

**Standardized and apparent ileal digestibility of protein and amino acids
in several feed ingredients fed to broilers**

By:

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Submitted to the Faculty of Graduate Studies

In Partial Fulfillment of the Requirement

For the Degree of

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Winnipeg, Manitoba

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**Standardized and Apparent ileal Digestibility of Protein and Amino Acids
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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of

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Of

Master of Science

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ABSTRACT

Five experiments were conducted to determine the apparent and standardized ileal digestibilities (AID and SID, respectively) of protein and amino acids (AA) in several unprocessed and processed feed ingredients fed to broilers.

Using direct or indirect method of estimating AA digestibility, day-old male Ross 308 broiler chicks were fed test diets balanced for vitamins and minerals, containing chromic oxide (0.3%) as a digestibility marker.

The average SID estimates of CP in pea, DESBM, wheat, barley, PBPM (calculated as the average digestibility value of each AA presented), ENRMAXTM, wheat DDGS, FM (calculated as the average digestibility value of each AA presented), and flaxseed were 88.3, 88.2, 87.2, 79.7, 75.8, 73.4, 69.1, 64.7 and 55.0, respectively.

The average CV associated with SID estimates of AA was highest in flaxseed (16.2%) and feather meal (12.1%) and lowest in wheat (1.8%) and ENERMAXTM (2.3%) for unprocessed and processed feed ingredients assayed.

DEDICATION

This thesis is dedicated to my parents, Mr. M. M. Bandegan and Mrs. M. Mousavi,
and my love, Loabat, thanks for all your support.

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FOREWORD

This thesis was written in a manuscript format and is composed of five Manuscripts, Manuscript II was presented at the Scientific Poultry Congress in Atlanta (2008) and was recognized with an outstanding research award and Manuscript III was presented at the Poultry Science Centennial meeting 2008 in Niagara Falls. All manuscripts were written according to the guidelines of Poultry Science manuscript preparation. Authors to all manuscripts are A. Bandegan, W. Guenter, D. Hoehler and C.M. Nyachoti.

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LIST OF ABBREVIATIONS

AA	Amino acid(s)
AID	Apparent ileal digestibility
Ala	Alanine
ANF	Anti-nutritional factor
Arg	Arginine
Asp	Aspartic acid
CP	Crude protein
CV	Coefficient of variation
Cys	Cystine
DDGS	Distillers' dried grains with solubles
DESBM	Dry-extruded expelled soybean meal
DM	Dry matter
ENERMAX TM	Extruded canola seed-pea blend
Exp.	Experiment
FM	Feather meal
Glu	Glutamic acid
Gly	Glycine
His	Histidine
IM	Indigestible marker
Ile	Isoleucine
Leu	Leucine
Lys	Lysine

ME	Metabolizable energy
Met	Methionine
NSP	Non-starch polysaccharides
PBPM	Poultry by-product meal
Phe	Phenylalanine
Pro	Proline
Ser	Serine
SID	Standardized ileal digestibility
Thr	Threonine
Val	Valine

1.0 GENERAL INTRODUCTION

Over 2000 different products (varietal differences excluded) have been characterized to some extent for animal feed (Pond et al., 1995; Kellem and Church, 2002). Composition of a given feedstuff may vary widely due to differences in climate, soil conditions, maturity, cultivar, management and processing factors (Miller et al., 1964; Lilburn et al., 1991; Ravindran and Blair, 1991; Pond et al., 1995; Dale, 1996; Bath et al., 1998). The supply of AA and protein in poultry diets represent a significant cost of production. Therefore, it is essential to optimize the utilization of these nutrients. Furthermore, the environmental consequences of over formulating the protein component of a diet and the lower production levels in animals that are fed an under formulated diet, in terms of its protein, makes it of vital importance to have information regarding the amounts of AA in the protein sources that are available to the animal for metabolism.

Although the total AA profile of a feed is a decent indicator of its potential nutritive value, a proportion of these AA will not be completely digested and absorbed by the animal. Additionally, this proportion will vary between sources of protein and even between sources or batches of the same ingredient (Hendriks et al., 2002). Accurate information is required on the amounts of AA in protein sources that are available to the animal to allow for a precise formulation of the diets.

The term “availability” refers to the amount of a nutrient that is absorbed in a form suitable for utilization by the animal (Stein et al., 2007). Ileal digestibility values for an AA can be used as a sensitive indicator of its availability (Ravindran et al., 1999b; Garcia et al., 2007) as most of the AA are absorbed in their native chemical form which then

renders them available to be utilized by the animal (Johns et al., 1986; Parsons, 1986). In fact, diet formulation based on digestible compared to total AA of the feed has been shown to result in better bird performance (Green, 1987; Fernandez et al., 1995; Rostagno et al., 1995; Wang and Parsons, 1998a; Douglas and Parsons, 1999). However, AID values are affected by the level of protein ingested and endogenous losses (Lemme et al., 2004; Kluth et al., 2005).

Endogenous protein and AA losses in ileal digesta can be divided into non-specific (basal or diet-independent) and specific (diet dependent) fractions. The non-specific fraction is related to the dry matter intake but is independent of the type of feedstuff or diet. In contrast, the specific loss is related to the composition of the feedstuff or diet (e.g. presence of inherent factors such as ANF) (Lemme et al., 2004). Based on the correction for these endogenous losses, digestibility estimates can be expressed as apparent, true or standardized. Ideally, true digestibility values are the most accurate to be used as they consider both non-specific and specific losses due to the diet, hence reflecting a fundamental property of the feedstuff which is independent of experimental and dietary conditions. However, lack of adequate, consistent data on specific losses and technical problems associated with their measurement are the limiting factors in creating a data set based on true ileal digestibility values. On the other hand, AID values are simply defined as a measure of the difference between the protein and AA intake and output and are affected by both the level of protein ingested and endogenous losses. It has been suggested that a correction for non-specific AA recoveries in ileal digesta will result in values which are independent of the protein level or AA content of the diet termed as “standardized” ileal digestibility (Stein et al., 2007). The SID values have been shown to

be independent of dietary protein and more additive when used in a mixed diet allowing for a better prediction of animal performance (Angkanaporn et al., 1996; Stein et al., 2001; Stein et al., 2005). Although several methods and their merit in quantifying basal endogenous losses have been explained and discussed in detail (Nyachoti et al., 1997; Lemme et al., 2004; Ravindran et al., 2004; Golian et al., 2007), it has been proposed that basal losses determined by feeding a nitrogen-free diet to the birds can be used as a measure of minimum losses for the correction of AID values (Stein et al., 2007; Golian et al., 2008). Besides the method of estimation, it has been recognized that digestibility values are affected by the age of the bird (Ten Doeschate et al., 1993; Garcia et al., 2007), therefore estimates derived using adult birds are not applicable to growing broilers. Most of the available published data on AA digestibility estimates in feed ingredients have been determined in the excreta of adult roosters using the precision feeding assay of Sibbald (1979) or modifications of this assay (Parsons, 1985). Digestibility values based on excreta analysis have the advantage of simplicity over the values based on ileal digesta analysis because the birds need not be sacrificed and assays can be carried out on a large number of birds. Nonetheless, the variable and modifying effects of caecal microflora on protein digestion (Ravindran et al., 1999b) and the fact that excreta contains AA from both feces and urine have favored the analysis of ileal digesta as a preferred method when assessing AA digestibility in poultry (Ravindran and Bryden, 1999; Lemme et al., 2004).

Ileal digestibility of protein and AA can be estimated using different approaches such as the direct, difference and regression methods (Fan and Sauer, 1995a; Kluth et al., 2005). The decision as to which approach to use depends on the protein content of the feedstuff and its palatability (Mosenthin et al., 2000). In general, the feedstuffs that are

high in protein content can be evaluated using any of the three approaches yet the difference and regression approaches are suggested to be more suitable for feedstuffs that are low in protein content (Fan and Sauer, 1995a).

It is obvious that there are large differences in ileal protein and AA digestibility values between feedstuffs. In addition, there are also large differences in ileal AA digestibilities among different samples of the same feedstuff. The variation observed in apparent ileal protein and AA digestibility results can be explained in part due to the differences in feed samples, birds or methodology used. For an effective formulation of the diet it is important to account for and report these variables in order to lower the cost associated with wider safety margins. Reports regarding these variations in the literature are very scarce and the results are confounded by the lack of a clear explanation for the coefficient of variation (CV%) presented. As the simplicity of a corn-soybean meal diet is being displaced by the use of very variable and inexpensive alternative protein supplements (e.g. wheat DDGS, extruded products, etc.), it becomes increasingly important to have adequate information not only on protein and AA digestibility estimates but also on their associated CV to improve the precision of least cost diet formulation and to reduce a wide and costly safety margin.

Therefore, standardized and apparent ileal digestibility of protein and AA need to be determined in different feedstuffs using broilers as the experimental animals.

The overall objective of these studies were to create a data set of apparent and standardized protein and AA ileal digestibility in several feed ingredients in Canada and to account for and report their associated variation in broiler chickens.

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

The quality of protein in feed ingredients is largely determined by the total protein content and availability of the AA. Describing the AA profile of a feed ingredient in terms of digestible AA content is closer than total AA content in reflecting the amount that actually becomes available for maintenance and production purposes (Rostagno et al., 1995). Although, digestible AA values are becoming important as the basis of poultry feed formulation, some commercial nutritionists are still hesitant to formulate diets based solely on digestible estimates. The major reasons might include: a) a wide variation in published digestible AA values from different sources, arising from differences in sample, type of birds, assay diets and assay methodology; b) inadequate knowledge of batch to batch variation of AA digestibility values and c) limited published information on broiler responses to diets formulated on the basis of digestible AA (Bryden et al., 2000; Lemme et al., 2004). Availability of AA in feedstuffs is an important feature of protein quality in animal nutrition. Many attempts have been made to determine AA availability in feed ingredients, using in-vitro (enzymatic and chemical assays) or indirect (microbiological or plasma amino acids) or direct (growth and digestibility assays) methods (Ravindran and Bryden, 1999). Studies in this review were confined to the digestibility assay.

2.2 Availability versus digestibility

Despite the fact that the term “digestibility” is often used synonymously with “availability”, there is a distinct difference in their definitions. Availability is a function of two processes: digestion and metabolism whereas digestibility is defined as the net disappearance of the ingested dietary nutrient from the digestive tract (Stein et al., 2007).

The relationship between availability and digestibility is shown schematically in Figure 2.1 (Johnson, 1992). Amino acid digestibility relates to enzymatic hydrolysis and microbial fermentation of ingested protein and peptides and their absorption from the gastrointestinal lumen (Fuller, 2003). Digestion, fermentation and absorption involve nutrients that are of dietary origin or from endogenous secretions into the digestive tract. Ileal digestibility is a more accurate estimate of AA bioavailability than total tract digestibility because of two main reasons. First, because AA are absorbed only from the small intestine and second, because of the effect of hindgut fermentation on AA metabolism (Sauer and Ozimek, 1986). Therefore, for the practical purposes, digestibility estimates are being used as a sensitive indicator of AA availability in feed ingredients (Ravindran et al., 1999b; Garcia et al., 2007). Although several biological methods have been used to measure available protein (nitrogen balance, biological value, net protein utilization and protein efficiency ratio) digestibility assays have become the most favored techniques for estimating availability, mainly because the values apply directly to the bird and all AA can be measured in one assay.

2.3 DIGESTIBILITY ASSAYS

Digestibility assays have become the most common approach for estimating AA availability. This assumes that the difference between the input and output is a valid indicator of bioavailability such that digestibility is likely the rate limiting step in AA availability (Johnson, 1992). Digestibility assays are divided into excreta and ileal procedures.

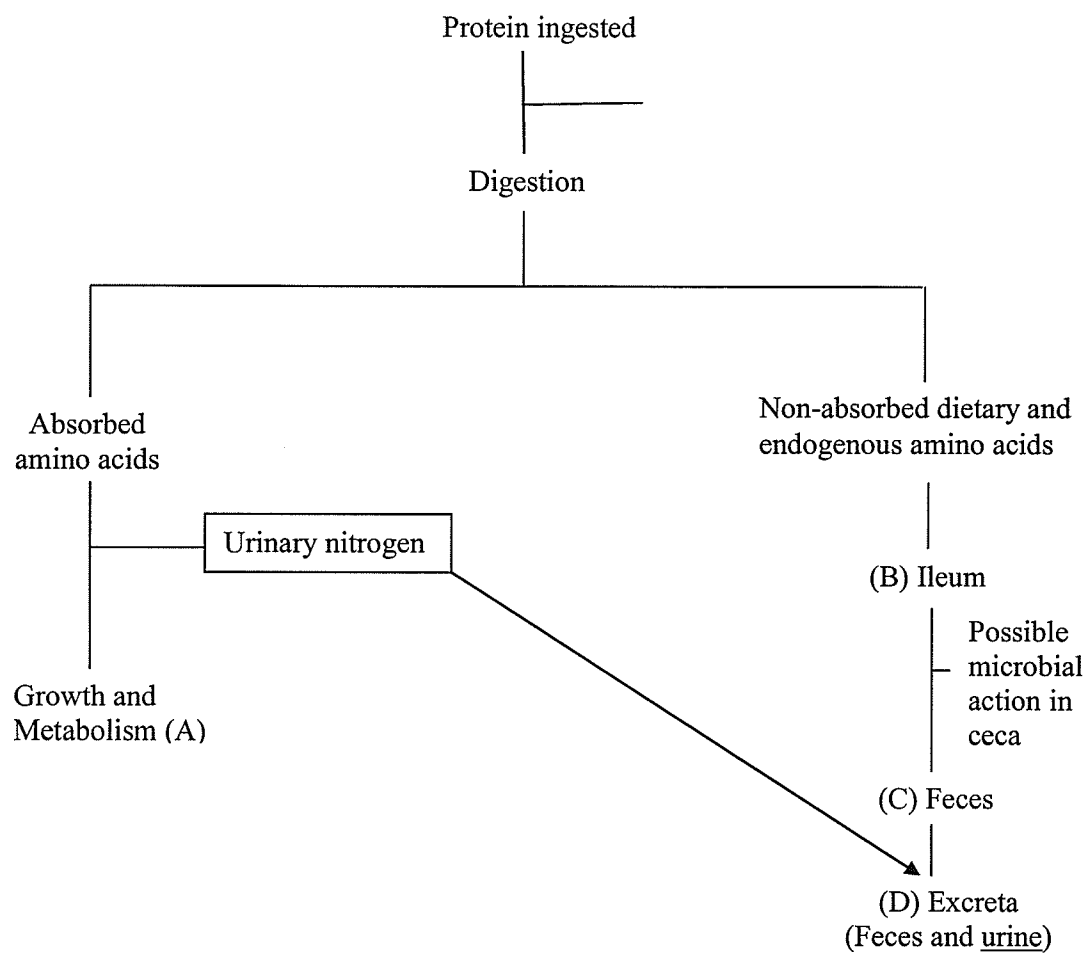


Figure 2.1 schematic diagrams of the digestion, absorption and metabolism of ingested protein in animals. Areas for the measurement of (A) amino acid availability, (B) ileal amino acid digestibility, (C) fecal amino acid digestibility and (D) excreta amino acid digestibility.

Adapted and modified from: Johnson, 1992.

2.3.1 Total tract digestibility

Digestibility based on excreta losses has been used by numerous researchers in the past mainly due to its simplicity. Estimates derived using this method are erroneous because excreta of intact birds contain nitrogenous compounds from both urinary and fecal sources (Ravindran et al., 1999b). However, the urinary AA contribution to poultry excreta was reported to be small and its AA concentration is unaffected by the nature of the diet (McNab, 1994). Nonetheless, critical examination of the technique, particularly when comparisons are being drawn between data derived from ileal and excreta balance studies, was recommended (McNab, 1994). Determination of AA digestibility in excreta has been widely criticized because intestinal microflora in the hind gut has a substantial effect on the amount of individual AA excreted in feces. In fact, Parsons et al. (1982) reported that this estimate can be as high as 25% of protein content in excreta. Consequently, the use of cecectomized birds for AA digestibility studies was recommended to overcome the problem of microbial modification of dietary protein and microbial protein synthesis in the hind gut (Parsons, 1986). However, the influence of cecectomy on AID of AA reported in the literature appears to be quite variable (Sibbald, 1979; Parson, 1984; Raharjo and Farrell, 1984b; Johns et al., 1986; Green et al., 1987; Green and Kiener, 1989). Moreover, there is a report showing that hind gut microbial effect is reduced following cecectomy but may not be eliminated (Whitacre and Tanner, 1989). Also removal of ceca may lead to digestion disturbances in the upper tract as cecal activity may be important in control of the digestive process (Sakata, 1987). These studies show that the effect of the ceca in poultry is dependent on the feedstuff being measured, thus resulting in inconsistent values. For example, when carbohydrate is limiting, such as

with fasted birds or birds given highly-digestible diets, AA excretion is probably reduced due to bacterial deamination in the ceca (Parsons et al., 1983b) that varies according to the type of ingredient and the individual AA (Ravindran et al., 1999b). Impaired bird welfare because of the force-feeding in the precision rooster assay and fasting of the birds to estimate the endogenous losses to correct the AID estimates are among the relevant constraints in this method. A significant amount of AA digestibility data published in the literature (Sibbald, 1986; Green, 1987; Parsons, 1991; Rhone-Poulenc, 1995; Heartland Lysine, 1996) and NRC (1994) has been generated using adult cockerels in the precision rooster assay, yet the question is whether these values can be applied to growing chicks with limited digestive capabilities. There is recent evidence that AA digestibility in a corn-soybean meal diet is lower during the first 7 d of age (Batal and Parsons, 2002) than at later stages. This observation has been further confirmed by Huang et al. (2005) stating that AA digestibility coefficients determined using the chick assay for different feed ingredients were lower in 14-d-old chicks than in 28- or 42-d-old chickens. Garcia et al. (2007) also reported that true lysine digestibility as determined using the rooster assay was approximately 20% greater in soybean meal and 35% greater in cottonseed meal than the SID values determined in chicks at 7 d of age indicating that the AA digestibility values obtained by the rooster assay might overestimate those for young chicks. These and previously published results (Ten Doeschate et al., 1993; Pertilla et al., 2001) on the effect of age on AA digestibility clearly suggest that the AID estimates of feeds for adult cockerels and growing birds are different.

2.3.2 Ileal digestibility

Since microbial activity on protein and AA fermentation is concentrated in the hind gut and the main sites of absorption of AA are the jejunum and ileum, Payne et al. (1968) suggested that the analysis of ileal contents rather than excreta might be a reliable method for assessing protein and AA digestibility eliminating the source of error from urine AA and the modifying effects of hind gut microbial fermentation. Ileal digesta collection can be performed in two ways depending on the technique of sample collection used - i.e. through an intestinal cannula or the slaughter of the birds. Ileal cannulation has been developed for adult cockerels (Raharjo and Farrell, 1984a; Gurnsey et al., 1985; Gurnsey and Johns, 1986). Although ileal cannulation seems to offer some theoretical advantages over the other method, it is a sophisticated technique for practical application. Some of its drawbacks include the rejection of cannula, type and placement of the cannula, the free flow of the digesta through the cannula or the use of an appropriate marker (Sauer et al., 1990). Moreover, this methodology can be only used in mature birds and therefore there remains a criticism that digestibility values measured in adult birds do not reflect the digestibility in growing broiler chickens. In the slaughter method the birds are killed humanely, intestine is exposed surgically and the content of the ileum is collected for analysis. The ileum is defined as the portion of the small intestine extending from the Meckel's diverticulum to a point 4 centimeters proximal to the ileo-cecal junction (Ravindran et al., 1999b; Lemme et al., 2004). Digesta from several birds within a cage are often pooled to obtain sufficient quantities for analysis. Obviously, quantitative collection of digesta in this method is not possible therefore, an indigestible marker (e.g. chromic oxide, titanium oxide, acid-insoluble ash) which does not affect nutrient digestibility and which yields a high recovery rate of almost 100% must be added to the

test diets. For AID calculation, AA concentrations in the diet and digesta are compared with the corresponding marker concentration using the following formula:

$$AID = \frac{(AA/IM)_d - (AA/IM)_{id}}{(AA/IM)_d}$$

Where, $(AA/IM)_d$ = ratio of AA to indigestible marker (IM) content in diet and $(AA/IM)_{id}$ = ratio of AA to indigestible marker (IM) content in ileal digesta.

2.3.2.1 Techniques for estimating nutrient digestibility

Three major approaches of direct, difference and regression are used to assess ileal AA digestibility in broilers. In the direct approach, the feed ingredient under test provides the only source of dietary protein. The diet is balanced for vitamins and minerals and purified carbohydrates (starch or dextrose) and oils can be added as energy sources.

The difference method is often used to determine AA digestibility values in feedstuffs of poor palatability where there is feed intake limitations (Kreienbring et al., 1988; Knabe et al., 1989). Whereas in the direct method the feed ingredient provides the sole source of protein, in the difference method the assay diet consists of a mixture of a basal diet and the test feed ingredient. Assuming there are no interactions between the digestibility values of AA in the basal diet and test feed ingredient, then the relationship between digestibility values can be expressed according to equation [1]. The digestibility

values of AA in the assay feed ingredient can then be determined according to equation

[2] (Van Leeuwen et al., 1987).

$$[1] \quad D_D = D_B \times S_B + D_A \times S_A$$

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

D_D = Apparent ileal digestibility of an AA in the assay diet (%)

D_B = Apparent ileal digestibility of an AA in the basal diet (%)

S_B = Contribution level of an AA from the basal diet to the test diet

$S_B = 1 - S_A$ (%)

D_A = Apparent ileal digestibility of an AA in the test feed ingredient (%)

And S_A = Contribution level of an AA from the test feed ingredient to the assay diet (%)

The regression method is another widely used approach and like the difference method, it is based on feeding the test and the basal feed ingredients simultaneously and provided that there is no interaction between the two ingredients, the ingredients can be mixed at various graded levels. As a result more than two assay diets are formulated for digestibility measurements. Detailed estimation of apparent nutrient digestibility, including mathematical equations, using the regression method has been explained previously (Fan and Sauer, 1995a; Short et al., 1999; Mosenthin et al., 2000; Rodehutscord et al., 2004).

The choice of the approach to use depends largely on palatability and nutrient content of the test feedstuff. For example, in the case of some protein supplements (blood meal) or legume seeds (faba beans) of poor palatability and a high content of anti-nutritional factors the difference or regression methods might be desirable while most of the cereal grains can be assayed by using a direct method because of a very high

palatability allowing a high inclusion level in the diet. Comprehensive studies conducted by Fan and Sauer (Fan and Sauer, 1995b) concluded that with high protein level feedstuffs any of these approaches can be used, however, the difference and regression approaches are suitable for feedstuffs with low protein content and the regression method is more appropriate for less palatable feedstuffs.

2.4 VARIATION IN ILEAL PROTEIN AND AMINO ACID DIGESTIBILITY VALUES

A number of factors can influence the AA digestibility. The nature and digestion of dietary protein is governed by factors such as genetics, agronomic condition, presence of anti-nutritional factors and processing conditions (Sibbald, 1987). Variation in digestibility values can also stem from difficulties associated with the conduct of assay procedures and the measurement of endogenous losses.

2.4.1 Dietary protein digestion

The dietary supply of protein is composed of a mixture of different protein sources. It would be anticipated that different proteins would be digested at different rates and this in turn would cause a variation in the rate at which different AA are taken up from the gut. However, this situation will be more complex if we agree that proteins are not isolated entities but have various linkages with carbohydrates, lipids and other proteins so that these interactions and composition of the diet may affect the digestibility of dietary protein (Hughes and Choct, 1999). In addition, protein digestion and absorption can be impaired by the presence of ANF in the diet. Protease inhibitors, lectins, polyphenolic compounds, saponins, tannins and NSP are examples of ANF that depress protein digestion and utilization (Dale, 1996; Hughes and Choct, 1999). Ironically, the

major feedstuffs used extensively as sources of dietary protein (e.g. grain legumes and oil seed meals) also contain the highest concentration of ANF. For example, soybean meal contains a number of ANF many of which are heat labile and destroyed during the heating process (Grant, 1989; Dale, 1996). Nonetheless, heat treatment required for inactivation of many anti-nutrients, may reduce protein quality in the presence of carbohydrates by the Maillard type reactions (Parsons et al., 1992). Processing, particularly heat treatment, may contribute to the variability of feed ingredients such as animal protein meals (Dale, 1996). Lysine and several other AA are reported to be heat labile (Papadopoulos et al., 1985) and their low digestibility in processed meals may be reflective of this fact. Extrusion is also one of the methods used efficiently to enhance the nutritional value of some feedstuffs (pea, canola and soybean). The efficiency seen in using heat and mechanical shear during extrusion is explained by the inactivation of heat-labile ANF, denaturation of protein and gelatinization of starch in the subject feed ingredients which subsequently increases their susceptibility to digestive enzymes and thus higher digestibility (Asp and Bjorck, 1989; Alonso et al., 2000; O'Doherty and Keady, 2000). It has been shown that the major influence of ANF on protein nutrition has been a reduction of AID of protein and AA (Herkelman et al., 1992; Grosjean et al., 2000; Wiseman et al., 2003). The effect of ANF on reduced digestion of protein due to both reduced digestion of endogenous and exogenous protein and increased endogenous secretions has been documented (Heinz et al., 1991; Choct and Annison, 1992; Angkanaporn et al., 1994; Gatel, 1994; Wiseman et al., 2003). It is obvious that the relative importance of these two factors leading to AA losses from the bird will vary with different ANF. The impact of ANF on AID of protein has also been demonstrated by the application of feed enzymes into poultry diets

(Ravindran et al., 1999a; Ravindran et al., 2001; Rutherford et al., 2004). The application of enzymes in these studies has been associated with an improved response in AA digestibility estimates. This positive effect of enzymes on AA digestibility highlights the impact of anti-nutritive factors on either reducing protein digestion or increasing endogenous AA losses with a net result of decreased apparent AA digestibility.

2.4.2 Assay procedures

Needless to say that values derived using different procedures would yield different results. There is a number of protein and AA digestibility reports in the literature yet these values have been derived using a number of different assay procedures which vary in terms of the age of the birds used, feeding procedures, basal diet, dietary inclusion level of test ingredients and the collection site for digesta (Ravindran and Bryden, 1999). Consistent use of assay procedures will be required to enable a comparison between the results of different studies and to evaluate the repeatability of the assay itself.

