TYR, the apparent ileal digestibilities of CP and amino acids did not reach their initial plateau values simultaneously at the same dietary CP content. These results tend to suggest that the dietary amino acid contents affect their respective apparent ileal amino acid digestibility values, possibly independent of the dietary CP content. Therefore, in order to obtain the plateau values, the level of inclusion of a feedstuff in the assay diet should be such that the amino acid contents in the assay diet are equal to or exceed the corresponding threshold levels. This consideration is especially important for the determination of the apparent ileal digestibilities of the limiting amino acids in protein-containing ingredients. It may not be practical, due to cost and time restraints, to determine the dietary threshold levels for the limiting amino acids in the many protein-containing ingredients that are available. However, it can be inferred from these studies that the higher the inclusion level of a protein-containing ingredient in the assay diet, the more likely the threshold levels for the limiting amino acids will be reached.

The apparent ileal amino acid digestibilities have been determined in many high-protein supplements including SBM. As was summarized by Sauer and Ozimek (1986), there was considerable variation in the apparent ileal amino acid digestibilities among different samples of SBM. For example, the apparent ileal digestibilities of LYS, MET, and THR ranged from 80.1 to 90.7%, 74.5 to 96.7%, and 70.7 to 82.2%, respectively. A review of these studies reveals that the digestibilities were determined in diets with varying amino acid contents. For instance, the apparent ileal digestibility of LYS in SBM (48%) was 90.7% in studies in which the dietary LYS content was 1.46% (Holmes et al., 1974), whereas Rudolph et al. (1983) reported a value of 83.5%, in studies in which the dietary LYS content was .70%. Although differences in experimental conditions between the aforementioned studies may have contributed to the difference in LYS digestibility, it is likely that the dietary LYS content was mainly responsible. The relatively large variation that was observed for the apparent ileal digestibility of MET among different samples of SBM may result, in part, from the fact that MET is the limiting amino acid in SBM. A relatively small change in the MET content in the diet may, therefore, causes a relatively large change in its apparent ileal digestibility. Notwithstanding, differences in analytical techniques for measuring MET may also have contributed to this variation. Other factors that may result in differences in digestibilities, including inherent factors in different samples, processing methods, and cannulation techniques, were previously discussed by Sauer and Ozimek (1986).

There is an increasing amount of information in the literature on apparent ileal amino acid digestibilities in feedstuffs with a medium protein content. Gatel (1992) recently summarized the apparent ileal amino acid digestibilities in legume seeds, including peas. Compared with the other amino acids, there was much more variation in the apparent ileal digestibilities of MET, cysteine (**CYS**), and tryptophan (**TRP**) among different samples of peas. The digestibilities of MET, CYS, and TRP ranged from 58.0 to 80.7%, 44.0 to 85.0%, and 46.6 to 78.0%, respectively. The sulfur-containing amino acids and TRP are

first and second limiting in protein from peas, respectively (e.g., Palisse-Roussel et al., 1984; Gatel et al., 1989). Therefore, small differences in the dietary contents of these amino acids may have been responsible for a large proportion of the variation in their apparent ileal digestibilities.

There is an abundance of information in the literature on apparent ileal amino acid digestibilities in feedstuffs with a low protein content. As discussed by Sauer and Ozimek (1986), there is much more variation in the apparent ileal amino acid digestibilities within each of the cereal grains compared with high-protein supplements. For example, the apparent ileal digestibilities of LYS and THR in barley ranged from 64.9 to 79.0% and from 64.4 to 76.0%, respectively; the apparent ileal digestibilities of LYS and THR in corn ranged from 71.0 to 82.0% and from 53.8 to 78.9%, respectively; the apparent ileal digestibilities of LYS and THR in wheat ranged from 62.3 to 81.0% and from 61.9 to 78.4%, respectively. The determination of apparent ileal amino acid digestibilities in cereal grains is usually carried out with the direct method in which the cereal grain in question provides the sole amino acids in the assay diet. However, when the direct method is employed, one can expect that the dietary levels of some of the amino acids, which include the limiting ones, are lower than their respective threshold levels. As a result, small differences in the dietary contents of these amino acids will elicit a relatively large change in their apparent ileal digestibilities, as was illustrated in Figure V-1. For instance, the apparent ileal digestibilities of LYS and THR in corn (10.5% CP) were 77.5 and 72.4% in studies in which their dietary levels were .31 and .35%, respectively (Lin et al., 1987). On the other hand, Green et al. (1987) reported LYS and THR digestibilities in corn (9.7% CP) of 56.8 and 60.2% in which the dietary contents were .21 and .24%, respectively. Apart from other factors that were referred to previously, the large difference in LYS (20.7 percentage units) and THR digestibilities (12.2 percentage units) between the two studies may have resulted, in part, from differences in the dietary contents of LYS (.10 percentage units) and THR (.11 percentage units), respectively. Based on the aforementioned

discussion, it is questionable whether the direct method is valid for determining the digestibilities of the limiting amino acids in cereal grains. Other methods, the difference method (Van Leeuwen et al., 1987) and regression method (Giger and Sauvant, 1983), should perhaps be used to determine apparent ileal amino acid digestibilities in low-protein feedstuffs.

In conclusion, there are quadratic with plateau relationships between the dietary amino acid contents and their respective apparent ileal amino acid digestibilities. The plateau values for the apparent ileal amino acid digestibilities should be determined. To determine the plateau apparent ileal amino acid digestibility value, the content of an amino acid in the assay diet should be equal to or exceed the corresponding threshold level. This consideration is especially important for the determination of the apparent ileal digestibilities of the limiting amino acids in protein-containing feedstuffs.

Table V-1. Formulation (%) of the experimental diets

	Dietary CP levels, %					
Ingredients	4	8	12	16	20	24
Soybean meal	8.4	16.8	25 2	33.6	42.0	50.4
Corn starch[a]	72.2	64.2	56.0	47.8	39.0	31.4
Dextrose[b]	10.0	10.0	10.0	10.0	10.0	10.0
Canola oil	3.5	3.5	3.5	3.5	3.5	3.5
Trace-mineralized salt[c]	.5	.5	.5	.5	.5	.5
Calcium carbonate	.7	.8	.9	.9	1.6	1.1
Dicalcium monophosphate	2.6	2.3	2.0	1.8	1.5	1.2
Potassium monobasic carbonate	.2	-	-	-	-	-
Vitamin premix[d]	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[e]	.5	.5	.5	.5	.5	.5
Chromic oxide[f]	.4	.4	.4	.4	.4	.4

[a]St. Lawrence Starch Company, Mississauga, ON.

[b]Corn Products, Englewood Cliffs, NJ.

[c]Supplied by Windsor Salt, Toronto, Canada. Composition (percentage): NaCl, 96.5; ZnO, .40; $FeCO_3$, .16; MnO, .12; CuO, .033; $Ca(IO_3)_2$, .007; CaO, .004.

[d]The vitamin premix supplied the following vitamins (mg/kg diet): retinyl palmitate, 5.2; cholecalciferol, .38; all-rac-α-tocopherol acetate, 44.0; menadione, 3.0; riboflavin, 2.2; niacin, 12.0; d-pantothenic acid, 11.0; vitamin B_{12}, .012; choline, 550; thiamine, 1.1; pyridoxine, 1.1; d-biotin .1; folic acid, .6; corn starch was used as carrier.

[e]The trace-mineral premix supplied the following minerals (mg/kg diet): Fe, 50; Zn, 50; Mn, 2; Cu, 3; I, .14; Se, .15; corn starch as was used carrier.

[f]Fisher Scientific, Fair Lawn, NJ.

Table V-2. The chemical composition[a] (%) of SBM and the experimental diets

		Dietary CP levels, %					
Items	SBM	4	8	12	16	20	24
DM	90.8	89.7	90.1	89.7	90.1	90.1	[illegible]
Ether extract	2.3	3.7	3.8	4.0	4.2	4.3	4.3
NDF	7.5	.6	1.3	1.9	2.6	[illegible]	[illegible]
ADF	4.8	.4	.8	1.3	1.6	2.0	[illegible]
Cellulose	3.6	.3	.6	.9	1.2	1.5	1.8
Lignin	1.1	.1	.2	.3	.4	.5	.6
CP	47.4	4.0	7.9	11.7	16.1	20.2	24.1
Amino acids							
Indispensable							
Arginine	3.21	.27	.53	.81	1.06	1.33	1.65
Histidine	1.15	.10	.19	.30	.38	.46	.59
Isoleucine	2.17	.18	.36	.55	.73	.91	1.14
Leucine	3.49	.30	.59	.89	1.16	1.46	1.78
Lysine	2.74	.23	.47	.70	.92	1.14	1.39
Methionine	.76	.06	.13	.19	.25	.32	.38
Phenylalanine	2.39	.20	.40	.60	.79	1.00	1.23
Threonine	1.74	.15	.30	.45	.58	.74	.89
Valine	2.18	.19	.37	.56	.73	.91	1.14
Dispensable							
Alanine	1.91	.16	.34	.49	.65	.80	.97
Aspartic acid	5.21	.44	.88	1.33	1.74	2.19	2.69
Cysteine	.73	.06	.12	.18	.25	.31	.37
Glutamic acid	8.96	.75	1.50	2.25	3.00	3.72	4.64
Glycine	1.78	.15	.31	.47	.61	.76	.94
Serine	2.36	.19	.40	.60	.79	1.00	1.21
Tyrosine	1.63	.14	.27	.40	.51	.68	.83

[a]As-fed basis.

Table V-3. The apparent ileal DM, CP, and amino acid digestibilities (%) in the experimental diets

	Dietary CP levels, %						
Items	4	8	12	16	20	24	SEM[a]
DM[b]	90.4	86.7	84.8	82.5	79.7	78.4	.57
CP[c]	56.7	68.6	77.3	81.3	83.2	83.4	1.05
Amino acids[c]							
Indispensable							
Arginine	76.9	82.9	88.7	90.9	91.8	92.4	.87
Histidine	75.6	78.3	86.2	87.3	88.6	88.7	.63
Isoleucine	72.6	78.4	84.6	85.5	87.1	88.2	.65
Leucine	73.3	78.3	84.0	85.4	86.5	87.2	.63
Lysine	74.5	79.4	85.6	86.3	87.4	87.4	.75
Methionine	76.3	83.4	88.0	89.4	90.1	90.5	.49
Phenylalanine	78.0	82.8	86.8	88.5	89.0	89.8	.52
Threonine	48.5	63.7	73.0	76.6	79.0	79.4	1.22
Valine	67.9	74.6	81.5	83.2	84.4	85.9	.74
Dispensable							
Alanine	60.2	70.6	78.1	80.1	81.7	82.6	1.10
Aspartic acid	70.7	77.5	83.0	83.7	85.2	84.7	.72
Cysteine	56.8	68.1	76.2	80.0	80.2	80.3	1.26
Glutamic ac	79.0	83.6	87.6	87.6	88.0	86.1	.76
Glycine	27.8	47.1	64.6	72.1	76.3	75.5	2.40
Serine	59.9	71.8	79.6	82.1	84.1	84.8	.83
Tyrosine	73.6	79.0	84.8	86.5	88.6	88.8	.47

[a]Standard error of mean (n = 6).

[b]Linear effect ($P < .01$).

[c]Quadratic effect ($P < .01$).

Table V-4. The segmented quadratic with plateau relationships between the apparent ileal amino acid digestibilities and dietary contents

Items	Regression equations[a, b, c]	P[d]	X[e]	
CP	Y = 41.6 + 3.9X - .08X²	±.	±	
Amino acids				
Indispensable				
Arginine	Y = 66.1 + 31.0X - X²	±	±	
Histidine	Y = 67.8 + 11.4X - X²	±	±	
Isoleucine	Y = 64.7 + 41.8X - X²	±	±	
Leucine	Y = 68.7 + 24.3X - X²	±	±	
Lysine	Y = 66.1 + 34.8X - X²	±	±	
Methionine	Y = 67.1 + 149.3X - X²	±	±	
Phenylalanine	Y = 71.4 + 41.4X - X²	±	±	
Threonine	Y = 51.0 + 124.3X - X²	±	±	
Valine	Y = 56.1 + 18.7X - X²	±	±	
Dispensable				
Alanine	Y = 47.8 + 16.8X - X²	±	±	
Aspartic acid	Y = 61.1 + 21.4X - X²	±	±	
Cysteine	Y = 43.3 + 218.4X - X²	±	±	
Glutamic acid	Y = 71.1 + 11.4X - X²	±	±	
Glycine	Y = 31.7 + 171.4X - X²	±	±	
Serine	Y = 46.3 + 73.2X - X²	±	±	
Tyrosine	Y = 66.1 + 51.2X - X²	±	±	

[a]Y = apparent ileal CP or amino acid digestibility (percentage).

[b]X = CP or amino acid content (percentage, DM basis).

[c]The intercept and linear and quadratic slopes of the equations are significant ($P < .01$, n = 36).

[d]The plateau digestibility values of apparent ileal CP and amino acids up to which the quadratic regression equations are valid and their 95% confidence intervals.

[e]The breakpoints on the X-coordinate corresponding to the plateau values (P) and their 95% confidence intervals.

Table V-5. The initial plateau apparent ileal digestibilities of CP and amino acids and the threshold levels of dietary CP and amino acids

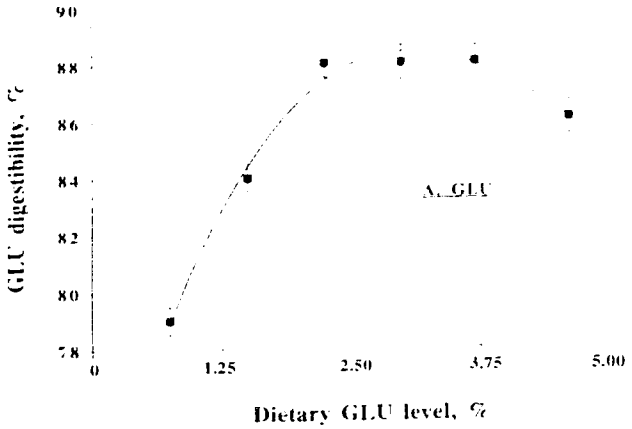
Items	Initial plateau[a]	Threshold level[b]	Corresponding CP level[c]
CP	80.9	17.07	15.36
Amino acids			
Indispensable			
Arginine	90.6	1.15	15.36
Histidine	87.2	.43	16.08
Isoleucine	86.2	.83	16.36
Leucine	85.6	1.33	16.30
Lysine	86.0	.94	14.70
Methionine	89.0	.24	13.70
Phenylalanine	87.9	.82	14.68
Threonine	77.0	.63	15.53
Valine	83.7	.84	16.53
Dispensable			
Alanine	80.2	.69	15.39
Aspartic acid	83.5	1.71	14.01
Cysteine	78.5	.24	14.29
Glutamic acid	86.2	2.31	11.00
Glycine	71.2	.67	15.98
Serine	82.6	.87	15.67
Tyrosine	87.6	.62	16.29

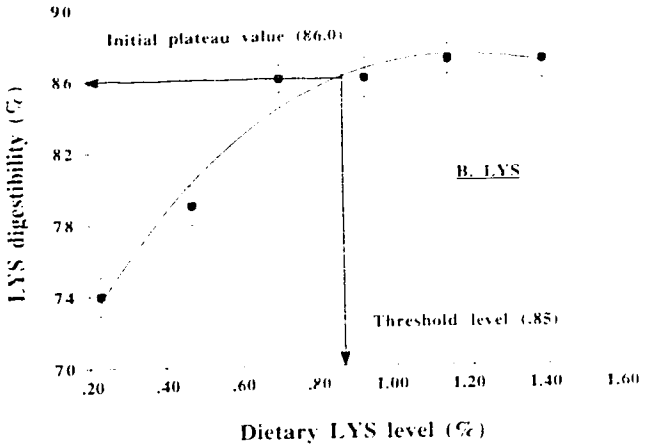
[a]Lower endpoints of 95% confidence intervals of the estimated plateau apparent ileal digestibilities.

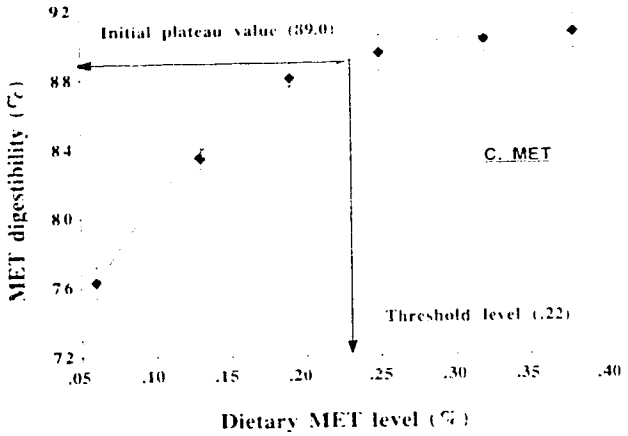
[b]Percentage on DM basis.

[c]The dietary levels of CP (percentage, as-fed basis) corresponding to the threshold levels of each amino acids.

Figure V-1. The quadratic with plateau relationship between apparent ileal amino acid digestibility (Y: percentage, mean ± SE) and dietary amino acid content (X: percentage, as-fed basis). A. GLU; B. LYS; C. MET; D. THR.