2.4.3 Endogenous AA losses

Two major areas of contention in digestibility assays are the use of ileal versus excreta collection procedures and the correction of digestibility values for endogenous secretions. Differences observed between the ileal and excreta digestibilities reported by Ravindran et al. (1999b) shows that there is a greater variation in excreta values than there is in ileal values. The difference observed between the results of the two digestibilities (excreta vs. ileal) in this report refers to the fact that AA metabolism by the microflora in the hind gut of chickens may be substantial. If feed intake is low, as it is in the precision-fed rooster assay, endogenous AA becomes a greater proportion of the AA

measured in digesta and excreta, depressing apparent digestibility values accordingly. The relative significance of these endogenous AA losses to AA levels in excreta or digesta decreases as dietary AA intake is increased. Therefore, the AA digestibility of feed ingredients with low AA levels, such as cereals, is most affected. However, the AID values can be corrected for basal endogenous AA losses to obtain SID values which are independent of dietary AA intake. The difference between the apparent and standardized ileal AA digestibility measurements relative to dietary AA intake is demonstrated in Figure 2.2 (Lemme et al., 2004). Thus, most digestibility assays include a correction for endogenous AA losses to provide a more accurate set of values for comparing different diets or protein sources. Secretion of nitrogenous compounds from the digestive tract into the gut lumen can be divided into two fractions, basal and specific. While the basal losses are related to the dry matter intake and independent of diet composition, specific losses are the additional secretions that are dependent on the composition of the test material (Donkoh and Moughan, 1994; Lemme et al., 2004). The relationship between dietary protein or AA intake and the recovery of AA at the distal ileum is demonstrated in Figure 2.3 (Lemme et al., 2004). Several different approaches for the estimation of endogenous AA in poultry have been reviewed in detail elsewhere (Ravindran and Bryden, 1999). Although, different methods has been suggested and applied for the estimation of basal endogenous losses, it appears that the methods used in direct measurement of specific losses are very few and the results are not consistent. As basal endogenous losses represent the minimum quantities of AA loss and are not influenced by the dietary composition, it has been suggested that a correction based on these values for the AID estimates can be used to overcome the technical problems of determining the specific

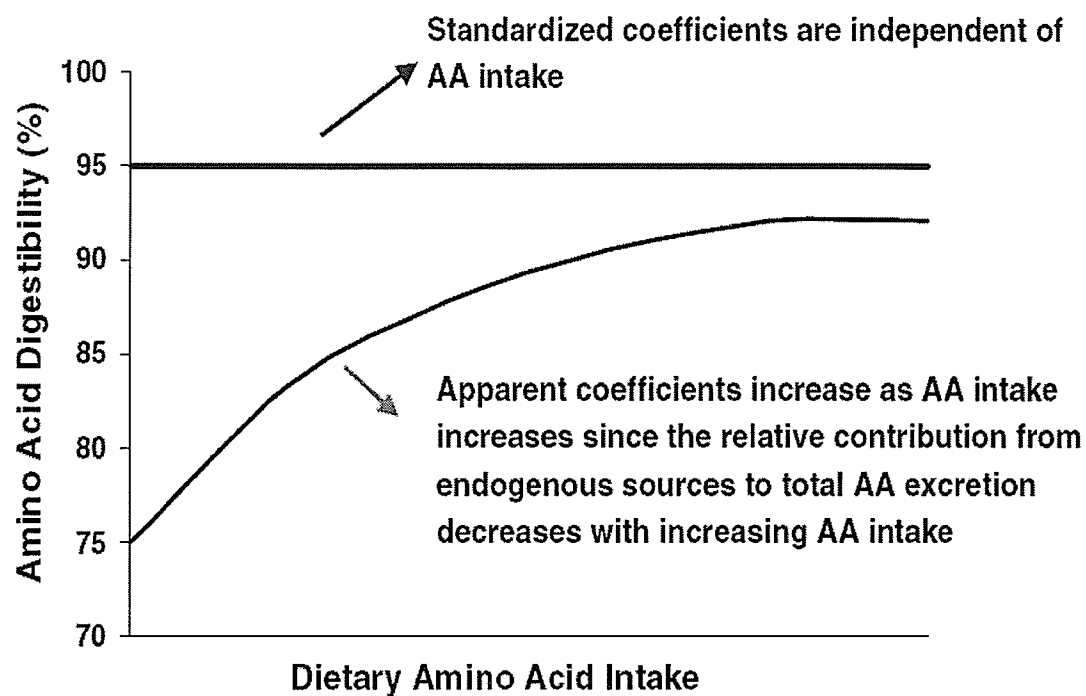
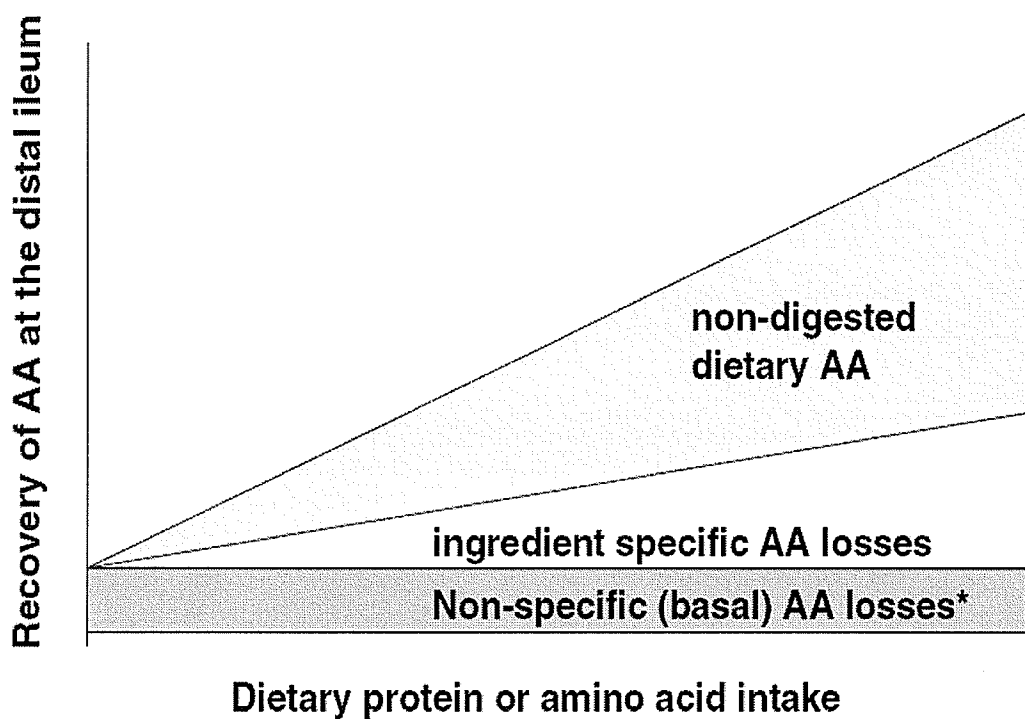


Figure 2.2 Relationship between apparent and standardized AA digestibility and dietary AA intake.

Source: Lemme et al. (2004).



* Level of basal endogenous losses is constant at different AA intake (g/kg DMI)

Figure 2.3 Various fractions that make up the AA recovered at the distal ileum.

Source: Lemme et al. (2004).

losses (Nyachoti et al., 1997; Ravindran and Bryden, 1999; Stein et al., 2001; Lemme et al., 2004; Golian et al., 2008).

2.5 APPARENT VERSUS STANDARDIZED ILEAL DIGESTIBILITY

As has been shown in several previous studies (Fernandez et al., 1995; Rostagno et al., 1995; Douglas and Parsons, 1999) one of the major advantages of using digestible AA in feed formulation is that it enables effective use of alternate cheaper ingredients (especially low quality protein sources) in poultry diets. Concurrently, it will increase the range of ingredients that can be incorporated, improve the precision of feed formulation and ensure more predictable bird performance. Another important aspect in using single feedstuffs in formulation of a complete diet is their additivity. Angkanaporn et al. (1996) demonstrated that the digestible AA supply in a complete diet can be predicted, with reasonable accuracy, based on AID values determined for individual feed ingredients (soybean meal, sunflower meal, meat and bone meal). However, more research on this issue is warranted as there might be associative effects when high levels of poorly digestible ingredients are used (Kadim and Moughan, 1997). As mentioned earlier, compared with high protein feed ingredients, when birds are fed low protein ingredients the AID values will be underestimated as a result of increased proportion of endogenous AA in the digesta. The other concern will be the applicability of the ideal protein concept in formulation based on AID estimates which may be questioned because ideal protein ratios are determined in a way that reflects true rather than AID (Ravindran and Bryden, 1999). These limitations of AID values can be overcome by using SID values. The obvious advantage of the SID system is that AID and basal endogenous

losses need not to be determined in the same experiment and SID values have been shown to be more additive when used in complete diets (Lemme et al., 2004; Stein et al., 2005; 2007). Using the result of basal endogenous AA losses of birds fed a nitrogen free diet which represents the minimum quantity of loss, the SID values can be calculated from AID values as follows:

$$\text{SID}\% = \text{AID} + [(\text{basal IAA}_{\text{end}} / \text{AA}_{\text{diet}}) \times 100]$$

Where, IAA_{end} = basal ileal endogenous AA loss, AA_{diet} = Amino acid content of the diet

2.6 CONCLUSION

Optimum use of feedstuffs to supply AA required by animals is of great importance. To accomplish this, the bioavailability or digestibility of the AA in the feed ingredient must be known. While the growth assay remains the only direct means of estimating the bioavailability of nutrients, digestibility assays appear to be the most practical as sensitive indicators of AA availability in the diet. Estimation of ileal protein and AA digestibility is a more reliable method for assessing AA digestibility in poultry. Although the debate among nutritionists will continue as to the relative merits of apparent and true digestible AA systems, there is no doubt that all digestible AA systems are superior to the use of total AA content in feed formulation. As it was explained above, the AID values are affected by the amount of the dry matter intake and the dietary level of protein, however, the use of the SID values in diet formulation can overcome the shortfalls associated with the application of the AID values. For optimum use of ileal digestibility values, the age of the target birds needs to be considered. Most data cited in the literature have been determined with roosters which are physiologically different from growing broilers. Another important point to be considered is the variability

observed in estimates which can stem from different factors such as assay condition, the inherent quality of the feed or the methodology used. To improve the degree of accuracy and reliability, the variance of the estimates needs to be accounted for.

This project was initiated in an attempt to create a data set which could be used by the broiler feed industry for a more accurate formulation of the diets. Moreover, the use of the data generated will result in a more sustainable agriculture by decreasing the excretion of nitrogenous compounds to the environment and subsequent pollution. The use of the SID values in diet formulation not only will increase the respective accuracy but also has the added advantage of lower feed cost due to a lower required safety margin.

3.0 OBJECTIVES

To determine the apparent and standardized ileal digestibilities of protein and AA and their associated variability for several feed ingredients using broiler chicks in a series of experiments.

4.0 MANUSCRIPT I

Standardized Ileal Amino Acid Digestibility in Wheat Distillers' Dried Grains with Solubles for Broilers

4.1 ABSTRACT

This study was conducted to estimate the ileal digestibility of AA in five different samples of wheat DDGS fed to broilers. A reference wheat sample was also evaluated for comparison purposes. Two hundred and sixteen male Ross broiler chicks were fed a commercial starter diet from d 1 to 14 of age followed by the test diets from d 15 to 21. The six test diets consisted of a DDGS sample or wheat (as the sole source of AA), dextrose, minerals and vitamins. Chromic oxide (0.3%) was included in all diets as a digestibility marker. Each test diet was randomly assigned to six replicate cages, each with 6 birds. On d 21, birds were killed to sample ileal digesta for determining the AID and SID. The SID values were calculated using ileal endogenous AA losses previously determined in our laboratory. Among the indispensable AA, the lowest AID value was observed for lysine averaging 35.6% whereas the respective value for phenylalanine (79.2%) was the highest for wheat DDGS. The most variable AID estimates of wheat DDGS samples were observed for lysine (24.4% - 45.7%), threonine (48.2% - 60.9%), histidine (57.4% - 69.1%) among indispensable AA and aspartic acid (32.5% - 50.9%), glycine (49.6% - 63.1%) and alanine (53.6% - 66.8%) among dispensable AA. The AID estimates of the wheat sample for lysine, threonine, histidine, glycine and alanine were 77.5%, 74%, 83.6%, 79.3% and 78.9%, respectively. All AA digestibility estimates for

both AID and SID determined in wheat were significantly higher than in wheat DDGS samples ($P<0.05$). Considering both AID and SID coefficients of wheat DDGS samples, lysine was the least digestible AA averaging 35.6% and 40.0%, respectively.

4.2 INTRODUCTION

There has been a marked increase in the availability of DDGS in North America primarily from corn as the feedstock. Various studies have demonstrated that corn-derived DDGS can be utilized in broiler diets as a source of protein and other nutrients. For example, Runnels (1966; 1968) reported a 20% incorporation of DDGS into broiler diets with performance equal or superior to that of chicks fed diets with corn-soybean meal and fish meal. In another report, up to 25% DDGS was included in isocaloric broiler diets without any reduction in body weight or feed utilization (Waldroup et al., 1981). Parsons et al. (1983a) found that DDGS can replace up to 40% of soybean meal protein when lysine content was adjusted. More recently, Lumpkins et al. (2004) reported that DDGS can be safely used at 6% in the starter period and 12 to 15% in the grower and finisher periods. Thacker and Widyaratne (2007) concluded that wheat-derived DDGS can be incorporated into diets at levels as high as 15% without detrimental effects on broiler performance.

A major concern regarding the use of DDGS as a feedstuff is that it tends to be variable in terms of its nutritive quality depending on various factors including composition of the grain used, amount of solubles added back and the drying technology used in its production (Martinez-Amezcu et al., 2007). Thus, it is not only critical to

evaluate the nutritive value of DDGS but also to understand the variability inherent in its quality.

In Western Canada, wheat is the preferred feedstock for ethanol production. Studies with swine have confirmed that the nutritive value of wheat-derived DDGS relative to wheat follows the same relationship established for corn and corn-derived DDGS (Salo, 1978; Nyachoti et al., 2005; Lan et al., 2008). These studies have shown that the digestibilities of some AA, especially lysine, are very low in wheat DDGS; a factor that will compromise its nutritive value for the non-ruminant animal. Thus far, there has been no study on the ileal digestibility of AA in wheat-derived DDGS for poultry despite evidence that it can be included in broiler diets up to 15% without compromising performance as long as the low lysine and energy content is compensated for in diet formulation (Thacker and Widyaratne, 2007). However, to effectively utilize wheat DDGS as a source of AA in broiler diets, it is critical that its digestible AA contents are determined in a study with broiler chicks as the experimental animal as has been suggested elsewhere (Ten Doeschate et al., 1993; Huang et al., 2005; Garcia et al., 2007). Apparent ileal digestibility estimates ignore the contribution of endogenous AA losses to digesta hence, underestimating the digestibility of AA in feedstuff. Accurate determination of AA digestibility in feed ingredients necessitates the consideration for AA of endogenous origin in the digesta. A correction based on obligatory basal loss for AID coefficients results in digestibilities termed as “standardized” ileal digestibility. In addition to being a closer representative of true digestibility values, SID values are more additive in a mixture of feed ingredients than AID (Angkanaporn et al., 1996; Stein et al., 2005).

4.3 OBJECTIVES

To determine the apparent and standardized ileal AA digestibilities and their associated CV in wheat DDGS for broilers.

4.4 MATERIALS AND METHODS

4.4.1 DIETS

The wheat DDGS samples assayed in the present study were obtained from Husky's Mohawk Ethanol plant at Minnedosa, Manitoba, Canada. Samples were collected from five different fermentation batches with different grain source and processing days. The sample of reference wheat was obtained from a local farm. Each test diet contained a wheat DDGS sample at 50% or wheat at 91.7% as the sole source of protein. All test diets were balanced for minerals and vitamins to meet NRC (1994) requirement specifications and contained chromic oxide (0.3%) as a digestibility marker.

4.4.2 BIRDS AND EXPERIMENTAL CONDUCT

The experimental protocol for the present study was reviewed and approved by the Animal Care Protocol Management and Review Committee of the University of Manitoba and birds were cared for according to the guidelines of the Canadian Council on Animal Care (1993).

Two hundred and sixteen day-old male broiler chicks (Ross 308, Aviagen) were obtained from a local hatchery (Carlton Hatchery, Grunthal, Manitoba, Canada) and housed in Petersime battery brooders (Petersime Incubator Co., Gettysburg, Ohio) in a

room with continuous fluorescent lighting. Room temperature was maintained at 32, 28, and 24°C during week 1, 2, and 3, respectively. From day 1 to 14, birds were fed a commercial starter diet (FeedRite, Ridley Inc., Winnipeg, Manitoba, Canada) in crumble form (3100 Kcal/Kg, 19% CP). On day 14, birds were fasted for 3 h, weighed and distributed (6 birds/cage) into 36 cages with similar body weights. Six replicate cages were randomly assigned to each of the six test diets and fed from day 15 to day 21. Birds had free access to feed and water throughout the study period. On day 21, birds were killed by cervical dislocation and the digesta samples from the ileum (from Meckel's diverticulum to a point 4 cm proximal to the ileo-cecal junction) were collected by gently squeezing the contents of the ileum into sample bags. Digesta from birds within a cage were pooled into one bag and frozen immediately after collection and subsequently freeze-dried. The dried ileal digesta were stored in airtight bags at -4°C until required for chemical analysis.

4.4.3 CHEMICAL ANALYSIS

Raw feed ingredients, test diets and digesta samples were finely ground using a coffee grinder (CBG5 Smart Grind; Applica Consumer Products, Inc., Shelton, CT). Dry matter was determined according to the method (procedure # 934.01) of AOAC (2000), and nitrogen was analyzed using the Leco nitrogen analyzer (Model CNS-2000; Leco Corp., St. Joseph, MI). Amino acid concentrations of the diet and digesta were determined by Evonik (Hanau-Wolfgang, Germany). Dietary AA contents were determined by using ion-exchange chromatography with postcolumn derivatization with ninhydrin (Llames and Fontaine, 1994). Sulfur AA (Methionine and Cystine) were first

oxidized with performic acid, which were subsequently neutralized with sodium metabisulfite (Commission Directive, 1998). Amino acid concentrations were quantified with the internal standard method by measuring the absorption of reaction products with ninhydrin at 570 nm. Chromium was analyzed, after the samples were ashed at 600 °C for 12 h in a muffle furnace, using inductively coupled plasma mass spectrometry (ICP-AES Vista, Varian) according to the method (procedure # 985.01) of AOAC (2005).

4.4.4 DIGESTIBILITY CALCULATION

The AID coefficient of AA was calculated using the indigestible marker as follows.

$$\text{AID} = \frac{(\text{AA/IM})_d - (\text{AA/IM})_{id}}{(\text{AA/IM})_d}$$

Where, $(\text{AA/IM})_d$ = ratio of AA to indigestible marker (IM) content in diet and

$(\text{AA/IM})_{id}$ = ratio of AA to indigestible marker (IM) content in ileal digesta

Basal endogenous loss estimates determined with a nitrogen-free diet (Golian et al., 2008) were used for the correction of AID coefficients. The SID coefficients were calculated as follows:

$$\text{SID}\% = \text{AID} + [(\text{basal IAA}_{\text{end}} / \text{AA}_{\text{diet}}) \times 100]$$

Where, IAA_{end} = basal ileal endogenous AA loss and AA_{diet} = Amino acid content of the diet.

The endogenous ileal AA loss values used to calculate the SID are presented in the Appendix.

4.4.5 STATISTICAL ANALYSIS

The data for digestibility estimates of wheat and wheat DDGS samples were analyzed using the GLM procedure of SAS Institute, Inc. (1990) as a completely randomized design. The Nested procedure was also applied to further analyze the random feed samples and bird/cage sub-sampling effects on the total variation observed. Tukey's test was used to separate means when a significant *F*-value was detected. For a better demonstration of variability, the components of total coefficients of variation (CV%) associated with the AID and SID estimates for each AA across different wheat DDGS samples are included as variance due to the samples (σ_s^2) and cages within samples (σ_e^2).

4.5 RESULTS AND DISCUSSION

Crude protein and AA composition of the wheat DDGS and wheat samples and diets are presented in Tables 4.1 and 4.2, respectively. The data show that the AA concentration in DDGS is two to three-folds higher than the content in wheat, which is consistent with previous reports (NRC, 1994; Nyachoti et al., 2005; Thacker and Widyaratne, 2007; Lan et al., 2008). Also, the AA profiles of the wheat DDGS and wheat samples tested were similar to those reported in previous studies (Thacker and Widyaratne, 2007; Lan et al., 2008). In wheat DDGS samples, average methionine (0.6%) and lysine (0.7%) contents relative to other AA were lowest when expressed

Table 4.1. Crude protein and amino acid composition (%) of wheat and wheat DDGS samples (DM basis)

Items	Wheat ¹	Wheat DDGS					Mean ²	CV% ³
		1	2	3	4	5		
DM	92.4	95.9	95.5	96.1	95.8	95.6	95.78	0.2
CP	15.6	41.3	39.2	39.9	38.2	40.9	39.90	3.2
<i>Indispensible amino acids</i>								
Arg	0.71	1.67	1.61	1.58	1.53	1.67	1.61	3.7
His	0.36	0.84	0.81	0.81	0.78	0.85	0.82	3.4
Ile	0.54	1.41	1.35	1.36	1.30	1.41	1.37	3.4
Leu	1.03	2.68	2.62	2.59	2.51	2.77	2.63	3.7
Lys	0.42	0.77	0.73	0.72	0.69	0.79	0.74	5.4
Met	0.26	0.61	0.62	0.60	0.59	0.62	0.61	2.1
Met+Cys	0.62	1.39	1.38	1.36	1.30	1.38	1.36	2.7
Phe	0.73	1.90	1.76	1.79	1.72	1.86	1.81	4.1
Thr	0.44	1.19	1.17	1.18	1.13	1.22	1.18	2.8
Val	0.67	1.72	1.70	1.69	1.63	1.74	1.70	2.5
<i>Dispensable amino acids</i>								
Ala	0.53	1.42	1.42	1.38	1.37	1.50	1.42	3.6
Asp	0.76	1.89	1.85	1.83	1.79	1.94	1.86	3.1
Cys	0.36	0.77	0.75	0.76	0.72	0.76	0.75	2.6
Glu	4.57	11.75	10.64	11.20	10.40	11.18	11.03	4.8
Gly	0.63	1.62	1.55	1.58	1.52	1.56	1.57	2.4
Pro	1.49	3.78	3.42	3.54	3.37	3.58	3.54	4.5
Ser	0.69	1.82	1.73	1.78	1.66	1.83	1.76	4.0
Overall	0.87	2.19	2.07	2.10	2.00	2.16	2.10	3.5

1. Reference wheat (AC Barrie from James farm, Manitoba).

2. Mean of five wheat DDGS samples.

3. Coefficient of variation (CV%) across five wheat DDGS samples.

Table 4.2. Analyzed crude protein and amino acid composition (%) of wheat and wheat DDGS diets (DM basis)

Item	Wheat ¹	Wheat DDGS					Mean ²	CV% ³
		1	2	3	4	5		
DM	92.4	92.9	92.5	92.9	93.3	92.6	92.84	0.3
CP	14.6	21.8	18.6	20.7	20.8	20.8	20.54	5.7
<i>Indispensable amino acids</i>								
Arg	0.41	0.86	0.82	0.80	0.86	0.86	0.84	3.4
His	0.28	0.46	0.44	0.43	0.46	0.46	0.45	3.1
Ile	0.40	0.75	0.71	0.70	0.74	0.76	0.73	3.5
Leu	0.96	1.41	1.37	1.32	1.45	1.47	1.40	4.3
Lys	0.50	0.42	0.40	0.39	0.40	0.43	0.41	4.0
Met	0.67	0.34	0.34	0.33	0.35	0.35	0.34	2.4
Met+Cys	0.34	0.75	0.73	0.73	0.77	0.74	0.74	2.2
Phe	0.62	0.99	0.92	0.91	0.99	0.97	0.96	4.0
Thr	0.67	0.61	0.61	0.59	0.64	0.63	0.62	3.2
Val	0.62	0.93	0.90	0.87	0.93	0.95	0.92	3.4
<i>Dispensable amino acids</i>								
Ala	0.51	0.74	0.75	0.71	0.78	0.79	0.75	4.3
Asp	0.70	1.00	0.98	0.95	1.04	1.04	1.00	3.9
Cys	0.33	0.41	0.40	0.39	0.42	0.40	0.40	2.8
Glu	4.22	6.01	5.45	5.60	5.87	5.75	5.74	3.8
Gly	0.57	0.84	0.80	0.81	0.87	0.82	0.83	3.4
Pro	1.39	2.00	1.80	1.82	1.94	1.89	1.89	4.4
Ser	0.64	0.91	0.89	0.90	0.95	0.93	0.92	2.6
Overall	0.81	1.14	1.08	1.07	1.14	1.13	1.11	3.4

1. Reference wheat (AC Barrie from James farm, Manitoba).

2. Mean of five wheat DDGS diets.

3. Coefficient of variation (CV%) across five wheat DDGS diets.

either on a total basis or as a percentage of total protein. In contrast, among all indispensable AA, leucine (2.6%) and phenylalanine (1.8%) contents ranked as the highest in the samples tested. This trend of AA profile observed in wheat DDGS samples compare well with that of wheat. Calculated CV for each of the AA presented among the wheat DDGS samples, positioned lysine as the most variable and methionine as the least variable AA with a CV of 5.4 and 2.1%, respectively.

A higher nutrient content in DDGS relative to the source cereal coupled with increasing availability of this co-product encourages its use at higher inclusion levels in poultry diets than has been the case in the past. Indeed, it has been shown that DDGS can be used successfully in broiler diets as a substitute for highly digestible and expensive feedstuffs without compromising growth performance (Parsons et al., 1983b; Lumpkins et al., 2004; Thacker and Widyarante, 2007). Studies have also shown that in order to maintain bird performance, diet formulation to contain DDGS must be done carefully to take the lower metabolizable energy and digestible lysine content in DDGS into consideration (Waldroup et al., 1981; Thacker and Widyarante, 2007). Amino acid digestibility studies with corn-derived DDGS have shown that lysine is the least digestible AA that is also associated with the greatest variability (Batal and Dale, 2006; Fastinger et al., 2006; Feine et al., 2006; Parsons et al., 2006). However, to date there has been no report on the protein and AA digestibility in wheat-derived DDGS for poultry. In experiments with pigs, we have shown that lysine is poorly digested in wheat-derived DDGS obtained from the same ethanol plant as the samples used in the present study (Nyachoti et al., 2005; Lan et al., 2008). To effectively utilize wheat DDGS in poultry diets, it is critical that its digestible AA content is determined. Thus, the present study

sought to determine the AID and SID of AA in wheat DDGS and to assess the variability of these estimates in five samples collected over time from the same ethanol plant.

Although, AA digestibility values determined with the rooster assay at the excreta level have been routinely used in formulating poultry diets, it has become increasingly clear that estimates determined with the broiler chicken at the ileal level should be used as they provide a better estimate of AA availability (Ten Doeschate et al., 1993; Kadim and Moughan, 1997; Ravindran and Bryden, 1999; Karakas et al., 2001; Kadim et al., 2002; Lemme et al., 2004).

The AID and SID of crude protein and AA in the wheat and wheat DDGS samples evaluated are presented in Tables 4.3 and 4.4, respectively. The average AID and SID digestibility estimates of AA determined in wheat were 84.3 and 88.9%, respectively. With slight changes in the ranking order, the AID and SID estimates for indispensable AA followed the same pattern with sulfur amino acids (89, 92%) and phenylalanine (89, 99%) having the highest and lysine (77.5, 82%), arginine (81, 84%) and threonine (74, 85%) representing the lowest digestible AA. These results are very similar to the AID values of wheat published previously (Ravindran et al., 1999b; Huang et al., 2005; Ravindran et al., 2005). Overall average AID and SID digestibilities in wheat DDGS were significantly lower ($P<0.05$) than in wheat, at 64.8 and 68.2% compared with 84.3 and 88.9%, respectively. Phenylalanine (79.2%, 86.4%), methionine (73.7%, 75.7%) and leucine (73.7%, 75.6%) were the most digestible indispensable AA in both AID and SID estimates in wheat DDGS. Among the indispensable AA in wheat DDGS, lysine represented the least digestible and most variable AA with AID and SID estimates of 35.6 and 40.0%, followed by threonine (54.8%, 62.2%) and histidine (63.7%, 65.7%),

Table 4.3. Apparent ileal digestibility coefficients¹ (%) of crude protein and amino acids in chicks fed diets containing wheat² (n = 1) and wheat DDGS² (n = 5)

Item	wheat	Wheat DDGS					Average ³
		1	2	3	4	5	
CP	85.4 ^a	69.9 ^b	64.3 ^b	64.1 ^b	65.7 ^b	71.0 ^b	67.0
<i>Indispensable amino acids</i>							
Arg	80.7 ^a	68.8 ^b	67.5 ^b	63.3 ^b	68.3 ^b	73.3 ^b	68.2
His	83.6 ^a	65.7 ^b	63.5 ^b	57.4 ^b	62.8 ^b	69.1 ^b	63.7
Ile	86.9 ^a	72.4 ^b	68.8 ^b	63.5 ^b	67.3 ^b	72.2 ^b	68.8
Leu	87.6 ^a	75.1 ^b	73.3 ^b	68.8 ^b	72.7 ^b	77.0 ^b	73.4
Lys	77.5 ^a	41.1 ^{bc}	35.0 ^{bc}	24.4 ^c	31.7 ^{bc}	45.7 ^b	35.6
Met	89.9 ^a	75.7 ^b	73.4 ^b	69.3 ^b	73.5 ^b	76.4 ^b	73.7
Met+Cys	88.5 ^a	69.9 ^b	66.6 ^b	62.6 ^b	66.4 ^b	71.0 ^b	67.3
Phe	89.0 ^a	80.7 ^b	79.0 ^b	76.0 ^b	78.6 ^b	81.6 ^b	79.2
Thr	74.0 ^a	56.7 ^b	54.6 ^b	48.2 ^b	53.5 ^b	60.9 ^b	54.8
Val	82.4 ^a	67.4 ^b	64.6 ^b	58.6 ^b	63.3 ^b	69.7 ^b	64.7
<i>Dispensable amino acids</i>							
Ala	78.9 ^a	62.0 ^b	61.0 ^b	53.6 ^b	59.7 ^b	66.8 ^b	60.6
Asp	76.1 ^a	45.7 ^b	42.1 ^b	32.5 ^b	41.3 ^b	50.9 ^b	42.5
Cys	87.1 ^a	65.0 ^b	61.4 ^b	55.6 ^b	60.3 ^b	66.3 ^b	61.7
Glu	94.8 ^a	84.9 ^b	82.9 ^b	80.8 ^b	82.9 ^b	84.8 ^b	83.2
Gly	79.3 ^a	59.9 ^b	56.4 ^b	49.6 ^b	56.8 ^b	63.1 ^b	57.1
Pro	93.3 ^a	83.2 ^b	80.7 ^b	78.4 ^b	80.1 ^b	83.3 ^b	81.1
Ser	83.1 ^a	66.6 ^b	64.9 ^b	61.6 ^b	64.8 ^b	70.4 ^b	65.7

1. All means with different superscripts within the same row are significantly different ($P < 0.05$).

2. One reference wheat (AC Barrie) and five wheat DDGS samples were fed to six replicate cages, each with 6 birds.

3. Overall average of five wheat DDGS digestibility coefficients.

Table 4.4. Standardized ileal digestibility coefficients¹ (%) of crude protein and amino acids in chicks fed diets containing wheat² (n = 1) and wheat DDGS² (n = 5)

Item	Wheat	Wheat DDGS					Average ³
		1	2	3	4	5	
CP	88.4 ^a	71.9 ^b	66.7 ^b	66.2 ^b	67.8 ^b	73.1 ^b	69.1
<i>Indispensable amino acids</i>							
Arg	83.9 ^a	71.3 ^b	70.1 ^b	66.0 ^b	70.8 ^b	75.7 ^b	70.8
His	86.3 ^a	67.7 ^b	65.6 ^b	59.5 ^b	64.8 ^b	71.1 ^b	65.7
Ile	91.1 ^a	75.2 ^b	71.7 ^b	66.5 ^b	70.2 ^b	74.9 ^b	71.7
Leu	90.8 ^a	77.3 ^b	75.6 ^b	71.2 ^b	74.9 ^b	79.2 ^b	75.6
Lys	82.1 ^a	45.4 ^{bc}	39.5 ^{bc}	29.0 ^c	36.3 ^{bc}	49.9 ^b	40.0
Met	92.4 ^a	77.7 ^b	75.5 ^b	71.4 ^b	75.4 ^b	78.4 ^b	75.7
Met+Cys	92.0 ^a	72.8 ^b	69.5 ^b	65.5 ^b	69.1 ^b	73.9 ^b	70.2
Phe	99.3 ^a	87.7 ^b	86.6 ^b	83.6 ^b	85.6 ^b	88.7 ^b	86.4
Thr	85.1 ^a	64.1 ^b	62.1 ^b	55.9 ^b	60.5 ^b	68.2 ^b	62.2
Val	87.0 ^a	70.4 ^b	67.7 ^b	61.8 ^b	66.3 ^b	72.7 ^b	67.8
<i>Dispensable amino acids</i>							
Ala	83.4 ^a	65.1 ^b	64.0 ^b	56.8 ^b	62.6 ^b	69.7 ^b	63.6
Asp	82.5 ^a	50.2 ^b	46.7 ^b	37.2 ^b	45.6 ^b	55.2 ^b	47.0
Cys	91.6 ^a	68.7 ^b	65.2 ^b	59.4 ^b	63.9 ^b	70.0 ^b	65.4
Glu	96.0 ^a	85.8 ^b	83.8 ^b	81.7 ^b	83.7 ^b	85.7 ^b	84.1
Gly	83.8 ^a	63.0 ^b	59.6 ^b	52.8 ^b	59.7 ^b	66.2 ^b	60.3
Pro	95.5 ^a	84.8 ^b	82.4 ^b	80.1 ^b	81.6 ^b	84.9 ^b	82.8
Ser	88.7 ^a	70.5 ^b	68.9 ^b	65.6 ^b	68.6 ^b	74.2 ^b	69.6

1. All means with different superscripts within the same row are significantly different ($P < 0.05$).

2. One reference wheat (AC Barrie) and five wheat DDGS samples were fed to six replicate cages, each with 6 birds.

3. Overall average of five wheat DDGS digestibility coefficients.

respectively. Lysine, as reported in the studies conducted using corn DDGS in poultry (Lumpkins et al., 2004; Lumpkins and Batal, 2005; Batal and Dale, 2006) and wheat DDGS in pigs (Nyachoti et al., 2005; Lan et al., 2008) was also found to be the least digestible AA in wheat DDGS fed to broilers. Irrespective of the methodology and the age of the animals used, which are important factors influencing the results, comparing the digestibility results reported for corn DDGS (Batal and Dale, 2006; Fastinger et al., 2006; Feine et al., 2006; Parsons et al., 2006) and wheat DDGS, would rank the latter as a less digestible feed. Interestingly enough, with a slight difference, AID results reported by Lan et al. (2008) for the finishing pigs fed wheat DDGS and the results of the current study are closely comparable. Although there has been reports of almost the same large variation within a source as between different plants with corn DDGS (Spiehs et al., 2002; Feine et al., 2006), the AID coefficients of AA determined among the wheat DDGS samples from the same source in this study were not significantly different except for lysine ($P=0.0422$).

As presented in Table 4.5, with respect to the CV for AID and SID estimations of indispensable AA across the diets, lysine, threonine and histidine had the highest variability while phenylalanine, leucine and methionine had the lowest with lysine marked as the least digestible, most variable AA among all. Studying the variance components of the total CV showed that the variation observed among the different samples of wheat DDGS was more affected by within rather than between samples variation. This can partly be explained by the adverse effects of feeding a high level of wheat DDGS to the birds on their variable feed intake responses (bird to bird differences) which could result in higher within sample variability.

With respect to AA nutrition, it should be noted that AA profile, balance and availability in a feedstuff are the factors which could determine its suitability as a protein source. Furthermore, a consistent AA profile of a feed is the key for its routine use in commercial feed formulation. As during the process of drying DDGS, the material is typically exposed to temperatures of approximately 315°C or (600°F), the bioavailability of lysine and several other AA is compromised (Lumpkins and Batal, 2005). Indeed, the adverse effect of excess heat on AA availability is well documented (McGinnis and Evans, 1947; Warnick and Anderson, 1968). Because lysine is the first or second limiting AA in poultry diets and because of its susceptibility to heat damage during the drying process, its digestibility is a major concern in the use of wheat DDGS. Performance and breast meat yield is greatly impacted by intake of lysine, methionine and some other AA (Schutte and Pack, 1995; Kerr et al., 1999; Wallis, 1999) moreover, in this context; lower content of these AA together with their low digestibility poses a negative implication if a higher inclusion level of wheat DDGS is to be used in the diet. However, satisfactory performance can be expected only if wheat DDGS is included in nutritionally-adequate diets.

Use of wheat DDGS as a feedstuff in broiler diets could replace a considerable quantity of highly digestible and expensive feed ingredients such as soybean meal protein but formulating diets with high inclusion levels of wheat DDGS will need to consider potential excesses and deficiencies of certain AA. This study was carried out in an attempt to create a data set which could be used for a more accurate formulation of diets. Because SID coefficients are more additive when used in formulation of a mixed diet (Stein et al., 2005; 2007), the AID estimates were transformed into SID values by correcting for basal

Table 4.5. Coefficient of variation (CV%) associated with apparent and standardized ileal digestibility estimates determined in chicks fed diets containing wheat DDGS¹ (n = 5)

Item	AID			SID		
	CV% ²	Variance components ³		CV% ²	Variance components ³	
		Between samples	Within samples		Between samples	Within samples
CP	9.6	10.2	89.8	9.2	9.2	90.8
<i>Indispensable amino acids</i>						
Arg	9.3	16.2	83.8	8.9	15.1	84.9
His	12	16.7	83.3	11.5	16.0	84.0
Ile	9.1	19.8	80.2	8.7	18.6	81.4
Leu	7.4	17.9	82.1	7.0	16.6	83.4
Lys	36.9	25.4	74.6	32.6	24.1	75.9
Met	7.1	12.4	87.6	6.9	12.0	88.0
Met+Cys	8.9	11.8	88.2	9.1	11.5	88.5
Phe	5.5	8.4	91.6	5.0	4.9	95.1
Thr	15.7	14.5	85.5	13.7	13.2	86.8
Val	11.0	20.3	79.7	10.5	19.2	80.8
<i>Dispensable amino acids</i>						
Ala	13.3	19.8	80.2	12.6	18.6	81.4
Asp	27.6	18.6	81.4	24.8	17.6	82.4
Cys	12.7	14.2	85.8	11.9	13.6	86.4
Glu	4.4	6.5	93.5	4.3	6.0	94.0
Gly	15.6	16.6	83.4	14.8	16.4	83.6
Pro	5.2	9.3	90.7	5.1	8.5	91.5
Ser	10.4	6.0	94.0	9.8	5.6	94.4
Overall	12.3	14.7	85.3	10.8	13.7	86.3

1. Five samples of wheat DDGS were assayed in six replicates cages with 6 birds in each and variance components for samples (σ_s^2) and cages within samples (σ_e^2) was determined.

2. Total coefficient of variation, $CV\% = \frac{\sqrt{\sigma_s^2 + \sigma_e^2}}{\bar{y}} \times 100\%$.

3. Percentage contribution to the total variation calculated as $\sigma_s^2 / (\sigma_s^2 + \sigma_e^2)$ and $\sigma_e^2 / (\sigma_s^2 + \sigma_e^2)$ for samples and cages within samples, respectively.

endogenous losses.

4.6 Conclusion

In terms of digestible AA estimates (AID and SID) in wheat DDGS, lysine and phenylalanine were the least and the most digestible AA, respectively. Among indispensable AA in wheat DDGS, lysine, threonine and histidine were the most variable. The AID estimates of the wheat sample for lysine, threonine, histidine, glycine and alanine were 77.5%, 74%, 83.6%, 79.3% and 78.9%, respectively. Protein and AA digestibility estimates for both AID and SID values determined in wheat were significantly higher than in wheat DDGS samples ($P < 0.05$). There was no significant ($P < 0.05$) difference for AID and SID estimates of protein and AA among wheat DDGS samples from the same plant except for lysine ($P = 0.0422$). Considering both AID and SID coefficients of wheat DDGS samples, lysine was the least digestible AA averaging 35.6% and 40.0%, respectively.

5.0 MANUSCRIPT II

Standardized Ileal Amino Acid Digestibility in Full Fat Flaxseed for Broilers

5.1 ABSTRACT

This study was conducted to estimate the ileal digestibility of AA in five different samples of flaxseed fed to broilers. Concurrently, a reference wheat sample was also evaluated for comparison purposes. Two hundred and sixteen male Ross broiler chicks were fed a commercial starter diet from d 1 to 14 of age followed by the test diets from d 15 to 21. The six test diets consisted of equal amounts of a full fat flaxseed sample and wheat (50:50) or reference wheat as the sole source of dietary protein, minerals and vitamins. Chromic oxide (0.3%) was included in all diets as a digestibility marker. Each test diet was randomly assigned to six replicate cages, each with 6 birds. On d 21, birds were killed to sample ileal digesta for determining the AID and SID of AA. The SID values were calculated using ileal endogenous AA losses previously determined in our laboratory. Among the indispensable AA, the lowest AID value was observed for threonine averaging 40.2% whereas the respective value for methionine (64.9%) was the highest for flaxseed samples. The most variable AID estimates of flaxseed samples were observed for threonine (37.1% - 42.5%), valine (47.7% - 56.4%), histidine (50.6% - 57.5%) as indispensable and aspartic acid (48.4% - 57.7%), alanine (49.2% - 62.1%) and serine (47% - 54.8%) as dispensable AA, respectively. The AID estimates of the wheat sample for threonine, valine, histidine, alanine and serine were 76.6%, 82.8%, 84.6%, 79.9% and 84.8%, respectively. Amino acid digestibility estimates for both AID and SID

determined in wheat were significantly higher than for flaxseed samples ($P<0.05$). Considering both AID and SID coefficients of flaxseed samples, threonine was the least digestible AA averaging 40.2% and 47.4%, respectively.

5.2 INTRODUCTION

Flax (*Linum usitatissimum*), like other oilseeds, is a valuable source of protein (20-23%) and oil (40-43%) (Lee et al., 1991). In addition, out of its total fatty acids, about 46 to 71% is α -linolenic acid which is currently of interest both in human and animal nutrition because of its health promoting effects (Cunnane, 1995; Sim and Qui, 1995; Bhatta, 1997; Wood and Enser, 2007). These two main characteristics of flaxseed together with its availability render it as a potential feedstuff to be used in broiler diets. Although its use in dairy cow rations to provide a source of fiber, protein and to enhance the milk composition is common (Petit, 2002; Ward et al., 2002), flaxseed is not as valued in monogastric diets due to its high mucilage content and deficiency in the essential amino acid, lysine (McDonald et al., 1995). Besides mucilage, the presence of linatine (an antipyrodoxine factor) and moderate amounts of cyanogenic glycosides (primarily linamarin) limit its use in poultry diets (Peterson, 1958; Klosterman et al., 1967; Madhusudhan et al., 1986; Ravindran and Blair, 1992; Bhatta, 1997). Nonetheless, there are reports of implementing effective processing methods to allow usage of higher amounts of flaxseed or meal in substitution for expensive protein sources in broiler diets while maintaining satisfactory performance. Kratzer et al. (1947) reported that the growth of chicks fed an AA-supplemented flaxseed meal diet was as good as chicks fed a conventional control diet, even though the mucilage caused sticky droppings. Wylie et al.

(1972) supplemented starter diets with 26 mg of pyridoxine per kg and growing diets with 6 mg pyridoxine per kg and found that flaxseed meal could be used satisfactorily at 17 to 18% of the diet and could replace half of the soybean meal in the ration for starting and growing pullets. Madhusudhan et al. (1986) successfully used water-treated flaxseed meal to supply 50 to 75% of the protein in a diet for chickens. However, information on the ileal AA digestibility values and the associated variability of flaxseed as a protein source in broiler diets is limited and studies conducted in the past mostly related to the effects of flaxseed on enhancing the fatty acid composition of meat (Madhusudhan et al., 1986; Ajuyah et al., 1991; Lee et al., 1991; Bond et al., 1997; Roth-Maier et al., 1998).

The definition of nutritional value of a feed ingredient has changed over time, and is now defined as available rather than total nutrient content. In fact, availability of protein and AA are greatly impacted by the inherent quality of a given feed ingredient leading to variable digestibility estimates in different sources of a feedstuff. It has been documented and discussed in detail that AA digestibility results are greatly impacted by the age of an animal and the methodology being used (Ten Doeschate et al., 1993; Lemme et al., 2004; Huang et al., 2005; Garcia et al., 2007); therefore, results obtained from mature birds at the fecal level (Lee et al., 1995; Leeson and Summers, 2005) are not applicable to broiler chickens. For an accurate determination of AA digestibility, basal endogenous losses at the ileal level should be used to transform the AID into the SID values (Donkoh and Moughan, 1994; Angkanaporn et al., 1996; Lemme et al., 2004). However, to the best of the authors' knowledge there has been no report on ileal protein and AA digestibility of flaxseed and their associated CV in broilers. Thus, the objective of this study was to determine the AID and SID of protein and AA and their associated CV in full fat flaxseed

fed to broilers. As used in our previous studies, a sample of our reference wheat was also evaluated for comparison purposes.

5.3 MATERIALS AND METHODS

5.3.1 DIETS

Five full fat brown flaxseed samples were obtained from different local farms. Each flaxseed sample was assayed in a test diet containing flaxseed and wheat at an equal ratio such that each of them was included at 47.45% of the diet. The reference wheat sample was also tested separately at 91.7% inclusion rate as the sole source of protein. Test diets were prepared in mash form, balanced for mineral and vitamin levels according to NRC (1994) requirements with chromic oxide (0.3%) used as digestibility marker.

5.3.2 BIRDS AND EXPERIMENTAL CONDUCT

The experimental protocol used in the present study was reviewed and approved by the Animal Care Protocol Management and Review Committee of the University of Manitoba and birds were cared for according to the guidelines of the Canadian Council on Animal Care (1993).

Day-old male broiler chicks (Ross 308, Aviagen) were obtained from a local hatchery (Carlton Hatchery, Grunthal, Manitoba, Canada) and housed in Petersime battery brooders (Petersime Incubator Co., Gettysburg, Ohio) in a room with continuous fluorescent lighting. Room temperature was maintained at 32, 28, and 24°C during week 1, 2, and 3, respectively. From day 1 to 14, birds were fed a commercial starter diet

(FeedRite, Ridley Inc., Winnipeg, MB) in crumble form (3100 Kcal/Kg, 19% CP). On day 14, birds were fasted for 3 h, weighed and distributed into 36 cages with similar body weights. Six replicate cages (6 birds/cage) were randomly assigned to each of the six test diets and fed from day 15 to day 21. Birds had free access to feed and water throughout the study period. On day 21, birds were killed by cervical dislocation and the digesta samples from the ileum (from Meckel's diverticulum to a point 4 cm proximal to ileo-cecal junction) were collected by gently squeezing the contents of the ileum into sample bags. The digesta from birds within a cage were pooled into one bag and frozen immediately after collection and subsequently freeze-dried. The dried ileal digesta were stored in airtight bags at -4°C until required for chemical analysis.

5.3.3 CHEMICAL ANALYSIS

The flaxseed, wheat, test diets and digesta samples were finely ground using a coffee grinder (CBG5 Smart Grind; Applicia Consumer Products, Inc., Shelton, CT). Dry matter was determined according to the methods of AOAC(2000), and nitrogen was analyzed using the Leco nitrogen analyzer (Model CNS-2000; Leco Corp., St. Joseph, MI). Amino acid contents of the diet and digesta were determined by Evonik (Hanau-Wolfgang, Germany). Dietary AA contents were determined by using ion-exchange chromatography with postcolumn derivatization with ninhydrin (Llames and Fontaine, 1994). Sulfur AA (methionine and cystine) were oxidized with performic acid, and neutralized with sodium metabisulfite for analyses (Commission Directive, 1998). Samples were quantified with the internal standard method by measuring the absorption of reaction products with ninhydrin at 570 nm. Chromium was analyzed, after the

samples were ashed at 600 °C for 12 h in a muffle furnace, using inductively coupled plasma mass spectrometry (ICP-AES Vista, Varian) according to the method (procedure # 985.01) of AOAC (2005).

5.3.4 DIGESTIBILITY CALCULATION

The AID coefficient of AA was calculated using the indigestible marker as follows.

$$\text{AID} = \frac{(\text{AA/IM})_d - (\text{AA/IM})_{id}}{(\text{AA/IM})_d}$$

Where, $(\text{AA/IM})_d$ = ratio of AA to indigestible marker (IM) content in diet and

$(\text{AA/IM})_{id}$ = ratio of AA to indigestible marker (IM) content in ileal digesta

Basal endogenous loss estimates determined with a nitrogen-free diet (Golian et al., 2008) were used for the correction of AID coefficients. The SID coefficients were calculated as follows:

$$\text{SID}\% = \text{AID} + [(\text{basal IAA}_{\text{end}} / \text{AA}_{\text{diet}}) \times 100]$$

Where, IAA_{end} = basal ileal endogenous AA loss and AA_{diet} = Amino acid content of the diet.

The endogenous ileal AA loss values used to calculate the SID are presented in the Appendix.

5.3.5 STATISTICAL ANALYSIS

The data for digestibility estimates of full fat flaxseed and wheat were analyzed using the GLM procedure of SAS Institute, Inc. (1990) as a completely randomized design. Tukey's test was applied to separate means when a significant *F*-value was detected. To examine the effects of different feed samples on the animal assay, the Nested procedure was also applied to further analyze the random feed sample and bird/cage sub-sampling effects. For a better demonstration of variability, the components of total coefficients of variation (CV%) associated with the AID and SID estimates for each AA across different flaxseed samples are included as variance due to the samples (σ_s^2) and cages within samples (σ_e^2).