GLU digestibility, %
90
88
86
84
82
80
78
0
1.25
2.50
3.75
5.00
A. GLU
Dietary GLU level, %


LYS digestibility (%)
90
86
82
78
74
70
Initial plateau value (86.0)
B. LYS
Threshold level (.85)
.20
.40
.60
.80
1.00
1.20
1.40
1.60
Dietary LYS level (%)


MET digestibility (%)
92
88
84
80
76
72
Initial plateau value (89.0)
C. MET
Threshold level (.22)
.05
.10
.15
.20
.25
.30
.35
.40
Dietary MET level (%)

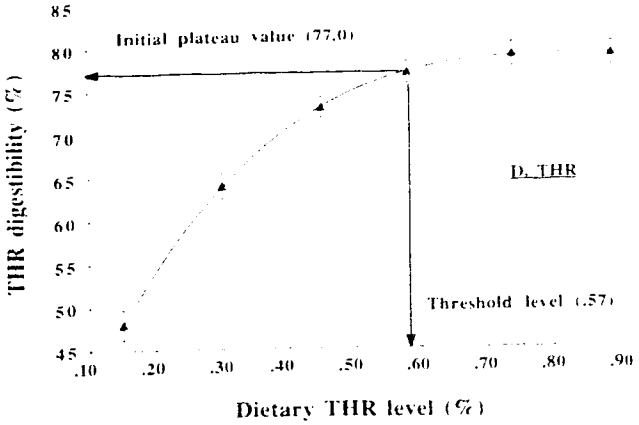
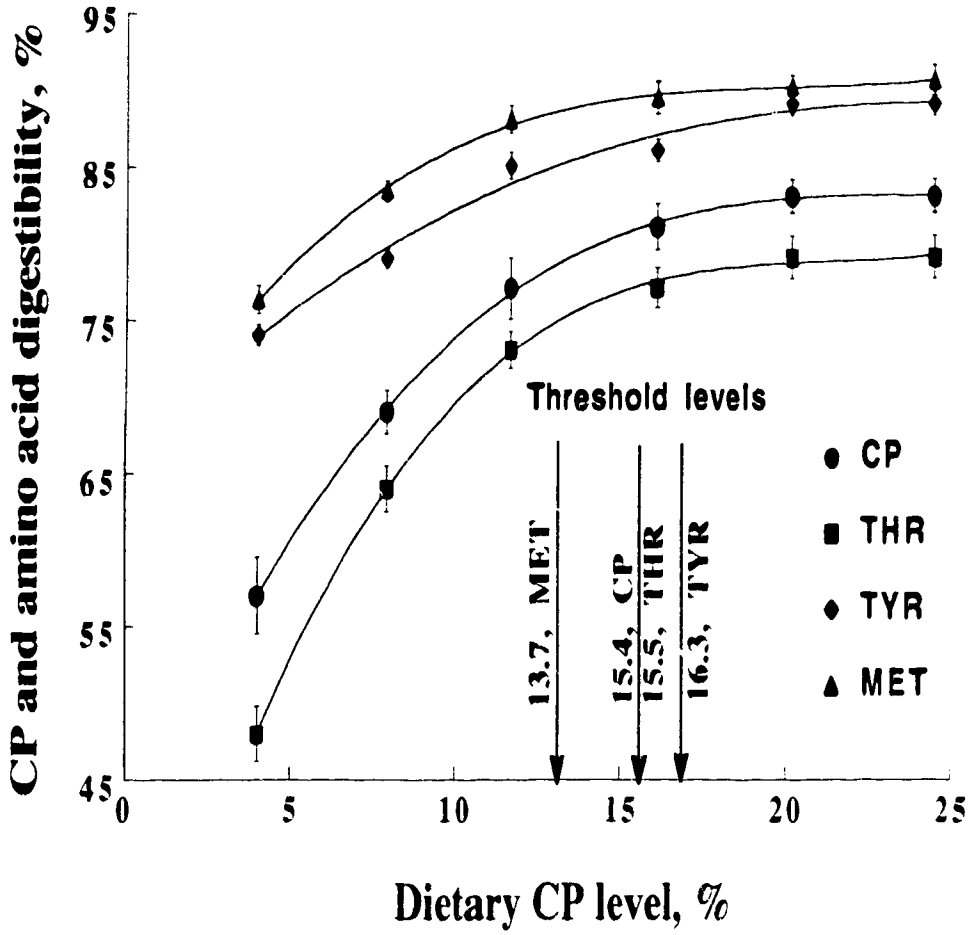

THR digestibility (%)
85
80
75
70
65
60
55
50
45
Initial plateau value (77.0)
D. THR
Threshold level (.57)
.10
.20
.30
.40
.50
.60
.70
.80
.90
Dietary THR level (%)

Figure V-2. The quadratic with plateau relationships between the apparent ileal CP and amino acid digestibilities (Y: percentage, mean ± SE) and dietary CP contents (X: percentage, as-fed basis).


CP and amino acid digestibility, %
95
85
75
65
55
45
0
5
10
15
20
25
Dietary CP level, %
Threshold levels
13.7, MET
15.4, CP
15.5, THR
16.3, TYR
CP
THR
TYR
MET

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CHAPTER VI

DETERMINATION OF APPARENT ILEAL AMINO ACID DIGESTIBILITY IN BARLEY AND CANOLA MEAL FOR PIGS WITH THE DIRECT, DIFFERENCE, AND REGRESSION METHOD

A. Introduction

The ileal rather than the fecal analysis method should be used to determine amino acid digestibility in feedstuffs for pigs because of the modifying action of the microflora in the large intestine (e.g., Zebrowska, 1973). Many studies have been carried out during the last two decades to determine the apparent ileal digestibility values of amino acids in feedstuffs. These results were summarized in several reviews (e.g., Tanksley and Knabe, 1984; Sauer and Ozimek, 1986).

It is hardly surprising to recognize large differences in amino acid digestibility between feedstuffs. These differences can be attributed to several factors, some not yet identified (e.g., Sauer and Ozimek, 1986; Knabe, 1991). However, it comes somewhat as a surprise to notice a large variability in amino acid digestibility values between samples of the same feedstuffs (in name). As was suggested by Sauer et al. (1990), this variability may result, in part, from differences in the methods used to determine amino acid digestibility.

Amino acid digestibility values in feedstuffs are usually determined with the direct method (e.g., Lin et al., 1987). The difference method is often used to determine amino acid digestibility values in feedstuffs of poor palatability (e.g., Kreienbring et al., 1988). With respect to the direct method, the study in Chapter V showed that the apparent ileal

digestibility values of amino acids in a feedstuff are dependent on their respective dietary contents.

The objectives of this study were to compare the direct, difference, and regression method for the determination of amino acid digestibility. Barley and canola meal, representing low- and high-protein feedstuffs, respectively, were selected for this study.

B. Experimental Procedures

Determination Methods

The direct, difference, and regression methods were compared for the determination of apparent ileal digestibility values of amino acids in barley and canola meal.

(1). Direct Method: the assay diet is formulated in such a manner that the assay feedstuff provides the sole source of dietary amino acids (e.g., Lin et al., 1987).

(2). Difference Method: this method involves the formulation of both a basal and an assay diet. The basal diet contains the basal feedstuff which provides the sole source of dietary amino acids; the assay diet consists of a mixture of the basal and the assay feedstuffs. If there are no interactions between the digestibility values of amino acids in the basal and assay feedstuffs, then the digestibility values can be calculated with the difference method (e.g., Van Leeuwen et al., 1987).

(3). Regression Method: the principle of the regression method for the determination of nutrient digestibility in feedstuffs for ruminants was briefly described by Giger and Sauvant (1983). This method measures simultaneously the digestibility values of amino acids in a basal and an assay feedstuff. The basal and assay feedstuffs are mixed at graded levels in a series of assay diets. The relationships between the ileal digestibility values of amino acids in the assay diets and the contribution levels of amino acids from the basal and assay feedstuffs to the assay diets can be expressed according to equation [1].

$$D_{Di} = D_B \times S_{Bi} + D_A \times S_{Ai}$$
$$= D_B \times S_{Bi} + D_A \times (1 - S_{Bi})$$
$$= D_A + (D_B - D_A) \times S_{Bi} \quad [1]$$

D_{Di} : apparent ileal amino acid digestibility in the ith assay diet (percentage); D_B: apparent ileal amino acid digestibility in the basal feedstuff (percentage); S_{Bi}: contribution level of an amino acid from the basal feedstuff to the ith assay diet (percentage), $S_{Bi} = 100\% - S_{Ai}$; D_A: apparent ileal amino acid digestibility in the assay feedstuff (percentage); S_{Ai}: contribution level of an amino acid from the assay feedstuff to the ith assay diet (percentage).

As D_B and D_A are the apparent ileal digestibility values of amino acids to be determined for the basal and the assay feedstuffs and we define $A = D_A$ and $B = D_B - D_A$, then equation [2] can be derived from equation [1] as follows:

$$D_{Di} = A + B \times S_{Bi} \quad [2]$$

Equation [2] is, in fact, a simple linear regression model in which D_{Di} and S_{Bi} are the dependent and independent variables, respectively. The regression coefficients of A and B can be estimated by fitting the simple linear regression model.

Animal Trial Procedures

Five barrows (Lacombe x Yorkshire), average initial BW 40 kg, were surgically fitted with a simple T-cannula at the distal ileum according to procedures adapted from Sauer et al. (1983). The cannulas were modified according to De Lange et al. (1989). After surgery, the barrows were individually housed in stainless steel metabolic crates in a temperature-controlled barn (20 to 22°C). During the 14-d recovery period, the barrows were fed a 16% CP grower diet (Sauer et al., 1983). A detailed description of pre- and post-operative care was previously presented by Sauer (1976).

After recovery, the barrows were fed one of the five experimental diets (Table VI-1) according to a 5 x 5 Latin square design. They were fed twice daily, equal amounts each meal, at 0800 and 2000 h. The dietary allowance was 1,800 g/d throughout the

experiment. Water was freely available from a low-pressure drinking nipple. At the conclusion of the experiment, the barrows, average final BW 56.8 kg, were killed by electrical stunning, bled out, and dissected to determine whether cannulation had caused intestinal abnormalities.

Five diets were formulated (Table VI-1). Diet 1 contained 42.7% canola meal, which was the sole dietary source of amino acids. Diets 2, 3, and 4 contained graded levels of barley (22.5, 45.0, and 67.5%, respectively) and canola meal (36.5, 30.5, and 24.4%, respectively). Diet 5 contained 90.0% barley, which was the sole source of dietary amino acids. With the exception of diet 5, the diets were formulated to contain 16% CP (%N x 6.25, as-fed basis). Canola oil was included in the diet at a level of 2.5% to reduce the dustiness of the diets. Vitamins and minerals were supplemented according to NRC (1988) standards. Chromic oxide (.4%) was included in the diets as the marker for the determination of digestibility values of the nutrients that were measured. Barley and canola meal were ground through a 3.2-mm mesh screen prior to diet formulation.

Each experimental period comprised 8 d. Ileal digesta were collected for a total of 24 h: from 0800 to 1000 h on d 7 and every other 2 h thereafter until 0800 h on d 8 and from 1000 to 1200 h on d 8 and every other 2 h thereafter until 0800 h on d 9. Ileal digesta were collected in soft plastic tubing (length, 15 cm; i.d., 2.5 cm), which was attached to the barrel of the cannula with Velcro tape. The tubing contained 10 mL of a solution of formic acid (10%, vol/vol) to minimize further bacterial activity. The tubing was removed and replaced as soon as it was partially filled with digesta. Digesta were immediately frozen at -20°C.

The experimental proposal, surgical procedures, and procedures for care and treatment of the barrows were reviewed and approved by the Faculty of Agriculture and Forestry Animal Care Committee of the University of Alberta. The barrows used in this experiment were cared for in accordance with the guidelines established by CCAC (1980).

Chemical Analyses

After the conclusion of the experiment, the digesta were freeze-dried, pooled within the barrow and the period for the same diet, ground in a Wiley mill through a 1-mm mesh screen, and mixed before analysis. The samples of the diets and ingredients were ground similarly. All analyses were performed in duplicate.

Analyses for DM, CP, and ether extract were carried out according to AOAC (1984) methods. Analyses for ADF, NDF, and lignin were carried out according to principles outlined by Goering and Van Soest (1970). Chromic oxide content was determined according to the procedure of Fenton and Fenton (1979).

For amino acid analyses, approximately .1 g of sample was weighed out into screw-capped test tubes, mixed with 3 mL of 6 *N* HCl, flushed with nitrogen, and then 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 1,110 x *g* for 15 min at room temperature. The samples were analyzed according to the method of Jones and Gilligan (1983) using a Varian 5000 HPLC system (Varian Associates, Sunnyvale, CA.) with a reversed-phase column previously described by Dugan et al. (1989). The amino acids in the sample were derivatized with an *o*-phthaldialdehyde reagent solution and detected spectrofluorometrically. The sulfur-containing amino acids and tryptophan contents were not determined.

Calculations and Statistical Analyses

The apparent ileal digestibility values of CP and amino acids in the five experimental diets were calculated with the indicator technique (Schneider and Flatt, 1975).

The apparent ileal digestibility values of CP and amino acids in canola meal and barley were determined with the direct method from diets 1 and 5, respectively. The digestibility values of CP and amino acids in barley and canola meal, included at three

levels in diets 2, 3, and 4, were determined with the difference method. The mean digestibility values in barley and canola meal, determined with the direct method, were used to calculate the digestibility values in barley and canola meal, respectively, with the difference method. In addition, the digestibility values of CP and amino acids in barley and canola meal were determined with the regression method from diets 1, 2, 3, and 4, respectively. Diet 5 was very low in CP and amino acid content and, therefore, excluded in the calculation of digestibility values with the regression method.

The apparent ileal digestibility values in the diets were first subjected to ANOVA for a 5 x 5 Latin square design. Means of treatments, periods, and barrows were compared using the Student-Newman Keul's multiple range test. Periods and barrows were the controlled factors in the 5 x 5 Latin square design. For the determination of the digestibility values of amino acids in barley and canola meal with the regression method, the simple linear relationships between the digestibility values of CP and amino acids in the diets and the contribution levels of CP and amino acids in canola meal or barley to the experimental diets were analyzed. The comparison of the digestibility values in barley or canola meal determined with the difference method, at three inclusion levels, and the comparison between the three determination methods were carried out with the one- or two-tailed Student's *t*-test. All statistical analyses were carried out according to principles described by Steel and Torrie (1980). The ANOVA, the multiple comparison, and the simple linear regression analyses were conducted using the GLM procedures of SAS (1990).

C. Results and Discussion

Composition and Digestibility of the Experimental Diets

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

The chemical composition of barley, canola meal, and the diets are presented in Table VI-2. The contents of NDF, ADF, cellulose, and lignin in the diets were calculated from the analyzed values in barley and canola meal. The analyzed values of CP and the majority of amino acids in the diets were very close to the calculated values based on the analyzed values in barley and canola meal.

The apparent ileal digestibility values of CP and amino acids in the experimental diets are presented in Table VI-3. Barley and canola meal provided the sole sources of protein in diets 1 and 5, respectively (Table VI-1). Therefore, the digestibility values of CP and amino acids in diets 1 and 5 correspond to the digestibility values in canola meal and barley, as determined with the direct method, respectively. The amino acid digestibility values in barley and canola meal are within the range of values summarized by Sauer and Ozimek (1986) and Knabe (1991) for these feedstuffs. There were no differences ($P > .05$) in the digestibility values of isoleucine, leucine, phenylalanine, valine, and serine between barley and canola meal. The digestibility values of CP and the other amino acids were lower ($P < .05$) in barley than in canola meal, which resulted in a decrease ($P < .05$) in their respective digestibility values as the level of inclusion of barley was increased from 22.5 (diet 2) to 67.5% (diet 4) (Table VI-3).

Determination of Apparent Ileal Amino Acid Digestibility in Barley with the Direct, Difference, and Regression Method

The apparent ileal digestibility values of amino acids in barley determined with the difference method, based on results obtained from diets 1, 2, 3, and 4 and the contributions of amino acids in barley to the diets are presented in Table VI-4. There were increases ($P < .05$) in the digestibility values of the majority of the amino acids in barley as its level of inclusion in the diets was increased from 22.5 to 67.5%. Of the indispensable amino acids, the increase was largest for lysine, from 23.5 to 61.3%, and, smallest for phenylalanine, from 56.8 to 72.1%. Among the amino acids, lysine and phenylalanine from barley contributed the lowest and highest percentage to the total dietary content, respectively. These results show that, at the lower levels of inclusion, the digestibility values in barley are underestimated with the difference method. This underestimation is greater for amino acids that are present in small amounts in barley, which often include the limiting amino acids, as was illustrated in this case for lysine. Furthermore, there were large decreases in the standard errors of the amino acid digestibility values as the level of inclusion of barley was increased from 22.5 to 67.5%.

At this point, it is of interest to refer to the study by Knabe et al. (1989) who determined the apparent ileal digestibility values of isoleucine, the limiting amino acid, in three blood meals. The digestibility values were 60, 70, and 80%, respectively. However, as these authors pointed out, these values might misrepresent the variation in isoleucine digestibility among the blood meals because these resulted from a difference of only 3 percentage units in digestibility among the diets containing both blood meal and soybean meal. In these diets, blood meal provided only 15% of the total dietary isoleucine. A 1-percentage-unit change in digestibility in the mixed diet resulted in a corresponding change in the digestibility of isoleucine of 10 percentage units. They concluded that differences in isoleucine digestibility among the blood meals might simply be a reflection of experimental error. On the other hand, studies by Van Leeuwen et al. (1987) with corn and soybean

meal diets showed that the levels of inclusion of soybean meal, 20 and 40%, had no effect on the apparent ileal digestibility values of amino acids (with the exception of serine) in soybean meal when these were determined with the difference method. However, in these studies, soybean meal contributed a large proportion to the total dietary CP, approximately 60 and 75%, respectively. Based on these and the aforementioned studies, it is evident that the reliability of the determination of the apparent ileal digestibility values of amino acids with the difference method is dependent on the contributions of amino acids in the assay feedstuff to their total dietary content. The higher the contribution of a particular amino acid to the total dietary contents, the more reliable the measurement of its digestibility with the difference method.

In order to determine the apparent ileal digestibility values of CP and amino acids in barley with the regression method, linear relationships were established between the digestibility values in diets 1, 2, 3, and 4 and the contribution levels of amino acids in canola meal to these diets (Table VI-5). Linear relationships could not be established ($P > .05$) for aspartic acid, isoleucine, leucine, phenylalanine, serine, threonine, and valine; differences in the digestibility values of these amino acids between barley and canola meal were not large enough to create a linear response. Therefore, in order to apply the regression method successfully to all amino acids, it is necessary that there are sufficiently large differences in the digestibility values of all amino acids between the two feedstuffs. To our knowledge, this is the first time that the regression method was used for the determination of ileal amino acid digestibility values in feedstuffs for swine.

The apparent ileal digestibility values of amino acids in barley, determined with the direct, difference and regression method, are summarized in Table VI-6. With the difference method, the digestibility values for barley, determined at the inclusion level of 67.5%, were referred to. As was mentioned previously, these digestibility values were associated with the smallest standard errors and therefore considered the most reliable for the difference method. There were no differences ($P > .05$) between the digestibility values

in barley when these were measured with the difference and regression method. With the exception of glutamic acid, the digestibility values measured with the direct method were lower than those measured with the difference or regression method. Of the indispensable amino acids, the differences were significant ($P < .05$ or $P < .10$) for arginine, lysine, and threonine. With the exception of glutamic acid, the differences were significant ($P < .05$ or $P < .10$) for all the dispensable amino acids.

These results suggest that the direct method is not suitable for the determination of apparent ileal digestibility values of amino acids in feedstuffs, such as barley, that are low in protein (and amino acid) content. This results from the fact that the endogenous amino acid contribution is relatively high at a low dietary amino acid intake. As was demonstrated in Chapter V, there are quadratic with plateau relationships between the dietary amino acid contents and their respective apparent ileal digestibility values.

Determination of Apparent Ileal Amino Acid Digestibility in Canola Meal with the Direct, Difference, and Regression Method

The apparent ileal digestibility values of amino acids in canola meal, determined with the difference method based on the results obtained from diets 2, 3, 4, and 5, and the contributions of amino acids in canola meal to the diets are presented in Table VI-7. Of the indispensable amino acids, the increase in contribution was smallest for lysine, from 67.9 to 90.5%, and largest for phenylalanine, from 49.4 to 81.5%. The digestibility values of most of the amino acids were higher ($P < .05$), when these were determined by difference from the diet with the lowest level of inclusion (24.4%) of canola meal. At first, these results seem contradictory. However, the digestibility values in canola meal were determined by difference using the mean digestibility values in barley determined with the direct method. As was shown previously, the direct method underestimates the digestibility values in barley. As a result, the digestibility values in canola meal will be overestimated when these are determined by difference from the diet with the lowest level of inclusion.

However, if valid digestibility values of amino acids in barley (67.5% inclusion) are used to determine the digestibility values in canola meal (24.4% inclusion), then similar values to those determined from the other inclusion levels (30.5 and 36.6%) will be obtained. Furthermore, there were no differences ($P > .05$) in the digestibility values of amino acids in canola meal when these were calculated from the diets that contained 30.5 and 36.6% canola meal; at these levels of inclusion the overestimation in amino acid digestibility values was eliminated. In addition, there were large decreases in the standard errors of the amino acid digestibility values as the dietary inclusion level of canola meal was increased from 24.4 to 36.6%. These results show, once more, that the determination of the apparent ileal digestibility values of amino acids with the difference method is dependent on the contributions of amino acids in the assay feedstuff to their total dietary contents.

For measurement of the apparent ileal digestibility values of amino acids in canola meal with the regression method, linear relationships were established between the digestibility values in diets 1, 2, 3, and 4 and the contribution levels of amino acids in barley to the diets (Table VI-8). For reasons explained previously, linear relationships could not be established ($P > .05$) for aspartic acid, isoleucine, leucine, phenylalanine, serine, threonine, and valine.

The apparent ileal digestibility values of amino acids in canola meal, determined with the direct, difference, and regression method, are summarized in Table VI-9. With the difference method, the digestibility values for canola meal determined at the inclusion level of 36.6%, were referred to. These digestibility values were associated with the smallest standard errors (Table VI-7). There were no differences ($P > .05$) in the digestibility values of amino acids in canola meal when these were determined with the different methods. These results suggest that the apparent ileal digestibility values of amino acids in high-protein feedstuffs, such as canola meal, can be determined with either method, including the difference method when its dietary inclusion level is relatively high.

In conclusion, these studies show that the direct method is not suitable for the measurement of apparent ileal digestibility values of amino acids in low-protein feedstuffs, as was illustrated for barley. On the other hand, the direct method is suitable for the measurement of digestibility values of amino acids in high-protein feedstuffs, such as canola meal. Furthermore, the difference method is suitable for the measurement of digestibility values of amino acids in both low- and high-protein feedstuffs when their inclusion levels in the assay diets are relatively high. Lastly, the regression method is suitable for the measurement of digestibility values of amino acids in both types of feedstuffs.

Table VI-1. Formulation (%) of the experimental diets

Diets	1	2	3	4	5
Ingredients					
Barley	-	22.5	45.0	67.5	90.0
Canola meal	42.7	36.6	30.5	24.4	-
Corn starch[a]	51.7	35.2	18.7	2.2	2.9
Canola oil	2.5	2.5	2.5	2.5	2.5
Trace-mineralized salt[b]	.5	.5	.5	.5	.5
Calcium carbonate	.7	.8	.9	1.0	1.4
Sodium monobasic phosphate	-	-	-	-	.8
Vitamin premix[c]	1.0	1.0	1.0	1.0	1.0
Mineral premix[d]	.5	.5	.5	.5	.5
Chromic oxide[e]	.4	.4	.4	.4	.4

[a]St. Lawrence Starch Company, Mississauga, ON.

[b]Supplied by Windsor Salt, Toronto, Canada. Composition (percentage): NaCl, 96.5; ZnO, .40; $FeCO_3$, .16; MnO, .12; CuO, .033; $Ca(IO_3)_2$, .007; CaO, .004.

[c]The vitamin premix supplied the following vitamins (mg/kg diet): retinyl palmitate, 5.2; cholecalciferol, .38; all-rac-α-tocopherol acetate, 44.0; menadione, 3.0; riboflavin, 2.2; niacin, 12.0; d-pantothenic acid, 11.0; vitamin B_{12}, .012; choline, 550; thiamine, 1.1; pyridoxine, 1.1; d-biotin .1; folic acid, .6; corn starch was used as carrier.

[d]The trace-mineral premix supplied the following minerals (mg/kg diet): Fe, 50; Zn, 50; Mn, 2; Cu, 3; I, .14; Se, .15; corn starch was used as carrier.

[e]Fisher Scientific, Fair Lawn, NJ.

Table VI-2. The chemical composition[a] (%) of barley, canola meal, and the experimental diets

Ingredients[b] and diets	B	CM	1	2	3	4	5
DM	89.8	90.4	89.6	89.1	88.3	87.6	86.8
Ether extract	1.9	4.1	3.2	3.4	3.4	3.9	3.3
NDF	14.4	35.7	15.4	16.5	17.8	18.9	13.4
ADF	3.9	19.7	8.5	8.2	7.9	7.7	3.7
Cellulose	2.0	18.3	7.9	7.2	6.6	5.9	1.8
Lignin	.7	1.3	.6	.8	.7	.7	.6
CP	11.4	42.2	18.1	18.2	18.4	18.5	10.6
Amino acids							
Indispensable							
Arginine	.52	2.48	1.06	1.04	1.01	.99	.49
Histidine	.27	1.08	.47	.46	.46	.46	.25
Iscleucine	.43	1.80	.77	.77	.76	.75	.40
Leucine	.79	2.86	1.23	1.24	1.25	1.27	.74
Lysine	.42	2.47	1.06	1.01	.96	.91	.39
Phenylalanine	.46	1.23	.53	.56	.59	.62	.43
Threonine	.37	1.65	.71	.69	.68	.67	.34
Valine	.58	2.18	.94	.94	.94	.95	.54
Dispensable							
Alanine	.46	1.82	.78	.78	.78	.78	.43
Aspartic acid	.66	2.95	1.27	1.24	1.22	1.20	.61
Glutamic acid	2.67	7.47	3.21	3.38	3.55	3.73	2.49
Glycine	.43	1.91	.82	.81	.80	.78	.40
Serine	.41	1.53	.66	.66	.66	.67	.38
Tyrosine	.19	1.05	.45	.43	.41	.40	.18

[a]DM basis.

[b]B: barley; CM: canola meal.

Table VI-3. Apparent ileal digestibility values (%) of CP and amino acids in the experimental diets

Diets	1	2	3	4	5	SEM[a]
CP	65.0[b]	63.4[bc]	62.5[c]	61.7[c]	56.6[d]	1.32
Amino acids						
Indispensable						
Arginine	79.8[b]	78.1[bc]	76.2[c]	75.8[c]	64.7[d]	1.14
Histidine	78.4[b]	77.4[b]	76.1[bc]	75.2[c]	69.5[d]	1.25
Isoleucine	69.3	64.8	63.9	65.4	61.8	1.83
Leucine	70.8	67.6	66.0	70.6	66.6	1.73
Lysine	71.7[b]	70.7[b]	68.8[c]	66.7[d]	54.1[e]	1.58
Phenylalanine	70.8	68.2	67.9	70.4	69.6	1.90
Threonine	63.1[b]	59.8[bc]	57.7[c]	62.8[d]	53.3[e]	1.75
Valine	67.5	64.6	65.9	67.4	62.6	1.73
Dispensable						
Alanine	66.9[b]	64.8[bc]	63.0[c]	60.8[d]	48.8[e]	1.59
Aspartic acid	64.1[b]	63.3[b]	59.3[b]	56.3[b]	50.9[c]	1.86
Glutamic acid	80.3[b]	78.6[bc]	77.7[c]	75.1[d]	75.1[d]	1.62
Glycine	63.4[b]	59.8[c]	57.3[cd]	54.5[d]	31.7[e]	3.23
Serine	65.0	61.5	61.9	64.9	58.4	1.66
Tyrosine	66.0[b]	64.3[bc]	63.4[c]	62.4[c]	51.9[d]	1.68

[a]Standard error of mean (n = 5).

b, c, d, [e]Means in the same row with different superscript letters differ ($P < .05$).

Table VI-4. Apparent ileal digestibility values[a] (%) of CP and amino acids in barley determined with the difference method and the contribution levels[b] (%) of CP and amino acids from barley to the corresponding diets

	Inclusion levels of barley[c], %					
Item	[illegible]		[illegible]		[illegible]	
CP	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Amino acids						
Indispensable						
Arginine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Histidine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Isoleucine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Leucine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Lysine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Phenylalanine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Threonine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Valine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Dispensable						
Alanine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Aspartic acid	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Glutamic acid	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Glycine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Proline	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Serine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[a]Mean and standard error (n = 5).

[b]Values in parentheses give the contribution levels (percentage) of CP and amino acids from barley to the corresponding diets.

[c]Inclusion levels of barley in diets 2, 3, and 4 from which the digestibility values were calculated.

[d, e]Means in the same row with different superscript letters differ ($P < .05$).

Table VI-5. The linear relationships between the apparent ileal digestibility values of CP and amino acids and the contribution levels of CP and amino acids from canola meal to the experimental diets

Items	Regression equations[a] [b]	r^2	S_{yx}	Probability[c]	
CP[d]	Y = 57.7 + 6.6X	.52	3.39	.0001	.0493
Amino acids					
Indispensable					
Arginine[d]	Y = 68.4 + 11.1X	.69	2.49	.0001	.0138
Histidine[d]	Y = 70.5 + 8.2X	.48	2.71	.0001	.0467
Isoleucine	Y = 64.5 + 2.6X	.01	3.81	.0001	.6552
Leucine	Y = 67.6 + 1.5X	.01	3.75	.0001	.7780
Lysine[d]	Y = 59.3 + 12.5X	.72	3.54	.0001	.0056
Phenylalanine	Y = 68.6 + .6X	.01	4.41	.0001	.9064
Threonine	Y = 62.3 + 1.9X	.01	3.68	.0001	.5235
Valine	Y = 63.9 + 1.5X	.01	3.94	.0001	.7893
Dispensable					
Alanine[d]	Y = 53.9 + 12.7X	.74	4.04	.0001	.0467
Aspartic acid	Y = 60.1 + 1.9X	.01	3.75	.0001	.7515
Glutamic acid[d]	Y = 72.2 + 7.4X	.55	3.02	.0001	.0286
Glycine[d]	Y = 43.5 + 18.5X	.76	5.51	.0001	.0049
Serine	Y = 61.8 + 1.3X	.01	3.45	.0001	.7905
Tyrosine[d]	Y = 58.2 + 6.3X	.54	3.54	.0001	.0370

[a]Y = apparent ileal digestibility values of CP and amino acids in the experimental diets (percentage).

[b]X = the contribution levels of CP and amino acids from canola meal to the experimental diets (percentage).

[c]The probabilities of significance for the intercepts and the slopes of the regression equations.

[d]Linear effect ($P < .05$, n = 20).

Table VI-6. Comparison of the apparent ileal digestibility values[a] (%) of CP and amino acids in barley determined with the direct, difference, and regression method

Methods	Direct	Difference[b]	Regression
CP	56.6±1.88	58.9±3.36	57.7±2.83
Amino acids			
Indispensable			
Arginine[c]	64.7±1.93	69.8±3.15	68.4±2.39
Histidine	69.5±2.68	71.9±3.56	70.5±2.27
Isoleucine	61.1±3.29	66.9±4.80	-
Leucine	66.6±2.80	70.3±3.79	-
Lysine[c]	54.1±4.18	61.3±4.93	59.3±4.65
Phenylalanine	69.6±3.41	72.1±4.28	-
Threonine	53.3±3.12[d]	62.4±3.90[e]	-
Valine	62.6±2.96	67.2±3.94	-
Dispensable			
Alanine	48.8±2.96[d]	57.3±3.39[e]	53.9±2.81[e]
Aspartic acid	50.5±3.83[d]	62.1±4.68[e]	-
Glutamic acid	75.1±2.52	73.9±2.10	72.2±2.89
Glycine	31.7±3.41[d]	48.1±5.64[e]	43.5±5.08[e]
Serine	58.4±3.19[d]	64.8±2.67[e]	-
Tyrosine[c]	51.5±3.88	61.2±5.62	58.2±3.43

[a]Mean and standard error (n = 5).

[b]Digestibility values calculated from diet 4 (67.5% inclusion of barley).

[c]Means in the same row show a trend to increase ($P < .10$, by one-tailed Student's *t*-test).

d, [e]Means in the same row with different superscript letters differ ($P < .05$).

Table VI-7. Apparent ileal digestibility values[a] (%) of CP and amino acids in canola meal determined with the difference method and the contribution levels[b] (%) of CP and amino acids from barley to the corresponding diets

Item	Inclusion levels of canola meal[c], %			
	44.4	33.3	[illegible]	[illegible]
CP	[illegible]	[illegible]	[illegible]	[illegible]
Amino acids				
Indispensable				
Arginine	[illegible]	[illegible]	[illegible]	[illegible]
Histidine[d]	[illegible]	[illegible]	[illegible]	[illegible]
Isoleucine	[illegible]	[illegible]	[illegible]	[illegible]
Leucine	[illegible]	[illegible]	[illegible]	[illegible]
Lysine[d]	[illegible]	[illegible]	[illegible]	[illegible]
Phenylalanine[d]	[illegible]	[illegible]	[illegible]	[illegible]
Threonine	[illegible]	[illegible]	[illegible]	[illegible]
Valine	[illegible]	[illegible]	[illegible]	[illegible]
Dispensable				
Alanine	[illegible]	[illegible]	[illegible]	[illegible]
Aspartic acid	[illegible]	[illegible]	[illegible]	[illegible]
Glutamic acid	[illegible]	[illegible]	[illegible]	[illegible]
Glycine	[illegible]	[illegible]	[illegible]	[illegible]
Serine	[illegible]	[illegible]	[illegible]	[illegible]
Tyrosine	[illegible]	[illegible]	[illegible]	[illegible]

[a]Mean and standard error (n = 5).

[b]Values in parentheses give the contribution levels (percentage) of CP and amino acids from barley to the corresponding diets.