5.4 RESULTS AND DISCUSSION

Crude protein and AA composition of flaxseed and wheat samples and diets are presented in Tables 5.1 and 5.2, respectively. Reported values for the AA profile of flaxseed in some previous studies are comparable with the data presented here (Barbour and Sim, 1991; Lee et al., 1995; Rodriguez et al., 2001; Alzueta et al., 2002). Amino acid profile of the wheat sample was similar to that reported in Manuscript I, but slightly higher compared with previous reports by others (Ravindran et al., 1999b; Nyachoti et al., 2005; Ravindran et al., 2005; Huang et al., 2006; Thacker and Widyaratne, 2007). Compared with the wheat, the average CP content of flaxseed samples was 1.5-fold higher. In the flaxseed samples, average sulfur AA and histidine were the lowest relative to other AA. The same trend was observed for the wheat, with sulfur AA, histidine and lysine contents being lower compared with other AA. Arginine (2.31%), leucine (1.40%) and valine (1.20%) in flaxseed and leucine (1.04%), phenylalanine (0.73%) and arginine

Table 5.1. Crude protein and amino acid composition (%) of wheat¹ and flaxseed² samples (DM basis)

Items	Wheat	Flaxseed					Mean ³	CV% ⁴
		1	2	3	4	5		
DM	91.5	95.9	95.7	95.6	95.2	95.5	95.6	0.3
CP	15.7	22.5	20.1	25.9	26.8	25.9	24.2	11.7
<i>Indispensable amino acids</i>								
Arg	0.72	2.08	1.94	2.47	2.56	2.52	2.31	12.3
His	0.35	0.48	0.45	0.52	0.55	0.52	0.50	7.8
Ile	0.52	0.91	0.85	1.07	1.12	1.04	1.00	11.3
Leu	1.04	1.30	1.21	1.47	1.52	1.48	1.40	9.6
Lys	0.43	0.87	0.84	0.98	1.02	0.96	0.93	8.2
Met	0.26	0.45	0.41	0.47	0.48	0.47	0.46	6.1
Met+Cys	0.62	0.81	0.77	0.91	0.94	0.92	0.87	8.6
Phe	0.73	1.05	0.99	1.20	1.25	1.23	1.14	10.2
Thr	0.46	0.85	0.79	0.90	0.93	0.94	0.88	7.1
Val	0.66	1.11	1.03	1.27	1.33	1.25	1.20	10.3
<i>Dispensable amino acids</i>								
Ala	0.54	0.98	0.93	1.11	1.17	1.13	1.06	9.7
Asp	0.77	2.09	1.95	2.39	2.49	2.46	2.28	10.6
Cys	0.36	0.37	0.37	0.45	0.46	0.45	0.42	10.9
Glu	4.62	4.19	4.01	5.03	5.22	5.16	4.72	12.2
Gly	0.62	1.30	1.26	1.48	1.56	1.50	1.42	9.3
Pro	1.50	0.76	0.71	0.84	0.88	0.86	0.81	8.9
Ser	0.72	1.04	0.98	1.14	1.17	1.23	1.11	9.1

1. Reference wheat sample (AC Barrie) obtained from James farm, Winnipeg, MB, Canada.

2. Five full fat flaxseed samples were obtained from local farms.

3. Mean of five full fat flaxseed samples.

4. Coefficient of variation (CV%) across five full fat flaxseed samples.

Table 5.2. Analyzed crude protein and amino acid composition (%) of wheat and flaxseed diets (DM basis)

Items	Wheat ¹	Flaxseed					Mean ²	CV% ³
		1	2	3	4	5		
DM	90.0	91.5	91.7	91.7	91.4	91.6	91.6	0.1
CP	14.6	17.9	17.4	19.8	20.1	20.1	19.1	6.8
<i>Indispensable amino acids</i>								
Arg	0.65	1.26	1.22	1.47	1.52	1.57	1.41	11.2
His	0.33	0.40	0.38	0.42	0.43	0.43	0.41	5.3
Ile	0.48	0.66	0.64	0.73	0.75	0.78	0.71	8.4
Leu	0.94	1.09	1.06	1.17	1.20	1.20	1.14	5.7
Lys	0.38	0.59	0.59	0.66	0.67	0.67	0.64	6.6
Met	0.30	0.39	0.35	0.39	0.40	0.40	0.39	5.4
Met+Cys	0.62	0.72	0.69	0.76	0.78	0.78	0.75	5.3
Phe	0.66	0.81	0.80	0.90	0.92	0.94	0.87	7.4
Thr	0.42	0.60	0.57	0.65	0.66	0.65	0.63	6.2
Val	0.59	0.81	0.78	0.89	0.91	0.95	0.87	8.2
<i>Dispensable amino acids</i>								
Ala	0.49	0.72	0.68	0.78	0.80	0.81	0.76	7.4
Asp	0.70	1.32	1.25	1.48	1.51	1.56	1.42	9.3
Cys	0.32	0.33	0.34	0.39	0.39	0.39	0.37	8.2
Glu	4.25	4.07	4.16	4.69	4.69	4.82	4.49	7.7
Gly	0.57	0.89	0.88	0.99	1.02	1.02	0.96	7.3
Pro	1.43	1.09	1.14	1.22	1.19	1.22	1.17	4.8
Ser	0.65	0.80	0.77	0.89	0.89	0.88	0.85	6.7

1. Reference wheat (AC Barrie) from James farm, Manitoba.

2. Mean of five full fat flaxseed diets.

3. Coefficient of variation (CV%) across five flaxseed diets.

(0.72%) in wheat were the indispensable AA with the highest content. The calculated CV among flaxseed samples demonstrated an overall average of 9.5% variability for the AA presented. While methionine (6.1%), threonine (7.1%) and histidine (7.8%) were the least variable, arginine (12.3%), glutamic acid (12.2%) and isoleucine (11.3%) were the most variable indispensable AA. The AA profile of flaxseed in this study revealed an overall average of 1.8-fold higher AA concentration compared with wheat.

Being grown and widely available in northern United States and southern Canada, there is a potential in using full fat flaxseed in broiler diets to enrich meat with ω -3 fatty acids and to substitute for expensive protein sources commonly used in broiler diets. Although not considered as a quality protein resource mainly due to its high mucilage and hull content (40% of the total seed) (Mandokhot and Singh, 1979), there are reports on positive qualitative effects of incorporating flaxseed in broiler diets. Ajuyah et al. (1991) included either 10 or 20% full fat flaxseed in broiler diets and reported less carcass fat, larger leg weights and increased ω -3 fatty acids in chickens. In a companion study, Ajuyah et al. (1993) fed 15% full fat flaxseed to broilers and determined higher levels of α -linolenic acid in dark meat than in white meat, with meat from flax-fed broilers having higher ω -3 fatty acids, compared with meat from chickens fed the control diet. Kratzer (1946) and McGinnis and Polis (1946) reported that the growth of chicks fed linseed meal could be greatly improved if the meals were treated with water and dried before being mixed in the feed. Studies with water-treated linseed meal as the only protein source in a diet for chicks showed that the major AA deficiency was observed for lysine (Kratzer, 1947). However, data regarding AA appraisal of full fat flaxseed in terms of its digestibility in monogastric animals is limited. While Forbes et al. (1914) reported 68%

total digestible nutrients in flaxseed meal, Bell and Keith (1993) reported a 70 to 76% of apparent digestibility of flaxseed under typical feeding conditions for growing pigs. Few previous studies regarding AA digestibility determination in flaxseed were carried out using adult roosters (Barbour and Sim, 1991; Lee et al., 1995; Leeson and Summers, 2005) and the total fecal collection method. However, physiologically mature birds do not reflect the digestive capabilities of younger chickens (Ravindran and Bryden, 1999) and moreover, it is well accepted that the ileal digestibility results, compared with excreta analysis, are a more sensitive and accurate indicator of AA availability. (Ten Doeschate et al., 1993; Kadim and Moughan, 1997; Ravindran and Bryden, 1999; Ravindran et al., 1999b; Karakas et al., 2001; Kadim et al., 2002; Lemme et al., 2004).

The results for AID and SID coefficients of wheat and flaxseed samples are presented in Tables 5.3 and 5.4, respectively. The average total AID and SID of AA estimated for wheat were 85.1 and 89.8%, respectively. Threonine, lysine and arginine were the least digestible indispensable AA in the wheat sample whereas sulfur amino acids, phenylalanine and leucine were the most digestible. The AID results determined for the wheat sample are in close agreement with ours (Manuscript I) and other previous reports (Ravindran et al., 1999b; Ravindran et al., 2005; Huang et al., 2006).

The AID coefficients of protein and AA in flaxseed were calculated using the difference method according to Fan and Sauer (1995a). The difference method is often used to determine AA digestibility values in feedstuffs of poor palatability where there is feeding limitation (Kreienbring et al., 1988; Knabe et al., 1989). In the difference method, assuming there are no interactions between the digestibility values of AA in the basal diet (wheat) and test feed ingredient (flaxseed), the relationship between

Table 5.3. Apparent ileal digestibility coefficients¹ (%) of crude protein and amino acids in chicks fed diets containing wheat² (n = 1) and flaxseed² (n = 5)

Item	Wheat	Flaxseed					Average ³
		A	B	C	D	E	
CP	83.3 ^a	51.5 ^b	56.3 ^b	56.5 ^b	47.9 ^b	51.5 ^b	52.7
<i>Indispensable amino acids</i>							
Arg	81.4 ^a	56.5 ^b	62.1 ^b	75.0 ^b	58.5 ^b	63.6 ^b	63.1
His	84.6 ^a	52.7 ^b	55.4 ^b	57.5 ^b	50.6 ^b	55.7 ^b	54.4
Ile	86.7 ^a	50.7 ^b	55.5 ^b	58.4 ^b	49.7 ^b	56.4 ^b	54.1
Leu	88.0 ^a	55.2 ^b	60.0 ^b	60.3 ^b	54.2 ^b	58.8 ^b	57.7
Lys	77.8 ^a	60.8 ^b	67.3 ^b	63.3 ^b	59.9 ^b	66.5 ^b	63.6
Met	92.3 ^a	62.3 ^b	64.2 ^b	72.3 ^b	61.4 ^b	64.4 ^b	64.9
Met+Cys	89.6 ^a	58.8 ^b	63.5 ^b	66.3 ^b	58.1 ^b	60.6 ^b	61.5
Phe	88.2 ^a	53.1 ^b	57.9 ^b	61.0 ^b	50.7 ^b	56.6 ^b	55.9
Thr	76.6 ^a	41.8 ^b	42.5 ^b	40.6 ^b	37.1 ^b	38.8 ^b	40.2
Val	82.8 ^a	49.7 ^b	54.2 ^b	56.4 ^b	47.7 ^b	54.9 ^b	52.6
<i>Dispensable amino acids</i>							
Ala	79.9 ^a	50.5 ^b	54.9 ^b	62.1 ^b	49.2 ^b	55.3 ^b	54.4
Asp	78.1 ^a	49.9 ^b	52.7 ^b	57.7 ^b	48.4 ^b	54.4 ^b	52.6
Cys	86.9 ^a	55.6 ^b	60.8 ^b	61.0 ^b	53.8 ^b	56.1 ^b	57.5
Glu	95.1 ^a	62.4 ^b	69.1 ^b	76.0 ^b	62.8 ^b	66.4 ^b	67.3
Gly	80.4 ^a	50.6 ^b	57.3 ^b	64.9 ^b	51.7 ^b	56.6 ^b	56.2
Pro	94.0 ^a	63.5 ^b	69.1 ^b	67.8 ^b	59.2 ^b	62.9 ^b	64.5
Ser	84.8 ^a	49.8 ^b	52.6 ^b	54.8 ^b	47.0 ^b	50.0 ^b	50.8

1. Means with different superscripts within the same row are significantly different ($p < 0.05$).

2. One reference wheat (AC Barrie) and five full fat flaxseed samples were fed to six cages, each with 6 birds.

3. Overall average of five full fat flaxseed digestibility coefficients.

Table 5.4. Standardized ileal digestibility coefficients¹ (%) of crude protein and amino acids in chicks fed diets containing wheat² (n = 1) and flaxseed² (n = 5)

Item	Wheat	Flaxseed					Average ³
		A	B	C	D	E	
CP	86.3 ^a	53.9 ^b	58.7 ^b	58.7 ^b	50.0 ^b	53.6 ^b	55.0
<i>Indispensable amino acids</i>							
Arg	84.6 ^a	58.1 ^b	63.8 ^b	76.4 ^b	59.9 ^b	64.9 ^b	64.6
His	87.4 ^a	54.9 ^b	57.7 ^b	59.6 ^b	52.7 ^b	57.8 ^b	56.5
Ile	91.1 ^a	53.8 ^b	58.7 ^b	61.2 ^b	52.4 ^b	59.0 ^b	57.0
Leu	91.4 ^a	58.0 ^b	62.9 ^b	62.9 ^b	56.7 ^b	61.4 ^b	60.4
Lys	82.6 ^a	63.8 ^b	70.3 ^b	66.0 ^b	62.5 ^b	69.1 ^b	66.3
Met	94.7 ^a	64.1 ^b	66.2 ^b	74.0 ^b	63.0 ^b	66.1 ^b	66.7
Met+Cys	93.1 ^a	61.8 ^b	66.5 ^b	69.1 ^b	60.8 ^b	63.3 ^b	64.3
Phe	98.7 ^a	61.6 ^b	66.6 ^b	68.7 ^b	58.1 ^b	63.9 ^b	63.8
Thr	87.3 ^a	49.3 ^b	50.5 ^b	47.5 ^b	44.0 ^b	45.8 ^b	47.4
Val	87.6 ^a	53.1 ^b	57.7 ^b	59.5 ^b	50.7 ^b	57.8 ^b	55.8
<i>Dispensable amino acids</i>							
Ala	84.6 ^a	53.6 ^b	58.2 ^b	64.9 ^b	52.0 ^b	58.1 ^b	57.4
Asp	84.5 ^a	53.3 ^b	56.3 ^b	60.7 ^b	51.3 ^b	57.2 ^b	55.8
Cys	91.6 ^a	60.1 ^b	65.2 ^b	64.8 ^b	57.7 ^b	59.9 ^b	61.5
Glu	96.4 ^a	63.6 ^b	70.3 ^b	77.0 ^b	63.8 ^b	67.5 ^b	68.4
Gly	84.9 ^a	53.5 ^b	60.2 ^b	67.4 ^b	54.1 ^b	59.1 ^b	58.9
Pro	96.1 ^a	66.2 ^b	71.7 ^b	70.3 ^b	61.6 ^b	65.4 ^b	67.0
Ser	90.3 ^a	54.2 ^b	57.2 ^b	58.8 ^b	51.0 ^b	54.0 ^b	55.0

1. Means with different superscripts within the same row are significantly different ($p < 0.05$).

2. One reference wheat (AC Barrie) and five full fat flaxseed samples were fed to six cages, each with 6 birds.

3. Overall average of five full fat flaxseed digestibility coefficients.

digestibility values can be expressed according to equation [1]. The digestibility values of AA in the test feed ingredient was determined according to the following equation [2] (Van Leeuwen et al., 1987).

$$[1] \quad D_D = D_B \times S_B + D_A \times S_A$$

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

D_D = Apparent ileal digestibility of an AA in the test diet (%)

D_B = Apparent ileal digestibility of an AA in the basal feed ingredient (%)

S_B = Contribution level of an AA from the basal feed ingredient to the test diet

$S_B = 1 - S_A$ (%)

D_A = Apparent ileal digestibility of an AA in the test feed ingredient (%)

And S_A = Contribution level of an AA from the test feed ingredient to the test diet (%)

Estimates of AID in flaxseed ranged from 40.2 to 67.3% for threonine and glutamic acid, respectively. The SID values followed the same trend with the least and the most digestible value of 47.4 and 68.4% for threonine and glutamic acid, respectively. The overall average of AID and SID for AA determined in flaxseed samples were 57.1 and 60.4%, respectively. In contrast, previous estimates of true AA availability of full fat flaxseed determined with the total excreta collection assay using mature leghorn roosters (Barbour and Sim, 1991) ranged from as low as 79% to as high as 91% for methionine and arginine, respectively. Lee et al. (1995) evaluated the apparent and true AA digestibility in full fat flaxseed using the same adult rooster protocol and reported apparent AA availability estimates of 60.8 to 84.4% for threonine and glutamic acid and true digestibility of 70.8 to 88.7% for threonine and glutamic acid, respectively. Also, some AA digestibility in whole flaxseed, estimated using the force-feeding method

reported by Leeson and Summers (2005) was 65 and 85% for threonine and tryptophan, respectively. Comparing the published data with results of the current study, a clear variability and large differences among AA digestibility values in flaxseed is evident. It has been shown that the method used (ileal vs. fecal) and age of the bird have significant effects on AA digestibility values (Ten Doeschate et al., 1993; Kadim and Moughan, 1997; Ravindran and Bryden, 1999; Ravindran et al., 1999; Karakas et al., 2001; Kadim et al., 2002; Lemme et al., 2004; Huang et al., 2006; Garcia et al., 2007). Indeed, Garcia et al. (2007) reported differences as large as 20 to 25% in the same diet for some AA in ileal digestibility estimates determined in 7-d-old compared with 21-d-old birds. Although differences in AA digestibility values among the flaxseed samples were not statistically significant ($P>0.05$), there was a distinctively significant difference ($P<0.05$) between AID values determined for wheat and flaxseed samples. Interestingly, in both wheat and flaxseed samples the lowest and highest AID estimates were observed for threonine and glutamic acid at 76.6 and 40.2% and 95.1 and 67.3%, respectively.

To demonstrate the variability associated with the AID and SID coefficients of flaxseed samples the total CV associated with each AA across the samples and their variation components (between and within samples) are presented in Table 5.5. Among the indispensable AA the AID and SID values were least variable for sulfur amino acids and threonine averaging 14.2 and 13.6%, respectively. Threonine had the highest variability for AID and SID values, averaging 23.7 and 20.2%, respectively. For most AA, there was a large effect (100%) of within sample variation on the observed total CV, which could be attributed to the varied feed intake of the birds due to the presence of ANF in flaxseed. In fact, the contents of mucilage, hull and cotyledon in flaxseed are reported

Table 5.5. Coefficient of variation (CV%) associated with apparent and standardized ileal digestibility estimates determined in chicks fed diets containing full fat flaxseed¹ (n = 5)

Item	AID			SID		
	CV% ²	Variance components ³		CV% ²	Variance components ³	
		Between samples	Within samples		Between samples	Within samples
CP	17.8	0.0	100.0	17.1	0.0	100.0
<i>Indispensable amino acids</i>						
Arg	19.2	17.3	82.7	18.7	16.9	83.1
His	17.8	0.0	100.0	17.1	0.0	100.0
Ile	18.1	0.0	100.0	17.1	0.0	100.0
Leu	15.5	0.0	100.0	14.8	0.0	100.0
Lys	15.0	0.0	100.0	14.4	0.0	100.0
Met	14.3	2.9	97.1	13.8	3.0	97.0
Met+Cys	14.2	0.0	100.0	13.6	0.0	100.0
Phe	16.0	3.0	97.0	14.1	3.8	96.2
Thr	23.7	0.0	100.0	20.2	0.0	100.0
Val	18.5	0.0	100.0	17.4	0.0	100.0
<i>Dispensable amino acids</i>						
Ala	19.5	4.7	95.3	18.4	4.6	95.4
Asp	20.5	0.0	100.0	19.3	0.0	100.0
Cys	16.9	0.0	100.0	15.8	0.0	100.0
Glu	13.5	19.9	80.1	13.2	19.7	80.3
Gly	19.1	9.9	90.1	18.2	9.6	90.4
Pro	12.1	10.5	89.5	11.7	10.7	89.3
Ser	18.3	0.0	100.0	17.0	0.0	100.0

1. Five samples of full fat flaxseed were assayed in six replicates cages with 6 birds in each and variance components for samples (σ_s^2) and cages within samples (σ_e^2) was determined.

2. Total coefficient of variation, $CV\% = \frac{\sqrt{\sigma_s^2 + \sigma_e^2}}{\bar{y}} \times 100\%$.

3. Percentage contribution to the total variation calculated as $\sigma_s^2 / (\sigma_s^2 + \sigma_e^2)$ and $\sigma_e^2 / (\sigma_s^2 + \sigma_e^2)$ for samples and cages within samples, respectively.

to be variable (Peterson, 1958). Mucilage significantly contributes to the high fiber content in flaxseed and its variable content could explain the differences observed in the digestibility of AA and CP. For example, Bell and Keith (1993) tested two different lines of yellow and brown flaxseed at 10, 20 and 30% of a wheat-barley based diet with growing pigs and found that apparent digestibility of dietary CP in yellow hull was higher compared to brown hull seeds (75.2% vs. 74.7%). In the same study it was reported that yellow-seeded flaxseed contained less neutral detergent fiber (24% vs. 29%), less crude fiber (8.7% vs. 11.7%) and more CP (41% vs. 38%) than brown-seeded flaxseed. Mucilage has a high water-absorption capacity and can increase the viscosity of digesta leading to reduced nutrient availability (Bhatta and Cherdkiatgumchai, 1990; Rodriguez et al., 2001). Furthermore, incorporation of flaxseed in poultry diets is limited by the presence of a number of deleterious factors such as phytic acid, cyanogenic glycosides, goitrogens, allergens and anti-pyridoxin compounds (Madhusudhan et al., 1986). It appears that the feeding value of flaxseed to chickens can not be improved unless effective processing procedures are used to decrease the effect of ANF. There are reports of demucilaging and/or dehulling (Mandokhot and Singh, 1978), wet autoclaving (Singh and Punia, 1979; Mandokhot and Singh, 1983) or water boiling (Madhusudhan et al., 1986) to improve the nutritive value of flaxseed.

Improvement in protein utilization following these processing procedures can be explained mainly by at least the two factors of denaturation of storage protein and reduction of ANF (Feng et al., 2003). Heat processing of plant protein sources results in protein denaturation and a subsequent higher protein utilization (Beumer et al., 1998). Toxicity of flaxseed in broiler diets is mainly manifested by the presence of cyanogenic

glycosides and an anti-pyridoxin factor (Kratzer et al., 1947; Conn, 1969; Klosterman, 1974). The catabolism of cyanogenic glycosides in the animal body is a process that consumes sulfur AA (Tewe and Iyayi, 1989) resulting in growth depression of the animal. Without processing, the presence of trypsin inhibitor in flaxseed (Bhatta, 1993) can also contribute to a reduction in nitrogen absorption and an increase in the endogenous nitrogen losses. Heat processing can be beneficial in reducing these heat labile ANF thus further improving nitrogen utilization by monogastric animals.

It should be noted that the protein requirement of chickens is, in fact, a requirement for AA (NRC, 1994; Leeson and Summers, 2005). However, not all AA in a feedstuff are equally digested and available to the animal to meet the requirement. As it was seen in the present study, despite a higher content of CP and AA compared with wheat, digestibility values (AID and SID) of flaxseed were significantly ($P < 0.05$) lower. Therefore, the use of AA digestibility values as a sensitive indicator of AA biological availability (Han and Parsons, 1990; Lemme et al., 2004) is preferable to total AA basis in ensuring an effective feed formulation. For full fat flaxseed to be effectively incorporated into broiler diets to replace highly digestible protein sources, its available AA contents need to be estimated. To the authors' knowledge, thus far, there has been no study indicating AA digestibility of full fat flaxseed using the chick ileal digestibility assay to demonstrate both availability and variability of AID and SID among different flaxseed samples. Thus, this study was conducted to determine the AID and SID coefficients and their associated variation in full fat flaxseed with broilers, aiming to provide a data set to be used for more accurate diet formulation.

5.5 Conclusion

Among the indispensable AA in flaxseed, threonine and methionine were the least and the most digestible AA, respectively. The associated total variability in AID and SID estimates for most AA was greatly affected by within sample (bird/cage) variation which demonstrates a different response of the birds to accepting flaxseed as feed. Amino acid digestibility estimates for both AID and SID determined in wheat were significantly higher than for flaxseed samples ($P < 0.05$) but there was no difference in AID and SID estimates of protein and AA among the flaxseed samples assayed ($P > 0.05$). The AID and SID of protein and AA in wheat conformed very well to those reported in manuscript I. Considering both AID and SID coefficients of flaxseed, threonine was the least digestible AA.

6.0 MANUSCRIPT III

Standardized Ileal Amino Acid Digestibility in Barley, pea and wheat for Broilers

6.1 ABSTRACT

Three separate experiments (Exp.) were conducted to estimate ileal digestibility of AA in 7 barley (Exp.1), 5 pea (Exp.2) and 6 wheat (Exp.3) samples fed to broilers. A sample of reference wheat was also assayed separately in each Exp. Two hundred and eighty eight day-old male Ross broiler chicks in each of Exp. 1, 2 and 336 were used in Exp.3, respectively. Birds were fed a commercial starter diet from d 1 to 14 of age followed by the test diets from d 15 to 21. Each test diet for barley, wheat, pea and the reference wheat samples contained 89, 92, 84 and 91.7% of the test ingredient as the sole source of protein and was balanced for minerals and vitamins. Chromic oxide (0.3%) was used in all diets as a digestibility marker. Each test diet was randomly assigned to six replicate cages, each with 6 (Exp. 1 and reference wheat) or 8 (Exp. 2 and 3) birds. On d 21, birds were killed to sample ileal digesta for determining the apparent (AID) and standardized (SID) ileal AA digestibilities. The SID values were calculated using ileal endogenous AA losses previously determined in our laboratory. Pea samples demonstrated higher AID and SID values of CP (86.1 and 88.3%) compared to the respective values for wheat (84.3 and 87.2%) and barley (76.3 and 79.7%). The AID values of indispensable AA in wheat and barley indicated low threonine (74.6 and 69.6%), lysine (79.3 and 76.3%) and arginine (82.2 and 76.8%) digestibilities. The AID estimates of indispensable AA in pea samples were evident with arginine (92.9%), lysine

(91.7%) and histidine (88.5%) as the most digestible and cystine (78.7%), methionine+cystine (81.4%) and threonine (83.0%) as the least digestible AA. The trend observed in the variability of either AID and SID estimates for indispensable AA in wheat was very similar to those of barley with sulfur AA and glutamic acid being the least and lysine and threonine as the most variable of all AA in both. The AID results of the reference wheat compared closely with the wheat samples assayed and conformed very well to the results from Manuscripts I and II. Coefficient of variation for AID and SID estimates of AA in pea was marked with arginine and lysine as the least and sulfur AA as the most variable of all.

6.2 INTRODUCTION

Cereal grains are major ingredients used in poultry diets to provide energy and protein. Depending on the age of the bird, poultry diets typically contain between 30 to 60% of grains. While corn is a major constituent of poultry diets in the USA and in South America, wheat and barley are commonly used in Europe and Canada. Compared with corn or wheat, barley generally has a lower nutritional value for monogastric animals. This is mainly due to a relatively high fiber content and cell wall NSP resulting in increased intestinal viscosity and lower level of available nutrients (Herstad and McNab, 1975; Hesselman and Aman, 1986; Classen et al., 1988; Ravindran et al., 2007). However, there are reports of incorporating increased amounts of whole barley in broiler diets with equal or better performance compared with a conventional corn-soybean diet (Brake et al., 1997; Nahas and Lefrancois, 2001). Protein content of wheat and barley is very variable, ranging from 10-18% and 11-16%, respectively (Scott et al., 1982; Scott,

1987; McDonald et al., 1995; Leeson and Summers, 2005). This wide variability is attributed to differences in genotype, location, agronomic practices and season (Jeroch and Danicke, 1995; Gutierrez-Alamo et al., 2008). Furthermore, their nutritional value and digestibility are affected by anti-nutritive activities of the NSP. For example, Choct and Annison (1990) reported that wheat pentosans cause a general inhibition of nutrient digestion affecting starch, fat and protein digestibility. For the same reason it has been shown that feeding barley-based diets to chickens with high levels of extractable viscous β -glucans, significantly depresses ileal digestibility of protein (Hesselman and Aman, 1986).

Although soybean meal is the predominant source of protein for use in the diets of non-ruminants, climatic and environmental factors considerably limit its cultivation in Canada, where alternative plants such as peas are grown prevalently as protein source. The reported protein content in peas is widely variable falling into the range of 15.5-39.7% (Monti, 1983). Amino acid composition of protein in pea is characterized by high lysine and low methionine levels whereas cereals are universally acknowledged to be deficient in lysine making the combination of the two, nutritionally complementary. Peas are also rich sources of energy, generally containing as much or slightly less energy than most cereals. However, problems with pea protein include high variability in chemical and nutritional composition which limits their use in poultry diets (Conan and Carre, 1989; Grosjean et al., 1999). For instance, it has been reported that ANF such as protease inhibitors, lectins, saponins and tannins are responsible for the variability observed in pea protein digestibility (Gatel, 1994).

The success of diet formulation is measured by the accuracy with which nutrient requirements can be met by nutrient supply to achieve a target performance. Ileal digestibility versus fecal digestibility estimates offer a rapid method of improving the accuracy of diet formulation by overcoming the inaccuracy incurred as a result of hind gut microbial fermentation in the ceca of the birds (Kadim et al., 2002). In addition, due to the reported difference in protein digestibility between young and adult birds, digestibility studies must be performed on growing broilers as target animals (Ten Doeschate et al., 1993; Huang et al., 2005). The AID values as practical estimates of AA availability are affected by both the level of protein intake in the diet and ileal endogenous AA losses (Lemme et al., 2004). A correction for basal endogenous AA loss results in a more additive and accurate estimation of ileal protein digestibility as “standardized” ileal digestibility (Angkanaporn et al., 1996; Stein et al., 2007). For effective use of AID and SID estimates for different ingredients, it is important to understand the variability associated with the estimates in different samples of the same feedstuff.

Hence, the present experiments were conducted to determine the AID and SID estimates of protein and AA and their associated variability in barley, pea and wheat samples fed to broilers.

6.3 MATERIALS AND METHODS

6.3.1 DIETS

Seven barley, five pea and six wheat samples obtained from different locations in Western Canada were assayed in three separate experiments; one for each feedstuff. Test diets contained 89, 92 and 84% of barley, wheat and pea samples as the sole source of protein, and were balanced for minerals and vitamins based on NRC (1994) recommendations. Chromic oxide (0.3%) was added as a digestibility marker.

To determine assay repeatability, a sample of the reference wheat at 91.7% inclusion rate was also assayed in each experiment.

6.3.2 BIRDS AND EXPERIMENTAL CONDUCT

The experimental protocol for the studies was reviewed and approved by the Animal Care Protocol Management and Review Committee of the University of Manitoba and birds were cared for according to the guidelines of the Canadian Council on Animal Care (1993).

Two hundred and eighty eight and 336 day-old male broiler chicks were used in each of Exp. 1, 2 and 3, respectively. The chicks (Ross 308, Aviagen) were obtained from a local hatchery (Carlton Hatchery, Grunthal, Manitoba, Canada) and housed in Petersime battery brooders (Petersime Incubator Co., Gettysburg, Ohio) in a room with continuous fluorescent lighting. Room temperature was maintained at 32, 28, and 24°C during week 1, 2, and 3, respectively. From day 1 to 14, birds were fed a commercial starter diet (FeedRite, Ridley Inc., Winnipeg, Manitoba, Canada) in crumble form (3100 Kcal/Kg, 19% CP). On day 14, birds were fasted for 3 h, weighed and distributed (6 birds/cage in Exp.1 or 8 birds/cage in Exp. 2 and 3) into 48, 36 or 42 cages with similar body weights for Exp. 1, 2 and 3, respectively. Six replicate cages were randomly assigned to each of

the test diets and fed from day 15 to day 21. Birds had free access to feed and water throughout the study period. On day 21, birds were killed by cervical dislocation and the digesta samples from the ileum (from Meckel's diverticulum to a point 4 cm proximal to the ileo-cecal junction) were collected by gently squeezing the contents of the ileum into sample bags. Digesta from birds within a cage were pooled into one bag and frozen immediately after collection and subsequently freeze-dried. The dried ileal digesta were stored in airtight bags at -4°C until required for chemical analysis.

6.3.3 CHEMICAL ANALYSIS

Test ingredients and diets along with digesta samples were finely ground using a coffee grinder (CBG5 Smart Grind; Applica Consumer Products, Inc., Shelton, CT). Dry matter was determined according to AOAC (2000) procedure # 934.01, and nitrogen was analyzed using the Leco nitrogen analyzer (Model CNS-2000; Leco Corp., St. Joseph, MI). Amino acid content of the feed ingredients, diet and digesta were determined by Evonik (Hanau-Wolfgang, Germany). Dietary and digesta AA contents were determined using ion-exchange chromatography with postcolumn derivatization with ninhydrin (Llames and Fontaine, 1994). Sulfur AA (methionine and cystine) were first oxidized with performic acid, and subsequently neutralized with sodium metabisulfite (Commission Directive, 1998). Amino acid contents were quantified with the internal standard method by measuring the absorption of reaction products with ninhydrin at 570 nm. Chromium was analyzed, after the samples were ashed for 12 h at 600 °C in a muffle furnace, using inductively coupled plasma mass spectrometry (ICP-AES Vista, Varian) according to the method (procedure # 985.01) of AOAC (2005).

6.3.4 DIGESTIBILITY CALCULATION

The AID coefficients are calculated using the indigestible marker as follows:

$$AID = \frac{(AA/IM)_d - (AA/IM)_{id}}{(AA/IM)_d}$$

Where, $(AA/IM)_d$ = ratio of AA to indigestible marker (IM) content in diet and

$(AA/IM)_{id}$ = ratio of AA to indigestible marker (IM) content in ileal digesta

Basal endogenous AA loss estimates determined with a nitrogen-free diet were used for the correction of AID coefficients (Golian et al., 2008). The SID coefficients were calculated as follows:

$$SID\% = AID + [(basal\ IAA_{end} / AA_{diet}) \times 100]$$

Where, IAA_{end} = basal ileal endogenous AA loss and AA_{diet} = Amino acid content of the diet.

The endogenous ileal AA loss values used to calculate the SID are presented in the Appendix.

6.3.5 STATISTICAL ANALYSIS

The data for digestibility estimates of feed samples were analyzed using the GLM procedure of SAS (SAS Institute, 1990) as a completely randomized design. The Nested procedure was also applied to further analyze the random effect of the feed sample and

bird/cage sub-sampling on the total variation observed. For a better clarification of variability associated with estimates, components of total coefficients of variation (CV %) associated with the AID and SID estimates for each AA across different barley, pea and wheat samples are included as variance due to the samples (σ_s^2) and cages within samples (σ_e^2).

6.4 RESULTS AND DISCUSSION

Crude protein and AA concentration of barley, pea, wheat and the reference wheat samples are presented in Table 6.1. The AA composition of the reference wheat, wheat and barley showed a close association with methionine, cystine and histidine as the lowest and glutamine, proline and leucine reflecting the highest content in these samples, respectively which is consistent with NRC (1994) values. The protein and AA content of the reference wheat were in a very close agreement with the results reported in manuscripts I and II. Generally, barley samples showed more variability in crude protein and AA content than wheat samples. Lysine in both wheat and barley samples was among the least variable AA with a CV of 7.93 and 8.72%, respectively. However, lysine in both wheat and barley was among the lowest contents of indispensable AA (0.44 and 0.49%). This feature is linked to the composition of storage protein in cereals, for example, wheat storage protein is characterized by containing a high proportion of glutamic acid and proline whereas lysine, arginine, threonine and tryptophan make up a small proportion of it (Gutierrez-Alamo et al., 2008). While the most variable indispensable AA in wheat samples were isoleucine and histidine with a CV of 11.4 and 11.0%, phenylalanine and

Table 6.1. Crude protein and amino acid composition (%) of wheat (n = 6), pea (n = 5) and barley (n = 7) samples^{1,2} (DM basis)

Item	Wheat			Pea			Barley		
	Range	Mean	CV% ³	Range	Mean	CV% ³	Range	Mean	CV% ³
DM	92.1 – 96.0	94.0	1.5	92.7 – 95.2	94.2	1.1	91.1 – 92.5	92.1	0.6
CP	14.8 - 18.4	16.2	9.2	20.6 - 26.3	23.6	9.1	12.1 - 18.0	14.3	14.1
<i>Indispensable amino acids</i>									
Arg	0.68 - 0.86	0.76	9.6	1.63 - 2.45	2.0	14.8	0.61 - 0.85	0.68	12.2
His	0.33 - 0.44	0.38	11.0	0.52 - 0.64	0.59	8.0	0.26 - 0.38	0.30	14.1
Ile	0.45 - 0.61	0.53	11.4	0.89 - 1.07	1.01	7.1	0.42 - 0.62	0.48	15.9
Leu	0.95 - 1.18	1.05	8.7	1.49 - 1.84	1.70	8.1	0.83 - 1.24	0.99	14.4
Lys	0.40 - 0.48	0.44	7.9	1.55 - 1.89	1.73	7.3	0.45 - 0.58	0.49	8.7
Met	0.22 - 0.27	0.25	7.5	0.18 - 0.22	0.20	7.5	0.21 - 0.28	0.24	10.6
Met+Cys	0.51 - 0.66	0.59	9.0	0.52 - 0.56	0.54	3.0	0.46 - 0.63	0.52	11.1
Phe	0.67 - 0.86	0.74	10.2	1.03 - 1.25	1.15	7.2	0.62 - 1.03	0.76	17.8
Thr	0.41 - 0.50	0.45	8.1	0.80 - 0.95	0.86	6.8	0.43 - 0.56	0.47	10.1
Val	0.57 - 0.69	0.66	10.6	0.98 - 1.17	1.11	6.9	0.59 - 0.86	0.70	14.8
<i>Dispensable amino acids</i>									
Ala	0.51 - 0.61	0.55	8.0	0.91 - 1.07	1.00	6.2	0.49 - 0.67	0.55	10.3
Asp	0.72 - 0.89	0.80	9.5	1.94 - 2.35	2.14	8.5	0.73 - 0.93	0.81	8.0
Cys	0.31 - 0.39	0.35	8.0	0.33 - 0.34	0.34	1.6	0.26 - 0.35	0.28	11.1
Glu	4.11 - 5.49	4.65	11.0	3.38 - 4.32	3.87	8.9	2.74 - 4.82	3.58	21.2
Gly	0.59 - 0.73	0.65	8.3	0.94 - 1.08	1.02	5.4	0.49 - 0.63	0.54	8.7
Pro	1.48 - 1.97	1.64	12.0	0.90 - 1.05	0.98	6.2	1.19 - 2.15	1.59	22.2
Ser	0.67 - 0.82	0.72	8.6	0.98 - 1.23	1.09	8.7	0.53 - 0.78	0.61	14.3

1. n = number of different samples of a feed.

2. The average of the 3 reference wheat samples for DM, CP, Arg, His, Ile, Leu, Lys, Met, Met+Cys, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro and Ser were 93.5, 16.4, 0.75, 0.37, 0.54, 1.07, 0.44, 0.26, 0.62, 0.75, 0.46, 0.67, 0.56, 0.80, 0.36, 4.72, 0.65, 1.63 and 0.73, respectively.

3. Coefficient of variation between different samples of the same ingredients.

isoleucine were the most variable indispensable AA in barley with a CV of 17.8 and 15.9%.

The average crude protein and AA profile of the pea samples analyzed were in agreement with the values reported previously (Igbasan et al., 1997; Ravindran et al., 2005). Crude protein content in pea samples averaged 23.6% with a variability of 9.1%. Previous reports (Holt and Sosulski, 1979; Monti, 1983; Conan and Carre, 1989) also indicated a wide variability of protein content among peas which is attributed to differences in genotype, location, agronomic practices and season (Igbasan and Guenter, 1996). Concurring with the earlier reports (Guegen and Barbot, 1988; Gatel, 1994; NRC, 1994) indispensable AA profile of pea samples was marked with a high arginine and lysine, low sulfur AA contents which is explained by AA composition of the main storage protein in peas being low in sulfur AA and high in lysine (Croy et al., 1980). Because lysine is the first limiting AA in cereal-based diets for poultry, AA profile of pea protein could have a complementary effect in balancing AA composition of these diets (Marquardt and Bell, 1988; Gatel, 1994). In terms of variability observed for the indispensable AA profile in pea samples, arginine and methionine+cystine were among the most and least variable AA with an average CV of 14.8 and 3%, respectively. A comparison of protein and AA variability among feed ingredients analyzed, presents barley as the most variable followed by wheat and pea in that order.

Apparent and standardized ileal digestibilities of CP and AA in the feed samples evaluated are summarized in Tables 6.2 and 6.3, respectively. The average AID estimates of CP and AA obtained for wheat and the reference wheat samples compares well with those reported in Manuscripts I, II and some previous reports

Table 6.2. Apparent ileal digestibility coefficients of protein and amino acids determined in chicks fed diets containing wheat (n = 6), pea (n = 5) and barley (n = 7) samples^{1, 2}

Item	Wheat		Pea		Barley	
	Range	Mean	Range	Mean	Range	Mean
CP	83 - 85	84.3	84 - 87	86.1	71 - 79	76.3
<i>Indispensable amino acids</i>						
Arg	81 - 83	82.2	92 - 94	92.9	69 - 81	76.8
His	84 - 85	84.4	86 - 90	88.5	71 - 82	77.4
Ile	85 - 87	86.3	84 - 88	86.4	73 - 82	79.1
Leu	87 - 88	87.4	85 - 89	87.5	75 - 85	81.1
Lys	78 - 80	79.3	90 - 93	91.7	69 - 79	76.3
Met	88 - 89	88.4	83 - 88	85.9	81 - 88	85.6
Met+Cys	86 - 88	87.1	79 - 83	81.4	77 - 85	81.8
Phe	87 - 89	87.5	86 - 89	87.8	75 - 84	80.3
Thr	72 - 78	74.6	81 - 84	83.0	63 - 73	69.6
Val	82 - 85	83.2	83 - 87	85.9	72 - 81	77.9
<i>Dispensable amino acids</i>						
Ala	79 - 81	79.4	86 - 89	87.6	66 - 76	73.4
Asp	77 - 79	77.8	86 - 89	87.9	65 - 75	71.6
Cys	85 - 87	86.1	77 - 81	78.7	71 - 81	77.9
Glu	94 - 95	94.7	90 - 93	91.8	81 - 90	85.9
Gly	79 - 81	79.9	84 - 88	86.3	64 - 74	71.4
Pro	93 - 94	93.4	84 - 87	85.4	81 - 88	84.4
Ser	83 - 85	83.7	84 - 87	85.5	70 - 78	75.1

1. n = number of different samples of a feed, fed to 6 cages, each with 6 (barley) or 8 (wheat, pea) birds.

2. The average apparent ileal digestibility coefficients of CP, Arg, His, Ile, Leu, Lys, Met, Met+Cys, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro and Ser for the 3 reference wheat samples were 84.9, 80.8, 84.1, 87.1, 87.9, 78.0, 90.6, 89.1, 88.2, 76.2, 82.9, 79.4, 77.4, 87.4, 94.9, 80.0, 93.8 and 84.3, respectively.

Table 6.3. Standardized ileal digestibility coefficients of protein and amino acids determined in chicks fed diets containing wheat (n = 6), pea (n = 5) and barley (n = 7) samples^{1,2}

Item	Wheat		Pea		Barley	
	Range	Mean	Range	Mean	Range	Mean
CP	86 - 89	87.2	86 - 89	88.3	74 - 83	79.7
<i>Indispensable amino acids</i>						
Arg	84 - 87	85.2	93 - 95	94.2	72 - 84	80.4
His	86 - 88	87.0	88 - 92	90.3	74 - 85	80.7
Ile	89 - 92	90.4	86 - 90	88.8	77 - 87	83.9
Leu	90 - 91	90.5	87 - 91	89.6	79 - 88	84.8
Lys	83 - 85	83.7	91 - 94	92.9	73 - 83	80.5
Met	91 - 92	91.4	87 - 91	89.8	84 - 91	88.3
Met+Cys	90 - 92	91.1	84 - 87	86.0	81 - 89	86.1
Phe	93 - 95	93.8	90 - 93	92.2	85 - 95	90.9
Thr	84 - 88	85.4	87 - 90	89.1	74 - 84	80.6
Val	87 - 89	87.7	86 - 90	88.8	76 - 86	82.5
<i>Dispensable amino acids</i>						
Ala	83 - 85	83.8	88 - 91	90.2	70 - 81	78.1
Asp	83 - 85	83.8	88 - 91	89.9	71 - 82	78.1
Cys	90 - 92	90.8	82 - 86	83.7	77 - 87	83.9
Glu	96 - 97	96.6	92 - 95	94.2	83 - 91	87.6
Gly	83 - 86	84.1	87 - 90	89.1	69 - 80	76.7
Pro	85 - 96	95.4	87 - 90	88.9	83 - 91	86.6
Ser	88 - 91	89.1	87 - 91	89.3	77 - 85	82.2

1. n = number of different samples of a feed, fed to 6 cages, each with 6 (barley) or 8 (wheat, pea) birds.

2. The average standardized ileal digestibility coefficients of CP, Arg, His, Ile, Leu, Lys, Met, Met+Cys, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro and Ser for the 3 reference wheat samples were 87.9, 84.0, 86.8, 91.3, 91.1, 82.6, 93.1, 92.6, 98.5, 87.3, 87.5, 83.9, 83.8, 91.9, 96.1, 84.5, 96.0 and 89.9, respectively.

(Ravindran et al., 1999b; Huang et al., 2005; Ravindran et al., 2005). Pea protein had higher AID and SID values (86.1 and 88.3%) compared to the respective values for wheat (84.3 and 87.2%) and barley (76.3 and 79.7%). The AID values of indispensable AA in wheat and barley indicated a low threonine (74.6 and 69.6%), lysine (79.3 and 76.3%) and arginine (82.2 and 76.8%) digestibility. On the other hand, methionine (88.4%), phenylalanine (87.5%) and leucine (87.4%) in wheat and methionine (85.6%), methionine+cystine (81.8%) and leucine (81.1%) in barley were estimated to be the most digestible indispensable AA. Among all AA in wheat and barley, threonine was the least digestible. It has been observed previously (Low, 1989) that elevated levels of NSP in feed (such as cereals) can markedly increase the secretory response of the gut. Therefore, poor digestibility of threonine is reflective of the fact that it is a major indispensable AA found in endogenous secretions (Siriwan et al., 1993; Ravindran and Hendriks, 2004a; Ravindran et al., 2004). In contrast, glutamic acid was the most digestible AA of all in both wheat (94.7%) and barley (85.9%). The AID and SID results of different AA in pea samples ranged from 78.7% to 92.9% and 83.7% to 94.2%, respectively. Igbanesan et al. (1997) using a precision-feeding rooster technique for estimation of AA availability in pea, reported values ranging from 75% to 89.6%. In contrast, the AID and SID values for different AA in the present study were higher, ranging from 78.7% to 92.9% and 83.7% to 94.2%, respectively. However, the trend observed among the digestibility values for AA followed the same pattern in both studies. The AID estimates of indispensable AA in pea samples were reported with arginine (92.9%), lysine (91.7%) and histidine (88.5%) as the most digestible and cystine (78.7%), methionine+cystine (81.4%) and threonine (83.0%) as the least digestible AA. The low digestibility of sulfur AA has been shown to be related

to the protease inhibitor content of peas, leading to hyper-secretion of trypsin and subsequently a pancreatic hypertrophy (Gatel, 1994; Wiseman et al., 2003). This increased secretion has two main consequences. First, because pancreatic enzymes are rich in sulfur AA their hyper-secretion diverts methionine and cystine from body tissue synthesis creating an increased nutritional requirement for them (Gatel, 1994; Wiseman et al., 2003). Second, increased secretion of sulfur AA-rich compounds results in a greater presence of these AA in the gut leading to their lower digestibility estimates. On the other hand, arginine and lysine in peas were present in the highest amount and were the most digestible AA.

The total CV and its components calculated for AID and SID estimates in wheat, pea and barley are shown in Tables 6.4 and 6.5, respectively. Of the three feed ingredients assayed, barley showed the widest range of estimates and CV for each AA. The trend observed in variability of estimates for indispensable AA digestibility in wheat and barley was very similar with sulfur AA being the least and lysine and threonine as the most variable. Coefficient of variation for AID and SID estimates of AA in pea samples was marked with arginine and lysine as the least and sulfur AA as the most variable. Reasons for the variability observed in different samples of cereals and peas can be classified as intrinsic (variety, chemical composition) and extrinsic factors (growing conditions, storage, etc.), both of which affect nutrient digestibility and availability (Gatel, 1994). Although there is a potential for peas to be included as an alternative protein source in poultry diets, the high variability in their chemical and nutritional composition limit their use. This may explain the lack of consistency in the findings of various studies examining pea inclusion rates in broiler diets

Table 6.4. Coefficient of variation (CV%) associated with apparent ileal protein and amino acid digestibility estimates determined in chicks fed diets containing wheat (n = 6), pea (n = 5) and barley (n = 7) samples¹

Item	Wheat			Pea			Barley		
	CV% ²	Variance components ³		CV% ²	Variance components ³		CV% ²	Variance components ³	
		Between	Within		Between	Within		Between	Within
CP	2.0	2.4	97.6	2.1	30.7	69.3	4.5	51.5	48.5
<i>Indispensable amino acids</i>									
Arg	2.5	0.0	100.0	1.5	10.3	89.7	5.5	66.6	33.4
His	2.1	0.0	100.0	2.1	41.4	58.6	5.2	58.1	41.9
Ile	1.8	9.7	90.3	2.7	38.8	61.2	4.8	60.6	39.4
Leu	1.5	0.0	100.0	2.6	37.5	62.5	4.5	61.1	38.9
Lys	2.9	0.0	100.0	1.9	39.3	60.7	5.6	52.6	47.4
Met	1.6	0.0	100.0	3.0	32.5	67.5	3.6	61.4	38.6
Met+Cys	1.7	4.8	95.2	2.8	29.8	70.2	4.0	57.8	42.2
Phe	1.7	0.0	100.0	2.3	13.4	86.6	5.0	54.1	45.9
Thr	3.9	7.8	92.2	2.5	26.8	73.2	6.2	47.7	52.3
Val	2.0	0.0	100.0	2.6	33.7	66.3	4.8	57.1	42.9
<i>Dispensable amino acids</i>									
Ala	2.8	0.0	100.0	2.2	33.0	67.0	5.9	55.0	45.0
Asp	2.9	0.0	100.0	2.2	28.9	71.1	5.9	52.6	47.4
Cys	1.7	9.4	90.6	3.2	29.8	70.2	5.1	47.8	52.2
Glu	0.6	1.9	98.1	1.8	27.9	72.1	3.8	67.4	32.6
Gly	2.5	0.0	100.0	2.2	28.6	71.4	6.0	53.0	47.0
Pro	0.8	12.4	87.6	2.5	24.8	75.2	3.9	57.9	42.1
Ser	1.9	4.8	95.2	2.3	22.4	77.6	4.7	47.1	52.9

1. Six, five and seven respective samples of wheat, pea and barley were assayed in six replicates cages with 6 (barley) or 8 (pea, wheat) birds in each and variance components for samples (σ_s^2) and cages within samples (σ_e^2) were determined.

2. Total coefficient of variation, $CV\% = \frac{\sqrt{\sigma_s^2 + \sigma_e^2}}{\bar{y}} \times 100\%$.

3. Percentage contribution to the total variation calculated as $\sigma_s^2 / (\sigma_s^2 + \sigma_e^2)$ and $\sigma_e^2 / (\sigma_s^2 + \sigma_e^2)$ for samples and cages within samples, respectively.

Table 6.5. Coefficient of variation (CV%) associated with standardized ileal protein and amino acid digestibility estimates determined in chicks fed diets containing wheat (n = 6), pea (n = 5) and barley (n = 7) samples¹

Item	Wheat			Pea			Barley		
	CV% ²	Variance components ³		CV% ²	Variance components ³		CV% ²	Variance components ³	
		Between	Within		Between	Within		Between	Within
CP	1.8	29.3	70.7	2.1	30.9	69.1	4.5	54.6	45.4
<i>Indispensable amino acids</i>									
Arg	2.3	23.5	76.5	1.4	11.1	88.9	5.5	67.1	32.9
His	1.7	13.3	86.7	2.1	40.2	59.8	5.0	59.6	40.4
Ile	1.6	25.8	74.2	2.6	40.2	59.8	4.7	63.0	37.0
Leu	1.5	22.2	77.8	2.5	38.4	61.6	4.4	63.6	36.4
Lys	2.4	16.4	83.6	1.9	40.4	59.6	5.3	53.2	46.8
Met	1.5	24.0	76.0	3.0	33.9	66.1	3.6	63.2	36.8
Met+Cys	1.5	34.2	65.8	2.6	26.8	73.2	3.9	60.4	39.6
Phe	1.4	24.0	76.0	2.2	18.6	81.4	5.0	63.6	36.4
Thr	3.1	26.1	73.9	2.3	29.2	70.8	5.7	53.0	47.0
Val	1.9	19.7	80.3	2.5	34.7	65.3	4.7	59.6	40.4
<i>Dispensable amino acids</i>									
Ala	2.3	17.5	82.5	2.1	34.0	66.0	5.6	56.5	43.5
Asp	2.4	12.3	87.7	2.1	29.5	70.5	5.5	53.8	46.2
Cys	1.6	15.6	84.4	2.9	24.5	75.5	4.9	50.2	49.8
Glu	0.6	27.0	73.0	1.8	31.0	69.0	3.9	69.3	30.7
Gly	2.3	21.8	78.2	2.1	28.8	71.2	5.7	54.5	45.5
Pro	0.8	21.6	78.4	2.3	22.6	77.4	4.0	61.3	38.7
Ser	1.8	27.1	72.9	2.1	20.2	79.8	4.5	51.8	48.2

1. Six, five and seven respective samples of wheat, pea and barley were assayed in six replicates cages with 6 (barley) or 8 (pea, wheat) birds in each and variance components for samples (σ_s^2) and cages within samples (σ_e^2) were determined.

2. Total coefficient of variation, $CV\% = \frac{\sqrt{\sigma_s^2 + \sigma_e^2}}{\bar{y}} \times 100\%$.

3. Percentage contribution to the total variation calculated as $\sigma_s^2 / (\sigma_s^2 + \sigma_e^2)$ and $\sigma_e^2 / (\sigma_s^2 + \sigma_e^2)$ for samples and cages within samples, respectively.

(Igbasan and Guenter, 1996; Farrell et al., 1999; Moschini et al., 2005). Thus, for an effective inclusion of a feed ingredient in a diet, in terms of its AA nutrition, it is necessary to use an accurate methodology not only to measure its AA availability but also to account for the variability observed for each AA within different samples of that feed ingredient. Therefore, to better explain the variability, the components of total CV associated with each AA digestibility estimate, due to the samples or assay, were calculated (Tables 6.4 and 6.5). While the total CV for each estimated digestible AA in wheat and pea was more affected by the within sample variation, the reverse was true for barley.

The ileal digestibility determination of protein and AA in a feed ingredient is an evaluation of its nutritional value in terms of its available protein and AA. However, protein evaluation based on AID may be biased due to endogenous AA secretions which are influenced by the level of dry matter intake and the nature of the protein source under study (Angkanaporn et al., 1997; Souffrant, 2001). The proportion of this endogenous loss that is independent from the nature of the protein source is referred to as basal nitrogen loss. A correction of AID values for the basal nitrogen loss has been used as a means to obtain digestibility values that are more accurate for practical feed formulation termed as SID (Lemme et al., 2004; Stein et al., 2007). Thus, the AID values in the present study were transformed to SID values as the latter has been reported to be more additive in a complete diet (Angkanaporn et al., 1996; Stein et al., 2001).