[c]Inclusion levels of canola meal in diets 4, 3, and 2 from which the digestibility values were calculated.

[d]Means in the same row show a trend to decrease ($P < .10$).

[e, f]Means in the same row with different superscript letters differ ($P < .05$).

Table VI-8. The linear relationships between the apparent ileal digestibility values of CP and amino acids and the contribution levels of CP and amino acids from barley to the experimental diets

Items	Regression equations[a] [b]	r^2	S_{yx}	Probability[c]	
CP[d]	Y = 64.3 - 6.6X	.52	3.39	.0001	.0493
Amino acids					
Indispensable					
Arginine[d]	Y = 79.5 - 11.1X	.69	2.49	.0001	.0138
Histidine[d]	Y = 78.7 - 8.2X	.48	2.71	.0001	.0467
Isoleucine	Y = 67.1 - 2.6X	.01	3.81	.0001	.6552
Leucine	Y = 69.1 - 1.5X	.01	3.75	.0001	.7780
Lysine[d]	Y = 71.8 - 12.5X	.72	3.54	.0001	.0056
Phenylalanine	Y = 69.2 - .6X	.01	4.41	.0001	.9064
Threonine	Y = 64.2 - 1.9X	.01	3.68	.0001	.5225
Valine	Y = 65.4 - 1.5X	.01	3.94	.0001	.7893
Dispensable					
Alanine[d]	Y = 66.6 - 12.7X	.74	4.04	.0001	.0467
Aspartic acid	Y = 62.0 - 1.9X	.01	3.75	.0001	.7515
Glutamic acid[d]	Y = 79.6 - 7.4X	.55	3.02	.0001	.0286
Glycine[d]	Y = 62.0 - 18.5X	.76	5.51	.0001	.0049
Serine	Y = 63.1 - 1.3X	.01	3.45	.0001	.7905
Tyrosine[d]	Y = 64.5 - 6.3X	.54	3.54	.0001	.0370

[a]Y = apparent ileal digestibility values (percentage) of CP and amino acids in the experimental diets.

[b]X = the contribution levels (percentage) of CP and amino acids from barley to the experimental diets.

[c]The probabilities of significance for the intercepts and the slopes of the regression equations.

[d]Linear effect ($P < .05$, n = 20).

Table VI-9. Comparison of the apparent ileal digestibility values[a] (%) of CP and amino acids in canola meal determined with the direct, difference, and regression method

Methods	Direct	Difference[b]	Regression
CP	66.0±0.85	62.5±1.44	64.3±1.46
Amino acids			
Indispensable			
Arginine	80.8±1.19	79.4±1.14	79.5± .95
Histidine	80.0±0.78	77.4±1.19	78.7±1.09
Isoleucine	69.3±0.77	65.3±1.58	-
Leucine	70.8±1.15	67.7±1.68	-
Lysine	73.7±0.79	70.7±1.10	71.8±1.38
Phenylalanine	70.8±1.18	67.9±1.90	-
Threonine	63.1±0.88	60.7±1.64	-
Valine	67.5±0.84	63.8±1.72	-
Dispensable			
Alanine	68.9±0.60	65.2±1.95	66.6±1.51
Aspartic acid	64.1±1.04	61.2±1.65	-
Glutamic acid	80.3±1.17	75.8±1.42	79.6±1.19
Glycine	63.4±1.95	63.7±1.28	62.0±1.89
Serine	65.0±0.64	62.0±1.65	-
Tyrosine	66.0±0.81	64.5±1.53	64.5±1.49

[a]Mean and standard error (n = 5).

[b]Digestibility values calculated from diet 2 (36.6% inclusion of canola meal).

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CHAPTER VII

THE ADDITIVITY OF THE DIGESTIBLE ENERGY AND AMINO ACID SUPPLY IN SINGLE INGREDIENTS IN THE FORMULATION OF COMLETE DIETS FOR PIGS[1]

A. Introduction

The nutritive value of a feed ingredient is, in part, not only determined by its total energy and nutrient content but also by the digestible energy and nutrient content, in particularly the digestible amino acid supply. Presently, it is generally accepted that the digestible amino acids supply in a feedstuffs should be determined with the ileal rather than fecal analysis method (e.g., Tanksley and Knabe, 1984; Sauer and Ozimek, 1986).

In the formulation of diets, it is usually assumed that the supply of digestible energy and amino acids in a mixture of feedstuffs is equal to the total of the supply based on the digestible supply determined in the single feed ingredients. This assumption, however, may be questioned as there is a scarcity of information on the additivity of the digestible amino acid and energy supply. In addition, the information that is available, is contradictory. Imbeah et al. (1988) reported that the digestible amino acid supply in a diet can be predicted from amino acid digestibilities determined in the single ingredients; the calculated digestibilities were equal to or slightly lower than those directly determined. On the other hand, Furuya and kaji (1991) found significant differences between the observed and calculated digestibilities for most of the amino acids in a barley and soybean meal but

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[1]A version of this chapter has been published. Fan, M. Z., W. C. Sauer, and S. Li. J. Anim. Physiol. Anim. Nutr. 1993. 70:72.

not in a corn and soybean meal diet.

The objective of the present study was to determine if the digestible energy and amino acid supply in a diet can be predicted from the digestible supply (dietary content x apparent digestibility) in the ingredients. Diets consisting of barley, wheat, and canola meal or soybean meal were evaluated in these studies.

B. Experimental Procedures

Animals and Diets

Six PIC barrows (Camborough x Canabrid), with an average initial BW of 35 kg, were obtained from the University of Alberta swine herd. The pigs were housed in stainless steel crates 1 wk before surgery and fed a 16% CP grower diet (Sauer et al., 1983). Water was freely available from a low-pressure drinking nipple.

The barrows were fitted with a simple T-cannula, approximately 5 cm anterior to the ileo-cecal sphincter, according to procedures adapted from those described by Sauer et al. (1983). The cannulas were prepared according to methods described by McBride et al. (1983), modified slightly according to De Lange et al. (1989). The pigs were immediately returned to the metabolic crates after surgery and fasted that same day. Starting the next day, they were given approximately 100 g of the grower diet twice daily. The feed allowance was increased slowly until the pigs consumed 1,800 g of diet daily. The pigs were allowed a 14-d recuperation period. A detailed description of pre- and post-operative care was previously presented by Sauer (1976) and Sauer et al. (1983). Once recovered from the surgery, the pigs were fed 900 g of the experimental diets at each meal twice daily, at 0800 and 2000 h.

Six diets were formulated (Table VII-1). The barley and wheat diets contained 94% of the respective cereal grains. The canola meal and soybean meal diets were corn starch-based and formulated to contain 16% CP. Dextrose was included at a level of 10% in these

diets to possibly improve the palatability. The barley-wheat-canola meal and barley-wheat-soybean meal diets were formulated to contain 16% CP. All diets contained 3% canola oil. Chromic oxide was included at .3% in the diets as a marker to determine digestibility. Soybean meal and canola meal were solvent-extracted. All the ingredients were ground through a 3.2-mm mesh screen prior to diet incorporation.

The experiment was carried out according to a 6 x 6 Latin square design with six diets and six periods. Each experimental period lasted 9 d. Feces were collected for 48 h from 0800 on d 6 to 0800 h on d 8. Ileal digesta were collected for a total of 24 h from 0800 on day 7 to 0800 h on day 9 at 2-h intervals according to procedures adapted from Imbeah et al. (1988). Ileal digesta were collected in soft plastic tubing (length, 15 cm; i.d., 2.5 cm), which was attached to the barrel of the cannula with Velcro tape. The plastic tubing was removed and replaced as soon as it was partially filled with digesta. The tubes contained about 15 mL of formic acid solution (10%, vol/vol) to minimize further bacterial activity. Digesta and feces were immediately frozen at -20°C after collection.

Chemical Analyses

The digesta and feces samples were freeze-dried, pooled within the pig and the period for the same dietary treatment, ground through a .8-mm mesh screen, and mixed before analyses. The feed samples were ground similarly.

Analyses for DM and CP were carried out according to AOAC (1984) methods. Chromic oxide content was determined according to the procedure of Fenton and Fenton (1979). GE content in feed, digesta, and feces was measured with a Parr Adiabatic Bomb Calorimeter.

For amino acid analyses, approximately .1 g of sample was weighed into screw-capped test tube and mixed with 3 mL of 6 *N* HCl. The tubes were flushed with nitrogen and then heated 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 1,110 x *g* for 15 min at

room temperature. The samples were analyzed according to the method of Jones and Gilligan (1983) using the high performance liquid chromatography column and equipment previously described by Dugan et al. (1989). The amino acids were derivatized with an *o*-phthaldialdehyde reagent solution, subjected to reversed-phase high-performance liquid chromatography and detected spectrofluorometrically.

Calculations and Statistical Analyses

The apparent fecal digestibility of energy in the diets and the apparent ileal digestibilities of CP and amino acids in the diets (and also in the ingredients) were determined with the direct method using formula [1].

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

Where D_D: apparent digestibility of a nutrient in the assay diet (percentage); I_D: marker concentration in the assay diet (percentage); A_F: nutrient concentration in ileal digesta or in feces (percentage); I_F: marker concentration in ileal digesta or in feces (percentage). A_D: nutrient concentration in the assay diet (percentage).

The fecal digestibility of energy in the ingredients (barley, wheat, soybean meal, and canola meal) were determined with the difference method using formula [2].

$$D_I = (D_D - S_B \times D_B) / S_A \quad [2]$$

Where D_I: apparent digestibility of a nutrient in the assay feed ingredient (percentage); D_D: apparent digestibility of a nutrient in the assay diet (percentage); S_B: contribution of a nutrient from the basal diet to the assay diet (percentage); D_B: digestibility of a nutrient in the basal diet (percentage); S_A: contribution of a nutrient from the assay feed ingredient to the assay diet (percentage); For these calculations, the DE values of corn starch, dextrose, and canola oil were assumed to be 16.9, 15.7, and 31.4 MJ/kg DM, respectively (NRC, 1988).

Analyses of variance were carried out according to the principles described by Steel and Torrie (1980). Where appropriate, means for treatments, periods, and animals were

compared using the Student-Newman Keuls' multiple range test. All the statistical analyses were carried out with the GLM procedures of SAS (1990). Animals and periods are the controlled factors in the 6 x 6 Latin square design. There were no significant ($P > .05$) animal and period effects, thus the calculated amino acid digestibilities in the barley-wheat-canola meal and in the barley-wheat-soybean meal mixtures were based on the individually determined values in each of the three ingredients and the contributions of the amino acids in these ingredients to the complete diets. The observed amino acid digestibilities and those calculated, based on determinations in the single ingredients, for barley-wheat-canola meal and for barley-wheat-soybean meal were compared by means of a *t*-test.

C. Results and Discussion

The pigs seemingly remained healthy and consumed their meal allowances throughout the study. Post mortem examinations, carried out at the conclusion of the experiment, revealed no intestinal adhesions.

The DM, GE, CP, and amino acid contents of barley, wheat, canola meal, and soybean meal are presented in Table VII-2. The CP and amino acid contents for barley, canola meal, and soybean meal are within the range of values reported by NRC (1988). The contents of CP, lysine, and threonine reported for wheat in the present studies were 20.5, .58 and, .54 %, respectively; these values were considerably higher than the average values in wheat compiled by NRC (1988) for wheat.

The DM, GE, CP, and amino acid contents of the diets, in addition to diets consisting of barley-wheat-canola meal or soybean meal are presented in Table VII-3. The directly determined CP and amino acid contents of these diets were very close, within a range of 5%, to the values calculated on the basis of the contributions of the ingredients to these diets and their CP and amino acid contents.

The DE content in the ingredients and in the mixtures of barley-wheat-soybean meal and barley-wheat-canola meal are presented in Table VII-4. The DE content was higher ($P < .05$) in wheat than in barley and higher in soybean meal than in canola meal, which is in agreement with the results summarized by Wiseman and Cole (1979) and NRC (1988). The DE content was higher ($P < .05$) in the mixture of barley-wheat-soybean meal than in the mixture of barley-wheat-canola meal due to the lower DE content in canola meal than in soybean meal. The directly determined and calculated DE contents in the barley-wheat-canola meal and barley-wheat-soybean meal diets are presented in Tables VII-5 and 6, respectively. There were no differences ($P > .05$) between the directly determined and calculated values.

The apparent ileal digestibilities of CP and amino acids in the ingredients and the mixtures are presented in Table VII-4. The digestibilities of amino acids were higher ($P < .05$) in wheat than in barley which is in agreement with studies reported by Sauer et al. (1977a), Sauer et al. (1981), and Zebrowska et al. (1981). In the present studies, of the indispensable amino acids, the differences ranged from 11.5 (valine) to 21.9 (phenylalanine) percentage units. Of the dispensable amino acids, the differences ranged from 12.4 (glutamic acid) to 34.6 (glycine) percentage units. The apparent ileal amino acid digestibilities were higher ($P < .05$) in soybean meal than in canola meal. Of the indispensable amino acids, the differences ranged from 10.5 (arginine) to 18.2 (phenylalanine) percentage units. Of the dispensable amino acids, the differences ranged from 5.6 (glutamic acid) to 19.4 (serine) percentage units. These results are in agreement with those previously reported by Sauer et al. (1982) and Imbeah and Sauer (1991). Some of the factors that may be responsible for the lower amino acid digestibilities in canola meal than in soybean meal include the higher fiber and tannin contents in canola meal (Sauer et al., 1982; Sauer and Thacker, 1986) and perhaps the faster rate of passage of diets containing canola meal rather than soybean meal (Imbeah and Sauer, 1991). As was expected, the apparent ileal amino acid digestibilities were higher ($P < .05$) in the barley-

wheat-soybean meal than barley-wheat-canola meal diets. Of the indispensable amino acids, the differences ranged from 5.8 (arginine) to 9.9 (threonine) percentage units. Of the dispensable amino acids, these differences ranged from 3.7 (glutamic acid) to 11.2 (glycine) percentage units. These results are in agreement with studies reported by Imbeah et al. (1988) who determined the amino acid digestibilities in barley-soybean meal and barley-canola meal diets. As was also reported by Imbeah et al. (1988), differences between soybean meal and canola meal in the apparent ileal digestibilities of amino acids were higher than those observed between the cereal-soybean meal and cereal-canola meal diets. The smaller differences would seem to be the results of dilution by cereal grains.

The directly determined and calculated apparent ileal digestibilities of CP and amino acids in the barley-wheat-canola meal and barley-wheat-soybean meal diets are presented in Tables VII-5 and 6. There were no differences ($P > .05$) between the directly determined and calculated apparent ileal digestibilities of CP and amino acids in the barley-wheat-canola meal and barley-wheat-soybean meal diets, respectively. Of the indispensable amino acids, the differences ranged from .1 (threonine) to 2.4 (histidine) percentage units in barley-wheat-canola meal diet and from .6 (phenylalanine) to 3.1 (threonine) percentage units in barley-wheat-soybean meal diet. Of the dispensable amino acids, there was a significant difference ($P < .05$) in one instance, namely for glycine ($P < .05$) in the barley-wheat-soybean meal diet (Table 6). Garren (1987) also reported no differences ($P > .05$) between the directly determined and calculated ileal amino acid digestibilities in a corn starch-based diet that contained a mixture of soybean meal and cotton seed meal. Furthermore, Imbeah et al. (1988) reported no significant differences ($P > .05$) between the directly determined and calculated apparent ileal amino acid digestibilities for most of the indispensable amino acids in barley-canola meal and barley-soybean meal diets. On the other hand, Furuya and Kaji (1991) reported differences ($P < .05$) between the observed and calculated apparent ileal digestibilities for most of the amino acids in a barley-soybean meal diet but not in a corn-soybean meal or wheat-soybean meal diet. Of the indispensable

amino acids in the barley-soybean meal diet, the difference was largest for threonine (7.6 percentage units); of the dispensable amino acids largest for glycine (12.3 percentage units). It is rather difficult to reconcile the results obtained in these studies and by Imbeah et al. (1988) and Furuya and Kaji (1991). However, in the aforementioned studies for all diets the calculated digestibilities were not overestimated; the calculated digestibilities were equal to or slightly lower than those determined directly.

The apparent ileal amino acid digestibilities in barley were lower (Table VII-4) than those reported previously (e.g., Sauer et al., 1977a) and those compiled by Sauer and Ozimek (1986). For example, the lysine and threonine digestibilities were 53.3 and 51.5% in these studies. The lysine and threonine digestibilities in the results from studies compiled by Sauer and Ozimek (1986) ranged from 64.9 to 79% and from 64.4 to 76%, respectively. However, two recent studies also showed very low apparent ileal digestibilities of lysine and threonine in barley; 37.6 and 44.2%, respectively in studies by Imbeah et al. (1988) and 55.0 and 51.0%, respectively in studies by Furuya and Kaji (1991). The barleys used in these studies, by Imbeah et al. (1988), and by Furuya and Kaji (1991) were very low in CP, ranging from 9.5 to 10.0%, and amino acid contents. As was shown by Eggum (1973) in studies with rats, the apparent fecal digestibility of CP was curvilinearly related to the CP content in the assay diet and the lower the CP content in the assay diet, the lower the apparent CP digestibility. A similar relationship can be expected between the amino acid content and the ileal amino acid digestibility. Furthermore, Buraczewska et al. (1987), in studies with barley samples varying in CP (amino acid) content, showed that a positive and significant relationship between the apparent ileal N digestibility and the CP content in the diet. It was also observed that the higher the CP content, the higher the ileal amino acid digestibilities. Furthermore, in addition to the CP (amino acid) content in barley, fineness of grinding may affect amino acid digestibility as was originally demonstrated by Sauer et al. (1977b) in studies with wheat. The aforementioned may explain the very low amino acid digestibilities, lower than in these

studies and by Furuya and Kaji (1991), which were reported by Imbeah et al. (1988) who used barley that was coarsely ground. In this context, studies by Wünsche et al. (1987) showed large differences between the apparent ileal amino acid digestibilities in pigs fed coarsely, medium, and finely ground barley. For example, the apparent ileal digestibilities of lysine were 43.6, 54.2, and 62.0% in coarsely, medium, and finely ground barley, respectively.