Use of highly nutrient-dense diets to maximize broiler performance implies that there is little room for wider safety margins in the diets; therefore the accuracy in determination of nutrient availability becomes very important in supplying the required

nutrients at a lower feed cost. Broiler diets are characterized by formulations made from a selection of a relatively narrow range of classical ingredients. Use of a more accurate protein and AA digestibility data in diet formulation increases the range of ingredients that can be used in the diet while improving the accuracy with which they can be used, permitting increased accuracy in predicting performance of the bird.

6.5 Conclusion

Pea samples demonstrated higher AID and SID values of CP (86.1 and 88.3%) compared to the respective values for wheat (84.3 and 87.2%) and barley (76.3 and 79.7%). The AID values of indispensable AA in wheat and barley indicated low threonine (74.6 and 69.6%), lysine (79.3 and 76.3%) and arginine (82.2 and 76.8%) digestibilities. The AID estimates of indispensable AA in pea samples were evident with arginine (92.9%), lysine (91.7%) and histidine (88.5%) as the most digestible and cystine (78.7%), methionine+cystine (81.4%) and threonine (83.0%) as the least digestible AA. The trend observed in the variability of AID and SID estimates for indispensable AA in wheat was very similar to those of barley with sulfur AA and glutamic acid being the least and lysine and threonine as the most variable of all AA in both. Coefficient of variation for AID and SID estimates of AA in pea was marked with arginine and lysine as the least and sulfur AA as the most variable of all. The results of the reference wheat in these studies compared very closely to those reported in manuscript I and II.

7.0 MANUSCRIPT IV

Standardized Ileal Amino Acid Digestibility in Dry-extruded Expelled Soybean Meal and Extruded Canola seed-Pea (ENERMAXTM) for Broilers

7.1 ABSTRACT

Two studies were conducted to estimate the ileal digestibility of AA in five different batches of dry-extruded expelled soybean meal (DESBM) and ENERMAXTM (a co-extruded full fat canola seed-pea blend, 50:50 wt/wt basis) fed to broilers. In each of these studies a sample of the reference wheat was also assayed separately for comparison purposes. In each study, two hundred and sixteen male Ross broiler chicks were fed a commercial starter diet from d 1 to 14 of age followed by the test diets from d 15 to 21. The twelve test diets consisted of an ENERMAXTM, a DESBM or the reference wheat sample as the sole source of AA, dextrose, minerals and vitamins. Chromic oxide (0.3%) was included in all diets as a digestibility marker. Each test diet was randomly assigned to six replicate cages, each with 6 birds. On d 21, birds were killed to sample ileal digesta for determining the apparent (AID) and standardized (SID) ileal AA digestibilities. The SID values were calculated using ileal endogenous AA losses previously determined in our laboratory. The AID results for DESBM ranged from 78.0% to 91.4% for cystine and arginine, respectively. The SID values also followed the same trend with cystine (82.5%) and arginine (92.9%) as the least and most digestible AA. The least and most digestible indispensable AA were threonine (82.6%), methionine+cystine (84.3%), valine (85.5%) and lysine (89.4%), methionine (90.5%) and arginine (91.4%). The AID and SID values

determined for AA in ENERMAXTM ranged from 67.8% to 83.3% and from 71.8% to 84.7%, respectively. Considering both AID and SID values in ENERMAXTM, arginine (83.3 and 84.7%) and methionine (81.6 and 83.4%) were the most digestible AA of all while threonine (67.8 and 72.7%) and cystine (68.4 and 71.8%) were the least digestible. The AID and SID of protein and AA in the reference wheat were consistent with those reported in previous manuscripts (I, II and III).

7.2 INTRODUCTION

Pea and canola meal contain approximately 23.8 and 38.0% protein and their AA composition are complementary, each contributing to overall lysine and methionine balance (NRC, 1994). Full-fat canola has a high oil content (40%) (Aherne and Bell, 1990) which can replace some amount of dietary fat in poultry diets and peas are a good source of energy, containing as much or slightly less energy than cereals (NRC, 1994). A blend of the two should provide an energy- and protein-dense product for poultry nutrition.

Although some previous reports showed improvements in protein and energy digestibilities in extruded pea, soybean and rapeseed (Wang and McIntosh, 1996; Alonso et al., 2000; Lichovnikova et al., 2004), depending on the technology and conditions involved (Webster et al., 2003), extrusion of feed ingredients has not always been associated with increased AA digestibilities. Apart from the observed influence of the grain variety on AA digestibility results (Herkelman et al., 1992; Grosjean et al., 1999; Grosjean et al., 2000), the heating process may impair the quality and availability of AA in feed (Asp and Bjorck, 1989). In fact, there are reports of varied, insignificant or

decreased AA digestibilities in extruded feed compared with untreated ones (Lichovnikova et al., 2004; Opapeju et al., 2006; Golian et al., 2007).

The variation observed in apparent ileal protein and AA digestibility results can be accounted for in part due to the differences in feed samples, birds or methodology used. For an effective formulation of the diet it is important to account for these variables in order to lower the cost associated with wider safety margins. Reports regarding these variations in the literature are very scarce and the results are confounded by the lack of a clear explanation for the CV% presented. Hence, the aim of this study was to determine the apparent and standardized ileal digestibility estimates in ENERMAXTM (a co-extruded blend of full-fat canola seed and pea, 50:50 wt/wt) and dry-extruded expelled soybean meal (DESBM) and their associated CV for broilers.

7.3 MATERIALS AND METHODS

7.3.1 DIETS

Five batches of ENERMAXTM (a co-extruded blend of full-fat canola seed and pea, 50:50 wt/wt; EXP Feeds Inc., Shoal Lake, Manitoba, Canada) and five batches of DESBM (Jordan Mills Inc., Delmar Commodities Ltd., Winkler, Manitoba, Canada) were assayed in two separate studies. A sample of the reference wheat was also assayed for each experiment. ENERMAXTM and DESBM samples were collected from five different processing days of the same plant. Each test diet contained a canola seed-pea blend, a DESBM or a reference wheat sample at 95.3, 95.3 or 91.7% as the sole source of protein.

All test diets were balanced for mineral and vitamin levels to meet NRC (1994) requirement specifications and contained chromic oxide (0.3%) as a digestibility marker.

7.3.2 BIRDS AND EXPERIMENTAL CONDUCT

The experimental protocol for the present study was reviewed and approved by the Animal Care Protocol Management and Review Committee of the University of Manitoba and birds were cared for according to the guidelines of the Canadian Council on Animal Care (1993).

In each study, two hundred and sixteen day-old male broiler chicks (Ross 308, Aviagen) were obtained from a local hatchery (Carlton Hatchery, Grunthal, Manitoba, Canada) and housed in battery brooders (Petersime Incubator Co., Gettysburg, Ohio) in a room with continuous fluorescent lighting for each experiment. Room temperature was maintained at 32, 28, and 24°C during week 1, 2, and 3, respectively. From day 1 to 14, birds were fed a commercial starter diet (FeedRite, Ridley Inc., Winnipeg, Manitoba, Canada) in crumble form (3100 Kcal/Kg, 19% CP). On day 14, birds were fasted for 3 h, weighed and distributed (6 birds/cage) into 36 cages with similar body weights. In each experiment, six replicate cages were randomly assigned to each of the test diets and fed from day 15 to day 21. Birds had free access to feed and water throughout the study period. On day 21, birds were killed by cervical dislocation and the digesta samples from the ileum (from Meckel's diverticulum to a point 4 cm proximal to the ileo-cecal junction) were collected by gently squeezing the contents of the ileum into sample bags. Digesta from birds within a cage were pooled into one bag and frozen immediately after collection

and subsequently freeze-dried. The dried ileal digesta were stored in airtight bags at -4°C until required for chemical analysis.

7.3.3 CHEMICAL ANALYSIS

Feed ingredients, test diets and digesta samples were finely ground using a coffee grinder (CBG5 Smart Grind; Applica Consumer Products, Inc., Shelton, CT). Dry matter was determined according to the method of AOAC (2000) procedure # 934.01, and nitrogen was analyzed using the Leco nitrogen analyzer (Model CNS-2000; Leco Corp., St. Joseph, MI). Amino acid concentrations of the feed ingredients, diet and digesta were determined by Evonik (Hanau-Wolfgang, Germany). Dietary and digesta AA contents were determined by using ion-exchange chromatography with postcolumn derivatization with ninhydrin (Llames and Fontaine, 1994). Sulfur AA (methionine and cystine) were first oxidized with performic acid, and subsequently neutralized with sodium metabisulfite for analysis (Commission Directive, 1998). Amino acid concentrations were quantified with the internal standard method by measuring the absorption of reaction products with ninhydrin at 570 nm. Chromium was analyzed, after the samples were ashed for 12 h at 600 °C in a muffle furnace, using inductively coupled plasma mass spectrometry (ICP-AES Vista, Varian) according to the method (procedure # 985.01) of AOAC (2005).

7.3.4 DIGESTIBILITY CALCULATION

The AID coefficient of AA was calculated using the indigestible marker as follows.

$$AID = \frac{(AA/IM)_d - (AA/IM)_{id}}{(AA/IM)_d}$$

Where, $(AA/IM)_d$ = ratio of AA to indigestible marker (IM) content in diet and

$(AA/IM)_{id}$ = ratio of AA to indigestible marker (IM) content in ileal digesta

Basal endogenous loss estimates determined with a nitrogen-free diet were used for the correction of AID coefficients (Golian et al., 2008). The SID coefficients were calculated as follows:

$$SID\% = AID + [(basal\ IAA_{end} / AA_{diet}) \times 100]$$

Where, IAA_{end} = basal ileal endogenous AA loss and AA_{diet} = Amino acid content of the diet.

The endogenous ileal AA loss values used to calculate the SID are presented in the Appendix.

7.3.5 STATISTICAL ANALYSIS

The data for digestibility estimates of DESBM and canola seed-pea blend (ENERMAXTM) samples were analyzed using the GLM procedure of SAS Institute, Inc. (1990) as a completely randomized design. The Nested procedure was also applied to further analyze the random feed and bird/cage sub-sampling effects on the total variation observed. To better explain the CV associated with the AID and SID estimates for each AA, the components of total variation were further calculated and expressed as variance due to the feed samples (σ_s^2) and cage within samples (σ_e^2).

7.4 RESULTS AND DISCUSSION

Crude protein and AA composition of ENERMAXTM, DESBM and the reference wheat samples are presented in Table 7.1. The CP and AA composition of ENERMAXTM and DESBM compare well with the previous published values (Woodworth et al., 2001; Opapeju et al., 2006; Golian et al., 2007). Results of the reference wheat also conform very well to the ones reported in manuscript I, II and III. The AA composition in both feed ingredients (ENERMAXTM, DESBM) followed the same pattern with methionine, cystine, histidine and leucine, arginine and lysine ranking the lowest and highest indispensable AA content, respectively. Previous reports indicate a significant effect of extrusion on decreasing the content of several heat-sensitive AA (histidine, tryptophan, methionine and cysteine) in pea, soybean meal and field bean (Jeunink and Cheftel, 1979; Cheftel, 1986; Prudencio-Ferreira and Areas, 1993; Alonso et al., 2000). The respective AA are known to be low in the original grains used in these products and a comparison between raw and processed products for the effect of extrusion on AA content was not made in this study. Nonetheless, Golian et al. (2007) using the same extruded canola seed-pea product (ENERMAXTM) made a comparison between the raw and processed blend to conclude that the AA concentration in ENERMAXTM was not affected by the extrusion process except for a decrease in glutamic acid and glycine contents. The DESBM batches demonstrated a more consistent AA profile compared with ENERMAXTM. The calculated variation for each AA between the batches of ENERMAXTM and DESBM ranged from 1.1% to 5% and 1.2% to 2%, respectively. Methionine (1.3%), isoleucine (1.3%), arginine (1.4%) and lysine (1.9%), histidine (2.0%), phenylalanine (2.0%) were the least and the most variable indispensable AA observed among the batches of DESBM, respectively.

Table 7.1. Crude protein and amino acid composition (%) of dry-extruded expelled soybean meal (n = 5) and ENERMAX™ (n = 5) samples^{1,2} (DM basis)

Item	Dry-extruded soybean meal			ENERMAX™		
	Range	Mean	CV% ³	Range	Mean	CV% ³
DM	92.3 - 93.9	93.3	0.7	92.9 - 94.6	93.5	0.7
CP	44.6 - 46.7	45.8	1.9	23.7 - 24.2	24.0	0.7
<i>Indispensable amino acids</i>						
Arg	3.19 - 3.31	3.24	1.4	1.52 - 1.61	1.58	2.3
His	1.14 - 1.20	1.16	2.0	0.55 - 0.59	0.57	2.5
Ile	2.01 - 2.07	2.04	1.3	0.94 - 0.98	0.96	2.2
Leu	3.37 - 3.49	3.43	1.6	1.64 - 1.70	1.68	1.4
Lys	2.75 - 2.88	2.81	1.9	1.28 - 1.37	1.34	2.8
Met	0.66 - 0.68	0.67	1.3	0.34 - 0.39	0.37	4.8
Met+Cys	1.37 - 1.42	1.38	1.6	0.76 - 0.85	0.81	4.8
Phe	2.21 - 2.32	2.26	2.0	1.03 - 1.10	1.08	2.4
Thr	1.80 - 1.85	1.82	1.8	0.94 - 0.96	0.95	1.1
Val	2.12 - 2.18	2.15	1.2	1.12 - 1.20	1.16	2.4
<i>Dispensable amino acids</i>						
Ala	1.92 - 1.99	1.96	1.5	1.00 - 1.04	1.02	1.6
Asp	5.08 - 5.28	5.18	1.8	2.02 - 2.21	2.14	3.6
Cys	0.70 - 0.74	0.71	2.0	0.42 - 0.47	0.44	5.0
Glu	7.90 - 8.17	8.04	1.9	3.83 - 4.01	3.90	1.8
Gly	1.90 - 1.96	1.93	1.6	1.03 - 1.08	1.06	1.6
Pro	2.09 - 2.19	2.14	1.7	1.08 - 1.17	1.11	3.1
Ser	2.27 - 2.36	2.32	1.9	1.05 - 1.13	1.10	2.9

1. n = number of different samples of a feed.

2. The average of the 2 reference wheat samples for DM, CP, Arg, His, Ile, Leu, Lys, Met, Met+Cys, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro and Ser were 92.4, 15.6, 0.72, 0.36, 0.54, 1.03, 0.42, 0.26, 0.62, 0.72, 0.44, 0.68, 0.53, 0.76, 0.36, 4.57, 0.63, 1.48 and 0.68, respectively.

3. Coefficient of variation among different samples.

In contrast, methionine (4.8%), lysine (2.8%), histidine (2.5%) and threonine (1.1%), leucine (1.4%), isoleucine (2.2%) were found to be the most and the least variable indispensable AA in ENERMAXTM, respectively.

The AID and SID estimates of CP, AA and their associated CV for DESBM and ENERMAXTM are presented in Tables 7.2 and 7.3. Also the results for the AID and SID estimates of protein and AA in the reference wheat are included as footnotes. The AID results for DESBM ranged from 78.0% to 91.4% for cystine and arginine, respectively. The least and most digestible indispensable AA were threonine (82.6%), methionine+cystine (84.3%), valine (85.5%) and lysine (89.4%), methionine (90.5%) and arginine (91.4%). Threonine is a major indispensable AA found in endogenous secretions which explains the reason for its lower AID (Siriwan et al., 1993; Ravindran and Hendriks, 2004a). Among other AA, available lysine is known to be affected by heating (Parsons et al., 1992) and the fact that lysine was among the most digestible AA reflects proper heating conditions during the extrusion process. The CV associated with the AID values for arginine (2.7%), lysine (2.9%) and methionine (2.9%) were lower compared to the CV associated with cystine (6.9%) and threonine (4.8%). Further dissection of total CV components for AID estimates of cystine and threonine revealed a different behavior of the data for each AA in terms of variation contribution from both within and between the samples where 36.1 and 50.5% of the total CV reported for cystine and threonine, respectively, was due to the variation observed between the samples. This means that the total variation reported for cystine was more affected by the assay condition than the feed samples whereas in threonine it was vice versa. Irrespective of the age of the birds and methodology used, the SID values determined in this study

Table 7.2. Apparent ileal digestibility coefficients and their associated coefficient of variation (CV%) determined in chicks fed dry-extruded expelled soybean meal (n = 5) and ENERMAX™ (n = 5) samples¹

Item	Dry-extruded soybean meal					ENERMAX™				
	Range	Mean	CV% ²	Variance components ³		Range	Mean	CV% ²	Variance components ³	
				Between	Within				Between	Within
CP	82.1 - 89.2	86.0	4.2	58.7	41.3	70.3 - 73.3	71.5	2.3	40.8	59.2
<i>Indispensable amino acids</i>										
Arg	88.8 - 93.6	91.4	2.7	49.1	50.9	82.2 - 84.4	83.3	1.5	39.6	60.4
His	85.0 - 92.0	89.1	3.2	44.3	55.7	72.6 - 76.6	75.2	3.8	21.5	78.5
Ile	84.0 - 90.7	87.3	3.9	52.1	47.9	71.7 - 74.1	72.8	2.3	23.1	76.9
Leu	84.2 - 90.6	87.6	3.7	57.2	42.8	73.9 - 76.3	74.9	2.0	30.4	69.6
Lys	86.7 - 91.6	89.4	2.9	44.2	55.8	76.1 - 78.8	77.4	2.1	40.2	59.8
Met	88.0 - 92.6	90.5	2.9	45.1	54.9	79.4 - 83.5	81.6	2.6	38.0	62.0
Met+Cys	82.0 - 86.8	84.3	4.6	39.1	60.9	72.9 - 76.2	74.5	2.5	36.6	63.4
Phe	86.2 - 91.9	89.1	3.3	55.2	44.8	73.3 - 76.1	74.2	2.7	21.5	78.5
Thr	78.4 - 85.5	82.6	4.8	50.5	49.5	66.6 - 70.3	67.8	3.1	39.3	60.7
Val	82.1 - 89.0	85.5	4.1	53.2	46.8	70.3 - 73.1	71.6	2.4	40.7	59.3
<i>Dispensable amino acids</i>										
Ala	83.2 - 89.1	86.5	3.6	53.0	47.0	74.0 - 76.2	74.8	2.0	27.2	72.8
Asp	81.9 - 89.0	85.8	4.1	46.5	53.5	72.1 - 75.5	73.4	2.5	44.6	55.4
Cys	72.1 - 81.1	78.0	6.9	36.1	63.9	67.4 - 69.4	68.4	2.7	12.3	87.7
Glu	87.2 - 92.3	90.0	3.1	41.2	58.8	79.8 - 82.0	80.8	1.6	34.2	65.8
Gly	79.2 - 86.0	83.1	4.3	46.3	53.7	70.0 - 72.8	71.2	2.4	32.1	67.9
Pro	83.1 - 89.0	86.4	3.7	46.2	53.8	71.1 - 73.4	71.9	2.0	26.3	73.7
Ser	81.3 - 87.7	85.3	4.0	53.9	46.1	68.5 - 71.8	69.5	2.9	30.4	69.6

1. Five samples of each feed ingredient (n = 5) were assayed in six replicates cages with 6 birds in each and variance components for samples (σ_s^2) and cages within samples (σ_e^2) was determined. The average apparent ileal digestibility coefficients of CP, Arg, His, Ile, Leu, Lys, Met, Met+Cys, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro and Ser for the 2 reference wheat samples were 84.1, 82.1, 84.8, 86.9, 87.8, 78.8, 89.9, 88.7, 88.0, 77.3, 83.6, 79.9, 78.0, 87.0, 94.9, 80.6, 93.9 and 84.9, respectively.

2. Total coefficient of variation, $CV\% = \frac{\sqrt{\sigma_s^2 + \sigma_e^2}}{\bar{y}} \times 100\%$.

3. Percentage contribution to the total variation calculated as $\sigma_s^2 / (\sigma_s^2 + \sigma_e^2)$ and $\sigma_e^2 / (\sigma_s^2 + \sigma_e^2)$ for samples and cages within samples, respectively.

Table 7.3. Standardized ileal digestibility coefficients and their associated coefficient of variation (CV%) determined in chicks fed dry-extruded expelled soybean meal (n = 5) and ENERMAXTM (n = 5) samples¹

Item	Dry-extruded soybean meal					ENERMAX TM				
	Range	Mean	CV% ²	Variance components ³		Range	Mean	CV% ²	Variance components ³	
				Between	Within				Between	Within
CP	84.4 - 91.4	88.2	4.0	42.3	57.7	72.2 - 75.2	73.4	2.3	40.4	59.6
<i>Indispensable amino acids</i>										
Arg	90.3 - 95.1	92.9	2.7	51.1	48.9	83.7 - 85.7	84.7	1.5	37.0	63.0
His	86.7 - 92.0	89.6	3.2	55.7	44.3	74.2 - 78.3	76.8	3.7	20.9	79.1
Ile	86.4 - 92.9	89.5	3.7	48.2	51.8	74.0 - 76.4	75.0	2.2	22.1	77.9
Leu	86.3 - 92.6	89.6	3.6	43.0	57.0	75.8 - 78.2	76.8	1.9	30.2	69.8
Lys	88.1 - 93.1	90.8	2.8	55.8	44.2	77.5 - 80.2	78.8	2.0	38.7	61.3
Met	90.1 - 94.7	92.5	2.9	54.6	45.4	81.3 - 85.2	83.4	2.4	35.5	64.5
Met+Cys	83.4 - 90.2	87.5	4.5	60.9	39.1	75.6 - 78.8	77.1	2.4	35.1	64.9
Phe	93.2 - 98.9	95.9	3.1	45.1	54.9	80.2 - 82.9	80.9	2.4	17.7	82.3
Thr	84.0 - 91.1	88.0	4.4	49.8	50.2	71.5 - 75.2	72.7	2.9	39.7	60.3
Val	85.1 - 91.9	88.4	4.0	47.0	53.0	72.8 - 75.7	74.1	2.3	39.5	60.5
<i>Dispensable amino acids</i>										
Ala	85.7 - 91.7	89.0	3.5	46.9	53.1	76.3 - 78.5	77.1	1.9	27.4	72.6
Asp	83.9 - 90.9	87.7	4.0	53.7	46.3	74.3 - 77.7	75.5	2.4	43.4	56.6
Cys	76.7 - 85.6	82.5	6.5	64.5	35.5	70.8 - 72.9	71.8	2.6	13.3	86.7
Glu	88.6 - 93.8	91.4	3.1	58.8	41.2	81.2 - 83.4	82.1	1.5	33.8	66.2
Gly	82.2 - 89.0	86.0	4.2	53.7	46.3	72.5 - 75.3	73.6	2.4	32.1	67.9
Pro	86.2 - 92.1	89.4	3.6	54.3	45.7	73.6 - 76.0	74.4	2.0	27.0	73.0
Ser	84.8 - 91.2	88.6	3.8	46.8	53.2	71.9 - 75.2	72.9	2.8	30.4	69.6

1. Five samples of each feed ingredient (n = 5) were assayed in six replicates cages with 6 birds in each and variance components for samples (σ_s^2) and cages within samples (σ_e^2) was determined. The average standardized ileal digestibility coefficients of CP, Arg, His, Ile, Leu, Lys, Met, Met+Cys, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro and Ser for the 2 reference wheat samples were 87.1, 85.3, 87.5, 91.1, 91.0, 83.4, 92.4, 92.2, 98.3, 88.4, 88.2, 84.4, 84.4, 91.5, 96.1, 85.1, 96.1 and 90.5, respectively.

2. Total coefficient of variation, $CV\% = \frac{\sqrt{\sigma_s^2 + \sigma_e^2}}{\bar{y}} \times 100\%$.

3. Percentage contribution to the total variation calculated as $\sigma_s^2 / (\sigma_s^2 + \sigma_e^2)$ and $\sigma_e^2 / (\sigma_s^2 + \sigma_e^2)$ for samples and cages within samples, respectively.

were lower than the true AA digestibility values determined in cecectomized roosters for DESBM (Opapeju et al., 2006). However, previous studies indicate a significant effect of age of the bird and methodology used in digestibility assays on the results derived (Huang et al., 2005; Garcia et al., 2007).

The AID and SID of AA determined for ENERMAXTM ranged from 67.8% to 83.3% and from 71.8% to 84.7%, respectively. The AID and SID values for arginine (83.3 and 84.7%) and methionine (81.6 and 83.4%) were the highest whereas threonine (67.8 and 72.7%) and cystine (68.4 and 71.8%) values were the lowest among all AA. Results from a previous study of AA digestibility with the same blend in our lab (Golian et al., 2007) using a cecectomized rooster assay, documented somewhat higher values ranging from 72% to 91.1% for valine and arginine, respectively. The total CV for AID and SID estimates followed the same pattern ranging from 1.5 to 3.8% for arginine and histidine, respectively. Considering the between and within sample variation, the total CV associated with histidine was more affected by within (78.5%) than between (21.5%) variation.

The AID and SID results of the reference wheat are very consistent with the previous results indicating a high repeatability of the assay.

The use of the canola seed, peas and soybean meal in poultry diets are associated with the presence of a plethora of ANF, of which protease inhibitors in soybean meal and peas, and glucosinolates in canola seed are among the major ones which depress nutrient availability to the birds (Gatel, 1994; Wiseman et al., 2003; Clarke and Wiseman, 2007). Extrusion has been shown to be one of the most effective methods used to minimize the presence of ANF in feed (Cheftel, 1986; Danicke et al., 1998; Alonso et al., 2000;

Lichovnikova et al., 2004; Clarke and Wiseman, 2007). It has been suggested that the use of an adequate heat treatment during the extrusion process results in inactivation of heat-labile ANF and denaturation of storage protein which subsequently renders the final product more digestible (Camire et al., 1990; O'Doherty and Keady, 2000). However, the magnitude of an increased response in protein availability due to the reduction in the level of ANF in feed is a result of processing severity (Salmon et al., 1988; Mansour et al., 1993; Zhang et al., 1993; Clarke and Wiseman, 2007). Although the effect of heat treatment on the available energy in several feed ingredients has been associated with a consistent improvement (Zhang et al., 1993; Wang and McIntosh, 1996; Alonso et al., 2000; Lichovnikova et al., 2004; Stein and Bohlke, 2007), it has not always been the same for AA availability. For example, Leeson and Atteh (1996) used temperatures up to 140°C in extruding soybean and incorporating it into broiler diet (30% inclusion level) to conclude that regardless of the decreased trypsin inhibitor activity seen at higher temperatures, there was no significant effect of extrusion temperature on nitrogen retention in birds. Danicke et al. (1998) found that increasing the temperature of the thermal treatment for rapeseed fed to broilers and laying hens was associated with a decrease in the digestibility of crude protein. Golian et al. (2007) made a comparison between raw and extruded samples of ENERMAXTM and showed that extrusion did not improve true AA digestibility in adult roosters. A report of true AA digestibility in two DESBM batches determined with the precision-fed cecectomized rooster assay compared with that of regular soybean meal (solvent-extracted) revealed somewhat different results (Opapeju et al., 2006). While there was no difference ($P > 0.05$) in AA digestibility between soybean meal and one of the DESBM batches, there was a greater AA

digestibility ($P \leq 0.05$) in soybean meal than that of the other DESBM batches. Parsons et al. (1992) reported lower digestibility of histidine, cysteine and lysine in heat processed soybean meal which was explained as a result of Maillard reactions.