Of the indispensable amino acids in the cereal grains, the apparent ileal digestibilities were lowest for lysine and threonine (Table VII-4). Lysine and threonine are also considered to be the first and second limiting amino acids, respectively, in both barley and wheat. Of the dispensable amino acids, the digestibility of glycine was lowest. Relatively low digestibilities of threonine and glycine were also observed in canola meal, soybean meal, and the complete diets. The very low apparent ileal digestibilities of lysine in barley and wheat result, in part, from the low contents of these amino acids in these cereal grains. The relatively low apparent ileal digestibilities of threonine and glycine, in addition to their low dietary contents, also result from their relatively high concentrations in endogenous protein. Previous studies (e.g., Holmes et al., 1974; Sauer et al., 1977b) showed relatively high contents of threonine and glycine in endogenous protein in digesta collected from the distal ileum of growing pigs fed a protein-free diet. Glycine, a major constituent base of the bile salt conjugates, accounts for more than 90% of the total amino acids secreted in porcine bile juice (Souffrant, 1991). The bile salt conjugates are degraded in the distal ileum by intestinal bacteria; 90% of the bile salts is re-absorbed via active transport and enters the enterohepatic circulation. However, the deconjugated glycine escapes re-absorption and enters large intestine (Newsholme and Leech, 1984; Shiau, 1987). Furthermore, the small intestinal secretions, which include mucins, supply the largest proportion of N to the endogenous N secretions in the small intestine (Auclair, 1986). As was shown by Neutra and Forstner (1987), "native" mucin, which represents over 95% of mucin glycoprotein, is very rich in threonine in addition to serine and proline.

In conclusion, the present studies show no differences between the directly determined and calculated DE content and apparent ileal digestibilities of the indispensable amino acids in diets consisting of a mixture of barley-wheat-canola meal or barley-wheat-soybean meal. In other words, the DE content and apparent ileal digestible supply of the indispensable amino acids in these diets can be predicted from digestibilities determined in the single ingredients.

Table VII-1. Formulation of the experimental diets (%)

Diets[a]	B	W	CM	SBM	B-W-CM	B-W-SBM
Ingredients						
Barley	94.0				44.5	49.0
Wheat		94.0			30.5	30.9
Canola meal			45.7		19.5	
Soybean meal				36.0		14.3
Cornstarch[b]			39.4	47.9		
Dextrose[c]			10.0	10.0		
Canola oil	3.0	3.0	3.0	3.0	3.0	3.0
Calcium carbonate	1.1	1.2	.8	.7	1.3	1.1
Dicalcium phosphate	.8	.7		1.3	.1	.6
Trace-mineralized salt[d]	.5	.5	.5	.5	.5	.5
Vitamin premix[e]	.2	.2	.2	.2	.2	.2
Mineral premix[f]	.1	.1	.1	.1	.1	.1
Chromic oxide[g]	.3	.3	.3	.3	.3	.3

[a]B: Barley diet; CM: Canola meal diet; SBM: Soybean meal diet; B-W-CM: Barley-Wheat-Canola meal diet; B-W-SBM: Barley-Wheat-Soybean meal diet.

[b]St. Lawrence Starch Company, Mississauga, ON.

[c]Corn Products, Englewood Cliffs, NJ.

[d]Supplied by Windsor Salt, Toronto, Canada. Composition (percentage): NaCl, 96.5; ZnO, .40; $FeCO_3$, .16; MnO, .12; CuO, .033; $Ca(IO_3)_2$, .007; CaO, .004.

[e]The vitamin premix supplied the following vitamins (mg/kg diet): retinyl palmitate, 5.2; cholecalciferol, .38; all-rac-α-tocopherol acetate, 44.0; menadione, 3.0; riboflavin, 2.2; niacin, 12.0; d-pantothenic acid, 11.0; vitamin B_{12}, .012; choline, 550; thiamine, 1.1; pyridoxine, 1.1; d-biotin .1; folic acid, .6; corn starch was used as carrier.

[f]The trace-mineral premix supplied the following minerals (mg/kg diet): Fe, 50; Zn, 50; Mn, 2; Cu, 3; I, .14; Se, .15; corn starch was used as carrier.

[g]Fisher Scientific, Fair Lawn, NJ.

Table VII-2. The chemical composition[a] of the experimental feed ingredients

Ingredients[b]	B	W	CM	SBM
DM, %	87.1	88.3	91.2	91.0
GE, MJ/kg	18.0	18.9	19.5	19.6
CP, %	11.4	20.5	40.9	52.4
Amino acids, %				
Indispensable				
Arginine	.49	.87	2.20	3.21
Histidine	.24	.43	.99	1.14
Isoleucine	.40	.67	1.49	2.10
Leucine	.74	1.22	2.61	3.56
Lysine	.41	.58	2.46	3.47
Phenylalanine	.47	.82	1.27	1.97
Threonine	.37	.54	1.60	1.81
Valine	.54	.79	1.84	2.12
Dispensable				
Alanine	.44	.63	1.61	1.96
Aspartic acid	.66	.99	2.81	5.47
Glutamic acid	2.68	6.22	6.97	9.04
Glycine	.32	.57	1.36	1.43
Serine	.41	.77	1.38	2.04
Tyrosine	.23	.41	1.07	1.45

[a]DM basis.

[b]B: barley; W: wheat; CM: canola meal; SBM: soybean meal.

Table VII-3. The chemical composition[a] of the experimental diets

Diets[b]	B	W	CM	SBM	B-W-SBM	B-W-CM
DM	87.2	88.4	90.2	89.8	88.0	88.6
GE, MJ/kg	18.7	16.7	18.9	18.3	19.1	19.1
CP, %	10.6	17.1	19.1	18.8	19.8	19.8
Amino acids, %						
Indispensable						
Arginine	.48	.80	1.04	1.21	.99	.99
Histidine	.23	.41	.43	.43	.41	.41
Isoleucine	.37	.62	.70	.80	.72	.68
Leucine	.68	1.36	1.18	1.35	1.37	1.19
Lysine	.39	.52	1.13	1.30	.90	.88
Phenylalanine	.45	.77	.61	.79	.77	.71
Threonine	.34	.51	.75	.69	.61	.66
Valine	.51	.74	.87	.81	.80	.84
Dispensable						
Alanine	.40	.59	.75	.75	.69	.70
Aspartic acid	.61	.91	1.30	2.06	1.42	1.13
Glutamic acid	2.44	5.85	3.23	3.34	4.51	4.41
Glycine	.31	.54	.64	.55	.55	.56
Serine	.38	.71	.64	.77	.75	.68
Tyrosine	.21	.37	.49	.54	.46	.41

[a]DM basis.

[b]Refer to Table VII-1.

Table VII-4. The DE and apparent ileal digestibilities of CP and amino acids in the experimental ingredients and mixtures

Diets[a]	B	W	CM	SBM	B-W-CM	B-W-SBM	SEM[b]
DE, MJ/kg DM	15.3^{c}	16.6^{c}	13.0^{e}	15.0^{d}	15.5^{d}	16.7^{c}	.08
CP, %	59.6^{e}	79.8^{c}	67.2^{d}	81.1^{c}	69.1^{d}	77.6^{c}	1.03
Amino acids, %							
Indispensable							
Arginine	69.0^{g}	81.8^{de}	79.5^{ef}	90.0^{c}	77.5^{f}	83.3^{d}	.85
Histidine	62.1^{d}	81.0^{d}	73.8^{e}	85.0^{c}	70.7^{e}	78.0^{d}	1.09
Isoleucine	68.2^{f}	83.4^{d}	72.1^{e}	87.5^{c}	74.3^{e}	81.8^{d}	1.01
Leucine	70.8^{f}	84.8^{cd}	74.0^{e}	87.4^{c}	75.4^{e}	82.9^{d}	1.08
Lysine	53.3^{f}	68.1^{e}	70.9^{e}	86.5^{c}	68.1^{e}	77.2^{d}	1.03
Phenylalanine	61.4^{f}	83.3^{c}	66.3^{e}	84.5^{c}	69.4^{e}	77.9^{d}	.41
Threonine	51.5^{f}	72.0^{d}	62.8^{e}	78.7^{c}	62.2^{e}	72.1^{d}	1.31
Valine	69.2^{e}	80.7^{d}	70.4^{e}	84.6^{c}	72.0^{e}	79.3^{d}	1.06
Dispensable							
Alanine	54.7^{f}	71.9^{d}	70.2^{d}	82.0^{c}	65.4^{e}	72.7^{d}	1.25
Aspartic acid	59.5^{g}	74.3^{e}	67.5^{f}	85.8^{c}	67.2^{f}	78.3^{d}	1.05
Glutamic acid	79.8^{f}	92.2^{c}	80.6^{f}	86.2^{d}	83.9^{e}	87.6^{d}	.77
Glycine	34.2^{g}	68.8^{cd}	59.4^{e}	72.7^{c}	54.8^{f}	66.0^{d}	1.52
Serine	60.6^{f}	81.1^{cd}	63.6^{f}	83.0^{c}	67.7^{e}	78.3^{d}	1.33
Tyrosine	55.3^{f}	76.9^{d}	69.1^{e}	85.5^{c}	66.4^{e}	77.5^{d}	1.11

[a]Refer to Table VII-1.

[b]Standard error of mean (n = 6).

c, d, e, f, gMeans in the same row followed by different superscript letters differ ($P <$ .05).

Table VII-5. The observed and calculated DE content and apparent ileal CP and amino acid digestibilities[a] in the barley-wheat-canola meal diet

Item	Observed	Calculated	Difference
DE, MJ/kg DM	15.5 ± .43	15.8 ± .29	.3
CP, %	69.1 ± 2.52	69.3 ± 1.89	.2
Amino acids, %			
Indispensable			
Arginine	77.5 ± 2.01	77.7 ± 1.99	.2
Histidine	70.7 ± 3.14	73.1 ± 1.29	2.4
Isoleucine	74.3 ± 2.10	74.5 ± 3.08	.2
Leucine	75.4 ± 2.51	76.5 ± 3.28	1.1
Lysine	68.1 ± 3.31	66.6 ± 2.58	1.5
Phenylalanine	69.4 ± 3.91	70.8 ± 2.58	1.4
Threonine	62.2 ± 5.57	62.3 ± 2.86	.1
Valine	72.0 ± 2.69	73.0 ± 3.19	1.0
Dispensable			
Alanine	65.4 ± 3.33	66.5 ± 2.42	1.1
Aspartic acid	67.2 ± 3.38	67.3 ± 3.15	.1
Glutamic acid	83.9 ± 1.68	85.3 ± 1.85	1.4
Glycine	54.8 ± 7.70	56.1 ± 1.94	1.3
Serine	67.7 ± 3.73	68.7 ± 1.83	1.0
Tyrosine	66.4 ± 2.52	68.2 ± 2.80	1.8

[a]Means ± standard deviation (n = 6).

Table VII-6. The observed and calculated DE and apparent ileal CP and amino acid digestibilities[a] in the barley-wheat-soybean meal diet

Item	Observed	Calculated	Difference
DE, MJ/kg DM	16.7 ± .29	16.0 ± .28	.7
CP, %	77.6 ± .88	74.6 ± 2.16	3.0
Amino acids, %			
Indispensable			
Arginine	83.3 ± 1.21	82.6 ± 1.35	.7
Histidine	78.0 ± 3.14	77.3 ± 2.44	.7
Isoleucine	81.8 ± 1.73	81.0 ± 2.14	.8
Leucine	82.9 ± 1.51	81.9 ± 2.28	1.0
Lysine	77.2 ± 1.74	75.3 ± 3.42	1.9
Phenylalanine	77.9 ± 2.47	77.3 ± 3.07	.6
Threonine	72.1 ± 1.53	69.0 ± 4.55	3.1
Valine	79.3 ± 2.01	78.5 ± 2.55	.8
Dispensable			
Alanine	72.7 ± 1.83	70.9 ± 2.63	1.8
Aspartic acid	78.3 ± 2.15	77.5 ± 2.17	.8
Glutamic acid	87.6 ± .84	86.9 ± 1.51	.7
Glycine	66.0 ± 3.26[b]	60.4 ± 3.50[c]	5.6
Serine	78.3 ± 1.52	76.3 ± 3.02	2.0
Tyrosine	77.5 ± 2.05	75.7 ± 3.29	1.8

[a]Means and standard deviation (n = 6).

[b, c]Means in the same row followed by different superscript letters differ ($P < .05$)

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CHAPTER VIII

ESTIMATION OF THE ENDOGENOUS AMINO ACID LEVELS IN DIGESTA COLLECTED FROM THE DISTAL ILEUM OF PIGS BY REGRESSION ANALYSIS

A. Introduction

The ileal rather than fecal analysis method should be used for the determination of amino acid digestibility in feedstuffs for pigs because of the modifying action of the microflora in the large intestine on amino acid metabolism (e.g., Zebrowska, 1973). However, measurements obtained with this method are confounded by the presence of endogenous amino acids in ileal digesta. The feeding of protein-free diets is the classical technique to determine the recoveries of endogenous amino acids in ileal digesta (e.g., Carlson and Bayley, 1970). While the feeding of protein-free diets remains a useful technique to study the effect of dietary factors other than protein on the recoveries of amino acids in ileal digesta (e.g., De Lange et al., 1989a), it is questionable whether results obtained with this technique are valid for determining true ileal amino acid digestibilities.

Using the ^{15}N-isotope dilution technique, a differentiation can be made between non-digested dietary and endogenous protein (e.g., Souffrant et al., 1981; De Lange et al., 1990; Lien et al., 1993). Nevertheless, this technique can only be used to estimate total endogenous protein, and not the recovery of each of the amino acids. A constant amino acid profile of endogenous protein is assumed to estimate true ileal amino acid digestibility (e.g., De Lange et al., 1990).

Alternatively, linear relationships between apparent ileal digestible and dietary input of amino acids can be used to estimate the levels of endogenous amino acids under normal

dietary conditions in pigs (Furuya and Kaji, 1986; 1989). However, this technique has been questioned, as it is not clear yet whether dietary levels of amino acids can influence the amount of endogenous amino acids and whether this amount is affected by differences in ranges of graded dietary amino acid levels.

The objectives of this study were to investigate the effect of different ranges of graded dietary levels of amino acids on the linear relationships and the estimation of the levels of endogenous amino acids and to study the effect of dietary levels of amino acids on levels of endogenous amino acids in ileal digesta and on true ileal amino acid digestibility.

B. Experimental Procedures

Principles of Extrapolation

The relationship between true and apparent ileal amino acid digestibility in an assay diet can be expressed by equation [1]. After further mathematical derivation, this relationship can also be expressed by equation [2].

$$D_T = (D_{Ai} / A_{Di} + A_E / A_{Di}) \times 100\% \quad [1]$$

$$D_{Ai} = -A_E + (D_T / 100\%) \times A_{Di} \quad [2]$$

D_T: true ileal amino acid digestibility in the ith assay diet (percentage); D_{Ai}: apparent ileal digestible amino acid content in the ith assay diet (g/kg DM intake, determined using equation [3]); i: the number of assay diets, i = 1, 2, ···· n; A_{Di}: amino acid content in the ith assay diet (g/kg DM); $(D_{Ai} / A_{Di}) \times 100\%$: apparent ileal amino acid digestibility in the ith assay diet (percentage); A_E: amount of an endogenous amino acid in ileal digesta (g/kg DM intake); $(A_E / A_{Di}) \times 100\%$: contribution of an endogenous amino acid (percentage).

Equation [2] is a simple linear regression model in which D_{Ai} and A_{Di} are the dependent and independent variables, respectively. A_E and D_T are the regression coefficients and can be estimated by fitting the simple linear regression model. If there are

linear relationships between dietary apparent ileal digestible and total contents of amino acids, then the quantity of endogenous amino acids in ileal digesta can be determined by extrapolating the dietary inputs of amino acids to zero, namely, obtaining intercepts of the linear regression equations (A_E). To determine true ileal amino acid digestibility in a protein-containing feedstuff, a series of assay diets are formulated to contain graded dietary levels of amino acids by including graded levels of the assay feedstuff in the assay diets. The contents of other dietary components, including antinutritive factors, fiber, and fat, which affect endogenous amino acid levels, should be controlled between the assay diets.

Animals, Diets, and Chemical Analyses

A detailed description of the experimental procedures and formulation and composition of the diets were presented previously in Chapter V. Six corn starch-based diets were formulated to contain 4, 8, 12, 16, 20, and 24% CP, respectively from soybean meal (**SBM**). Six barrows, average initial BW 35 kg, fitted with a simple T-cannula at the distal ileum, were fed the six diets according to a 6 x 6 Latin square design. They were fed twice daily, equal amounts each meal, at 0800 and 2000 h. The dietary allowance was 1,600 g/d during Period 1 and increased by 100 g/d each following period. Digesta were collected, at 2-h intervals, for a total of 24 h during d 7 and 8.

The experimental proposal, surgical procedures, and procedures for care and treatment of the animals were reviewed and approved by the Faculty of Agriculture and Forestry Animal Care Committee of the University of Alberta. The animals used in this experiment were cared for in accordance with the guidelines established by CCAC (1980).

Samples of digesta, diets, and SBM were prepared for analyses as described previously in Chapter V. The methods and procedures for analyses of DM, CP, amino acids, and chromic oxide in feed and digesta were described in detail previously in Chapter V.

Calculations and Statistical Analyses

The apparent ileal digestible CP and amino acid contents and the apparent ileal digestibilities of CP and amino acids in the diets (and also in SBM) were determined using equations [3] and [4], respectively.

$$D_{Ai} = A_D - [(I_D \times A_I) / I_I] \quad [3]$$

$$D_{Ai} = 100\% - [(I_D \times A_I) / (I_I \times A_D)] \times 100\% \quad [4]$$

D_{Ai}: contents of apparent ileal digestible CP and amino acids in the ith assay diet (g/kg DM, in equation [3]) and apparent ileal digestibilities of CP and amino acids (percentage, in equation [4]); A_D: concentrations of CP and amino acids in the assay diet (g/kg DM); I_D: marker concentration in the assay diet (percentage); A_I: concentrations of CP and amino acids in ileal digesta (g/kg DM intake); I_I: marker concentration in ileal digesta (percentage).

Based on the apparent ileal digestibilities of CP and amino acids in the diets (also in SBM) and the amounts of endogenous CP and amino acids extrapolated with regression analysis, the true ileal digestibilities of CP and amino acids in the diets (also in SBM) were determined using equation [5]. All the symbols in this equation were defined previously.

$$D_T = D_{Ai} + (A_E / A_{Di}) \times 100\% \quad [5]$$

Periods and animals were the controlled factors in the 6 x 6 Latin square design. The contents of apparent ileal digestible CP and amino acids and the true ileal digestibilities of CP and amino acids were first subjected to ANOVA for a 6 x 6 Latin square design. The intervals between the treatment levels of CP and amino acids were designed to be equal through increasing equal amounts of SBM (8.4%) in the diets at the expense of corn starch. The treatment effect was partitioned and tested according to equally-spaced orthogonal polynomial analysis based on principles described by Steel and Torrie (1980). The ANOVA and the orthogonal polynomial analyses were carried out using the GLM procedures of SAS (1990).

To determine the quantities of endogenous protein and amino acids in ileal digesta by regression analysis, simple linear relationships between dietary contents of apparent ileal

digestible and total CP and amino acids (g/kg DM) were established. The comparison between the amounts of endogenous CP and amino acids, i.e. the intercepts, extrapolated from different ranges of graded dietary levels of CP and amino acids was conducted by testing homogeneity of the linear regression equations. Analyses of simple linear regression and covariance analyses for testing homogeneity of the regression equations were carried out using the GLM procedures of SAS (1990) according to the principles described by Steel and Torrie (1980).

C. Results and Discussion

Extrapolation of Endogenous protein and Amino Acids

Six graded levels of CP were created in six diets using SBM as protein source. The contents of CP and amino acids in the experimental diets are presented in Table VIII-1.

The content of apparent ileal digestible amino acids, as g/kg DM intake, are presented in Table VIII-2. In response to increases in the dietary level of CP from 4 to 24%, there were corresponding linear increases ($P < .01$) in the content of apparent ileal digestible amino acids. Estimation of the amount of endogenous amino acids in ileal digesta by regression analysis is dependent on the presence of significant linear relationships between dietary contents of apparent ileal digestible and total amino acids. Linear relationships between dietary contents of apparent ileal digestible and total amino acids were established for ten ranges of graded dietary levels of CP and amino acids, from 4 to 24%, 4 to 20%, 4 to 16%, 4 to 12%, 8 to 24%, 8 to 20%, 8 to 16%, 12 to 24%, 12 to 20%, and 16 to 24% CP, respectively. Only linear relationships for the range of graded dietary levels from 4 to 24% CP are presented (Table VIII-3). The coefficients of linear determination (r^2) usually ranged from .95 to .99 and the linear relationships were significant ($P < .0001$) for all ranges of graded dietary levels. These results suggest that differences in the range of

graded dietary levels of amino acids have no effect on the linearity between dietary contents of apparent ileal digestible and total amino acids.

On the other hand, dietary factors that influence the amount of endogenous amino acids recovered from the distal ileum, including sources and levels of fiber (e.g., Sauer et al., 1977; Taverner et al., 1981), antinutritive factors (e.g., Begbie and Pusztai, 1989), and possibly fat (e.g., De Lange et al., 1989a), can alter the ratio of exogenous : endogenous amino acids, thereby likely affecting the linear relationships between dietary contents of apparent ileal digestible and total amino acids. In this study, there was only a small range in the contents of NDF (.6 to 3.8%) and fat (3.7 to 4.4%) among the diets (Chapter V). However, if feedstuffs high in fiber or antinutritive factors are used, then the assay diets should be be formulated in such a manner that they contain the same amount of fiber or antinutritive factors. Otherwise it may not be possible to create linear relationships.

Intercepts of the linear regression equations provide the estimated amount of the endogenous amino acids. The principle for estimating the amount of endogenous amino acids with the regression technique is illustrated in Figure VIII-1 for methionine (**MET**). Mathematically, the dietary contents of amino acids were designated to be positive (+); the amount of endogenous amino acids were designated negative (-). The estimated amounts of endogenous amino acids and their corresponding standard errors, extrapolated from different ranges of ten graded dietary levels are summarized in Table VIII-4. There were large differences in the estimates of endogenous amino acid levels between different ranges of graded dietary levels. However, these differences were not significant ($P > .05$), as a result of the large standard errors associated with some of the extrapolated values of endogenous amino acids (Table VIII-4). Furthermore, differences in the amount of endogenous amino acids estimated from different ranges of graded dietary levels were unlikely to be of a physiological nature and likely resulted from the statistical deviation of the regression analyses of different ranges of data. To maintain a normal physiological condition of the animal, it is advisable to carry out the estimation under ranges of higher

graded dietary levels of amino acids. However, from a statistical point of view, the closer the graded dietary levels of amino acids to the origin of the coordinate , the more reliable the estimation of the amount of endogenous amino acids. Therefore, a major consideration in methodology for the determination of endogenous amino acid levels is the choice of range of graded dietary levels. The results of this study suggest that ranges of graded dietary levels of 4 to 24%, 4 to 20%, 4 to 16%, and 4 to 12% CP are most reliable for estimating the levels of endogenous amino acids. The results determined from these ranges were usually associated with relatively small standard errors. The amounts of endogenous amino acids, determined from the range of graded dietary levels of 4 to 24% CP, represented all dietary levels of amino acids and were, therefore, considered the most reliable values and will be referred to from now on.

Effect of Dietary Levels of Amino Acids on the amounts of endogenous Protein and Amino Acids

The original studies with the ^{15}N-isotope dilution technique showed a positive linear relationship between protein intake and excretion of endogenous protein in feces of rats, which varied depending on the protein source (Krawielitzki et al., 1977). However, the effect of an increase in dietary fiber content, in association with an increase in CP content, was not taken into account in their studies. Studies with pigs fed protein-free diets showed an increase in the recoveries of endogenous protein and amino acids (both in ileal digesta and feces) with an increase in dietary fiber content (e.g., Sauer et al., 1977; Taverner et al., 1981). Therefore, differences in dietary fiber content may explain at least some of the increase in the recovery of endogenous protein as reported by Krawielitzki et al. (1977).

Several studies showed that an increase in dietary protein content resulted in an increase in endogenous protein secretions from the pancreas into the digestive tract of pigs (e.g., Corring and Saucier al., 1972; Partridge et al., 1982; Hee et al., 1988). Endogenous protein is partially digested and the end-products are re-absorbed; approximately 75% is

digested in the small intestine and 85% over the whole digestive tract (Souffrant et al., 1986). An increase in dietary protein content will likely lead to an increase in the levels of endogenous amino acids in ileal digesta. On the other hand, the absorptive capacity of the small intestine, which is determined by the absorptive area, absorption rate, and the total number of transporters, will increase as the dietary amino acid content is increased (Karasov and Diamond, 1987; Scharrer, 1987). As a result, the efficiency of absorption of amino acids and small peptides, both of dietary and endogenous origin, is expected to increase. Based on the previous discussion, there is no direct evidence in the literature to conclude whether dietary levels of amino acids increase the amount of endogenous amino acids in digesta collected from the distal ileum.

The linear relationships between dietary contents of apparent ileal digestible and total amino acids (Table VIII-3) imply that the contributions of endogenous amino acids, as g/kg DM intake, are independent of their respective dietary levels as is illustrated for MET in Figure VIII-1. These results further indicate that when the source of dietary protein and the contents of other dietary components, including fiber and antinutritive factors, are controlled between assay diets, i.e. the amounts of endogenous amino acids and true digestibilities will be constant. In contrast, if the amounts of endogenous amino acids are not constant at different dietary levels, then the relationships between contents of apparent ileal digestible and total amino acids will be curvilinear rather than linear.

The estimated amounts of endogenous amino acids and their percentage contributions to the total dietary contents are presented in Table VIII-5. The contributions of endogenous amino acids decreased curvilinearly in response to increases in the dietary levels of amino acids. This curvilinear relationship is illustrated for MET in Figure VIII-2. These results show that endogenous amino acid contributions have very little effect on the respective apparent digestibilities of amino acids at higher dietary levels.

Estimates of the recovery of endogenous protein at the distal ileum of growing and finishing pigs vary considerably, ranging from 10.0 to 30.5 g/kg DM intake (Holmes et

al., 1974; Sauer et al., 1977; Wüensche et al., 1979; Taverner et al., 1981; Furuya and Kaji, 1989; De Lange et al., 1989a, b, and Chung and Baker, 1992). Values observed in this study (Table VIII-5) fall within the range of those reported in the aforementioned studies. The difference in the estimates of endogenous protein between these studies may result from differences in diet composition, techniques used for estimation and other factors.

Estimates of the levels of endogenous amino acids in ileal digesta in this study are in general agreement with previous estimates reported by Holmes et al. (1974), Sauer et al. (1977), Taverner et al. (1981), Furuya and Kaji (1989), De Lange et al. (1989a,b), and Chung and Baker (1992). Of the indispensable amino acids, the three most abundant were threonine, leucine, and arginine; of the dispensable amino acids, the six most abundant in decreasing order, were proline, glycine, glutamic acid, aspartic acid, serine, and alanine. For the sulfur-containing amino acids, cysteine was more abundant than methionine. Differences in abundance among the endogenous amino acids in ileal digesta result mainly from differences in their concentrations in the various endogenous secretions into the digestive tract. Auclair (1986) concluded that the small intestinal secretions, which include mucins, supply the largest proportion of the endogenous N secretions. As was shown by Neutra and Forstner (1987), "native" mucin, which represents over 95% of mucin glycoprotein, is very rich in aspartic acid, alanine, glycine, glutamic acid, proline, serine, and threonine. Furthermore, studies by Corring and Jung (1972) and Pöhland et al. (1993) showed a relatively high abundance of aspartic acid, glutamic acid, and leucine in porcine pancreatic juice. Although the contents of sulfur-containing amino acids in both mucins and pancreatic juice were usually lower than those of the other amino acids, the abundance of cysteine was higher than that of methionine in both sources of secretions (e.g., Corring and Jung, 1972; Neutra and Forstner, 1987; Pöhland et al., 1993). Glycine, a major constituent base of the bile salt conjugates accounts for more than 90% of the total amino acids secreted in porcine bile juice (Souffrant, 1991). These bile salt conjugates are degraded in the distal

ileum by intestinal bacteria; 90% of the salts is re-absorbed via active transport and enters the enterohepatic circulation. However, the deconjugated glycine escapes re-absorption and enters the large intestine (Newsholme and Leech, 1984; Shiau, 1987).

Effect of Dietary Levels of Amino Acids on True Ileal Digestibilities of Amino Acids

The slope of the linear regression equation provides a direct estimation of true ileal amino acid digestibility. True ileal digestibilities of amino acids in SBM and their corresponding standard errors were determined directly from the regression equations presented in Table 3. In order to examine the effect of dietary levels of amino acids on true digestibilities, the true digestibilities were determined from their respective apparent ileal digestibilities at different dietary levels (Table VIII-6). With the exception of arginine, aspartic acid, and glutamic acid, small but significant curvilinear effects ($P < .05$) of dietary levels of amino acids on the true ileal digestibilities were observed. However, these effects actually resulted from the large differences in the true ileal digestibilities between the dietary levels at 4 and 8% CP. Furthermore, the true ileal digestibilities of amino acids, determined from their apparent values at higher dietary levels (12, 16, 20, and 24% CP), were relatively constant and close to the values directly determined with regression analysis. Whereas, the true ileal digestibilities determined from the apparent values at the lower dietary levels (4 and 8% CP) were quite variable. The effect of dietary levels of amino acids on their respective apparent and true ileal digestibilities are illustrated for MET in Figure VIII-3. The quadratic with plateau relationships between dietary amino acid levels and their respective apparent ileal digestibilities and the determination of the plateau digestibilities were previously described in Chapter V. The true ileal digestibilities of amino acids seem to be independent of their dietary contents (Table VIII-6).

It can be implied from the previous discussion that the true ileal digestibilities of amino acids, determined from their apparent ileal values at high dietary levels (12, 16, 20, and 24% CP), are more reliable than values measured at low dietary levels. As the

contributions of endogenous amino acids decrease curvilinearly with increasing dietary levels (Figure VIII-2), the effect of endogenous amino acids on apparent ileal digestibilities is considerably reduced at the higher dietary levels. Therefore, the true ileal digestibilities of amino acids should be determined from the valid apparent digestibilities measured at higher dietary levels, ideally from the plateau apparent ileal amino acid digestibilities.

In conclusion, there were linear relationships ($P < .0001$) between dietary contents of apparent ileal digestible and total amino acids, irrespective of ranges of different graded dietary levels of amino acids. Differences in ranges of graded dietary levels of amino acids resulted in large differences between the estimated amounts of endogenous amino acids. Furthermore, it seems that the amount of endogenous amino acids, as g/kg DM intake, is constant at different dietary amino acid levels, while contributions of endogenous amino acids, as a percentage of total dietary content, decrease curvilinearly with increasing dietary contents. Therefore, the true digestibilities of amino acids seem to be independent of their respective dietary levels.

Table VIII-1. The dietary levels (g/kg DM) of CP and amino acids in the experimental diets

	Dietary CP levels, %					
Items	4	8	12	16	20	24
CP	44.06	88.16	130.35	178.77	224.26	272.80
Amino acids						
Indispensable						
Arginine	3.01	5.83	8.99	11.78	14.72	18.40
Histidine	1.10	2.08	3.29	4.21	5.35	6.58
Isoleucine	2.02	4.03	6.13	8.06	10.07	12.57
Leucine	3.31	6.54	9.94	12.91	16.21	19.86
Lysine	2.51	5.17	7.82	10.18	12.63	15.52
Methionine	.70	1.40	2.10	2.80	3.50	4.30
Phenylalanine	2.18	4.46	6.66	8.80	11.09	13.75
Threonine	1.65	3.29	4.96	6.47	8.23	9.91
Valine	2.08	4.15	6.20	8.13	10.15	12.55
Dispensable						
Alanine	1.79	3.73	5.48	7.18	8.91	10.84
Aspartic acid	4.95	9.76	14.82	19.29	24.34	29.94
Cysteine	.70	1.40	2.10	2.70	3.40	4.10
Glutamic acid	8.39	16.65	25.12	33.30	41.27	51.58
Glycine	1.67	3.39	5.19	6.72	8.44	10.42
Serine	2.17	4.48	6.73	8.80	11.07	13.44
Tyrosine	1.55	2.97	4.47	5.71	7.59	9.27

Table VIII-2. The contents (g/kg DM intake) of apparent ileal digestible CP and amino acids in the experimental diets

	Dietary CP levels, %						
Items	4	8	12	16	20	24	SEM[a]
CP[b]	25.43	62.60	103.28	150.17	193.33	233.50	1.62
Amino acids[b]							
Indispensable							
Arginine	2.38	4.97	8.16	11.09	13.95	17.47	.05
Histidine	.83	1.64	2.93	3.84	4.87	5.95	.09
Isoleucine	1.53	3.29	5.33	7.18	9.06	11.37	.05
Leucine	2.49	5.33	8.57	11.44	14.53	17.79	.08
Lysine	1.94	4.29	6.85	9.15	11.46	13.90	.09
Methionine	.51	1.20	1.86	2.48	3.20	3.83	.02
Phenylalanine	1.72	3.81	5.90	8.05	10.24	12.62	.06
Threonine	.83	2.17	3.72	5.13	6.72	8.10	.07
Valine	1.43	3.21	5.14	6.97	8.86	11.08	.06
Dispensable							
Alanine	1.08	2.75	4.38	6.01	7.52	9.17	.08
Aspartic acid	3.53	7.83	12.62	16.75	21.47	26.02	.16
Cysteine	.38	.90	1.53	2.22	2.76	3.30	.04
Glutamic acid	6.71	14.45	22.48	30.32	37.56	45.53	.29
Glycine	.47	1.65	3.42	5.05	6.64	8.08	.14
Serine	1.32	3.30	5.50	7.47	9.67	11.53	.08
Tyrosine	1.18	2.45	3.90	5.10	7.00	8.44	.04

[a]Standard error of mean (n = 36).