Reports with varied results in terms of protein and AA availability between different batches of processed feed, prompts the need for an accurate determination of AA availability where these variations are accounted for. In fact, a wide variation between the processing conditions means that the birds are ingesting considerably different amounts of ANF, as a consequence. The significance of these fluctuations becomes clearer when a larger proportion of a diet consists of processed feed which will subsequently lead to an altered bird performance and uniformity of the end product.

It is well accepted that the AA digestibilities determined at the ileal level are more accurate than digestibilities determined at the excreta level (Ravindran et al., 1999b; Lemme et al., 2004). Furthermore, it is now clear that ileal AA digestibility values are affected by the methodology and age of the birds used (Ten Doeschate et al., 1993; Ravindran and Hendriks, 2004b; Garcia et al., 2007). A large body of AA digestibility data in the literature has been created (Parsons, 1991; NRC, 1994; Rhone-Poulenc, 1995; Heartland Lysine, 1996) using the cecectomized rooster assay which renders them inapplicable to broilers. Moreover, these results suffer from a clear expression of variation associated with each of the observations within the assay condition. It seems plausible to argue that the existing nutrient density in broiler diets does not leave enough room for the required safety margins if the nutrient availability in a processed feed is not known. Thus, for an effective diet formulation and optimum use of feedstuff to meet the requirements it is important not only to determine ileal digestibility of AA in feed using the birds at the

right age but also to account for and report the variation observed for the feed and methodology separately. As the importance of plant protein sources is being recognized by the controlled use of animal protein sources in the diet, it becomes increasingly necessary to improve the accuracy with which they can be used in boiler diets. To the best of this authors' knowledge this is the first report of SID determined in DESBM and ENERMAXTM fed to broilers. Further studies are warranted to provide sufficient data for comparison purposes.

7.5 Conclusion

The AID results for DESBM ranged from 78.0% to 91.4% for cystine and arginine, respectively. The SID values also followed the same trend with cystine (82.5%) and arginine (92.9%) as the least and most digestible AA, respectively. The least and most digestible indispensable AA of AID estimates in DESBM were threonine (82.6%), methionine+cystine (84.3%), valine (85.5%) and lysine (89.4%), methionine (90.5%) and arginine (91.4%). The AID and SID values determined for AA in ENERMAXTM ranged from 67.8% to 83.3% and from 71.8% to 84.7%, respectively. Considering both AID and SID values in ENERMAXTM, arginine (83.3 and 84.7%) and methionine (81.6 and 83.4%) were the most digestible AA of all while threonine (67.8 and 72.7%) and cystine (68.4 and 71.8%) were the least digestible. Threonine and methionine+cystine in DESBM and histidine and threonine in ENERMAXTM had the most variable estimates of AID and SID. The AID and SID results of protein and AA in the reference wheat determined in these studies compared very well with those reported in previous manuscripts (I, II and III).

8.0 MANUSCRIPT V

Standardized Ileal Amino Acid Digestibility in Feather Meal (FM) and Poultry By-Product Meal (PBPM) for Broilers

8.1 ABSTRACT

This study was conducted to estimate the ileal digestibility of AA in five different samples of each FM and PBPM fed to broilers. A sample of the reference wheat was also assayed, concurrently. Three hundred and ninety six male Ross broiler chicks were fed a commercial starter diet from d 1 to 14 of age followed by the test diets from d 15 to 21. The eleven test diets consisted of a FM, PBPM or reference wheat sample as the sole AA source, dextrose, minerals and vitamins. Chromic oxide (0.3%) was included in all diets as a digestibility marker. Each test diet was randomly assigned to six replicate cages, each with 6 birds. On d 21, birds were killed to sample ileal digesta for determining the AID and SID ileal AA digestibilities. The SID values were calculated using ileal endogenous AA losses previously determined in our laboratory. The AID results show a wider AA digestibility range for PBPM (from 50.6% to 81.2%) compared with FM (from 39.4% to 74.1%). Cystine (50.6%) and aspartic acid (39.4%) were the least digestible AA in PBPM and FM, respectively. Among indispensable AA, threonine (70.2%) and methionine+cystine (70.9%) for PBPM and methionine+cystine (53.1) and lysine (56.4%) for FM were the least digestible. In contrast, valine (71.3%), phenylalanine (73.3%) and isoleucine (74.1%) in FM and alanine (78.9%), methionine (80.3%) and arginine (81.2%) in PBPM were the most digestible indispensable AA. Regarding SID estimates the same trend as in AID estimates was observed with cystine (57.6%) in PBPM and aspartic acid

(41.7%) in FM as the least digestible AA whereas phenylalanine was the most digestible in both test materials. The AID and SID results of protein and AA determined in the reference wheat were in accordance with those obtained previously (Manuscript I, II, III and IV).

8.2 INTRODUCTION

Use of animal protein sources in poultry diets is an integral part of poultry nutrition throughout the world. Protein concentrates derived from animal tissues originate primarily from inedible tissues from meat packaging or rendering plants. Dramatic improvement of poultry rendering technology has resulted in the availability of large quantities of by-products such as feather and poultry by-product meal. Feather meal has been of interest in nutritional studies because of its high protein content (over 85%) which could potentially substitute for other expensive feed protein sources (Papadopoulos et al., 1985; Han and Parsons, 1991; Ravindran and Blair, 1993). Owing to its high keratin content it is very rich in sulfur containing AA, particularly cysteine (Papadopoulos et al., 1986). The keratin proteins are of little nutritive value in their natural state because of their chemical structure containing cysteine disulphide bonds which makes them insoluble and poorly digestible for the birds (Naber et al., 1961; Eggum, 1970; Papadopoulos et al., 1986). However, hydrolyzing feathers in order to enhance their nutritive value has resulted in widely varying nutrient composition in these products (Johnston and Coon, 1979; Papadopoulos et al., 1985). Manifested with AA imbalances and varying quality due to processing conditions, discrepancies exist in terms of the amount of feather meal which can be effectively used under practical conditions (Moran et al., 1966; Morris and Balloun, 1973; Luong and Payne, 1977; MacAlpine and Payne, 1977; Baker et al., 1981).

The terminology of PBPM has been somewhat confusing in the literature (Daghir, 1975). Scott (1982) defined PBPM as the product which consists of the ground dry rendered or wet rendered clean parts of the carcass of slaughtered poultry such as heads, feet, undeveloped eggs and intestines exclusive of feathers except in such trace amounts as might occur unavoidably in good manufacturing practices. Because only one animal species is used in production of PBPM, it is expected to be more homogenous compared with meat meal (Leeson and Summers, 1997). However, variability seen in this product can occur as a result of the inclusion of feather, blood and viscera with the former product necessitating higher cooking temperature in order to hydrolyze the keratin proteins. In fact, nutritional quality of PBPM has been shown to markedly vary depending on the amount of feather in the meal (Main and Daghir, 1981; 1982; Wang and Parsons, 1998b). Consequently, it has been shown that the digestibility of AA determined in PBPM can vary greatly (Burgos et al., 1974; Johnston and Coon, 1979; Han and Parsons, 1990). The variability in protein and AA quality of FM and PBPM is one of the greatest concerns regarding their use in poultry diets.

Although total AA profile of a feed protein source is a good indicator of its potential nutritive value, it does not reflect its availability to the animal. It has been extensively discussed and reviewed elsewhere that the ileal AA digestibility assay is preferable to the excreta analysis for estimating the protein and AA availability of poultry feeds (Ravindran et al., 1999b; Lemme et al., 2004). Reports indicate that the AID values are affected by the level of protein ingested and endogenous losses (Lemme et al., 2004; Kluth et al., 2005). It has been suggested that a correction for non-specific endogenous AA recoveries in ileal digesta will result in “standardized” ileal digestibility (SID) values

which are independent from the protein level or AA content of the diet. Although several methods and their merit in quantifying basal endogenous AA losses have been proposed and discussed in detail (Nyachoti et al., 1997; Ravindran et al., 2004; Golian et al., 2008), it has been suggested that basal losses determined by feeding a nitrogen-free diet to the birds can be used as a measure of minimum losses for the correction of AID values (Stein et al., 2007; Golian et al., 2008). Furthermore, besides the method of estimation, it has been recognized that digestibility values are affected by the age of the birds used (Ten Doeschate et al., 1993; Garcia et al., 2007), therefore estimates derived using adult birds are not applicable to growing broilers. Reports regarding protein and AA digestibility of FM in broilers are very limited and there are no studies reporting SID estimates for AA in FM and PBPM.

Thus, this study was conducted to determine the apparent and standardized ileal digestibility estimates in FM and PBPM and their associated CV for broilers.

8.3 MATERIALS AND METHODS

8.3.1 DIETS

The FM and PBPM samples assayed in the present study were obtained from Rothsay's rendering plant (Dawson Road, Winnipeg, Manitoba, Canada). Five samples of each meal were collected from five different batches processed on different days. Each test diet contained a FM sample at 28.4% or PBPM at 38.4% as the sole source of protein. Dextrose and canola oil were used at 59.5%, 3.1% and 51.2%, 1.4% for the FM and PBPM diets, respectively. Solkaflor (MP Biochemicals, Solon, OH) was included at 3%

in all test diets as a source of fiber. The diets were balanced for mineral and vitamin contents to meet NRC (1994) requirement specifications and contained chromic oxide (0.3%) as a digestibility marker. A reference wheat sample (at 91.7% of the test diet) was also assayed in this experiment for comparison purposes.

8.3.2 BIRDS AND EXPERIMENTAL CONDUCT

The experimental protocol for the present study was reviewed and approved by the Animal Care Protocol Management and Review Committee of the University of Manitoba and birds were cared for according to the guidelines of the Canadian Council on Animal Care (1993).

Three hundred and ninety six day-old male broiler chicks (Ross 308, Aviagen) were obtained from a local hatchery (Carlton Hatchery, Grunthal, Manitoba, Canada) and housed in battery brooders (Petersime Incubator Co., Gettysburg, Ohio) in a room with continuous fluorescent lighting. Room temperature was maintained at 32, 28, and 24°C during week 1, 2, and 3, respectively. From day 1 to 14, birds were fed a commercial starter diet (FeedRite, Ridley Inc., Winnipeg, Manitoba, Canada) in crumble form (3100 Kcal/Kg, 19% CP). On day 14, birds were fasted for 3 h, weighed and distributed (6 birds/cage) into 66 cages with similar body weights. Six replicate cages were randomly assigned to each of the eleven test diets and fed from day 15 to 21. Birds had free access to feed and water throughout the study period. On day 21, birds were killed by cervical dislocation and digesta samples from the ileum (from Meckel's diverticulum to a point 4 cm proximal to the ileo-cecal junction) were collected by gently squeezing the contents of the ileum into sample bags. Digesta from birds within a cage were pooled into one bag

and frozen immediately after collection and subsequently freeze-dried. The dried ileal digesta were stored in airtight bags at -4°C until required for chemical analysis.

8.3.3 CHEMICAL ANALYSIS

Feed ingredients, test diets and digesta samples were finely ground using a coffee grinder (CBG5 Smart Grind; Applica Consumer Products, Inc., Shelton, CT). Dry matter was determined according to AOAC (2000) procedure # 934.01, and nitrogen was analyzed using the Leco nitrogen analyzer (Model CNS-2000; Leco Corp., St. Joseph, MI). Amino acid concentration of the feed ingredients, diet and digesta were determined by Evonik (Hanau-Wolfgang, Germany). Dietary AA contents were determined by using an ion-exchange chromatography with postcolumn derivatization with ninhydrin (Llames and Fontaine, 1994). Sulfur AA (methionine and cystine) were oxidized with performic acid prior to acid hydrolysis and subsequently neutralized with sodium metabisulfite (Commission Directive, 1998). Amino acid concentrations were quantified using the internal standard method by measuring the absorption of reaction products with ninhydrin at 570 nm. Chromium was analyzed, after the samples were ashed for 12 h at 600°C in a muffle furnace, using inductively coupled plasma mass spectrometry (ICP-AES Vista, Varian) according to the method (procedure # 985.01) of AOAC (2005).

8.3.4 DIGESTIBILITY CALCULATION

The AID coefficient of AA was calculated using the indigestible marker as follows:

$$AID = \frac{(AA/IM)_d - (AA/IM)_{id}}{(AA/IM)_d}$$

Where, $(AA/IM)_d$ = ratio of AA to indigestible marker (IM) content in diet and

$(AA/IM)_{id}$ = ratio of AA to indigestible marker (IM) content in ileal digesta

Basal endogenous AA losses determined with a nitrogen-free diet were used for the correction of AID coefficients (Golian et al., 2008). The SID coefficients were calculated as follows:

$$SID\% = AID + [(basal\ IAA_{end} / AA_{diet}) \times 100]$$

Where, IAA_{end} = basal ileal endogenous AA loss and AA_{diet} = Amino acid content of the diet.

The endogenous ileal AA loss values used to calculate the SID are presented in the Appendix.

8.3.5 STATISTICAL ANALYSIS

Digestibility data were analyzed using the GLM procedure of SAS Institute, Inc. (1990) as a completely randomized design. The Nested procedure was used to account for the random feed sample and bird/cage sub-sampling effects. To better explain the CV associated with the AID and SID estimates of each AA, the components of the total variation were further calculated and expressed as variance due to the samples (σ_s^2) and cages within samples (σ_e^2).

8.4 RESULTS AND DISCUSSION

Crude protein and AA composition of FM, PBPM and the reference wheat sample are presented in Table 8.1. Although some variation was evident, generally the results of the CP and AA composition of FM and PBPM samples compare well with previously published values for FM and PBPM (Daghir, 1975; Johnston and Coon, 1979; Baker et al., 1981; Papadopoulos et al., 1985; Wang and Parsons, 1998b; Ravindran et al., 2005). The CP and AA profile of the reference wheat sample are also in close agreement with the previous reports (manuscript I, II, III and IV). A comparison between the AA profile of FM and PBPM showed a somewhat similar trend where sulfur AA and histidine in PBPM and sulfur AA, histidine and lysine in FM had the lowest content of all AA. On the other hand, leucine and arginine in PBPM and leucine and valine in FM ranked as the highest in content of indispensable AA. Generally, the CV calculated for each AA content in FM demonstrated a wider range (from 3.5 to 8.1%) compared to PBPM (from 0.6% to 3.5%). Cystine was the most variable AA of all in both FM and PBPM with a CV of 8.1% and 3.5%, respectively. It has been shown that processing conditions have a substantial effect on reducing cystine, methionine, lysine and histidine contents as heat-labile AA in feather meal (Moran et al., 1966; Eggum, 1970; Papadopoulos et al., 1986) which agrees well with our data.

Apparent and standardized ileal digestibility of AA in FM, PBPM and the reference wheat samples are presented in Tables 8.2 and 8.3, respectively. The AID and

Table 8.1. Crude protein and amino acid concentration (%) of poultry by-product meal (n = 5) and feather meal (n = 5) samples^{1,2} (DM basis)

Item	Poultry by-product meal			Feather meal		
	Range	Mean	CV% ³	Range	Mean	CV% ³
DM	96.5 - 97.0	96.8	0.19	91.8 - 95.6	93.8	1.65
CP	61.6 - 63.5	62.6	1.1	88.7 - 98.3	95.0	4.1
<i>Indispensable amino acids</i>						
Arg	4.04 - 4.16	4.11	1.2	6.04 - 6.69	6.37	4.5
His	1.25 - 1.34	1.29	3.1	0.69 - 0.76	0.74	4.0
Ile	2.03 - 2.13	2.08	1.8	4.09 - 4.67	4.40	6.2
Leu	4.02 - 4.18	4.07	2.0	6.96 - 7.80	7.41	5.4
Lys	3.53 - 3.68	3.59	1.8	1.89 - 2.14	1.98	5.2
Met	1.05 - 1.09	1.07	1.5	0.59 - 0.64	0.61	3.5
Met+Cys	1.54 - 1.62	1.58	2.0	4.47 - 5.10	4.72	6.7
Phe	2.24 - 2.32	2.28	1.6	4.14 - 4.86	4.53	7.4
Thr	2.16 - 2.24	2.20	1.7	3.99 - 4.44	4.24	4.8
Val	2.70 - 2.86	2.78	2.2	6.22 - 7.37	6.82	7.6
<i>Dispensable amino acids</i>						
Ala	4.16 - 4.28	4.21	1.2	3.89 - 4.35	4.14	4.8
Asp	4.76 - 4.96	4.85	1.6	5.98 - 6.61	6.30	4.7
Cys	0.48 - 0.53	0.51	3.5	3.84 - 4.51	4.11	8.1
Glu	7.60 - 7.90	7.74	1.5	9.40 - 10.13	9.79	3.7
Gly	6.53 - 6.63	6.57	0.6	6.82 - 7.69	7.26	5.4
Pro	3.94 - 4.08	3.99	1.4	8.11 - 9.13	8.60	6.5
Ser	2.32 - 2.44	2.37	2.2	9.25 - 10.93	10.24	7.1

1. n = number of different samples of a feed ingredients.

2. Mean of the reference wheat sample for DM, CP, Arg, His, Ile, Leu, Lys, Met, Met+Cys, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro and Ser were 92.1, 15.6, 0.72, 0.35, 0.53, 1.04, 0.42, 0.26, 0.62, 0.70, 0.45, 0.66, 0.53, 0.77, 0.36, 4.58, 0.63, 1.52 and 0.71, respectively.

3. Coefficient of variation among different samples.

Table 8.2. Apparent ileal amino acid digestibility coefficients and their associated coefficient of variation (CV%) determined in chicks fed poultry by-product meal (n = 5) and feather meal (n = 5) samples¹

Item	Poultry by-product meal (n = 5)					Feather meal (n = 5)				
	Range	Mean	CV% ²	Variance components ³		Range	Mean	CV% ²	Variance components ³	
				Between	Within				Between	Within
<i>Indispensable amino acids</i>										
Arg	74.2 - 84.9	81.2	5.7	75.2	24.8	75.2 - 66.6	70.5	8.6	3.7	96.3
His	66.1 - 80.9	75.5	8.0	83.3	16.7	52.3 - 63.2	57.7	10.7	18.2	81.8
Ile	65.9 - 80.5	75.2	8.0	80.8	19.2	70.9 - 78.8	74.1	7.6	3.0	97.0
Leu	69.2 - 82.7	77.8	7.1	81.5	18.5	65.8 - 75.7	70.0	9.4	6.4	93.6
Lys	67.8 - 81.9	76.7	7.5	82.9	17.1	51.5 - 60.5	56.4	10.4	12.6	87.4
Met	73.0 - 84.5	80.3	6.0	79.1	20.9	63.3 - 71.0	67.9	7.0	16.2	83.8
Met+Cys	60.6 - 76.7	70.9	9.4	80.9	19.1	43.4 - 59.7	53.1	18.6	20.4	79.6
Phe	69.8 - 83.3	78.2	7.1	79.7	20.3	69.4 - 78.6	73.3	8.3	5.9	94.1
Thr	59.4 - 76.2	70.2	10.0	81.3	18.7	48.8 - 63.0	56.5	13.9	24.1	75.9
Val	66.6 - 80.8	75.7	7.8	80.3	19.7	66.5 - 76.6	71.3	9.6	6.6	93.4
<i>Dispensable amino acids</i>										
Ala	71.1 - 83.2	78.9	6.4	78.8	21.2	63.9 - 74.3	68.1	10.3	5.0	95.0
Asp	44.7 - 67.6	59.0	15.7	82.8	17.2	26.6 - 48.3	39.4	27.7	40.8	59.2
Cys	34.0 - 59.8	50.6	21.5	77.2	22.8	38.9 - 58.0	50.4	22.5	23.9	76.1
Glu	66.6 - 80.8	75.8	7.8	79.4	20.6	67.8 - 55.2	62.3	12.7	14.0	86.0
Gly	66.4 - 80.6	75.7	8.4	67.9	32.1	62.7 - 74.4	68.7	11.1	9.0	91.0
Pro	61.4 - 76.8	71.3	9.1	78.5	21.5	52.3 - 66.4	59.9	13.2	21.7	78.3
Ser	57.3 - 74.9	68.4	10.6	81.1	18.9	58.5 - 71.5	65.2	11.3	19.2	80.8

1. Five samples of each feed ingredient were assayed in six replicate cages with 6 birds in each and variance components for samples (σ_s^2) and cages within samples (σ_e^2) was determined. The apparent ileal digestibility coefficients of Arg, His, Ile, Leu, Lys, Met, Met+Cys, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro and Ser for the reference wheat sample were 81.5, 82.8, 84.3, 85.8, 76.5, 90.8, 89.0, 88.5, 74.2, 83.4, 76.2, 75.1, 86.0, 94.3, 77.8, 93.0 and 83.0, respectively.

2. Total coefficient of variation, $CV\% = \frac{\sqrt{\sigma_s^2 + \sigma_e^2}}{\bar{y}} \times 100\%$.

3. Percentage contribution to the total variation calculated as $\sigma_s^2 / (\sigma_s^2 + \sigma_e^2)$ and $\sigma_e^2 / (\sigma_s^2 + \sigma_e^2)$ for samples and cages within samples, respectively.

Table 8.3. Standardized ileal amino acid digestibility coefficients and their associated coefficient of variation (CV%) determined in chicks fed poultry by-product meal (n = 5) and feather meal (n = 5) samples¹

Item	Poultry by-product meal					Feather meal				
	Range	Mean	CV% ²	Variance components ³		Range	Mean	CV% ²	CV (%) Contribution ³	
				Between	Within				Between	Within
<i>Indispensable amino acids</i>										
Arg	75.5 - 86.2	82.5	5.6	75.0	25.0	67.7 - 76.3	71.6	8.4	3.2	96.8
His	67.8 - 82.5	77.1	7.8	83.1	16.9	55.7 - 66.9	61.3	10.2	19.9	80.1
Ile	68.5 - 83.0	77.7	7.7	80.6	19.4	72.5 - 80.3	75.7	7.4	2.4	97.6
Leu	71.1 - 84.5	79.7	6.9	81.3	18.7	67.2 - 77.0	71.4	9.2	5.8	94.2
Lys	69.1 - 83.1	78.0	7.4	82.8	17.2	54.3 - 63.7	59.5	10.0	15.3	84.7
Met	74.4 - 85.9	81.7	5.9	79.1	20.9	66.2 - 74.2	71.0	6.8	18.3	81.7
Met+Cys	79.7 - 63.8	74.1	9.0	80.6	19.4	44.9 - 61.2	54.7	18.1	20.4	79.6
Phe	77.5 - 90.6	85.8	6.3	78.8	21.2	74.7 - 83.3	78.4	7.7	3.4	96.6
Thr	64.6 - 81.2	75.3	9.2	80.9	19.1	52.3 - 66.4	59.9	13.0	23.2	76.8
Val	69.1 - 83.3	78.2	7.5	80.1	19.9	68.0 - 77.9	72.7	9.4	5.9	94.1
<i>Dispensable amino acids</i>										
Ala	72.4 - 84.4	80.2	6.3	78.7	21.3	65.7 - 76.0	69.9	10.1	4.5	95.5
Asp	47.0 - 69.8	61.3	15.1	82.7	17.3	29.0 - 50.6	41.7	26.1	40.4	59.6
Cys	41.4 - 66.5	57.6	18.5	76.4	23.6	40.1 - 59.3	51.6	21.9	23.8	76.2
Glu	68.3 - 82.4	77.4	7.7	79.3	20.7	56.9 - 69.5	64.1	12.3	13.8	86.2
Gly	67.3 - 81.5	76.7	8.3	67.8	32.2	63.9 - 75.5	69.8	10.9	8.6	91.4
Pro	63.2 - 78.5	73.1	8.8	78.4	21.6	53.5 - 67.4	61.0	12.9	21.1	78.9
Ser	61.1 - 78.4	72.1	9.9	80.7	19.3	59.7 - 72.5	66.3	11.1	18.5	81.5

1. Five samples of each feed ingredient were assayed in six replicate cages with 6 birds in each and variance components for samples (σ_s^2) and cages within samples (σ_e^2) was determined. The standardized ileal digestibility coefficients of Arg, His, Ile, Leu, Lys, Met, Met+Cys, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro and Ser for the reference wheat sample were 84.8, 85.6, 88.7, 89.1, 81.3, 93.1, 92.5, 99.0, 85.0, 88.1, 80.9, 81.5, 90.7, 95.5, 82.3, 95.1 and 88.5, respectively.

2. Total coefficient of variation, $CV\% = \frac{\sqrt{\sigma_s^2 + \sigma_e^2}}{\bar{y}} \times 100\%$.

3. Percentage contribution to the total variation calculated as $\sigma_s^2 / (\sigma_s^2 + \sigma_e^2)$ and $\sigma_e^2 / (\sigma_s^2 + \sigma_e^2)$ for samples and cages within samples, respectively.

SID of AA determined in wheat compares very well with the results presented elsewhere (Manuscript I, II, III and VI) which indicates a high level of repeatability among the assays. The AID results show a higher AA digestibility range for PBPM (from 50.6% to 81.2%) compared with FM (from 39.4% to 74.1%). Cystine (50.6%) and aspartic acid (39.4%) were the least digestible AA in PBPM and FM, respectively. Among indispensable AA, threonine (70.2%) and methionine+cystine (70.9%) for PBPM and methionine+cystine (53.1) and lysine (56.4%) for FM were the least digestible. In contrast, valine (71.3%), phenylalanine (73.3%) and isoleucine (74.1%) in FM and alanine (78.9%), methionine (80.3%) and arginine (81.2%) in PBPM were the most digestible indispensable AA. With a slight change of order due to the correction made, the SID values followed the same trend of digestibility for all AA as those of AID. Cystine (57.6%) in PBPM and aspartic acid (41.7%) in feather meal were found to be the least digestible AA whereas phenylalanine was the most digestible in both. Comparing SID values of both FM and PBPM shows that lysine was among the least digestible AA in FM (59.5%) while it was better digested in PBPM (78%). Results published for the average true AA digestibility estimates for FM and PBPM in NRC (1994) are somewhat higher than those found in the present study. This could be partly explained by the fact that the methodology and age of the birds used in experiments can affect digestibility results from different assays (Ten Doeschate et al., 1993; Ravindran and Hendriks, 2004b; Garcia et al., 2007).

As mentioned earlier, digestibility estimates of processed products are very variable and this might be due to the feed samples used or assay condition yet the pooled variation reports in the literature lacks the transparency required to explain them

separately. Since total variation (CV%) observed in estimating AID and SID values are affected by both the feed samples (between) and assay condition (within), it is of benefit to separate the total variance into these components to demonstrate their contribution separately. This will find significance when reporting the variability observed due to the effect of feed samples (between) for each AA estimate. Overall, the CV associated with the AID estimates of AA in PBPM was less variable (from 5.7 to 21.5%) and was mostly due to the feed sample whereas those of FM varied more (from 7 to 27.7%) and were mainly due to the assay condition (cage to cage differences). The CV associated with AID estimates of cystine and aspartic acid was the most variable of all AA in both PBPM and FM. Low methionine, lysine and histidine contents together with their lower digestibility in FM reflect its severe deficiency in the respective AA for broiler nutrition. Indeed, a severe deficiency for these AA in FM for the chicken has been reported by other researches (Naber et al., 1961; Moran et al., 1966; Morris and Balloun, 1973; MacAlpine and Payne, 1977; Baker et al., 1981; Scott et al., 1982). It has been shown that AA imbalance in feed can depress appetite (Sanahuja et al., 1965; Peng and Harper, 1970; Payne, 1972). If the CV associated with AID values for each FM sample is because of depressed appetite and varied feed intake of the birds, then the total CV of each AA determined in FM is expected to be affected by within sample component. Therefore, as it was expected a wider variation of the total CV was due to cage to cage difference (within) than the samples themselves (between). On the contrary, PBPM is a more digestible feed than FM and it was shown that mixing PBPM with FM resulted in superior bird performance than using FM alone (Bhargava and O'Neil, 1975; Daghir, 1975). Thus, the

total variation observed for each AA estimate in PBPM was more affected by the samples (between) than the assay condition (within).

Although routinely used in poultry diets, animal protein supplements can vary widely in protein and AA quality mainly due to the nature of the raw material and the processing methods employed (Parsons et al., 1997). It has been shown that the loss of AA due to thermal processing may be followed by the formation of unusual AA (lanthionine, lysinoalanine, ornithinoalanine) (Davis et al., 1961; Baker et al., 1981; Papadopoulos et al., 1986). Extensive hydrolysis causes the conversion of cystine to lanthionine which in several stereoisometric forms, can spare cystine in an animal's diet but, on a weight basis, is not as effective as cystine (Jones et al., 1948; Robbins et al., 1980). In contrast, the indispensable AA, valine, leucine and phenylalanine were shown to be rather stable in thermal processing (Papadopoulos et al., 1986) thus maintaining a relatively higher content which is in agreement with our results. Reports indicate that AA imbalances in these processed feeds has resulted in discrepancies in regard to deciding their maximum level of inclusion in chicken diets without producing a depressed growth rate or feed conversion (Moran et al., 1966; Portsmouth et al., 1970; Daghir, 1975; Baker et al., 1981; Escalona and Pesti, 1987).

It can be substantiated from the above literature reports that, apart from different methodology used, for an effective incorporation of these feed ingredients as protein supplements in broiler diets, it is necessary not only to determine their available AA profile but also to account for their associated variation. As explained earlier SID values are shown to be a more accurate estimate of the AA availability in feedstuff and when used in a mixed diet, they are shown to be more additive for different feed ingredients

(Angkanaporn et al., 1996; 2001; Stein et al., 2005). The reports regarding AA digestibility of FM and PBPM are very scarce and determined using the rooster assay. Thus, this study was conducted to investigate and report a data set which can be used when incorporating FM and PBPM in broiler diets. To better express the variability, CV was further partitioned and expressed as the source of the variation due to the feed samples and the assay condition (cage to cage variation).

8.5 Conclusion

The AID results in this study showed a slightly wider AA digestibility range for PBPM (from 50.6% to 81.2%) compared with FM (from 39.4% to 74.1%). Cystine (50.6%) and aspartic acid (39.4%) were the least digestible AA in PBPM and FM, respectively. Among indispensable AA, threonine (70.2%) and methionine+cystine (70.9%) for PBPM and methionine+cystine (53.1) and lysine (56.4%) for FM were the least digestible. In contrast, valine (71.3%), phenylalanine (73.3%) and isoleucine (74.1%) in FM and alanine (78.9%), methionine (80.3%) and arginine (81.2%) in PBPM were the most digestible indispensable AA. Regarding SID estimates the same trend as in AID estimates was observed with cystine (57.6%) in PBPM and aspartic acid (41.7%) in FM as the least digestible AA whereas phenylalanine was the most digestible in both test materials. The total CV associated with FM AA digestibility estimates was greatly affected by within sample variation which can reflect a variable bird feed intake response, whereas PBPM was more affected by between sample variation. Both the AID and SID results of protein and AA in the reference wheat were in agreement with the previous

reports (Manuscripts I, II, III and IV), highlighting a high repeatability in the conduct of the assays.

9.0 GENERAL DISCUSSION

The main goal of nutritionists in terms of AA and protein feeding is to satisfy the respective requirements for a specific production target in a sustainable and economic manner. The high cost associated with providing dietary protein has sparked attempts to increase the incorporation of an array of cheaper, alternative protein supplements in poultry diets without depressing animal performance. Protein and AA composition of both intact and processed feeds vary widely due to several factors as reported earlier (Papadopoulos et al., 1986; Gatel, 1994; Ravindran and Bryden, 1999; Gutierrez-Alamo et al., 2008). Thus, their effective and efficient use in diet formulation as protein sources will require an appraisal of both their AA availability and the associated variation from batch to batch. Furthermore, an improvement in the accuracy of diet formulation for the target requirement will make a major contribution towards minimizing the nitrogen output and subsequent release into the environment.

Although the AA content of protein sources can be determined by chemical analysis, a proportion of these AA will not be completely digested and absorbed by the animal. As has been explained elsewhere (Ravindran and Bryden, 1999; Lemme et al., 2004), ileal digestibility estimation of protein and AA in broilers is a preferable method to express the AA availability in a given feed ingredient (Stein et al., 2007). Nonetheless, this estimation (AID) is affected by the level of dietary protein intake and endogenous losses. A correction based on basal endogenous losses has been suggested to transform the former values (AID) into a new set of values which are more diet independent and accurate (SID) (Lemme et al., 2004). Use of SID values in complete diets has been shown

to be more additive than AID values (Angkanaporn et al., 1996; Stein et al., 2005).

Therefore, this thesis represents a series of studies conducted to calculate and determine the standardized and apparent ileal digestibility of protein and AA in several selected feed ingredients fed to broilers.

Digestibility estimates are affected by various factors including assay conditions. To assess the repeatability of the broiler ileal digestibility assay as used in our laboratory, ileal AA digestibilities in a sample of wheat (AC Barrie) were determined in a series of six independent assays as a reference ingredient. The total inter-assay CV ranged from 0.8 to 4.7% for glutamic acid and lysine, respectively (Table 9.1). No significant ($P \leq 0.05$) differences were detected in the AID of CP and all AA among assays. As expected, assuming that the use of the same wheat at the same level of diet would have the same effect on bird digestibility, the calculated breakdown of the determined total CV into its components pointed to a high variation contribution from within assay rather than between assays. These findings prove the reproducibility of the ileal AA digestibility assay and confirm that the digestibility values obtained in different assays are comparable. A less than 5% CV and no significant differences ($P \leq 0.05$) among the AID results of these assays for wheat give us enough confidence to say that the digestibility values obtained in different assays are comparable. For comparison purposes the results of protein and AA composition, SID and total CV associated with protein and AA digestibility estimates for each ingredient are summarized in Tables 9.2, 9.3 and 9.4.

As it is evident from Table 9.2, among unprocessed plant protein sources studied, flaxseed had a higher CP and AA content compared to wheat and barley. The protein and AA profile of flaxseed was slightly different but comparable to that of pea. In terms of the

Table 9.1. Apparent ileal crude protein and amino acid digestibility coefficients and their associated coefficient of variation (CV%) determined in chicks fed diets containing reference wheat sample in six different assays¹

Item	Assay						CV% ²	Variance components ³		P-value ⁴	
	1	2	3	4	5	6		Average	Between samples		Within samples
CP	86.0	83.3	85.4	MD ⁵	84.8	86.0	85.2	2.3	13.2	86.8	0.15
<i>Indispensable amino acids</i>											
Arg	80.3	81.4	80.7	81.5	82.7	84.3	81.8	3.9	9.9	90.1	0.21
His	84.0	84.6	83.6	82.8	84.9	85.9	84.3	2.8	0.0	100.0	0.31
Ile	87.8	86.7	86.9	84.3	87.0	87.7	86.7	2.3	0.0	100.0	0.05
Leu	88.0	88.0	87.6	85.8	87.6	88.9	87.6	2.1	0.0	100.0	0.13
Lys	78.8	77.8	77.5	76.5	79.7	81.2	78.6	4.7	0.0	100.0	0.36
Met	90.8	91.1	89.9	90.8	88.7	89.9	90.2	2.0	33.7	66.3	0.12
Met+Cys	89.1	89.6	88.5	89.0	87.8	89.0	88.8	1.8	0.0	100.0	0.56
Phe	87.9	87.7	89.0	88.5	88.2	88.8	88.4	1.9	0.0	100.0	0.76
Thr	77.9	76.6	74.0	74.2	77.9	77.6	76.3	4.2	10.9	89.1	0.09
Val	83.5	82.8	82.4	83.4	84.3	85.0	83.6	2.9	0.7	99.3	0.48
<i>Dispensable amino acids</i>											
Ala	79.3	79.9	78.9	76.2	79.9	81.6	79.3	3.8	0.0	100.0	0.09
Asp	78.1	78.1	76.1	75.1	77.9	79.3	77.4	4.1	0.0	100.0	0.23
Cys	88.1	86.9	87.1	86.0	87.0	87.7	87.1	2.1	0.0	100.0	0.45
Glu	94.9	95.1	94.8	94.3	94.7	95.3	94.9	0.8	0.0	100.0	0.32
Gly	80.2	80.4	79.3	77.8	80.7	81.9	80.0	3.5	0.0	100.0	0.19
Pro	94.2	94.0	93.3	93.0	93.7	94.0	93.7	1.0	0.0	100.0	0.32
Ser	85.0	84.8	83.1	83.0	84.9	85.5	84.0	2.5	1.6	98.4	0.25

1. Same sample of wheat was fed to six cages (6 or 8 birds/cage) in each assay.

2. Total coefficient of variation, $CV\% = \frac{\sqrt{\sigma_s^2 + \sigma_e^2}}{\bar{y}} \times 100\%$.

3. Percentage contribution to the total variation calculated as $\sigma_s^2 / (\sigma_s^2 + \sigma_e^2)$ and $\sigma_e^2 / (\sigma_s^2 + \sigma_e^2)$ for samples and cages within samples, respectively.

4. P-values of comparison of crude protein and amino acid values across six different assays.

5. Missing data.

Table 9.2. Crude protein and amino acid composition (%) of different feed ingredients¹ (DM basis)

Ingredient	Wheat DDGS ²	Flaxseed	Wheat	Pea	Barley	DESBM ²	ENERMAX TM	PBPM ²	FM ²
DM	95.8	95.6	94.0	94.2	92.1	93.3	93.5	96.8	93.8
CP	39.9	24.2	16.2	23.6	14.3	45.8	24.0	62.6	95.0
<i>Indispensable amino acids</i>									
Arg	1.61	2.31	0.76	2.03	0.68	3.24	1.58	4.11	6.37
His	0.82	0.50	0.38	0.59	0.30	1.16	0.57	1.29	0.74
Ile	1.37	1.00	0.53	1.01	0.48	2.04	0.96	2.08	4.40
Leu	2.63	1.40	1.05	1.70	0.99	3.43	1.68	4.07	7.41
Lys	0.74	0.93	0.44	1.73	0.49	2.81	1.34	3.59	1.98
Met	0.61	0.46	0.25	0.20	0.24	0.67	0.37	1.07	0.61
Met+Cys	1.36	0.87	0.59	0.54	0.52	1.38	0.81	1.58	4.72
Phe	1.81	1.14	0.74	1.15	0.76	2.26	1.08	2.28	4.53
Thr	1.18	0.88	0.45	0.86	0.47	1.82	0.95	2.20	4.24
Val	1.70	1.20	0.66	1.11	0.70	2.15	1.16	2.78	6.82
<i>Dispensable amino acids</i>									
Ala	1.42	1.06	0.55	1.00	0.55	1.96	1.02	4.21	4.14
Asp	1.86	2.28	0.80	2.14	0.81	5.18	2.14	4.85	6.30
Cys	0.75	0.42	0.35	0.34	0.28	0.71	0.44	0.51	4.11
Glu	11.03	4.72	4.65	3.87	3.58	8.04	3.90	7.74	9.79
Gly	1.57	1.42	0.65	1.02	0.54	1.93	1.06	6.57	7.26
Pro	3.54	0.81	1.64	0.98	1.59	2.14	1.11	3.99	8.60
Ser	1.76	1.11	0.72	1.09	0.61	2.32	1.10	2.37	10.24

1. Except for wheat and barley where 6 and 7 different samples of each were assayed, respectively, all other ingredients had 5 different samples analyzed.

2. Wheat DDGS (wheat distillers dried grain with soluble); DESBM (dry-extruded expelled soybean meal); PBPM (poultry by-product meal); FM (feather meal).

Table 9.3. Standardized ileal protein and amino acid digestibility in chicks fed diets containing different feed ingredients¹

Item	Raw				Processed				
	Flaxseed	Wheat	Pea	Barley	Wheat DDGS ²	DESBM ²	ENERMAX TM	PBPM ²	FM ²
CP	55.0	87.2	88.3	79.7	69.1	88.2	73.4	MD ³	MD ³
<i>Indispensable amino acids</i>									
Arg	64.6	85.2	94.2	80.4	70.8	92.9	84.7	82.5	71.6
His	56.5	87.1	90.3	80.7	65.7	89.6	76.8	77.1	61.3
Ile	57.0	90.4	88.8	83.9	71.7	89.5	75.0	77.7	75.7
Leu	60.4	90.5	89.6	84.8	75.6	89.6	76.8	79.7	71.4
Lys	66.3	83.7	92.9	80.5	40.0	90.8	78.8	78.0	59.5
Met	66.7	91.4	89.8	88.3	75.7	92.5	83.4	81.7	71.0
Met+Cys	64.3	91.1	86.0	86.1	70.2	87.5	77.1	74.1	54.7
Phe	63.8	93.8	92.2	90.9	86.4	95.9	80.9	85.8	78.4
Thr	47.4	85.4	89.1	80.6	62.2	88.0	72.7	75.3	59.9
Val	55.8	87.7	88.8	82.5	67.8	88.4	74.1	78.2	72.7
<i>Dispensable amino acids</i>									
Ala	57.4	83.8	90.2	78.1	63.6	89.0	77.1	80.2	69.9
Asp	55.8	83.8	89.9	78.1	47.0	87.7	75.5	61.3	41.7
Cys	61.5	90.8	83.7	83.9	65.4	82.5	71.8	57.6	51.6
Glu	68.4	96.6	94.2	87.6	84.1	91.4	82.1	77.4	64.1
Gly	58.9	84.1	89.1	76.7	60.3	86.0	73.6	76.7	69.8
Pro	67.0	95.4	88.9	86.6	82.8	89.4	74.4	73.1	61.0
Ser	55.0	89.1	89.3	82.2	69.6	88.6	72.9	72.1	66.3

1. Except for wheat and barley where 6 and 7 different samples of each were assayed, respectively, all other ingredients had 5 samples assayed.

2. Wheat DDGS (wheat distillers dried grain with soluble); DESBM (dry-extruded expelled soybean meal; PBPM (poultry by-product meal); FM (feather meal).

3. Missing data.

Table 9.4. Total coefficient of variation associated with standardized ileal protein and amino acid digestibility estimates in different feed ingredients¹

Item	Raw				Processed				
	Flaxseed	Wheat	Pea	Barley	Wheat DDGS ²	DESBM ²	ENERMAX TM	PBPM ²	FM ²
CP	17.1	1.8	2.1	4.5	9.2	4.0	2.3	MD ³	MD ³
<i>Indispensable amino acids</i>									
Arg	18.7	2.3	1.4	5.5	8.9	2.7	1.5	5.6	8.4
His	17.1	1.7	2.1	5.0	11.5	3.2	3.7	7.8	10.2
Ile	17.1	1.6	2.6	4.7	8.7	3.7	2.2	7.7	7.4
Leu	14.8	1.5	2.5	4.4	7.0	3.6	1.9	6.9	9.2
Lys	14.4	2.4	1.9	5.3	32.6	2.8	2.0	7.4	10.0
Met	13.8	1.5	3.0	3.6	6.9	2.9	2.4	5.9	6.8
Met+Cys	13.6	1.5	2.6	3.9	9.1	4.5	2.4	9.0	18.1
Phe	14.1	1.4	2.2	5.0	5.0	3.1	2.4	6.3	7.7
Thr	20.2	3.1	2.3	5.7	13.7	4.4	2.9	9.2	13.0
Val	17.4	1.9	2.5	4.7	10.5	4.0	2.3	7.5	9.4
<i>Dispensable amino acids</i>									
Ala	18.4	2.3	2.1	5.6	12.6	3.5	1.9	6.3	10.1
Asp	19.3	2.4	2.1	5.5	24.8	4.0	2.4	15.1	26.1
Cys	15.8	1.6	2.9	4.9	11.9	6.5	2.6	18.5	21.9
Glu	13.2	0.6	1.8	3.9	4.3	3.1	1.5	7.7	12.3
Gly	18.2	2.3	2.1	5.7	14.8	4.2	2.4	8.3	10.9
Pro	11.7	0.8	2.3	4.0	5.1	3.6	2.0	8.8	12.9
Ser	17.0	1.8	2.1	4.5	9.8	3.8	2.8	9.9	11.1

1. Except for wheat and barley where 6 and 7 different samples of each were assayed, respectively, all other ingredients had 5 samples assayed.

2. Wheat DDGS (wheat distillers dried grain with soluble); DESBM (dry-extruded expelled soybean meal; PBPM (poultry by-product meal); FM (feather meal).

3. Missing data.

content of each AA relative to CP, when comparing the AA profile of wheat and barley there is a close association between the two. Defined by their storage protein composition, wheat and barley are acknowledged to be low in lysine content unlike peas (D'Mello, 1993). The lysine content in flaxseed was approximately double as much as wheat and barley but slightly less than pea.

Among the processed feed ingredients studied, FM had the highest and ENERMAXTM had the lowest content of CP and AA compared to the others. Apart from the main ingredients used, the protein and AA content of processed feedstuff are known to be greatly affected by the processing conditions (Papadopoulos et al., 1986; Woodworth et al., 2001). Effect of heating on reducing methionine, cystine, lysine and histidine contents as heat-labile AA is well documented (McGinnis and Evans, 1947; Papadopoulos et al., 1986; Parsons et al., 1992). In fact, comparing AA profile of processed ingredients in the present study revealed a lower content of methionine, cystine, lysine and histidine relative to other AA. Relative to CP, the AA profile of ENERMAXTM was evident with approximately a 1.5-fold higher sulfur AA but 0.8-fold lower lysine content compared with that of pea. On a DM basis, the AA concentration in wheat DDGS was two to three-fold higher than the content in wheat. Lower sulfur AA and lysine content relative to other AA was maintained in both wheat DDGS and wheat.

The SID values for different feed ingredients assayed are summarized in Table 9.3. Among the unprocessed feed grains, pea had the highest SID values followed by wheat, barley and flaxseed. Although, wheat was slightly less digestible than pea, its SID value of sulfur AA was higher than those in pea. Conversely, lysine was more digestible in pea than it was in wheat. Barley had a lower SID value for protein and AA compared to

wheat. Relative to other AA, lysine in wheat (83.7%) and alanine (78.1%) in barley were the least digestible. Higher fiber content and NSP in barley are the main factors for its lower digestibility (Classen et al., 1988; Ravindran et al., 2007). Flaxseed was the least digestible compared to pea, wheat and barley. Its AA digestibility (SID) ranged from 47.4 to 68.4% for threonine and glutamic acid, respectively. Lysine (66.3%) and methionine (66.7%) in flaxseed were the most digestible indispensable AA relative to others. As explained in detail (Manuscript II), high content of mucilage and fiber in flaxseed are the main factors depressing its digestibility (Ravindran and Blair, 1992).

As it has been discussed before (Manuscript IV and V), AA availability in processed feed ingredients is greatly affected by processing conditions. Among the processed feed ingredients studied, in terms of protein and AA, DESBM was the most digestible with its SID values ranging from 82.5 to 92.9% for cystine and phenylalanine, respectively. The SID values for protein and AA in wheat DDGS compared with wheat were very low ranging from 40 to 86.4% for lysine and phenylalanine, respectively. ENERMAXTM had an overall lower AA digestibility compared to pea. Although ENERMAXTM had a higher sulfur AA content compared to pea, its SID value for sulfur AA was lower than that of pea (77.1% vs. 86.0%). Poultry by-product meal was more digestible with lesser variability compared to FM in both the AID and SID estimates.

A common point among all processed feeds was that they all had the heat-labile AA including lysine (in wheat DDGS and FM), sulfur AA, glycine and cystine ranked among the lowest SID values whereas phenylalanine as reported to be stable in thermal processing (Papadopoulos et al., 1986) was rated as the most digestible AA of all, except in ENERMAXTM where arginine was the most digestible AA.

For general comparison of total CV associated with SID estimates, the data are summarized in Table 9.4. The CV associated with SID estimates of protein and AA in flaxseed was the most variable among the unprocessed feed ingredients tested followed by barley, pea and wheat. The high variability in flaxseed could be attributed to the presence of varied amounts of ANF (e.g. high and varied fiber and mucilage content) and their subsequent effect on bird's feed intake which is discussed in detail in Manuscript (II). Barley, as mentioned earlier, contains high amount of fiber and NSP varying based on its inherent quality which can translate into a high and variable CV. In fact, further analysis of total CV in barley points at a more between samples variation than within (Manuscript III). The total CV associated with the SID estimation of threonine in flaxseed, barley and wheat was the most variable of all AA, whereas methionine was the most variable in pea.

The CV associated with SID estimates of protein and AA in processed feeds was generally high. Varied effect of heating on AA content during processing and the effect of AA imbalances on the feed intake can affect the results of protein and AA digestibility. The CV associated with the SID estimates of AA was higher in wheat DDGS and FM compared to ENERMAXTM and PBPM with ENERMAXTM as the least variable of all. This could reflect more consistent processing conditions for the different batches of the product assayed which ultimately resulted in more consistent estimates. Apart from the added-back soluble, excessive heat treatment on wheat DDGS seems to have exacerbated the already variable content of available lysine in wheat to a greater extent, such that the total CV associated with the SID estimate of lysine was the most variable of all. In both FM and PBPM, aspartic acid and cystine estimates of SID were the most variable. Higher

CV associated with the estimates of cystine in FM and PBPM can have two main reasons. First the source materials used in their production and second the processing condition, each of which has been discussed in detail in Manuscript V.

It can be substantiated from these studies that in terms of the CP digestibilities among the unprocessed and processed feed ingredients tested, pea (88.3%) and DESBM (88.2%) were the most digestible, whereas flaxseed (55%) and feather meal (the CP digestibility of FM was calculated by averaging the digestibilities of each of the AA presented) (64.7%) were the least digestible, respectively. The average CV associated with SID estimates of AA was highest in flaxseed (16.2%) and feather meal (12.1%) and lowest in wheat (1.8%) and ENERMAXTM (2.3%) for unprocessed and processed feed ingredients assayed, respectively.

10.0 SUMMARY AND CONCLUSIONS

The determination of protein and AA digestibility in feedstuffs is an important criterion in feed evaluation. Total protein and AA content of feedstuffs determined by chemical analysis can not be used as an indication for the amount which is actually available to the animal. As peptides and AA are absorbed in their native chemical form, AA digestibility of a feedstuff can give a good indication of their availability. Digestibility of protein and AA determined at the ileal level with growing chicks has been shown to be superior to their total tract digestibility determined with adult roosters. Furthermore, standardized ileal digestibility estimates of feedstuffs are more additive when used in formulating mixed diets. It should be noted that AA digestibility varies considerably among different feedstuffs and between samples within a feedstuff. Thus, for an efficient diet formulation, it is important to account for this variability. Hence, a series of experiments were conducted to determine the standardized ileal digestibility of protein and AA and their associated variability in several selected feedstuff fed to broilers. As it has been recognized that digestibility assays are subject to variation due to the assay condition, a sample of a reference wheat was assayed separately and concurrently with each experiment and the results were compared to conclude a high repeatability of the assays. This will allow for comparisons of the digestibility results for feedstuffs assayed in different experiments to be made.

Based on the results obtained in these studies, it can be concluded that:

1. The results obtained in the direct method of conducting six separate ileal protein and AA digestibility assays using broiler chicks fed wheat are reproducible which proves the

repeatability of ileal digestibility assays and therefore allowing for the comparisons among the results of different assays to be made.

2. Digestibility values (AID and SID) are different in various feed ingredients and their total protein and AA content is merely a reflection of their potential nutritive value. For example, despite higher protein and AA concentration, AA wheat DDGS and flaxseed are significantly less digestible than wheat.

3. The average SID estimates of CP in pea, DESBM, wheat, barley, PBPM (calculated as the average digestibility value of each AA presented), ENRMAXTM, wheat DDGS, FM (calculated as the average digestibility value of each AA presented), and flaxseed are 88.3, 88.2, 87.2, 79.7, 75.8, 73.4, 69.1, 64.7 and 55.0, respectively.

4. The average CV associated with SID estimates of AA is highest in flaxseed (16.2%) and feather meal (12.1%) and lowest in wheat (1.8%) and ENERMAXTM (2.3%) for unprocessed and processed feed ingredients assayed.

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10.0 APPENDIX

Ileal endogenous amino acid flow (mg/kg of DM intake) in chicks fed a nitrogen-free diet

CP	4368
<i>Indispensable amino acids</i>	
Arg	203
His	91
Ile	200
Leu	298
Lys	173
Met	65
Phen	420
Thr	434
Trp	71
Val	270
<i>Dispensable amino acids</i>	
Ala	217
Asp	430
Cys	143
Glu	492
Gly	245
Pro	289
Ser	343

Adapted and modified from Golian et al. (2008)