[b]Linear effect ($P < .01$).

Table VIII-3. The linear relationships[a] between dietary contents of apparent ileal digestible and total CP and amino acids

Items	Regression equations[b] [c]	r^2	S_{yx}	Probability[d]	
CP	Y = -16.52 + .8968X	.99	5.55	.0001	.0001
Amino acids					
Indispensable					
Arginine	Y = -.64 + .9622X	.99	2.22	.0001	.0001
Histidine	Y = -.23 + .9231X	.99	.09	.0001	.0001
Isoleucine	Y = -.46 + .9208X	.99	2.20	.0001	.0001
Leucine	Y = -.69 + .9051X	.99	2.64	.0001	.0001
Lysine	Y = -.47 + .9069X	.99	2.52	.0001	.0001
Methionine	Y = -.13 + .9359X	.99	.06	.0001	.0001
Phenylalanine	Y = -.31 + .9079X	.99	2.62	.0029	.0001
Threonine	Y = -.69 + .8655X	.99	2.15	.0001	.0001
Valine	Y = -.54 + .8978X	.99	1.91	.0001	.0001
Dispensable					
Alanine	Y = -.59 + .8831X	.99	2.24	.0001	.0001
Aspartic acid	Y = -.90 + .8815X	.99	.48	.0001	.0001
Cysteine	Y = -.20 + .8573X	.99	.10	.0001	.0001
Glutamic acid	Y = -1.06 + .8829X	.99	.94	.0031	.0001
Glycine	Y = -1.17 + .8763X	.98	4.21	.0001	.0001
Serine	Y = - .68 + .8916X	.99	.22	.0001	.0001
Tyrosine	Y = - .36 + .9270X	.99	1.11	.0001	.0001

[a]Established from the range of graded dietary levels of CP from 4 to 24%.

[b]Y = dietary contents of apparent ileal digestible CP and amino acids (g/kg DM intake).

[c]X = dietary contents of CP and amino acids (g/kg DM).

[d]The probabilities for the intercepts and slopes of the regression equations (n = 36).

Table VIII-4. Effect of different ranges of graded dietary levels of CP and amino acids on the estimation of the amounts[a] (g/kg DM intake) of endogenous CP and amino acids in ileal digesta

Range[b]	[illegible]	4-20	4-16	4-12	[illegible]	[illegible]	[illegible]	12-24	12-20	16-24
n[c]	[illegible]	30	24	18	30	24	18	24	18	18
CP	[illegible]	17.4±2.2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]±4.7	[illegible]	[illegible]	[illegible]
Amino acids										
Indispensable										
Arginine	[illegible]	.75± .09	.77± .10	[illegible]	.70± [illegible]	1.19± .12	1.17± .16	.40± .19	[illegible]	[illegible]
Histidine	[illegible]	[illegible]± .03	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]± .07	.18± .08	[illegible]± .10	[illegible]
Isoleucine	[illegible]	[illegible]± .09	[illegible]± .07	[illegible]± .07	[illegible]	[illegible]± .14	[illegible]± .14	[illegible]	1.0[illegible]± .30	1.34± [illegible]
Leucine	[illegible]± .10	[illegible]± .11	[illegible]	[illegible]± .11	[illegible]	.87± [illegible]	[illegible]	[illegible]	[illegible]	.71± .48
Lysine	.47± .10	.51± .09	[illegible]± .10	[illegible]± .10	[illegible]	[illegible]	[illegible]	.31± [illegible]	[illegible]	[illegible]
Methionine	[illegible]	[illegible]	[illegible]	[illegible]	.14± .04	[illegible]± .04	[illegible]	.11± [illegible]	.11± .07	[illegible]± .11
Phenylalanine	[illegible]± .10	[illegible]± .09	[illegible]	[illegible]± .10	[illegible]	[illegible]	[illegible]± .15	[illegible]	[illegible]	.44± [illegible]
Threonine	[illegible]	.72± [illegible]	.70± [illegible]	[illegible]	[illegible]	.85± .13	[illegible]	[illegible]	.77± [illegible]	[illegible]± .40
Valine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]± .11	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Dispensable										
Alanine	[illegible]	[illegible]± .09	[illegible]	[illegible]± .09	.71± .14	.70± [illegible]	.74± [illegible]	[illegible]	[illegible]± .30	.79± [illegible]
Aspartic acid	[illegible]	[illegible]	[illegible]± .15	[illegible]± .17	[illegible]	1.19± [illegible]	[illegible]	[illegible]± .48	[illegible]	[illegible]
[illegible]	[illegible]± .04	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]± .10	[illegible]± .16	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]± .48	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]± .34	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[a]Estimate ± standard error.

[b]Ranges of graded dietary levels of CP and amino acids.

[c]Number of observations.

Table VIII-5. The amounts and contributions[a] of endogenous CP and amino acids in ileal digesta

		Dietary CP levels, %					
Items	4-24%[b]	4	8	12	16	20	24
CP	16.5	37.5	18.7	12.7	9.2	7.4	6.1
Amino acids							
Indispensable							
Arginine	.64	21.3	11.0	7.1	5.4	4.4	3.5
Histidine	.23	20.9	11.1	7.0	5.5	4.3	3.5
Isoleucine	.46	22.8	11.4	7.5	5.7	4.6	3.7
Leucine	.69	20.9	10.6	6.9	5.3	4.3	3.5
Lysine	.47	18.7	9.1	6.0	4.6	3.7	3.0
Methionine	.13	18.6	9.3	6.2	4.6	3.7	3.0
Phenylalanine	.31	14.2	7.0	4.7	3.5	2.8	2.3
Threonine	.69	41.8	21.0	13.9	10.7	8.4	7.0
Valine	.54	26.0	13.0	8.7	6.6	5.3	4.3
Dispensable							
Alanine	.59	33.0	15.8	10.8	8.2	6.6	5.4
Aspartic acid	.90	18.2	9.2	6.1	4.7	3.7	3.0
Cysteine	.20	28.6	14.3	9.5	7.4	5.9	4.9
Glutamic acid	1.06	12.6	6.4	4.2	3.2	2.6	2.1
Glycine	1.17	70.1	34.5	22.5	17.4	13.9	11.2
Serine	.68	31.3	15.2	10.1	7.7	6.1	5.1
Tyrosine	.36	23.2	12.1	8.1	6.3	4.7	3.9

[a]The contributions of endogenous CP and amino acids (percentage) = [(amount of endogenous CP or amino acids, g/kg DM intake) / (contents of dietary CP or amino acids, g/kg DM)] x 100%.

[b]The amounts (g/kg DM intake) of endogenous CP and amino acids extrapolated from the range of graded dietary levels of 4 to 24% CP from the equations presented in Table VIII-3.

Table VIII-6. Effect of dietary levels of CP and amino acids on the true ileal digestibilities of CP and amino acids in SBM

Items	Dietary CP levels, %						SEM[a]	Linear[b]
	4	8	12	16	20	24		
CP[c]	83.3	87.2	90.1	90.9	91.6	89.4	1.44	+++ ±1.1
Amino acids								
Indispensable								
Arginine	85.3	91.6	95.8	96.2	96.1	95.0	.	+ . ± . +
Histidine[g]	85.7	89.3	93.2	92.8	93.9	92..	.99	+.. ± . +
Isoleucine[g]	85.4	89.8	93.1	93.2	93.6	93..	.91	+.. ± . .
Leucine[f]	84.2	88.9	93.2	93.7	93.8	93.	..	+ .. ± .
Lysine[f]	83.2	88.8	93.7	93.6	93.2	92.3	.7.	+ . ± .
Methionine[f]	84.9	91.6	94.2	94.3	95.9	93.7	.9.	+ .. ± .
Phenylalanine[f]	85.2	89.8	93.4	94.2	93.9	93.	..	+ . ± .
Threonine[g]	80.3	84.7	88.9	87.3	87.4	86.7	1..	++ .. ± . .
Valine[f]	83.9	87.9	91.2	90.9	90.8	9. .	. 1	. . ±
Dispensable								
Alanine[g]	83.1	88.4	93.9	94.3	93.4	9..1	1. .	+ . ± .
Aspartic acid[g]	84.9	88.8	93.1	93.4	94.3	93.7	.9.	+ . ± .
Cystine[f]	75.4	82.4	86.7	87.4	86.1	85.1	1..	++ .. ± . .
Glutamic acid[g]	91.7	93.9	96.9	96.7	96.7	96..	. .	. ± . .
Glycine[g]	81.9	87.4	91.1	93.7	94..	92.	..1	. . ±
Serine[g]	80.4	87.0	89.7	93.9	93..	9..	. .	+ . ± . .
Tyrosine[g]	86.8	91.1	95.9	96.8	96.4	9..7	..	+ . ± . .

[a]Standard error of mean (n = 6).

[b]True ileal CP and amino acid digestibilities estimated directly with regression analysis from the range of graded dietary levels of 4 to 24% CP from the regression equations presented in Table VIII-3.

[c]Linear effect ($P < .05$).

[d]Quadratic effect ($P < .05$).

[e]Cubic effect ($P < .05$).

[f]Quartic effect ($P < .05$).

[g]Quintic effect ($P < .05$).

Figure VIII-1. Extrapolation of endogenous MET (g/kg DM intake) in ileal digesta from the linear relationship between dietary content of apparent ileal digestible (Y: g/kg DM intake, means ± SE) and total MET (X: g/kg DM) derived from the range of graded dietary levels of 4 to 24% CP.

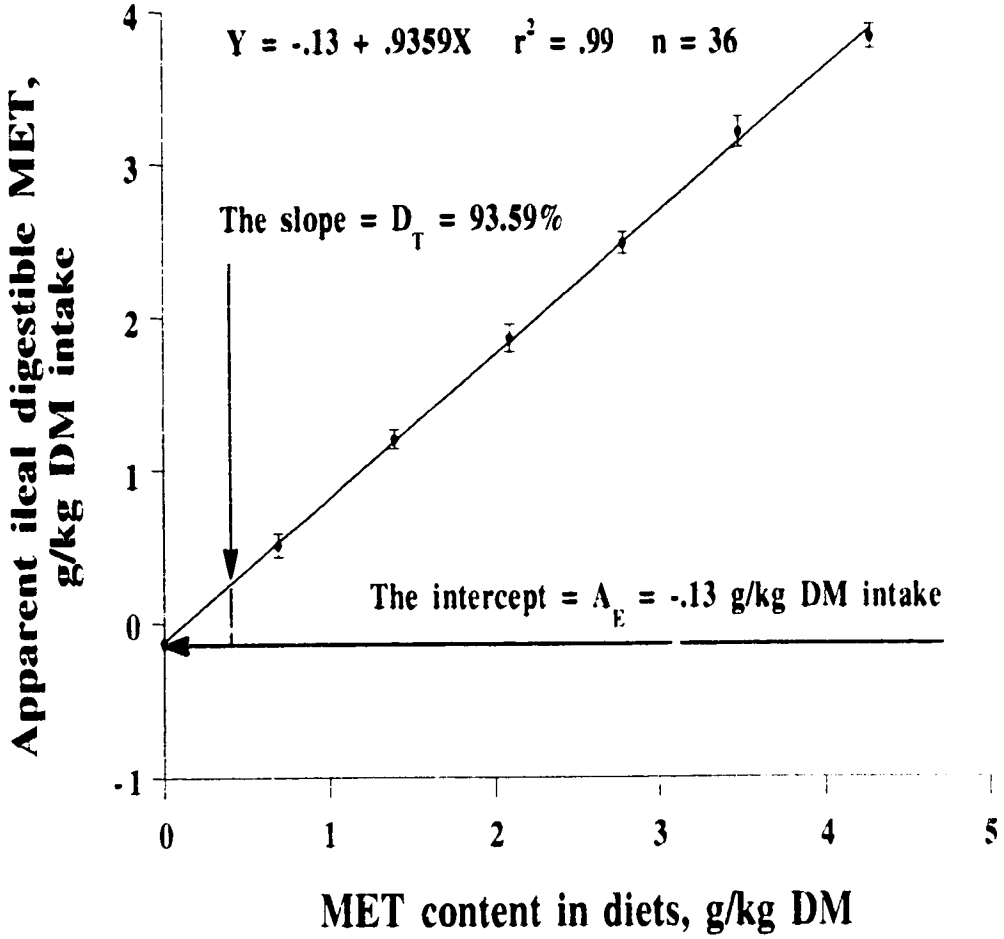

Y = -.13 + .9359X r² = .99 n = 36
The slope = D_T = 93.59%
The intercept = A_E = -.13 g/kg DM intake
Apparent ileal digestible MET, g/kg DM intake
MET content in diets, g/kg DM
4
3
2
1
0
-1
0
1
2
3
4
5

Figure VIII-2. Relationship between dietary MET content (percentage, DM basis) and the contribution (percentage) of endogenous MET in ileal digesta.

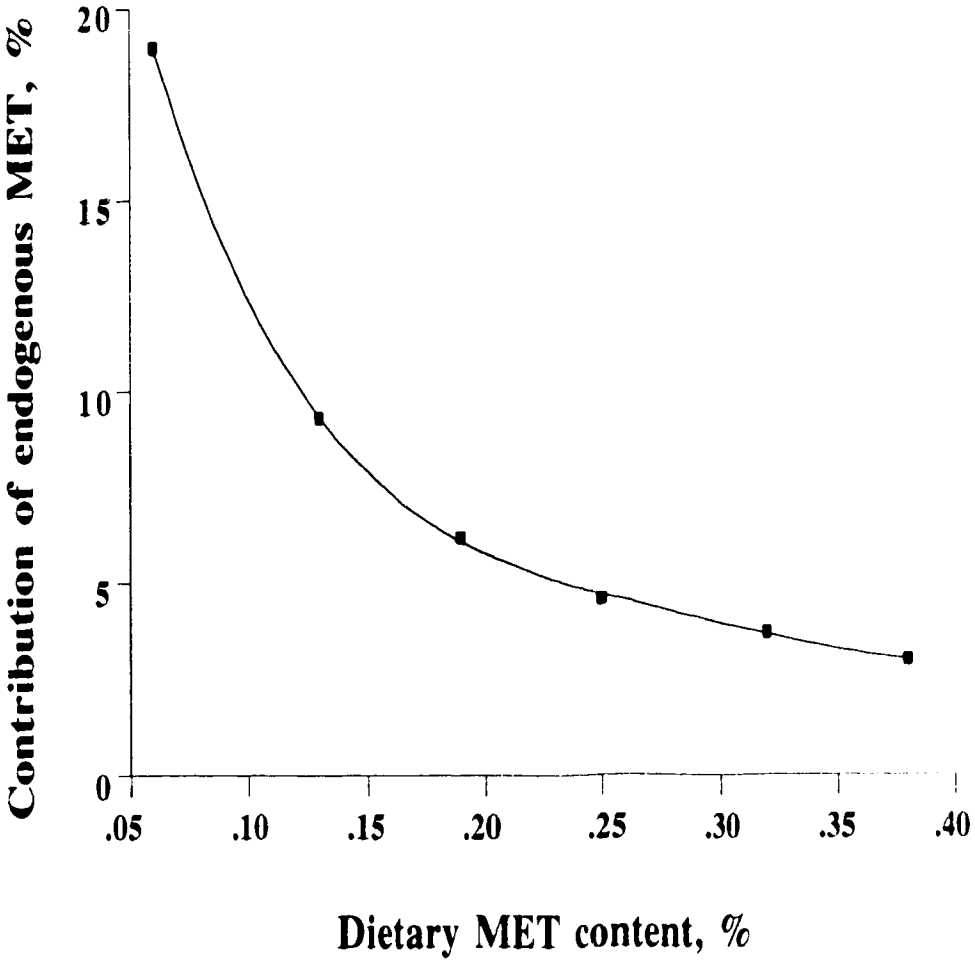
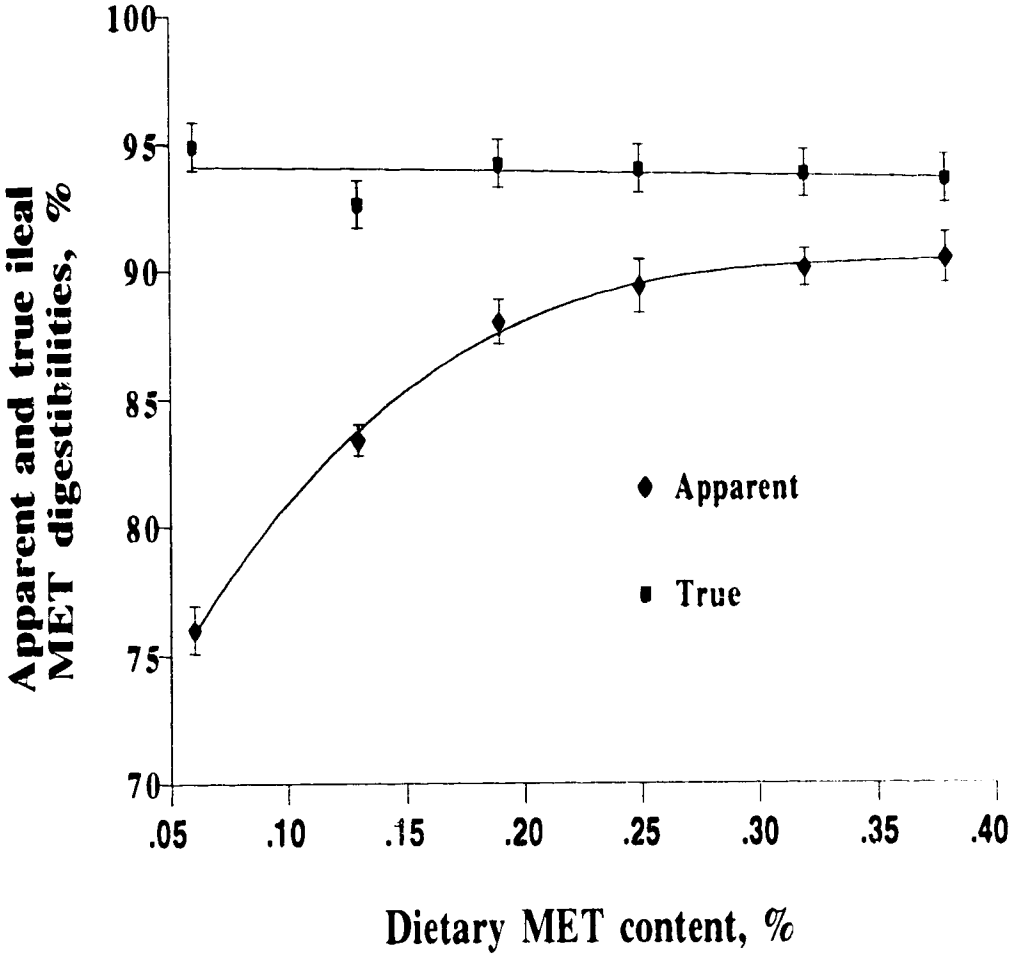

Contribution of endogenous MET, %
20
15
10
5
0
.05
.10
.15
.20
.25
.30
.35
.40
Dietary MET content, %

Figure VIII-3. Relationship between dietary MET content (percentage, DM basis) and apparent and true ileal digestibilities of MET (percentage) in SBM.


Apparent and true ileal MET digestibilities, %
100
95
90
85
80
75
70
.05
.10
.15
.20
.25
.30
.35
.40
Apparent
True
Dietary MET content, %

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CHAPTER IX

GENERAL DISCUSSION AND CONCLUSIONS

Amino acid digestibilities are the most important single determinant of the utilization of amino acids in feedstuffs by pigs. As such, amino acid digestibilities are widely used to estimate amino acid bioavailability in feedstuffs for pigs (e.g., Tanksley and Knabe, 1984; Sauer and Ozimek, 1986). Amino acid requirements determined on the basis of digestible rather than total supply should lead to more accurate formulation of diets and improved efficiency of utilization of dietary amino acids in swine production.

Sufficient evidence suggests that the ileal rather than the fecal analysis method should be used for the determination of amino acid digestibility in feedstuffs for pigs because the modifying action of the microflora in the large intestine on amino acids usually results in overestimation of amino acid digestibility (e.g., Zebrowska, 1973; Low, 1982). However, measurements obtained with the ileal analysis method are often confounded by the presence of non-reabsorbed endogenous amino acids. Therefore, amino acids digestibilities determined with the ileal analysis method, without correction for ileal endogenous amino acid contributions, are referred to as apparent ileal amino acid digestibilities. In principle, true ileal amino acid digestibilities, determined from the corresponding apparent values by correcting for the endogenous amino acid contributions, should be determined in feedstuffs for pigs.

Although several techniques for quantifying endogenous amino acids in ileal digesta of pigs are developed, a suitable technique has not yet been validated. As a result, the apparent ileal amino acid digestibilities in feedstuffs are currently used in diet formulation. There is considerable information in the literature on apparent rather than true ileal amino acid digestibilities in feedstuffs for pigs. It is hardly surprising to recognize large

differences in the apparent amino acid digestibilities between feedstuffs. However, it comes somewhat as a surprise to notice large differences in the digestibilities between samples of the same feedstuff (in name), evident by the relatively large standard deviations (Sauer et al., 1990). This considerable variability may result from inherent factors or the methodology used to determine ileal digestibility, and the conditions and manner in which the feedstuffs were processed.

In fact, with the exception of soybean meal, only a limited number of samples for each feedstuffs have been evaluated simutaneously under the same experimental conditions. Possible inherent factors responsible for the variation in apparent ileal amino acid digestibilities among different samples of same feedstuff (in name) were not identified in most feedstuffs.

On the other hand, as was suggested by Sauer et al. (1990), large variability within the same feedstuff may, result, in part, from differences in the methodological aspects, i.e. methods used to determine amino acid digestibility and levels of amino acids in the assay diets. The possible variation in the amino acid digestibilities, associated with these two methodological aspects contributes, in part, to the experimental error and should be controlled through appropriate use of experimental methodology. Furthermore, because true ileal amino acid digestibilities are usually determined from their corresponding apparent values, accurate determination of apparent ileal digestibilities of amino acids is also important for the determination of valid true ileal amino acid digestibilities in feedstuffs.

Additivity of apparent ileal amino acid digestibilities, determined in single feedstuffs, is a crucial consideration in the formulation of diets for pigs. However, there is a scarcity of information in the literature in this regard. Furthermore, the information that is available, as reported by Imbeah et al. (1988) and Furuya and Kaji (1991), is contradictory. A study was therefore conducted to investigate this fundamental issue.

True rather than apparent ileal amino acid digestibilities should be determined in feedstuffs for pigs. The key issue for determination of true ileal amino acid digestibilities is

to quantify the levels of endogenous amino acids in digesta collected from the distal ileum. The feeding of protein-free diets is the classical technique for determining the amount of endogenous amino acids (e.g., Carlson and Bayley, 1970). However, because the measurements are carried out under protein-free feeding conditions, results obtained with this technique are not valid for the purpose of determination of true ileal amino acid digestibilities. A differentiation can be made between non-digested dietary and endogenous protein using the ^{15}N-isotope dilution techniques (e.g., Souffrant et al., 1981; De Lange et al., 1990; Lien et al., 1993). However, several questions have arisen concerning the methodology of ^{15}N-isotope dilution technique (De Lange et al., 1990, 1992; Lien et al., 1993) and this technique is not yet suitable for routine use. Alternatively, linear relationships between apparent ileal digestible and dietary amino acid contents can be used to estimate the levels of endogenous amino acids under normal dietary conditions in pigs (Furuya and Kaji, 1986, 1989). However, this technique has also been questioned, as it is not clear yet whether dietary levels of amino acids can influence the amount of endogenous amino acids and whether this amount and the linear relationships are affected by differences in ranges of graded dietary amino acid levels (De Lange et al., 1989; Souffrant, 1991). Therefore, studies were conducted to validate this technique for the determination of true ileal amino acid digestibilities in feedstuffs for pigs.

A. Factors Responsible for Variability in Apparent Ileal Amino Acid Digestibility among Different Samples of the Same Feedstuff

Three experiments were carried out to investigate the variability in apparent ileal digestibilities of amino acids in six pea, six canola meal, and six wheat samples, respectively.

As was discussed in Chapter II, with the exception of arginine, cysteine, histidine, proline, and methionine, the apparent ileal digestibilities of amino acids were different ($P < .05$) among the pea samples. The ileal digestibilities of amino acids, with the exception of arginine, cysteine, proline, and tryptophan, were negatively correlated ($P < .05$) with the NDF content in the pea samples. These results suggest that differences in NDF were partly responsible for the variability in the digestibilities of the majority of amino acids among the pea samples. Gdala et al. (1992) also reported negative correlation between NDF content and amino acid digestibilities in peas. The mechanism(s) by which differences in NDF content contribute to the variability in amino acid digestibilities among pea samples is not well defined. It is possible that NDF is associated with protein in peas, making it resistant to hydrolysis by the proteolytic enzymes. Therefore, differences in the amount of indigestible protein associated with NDF among the pea samples may be linked to the variation in digestibilities. Furthermore, of all the amino acids, only the digestibility of tryptophan was negatively correlated ($P < .05$) with the trypsin inhibitor activity in the pea samples. The mechanism by which trypsin inhibitors exert their effect on tryptophan digestibility is not clear. However, further studies on the distribution of tryptophan in peas may provide an answer. On the other hand, with the exception of arginine, cysteine, and proline, the apparent ileal digestibilities of the amino acids were positively correlated ($P < .05$) with their respective dietary levels. The study in Chapter V showed that apparent ileal digestibilities of amino acids were quadratically affected by their dietary levels. Therefore, differences in the dietary levels of amino acids were also, in part, responsible for the variability in amino acid digestibilities among the pea samples. Further research should be conducted to identify the distribution of amino acids in peas, e.g., hulls, cotyledons, and amino acid digestibilities in these fractions.

As investigated in Chapter III, with the exception of proline, there were differences ($P < .05$) in the digestibilities of all amino acids among the canola meal samples. The apparent ileal digestibilities of amino acids, with the exception of arginine, were negatively

correlated ($P < .05$) with the NDF content in the canola meal samples. These results indicate that differences in the NDF content were, in part, responsible for the variability in the digestibilities of amino acids among the canola meal samples. The NDF fraction comprises cell wall material, including cellulose, hemicellulose, and lignin (Goering and Van Soest, 1970). Pectins, which are soluble in water and neutral detergent, are not included in the NDF fraction. As pectins are present in both hulls and cotyledons (Naaczk and Shadidi, 1990), one can postulate that there may be a positive correlation between the NDF and pectin content in hulls. Water-insoluble fiber, i.e. NDF, can decrease apparent ileal amino acid digestibilities by increasing the recovery of endogenous amino acids at the distal ileum (e.g., Li et al., 1994). Studies by Anderson et al. (1990) showed that inclusion of water-soluble fibers, including pectins, decreased the digestion and absorption of nutrients. In addition, fiber components contained in the protein-containing feedstuffs, such as these in canola meal, can decrease amino acid digestibilities by physically hindering the access of proteolytic enzymes to proteins and by directly binding to proteins. Therefore, differences in NDF and pectin contents among the canola meals may, in part, result in the variability in the digestibilities of amino acids through the aforementioned mechanisms. Furthermore, the variability in amino acid digestibilities may also, in part, be related to differences in the content of hulls among the canola meals, because hulls contain a large proportion of protein which is of very low digestibility (Cichon and Sauer, 1980; Bell and Shires, 1982; Bell, 1993). Further research should be conducted to clarify effect of pectin content in canola meal on amino acid digestibilities. In addition, more information regarding amino acid composition and digestibilities in different fractions of canola meal, i.e. in hulls and cotyledons, and the relationship between the content of NDF and hull fraction in canola meal samples will be useful to predict the variability of amino acid digestibilities among canola meal samples. On the other hand, the apparent ileal digestibilities of amino acids, with the exception of aspartic acid, glycine, proline, serine, threonine, tryptophan, and valine, were positively correlated ($P < .05$) with their dietary

levels. Therefore, differences in the dietary levels of these amino acids were also, in part, responsible for the variability in the digestibilities among the canola meal samples as was demonstrated in Chapter V.

With respect to the studies on wheat samples in Chapter IV, there were differences ($P < .05$) in the apparent ileal digestibilities of all amino acids among the wheat samples. With the exception of arginine, glutamic acid, proline, and tyrosine, the digestibilities of the remaining amino acids were negatively correlated ($P < .05$) with the NDF content in the wheat samples. These results suggest that the differences in NDF content among the wheat samples were, in part, responsible for the variation in the digestibilities of amino acids. The variability in amino acid digestibilities in association with differences in the NDF content among the wheat samples may result from their differences in aleurone layer contents. Further studies should be carried out to determine the contents and digestibilities of amino acid in different fractions of wheat, i.e. the fractions of aleurone layer and endosperm. Furthermore, although there were differences in the dietary levels of amino acids among the wheat diets, the apparent ileal digestibilities of amino acids were not correlated ($P < .05$) with their respective dietary contents. These results indicate that differences in NDF content largely dominate the variability in the amino acid digestibilities among the wheat samples. The effect of dietary levels of amino acids on digestibilities becomes negligible in this case.

B. Methodological Aspects for the Determination of Apparent Ileal Amino Acid Digestibility in Feedstuffs

Effect of experimental dietary levels of amino acids on the determination of apparent ileal digestibilities of amino acids is a major methodological consideration. Surprisingly, this effect has been simply neglected in many studies reported in the literature regarding the determination and comparison of apparent ileal digestibilities of amino acids in feedstuffs. In Chapter V, effect of dietary levels of amino acids on their respective apparent ileal

digestibilities was investigated with six corn starch based soybean meal "model" diets containing six levels of amino acids. Results from this study demonstrated that dietary amino acid level quadratically affected ($P < .05$) their respective apparent ileal digestibilities until the plateau digestibilities were reached. The plateau apparent ileal digestibilities of amino acids, which are independent of dietary amino acid contents, should be determined in feedstuffs. Differences in amino acid contents in the assay diet explained, in part, the variation in their respective apparent ileal digestibilities, especially of the limiting amino acids, among different samples of the same feedstuff (in name) that were determined in Chapters II, III, and reported in the literature (e.g., Sauer and Ozimek, 1986).

Inappropriate use of different methods may result in variation in the apparent ileal digestibilities of amino acids within the same feedstuff. In Chapter VI, effect of the direct, difference, and regression method on the determination of apparent ileal amino acid digestibilities was investigated with barley and canola meal, representing low- and high-protein feedstuffs, respectively. Different determination methods did result in variation in the amino acid digestibilities in both types of feedstuffs. Amino acid digestibilities in feedstuffs with a low protein content should be determined with difference and regression method rather than with the direct method. Amino acid digestibilities in feedstuffs with a high protein content can be determined with either method.

C. Additivity of Apparent Ileal Amino Acid Digestibility Determined in Single Feedstuffs

The additivity of apparent ileal digestibilities of amino acids determined in single feedstuffs were investigated with barley, wheat, canola meal, and soybean meal and their mixtures in Chapter VII. There were no differences ($P < .05$) between the directly determined and calculated apparent ileal amino acid digestibilities in the diet mixtures. These results show that the apparent ileal digestible amino acid supply in a mixture of

feedstuffs can be predicted from amino acid digestibilities determined in single feedstuffs. However, it can not be ruled out that some dietary factors including fat, fiber, and antinutritive factors may affect the additivity of apparent ileal digestibilities of amino acids determined in single feedstuffs. Furthermore, true ileal amino acid digestibilities determined in single feedstuffs, which are independent of endogenous amino acid contributions, should be more additive than their apparent values. Therefore, true ileal amino acid digestibilities should be eventually determined in feedstuffs for pigs.

D. Methodological Considerations for Estimating True Ileal Amino Acid Digestibility in Feedstuffs by Regression Analysis

Results of the final study in Chapter VIII demonstrated that there were linear relationships ($P < .05$) between the dietary contents of apparent ileal digestible and total amino acids, irrespective of the ranges in graded dietary levels of amino acids. The amount of endogenous amino acids in the ileal digesta can be reliably determined with the linear relationships. Differences in ranges of graded dietary levels of amino acids did result in large variation in the estimated amount of endogenous amino acids. Therefore, appropriate design of the graded dietary levels of amino acids is an important methodological consideration. Furthermore, the amounts of endogenous amino acids in ileal digesta is constant at different dietary amino acid levels. Therefore, true ileal amino acid digestibilities appear to be independent of the respective dietary amino acid levels. This regression analysis technique seems to be very promising for the determination of true ileal amino acid digestibilities.

E. General Conclusions

In conclusion:

1. there was large variability in the apparent ileal digestibilities of amino acids among six pea, six canola meal, and six wheat samples, respectively.

2. differences in the NDF content among different samples within the same feedstuff were inherently, in part, responsible for the variation.

3. differences in the dietary levels of amino acids were also methodologically, in part, responsible for the variation.

4. differences in dietary levels of amino acids were usually, in part, responsible for the variability in apparent ileal digestibilities of amino acids among different samples of the same feedstuff (in name) reported in the literature. Therefore, dietary level of amino acids is an important methodological consideration for the determination of apparent ileal amino acid digestibilities.

5. Apparent ileal amino acid digestibilities in feedstuffs with a low protein content should be determined with the difference or regression method rather than with the direct method. Apparent ileal amino acid digestibilities in feedstuffs with a high protein content can be determined with either method, i.e. the direct, difference, and regression method.

6. The apparent ileal digestibilities of amino acids determined in single feedstuffs are additive when used in the formulation of complete diets.

7. Both quantities of endogenous amino acids in ileal digesta and true ileal amino acid digestibilities in feedstuffs can be reliably determined with the linear relationships between dietary contents of apparent ileal digestible and total amino acids. Appropriate design of the graded dietary levels of amino acids is an important methodological consideration for using this regression analysis technique.

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